

Control of Enzymatic Browning of Foods

Vlasta Piližota* and Drago Šubarić

Faculty of Food Technology, F. Kuhača 18, HR-31 000 Osijek, Croatia

Received: November 3, 1997

Accepted: June 17, 1998

Summary

The mechanism of enzymatic browning of foods, which in most cases affects adversely the quality of food, is briefly described. Enzymatic browning is in most cases connected with the action of the monooxygenase (polyphenol oxidase, PPO), which in the presence of oxygen hydroxylates the colorless monophenols to o-diphenols which are subsequently oxidized to colored o-quinones. Through a series of nonenzymatic reactions, o-quinones are often converted irreversibly to brown polymeric pigments. In order to prevent the deteriorating effect of enzymatic browning, various inhibitors are applied during processing and storage of foods. The inhibition of enzymatic browning generally proceeds via the following three mechanisms: (1) direct inhibition of polyphenol oxidase, (2) nonenzymatic reduction of o-quinones which are formed by enzymatic oxidation of o-phenols to the derivatives of o-diphenols, and (3) chemical modification or removal of phenolic substrates of polyphenol oxidase. The most efficient multifunctional agents in control of enzymatic browning of foods are various sulfites. However, their use is in many cases limited by sanitary regulations. For this reason a very intensive research in the development of sulfite alternatives is under way. The best known alternative to sulfite in control of browning of foods is L-ascorbic acid and its cheaper stereoisomer D-ascorbic acid commercially known as erythorbic acid. The other well-known substitute to sulfites is 4-hexylresorcinol, a part of EverFresh formulation. Very active inhibitors of enzymatic browning are various chelating agents, which either directly form complexes with PPO, or react with its substrates. Example of an inhibitor which reacts with the copper containing prosthetic group of PPO is β -cyclodextrin. Other inhibitors of enzymatic browning which are commercially used are: sulfhydryl containing amino acids, specially cysteine and reduced glutation, inorganic salts, like sodium and zinc chloride, and honey which inhibits the browning of white grapes. As a part of our own work, the effect of erythorbic acid applied under pressure or vacuum to the discoloration of fruit and vegetables and the importance of proper selection of cultivar are briefly described in this review.

Keywords: browning, enzymatic, nonenzymatic, fruits, vegetables, food, browning inhibitors

Introduction

The increasing interest in the production and distribution of fresh commodities and minimally processed (fresh-cut) fruits and vegetables has led to the discovery and application of a large number of various additives. The function of these additives is to ensure stability of color and taste, appearance and nourishing value, but at the same time the additive must satisfy the stringent sanitary regulations. The large variety of food products and increasing regulatory limitations concerning the quality of foods have stimulated considerable research activity, resulting in the development of a large number of new additives capable of solving complex problems

of the production of healthy food. Modern production, storage and transportation of food products would not be possible without the use of various ingredients (ascorbic acid, D-isoascorbic acid, citric acid, calcium salt, sodium chloride, cysteine, sodium benzoate or potassium sorbate) and without the application of various processes (pasteurization and sterilization). It is often emphasized that »technology is a part of every menu«.

Many food products undergo various desired and undesired changes as a consequence of enzymatic and nonenzymatic reactions. Both enzymatic and nonenzymatic reactions often lead to browning and deterioration

* To whom correspondence should be addressed

of the quality of foods. In order to prevent the detrimental effect of enzymatic reactions, various inhibitors have been developed and commercially applied.

In the present article we wish to review the results published during the last few years concerning the scientific status of the use of enzymatic and partially nonenzymatic browning inhibitors. A part of the results obtained in the research laboratories of the Faculty of Food Technology in Osijek will be also included in this review.

Measuring of Color of Foods

Selection of food products by consumers is usually based on color, shape, texture, surface defects and size of particles. However, in spite of the large number of factors which are of importance in evaluation of food, color is usually the most influential, and often the off-color products are non acceptable in spite of good taste and flavor.

The color of foods is generally related to two classes of pigments; first are natural pigments like hemes, myoglobins, chlorophylls, anthocyanins, carotenoids and betacyanins, and second are added colorants. During the handling, processing and storage, the degradation and variations caused by desired and undesired chemical reactions to which pigments are subjected, change the color of food. In addition, the browning of food as a result of enzymatic and nonenzymatic interactions of food components can also occur (1–4). Brown pigmentation following this enzymatic reaction and subsequent nonenzymatic reaction, is generally considered to be detrimental to food quality from both the organoleptic and nutritional points of view. Therefore, the control of enzymatic browning has always been a challenge to food scientists owing to the losses that it causes in many food products (2).

Due to the complexity of the term »color of foods«, and the changes of color which are inevitable, it is very difficult to develop and standardize the method for the determination of color. Color quality of food can be determined by visual inspection. However, although human visual sensory for color is very sensitive, and often reproducible, this type of inspection is often subjective and depends on the inspector's mood. Objective color measurements by the use of colorimeters and spectrophotometers have been developed and used for color determination (2,5). However, these methods are suitable for laboratory, but not for fast routine monitoring. Furthermore, because of the complicated sampling procedure, the results often misrepresent food production quality.

In order to improve the color measurements of food, a sensing technology that can rapidly monitor color during processing was recently developed. This procedure is called »color machine vision«. Machine vision has a significantly higher resolution than colorimeter or spectrophotometer which can investigate only one location at a time, while a common commercial red-green-blue camera has more than 240 000 sensing elements and can collect color information from more than 80 000 locations in its field of view. Color machine vision is

more and more used as a fast noncontact and nondestructive method for determination and prediction of color quality of processed food. In principle, the instrumental color measurement by color machine vision simulates physical transformations which take place in a human visual system. A short description of the instrument is presented in a review article published in *Chemtech* in 1996 (6) and elsewhere (7).

Enzymatic Browning

Enzymatic browning is a discoloration of fruits, vegetables and marine animals, especially crustaceas, after being exposed to air. PPO represents a large family of enzymes which in the presence of oxygen hydroxylates the colorless monophenols to colorless *o*-diphenols, which are subsequently oxidized to the corresponding *o*-quinon (8–12). The *ortho*-quinones formed *via* the enzymatic reaction polymerize by nonenzymatic reaction to high molecular dark brown products (Fig. 1).

Polyphenol oxydase (PPO) is an oligomeric enzyme containing a metaloporphyrine ring with copper as prosthetic group, so that chelating agents which form complexes with copper may be of importance as browning inhibitors. Some of the compounds which have found practical application as enzyme inhibitors are cyanides, carbon monoxide, sodium diethyldithiocarbamate, mercapto benzothiazol, azides, methyl xantates and specially ascorbic acid. Ascorbic acid can react directly with the enzyme by forming complex with the prosthetic group, thus preventing the enzymatic browning (13, 14). Inhibition of enzymatic browning can also be achieved by removal of oxidizable substrates like catecholes, cinamic acid esters, 3,4-dihydroxy phenylalanine (DOPA) and tyrosine.

Polyphenol oxydase is most active at pH = 5–7, so that acidifiers which decrease pH below 3 inhibit the enzymatic activity of PPO. However it is often impossible to change pH of fresh foods without affecting adversely its flavor. PPO can also be inhibited by heat treatment which causes denaturing of the enzyme but often causes the degradation of food components and impaires the taste and quality of the products. It is also known that PPO can be partially inhibited by acids, halogens, phenolic acids, complexing agents and reducing additives like sulfites, ascorbic acid and cysteine. The addition of reducing additives like sulfites and ascorbates is the most commonly applied method for controlling enzymatic browning. It is well-known that the mechanism of inhibition of enzymatic browning caused by reducing agents is the nonenzymatic reduction of colored *o*-quinones to colorless *o*-diphenols.

Concerning the enzymatic browning, it must be emphasized that in some cases, browning is not a defect but is a desirable property since it contributes to improved color and flavor in products like raisins, prunes, coffee, tea and cocoa (15). Inactivation of PPO can be sometimes controlled by blanching. However with some products it cannot be applied since it affects adversely flavor and texture (16,17). For this reason exclusion of oxygen or application of browning inhibitors must be used.

Nonenzymatic Browning

Nonenzymatic discoloration of foods, often called Maillard reaction, is a series of complex reactions, in most cases reactions of aldose sugars with free amino groups, leading to the formation of brown melanoidin pigments. Maillard reaction represents a series of reactions like sugar dehydration, sugar fragmentation, Strecker decarboxylation of amino acid, aldol condensation, and finally aldehyde-amine polymerization under the formation of heterocyclic nitrogen compounds (18). It is considered that about 20% of the nonenzymatic browning reactions occurring during storage can be attributed to nonoxidative, or Maillard reactions, and 70% to oxidation (19).

Nonenzymatic browning is an undesirable reaction not only because of discoloration of foods, but it also results in degradation of nutrients like essential amino acids and ascorbic acid, it decreases protein digestibility, inhibits digestive enzymes and sometimes leads to the formation of toxic and mutagenic products (20). Nonenzymatic browning can be effectively inhibited by refrigeration, by decrease of amino nitrogen content in juices, by the use of sulfites (20), packaging with oxygen scavengers and by the use of ion exchange and adsorbent technologies (21).

Although each of the mentioned procedures for the inhibition of nonenzymatic browning is important in specific applications, the ion exchange and adsorbent technologies are of special interest in improvement of a wide variety of juices.

Most important application of ion exchange in enhancing fruit juices is the recovery of fruit sugars. Most fruit juices contain approximately 10–15% of sugars with varying proportions of sucrose, glucose and fructose, depending upon the types of fruit. Examples which illustrate the fruit juices enhancement by the application of ion exchange technology are: grape juice, apple and pear juice, pineapple mill juice, reduced acid frozen concentrated orange juice and debittered orange juice (21).

While nonenzymatic browning often limits the use of some products, it is a desirable attribute in products like snack foods, bakery products, nuts and roast meat. The lack of color developed in microwave heated products is often improved by incorporation of the browning precursors. These additives sometimes improve the flavor of cooked products (15,22).

Inhibitors of Enzymatic Degradation of Foods

It is generally considered that the inhibition of browning of foods proceeds *via* one of the following three mechanisms: 1. direct inhibition of polyphenol oxidase, 2. nonenzymatic reduction of *o*-quinone to derivatives of *o*-diphenols, and 3. chemical modification or removal of phenolic substrates of PPO. Most commonly used inhibitors of polyphenol oxidase according to the first two mechanisms are various sulfites, ascorbic acid and derivatives, cysteine and often mercaptanes, honey and 4-hexyl resorcinol. Sapers and Hicks have shown in 1996 that β -cyclodextrin inhibits PPO by the third mechanism (23).

Sulfites

Sulfites are among the most efficient agents in the control of discoloration of foods, but their application is limited by sanitary regulations due to the adverse effects on health. As food additives the most important are sulfur dioxide, sodium sulfite, sodium or potassium bisulfites. Sulfites are unique additives to food since they can perform many functions. They can control enzymatic and nonenzymatic browning, they can act as antimicrobial agents, thus preventing the growth of microorganisms in foods, but can also act as bleaching agents, antioxidants and reducing agents (24).

The sulfites may control enzymatic browning either by direct inhibition of PPO or by reacting with intermediates of enzyme reactions, thus preventing the formation of brown polymeric pigments. Sayavedra-Soto and Montgomery (25) have shown that sulfites can form complexes with diphenols and quinones thus removing them from the reaction. And finally sulfites are active reducing agents capable to transform by nonenzymatic mechanism *o*-quinones to colorless diphenols (26).

In most cases the sulfites do not inhibit enzymatic browning irreversibly, so that they are consumed in the reaction. For this reason the required concentration of sulfites is dependent on the length of time during which the browning of food must be inhibited. Residual levels of sulfites in foods usually do not exceed several hundred parts per million (ppm), but sometimes approach 1000 ppm in certain fruit and vegetable products (24).

Although sulfites as enzymatic browning inhibitors are widely used, there are some disadvantages to their use. FDA received several documented reports of allergic reactions in individuals who consumed sulfited food. These reports indicated that sulfites can induce asthmatic episodes in some asthmatic persons. In some cases anaphylactic-like reactions may occur in a segment of asthmatic population (24). In 1988 FDA proposed the maximal residual sulfur dioxide levels of 300, 500 and 2000 ppm in fruit juices, dehydrated potato, and dried fruit, respectively. Additionally, new rules were proposed by FDA, which determine specific labelling criteria for sulfite treated foods (27). The label must also declare that food contains detectable level of sulfite (*e.g.* 100 ppm or more) as defined by FDA. Sulfiting agents are not allowed in meats, foods used as major source of vitamin B₁, or fruits and vegetables presented to consumers as fresh (19,28). It is of interest to mention that in 1984 FDA established an *ad hoc* review panel to examine the «Generally Recognized As Safe» (GRAS) status of sulfiting agents in foods. As stated in ref. 29: «The panel found no new need to change the no-observed-adverse-effect level, which is about ten times the level of sulfur dioxide ingested by high-intake consumers and 180 times the mean *per capita* intake». In 1990 (30), FDA revoked the GRAS status to fresh potatoes served or sold to customers unpackaged and unlabelled, but since it was found that sulfiting agents are not teratogenic, mutagenic or carcinogenic, this revocation was not accepted.

Sulfite Alternatives

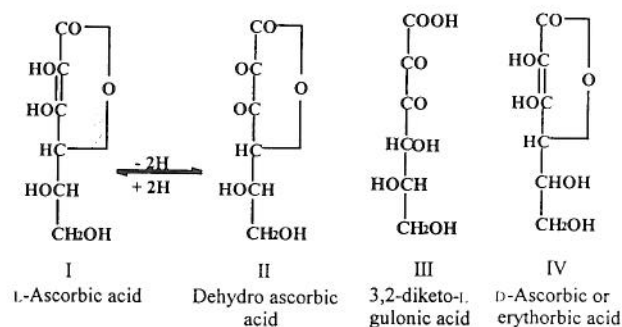
Restrictions on the use of sulfites as stabilizers in fruit and vegetable products and concern that other uses

might be subject to further limitations have prompted scientists and additive producers to develop various sulfite alternatives (31). However, sulfites have been known for centuries as cheap and efficient multifunctional reagents, and it is very unlikely that any of the substituent to sulfites will also be a multifunctional additive. Probably a combination of several compatible formulated ingredients must be developed in order to meet specific requirements for specific products.

Formulations based on ascorbic acid and derivatives

Ascorbic acid or vitamin C is the best known alternative to sulfites in the control of browning of foods. Vitamin C is a very active inhibitor of enzymatic browning. This inhibition is based on the ability of ascorbic acid to reduce *o*-quinones formed by the oxidation of polyphenols by PPO. As a result of the reduction of quinones, ascorbic acid is oxidized to dehydro-2-ascorbic acid (II). The oxidation of ascorbic acid to dehydro-ascorbic acid is a reversible reaction, but under the conditions which exist in foods, the reaction proceeds only in the direction of oxidation. The dehydro-L-ascorbic acid is further oxidized to 2,3-diketo-L-gulonic acid (III) which by decarboxylation forms oxalic acid, L-xylose and a large number of other products (32). When all added ascorbic acid is oxidized to dehydroascorbic acid, quinones can accumulate thus leading to browning. Besides, the dehydroascorbic acid can form brown pigments through the nonenzymatic aldol reaction, or by reaction with amino acids.

Ascorbic acid is stable in dry form but it gradually darkens on exposure to light. Solutions of vitamin C are more sensitive to bases than to acids. Heat alone without oxygen does not destroy the vitamin C. Very rapid deterioration takes place in the presence of copper or silver as catalysts.



In the presence of a higher concentration of ascorbic acid, the direct inhibition of PPO occurs. It is known that PPO is a metaloporphyrine with copper as prosthetic group (8a), but that ascorbic acid oxidase also contains copper bound to protein (32). The role of copper in ascorbic acid oxidase is to transfer hydrogen from ascorbic acid to molecular oxygen. Based on a large number of studies concerning the mechanism of oxidation of ascorbic acid it can be proposed that its oxidation in plant tissues in the presence of oxidase and the direct oxidation of PPO in foods, respectively, are based on the same mechanism in which copper as the prosthetic group plays an important role. Ascorbic acid based browning inhibitors are not as effective as sulfites be-

cause of greater stability and penetration of sulfites. Stable derivatives of ascorbic acid like ascorbic acid-2-phosphates or ascorbyl palmitate are effective browning inhibitors of fruits. The mixture of ascorbic acid with synergists like citric acid, phosphoric acid, tartaric acid, monoglyceride citrate, stearyl citrate and monoisopropyl citrate have also been used in experimental formulations. The mentioned additives to ascorbic acid have low antioxidant action but they have ability to enhance the antioxidant activity of ascorbic acid in mixture (33). As sulfite substitute, ascorbic acid is often used in combination with other preservatives like sodium benzoate or potassium sorbate (29). Due to relatively high price of ascorbic acid, its stereoisomer D-ascorbic acid (iso-ascorbic acid, commercially known as erythorbic acid (IV)), is often used as substitute to sulfites. Erythorbic acid has only 5–7% of antiscorbutic activity of vitamin C, but has similar antioxidant properties. Production of erythorbic acid is simpler and cheaper than the production of vitamin C, so that using erythorbates as antioxidants in place of ascorbates can give savings of as much as 35–40% (34).

Erythorbates have three functions in controlling enzymatic browning in foods. First, they can act as free radical scavengers and chelators, second, erythorbates can alter the redox potential of the system, and finally, they act as reducing agents (9). Combination of erythorbic acid and citric acid are commonly used for anti-browning treatment. Erythorbic acid or combination of erythorbic and citric acid can be directly added to the water media (juices, syrups, etc.); it can be used as dip solution or it can be directly added to the food product (35). After that the product is packed in vacuum. However, the product protected by vacuum packing will brown rapidly when the customer opens the package. There is a safety concern about vacuum packaging (e.g. pre-peeled potatoes) in high-barrier film, because of potential growth of *Clostridium botulinum* and production of dangerous toxin under anaerobic conditions (36).

During the course of our studies, performed in cooperation with Professor Sapers from the Eastern Regional Research Center, Philadelphia, vacuum and pressure infiltration of ascorbic acid and erythorbic acid as browning inhibitors to cut apples and potato were investigated (35). It is known that serious shortcoming of ascorbic and erythorbic acid or their calcium salts in combination with citric acid is their limited penetration into the cut fruit and vegetable, compared with that of sulfite (24). In our investigation (35), selected apple and potato cultivars were cut in a controlled way and immersed in 0.25% NaCl solution to prevent browning during dicing.

Vacuum infiltration treatment was applied by placing the beaker which contains half-plugs or dices of apples into a vacuum oven operated at room temperature. The oven was evacuated to 169–980 mbar and held at the desired vacuum for 0.5 to 2 min until the vacuum was broken by admission of air. The uptake of ascorbic acid (1.12 and 2.25%) by vacuum infiltration at 169 mbar and dipping of apple dice or plugs was not significantly different, and no further work was done in our investigations of the vacuum infiltration of apple. Similarly, the vacuum infiltration of potato plugs with solution of

ascorbic acid (4%) at vacuum as high as 980 mbar for 5 min, resulted in no additional mass gain or improvement in storage life of potato at 4 °C. Colorimetry was performed on the transversely cut surface of treated and half-plugs with tristimulus colorimeter. The color of apple and potato dices were evaluated with a colorimeter. Visual observations of sample appearance were made by two of the investigators at the same time as the instrumental measurements.

The application of browning inhibitor solutions by pressure infiltration was studied in order to determine if gains in storage life could be obtained without substantial sample darkening or water-logging. Mass gains in pressure-infiltrated apple plugs were correlated with the application pressure ($r = 0.92$) but not with infiltration time (2 vs. 5 min). Systematic studies of vacuum and pressure infiltration of browning inhibitor solutions to cut apples has in general shown many advantages of pressure over vacuum infiltration. The advantage of pressure over vacuum infiltration can be explained in the following manner; with vacuum infiltration more gas is removed from apple plugs, and when vacuum is released, gas is rapidly replaced by treatment solution. With pressure infiltration, this gas is compressed, permitting the absorption of a smaller amount of treatment solution and resulting in less water-logging.

Comparison of plugs, treated with solutions containing 2.25 or 4.5% sodium erythorbate, applied by dipping at atmospheric pressure or at a pressure of 34 kPa for 5 min, indicated that samples infiltrated under pressure contained 50–60% more erythorbate irrespective of concentration of inhibitor or cultivar. The samples dipped under atmospheric pressure showed significantly more browning than the pressure infiltrated samples during the second week of storage with 4.5% of sodium erythorbate. Our studies with plugs and dice indicate that the storage life of cut apples (35) is extended with browning inhibitors applied by pressure infiltration.

On the other hand, attempts to improve the storage life of potato by pressure infiltration were not successful.

It may be the lower surface-to-volume ratio of plugs that limits the quantity of treatment solution adhered to surface after dipping. Ascorbic acid based browning inhibitors are not as effective as sulfites which have greater

stability and better penetration than ascorbic acid. Use of stable ascorbic acid-2-phosphates in browning-inhibitor formulations for apples and potatoes can significantly improve the performance of ascorbic acid (37). Another derivative which shows greater stability than ascorbic acid is α -glucosyl-ascorbic acid (38).

Inhibition of polyphenol oxidase

A large number of polyphenol oxidase (PPO) inhibitors are known, but only few of them are used commercially as substitutes for sulfites. Particular attention has been paid to acidulants as different organic acid (acetic, formic, propionic, benzoic, cinnamic, malonic, oxalic and lactic acid) (39), which are thought to reduce the PPO activity, known to be optimal between pH = 5 and 8 (40). Recently, very promising results were reported in controlling »Iceberg« lettuce-butt discoloration (browning of the cut stem) by washing the cut stems with organic acids, without antioxidant activity (41). It has been suggested that the inhibitory mechanism of organic acids is to maintain the pH well below that necessary for optimal PPO activity (9). Cinnamic acid and benzoic acid are effective as browning inhibitors in apple juice, especially used in combination with ascorbic acid (37). Carbon monoxide is effective as browning inhibitor for mushrooms, but the use of this compound would require special measures to ensure the safety of packaging (43). 4-hexylresorcinol, which is a part of »EverFresh« formulation, has been used for a long time as browning inhibitor of shrimp. »EverFresh« formulation was patented in 1991 (44).

The scheme in the Fig. 1. illustrates the mechanism of inhibition of enzymatic browning by 4-hexylresorcinol. As already mentioned, sulfites control the enzymatic browning by reducing *o*-quinones back to diphenols. In contrast, 4-hexylresorcinol reacts with PPO and stops its enzymatic reaction in its earliest phase. However, as shown in Fig. 1, the addition of 4-hexylresorcinol prevents the formation of *o*-quinones even if the oxidation of phenols has already started.

»EverFresh«, which contains 4-hexylresorcinol as active ingredient and sodium chloride as a carrier agent, is used for controlling enzymatic browning in decapitated shrimp. Stability of three series of freshly harvested beheaded shrimps stored for 7, 14 and 30 days, respectively

Table 1. Effects of browning inhibitors, applied to apple plugs by pressure infiltration at 34 kPa vs. dipping at atmospheric pressure for 5 min

Cultivar	Formulation	Treatment	w (erythorbic acid)	Percent inhibition / day		
			mg/kg	3/4	7	14
Delicious	C	Dip	2470 ^d	93 ^c	84 ^c	66 ^d
		34 kPa	3590 ^c	92 ^c	86 ^c	82 ^c
	D	Dip	1250 ^f	66 ^e	54 ^e	–
		34 kPa	1910 ^e	80 ^c	70 ^d	–
Winesap	C	Dip	2850 ^d	92 ^c	74 ^c	63 ^c
		34 kPa	4170 ^c	84 ^{cd}	79 ^c	77 ^c
	D	Dip	1340 ^f	53 ^e	34 ^e	–
		34 kPa	2220 ^e	72 ^d	52 ^d	–

^aC=4.5% Na-erythorbate + 0.2% CaCl₂ (pH=7.8); D=2.25% Na-erythorbate + 0.2% CaCl₂ (pH=7.8), ^b 3 days for formulation C; 4 days for D, ^{c-f} Means of 4 replicates; means within columns for each cultivar, followed by different superscripts, are significantly different at $p < 0.05$ by the Bonferroni LSD test.

Table 2. Effect of browning inhibitors applied to potato plugs by pressure infiltration *vs.* dipping at atmospheric pressure

Cultivar	Formulation	Treatment	w (ascorbic acid)	Percent inhibition / day		
			mg/kg	2	4	6
Russet Burbank	E	Dip- 5 min	1280 ^d	70 ^{bc}	42 ^c	29 ^c
		34 kPa- 5 min	1890 ^b	86 ^b	60 ^{bc}	44 ^{bc}
	F	Dip- 5 min	1320 ^d	72 ^c	44 ^c	35 ^c
		34 kPa- 5 min	1710 ^c	85 ^b	68 ^b	55 ^b
Katahdin	E	Dip- 5 min	1010 ^c	81 ^c	55 ^c	51 ^b
		34 kPa- 5 min	1330 ^b	94 ^b	80 ^b	71 ^b
	F	Dip- 5 min	1120 ^c	71 ^d	52 ^e	49 ^b
		34 kPa- 5 min	1350 ^b	92 ^b	83 ^b	75 ^b

^aE=4% ascorbic acid + 1% citric acid + 0.2% CaCl₂ (pH=2.1); F=4% ascorbic acid + 1% citric acid + 0.8% ascorbic acid 2-phosphate (pH=3.0) and (pH=2.1), ^{b-d} Means of 4 replicates; means within columns for each cultivar, followed by different superscripts, are significantly different at $p < 0.005$ by the Bonferroni LSD test.

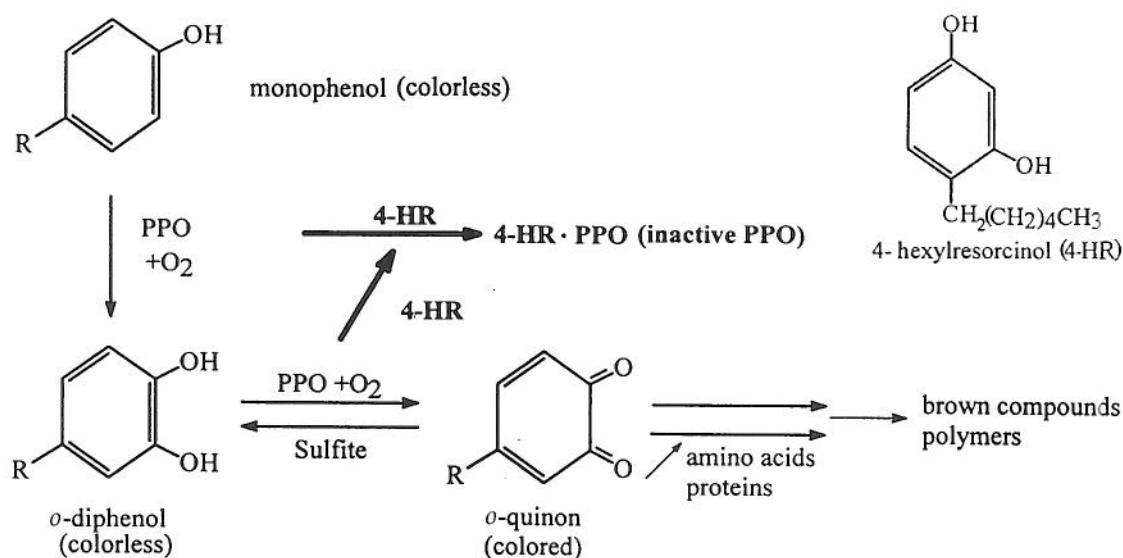


Fig. 1. Effect of 4-hexylresorcinol (4-HR) on inhibiting polyphenol oxidase and enzymatic browning (34)

Table 3. Percent fraction of blackspots in raw brown shrimps stored at 2 °C for 7, 14 and 30 days (34)

	Water	Na-metabisulfite	4-hexylresorcinol
7 days at 2 °C	54	11	3.6
14 days at 2 °C	75	25	3.6
30 days at -20 °C	58	34	9.0

vely, is shown in Table 3. Stability was determined by visual inspection of blackspots on the shrimps. One group of shrimps was dipped for 1 minute into a solution which contains 50 ppm of 4-hexylresorcinol, second group of shrimps was dipped into a solution of 1.25% of sodium metabisulfite, and the third, control, group was dipped in water. Results in Table 3 indicate that 4-hexylresorcinol is a much more effective inhibitor of PPO than sodium metabisulfite (34,45). After 30 days at -20 °C, the water treated shrimps had 58% of blackspot, the sulfite-treated shrimps had 34% of blackspot, and the »EverFresh«-treated shrimps had only 9% of black-

spot. A combination of 0.01% of 4-HR, 0.5% of ascorbic acid (AA) and 0.2% of CaCl₂ prevented browning of sliced apple fruit stored under refrigeration for 50 days (46–49).

Complexing agents

As previously mentioned copper is essential to the function of PPO, and for this reason it is obvious that chelating agents which complex copper are of interest as potential browning inhibitors. Examples of chelating reagents which react with copper are ethylenediamine tetraacetic acid, sodium acid pyrophosphate and specially citric acid, often used in many browning inhibitor formulations, which act as chelating agent and acidulant, thus inhibiting PPO.

Besides the compounds which directly complex PPO, several compounds which bind or complex the substrates of PPO are also valuable as browning inhibitors. Among these compounds of special interest are cyclodextrins which inhibit the enzymes that cause browning faster than monophenol oxidase.

Cyclodextrins are natural oligosaccharides discovered almost 100 years ago, but due to the difficulties in the separation of cyclic sugars of the controlled molecular mass, and because of high cost of these compounds, they had not found industrial application for more than fifty years. However, recent biotechnological advancements have resulted in improvements of manufacture of cyclodextrins at low cost and of high purity. Due to the availability of pure and relatively cheap cyclodextrins, their application in pharmaceutical and recently in food industry is growing very fast. Biotechnological production of cyclodextrin is based on enzymatic cyclization of starch by a group of amylases known under the name »glucosyltransferases«. Due to the growing interest in the application of cyclodextrins, more than 1000 scientific papers and almost the same number of patents are published yearly. Several books and monographs are also published (49).

Cyclodextrins consist of (α -1,4)-linked α -D-glucopyranose units, with a somewhat lipophilic central cavity and a hydrophilic outer surface. Due to restricted rotation around the bonds connecting the glucopyranose units, the cyclodextrins are cone-shaped rather than perfectly cylindrical molecules. Primary hydroxyl groups are located on the narrow part of the cone, while the secondary hydroxyl groups are distributed around the broader ring of the cone.

The most important naturally-occurring cyclodextrins are α -, β -, and γ -cyclodextrins, consisting of six, seven and eight glucopyranose units, respectively. However, due to its low cost, availability and cavity size, β -cyclodextrin is the most useful complexing agent in pharmaceutical and food industry (50).

Cyclodextrins belong to the class of cryptands or spherands and like other crown compounds they form with »guest molecules« stable complexes inserted in the cavity of macrocyclic compounds. Since the cavity of cyclodextrins consists of skeletal carbon atoms bound with other oxygen atoms, molecules of water within the cavity cannot satisfy their hydrogen bonding potential. For this reason they have higher enthalpy than the bulk water molecules in the solution. The complex formation in the case of β -cyclodextrin and its derivatives is based on the substitution of enthalpy-rich water molecules with »guest molecules«, thus decreasing the energy of the system. Greatest stability seems to be a guest molecule orientation in the cyclodextrin which allows maximum interaction with the groups lining the interior. The diameter of the inner ring of β -cyclodextrin is 0.75 nm (51).

Matsui and coworkers (52) revealed that substituted cyclodextrin forms complex with Cu^{2+} . It is therefore likely that inhibition of PPO with β -cyclodextrin proceeds through the complex formation of copper from metalloporphyrin with β -cyclodextrin. In a recent paper Hicks *et al.* (23) have described the mechanism of enzymatic browning inhibition of fresh raw apple, pear, white grape and celery by soluble and insoluble polymer immobilized β -cyclodextrin. Small amount of β -cyclodextrin in solution prevents the discoloration of apple juice for 3 to 4 weeks at 4 °C, while treatment of apple, pear, white grape and celery juice with an insoluble polymer-bound

β -cyclodextrin in a batch-wise or flow-through process, resulted in juices free of β -cyclodextrin, that were indefinitely resistant to browning.

Sulfhydryl containing amino acid

It has been known for a long time that cysteine inhibits PPO and prevents formation of brown pigments by reacting with intermediates of quinones which are converted to colorless and stable compounds (53). At the same time Bolin and Steele (19) have found that cysteine does not reduce the nonenzymatic browning of dried apples during the storage. Recently Molnar-Perl and Friedman (54) found that reduced glutathione and *N*-acetylcysteine are almost as active inhibitors as sulfites in controlling browning of apple, potato and fresh fruit juices.

Other Inhibitors and Various Processes for Inhibition of Enzymatic Browning

Inorganic halides, especially sodium chloride, are well-known inhibitors of PPO (8b). Use of sodium chloride is »Generally Recognized As Safe« (GRAS), but its use is limited since it has effect on product taste. Zinc chloride is also a highly effective browning inhibitor, especially in combination with calcium chloride, ascorbic acid and citric acid (55). It is of interest to mention that honey is an effective inhibitor of enzymatic browning of white grape and cut fruits. Research on the mechanism of action of honey and its practical application are in progress (56).

Since oxygen is essential in enzymatic browning, the inhibition of browning can be achieved by exclusion of oxygen. This procedure was developed more than 50 years ago by vacuum filled sliced fruits with syrup containing ascorbic acid. The process is suitable with the products to be frozen, but is not applicable to slices kept in refrigerator, since they would become objectionably water-logged (35). As previously mentioned, the removal of oxygen during the packaging of food can result in the loss of aroma and deterioration of taste, and besides, under the anaerobic conditions, a risk exists of the growth of dangerous pathogenic bacteria (36).

Finally, it must be emphasized that the reactivity of enzymatic browning depends on the manner of cutting and peeling of fruits and vegetables. Peeling potato with sharp knife is less injurious than peeling with abrasion or steam. A water-jet cutting system causes more sub-surface cellular damage in sliced potato than sharp knife blade. One factor which is of the utmost importance in production of food resistant to enzymatic and nonenzymatic browning is selection of proper cultivar of fruits and vegetables. Also, enzymatic and nonenzymatic browning can be related to degree of ripeness of cultivar (57). Since different cultivars have different tendency to browning (58), it is expected that genetic engineering will be the most useful process in developing low browning fruits and vegetables.

Conclusions

During processing, transportation and storage, food products often undergo browning discoloration through enzymatic and nonenzymatic reactions. Browning disco-

loration is a very complex reaction which in most cases has adverse effect on the quality of foods. Enzymatic browning in fruits and vegetables is usually connected with oxidation of *o*-phenols with oxygen in the presence of polyphenol oxidase. In order to prevent the deteriorative effect of enzymatic discoloration of foods various inhibitors of browning reaction are applied during processing and storage. The most important inhibitors are various sulfites. However, due to the sanitary limitations, a large number of sulfite alternatives have been developed and commercially applied: 4-hexylresorcinol, ascorbic and erythorbic acid, sulfhydryl containing amino acids, honey, ion exchangers and adsorbants, and various chelating agents.

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Sprječavanje enzimskog posmeđivanja hrane

Sažetak

U ovom su revijalnom pregledu ukratko opisani neki mehanizmi enzimskog posmeđivanja koji negativno utječu na kakvoću hrane. Enzimsko je posmeđivanje u svezi s djelovanjem monooksigenaze (polifenol-oksidadza, PPO), koja u prisutnosti kisika hidroksilira bezbojne monofenole u o-difenole, a ovi se spontano oksidiraju do obojenih o-kinona. Preko niza reakcija neenzimskog posmeđivanja o-kinoni se često nepovratno pretvaraju u polimere smeđe boje. Da bi se spriječilo enzimsko posmeđivanje, primjenjuju se različiti inhibitori tijekom preradbe i skladištenja hrane. Sprječavanje enzimskog posmeđivanja najčešće se provodi s pomoću tri mehanizma: 1. izravnom inhibicijom polifenol-oksidadze, 2. neenzimskom redukcijom o-kinona koji su nastali enzimskom oksidacijom o-fenola u derivate o-difenola i 3. kemijskom modifikacijom ili uklanjanjem fenolnih supstrata polifenol-oksidadze. Različiti su sulfiti najdjelotvorniji višefunkcionalni spojevi koji sprječavaju enzimsko posmeđivanje hrane. Međutim, njihova je primjena uglavnom ograničena zakonskim normama. Zbog toga se intenzivno istražuju zamjene za sulfite. Najpoznatija je alternativa sulfitima u sprječavanju posmeđivanja hrane L-askorbinska kiselina i njezin jeftiniji stereoizomer D-askorbinska kiselina, komercijalno poznata pod nazivom eritorbinska kiselina. Dobro poznata zamjena sulfita je 4-heksilresorcinol, dio pripravka EverFresh. Vrlo aktivni inhibitori posmeđivanja su različiti kelatni spojevi koji se ili izravno vežu s polifenol-oksidadzom ili reagiraju s njezinim supstratima. Primjer inhibitora koji reagira s bakrom što je sastavni dio prostetske skupine polifenol-oksidadze je β -ciklodekstrin. Drugi inhibitori enzimskog posmeđivanja što se komercijalno koriste jesu: aminokiseline koje sadržavaju sulfhidrilnu skupinu, posebno cistein i reducirani glutation, anorganske soli, kao što su natrijev i cinkov klorid te med koji inhibira posmeđivanje bijeloga grožđa. U ovom je revijalnom prikazu opisan dio našega rada u proučavanju inhibitora posmeđivanja djelovanjem eritorbinske kiseline na voće i povrće, i to pri povišenom tlaku ili u vakuumu.