**ENZYMATIC BROWNING: BROWNING REACTION, INHIBITION OF ENZYMATIC BROWNING BY VARIOUS METHODS; REVIEW**

**INTRODUCTION:**

The agricultural produce of fruits and vegetables such as apples, apricots, bananas, grapes, peaches, strawberries, brinjal, and potatoes cut and exposed to air leads to the development of a brown color on the surfaces. Enzymatic browning is due to the development of a brown color with the help of an enzyme called polyphenol oxidase. Enzymatic browning is undesirable for food industries and leads to off-flavor development and loss of nutritional quality. There are about 50% losses of fruits and vegetables due to enzymatic browning (Daniel et al., 2010). So, it can be prevented by various processing methods including chemical treatment, and thermal and non-thermal methods. Polyphenols are naturally occurring in fruits, vegetables, cereals, and beverages. In some cases, such as the processing of prunes, black raisins, black figs, tea, coffee, and cocoa, PPO activities are desirable and act as protection against insects and micro-organisms (Whitaker, 1995). These polyphenols are classified based on the number of phenol rings broadly divided into four classes- phenolic acids, flavonoids, stilbenes, and Lignans (Pandey et al., 2009). Secondary metabolites of polyphenols are little responsible for color, astringency, bitterness, flavor, and nutritional qualities in agricultural produce (Macheix et al., 1990).

**Enzymatic browning reaction:**

Polyphenol oxidase (PPO) are enzyme belonging to the members of oxidoreductases. This PPO, also called metalloproteinase, containing a group of copper catalyzes the hydroxylation of monophenols to O-diphenols and forms Ortho-quinone by undergoing oxidation. (Mizobutsi et al., 2010). Phenolic compounds present in fruits and vegetables undergoing an oxidation reaction in the presence of polyphenol oxidase enzymes form a brown color on the cut surfaces. Compared to high-acidic fruits, low-acidic fruits oxidize and form a brown color when they are exposed to the atmospheric air. Brown formation occurs when the fruits are immediately exposed to air and the process is called enzymatic browning or oxidation. The discoloration of agricultural produce is due to an enzyme known as polyphenol oxidase which oxidizes the phenolic compounds in the tissues and causes them to form a brown color. Enzymatic browning is an oxidative reaction that involves two oxidoreductase enzymes: known as polyphenol oxidase (PPO) and peroxidase (POD). PPO catalyzes two reactions:

The first step is slow compared to the next and forms a colorless compound by undergoing hydroxylation of monophenols to diphenols.

The second step is the fastest and gives a rapid-colored product by converting diphenols to quinones through oxidation.

**Step -1**

 +Cu 2+ Prosthetic group oxidation, phenols

PPO O- Quinone

**Step- 2**

 Spontaneous polymerization

O- Quinone Melanins

 (Brown pigment)

**Step -3**

Melanin+ Amino acids or proteins = Enhancement of brown color/pigment

**Prevention of enzymatic browning:**

Enzymatic browning occurs because PPO enzymes react to one or more substrates such as oxygen. We can prevent the formation of enzymatic browning by heat treatment, chemical, physical, and combination of several methods by removing one or both of the substrates, by lowering the pH to the optimum below, by inactivation of the enzymes, or by preventing the melanin formation.

There are different methods used to prevent enzymatic browning in fruits and vegetables are

1. Chemical methods such as anti-oxidation, acidifying agent, firmness agent, or chelating agent
2. Physical methods- blanching, freezing, and modifying atmospheric packaging (MAP)
3. Coating methods
4. A combination of several preservation methods

**Pre-treating of fruits:** Fruit cuticles have the hydrophobic nature of biopolymers between which there are waxes, representing the limitation to the diffusion of molecules used in chemical treatments or to the physical treatment efficiency such as blanching. Permeabilisation is one of the pre-treatment techniques for the cuticle which helps for better treatment in the core of the products (Irina Ioannou, 2013).

**Antioxidant:** Anti-oxidants are substances that help to prevent the initiation of a browning reaction by reacting with oxygen. These anti-oxidants also react with the intermediate products, thus breaking the chain reaction and preventing the formation of melanin (Lindley et al., 1998). The anti-oxidants mainly used are hexylresorcinol, erythorbic acid, N-acetyl cysteine, cysteine hydrochloride, ascorbic acid, and glutathione. Their effectiveness depends on pH, water activity, temperature, light, and atmospheric composition (Irina Ioannou, 2013).

**Chelating agents:** Chelating substances are compounds that form complexes with metal ions. PPO is a metal proteinase of copper. Chelating substances help to bind the copper metal and form a complex, thus helping to prevent browning. The important chelating agents used in enzymatic browning are kojic acid, citric acid, and EDTA (Irina Ioannou, 2013).

**Firmness agents:** Firmness agents are used for strengthening cell walls, thus preventing the destruction of cell compartments, and also react with PPO with polyphenols. The main firmness agents are calcium salts such as calcium lactate, calcium propionate, calcium chloride, calcium ascorbate, and sodium chloride (Irina Ioannou, 2013)

**Acidifying agents:** Fruits are acidic. Moreover, when adding the acidifying agent, it helps to increase the acidic condition and maintain the optimal pH 2. PPO is sensitive to pH variations. The main acidifying agents are citric acid, erythorbic acid, ascorbic acid, and glutathione (Irina Ioannou, 2013).

**Blanching:** Blanching is a heat treatment method to inactivate the enzymes responsible for sensory, prevent oxidation, improve the color, and destroy the micro-organisms. Blanching is a method of dipping the products in boiling water or steaming for 2-3 minutes. Blanching is used as pre-treatment for the process of freezing, sterilization, and lyophilization. PPO is an enzyme sensitive to high temperatures. The blanching of plums above 80 0C (Ozel and others, 2010) and pineapples above 40 0 C inactivates polyphenol oxidase. Oxidative activity of polyphenol oxidase varies according to temperature; as the temperature increases the activity of enzymes increases. Once it reaches the optimal activity of enzymes, it lowers the activity of enzymes with an increase in temperature.

**High Hydrostatic Pressure Treatment (HHP):** HHP is a non-thermal treatment that helps to reduce microbial growth by inactivating the microbial cells (Bayindirli, A., 2006). This method has advantages over thermal processing because of minimal effects on the food compounds such as flavoring agents, pigments, and vitamins (Butz, P 2003).Messens., W 1997). HHP treatment of fruit and vegetable products produces high quality, and safety and increases the shelf life. By applying pressure and temperature, it affects the protein conformation and leads to protein denaturation. Pressure influences biochemical reactions by reducing molecular spacing and increasing interchain reactions. The efficacy of treatment depends on the type of enzyme, pH, temperature, time, duration of pressure, and medium composition (Hendrickx, M 1998). There is 90% inactivation of (Kim., 2001). HHP inactivates the enzymes by affecting the protein confirmation using applied pressure, the temperature, and the duration of the pressure treatment. The efficacy of treatment depends on the type of enzymes, temperature, medium of composition, time, and pressure level (Hendrickx, M 1998). PPO when treated gets inactivated at 600 MPa, a pressure higher than 400MPa combined with a mild heat temperature of 50 0 C. Inactivation of PPO was observed at 90% in lychee by treating HHP at 600MPa and 60 0C for 20 minutes for lychee (Queiroz., 2008). PPO is more impervious to pressure than thermal treatment. HHP is used to obtain analogue products with negligible effect on flavor, color, and nutritional value and deprived of any thermal degradation (Messens, W 1997).

**Pulsed Electric Field:** PEF is a non-thermal food preservation technology conducted by introducing the food into a chamber containing two electrodes that apply high voltage pulses of 20-80kV for microseconds. PEF causes irreversible loss of cell membrane functionality in food. That process, known as electroporation, helps to inactivate the microbial cells (Cserhalmi, Z 2006; Zhong K 2005). This treatment is used in the processing of liquid foods (Garcia, D 2005; Evrendilek, G A 2004; Li, S-Q 2004; Queroz., 2008).

**Gamma Irradiation:** Agricultural produce can be treated by ℽ irradiation to inactivate micro-organisms such as bacteria, molds, and yeast by directly exposing them to electrons or electromagnetic rays, helps to preserve the food by increasing the shelf-life, improves quality, and safety, delays the ripening process and senescence (Lemma, J 1999). Low-dose irradiation treatment can be used to apply to fruit and vegetable products. Low-dose treatment does not affect the sensory characteristics such as visual quality and off-flavor development (Prakash A 2006; Zhang et al 2006) but helps in reducing the decay of micro-organisms at a dose of 1.0kGy in fresh-cut lettuce and the shelf life was 9 days based on microbial safety. At the dose of 1kGy, PPO activity from lettuce reduces to 31% more than the untreated stored at 4 0C for 3 days. Irradiation treatment is used to combine with other methods or chemical anti-browning effectors. An amalgamation of calcium ascorbate dipping and low-dose ionizing radiation resulted in microbiologically safe and high-quality fresh-cut apples (Fan X 2005; Queiroz., 2008).

**Freezing:** Freezing is a technique used to convert liquid water to a solid form by lowering the temperature to less than zero degrees Celsius. Freezing not only helps to reduce microbial growth by building up water availability but is used to stop browning reactions in fruit by reducing the availability of water for enzymatic reactions. PPO is no longer active in apples when it is treated with freezing and water activity lies below 0.3. Freezing leads to irreversible changes in the food product such as firmness loss during thawing ( Irina Ioannou, 2013).

**Modified Atmospheric Packaging:** For enzymatic browning, oxygen is the substrate for oxidation of PPO. Controlling the enzymatic browning by lowering the oxygen concentration of the storage atmosphere. Oxygen is vital for the oxidation reaction and PPO activity, by reducing the oxygen concentration. It can be able to control the enzymatic browning reaction. Some studies show that by modifying the composition of the atmosphere, it shows that the enzymatic reaction is delayed without altering product quality. Some studies show that modifying oxygen by replacing it with CO2 or N2 and using Ar or NO2 to control the atmosphere. It shows the good anticipation of enzymatic browning without quality loss (Irina Ioannou, 2013).

**Edible Coating:** The coating method is the application of layering of any edible material on the surface of the fruits. Edible coating deals with the reduction of moisture and aroma losses, the delaying of color changes and gas transfer, and the enhancement of the general appearance of the product through storage. Coating agents permit delaying enzymatic browning because they produce a modified atmosphere on coated fruits by separating the coated product from the environment (P K Raghav 2016). There are different methods of coating, The gel coating method is better than the immersion coating by inhibiting the enzymatic browning. The immersion method is dipping the products into a solution of an anti-browning bath (Oms-Oliu et al., 2010b). The most commonly used coating agents are chitosan, alginate, or carrageenan (Irina Ioannou, 2013).

**A combination of chemical and physical processes:** In many cases, a combination of several techniques is used to prevent oxidation, and to improve the protection of vegetables against oxidation. Thus, a combination of agricultural produce is often combined with physical methods such as blanching and coating to prevent enzymatic browning and the loss of firmness. The protection brought by blanching and coating is time-dependent, whereas dipping is instantaneous. Thus, a combination with dipping decreases the efficiency of food protection against quality losses (Irina Ioannou, 2013).

**Non-thermal methods:**

 Thermal treatments are efficient in avoiding enzymatic browning but they modify the product parameters such as texture and taste. To maintain the quality of the product through no changes in the sensory parameters, follow non-thermal processing such as high hydrostatic pressure (HHP), irradiation, ultrasonication, and pulsed electric field. Among these, the main objective is to inactivate the enzymatic browning by using different techniques; light, pressure, or electricity. This method is more efficient for inactivating microorganisms.

**Other technologies:**

**Supercritical carbon dioxide** physically destroys the microbial cells by applying pressurized gas that penetrates the microbial cells and helps to subsequent explosive decompression resulting in rapid gas expansion within the cells. Beyond the lethal effect on micro-organisms, supercritical carbon dioxide also affects enzyme inactivation by affecting the secondary and tertiary structure (Gui, F 2007; Queiroz., 2008).

**Ohmic heating** is a process in which electric currents are passed through foods or materials to heat the products. The principle of ohmic heating is the conversion of electrical energy into heat energy by using an electrical conductor. The electrical conductor generates heat when an alternating current passes through the food, which results in the generation of heat from the center part. The amount of heat generated is equal to the current induced by the voltage gradient in the field, and the electrical conductivity. The electrical conductivity of the food material depends on various factors such as temperature, voltage gradient, and food composition such as sugar content (Kurnia Aurina et al. 2021).

**Bioavailability of polyphenol:**

Polyphenols are present in fruits, vegetables, cereals, and beverages. Polyphenols generally contribute to bitterness, astringency, color, flavor, odor, and oxidative stability. Polyphenols are the secondary metabolites of plants involved in protecting against pathogens. Polyphenols are classified based on their structures and are phenolic acids, flavonoids, stilbenes, and lignans. Polyphenol compounds are distributed non-uniformly in the tissue, cellular, and subcellular levels. Quercetin is found in all plant products; fruits, vegetables, cereals, tea, wine, etc. The phenolic component of plants is affected by numerous factors including the degree of ripeness at the time of harvesting, environmental factors, processing, and storage. Bioavailability is the quantity of the nutrient that is digested, absorbed, and metabolized through normal pathways. Most of the polyphenols are present in food in the form of esters, glycosides, or polymers that cannot be absorbed in their native form. Before absorption, these compounds must be hydrolyzed by intestinal enzymes or by colonic microflora. During the period of absorption, polyphenols undergo modification in intestinal cells and later enter the liver by methylation, sulfation, and/or glucuronidation. It is very difficult to identify the metabolites and to evaluate their biological activity reaching the blood and tissues the different forms. Polyphenols differ in their site of absorption in humans. Some polyphenols are well absorbed in the GI tract, others are in the intestine or other parts of the digestive tract. Glycosides resist the acid hydrolysis in the stomach and reach the intestine where only a few aglycone and glucosides can be absorbed (Pandey et al., 2009).

**Application of PPO in industries:** Most of the fungal strains contain polyphenol oxidases considered to be excellent sources for industrial production. PPO is found in Bacillus species. PPO is oxidizing the aromatic compounds. This helps to make use of biotechnological applications in the food industry, the pulp and paper industry, the textile industry, medicine, and environmental technology (Ziyan E 2003; Motoda, S 1998).

1. Applications of PPO in food industries include color development and flavor augmentation of tea, cocoa, and coffee, Ascorbic acid determination, sugar beet pectin gelation, and a biosensor. In some cases, in food industries, PPO is undesirable and plays an important role in the determination of quality.
2. In environmental technology, industrial practices waste-waters from coal conversion, petroleum refining wood preservation, textile, paper, food, and chemical industries having hazardous phenolic compounds and their derivatives. The government sets rules and regulations for industries to remove toxic compounds from wastewater. The use of peroxidases and polyphenol oxidases helps to remove the phenolic compounds from industrial effluents. (Maloveryan A 2001; Kuwabara, T 1997).
3. In the medical field, PPO is used for the treatment of Parkinson's disease, inhibits the adhesion of streptococcus bacteria responsible for cavity formation, is used as a prodrug therapy agent, as a tumor suppresser, controls melanin synthesis (Kaml A, 2015).
4. PPO is also used in the development of biosensors for immunoassays and for the detection of phenols and phenolic compounds in wastewater, food, and beverages.
5. In cosmetics, PPO is used in some hair dyes and dermatological skin-lightening preparations.
6. In the textile industry, PPO is used for bleaching and dye decolorization (Kaml A 2015).

**Conclusions:**

Polyphenols are abundantly found in fruits and vegetables, when exposed to the atmospheric air form a brown color on the surface of the food. Easily observed in cut apple, potato, and brinjal formation of brown color enzymatic browning is due to polyphenol oxidase, some food industries are not accepting the product due to browning reaction caused by enzymes. Controlling the enzymatic browning is done by various methods such as chemical, physical, coating, and non-thermal treatment. PPO is also applicable in various industries other than food such as medical, cosmetics, textiles, and in the environment. Apart from that PPO is also used in biosensors for immunological assays.

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