

AN ABSTRACT OF THE THESIS OF

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presented on September 25, 1998. Title: Extending Shelf-Life of Fresh-Cut Pears

Abstract approved: _____

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Different concentrations of various browning inhibitors and a firming agent were evaluated to prevent browning and softening of Bartlett, Bosc and Anjou pear slices during cold storage at 2-4°C. Browning inhibition effect of Semperfresh, Snow Fresh and cysteine were compared with that of 4-hexylresorcinol (4-HR). The effect of ripeness on color and texture changes as well as the influence of storage temperature on texture and color retention was investigated. 4-HR residual content under different treatment conditions and its sensory properties were analyzed. 4-HR was an effective browning inhibitor at concentration as low as 0.005% when combined with 0.5% ascorbic acid (AA). Cysteine (0.5%) with 0.5% ascorbic acid (AA) effectively inhibited Anjou and Bosc pear slice browning for 25 days and its sensory properties merit further study. A combination of 1.0% AA and 1.0% calcium lactate prevented Anjou pear slices from discoloration, but also caused tissue leaking and softening. Semperfresh and Snow Fresh did not maintain flesh color effectively. 1.0% calcium lactate maintained slice firmness at an acceptable level for 30 days when the average firmness of whole pears at slicing was 49, 38 and 43 Newton (N) for Bartlett, Bosc and Anjou respectively. A storage temperature of 0°C significantly retained better texture than the control of 2-4°C. Slices

with acceptable texture and color were achieved by treatment with a solution of 0.01% 4-HR, 0.5% AA, and 1.0% calcium lactate for 2 min, vacuum packaging, and storage at 2-4°C for 30 days for Bartlett and Bosc, and 15 days for Anjou.

4-HR residual content increased with increasing dipping times and concentrations, with the latter being the major factor. The 4-HR residual decreased with storage time for all the treatments. Sensory tests indicated that panelists detected the overall differences between 0.01% 4-HR treated samples and the controls for both Bartlett and Anjou pears ($P < 0.01$). There were no significant differences in preference between treated sample and control for Anjou pears.

Extending Shelf-Life of Fresh-Cut Pears

By

Xiaoling Dong

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirement for the
Degree of

Master of Science

Completed September 25, 1998
Commencement June 1999

Master of Science thesis of Xiaoling Dong presented on September 25, 1998.

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Xiaoling Dong, Author



ACKNOWLEDGMENT

I would like to thank my major professors and advisors Ronald E. Wrolstald and David Sugar, who give me the opportunity to work on this thesis, for their support, advice, and encouragement. I also thank David Sugar for providing experimental materials.

I want to thank Bob Durst for his technical support. My thanks also go to Marcia Walker for her allowing me to use the equipment critical to the experiment. I would like to thank Cindy Lederer for her contribution to the sensory evaluation.

I want to thank the graduate students studying in Food Science and Technology Department, and my lab-mates for their help and advice. Among them, I would like to address my special thanks to Monica M. Giusti and Luis E. Rodriguez-Saona, not only for their interest, advice, encouragement and support to my research and writing, but also for their valuable friendship and enthusiasm.

I would like to acknowledge and thank the Winter Pear Control Committee and Agriculture Research Foundation that support this research.

I thank in a very special way to my husband and son, for their love, understanding and support. I thank my parents to encourage me to fulfill my study.

CONTRIBUTION OF AUTHORS

Ronald E. Wrolstad was involved in the experimental design, data interpretation and revision of each chapter. David Sugar was involved in the experimental design, revision of chapter 2 and providing experimental materials.

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EXTENDING SHELF-LIFE OF FRESH-CUT PEARS

Chapter 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Fresh-cut or minimally processed fruits and vegetables is a rapid growing segment of the U.S. fresh produce industry (Gorny et al., 1997). They have the attributes of convenience and fresh-like quality. The preparation of minimally processed fruits and vegetables includes peeling, trimming, coring and slicing. Their forms vary widely, depending upon the nature of the unprocessed commodity and how it is normally consumed. In many cases, the minimally processed products are raw and cells of the tissue are alive (Huxsoll and Bolin, 1989). Two basic problems confront the extension of shelf-life of minimally processed fruits and vegetables, which are due to processing and wounding: accelerated texture deterioration and cut surface discoloration.

Tissue wounding stimulates ethylene production in fruits and vegetable tissue (Abeles, 1973) and induces a higher respiration rate, in most of the cases, 3-5 times that of the intact organ, and accelerated senescence (Rosen and Kader, 1989), which are principle causes of rapid texture deterioration. Tissue wounding also induces undesirable (in most cases) enzymatic browning which is caused by oxidation of phenolic compounds by endogenous polyphenol oxidase (PPO) and diffusion of atmosphere O₂ into the tissue (Lee, 1993).

Combinations of controlled atmosphere packaging and /or texture firming agents such as calcium salts have been reported to successfully retard texture softening (Gorny et al., 1997; Rosen and Kader, 1989; Poovaiah, 1986; Ponting et al., 1972). Sulfites have been applied to inhibit both enzymatic and nonenzymatic browning in foods. However, sulfites have been associated with severe allergy-like reactions in some asthmatic, prompting the Food and Drug Administration (FDA) to limit their use to certain categories of food products (Sapers, 1993). A long-standing goal of food technology has been to find effective substitutes to sulfites to prevent enzymatic browning and extend the shelf-life of fresh products. Numerous potential browning inhibitors have been tested on fresh-cut fruits and vegetables. Ascorbic acid and its isomer erythorbic acid (Ponting et al., 1972; Sapers et al., 1987, 1989 & 1990; Santerre et al., 1988; Gil et al., 1998), L-cysteine (Molnar-perl and Friedman, 1990; Dorantes-Alvarez et al., 1996), 4-hexylresorcinol (Luo and Barbosa-Canovas, 1995; Monsalve-Gonzalez et al., 1993, 1995; Ibarz et al., 1996; Sapers and Miller, 1997), and pineapple juice (Lozano-de-Gonzalez et al., 1993) have been reported to be effective browning inhibitors for various fresh-cut fruits.

Pears are one of the major fruit crops in the USA. Its total production in 1997 was 1,044,000 ton with almost half of the production consumed in processed form, mostly as canned (Agricultural Statistics, 1998). Consumers are putting increased emphasis on consumption of convenience and fresh-like quality of fruits and vegetables (King and Bolin, 1989). Pear growers and marketers have shown high interest in developing fresh-cut pear products to stimulate the fresh utilization of pears. However, fresh-cut pears offer a unique challenge due to dramatic texture softening in addition to

high potential for browning during storage. Efforts have been made to inhibit or reduce browning of fresh-cut pears during storage by controlled atmosphere in combination with or without ascorbic acid and/or calcium dipping (Rosen and Kader, 1989; Gorny et al, 1996). Sapers and Miller (1997) reported that a shelf life of 12 to 14 days for fresh-cut pears was obtained with a combination of sodium erythorbate/calcium/4-HR and modified atmosphere package.

Our objective was to develop effective methods to extend the shelf life of fresh sliced pears with retention of visually appealing color and acceptable texture.

Literature Review

Enzymatic browning is the result of polyphenol oxidase (PPO) catalyzed oxidation of mono- and diphenols to o-quinones with o-quinones undergoing further oxidative polymerization to yield brown-black melanin pigments (Lee and Whitaker, 1995).

CHEMISTRY OF ENZYMATIC BROWNING

Polyphenol oxidase (PPO) in plants

Polyphenol oxidase is a generic term for the group of enzymes that catalyze the oxidation of phenolic compounds to produce brown color on cut surfaces of fruits and vegetables. Based on the substrate specificity, enzyme nomenclature has designated monophenol monooxygenase, cresolase or tyrosinase as EC1.10.3.1; diphenol oxidase, catechol oxidase or diphenol oxygen oxidoreductase as EC1.10.3.2; Laccase or p-diphenol oxygen oxidoreductase as EC1.10.3.1 (Walker, 1995).

The first two enzymes, tyrosinase and catechol oxidase, occur in practically all plants. Phenolase from banana, tea leaves, tobacco leaves and clingstone peaches has been reported to oxidize o-diphenols only, while the enzymes from potatoes, apples, sugar beet leaves, broad bean leaves and mushroom have both types of activity. Laccase is less frequently encountered as a cause of browning in fruits and vegetables than catechol oxidase, although laccase-like enzymes have recently been reported in peach and apricot fruit (Walker, 1995).

Mode of action of PPO

Catecholases

Catecholases contain copper as the prosthetic group and for the enzymes to act on its substrate phenols, the Cu^{2+} must first be reduced to Cu^+ in which state the enzyme can then bind O_2 . The phenolic substrates bind only to the oxy-DPO moiety and, as a result of this binding, hydroxylation of monophenol or oxidation of the diphenol occurs (Walker, 1995). Most catechol oxidase preparations from potatoes, apples and beans possess both activities, while those from tea leaves, tobacco, mango, banana, pears and sweet cherries have been reported not to act on monohydroxyphenols (Lee, 1993).

Laccase

Laccase oxidizes p-diphenols. In addition, it oxidizes various m-diphenols and p-phenylenediamine, but does not oxidize tyrosine (Lee, 1993).

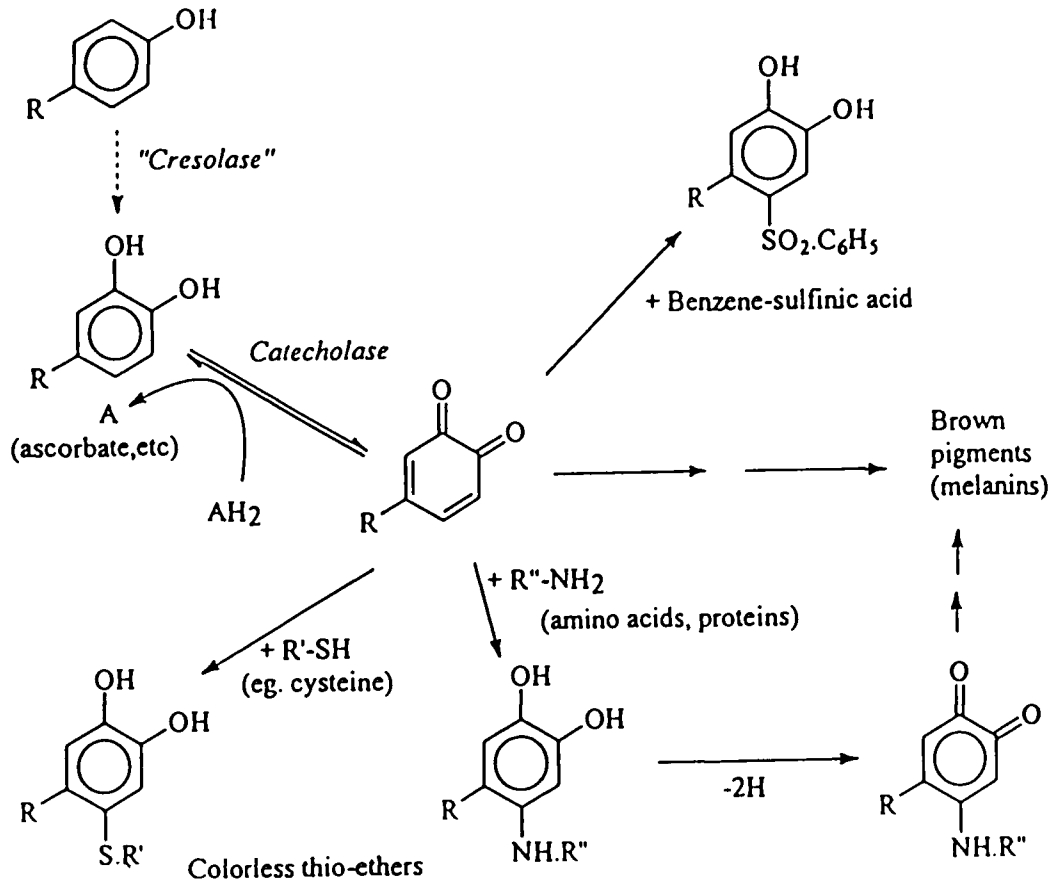


Fig. 1.1 —Reactions catalyzed by cresolase and catecholase (o-DPO). Source: Walker, 1995.

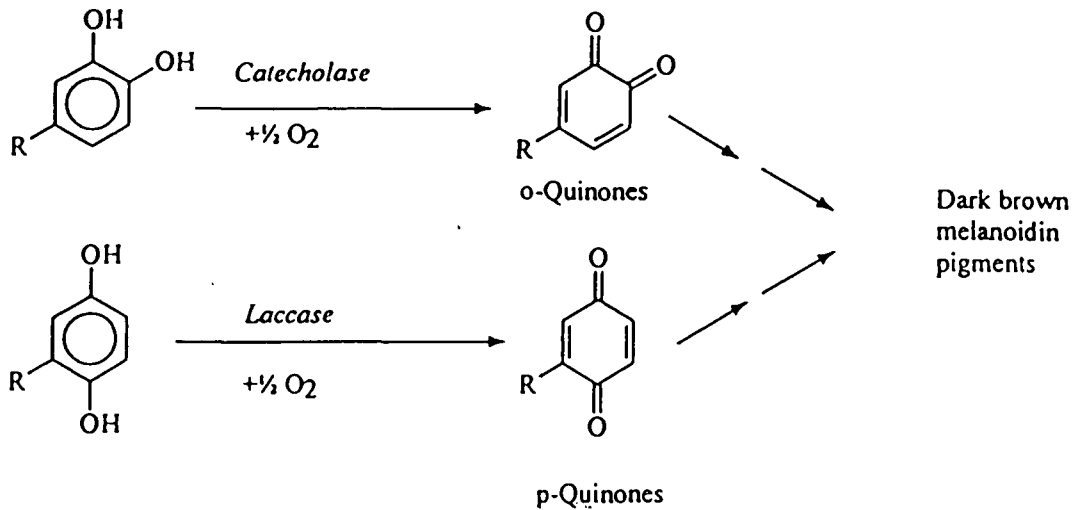


Fig. 1.2 —Comparison of reactions catalyzed by catecholase (o-DPO) and laccase (p-DPO). Source: Walker, 1995.

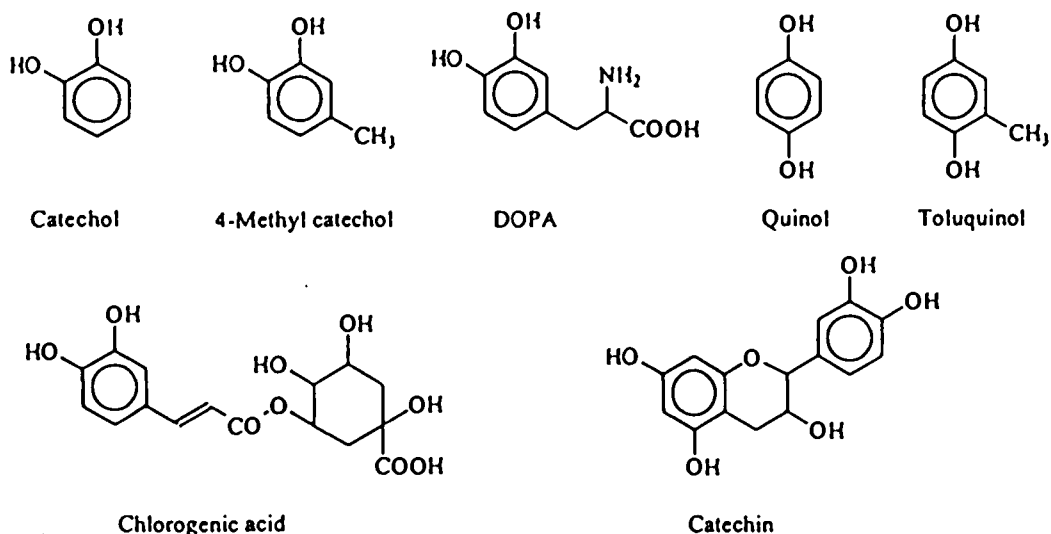


Fig. 1. 3 —Common substrates for catecholases and laccases. Source: Walker, 1995.

Substrate specificity of o-diphenol oxidases

All o-PPO's require the basic o-dihydroxyphenol structure for oxidase activity so that catechol is the simplest, but not necessary the best substrate; 4-methylcatechol is usually the fastest. The structure of some natural and artificial diphenol oxidase substrates are shown in Fig. 1.3. It was found that the nature of substituent groups has profound effects on oxidation rate and interaction between substrate and the active-site for o-DPO's (Walker, 1995).

With respect to enzymatic browning, catecholase activity is the most important and the commonest natural substrate for this reaction is chlorogenic acid, which is widely distributed in plants (Walker, 1995).

POLYPHENOL OXIDASE (PPO)

Enzymatic browning, which occurs rapidly on the cut surface of fruits, determines the suitability of fruits for processing with respect to the color of the derived products.

The degree to which fruits turn brown depends on O₂ and endogenous polyphenol oxidase contents, activity and phenolics content (Amiot et al., 1995).

Localization of polyphenol oxidase

o-DPO is located in a variety of plant cells and organelles (chloroplasts, mitochondria, peroxisomes) where it may be tightly bound to membrane, and/or in the soluble fraction of the cell. However, many experimenters, particularly immunocytochemists, agree that active o-DPO is located exclusively in plastids in healthy cells. In fruits, subcellular localization of o-DPO has mainly been reported in chloroplasts, but it has also been found in mitochondria or in microbodies for some fruit species (Macheix et al., 1990)

In most fruits, the soluble form of the enzymes was found to be dominant and the activity of the soluble fraction amounted to 10-30% of the total activity depending on the fruit. In some fruits, such as in olive, banana, peach, grape, and plum, the simultaneous presence of “soluble” and “bound” forms has been reported (Macheix et al., 1990).

One of the main causes of the diversity of o-DPO activity lies in their localization. In plum, o-DPO activity was two or three times as high as in interior pulps than in exterior pulps, whatever the physiological stage of the fruit. The opposite applies in apple and pear where o-DPO activity is substantially higher in the peel than that in the flesh (Macheix et al., 1990). However, Murata et al. (1995) indicated that active PPO was mainly localized around the core in mature apples and this localization of PPO corresponding to intense browning around the core.

o-DPO activity change during fruit development

o-DPO activity is generally more important in young green fruits than in ripe fruits. This has been observed in apple, olive, grape, plum, banana, mango, tomato, coffee and berries. The changes are not the same for the different forms of o-DPO and there is a general transformation from 'bound' to more 'soluble' form during maturation (Macheix et al., 1990). Murata et al (1995) reported that both polyphenol content and PPO activity in immature apples were much higher than in mature apples, and the active PPO was mainly localized near the core, while, it was uniformly distributed in immature apple. PPO activity in peaches followed a similar pattern (Lee, 1990).

o-DPO activity with different varieties

PPO activity in peaches varied greatly among different cultivars. It was observed that the degree of actual browning of individual peach cultivar was correlated to its PPO activity: those varieties having higher PPO activity showed a higher rate of browning. Conversely, peaches low in PPO activity showed a reduced rate of browning (Lee, 1990). It was found that PPO activities were different in pear varieties of Bosc and Red pears. Bosc pear had only 81.6% of the specific activity exhibited by Red pears (Siqqid et al., 1994). In apple, PPO activity is also variety dependent. Red Delicious always had the highest PPO activity compared with other cultivars (Nicolas, 1994).

PPO relative activity with different natural substrates

Catechin, epicatechin and caffeic acid derivatives appear to be the best substrates for o-DPO. Chlorogenic acid and its isomers, in numerous fruits, and other caffeic esters are easily degraded by o-DPO. It seems that esterification of the carboxyl group of

caffeic acid with quinic or tartaric acids leads to an increase in o-DPO activity in all cases (Macheix et al., 1990).

Substrates with smaller substituent groups on the ring of diphenols were oxidized by banana and cherry o-DPOs at a faster rate than those with larger substituent groups. This shows that the nature of the side chain is critical and may, therefore, play a part in the enzyme-substrate interaction (Macheix et al., 1990). Other phenolics, such as Procyanindins and anthocynins which are present in substantial quantities in some fruits do not appear to be direct substrates of o-DPO, but their degradation is strongly increased through coupled oxidation phenomena. Flavonols and their derivatives are very poor o-DPO substrates (Macheix et al., 1990). Other phenolic compounds found in fruits which are very close to o-DPO substrates do not contribute to browning and even act as

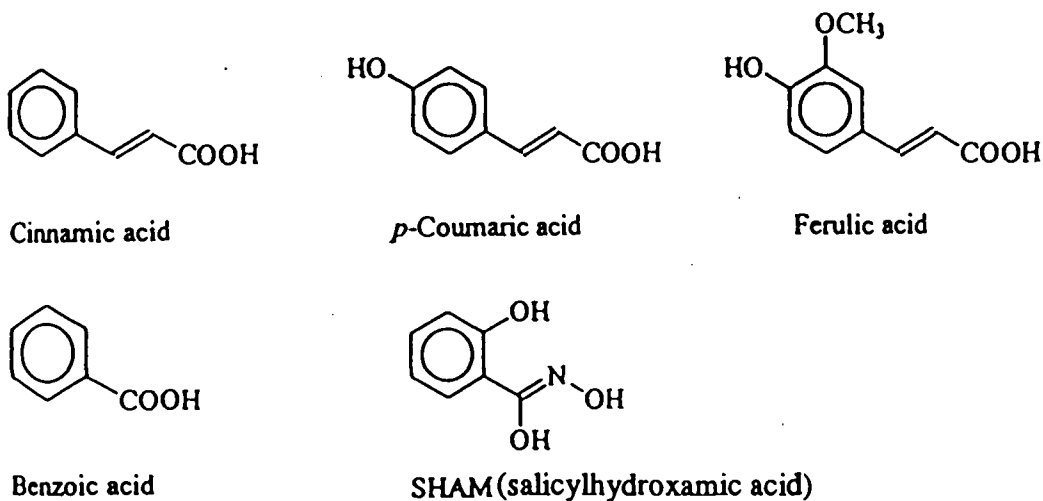


Fig. 1.4 —Inhibitors of catecholase and laccase activity. Source: Walker, 1995.

inhibitors. These include in particular substituted benzoic and cinnamic acids, such as p-coumaric, ferulic and sinapic acids (Fig. 1.4) (Walker, 1995; Macheix et al., 1990; Sapers et al., 1989; Vamos-Vigyazo, 1995; Gunata et al., 1987; McEvily and Iyengar, 1992).

Effect of pH and temperature on PPO activity

The optimum pH was found to be 5.0 for Bosc pear and 5.5 for Red pear PPO (Siddiq et al., 1994). The activity of the enzymes from both cultivars declined sharply above 5.5 with no activity at pH 7.0. Activity of Red pear PPO decreased below pH 5.0. However, Bosc pear PPO retained about 62% of its activity even at pH as low as 3.2. Smith and Montgomery (1985) had similar reports for d'Anjou pear PPO, who found that the enzyme was unstable at pH above 6.5. Those findings were in good agreement with Sapers and Millers (1997) results for fresh cut pear browning inhibition that neutral formulations containing sodium ascorbate or erythorbate were more effective than corresponding acidic formulations.

In apple, most studies indicated that the bulk of apple PPO had a pH optimum between 4.5 and 5.5. Moreover, the enzyme seemed relatively tolerant to acid pH, because the activity at pH 3.0 still represented 40% of the maximum activity. Thus, control of enzymatic browning by acidification only is difficult unless a very low pH is obtained (Nicolas, 1994).

Optimum temperature was found to be 20°C for Bosc and 23°C for red pears PPO. Red pear PPO was active (80% activity) over a wide temperature range (25-45°C), whereas Bosc pear PPO was more active around 20-30°C only. Heating at 75°C for 30 min completely inactivated the enzyme from both cultivars. Heating at 55°C and 65°C for the same duration resulted in partial inactivation (45-60%) of this enzyme (Siddiq et al., 1994).

PHENOLIC COMPOUNDS

Compartmentation of phenolics

Phenolic compounds accumulate at two major sites in plant cells: the cell wall, where lignin is deposited along with simpler molecules, such as flavonoids and esterified ferulic acid; and the vacuole where various soluble phenolic compounds and derivatives accumulate. In fresh fruits, lignin is found only in stones and in certain cells of pear pulp or is formed as a reaction to wounding. However, as in all parts of plants, the vacuoles in fruit cells form the main compartment in which phenolic compounds accumulate (Machiex et al., 1990).

In most cases, levels of soluble phenolic compounds are higher in the external tissues of fleshy fruits (epidermal and subepidermal layers) than internal tissues (mesocarp, pulp). Apple peel contains 10 times as much total phenols as flesh and pear peel contains 27 times as much total phenols as flesh (Macheix, 1990).

Phenolic content changes during fruit development

In general, the propensity for browning is particularly high in young fruits and then falls rapidly to a low level during maturation (Machiex et al., 1990). Total phenolics quickly decreased up to 3.5 months after flowering for apples (Murata, 1995). After that, phenolic levels were almost constant or decreased slightly. For pear fruits picked at dates close to the commercial maturity stage, the phenolics content was not greatly different (Amiot, 1995). However, the levels in phenolics tended to decrease with delayed harvested time.

Phenolics variation at the cultivar level

Great differences in the phenolic composition of pears has been reported (Amiot et al. 1995). More quantitative differences were due to cultivar than to the stage of maturity at which fruits were harvested. Siddiq et al (1994) found that there was a large variation in phenolic content in Bosc and Red Pears, with Red pears having much higher phenolic content than Bosc. Analysis of phenolic content and composition by Amiot et al (1993) indicated there was huge variation among varieties for both apples and pears. In apples, the extent of browning is closely correlated to the amount of hydroxycinnamic derivatives (HCD) and flavanols(FLA) degraded. In pears, L* value was correlated to the amount of two flavanols. A better control of browning would probably come from selection of cultivars with a low content of chlorogenic acid. Secondly, flavanol content and the relatively balanced HCD and FLA are of great importance to the susceptibility to browning, during conservation (Machiex et al. 1990). A large difference in concentration of certain phenolics was also observed among different peach cultivars (Lee et al., 1990).

Influence of postharvest storage on phenolics content

The total phenolic content of nine pear cultivars increased under air storage condition (Amiot, 1995). During the first month of air storage, total phenolics slightly increased, and a second month of storage resulted in a further large increase in total phenolic content; most affected were flavanols, followed by flavonols and cinnamics). In the case of d'Anjou pears, chlorogenic acid and total phenols increased in both skin and flesh during storage at 1°C (Meadows, 1983). Increase in the chlorogenic acid and total phenol concentrations in 'd'Anjou pear leads to much greater sensitivity to friction discoloration although other factors are involved. In contrast, in certain other pear

cultivars (e.g. Passe Crassane), the appearance of internal browning can not be accounted for by small variations in chlorogenic acid and o-diphenol contents but is related to an increase in o-DPO activity (Macheix, 1990).

Controlled atmosphere storage strongly reduced the ability of pears to synthesize phenolics (Amiot et al., 1995). The storage of William pears under low concentrations of O₂ and CO₂ seemed to be successful at reducing the susceptibility of the pears to browning. It has been reported (Yoshida et al., 1986, Chen et al., 1986) that the risk of brown core decreased in pears stored under low-oxygen conditions.

In apple stored at 0°C, browning potential varied little in most cultivars or increased over a period of about 2 months and then decreased. The influence of controlled atmosphere on flesh browning of apple varies considerably according to cultivars: it is either increased in the presence of CO₂ or no effect is observed (Macheix et al., 1990).

INHIBITION OF ENZYMATIC BROWNING

Inhibition of o-DPO may be grouped according to their mode of action, although some compounds may belong to more than one groups: inhibition may be achieved by chelation of the prosthetic group, competition for the substrates, or by interaction with the products of the reaction. Both o and p-DPO's utilize Cu²⁺ as a tightly bound prosthetic group so that the first category includes many Cu-chelating agents, which will inhibit these enzymes. Carbon-monoxide, cyanide, Na-diethyldithiocarbamate (DIECA), azide, tropolone, methimazole, mercaptobenzothiazole and PVP are all potent inhibitors of o-DPO's and are frequently used for this purpose by biochemists when endeavoring to isolate other plant enzymes and organelles (Walker, 1995).

Probably the most common method of controlling enzymatic browning both in industry and laboratories is by the addition of reducing agents such as SO₂, metabisulfite and /or ascorbic acid. These compounds prevent browning by reducing the enzymatically formed quinones back to their parent o-diphenols; therefore, they are consumed in the process (Walker, 1995).

Sulfites

Sulfites can act as a reducing agent, and they also can react with quinone intermediates to form sulfoquinones and may irreversibly inhibit o-PPO's (Walker, 1995). Both enzymatic and nonenzymatic browning in foods could be inhibited by application of sulfites (Taylor et al., 1986; Andres, 1985). However, sulfites have been associated with severe allergy-like reactions in some asthmatics, prompting the Food and Drug Administration (FDA) to limit them to certain categories of food (Sapers, 1993). Because of the restriction, food processors and scientists have turned to a number of sulfite alternatives, mostly formulations effective only against enzymatic browning, with varying success. The demand for more effective browning inhibitors has stimulated considerable research activity in this area, resulting in the development of a number of promising browning inhibitor treatments.

Ascorbic acid, erythorbic acid and their derivatives

Ascorbic and its isomer, erythorbic acid (Fig. 1.5), have frequently been used as antioxidants in the food industry. The main role of ascorbic and erythorbic acids in the prevention of enzymatic browning is their ability to reduce the quinones to diphenols (Fig. 1.1). Hsu et al. (1988) reported that AA inhibited mushroom polyphenol oxidase by

reducing Cu^{2+} of PPO to Cu^+ . Usually, ascorbic acid was used in combination with other reagents such as calcium chloride (Ponting et al., 1972), or citric acid (London, 1987; Santerre, et al., 1988; Sapers et al., 1987 and 1989).

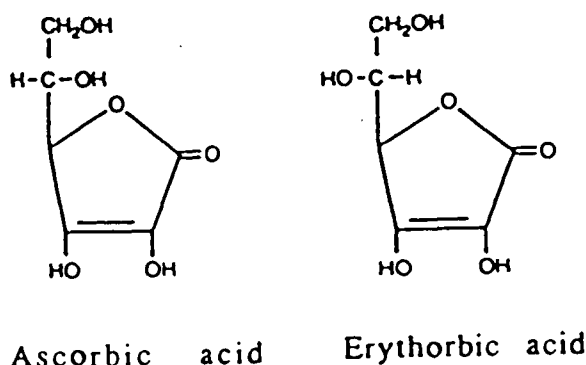


Fig. 1.5 —Structure of ascorbic acid and erythorbic acid. Source: McEvily and Iyengar, 1992.

The effectiveness of ascorbic acid (AA) and erythorbic acid (EA) in inhibiting enzymatic browning at cut surface of apple and in raw apple juice was compared (Sapers and Ziolkowski, 1987). It was found that AA was consistently more effective than EA in prevention of apple cut surface discoloration. With apple juice, AA and EA were similar in effectiveness. Therefore, they pointed out that because the relative effectiveness of AA and EA depends on the system in which they are compared, they should not be used interchangeably as sulfite alternatives without experimental verification of equivalency.

The response of “Fuji” apple slices to ascorbic acid treatments and low oxygen atmosphere has been tested (Gil et al., 1998). Ascorbic acid-treated fruit slices were significantly lighter than non-ascorbic acid-treated fruit slices, irrespective of the atmosphere tested. To inhibit fresh-cut pear browning, Sapers and Miller (1997) applied 4% sodium ascorbate or erythorbate dipping and found that neutral formulations were

more effective than corresponding acidic formulations. The combination of ascorbic acid with calcium chloride was reported to be effective for browning inhibition of apple slices (Ponting et al., 1972) and fresh-cut pears (Gorny et al., 1997).

Due to limited penetration strength of ascorbic and erythorbic acid into fruit and vegetable matrix, Sapers et al. (1990) applied vacuum and pressure infiltration techniques. Apple plugs infiltrated at 34 KPa pressure showed more uniform uptake of treatment solution and less extensive water-logging than plugs vacuum-infiltrated at 169-980 mB. Delicious and Winesap plugs and dices gained 3-7 days of storage life at 4°C when treated by pressure infiltration, compared to dipping. However, infiltration required dewatering by centrifugation or partial dehydration to prevent water-logging. Pressure infiltration at 108 KPa extended the life of potato plugs by 2-4 days, compared to dipping, but was ineffective with diced potatoes.

Because ascorbic acid functions as a browning inhibitor chiefly by its reducing ability and is consumed in the process, it provides limited protection against discoloration, depending on the specific system and the concentrations used (Vamos-Vigyazo, 1981; Sapers, 1993). Therefore, some contradictory results for ascorbic acid have been obtained. Rosen and Kader (1989) reported that citric acid and /or ascorbic acid dips were not effective in controlling pear slices browning. Landon (1987) also found that mixtures of citric and ascorbic acids plus potassium appeared to be of limited value for browning inhibition of apples and potatoes. They were acceptable when measured immediately after slicing. However, during storage of the food the treatments seemed to lose their effectiveness (Molnar-Perl and Friedman, 1990).

As an anti-browning reagent, ascorbic acid and erythorbic acid are easily oxidized by endogenous enzymes, as well as decomposed by iron or copper-catalyzed autoxidation to form dehydroascorbic acid. These reducing agents also can react with other compounds in the food system, resulting in deteriorate effects (McEvily and Iyengar, 1992).

Ascorbyl esters

The rapid oxidation of ascorbic acid to dehydroascorbic acid has led to the development of ascorbic acid derivatives with increasing stability. Ascorbic acid-2-phosphate (AAP) and ascorbic acid-2-triphosphate (AATP) have been investigated as a stable alternative source of ascorbic acid for the inhibition of browning at the cut surfaces of raw apples, potatoes and apple juice (Sapers et al., 1989). Both AAP and AATP showed considerable activity as browning inhibitors when applied as dips at concentrations of 45.4 mM (0.8% AA) to the cut surfaces of Red Delicious plugs. It was found that the AA derivatives were more effective than equivalent concentrations of AA as browning inhibitors. Similar results were obtained with Winesap plugs. Browning inhibition was not improved significantly by the addition of 22.7 mM AA (0.04%) in combination with AAP or AATP. Addition of 1% citric acid decreased the effectiveness of AATP as a browning inhibitor. AAP and AATP were also proved to be less effective than AA at concentrations as high as 1.14 mM on apple juice browning inhibition. It was hypothesized that the failure of the AA-2-phosphates in apple juice resulted from insufficient endogenous acid phosphatase activity due to enzyme inactivation during juice preparation and / or to the low pH (3.3), which is substantially less than the optimal pH for acid phosphatase from plant tissue. The poor performance of AATP in combination

with citric acid probably resulted from acid inhibition of phosphatase activity as in juice. The success of AA-2-phosphates in inhibiting browning of apple plugs was due primarily to their stability. Presumably, sufficient acid phosphatase was present at the cut surface of apple fruit to permit hydrolysis of the AA-2-phosphates at a rate sufficient to prevent browning but not great enough to generate a large excess of AA that would be subject to oxidation. Under favorable conditions, AATP is hydrolyzed more slowly than AAP. The suitability of the AA-2-phosphates as browning inhibitors for commodities other than apple will depend on their acidity and endogenous acid phosphatase activity.

Ascorbyl-6-fatty acid-esters (ascorbyl palmitate, Laurate and deconoate) are other alternative stable sources of ascorbic acid. When those esters were added to Granny Smith juice at concentrations as high as 1.14 mM (equivalent to 200ppm AA), demonstrated that these esters were less effective than, or similar to AA initially, but surpassed AA as browning inhibitors after longer periods of storage (Sapers et al., 1989). The addition of AA with ascorbyl palmitate (AP) or ascorbyl Laurate (AL) had little or no effect on browning inhibition. However, the combination of AA with ascorbyl decanoate (AD) was significantly more effective than AD alone, the former providing protection against browning for at least 24 hr.

AP dispersions in pH 7 phosphate buffer, stabilized with lipophilic emulsifying agents such as Durkee's EC-25 or Dulac 100, usually were more effective in controlling browning than equivalent concentrations of AA (Sapers et al. 1989). However, the degree of inhibition was not consistent, probably because of AP precipitation on the cut surface during storage. 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, tend to induce the browning of apple plugs. The adverse effect of the addition of the emulsifiers may be due to the disruption of membrane in cells near the cut surface of the fruit, causing leakage of PPO and its substrates and thereby increasing the extent of browning

Sulfur amino acids

The primary mode of reaction of sulfur amino acids in the prevention of browning is to react with o-quinones formed by enzymatic catalysis to produce stable, colorless adducts (Fig. 1.1). The cysteine addition compounds with 4-methylcatechol, chlorogenic acid, (-) epicatechin and (+) catechin were demonstrated by HPLC and the structure of each cysteine conjugate was determined by NMR Spectroscopy (Richard F.C. et al., 1991). However, some authors indicated that cysteine also reduces the o-quinones to their phenol precursors (Cilliers and Singleton, 1990). The direct inhibition of PPO by cysteine through the formation of stable complexes with copper has also been proposed by Kahn (1985).

L-cysteine was reported to inhibit enzymatic browning catalyzed by mushroom, apple and pear polyphenol oxidase activity (Dudley and Hutchkiss, 1989; Richard et al., 1992; Siddiq et al., 1994). The kinetic mechanism was investigated and it was concluded that L-cysteine had no direct effect on apple PPO. Rather, it was proposed that during enzymatic oxidation, it traps the o-quinone by forming cysteinyl adducts. These cysteine-quinone addition compounds are not substrates, but they show competitive inhibition properties with an affinity for the enzyme higher than that of their precursors. With a sufficient amount of cysteine (i.e., for cysteine : phenol ratios above 1), the phenol is fully degraded in cysteine-quinone addition compounds without color formation. For

cysteine to phenol ratios below 1, o-quinone are formed in excess and are able to react with cysteine-quinone addition compounds with regeneration of phenols and formation of highly colored pigments. Therefore, provided this critical level in cysteine concentration is exceeded, this thiol could in theory allow a permanent protection against enzymatic browning. However, this level may often be non-consistent with good sensory properties of the final product. Therefore, formulations including other innocuous additives have to be optimized to succeed in the control of enzymatic browning (Richard et al., 1992).

Dorantes-Alvarez et al. (1996) reported that cysteine and tetrasodic pyrophosphate preserved better original color of fresh-cut avocado. The effect of tetrasodic pyrophosphate/cysteine mixture, water activity and the pH of the solution were evaluated through surface response analysis. It was found that the best combination for the solution was 0.2% cysteine, $A_w = 0.8$ and pH 5.5 from the evaluation of the quality parameters of color and overall flavor. Kahn (1985) found that 0.32 mM L-cysteine to be very effective for the inhibition of avocado and banana homogenate browning. Walker (1995) reported the use of cysteine in the prevention of browning of apple products for over 24 hours without the introduction of undesirable off-flavor. However, McEvily and Iyengar (1992) indicated that the concentrations of cysteine necessary to achieve acceptable levels of browning inhibition have negative effects on the taste of the treated foods.

Molnar-Perl and Friedman (1990) investigated browning inhibition effect by sulfur amino acids on fresh-cut apples and potatoes. N-acetyl-L-cysteine and reduced glutathione were excellent inhibitors of browning of apples and potatoes. These two SH-containing compounds were more efficient inhibitors than L-cysteine and approached the

effectiveness of sodium sulfite. The optimum pH for the sulfur amino acids was in the range of 7.2-7.55. The preliminary taste experiments suggested that N-acetyl-L-cysteine and reduced glutathione have a lower flavor threshold than cysteine, which merits further study.

Although sulfhydryl compounds, such as reduced glutathione was reported much more effective than ascorbic acid and other inhibitors, it is considered too expensive to be a practical commercial alternative (McEvily and Iyengar, 1992).

Aromatic carboxylic acids

Aromatic carboxylic acids are inhibitors of PPO due to their structure similarity with phenolic substrates. In model systems, the type of inhibition observed is dependent on the substrate being assayed and is competitive, noncompetitive, or mixed (McEvily and Iyengar, 1992). Using 4-methylcatechol as the substrate, the inhibition of the grape PPO by cinnamic and benzoic acids was competitive, but with caffeic acids as the substrate the mode of inhibition was non-competitive (Gunata et al., 1987). Various cinnamic acids (Fig. 1.4) were found to be powerful inhibitors of apple o-DPO and inhibitory action decreased in the order of cinnamic acid > p-coumaric acid > ferulic acid > m-coumaric acid > coumaric acid >> benzoic acids. It was also significant that sinapic (3,5-dimethoxy-4-hydrocinnamic) and hydro-cinnamic (phenylpropionic) acids together with the lower homologous of p-coumaric acid were all without inhibitory action. It seemed that the unsaturated side-chain of the cinnamic acid derivatives was essential for inhibition of o-DPO action (Walker, 1995). Sapers et al. (1989) reported that cinnamic acid inhibited enzymatic browning in Granny Smith juice when added as sodium cinnamate (CINN) at concentrations between 0.67 and 2.67 mM (114-454ppm). The

combination of CINN with AA appeared to be only slightly more effective than CINN applied alone. With apple plugs, 10 mM CINN inhibited browning for several hours, but then induced severe browning over extended storage time. The combination of CINN with AA in dips was more effective on inhibiting browning than AA alone, and significantly extending lag times. However, the combination of CINN with ascorbic-2-phosphate (AAP) showed no advantage over AAP alone. The tendency of CINN to induce browning indicated a potential problem with the use of this compound. Such behavior suggested that exogenous CINN might undergo gradual conversion at the cut surface to a PPO substrate by cinnamate-hydroxylase and other enzymes involved in the biosynthesis of phenols.

Sodium benzoate exhibited anti-browning in preliminary experiments with the juice system. The effect appearing to be concentration dependent. The combination of benzoate with AA or AAP inhibited browning to a greater extent than either treatment alone, the effect appearing to be synergistic rather than additive in samples stored 24 hours. Sodium benzoate alone or in combination with AA, provided short-term protection against browning in Red Delicious and Winesap plugs, but induced browning in samples after 6 or more hours. Because of the probability that benzoic acid and cinnamic acid may induce browning under some conditions, neither of them is recommended to be used as a PPO inhibitor as a component of anti-browning formulations (Sapers, et.al., 1989).

4-substituted resorcinols

Three inhibitors were isolated from ficin preparations by conventional and high-performance liquid chromatography (HPLC) (McEvily and Iyengar, 1992). Based on

analytical data for homogeneous preparations, the inhibitors present in the fig extract were found to be analogous 4-substituted resorcinols. Their I_{50} values were determined using mushroom PPO in an in vitro assay system (I_{50} is defined as the inhibitor concentration at which 50% inhibition of PPO activity is obtained).

In addition to the natural compounds, synthetic 4-substituted resorcinols were screened for efficacy as PPO inhibitors. The I_{50} values were determined and 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 (McEvily and Iyengar, 1992).

Of the 4-substituted resorcinols, 4-hexylresorcinol (4-HR) may have the greatest potential for use in the food industry. It has low I_{50} from the spectrophotometric assay system, positive preliminary results from tests for food safety, and the fact that this compound has a long, safe history of human use in cosmetics and pharmaceuticals (McEvily and Iyengar, 1992). The use of 4-hexylresorcinol in over-the counter drug products for more than 40 years has produced no evidence of systematic toxicity. The available human data indicated that 4-hexylresorcinol acted as a local irritant only at very high concentrations. Although human data on 4-hexylresorcinol are limited, there is no evidence the 4-hexylresorcinol produces hypersensitivity reactions in human. The animal toxicity tests indicated that there was no evidence for an association between 4-hexylresorcinol and teratogenicity in humans and it does not possess a chemical structure with the potential to interact with DNA. The National Toxicology Program yields an acceptable daily intake of 4-hexylresorcinol of 0.11 mg/Kg/day (Frankos et al., 1991).

The initial 4-hexylresorcinol food application targeted for intensive investigation was prevention of shrimp melanosis. 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 (McEvily et al., 1991; Otwell et al., 1991). This highly effective inhibitor is substantially more effective than bisulfite on a weight-to-weight basis. The use of 4-hexylresorcinol for the inhibition of shrimp melanosis resulted in extremely low residuals in shrimp meat as determined by ¹⁴C-labelled 4-hexylresorcinol (Iyengar et al., 1991) and HPLC residual analysis (King et al., 1991). Residuals are typically ≤ 1 ppm when shrimp are dipped under proposed use conditions: a 1 minute dip in 50 ppm 4-hexylresorcinol. Residual levels were determined for head-on and headless shrimp that had been dipped for extended dip times, exposed to excessive inhibitor concentrations, or received more than one dip. In no case did the residuals exceed 3 ppm (McEvily et al., 1991). It was demonstrated that 4-hexylresorcinol presented no risk of toxicity at the levels proposed for treatment of shrimp, and the use of 4-hexylresorcinol as a processing aid for preventing melanosis in shrimp is GRAS (Frankos et al., 1991).

Effectiveness of 4-hexylresorcinol to inhibit enzymatic and non-enzymatic browning in apple slices preserved by combined methods was assessed during storage at four temperatures by Monsalve-Gonzalez et al. (1993). Of the three treatments, sodium sulfite and 4-hexylresorcinol were most effective in preventing browning at 25°C without refrigeration. However, a 4 hour immersion in 0.02% (200 ppm) 4-HR solution resulted in 100 ppm residual in the fruits (Luo and Barbosa-Canovas, 1995).

The effect of 4-hexylresorcinol on the browning inhibition of Delicious apple slices during cold storage (0.5 or 4.4°C) was studied by Luo and Barbosa-Canovas (1995). Significant inhibition in browning of apple slices was obtained with 4-HR solution concentrations as low as 0.005%. Discoloration was observed on vascular bundles of the fruits two days after slicing. A combination of 0.5% ascorbic acid (AA) with 4-HR eliminated vascular discoloration and synergistically enhanced the browning inhibition. With partial vacuum (20 inch Hg vacuum) packaging and low temperature storage, more than 50 days of browning free storage life of the apple slices were obtained with a 5 min dipping in a solution containing 0.01% 4-HR, 0.5% AA and 0.2% calcium chloride. To prevent browning in High Hydrostatic Pressure treated apple slices of the varieties, 4-HR solutions or 100,000 psi range should be considered (Ibarz et al., 1996). Sapers et al. (1997) reported that 50 ppm 4-HR retarded browning of core tissue of fresh-cut pears.

Protein, peptides and amino acids

Protein, peptides and amino acids can affect PPO activity in at least two ways: by reacting with the o-quinones, the products of PPO activity; and by chelating the essential copper at the active site of PPO. The effect of several amino acids has been investigated (Kahn, 1985). It was found that L-lysine, L-histidine and L-cysteine inhibited o-DPO activity of mushroom tyrosinase, with L-cysteine being the most effective; and glycine and triglycine decreased the formation of colored o-quinones, related intermediates and melanin. Application of the amino acids to fresh cut slices of avocado and banana showed that L-cysteine effectively prevented any discoloration of the tissue even 6 hours after cutting the slices. Diglycine and triglycine offered mild protection against

discoloration only in the case of avocado. The experimental results demonstrated that L-cysteine was a most effective inhibitor of mushroom tyrosinase as well as of browning in avocado and banana tissue. Glycine, diglycine and triglycine, at much higher concentrations than L-cysteine, offered a reasonable protection against browning of avocado with the protection being expressed for a relatively long incubation.

The inhibition effect of honey on polyphenol oxidase (PPO) and on browning reaction was studied in apple slices, grape juice and model systems (Oszmianski and Lee, 1990). The results showed that apple slices treated with 10% honey (30 min immersion at room temperature) exhibited the lowest browning rate compared with sucrose or water treatments. The percent inhibition in apple slices after 2 hours with honey and sucrose was 62% and 23%, respectively. Grape juice preparation with added honey exhibited less browning and retained a higher concentration of polyphenols than juices made with no added honey. In model solutions of caffeoyl tartarate and epicatechin, the browning rate was inversely proportional to the added honey concentration. A kinetic study showed that honey is non-competitive with epicatechin. Preincubation of PPO with honey progressively decreased the PPO activity with time. The compound responsible for this inhibitory effect of honey appeared to be a small peptide with an approximate MW of 600. The further studies are underway to obtain a better understanding of the methods for the control of oxidation of polyphenol and for the development of practical uses for honey.

Other browning inhibitors

Anions

Inorganic halides have been reported to be inhibitors of PPO (McEvily and Iyengar, 1992). The mode of inhibition of apple PPO by the halide has been investigated by Janovitz-Klapp et al. (1990). The inhibition of sodium chloride at pH 4.5 was noncompetitive as determined by Lineweaver-Burk analysis. Rouet-Mayer and Philippon (1986) found that the catechol oxidase activity extracted from apple is pH dependent. Addition of neutral salt to a buffer solution decreased the pH by modification of the equilibrium constants of the buffer system. The slight decrease in pH resulting from an addition of sodium chloride to the buffer medium enhances its inhibitory effect, and simultaneously increases or decreases the potential activity of the enzyme depending on whether the initial pH is above or under pH 5.0, which was found to be optimum pH for the enzyme.

Of the halide salts, sodium and calcium chlorides at concentrations of 2-4% (w/v) are often used in the industry for the inhibition of browning (Steiner and Reith, 1989). Use of 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 foods (Gorny et al., 1997)

Kojic acid

Kojic acid [5-hydroxy-2-(hydroxymethyl)- γ -pyrone] exhibited competitive inhibition for the oxidation of chlorogenic acid and catechol by potato PPO and of 4-methylcatechol and chlorogenic acid by apple PPO (Chen et al., 1991). It interacts with quinone formation from di-phenols by decreasing O₂ uptake by the enzyme. It also

inhibits monophenolase activity of PPO. Kojic acid was found to be effective in inhibiting the development of melanosis in pink shrimp (McEvily and Iyengar, 1992). Kojic acid showed a mixed type inhibition of PPO in oxygen uptake studies, and also bleached the preformed melanin. The results indicated that the mode of action of kojic acid in prevention of shrimp melanosis was twofold: direct inhibition of PPO and chemical reduction of the pigment or pigment precursors to colorless compounds. However, the practical application of kojic acid is limited because it has been found to exhibit acute toxic effects in several animal models and also presents difficulties for large-scale production and high cost (McEvily and Iyengar, 1992).

Citric acid

Citric acid has a double inhibitory effect on phenolase by lowering the pH of the media and by chelating the copper portion of certain phenolases. When used with ascorbic acid, it provides a protective effect on ascorbic acid and tends to slow its autoxidation (Landon, 1987). Potato slices treated with a combination of ascorbic acid and citric acid, and packed in B-900 bags looked, smelled and had the texture of freshly sliced potatoes and were as white as the sulfited potatoes (Landon, 1987). McCord and Kilara (1983) reported that citric acid was effective at pH 3.5 and it could inhibit both enzymatic and non-enzymatic browning. Mushrooms showed no improvement in color when they were washed and soaked in water at pH 3.5; whereas when the pH was lowed with citric acid in vacuum or blanching operations significant improvement in color over non-acidified controls was observed. On the other hand, Rosen and Kader (1989) found that citric acid and /or ascorbic acid dips were not effective in controlling fresh-cut pear browning.

Cyclodextrins

The cyclodextrins inhibit browning by formation of inclusion complexes with or entrapment of PPO substrates or products. Preliminary experiments with cyclodextrins dissolved in Granny Smith juice indicated that β -cyclodextrin (β -CD) inhibited enzymatic browning, the degree of inhibition increasing with β -CD concentration. Substantially higher concentrations could not be used because of the limited solubility of this compound, 15.8mM for a saturated solution. However, solutions containing 8.8mM (1%) β -CD applied to Winesap and Red Delicious plugs by dipping for 90 seconds were ineffective in controlling browning (Sapers et al., 1989).

Sporix

Sporix, an acidic polyphosphate described as having a three dimensional network structure, has been recommended for use with acidic foods such as fruit juices, nectars, and carbonated beverage (McEvily and Iyengar, 1992). Sapers et al. (1989) found that addition of about 0.6% sporix to Granny Smith juice effectively controlled browning during 24 hours at 20°C, while 0.57 mM AA (100 ppm) failed after 1 hour. If added in combination with 0.57 mM AA, a lower concentration of sporix and AA were highly effective in inhibiting enzymatic browning on the cut surface of apple plugs.

Browning inhibition by sporix combinations can be attributed to two effects: a greatly extended lag time compared to that obtained with the individual inhibitors, and a reduced rate of browning once the lag time has been exceeded. It was postulated that the lag time effect could result from inhibition of copper-containing oxidases and other copper-catalyzed oxidative processes in apple by sporix, which is a powerful chelating

agent. Sporix would also inhibit PPO directly by chelation of its copper, thereby decreasing the rate of polyphenol oxidation and subsequent browning (Sapers et al. 1989).

Pineapple juice

Pineapple juice was reported as an effective browning inhibitor in both fresh and dried apple (Lozano-De-Gonzalez et al., 1993). Pineapple juice was fractionated using various size and charged separation procedures. All fractions inhibited enzymatic browning of crude apple extracts by at least 26%. The results indicated that the inhibitor was a neutral compound of low molecular weight.

Physical approaches to browning inhibition

Elimination or reduction of oxygen

The storage life of fresh-cut fruit products may also be extended by a combination of oxygen exclusion or reduction and enzymatic browning inhibitors. Reduced O₂ storage atmosphere could have two benefits: retarded browning reaction and reduced respiration rate (Kader, 1986; Rosen and Kader, 1989; Gil et al., 1998). Gil et al. (1998) Reported that apple slices stored in 0 KPa O₂ were significantly lighter in color than those stored in either air or 0.25 KPa O₂ after the storage period. The texture and color change of sliced strawberries and pears dipped in various solutions and stored in air and controlled atmosphere (CA) have been investigated by Rosen and Kader (1989). CA storage suppressed respiration and ethylene production rates of sliced fruits. Firmness of strawberry and pear slices was maintained by storage in air + 12% CO₂ and in a 0.5% O₂

atmosphere, respectively. These treatments also resulted in lighter colored pear slices than the air-controlled treatment.

Luo et al. (1995) reported that with partial vacuum (20 inch Hg vacuum) packaging and low temperature, more than 50 days browning free storage life of apple slices were obtained with 5 min dipping in a solution containing 0.01% 4-hexylresorcinol, 0.5% AA and 0.2% calcium chloride. Sapers and Miller (1997) also indicated that control of browning and suppression of mold growth of fresh-cut pears were enhanced by use of modified atmosphere packaging with a 90 oxygen transmission rates from a laminated polyethylene film.

Cultivar selection

It was noticed that there is a positive correlation between browning and PPO or polyphenol content among different cultivars, but not with both (Lee, 1993). To minimize browning in fresh fruits and vegetables, many attempts were made to select cultivars that display minimum browning.

Intercultivar differences in deterioration rate of fresh-cut pear slices were investigated by Gorny and Kader (1998). Based on visual quality, 'Bosc' and 'Bartlett' pear slices had the longest shelf-life in air at 10°C, of 3 and 4 days, respectively. 'Anjou' and 'Red Anjou' pear slices had very short shelf-life of less than 2 days each, due to severe cut surface enzymatic browning. 'Bartlett' and 'Bosc' pear slices experienced a much greater loss in fresh firmness after slicing and storage in air at 10°C than 'Anjou' and 'Red Anjou' pear slices. 'Bartlett' pear slices had the highest rates of respiration and ethylene production among the cultivars tested. Sapers and Miller (1997) found that with use of sodium erythorbate/CaCl₂/4-HR combination and modified atmosphere packaging,

a shelf-life of 12-14 days with d'Anjou and Bartlett fresh cut pears could be obtained, but fresh-cut Bosc showed unacceptable browning with all treatments.

The investigation of quality of minimally processed apple slices from 12 cultivars indicated that the respiration rate varied widely among cultivars. Change in color of slices was rapid during the first 3 days. Cortland, Empire, Golden Delicious and New York 674 showed the least browning and presented more desirable attributes for minimal processing (Kim et al., 1993).

Amiot et al. (1995) investigated the influence of cultivar, maturity stage, and storage conditions on phenolic composition and enzymatic browning of pear fruits. It was found that differences in phenolic composition and in the degree of browning were more influenced by cultivar than by maturity stage of the fruit. Large cultivar variations in certain phenolic compounds and PPO activity were also observed among peach varieties, and the degree of browning was closely correlated with phenolic content and enzymatic activity (Lee et al., 1990). The extent of enzymatic browning at cut surfaces of Atlantic potato, its siblings and parents, and Russet Burbank was investigated (Sapers et al., 1989). The degree of browning was compared with cultivar variation in PPO, PPO substrates and ascorbic acid. Atlantic potato was much less subject to browning at cut and peeled surfaces than Russet Burbank. Browning in Atlantic potato could be almost eliminated by dipping in water. Belchip and Chipbelle potato (siblings) and Wauseon and Lenape (parents) were similar to Atlantic potato in browning behavior. The tendency to browning in these cultivars and Russet Burbank was found to correlate with total phenolic compounds and tyrosine, to a lesser extent, PPO activity.

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Chapter 2

EXTENDING SHELF-LIFE OF FRESH-CUT PEARS

Abstract

Various browning inhibitors and a firming agent were evaluated to prevent browning and softening of pear slices during 2-4°C storage. 4-Hexylresorcinol (4-HR) was an effective browning inhibitor at a concentration as low as 0.005% when combined with 0.5% ascorbic acid (AA). A combination of 1.0% AA with 1.0% calcium lactate prevented Anjou pear slices discoloration, but caused tissue leaking and softening. 1.0% calcium lactate maintained slice firmness at an acceptable level for 30 days when the average firmness of whole pears at slicing was 49, 38 and 43 Newton (N) for Bartlett, Bosc and Anjou respectively. Slices with acceptable texture and appealing color were achieved by dipping in the solution of 0.01% 4-HR, 0.5% AA, and 1.0% calcium lactate for 2 min, vacuum packaging, and storing at 2-4°C for 30 days for Bartlett and Bosc, and 15 days for Anjou.

Introduction

Fresh-cut or minimally processed fruits and vegetables is a rapid growing segment of the U.S. fresh produce industry (Gorny et al., 1997). These products have the attributes of convenience and fresh-like quality. Two basic problems confront the extension of shelf-life of fresh-cut fruit and vegetable products. First, the processing procedures of peeling and cutting cause decompartmentalization of polyphenol oxidase

and phenolics, which will undergo enzymatic browning to produce undesirable brown color (Macheix et al., 1990; Walker, 1995). Second, tissue wounding induces high respiration rate, which triggers faster texture deterioration compared to intact tissue (Rosen and Kader, 1989). A combination of controlled atmosphere packaging and/or firming agents like calcium salts have been reported to successfully retard texture softening (Ponting et al., 1972; Poovaiah, 1986; Rosen and Kader, 1989; Gorny et al., 1997). Sulfites have been used to inhibit both enzymatic and nonenzymatic browning in foods. However, sulfites have been associated with severe allergy-like reactions in some asthmatics, prompting the Food and Drug Administration (FDA) to limit their use to certain categories of food products (Sapers, 1993). A long-standing goal of food technology has been to find effective substitutes to prevent cut surface browning to extend the shelf-life of fresh products. Numerous potential browning inhibitors have been tested on fresh-cut fruits and vegetables. Ascorbic acid and its isomer erythorbic acid (Ponting et al., 1972; Sapers et al., 1987 & 1989), L-cysteine (Molnar-perl and Friedman, 1990), 4-hexylresorcinol (Luo and Barbosa-Canovas, 1995; Monsalve-Gonzalez et al., 1993, 1995), and pineapple juice (Lozano-de-Gonzalez et al., 1993) have been reported to be effective browning inhibitors for fresh-cut apples.

Pear growers and marketers have shown a high interest in developing fresh-cut pear products to stimulate the consumption of pears. However, fresh-cut pears offer a unique challenge due to dramatic texture softening in addition of high browning potential during storage. Efforts have been made to inhibit or reduce browning of fresh-cut pears during storage by controlled atmosphere storage in combination with/without ascorbic acid and/or calcium dipping (Rosen and Kader, 1989; Gorny et al., 1996). Sapers and Miller

(1997) reported that a shelf-life of 12 to 14 days for fresh-cut pears was obtained with a combination of sodium erythorbate/ calcium/4-HR and modified atmosphere packaging.

Our objective was to develop effective methods to extend the shelf life of fresh sliced pears with visually appealing color and acceptable texture.

Materials and Methods

Preparation of Pear Slices

Three pear varieties, Bartlett, Bosc and Anjou were harvested at optimum maturity at Southern Oregon Research and Extension Center, Medford, Oregon, and stored at 0°C until transported to Corvallis for further processing. Fruits were stored at 2-4°C in Corvallis. Whole pears were partially ripened at room temperature (20-25°C) and rinsed with 0.05% sodium hypochlorite before slicing. Pears were peeled and cut into uniform, 5 mm thick rings with a mechanical apple slicer. Pear slices were obtained by cutting the fruit along the stem-calyx axis with a stainless steel knife. Slices from two pears were placed in the inner bowl of a salad spinner, dipped in 3 L of a test solution for 2 min, and drained in a perforated plastic container for 1 min. Slices were then placed into 3 mm thick nylon coated 15.5×21.5 cm plastic pouches (Kapak Corp.), vacuumed, heat sealed, and stored at 2-4°C. Color and texture were evaluated every 5 days during 30 days storage. A complete factorial design with repeated measurements was conducted in three replicates. All processing operations were conducted at 2-4°C.

Testing solutions for browning inhibition and texture maintenance

Potential browning inhibitors

Pear slices were dipped in the following solutions: 1) canned pineapple juice (Dole); 2) 1.0 % ascorbic acid (AA) and 1.0% citric acid; 3) 0.01% 4-HR (Sigma Chemical Co., ST. Louis, MO), 0.5% AA and 0.2% calcium lactate; 4) 0.2% L-cysteine. Distilled water was used as a control.

Treating pear slices with calcium lactate

Bartlett and Bosc pear slices were dipped in solutions containing different concentrations of calcium lactate: 0, 0.2%, 0.5% and 1.0%. Anjou pear slices were dipped in solutions containing 1.0% calcium lactate plus 0, 0.5%, or 1.0% ascorbic acid.

Treating pear slices with 4-HR, AA and calcium lactate

Bartlett, Bosc and Anjou pear slices were dipped in solutions of 0.5% AA and 1.0% calcium lactate combined with 0.005%, 0.01%, 0.02%, 0.03% 4-HR. Bosc pear slices were dipped in solutions of 1.0% calcium lactate plus the four concentrations of 4-HR listed above, without AA.

Effect of ripeness of whole pears at slicing on color and texture change

Bartlett and Bosc pears were sliced at three levels of firmness. Bartletts were sliced at an average of 85 N (no ripening), or ripened to 49 N, and 37 N. Bosc pears were sliced at an average of 71 N (no ripening), or ripened to 60 and 38 N. Slices were dipped in a solution containing 0.01% 4-HR, 0.5% AA and 1.0% calcium lactate.

Color measurement

Color characteristics (Hunter CIE L*, a*, b*, chroma and hue angle) were measured using a ColorQuest Hunter colorimeter (HunterLab, Hunter Associates Laboratories Inc., Reston, VA). The equipment was set up for reflectance 45/0 with specular included, Illuminant C, 10° observer angle. The plastic pouches containing pear slices were opened and the slices were evenly placed in an optical glass cell (13 cm dia × 5 cm ht) (HunterLab, Hunter Associates Laboratories Inc., Reston, VA). Color values of L* (lightness), a* (red to green), b* (yellow to blue), chroma (color intensity) and hue angle (color itself, 0° = red-purple, 90° = yellow, 180° = bluish – green, and 270° = blue) were obtained from the average of 9 readings for each treatment, 3 replicates with 3 readings for each replicate. Color and firmness values measured just after slicing without any treatments (considered as original fresh color and texture values) and within 24 hours of treatments were recorded as time 0 and 1, respectively. Measurements were taken every 5 days for 30 days.

Texture determination

The firmness of whole pears was measured with a penetrometer with a diameter of 8 mm tip. The penetration strength was determined by taking measurements at two opposite spots of each pear without peel and close to the calyx end. The average penetration strength of 10 whole pears was considered as the firmness before slicing. Firmness between 45-67 N for Bartlett, 27-45 N for Bosc and 36-45 N for Anjou was used for all treatments except when studying the effect of ripeness of whole pears at slicing on color and texture change. The firmness of slices was measured with an U.S. fruit

firmness tester equipped with a 3 mm tip. One measurement per slice was performed. The penetration strength of 20 slices were averaged and considered as the firmness of the treatment.

Statistic analysis

The data was submitted to General Linear Model Procedure and Tukey's multiple comparison (LSD) with significance level of $P < 0.05$, by using SAS[®] (SAS Institute, Inc.) for Windows software.

Results and Discussion

Potential browning inhibitors

The mean color values of the slices treated with different browning inhibitors at 30 days storage are presented in Table 2.1. Slices treated with 0.01% 4-HR, 0.5% AA and 0.2% calcium lactate maintained original fresh color significantly better ($P < 0.05$) than any other treatments. At 30 days after slicing, color values of L^* , a^* and hue angle were nearly the same as at the day of slicing. Luo and Barbosa-Canovas (1995) and Monsalve-Gonzalez et al. (1995) found that 0.01% 4-HR treatment was effective in preventing discoloration of apple slices in the presence of 0.2-0.5% AA.

With Bartlett slices, color in all other treatments consistently browned over time. Treatments with 1.0% AA and 0.2% cysteine on Bosc and Anjou pear slices showed some browning inhibition, but browning was not satisfactorily controlled. Bosc and Anjou slices with these two treatments also had a tendency of increase in redness (higher a^* values).

Table 2.1 —Mean values of CIELAB, chroma and hue angle of Bartlett, Bosc and Anjou pear slices dipped in various solutions and stored for 30 days at 2-4°C. Water = distilled water; CPJ = canned pineapple juice; AA = 1.0% ascorbic acid + 1.0% citric acid; 4-HR = 0.01% 4-HR + 0.5% ascorbic acid + 0.2% calcium lactate; CY = 0.2% cysteine.

Variety	Bartlett					Bosc					Anjou				
	CIE L*	CIE a*	CIE b*	Chroma (c)	Hue (h)	CIE L*	CIE a*	CIE b*	Chroma (c)	Hue (h)	CIE L*	CIE a*	CIE b*	Chrom a(c)	Hue (h)
Fresh	75.1	0.28	26.2	26.2	88.5	75.0	0.71	24.7	24.7	88.4	76.3	-0.12	22.1	22.03	90.29
Water	48.4 (3.62)	11.52 (2.44)	32.5 (2.31)	34.5 (2.92)	70.6 (2.82)	59.9 (1.00)	6.33 (0.65)	27.7 (1.09)	28.4 (1.20)	77.2 (0.83)	51.0 (4.63)	7.08 (1.00)	27.0 (0.52)	27.9 (0.71)	75.3 (1.79)
CPJ	47.8 (2.38)	11.68 (1.50)	32.0 (1.49)	33.8 (1.59)	70.0 (1.60)	55.3 (1.02)	8.95 (1.24)	26.2 (0.11)	27.7 (0.50)	71.2 (2.34)	51.8 (0.93)	7.93 (0.18)	26.7 (1.00)	28.0 (1.03)	73.5 (0.78)
AA	54.9 (6.51)	9.61 (4.06)	32.7 (1.43)	34.2 (2.40)	73.9 (6.06)	65.2 (5.42)	4.99 (3.72)	31.3 (0.32)	32.1 (0.61)	81.1 (6.51)	61.6 (7.49)	2.91 (4.77)	27.9 (2.30)	28.3 (2.91)	84.7 (8.80)
4-HR	72.9 (1.56)	-0.05 (0.65)	32.1 (0.49)	32.3 (0.61)	90.3 (0.88)	70.4 (0.85)	-0.09 (0.59)	26.5 (0.43)	26.5 (0.43)	90.2 (1.24)	67.0 (3.17)	0.61 (1.42)	23.7 (1.12)	23.7 (1.15)	88.7 (3.36)
CY	49.9 (0.70)	12.0 (0.53)	22.5 (1.51)	25.6 (1.46)	61.9 (1.22)	65.9 (2.04)	5.14 (1.12)	26.3 (1.30)	26.9 (1.04)	78.9 (2.90)	61.6 (3.55)	3.55 (0.64)	24.1 (1.81)	24.4 (1.88)	82.3 (2.59)

Standard deviations in parenthesis.

Effect of calcium lactate on color and texture change

We used calcium lactate as the texture firming agent and found that calcium lactate treatment helped Bartlett and Bosc pear slices maintain firmness, with increasing benefit by increasing concentration (Fig. 2.1). Bosc pear slices treated with 1.0% calcium lactate had a significant firmer texture than the control. No significant firming effect was obtained for Bartlett slices with calcium dipping treatments. However, visual observations revealed that the surfaces of Bartlett and Bosc pear slices dipped with 1.0% calcium lactate were smooth, while slices from other treatments had varying degrees of stickiness and mushiness on the surfaces.

Rosen and Kader (1989) found that treatment of 1.0% calcium chloride maintained pear slice firmness and resulted in lighter colored pear slices than water dipped slices after 7 days at 2.5°C. Ponting et al. (1972) noted that calcium lactate (as calcium resource) gave a somewhat better flavor than calcium chloride in canned apples.

Bartletts showed similar behavior to Bosc slices with respect to color change. Therefore, only Bosc data is presented. L^* and h values decreased similarly in all treatments, indicating that no significant browning inhibition was achieved with any calcium lactate concentrations. This was in agreement with the observation of Ponting et al. (1972) that calcium treatment alone resulted in poor color on apple slices. Calcium has been reported to maintain the cell wall structure in fruits by interacting with pectic acid in the cell wall to form calcium pectate which firms molecular bonding between constituents of cell wall (Fenemma, 1985). Thus, fruits treated with calcium are generally firmer than controls (Poovaiah, 1986). Gorny et al. (1996) also reported that pear fruit

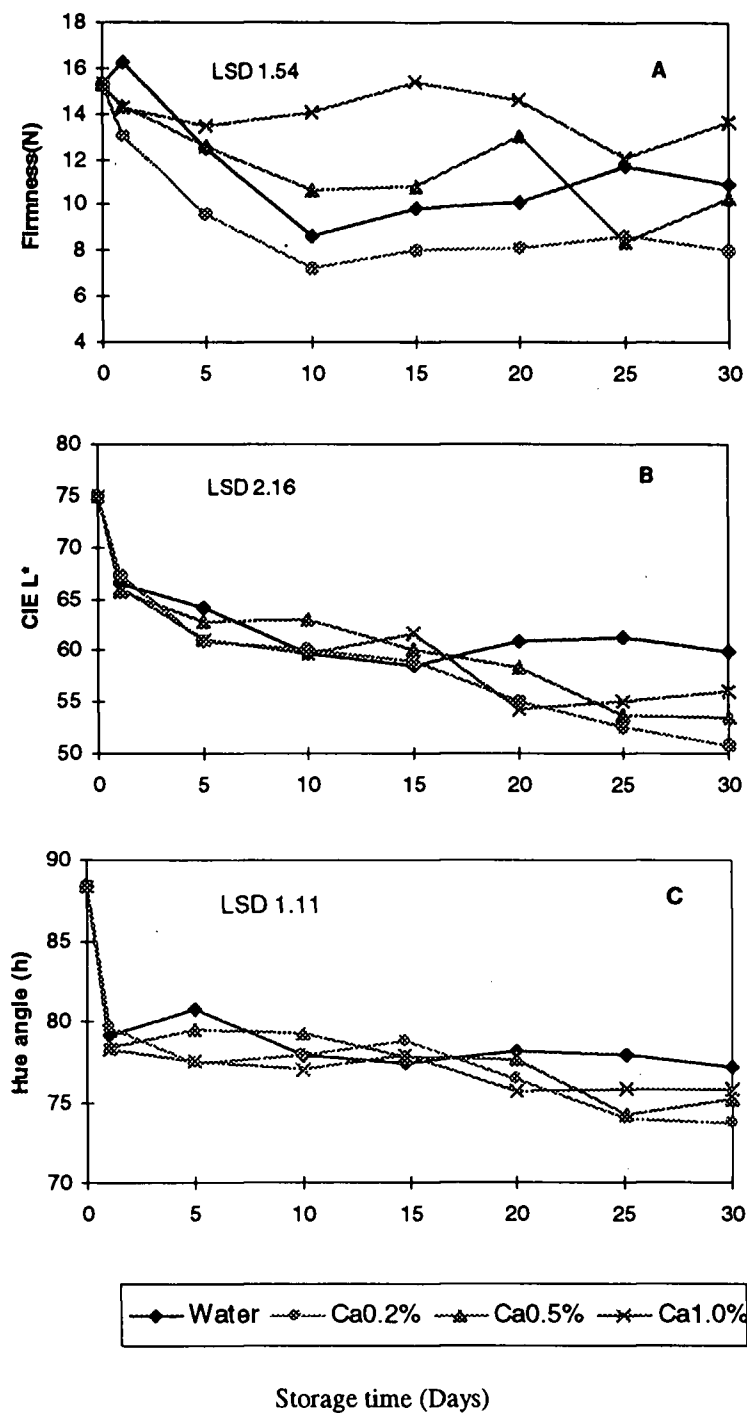


Fig. 2.1 —Firmness (A), L* (B) and hue angle (C) changes of Bosc pear slices dipped in calcium lactate solutions and stored at 2-4°C for 30 days. Pooled LSD is shown.

slices treated with 1.0% CaCl_2 after cutting had significantly higher flesh firmness than non- CaCl_2 treated slices irrespective of the storage atmosphere.

Effect of calcium lactate and ascorbic acid treatments on Anjou pear slices color and firmness

Gorny et al. (1996) reported that 1.0% calcium lactate and 2.0% ascorbic acid applied as a dip for 1 min was effective in reducing pear slice surface browning. Ponting et al. (1972) also found that the combination of treatments with 1.0% ascorbic acid and 0.1% calcium lactate was very effective in apple slice browning inhibition. We found that the combination of 1.0% calcium lactate with 1.0% ascorbic acid inhibited discoloration, yet caused firmness loss in Anjou pear slices (Fig. 2.2). Calcium lactate alone had no effect on color change. The L^* and h values of the slices treated with 1.0% calcium lactate and 0.5% AA were significantly higher than those of the control or 1.0 % calcium lactate alone. However, even within one day after slicing, the L^* and h values had decreased from Time 0 (fresh) of 76.3 and 90.3 to 71.8 and 85.8 respectively, and continued decreasing. Some browning was noticeable just after vacuum packaging. Increasing the ascorbic acid concentration to 1.0% and combining with 1.0 % calcium lactate was more effective in browning inhibition. Browning inhibition by the combination of 1.0% AA and 1.0% calcium lactate could be due to the calcium stabilizing the cell membrane (Gorny et al., 1996), which in turn could limit the free release of polyphenoxidase and the substrates of phenolics. At the same time, the oxidation products of quinones would be converted back to diphenols by the presence of AA. Therefore, the browning inhibition effect would be dependent on the sufficient amount of AA.

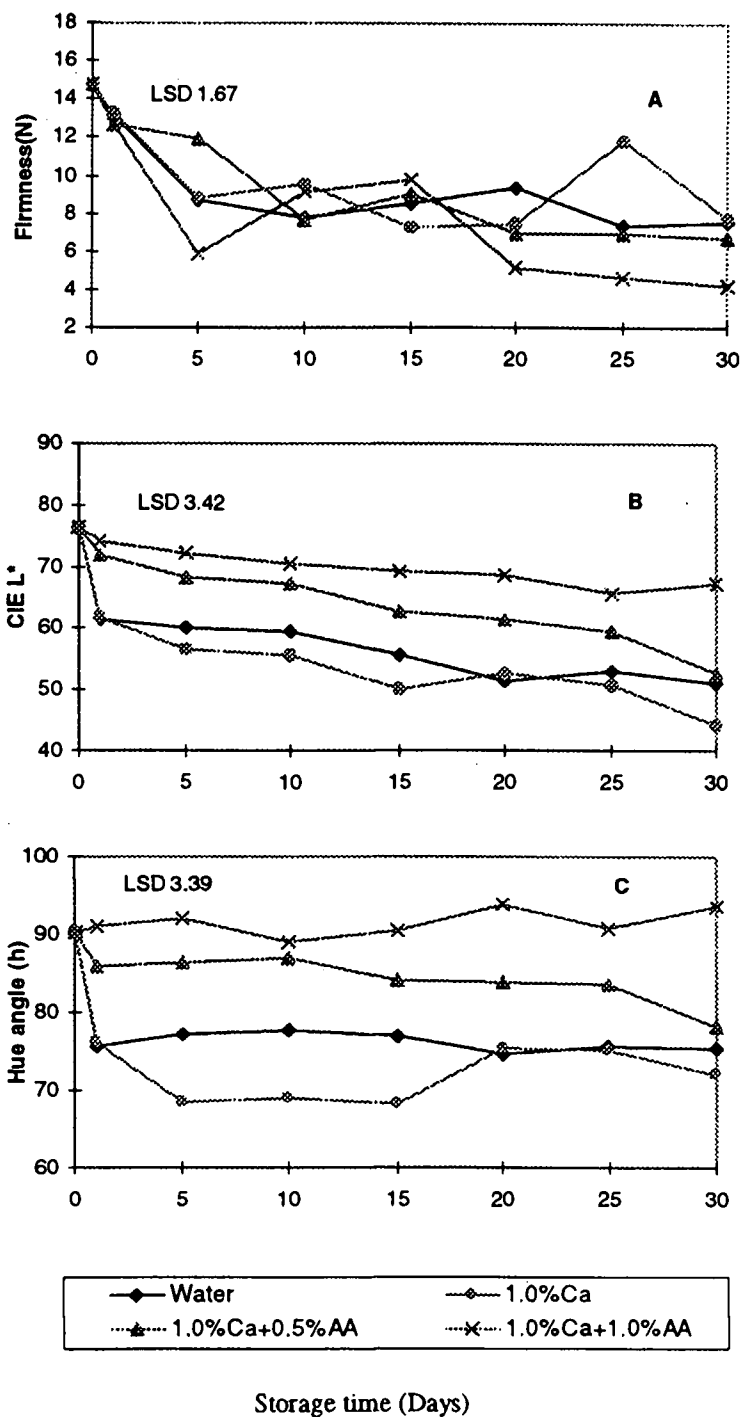


Fig. 2.2 —Firmness (A), L* (B) and hue angle (C) changes of Anjou pear slices dipped in the solutions containing 1.0% calcium lactate and various concentrations of AA and stored at 2-4°C for 30 days. Pooled LSD is shown.

Anjou slices treated with 1.0% ascorbic acid were significantly softer than other treatments, and tissue leakage was observed. Similar phenomena were observed on the slices treated with 1.0% AA and 1.0% citric acid. Sapers et al. (1992) also observed that treatment with dips containing AA-2-phosphate (which served as an AA reservoir) caused some leakage on cut potato surfaces. The reasons for the softening and leaking are not understood, but gradual cleavage of pectin glycosidic linkage in acidic media or osmotic leaking could be possible explanations.

Treatments of 1.0% calcium lactate and 0.5% AA combined with different concentrations of 4-HR

The concentration of 4-HR at 0.005% effectively inhibited browning of Bartlett slices for 20 days in the presence of 0.5% AA. After 20 days, some browning was observed on the slices treated with 0.005% 4-HR concentrations, or higher than 0.01%. The 4-HR at 0.005% also kept Anjou slices from discoloration for 15 days storage as effectively as did other higher concentrations of 4-HR. After 15 days, some brown color was noticed on Anjou pear slices in all the concentration treatments, with the least browning with 0.01% 4-HR (Table 2.2).

Within 20 days, all concentrations of 4-HR inhibited browning of Bosc slices equally well. After 20 days, browning occurred slowly on the slices treated with 0.005%. The concentration of 0.01% 4-HR effectively kept Bartlett and Bosc pear slices from browning for 30 days.

Table 2.2 — Mean values of CIELAB, chroma and hue angle of Bartlett, Bosc and Anjou pear slices dipped in solutions combining 1.0% calcium lactate, 0.5% ascorbic acid with various concentrations of 4-HR, and stored for 30 days at 2-4°C.

Variety	Bartlett					Bosc					Anjou				
	CIE L*	CIE a*	CIE b*	Chroma (c)	Hue (h)	CIE L*	CIE a*	CIE b*	Chroma (c)	Hue (h)	CIE L*	CIE a*	CIE b*	Chroma (c)	Hue (h)
Fresh	75.1	0.28	26.2	26.2	88.5	75.0	0.74	24.7	24.7	88.4	76.3	-0.12	22.1	22.1	90.3
4-HR 0.005%	69.4 (4.22)	2.03 (1.84)	29.4 (1.65)	29.7 (1.61)	83.5 (4.58)	69.3 (0.59)	0.37 (0.10)	25.7 (0.50)	25.6 (0.44)	89.2 (0.27)	66.5 (0.92)	0.50 (2.14)	24.9 (0.75)	24.9 (0.82)	89.0 (4.73)
4-HR 0.01%	74.6 (0.52)	0.08 (0.44)	29.3 (0.28)	29.3 (0.28)	89.9 (0.87)	69.4 (0.83)	0.10 (0.25)	25.2 (0.86)	25.2 (0.86)	89.8 (0.54)	64.8 (0.85)	0.48 (1.42)	23.7 (0.99)	23.7 (1.01)	88.9 (3.32)
4-HR 0.02%	73.8 (2.29)	0.17 (1.05)	28.1 (0.93)	28.1 (0.93)	89.4 (2.47)	70.7 (1.23)	-0.41 (0.07)	26.2 (1.01)	26.2 (1.01)	90.9 (0.15)	64.0 (0.60)	1.21 (0.76)	25.2 (1.67)	25.3 (1.69)	87.3 (1.64)
4-HR 0.03%	69.9 (2.29)	2.79 (1.96)	28.3 (1.77)	28.5 (1.67)	84.2 (4.26)	70.4 (1.18)	-0.50 (0.45)	27.3 (0.67)	27.3 (0.67)	91.1 (0.94)	64.8 (0.85)	0.48 (1.42)	24.8 (0.71)	24.8 (0.72)	88.9 (3.32)

Standard deviations in parenthesis.

Treatments of 1.0% calcium lactate combined with 4-HR, without ascorbic acid

In the absence of AA, browning in all 4-HR treatments increased with time (Fig. 2.3). It is apparent that the synergistic browning inhibition was obtained by a combination of 4-HR with 0.5% ascorbic acid. Luo and Barbosa-Canovas (1995) also reported similar results for apple slices.

AA is a reducing agent which mainly reverses the quinones formed during browning reaction back to diphenols (McEvily et al., 1992). 4-HR has been found to act as a selective inhibitor of polyphenoxidase (PPO) of shrimp (McEvily et al., 1991) and mushroom (Dawley and Flurkey, 1993). Luo and Barbosa-Canovas (1995) proposed that a mixed type of competitive and uncompetitive inhibition might be expected. The combination of AA and 4-HR prolonged the retention of AA due to the suppression of PPO activity by 4-HR, and consequently, a better browning inhibition was achieved in the presence of AA to reduce the quinones formed during browning reaction catalyzed by any residual PPO activity.

Although the penetrometer did not show significant softening with 0.02 and 0.03% 4-HR treatments, tissue leakage and loss of cell turgor pressure were noticed visually. No tissue leakage was observed with the concentrations of 0.005 and 0.01% 4-HR treatments. Luo and Barbosa-Canovas (1995) reported similar observations on apple slices and it was suggested that higher concentrations of 4-HR impaired the texture of the fruits.

Effect of firmness at slicing on browning and texture change

The combination of 0.01% 4-HR, 0.5% ascorbic acid and 1.0% calcium lactate was found to be the most effective treatment for browning inhibition. Therefore it was

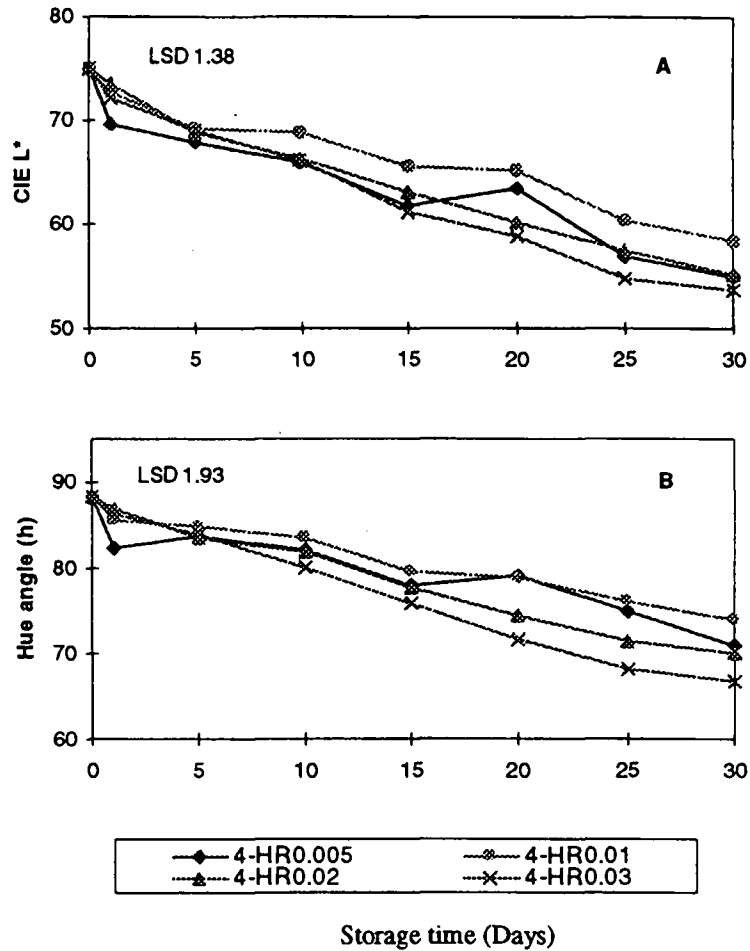


Fig. 2.3 —L* (A) and hue angle (B) changes of Bosc pear slices dipped in solutions containing 1.0% calcium lactate and various concentrations of 4-HR without AA, and stored at 2-4°C for 30 days. Pooled LSD is shown.

applied as a dipping solution to test the effect of ripeness of whole pears at slicing on color and texture change during 30 days storage. Bartlett pears sliced at 37 N were too soft for mechanical slicing with our equipment. Browning at this firmness was inhibited for 20 days. However, the texture was unacceptably soft and mushy. The firmness of 49 N (range 45-67 N) was appropriate for slicing Bartlett. After 30 days, the color of the slices was almost as good as on the day of slicing, with acceptable texture. This result is in agreement with the observation of Sapers and Miller (1997) that fruit firmness values of at least 6 Kg (59 N) were required for successful treatment of Bartlett pears.

Bosc pears at 38 N (range 27-45 N) sliced well and had acceptable color and texture after 30 days storage. The texture of Bosc slices did not change over time as much as did Bartlett slices. Slicing at higher firmness (85 N for Bartlett, 60 N or 70 N for Bosc) resulted in lack of browning and high firmness retention. However, at 30 days the slices had too firm a texture for fresh eating, and lacked flavor.

Conclusions

4-HR was an effective browning inhibitor of fresh sliced pears in the presence of 0.5% ascorbic acid. Without AA, 4-HR alone did not inhibit browning of Bosc pear slices. Calcium lactate (1.0%) treatment resulted in better texture retention and kept slice surfaces from becoming sticky and mushy. The treatment of 1.0% AA in combination of 1.0% calcium lactate effectively maintained Anjou pear slices free from browning, also induced texture softening and leaking. Initial whole pear firmness of 45-67 N for Bartlett, 27-45 N for Bosc and 36-45 N for Anjou were essential for mechanical slicing and successful color and texture maintenance. Bosc and Bartlett pear slices with appealing

color and acceptable texture were obtained by 2 min dipping in the solution of 0.01% 4-HR, 0.5% ascorbic acid and 1.0% calcium lactate, vacuum packaging and 2-4°C storage for 30 days. Anjou pear slices could be stored for 15 days under the same treatment conditions. 1.0% AA and 1.0% citric acid, 1.0% calcium lactate alone, 0.2% cysteine or canned pineapple juice did not satisfactorily inhibit pear slices from discoloration.

Acknowledgment

We thank the Winter Pear Control Committee for funding this research. Thanks to Bob Durst for his technical support. We are grateful to Monica M. Giusti and Luis E. Rodriguez-Saona for their suggestion and revision of the paper.

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Chapter 3

4-HEXYLRESORCINOL-BROWNING INHIBITION OF FRESH-CUT PEARS AND ITS RESIDUAL AND SENSORY PROPERTIES

Abstract

The effectiveness of Semperfresh, Snow Fresh and cysteine for inhibition of browning in fresh-cut pears was compared with 4-hexylresorcinol (4-HR). The influence of storage temperature on texture and color retention was studied. Cysteine (0.5%) combined with 0.5% ascorbic acid (AA) effectively inhibited browning in pear slices for 25 days. Semperfresh and Snow Fresh did not maintain flesh color satisfactorily. A storage temperature of 0°C significantly retained better texture than the control (2-4°C). 4-HR residual content and its sensory properties were evaluated. Dipping time and concentration both contributed to an increase in 4-HR residual content in treated pear slices, with the latter being the major factor. The 4-HR residual content decreased with storage time for all the treatments. Sensory tests indicated that panelists detected the overall difference between 0.01% 4-HR treated samples and the controls for both Bartlett and Anjou pears. There was no significant difference in preference between treated sample and the control for Anjou pears.

Introduction

4-hexylresorcinol (4-HR) controls enzymatic browning in shrimp and has potential for applications in a variety of other foods and beverages (McEvily et al., 1991). 4-HR was shown to be a potent inhibitor of mushroom tyrosinase, the major enzyme

responsible in mushroom enzymatic browning (Dawley and Flurkey, 1993). The effect of 4-HR on browning inhibition of apple slices has been tested and demonstrated (Luo et al., 1995; Monsalve-Gonzalez et al., 1993 and 1995).

Sapers (1997) reported that 4% sodium erythorbate was required to control cut surface browning in Bartlett and d'Anjou pear, while 50 ppm 4-HR retarded browning of core tissue. At the same time, we found that 4-HR was an effective browning inhibitor at a concentration as low as 50 ppm in the presence of 0.5% AA (Dong et al., 1998). Bosc, Anjou and Bartlett pear slices with acceptable texture and color were achieved by dipping them in a solution of 50 ppm 4-HR, 0.5% AA and 1.0% calcium lactate for 2 min, followed by vacuum packaging and storage for 15 days. The shelf life of Bartlett and Bosc slices was extended to 30 days by increasing 4-HR concentration to 100 ppm.

Semperfresh and Snow Fresh are commercially available browning inhibitors. Semperfresh is an edible coating film (sucrose ester) which is proposed to inhibit browning by functioning as an oxygen barrier when it is coated on the surface of fresh-cut fruits. Snow Fresh (Formulation of calcium chloride, sodium acid pyrophosphate, citric acid and L-ascorbic acid) is reported to be effective for maintaining the color, texture and quality of most fresh fruits and vegetables (Monsanto, 1988). Cysteine has been reported to inhibit enzymatic browning catalyzed by mushroom, apple and pear polyphenol oxidase activity (Dudley and Hotchkiss, 1989; Richard et al., 1992; Siddiq et al., 1994). Kahn (1985) found cysteine (0.32 mM) to be very effective for inhibition of avocado and banana homogenate browning. Our objective was to compare the effectiveness of those three browning inhibitors for fresh-cut pears with 4-HR which we found to be very effective in the previous research (Dong et al., 1998).

4-HR is approved for use as an inhibitor of shrimp melanosis. It is presently not approved for use as a browning inhibitor in fresh-sliced fruits and vegetables. Measurement of 4-HR residual and determination of the sensory properties of treated samples is needed by processors and regulatory agencies for evaluation of its possible approval and use.

Materials & Methods

Reagents and Materials

Reagent grade 4-hexylresorcinol, cysteine, ascorbic acid and calcium lactate were purchased from Sigma Chemical Co. (ST. Louis, MO) and used for browning inhibition and 4-HR residual analysis. Snow Fresh™ was obtained from Monsanto Co. (ST. Louis, MO), and Semperfresh™ from Agricoat (A Mantrose-U.K. Company). Food grade 4-HR was received from Cultor Food Science, Inc. (Ardsley, NY), and ascorbic acid and calcium lactate from EM Science (a Division of EM Industries, Inc., Darmstadt, Germany), and used for sensory analysis.

Browning Inhibition in Sliced Pears

Preparation of pear slices

Anjou and Bosc pears were harvested at optimum maturity at the Southern Oregon Research and Extension Center (Medford, Oregon) and stored at 0°C until transported to Corvallis for further processing. The procedures of sample preparation were the same as described by Dong et al. (1998).

Testing solutions for browning inhibition

The following dipping solutions were tested for browning inhibitors of Anjou and Bosc pear slices.

Semperfresh. Anjou and Bosc pear slices were dipped in solutions containing 0.5% AA and 1.0% calcium lactate combined with Semperfresh concentrations of 0.2, 0.5 and 1.0%; A solution only containing 1.0% Semperfresh was tested on Anjou pear slices.

Cysteine. Testing solutions containing 0.5% AA and 1.0% calcium lactate with 0.2 or 0.5% cysteine were applied as dipping solutions to both Anjou and Bosc pear slices.

Snow Fresh. 1.0% Snow Fresh solution was used to test its browning inhibition effect on Anjou and Bosc pear slices.

Controls. A solution of 0.01% 4-HR with 0.5% AA and 1.0% calcium lactate, and distilled water were used as controls.

Effect of storage temperatures on texture and color maintenance

Anjou and Bosc pear slices were treated with 0.01% 4-HR, 0.5% AA and 1.0% calcium lactate dipping solution and stored under different temperatures of 0°C, 1.1°C and 2.2°C in an incubator (Fisher Scientific, Isotemp. Pittsburgh, PA). A cold storage room (2-4°C) served as a comparative control.

Color measurement and texture determination

The color was measured with HunterQuest Colorimeter and texture measured with penetrometer. Details were referred to Dong et al. (1998).

4-HR Residual Content Analysis

4-HR extraction recovery

Bartlett pears were obtained from a local grocery store (Corvallis, OR), and ripened at room temperature until the whole pear firmness was 44 to 66 Newton (N). Fresh pear slices (9.5 – 10 g) without 4-HR treatment were cut into small dices, spiked with 4 mL of known concentrations (10, 20 and 40 ppm) of 4-HR solutions (in 50% aqueous methanol), and blended with 10 - 15 mL 100% methanol for 5 - 10 min. The contents were transferred to a 500 mL glass beaker. The blender was rinsed 3 - 4 times with 100% methanol until all the pear particles were washed out into the beaker. The sample was let stand for 30 – 60 min with methanol, and then vacuum filtered with Whatman No. 1 paper. The filter-cake residual was reextracted twice with 100% methanol. The filtrates were combined and taken to dryness with a rotary evaporator (35°C). The residual was taken up in 4 mL of 60% methanol and transferred to a 10 mL volumetric flask. The rotovapor flask was rinsed with 50% aqueous methanol and was added to the volumetric flask to bring the volume to 10 mL. This extract was filtered through a 0.45 μ m HAVP millipore filter paper (Millipore Co.). The filtrate was collected and used for 4-HR quantitative analysis with HPLC/UV. Four concentrations (1, 4, 8, and 20 ppm) of standard 4-HR solutions were run with the samples, and used to make a standard calibration curve. The 4-HR sample content was calculated from the regression formula of the standard curve. The extraction recovery was obtained from the amount of 4-HR extracted from spiked samples divided by the known added quantity. Measurements were duplicated. Percentage recovery for three replicated extractions averaged 90%.

Pear slice preparation and 4-HR extraction

Three Bartlett pears were sliced, trimmed, mixed and separated into 4 portions for the following browning inhibition treatments. (1) 50 ppm 4-HR 1min dipping; (2) 50 ppm 4-HR 2 min dipping; (3) 100 ppm 1 min dipping; (4) 100 ppm 2 min dipping; dipping solutions contained 0.5% AA and 1.0% calcium lactate. After each dipping treatment, the slices were drained in a perforated plastic container for 1 min. Slices with same treatment were separated into three portions, two of them were placed into plastic pouches, vacuum packaged and stored at 2-4°C for 7 and 14 days, respectively, for 4-HR residual analysis. The other portion was analyzed immediately (considered as storage time 0). The extraction procedures were the same as those used for 4-HR recovery experiment. The experiment was replicated.

4-HR separation with HPLC/UV

Apparatus. A Perkin-Elmer Series 400 liquid chromatograph equipped with a Hewlett-Packard 1040A photodiode array detector and a Hewlett-Packard 9000 computer system was used. Detection was set at 280 nm. The spectra (detection wavelength from 200 to 600 nm) were recorded for 4-HR standard and samples to ascertain the 4-HR peak identity.

Column and mobile phase. Supelcosil LC-18 column (Supelco Inc. Bellefonte, PA) (5 micron particle size), 250 × 4.6 mm i.d., 1.5 cm × 4.6 mm id guard column. Solvent A: HPLC grade methanol, solve B: deionized water. Flow rate: 1 ml/min. 4-HR was analyzed isocratically with 60% methanol in 18 min.

Quantification of 4-HR

4-HR standard solutions of 1, 4, 16 and 20 ppm were distributed and run as external standards with samples with two injections for each vial. A standard curve of concentration versus peak area was obtained. 4-HR concentrations in the samples were calculated from the regression formula of the standard calibration curve and normalized with 90% extraction recovery.

Sensory Evaluation

Pear slice preparation

Bartlett and Anjou pears were obtained from Cub Foods Supermarket, Corvallis (OR). Bartlett pears were ripened at room temperature to 10 to 15 Lb. and Anjou 8 to 10 Lb. before slicing. Uniform, 5 mm thick pear slices were prepared with a mechanical apple slicer by cutting the fruit along the stem-calyx axis with a stainless knife. Bartlett and Anjou samples were prepared by dipping in the solution containing 100 ppm 4-HR, 0.5% AA and 1.0% calcium lactate for 2 min, draining for 1 min, vacuum packaging in plastic pouches and stored at 2-4°C for sensory analysis the following morning. Control samples were prepared just before sensory tests by dipping pear slices into drinking water for 2 min and draining for 1 min.

Sensory tests

The “Overall-Liking” and “Difference-from-Control” tests were conducted in the sensory science laboratory in the Department of Food science and Technology at Oregon State University. For each variety, “Overall-Liking” test was first conducted with

monodic sample presentation, and followed by the “Difference-from-Control” test. Tests were conducted under red lights to mask possible color differences between samples and controls. Samples and controls were coded with random 3 digit numbers and served in a randomized order. A total of 70 panelists participated in the tests and 68 valid ballots were collected. The ballots for “Overall-Liking” and “Difference-from-Control” tests are presented in appendices 1 and 2.

Statistical analysis

The ballots were decoded and the data was submitted to Analysis of Variance and Turkey multiple comparison with significant level of $P < 0.05$, by using SAS (Statistic Analysis System) for Windows software.

Results and Discussion

Browning Inhibition in Sliced Pears

Effect of Semperfresh treatments on browning inhibition

Anjou pear slices treated with Semperfresh solutions had less browning than those treated with distilled water. However, brown color developed within one day after dipping treatment and the color was visually not acceptable as the storage time continued (Fig. 3.1). Meanwhile, color of slices treated with 0.01% 4-HR and 0.5% AA was significantly better than that treated with Semperfresh and the color was similar to fresh one. There were no significant color differences among the four Semperfresh concentration treatments. However, as higher concentrations of Semperfresh were

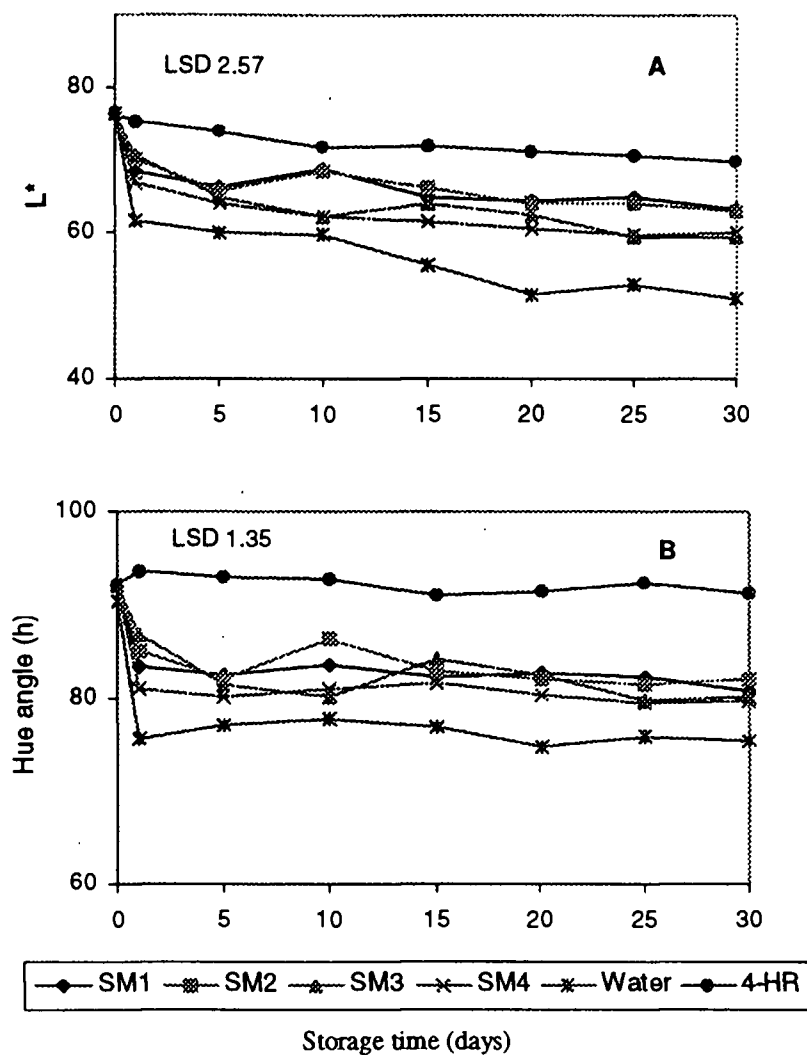


Fig. 3.1 —Changes of L^* (A) and hue angle (B) of Anjou pear slices dipped in the solutions of 0.5% AA and 1.0% calcium lactate combined with various concentrations of Semperfresh (SM), and stored at 2-4°C for 30 days. SM1=0.2%; SM2=0.5%; SM3=1.0%; SM4=1.0% SM alone. 4-HR and water were the controls. Pooled LSD is shown.

applied as dipping solutions (0.5% and 1.0%), white precipitation was observed on the surface of some pear slices. Similar observations were obtained with Bosc pear slices.

Effect of Snow Fresh treatment on browning inhibition

1.0% Snow Fresh dipping treatment did not inhibit browning of Bosc pear slices. Color values of L^* and h of Anjou pear slices treated with 1.0% Snow Fresh were significantly ($p < 0.05$) higher than those treated with distilled water. However, brown color developed even within one day after the dipping treatment (Fig. 3.2).

Snow Fresh is a mixture of calcium chloride, sodium acid pyrophosphate, citric acid and L-ascorbic acid. Rosen and Kader (1989) advised that ascorbic acid and/or citric acid were not effective for controlling browning of fresh-cut pears. It was reported that the combination of ascorbic acid and citric acid was only effective on apples and potatoes immediately after slicing, losing their effectiveness during storage (Molnar-Perl and Friedman, 1990).

Effect of cysteine treatments on browning inhibition

Anjou pear slices treated with 0.2% cysteine and 0.5% AA had continuous decreasing L^* and h values and increasing a^* values with storage time (Fig. 3.3). Cystiene (0.5%) and 0.5% AA effectively maintained the initial fresh color for 25 days. Bosc pear slices behaved similarly to Anjou. Slices treated with 0.01% 4-HR and 0.5% AA had the most acceptable color of all treatments over the 30 days.

Although 0.5% cysteine treatment maintained acceptable color for as long as 25 days for both Anjou and Bosc, McEvily and Iyengar (1992) reported that the

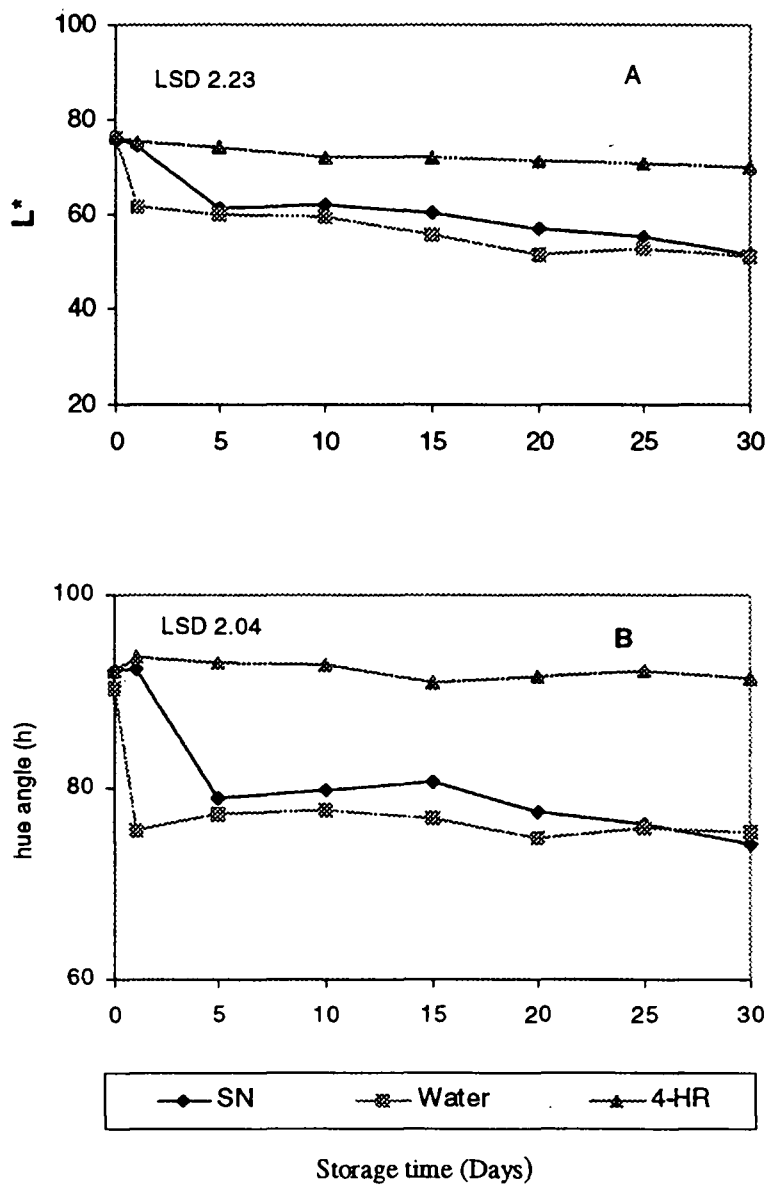


Fig. 3.2 —Changes of L* (A) and hue angle (B) of Anjou pear slices dipped in the solution of 1.0% Snow Fresh (SN) and stored at 2-4°C for 30 days. 4-HR and water were the controls. Pooled LSD is shown.

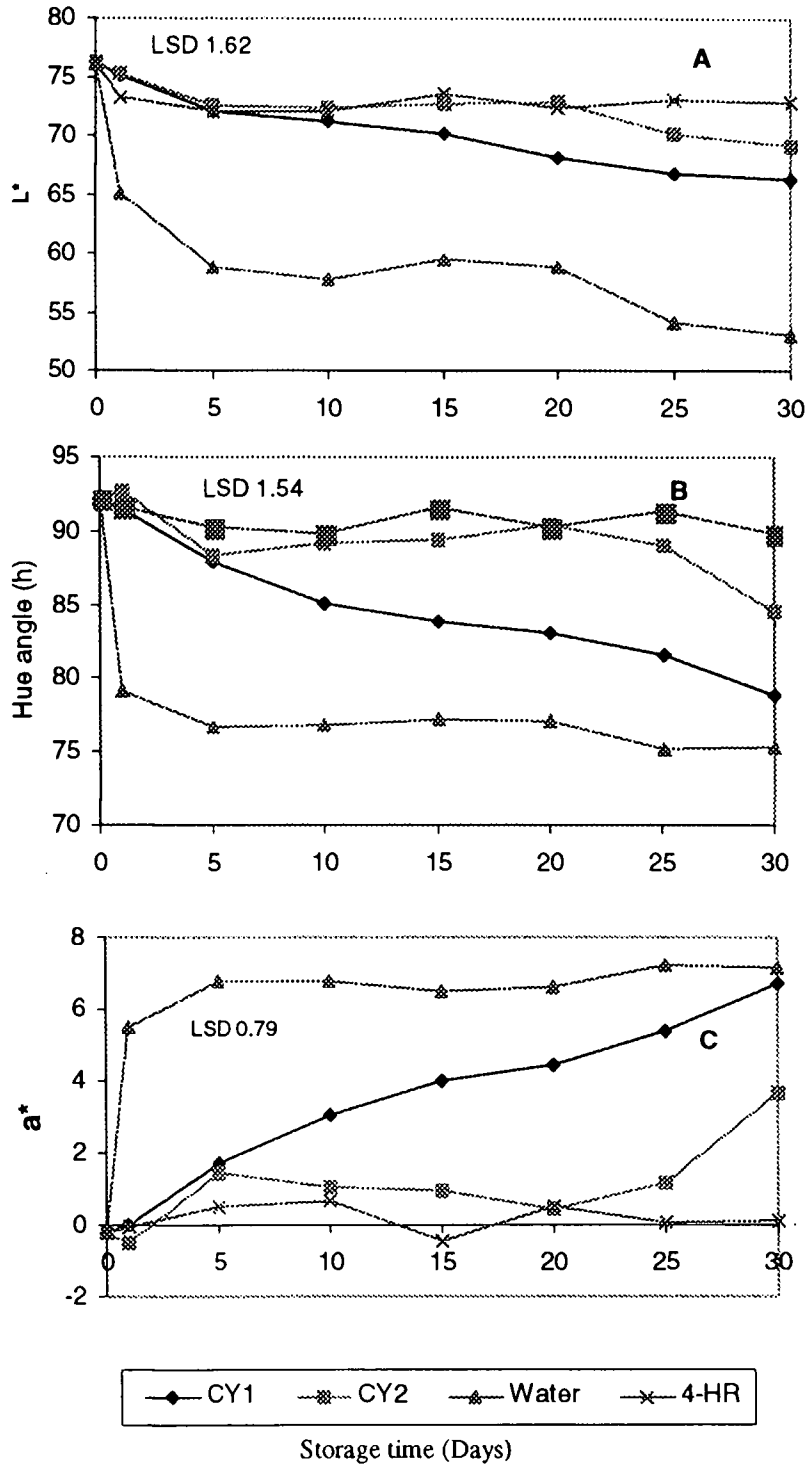


Fig 3.3 —Changes of L* (A), hue angle (B) and a* (C) of Anjou pear slices dipped in the solutions of 0.5% AA, 1.0% calcium lactate with cysteine 0.2% (CY1) or 0.5% (CY2)

concentrations of cysteine necessary to achieve acceptable levels of browning inhibition gave undesirable taste on the treated foods.

Effect of storage temperature on texture and color maintenance

Anjou and Bosc pear slices stored at 0°C had significant firmer texture than those stored at 2-4°C (control) (Fig. 3.4). No color benefit was obtained, however, with 0°C storage. The slices stored at 1.1°C and 2.2°C did not differ significantly in texture with those stored at 2-4°C. Since Anjou and Bosc pears used for 0°C treatment had a softer initial texture than those used for 1.1°C and 2.2°C treatments, it was speculated that pear slices with a relatively softer initial texture might have a stricter storage temperature requirement than those having a firmer initial texture. Pear slices with a firmer texture might not be influenced as much by small storage temperature differences and be able to accommodate a wider temperature range.

4-HR Residual Content Analysis

The extraction recovery

The extraction recovery of 4-HR from spiked samples ranged from 84 to 96% (Table 3.1) with an average recovery of 90% from three replicates.

Determination of 4-HR residual content in pear slices

4-HR concentrations of 50 ppm and 100 ppm, with dipping time of 1 and 2 min were used for pear slice dipping treatments since they were found to be effective in the previous experiment (Dong et al., 1998). A major objective was to determine the minimum 4-HR residual that would also provide an acceptable shelf life. We observed

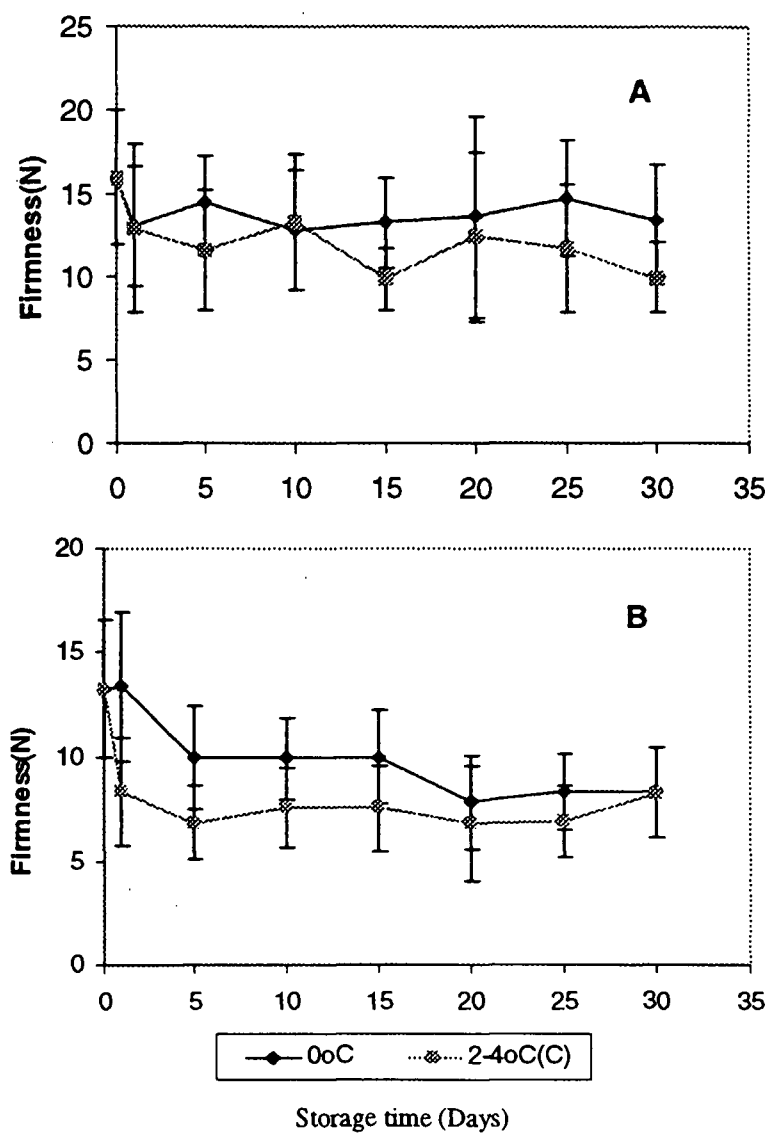


Fig. 3.4 —Changes of firmness values with storage time under 0°C and 2-4°C (control) storage temperatures. A — Anjou; B — Bosc.

Table 3.1 —Extraction recovery of 4-HR from treated pear slices.

Replicate	Conc. Of 4-HR spiked	% of Recovery
1	4 ppm	89.6
	8 ppm	86.6
	16 ppm	84.1
2	4 ppm	93.4
	8 ppm	88.6
	16 ppm	88.3
3	4 ppm	96.5
	8 ppm	91.1
	16 ppm	91.1
Average		89.9

that all 4-HR treatments gave a visually acceptable appearance for up to 14 days at 2-4°C storage. Good separation and resolution of 4-HR was obtained with HPLC under the condition used (Fig. 3.5), and the spectrum (Fig. 3.6) was used to confirm its identity. The residual content of 4-HR at different treatment conditions is shown in Fig. 3.7. The amount of 4-HR in slices treated with 50 ppm for 2 min was 6.5 ppm at storage time 0 day, and increased to 16.0 ppm when the dipping concentration of 100 ppm was applied. The residual of 4-HR also increased as the dipping time was increased from 1 to 2 min for both concentrations. However, increase of 4-HR dipping concentration was the major factor for increase in residual content.

The residual 4-HR content decreased with storage time for all the treatments (Fig. 3.7). It was speculated that the 4-HR absorbed on the surface might be consumed as an active browning inhibition agent which could interact with PPO or phenolics on the surface or inner tissue during the storage.

Sensory Evaluation

Results of sensory tests of “Overall-Liking” and “Difference-from-Control” for both Anjou and Bartlett are presented in Table 3.2. Panelists detected differences between treated samples and controls for both Bartlett and Anjou with “Difference-from-Control” tests ($P < 0.05$). However, there was no significant difference in preference between the sample and the control for Anjou pears from “Overall-Liking test”. The score of 5.04 and 4.59 indicate that panelists neither liked nor disliked the sample and the control. For Bartlett pears, the “Overall-Liking” test indicated that there was significant difference in acceptance between the control and the 4-HR treated sample. Bartlett and Anjou pears were only partially ripened for mechanical slicing purpose, panelists

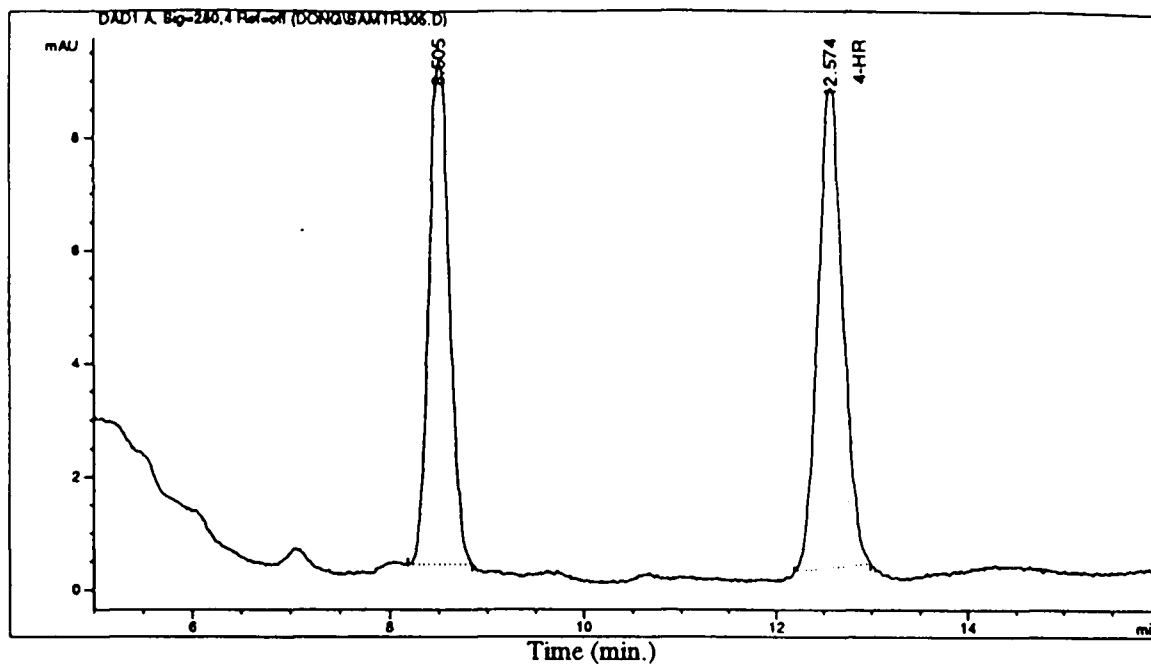


Fig. 3.5 —4-HR HPLC chromatogram. Supeclosil LC-18, 250 × 4.6 mm i.d. column. Solvent A: HPLC grade methanol; B: deionized water, run isocratically at 60% A for 18 min. Flow rate: 1 mL/min. Injection volume: 50 μ l.

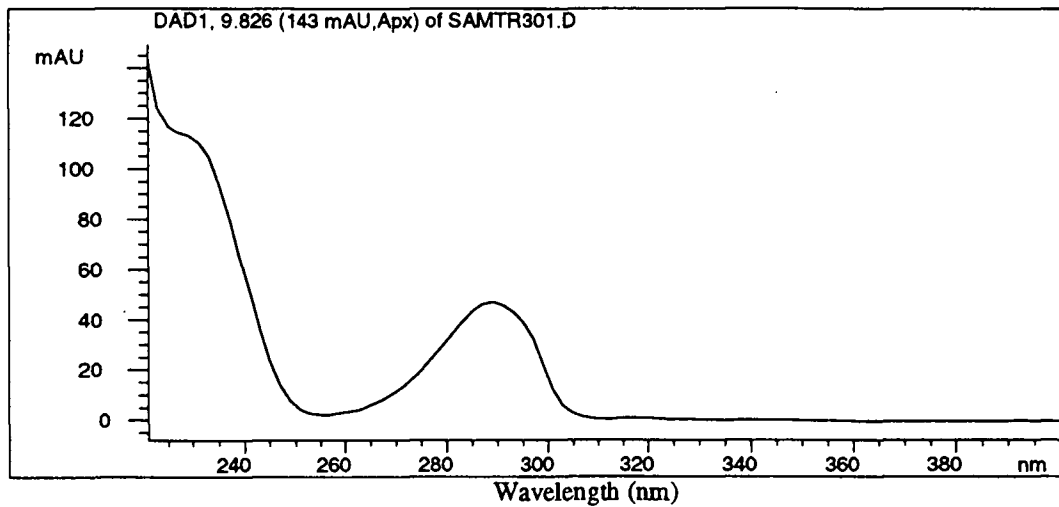


Fig. 3.6 —4-HR standard absorption spectrum. Same HPLC conditions as for Fig. 3.5.

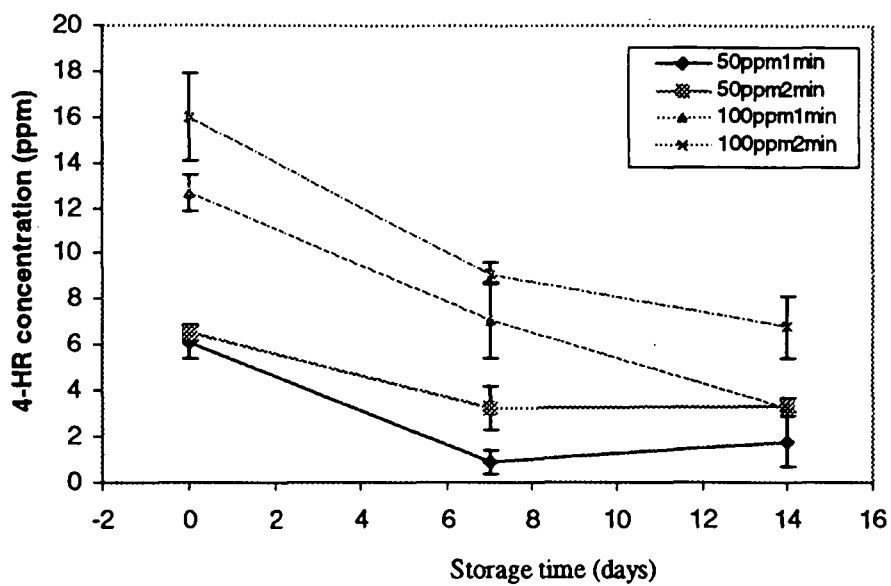


Fig. 3.7 —Residual content of 4-HR in pear slices stored at 2-4°C

Table 3.2 —Mean scores of sensory tests of “Overall-Liking” and “Different-from-Control”.

Varieties	Overall-liking Test		Different from Control test	
	Control	Sample	Blind control	Sample
Anjou	5.04 ^a	4.59 ^a	2.33 ^b	4.67 ^a
Bartlett	5.63 ^a	4.53 ^b	2.42 ^b	5.64 ^a

Means with different superscript are significantly different ($p < 0.05$) for same variety with same test.

commented that the texture was “woody” or “hard” and the pears “lacked flavor”. There was also considerable variation on texture from pear to pear, and even noticeable differences within a pear. Those could account or contribute to the low “Overall-Liking” scores given by panelists.

Conclusions

Cysteine (0.5%) with 0.5% ascorbic acid effectively inhibited pear slices browning for 20 to 25 days. Semperfresh concentrations of 0.2, 0.5 and 1.0% combined with 0.5% ascorbic acid, or 1.0% Semperfresh alone, and 1.0% Snow Fresh were not effective for inhibiting browning in both Anjou and Bosc fresh-slices. A solution of 0.01% 4-HR, 0.5% ascorbic acid and 1.0% calcium lactate was the most effective treatment on pear slices browning inhibition for all the solutions tested. Pear slices stored at 0°C had a firmer texture than those stored at 2-4°C. 4-HR residual content increased with increasing dipping time and 4-HR dipping concentration, with the latter one being the major contributor. The residual content of 4-HR decreased with storage time for all treatments. Sensory tests on 4-HR treated pears indicated that panelists detected overall difference between treated sample and the control for both Bartlett and Bosc. There were no significant differences in preference between 0.01% 4-HR treated sample and the control for Anjou pears. For Bartlett, however, panelists liked the control more than the 0.01% 4-HR treated sample.

Acknowledgment

We thank the Winter Pear Control Committee for funding this research. Thanks to Bob Durst for his technical support. We are grateful to M. Monica Giusti and Luis E. Rodriguez-Saona for their interest, suggestion and revision of the paper. We appreciated Cindy Lederer's suggestion and helps that made the sensory tests to run smoothly. Our thanks go also to David Lundale and Mina R. McDadiel for their advice to the sensory evaluation.

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APPENDICES

"Overall-Liking" Test on Fresh Sliced Pears

Panel # _____ Date _____

Sample _____

Overall evaluation: Please mark the scale which best describe how you like this product.

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Like Extremely				Neither like nor dislike				Dislike extremely

Comments: _____

Appendix 1. The Ballot for "Overall-Liking" Test

Difference-From-Control Test

Panel # _____ Date: _____

Type of Sample: Fresh Sliced pears**Instructions**

1. Taste the sample marked "Control" first.
2. Taste the samples marked with the three digit codes.
3. Assess the overall sensory difference between each sample and the control using the scale below.
4. Circle the scale to indicate the size of the overall difference from the control.

Sample1: _____

	Scale
No difference	0
	1
	2
	3
	4
	5
	6
	7
	8
Extremely different	9

Comments: _____
