PREVENTION OF ENZYMATIC BROWNING IN FRUIT AND VEGETABLES

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Abstract

Enzymatic browning is the second largest cause of quality loss in fruits and vegetables. Methods to prevent browning are the subject of a great deal of research in the field of the food industry. In this paper we review all the methods to prevent oxidation in fruit and vegetable. Studies developed along the last decade, like as chemical, physical (blanching, freezing), controlled atmosphere and coating methods, to prevent enzymatic browning are reported and discussed.

Keywords: Enzymatic browning, dipping, blanching, coating, preservation

Introduction

Fruit and vegetables have health benefits for consumers, due to their content of fiber, vitamins and antioxidant compounds. However, for the antioxidant compounds many changes occur during harvesting, preparation (fresh-cut fruits) and storage of these fruits. These changes induce a pronounced loss of the microbiological and antioxidant qualities (Lindley, 1998). Thus, preservation against oxidation in food during processing and storage has become an increasing priority in the food industry. In fact, oxidation is the second most important cause of food deterioration after that induced by microbiological contamination. The main oxidative reactions are enzymatic browning. They involve two oxidoreductases enzymes: polyphenoloxidase (PPO) and peroxydase (POD). PPO catalyzes two reactions; the first, a hydroxylation of monophenols to diphenols, which is relatively slow and results in colourless products. The second, the oxidation of diphenols to quinines, is rapid and gives coloured products (Queiroz, Lopes, Fialho & Valente-Mesquita, 2008). 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 (Toivonen & Brummell, 2008). 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.

Numerous methods and strategies for post harvest storage of fruits and vegetables are discussed in the literature. Artes (1998) reviewed the methods to prevent oxidation by chemical, controlled atmosphere and coating treatments. Several chemical treatments are used to preserve colour, Oms-Oliu (2010b) reviewed recent advances and underlined new strategies to use natural preservatives. Singh (2006) analyze the effect of controlled atmosphere during the storage of fruit and vegetables. Coating has also been largely discussed by Olivas (2005) and by Vargas (2008). Queiroz (2008) present PPO characteristics and some methods to control enzymatic browning. All the previous reviews deal only with one or two preservation methods. In this paper, we propose to gather and give the new advances in all the methods used to prevent enzymatic browning in fruit and vegetable during the last decade.

Chemical treatments will be presented by underlining the main action of each molecule (antioxidants, acidifying, agents of firmness or chelating agents). Then physical methods (blanching, freezing and the modification of product atmosphere) will be updated by introducing the new advances in this field. Coating methods will also be discussed in this paper as will the combination of several preservation methods. The last part of this paper will deal with the new methods of preservation.

1. Pre-treatment of fruit

In the case of an entire product, the action of chemical and physical treatments can be limited by the presence of the cuticle of the fruit and vegetables. The fruit cuticle is composed of hydrophobic biopolymers (cutin) between which there are waxes. It is a natural barrier to external attacks and also to water and solutes transported to and out of the plant. It represents the main limitation to the diffusion of molecules used in chemical treatments or to the efficiency of physical treatments such as blanching. Therefore techniques of pre-treatments were elaborated, such as permeabilisation of the cuticle which may allow a better treatment in the core of the product. Several authors suggest strategies to break down the cuticle and promote trade. The different permeabilisation methods found in the literature are mechanical or chemical pre-treatments.

Mechanical permeabilisation methods

One possibility is the perforation with a set of fine needles mounted on a vertical metal base to create micro holes (density: 80 to 120 holes/cm²) (Shi, Le Maguer, Wang & Liptay, 1997). This solution, however, seems to be not applicable on an industrial scale. Di Matteo and others (2000) proposed a treatment by mechanical abrasion on the skin of grapes. The abrasion of the grape skin is carried out in an agitator whose walls are covered with an abrasive surface, for duration of 10 min. This method improves the mass transfer coefficient by a factor of 4 (Di Matteo, Cinquanta, Galiero & Crescitelli, 2000). Permeabilisation can be achieved by vacuum impregnation, so the effect of ascorbic acid is enhanced by vacuum impregnation rather than dipping (Joshi, Rupasinghe & Pitts, 2010; Shao et al., 2011).

Chemical permeabilisation

The cuticle can be degraded by treatment with a bath of 1-8% (v / v) NaO and 2-8% (v / v) ethyl oleate at T = 35 ° C (Shi et al., 1997). Similarly, Di Matteo and others (2000) use an aqueous 2% (v / v) ethyl oleate and 2.5% (v / v) K_2CO_3 at 40 ° C for 3 min.

Other techniques have been identified, based on a dispersion of arabic gum (Vogg et al., 2004). The dried gum can be peeled with forceps. But again, this solution does not seem feasible on an industrial scale.

2. Chemical treatments

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 summarized in Table 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). Their effectiveness depends on environmental factors such as pH, water activity (a_w), 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 (Oms-Oliu, Aguilo-Aguayo & Martin-Belloso, 2006; Arias, Gonzalez, Oria & Lopez-Buesa, 2007). 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 agent.

Treatment with chelating agents

PPO requires copper ions to be active (Du, Dou & Wu, 2012). 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.

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, Hernando, Perez-Munuera & Lluch, 2007; Guan & Fan, 2010; Khunpon, Uthaibutra, Faiyue & Saengnil, 2011). The main agents of firmness are calcium lactate E327, calcium propionate E282, calcium chloride E509, calcium ascorbate E302 and sodium chloride.

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 (Grimm, Khanal, Winkler, Knoche & Koepcke, 2012). The main acidifying agents are citric acid E330, erythorbic acid E315, ascorbic acid E300 and glutathione.

The chemical treatments shown in table 1 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 product.

Table 1 Studies on chemical treatments used to prevent enzymatic browning (Room Temperature: RT)

Products	Chemical agents	Time / T°	Results	References
Apple	Phytic acid (0.08%)	RT	Inhibition of the PPO (99.2%)	(Du et al., 2012)
	Ascorbic acid (0.3 mM)	10 min	Decrease of the browning.	(Grimm et al., 2012)
	Immersion into1% (w/v) ascorbic acid + 0.1% (w/v) calcium chloride pH 3.5	4°C/5 min	Preservation of the apple texture after UV-C irradation and storage at 5 °C	(Gomez, Garcia-Loredo, Salvatori, Guerrero & Alzamora, 2011)
	Sodium chloride (300 mg /L), acidified sodium chlorite (300 mg /L), citric acid (20 g/ L), calcium chloride (20 g/l)	RT/1 min	The most effective treatment is with sodium chlorite.	(Luo, Lu, Zhou & Feng, 2011)
	Sodium chloride and/ or calcium propionate at different concentrations (0-2%)	5 min	Each chemical agent is not sufficient to inhibit browning, a combination of both is necessary.	(Guan & Fan, 2010)
	Sodium chloride + citric acid at different concentrations	1 min	0.5 g/l sodium chlorite with a pH from 3.9 to 6.2 adjusted using citric acid is the most effective treatment to prevent browning	(Lu, Luo, Turner & Feng, 2007)
	4% calcium propionate	RT/30 min	Preservation of the parenchyma structure and minimization of the degradation of fresh-cut apples	(Quiles et al., 2007)
	1% N-acetyl-cysteine + 1% glutathione + 1% calcium lactate	RT /1 min	Preservation of the firmness and the colour during a storage of 30 days at 5°C	(Raybaudi-Massilia, Mosqueda-Melgar, Sobrino-Lopez, Soliva-Fortuny & Martin-Belloso, 2007)
	0.5% ascorbic acid + 1% calcium chloride + 0.1% propionic acid pH 2.74	20 ° C / 3 min	Preservation of the texture and prevention of enzymatic browning	(Varela, Salvador & Fiszman, 2007)
	0.5 % Ascorbic acid + 0.5% calcium chloride	5 min	The most effective treatment for delaying browning	(Zhu, Pan & McHugh, 2007)
	Sodium benzoate (0.03 %) + Potassium sorbate (0.03 %) with or without calcium lactate (0.5 %) +	10°C/1 min	Increase of apple structure stability with calcium lactate. Preservation of	(Alandes, Hernando, Quiles, Perez-Munuera & Lluch, 2006)

			apple texture for 3 weeks at 4°C	
	Sodium metabisulfite, 4-Hexylresorcinol, Ascorbic acid, L-Cysteine, Reduced gluthatione, Maillard Reaction Products.	5 min	Proportional correlation between agent antibrowning concentration and their inhibitory effect.	(Eissa, Fadel, Ibrahim, Hassan & Abd Elrashid, 2006)
	7% calcium ascorbate	8°C/2 min	Preservation of the firmness and decrease of the browning reactions.	(Fan, Niemera, Mattheis, Zhuang & Olson, 2005)
Kiwi	2% ascorbic acid + 2% calcium chloride	RT/2 min	Treatment effective at delaying softening and browning	(Antunes, Dandlen, Cavaco & Miguel, 2010)
Watermelon	2% sodium chloride	RT	Preservation of the firmness of fresh cut tissue throughout storage	(Mao, Jeong, Que & Huber, 2006)
Pear	1-Methylcyclopropene (300nL/L) then 2% ascorbic acid + 0.01% 4-hexylresorcinol + 1% calcium chloride	0°C/24h 4°C/15 min	Browning and softening are delayed	(Arias, Lopez-Buesa & Oria, 2009)
	Ascorbic acid + 4 - hexylresorcinol	30°C	Synergistic effect between ascorbic acid and 4-hexylresorcinol for the inhibition of the polyphenoloxidase	(Arias et al., 2007)
	0.75% N-acetylcysteine or 0.75% glutathione	15°C/2 min	Prevention of browning of pear wedges during storage	(Oms-Oliu et al., 2006)
Mango	3% sodium chloride	10 ° C / 2 min	Significant decrease of the loss of tissue firmness.	(De Souza, O'Hare, Durigan & de Souza, 2006)
Eggplant	Calcium ascorbate or citrate (0.4%)	60°C / 1 min	Calcium ascorbate was the best treatment to inactivate enzymes	(Barbagallo, Chisari & Caputa, 2012)
Artichoke	Ascorbic acid, citric acid, cysteine and their combination, ethanol, sodium chloride, 4-hexylresorcinol	RT/1 min	Cysteine (0.5%) was the most effective treatment to prevent browning	(Amodio, Cabezas-Serrano, Peri & Colelli, 2011)
Longan fruit	0.01% Sodium chlorite	RT/10 min	0.01% is the optimal concentration to reduce browning and	(Khunpon et al., 2011)

			polyphenoloxidase and peroxidase activities	
	1.5 N hydrochloric acid then rinsing	RT/20 min	Pericarp browning is delayed	(Apai, 2010)
Potato	1% sodium acid sulfate + 1% citric acid and 1% ascorbic acid	RT	Polyphenoloxidase activity and browning are reduced	(Calder, Skonberg, Davis-Dentici, Hughes & Bolton, 2011)
Chestnut	0.5 µM Nitric oxide	10 min	Treatment effective on delaying browning Decrease of the polyphenoloxidase and peroxidase activities	(Shi, Li, Zhu & Zhou, 2011)
Mushroom	DETANO (2,2'-(hydroxynitrosohydrazino)-bisethnamine at 0.5, 1 or 2 mM	20°C/10 min	1mM of DETANO is sufficient to maintain a high level of firmness, to delay browning.	(Jiang et al., 2011)

3. Approach by physical processes

The literature mentions various physical treatments with different actions: either a modification of the temperature of the product or a decrease of the availability of oxygen.

In blanching and freezing methods, temperature plays a key role. Indeed, polyphenoloxidase is sensitive to temperature variations, notably to high temperatures. Özel and others (2010) report that the blanching of plums above 80°C inactivates polyphenoloxidase; whereas freezing induces a decrease of available water for the enzymatic reactions leading to less activity of polyphenoloxidase (Lavelli & Caronni, 2010). According to the Arrhenius law, a temperature decrease leads to a decrease of the rate of browning reactions (Mastrocola, Manzocco & Poiana, 1998). Conservation under modified atmosphere reduces oxygen content and avoids the reaction of enzymatic browning (Ingraham, 1955).

All these treatments will be detailed and discussed in the following paragraphs.

3.1 Blanching

Blanching food is a heat treatment. Blanching treatments are presented according to the heat medium used: blanching in boiling water and/or in steam; blanching by using microwave was also developed the last years. The blanching time varies depending on the technique used, the type of product, size or maturity status. It is often used before the process of appertization, freezing and lyophilization. This process inactivates the enzymatic systems responsible for sensory and vitaminic alterations and thus

limits the oxidation. In addition, the colours of plants are heightened, for better presentation. Indeed, oxidative activity of polyphenoloxidase varies according to temperature; it increases with temperature to reach a plateau. Once the optimal activity of the enzyme is reached, the relative activity of the enzyme drops with a temperature increase (Özel, Colak, Arslan & Yildirim, 2010). Blanching has also some disadvantages. It alters, in part, the consistency of treated product and sometimes gives a cooked flavor. It also generates losses of nutrients and results in decreased weight of the product. For this latter reason, the choice of the optimum combination time - temperature of the heat treatment has to be made by minimizing nutritional and textural losses. Table 2 summarizes the studies dealing with blanching treatments in the literature.

Blanching in water

Blanching in water has the advantage of a homogenous treatment of food and the possibility of modulating the temperature of blanching. For example, the conditions of carrot blanching are a temperature of 95°C for 1 minute to inactivate polyphenoloxidase and peroxidase (Shivhare, Gupta, Basu & Raghavan, 2009). For Salak blanching, temperatures below 70°C must be used during 5 minutes (Ong & Law, 2011). A drawback of water blanching is the low energy yield and the leaching of many soluble substances (Mazzeo et al., 2011). To overcome this drawback, chemical agents are added to avoid nutritional losses (Gupta, Kumar, Sharma & Patil, 2011; Gonzalez-Cebrino, Garcia-Parra, Contador, Tabla & Ramirez, 2012). The combination of osmotic dehydration with conventional water blanching before the process of drying was studied on the Indian gooseberry (Gudapaty et al., 2010). The objective was to reduce the drying time and obtain a product with better preservation quality. This reduction of drying time leads to a less degradation of vitamin C. However, the fruit segments osmotically dehydrated with salt (2%) retained a higher content of vitamin C compared to those subjected to a supplementary pretreatment by blanching process.

Table 2: Summary of parameters t / T for blanching

Fruit/Vegetable	Blanching method	Blanching conditions	Results	References
Plum	• Water • Ascorbic acid (400ppm)	80°C, 40 s	Blanching is necessary to inactivate browning enzymes.	**(Gonzalez-Cebrino et al., 2012)**
Red beet	• Microwave	5 min, 250-450W, red beet immersed in water	Beet must be immersed in water to avoid product shrinkage. Inactivation of polyphenoloxidase and peroxidase activities at 90%.	**(Latorre, Bonelli, Rojas & Gerschenson, 2012)**
Watercress	• Thermosonication	86°C, 30 s	Inactivation of polyphenoloxidase and peroxidase activities at 90%. Loss of the watercress microstructure.	**(Cruz, Vieira, Fonseca & Silva, 2011)**
Pineapple	• Steam	100°C for 3 min	Prevention of enzymatic browning but apparition of cell shrinkage after frying.	**(Hasimah, Zainon & Norbaiti, 2011)**
Aonla	• Water • Potassium metabisulphite (0.3%)	80°C for 3 min	The addition of potassium prevents the leaching of nutrients, blanching is necessary to inactivate enzymes.	**(Gupta et al., 2011)**
Carrot, cauliflower, spinach	• Water • Steam	100°C, 10 min (spinach), 12 min (carrot), 9 min (cauliflower). 100°C, 20 min (spinach, carrot), 12 min (cauliflower).	Water blanching leads to nutritional losses in comparison with steam blanching.	**(Mazzeo et al., 2011)**
Indian gooseberry	• Water	100°C for 7 min (water)	Increase of quality after drying (colour, texture and taste) but decrease of vitamin C content.	**(Gudapaty et al., 2010)**
Salak fruit	• Water	50, 60, 70°C for 5 min	Colour changes during drying were minimized for blanched samples.	**(Ong & Law, 2011)**
Indian gooseberry	• Hot water	100°C for 3 min	Blanching affects all chemical properties except ascorbic acid content and preserves colour.	**(Prajapaty, Nema & Rathore, 2011)**
Mango	• Steam	94 ° C for 1, 3, 5 and 7 min	Peroxidase was inactivated after 5 min and polyphenoloxidase after 7 min.	**(Ndiaye, Xu & Wang, 2009)**

Carrot	• Water • 0.05N acetic acid solution • 0.2% calcium chloride solution	80 to 100°C for 1 to 10 min 80 to 100°C for 1 to 10 min 80 to 100°C for 1 to 10 min	At 95 °C for 1 min in water, catalase and peroxidase were totally inactivated without affecting carrot quality.	**(Shivhare et al., 2009)**
Potato	• Superheated steam (SHS) and a spray of micro drops hot water (WMD)	115 ° C (steam) for 11 min or 100 ° C (microdrops) 11 min	Changes in texture and colour were reduced by the combined treatment	**(Sotome et al., 2009)**
Green coconut water	• Microwave	Seventeen different conditions heating with maxima temperature between 52.5 and 92.9°C	Thermal inactivation of polyphenoloxidase and peroxidase was significantly faster with microwave blanching than conventional blanching	**(Matsui, Gut, de Oliveira & Tadini, 2008)**
Potato	• Water	Hot water bath, shaken at 120 rpm	The blanching optimum conditions to prevent enzymatic browning are : a concentration of ascorbic acid of 2g/kg potato, a time of 5.5 min and a temperature of 69°C.	**(Reis, Masson & Waszczynskyj, 2008)**
Brussels sprouts	• Water • Microwave/water	50°C for 5 min then 100°C for 3 min 700 W for 5 min then 100°C for 2 min	A pre treatment by microwave is better to preserve all the product properties	**(Vina et al., 2007)**
Pea puree	• Ohmic • Water	20-50 V/cm to reach 100°C 100°C	Ohmic blanching allows a decrease in blanching time to inactivate the peroxidase. Colour is better preserved with ohmic blanching.	**(Icier, Yildiz & Baysal, 2006)**
Peas	• Water • Steam • Microwave	No conditions	No differences between blanching methods for enzyme inactivation. Losses of nutrients are higher with water blanching.	**(Lin & Brewer, 2005)**

Steam blanching

Steam blanching is used for highly fragmented products. This is carried out in a tunnel of about 15 meters long in which the product is exposed to a steam atmosphere. The residence time of products in the steam is varied depending to the nature and maturity of the vegetable raw material. This method reduces the release of soluble substances. However, blanching is less homogeneous, takes 20% to 40% more time and has a higher cost.

The stability of peroxydase (POD) and polyphenol oxydase (PPO) was studied in mango slices (Mangifera indica L.), as well as the colour after different times of blanching at 94 ° C with saturated steam (Ndiaye et al., 2009). The POD was totally inactivated after 5 min, and the PPO after 7 min. Three minutes of steam blanching lead to a residual activities less than 2.85% and 8.33% of PPO and POD, respectively.

Steam blanching gave worse results in terms of inactivation of POD and heterogeneity in comparison with water blanching (Shivhare et al., 2009).

An alternative is to use a process with superheated steam (SHS) and a spray of micro drops of hot water (WMD) (Sotome et al., 2009). This process was tested on potatoes in comparison to conventional methods of blanching and good results were obtained.

For the process where water and steam are combined, a mixture of SHS at 115 ° C (3.0 kg / h) and WMD 100 ° C (0.54 kg / h) was used. Changes in texture and colour were reduced by the combined treatment. This process prevents the absorption of water in the product and the dissolution of potato substances in water. In addition, dehydration is limited by the sprinkling of water on the surface of the potato, thus limiting weight loss.

Microwave blanching

Microwave energy can be used for blanching because it causes an inactivation of enzymatic systems in foods (Matsui et al., 2008). It is mentioned that microwave blanching preserves better the nutritional value of the product (Ramesh, Wolf, Tevini & Bognar, 2002). However, Latorre (2012) showed that blanching by microwave leads to large weight losses and product shrinkage. The use of microwaves combined with conventional water blanching was studied for the Brussels sprouts; immersing food products in water before microwave heating, maintains product structure (Vina et al., 2007). However, the main problem in microwave heating is the non-uniformity of heating (Vadivambal & Jayas, 2007).

Microwave blanching is often combined with another technique to prevent the disadvantages of this method. Time-temperature combinations of microwave blanching must be specific to each product depending on their composition and their structure.

Blanching is a widespread technique used to prevent enzymatic browning. Recent studies have attempted to develop new physical approaches of blanching, such as ohmic heating (Icier et al., 2006) or thermosonication (Cruz et al., 2011). In other studies, blanching is often combined with a chemical approach such as dipping or coating. These studies will be developed in the last part of this paper.

3.2 Freezing

Freezing is a technique often used to stop browning reactions in fruit. Indeed, freezing causes a decrease in available water for enzymatic reactions. It is confirmed by Lavelli (2010) who found that in the apple a water activity below of 0.3, the PPO is no longer active. As the water activity decreases with temperature, the storage temperature would be -24°C to reach an a_w of 0.3 (Heldman & Taylor, 1997). Few studies deal with the optimization of freezing as conservation method because freezing leads to irreversible changes in the food product such as firmness loss during thawing (Shomer, Borochov-Neori, Luzki & Merin, 1998). Freezing can be a useful technique if the product does not need to be thawed. Indeed, after thawing, food quality is often altered and enzymatic reactions take place very rapidly in the product. Thus, freezing can be used to increase product shelf-life, but in association with other conservation methods such as dipping or blanching (Prestamo, Palomares & Sanz, 2005; Van Buggenhout, Messagie, Van der Plancken & Hendrickx, 2006; Gossinger et al., 2009; Hasimah et al., 2011) .

3.3 Conservation in modified atmosphere

Oxygen is essential for the oxidation reaction and PPO activity, a solution to control enzymatic browning reactions would be to change the oxygen content of the storage atmosphere (Ingraham, 1955). The studies dealt with modified atmosphere packaging, by modifying the composition of atmosphere, showed that the enzymatic systems are delayed without altering product quality (Table 3). The first studies modified the O_2 content by replacing it with CO_2 or N_2 (De Souza et al., 2006; Teixeira, Durigan, Alves & O'Hare, 2008; Wang et al., 2011). Recent studies used Argon or NO_2 to control the atmosphere. The efficiency of these two gases was shown in comparison with N_2 (Rocculi, Romani & Dalla Rosa, 2005; O'Beirne, Murphy & Eidhin, 2011). It allows a better preventing browning without quality loss. However, more and more studies combine atmosphere modification with a chemical treatment of the fruit to increase the duration of the storage without quality loss. These studies will be discussed in the last part of this review.

4. Coating

The coating agents are usually used to extend the shelf-life of fruits during their storage. It consists on the application of a layer of any edible material on the surface of fruit. Actions of these agents deal with the decrease of moisture and aroma losses, the delaying of colour changes and gas transfer, and the improvement of the general appearance of the product through storage (Olivas & Barbosa-Canovas, 2005). The coating agents allow delaying enzymatic browning because they produce a modified atmosphere on coated fruits by isolating the coated product from the environment. The use of gels coating instead of a bath solution of anti browning has been widely discussed in the literature. The general conclusion is that the application of a gel works better against the enzymatic browning than immersion in a bath (Oms-Oliu et al., 2010b) due to the selective permeability to gases of gel coatings.

Table 4 provides a summary of the various coating solutions in the literature. The most used coating agent is chitosan. Alginate or Carrageenan are also used. Most of the studies dealing with fruit coating discuss the prevention of microbial degradations. With an objective of preventing enzymatic browning, few studies used coating alone. Indeed, most of coating methods are used in combination with dipping; the properties of the coating film can be improved by the use of additives such as antibrowning agents, preservatives, firming agents, plasticizers, nutraceuticals,…Studies dealing with the combination of dipping and coating are presented below.

Table 3: Studies dealing with modified atmosphere to prevent enzymatic browning

Fruit/Vegetable	Conditions	Results	Reference
Apple	High Pressure (150MPa) Argon treatment	Enzymatic browning is delayed but conditions are not sufficient to prevent it.	(Wu, Zhang & Wang, 2012)
Apple/Mushroom	Different content in O_2 are tested, balanced either by Argon or by N_2	Products with highest sensory quality after storage are products with an atmosphere 21% O_2/79% Ar.	(O'Beirne et al., 2011)
Mushroom	Modified Atmosphere Package : 100% N_2/20%O_2-80% N_2/ high oxygen modified atmosphere (50 to 100% O_2)	The best treatment to prevent enzymatic browning is a high oxygen modified atmosphere at 80% O_2 (balance N_2).	(Wang et al., 2011)
Carambole	Controlled atmospheres (0.4-20.3% O_2)	Use of low O_2 atmosphere is not sufficient to prevent browning reactions.	(Teixeira et al., 2008)
Mango	10 atmosphere combinations of oxygen (2.5 and 21%) and carbon dioxide (0.5, 10, 20 and 40%)	The most significant effect is due to a reduced O_2 atmosphere (2.5%).	(De Souza et al., 2006)
Kiwi	Modified Atmosphere Package : O_2 5%, CO_2 5% balanced with N_2, Ar, NO_2	Modified atmosphere with 90% NO_2 is the best mixture to maintain the quality of kiwi fruit slices.	(Rocculi et al., 2005)

Table 4: Summary of coating compositions

Products	Coating Agents	Treatment	Results	References
Pomegranate	Starch + glycerol	Starch + glycerol (2 :1) Seed oil (300/600 ppm) 15 min at room temperature	Significant delay of browning with a starch coating with 300ppm seed oil.	(Oz & Ulukanli, 2012)
	Putrescine + Carnauba wax	Putrescine + carnauba wax treatment Cold storage at 2°C Exposure at 20 ◦C for 3 days	Delay of chilling damage, and browning during storage Decrease of respiration and ethylene evolution rate	(Barman, Asrey & Pal, 2011)
Melon	Pectin	Osmotic dehydratation of fresh-cut melon in 40°Bx sucrose solution containing 0.5% calcium lactate solution + Coating with 1% pectin	Enhancement of shelf-life to 14 days Improvment of firmness and preservation of cellular structure by calcium lactate Reduction of the mechanical damage during storage Increasing of soluble solid content of product, improvement of the sensory acceptance of coated melon by osmotic dehydratation	(Ferrari, Sarantopoulos, Carmello-Guerreiro & Hubinger, 2011)
	Alginate	Alginate + (2.5% + 0.7% MA lemongrass, cinnamon oil or 0.3%)	Prevention of browning and firmness loss	(Raybaudi-Massilia, Rojas-Graue, Mosqueda-Melgar & Martin-Belloso, 2008)
Apple	Konjac glucomannan	Konjac glucomannan + pineapple fruit extract at different concentrations in distillated water	Delay of enzymatic browning. the best result is obtained with pineapple fruit extract (1:1).	(Supapvanich, Prathaan & Tepsorn, 2012)
	Alginate	Alginate solution with lemongrass or oregano oil or vanillin + Dipping in calcium chloride	Prevention of browning and firmness loss	(Rojas-Graue, Soliva-Fortuny & Martin-Belloso, 2009)

	Trehalose	Trehalose 0.8%, sucrose 0.1% and sodium chloride at 0.1%	This coating reduces the browning phenomena. However, decrease of weight loss is observed.	(Albanese, Cinquanta & Di Matteo, 2007)
Mushroom	Aloe vera gel and/or Gum tragacanth	Aloe vera gel (30% w/w) Gum tragacanth (10% w/w) Aloe vera gel + gum tragacanth (50% w/w) + calcium chloride (0.2 g/l) and citric acid (40 g/l)	The combination of both is more efficient to delay browning.	(Mohebbi, Ansarifar, Hasanpour & Amiryousefi, 2012)
Papaya	Chitosan	Chitosan solution at 0.02 g/ml	Prevention of browning and firmness loss	(Gonzalez-Aguilar et al., 2009)
Mangos in slice	Chitosan	Washing, peeling, cutting and soaking for 1 min in aqueous solution at 0, 0.5, 1 and 2% chitosan pH = 5 using 0.1 M NaOH Drying at 25 ° C 30 min Storage at 6°C	Coating increases shelf life, reduces weight loss, delay browning (7 days without significant colour change) Differences between 0.5 and 1 / 2% chitosan solution for the weight loss.	(Chien, Sheu & Yang, 2007)
Strawberry	Comparison between starch, Carrageenan and Chitosan	Carrageenan 0.3% (w / v) pH = 5.6 with 50% citric acid + Tween 80 (surfactant) between 0.01 and 0.1%	Coating with Carrageenan gives the best result to prevent colour changes and loss of firmness.	(Ribeiro, Vicente, Teixeira & Miranda, 2007)

Table 5: Combination of several treatments

Product	Treatments	Conditions	Results	Reference
Plum purée	Blanching High pressure	80°C/40s 400/600 MPa 7 min	Combination with high pressure treatment increases the efficiency of blanching to inactivate PPO.	(Gonzalez-Cebrino et al., 2012)
Litchi	Dipping (Sodium hypochlorite, potassium metabisulfite, hydrochloric acid and ascorbic acid) Radiation	Different concentrations and conditions are tested. 0.5 kGy Gamma radiation	The best treatment to avoid enzymatic browning is a sequential dip treatment : sodium hypochloride (0.2%, 4min, 52°C), potassium metabisulfate (3%, 30 min, 26°C) and hydrochloric acid (0.25N) containing ascorbic acid (2%, 10 min, 26°C) followed by gamma irradiation.	(Kumar et al., 2012)
Apple	Heat treatment Coating	38°C during 4 days Chitosan (1%)	Combination of these two treatments is better to prevent colour degradation of apples.	(Shao, Tu, Tu & Tu, 2012)
Apple	Dipping High pressure argon treatment	Ascorbic acid (0.5%), citric acid (0.5%) and calcium chloride (0.5%) 150MPa for 10 min	Dipping reduces the changes of colour and firmness of apple during high pressure treatment. High pressure Argon treatment delays litchi browning.	(Wu et al., 2012)
Broccoli	Coating Heat treatment	Chitosan (20g/kg + 1% acetic acid and 1% glycerol solution) Carboxymethyl-cellulose (0.75% in a water-ethyl alcohol mixture at 75°C) 50°C during 1.5 min	Thermal treatment decreases the enzymatic browning and firmness loss but the addition of a coating agent allows better product stability during cold storage	(Ansorena, Marcovich & Roura, 2011)
Apple	Dipping Ultrasonics	Ascorbic acid (1%) Frequency of 40 Hz	Combination of these two methods inactivates enzymes while each method used separately does not.	(Jang & Moon, 2011)
	Blanching+syruping+freezing	100°C for 3 min (steam) 25% glucose syrup solution (20h)		(Hasimah et al., 2011)

		Freezing at – 18°C during 20h		
Indian gooseberry	Hot water blanching KMS (potassium metabisulphate)	100°C for 3 min Normal water with 0.1% KMS for 3 min	Blanching with KMS exhibited superior quality over hot water blanching	(Prajapaty et al., 2011)
Apple	Dipping Coating	Ascorbic acid (2%) + $CaCl_2$ (0.5%) Chitosan (1%)	Combination of chitosan-coating and dipping delays enzymatic browning of apple slices during storage and avoids loss of firmness.	(Qi, Hu, Jiang, Tian & Li, 2011)
Pear	Dipping Coating	Sodium chlorite (0-1000 mg/l) for 1 min. Chitosan or Carboxymethyl chitosan solutions (1%) for 2 min.	The combination of dipping and coating allows preventing browning and maintaining tissue firmness.	(Xiao, Luo, Luo & Wang, 2011)
Mango	Dipping Coating	Citric acid (5g/l) Cassava starch (10g/l)	Better preservation of texture and colour. Delay browning during storage.	(Chiumarelli, Pereira, Ferrari, Sarantopoulos & Hubinger, 2010)
Indian gooseberry	Water blanching Osmotic dehydration	100°C for 7 min (water) Hypertonic solution of 2% salt solution or 40% sucrose solution for 12h (OD).	Increase quality after drying (colour, texture and taste)	(Gudapaty et al., 2010)
Banana	Dipping and/or Coating and/or Controlled atmosphere	Calcium chloride (1%), ascorbic acid (0.75%) and Cysteine (0.75%) during 2 min. Carageenan coating (5g/l) 3% O_2 + 10% CO_2	The best combination to delay browning and firmness loss is dipping combined with controlled atmosphere.	(Bico, Raposo, Morais & Morais, 2009)

Mushroom	Blanching Dipping	Water or Steam for 1, 3 or 7 min. SO_2 or H_2O_2 or EDTA or citric acid for 10 min at different concentrations	Mushrooms pretreated with sulphites or H2O2 and steam blanching had the best colour values.	(Eissa, Fouad & Shouk, 2009)
Carambola	Dipping Modified atmosphere	Ascorbic acid or citric acid or Ca-EDTA at different concentrations for 5 min 0.4-20.3% O_2	Treatment with 1% ascorbic acid in association with 0.4% oxygen did not present significant browning or loss of visual quality for up to 12 days.	(Teixeira et al., 2008)
Melon	Dipping Modified atmosphere	Ascorbic acid 1% +calcium chloride 0.5% for 1 min 2.5 kPa O_2 + 7kPa CO_2	A treatment with ascorbic acid and calcium chloride in combination with modified atmosphere packaging contributes to a greater extension of the shelf-life of fresh-cut melon	(Oms-Oliu, Soliva-Fortuny & Martin-Belloso, 2007)
Mango	Dipping Modified atmosphere	Organic acids or calcium chloride (3%) Reduced oxygen (2.5%), enhanced carbon dioxide (5-40%)	A combination of low oxygen and calcium extends the shelf-life during cold storage. Reduced oxygen was effective at controlling tissue darkening whereas calcium slows down the loss of tissue firmness.	(De Souza et al., 2006)
Apple	Dipping Coating	Ascorbic acid (0.5/1%), cysteine (0.1/0.3/0.5%), 4-hexylresorcinol (0.005/0.02%) Whey protein concentrate or beeswax	Combined action is more efficient to prevent browning than dipping or coating alone. The most effective treatments are whey protein concentrate coatings with 1% ascorbic acid or 0.5% cysteine.	(Perez-Gago, Serra & del Rio, 2006)

5. Approach combining chemical and physical processes

In many studies, combination of several techniques is used to prevent oxidation. These studies are presented in Table 5.

Indeed, the use of one chemical or physical method is often not sufficient to protect food against enzymatic browning without altering food quality. To improve the protection of vegetable against oxidation several techniques were combined. Thus dipping is often combined with a physical method (blanching, coating, and modified atmosphere) to prevent enzymatic browning and the loss of firmness. The protection brought by dipping is instantaneous whereas the protection with blanching or coating is time dependant. Thus, a combination with dipping increases the efficiency of the food protection against quality losses.

6. Alternative methods to replace thermal methods

Thermal methods are very efficient to avoid enzymatic browning but it leads to a modification of some product parameters as texture, taste. Find some non thermal methods is a relevant challenge in this field. Methods actually studied are high hydrostatic pressure, irradiation, ultrasonication and pulsed electric fields. Among these alternative methods, the main objective is to inactivate browning enzymes by different techniques: light, pressure or electricity. Queiroz and others (2008) review studies until 2007 on these alternative methods. Recent studies from 2008 on the development of these methods are gathered in table 6.

The most used method is high hydrostatic pressure (HHP), Guerrero-Baltran and others (2005) reviewed the different studies on the efficiency of HHP to prevent enzymatic browning in fruit and its derivatives. However, this method is more efficient to inactivate microorganisms than to totally inactivate enzymes which are more resistant. Its use allows decreasing the temperature of thermal treatment but it seems difficult to replace thermal methods by high hydrostatic pressure method. Same conclusions are given for the use of light methods; different sources are used: Ultraviolet, gamma, visible light. For example, exposure to UV-C light leads to protein aggregations and thus a decrease of enzymatic system activities (Manzocco, Quarta & Dri, 2009). Another method is the pulsed electric field; this one is more used to avoid microbiological degradation than to prevent enzymatic browning.

Conclusion

The different methods of preservation against enzymatic browning in fruits and vegetables are in constant development. The different data analyzed in this review allow us to distinguish two trends in this field. In one hand, the optimization of the prevention by combining different techniques,

however the optimized parameters depend on the kind of fruit or vegetable. On the other hand, the development of alternative methods to replace thermal methods is in constant increase.

In spite of the progress realized in non thermal treatments, the thermal methods remain the most effective for protecting foods against oxidation. So, more research is needed to replace thermal methods by non-thermal methods.

Table 6: Alternative methods

Product	Methods	Conditions	Results	Reference
Spinach purée	HHP	200, 400 and 600 MPa for 5, 15 and 25 min at room temperature	PPO activity decreases when pressure increases. The time has no effect.	(Wang et al., 2012)
Watermelon juice	HPCD	10, 20, and 30 MPa at 50 °C, for 5, 15, 30, 45 and 60 min	Browning degree decreased with pressure and treatment time	(Liu, Hu, Zhao & Song, 2012)
Watermelon juice	HHP Ultraviolet Blanching	300, 600, and 900 MPa for 5, 20, 40, and 60 min at 60°C 2421, 4843, 7264, and 9685 J/l 60°C for 5, 20, 40, and 60 min	HHP treatment showed the lowest changes in colour, dynamic viscosity, browning degree, and lycopene content in comparison with other methods	(Zhang et al., 2011)
Apple	HHP + pineapple juice	600 MPa for 1-5 min at 22°C	The combined treatment significantly reduced residual PPO activity	(Perera, Gamage, Wakeling, Gamlath & Versteeg, 2010)
Ginger	HHP	400 MPa for 5 min	HHP allows reducing PPO activity of 37 % whereas thermal treatment (100°C, 10 min) reduces it of 10%.	(Yamaguchi, Kato, Noma, Igura & Shimoda, 2010)
Litchi	Gamma Irradiation	0.3 and 0.5 kGy was performed at 26°C	Inhibition of the PPO and POD activities	(Mishra et al., 2012)
Lettuce	High intensity light	500 and 2500 lux	Intensity of 2500 lux protected from browning and quality decay by inhibiting browning-related enzyme activity	(Zhan, Li, Hu, Pang & Fan, 2012)
Apple	UV-C irradiation	5.6 ± 0.3; 8.4 ± 0.5 and 14.1 ± 0.9 kJ/m2	Irradiation is efficient to inactivate microorganisms but increase enzymatic browning. Pretreatment are necessary to avoid browning.	(Gomez, Alzamora, Castro & Salvatori, 2010)

Mushroom	Pulsed light treatment	4.8,12 and 28 J/cm²	The use of high pulsed light (12 and 28 J:cm²) promoted enzymatic browning by an increase in PPO activity.	(Oms-Oliu, Aguilo-Aguayo, Martin-Belloso & Soliva-Fortuny, 2010a)
Apple	UV-C irradiation Visible light	Irradiances : 0, 3.9, 5.4, 7.5 and 13.8 W/m² Irradiances : 0, 12.7, 13.0, 11.7 and 9.3 Wm/m²	UV-C light promoted enzyme inactivation in the entire range of irradiance and exposure time tested whilst visible light was effective only at high doses since lower intensity treatments were associated to enzyme activation.	(Manzocco et al., 2009)
Apple juice	Ultraviolet irradiation PEF Thermal treatment (control)	30 W, 30 min 100 square-wave pulses (1 µs, 15 Hz) at 40 kV/cm. 72 and 94 °C for 26 s	Reduction of PPO and POD activity was greater with the PEF method or with the combination ultraviolet irradiation and PEF than a thermal treatment at 72 °C.	(Noci et al., 2008)
Litchi	Ultrasonication	120 W, 10 min	Application of ultrasonic inhibits the activities of PPO and POD.	(Chen et al., 2011)
Apple	PEF Thermal treatment	15, 25 and 35 kV/cm From 20 to 60 °C	To have a total inhibition of browning enzymes, it is necessary to use a pretreatment at 60 °C.	(Schilling et al., 2008)

HHP: High hydrostatic Pressure; HPCD: High pressure carbon dioxide; PEF: Pulsed electric fields

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