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Understanding and Management of Browning in Fresh Whole and Lightly Processed Fruits

Riccardo Massantini* • **Fabio Mencarelli**

Department of Food Science and Technology, Postharvest Laboratory, Università della Tuscia, Via De Lellis, snc 01100 Viterbo Italy

Corresponding author: * massanti@unitus.it, www.postharvestlab.eu

ABSTRACT

Browning is the result of appearance of dark colored pigments formed by enzymatic and non enzymatic reactions. In this review AA, first overview the biochemistry of browning and fruits response and protection, successively touch the topic in the lightly processed fruits. Non enzymatic reaction is mainly due to Maillard reaction compounds in which concentration of amino acid (nitrogen supply) and sugars content plays the important role. Enzymatic reaction is driven mainly by polyphenols oxidase (PPO) but PPO is not the only factor, indeed the decline in the concentration of some browning activators, such as fatty acids and organic acids, and a decrease in phenolic substrate synthesis with increasing ripening, could be even important. Other enzymes are involved in the browning process, more or less intensively, depending on the product. The action of PPO as well as the other enzymes is favoured by membrane degradation which permits the contact between the enzymes and the substrate (PPO and phenols). Thus, every factor affecting membrane layers stability will result in a browning symptom. In some cases, anoxic conditions for short time prevent the appearance of browning because of reduction energy available, or because of the formation of ethanol and acetaldehyde. Carbon dioxide can inhibit browning but it can accelerate. 1-methylcyclopropene, ethylene action inhibitor, can inhibit superficial scald in apple but even provokes browning (greyish peel) in banana. In conclusion there is no prevention action against browning which can be recommended totally safe because the system is very complex and affected from many factors.

Keywords: colour retention, fresh-cut, LOX, oxidation, POX, PPO, SOD

CONTENTS

UNDERSTANDING BROWNING DISORDER OF FRESH FRUITS

Browning of fruit and vegetables is a chemical reaction which involves non enzymatic and enzymatic oxidation of phenolic compounds. Whatever the reason for browning, the quality of food is negatively affected because of the associated changes of colour, flavour, and softening. The major enzyme involved in browning process is polyphenol oxidase (PPO; EC 1.14.18.1). PPO is a copper-containing enzyme located mainly in plastids (Nicolas 1994), also known as tyrosinase, cresolase, catecholase, diphenolase, and phenolase. The PPO gene is encoded in the nucleus and translated in the cytoplasm; proPPO formed is then transported to the chloroplast where it is cleaved by a protease, producing the active form. This active form is responsible for the phenol oxidation to *o*-quinones and for condensation to brown polymers (melanins), through the first step which is the hydroxylation of monophenols to *o*-diphenols (monophenolase, cresolase or hydroxylase activity), and successively the oxidation of *o*-diphenols to *o*-quinones (diphenolase, catecholase or oxidase activity). pH, temperature, but, above all, oxygen are the main factors that trigger the oxidation process (Martinez and Whitaker 1995). Non enzymatic browning is generally referred to as the Maillard reaction, auto-oxidation reactions involving phenols with the formation of iron-phenol complexes, occurring generally in dried fruits such as 'Sultana' grapes and it is strongly dependent on the level of amino acids above all arginine at values of 3.8 and 7.7 mg/g, respectively in skin and flesh (Frank *et al*. 2005). Regarding PPO and its role in the phenol oxidation, an exhaustive review was done by Martinez and Whitaker (1995). Fresh fruits and vegetables are living cells in continuous and dynamic changes thus enzymatic browning, occurring in different fruits and in different situations (Tomas-Barberan and Espin 2001), even when products are intact and not subject to dramatic forms of stress i.e. cutting (fresh cut products), chilling injuries, high temperatures. Even though browning of peach (Luh and Phithakpol 1972), banana (Jayaraman *et al*. 1982), lettuce (Fukumoto *et al*. 2002), and strawberry (Chisari *et al*. 2007) has been related to PPO activity, browning cannot be seen as only PPO-dependent but it must be faced as a global, complex enzymatic process depending on the kind of stress which the product is subjected to. In apples, Valentines *et al*. (2005) found that the browning potential (measured by immersion of portion of peeled tissue under antioxidant or oxidant solution and then reading the colour change by chro-

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mameter) was higher in earlier harvested fruit and there was no relation to PPO activity. The Authors concluded this enzyme is not a limiting factor, but the decline in the concentration of some browning activators, such as fatty acids and organic acids, and a decrease in phenolic substrate synthesis with increasing ripening, could be more important. Peroxidase (POX; EC 1.11.1.7) has been assumed to play a role in enzymatic browning, after the activity of PPO in the oxidation of phenols where H_2O_2 is released (Richard-Forget and Gaillard 1997; Subramanian *et al*. 1999). H_2O_2 is continuously generated from various sources (chloroplast, mitochondria, peroxisomes) during the normal metabolism of cells but the high rate is normally balanced by very efficient antioxidant systems which involve the ascorbate and glutathione cycle and catalase activity (Neill *et al*. 2002). A biotic stress such as dehydration, low and high temperatures, and excess irradiation can accelerate H_2O_2 generation, which becomes a signal for enzyme activation, gene expression, programmed cell death (PCD) and cellular damage. The reduction of the antioxidant system more than the increased H_2O_2 production is responsible for a disastrous oxidative effect. For instance, in litchis (*Litchi chinensis* Sonn.), browning, which is the major problem in the distribution chain for this fruit, POX has been seen to be involved in this disorder together with PPO (Gong and Tian 2002; Jiang *et al*. 2004). In strawberry, the role of POX on browning was partial depending on the variety (Chisari *et al*. 2007). Superoxide dismutase (EC 1.15.1.1) can convert the superoxide ion to H_2O_2 ($2O_2^{\bullet-} + 2\ H+ > H_2O_2 + O_2$) and it is found in mitochondria and cytosol. SOD activity together with APX (ascorbate peroxidase; EC 1.11.1.11), GR (glutathione reductase; EC 1.6.4.2), and CAT (catalase; EC 1.11.1.6) showed lower activity in mandarins with a high incidence of rindstaining, a browning disorder (Sala and La Fuente 2004). In bruised apricots (*Prunus armeniaca* L.) where external light browning occurred after 3 days, SOD activity increased greatly in injured and sound tissues of the same bruised fruit, while POX activity did not rise, indicating an inability of POX to detoxify H_2O_2 and this could be the cause of the browning reaction (De Martino *et al*. 2006). In our laboratory, drying red grapes (*Vitis vinifera* L.) for wine production, we observed oxidation of phenolic compounds occurring with the progress of water loss, and brown discoloration took place; phenolic acids (*trans* caftaric, gallic, ferulic, caffeic acids) disappeared while anthocyanin content was reduced significantly at 20°C but not at 10°C; in contrast PPO activity, at 10% of weight loss, was 5-fold higher in berries dehydrated at 10°C than at 20°C (unpublished data). In white grapes, darkening of epicarp occurs with ripening due to the oxidation of carotenoids with the development of C13 nor-isoprenoids, pleasant volatiles compounds such as β-damascenone and β-ionone (Wachè *et al*. 2003). During ripening and senescence of loquat (*Eriobotrya japonica* Lindl), browning is a characteristic feature; phenols loss and increase of PPO activity has been found (Cai *et al*. 2006). The Authors found increase of electrical conductivity parallel to the rise of lipooxygenase (LOX) which is known to contribute to membrane deterioration with formation of hydroperoxides (Paliyath and Droillard 1992). In 'Malvasia' grapes, the increase of LOX and release of C6 volatiles compounds (aldehydes and alcohols) occurred immediately with water loss as the first marker of water stress, before skin browning appeared (Costantini *et al*. 2006). In nitrogen-treated peaches (*Prunus persica* L.), LOX activity was higher than in the air-treated sample (Bellincontro *et al*. 2005) because even in the absence of oxygen membranes are subjected to degradation (Rawyler *et al*. 2002). Recently, Franck *et al*. (2007) showed a model that assumes that browning disorder in pears (*Pyrus malus* L.) is caused by an imbalance between oxidative and reductive processes due to metabolic gas gradients inside the fruit, leading to an accumulation of reactive oxygen species (ROS), which, in turn, induce the loss of membrane integrity which becomes visible through the enzymatic oxidation of phenolic compounds. It has been postulated that browning disorder does not occur unless the L-AA (ascorbic acid) concentration falls below a certain threshold (Zerbini *et al*. 2002; Franck *et al*. 2003) but it is doubtful that only one compound can be responsible for a complex disorder such as browning. Larrigaudiere *et al*. (2004) showed that 1-MCP–treated fruits had a lower content of ascorbic acid and hydrogen peroxide but higher activities of enzymatic antioxidant potential (superoxide dismutase, ascorbate peroxidase, catalase, and peroxidase). Application of anti-browning compounds with a specific function such as AA (ascorbic acid) and DPA (diphenylamine) as antioxidants or citric acid (reducing pH) halts the appearance of browning but only rarely they are able to control the browning generative metabolism. This is the case of superficial scald (a kind of superficial browning) of apple (*Malus domestica* L.) and pear, the major storage disorders for these fruits, which generally intensifies after the removal from storage; it is due to necrosis of the hypodermal cortical tissue and it is thought that this cell damage is induced by oxidation products of a sesquiterpene (*E*,*E*-α-farnesene) and its conjugated triols (CTols) (Rowan *et al*. 1995, 2001; Gapper *et al*. 2006). Today scald is commercially controlled by ethoxiquin (1,2 dihydroxy-6-2,2,4-trimethylquinoline), DPA, LO (low oxygen) or ULO (ultra low oxygen). The first two products act by delaying the accumulation of *E*,*E*-α-farnesene or by halting its oxidation; the ULO or LO atmospheres delayed the accumulation of *E*,*E*-α-farnesene and CTols and even nearly completely suppressed ethylene (Whitaker *et al*. 1997). The role of ethylene has been confirmed recently by the application of 1-MCP, ethylene action blocker, which greatly curtails *E*,*E*-α-farnesene production and markedly reduces scald incidence and severity (Chen and Spots 2005; Lurie *et al*. 2005; Gapper *et al*. 2006) indicating that ethylene synthesis and perception is involved in the regulation of the synthesis. The involvement of ethylene would explain why apples harvested earlier are more susceptible than those harvested later but this is not always the case for storage disorder, i.e., breakdown of apples appears more frequently in late harvested apples.

As it has been described above, generally speaking browning is the sensorial response to an oxidation system due to the direct contact between an enzymatic pool and an easy oxidising group of compounds such as, primarily, phenols which after oxidation provide brown and black pigmentation. The initial reaction, catalysed by PPO, uses O_2 as co-substrate but other important factors are also phenols concentration, PPO concentration and activity, and ascorbic acid and peroxidases. As PPO and its substrate are located in different cell compartments (cytoplasm/plastids and vacuole, respectively) enzymatic browning is a direct consequence of membrane disintegration. Thus membrane integrity plays an important role in the browning manifestation. When degradation (catabolic) processes exceed the maintenance (anabolic) processes, membrane disruption occurs. Changes in membrane fatty acid synthesis can lead to membrane alteration and the energy storage under ATP (adenosine triphosphate) is the first step of fatty acid biosynthesis (Ohlrogge and Browse 1995). High ATP and ADP (adenosine diphosphate) concentrations and high adenylate energy charge levels are associated with low pericarp browning and low membrane permeability (high membrane integrity) in N_2-pretreated (nitrogen for 6 hours at 20°C and then in air) litchi fruits (Liu *et al*. 2007). In this paper, anoxia treatment inhibited AMP (adenosine monophosphate) degradation. Storage of pears under low O_2 conditions may induce metabolic adaptations to survive (induction of fermentation) or avoid anoxia (i.e. O_2 and ATP consuming pathways are retarded). It appears that plant cell have a low oxygen sensing system to adapt to low oxygen condition (Geingenberger 2003). If low o no oxygen can have positive effect on controlling browning the formation of metabolites such as ethanol and acetaldehyde can drive to browning disorders (Ke and Kader 1992). Low limits for oxygen varies depending on fruits and variety i.e. in apple the limits are 0.7, 0.9, and 1.9% respectively for 'Delicious', 'Law Rome', and

Fig. 1 High carbon dioxide enhances the browning due to chilling injury (3°C) in eggplants.

Fig. 2 Senescent banana (top) treated only with ethylene and senescent banana treated with 1-MCP after the ethylene treatment (bottom). It is possible to observe the greyish peel with black spots and green parts on the bottom picture.

'McIntosh' (Gran and Beaudry 1993) but fruits can try advantage from the formation of ethanol and acetaldehyde (Pesis 2005). Not only lack of oxygen can be responsible of browning disorder but even high carbon dioxide. Brown core in pear increased with the increase of carbon dioxide (Pinto *et al*. 2001) but it is not always clear if this disorder is related to accumulation of ethanol and acetaldehyde. Short exposure to CO_2 decreases the pH of avocado (Lange and Kader 1997) and this could justify the formation of acetaldehyde and ethanol (Yanez *et al*. 2001) which in turn can cause internal browning. Ethanol is known to have a strong effect on membrane lipids disordering that results in reduction of membrane viscosity (Chin and Goldstein 1981). The entity of this effect can have positive or negative results. Ethanol treatment have shown to reduce blackening of protea (*Protea* L.) leaves (Crick and McConchie 1999) and superficial scald in apple is reduced by ethanol treatment (Gharamani and Scott 1998). High CO_2 has been seen to modulate the phenolic response (less susceptibility to chilling injury and browning) in cherimoya (*Annona cherimola* Mill.) (Maldonado *et al*. 2002). In contrast, in eggplants, high CO_2 (2-12%) increased the symptoms of chilling injury (**Fig. 1**) (Mencarelli *et al*. 1989) and in apple, concentration of 20% for 1-3 days at harvest induced flesh browning (Volz *et al*. 1998). Acetaldehyde has shown positive effect in controlling browning on avocado due to higher concentration of free SH groups (Pesis *et al*. 1998) but higher concentration can induce strong oxidation of the tissue (Pesis 2005). Ethylene induces browning on different tissue due to acceleration of senescence process with membrane and cell wall degradation (Saltveit 1999). The positive effect of low O_2 and/or high CO_2 on browning control can also be attributed to the inhibition of ethylene biosynthesis and action. The positive effect of 1-MCP to inhibit superficial scald in apple has been attributed not only to inhibition of ripening but to the influence on peroxidation and POX activity (Vilaplana *et al*. 2006). In contrast, in banana, treatment with 1-MCP after ethylene treatment, even though delayed softening, yellowing, and sugars accumulation, it induced an uniformly diffused grey colour to the peel which masks partially the yellow colour, compromising the banana appearance (De Martino *et al*. 2007) (**Fig. 2**).

Molecular approach to control browning in fruits has not been well elucidated. Allen *et al*. (1997) discussed on the potential to use transgenic plant to protect plant against oxidative stress but up to date not many papers have been published on this topic especially in relationship to fruit. Overexpression of PPO in transgenic sugarcane has provided darker juice showing the influence of PPO on browning (Vickers *et al*. 2005). In potato, antisense PPO reduced enzymatic browning (Coetzer *et al*. 2001) and in transgenic apple calli prepared by using *Agrobacterium tumefaciens* with PPO antisense, PPO acitivity was reduced of 50% as well as browning.

In conclusion, browning word includes a large number of disorders, the complexity of which is hard to describe in few lines. Browning is a result of field management, harvest management, shipping management, storage conditions all

of them which can integrate to develop the disorder or act alone. This the reason for which sometimes the results are in contrast.

CONTROL OF BROWNING IN LIGHTLY PROCESSED FRUITS

Food statistics show that minimally processed fruits are becoming more and more popular because the wealthier US and European consumers, with their busy and comfortable lifestyle and increasing purchasing power, are demanding more ready-to-eat fruit. Even if fresh fruit costs less, consumers are willing to pay more for minimally processed fruit, due to the additional services provided such as: selection, washing, sanitation, cutting and packaging that increase the value.

The most important aspects of fresh-cut produce are its colour, texture, flavour and smell. Apart from these comercial characteristics, consumers expect minimally processed fruits to be safer and more nourishing than fresh fruit. Fresh cut produce has a shorter shelf life compared to whole produce. Peeling and cutting essentially cause a wound response in the physiology of processed fruits with increase both in the respiration rate and ethylene production. As a consequence deteriorating enzyme reactions take place in addition to tissue cut surface, dehydration and microbial spoilage.

For consumers the most important requirement of a fresh-cut product is probably its colour, and therefore browning shortens the shelf-life of minimally processed products considerably. The enzymatic oxidation of phenolic compounds by PPO is usually the main but not the unique cause of enzymatic browning as discussed above. Many cells are damaged during cutting and peeling causing intracellular products such as oxidizing enzymes to be liberated and put in contact with their substrates. In order to prevent or at least hinder enzymatic browning, it is necessary to eliminate oxygen and/or inhibit enzyme deterioration. There are both physical and/or chemical methods that can be used to control enzymatic browning and researchers have suggested several different approaches.

Sharp knives and blades should be used during peeling and cutting in order to avoid cell damage. Mencarelli *et al*. (1993) showed that using a non-serrated blade for cutting potatoes reduced the browning effect. After cutting, fruits and vegetables are washed to remove intracellular residues and micro-organisms; moreover, additives can be added to the water for preserving the produce. It can then be gently dried to avoid further stress to the tissues.

Use of chemicals for controlling browning

There are also some natural products and their derivatives that can be positively used to prevent the browning of cut tissues. Moline *et al*. (1999) tested several natural compounds such as canned pineapple juice, lemon and lime juices, ascorbic, isoascorbic, galatturonic, gallic, malic, mucic and many others acids in order to prevent browning on sliced banana (*Musa acuminata)*. Only citric acid and *N*-acetylcysteine did not reveal any objectionable flavours. Also Buta *et al*. (1999) attempted to lengthen the time in cold storage of minimally processed apple slices (*Malus domestica* cv. 'Delicious') using enzymatic inhibitors like 4-hexylresorcinol, isoascorbic acid, *N*-acetylcysteine and calcium propionate. Slices treated with the combination of the above mentioned compounds retained higher levels of malic acid and had no deterioration in sugar levels (fructose, glucose, sorbitol and sucrose).

Raybaudi-Massilia *et al*. (2007) confirmed the possibility of shelf life extension on fresh-cut 'Fuji' apples (*Malus domestica*) at two stage of ripeness using natural substances enclosing *N*-acetylcysteine. Fresh-cut apples were immersed for one minute in two different solutions, one contained *N*-acetyl-L-cysteine at 1% (w/v), glutathione at 1% (w/v) and calcium lactate at 1% (w/v) (CGLW), and the other one contained CGLW and L-malic acid at 2,5% (w/v). Both solutions prevented browning of cut apples and maintained the fresh-cut apple colour for 14 days at 5°C.

The best way to study colour control is to carry out various tests on different browning agents and their mixtures. Dipping fruit into water not only rinses off the enzymes and substrate released by damaged cells after cutting the surface off the fruit, but it is also a means of adding additives to minimally processed products. Legislation regarding additives may differ from country to country and therefore the choice of anti browning agents must comply with local legislation.

A common procedure after processing is to dip freshly-cut fruits in solutions containing ascorbic acid (AA). Soliva-Fortuny *et al*. (2002) showed that the phase of ripeness of 'Golden delicious' apple (*Malus domestica*) can cause a change of colour on freshly-cut apple slices. AA is probably the most widely used anti-browning agent: it reduces the risk of browning but it also slightly lowers the pH of the fruit. Thiol-containing compounds like cysteine are also reducing agents that control enzymatic browning (Buta *et al*. 1999; Moline *et al*. 1999). Citric acid is an acidulating substance which is usually used in combination with other anti-browning agents. Of all chemical preservatives, the most widely used is 4-hexylresorcinol because it hinders PPO but even so, its effectiveness in the prevention of enzymatic browning can be improved by combining it with other additives (Dong *et al*. 2000).

Buta *et al*. (1999) tested a combination of enzymatic inhibitors, reducing agents and antimicrobial compounds containing calcium obtained from natural products, which extended shelf life preventing the browning and microbial spoilage of 'Red Delicious' apple slices. It was found that the combination of 0.001 M 4-hexylresorcinol (4-HR) + 0.5 M isoascorbic acid + 0.05 calcium propionate + 0.025 M homocysteine maintained minimally processed apples for 4 weeks at 5°C. The most effective treatment for preventing the browning of banana slices (*Musa acuminata)* for 7 days of storage was a combination of 0.5 M citric acid and 0.05 M *N*-acetylcystene. Other treatments with compounds applied alone or mixed together such as citric acid, cysteine, AA, 4-HR, neutral sugars, sulphur-containing amino acids and their derivates, and fruit juices were effective (Moline *et al*. 1999). Dong *et al*. (2000) found a combination of 0.01% 4-HR, 0.5% AA and 1.0% calcium lactate effective in maintaining the surface colour of fresh-cut 'Anjou', 'Bartlett' and 'Bosc' pears (*Pyrus malus)* for 30 days in refrigerated storage. By dipping 'Bartlett' pear slices in a solution containing 2% (w/v) AA, 1% (w/v) calcium lactate, and 0.5% (w/v) cysteine adjusted with NaOH to pH 7.0, kept them firm and prevented surface cutting thus extending their shelf life up to 8 days at 0°C (Gorny *et al*. 2002).

The above studies show us how different species have to be treated differently in order to maintain their colour.

Use of MAP to control browning

Regarding preservation by means of the modification of the atmosphere, although fresh-cut fruit can tolerate a more extreme level of O_2 and CO_2 concentrations, exposure beyond its limit of tolerance may lead to anaerobic respiration developing off-flavour and stimulating growth of food-born pathogens. The subject of many articles published in the past was how to preserve the quality of minimally processed fruits through the study of a proper storage atmosphere. Bai *et al*. (2003) compared the quality standards of fresh-cut honeydew melon cubes (*Cucumis melo* var. inodorus) held in passive modified atmosphere packaging (nMAP), an active MAP with 4 kPa O_2 plus 10 kPa CO_2 (fMAP) and perforated film packaging (PFP). Both passive MAP and active MAP positively affected visual quality and aroma keeping the shelf life of the cubes for 9 days at 5°C while the shelf life of the cubes in PFP was from 5 to 7 days due to tissue translucency and/or off odour development. fMAP preserved quality better than nMAP in

terms of colour retention, development of translucency, respiration rate and microbial population. Since active packaging costs more than passive packaging, (cost of gas packaging machinery, gases and packaging material), it is important to compare how active and passive MAP influence quality standards and eventually decide if the market price is able to cover the extra expense of active packaging. On designing a MAP we must keep in mind the quality characteristics of the raw material (Bai *et al*. 2003) as well as the storage temperature at retail markets (Qi *et al*. 1999). The shelf life of fruits and vegetables is greatly influenced by the storage temperature but keeping a steady temperature is always a critical point. In supermarkets the usual storage temperature of commodities is higher than the optimal temperature and the rise in respiration rate may lead to a rapid O_2 loss and the development of an anaerobic condition inside packaging. A gas mixture of 2% O_2 plus 10% CO_2 maintains the quality of fresh-cut honeydew melons at the optimal temperature of 5°C while a 4% O_2 plus 10% CO_2 CA was beneficial at 10°C (Qi *et al*. 1999). The authors underlined that "these combination of gases are desirable only when temperature is strictly controlled" and a higher O_2 level in the gas mixture of modified atmosphere packaging should be considered if the commercial storage temperature is higher.

Appropriate MAP can strengthen the effectiveness of anti-browning agents, in fact the right mixture of gases combined with plastic film decreased the PPO activity in 'Golden Delicious' apple slices (Soliva Fortuny *et. al*. 2001). 4-hexylresorcinol, 4-HR, (0.001 M) plus D-isoascorbic acid (=ES) (0.5 M) plus potassium sorbate (=KS) (0.05 M, an antimicrobial compound) combined with nMAP inhibit browning, decay and deterioration of minimally processed mangos (*Mangifera indica* L. cv. 'Kent'). 4-HR+ES+KS reduced changes in colour, microbial growth and did not affect the sensory characteristics of mango slices, and a high percentage of humidity inside the packaging reduced water loss in the tissues (Gonzalez-Aguilar *et al*. 2000).

The paper by Gorny *et al*. (2002) showed that using MAP alone did not prevent cut surface browning of 'Bartlett' pear slices. Minimally processed pears kept in different low O_2 and/or high CO_2 showed similar rates of browning and 20 kPa CO_2 accelerated browning and necrosis in the flesh tissue.

In the past few years the effect of non conventional atmosphere packaging on the shelf life of minimally processed kiwifruit (*Actinidia deliciosa* var. 'Hayward') (Rocculi *et al*. 2005), 'Granny Smith' (Corbo *et al*. 2000) and 'Golden Delicious' apples (Soliva-Fortuny, *et al*. 2002), have been examined with interesting results. Apples slices that were dipped in a solution of 1% AA and 0.5% calcium chloride and stored under 100% N_2 atmosphere preserved their colour with minor changes compared to nMAP (Soliva-Fortuny *et al*. 2002). Rocculi *et al*. (2004) tested different mixtures of gases in order to preserve the quality of 'Golden Delicious' apples slices. Prior to pack-aging they were dipped into a solution containing 0.5% AA + 0.5% citric acid + 0.5% calcium chloride. Minimally processed apples packed in 25% Ar, 65% N_2O, 5% CO_2, 5% O_2 and packed in 90% N_2O, 5% CO_2, 5% O_2, preserved their original colour better. In particular, slices packaged with Ar showed the lowest rate of browning and maintained their quality for 12 d at 4°C. As suggested by authors (Rocculi *et al*. 2004) Ar could inhibit PPO replacing O_2 due to the fact that has the same solubility. The gas mixture containing 90% N_2O, 5% CO_2, 5% O_2, showed a better colour retention in kiwifruit stored for 12 d at 4°C (Rocculi *et al*. 2005).

Lanciotti *et al*. (1999) tested the effect of hexanal on the shelf life of fresh-cut 'Granny Smith' apples previously dipped in a solution with AA and citric acid. The atmosphere containing 70% N_2, 30% CO_2 and hexanal was found to be very effective in controlling browning for 17 days at 15°C. At 4°C there were no changes observed in hue angle value. The authors (Lanciotti *et al*. 1999) examined the residual enzymatic activity of PPO expressed as a lag phase and also found that the gas mixture with hexanal, delayed the enzymatic reaction after the opening of the packaging. It was found that hexanal tended to disappear in 2-3 days during storage due to both its conversion in hexanol and hexyl acetate and its partition in the apple tissues. These authors suggested that the conversion of hexanal to hexanol could explain its browning prevention. This is because aliphatic alcohols are regarded as inhibitors of polyphenol oxidase. They also suggested that phenylalanine ammonia lyase (a key enzyme involved in polyphenol biosynthesis activated by tissue wounding and ethylene) could be a possible target for hexanal. Corbo *et al*. (2000) added *trans*-2-hexenal to the gas mixture described above, and found that this compound had a negative effect on colour retention of 'Granny Smith' apple slices. By increasing the concentration of hexanal there was a reduction of browning which was directly correlated to the rise in temperature. The use of natural volatile compounds to maintain the quality of fresh-cut fruit seems to be a very promising field of research since minimally processed fruits sold at retail markets are at least packed in MAP and do not usually contain any preservatives or antimicrobial substances. C6 compounds have a very low perception threshold and it would be interesting to assess if and how they affect the quality of cut fruit in terms of aroma.

Use of edible coating

A promising tool to improve the quality and extend the shelf life of minimally processed fruit is by applying edible coating and fruit-based edible film made from fruit puree. Further investigation is required due to contrasting results. An edible coating is a thin layer of edible material applied to food in liquid form by dipping, panning or spraying while film wrap is a preformed thin layer of edible material placed on or between food components (McHug and Senesi 2000). By using edible coatings and films on minimally processed fruits, we can see their capacity to regulate the transfer of moisture, and the transmission of O_2 and CO_2, and so it can be considered a physical method for controlling enzymatic browning. Dong *et al*. (2000) tried sucrose ester (Semperfresh^{TM}), which is a commercial browning inhibitor proposed for its oxygen barrier properties on 'Anjou' and 'Bosc' pear slices, but it did not satisfactorily inhibit browning. The use of Semperfresh^{TM} and Freshseal on fresh-cut apricots (*Prunus armeniaca* var. tirynthos) gave contrasting results (Massantini *et al*. 2000).

Chitosan-based edible coating was successfully used on peeled litchi fruits (*Litchi chinensis* var, Huaizhi) (Dong *et al*. 2004) at different concentrations maintaining quality attributes and extending shelf life. PPO and POX activity in treated litchis was inhibited after 6 days at -1°C.

An interesting research was carried out concerning edible coatings and films made from apple puree with different concentrations of fatty acids, fatty alcohols, beeswax and vegetable oil (McHugh and Senesi 2000). Concerning the control of browning they found that wrappings completely inhibited browning of 'Granny Smith' apple slices for up to 10 days at 5°C while coating was only able to control it for 3 days, due to the fact that wrapping was a more effective oxygen barrier than coating. Moreover wrapping preserved other qualities such as texture and flavour and it also prevented water loss better than coating.

Rojas-Graü *et al*. (2007) tested apple-puree alginate edible coatings to prolong shelf life of fresh-cut 'Fuji' apples (*Malus domestica*). Coatings solutions were prepared by mixing apple puree (26% w/w) with alginate solution (2% w/w), glycerol (1.5% w/w) and different concentration of essential oils. After dipping in coating solutions, apples cylinders were dipped in a solution containing *N*-acetylcysteine (1% w/w) and calcium chloride (2% w/w). The results showed that alginate-apple puree edible coating containing essential oils (lemongrass, oregano and vanillin) can extend the shelf-life of apples slices and *N*-acetylcysteine is an effective antibrowing agent to be incorporated in the for-

mulation of edible coating as confirmed by Raybaudi-Massilia *et al*. (2007).

Alginate coatings were successfully tested also on 'Gala' apples (*Malus domestica*) (Olivas *et al*. 2007). Apples slices immersed in acqueous solution of 10% calcium chloride followed by application of alginate based coating (Alg-Ca) or alginate-acetylated monoglyceride-linoleic acid based coating (Alg-Ca-AMG) or butter linoleic acid acid based coating (Alg-Ca-MF), showed less browning compared to non-treated apple slices.

CONCLUDING REMARKS

Browning control can be done with physical and several antioxidant chemical compounds which can behave as quenchers or scavengers or with enzymes inhibitors even from natural products, e.g., new derivatives of licorice roots (glabrene, glabridin) or 2,4 dihydroxyphenilpropionic acid from figs leaves (Nerya *et al*. 2006) used for mushrooms. In whole fruits control of browning disorders is performed by physical treatment such as controlled atmosphere, ethanol, acetaldehyde, ethylene control, and only for superficial scald chemical compounds are used. In lightly processed fruits, where the problem is very great, chemical compounds are widely used. Anyway, no chemicals and physical treatment can guarantee a total success of the treatment due to the complexity of the phenomenon. The external factors which can contribute to protect this degradation (temperature control, relative humidity control, right time for harvest of fruits or vegetables and not only for the market, right use of atmosphere and not extreme long-term storage) can avoid the use of unhealthy chemical compounds. Use of modified atmosphere for lightly processed fruits and vegetables is very often limited by the cost of the application and by the inaccurate cold chain which is the keyword for the market success of these products.

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