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Enzymatic browning of apple and its control by chemical treatment: A review

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Abstract
This review paper is aimed at providing a comprehensive information of issues associated with minimally processed fresh cut apple slices and the quality changes associated with it. Physiological and biochemical changes in such products occur at a faster rate than in intact fruits. Mechanical injury sets off a complex series of events which result in loss of quality. Wounding stimulates respiration rate, induces ethylene synthesis, oxidation of phenols, enzymatic activity, and microbiological development, leading to an accelerated loss of quality, especially colour and firmness attributes. Appropriate steps must be taken during fruit processing against the development of this type of reaction. Browning damages the appearance, organoleptical properties, nutritional quality and, occasionally, safety of the commodities and apple is not an exception. Browning of cut surface of apple is on account of activity of polyphenol oxidase enzyme which during physical damage comes in contact with the phenolic substrates forming undesirable, brown colouring melanin pigment. Further this review paper elaborates the treatments employed for curbing such reactions with the help of anti-browning chemical treatments viz., anti-oxidant agents, acidifying agents, chelating agents and agents of firmness and also provides the comprehensive information of work being conducted globally for accessing the efficacy of browning inhibitors either alone or in combination, so as to provide an effective control of cut surface browning of the apple and thus maintain its quality.

Keywords: apple, enzymatic browning, quality, fruit juices

1. Introduction
Apple (*Malus domestica* Borkh.) is a highly appreciated fruit grown in many countries of the world and economically, it is the fourth most important fruit crop after citrus, grapes and banana (Affzadi,2014) [1]. The total area under apple cultivation in the world is 4860010 hectares with an annual production of more than 77.3 million tons (Anonymous, 2018) [3]. Economically, apple is the fourth most important fruit crop after citrus, grapes and banana (Affzadi, 2014) [1]. China is the leading producer of apples in the world with an annual production of 44.5 million tons followed by United States, Turkey and Italy (Anonymous, 2018) [3]. India is the fifth largest producer of apples in the world. In India, the total area under apple cultivation is 274,000 hectares with an overall production of 2300000 Metric tons which accounts for 2.97% of the total worlds production (Anonymous,2018) [3].
Fresh apples are considered as a food of moderate energy value among common fruits. Apple is composed of 84% water and the remaining 16% are total solids. This 16% contains nitrogen, fatty materials, minerals, carbohydrates, astringents, colour compounds, enzymes, volatiles, vitamins A, C and flavonoids (Priyadarshani, 2013) [45]. Apple is cholesterol free, low calorie food and has less than 1% fat, and contains most of the essential vitamins and minerals. Apple is an excellent source of fiber. A medium sized apple has about 5 g of fiber which is 25% of the recommended daily dietary intake of fiber (20 g). Apples are not bursting with vitamins and minerals like some other fruits, though they do provide a bit of vitamin C and potassium. How ever apples are good source of soluble fibres, especially pectin, which help control insulin levels by slowing the release of sugar in to the blood stream(Low glycemic index).Pectin is also known to help reduce cholesterol levels by lowering insulin secretion (Lee,2012) [34].
Due to high moisture content apples are perishable in nature. The storage problems, poor marketing and lack of appropriate processing technologies, therefore leads to high economic losses in apples (Hodges *et al.*, 2011) [22]. About 60-70 per cent of the total Apple production of the world is marketed fresh and 30-40 per cent is utilized for processing. A variety of processed products are made from apples the main products being juice, canned sauce, canned apple slices, dried apple slice, frozen slices and Apple juice concentrate, vinegar, jam, jelly and fresh apple slices. Now-a-days dried apples are being used as important ingredients in baking, snack and confectionery industry.

2. Quality changes during minimal processing of apples
Minimally processed (MP) fruits are new forms of product marketing intended to meet the consumers desires for convenience and fresh-like quality. Minimal processing includes operations such as washing, sorting, peeling, coring and cutting, although the product is still unavoidably wounded and its shelf life greatly diminished compared to the intact fruit. The physiology of minimally processed fruits is essentially the physiology of wounded tissue (Brecht, 1995) [8]. Physiological and biochemical changes in such products occur at a faster rate than in intact fruits. Mechanical injury sets off a complex series of events which result in loss of

quality. Wounding stimulates respiration rate, induces ethylene synthesis, oxidation of phenols, enzymatic activity, and microbiological development, leading to an accelerated loss of quality, especially colour and firmness attributes (Rolle and Chism, 1987; Kim *et al*., 1993) [50, 27]. Control of wounding is therefore the major obstacle that must be overcome for extension of the shelf life of MP fruits.

The rapid darkening of many fruits such as apples, bananas and avocados, is a serious problem during minimal processing operations. Appropriate steps must be taken during fruit processing against the development of this type of reaction. Browning damages the appearance, organoleptical properties, nutritional quality and, occasionally, safety of the commodities (Molnar-Perl and Friedman, 1990) [40]. Extensive research exists in literature on the control of enzymatic browning of apple (Sapers *et al*., 1990; Mc-Evile *et al*., 1992; Pizzocaro *et al*., 1993) [54, 37, 43]. Physiological, biochemical and microbiological storage stability of minimally processed fruits may vary depending upon cultivar (Brecht, 1995; Roming, 1995) [8, 51]. Relatively little information regarding quality changes of minimally processed apple is available in the literature.

3. An Overview of enzymatic browning in apple

When an apple *(Malus domestica* Borkh.) is cut or injured, the flesh oxidizes and turns brown. This process is known as enzymatic browning, and is a reaction catalyzed by the enzyme polyphenoloxidase (PPO). In the reaction, phenolic compounds present in the apple flesh oxidize to form slightly colored o-quinones, which then polymerize to form pigments of varying hues and intensity (Le Bourvellec *et al*. 2004b) [30]. The browning reaction is undesirable, leading to an unpleasant appearance, the possible development of off-flavors, and limiting the shelf life of fresh-cut or processed apples (Gámbaro *et al*. 2006) [15]. The rate and extent of apple flesh browning varies by cultivar, and may be affected by the activity level of the enzyme polyphenoloxidase as well as the total phenolic content of the fruit.

The basic enzymatic browning reaction involves the oxidation of colorless monophenols to diphenols, which are also colorless. These then further oxidize to form o-quinones, which are slightly colored. In the presence of amino acids and other proteins, these o-quinones polymerize into complex brown pigments (Grotheer *et al*. 2005) [20]. Additionally, quinones produced in the initial browning reaction may participate in coupled oxidation reactions, enabling them to oxidize other polyphenols that cannot be directly enzymatically oxidized.

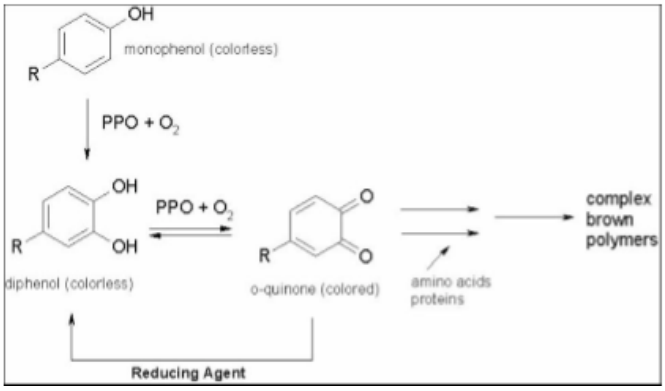

Fig 1.1 Simple diagram of enzymatic browning source (Grotheer *et al*., 2005)

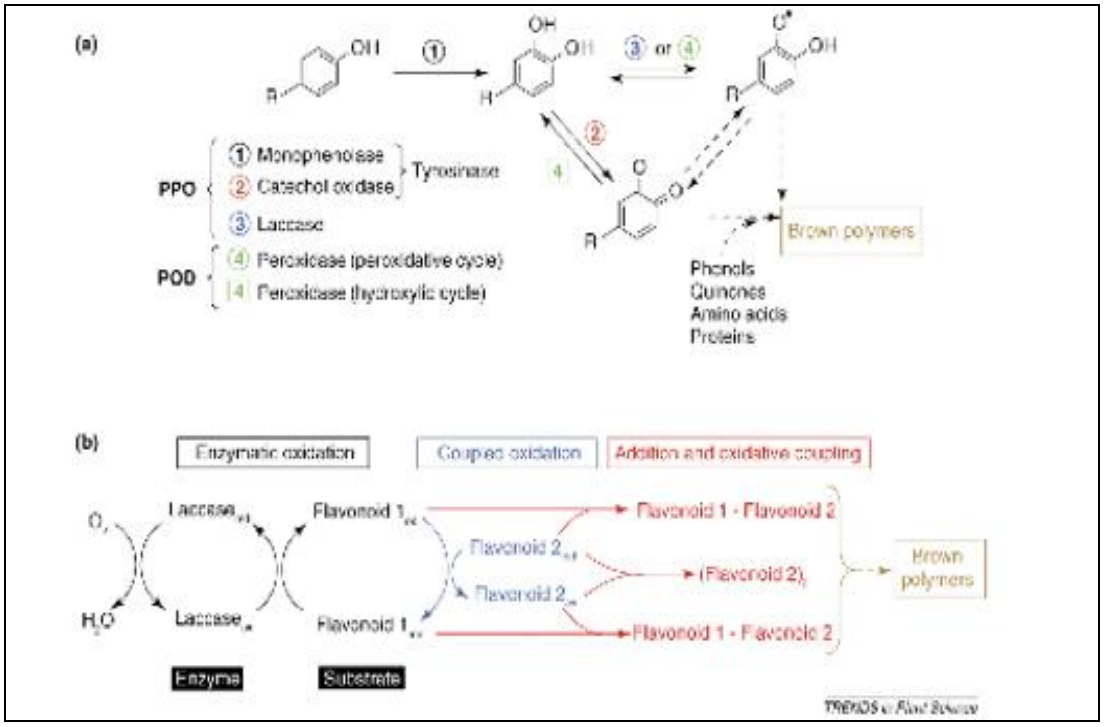

Fig 2: Comprehensive diagram of the enzymatic browning reaction, illustrating the initial conversion od moniphnols to o-quinones, as well as the coupled oxindation reactions performed by o-quinones on other flavonoids (Pourcel *et al*. 2006)

Enzymatic browning is a significant problem in the juice, processing, dehydrated fruits and the fresh cut industries. Market reports estimate that increasing the availability of pre-cut apples could expand the apple market in the same way that baby carrots expanded the carrot market - doubling consumption, while at the same time doubling its price (Jellie, 2006) [24]. Approximate consumers said they would purchase fresh cut apple slices over whole apples; 55% would purchase both (Jellie, 2006) [24].

Currently, fresh cut apples are carefully processed to maintain fruit texture, flavor and appearance. The apples are washed and then sliced by a robotic slicing machine designed to minimize cell damage and therefore decrease browning and water loss. Slices are then treated with a calcium mineral solution such as Nature Seal TM (calcium ascorbate) to preserve texture, crispness and color for up to 21 days. Finally, slices are packed in modified atmosphere packaging and shipped and handled at 34^0 F to optimize storage life (Rupasinghe *et al*., 2005) [52]. Modified atmosphere packaging consists of micro-perforated packaging films that allow for gas exchange with specified oxygen transmission rates (Bliss, 2006) [7]. Gas exchange is critical, as vacuum packaging leads to a significant decrease in firmness over time and immediate browning upon opening (Lee and Smith, 1995) [32]. Additionally, pre-slicing storage conditions have a significant effect on the quality of fresh-cut products. Apples stored under controlled atmosphere (Carbon dioxid storage) exhibited significantly lower browning than apples stored in refrigerated air (Chung and Moon, 2009) [10].

Several factors affect the rate and extent of apple enzymatic browning. Factors with a strong genetic component, such as specific enzyme activity and substrate availability, will vary at the cultivar level. Rocha & Morais found that the activity of polyphenoloxidase (PPO), the enzyme that catalyzes the reaction, was highly correlated to color changes on minimally processed 'Jonagored' apples over several days, with higher enzymatic activity leading to a greater overall color change on the fruit (Rocha and Morais, 2002) [48]. Browning was directly correlated with PPO activity in the cultivars Classic Delicious, Rhode Island Greening, McIntosh, Cortland, 'Arengeh', and 'Granny Smith' (Coseteng and Lee, 1987; Milani and Hamedi 2005) [11, 39]. The activity of the enzyme peroxidase can also contribute to enzymatic browning (Pourcel *et al*., 2006) [44]. Additionally, the quantity and type of phenolic substrates available in the apple may affect the rate and extent of the browning reaction, with catechins, chlorogenic acids and caffeic acids most reactive. A high positive correlation was found between the rate of browning and total phenolic concentration in the apple cultivars 'Empire', 'Rome', 'Golden Delicious', and 'Delicious' (Coseteng and Lee, 1987; Milani and Hamedi, 2005) [11, 39]. In Japanese pear (Pyrus pyrifolia), immature fruits with high browning potential had high PPO activity and a high level of phenolics, while mature fruits with little browning had high PPO activity but a low level of phenolics (Nishimura *et al*., 2003) [41]. In peach (Prunus persica), the degree of browning was correlated with total phenolics (r = 0.67) (Lee *et al*. 1990) [33]. PPO activity and phenolic content tend to vary at the cultivar level because of the genetic makeup of the different cultivars as well as genotype by environment interactions.

Apple cultivars displaying relatively high levels of enzymatic browning include 'Red Delicious', 'Liberty', 'McIntosh', 'Macoun', 'Rome', 'Rhode Island Greening', 'Stayman', and 'Idared' (Coseteng and Lee 1987; Lee and Smith 1995; Milani and Hamedi 2005) [11, 32, 39]. Cultivars with relatively lower rates of browning include 'Granny Smith', 'Jonagold', 'Empire', 'Cortland', 'Golden Delicious', 'AutumnCrisp' (NY674), and 'Gala' (Coseteng and Lee, 1987; Sapers and Douglas, 1987; Lee and Smith 1995; Milani and Hamedi 2005;) [11, 53, 32, 39]. Cultivars such as 'Gala', 'Pink Lady', 'Granny Smith', and 'Empire' are some of the cultivars commonly used in the fresh-cut apple market due to their low browning rates and maintenance of firmness over time. 'Autumn Crisp', a cultivar known for its low browning potential, does not maintain firmness well in storage, so while not ideal for the fresh cut market, it is still being used (Lee and Smith 1995) [32].

3.1 Polyphenol oxidase enzyme and its relation with browning

Polyphenoloxidase is the catalyst in the enzymatic browning reaction. As a class of enzymes, the polyphenoloxidases contain a dinuclear copper center bound to histidine residues and function to insert an oxygen in the ortho- position to an existing hydroxyl group of an aromatic ring (Mayer,2006) [36]. Polyphenoloxidases are wide-spread in animals, plants, fungi and bacteria (Mayer, 2006) [36]. The two main classes of polyphenoloxidases are catechol oxidases and laccases (Pourcel *et al*. 2006) [44]. Peroxidase (POD) is a similar enzyme that belongs, along with the polyphenoloxidases, to a larger grouping of enzymes known as the oxidoreductases (Pourcel *et al*. 2006) [44]. Catechol oxidases, also known as tyrosinase or monophenol oxygenase, are the class of PPO most common in apple fruit. They catalyze the hydroxylation of monophenols to o-diphenols and the oxidation of odiphenols to o-quinones, both of which consume molecular oxygen (Pourcel *et al*. 2006) [44]. Figure 1.3 illustrates the hydroxylation and oxidation catalyzed by PPO.

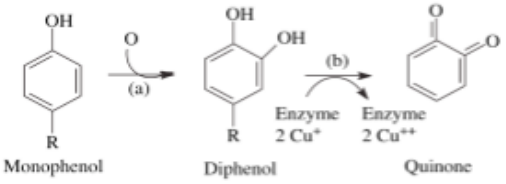

Fig 3: The (a) hydroxylation and (b) oxidation reactions catalyzed by PPO (Queiroz *et al*. 2008.)

PPO's primary function is to serve as a catalyst of the enzymatic browning reaction. The substrates involved in these reactions are located in the vacuoles while enzymes are in the cytoplasm; the reactions can take place only if they are mixed and in the presence of oxygen. So, all phenomena (cutting, shock, loss of firmness) lead to the starting of browning reactions which induce losses or changes of flavor. Odor and nutritional value. To avoid this phenomenon various methods are developed. The role of these methods is either to inactivate polyphenol oxidase (PPO) or to avoid contact between the enzyme and its substrate, either by adding antioxidants or by

maintaining the structural integrity of the food.

3.2 Prevention of enzymatic browning by chemicals

Now a days, several methods have been used to inhibit the PPO activity, such as the use of Antibrowning agents, removal of its one of the necessary component: O2, Cu2+ etc or by thermal processing to inactivate PPO activity is limited due to loss of sensory and nutritional quality of fruits and vegetables (Sun *et al*., 2002) [56]. Removal of oxygen from PPO can also check enzymatic browning but browning may restart when oxygen is available (Langdon, 1987) [29]. So, the best way to prevent enzymatic browning is the use of anti-browning agents. These agents act on the enzyme or react with the substrate and/or products of enzymatic catalysis and thereby formation of browning pigment is inhibited (Arslan and Dogan, 2005) [6]. To limit the oxidation phenomenon of the fruit, various chemical treatments are used in the literature. They differ by their action depending on the used chemical agents: antioxidant agent, chelating agent, firmness agent and acidifying agent. The main used chemical treatments are as under.

3.2.1 Treatment with antioxidant agents

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, 1998) [35]. Their effectiveness depends on environmental factors such as pH, water activity (aw), temperature, light and composition of the atmosphere. The main antioxidants reported in the literature are hexylresorcinol E586, erythorbic acid E315, N-acetyl cysteine E920, cysteine hydrochloride E920, ascorbic acid E300 and glutathione (Arias *et al*., 2007) [5]. The antioxidant properties of glutathione are very relevant but its use is not yet generalized in the food industry; while the ascorbic acid is traditionally the most widely used.

3.2.2 Treatment with chelating agents

PPO requires copper ions to be active (Du, and Wu, 2012) [13]. Thus, the presence of a substance capable of binding divalent cations present in the medium reduces the enzymatic activity of PPO. There are several chelators in the literature. The principal chelating agents are kojic acid, citric acid E330 and EDTA E385. The legislation is very elusive on kojic acid. Usually citric acid is used for its chelating role, but also for acidifying the medium

3.2.3 Treatment with agents of firmness

Calcium salts are the best known; they are used in the strengthening of cell walls. The cell walls are more stable to different treatments. This prevents the destruction of cell compartments and also the contact of PPO with polyphenols in the vacuole (Quiles *et al*., 2007; Guan and Fan, 2010) [47, 21]. The main agents of firmness are calcium lactate E327, calcium propionate E282, calcium chloride E509, calcium ascorbate E302 and sodium chloride**.**

3.2.4 Treatment with 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 E330, erythorbic acid E315, ascorbic acid E300 and glutathione. The chemical treatments are often a mix of different molecules, for example an agent of firmness with an antioxidant and an acidifying agent. Each molecule contributes to the prevention of enzymatic browning. The concentrations of the chemical solutions used depend on the kind of fruit and the conditions of storage. Indeed, different fruits have a varying sensitivity to oxidation due to their structure and composition. Moreover, conditions of storage also affect oxidation reactions and the efficiency of chemical agent's combination, depending on the storage time and temperature, the kind of packaging and the oxygen content of the packaging. In general, chemical treatments are used to treat fresh-cut foods. For entire fruit, chemical agents are less efficient because they are limited by the presence of the cuticle. Pre-treatment is then needed to allow the diffusion of chemical agents into the prod

4. Chemical treatments for cut surface browning in apples

Fresh fruit browning reaction is one of the main challenges for fruit processing industry. During fruit peeling or cutting fruit cell membranes excrete cell substrate, which contains polyphenol oxidases that at the presence of oxygen dehydrogenate polyphenols to unstable quinones. These compounds are responsible in further reactions for development of dark-coloured pigments (Arias *et al*., 2009) [4]. To prevent browning reactions different additives could be used – ascorbic acid, citric acid, calcium propionate, calcium lactate, calcium ascorbate, carboxylic acids, chelators, thiolcontaining compounds, cysteine, glutathione or specific enzyme inhibitors such as 4-hexylresorcinol (Chiabrando and Giacalone, 2013; Rojas-Graü *et al*., 2006; Oms-Oliu *et al*., 2010; Gomes *et al*., 2010) [9, 49, 42, 17]. Different substances and their compositions are described as anti-browning agents for fresh-cut fruits (e.g. Rojas-Graü *et al*., 2006; Suttirak and Manurakchinakorn, 2010) [49, 57]. Combined treatment methods are thought to be more effective (González-Aguila *et al*., 2000) [18].

Traditionally, commercial apple slices, processed mainly for commercial bakeries in refrigerated, frozen, or dehydrofrozen forms, are treated with sulfites (sulfur dioxide), a highly effective anti-browning agent. The use of a sulfur dioxide treatment is common practice on fresh sliced apples for the baking industry. Sulfur dioxide preserves the color of the slices and prevents microbial spoilage. Unfortunately, the safety of sulfites in foods has been questioned because of alleged health hazards to asthmatics and because of the allergic reaction that sulfites cause in a certain segment of the population. Since 1986, the Food and Drug Administration has banned the use of sulfites on fresh fruits and vegetables and required a label declaration on any processed food containing more than 10 ppm of sulfur dioxide. Therefore, sulfur dioxide cannot be used in minimally processed apple products. New methods that maintain fresh apple slice-like quality for a certain period of storage time are needed.

Labuza and schimdt (1986) [28] reported that 300 mg of ascorbic acid is required to prevent browning of one lb of Apples. Sulphiting agents which inhibit PPO and may combine with quinones or reduce quinones to phenols have

been conventionally used to prevent browning of fruits and vegetables. However sulphites can produce acute allergic reactions in some asthamatics.As a result the use of sulphites as inhibitors of enzymatic browning in foods have been restricted by the Food and drug administration.

Kaur and Kapoor (2000) [26] studied the effect of different browning inhibitors like ascorbic acid(AA),4-hexyl resorcinol and banana leaf extract(BLA) either alone or in different combinations on Apples, mushrooms and potatoes and the results were compared with samples treated with sulphites which are banned because of their toxic effect.A mixture of HRA,AA and BLA significantly inhibited the enzymatic browning during storage at 4^0 c.Similar decrease in the polyphenol oxidase activity was also observed.The colour and texture of treated samples were closer to the fresh samples.

Javdani *et al.* (2013) [23] treated fresh slices of apple with 1% ascorbic acid solution for one min and hot water with 50°C for two min. The results showed that both heat and ascorbic acid treatments could significantly reduce cut surface browning, although ascorbic acid treatment was some more effective. The both treatments showed inhibitory effects on PPOs and POD enzymes in related to enzymatic browning in fresh cut browning, although hot water treatments was some more effective than ascorbic acid on suppressing both monophenolase and diphenolase activity of PPOs and POD enzymes.

Francesco *et al.* (1993) [14] studied the inhibitory effect of ascorbic acid, citric acid and sodium chloride on Polyphenoloxidase (PPO) of Golden Delicious apple cubes. Dipping in ascorbic acid (0.2-10 g/L range) and in NaCl (0.2-1 g/L range) solutions for 5 min increases the PPO activity. Citric acid solutions (0.2-10 g/L range) have little or no inhibition of PPO. A 90-100% PPO inhibition was obtained with a 5 min dip in mixtures of ascorbic acid and citric acid (10 + 2 g/L), and of ascorbic acid and sodium chloride (I0 + 0.5 g/L).

Denoya *et al.*, (2012) [12] evaluated the PPO activity and chromatic characteristics of pulp in cv. Granny Smith apple slices. The slices were submitted to three treatments: I. 2% ascorbic acid + 1% citric acid + 0.5% EDTA; II. 1% ascorbic acid + 0.5% citric acid + 0.25% EDTA; and III. water, used as control. During the storage, parameters of the CIE L*a*b* color space of the slices were evaluated, indicating that both treatments containing additives were effective in preventing browning. The specific activity of PPO was determined spectrophotometrically in apple extracts obtained from each treatment. The results indicated that the stronger treatment (I) had induced the most effective inhibition of the enzyme. On view of the present results, It is proposed to evaluate the "in vitro" effectiveness of the inhibitors in order to compare these results with the ones obtained with apple slices.

Jeong *et al.* (2008) [25] studied the influence of antibrowning agents on the correlation between phenolics, and PPO activity and browning of fresh-cut 'Fuji' apple.Fresh Apples were treated with distilled water (WC), chlorinated water (CW, 0.01%, v/v), cysteine solution (CS, 0.5%, w/v) and ascorbic acid solution (AA, 0.5%, w/v). The WC treatment was considered as a control. All samples were stored in the dark at 4oC and RH 90% for 7 days. Color, browning index, total phenolics, and PPO activity of the samples were evaluated. PPO activity and browning index of all samples increased during storage. For total phenolics, WC and CW treatments did not show observed changes during storage, although CS and AA treatments showed an increase. Browning index of WC and CW treatments during storage was found to be highly correlated with PPO activity and color degradation, as indicated by changes in color parameters, but CS and AA treatments were not. Total phenolics of fresh-cut apples during storage were found to be moderately correlated with browning index and not correlated with color degradation.

Melo and Vilas Boas (2006) [38] evaluated the effect of ascorbic acid (AA), calcium chloride (CC), L-cysteine hydrochloride (Cys) and EDTA on prevention of enzymatic browning of fresh-cut apple and banana. The following combinations were used: (i) AA 1%+CC 1%+Cys 0.5%, (ii) AA 1%+CC 1%+Cys 1% (iii) AA 1%+CC 1%+Cys 1.5% e (iv) EDTA 1%, building up four treatments of a completely randomly design. Fresh-cut products without chemical treatment were not analyzed because they browned quickly and presented less than 6 h of shelf life. The bananas were treated with sodium hypochlorite, sliced, dipped in chemical treatments, put in packages sealed with 30 µm PVC film and stored for five days at 5+1°C and 85+3% RH. Samples were evaluated everyday, during five days of storage. The treatments containing AA 1%+CC 1%+Cis 1% and AA 1%+CC 1%+Cis 1.5% determined the higher values of titratable acidity and lower values of pH. Increasing in a* value and decreasing in b* and L* values on fresh-cut apple banana were observed, in spite of chemical treatment, during the storage. AA 1% + CC1% + Cis1.5% treatment was the most effective on prevention of changes in a*, b* and L* values, associated to color of slices. Increasing in polyphenoloxidase (PPO) and peroxidase (POD) activity was observed during the storage of banana slices, despite the treatment, except a decreasing observed on PPO activity, on products treated with EDTA. Treatments contenting EDTA and AA 1%+CC1%+Cis1.5% were the most effective on contention of increasing of PPO and POD activities, respectively.

Pizzocaro *et al*. (1993) [43] studied the inhibitory effect of citric acid, ascorbic acid and sodium chloride on Polyphenoloxidase (PPO) of Golden Delicious apple cubes. Dipping in ascorbic acid (0.2-10 g/L range) and in NaCl (0.2-1 g/L range) solutions for 5 min increases the PPO activiry. Citric acid solutions (0.2-10 g/L range) have little or no inhibition of PPO. A 90-100% PPO inhibition was obtained with a 5 min dip in mixtures of ascorbic acid and citric acid (10 + 2 g/L), and of ascorbic acid and sodium chloride (I0 + 0.5 g/L).

Giacalone and Chiabrando, (2013) [16] compare a commercial product, Natureseal (AgriCoat, Great Shefford, United Kingdom, control), with 2 different Ca salts (Calcium propionate 1%, w/v, and CaCl2 1%, w/v) combined with citric acid (1%, w/v) on 'Golden Delicious' apple diced, stored 5 days at 1 °C. The results obtained showed that Natureseal was highly effective in maintaining colour and firmness of fresh-cut apples, but also application of CaCl2 + citric acid (CA) could be a good method to preserve the same product for 5 days. On the contrary, application of Ca propionate + CA resulted in acceptable values of firmness, but high browning,

sometimes associated with off-flavours. The treatment with CaCl2 + CA could be used in small fresh-cut industries as a cheap alternative to commercial products.

Wessels *et al*. (2014) [58] evaluated the anti-browning effect of 36 plant extracts, divided into three groups: (1) fruits, vegetables, and oil seeds, (2) herbs and tea plants, and (3) medicinal plants, on minimally processed fresh apples. The extracts were applied to freshcut apple slices as dipping solutions. Development of browning was analyzed by measuring L*, a*, and b* values. The greatest inhibition of browning was caused by extracts from pumpkin seed (group 1), hibiscus flower (group 2), and pelargonium root (group 3). However, the latter caused intense passive staining. The inhibitory potential might be attributable to the antioxidative activity of secondary plant metabolites, especially phenolic compounds. Furthermore, these bioactive substances might influence enzyme activity directly by acting as competitive or non-competitive inhibitors.

Synergistic effects of AA, CA and OA were found on apple slices by mixing the antibrowning agents. The mixed solution of 1 % CA and 0.02 % OA and the mixed solution of 1 % AA and 0.02 % OA clearly showed higher antibrowning efficiency than individual solutions of 1 % AA, 1 % CA and 0.02 % OA (Son *et al*., 2001) Similarly, a mixed solution of 1 % AA and 0.1 % CA exhibited higher PPO inhibition in apple slices, compared with the solutions of 1 % AA and 1 % CA. Moreover, when 0.2 % CA instead of 0.1 % CA was added to 1 % AA, the degree of PPO inhibition increased from 36.3 % to 87.1 % (Pizzocaro *et al*., 1993) [43]. The use of mixtures of various antibrowning agents conducive to increased antibrowning efficiency is presumably due to the collaborative inhibitory mechanism of the constituents.

The effectiveness of AA, CA and OA for controlling browning in fresh-cut apples has been reported to differ among apple cultivars. In Liberty apples, 1 % OA showed the highest inhibitory activity on browning followed by 1 % CA and 1 % AA, respectively (Son *et al*., 2001) [55] while 1 % AA possessed better antibrowning efficiency than 1 % CA in Fuji apples (Lee *et al*., 1990) [33]. Surprisingly, 1 % CA exhibited higher inhibitory activity on Golden Delicious apples PPO than 1 % AA, which did not inhibit, but activated PPO activity (Pizzocaro *et al*., 1993) [43].

5. Conclusion

Browning of apple is an economically important physiological disorder that degrades the sensory properties and discourages consumer purchase of fresh-cut apple slices. Control of browning on fresh-cut apple has been the focus of extensive research and many technologies have been explored with successful results. However, concerns over off-flavours and off-odours, food safety, economic feasibility and effectiveness of inhibition, result in few browning inhibitors demonstrating the potential for use in the fresh-cut industry. An important future goal in this field is the discovery of new compounds from natural sources that have health benefits for consumers, as well as providing safe and effective control of browning in fresh-cut apples. Studies on effective combinations of different treatments reviewed above needs to be undertaken since no single treatment can effectively prolong the shelf-life of fresh-cut products, while preventing browning and maintaining product quality and safety for consumers

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