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Polyphenol oxidase inhibition using chemical methods during minimal processing

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Abstract— Minimal processing means the operations such washing, sorting, peeling, slicing or chopping and preventing browning in order to keep the freshness of vegetables. In this case, Mechanical injury and ethylene can stimulate phenolic metabolism in present of polyphenol oxidase enzyme caused browning of ready to eat product. Chemical methods in immersion form such as acidulates, reducing agents, chelating agents, complexing agents, enzyme inhibitors act to inhibit the enzyme with remove or function with its substrates (oxygen and phenolic compounds). In attention to chemical base of these methods, Identification of them in order to achieve high quality commodity is important.

***Keywords*—**Minimal processing, Browning, Phenolic metabolism, Polyphenol oxidase, Chemical methods.

I. INTRODUCTION

Minimal processes definition as those which 'minimally influence the quality characteristics of a food and giving the food sufficient shelf-life during storage and distribution [1]. An even more precise definition, which situates minimal processing methods within the context of more conventional technologies, describes them as techniques that 'preserve foods but also retain to a greater extent their nutritional quality and sensory characteristics by reducing the reliance on heat as the main preservative action' [2]. Minimal processing can, therefore, be seen in the context of the traditional concern of food processing to extend the shelf-life of food.

Minimal or lightly processing means the operations like washing, sorting, trimming, peeling, slicing or chopping and preventing browning that affect the freshness of vegetables . These types of products are subject to susceptibility to undesirable browning reactions during their relatively short shelf life. The browning reaction is often accompanied by a reduction of flavor, texture, and nutrients [3]. Enzymatic browning is a biochemical process that takes place in many higher plants and is responsible for a significant loss of product in the food industry. It is estimated that over 50% of losses in fruits and vegetables are due to enzymatic browning. The rate of browning is primarily determined by the PPO activity and the concentration of phenolic content [4]. Peroxidase is an enzyme widely distributed in plants. Changes in peroxidase may be brought about by wounding, physiological stress and infections. Many reactions can be promoted by peroxidase, and in the presence of small amounts of hydrogen peroxide, it can oxidize a number of naturally occurring phenolics. Mono- and diphenols are potential substrates for peroxidase [5]. It is believed that although peroxidase may also contribute to enzymatic browning, its role remains questionable [6] and limited by hydrogen peroxide availability [7]. Mechanical injury (wounding) and ethylene can stimulate phenolic metabolism in fresh-cut tissue. Wounding and ethylene induce the activity of the enzyme phenylalanine ammonia lyase (PAL), a key enzyme for phenolic biosynthesis. Accumulated phenolic compounds can be used as substrates by PPO, leading to browning. It has been suggested that lettuce storage life is related to the activity of stressinduced PAL [8].

II. ACTION MECHANISM OF POLYPHENOL OXIDASE

Polyphenol oxidase, also known as catechol oxidase, is a monomeric enzyme, which is ellipsoid in shape with a secondary structure made primarily by α-helicals surrounding the catalytic dinuclear copper center. Disulfite bridges hold the loop-rich N-terminal region to one of the helices. Both active-site coppers are coordinated by three histidine residues from the alpha-helices [9]. Polyphenol oxidase has two catalytic activities; one of them involves the conversion of monophenols into *o*-diphenol and further oxidation to *o*-quinones known as monophenolase and diphenolase activities respectively [10]. In this paper, how occurrence of this reactions explained briefly.

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A. Monophenolase Activity

The monophenolase activity is characterized by the first step in the melanization pathway and involves hydroxylation of monophenol to o-diphenol. In higher plants it is also referred to as cresolase because of its ability to utilize the monophenolic substrate, cresol (Figure 1).

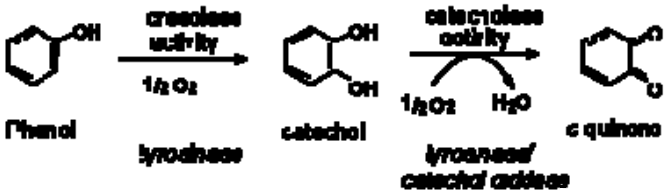

Figure 1. Cresolase activity and catechol activity of tyrosinase and catechol oxidase. adapted from [20].

Tyrosinase is often used for describing both monophenol and diphenol oxidase activities although L-tyrosine is not the only substrate for the enzyme that is present in the plants. The enzyme is probably referred to as polyphenol oxidase due to the abundance of phenolics in plants. The monophenolase activity reaction separates PPO from other phenol-oxidizing enzymes. Monophenolase activity has a distinguishing lag period showing before the hydroxylation step reached its maximum velocity depending on the nature of the enzyme, monophenol and enzyme concentration, o-diphenol or transition metal ions. Since the hydroxylation reaction is slower than the oxidation reaction for quinone production, the monophenol oxidase reaction is often overlooked. The enzyme also can metabolize aromatic amines and o-aminophenols [11] (Figure 2).

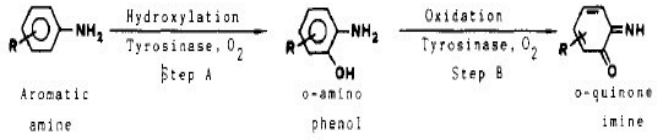

Figure 2. Polyphenol oxidase activity for the aromatic amines and o-aminophenols. adapted from [11].

B. Diphenolase Acitivity

The diphenolase activity is characterized by the formation of the *o*-quinones, reduction of oxygen and two molecules of water (Figure 3). This activity is characterized by the fact that if noncyclizable diphenol is used, quinone production and decrease of oxygen concentrations are linear with no lag period, meaning a high catalytic rate. In the case of cyclizable diphenol, although appearance of cyclic quinone is pH dependent, the oxygen is decreasing in a linear fashion at any pH.

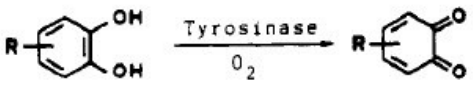

Figure 3. Production of the quinones. (o-Benzoquinone). adapted from [11].

III. CONTROL OF ENZYMATIC BROWNING

Enzymatic browning may be controlled through the use of physical and chemical methods, and, in most cases, both are employed. Physical methods may include reduction of temperature and/or oxygen, use of modified atmosphere packaging or edible coatings or treatment with gamma irradiation or high pressure. Chemical methods utilize compounds that act to inhibit the enzyme, remove its substrates (oxygen and phenolic compounds) or function as preferred substrate.

In current paper, since in minimal processing, identification of chemical compounds effects on enzyme activity and food safety is important, chemical methods such as acidulates, reducing agents, chelating agents, complexing agents and enzyme inhibitors are explained. In general, chemical compounds used for inhibition or control of browning in solution form (Mostly formulation of one or several compounds) for immersion of fruits and vegetables fresh-cuts.

A. Acidulants

The use of chemicals that lower the product pH, or acidulants, finds widespread application in the control of enzymatic browning. The most commonly used acidulant is citric acid. Acidulants are frequently used in combination with other types of antibrowning agents, because it is difficult to achieve efficient browning inhibition solely through pH control. In addition, there are variations in the effect of different acids on PPO; as an example, malic acid has been reported to be more efficient in preventing apple juice browning than citric acid. While the optimum pH for PPO has been reported as ranging from acid to neutral, in most fruits and vegetables, optimum PPO activity is observed at pH 6.0–6.5, while little activity is detected below pH 4.5 [12]. It has also been reported that irreversible inactivation of PPO can be

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achieved below pH 3.0. Nevertheless, it has also been reported that apple PPO is quite tolerant to acidity, and at pH 3.0, it retains 40% of its maximum activity [13].

B. Reducing Agents

This type of antibrowning agent causes chemical reduction of colorless O –quinones resulting from the PPO reaction back to o-diphenols [14]. Reductants are irreversibly oxidized during the reaction, which means that the protection they confer is only temporary, because they are consumed in the reaction. When all the reducing agent added is oxidized, the O-quinones from the PPO reaction may undergo further oxidation reactions (not involving PPO) and finally rapid polymerization leading to the formation of brown pigments. Due to the oxidative nature of enzymatic browning, reducing agents can also be applied in the prevention of discoloration. Ascorbic acid is probably the most widely used antibrowning agent, and in addition to its reducing properties, it also slightly lowers pH. Ascorbic acid reduces the *o*-benzoquinones back to *o*-diphenols, and it also has a direct effect on PPO [12], [15]. Thiol-containing compounds, such as cysteine, are also reducing agents that inhibit enzymatic browning. However, for complete browning control, the amount of cysteine required (cysteine-to-phenol ratios above 1) is often incompatible with product taste [6].

C. Chelating Agents

By complexing copper from the PPO active site, chelating compounds, such as ethylenediamine tetraacetic acid (EDTA) can inhibit PPO, which is a metalloenzyme containing copper in the active site. Sporix is a powerful chelator, and also an acidulant. Browning prevention in apple juice and cut surfaces was obtained with combinations of Sporix and ascorbic acid [17].

D. Complexing Agents

This category includes agents capable of entrapping or forming complexes with PPO substrates or reaction products. Examples of this category are cyclodextrins or cyclic nonreducing oligosaccharides of six or more D-glucose residues. In aqueous solution, the central cavity of cyclodextrins can form inclusion complexes with phenolics, consequently depleting PPO substrates. β-Cyclodextrin has the most appropriate cavity size for complexing phenolic compounds, but its water solubility is low [18]. Effect of β-Cyclodextrin on cooked and vacuum packaged potato, storage in 4 ˚C for 14 days, investigated. Immersion in β-Cyclodextrin solution created less color and attractive appearance [16]. β-Cyclodextrin was not effective in controlling browning of diced apples, presumably due to its low diffusion [17]. Large variations in the inhibitory properties of cyclodextrins have been found with different phenols tested.β-Cyclodextrin binding strength varies with different phenols. In model systems containing a single phenolic compound, β-cyclodextrin always works as a PPO inhibitor. When mixtures of phenolic compounds were tested, the results were variable, and the balance among the PPO substrates present can be modified, resulting in color changes after enzymatic oxidation catalyzed by PPO [18].

E. Enzyme Inhibitors

One of the antibrowning agents with the most potential for application to fresh-cut products is 4-hexylresorcinol, a chemical that has been safely used in medications for a long time and has been granted FDA GRASS (generally regarded as safe) status for use in the prevention of shrimp discoloration (melanosis), where it proved to be more effective than sulfite on a weight-to-weight basis. Currently, its use on fruit and vegetable products has been delayed while awaiting FDA approval. The efficiency of 4-hexylresorcinol has been demonstrated in preliminary tests carried out using cut apples and potatoes [14]. The combination of 4-hexylresorcinol with ascorbic acid improved browning control in apple slices [19].

Sodium chloride (as other halides) is known to inhibit PPO; its inhibition increases as pH decreases. Chloride is a weak inhibitor; some authors report that the chloride levels required for PPO inhibition are elevated and may compromise product taste [20]. Nevertheless, other authors believe that browning control may be possible provided that the dipping solutions are acidic; a pH of at least 3.5 has been suggested [21].

Browning inhibition (62%) in slices of peeled apples has been achieved by dipping in a 10% honey solution for 30 minutes at room temperature. Comparison with a control sucrose solution at the same sugar level as the honey preparation showed only a 23% inhibition of browning [22].

Although benzoic and cinnamic acids (aromatic carboxylic acids) are PPO inhibitors [26], they have not given prolonged protection as antibrowning agents. When solutions of sodium cinnamate were used to dip apple plugs, browning prevention was obtained on a short term, but over prolonged storage (> 24 hr), a severe browning developed [23]. It has been suggested that cinnamates and benzoates may undergo a slow but gradual conversion to PPO substrates [24].

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F. Application of Antibrowning Agents

Sapers and Miller [25] reported that lye digestion treatment given to remove surface tissues from peeled potatoes prior to treatment with browning inhibitors. Solution used for lye digestion likely penetrated, disrupted layers of cells at the peeled surface, digesting the middle lamellae crouching parenchyma cells, so that tissue could be separated from underlying undamaged tissue, thereby, influenced in preventing browning as compared to conventional treatment. It has been reported that with some chemicals, such as ascorbic and erythorbic acid or their salts, limited penetration into the plant tissue is an issue. A comparison of the effect of dipping vs. pressure or vacuum infiltration on the penetration of ascorbic and erythorbic acids showed that pressure infiltration was ineffective with potato dice but extended the shelf life of potato plugs by two to four days when compared to dipping [26].

There are consumers who want to avoid any type of food preservative [27]. It is recognized that the consumer perceives fresh-cut products as minimally processed products with characteristics close to their raw unprocessed material. Flavor, color and texture characteristics are probably an added appeal of fresh-cut products, and as a consequence, some processors would rather not use chemical additives that could change that perception of a "natural" product. This may be one of the reasons that ascorbic acid, which may be labeled as vitamin C, is frequently preferred as an antibrowning agent, an added value to the product. Other chemicals of natural origin or identical to natural compounds are also frequently preferred, an example of which is citric acid.

IV. CONCLUSION

This paper was a review for polyphenol oxidase enzyme inhibitors. Certainly, application of one chemical method cannot be effective on reducing the enzyme activity and along with other chemical or physical methods such as packaging seems to be necessary. In other side, consumer's trend is not for food additives, thus, optimization of dose and chemical compound content, so that maximum inhibition of enzyme activity and the lowest rate of adverse reactions to food, it is necessary and the need to research. Understanding the details of browning reaction in the presence of enzyme achieves the efficient methods of food minimal processing. For this purpose, at least as regards the processing of foodstuffs, especially fruits and vegetables should be reserved so that natural quality food, chemical compound using based on commodity and its shelf life of and sensitivity could be effective in minimal processing, commercially.

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