Core course 13: Unit 3: Food Chemistry II

Browning reactions in food (part 2)

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Dear viewers, in today's session under Food technology we will be learning about enzymatic browning reactions.

Browning of foods is defined as the process in which there is a change in the color of the food due to a chemical process. Browning of foods is desirable for caramelization of sugars, to impart flavor in tea, to form crust in bread. Browning is essential to produce raisins and prunes. However, browning of fruits and vegetables is undesirable. Browning reaction is broadly classified into two types i.e., Enzymatic browning and non-enzymatic browning. Fruits and vegetables undergo enzymatic browning whereas sugars individually undergo caramelization. Sugars along with proteins undergo Maillard reaction which is a non-enzymatic browning. Enzymatic browning is the second largest cause for loss of quality in fruits and vegetables. The chapter is divided into five sub-divisions namely

1. Enzymes responsible for browning
2. Mechanism of enzymatic browning
3. Factors influencing enzymatic browning reaction
4. Prevention of enzymatic browning
5. Recent advances in research for prevention of browning reactions

1. Enzymes responsible for browning:

Enzymatic browning of fruits and vegetables is defined as a process wherein enzymes such as phenolase oxidize phenols to orthoquinones which rapidly polymerize to form undesirable brown or black pigments such as melanins.This process takes place in the presence of oxygen. Enzymatic browning becomes evident when fruits and vegetables are subjected to processing or to mechanical injury. Phenolase include a group of enzymes such as phenoloxidase, cresolase, dopa oxidase, catecholase, polyphenoloxidase, potato oxidase, laccase, tyrosinase, sweet potato oxidase, grape peroxidase, and phenolase complex. Out of these polyphenol oxidases (PPO) are studied extensively for browning of fruits and vegetables. Polyphenol oxidase, a copper-containing metalloprotein, catalyzes the oxidation of phenolic compounds to quinones. These enzymes are present in fruits such as apples, banana, pears, gooseberry and kiwi and; in vegetables such as lettuce, brinjal, mushrooms, asparagus, broccoli, carrot and potato.

2. Mechanism of enzymatic browning:

Browning occurs only when fruits and vegetables are exposed to processes such as cutting and peeling which causes destruction of cell membrane exposing the cell components to the environment. The mechanism of action proposed for PPO is based on its capacity to oxidize phenolic compounds. Damage to the tissue leads to the rupture of plastids, the cellular compartment where PPO is located. This facilitates the enzyme to come in contact with the phenolic compounds released by rupture of the vacuole which is the main storage organelle of these substrates. The active site of PPO consists of two copper atoms and the enzyme catalyzes two different reactions in the presence of molecular oxygen firstly, the hydroxylation of monophenols (monophenolase activity) and then the oxidation of *o*-diphenols to *o*-quinones (diphenolase activity). This reaction is followed by non-enzymatic polymerization of the quinones giving rise to melanins, pigments of high molecular mass and dark color.

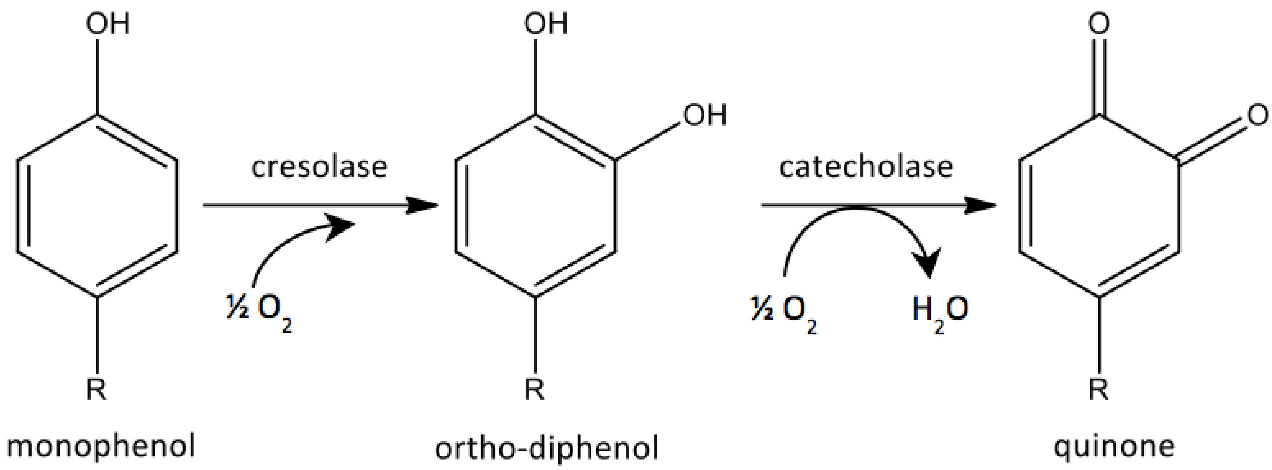

Figure 1: Conversion of ortho-diphenol to quinone.

Image source: http://www.bio-protocol.org/e1213

3. Factors influencing enzymatic browning reaction:

Enzymatic browning requires four different components i.e., oxygen, enzyme, copper and a substrate.

Catalyst: Presence of transition metals such as copper will act as a catalyst to the browning reaction.

Substrates and enzymes: The rate of browning reaction depends on the availability of substrates such as phenols, catechols, caffeic acid, chlorogenic acid, catechins etc. Availability of enzymes is important to convert them to secondary products. It also depends on the substrate specificity of enzymes. For example, PPOs oxidize mono, di and polyphenols.

Processing: Processes such as cutting, peeling causes destruction of cell membrane and exposes the cell components to air to initiate enzymatic browning. Delayed processing of minimally processed fruits and vegetables also influences enzymatic browning since the exposure of food to components causing browning is longer.

Oxygen: Presence of oxygen accelerates enzymatic browning by conversion of phenols to orthoquinones which rapidly polymerize to form brown or black pigments such as melanin. Higher the concentration of oxygen availability faster is the browning reaction.

pH: The optimum pH of PPO activity depends varies widely over the type of enzyme and the substrate. pH ranging between 4 to 7 is optimum for activity of PPO and hence adjusting pH lower than 4 and higher than 7 will reduce browning.

Temperature: The temperature stability of PPO varies with the species and the cultivar. The enzyme is heat labile and its activity is completely destroyed at 80°C. Heat inactivation of PPO is feasible at temperatures more than 50°C but can impart undesirable changes in flavor, texture and color.

Packaging materials: Permeability of packaging materials is of importance to prevent the direct contact of the food material to the atmosphere.

Others: Browning tendency of fruits and vegetables differs widely between cultivars due to the compositional differences. Therefore correct choice of cultivars is important. Post-harvest techniques such as transport and storage greatly influence browning reaction. Method of pre-processing is also vital. For example, pre-peeled potatoes have better stability against browning than sliced potatoes since the surface area exposed is lesser in peeling than in slicing.

4. <u>Prevention of enzymatic browning:</u>

4.a Conventional methods:

Addition of pineapple juice, lemon juice, honey prevents browning and inhibits PPO activity. Honey contains a number of components known to act as preservatives; these include α-tocopherol, ascorbic acid, flavonoids and other phenolic compounds. Honey from different floral sources reduced PPO activity over a range of 2–45% in fruit and vegetable homogenates. Also, combination with ascorbic acid enhances the inhibitory effect.

Addition of sugar: In this method, fruits are partially dehydrated by reducing to 50% of its original weight by osmosis in sugar or syrup. After draining, the fruits are either frozen or dried further in an air or vacuum dryer. Sugar inhibits enzymatic browning through complete dehydration. In addition, it adds to the flavor.

4.b Physical methods:

4.b.1: Thermal processes: Heat treatment is the most widely used method for stabilizing foods because of its capacity to destroy microorganisms and to inactivate enzymes.

Boiling or Steaming: Boiling water or steam is used to inactivate the enzymes and control enzymatic browning in vegetables. This method may not be satisfactory for fruits because of the development of cooked flavor and softening of the tissue.

Blanching: It is the most common method used to inactivate vegetable enzymes. It causes denaturation and therefore inactivation of the enzymes. In general, exposure of PPO to temperatures of 70–90°C destroys their catalytic activity, but the time required for inactivation depends on the product.

Ohmic heating: It is defined as a process in which electric currents are passed through foods with the main purpose of heating them. Ohmic heating is distinguished from other electrical heating methods by the presence of electrodes contacting the foods, the frequency, and the waveform of the electric field imposed between the electrodes. Treatment of pea puree by ohmic blanching inactivates the peroxidase enzyme at lower processing time than conventional water blanching.

Microwave heating: It is an alternative method for liquid food pasteurization. Compared to conventional heating methods, microwaves are able to heat products internally, have greater penetration depth and faster heating rates. This potentially improves retention of thermolabile constituents in the food. Microwave energy induces thermal effects over microorganisms and enzymes similar to those of conventional heating mechanisms.

4.b.2 Non-thermal processes:

Refrigeration and Freezing: Refrigeration delays the browning reaction whereas freezing technique is used often to stop browning reactions in fruit. Freezing makes water less available for enzymatic reactions. In apples, a water activity below 0.3 inactivates the PPO. As the water activity decreases with temperature, the storage temperature would be -24°C to reach water activity of 0.3.

High hydrostatic pressure (HHP): Treatment of fruit and vegetable products with HHP offers a chance of producing foods of high quality, greater safety, and increased shelf life. HHP reduces microbial counts and inactivates enzymes. The treatment is expected to be less detrimental than thermal processes to low molecular mass food compounds such as flavouring agents, pigments and vitamins as covalent bondings are not affected by pressure. HHP can affect protein conformation and lead to protein denaturation, aggregation or gelation, depending on the protein system, the applied pressure, the temperature and the duration of the pressure treatment.

γ-irradiation: Fruits and vegetables can be treated with γ irradiation to extend shelf life. Irradiation is a physical treatment involving direct exposure to electrons or electromagnetic rays, for food preservation and improvement of safety and quality.

Modified atmosphere: Oxygen is essential for the oxidation reaction and PPO activity. To control enzymatic browning reactions change in the oxygen content of the storage atmosphere, can be of help. The studies dealt with modified atmosphere packaging, by modifying the composition of atmosphere. This results in the delayed enzymatic reactions without altering product quality.
The initial studies modified the O_2 content by replacing it with CO_2 or N_2. Now, Argon or NO_2 are used to control the atmosphere which is more efficient in preventing browning reactions in fruits and vegetables.

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. The coating agents allow delay in enzymatic browning because they produce a modified atmosphere on coated fruits by isolating the coated product from the environment. Examples of coating agents are starch, glycerol, pectin, alginate, trehalose and chitosan.

4.c Chemical methods:

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. Their effectiveness depends on environmental factors such as pH, water activity, temperature, light and composition of the atmosphere. The main antioxidants reported in the literature are hexylresorcinol, erythorbic acid, N-acetyl cysteine, cysteine hydrochloride, ascorbic acid and glutathione. Ascorbic acid is traditionally the most widely used agent. Ascorbic acid reduces quinones back to phenolic compounds before they can undergo further reaction to form pigments. However, ascorbic acid at 0.75% level can impart unpleasant taste to fruits.

Oxidizing agents: Sodium chloride is a strong oxidizing agent, which can generate chlorine dioxide under acidic conditions. Below pH values of ∼5, a strong pH dependent PPO inhibitory effect is observed. The degree of inhibition increases with the acidity of the reaction medium. On the other hand, an activation effect is observed at higher pH values. These differences in the mechanisms allow the use of combinations of anti-browning agents that may result in enhanced inhibition. Ascorbic acid, cysteine and cinnamic acid in combination show a synergistic effect compared to the individual compounds in browning of cloudy apple juice.

Chelating agents: PPO requires copper ions to be active. 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 and EDTA. Usually citric acid is used for its chelating role, but also for acidifying the medium. Kojic acid in apple juice at concentrations ranging from 1 to 4 mM inhibits PPO and bleaches melanin due to chemical reduction of the browning pigment to colourless compounds. Sulphites have multifunctional role as they prevent enzymatic and non-enzymatic browning. They act as chelating agents, antioxidants and reducing agents. However, sulphites may cause tissue softening, off-flavor, could be toxic at higher levels and are corrosive in nature. According to the current indications of WHO, the acceptable daily intake of SO_2 in foodstuffs has been established as 0.7 mg/kg of body weight. Other sulphur-containing agent used to prevent browning of fruits is cysteine which is used to prevent browning of mangoes. A concentration of 0.2 mg/g has been effective in preventing the browning of apple juice.

Firming agents: Calcium salts are the best known to strengthen the cell walls. This prevents the destruction of cell compartments and also the contact of PPO with polyphenols in the vacuole. The main agents of firmness are calcium lactate, calcium propionate, calcium chloride, calcium ascorbate and sodium chloride.

Acidifying agents: PPO is sensitive to pH variations. Fruits have a natural acidic environment. Additional acidification may reduce the PPO activity or inactivate it below pH 3. The main acidifying agents used are citric acid, erythorbic acid, ascorbic acid and glutathione. Ascorbic acid has shown to be more effective than its isomer iso-ascorbic acid. Since optimum pH to PPO reactions range from 5 to 7.5 lower values inhibit enzymatic activity. Mango puree with the addition of ascorbic acid inhibited more PPO activity than puree containing cysteine or 4-hexylresorcinol (4-HR).

Enzyme inhibitors: Aromatic carboxylic acids such as benzoic acid and cinnamic acids are PPO inhibitors due to their structural similarities with the phenolic substrates. The undissociated forms of these acids are able to inhibit PPO by forming complexes with copper at the active site of the enzyme. Benzoic acid exhibits weak inhibition at 0.5 mM in grapes.

Complexing agents: β-cyclodextrins bind substrate in their hydrophobic core. The most important functional property of cyclodextrins is their ability to form inclusion complexes with a wide range of organic guest molecules, including PPO substrates. β-cyclodextrins prevent browning at concentrations greater than 1.8 mM.

Use of chemical compounds individually or in combinations to prevent browning reactions is given in Table 1 and Table 2.

Table 1: Use of chemical compounds to prevent browning reactions in fruits.

Fruits	**Chemical**	**Time/temperature**	**Effect**
Apple	Phytic acid (0.08%)	RT	Inhibition of the PPO (99.2%)
	Ascorbic acid (0.3 mM)	10 min	Decrease of the browning.
	Sodium chloride (300 mg /L), acidified sodium chlorite (300 mg /L), citric acid (20 g/ L), calcium chloride (20 g/l)	RT/1 min	Prevention of browning
	0.5 g/L Sodium chloride with pH from 3.9 to 6.2 using citric acid	1 min	Most effective treatment to prevent browning
	0.5 % Ascorbic acid + 0.5% calcium chloride	5 min	The most effective treatment for delaying browning
Pear	1-Methylcyclopropene (300nL/L) then 2% ascorbic acid + 0.01% 4-hexylresorcinol + 1%	0°C/24h 4°C/15 min	Browning and softening are delayed

	calcium chloride		
	Ascorbic acid + 4 -hexylresorcinol	30°C	Synergistic effect between ascorbic acid and 4-hexylresorcinol for the inhibition of the polyphenoloxidase
	0.75% N-acetylcysteine or 0.75% glutathione	15°C/2 min	Prevention of browning of pear wedges during storage
Kiwi	2% ascorbic acid + 2% calcium chloride	RT/2 min	Treatment effective at delaying softening and browning

Table 2: Use of chemical compounds to prevent browning reactions in vegetables

Vegetables	Chemical	Time/temperature	Effect
Eggplant	Calcium ascorbate or citrate (0.4%)	60°C / 1 min	Calcium ascorbate was the best treatment to inactivate enzymes
Artichoke	Cysteine (0.5%)	RT/1 min	Most effective treatment to prevent browning
Potato	1% sodium acid sulfate + 1% citric acid and 1% ascorbic acid	RT	Polyphenoloxidase activity and browning are reduced
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.

5. Recent Advances in research for prevention of browning reactions

Pulsed electric field (PEF) is an emerging non-thermal food-preservation technology that has been researched and developed close to the commercial stage. This process is conducted by introducing the food in a chamber containing two electrodes that apply high voltage pulses in the order of 10–80 kV for microseconds. Studies have been reported in apple and peaches. PEF treatment also induces changes in secondary structure of PPO and peroxidase.

Supercritical carbon dioxide is a non-thermal technology with a pressurization step. An addition of carbon dioxide in the product before HHP treatment can also be used to inhibit PPO activity.

Conclusion:

Browning of fruits and vegetables can result in quality losses. Enzymatic browning can be prevented by various physical, chemical and conventional methods. These methods are used individually or in combinations to prevent browning. Inactivation of enzymes is the prime objective of any of these processes. In spite of the progress realized in non-thermal treatments, the thermal methods remain the most effective for protecting foods against oxidation. Advances in research and techniques are needed to find better approaches to prevent enzymatic browning. Thank you viewers.