

## **Foods Browning and Its Control**

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### **SUMMARY**

Browning of foods during processing and storage, especially during manufacture of meat, fish, fruit and vegetable products decreases the sensory properties of products due to associated changes in the colour, flavour, and softening besides nutritional properties. Therefore, its control is essential to preserve the quality of the food. In the present article, browning mechanisms, and browning inhibitors are reviewed.

### **ÖZET**

Gıdalarda işleme ve depolama da meydana gelen esmerleşme, gıdaların renk, koku, yumuşama gibi duyuşal özellikleri yanında besin değerlerinde de kayıplara neden olmaktadır. Bu yüzden, özellikle et, balık, meyve ve sebze üretiminde gözlenen esmerleşmeyi önlemek gıda kalitenin korunması açısından önemlidir. Bu derleme makalede, esmerleşme mekanizmaları ve önleyicileri tartışılmıştır.

### **INTRODUCTION**

Browning of food are widespread which take place during processing and storage, especially during manufacture of meat, fish, and vegetable products, as well as when fresh fruits and vegetables are subjected to mechanical injury (Eskin 1990). Browning usually impairs the sensory properties of products due to associated changes in the colour, flavour, and softening (due probably to the action of peptic enzymes) besides nutritional properties (Eskin, 1990; Martinez and Whitaker, 1995).

Browning of food results from both enzymatic and non-enzymatic oxidation of phenolic compounds as well as from Maillard reaction that occurs when mixtures of amino acids and reducing sugars are heated (Mcevely and Iyengar, 1992). It is generally difficult to ascertain whether the mechanism has been enzymatic or nonenzymatic unless the enzymes in the food that

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are responsible for the enzymatic browning is inactivated, then only non-enzymatic reaction is said to occur, although, strictly, colour could develop non-enzymatically from intermediates formed through enzyme-mediated oxidations which have taken place before the enzymes were inactivated (Wedzicha, 1984).

### **Enzymatic browning**

Enzymatic browning is a significant problem in a number of important commodities, specially fruits such as apricots, apples, pears, peaches, bananas, and grapes; vegetables such as potatoes, mushrooms, and lettuce; and seafood such as shrimp, spiny lobsters, and crabs. This discoloration limits the shelf life of many minimally processed foods (Huxsoll et al., 1989; cited by Sapers, 1993) and also a problem in the production of dehydrated and frozen fruits and vegetables (Shewfelt, 1986; Hall, 1989; cited by Sapers, 1993). If it is necessary to retain light colour of the product that is cut or dried, some type of pre-treatment is required. The treatment not only inhibits enzymatic browning during processing but also prevent nonenzymatic browning during storage (Bologna and Steele, 1987). Enzymatic browning is not always a defect: but contributes to the desirable colour and flavour of such products as raisins, prunes, coffee, tea, and cocoa (Sapers, 1993).

Enzymatic browning is the discoloration that results when monophenolic compounds of plants or shellfish, in the presence of atmospheric oxygen and polyphenol oxidase (PPO), are hydroxylated to o-diphenols, and the latter are oxidised to o-quinones (Vamos-Vigyazo, 1981, cited by Sapers, 1993; McEvily et al., 1992). The quinones condense and react nonenzymatically with other phenolic compounds, amino acids, etc., to produce dark brown, black or red pigments of indeterminate structure (Fig. 1) (Sapers and Hicks, 1989; Sapers, 1993).

PPO (1,2-benzenediol:oxygen oxido-reductase) is a Cu-containing enzyme which is also known as tyrosinase, diphenol oxidase, o-diphenolase catechol oxidase, catecholase, phenolase (Martinez and Whitaker, 1995). PPO is present in some bacteria and in some fungi, in most plants, some arthropods and all mammals. In all cases, the enzyme is associated with dark pigmentation in the organism, and seem to have a protective function, but a vital one (Mayer and Harel, 1991; cited by Martinez and Whitaker, 1995). PPOs are found in almost all higher plants, including

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wheat, tea, potato, cucumber, artichoke, lettuce, pear, papaya, grape, peach, mango and apple as well as seed such as cocoa (Martinez and Whitaker, 1995).

In plants, both soluble and membrane-bound PPOs have been described. The PPO gene is encoded in the nucleus and translated in the cytoplasm; the proPPO formed is then transported to the chloroplast where it is cleaved by a protease, producing an active form (Martinez and Whitaker, 1995).

*Neurospora crassa* and *St. glaucescens* PPOs are single polypeptide enzymes. Mushroom PPO is generally thought to contain four subunits, although under some conditions monomeric through to octameric forms are found.

So far, all of the PPOs discovered have the ability to convert o-dihydroxyphenol to o-benzoquinones, using oxygen as the secondary substrate (catecholase activity), but not all PPOs hydroxylate monophenols. The proposed mechanism of oxidation of both monophenols and diphenols as shown in Fig. 2 (Martinez and Whitaker, 1995).

A wide range of o-dihydroxyphenols are substrates for the PPOs in higher plants; therefore there is a great deal of potential for browning because of the presence of oxidizable OH groups (those phenolic OHs that are adjacent, *ortho*, to each other) (Fig. 3) besides 3,4-hydroxyphenonylalanine (DOPA), and tyrosine. The enzyme phenylalanine ammonia lyase (PAL) is involved in the biosynthesis pathway of phenolic compounds. Control of PAL activity, and thereby the biosynthesis of phenolic compounds at the site of injury to the fruit and vegetables, is also important in controlling enzymatic browning caused by postharvest treatments (Martinez and Whitaker, 1995).

The enzyme is relatively heat labile. Heat inactivation of PO is feasible by applying temperatures of  $>50^{\circ}\text{C}$  but may produce undesirable colours and/or flavours as well as undesirable changes in texture (Nicoli et al., 1991; Saper, 1993; Martinez and Whitaker, 1995). Temperatures of  $>60^{\circ}\text{C}$  for 3 min are sometimes used to heat treat red grapes before vinification.

Consequently, exclusion of oxygen and/or application of inhibitors such as: acids, halides, phenolic acids, sulfites, chelating agents such as ascorbic acid, quinone couplers such as cysteine, and various substrate-binding compounds must be used (Sapers, 1993).

The most important factors that determine the rate of the enzymatic browning of fruit and vegetables are the concentrations of both active PPO and phenolic compound present, the pH, the

temperature and the oxygen availability of the tissue. pH and Oxygen also influence subsequent nonenzymatic browning (Martinez and Whitaker, 1995). The optimum pH for PPO activity is between pH 5 and 7. Adjustment of the pH with citric (lemon juice is frequently used), malic or fumaric acids to pH 4, or below can be used to control browning in juices, fruit slices, avocado, guacamole, etc., as long as the acidity can be tolerated taste-wise. There may be a further decrease below pH 4 due to less tight binding of copper in the active site of the enzyme, permitting chelators, for example citric acid, to remove the copper (Osuga et al., cited by Martinez and Whitaker, 1995).

### **Nonenzymatic Browning**

Nonenzymatic Browning is discoloration resulting from

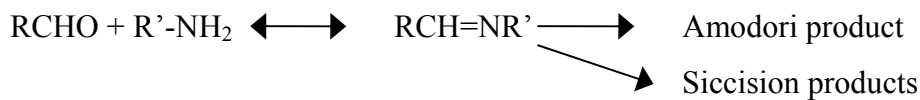
(a) the reaction of carbonyl groups (reducing sugar, aldehydes, ketones, lipid oxidation products ) and amino compound (lysine, glycine, peptide, amine, ammonia proteins) (Namiki, 1988)

(b) include caramelisation or pyrolysis of food carbohydrate (Wedzicha, 1984) due to heat treatment above the melting point of the sugar under alkaline or acidic conditions (Namiki, 1988)

(c) ascorbic acid browning, that is the spontaneous thermal decomposition of ascorbic acid under both aerobic and anaerobic conditions and either in the presence or absence of amino-compound (Wedzicha, 1984)

(d) lipid browning, which is probably oxidative of unsaturated deterioration of glyceride components followed by polymerisation which is accelerated by the presence of ammonia, amines or proteins (Wedzicha, 1984)

Maillard reaction involves three stages (Fig. 4). (1) An initial stage (reactions A and B) involving formation of glycosyl-amino products followed by Amadori rearrangement. (2) An intermediate stage (reactions C, D, and E) involving dehydration and fragmentation of sugars, amino acid degradation, and other. (3) A final stage involving aldol condensation, polymerisation, and the formation of heterocyclic nitrogen compounds and coloured products (Namiki, 1988).



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Maillard reaction



products

The key step is the Amodori rearrangement (reaction B), which irreversibly produces ketosyl compounds that enolize and lead to the complex reactions in the intermediate stage (Namiki, 1988). Maillard products appear to have an important role in preventing enzymatic browning. Their strong antibrowning effect could be related to their antioxidant properties (Nicoli et al., 1991) which may also protect lipids from oxidation (Whitfield, 1992).

Maillard reaction limits the shelf life of various dehydrated fruits and vegetables, citrus products, and juices (Hodge, 1953; cited by Sapers, 1993; Handwerk and Coleman, 1988). Sugar-amino acid reactions of the Maillard type are of minor importance in citrus juice browning because of the high acidity involved. However, acid-catalysed thermal decomposition of reducing sugars to reactive intermediates is an important element in citrus nonenzymatic browning (Lee, and Nagy, 1988). Ascorbic acid is the most reactive constituent of the orange drinks with respect to formation of the browning pigments which is accelerated in the presence of oxygen, and amino acids (Kacem et al., 1987).

Vitamin C browning is a special case, leading to the destruction and loss of this important vitamin and to the production of furfurals as a result of degradative transformations. Vitamin C and its degradation products also participate with amino acids in Maillard browning (Molnar, and Friedman, 1990a).

Oxidative nonenzymatic browning contributes 80% of the browning of dried apple during storage packed under vacuum while the rest is due to nonoxidative nonenzymatic reactions (Bolin and Steele, 1987).

Metal-ions (iron, copper and transition metals) are involved in nonenzymatic browning of citrus products which results from both oxidative and nonoxidative reactions. Oxygen required for oxidative type nonenzymatic browning may enter in aseptically packed products by dissolving from headspace or penetrate through packaging material such as plastic metallized laminate bags which are permeable to oxygen (Kanner or Shapira, 1989).

In addition to causing discoloration, non-enzymatic browning reactions also result in the destruction of nutrients such as essential amino acids and ascorbic acid, reduced protein

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digestibility, inhibition of digestive enzymes, and interference with mineral metabolism through metal ion complexation. Potentially toxic and mutagenic Maillard reaction products also may be formed, especially in cooked muscle foods (Namiki, 1988; O'Brien and Morissey, 1989; cited by Sapers, 1993).

While nonenzymatic browning is a defect in some products, it is a desirable attribute in others such as bakery products, snack foods, nuts, and roasted meats. To compensate for the lack of colour development during microwave cooking of certain foods, browning precursors may be incorporated into the product to induce browning during microwave heating (Fellenz and Moppett, 1991). Volatiles produced by non-enzymatic browning reactions during cooking contribute to the flavour of many foods (Whitfield, 1992).

The extent of nonenzymatic browning in foods depends on product composition, e.g., Maillard precursors or ascorbic acid; pH; water activity; exposure to oxygen; and storage time, temperature (Saper, 1993), moisture content, heavy metal ions, light, presence of inhibitors (Namiki, 1988). For example, the browning rates of aldoses in general are higher than those of ketoses, those of pentoses are higher than those of hexoses, and the two- and three-carbon sugar analogs brown very rapidly. Basic and amino acids generally brown more easily than acidic amino acids in the following order: lysine > B-alanine > A-alanine > glutamic acid. Alkaline pH and higher temperatures greatly enhance the reactions and result in changes in the product distribution (Namiki, 1988).

Nonenzymatic browning in fruit and vegetable products can be inhibited by refrigeration; control of water activity in dehydrated foods; reduction of reducing sugar content in potatoes by storage, or glucose oxidase treatment; reduction of amino nitrogen content in juices by ion exchange; packaging with oxygen scavengers; and use of sulfites. Sulfhydryl-containing amino acids are nearly as effective as bisulfite in inhibiting nonenzymatic browning in a model system (Saper, 1993). Although there are some effective alternatives to sulfites to retard enzymatic browning reactions, there are fewer effective options for nonenzymatic browning (Bolin and Steele, 1987; Sapers, 1993).

### **BROWNING INHIBITORS**

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Sulfiting agents are widely used browning inhibitors but they effects on health. Therefore, sulfites alternatives such as ascorbic acid based formulations, PPO inhibitors, complexing agents (e.g., EDTA, sodium acid pyrophosphate), sulfhydryl-containing amino acids, organic halides, edible coatings are being heavily investigated as a possible substitute. Moreover, exclusion of oxygen, ultrafiltration, better peeling systems, blanching of some foods, reducing certain amino acid content (e.g. lysine and glycine), cultivar selection and preharvest treatments may decrease extend of browning related quality losses.

### **Sulfites**

Sulfites are highly effective in controlling browning but they are subject to regulatory restrictions because of adverse effects on health. Sulfiting agents (sulfur dioxide, sodium sulfite, sodium and potassium bisulfites and metabisulfites) have been added to many foods since antiquity to prevent enzymatic and nonenzymatic browning; control of growth of micro-organisms in wine, grape and other products; act as bleaching agents such as in cherries, antioxidants, or reducing agents; carry out various technical functions (Sapers and Hicks, 1989; Taylor, et al., 1986; cited by Sapers, 1993). Sulfites act as PPO inhibitors and also react with intermediates to prevent pigment formation (Sayavedra-Soto and Montgomery, 1986; cited by Sapers, 1993). Sulfites inhibit nonenzymatic browning by reacting with carbonyl intermediates, thereby preventing their reaction to form brown pigments (Wedzicha, 1987; cited by Sapers, 1993).

Sulfite treatment levels vary widely, depending on the application. FDA proposed that maximum residual sulfur dioxide levels of 300, 500, and 2000 ppm be permitted in fruit juices, dehydrated potatoes, and dried fruit, respectively.

Sulfiting agents are not teratogenic, mutagenic, or carcinogenic in laboratory animals, but there are fraction of the public that is sulfite sensitive who are susceptible to a unpredictably severe effects of the agents due to acute allergic reactions (FDA, 1988; cited by Sapers, 1993). Sulfiting agents are not generally accepted as for use in meats, foods recognised as a major source of vitamin B1, or “fruits and vegetables intended to be served raw to consumers or sold raw to consumers or to be presented as fresh” (FDA, 1988; cited by Sapers, 1993). Sulfites are no longer used in salad bars as a result of Food and Drug Administration regulations in 1995 (Martinez and Whitaker, 1995).

### **Alternatives to Sulfites**

The current and future restrictions to the uses of sulfite agents in foods promoted the researchers to develop the sulfite substitutes. It is unlikely that a multifunctional sulfite substitute can be developed. Rather combinations of several active ingredients, formulated to meet the needs of specific commodities and product types will be developed (Sapers, 1993) i.e., use of browning inhibitors in combination with antimicrobials and modified atmosphere packaging (Sapers and Hicks, 1989). Such formulations must be cost-effective in their stated use, and they must be approved for food use by FDA. Moreover peeling, cutting, heating, and dehydration conditions should be improved to minimise the extent of both enzymatic and nonenzymatic browning (Sapers and Hicks, 1989).

### **Ascorbic Acid Based-Formulations**

Probably the best known alternative to sulfites is ascorbic acid which is highly inhibitor of enzymatic browning, primarily because of its ability to reduce quinones, generated by PPO-catalysed oxidation of polyphenols, back to phenolic compounds before they can undergo further reaction to form pigments (Fig. 1). However, once the added ascorbic acid has been completely oxidised to DHAA by this reaction, quinones can accumulate and undergo browning. Additionally, DHAA itself can brown non-enzymatically. At high concentrations, ascorbic acid also can directly inhibit PPO (Vamos-Vigyazo, 1981; cited by Sapers, 1993).

Ascorbic acid and its isomer erythorbic (d-isoascorbic) is commonly used as inhibitors of enzymatic browning in fresh-cut and frozen fruits such as peaches and apples. These compounds are added to syrups or applied by dipping the fruit in solutions containing the browning inhibitor, sometimes in combination with an organic acid such as citric acid and a calcium salt (Sapers, 1993). Browning-inhibitor penetration can be enhanced by vacuum infiltration of treatment solutions, which also removes air from within the product's void spaces.

Ascorbic acid-based formulations contain either ascorbic acid or erythorbic acid' or their sodium salts, usually in combination with one or more adjuncts such as citric acid or some other

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acidulants, such as calcium salts, a phosphate, sodium chloride, cysteine, or preservative such as sodium benzoate or potassium sorbate.

The storage life of treated fresh commodities is 4-7 days. A longer storage life can be obtained with pre-peeled potatoes by shipping them in a preservative solution (Santerre et al., 1991: cited by Sapers, 1993), or by vacuum packing treated potatoes to exclude oxygen (Langdon, 1987). However, products protected by vacuum packing will brown rapidly once the consumer opened the package. Furthermore, there is a safety concern about vacuum packaging pre-peeled potatoes in a high-barrier film because of the potential for *Cl. Botulinum* to grow and produce toxin under anaerobic conditions.

Ascorbic acid-based browning inhibitors are usually not as effective as sulfites because of the greater stability and better penetration of the latter. Use of ascorbic acid 2-phosphates in browning-inhibitor formulations for apples and potatoes can significantly improve their performance. Ascorbyl palmitate and other fatty acid esters of ascorbic acid are effective with fruit juices (Sapers, 1993).  $\alpha$ -glucosyl ascorbic acid may show greater stability than ascorbic acid and be suitable for use as browning inhibitor in systems where sufficient levels of  $\alpha$ -glucosidase enzyme are present (Ikai, 1990; cited by Sapers, 1993).

Penetration of ascorbic acid-based browning inhibitors can be improved by treating under pressure or vacuum instead of dipping or spraying. However, excessive absorption of browning-inhibitor solution by treated samples will result in water-logged appearance and premature spoilage of fresh commodities (Sapers and Hicks, 1989; Sapers, 1993).

### **PPO Inhibitors**

Cinnamic acid and benzoic acid is effective in apple juice, especially when used in combination with ascorbic acid (Sapers, 1993). Carbon monoxide has been proposed as browning inhibitor of mushrooms (Albisu et al., 1989; cited by Sapers, 1993). 4-hexylresorcinol is being used on shrimp, and proposed for use in some fruit and vegetables. Kojic acid, a fungal metabolite has been shown to be PPO inhibitor but has limited practical importance due to its mutagenic properties.

### **Complexing agents**

Since copper is essential to the function of PPO, chelating agents that complex copper may have value as browning inhibitors. Ethylenediamine tetraacetic acid (EDTA), widely used chelating agent, and sodium acid pyrophosphate are used to control after-cooking darkening in pre-peeled potato (Feinberg et al., 1987; cited by Sapers, 1993). EDTA also inhibits oxidative degradation of ascorbic acid, and browning of grapefruit juice (Kanner and Shapira, 1989). Citric acid acts as a chelating agent and acidulant, both functionalities inhibiting PPO (Mccord and Kilara, 1983; Santerre et al., 1988; cited by Sapers, 1993).

Compounds that bind or complex PPO substrates also may have potential value as browning inhibitors. Polyvinylpyrrolidone (PVPP), a product permitted for use as a finning agent for apple juice, can bind polyphenols and prevent their participation in enzymatic browning reactions (Van Buren, 1989; cited by Sapers, 1993). Cyclic polysaccharides (cyclodextrin) can form inclusion complexes with polyphenols substrates of PPO. Addition of this compound to juices or treatment of juices in insoluble cyclodextrin columns can prevent browning which is not approved yet (Sapers et al., 1989b; cited by Sapers, 1993).

### **Sulphydryl-Containing Amino Acids**

Cysteine prevents brown pigment formation by reacting with quinone intermediates to form stable, colourless compounds (Dudley and Hotchkiss, 1989; cited by Sapers, 1993) in milk, in pear concentrate (Montgomery, 1983; Bolin and Steele, 1987). Cysteine has been used as ingredient in commercial browning inhibitor (Cherry and Singh, 1990; cited by Sapers, 1993). Reduced glutathione and N-acetylcysteine are nearly as effective as sulfites in controlling browning in apple, potato and fresh fruit juices (Molnar-Perl and Friedman, 1990a,b).

### **Other Browning Inhibitors**

Inorganic halides are well known inhibitors of PPO (Vamos-Vigyazo, 1981; cited by Sapers, 1993), and sodium chloride is a commercial browning inhibitors. It is generally regarded as safe but

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limited by its effect on product taste. Zinc chloride is effective browning inhibitor, especially when used in combination with calcium chloride, ascorbic acid, and citric acid (Bolin and Huxsoll, 1989; cited by Sapers, 1993). Calcium is effective in retarding the nonenzymatic browning in dehydrated potatoes (Simon et al., 1995; Bolin and Steele, 1987).

Treatment of white grapes and cut fruit with honey has been shown to inhibit enzymatic browning due to presence in honey of a small peptide having a molecular weight of about 600 Da rather than a reduction in dissolved oxygen due to added sugar (Oszminski and Lee, 1990).

Edible coating reported to prevent enzymatic browning of mushroom slices (Nisperos-Carriedo; cited by Sapers, 1993). Various sulfated polysaccharides including carrageenans, amylose sulfate, xylan sulfate, were found to be effective as browning with apple juice and diced juice (Tong and Hicks, 1991; cited by Sapers, 1993).

Protease enzymes are effective browning inhibitors for apples, potatoes, shrimp, plum juice. The research is in progress. Protease-free extract from fig latex contain a component of less than 5000 Da which inhibits enzymatic browning (McEvily, 1991; cited by Sapers, 1993).

### **Exclusion of Oxygen**

As oxygen is required by PPO as the site of wounding to initiate the browning reaction, the use of oxygen impermeable packages or edible films may be useful in preventing the onset of browning (Martinez and Whitaker, 1995). The exclusion of oxygen is also used in juices and vines by bottling them under nitrogen. Prevention of mechanical bruising during the shipping of fresh fruit is important to prevent oxygen accessibility; compression and vibration can be prevented by the use of pulp board to cushion individual pieces (Martinez and Whitaker, 1995). Vacuum filling sliced fruits with syrup, sometimes containing ascorbic acid (Guadagni, 1949; cited by Sapers, 1993) would result in objectionably water-logged with slices preserved by refrigeration, but is effective in solutions with frozen products (Poninting and Jackson, 1972; Sapers et al., 1990).

Modified atmosphere packaging to reduce oxygen concentration in the atmosphere surrounding a product such as shredded lettuce or mushrooms can delay browning, but excessive reduction of oxygen will damage the product by inducing anaerobic metabolism, leading to breakdown and off-flavour formation (Ballantyne et al., 1988; cited by Sapers, 1993). The removal

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of oxygen also entails a risk that conditions in the product might become favourable for the growth of *Cl botulinum*. To avoid such a risk, overwrapped packages of fresh mushrooms must contain holes admitting air so that this product, which has a high respiration rate, will not become anaerobic (Nichols, 1985; cited by Sapers, 1993). Packaging headspace of nitrogen reduces browning rate in sulfured dried peaches (Bolin et al., 1976; Bolin and Steele, 1987). Oxygen plays the most important role in degradation of ascorbic acid and nonenzymatic browning of grapefruit juice stored at 23°C (Kanner and Shapira, 1989).

**Other Alternatives**

Ultrafiltration has been studied, with mixed results, as an alternative to sulfiting white wines. Presumably, the ultrafiltration membrane will remove PPO but not lower-molecular-weight polyphenols or Maillard reaction precursors which could undergo nonenzymatic browning during storage.

The severity of enzymatic browning at cut or peeled surfaces of fruits and vegetables will depend in part on the extent of damage done to surface tissues by peeling or cutting procedure (Sapers, 1993). Because once the cell wall and its cellular membranes lose their integrity, enzymatic oxidation proceeds much more rapidly (Martinez and Whitaker, 1995). Peeling potatoes with a sharp knife is less injurious than peeling by abrasion, water-jet cutting system or steam (Sapers et al., 1989a; Becker and Gray, 1992; cited by Sapers, 1993).

Blanching at 93°C for 2 min in water to inactivate PPO and improve the mass transfer of water vapour through the skin can be applied for light coloured raisins (Aguilera et al., 1986; cited by Sapers, 1993).

For Maillard reaction to occur, certain amino acids such as lysine and glycine, and also certain amino reducing sugars are required. Reducing availability of these reactants by modification of ε-amino groups of the fruit either by methylation with formaldehyde or by ethylation with acetylaldehyde is possible (Bodwell, 1975; cited by Bolin and Steele, 1987).

Different cultivars of fruits and vegetables may show large differences in their tendency to brown, because of cultivar variation in PPO activity and substrate content (Sapers, 1993).

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Direct treatment of the plant, prior to harvest, with inhibiting substances such as calcium salts gives satisfactory results (Smith and Cline, 1984; cited by Nicoli et al., 1991).

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