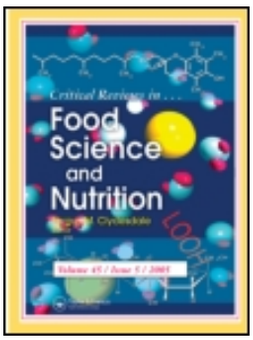

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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To cite this article: Arthur J. McEvily , Radha Iyengar & W. Steven Otwell (1992) Inhibition of enzymatic browning in foods and beverages, Critical Reviews in Food Science and Nutrition, 32:3, 253-273, DOI: 10.1080/10408399209527599

To link to this article: https://doi.org/10.1080/10408399209527599

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Critical Reviews in Food Science and Nutrition, 32(3):253–273 (1992)

Inhibition of Enzymatic Browning in Foods and Beverages

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ABSTRACT: Enzymatic browning is a major factor contributing to quality loss in foods and beverages. Sulfiting agents are used commonly to control browning; however, several negative attributes associated with sulfites have created the need for functional alternatives. Recent advances in the development of nonsulfite inhibitors of enzymatic browning are reviewed. The review fouses on compositions that are of practical relevance to food use.

KEY WORDS: enzymatic browning, polyphenol oxidase, inhibition, antibrowning agents, melanosis.

I. INTRODUCTION

Browning of raw fruits, vegetables, and beverages is a major problem in the food industry and is believed to be one of the main causes of quality loss during postharvest handling and processing.[1] The mechanism of browning in foods is well characterized and can be enzymatic or nonenzymatic in origin.[2] Nonenzymatic browning results from polymerization of endogenous phenolic compounds, as well as from the Maillard reaction that occurs when mixtures of amino acids and reducing sugars are heated. This article focuses on the various approaches taken to inhibit the enzymatic component of the browning reaction only. Note that several of the approaches described below may inhibit both components of the browning reaction.

The formation of pigments via enzymatic browning is initiated by the enzyme polyphenol oxidase (PPO; monophenol, L-DOPA: oxygen oxidoreductase; EC 1.14.18.10), also known as tyrosinase, phenol oxidase, monophenol oxidase, or cresolase. Endogenous PPO activity is present in foods that are particularly sensitive to oxidative browning, e.g., potatoes, apples, mushrooms, bananas, peaches, fruit juices, and wines. Browning is more severe when the food has been subjected to surface damage, which can result from cutting, peeling, comminuting, pureeing, pitting, pulping, or freezing. In uncut or undamaged fruits and vegetables, the natural phenolic substrates are separated from the PPO enzyme by compartmentalization, and browning does not occur. Browning can cause deleterious changes in the appearance and organoleptic properties of the food product, resulting in shorter shelf-life, decreased market value, and, in some cases, complete exclusion of the food product from certain markets. On the other hand, in certain situations, such as the manufacture of tea, coffee, cocoa, raisins, or cider, a specific degree of browning is desirable and is an essential part of the production process.

Enzymatic browning is the result of PPO-catalyzed oxidation of mono- and diphenols to

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o-quinones (Figure 1). PPO is a mixed function oxidase that catalyzes both the hydroxylation of monophenols to diphenols (cresolase activity) and the subsequent oxidation to *o*-quinones (catecholase activity). This enzyme is ubiquitous in fruits, vegetables, and animals.[3-5] The *o*-quinones are highly reactive compounds and can polymerize spontaneously to form high-molecular-weight compounds or brown pigments (melanin), or react with amino acids and proteins that enhance the brown color produced.[4,6,7]

The most effective method for controlling enzymatic browning in canned or frozen fruits and vegetables is to inactivate the PPO by heat treatment, such as by steam blanching, but this is not a practical alternative for treatment of fresh foods. As browning is an oxidative reaction it can be retarded by the elimination of oxygen from the cut surface of the fruit or vegetable, although browning will occur rapidly when oxygen is reintroduced. Exclusion of oxygen is possible by immersion in deoxygenated water, syrup, brine, or by vacuum deoxygenation,[8] or coating of the food with surfactants.[9] These processes can be relatively expensive or impractical. A more common approach for the prevention of browning of food and beverages has been the use of antibrowning agents. Antibrowning agents are compounds that either act primarily on the enzyme or react with the substrates and/or products of enzymatic catalysis in a manner that inhibits pigment formation. The use of antibrowning agents in the food industry is constrained by considerations such as toxicity, effects on taste, flavor, color, texture, and cost.

The most widespread methodology used in the food and beverage industries for control of browning is the addition of sulfiting agents. Sulfites are currently used to inhibit melanosis (blackspot) in shrimp, browning of potatoes, mushrooms, apples, and other fruits and vegetables, as well as to stabilize the flavor and color of wines. The major effect of sulfites on enzymatic browning is to reduce the *o*-quinones produced by PPO catalysis to the less reactive, colorless diphenols, thereby preventing the nonenzymatic condensations to precipitable pigments (Figure 2). In some instances, excessive concentrations of sulfiting agents are used to bleach brown or black pigments that may have developed prior to treatment. Sulfiting agents are also antimicrobial when used in sufficient concentration.

Although sulfites are very effective in the inhibition of both enzymatic and nonenzymatic browning reactions, there are several negative attributes associated with their use in foods and beverages. Sulfites are known to cause adverse health effects, especially in certain sensitive individuals such as steroid-dependent asthmatics. Several deaths have resulted due to consumption of sulfited foods among this highly sensitive

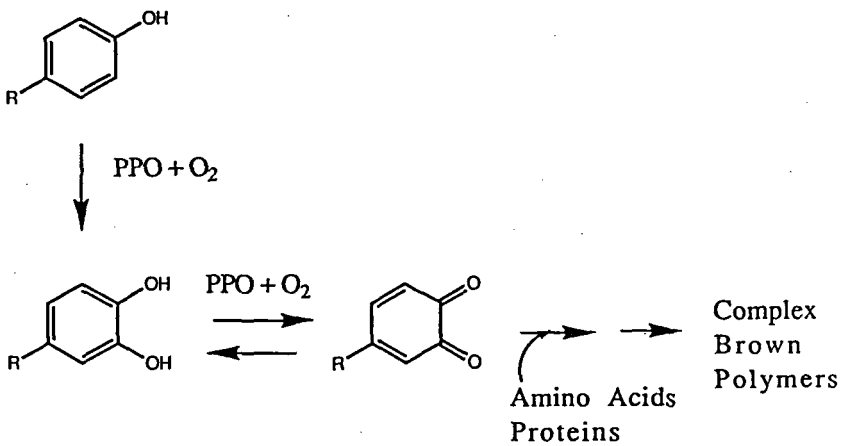

FIGURE 1. Simplified schematic of the initiation of browning by polyphenol oxidase. (Adapted from Walker, J. R. L., *Food Technol. N. Z.*, 19, 21, 1977. With permission.)

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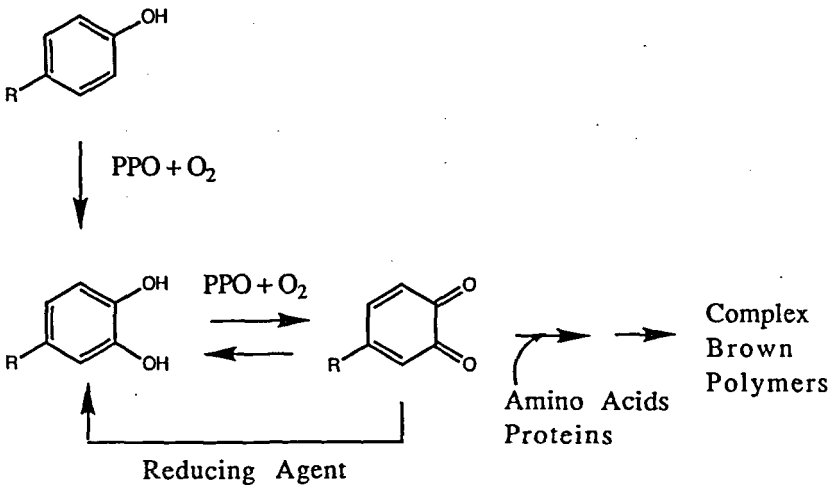

FIGURE 2. The primary role of reducing agents such as sulfiting agents or ascorbyl compounds in the inhibition of enzymatic browning is to reduce the pigment precursors (quinones) to colorless, less-reactive diphenols. (Adapted from Walker, J. R. L., *Food Technol. N. Z.*, 19, 21, 1977.)

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group. Sulfites can also liberate sulfur dioxide gas and in enclosed areas, such as the holds of fishing vessels, sulfur dioxide vapors have led to several deaths among fisherman.[10] Also, in certain foods, sulfite residuals are so high as to have a negative effect on the taste of the treated product. For more information on the use of sulfiting agents and associated health risks, the reader is referred to an excellent review by Taylor et al.[11]

In recent years, the Food and Drug Administration (FDA) has banned sulfites for use in salad bars,[12] moved to ban their use on fresh, peeled potatoes,[13,14] increased surveillance and seizure of imported products with undeclared or excessive sulfite residuals,[15,16] and has set specific limits on sulfite residuals allowable in certain foods.[17,18] A determination has been made by the Center for Food Safety and Applied Nutrition Health Hazard Evaluation Board of the FDA that a "four-ounce serving of shrimp containing 90 ppm sulfites presents an acute life threatening hazard to health in sulfite sensitive individuals".[15] The negative connotations associated with sulfited foods has led to decreased consumer acceptance. The adverse health effects, increased regulatory scrutiny, and lack of consumer acceptance of sulfited foods have created the need for practical, functional alternatives to sulfiting agents.

Section II reviews recent advances in the development of nonsulfite antibrowning agents, with particular emphasis on their use in the food industry. The agents have been classified according to their primary mode of action (Table 1). As can be seen in Table 1, there are many approaches available to food technologists to inhibit brown-

TABLE 1
Representative Inhibitors of Enzymatic Browning

Reducing agents	**Chelating agents**
Sulfiting agents	Phosphates
Ascorbic acid and analogs	EDTA
Glutathione	Organic acids
Cysteine	
Enzyme inhibitors	**Acidulants**
Aromatic carboxylic acids	Citric acid
Aliphatic alcohols	Phosphoric acid
Substituted resorcinols	
Anions	**Complexing agents**
Peptides	Cyclodextrins
Enzyme treatments	
Oxygenases	
o-Methyl transferases	
Proteases	

ing. The choice of one approach over another will result from an evaluation of inhibitor performance, treatment cost, organoleptic impact, and toxicity/regulatory concerns.

II. REDUCING AGENTS

The major role of reducing agents or antioxidants in the prevention of browning is their ability to chemically reduce the enzymatically formed or endogenous *o*-quinones to the colorless diphenols, or react irreversibly with the *o*-quinones to form stable colorless products analogous to the action of sulfites (Figure 2).[19-21] The effect of reducing agents can be considered temporary because these compounds are oxidized irreversibly by reaction with pigment intermediates, endogenous enzymes, and metals such as copper. Thus, reducing agents are effective for the time period determined by their rate of consumption. The nonspecificity of reducing agents can also result in products with off-flavors and/or off-colors.

A. Ascorbic Acid and Ascorbyl Derivatives

1. Ascorbic Acid and Erythorbic Acid

Ascorbic acid and its isomer, erythorbic acid (Figure 3), have frequently been used interchangeably as antioxidants in the food industry. Their function in food systems is (1) to act as a free radical scavenger and thereby prevent oxidation, (2) to alter the redox potential of the system, and (3) to reduce undesirable oxidative products. The main role of ascorbic acid and erythorbic acid in the prevention of enzymatic browning is their ability to reduce the *o*-quinones to diphenols (Figure 2).[22] The effect of these agents directly on the enzyme, PPO, has been controversial and remains to be proven.[21,23,24] Early studies indicated that ascorbic acid had no direct effect on the activity of PPO[25,26] and neither activated nor inhibited the enzyme;[27] however, activation of PPO by ascorbic acid was reported by Krueger.[28] Conversely, several reports claim inactivation of the enzyme by ascorbic acid.[29-31] Golan-Goldhirsh and Whitaker[24] reported decreased PPO activity upon incubation of the mushroom enzyme with ascorbic acid in the absence of phenolic substrates. A more detailed polarographic investigation of this phenomenon indicated that the inactivation was biphasic; there was an initial slow rate of inactivation followed by a fast rate of inactivation that decreased with time. The inactivation appeared to be irreversible, although after electrophoresis some isoenzymes regained activity. Janovitz-Klapp et al.[32] studied the effect of increasing concentrations of ascorbic and erythorbic acid on apple PPO both spectrophotometrically (color formation) and polarographically (O_2 uptake). As was reported previously concerning the use of PPO from other sources,[21,23,24,33] in the presence of either reducing agent, spectrophotometric assays exhibited an initial lag in the absorbance change that was followed by a slow increase in reaction rate, whereas immediate oxygen uptake was observed by polarography. The greater the reductant concentration, the longer the initial lag period. The rate of initial increase in the absorbance following the lag period reflects the effect of the reductant concentration on the inactivation of PPO, but the length of the lag period is due to the effect of the chemical reduction of the *o*-quinones. By spectrophotometry, the I_{50} value (the inhibitor concentration that yields 50% inhibition of enzyme activity) was 0.24 m*M* for ascorbic acid, whereas by polarography concentrations of less than 0.5 m*M* ascorbic acid had no effect on oxygen consumption. These results suggest that enzyme activity was unaffected by ascorbic acid at these

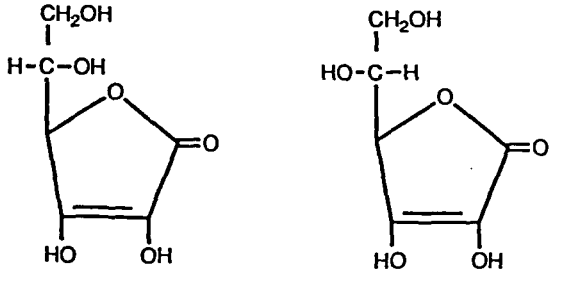

FIGURE 3. Comparison of the chemical structures of ascorbic and erythorbic acid.

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concentrations; however, the products of catalysis were reduced back to the nonabsorbing substrates. The decreased activity of PPO following the lag phase may be due to the decrease in oxygen concentration in the assay mixture. Therefore, the *observed* effects of reductants on PPO are dependent on the assay method, which may account for some of the apparently conflicting reports in the literature as to the effects of ascorbic and erythorbic acids on PPO.

Although the mode of action of ascorbic and erythorbic acid is the same, ascorbic acid has been reported to be a more effective inhibitor of browning than erythorbic acid.[34,35] Nevertheless, recommended-use concentrations of the two reducing agents are similar.[36] Erythorbic acid has been reported to undergo copper-catalyzed oxidation more readily than ascorbic acid in aqueous model systems and food products.[34] As copper is present in trace amounts in almost all food systems, the difference in efficacy of the two reducing agents can be attributed to the faster rate of oxidation of erythorbic acid. Sapers and Ziolkowski,[37] in a more recent comparison of erythorbic and ascorbic acid as inhibitors of enzymatic browning in apples, showed that both reducing agents were similar in effectiveness in apple juice (0.125 or 0.250% w/v ascorbic or erythorbic acid). However, under identical treatment conditions, plugs of Winesap and Red Delicious apples showed longer time periods before the onset of browning with ascorbic acid when compared with erythorbic acid. The performance of erythorbic and ascorbic acid as browning inhibitors appears to be dependent on the specific food system. Therefore, one compound cannot be substituted for the other without prior experimental evaluation of their equivalence.

Another serious shortcoming of either ascorbic or erythorbic acid as an antibrowning agent is that they are easily oxidized by endogenous enzymes,[38] as well as decomposed by iron or copper-catalyzed autoxidation to form dehydroascorbic acid. Ascorbic acid, when oxidized by these reactions or used at elevated concentrations, may exert prooxidant effects.[39]

Another major problem that limits the efficacy of ascorbic acid and erythorbic acid when compared with sulfites is their insufficient penetration into the cellular matrix of the fruit or vegetable pieces.[11] Sapers et al.[40] have investigated pressure and vacuum infiltration of ascorbic and erythorbic acid into the cut surfaces of raw apples and potatoes to improve the efficiency of inhibition. Comparison of apple plugs treated by pressure or vacuum infiltration with 2.25% sodium ascorbate or erythorbate, and 0.2% calcium chloride, showed that plugs infiltrated at pressures of about 34 kPa had more uniform uptake of the treatment solutions and less extensive water-logging than plugs vacuum-infiltrated at 169 to 980 mB. The storage life of Red Delicious and Winesap apple plugs and dice can be extended by 3 to 7 d when treated by pressure infiltration, when compared with dipping at atmospheric pressure for 5 min. There is a tradeoff between the concentration of inhibitor used and the choice of method of application: the more expensive pressure infiltration process would permit the use of lower concentrations of ascorbic or erythorbic acid to control browning than is required with dipping at atmospheric pressure, but infiltrated dice samples gradually became water-logged during storage and required dewatering by centrifugation or partial dehydration. The storage life of Brown Russet potato plugs was extended by 2 to 4 d when treated by pressure infiltration at 103 kPa with solutions containing 4% ascorbic acid, 1% citric acid, and 0.2% calcium chloride, when compared with dipping at atmospheric pressure for 5 min. The same pressure infiltration procedure has no effect on potato dice.

These reducing agents are relatively reactive compounds and can react with other components in the food system, resulting in deleterious effects. Golan-Goldhirsh and Whitaker[24] reported that although ascorbic acid inhibited browning in avocado extracts assayed spectrophotometrically, the addition of ascorbic acid enhanced browning of avocado pulp. In tests on shrimp to evaluate the efficacy of ascorbic acid in the prevention of PPO-catalyzed "blackspot", the ascorbic acid-treated samples were found to develop a distinct yellow off-color.[41]

2. Ascorbyl Phosphate Esters

The rapid oxidation of ascorbic acid to dehydroascorbic acid has led to the development of

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ascorbic acid derivatives with increased stability. Cutola and Larizza[42] reported the phosphorylation of ascorbic acid. Since then a number of 2- and 3-phosphate and phosphinate esters of ascorbic acid have been synthesized.[43] Ascorbic acid-2-phosphate and ascorbic acid-2-triphosphate have been investigated as stable alternative sources of ascorbic acid for the inhibition of browning at the cut surfaces of raw apples, potatoes, and in fruit juices.[44–47] These esters release ascorbic acid when hydrolyzed by acid phosphatases.[48] The phosphate esters were less effective than ascorbic acid in the prevention of browning of cut potatoes but were more effective than similar concentrations of ascorbic acid in the prevention of browning on the cut surfaces of Red Delicious or Winesap apple plugs.[45] The improved performance of the esters may be due primarily to their oxidative stability, as seen by the longer lag times for the onset of browning obtained with these derivatives when compared with equivalent concentrations of ascorbic acid.

Ascorbyl phosphate esters used in combination with citric acid (1% final concentration) were not as effective, probably due to the inhibition of the acid phosphatases at low pH.[49–51] Also, the failure of the esters to prevent browning of apple juice may result from low activity of endogenous acid phosphatase due to inactivation of the enzyme during preparation or the low pH (3.3) of the juice. Acid phosphatase activity in fruits and vegetables depends on the enzyme concentration, cellular location, pH, and concentration of multivalent cations.[50–52] Thus, suitability of the phosphate esters as browning inhibitors depends on the ability of the food system to absorb the compound, the acidity of the system, and the activity of endogenous acid phosphatase.[53]

3. Ascorbyl Fatty Acid Esters

Alternative stable sources of ascorbic acid are the ascorbyl-6-fatty-acid esters (ascorbyl palmitate, laurate, and decanoate).[26,44] The ascorbyl-6-fatty-acid esters, when added to Granny Smith apple juice at concentrations as high as 1.14 m*M* (equivalent to 0.02% ascorbic acid), inhibited browning for at least 6 h.[54] The performance of the esters was less effective or similar to that of free ascorbic acid initially but was superior to that of ascorbic acid after longer storage periods.[44] The combination of ascorbyl decanoate and ascorbic acid was significantly more effective than either agent alone and together they can prevent browning of apple juice for up to 24 h.

Cort[55] reported that the ascorbyl-fatty-acid esters needed to be solubilized, i.e., by adjusting the pH to 9.0, to act as antibrowning agents. Sapers et al.[54] investigated the effect of emulsifying agents as stabilizers of aqueous dispersions of esters at concentrations of 1.14 m*M* in apple juice. Stable dispersions could be prepared by using hydrophilic emulsifying agents such as Tween 60 (polyoxyethylenesorbitan monostearate), Santone 8-1-0 (a polyglycerol ester), Tween 80 (polyoxyethylenesorbitan monooleate), or EC-25 (a propylene glycol ester) at ratios in the range of 1:2 to 2:1 (ratio of emulsifying agent to ester). Highly lipophilic emulsifying agents such as Durlac 100 (a lactylated glycerol ester) and Dur-Em 114 (a mono- and diglyceride) tended to precipitate the esters. The combination of the esters and emulsifiers such as EC-25, Santone 8-1-0, or Tween 60 decreased the effectiveness of the esters in the prevention of browning of apple juice. The adverse effect of the addition of Tween may be due to its ability to solubilize significant quantities of the membrane- or organelle-bound PPO. Also, activation of PPO by detergents has been reported previously.[4,7]

Mixed results were obtained when the combination of ascorbyl-fatty-acid esters and emulsifying agents were evaluated as antibrowning agents for apple plugs. Ascorbyl palmitate dispersions at pH 7.0 in combination with EC-25 or Durlac 100 were more effective than equivalent concentrations of ascorbic acid. However, the ascorbyl palmitate tended to precipitate on the surface of the apples during storage, giving inconsistent results. Treatment of apple plugs with combinations of ascorbyl laurate or ascorbyl decanoate with EC-25, Durlac 100, or less lipophilic emulsifiers like Tween 60 or 80, tended to induce the browning of apple plugs. The adverse effect of the addition of the emulsifiers may be due to the disruption of the cell membranes at the cut surface of the fruit, resulting in leakage of PPO and its substrates, thereby increasing the

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browning reaction. In essence, emulsifying agents increase the stability of ascorbyl ester dispersions but have detrimental effects on their ability to function as antibrowning agents.

4. Miscellaneous Ascorbic Acid Derivatives

The preparation and use of L-5,6-*O*-isopropylidene-2-*O*-methylcarbo:methyl ascorbic acid[56] and ascorbic acid *vic*-glycols, produced by reaction of dioxalan-based compounds with organic acids such as acetic acid,[57] were described recently. Both of these types of derivatives were claimed to be more stable than ascorbic acid and useful for the prevention of browning of foods in addition to maintaining freshness and flavor.

B. Sulfhydryl Compounds

Many sulfhydryl-containing reducing agents such as β-mercaptoethanol, dithiothreitol, and thiourea will probably never be approved for food use as antibrowning agents. Although much more effective than ascorbic acid, use of other, more acceptable sulfhydryl compounds, such as reduced glutathione, is too expensive to be a practical commercial alternative.[24]

Practical alternatives in this category may be limited to sulfur-containing amino acids such as L-cysteine, L-cystine, and D,L-methionine.[58] The potential for the use of L-cysteine and other thiols has been recognized for a long time,[6] although relatively little attention has been devoted to these compounds. Walker and Reddish[59] reported the use of cysteine in the prevention of browning of apple products for over 24 h without the introduction of undesirable off-flavors. L-Cysteine (10 m*M*) was reported to be more effective than sodium bisulfite at the same concentration in the prevention of browning of Jerusalem artichoke extracts.[60] Kahn[61] found 0.32 m*M* L-cysteine to be very effective for the inhibition of avocado and banana homogenate browning. L-Cysteine retards the browning of pear juice concentrates when used at concentrations of 0.5 to 2 m*M*.[62] Unfortunately, the concentrations of cysteine necessary to achieve acceptable levels of browning inhibition have negative effects on the taste of the treated foods.

The primary mode of action of sulfhydryl compounds in the prevention of browning is to react with the *o*-quinones formed by enzymatic catalysis to produce stable, colorless adducts[63–65] (Figure 4). Richard et al.,[66] among others, have elucidated the structures of the adducts of cysteine with 4-methylcatechol, chlorogenic acid, (−)-epicatechin, (+)-catechin,[66] pyrocatechol, and L-dopa,[19,67] and the product of glutathione and caftaric acid condensation.[20] Cysteine was found to form a single addition product with 4-methylcatechol and chlorogenic acid, and two products with the epicatechin and catechin.[66] The latter two addition products differed in the position of the cysteine moiety in the B ring of the parent compound. The 2′- and 5′-positions were found to react with cysteine at equivalent rates. The *o*-diphenolic cysteine and glutathione adducts are not substrates for PPO,[19,68,69] whereas PPO inhibition has been reported for the cysteinylcatechol.[19,70]

III. CHELATING AGENTS

As mentioned previously, PPO contains copper in its active site. In the context of PPO-catalyzed browning, chelating agents are believed to either bind to the active site copper of PPO or reduce the level of copper available for incorporation into the holoenzyme.

A. EDTA

Ethylenediaminetetraacetic acid (EDTA) or its sodium salt is used widely in the food industry as a metal chelating agent. The log K_1 (stability constant) for binding of copper is 18.8. As an antibrowning agent, EDTA is generally used in combination with other agents to eliminate browning (see Section VIII).

B. Phosphate-based Compounds

Sodium acid pyrophosphate, polyphosphate, or metaphosphate are chelating agents and have

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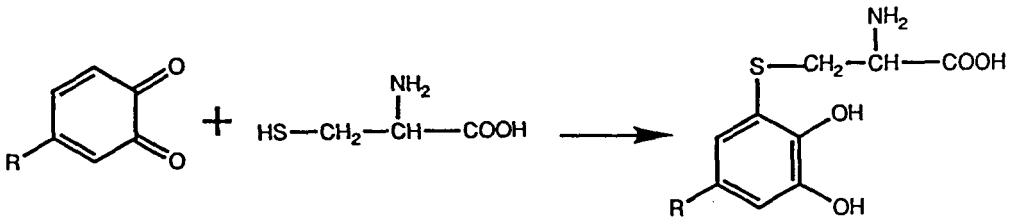

FIGURE 4. The mode action of sulfhydryl compounds in the inhibition of enzymatic browning.

been used as antibrowning agents for fresh-peeled fruits and vegetables.[71] The phosphate compounds have low solubility in cold water and, hence, are normally used by predissolving the compounds in water or at low concentration. Phosphate-based agents typically are used at levels of 0.5 to 2% (final concentration in the dip solution) in combination with other antibrowning agents (see Section VIII).

Sporix,™ an acidic polyphosphate mixture that has a three-dimensional network structure, has been evaluated as an antibrowning agent in combination with ascorbic acid.[72] Sporix™ is recommended for use on acidic foods such as fruit-based juices, nectars, and carbonated beverages.[73] Sporix™ at about 0.6% was more effective than ascorbic acid (0.01%) in preventing browning of Granny Smith apple juice for 24 h. If the two compounds were used in combination, a much lower concentration of Sporix™ was needed to obtain the same degree of browning inhibition. The effectiveness of the combination to delay the onset of browning was synergistic, not simply additive. The effect of the Sporix™-ascorbic acid mixture was pH dependent. Increasing the pH of the treated juice from 3.1 to 3.3 resulted in a more rapid onset of browning and an increase in the rate of the browning reaction. Winesap or Red Delicious apple plugs dipped into solutions containing Sporix™ (0.24%) and ascorbic acid (1%) showed little or no evidence of browning after 24 h at 20°C. Control samples that received no treatment browned within a few hours.

As noted above, the combination of ascorbic acid and Sporix™ as an antibrowning agent can extend the lag time before the onset of browning and also results in a reduced rate of browning after the lag time has been exceeded. The increased lag time effect most likely results from the inhibition of PPO- and copper-catalyzed oxidative reactions by chelation of copper by Sporix.™ The combination of Sporix™ with other antibrowning agents will be reviewed below (see Section VIII).

IV. ACIDULANTS

The pH optimum of polyphenol oxidase activity varies with the source of the enzyme and the particular substrate but in most cases it has an optimum pH in the range of pH 6 to 7.[74] PPO preparations from several sources are reported to be inactivated below pH 4.0.[75,76] By lowering the pH of the media below 3, the enzyme is effectively inhibited. Hence, the role of acidulants is to maintain the pH well below that necessary for optimal catalytic activity.

A. Citric Acid

The most widely used acid in the food industry for the prevention of browning is citric acid. Citric acid may have a dual inhibitory effect on PPO by reducing the pH and by chelating the copper at the enzyme-active site. This acidulant is often used in blended products in combination with other antibrowning agents (see Section VIII). Treatment of fresh fruits or vegetables with a solution of citric acid (typically, 0.5 to 2% w/v) helps control enzymatic browning. McCord and Kilara[77] studied the mechanism of the inactivation of PPO in processed mushrooms. They reported that citric acid was effective at pH 3.5 and that it could inhibit both enzymatic and nonenzymatic browning. Mushrooms showed no improvement in color when they were washed and soaked in water at pH 3.5, whereas when the pH

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was lowered in vacuum or blanching operations significant improvement in color over nonacidified controls was observed.

Reitmeier and Buescher[78] reported that treatment for up to 30 s with a 5% citric acid solution afforded a temporary reduction in the browning of snap bean cut-end-tissue homogenates. A 67% inhibition was seen after 24 h, which decreased to 13% inhibition after 48 h.

B. Other Acidulants

Other alternatives to citric acid are organic acids, such as malic, tartaric, and malonic, and inorganic acids such as phosphoric and hydrochloric. When compared with citric acid, the main disadvantages of these acids are factors such as availability, price, and taste of the food product after treatment.

V. PPO INHIBITORS

There are numerous reports on specific PPO inhibitors. Only those that are of practical relevance to food use are included in the following section.

A. Substituted Resorcinols

Protease preparations, especially ficin, the protease from fig (*Ficus* sp.) latex, appear to function as browning inhibitors in several food systems (see Section VII.C).[79] The ficin preparations employed were partially purified and the possibility existed that a nonprotease component of the preparation was responsible for the observed antibrowning effect. Indeed, preparations of either heat-inactivated ficin[79] or ultrafiltered ficin-free fig extract[80] were as effective in PPO inhibition as the preparation containing the active protease.

Three inhibitors were isolated from the ficin preparations by conventional and high-performance liquid chromatography.[81] Based on analytical data for homogeneous preparations, the inhibitors present in the fig extract were found to be analogous 4-substituted resorcinols. The compounds, identified as 2,4-dihydroxydihydrocinnamic acid, 2,4-dihydroxydihydrocinnamoyl putrescine, and *bis*-(2,4-dihydroxydihydrocinnamoyl)-spermidine, are novel, plant secondary metabolites (Figure 5). 2,4-Dihydroxydihydrocinnamic acid has also been isolated from the edible fig fruit, in addition to the fig latex from which the ficin preparation had been derived.[81]

A structurally related PPO inhibitor, *bis*-(2,4-dihydroxydihydrocinnamoyl)-putrescine was produced as a secondary reaction during the *in vitro* synthesis of 2,4-dihydroxydihydrocinnamoyl-putrescine (Figure 6).

The I_{50} values for the naturally occurring inhibitors and *bis*-(2,4-dihydroxydihydrocinnamoyl)-putrescine were determined using mushroom PPO in an *in vitro* assay system.[81] The I_{50} is defined as the inhibitor concentration at which 50% inhibition of PPO activity is obtained. The results are presented in Table 2.

In addition to the natural compounds, synthetic 4-substituted resorcinols were screened for efficacy as PPO inhibitors. I_{50} values were determined and are summarized in Table 3. Resorcinol is a poor inhibitor with an I_{50} in the millimolar range; however, substitutions in the 4-position yield decreased I_{50} values. The lowest values are obtained with hydrophobic substituents in the 4-position such as 4-hexyl-, 4-dodecyl-, and 4-cyclohexylresorcinol with I_{50} values of 0.5, 0.3, and 0.2, respectively.

Resorcinol derivatives with substitutions in the 5-, 2-, and 1,3-positions were also evaluated as PPO inhibitors. Resorcinols that were 5-substituted exhibited an inhibitory trend analogous to that seen with 4-substituted resorcinols: hydrophobic substituents of increasing chain length yield inhibitors with decreasing I_{50} values.[81] Although the 5-substituted resorcinols appear to be effective PPO inhibitors *in vitro* and several of these compounds also occur in nature,[82,83] their use in food applications was not pursued due to the toxic and irritant properties associated with this class of compounds.[84–89] Substitutions in the 2- and 1,3-positions led to greatly increased I_{50} values relative to resorcinol. These compounds exhibited only low levels of PPO inhibition even at the limit of their respective solubilities.[81]

Of the 4-substituted resorcinols, 4-hexylresorcinol may have the greatest potential for use

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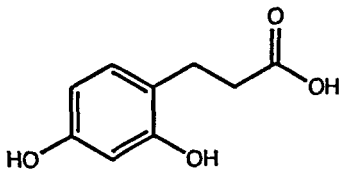
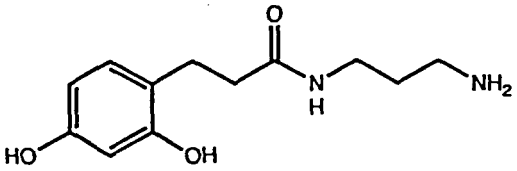
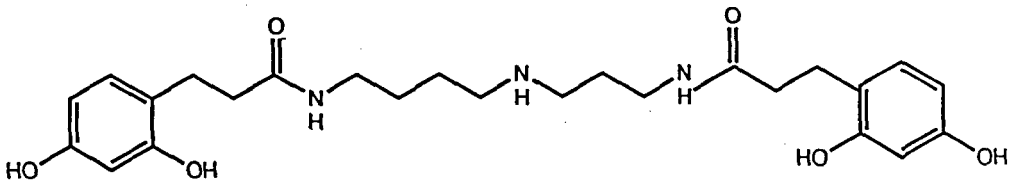


2,4-Dihydroxydihydrocinnamic Acid

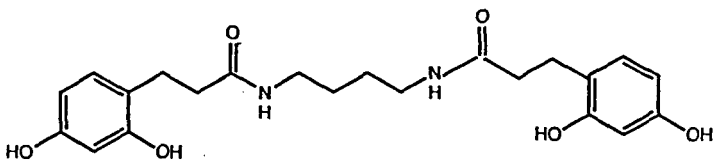


2,4-Dihydroxydihydrocinnamoylputrescine



***bis*-(2,4-Dihydroxydihydrocinnamoyl)-spermidine**

FIGURE 5. Structures of 4-substituted resorcinol PPO inhibitors isolated from fig extract. (From McEvily, A. J., Iyengar, R., and Gross, A. T., in *ACS Symposium Series,* Ho, C.-T., Ed., American Chemical Society, Washington, D. C., 1991, in press. With permission.)



***bis*-(2,4-Dihydroxydihydrocinnamoyl)-putrescine**

FIGURE 6. Structure of synthetic 4-substituted resorcinol PPO inhibitor produced as a byproduct in the synthesis of 2,4-dihydroxydihydrocinnamoylputrescine. (From McEvily, A. J., Iyengar, R., and Gross, A. T., in *ACS Symposium Series,* Ho, C.-T., Ed., American Chemical Society, Washington, D.C., 1991, in press. With permission.)

in the food industry due to its low I_{50} in the spectrophotometric assay system, positive preliminary results from tests in actual food systems (see below), and the fact that this compound has a long, safe history of human use in nonfood applications. Numerous toxicological studies on

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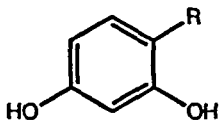
TABLE 2
I_{50} Values for 4-Substituted Resorcinols as PPO Inhibitors

Compound	I_{50} μ*M*
2,4-Dihydroxydihydrocinnamic acid	25
2,4-Dihydroxydihydrocinnamoyl putrescine	5
bis-(2,4-Dihydroxydihydrocinnamoyl)-putrescine	5
bis-(2,4-Dihydroxydihydrocinnamoyl)-spermidine	5

From McEvily, A. J., Iyengar, R., and Gross, A. T., in *ACS Symposium Series*, Ho, C.-T., Ed., American Chemical Society, Washington, D.C., 1991, in press. With permission.

TABLE 3
I_{50} Values for Synthetic 4-Substituted Resorcinols as PPO Inhibitors



R	I_{50} μ*M*
H	2700
Hexanoyl	750
Carboxyl	150
Ethyl	0.8
Hexyl	0.5
Dodecyl	0.3
Cyclohexyl	0.2

From McEvily, A. J., Iyengar, R., and Gross, A. T., in *ACS Symposium Series*, Ho, C.-T., Ed., American Chemical Society, Washington, D.C., 1991, in press. With permission.

4-hexylresorcinol support potential food use for this compound. These studies are the subject of a recent review relative to use of 4-hexylresorcinol in foods.[90]

The initial 4-hexylresorcinol food application targeted for intensive investigation was the prevention of shrimp melanosis (blackspot). Blackspot is a cosmetic discoloration caused by endogenous shrimp PPO and has a negative impact on the commercial value of the shrimp product. The efficacy of 4-hexylresorcinol in maintaining the high quality of landed shrimp has been shown in both laboratory and field trials under a variety of process conditions.[91,92] This highly effective inhibitor is substantially more effective than bisulfite on a weight-to-weight basis, it should prove to be competitive with bisulfite on a cost basis, and it will require no changes in the on-board or ex-vessel handling of the shrimp product.

In addition to being a water soluble, stable compound, 4-hexylresorcinol is also nontoxic, nonmutagenic, and noncarcinogenic and is generally recognized as safe (GRAS) for use in the prevention of shrimp melanosis.[90] The use of 4-hexylresorcinol as a processing aid for the inhibition of shrimp melanosis has no negative impact on taste, texture, or color of the treated shrimp product, due to very low residuals (<1 ppm) on shrimp meat.[93,94]

Preliminary results from laboratory studies indicate that 4-hexylresorcinol inhibits browning of fresh and hot-air dried apple and potato slices, avocado (guacamole), and in liquid systems such as apple and white grape juices (McEvily, A. J., unpublished results). Note that 4-hexylresorcinol appears to function well in the prevention of apple juice browning, whereas resorcinol has been reported to be neither a substrate nor an inhibitor of apple PPO and, in another study, was found to stimulate apple PPO-catalyzed chlorogenic acid oxidation.[95]

Resorcinols that are 4-substituted have several advantages over sulfites for use on foods. Among others, these include: (1) these compounds are specific, potent polyphenol oxidase inhibitors allowing use at much lower concentrations than sulfites; (2) 4-substituted resorcinols do not "bleach" pigments as excess sulfites can, and, therefore, use of excessive concentrations is not encouraged; and (3) the 4-substituted resorcinols are more chemically stable relative to sulfites. Because much lower concentrations of resorcinol derivative are required, these agents are also cost-competitive with sulfite.

B. Aromatic Carboxylic Acids

Aromatic carboxylic acids are inhibitors of

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PPO due to their structural similarities with the phenolic substrates.[96–98] In model systems, the type of inhibition observed is dependent on the substrate being assayed and was either competitive, noncompetitive, or mixed.[32] Using 4-methylcatechol as the substrate, the inhibition of grape PPO by cinnamic and benzoic acids was competitive, but with caffeic acid as the substrate the mode of inhibition was noncompetitive.[99] Cinnamic acid and its analogues, *p*-coumaric, ferulic, and sinapic acids, were found to be potent inhibitors of apple PPO,[100,101] with K_i values from 2 to 30 times lower than benzoic acid and its analogues, *p*-hydroxybenzoic, vanillic, and syringic acids. Unsaturation, such as in the side-chain of cinnamic acid, is an important structural determinant in the potency of inhibitors. The benzoic acid derivatives were more effective inhibitors than phenylacetic, phenylpropionic, and *p*-hydroxyphenylpropionic acids.[32] For the cinnamic and benzoic acid series of compounds, *p*-hydroxy substitution slightly enhances the inhibitory characteristics, whereas the addition of one or two methoxy groups in the meta-positions reduces the inhibitory properties of the compounds. Esterification of the carboxy group of benzoic acid or cinnamic acid results in a considerable decrease in inhibition.[99,100,102] The degree of inhibition by the acids is pH dependent, increasing as the pH is decreased. Robb et al.[102] postulated that the undissociated carboxylic group is necessary to form a complex with the copper at the enzyme active site.

The use of cinnamic, *p*-coumaric, and benzoic acid as antibrowning agents for apple juice was investigated by Walker.[103] Various concentrations of the acids were added to freshly prepared opalescent apple juice and the mixtures were aerated to promote browning. Cinnamic acid (or its more soluble sodium salt) at levels of 0.01% or less was reported to be the most effective antibrowning agent for providing long-term inhibition of browning.

Combinations of sodium cinnamate at concentrations between 0.01 and 0.04%, with ascorbic acid at 0.02%, were more effective than either compound alone in the prevention of browning of Granny Smith apple juice.[45] Treatment of Winesap apple plugs with sodium cinnamate (0.2%) resulted in short-term inhibition of browning but after storage for 24 h the treatment induced the browning of the plugs. The combination of cinnamate and ascorbic acid in dips was more effective than the use of ascorbic acid alone and resulted in significant extension of the lag time for the onset of browning of apple plugs. The tendency of cinnamic acid or its sodium salt to induce browning is a major problem with the use of these compounds. The slow increase in the browning of the food suggests that the exogenous cinnamate at the cut surface is gradually being converted to a PPO substrate by cinnamate hydroxylases or other enzymes involved in the biosynthesis of polyphenols.[104] Hydroxylation of cinnamate results in *p*-coumaric acid, a PPO inhibitor, which might be hydroxylated further to caffeic acid, a substrate.[105]

Sodium benzoate showed concentration-dependent antibrowning properties in Granny Smith apple juice.[45] Combinations of 0.1% sodium benzoate and 0.02% sodium ascorbate (the acid was not used to avoid precipitation of the benzoate) or ascorbic acid-2-phosphate appeared to have a synergistic effect in the prevention of browning of the juice for 24 h. The main effect of the combination was to increase the lag time for the onset of browning. Granny Smith apple plugs dipped into solutions containing benzoate alone or in combination with ascorbic acid showed short-term protection against browning but, subsequently, severe browning was induced in samples stored more than 6 h. As in the case of cinnamate, benzoate may be undergoing slow conversion to a PPO substrate.

C. Aliphatic Alcohols

Montedaro and Canterelli [106] and Kidron et al.[107] have reported the inhibition of PPO by ethanol, but inhibition by other aliphatic alcohols was not studied extensively until more recently. Valero et al.[108] studied the effect of natural aliphatic alcohols on grape PPO. The authors reported that inhibition increases with the number of carbon atoms of the aliphatic alcohol (from one to five carbon atoms). The order of effectiveness for various alcohols appeared to be primary>secondary>tertiary alcohols. The authors attempted to correlate the inhibitory effects with

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the hydrophobic nature of the alcohols as measured by their respective octanol/water partition. For primary alcohols, the relationship was nonlinear, suggesting that other factors must also be involved.

D. Amino Acids, Peptides, and Proteins

Inhibition of browning in apple slices, grape juice, and model systems by honey was studied by Lee and co-workers.[109,110] White grapes and sliced fruit dipped into a 20% solution of honey before conversion to yellow raisins or dried fruit maintained their natural flavor, texture, and color, when compared with sulfites, and did not have a honey taste.[111] As sugar solutions inhibit browning by reducing the concentration of dissolved oxygen and the rate of diffusion of oxygen into the fruit tissue,[112] the rates of browning of apple slices after treatment with 8% sucrose (level of sugar in 10% honey) and 10% honey were compared. The results showed that the apple slices treated with honey showed the least amount of browning. This suggested that the honey contains inhibitors of PPO in addition to sugars. Purification of the honey by Bio-Gel P-2 and Sephadex G-15 columns gave a fraction that had high inhibitory activity. The compound responsible for the inhibition of PPO appeared to be a small peptide with an approximate molecular weight of 600. Alternatively, Chang[79] suggested that a bee protein complexes with fruit tannins, thereby preventing oxidative discoloration.

Proteins, peptides, or amino acids can affect PPO-catalyzed browning by direct inhibition of the enzyme and by reaction with the quinonoid products of PPO catalysis. Kahn[61] studied the effect of proteins, protein hydrolyzates, and amino acids on the activity of mushroom, avocado, and banana PPO using D,L-dopa or 4-methylcatechol as substrate. Casein hydrolyzate and bovine serum albumin did not inhibit mushroom or avocado PPO. Millimolar concentrations of the L-amino acids, lysine, glycine, histidine, and phenylalanine (in increasing order of effectiveness) weakly inhibited mushroom PPO, with 60% maximal inhibition. Pigment formation by mushroom PPO was decreased by triglycine, diglycine, and glycine (in decreasing order of effectiveness). In *in vivo* experiments with banana and avocado homogenates, histidine (230 m*M*) exhibited slight inhibition only of avocado browning, whereas lysine (230 m*M*) was ineffective on both foods. Of all amino acids tested, L-cysteine was most effective (see Section II, B).

E. Anions

Inorganic halides have been reported to be inhibitors of PPO, but other anions, such as sulfate or nitrate, have no effect.[113] This could be due to the larger ionic radii of these latter anions. The inhibition by halides is pH dependent and decreases as the pH is increased, with maximum inhibition in the pH range 3.5 to 5.0.[97] The pH effect on the inhibition by halides was explained by the interaction between the negatively charged inhibitor and a positively charged imidazole group at the active site of PPO. The order of decreasing inhibitory power of the halides has been reported to be $F > Cl > Br > I$. This is exactly the order of decreasing ionic radii and, hence, steric effects may explain the differences. More recent investigations by Martinez et al.[114] have shown that the order of effectiveness for halides as PPO inhibitors was dependent on the source of the enzyme. The authors postulated that the observed effect was the combination of the accessibility of the active site copper to the halide, and the stabilization of the copper-halide complex thus formed. The mode of inhibition of apple PPO by the halides has been investigated by Janovitz-Klapp et al.[32] The inhibition of sodium chloride at pH 4.5 was noncompetitive as determined by Lineweaver-Burk analysis. Other halides tested at the same pH appeared to be competitive inhibitors. Sodium fluoride appeared the most potent, with an apparent K_i of 0.07 m*M*, whereas the values for bromide and iodide were 106 and 117 m*M*, respectively.

Of the halide salts, sodium and calcium chloride at concentrations of 2 to 4% (w/v) are the compounds most commonly used in the food industry for the inhibition of browning.[115] Use of the calcium salt has the added advantage of maintaining the firmness of the pulp tissue by interacting with pectin in the cell walls of the treated food. Recently, zinc chloride has been reported

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to be a more effective inhibitor of browning than calcium chloride.[116]

F. Kojic Acid

Kojic acid (5-hydroxy-2-hydroxymethyl-γ-pyrone) is a metabolite produced by several species of *Aspergillus* and *Penicillium*,[117,118] and is found in many fermented Japanese foods.[119] Kojic acid is an antibacterial and antifungal agent,[120] a reducing agent, and antioxidant,[121,122] which has been reported to also inhibit mushroom PPO activity.[123] A mixture of ascorbic acid and kojic acid has been patented for use as an antibrowning agent in foods.[124]

Applewhite et al.[125,126] have recently found that kojic acid inhibits the development of melanosis on pink shrimp (*Penaeus duorarum*). A 1-min dip into 1% kojic acid slowed blackspot formation to the same degree as the customary 1.25% bisulfite dip. Kojic acid was shown to exhibit mixed-type inhibition of PPO directly in oxygen uptake studies, as well as to bleach preformed melanin. These results indicated that the mode of action of kojic acid in the prevention of shrimp melanosis was twofold: direct inhibition of PPO and chemical reduction of the pigment or pigment precursors to colorless compounds. Subsequent studies with PPOs from several plant and crustacean sources have yielded similar results.[127]

Although the presence of kojic acid in certain foods occurs as a result of natural fermentation processes, its widespread use as a food additive for the inhibition of browning is doubtful because of associated toxicity. Kojic acid has been found to exhibit acute toxic effects in several animal models.[120,128,129] Effects included nephrosis and lethality.[118] Genetic toxicity in rat liver cells and teratogenicity of chicken embryos by kojic acid have also been observed.[119] Studies by Wehner et al.[130] and Wei et al.[131] indicate that kojic acid is mutagenic in a *Salmonella typhimurium* assay.

In addition to the problems with toxicity, the use of kojic acid in the food industry may be restricted due to the difficulty of large-scale production and high cost.[132]

VI. COMPLEXING AGENTS

A. Cyclodextrins

Hicks et al.[133] obtained a patent on the use of cyclodextrins, cyclic oligosaccharides, as inhibitors of enzymatic browning for raw fruit and vegetable juices. The cyclodextrins inhibit browning by formation of inclusion complexes with or entrapment of PPO substrates or products. The patent also claimed novel compositions of cyclodextrins in combination with other known antibrowning agents, such as reducing agents, acidulants, chelating agents, etc. This approach can be employed in solution by the use of soluble α-, β-, or γ-cyclodextrins or with insoluble cyclodextrins packed in a column or as a batch treatment process.

β-Cyclodextrin dissolved in Granny Smith apple juice inhibited enzymatic browning for more than 1 h with browning inhibition proportional to cyclodextrin concentrations between 5.9 and 13.6 m*M* in the juice. α- and γ-Cyclodextrins were less effective than the β-cyclodextrin. Samples were evaluated by tristimulus colorimetry. Browning inhibition by β-cyclodextrin was enhanced by ascorbic acid (1.14 m*M*) or addition of other ascorbyl derivatives. Mixtures containing maltosyl-β-cyclodextrin, dimaltosyl-β-cyclodextrin, and β-cyclodextrin were effective at concentrations of 1 and 4%, with enhancement of inhibition by addition of Sporix™, ascorbic acid, or citric acid. Treatment of green grape, Granny Smith apple, Anjou pear, and celery juices with insoluble cyclodextrin, using a column technique, greatly delayed the onset of browning. For example, the apple juice sample browned significantly within 1 h, whereas browning of the treated sample was prevented for 82 h, at which point the sample was discarded due to microbial growth.

Although cyclodextrins appear somewhat effective in retarding the browning reaction, there are several potential drawbacks to their use. The lack of specificity of inclusion complex formation could result in loss of flavor or color compounds present in low concentrations. The adsorption of flavor or color compounds may be

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minimized by use of derivatized cyclodextrins; however, the advantage of derivatization must justify the increased cost. Even for commercially available cyclodextrins at concentrations from 1 to 4%, cost considerations could become prohibitive. This concern would also apply to the insoluble approach if the cyclodextrin could not be recycled in high yield. From a regulatory standpoint, cyclodextrins are not food approved.

B. Chitosan

Chitosan, a naturally abundant polymer of *N*-acetylglucosamine, inhibits the enzymatic browning of apple and pear juices.[134] Browning inhibition was observed in McIntosh apple juice that had been treated with 200 ppm chitosan and subsequently filtered through diatomaceous earth. A much higher level was necessary to achieve comparable results in Bosc and Bartlett pear juices. Browning of juice obtained from very ripe Bartlett pears was not inhibited by chitosan treatment. The mode of action of chitosan in this application is undefined but may be due to adsorption of the PPO enzyme, substrates, or products, or a combination of these processes. As is the case with cyclodextrins, the use of chitosan as an antibrowning agent would be limited to liquid systems.

VII. ENZYME TREATMENTS

A. Ring-cleaving Oxygenases

An alternative approach to the development of browning, involving the irreversible modification of the phenolic substrates, was proposed by Kelly and Finkle.[135] The authors treated apple juice with the bacterial enzyme protocatechuate-3,4-dioxygenase (PC ase), which catalyzes the oxidative ring-opening reaction and the ortho-fission of catechols. Juice treated with PC ase in the presence of ascorbic acid did not darken when compared with a control sample of untreated juice. This would be a very expensive process for the inhibition of browning. Also, the authors reported that the rate of ring fission of chlorogenic acid, the major substrate responsible for the browning of apples,[136] was very slow.

B. *o*-Methyl Transferase

Finkle and Nelson[137] have proposed the use of *o*-methyl transferase for the prevention of browning of apple juice. The authors treated apple juice with *o*-methyl transferase and *S*-adenosylmethionine and showed that the PPO substrates, caffeic and chlorogenic acids, were converted to ferulic acid (an inhibitor of PPO) and feruloylquinic acids, respectively. Unfortunately, this procedure is too expensive to be of any commercial use.

C. Proteases

Taoukis et al.[138] and Labuza[139] reported that certain fruit extracts containing proteases, particularly ficin from fig, inhibit browning in fruit and shrimp. Preliminary studies showed that shrimp dipped for 5 min into a 0.5% (w/v) ficin solution, then stored at 4°C for 4 d on ice and examined for blackspot (melanosis) formation were comparable to sulfite (1.25% w/v) treated shrimp. The authors suggested that the mode of action of the ficin is to inactivate the PPO enzyme via proteolysis. Since the ability of ficin to inhibit browning is unaffected by its heat denaturation or ultrafiltration, other nonenzymatic factors are probably involved in the fig extract inhibition of browning (see Section V). Additionally, as noted by Labuza,[139] the cost of the proteases would narrow their use.

VIII. COMBINATIONS OF ANTIBROWNING AGENTS

The mechanism of inhibition is quite different for each of the categories of enzymatic browning inhibitors discussed above, such as chemical reduction, chelation, enzyme inhibition, etc. These mechanistic differences may al-

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TABLE 4
Commercial Antibrowning Ingredients

	Citric acid	Ascorbic acid	Calcium chloride	Sodium chloride	Phosphate	Dextrose
Pfizer CE-101P	X	X[a]				
Sexton Antioxidant	X	X	X			
Freshway	X	X	X		X	
Flavor Brite	X	X	X	X	X	X
Potato Fresh[b]	X	X			X	
Color Fresh	X	X				X
Salad Fresh[c]	X	X[d]			X	X
Crisp and Fresh	X[e]			X		

[a] Added as iso-ascorbic (erythorbic) acid.
[b] Also contains cysteine hydrochloride.
[c] Also contains aluminum sulfate.
[d] Added as sodium ascorbate.
[e] Added as sodium citrate.

From Kim, H.-J. and Taub, I. A., *Natick Army Res. Tech. Rep. No. TR-88/052,* 162, 1988.

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low the use of combinations of antibrowning agents that result in enhancements of activity relative to the use of any single agent individually. Due to the numerous factors that affect the efficacy of an antibrowning agent or combination thereof (i.e., penetration into the tissue, pH, competing processes, side reactions, etc.), the performance of the combined agents must be evaluated empirically for each food item treated. A typical combination may include a chemical reductant (ascorbic acid), an acidulant (citric acid), and a chelating agent (EDTA). In many cases, the enhanced activity of the combined ingredients is additive, although synergism has been claimed for several blends of antibrowning agents. The literature on combined antibrowning agents is too numerous to list here. Following are a representative sample of recent results regarding this category of inhibitors of enzymatic browning.

Most combinations of antibrowning agents cited in the literature or commercially available are ascorbic acid-based compositions. Ponting et al.[140,141] describe the use of a solution containing from 0.5 to 1% ascorbic acid and from 0.05 to 0.1% calcium chloride and bicarbonate to maintain a pH between 7 and 9 for the preservation of apple slices. This blend of agents was found to be synergistic and was claimed to be effective on Newtown Pippin apple slices stored for as long as 2 months. A combination of ascorbic acid and a thixotropic gum (i.e., xanthan, guar, tragacanth, etc.) was reported to be effective in reducing deterioration and browning of fruits and vegetables used in salad bars and prepared salads sold in fast food restaurants.[142] As mentioned above, mixtures of ascorbic acid and cyclodextrins were reported effective in the inhibition of Granny Smith apple juice browning.[133] Ascorbic and citric acid blends appeared to inhibit blackspot development in shrimp, but the use of these agents was precluded by the development of a yellow off-color.[41] A solution containing ascorbic acid (0.25 to 1%), calcium chloride (0.5 to 1%), citric acid (0.25 to 1%), and sodium acid pyrophosphate (0.5 to 2%) was claimed to inhibit browning of potatoes, pears, green peppers, apples, and lettuce.[143] A combination of ascorbic acid, citric acid, and potassium sorbate in conjunction with vacuum packaging appeared to slow browning of potato slices, but within 30 min after opening the package, onset of browning was observed.[144] Dipping of whole, peeled potatoes in a solution containing erythorbic acid, sodium chloride, and sodium pyrophosphate, followed

by packing in ascorbic acid, sorbic acid, and calcium chloride, resulted in potatoes that were less brown and had lower microbial counts than potatoes treated by the customary bisulfite dip protocol.[145]

Other blends that have been reported to be antibrowning agents but do not contain ascorbic or erythorbic acid include solutions of citric acid, sodium chloride, and calcium chloride,[115] cysteine and citric acid,[146] and Sporix™ and citric acid.[71] A partial listing of commercially available antibrowning blends and their constituent ingredients was compiled by Kim and Taub (Table 4).[147]

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