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Enzymatic browning and its control in fresh-cut produce

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Abstract

Purpose of review: Enzymatic browning of fruits and vegetables during postharvest handling and processing degrades the sensory properties and nutritional value and discourages consumer purchase of fresh-cut products. Consequently, enzymatic browning results in significant economic losses for the fresh produce industry. This paper discusses the biochemistry of enzymatic browning, and focuses on technologies that can be used to prevent browning of fresh-cut fruits and vegetables and maintain good product quality and safety for consumers.

Main findings: Enzymatic browning results from oxidation of phenolic compounds catalysed by polyphenol oxidase (PPO) followed by non-enzymatic formation of pigments. PPOs exhibit either mono- or di-phenol oxidase activity, or both types of activities. Peroxidase (POD) and phenylalanine ammonia lyase (PAL) are also found to be closely associated with the browning of fresh-cut fruits and vegetables. A range of physical and chemical treatments that have the potential to be adopted by fresh-cut industry for browning inhibition were reviewed. The effective treatments can be divided into three methods: 1) dipping in anti-browning solutions; 2) modified atmosphere packaging; and 3) heat shock and refrigerated storage. The importance of balancing browning inhibition and pathogen inactivation in fresh-cut produce was particularly emphasised.

Directions for future research: Understanding the details of enzymatic browning, which occurs during the processing of fresh-cut products, is necessary for improving browning control. The activities of PPO, POD and PAL, as well as their interactions during browning reactions in fresh-cut produce need more investigation. The relationship between enzymatic browning and the content of total phenolic compounds (or specific phenolic composition) also requires further research. In addition, the development of dual controls to prevent both browning and pathogen contamination in fresh-cut produce is critical to maintaining the quality and safety of fresh-cut produce.

Keywords: fresh-cut produce; enzymatic browning; fruits; vegetables; anti-browning

Abbreviations

4-HR	4-Hexylresorcinol
AA	Ascorbic Acid
CA	Controlled Atmosphere
FDA	Food and Drug Administration
GRAS	Generally Recognised as Safe
HSP	Heat Shock Protein
IAA	Isoascorbic Acid
MAP	Modified Atmosphere Packaging
PAL	Phenylalanine Ammonia Lyase
POD	Peroxidase
PPO	Polyphenol Oxidase
PQSL	Produce Quality and Safety Laboratory
SOD	Superoxide Dismutase

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Introduction

The fresh-cut produce industry is one of the fastest growing food industries in the USA. It has been rapidly expanding over the past two decades as a result of consumer demands for fresh, nutritious and convenient foods. The nutritional benefits of consuming fresh fruits and vegetables are well documented. Fresh fruits and vegetables provide valuable sources of antioxidants including vitamins A, C and E, carotenoids and flavonoids, as well as minerals. Growing evidence suggests that habitual inclusion of fresh produce in the diet may prevent or reduce the risk of several chronic diseases [1, 2]. In addition, fresh-cut produce also offers other advantages over bulk produce in terms of waste management, shipping and in-store labour cost reduction, etc. Fresh-cut products remain in a minimally-processed and fresh state. Minimal processing includes peeling, cutting, washing and sanitising, drying and packaging, so that the finished product is ready to eat. Fresh-cut products are characterised as pre-packaged fruits or vegetables that have been peeled or cut into 100% usable product. Lack of a microbial killing step and vulnerability of injured plant tissues to microbial growth result in a short shelf-life for fresh-cut products due to spoilage, as well as an increased risk of contamination by pathogens. High standards of food quality and safety are essential for sustained growth of the fresh-cut produce industry.

Enzymatic browning is a widespread colour reaction occurring in fruits and vegetables, which involves the interaction of oxygen, phenolic compounds and polyphenol oxidases (PPOs). Browning is usually initiated by the enzymatic oxidation of monophenols into *o*-diphenols and *o*-diphenols into quinones, which undergo further non-enzymatic polymerisation leading to the formation of pigments. Enzymatic browning is detrimental to quality maintenance of the fresh-cut fruit and vegetable industry, although it is beneficial to the development of colour and flavour in some food items such as tea, coffee and cocoa. A variety of fruits and vegetables, such as apple, pear, banana, peach, lettuce and potato, are especially susceptible to enzymatic browning during processing and storage. Browning not only has a negative effect on their appearance, but also may impair other sensory properties including taste, odour and texture, as well as nutritional value [3, 4]. Unlike traditionally processed foods, fresh-cut products consist of living tissues and sustain substantial tissue injury during processing [5]. Enzymatic browning does not occur in intact plant cells since phenolic compounds in cell vacuoles are separated from the PPO that is present in the cytoplasm. Once tissues are damaged by slicing, cutting or pulping, however, the mixture of PPO and phenolic compounds consequently results in a rapid browning reaction. Enzymatic browning of fresh-cut products can lead to considerable economic losses, especially if browning occurs early in the product's projected shelf-life, but after the costs of processing, packaging and storage have been incurred. The understanding of browning and its control from harvesting to consumption is therefore important for minimising losses and maintaining the economic profitability of the fruit and vegetable processors.

Polyphenol oxidase and phenolic substrates

PPO (1,2-benzenediol: oxygen oxidoreductase; EC 1.10.3.1) is also known as catechol oxidase, catecholase, diphenol oxidase, *o*-diphenolase, phenolase, tyrosinase and cresolase. It contains copper in its active site, which is essential for enzyme activity. PPO catalyses two basic reactions: 1) hydroxylation of the phenolic substrate at the *o*-position, adjacent to an existing hydroxyl group (monophenol oxidase activity); and 2) oxidation of diphenol to *o*-benzoquinones (diphenol oxidase activity). Both reactions use molecular oxygen as a co-substrate. So far, all of the PPOs discovered have the ability to convert *o*-dihydroxyphenols to *o*-benzoquinones, but not all PPOs hydroxylate monophenols. A mulberry PPO whose molecular weight is estimated to be 65 kDa was isolated and purified [6*]. The mulberry PPO has great reactivity towards catechol, 4-methyl catechol and pyrogallol, but shows no activity towards monophenols such as *p*-cresol and L-tyrosine. Gowda and Paul [7**] reported on the monophenolase and diphenolase activities of field bean PPO. Results show that the hydroxylation of ferulic acid and tyrosine by the field bean PPO does not take place in the absence of catechol, while the PPO can catalyse the oxidation of diphenols such as gallic acid, chlorogenic acid and caffeic acid to their quinones. When both monophenol- and diphenol oxidases are present in plants, the ratio of monophenol to diphenol oxidase activity is sometimes as low as 1: 40. Perez-Gilabert and Garcia [8*] purified PPO from eggplant and found that the catecholase/cresolase ratio is 41:1 at a pH close to the physiological, indicating that the diphenol oxidation predominates over the monophenol oxidation. Diphenol oxidases have received considerably more attention than monophenol oxidases owing to their high catalytic rate and their association with the formation of quinones, which lead to the production of brown pigment. Some PPOs occur with a few subunit modes and the subunits usually differ with respect to chemical, physical and kinetic properties. These subunit differences were believed to be responsible for the different catalysing activities of mono- and diphenolic substrates [9*].

There is much evidence indicating the involvement of peroxidase (POD, EC 1.11.1.7) and phenylalanine ammonia lyase (PAL, EC 4.3.1.5) in tissue browning of fresh-cut products. POD is another important oxidative enzyme besides PPO in the plant kingdom. It is a heme-containing enzyme, performing single-electron oxidation of phenolic compounds in the presence of hydrogen peroxide. The generation of hydrogen peroxide from the oxidation of some phenolics catalysed by PPO can induce synergistic action between PPO and POD, which suggests the involvement of POD in browning processes [10]. PAL is the first committed enzyme in phenylpropanoid metabolism, and an increase in PAL activity provokes an increase in the concentration of phenolic compounds, which are substrates for PPO and POD. Hisaminato *et al.* [11**] observed an apparent relationship between the enzymatic browning and PAL activity of cut lettuce during storage, and found that browning can be prevented by inhibiting PAL activity. Cantos *et al.* [12] reported an overall increase

in PPO, POD and PAL activities in minimally-processed potatoes, together with an increase of chlorogenic acid content. Kang and Saltveit [13*] found that the wounding sustained by lettuce tissue during fresh-cut preparation induces the increase of PAL, resulting in the synthesis and accumulation of phenolic compounds that contribute to subsequent tissue browning.

The phenolic compounds of fruits and vegetables are diverse and vary in accordance with species, cultivar, maturity and other plant physiological conditions. Structurally they contain an aromatic ring bearing one or more hydroxyl groups, together with a number of other substituents. Phenolic compounds and PPO are, in general, directly responsible for enzymatic browning reactions in damaged fresh-cut products during postharvest handling and processing. The substrate specificity of PPO varies according to the source of the enzyme. Robards *et al.* [14] reviewed phenolic compounds and their role in oxidative processes, and discussed the relationship of the rate of browning to phenolic content and PPO activity. Browning in peach and apple usually shows a positive correlation with chlorogenic acid content. However, no significant correlation was found between either the rate or degree of browning and PPO, POD or total phenolic content in fresh-cut potato [12].

Control of enzymatic browning

The most important factors that determine the rate of enzymatic browning of fruits and vegetables are the concentrations of both PPO and phenolic compounds present, pH, temperature and oxygen availability of the tissue. Understanding the effects of these factors on enzymatic browning is necessary in order to control it. So far, various techniques and mechanisms have been investigated to control the enzymatic browning of fresh-cut fruits and vegetables, and in theory all the techniques attempt to eliminate one or more of essential components (such as oxygen, enzyme, copper or substrate) from the browning reaction. Consequently, dips in anti-browning solutions, modified atmosphere packaging (MAP), and heat treatment and refrigeration storage are the most common methods used to help maintain the initial colour of fresh-cut fruits and vegetables.

Dipping in anti-browning solutions

Currently, the use of reducing compounds, including ascorbic acid (AA) and its derivatives, cysteine and glutathione, is most effective for controlling enzymatic browning and calcium ascorbate is already commercially adopted by the fresh-cut produce industry. Reducing compounds can play an important role in anti-browning either by reducing *o*-quinones to colourless diphenols, or by reacting irreversibly with *o*-quinones to form stable colourless products. Sulphites were found to be very effective in controlling browning, however the application of sulphites on fresh and fresh-cut produce was banned by the United States FDA due to its deleterious health effect to the allergenic populations. AA and its derivatives have been used in many studies in concentrations rang-

ing from 0.5 to 4%, and have been the leading generally recognised as safe (GRAS) antioxidants for use on fresh-cut products for the prevention of browning and other oxidative reactions. Generally, its inhibitory effect is also due to the reduction of the *o*-quinones, generated by the action of PPO enzymes, back to phenolic substrates. In addition, AA is also believed to have a chelating effect on the copper prosthetic group of PPO. NatureSeal, which contains mainly calcium ascorbate, has been widely used for browning control of cut apples. Abbott *et al.* [15] compared NatureSeal treatment and an anti-browning treatment developed by the Produce Quality and Safety Laboratory (PQSL) on apple slices. They found that after storage at 5°C for 7 days both treatments maintained cut-surface colour similar to that at the time of cutting; but NatureSeal-treated slices were rated slightly better for texture than those receiving the PQSL treatment. Gonzalez-Aguilar *et al.* [16] reported a better anti-browning effect of isoascorbic acid (IAA) than AA for fresh-cut pineapple. Gorny *et al.* [17*] reported that a post-cutting dip of 2% AA + 1% calcium lactate + 0.5% cysteine, combined with appropriate MAP treatment, significantly extended the shelf-life of pear slices by inhibiting the loss of slice flesh firmness and preventing cut surface browning.

Several thiol-containing compounds such as L-cysteine and glutathione can reduce *o*-quinones to their corresponding phenol precursors, so they are also effective inhibitors of enzymatic browning. Eissa *et al.* [18] compared the effect of thiol-containing compounds (cysteine and glutathione) and AA for their ability to inhibit enzymatic browning in *Red delicious* apple slices stored for 24 h at 4, 25 and 35°C. Cysteine and glutathione showed significantly higher inhibitory effects than AA under all experimental conditions. The inhibition of browning by thiol-containing compounds is thought to be due to the formation of colourless thiol-conjugated *o*-quinones. Concentrations of cysteine and other thiols required for the achievement of acceptable levels of browning inhibition have however been shown to have negative effects on taste.

Ionisable groups of the protein structure of PPO, POD and PAL enzymes are affected by environmental pH. These groups must be in the appropriate ionic form so as to maintain the conformation of the active site, bind substrates or catalyse the browning reaction. Changes in the ionisation status of the enzymes are generally reversible; however, irreversible inactivation can occur under conditions of extreme pH. Moreover, the stability of the phenolic substrate is also affected by pH, since they can undergo chemical breakdown under extreme conditions of pH. The resulting degradation products often behave as PPO inhibitors, since they share the molecular features of the phenolic substrate. PPO may be rendered inactive by adjusting the pH well below that required for optimum activity with acidulants such as citric, malic and phosphoric acids. Citric acid is one of the most widely used acidulants in the food industry. It is often used in combination with other anti-browning agents such as AA. Citric acid is typically applied at levels ranging from 0.5% to

2% for the prevention of browning in fresh-cut products. In addition to lowering the pH, citric acid also exerts its inhibitory effect on PPO by chelating the copper at the active site of the enzyme. Jiang *et al.* [19] found that citric acid can stimulate PPO activity at low concentration, but at 0.1 M or higher it can significantly inhibit PPO and extend the shelf-life of fresh-cut Chinese water chestnut. Ihl *et al.* [20] reported a positive effect on the shelf-life of minimally-processed lettuce by using an immersion solution (citric acid + calcium chloride + garlic extract), where the activities of chlorophyllase and PPO were both inhibited.

Chelating agents such as sorbic acid, polycarboxylic acids (citric, malic, tartaric, oxalic and succinic acids), polyphosphates (ATP and pyrophosphates), macromolecules (porphyrins, proteins) and ethylene diamine tetra-acetic acid (EDTA), which can inactivate enzymes by binding to transition metals in the metal-enzyme complex, have been used for a variety of food processing applications. PPO possesses copper at its active site and removal of the copper by chelation inevitably renders PPO inactive. A typical combination of anti-browning agents for fresh-cut products may consist of a chemical reducing agent, an acidulant and a chelating agent. AA has a chelating effect on the prosthetic group of PPO. Polyphosphates have been used as anti-browning agents for fresh-cut fruits and vegetables at concentrations as low as 0.5–2%. Some polysaccharides can also behave as effective browning inhibitors. Pectin, a naturally occurring anionic polysaccharide, inhibits apple juice browning by 5–10% when used at a concentration of 0.5% [21]. Carboxyl groups present in pectin are believed to be capable of chelating the copper moiety of PPO, thus preventing browning. Marshall *et al.* [22**] reviewed the application of chelating agents for the control of enzymatic browning in fruits and vegetables.

PPO activity can be competitively inhibited by 4-hexylresorcinol (4-HR), an *m*-diphenolic compound that is structurally related to phenolic substrates. As a chemically stable, water-soluble compound, 4-HR has been shown to be effective in controlling enzymatic browning in shrimp. The United States FDA has provided GRAS status to 4-HR for use on shrimp since toxicological, mutagenic, carcinogenic, and allergenic studies have shown that there are no risks associated with effective levels. Luo and Barbosa-Canovas [23] reported a significant inhibition in browning of apple slices by dipping treatment in 4-HR solution at concentrations as low as 0.005%. They also found that combining 0.5% AA with 4-HR could eliminate vascular bundle discoloration and synergistically enhanced the browning inhibition [24, 25]. In combination, 4-HR and AA have a synergistic effect on the prevention of browning. Several recent studies have suggested the use of 4-HR on minimally-processed fruits and vegetables. Saper and Miller [26] found that 4-HR alone can retard browning, but cannot prevent the darkening of cut pear edges. The combination of 0.001 M 4-HR + 0.5 M IAA + 0.05 M calcium propionate + 0.025 M homocysteine caused fresh-cut apple slices to have an essentially unchanged ap-

pearance for 4 weeks at 5°C [27*]. Saper and Miller [26] also found that 4-HR, in combination with sodium erythorbate, had a beneficial effect on preventing browning of fresh-cut pears. Dong *et al.* [28] reported colour stability of pear products refrigerated for 30 days, after dipping in 0.01% 4-HR + 0.5% AA + 1.0% calcium lactate solutions. Some other PPO enzyme inhibitors, like halide salts, honey, amino acids, peptides and proteins, were reviewed by Marshall *et al.* [22**].

Modified atmosphere packaging

The rapid growth of the packaged fresh-cut produce industry has been enabled largely by the development of MAP technology. With MAP, the desired balance of oxygen (O₂) and carbon dioxide (CO₂) is created through the control of O₂ and CO₂ transmission of the packaging film and the respiration rate of the produce. Studies have shown that low O₂ and elevated CO₂ atmospheres can slow down the browning reaction.

Tian's research group made some recent contributions towards the control of enzymatic browning via controlled atmosphere (CA) storage. They investigated the effects of different O₂ and CO₂ atmospheres on physiology, quality and decay of longan fruit (*Dimocarpus longan* Lour) stored in CAs at 2°C, and found that CA more effectively inhibits PPO activity, prevents peel browning and decreases fruit decay than MAP treatment does [29*]. Particularly, CA with 70% O₂ concentration was in reducing ethanol production in the flesh, retaining low pH in the peel and preventing peel browning, but stimulated flesh browning after 40 days of storage [30*]. CA with 15% CO₂ more significantly reduced fruit decay and extended storage life. Tian *et al.* [30*] also reported that CA with 5% O₂ + 10% CO₂ more significantly inhibited the activities of PPO and POD, reduced malondialdehyde content, effectively prevented flesh browning, decreased fruit decay and extended the storage life of sweet cherry fruit than other treatments did. Tian *et al.* [31**] considered that PPO, POD, anthocyanin and total phenols were involved in cellular browning of litchi fruit, and pointed out that CA conditions could be effective at reducing total phenol content, delaying anthocyanidin decomposition, preventing pericarp browning and decreasing litchi fruit decay. Recently, Zheng and Tian [32*] reported that treatment of litchi fruit with 2 and 4 mM oxalic acid could significantly inhibit pericarp browning, due to increased membrane integrity, inhibition of anthocyanin degradation, decline of oxidation and maintenance of relatively low peroxidase activity in the fruit during storage. This finding suggests that application of oxalic acid can effectively control the pericarp browning of litchi fruit during postharvest storage. Wang *et al.* [33*] determined the effects of different O₂ and CO₂ atmospheres on the activities of lipoxygenase, POD, superoxide dismutase (SOD) and catalase, as well as malondialdehyde content and membrane integrity of peach fruit. They found that a CA with 5% O₂ + 5% CO₂ reduced chilling injury and delayed the reduction of SOD, catalase and POD activities compared with the control, resulting in effective inhibition of flesh browning.

Edible films and coatings are gaining importance as approaches that can maintain the quality of fresh-cut products. Edible films can serve as semi-permeable barriers designed to extend shelf-life by reducing moisture and solute migration, gas exchange, respiration and oxidative reaction rates, as well as suppressing physiological disorders on fresh-cut products. Recently, some reports have proposed the use of edible coatings in combination with anti-browning compounds to improve the colour preservation of fresh-cut fruits. Through modification of CO₂, O₂ and ethylene transmission, the coating/film has the potential to retard water loss, form a barrier to oxygen and control the release of anti-browning compounds on the surface of cut tissues. Edible coatings may be used in combination with other methods such as low temperature and suitable packaging to achieve browning control in fresh-cut fruits and vegetables. McHugh and Senesi [34*] developed an edible film composed of 61% apple puree, 23% beeswax, 7% pectin, 7% glycerol, 1% AA and 1% citric acid that can prevent browning in apple. Lee *et al.* [35] reported that the initial respiration rate of minimally-processed apples decreased by 5 and 20% in carrageenan (0.5 g/100 mL)-coated and whey protein (5 g/100 mL)-coated apples, respectively, at 25°C. They also found that the edible coatings in combination with certain anti-browning agents (AA, citric acid or oxalic acid) effectively prolonged the shelf-life of the minimally-processed apple slices by 2 weeks when stored at 3°C. Flavour acceptance of the coated fresh-cut products is questioned and further investigation is required.

Heat shock and refrigeration

Heat treatment is the most widely used method for stabilising foods because of its capacity to kill microorganisms and inactivate enzymes. Enzymatic browning in canned or frozen fruits and vegetables may be effectively controlled by treatment with steam or hot water, at temperatures ranging between 70 and 105 °C. However, because of the damage caused to the living tissues and the requirement for “fresh” quality these methods are not suitable for the prevention of browning in fresh-cut fruits and vegetables. Mild heat treatment in the range from 45 to 60°C has been used to induce formation of heat shock proteins (HSPs) which afford a variety of benefits to fresh and fresh-cut produce ranging from browning inhibition to protection against plant pathogens. Loaiza-Velarde *et al.* [36**] reported that an increased PAL activity was induced by the minimal processing of celery, and found that a heat shock treatment at 50°C for 90 s significantly reduced the rise in PAL and subsequent browning. PAL is the first committed enzyme of the primary phenolic pathway, and its activity controls the rate through the phenolic pathway. The phenylalanine reacts with cinnamate and *p*-coumarate to produce caffeic acid, which conjugates with quinic acid to generate chlorogenic and isochlorogenic acid, and with tartaric acid to yield caffeoltartaric and dicaffeoltartaric. These four phenolic compounds accumulate in fresh-cut lettuce and celery, and are associated with subsequent tissue browning. Wounding induces synthesis and subsequent accumulation of phenolic compounds resulting in browning

of tissues, despite initially low levels of preformed phenolic compounds (eg, celery, lettuce). The mechanism by which heat shock treatment may reduce browning in fresh-cut celery or lettuce may entail the redirection of protein synthesis away from production of the wound-induced enzymes of phenolic metabolism, and toward the production of beneficial HSPs. Exposure of plant tissue to temperatures about 10°C above the normal growing temperature induces the synthesis of a unique set of proteins called HSPs. This response is ubiquitous to plants and protects the induced tissue from subsequent high temperature stress. Moreover, the synthesis of HSPs is accompanied by a general inhibition of normal protein synthesis, such as the synthesis of wound-induced PAL in fresh-cut celery and lettuce. Murata *et al.* [37*] studied the effects of heat shock treatment at 50°C for 90 s on the quality of cut lettuce during cold storage. They found that the heat shock significantly repressed the induction of PAL activity and phenolics accumulation and prevented browning in cut lettuce during storage. They also found that AA content was not affected by the heat shock, and that the sensory attributes of treated cut lettuce were superior to those of the control.

Cold storage during distribution and retailing are necessary for the prevention of browning in fresh-cut fruits and vegetables, since refrigerated temperatures are effective in lowering the activities of browning-related enzymes. The rate of enzyme-catalysed reactions is controlled to a great extent by temperature. For every 10°C temperature reduction (in biologically important ranges), there is a two-fold decrease in the rate of an enzyme-catalysed reaction. Reduced kinetic energy of the reactant molecules, at low temperatures, results in a decrease in both mobility and “successful collisions” needed to establish enzyme-substrate complexes. However, chilling injury can occur during the storage of some tropical or subtropical fruits. Therefore, commodities susceptible to chilling injury (bananas, mangoes, avocados, tomatoes, etc) should not be stored below their respective critical temperatures. Other vegetables (broccoli, berries, spinach, peas, etc) may be stored at chilling temperatures.

Balancing browning inhibition and pathogen inactivation

Fresh produce may be handled numerous times as it travels from farm field to dinner table. Contamination with human pathogens can potentially occur at any one of many stages, including in the field by animal carrier or irrigation water, during harvesting, transportation, packaging or distribution. Unlike intact fruits and vegetables, the physical and chemical barriers provided by the epidermis of fruits and vegetables are removed during preparation of produce for the fresh-cut market. This practice creates opportunities for spoilage or human pathogenic microorganisms to directly contact the edible portions of produce, thus leading to more rapid product decay and the potential for pathogen contamination. Wounding during processing releases plant cellular fluids which provide nutritive media on which pathogens, if present, may survive and grow. Therefore, produce safety is of great concern to the fresh-cut

produce industry, and sanitisers such as chlorine, ozone and chlorine dioxide are employed during the processing of fresh-cut fruits and vegetables. Unfortunately, most of the sanitisers used on fresh-cut products are incompatible with many anti-browning agents because the sanitisers tend to be oxidising agents, whereas the browning inhibitors used tend to be reducing agents. Consequently, in combination they usually cancel out each other's desired effects. Urgently needed to maintain safety and quality of fresh-cut apples is a sanitiser that is compatible with current widely used anti-browning solutions or preferably, a solution that can itself provide dual inhibition of browning reaction and microbial growth. Recent studies have shown that sodium chlorite, a known anti-microbial agent, strongly inhibits browning of fresh-cut apples [38**, 39]. Further research done by this group indicated that sodium chlorite exhibited a strong inhibition on PPO from both apples and mushrooms. Since sodium chlorite possesses strong anti-microbial activity, the discovery of the anti-browning effect of sodium chlorite suggests that sodium chlorite has the potential to be applied to food products to achieve a much-needed dual control for both browning and microbial contamination.

Conclusion

Browning is an economically important physiological disorder that degrades the sensory properties and discourages consumer purchase of fresh-cut fruits and vegetables. Control of browning on fresh-cut products 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 products. 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. Transgenic technology may be another option to prevent browning of fruits and vegetables. The introduction of products that do not require anti-browning treatment would alleviate the problem of finding a sanitiser that would not interfere with browning inhibitors and thus make it easier to provide high quality, safe, fresh-cut products.

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- 17 Gorny JR, Hess-Pierce B, Cifuentes RA and Kader AA. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biology and Technology* 2002; 24(3): 271–278.

* The article shows that low O₂, elevated CO₂ or superatmospheric O₂ atmospheres alone cannot effectively prevent cut-surface browning or softening of fresh-cut pear slices, whereas a post-cutting dip of 2% AA + 1% calcium lactate + 0.5% cysteine (pH 7.0) can significantly extend the shelf-life of pear slices by inhibiting the loss of slice flesh firmness and preventing cut-surface browning.

- 18 Eissa HA, Fadel HHM, Ibrahim GE, Hassan IM and Elrashid AA. Thiol containing compounds as controlling agents of enzymatic browning in some apple products. *Food Research International* 2006; 39(8): 855–863.
- 19 Jiang YM, Pen L and Li J. Use of citric acid for shelf life and quality maintenance of fresh-cut Chinese water chestnut. *Journal of Food Engineering* 2004; 63(3): 325–328.
- 20 Ihl M, Aravena L, Scheuermann E, Uquiche E and Bifani V. Effect of immersion solutions on shelf life of minimally processed lettuce. *Lebensmittel-Wissenschaft und-Technologie* 2003; 36(6): 591–599.
- 21 Tong CBS, Hicks KB, Osman SF, Hotchkiss AT and Haines RM. Oxalic acid in commercial pectins inhibits browning of raw apple juice. *Journal of Agricultural and Food Chemistry* 1995; 43: 592–597.
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** This is an excellent overview of enzymatic browning. This paper includes the biochemistry of enzymatic browning and extensive methods for the prevention of browning in fruits, vegetables and seafood.

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- 26 Sapers GM and Miller RL. Browning inhibition in fresh-cut pears. *Journal of Food Science* 1998; 63: 342–346.
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* This article shows that combinations of enzymatic inhibitors, reducing agents and antimicrobial compounds can be used successfully to decrease browning of apple slices stored at 5°C under normal atmospheric conditions. The dipping solutions consist of 4-HR, IAA, a sulphur-containing amino acid (N-acetylcysteine) and calcium propionate.

- 28 Dong X, Wrolstad RE and Sugar D. Extending shelf life of fresh-cut pears. *Journal of Food Science* 2000; 65: 181–186.
- 29 Tian SP, Xu Y, Jiang AL and Gong QQ. Physiological and quality responses of longan fruits to high-O₂ or high-CO₂ atmospheres in storage. *Postharvest Biology and Technology* 2002; 24: 335–340.

* In this study, the author found that CAs inhibited PPO activity, prevented peel browning and decreased fruit decay in comparison with MAP.

- 30 Tian SP, Jiang AL, Xu Y and Wang YS. Responses of physiology and quality of sweet cherry fruit to different atmospheres in storage. *Food Chemistry* 2004; 87: 43–49.

* The authors investigated physiological properties, quality attributes and storability of sweet cherry fruits stored in MAP and CA conditions during storage periods of 60 days, and found that a CA with 5% O₂ + 10% CO₂ more significantly inhibited the enzymatic activities of PPO and POD, reduced malondialdehyde content, effectively prevented flesh browning, decreased fruit decay and extended the storage life of sweet cherry fruits than other treatments.

- 31 Tian SP, Li BQ and Xu Y. Effects of O₂ and CO₂ concentrations on physiology and quality of litchi fruit in storage. *Food Chemistry* 2005; 91: 659–663.

** In this study, the authors examined the involvement of PPO, POD, anthocyanin and total phenols in cellular browning of litchi fruit, and revealed that CA conditions were effective in reducing total phenol content, delaying anthocyanidin decomposition, preventing pericarp browning and decreasing litchi fruit decay.

- 32 Zheng XL and Tian SP. Effect of oxalic acid on control of postharvest browning of litchi fruit. *Food Chemistry* 2006; 96: 519–523.

* In this article, the authors proved that oxalic acid at 2 and 4 mM could significantly inhibit pericarp browning of litchi fruit, suggesting that application of oxalic acid can effectively control pericarp browning of litchi fruit during postharvest storage.

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* This article provided a comprehensive overview of the considerable success obtained in controlling chilling injury and browning of peach fruit using CA storage, primarily as a result of delaying the reduction of antioxidant enzymes.

- 34 McHugh TH and Senesi E. Apple Wraps: A novel method to improve the quality and extend the shelf life of fresh-cut apples. *Journal of Food Science* 2000; 65(3): 480–485.

* In this study, edible coatings and films were made from apple puree with various concentrations of fatty acids, fatty alcohols, beeswax and vegetable oil, which were developed to extend the shelf-life and improve the quality of fresh-cut produce.

- 35 Lee JY, Park HJ, Lee CY and Choi WY. Extending shelf life of minimally processed apples with edible coatings and anti-browning agents. *Lebensmittel-Wissenschaft und-Technologie* 2003; 36: 323–329.

- 36 Loaiza-Velarde JG, Mangrich ME, Campos-Vargas R and Saltveit ME. Heat shock reduces browning of fresh-cut celery petioles. *Postharvest Biology and Technology* 2003; 27(3): 305–311.

** The authors found that the preparation of 5 mm segments of celery petioles induced an increase in the activity of PAL and subsequent tissue browning potential. They also found heat shock treatment at 50°C for 90 s significantly reduced PAL rise and browning, thus extending the storage life of the petiole segments.

- 37 Murata M, Tanaka E, Minoura E and Homma S. Quality of cut lettuce treated by heat shock: prevention of enzymatic browning, repression of phenylalanine ammonia-lyase activity, and improvement on sensory evaluation during storage. *Bioscience, Biotechnology and Biochemistry* 2004; 68(3): 501–507.

* In this study, the effect of heat shock treatment at 50°C for 90 s on the quality of cut lettuce during cold storage was examined. Result showed that the treatment is useful for prolonging the shelf-life of cut lettuce. It can significantly repress the induction of PAL activity and phenolics accumulation in cut lettuce during storage, and prevent browning of cut lettuce.

- 38 Lu S, Luo Y, Tumer E and Feng H. Efficacy of sodium chlorite as an inhibitor of enzymatic browning in apple slices. *Food Chemistry* 2007; 104(2): 824–829.

** This research investigated the efficacy of sodium chlorite as a browning control agent for use on fresh-cut apple slices, applied alone, or in conjunction with organic acids. Results showed that apple slices treated with acidified sodium chlorite or sodium chlorite solution had a significantly smaller decrease in *L* value indicating less browning than those treated with citric acid or water control.

- 39 Lu S, Luo Y and Feng H. Inhibition of apple polyphenol oxidase activity by sodium chlorite. *Journal of Agricultural and Food Chemistry* 2006; 54: 3693–3696.