THE EFFECTS OF SELECTED ANTIBROWNING AGENTS, SELECTED PACKAGING METHODS, AND STORAGE TIMES ON SOME CHARACTERISTICS OF SLICED RAW POTATOES

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CHAPTER I

INTRODUCTION

Research Problem

Sulfiting agents have been used as food preservatives for literally centuries. There are references in ancient Egyptian, Greek and Roman writings to the use of SO_2 to sanitize wine vessels (Roberts and McWeeny, 1972). When the term sulfiting agent is used, it refers to sulfur dioxide (SO_2) or the several forms of inorganic sulfite which liberate SO_2 under conditions of use in food products (Taylor, *et al.*, 1986). These inorganic sulfite compounds are sodium bisulfite ($NaHSO_3$), potassium bisulfite ($KHSO_3$), sodium sulfite (Na_2SO_3), potassium metabisulfite ($K_2S_2O_5$) and sodium metabisulfite ($Na_2S_2O_5$). For nonalcoholic food products, these agents were probably first used in processing dried fruits and vegetables (Taylor and Bush, 1986). Their use in food processing increased rapidly because of the apparent absence of toxicity and their functional effectiveness. A 30-70% increase in the use of several sulfiting agents was reported for the decade between 1960 and 1970 (Subcommittee on Review of the Generally Regarded as Safe (GRAS) List, 1972). Sulfiting agents serve many functions in food products such as inhibition of nonenzymatic

browning, inhibition of enzymatic reactions including browning, antimicrobial agents, antioxidants or reducing agents, and bleaching agents (Taylor, *et al.*, 1986).

Significance of the Problem

Sulfites have been used for many years on fresh, pre-cut potatoes and potato products to prevent discoloration and to extend storage life (Lecos, 1986). Since 1959, the Food and Drug Administration's(FDA) 'generally regarded as safe'(GRAS) list has included the sulfites for use in foods (Lecos, 1988). Increased concern about the safety of sulfites as food additives has resulted in actions taken by the Food and Drug Administration and the food industry to find appropriate alternatives for the functions served by added sulfites in foods (Langdon, 1987).

In August, 1985, the FDA issued a proposed regulation which would revoke the GRAS status of sulfites used on raw produce. The effect would be to ban the use of sulfites on fruits and vegetables intended to be eaten raw. This regulation would impact restaurants and other food service outlets as well as applying to raw produce sold by supermarkets and grocers. At the same time, the FDA was considering a regulation dealing with the extensive use of sulfites in processed potato products, and most packaged potato products used in restaurants were treated with sulfites (Dziezak, 1986). On August 8, 1987, the FDA issued the final rule on the revocation of GRAS status for the use

of sulfiting agents on fruits and vegetables intended to be served or sold raw to consumers (FDA, 1986). Potatoes were not included in this ban but would remain under investigation by the FDA (Langdon, 1987). On April 16., 1990, the FDA amended the regulations on the use of sulfiting agents on fresh potatoes that are intended to be served or sold unpackaged and unlabeled to the consumer. This final ruling revoked the GRAS status of the use of sulfites on fresh fruits and vegetables intended to be served or sold to the consumer raw (FDA, 1990). The challenge becomes finding alternatives to the sulfiting agents. Possible alternatives are usually less effective and more expensive. At present, ascorbic acid appears to be the best alternative to the sulfites(Langdon, 1987).

Description of Experiment

The objective of this experiment was to determine the effects of selected antibrowning agents, packaging methods, and storage time on degree of browning, total phenol content, polyphenol oxidase(PPO) activity, ascorbic acid and sugars of sliced raw potatoes. The fresh and processed produce market would benefit from an economical and chemically stable alternative to sulfiting agents as an anti-browning treatment for these foods.

Approximately 50 kg of potatoes (Russet Burbank) were obtained from a local food store. The potatoes were hand peeled under deionized water and mechanically sliced, using a

Rival commercial slicer, into 1 cm thick slices. The potato slices were divided into 7 equal portions. Each portion was dipped in a treatment solution for 10 minutes. The treated slices were divided equally and randomly, and packaged either with or without vacuum in polyolefin film and stored at 10°C. Samples from each treatment were randomly obtained at days 1, 8, 15 and 22. Changes in color, PPO activity, total phenols, ascorbic acid and sugars were measured to evaluate the effectiveness of antioxidant and chelating agents as chemically stable alternatives for sulfiting agents on raw potato products.

All treatments were prepared in two trials arranged in a randomized complete block design where each block represented one trial (Cox, 1958). The data were analyzed using the analysis of variance (ANOVA) for a split plot with the whole units in a randomized complete block design. The whole unit treatment factor was represented by the treatment solutions and the sub-unit treatment factor by storage times and packaging methods. The least significant difference (LSD) was used to compare pairs of means (Steel and Torrie, 1980).

Hypothesis

The hypothesis tested was that there were no effects of selected antibrowning agents, packaging methods and storage time on the degree of browning, total phenol content, PPO activity, ascorbic acid or sugar content of sliced raw potatoes stored at 10°C.

Format of Dissertation

The body of this dissertation is written in journal format to conform with the requirements of the Journal of Food Science.

CHAPTER II

REVIEW OF LITERATURE

Phenolic Compounds and Phenolases

In recent years, reports of asthmatic episodes in a number of patients have implicated sulfites ingested in foods and drugs (Kochen, 1976; Allen and Collett, 1981; Stevenson and Simon, 1981a & b). Several of these reports provide a history of asthmatic reactions to foods suspected of containing sulfite residues. Salad bar items and fresh fruits and vegetables account for almost half of all complaints of sulfite reactions reported to the Food and Drug Administration(FDA); potato products are responsible for about 12 percent of the complaints (Lecos, 1986).

The phenolic compounds in hotircultural food products can occur as small monomeric acids (tyrosine), or as large polymeric phenols (chlorogenic acid). The functional group in phenolic compounds is a benzene ring with one or more hydroxyl groups attached to carbon atoms at various positions. The most important naturally occurring polyphenols are catechins, cinnamic acid esters, 3,4-dihydroxyphenylalanine (DOPA) and tyrosine. Chlorogenic

acid, derived from a cinnamic ester, is the most widespread natural polyphenol (Vamos-Vigyazo, 1981).

The phenolic compounds found in raw potatoes are colorless but when potatoes are cut or peeled and exposed to air, they are involved in various discoloration reactions. In potatoes tyrosine, chlorogenic acid, and caffeic acid have been found to be involved in pigment formation resulting from enzyme action. The typical red-brown color observed in vivo in potatoes is probably produced by tyrosine. Thus, the discoloration of raw potatoes is considered essentially a tyrosine reaction (Vamos-Vigyazo, 1981). Chlorogenic acid is concentrated in the outer tissues of the potato whereas tyrosine is more generally distributed throughout the tuber. Both of these polyphenols are alcohol-extractable (Talburt, et al., 1987).

The discoloration of potatoes is carried out by a group of enzymes known as oxidoreductases, more specifically polyphenol oxidases. They utilize molecular oxygen and have a pair of copper ions as part of their functional group (Vamos-Vigyazo, 1981). There are several names commonly used for these enzymes, referring to the principal substrates involved, such as tyrosinase and catechol oxidase. For this discussion, the enzyme of primary interest is o-diphenol oxidase, and it will be referred to as polyphenoloxidase (PPO).

Almost all discoloration in plants involves PPO and the polyphenolic compounds. Browning in potato tissue has been

correlated with PPO activity and the concentration of PPO substrates (Sapers, Douglas, *et al.* 1989). Mondy, *et al.* (1985) observed that browning of potatoes is related to PPO activity, phenolic content and ascorbic acid content. Furthermore, in comparisons of browning and composition data, Sapers, Douglas, *et al.*(1989) found significant correlations between browning parameters and composition factors of the potato cultivars studied. Enzymatic browning in raw potatoes is correlated with tyrosine turnover which depends on the concentrations of PPO, tyrosine, chlorogenic acid and ascorbic acid rather than any one factor (Amiot, *et al.*, 1992). Browning occurs when the substrates, PPO enzymes and oxygen are together under appropriate conditions of pH, temperature and water activity (Hsu, *et al.*, 1988). The browning is accelerated when plant tissue is damaged in some way (Joslyn and Ponting, 1951). Indeed, peeling changes the potato from a relatively stable vegetable with a shelf life of many months to a highly perishable vegetable which must be kept chilled to hold up for two weeks (Feinberg, 1987). Bruising, cutting, peeling or other cellular disruption promote enzymatic browning in fruits and vegetables. For fresh or processed fruits and vegetables this represents important economic impact because of the undesirable colors which are produced during discoloration (Hsu, *et al.*, 1988). The discoloration of injured plant tissues containing high levels of phenolic compounds, collectively known as o-diphenols, is principally due to oxidation of these colorless

compounds to o-quinones which range in color from reddish-brown to black. O-quinones are very reactive, will readily polymerize and react with o-phenols and O_2 (Rouet-Mayer, *et al.* 1990). The formation of o-quinones is reversible, but subsequent polymerization reactions leading to melanin formation are not (Joslyn and Ponting, 1951).

The PPO enzyme complex is involved in two different reactions: 1) the hydroxylation of monohydroxyphenols to o-dihydroxyphenols, and 2) the oxidation of o-dihydroxyphenols to o-quinones (Mathew and Paroia, 1971). The oxidation of tyrosine to DOPA is an example of the first reaction. The further oxidation of DOPA to a quinone which rapidly undergoes cyclization and further oxidation and condensation to melanin, is an example of the second reaction (Haisman, 1974). Both of these reactions require the presence of copper ions in the enzyme. Removal of the copper ions from the enzyme leads to inactivation or reduction of activity which can be restored by the addition of copper salts (Vamos-Vigyazo, 1981). PPO also can become inactivated during the second reaction due to the o-quinones forming a covalent linkage with PPO near the active site (Vamos-Vigyazo, 1981). Enzymatic reactions such as these involving PPO are dependent on substrate concentration, pH of the medium and availability of oxygen. However, in practice, controlling these factors can be difficult during storage and processing of food products (Mathew and Paroia, 1971).

PPO activity and polyphenol content are not distributed uniformly in potato tubers (Sapers, Douglas, *et al.*, 1989). Substrate concentration and enzyme activity are highest in the eye and peel. During storage, PPO activity and polyphenol content increased in the outer parts of the tuber and chlorogenic acid content increased in the inner parts of the tuber (Vamos-Vigyazo, 1981).

Enzymatic Browning and Its Control

Discoloration in raw potatoes does not occur in normal, uninjured tissue. However, when the potato tuber is mechanically manipulated through peeling or cutting during processing, discoloration occurs as the phenols are rapidly converted to melanins (Vamos-Vigyazo, 1981). In this reaction the concentration of tyrosine appears to be a major factor (Mapson, *et al.*,1963). The intermediate compounds in this conversion of tyrosine to melanin include DOPA, dopaquinone, and dopachrome.

Enzymatic browning can be inhibited by any process that destroys or inactivates the enzyme (Taylor,*et al.*, 1986). There are a number of conditions or chemical treatments which have been used or proposed for use to retard or prevent this type of deterioration in raw potatoes: 1) the use of heat, acid, and/or low temperatures to retard enzymatic action or to inactivate the enzyme altogether; 2) the use of competitive inhibitors which specifically compete with polyphenolic substrates for the enzyme's active site; 3) the

use of chelating agents that react with the enzyme-bound copper; and 4) the use of reducing agents to oxidize the o-quinones to o-diphenols thus preventing the formation of melanin through polymerization (Talburt, *et al.*, 1987).

Blanching has been used as a method for preventing enzymatic browning (Mathew and Paroia, 1971). Heat will readily inactivate PPO. Blanching peeled ripe bananas in boiling water for 11 minutes resulted in inactivation of 96-100% of PPO (Cano, *et al.*,1990). This was a drastic treatment and produced an overcooked banana product. Thermotolerance of PPO depends on substrate specificity, on pH and temperature optima for activity as well as on the concentration of the enzyme. PPO however, is not noted for heat stability. In most cases, short exposure of tissues to temperatures of 70-90°C, is sufficient for partial or total irreversible inactivation of the enzyme (Cano, *et al.*,1990). Heat stability of PPO has not been studied as extensively in vegetables as it has been in fruits (Vamos-Vigyazo, 1981). In potatoes, PPO activity reaches maximum levels with the substrate cathechol at 22°C (Mathew and Paroia, 1971). The efficiency of thermal inactivation of PPO in plant tissues is directly related to heat penetration, which in turn is influenced by the size and shape of the particles (Vamos-Vigyazo, 1981). Rodriguez and Zaritsky (1986) described the uptake of sulfur dioxide by peeled potatoes of different geometry and size as a function of: 1) the product physical properties, and 2) the industrial operating parameters

(concentration of dipping solution and sample rectilinear velocity). Effects of sodium bisulfite solution concentration, immersion time, size, shape, dry matter, density and velocity were analyzed in terms of three potato shapes (spheres, cubes and parallelepipeds). The results showed that mathematical modeling could adequately describe the uptake of the treatment solution by the potatoes. This information can be applied to the optimization of the treatment under industrial conditions.

Sugar Content Of Potatoes

Sucrose, fructose and glucose are the major sugars found in potatoes (Smith, 1987). The two main factors which influence sugar content of potatoes during postharvest handling are cultivar and temperature. The Russet Burbank cultivar has a high specific gravity and tends to accumulate less sugar than cultivars with lower specific gravity (Talburt, *et al.*, 1987). Of the major sugars, fructose is the most responsive to changes in storage temperatures. Tubers stored at low temperatures (4°C) were high in fructose content. At 10°C storage, reducing sugars may increase from 0.2 to 1.0% in 3 days (Smith, 1987). At storage temperatures below 10°C, both total and reducing sugars increase, with the rate and extent of increase being greater as the temperature approaches the freezing point. During the early stages of storage at low temperatures sucrose appears to accumulate most rapidly (Talburt, *et al.*, 1987). The storage atmosphere

has an effect on the reducing sugar content of potatoes. Glucose concentration of potatoes held at 4.4°C in an atmosphere of high-CO_2 and low O_2 concentrations was much lower than potatoes stored in air (Smith, 1987).

Storage Temperature

During storage at 10°C ascorbic acid content decreases while at the same time the fructose and glucose content increases (Smith, 1987). However, for sucrose , the relationship is very different. A high correlation exists between ascorbic acid and sucrose content in potatoes. The reduction in sucrose content closely parallels the loss of ascorbic acid. One possible explanation for this relationship is that sucrose and ascorbic acid are formed from a common precursor, D-glucose (Smith, 1987).

Cold storage also has an effect on enzymatic browning in potatoes. At 4.4°C, the rate of browning increased for the first 100 days and then remained constant compared to potatoes stored at room temperature (Mapson, *et al.* 1963). Discoloration of potatoes increased with length of storage and was accompanied by an increase in phenolic content and a decrease in PPO activity (Mondy, *et al.*, 1966).

The use of low temperatures (0-5°C) to retard discoloration of sliced apples and potatoes was effective when combined with N-acetyl-L-cysteine and reduced glutathione (Molar-Perl and Friedman, 1990). At refrigeration temperatures, the treatment combination of 1.0%

ascorbic acid and 0.1% calcium (as $CaCl_2$) was very effective in retarding discoloration in sliced apples (Ponting, *et al.*, 1972). Chilled potato strips showed no discoloration for at least 2 weeks when vacuum packaged after ascorbic acid treatment (O'Beirne and Ballantyne, 1987).

Alterations In pH

Alterations of the pH affect PPO activity. The optimum activity for most of the PPOs is achieved at a pH of 6.0-7.0 (Aurand, *et al.*,1987). Vamos-Vigyazo (1981) reported that in potato tissue PPO is practically inactive at a pH <5.0. There can be changes in the form of the pH curve as a result of treating the enzyme with denaturing agents, elevating temperature or exposing briefly to acids (Mayer and Harel, 1979). In studies on apples, two pH optima(5.2 and 3.7)were observed. The activity at pH 5.2 was greater than that of pH 7.3 (Shannon and Pratt, 1967). In work done with apple juice,the activity of PPO was reduced to 34% and 98% after 20 minutes of adjusting the pH values to 2.5 and 2.0, respectively. The higher pH resulted in slower inactivation; the pH 2.0 sample showed almost complete inactivation after 20 minutes. After 24 hours, PPO levels had not significantly changed in any of the apple juice samples, thus showing the irreversibility of the inactivation of PPO with low pH (Zemel, *et al.*, 1990). When apple juice was acidified with HCl to pH 2.0 for 45 minutes and subsequently raised to the original pH of 5.0 with NaOH, the PPO activity was decreased

by 88% (Zemel, *et al.*, 1990). In work on strawberries, the optimum pH for maximum activity of PPO was pH 4.5 with the phenolic compound cathechol as substrate. Beyond pH 5.5 oxygen uptake increased from autoxidation of the substrate in addition to the remaining PPO (Wesche-Ebling and Montgomery, 1990).

PPO Inhibitors

The use of PPO inhibitors to control discoloration has been explored. In a study done with apples, phenolic acids were used to inhibit PPO. It was found that the cinnamic acid structure and the benzene nucleus were essential for inhibition of the enzyme (Walker and Wilson, 1975). Benzoic acid has been very effective in retarding browning in apples and combinations of benzoic acid and sodium ascorbate were even more effective. The primary effect of the combination treatments was an increase in lag time prior to the onset of browning (Sapers, Hicks, *et al.*, 1989).

Cinnamic acid, as sodium cinnamate, was effective in inhibiting browning in Granny Smith apples (Sapers, Hicks, *et al.*, 1989). Walker and Wilson (1975) demonstrated that sodium cinnamate added to Granny Smith apple juice retarded browning for over 7 hours. The long term stability of the inhibitory effects of cinnamic acid, in low concentrations, compared favorably with ascorbic acid as a treatment to prevent browning. Cinnamic acid functions either competitively or non-competitively to inhibit PPO, depending

on the substrate. Furthermore, the combination of cinnamic and ascorbic acids in dipping solutions was more effective in browning inhibition than ascorbic acid alone. Cinnamic acid may undergo conversion to p-coumaric acid by cinnamate-hydroxylase as well as by other enzymes involved in polyphenol biosynthesis. P-coumaric acid is a PPO inhibitor which may be hydroxylated further to caffeic acid, a substrate for PPO. Therefore, the use of cinnamic acid as a PPO inhibitor is not recommended because of the possibility that it may induce browning (Sapers, Hicks, *et al.*, 1989).

Ascorbic Acid

Ascorbic acid action on PPO is complex; it reduces o-quinones to o-diphenols (Vamos-Vigyazo,1981) and chelates the copper in the enzyme (Borenstein,1965). Ascorbic acid, as well as sodium metabisulfite, acts by reducing the quinones formed by PPO action back to polyphenols while they are themselves oxidized (Augustin, *et al.*,1985). It also inhibits the enzyme competitively by direct oxidation (Cort, 1982). The effectiveness of ascorbic acid as a PPO inhibitor is dependent on its concentration. Ponting *et al.* (1972) found 1.0% ascorbic acid and 0.1% $CaCl_2$ very effective in preventing discoloration of allpe slices. At low concentrations the formation of colored polymers is prevented for a limited time because the ascorbic acid has been quickly consumed (Liao and Seib, 1988). At high concentrations, ascorbic acid reduces the o-quinones formed to furnish new

substrate (o-diphenols) for PPO. As a result, ascorbic acid in high concentrations may permit long term protection against browning (Vamos-Vigyazo, 1981).

Ascorbic acid is very unstable and alternatives have been investigated as possible anti-browning agents. In studies conducted on apples (Sapers and Douglas, 1987; Sapers and Ziolkowski, 1987), the effectiveness of ascorbic acid and erythorbic acid (isoascorbic acid) in inhibiting enzymatic browning on cut apple surfaces was compared. Comparisons indicated that ascorbic acid was consistently more effective than erythorbic acid on cut apple surfaces. However, the use of the more stable ascorbic acid derivatives as anti-browning agents, substituted for the sulfiting agents, should be investigated with a variety of foods. In studies conducted on fresh mushrooms (Hsu, *et al.*, 1988), a number of ascorbic acid derivatives were tested as inhibitors of mushroom PPO. Ascorbic acid and isoascorbic acid were more effective than dehydroascorbic acid in PPO inhibition. Erythorbic acid is a strong reducing agent and its use in combination with citric acid as an alternative to sulfites has been proposed. This combination has been used to retard browning on salad vegetables and fresh apple slices (Dziezak, 1986).

In a study conducted by Santerre *et al.* (1991), whole abrasion-peeled potatoes were treated with a solution of erythorbic acid and packed in a citric acid solution. The combined treatments proved to be effective in reducing enzymatic browning. However, the added weight of the packing

solution could be viewed as a disadvantage of this method of pre-treatment of raw potatoes.

In another study using apples and potatoes, Sapers, *et al.*, (1990) explored the efficacy of using vacuum or pressure as a means of applying anti-browning treatments. There was apparently no advantage in using pressure infiltration for applying the inhibitors to 3/8 in. potato dice. The suggestion was made that this process combined with vacuum packaging may be effective in preventing browning of potatoes. Vacuum infiltration was not very successful on apples, nor did it result in any improvement in storage life at 4°C for potatoes.

Sulfites

The effect of sulfite on PPO is also complex. The mechanism of action of sulfites in preventing browning very likely involves several types of reactions (Joslyn and Braverman, 1954). Sulfites may directly inhibit the enzyme. When PPO was exposed to sulfite prior to substrate addition, inhibition of the enzyme was irreversible. A PPO-SO_3 complex probably could have occurred due to the interaction between sulfite and PPO, resulting in the formation of inactive PPO (Sayavedra-Soto and Montgomery, 1986). Sulfites may also interact with the intermediate o-quinones in the browning reaction and prevent their participation in the polymerization reactions. The sulfite is gradually consumed

in the quinone-coupling process; thus its action depends on concentration of the enzyme (Vamos-Vigyazo, 1981).

The sulfites may also act as reducing agents promoting the reaction which changes o-quinones back to the original phenols (Embs and Markakis, 1965). In the past, the most common commercial method of inhibiting enzymatic blackening of peeled potatoes was the use of sodium bisulfite or the combination of bisulfite and citric acid (Feinberg *et al.*, 1967). Bisulfite alone is an effective inhibitor of tyrosine oxidation but considerably less effective for inhibiting oxidation of 3,4-dihydroxyphenylalanine, the first product of tyrosine oxidation (Muneta, 1966). Furthermore, the use of a sodium bisulfite-citric acid treatment greatly increased the absorption of SO_2 as compared to sodium bisulfite alone (Ross and Treadway, 1961).

In another study, Francis and Amla (1961), observed that increasing the immersion times of peeled potatoes from 10 seconds to 10 minutes had no effects on the quantity of SO_2. They also observed that the abrasion-peeled potatoes had higher SO_2 residues than the hand-peeled potatoes, because the former had a rougher surface and thus a greater absorption surface area. However, Furlong (1961) observed that the uptake of metabisulfite increased both with concentration of the solution and the time of soaking. He also observed that the rough surface enhanced absorption. The level of sulfites required to prevent browning is dependent on the substrate available. When only monophenols

are present such as tyrosine in potatoes, low levels of sulfite are effective (Taylor, Higley, and Bush, 1986). Embs and Markakis (1965) found the presence of sulfite resulted in the appearance of a lag period in the development of browning. Their observations further suggested the formation of compounds between sulfite and the intermediary enzymatic browning products. In dried fruits, the concentration of absorbed SO_2 is a function of the concentration of sulfite in the treatment solution, the treatment time and the pH of the solution (Stafford *et al.*, 1972). Another consideration in determining the fate of sulfites in foods is the method of processing. Sulfites can be lost as SO_2 if the pH of the product drops below 4.0, particularly if the product is heated. Storage is another consideration in determining the fate of sulfites in foods and almost always diminishes the amount of inorganic sulfite or free SO_2 in the product. In dehydrated potatoes, 46-68% losses in residual sulfite occurred within 24 weeks of storage at 24°C, depending on the use of cans or cartons (Lisberg and Chen, 1973).

Amla and Francis (1961) treated hand-peeled potatoes with sodium bisulfite solutions ranging in concentration from 0-4000 ppm available sulfur dioxide, with varying pH levels, and storage temperatures (0-1°C or 4-5°C). They found the optimum pH for the dipping solutions to minimize discoloration was in the range of 5.5 to 6.5.

Chelating Agents

Chelating agents are synergistic compounds forming complexes with pro-oxidative metal ions such as copper. An unshared pair of electrons in their molecular structures allows the complexing to occur. Among the most common chelators used in foods are citric acid and calcium or disodium salts of ethylenediaminetetraacetic acid (EDTA). Citric acid is regarded as GRAS by the FDA and is very effective as a sequestrant. PPO contains copper and browning can be prevented when that enzymatic copper is chelated with citric acid. Potentiation of an antioxidant such as erythorbic acid and inactivation of PPO can be achieved when citric acid is used with the antioxidant to inhibit color deterioration. In this context, usage levels for citric acid are usually 0.1-0.3% with ascorbic acid at 1.0-2.0% (Dziezak, 1986).

EDTA is also considered GRAS as a chemical preservative in the form of calcium or disodium salts. Very stable complexes are formed when EDTA has sequestered the copper ions in the PPO enzyme molecule (Dziezak, 1986). In a study conducted by Friedman *et al.* (1986), metalloenzymes including tyrosinase, were treated with EDTA at varying concentrations and for varying lengths of time. Inactivation was both concentration and time dependent, with tryosinase inhibition increasing as concentration and exposure time increased.

Packaging Methods

Limited work has been done on the effects of different packaging methods to maintain quality and storage life of raw potato products. In a study conducted by O'Beirne and Ballantyne (1987), several packaging films (Surlyn-polyvinyledene chloride coated polyester, low-density polyethylene or plasticized polyvinyl chloride) for vacuum pack (VP), and a modified-atmosphere pack (MAP) gas-flushed system were tested on chilled potato strips. The MAP rapidly produced an equilibrium-modified atmosphere with O_2 levels below 3%. Even these low levels of oxygen were insufficient to prevent enzymatic browning in cut potato strips. Furthermore, treating potato strips in a relatively high concentration (10%) ascorbic acid solution was necessary to control browning for 7 days at a storage temperature of 5°C. Vacuum packaged potato strips retained excellent color for 14 days, either without any antioxidant treatment or with 1.0 or 5.0% ascorbic acid.

The effects of sodium bisulfite concentration and gas permeability of package film on the surface color of pre-peeled chilled potatoes were evaluated to determine the shelf-life of the product and the lowest level of the preservative which could be used (Giannuzzi, *et al.*, 1988). Results indicated that packaging in the polyolefin film allowed reduced levels of bisulfite to be used for treatment. Vacuum packaging of 50 ppm bisulfite treated samples doubled

the shelf-life of the potatoes as compared with aerobic polyethylene packaging.

In another study, the effect of three packaging materials (mesh, paper, and polyethylene) on discoloration, phenolic content and ascorbic acid content of unpeeled potatoes during storage was examined (Gosselin and Mondy, 1989). The potatoes packaged in polyethylene were significantly higher in phenolic content ($p \leq 0.01$) and discoloration ($p \leq 0.05$),followed by the mesh bag and the paper bags. Ascorbic acid loss was significantly higher ($p \leq 0.05$) in the potatoes stored in the polyethylene bags than either the mesh or paper bags. The high moisture environment inside the polyethylene bags may have produced a stress on the potatoes and resulted in the high phenolic levels and discoloration as well as influencing the loss of ascorbic acid (Gosselin and Mondy, 1989).

CHAPTER III

MATERIALS AND METHODS

Raw Materials

Russet Burbank potatoes were purchased from a local food store and stored at room temperature. The potatoes were washed with tap water to remove adhering soil. The tubers were hand-peeled with a stainless steel peeler and placed in deionized water for approximately 30 min. Using a Rival commercial electric slicer, the potatoes were sliced (1 cm in thickness) and held in deionized water for approximately 30 min awaiting treatment. These procedures were repeated in a second trial.

Application of Browning Inhibitors

Potato slices were randomly divided into seven (2 kg each) equal portions. The slices of each portion were submerged in one of the 7 pre-prepared solutions for 10 min. Citric acid was added to the treatment solutions to maintain pH but the concentration was probably too low to act as a chelating agent. The calcium chloride was added to the treatment solutions to maintain the texture of the potato slices. These antibrowning treatments were repeated in the second trial. The treatments are summarized in Table I.

TABLE I

LIST OF ANTIBROWNING TREATMENTS USED ON SLICED RAW POTATOES

TRT 1)	100 ppm potassium metabisulfite ($K_2S_2O_5$)
TRT 2)	2% L-ascorbic acid + 1% citric acid + 0.1% $CaCl_2$
TRT 3)	2% isoascorbic acid + 1% citric acid + 0.1% $CaCl_2$
TRT 4)	100 ppm EDTA
TRT 5)	2% L-ascorbic acid + EDTA + 1% citric acid + 0.1% $CaCl_2$
TRT 6)	2% isoascorbic acid + EDTA + 1% citric acid + 0.1% $CaCl_2$
TRT 7)	1% L-ascorbic acid + 1% isoascorbic acid + EDTA + 1% citric acid + 0.1% $CaCl_2$

Potato slices were removed from their solutions, drained and blotted using paper towels to remove excess treatment solution. The slices of each portion were immediately and randomly divided into 40 samples (50 gm each) and packaged in pre-made and randomly coded polyolefin bags (Clysar LLP shrink film, 75 gauge; oxygen permeability of 0.5624 $cc/cm^2/24$ hr; water vapor transmission rate of 1.928 $g/cm^2/24$ hr). Twenty bags were sealed under atmospheric pressure using a heat impulse sealer and the other 20 bags were sealed under vacuum using a vacuum heat impulse sealer. After sealing the bags were stored at 10°C. These procedures were repeated in the second trial.

Measurement of Variables

At day 1, 8, 15 and 22 of storage, five samples from each treatment were randomly obtained for evaluation. Two samples were used for determination of color and PPO activity, while the remaining three samples were frozen for analysis of total phenols, ascorbic acid and sugars at a later time.

Degree of Browning

Color was measured using the Minolta Chroma Meter (Series CR-200) tristimulus color analyzer after calibration with a white standard tile (L^*=97.78, a^*=-0.69, b^*=2.31). Value of the L^* coordinate at the center of each slice was measured. The soluble pigments were measured using a Gilford Response II Spectrophotometer set at a 475 nm wave length. The degree of browning was determined by the simultaneous measurements of soluble (absorbance at 475 nm) and insoluble (lightness, L^*) brown pigments. These were expressed as the normalized sum of the two parameters (A_{475} and L^*) after normalization (Amiot, *et al.*,1992). That is, the parameters: A_{475} and L^* normalized to give

$$[A475]_n = (A_{475}-A_{475min})/(A_{475max}-A_{475min})$$

and

$[L^*]_n = (L^*-L^*_{max})/(L^*_{min}-L^*_{max})$, respectively. They were summed, $S = [A475]_n + [L^*]_n$ and the degree of browning was expressed as the normalized sum $[S]_n = (S-S_{min})/(S_{max}-S_{min})$ (Amiot, *et al.*,1992).

PPO Activity

PPO activity was measured using a procedure outlined by Boyer (1977). A fifty-gram sample of potato slices was placed in a blender (Osterizer) with 100 ml of ice cold buffer solution with a pH of 6.6 (0.1 M sodium phosphate and 0.1 M NaF, sodium fluoride) and blended for 1 min at slow speed and 2 min at high speed. The mixture was filtered through 6 layers of cheesecloth and the resulting filtrate was centrifuged at 10,000 x g for 10 min at 4°C. The supernatant enzyme extract was held in an ice bath. A blank consisting of 2 ml of 0.1 M sodium phosphate buffer solution (pH 6.6) and 1 ml 1 mM tyrosine solution was prepared. Triplicate samples consisting of 1.5 ml buffer, 1 ml tyrosine solution as substrate and 0.5 ml PPO enzyme extract were prepared.

Absorbance which is related to the level of PPO activity, was determined using a Gilford Response II Spectrophotometer set at a 475nm wave length. Data were compiled as a mean of triplicate readings.

Total Phenols

Total phenols were determined with Folin-Ciocalteau reagent using a procedure outlined by Chien and Snyder (1983). A 50 g sample of potato slices was placed in a blender (Osterizer) with 100 ml of 80% methanol and blended for 1 min at slow speed and 2 min at high speed. After blending, the mixture was transferred to a 200 ml volumetric flask and brought to volume with the addition of 80%

methanol. The mixture was filtered through Whatman No.4 filter paper with the aid of a vacuum pump and 0.5 ml of the filtrate was combined with 10 ml 2% sodium bicarbonate solution. After 2 min, 0.5 ml of 50% Folin-Ciocalteau reagent was added, mixed on a vortex mixer and allowed to react at room temperature for 30 min. The samples were then centrifuged at 20,000 x g for 20 min. Three ml of supernatant was transferred to a cuvette and absorbance, which is positively correlated to the level of polyphenols present, was measured at 750 nm wave length using a Gilford Response II Spectrophotometer. A 1 mM solution of chlorogenic acid was prepared and used as the standard and all polyphenol data were reported as chlorogenic equivalents: chlorogenic acid equivalents=(AB)/C, where A = mg chlorogenic acid in standard, B = absorbance of sample, and C = absorbance of standard

Data were compiled as a mean of triplicate readings.

Ascorbic Acid.

Changes in L-ascorbic acid concentration in raw potato slices were measured by direct visual titration using the AOAC procedure (1990). In this procedure, quantitative determination of ascorbic acid is based on its properties as a reducing agent, using 2,6-dichlorophenol-indophenol dye as a specific reagent. A 0.0125% dye solution was prepared and standardized by titrating three 5.0 ml aliquots of ascorbic acid (0.05%) with the indophenol dye until a light rose color persisted for at least 5 sec. A dye factor was calculated as

follows: Dye Factor = A/B, where A = mg ascorbic acid in the 5ml solution(.0025mg), and B = ml dye solution used. A 20 g sample of potato slices, was placed in a blender (Osterizer) with 100 ml 1% oxalic acid and blended 1 minute at slow speed and 2 minutes at high speed. The mixture was transferred to a 200 ml volumetric flask. The blender was washed with an additional 75ml of 1% oxalic acid and added to the flask. The contents of the flask were brought to this volume by the addition of 1% oxalic acid solution. The mixture was filtered through Whatman No.4 filter paper with the aid of a vacuum pump. Three 10 ml aliquots of the filtrate were transferred to three 150 ml Erlenmeyer flasks and quickly titrated with indophenol solution until a faint pink end point lasting at least for 5 sec. was reached. The amount of dye used was recorded and the ascorbic acid content was calculated as follows: mg ascorbic acid/100 mg potatoes = (Dye Factor)(Y)(100), where Dye Factor = mg ascorbic acid titrated with 1ml of dye; Y = ml dye used in titrating the sample. Data were compiled as a mean of triplicate titration readings.

Sugars

Sucrose, fructose and glucose of potato slices were determined by high-pressure liquid chromatography and samples were prepared using the procedure outlined by Wilson, *et al.*, (1981). A twenty-five gram sample of potato slices was placed in a blender (Osterizer) with 75 ml HPLC grade methanol and blended 1 minute at slow speed and 2 minutes at high speed.

The mixture was filtered through Whatman No.4 filter paper with th aid of a vacuum pump and 10 ml aliquots of each sample filtrate were centrifuged at 20,000 x g for 10 min. Five ml of each sample supernatant was filtered through a Waters Sep-Pak C18 cartridge (Millipore Corp., Milford, MA). A 1ml aliquot from each sample was transferred to a 4 ml vial, and dried in the Speed Vac Concentrator (Savant, Farmingdale, NY). The residue was diluted with 1 ml HPLC grade water. A 10 μl injection of the standard was chromatographed prior to analysis of each randomly selected set of seven 10 μl samples. The prepared sugar standard contained 0.5 mg each of sucrose, fructose and glucose per 10 μl. Liquid chromatography was carried out on the DIONEX BioLC system Series 4500 (Houston, TX) equipped with the CarboPac PA 1 ion exchange column (flow rate: 1 mL/min.;chart speed: 0.2 in./min.) and Pulsed Electrochemical Detector (Wahem, 1992). The CarboPac PA1 column is a strong basic anion exchanger and the monosaccharide separations were carried out with an NaOH eluant with a pH of 13.0 because the hydroxyl groups on the sugars tend to ionize at this high pH and can be easily detected.

Sucrose, fructose and glucose concentrations were calculated by converting the area units of the peaks to (mg /25gm potato tissue sample)(2):

area units(standard)/0.5 mg = area units (sample)/X mg

Statistical Analysis

All treatments were prepared in two trials arranged in a randomized complete block design where each block represented one trial (Cox, 1958). The data were analyzed using the analysis of variance (ANOVA) for a split plot with the whole units in a randomized complete block design. The whole unit treatment factor was represented by the treatment solutions and the sub-unit treatment factor by storage times and packaging methods. The least significant difference (LSD) was used to compare pairs of means (Steel and Torrie, 1980).

CHAPTER IV

EFFECTS OF SELECTED ANTIBROWNING AGENTS, PACKAGING METHODS AND STORAGE TIME ON ASCORBIC ACID AND SUGAR CONTENT OF SLICED RAW POTATOES

JANE B. DENNIS, IBRAHIM A. WAHEM AND P. L. CLAYPOOL

Abstract

Potatoes were treated with antibrowning agents, packed in low density polyolefin film with or without vacuum, stored at 10°C and removed at days 1, 8, 15 and 22 for analysis of ascorbic acid and sugars. All antibrowning treatments used had significant effects on ascorbic acid concentration of raw potato slices. There was a loss of ascorbic acid with all treatments. Length of storage also had significant effects on ascorbic acid concentration, with a loss of ascorbic acid over time. Length of storage and packaging method interaction affected sucrose concentration. A steady loss of sucrose in the potatoes packaged without vacuum was observed between days 1 and 8 while vacuum packed potatoes showed an increase in sucrose between days 1 and 8. Length of storage and antibrowning treatment interaction had a significant effect on glucose concentration resulting in a steady loss over time. Ascorbic acid was more effective than isoascorbic acid for conserving glucose. A 3-way interaction existed between antibrowning treatment, length of storage and packaging method for fructose concentration. Vacuum packaging was more effective in conserving fructose.

Key Words: Potato, polyolefin film, ascorbic acid, isoascorbic acid, antibrowning agent.

INTRODUCTION

Sulfites have been used for many years on fresh, pre-cut potatoes and potato products to prevent discoloration and to extend storage life (Lecos, 1986). Concerns about the safety of sulfites as food additives have resulted in actions taken by the Food and Drug Administration and the food industry to find appropriate alternatives for the functions served by added sulfites in foods (Langdon, 1987).

The challenge becomes finding alternatives to the sulfiting agents and at present, ascorbic acid appears to be the best alternative to the use of sulfites on fruits and vegetables (Langdon, 1987).

Sucrose, fructose and glucose are the major sugars found in the potato (Smith, 1987). The two main factors which influence sugar content of potatoes during post harvest handling are cultivar and temperature. The Russet Burbank cultivar has a high specific gravity and tends to accumulate less sugar than low specific gravity cultivars (Talburt *et al.*, 1987). Of the three major sugars, fructose is the most responsive to changes in storage temperatures. Tubers stored at low temperatures (4°C) were high in fructose content. At 10°C storage, reducing sugars may increase from 0.2% to 1.0% in 3 days (Smith, 1987). At storage temperatures below 10°C, both total and reducing sugars increase, with the rate and extent of increase being greater as the temperature approaches the freezing point. During the early stages of storage at low temperatures, sucrose appeared to accumulate

most rapidly and with prolonged storage the ratio of sucrose to reducing sugars increased (Talburt *et al.*, 1987). The glucose content of potatoes held at 4.4°C. in an atmosphere of high-CO_2 low O_2 concentration was lower than potatoes stored in air (Smith, 1987).

During storage at 10°C the rate of loss of ascorbic acid is high while the fructose and glucose content increases in direct opposition to ascorbic acid content (Smith, 1987). For sucrose, however, the relationship is very different. A high correlation exists between ascorbic acid and sucrose content in potatoes. The reduction in sucrose content closely parallels the loss of ascorbic acid. One possible explanation of this relationship is that sucrose and ascorbic acid are formed from a common precursor, D-glucose (Smith, 1987).

Limited work has been done on the effects of different packaging methods to maintain quality and storage life of raw potato products. The effect of three packaging materials (mesh, paper, and polyethylene) on ascorbic acid content of unpeeled potatoes during storage was examined by Gosselin and Mondy (1989). Ascorbic acid loss was significantly higher ($p \leq 0.05$) in the potatoes stored in the polyethylene bags than either the mesh or paper bags.

The objective of this study was to determine the effects of selected antibrowning treatments, of packaging methods and of storage time on ascorbic acid and sugar concentration of sliced raw potatoes stored at 10°C.

MATERIALS & METHODS

Raw material

Russet Burbank potatoes were purchased from a local food store, washed with tap water to remove adhering soil, hand-peeled with a stainless steel peeler, sliced with a Rival commercial electric slicer, (1 cm in thickness) and held in deionized water. Potato slices were randomly divided into 7 (800 mg each) equal portions and immediately submerged in one of 7 pre-prepared solutions listed in Table I, for 10 min. Potato slices were removed from their solutions, drained and blotted using paper towels to remove excess treatment solution. The slices of each portion were immediately and randomly divided into 16 samples (50 g each) and packaged in pre-made and randomly coded polyolefin bags (Clysar LLP shrink film, 75 gauge; oxygen permeability of 0.5624 $cc/cm^2/24$ hr; water vapor transmission rate of 1.928 $g/cm^2/24$ hr). Eight bags were sealed without vacuum using a heat impulse sealer and the remaining 8 bags were sealed under vacuum using a vacuum heat impulse sealer. After sealing the bags were stored at 10°C. At day 1, 8, 15 and 22 of storage, two samples from each treatment were randomly obtained for evaluation of ascorbic acid and sugars. These procedures were repeated in the second trial.

Measurement of variables

Changes in L-ascorbic acid content in raw potato slices were measured by titration with 2,6-dichloroindophenol dye (AOAC, 1990). Twenty-gram samples of potato slices were blended (Osterizer) with 1% oxalic acid for 1 min at slow speed and 2 min at high speed. The mixture was transferred to a 200 ml volumetric flask and brought to the volume with additional 1% oxalic acid and filtered through Whatman No.4 filter paper with the aid of a vacuum pump. Three 10 ml aliquots of the filtrate were transferred to 3 150 ml Erlenmeyer flasks and quickly titrated with indophenol solution until a faint pink endpoint lasting at least for 5 sec was reached. Data were compiled as a mean of triplicate titration readings.

Sucrose, fructose and glucose of potato slices were determined by high-pressure liquid chromatography using the DIONEX BioLC system Series 4500 equipped with the CarboPac PA 1 ion exchange column (flow rate: 1 mL/min.;chart speed: 0.2 in./min.) and Pulsed Electrochemical Detector (Wahem, 1992). Samples were prepared using the procedure outlined by Wilson *et al.* (1981). Twenty-five gram samples of potato slices were blended (Osterizer) with 75 ml HPLC grade methanol for 1 minute at slow speed and 2 min at high speed and filtered through Whatman No.4 filter paper with the aid of a vacuum pump. Aliquots of each sample filtrate were centrifuged 20,000 x g for 10 min. Five ml of each sample supernatant

were filtered through a Waters Sep-Pak C18 cartridge (Millipore Corp., Milford, MA). A 1 ml aliquot for each sample was transferred to a 4 ml vial, and dried in the Speed Vac Concentrator (Savant, Farmingdale, NY). The residue was diluted with 1 ml of HPLC grade water. A 10 μl injection of the standard was chromatographed prior to analysis of each randomly selected set of seven 10 μl samples. The prepared sugar standard contained 0.5 mg each of sucrose, fructose and glucose per 10 μl. Data were compiled as a mean of duplicate readings. All treatments were prepared in two trials arranged in a randomized complete block design where each block represented one trial (Cox, 1958). The data were analyzed using the analysis of variance (ANOVA) for a split plot with the whole units in a randomized complete block design. The whole unit treatment factor was represented by the treatment solutions and the sub-unit treatment factor by storage times and packaging methods. The least significant difference (LSD) was used to compare pairs of means (Steel and Torrie, 1980).

RESULTS & DISCUSSION

Ascorbic Acid Content

Autoxidation accounts for most of the losses of L-ascorbic acid in foods (Liao and Seib, 1988). In the evaluation of ascorbic acid concentration there were no significant interactions among treatment factors. A summary of results is in Appendix B. There was no 3-way interaction

effect among antibrowning treatments, packaging methods and length of storage for ascorbic acid concentration. The antibrowning agents applied to raw potato slices had significant ($p \leq 0.0002$) effects on ascorbic acid concentration. The ascorbic acid concentrations for the 100 ppm $K_2S_2O_5$ (TRT 1) and the 100 ppm EDTA treatments (TRT 4) were significantly ($p \leq 0.05$) lower than for the other treatments and the ascorbic acid concentration for the 2% Isoascorbic acid + 100 ppm EDTA treatment (TRT 6) was the highest ($p \leq 0.05$) (Table II);(Figure 1.). The loss of ascorbic acid with TRT 1 was probably because the concentration of $K_2S_2O_5$ was too low to protect ascorbic acid from the action of ascorbate oxidase (Haisman, 1974). Inhibition of autoxidation of ascorbic acid was reported to be more complete when the sulfite concentration was 500 ppm (Joslyn and Braverman, 1954) by limiting available oxygen to ascorbic acid (Haisman, 1974). EDTA also reduces loss of ascorbic acid. The concentration of EDTA in our experiment (TRT 4) was probably too low to be an effective inhibitor of autoxidation (Liao and Seib, 1988).

Length of storage had a significant ($p \leq 0.0001$) effect on the concentration of ascorbic acid retained in potato slices. For all the antibrowning treatments, regardless of packaging method, there was a decline in ascorbic acid content after day 8 (Table III).

During storage at 10°C the rate of loss of ascorbic acid is high (Smith, 1987). In a study by Gosselin and Mondy

(1989), ascorbic acid loss was significantly higher ($p \leq 0.05$) in the potatoes stored in the polyethylene bags than either the mesh or paper bags. The high moisture environment inside the polyolefin bags may have produced a stress on the potatoes and resulted in the loss of ascorbic acid (Gosselin and Mondy, 1989). In this study, the rise in ascorbic acid content from day 1 to day 8 for several of the chemical treatments agrees with observations made by Smith (1987) that ascorbic acid concentration increased in raw potatoes stored in a dark humid atmosphere for 48-96 hours. This reflects the high correlation with sucrose concentration, thus reflecting the interaction between respiration in the potato and the formation or loss of ascorbic acid (Smith, 1987).

Sucrose Content

A close interaction ($p \leq 0.07$) existed between length of storage and packaging method for sucrose concentration in raw potatoes. There was a steady loss of sucrose in the potato slices packaged without vacuum while the potato slices packed in a vacuum showed a significant rise ($p \leq 0.05$) in sucrose concentration between day 1 and day 8. From day 8 to day 22 however, the rate of loss of sucrose was greater for the vacuum packed potato slices than with those packaged without vacuum. The concentration of sucrose was still higher for the vacuum packed potatoes at day 22 (Table IV.);(Figure 2.). The data indicated a significant ($p \leq 0.05$) loss of

sucrose between day 1 and day 22 regardless of packaging (Table III). These data support the fact that a reduction in sucrose content closely parallels the loss of ascorbic acid and that a similarity exists between the loss of ascorbic acid and sucrose in potatoes (Smith, 1987).

Glucose Content

The data on glucose content of potato slices showed a significant ($p \leq 0.001$) interaction between length of storage and antibrowning treatment. The data are summarized on Tables III and V. There were no significant differences among treatments for day 1. However, day 8 showed the greatest differences in glucose concentration among all treatments. The glucose concentration of the potato slices treated with the 2% isoascorbic acid + 1% citric acid + 0.1% $CaCl_2$ treatment (TRT 3) was the lowest of all treatments and was significantly different ($p \leq 0.05$) from all other treatment means. The highest glucose concentration was observed at day 8 for the 2% L-ascorbic acid + 1% citric acid + 0.1% $CaCl_2$ treatment (TRT 2), but was not different from TRT 4 and TRT 7 either practically or significantly ($p \leq 0.05$). For day 15, only the 2% L-ascorbic acid + 1% citric acid + 0.1% $CaCl_2$ treatment (TRT 2) and the 100 ppm EDTA treatment (TRT 4) were significantly different ($p \leq 0.05$). For day 22, the difference between TRT 2 and TRT 1 was significant ($p \leq 0.05$). These data agree with the observations made by Borenstein (1965) that L-ascorbic acid is more stable than isoascorbic acid in aqueous

systems. This could affect the respiration rate in potato tissue at low temperatures, which utilizes sugars by converting them to CO_2 and water (Smith, 1987).

Fructose Content

A close ($p \leq 0.055$) 3-way interaction existed between chemical treatment, length of storage and packaging method for fructose concentration of treated potato slices. The potato slices which were treated with 100 ppm EDTA (TRT 4) and packaged without vacuum, had the greatest increase in fructose concentration from day 1 to day 8. However, the fructose concentration of the TRT 4 potatoes declined after day 8. Potatoes treated with the 1% L-ascorbic acid + 1% isoascorbic acid + EDTA + 1% citric acid + 0.1% $CaCl_2$ treatment (TRT 7), and packed without vacuum also showed an increase in fructose level at day 8 and declined after day 8. The remaining treated potato samples packaged without vacuum followed a pattern of falling fructose levels from day 1 to day 8, rising at day 15 and falling again at day 22. Potatoes treated with the 2% isoascorbic acid + 1% citric acid, + 0.1% $CaCl_2$ treatment (TRT 3), or the 100 ppm EDTA treatment (TRT 4), and vacuum packaged showed a loss of fructose from day 1 to day 8. The potatoes treated with TRT 4 and vacuum packed increased fructose concentration at day 15 only to fall again at day 22. The data are summarized in Table VI and Figure 2. For treatments 1-3 and 5, vacuum packaging conserved fructose in the potato slices.

Treatments 4,6 and 7 showed a loss of fructose with vacuum packaging compared to packaging without vacuum.

Discussion and Conclusions

The objective of this study was to determine the effects of selected antibrowning agents, packaging methods and storage times on the ascorbic acid and sugar concentration of sliced raw potatoes. The endogenous ascorbic acid in sliced raw potatoes was reduced after antibrowning treatment and storage at 10°C for after day 8. However, it appeared that TRT 6 was most effective in minimizing the loss. Neither packaging method had a significant effect on the ascorbic acid concentration of raw potatoes. Length of storage and packaging method reduced the sucrose content of raw potatoes. There was a steady loss of sucrose in the potato slices packaged without vacuum while the potato slices packed in a vacuum showed an increase in sucrose concentration between day 1 and day 8. This was possibly due to the conversion of starch to sucrose. Length of storage and antibrowning treatment caused a loss of glucose over time. Ascorbic acid (TRT 2) was more effective than isoascorbic acid at conserving glucose in raw potatoes. Antibrowning treatment, length of storage and packaging method resulted in loss of fructose over time. The vacuum packaging method with ascorbic acid (TRT 2) was more effective in conserving fructose concentration of raw potatoes than the no vacuum pack.

In conclusion, although the treatment solutions which contained ascorbic acid or isoascorbic acid were effective in conserving endogenous ascorbic acid and sugars in raw potatoes, further research on different concentrations of these compounds as antibrowning treatments should be conducted.

TABLE I

LIST OF ANTIBROWNING TREATMENTS USED ON SLICED RAW POTATOES

TRT 1) 100 ppm potassium metabisulfite ($K_2S_2O_5$)
TRT 2) 2% L-ascorbic acid + 1% citric acid + 0.1% $CaCl_2$
TRT 3) 2% isoascorbic acid + 1% citric acid + 0.1% $CaCl_2$
TRT 4) 100 ppm EDTA
TRT 5) 2% L-ascorbic acid + EDTA + 1% citric acid + 0.1% $CaCl_2$
TRT 6) 2% isoascorbic acid + EDTA + 1% citric acid + 0.1% $CaCl_2$
TRT 7) 1% L-ascorbic acid + 1% isoascorbic acid + EDTA + 1% citric acid + 0.1% $CaCl_2$

TABLE II

MEAN ASCORBIC ACID CONCENTRATION OF RAW POTATOES BY ANTIBROWNING TREATMENT

TREATMENT	ASCORBIC ACID (mg/100g)*
1**	.00994[a]±.00238
2	.02400[b]±.00615
3	.02794[b]±.00537
4	.00956[a]±.00182
5	.02688[b]±.00689
6	.03344[c]±.00853
7	.02644[b]±.00681

* Means of triplicate titrations ± S.D.; averaged over 4 days and 2 blocks.
**Refer to Table I for list of antibrowning treatments.
[a-c]Means in the same column with the same superscript are not significantly different ($p \leq 0.05$).

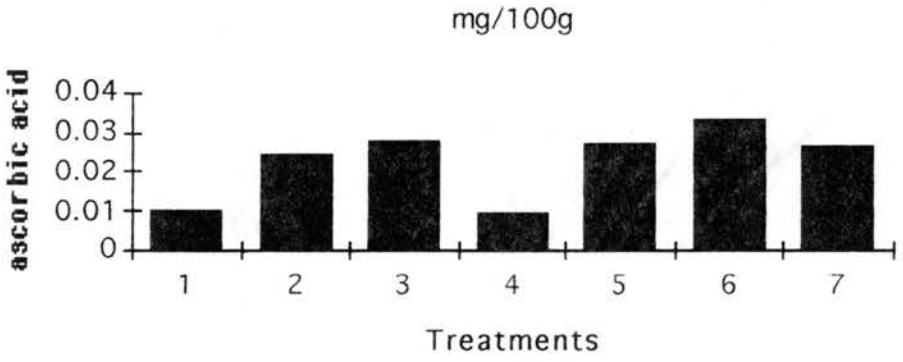

Figure 1. Comparisons of treatment means of ednogenous ascorbic acid content of sliced raw potatoes stored at 10°C.

Means; n=16; averaged over 4 days and 2 blocks.

TABLE III

THE EFFECT OF LENGTH OF STORAGE ON ASCORBIC ACID AND SUGAR CONCENTRATION (mg/100 g) OF SLICED RAW POTATOES STORED AT 10°C

	DAY 1	DAY 8	DAY 15	DAY 22
Asc. Acid	.024±.012^{b}	.026±.011^{bc}	.022±.009^{b}	.018±.009^{a}
Sucrose	261±115^{b}	2743±96^{b}	204±76^{a}	177±42^{a}
Glucose	409±170^{a}	448±259^{a}	507±191^{a}	444±143^{a}
Fructose	328±259^{a}	311±312^{a}	510±269^{a}	431±189^{a}

Means ± S.D.; n=28; averaged over 7 treatments, 2 packageing methods and 2 blocks.

$^{a-c}$ Means in the same row with the same superscripts are not significantly different ($p \leq 0.05$).

TABLE IV

MEAN SUCROSE CONCENTRATION OF SLICED RAW POTATOES STORED AT 10°C

PACK*	DAY 1	DAY 8	DAY 15	DAY 22
VO	261.43[b]	220.29[ab]	165.43[a]	156.00[a]
V	259.71[ab]	328.29[c]	242.57[a]	198.00[a]

Means; n=14; averaged over 7 treatments and 2 blocks.
*VO= without vacuum; V= with vacuum.
[a-c] Means in the same row with the same superscripts are not significantly different ($p \leq 0.05$).

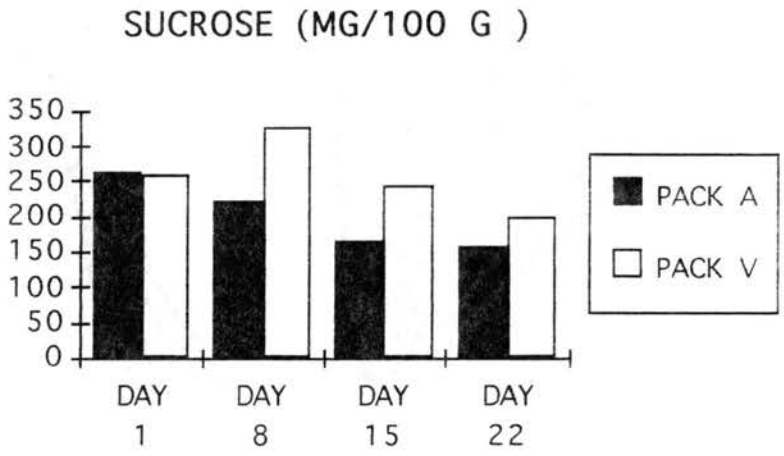

Figure 2. Comparison of length of storage and packaging means of sucrose concentration of sliced raw potatoes stored at 10°C.
Means; n=14; averaged over 7 treatments and 2 packaging methods.

TABLE V

COMPARISON OF TREATMENT AND LENGTH OF STORAGE MEANS OF GLUCOSE CONTENT OF SLICED RAW POTATOES STORED AT 10°C*

TRT**	DAY 1	DAY 8	DAY 15	DAY 22
1	438^{a}	483^{b}	444^{ab}	342^{a}
2	411^{a}	561^{bc}	591^{b}	504^{b}
3	417^{a}	123^{a}	528^{ab}	480^{ab}
4	450^{a}	555^{bc}	423^{a}	435^{ab}
5	414^{a}	348^{b}	531^{ab}	438^{ab}
6	363^{a}	504^{bc}	525^{ab}	447^{ab}
7	369^{a}	558^{bc}	510^{ab}	462^{ab}

*Means; n=4; averaged over 2 packaging methods and 2 blocks.
** Treatments: Refer to Table I for list of antibrowning treatments.
$^{a-c}$ Means in the same column with the same superscripts are not significantly different ($p \leq 0.05$).

TABLE VI

THE EFFECT OF ANTIBROWNING AGENTS, LENGTH OF STORAGE AND PACKAGING METHOD ON FRUCTOSE CONCENTRATION (mg/100g) OF SLICED RAW POTATOES STORED AT 10°C*

Day	Pack	TRT 1	TRT 2	TRT 3	TRT 4	TRT 5	TRT 6	TRT 7
1	VO**	414^{b}	360^{a}	150^{a}	342^{a}	372^{a}	330^{a}	360^{a}
	V	48^{a}	150^{a}	516^{ab}	564^{abc}	360^{ab}	318^{ab}	306^{ab}
8	VO	222^{a}	114^{a}	000^{a}	726^{abc}	292^{ab}	168^{a}	522^{abc}
	V	468^{ab}	408^{ab}	126^{a}	000^{a}	426^{ab}	336^{ab}	540^{ab}
15	VO	552^{a}	594^{a}	420^{a}	558^{a}	612^{a}	456^{a}	498^{a}
	V	468^{a}	576^{a}	384^{a}	564^{a}	492^{a}	468^{a}	492^{a}
22	VO	342ab	294^{a}	408^{ab}	552^{b}	396^{ab}	378^{ab}	516^{ab}
	V	474^{a}	576^{a}	468^{a}	426^{a}	426^{a}	372^{a}	408^{a}

*Means; n=2; averaged over 2 blocks.
**VO= without vacuum pack; V= vacuum pack.
Treatments:refer to Table I.
$^{a-c}$ Means in rows with the same superscripts are not significantly different ($p \leq 0.05$).

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CHAPTER V

THE EFFECTS OF SELECTED PACKAGING, STORAGE TIME, AND ANTIBROWNING AGENTS ON POLYPHENOL OXIDASE ACTIVITY, TOTAL PHENOL CONTENT AND DEGREE BROWNING OF SLICED RAW POTATOES

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Abstract

Potatoes treated with antibrowning agents were packed in low density polyolefin film with or without vacuum, stored at 10°C and removed at days 1, 8, 15 and 22 for analysis of color (browning), polyphenol oxidase (PPO) activity and total phenols. The antibrowning agents and packaging method had no significant interaction effects on PPO activity, total phenols or degree of browning. Length of storage had a significant effect on all dependent variables. There was a steady rise in enzyme activity and total phenolic content from day 1 through day 22, and a slight decline in degree of browning over time.

Key Words: Potato, polyolefin film, polyphenol oxidase, total phenols, antibrowning agents, storage time.

INTRODUCTION

Enzymatic browning in raw potatoes is correlated with the concentrations of polyphenol oxidase (PPO), tyrosine, chlorogenic acid and ascorbic acid (Amiot, *et al.*, 1992). The discoloration of injured plant tissues containing high levels of phenolic compounds, is principally due to oxidation of these colorless compounds to o-quinones which range in color from reddish-brown to black. O-quinones which are very

reactive, will readily polymerize and react with o-phenols and O_2 (Rouet-Mayer, *et al.*, 1990).

The PPO enzyme complex is involved in two different reactions: 1) the hydroxylation of monohydroxyphenols to o-dihydroxyphenols, and 2) the oxidation of o-dihydroxyphenols to o-quinones (Mathew and Paroia, 1971). Both of these reactions require the presence of copper ions in the enzyme. Removal of the copper ions leads to inactivation or reduction of enzyme activity (Vamos-Vigyazo, 1981).

Cold storage affects enzymatic browning in potatoes. At 4.4°C, the rate of browning increased for the first 100 days and then remained constant, compared to potatoes stored at room temperature (Mapson, *et al.*, 1963). When stored at 10°C, discoloration of potatoes increased with length of storage and was accompanied by an increase in phenolic content and a decrease in PPO activity (Mondy, *et al.*, 1966). The treatment combination of 1.0% ascorbic acid and 0.1% calcium (as $CaCl_2$) was very effective in retarding discoloration in sliced apples when stored at 1.1°C (Ponting, *et al.*, 1972). Chilled (5°C) potato strips showed no discoloration for at least 2 weeks when vacuum packaged after 1, 5 or 10% ascorbic acid treatment (O'Beirne and Ballantyne, 1987).

Enzyme inhibition was irreversible when PPO was exposed to sulfite prior to substrate addition. When only monophenols are present such as tyrosine in potatoes, low levels of sulfite are effective (Taylor, *et al.*, 1986). Amla

and Francis (1961) treated hand-peeled potatoes with sodium bisulfite solutions ranging in concentration from 0 to 4000 ppm as available sulfur dioxide, with varying pH levels (4.0-10.0), and storage temperatures (0-1°C or 4-5°C). They found discoloration was decreased with higher levels of sulfite, lower pH and lower storage temperature. They also found that the optimum pH of the dipping solutions to minimize discoloration was in the range of 5.5 to 6.5.

PPO contains copper and browning can be prevented when that enzymatic copper is chelated. Potentiation of an antioxidant such as erythorbic acid and inactivation of PPO can be achieved when citric acid is used with the antioxidant to inhibit color deterioration. In this context, usage levels for citric acid are usually 0.1-0.3% with ascorbic acid at 1.0-2.0% (Dziezak, 1986).

Very stable complexes are formed when EDTA has sequestered the copper ions in the enzyme molecule (Dziezak, 1986). In a study conducted by Friedman, *et al.* (1986), metalloenzymes including tyrosinase, were treated with EDTA at varying concentrations (0.4, 1.0 and 2.5 mM) and for varying lengths of time (15 or 60 min.). Inactivation was both concentration and time dependent, with tryosinase inhibition increasing as concentration and exposure time increased.

Ascorbic acid reduces o-quinones to o-diphenols (Vamos-Vigyazo, 1981) and chelates the copper in PPO (Borenstein, 1965). It also inhibits the enzyme competitively by direct

oxidation (Cort, 1982). The effectiveness of ascorbic acid as a PPO inhibitor is dependent on its concentration. Ponting, *et al.* (1972) found 1.0% ascorbic acid and 0.1% $CaCl_2$ very effective in preventing discoloration of raw apple slices. At low concentrations the formation of colored polymers is prevented for a limited time because the ascorbic acid is quickly consumed (Liao and Seib, 1988). At high concentrations, ascorbic acid reduces the o-quinones to o-diphenols, and inhibits PPO (Vamos-Vigyazo, 1981).

Ascorbic acid was reported to be consistently more effective in inhibiting browning on cut apple surfaces than isoascorbic acid (Sapers and Douglas, 1987);(Sapers and Ziolkowski, 1987). Furthermore, ascorbic acid and isoascorbic acid were reported to be more effective in inhibiting browning of fresh mushrooms than dehydroascorbic acid (Hsu, *et al.*, 1988).

Limited work has been done on the effects of different packaging methods to maintain quality and storage life of raw potato products. In a study conducted by O'Beirne and Ballantyne (1987), several packaging films (Surlyn-polyvinyledene chloride coated polyester, low density polyethylene, and plasticized polyvinyl chloride) for vacuum pack (VP), and a modified-atmosphere pack (MAP) gas-flushed system were tested on chilled potato strips. The MAP rapidly produced an equilibrium-modified atmosphere with O_2 levels below 3%. These low levels of oxygen were insufficient to prevent enzymatic browning in cut potato strips.

Furthermore, treating the potato strips in a relatively high concentration (10%) ascorbic acid solution was necessary to control browning for 7 days at 5°C. Vacuum packaged potato strips retained excellent color for 14 days, either without any antioxidant treatment or with 1.0 or 5.0% ascorbic acid.

The effects of sodium bisulfite concentration and gas permeability of package film on surface color of pre-peeled chilled potatoes were evaluated (Giannuzzi, *et al.*, 1988). Results indicated that packaging in polyolefin film allowed reduced levels of bisulfite to be used for treatment. Vacuum packaging of 50 ppm bisulfite treated samples doubled the shelf-life of the potatoes as compared with aerobic polyethylene packaging.

The objective of this study was to determine the effects of selected antibrowning agents, packaging methods and storage times on the degree of browning, PPO activity and total phenols of sliced raw potatoes.

MATERIALS & METHODS

Raw material

Russet Burbank potatoes were purchased from a local food store, washed with tap water to remove adhering soil, hand-peeled with a stainless steel peeler, sliced with a Rival commercial electric slicer, (1 cm in thickness) and held in deionized water for approximately 30 min awaiting treatment. These procedures were repeated in the second trial.

Application of Browning Inhibitors

Potato slices were randomly divided into 7 (1.2 kg each) equal portions and immediately submerged in one of the 7 pre-prepared solutions listed in Table I, for 10 min. The treated slices were removed from their solutions, drained and blotted using paper towels to remove excess treatment solution. The slices of each portion were immediately and randomly divided into 24 samples (50 gm each) and packaged in pre-made and randomly coded polyolefin bags (Clysar LLP shrink film, 75 gauge; oxygen permeability of 0.5624 $cc/cm^2/24$ hr; water vapor transmission rate of 1.928 $g/cm^2/24$ hr). Twelve bags were sealed without vacuum using a heat impulse sealer and the remaining 12 bags were sealed under vacuum using a and vacuum heat impulse sealer. After sealing the bags were stored at 10°C. At day 1, 8, 15 and 22 of storage, 3 samples from each treatment were randomly obtained for evaluation of color, PPO activity and total phenols. These procedures were repeated in the second trial.

Measurement of variables

Color was measured using a Minolta Chroma Meter (Series CR-200) after calibration with a white standard tile (L*=97.78, a*=-0.69, b*=2.31). Value of the L* coordinate at the center of each slice was measured. Data were compiled as a mean of triplicate readings.

PPO activity was measured using a procedure outlined by Boyer (1977). Fifty-gram samples of potato slices were blended (Osterizer) with 100 ml of ice cold buffer solution

with a pH of 6.6 (0.1 M sodium phosphate and 0.1 M sodium fluoride) for 1 min at slow speed and 2 min at high speed, filtered through 6 layers of cheesecloth. The filtrate was centrifuged at 10,000 x *g* for 10 min at 4°C. The supernatant enzyme extract was held in an ice bath. A blank consisting of 2 ml 0.1 M sodium phosphate buffer solution (pH 6.6) and 1 ml 1 mM tyrosine solution was prepared. Triplicate samples were prepared consisting of 1.5 ml buffer, 1 ml tyrosine solution as substrate and 0.5 ml PPO enzyme extract were prepared.

Absorbance which is related to the level of PPO activity, was determined using a Gilford Response II Spectrophotometer set at a 475 nm wave length. Data were compiled as a mean of triplicate readings.

Since browning tendency in potatoes is the result of the formation of soluble and insoluble pigments, the degree of browning can be expressed as the normalized sum of absorbance at 475 nm and L* after normalization. The degree of browning was determined by the simultaneous measurements of soluble (absorbance at 475 nm) and insoluble (lightness, L*) brown pigments. These were expressed as the normalized sum of the two parameters (A_{475} and L*) after normalization (Amiot, *et al.*, 1992). That is, the parameters: A_{475} and L* were normalized to give

$$[A_{475}]n = (A_{475}-A_{475min})/(A_{475max}-A_{475min})$$

and

$[L^*]_n = (L^*-L^*_{max})/(L^*_{min}-L^*_{max})$, respectively. They were then summed, $S = [A_{475}]_n + [L^*]_n$, and the degree of browning was expressed as the normalized sum $[S]_n = (S-S_{min})/(S_{max}-S_{min})$ (Amiot *et al.*, 1992).

Total phenols were determined with Folin-Ciocalteau reagent using a procedure outlined by Chien and Snyder (1983). Fifty-gram samples of potato slices were blended (Osterizer) with 100 ml 80% methanol for 1 min at slow speed and 2 min at high speed, transferred to a 200 ml volumetric flask and then brought to volume with additional 80% methanol and filtered through Whatman No.4 filter paper with the aid of a vacuum pump and 0.5 ml of the filtrate was combined with 2% sodium bicarbonate solution. After 2 min, 0.5 ml of 50% Folin-Ciocalteau reagent was added, mixed on a vortex mixer and allowed to react at room temperature for 30 min. Samples were centrifuged at 20,000 x *g* for 20 min. Three ml of supernatant were transferred to a cuvette and absorbance, which is positively correlated with the level of polyphenols present, was measured at 750 nm wave length using a Gilford Response II Spectrophotometer. A 1 mM solution of chlorogenic acid was prepared and used as the standard and all polyphenol data were reported as chlorogenic equivalents:

chlorogenic acid equivalents=(AB)/C, where A = mg chlorogenic acid in standard, B = absorbance of sample, and C = absorbance of standard

Data were compiled as a mean of triplicate readings. All treatments were prepared in two trials arranged in a

randomized complete block design where each block represented one trial (Cox, 1958). The data were analyzed using the analysis of variance (ANOVA) for a split plot with the whole units in a randomized complete block design. The whole unit treatment factor was represented by the treatment solutions and the sub-unit treatment factor by storage times and packaging methods. The least significant difference (LSD) was used to compare pairs of means (Steel and Torrie, 1980).

RESULTS & DISCUSSION

There were no significant interactions observed between antibrowning treatments, storage times or packaging methods for degree of browning, PPO activity or total phenols. Furthermore, there was no significant treatment effect or packaging effect for any of the independent variables. However, length of storage had a significant ($p \leq 0.0001$) effect on PPO activity. There was a steady rise in enzyme activity from day 1 through day 22. The data are in disagreement with Mondy, *et al.*(1966) who observed a decrease in PPO activity over time. This is probably because our storage temperature was 10°C compared to 4.4°C and allowed enzyme activity to remain fairly high. Total phenolic content was significantly ($p \leq 0.0001$) affected by length of storage. There was a steady rise in total phenolic content from day 1 through day 22, which agreeed with Mondy, *et al.*(1966) who observed increased total phenols over time in potatoes. Length of storage also had a significant

($p \leq 0.0001$) effect on degree of browning. Although the overall change in the degree of browning observed from day 1 and to day 22 was not significant, the decline in degree of browning between day 1 and day 8 was significant ($p \leq 0.05$), as was the rise on day 15, and a final decline at day 22. This is contrary to Amiot, *et al.* (1992) who stated that with increased absorbance and decreased L* readings the degree of browning should increase. Furthermore, Amiot, *et al.* (1992) showed that phenolic content was more closely related to degree of browning than PPO activity. However, O'Beirne and Ballantyne (1987) observed that chilled potato strips showed no discoloration for at least 2 weeks when vacuum packaged after ascorbic acid treatment. Browning in potato tissue has been correlated with PPO activity and the concentration of PPO substrates (Sapers,*et al.*, 1989) and other compounds, including ascorbic acid, and acidity may also influence browning. Data are summarized in Tables I and II.

In this experiment, the decline in the degree of browning from day 1 to day 8 was in part the result of an unavoidable delay of several hours after sample preparation in measuring absorbance for the PPO enzyme activity for day 1, block 1 samples. This delay resulted in higher absorbance readings than had the samples been read shortly after preparation. This meant the drop in the degree of browning from day 1 to day 8 was not a true indicator of the normal browning pattern. Furthermore, an error in instrument

calibration when recording L* values for day 8 samples resulted in higher than normal readings for block 1.

CONCLUSIONS

Neither packaging method nor treatment of the raw potato slices with antibrowning agents had a significant effect on PPO activity, total phenols or degree of browning. Length of storage had a significant ($p \leq 0.0001$) effect on PPO activity, total phenols and degree of browning. There was a steady rise in enzyme activity from day 1 through day 22. Total phenolic content was significantly ($p \leq 0.0001$) affected by length of storage, with a steady rise in total phenolic content from day 1 to day 22. Length of storage also had a significant ($p \leq 0.0001$) effect on degree of browning. A decline in the degree of browning was observed from day 1 to day 22. From this data it can be concluded that the antibrowning agents used in this study were about equal in their effect on browning, PPO activity and total phenols in raw sliced potatoes. Since potassium metabisulfite (TRT 1) was the treatment which was being replaced, the data suggests that any of the other treatments used would be a satisfactory substitute as an antibrowning agent.

The presence or absence of a vacuum as part of the packaging method appears to have very little if any influence on raw potato quality, based on this study. Additional research on packaging methods for raw potato products would be useful since exposure to air (O_2) accelerates browning on

cut raw potato surfaces. Finally,the data suggests that length of storage at 10°C had the greatest influence on raw potato quality and that the storage of raw potato products should probably be limited to 2 weeks.

TABLE I

LIST OF ANTIBROWNING TREATMENTS USED ON SLICED RAW POTATOES

TRT 1) 100 ppm potassium metabisulfite ($K_2S_2O_5$)
TRT 2) 2% L-ascorbic acid + 1% citric acid + 0.1% $CaCl_2$
TRT 3) 2% isoascorbic acid + 1% citric acid + 0.1% $CaCl_2$
TRT 4) 100 ppm EDTA
TRT 5) 2% L-ascorbic acid + EDTA + 1% citric acid + 0.1% $CaCl_2$
TRT 6) 2% isoascorbic acid + EDTA + 1% citric acid + 0.1% $CaCl_2$
TRT 7) 1% L-ascorbic acid + 1% isoascorbic acid + EDTA + 1% citric acid + 0.1% $CaCl_2$

TABLE II

COMPARISON OF LENGTH OF STORAGE MEANS OF TOTAL PHENOLS, PPO ACTIVITY AND DEGREE OF BROWNING OF SLICED RAW POTATOES STORED AT 10°C*

DAY	TOTAL PHENOL (mg/100g)	PPO ACTIVITY (A475)	LIGHTNESS** (L*)	DEGREE BROWNING
Day 1	.03004^{b}	.15429^{b}	79.64^{a}	.661^{ab}
Day 8	.07579^{bc}	.18693^{b}	100.53^{a}	.299^{a}
Day 15	.10368^{a}	.27971^{a}	80.13^{a}	.873^{c}
Day 22	.09164^{ac}	.27732^{a}	98.83^{a}	.494^{a}

*Means; n=28; averaged over 7 treatments, 2 packaging methods and 2 blocks.
**Lightness (L*) was used to calculate Degree of Browning only.
$^{a-c}$ Means in the same column with the same superscripts are not significantly different ($p \leq 0.05$)

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CHAPTER VI

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

This research evaluated the effects of antibrowning agents, of packaging methods and of storage time on PPO activity, on total phenol concentration, on degree of browning, on ascorbic acid and sugar concentration of sliced raw potatoes stored at 10°C.

The objective and hypothesis are listed in the introduction of this dissertation. These will be considered first; then other conclusions and recommendations will be discussed.

The hypothesis tested in this research was that there were no effects of selected antibrowning agents, packaging method or storage time on PPO activity, total phenol content, the degree of browning, ascorbic acid and sugar content of sliced raw potatoes stored at 10°C.

Length of storage had a significant ($p \leq 0.0001$) effect on PPO activity which increased over time. Total phenolic concentration likewise increased ($p \leq 0.0001$) over time. Length of storage also affected ($p \leq 0.0001$) degree of browning. The null hypothesis was rejected.

The antibrowning treatments used in this experiment had an effect ($p \leq 0.0002$) on ascorbic acid concentration, which was lower for the $K_2S_2O_5$ (TRT 1) and the EDTA (TRT 4) treatments than for the other treatments. Treatment 1 was assigned as the control because sulfiting agents must now be replaced as chemical food treatments to preserve quality in raw produce including potatoes. Length of storage also had an effect ($p \leq 0.0001$) on ascorbic acid content with a loss of ascorbic acid observed over time regardless of packaging method. The null hypothesis was rejected.

There was a length of storage and packaging method interaction effect on sucrose content, with a steady loss observed over time for both packaging methods. The null hypothesis was rejected.

The data on glucose content showed a length of storage and chemical treatment interaction effect ($p \leq 0.0001$). The antibrowning treatments containing L-ascorbic acid resulted in higher glucose content than the other treatments over time. The null hypothesis was rejected.

There was a close ($p \leq 0.055$) 3-way interaction among antibrowning treatment, length of storage and packaging method for fructose concentration. When separated by antibrowning treatments and packaging methods for each day, treatments 1-3 and 5 vacuum packed, conserved fructose content while the remaining treatments exhibited a loss of fructose. The null hypothesis was rejected.

Conclusions

The objective of this study was to determine the effects of selected antibrowning agents, packaging methods and storage times on the ascorbic acid and sugar concentration degree of browning, PPO activity, and total phenols of sliced raw potatoes. Length of storage had the most consistent and significant effect on all the dependent variables. Furthermore, the antibrowning treatments and length of storage had a more pronounced effect on endogenous ascorbic acid content of raw potatoes than did packaging method and it appeared that TRT 6 was most effective in minimizing the loss. Neither packaging method had a significant effect on the ascorbic acid concentration of raw potatoes. The length of storage and packaging method affected sucrose content more than antibrowning treatment whereas antibrowning treatment and length of storage influenced glucose content more than did packaging. There was a steady loss of sucrose in the potato slices packaged without vacuum while the potato slices packed in a vacuum showed an increase in sucrose concentration between day 1 and day 8. Ascorbic acid (TRT 2) was more effective than isoascorbic acid at conserving glucose in raw potatoes. Although fructose content was affected by antibrowning treatment, length of storage and packaging method, the vacuum pack was more effective in conserving fructose than the no vacuum pack. The vacuum packaging method with ascorbic acid (TRT 2) was most effective in conserving fructose in raw potatoes.

Neither packaging method nor treatment of the raw potato slices with antibrowning agents had any significant effect on PPO activity, total phenols or degree of browning. Length of storage had an effect on PPO activity, total phenols and degree of browning. There was a steady rise in enzyme activity from day 1 through day 22. Total phenolic content was affected by length of storage, with a steady rise in total phenolic content from day 1 to day 22. Length of storage also had an effect on degree of browning. A decline in the degree of browning was observed from day 1 to day 22. The antibrowning agents used in this study were about equal in their effect on browning, PPO activity and total phenols in raw sliced potatoes. Since potassium metabisulfite (TRT 1) was the treatment which was being replaced, the data suggests that any of the other treatments used would be a satisfactory substitute as an antibrowning agent. The data also suggests that length of storage at 10°C has the greatest influence on potato quality as defined by browning and that the storage of raw potato products should probably be limited to 2 weeks.

Although the treatment solutions which contained ascorbic acid or isoascorbic acid were effective in conserving endogenous ascorbic acid and sugars in raw potatoes, further research on different concentrations of these compounds as antibrowning treatments should be conducted.

Recommendations

The concentrations of the treatment solutions were probably too low to be effective antioxidants and chelating agents. Future experiments might test concentrations of ascorbic acid and isoascorbic acid of 5% in an effort to reduce PPO activity, browning and loss of endogenous ascorbic acid.

The low density polyolefin packaging material used in this experiment may have been inappropriate for controlling enzyme activity and autoxidation in disrupted potato tissue. Future research might look at more impermeable packaging materials as alternatives.

Finally, the storage temperature used in this research (10°C) should be considered as a factor in the overall pattern of the results. The antibrowning treatments and packaging might have possibly been more effective in controlling the dependent variables if the storage temperature had been lower. Future research might evaluate the use of lower temperatures (1°C to 4°C) to help control deterioration of treated raw potatoes. In conclusion, based on this study it is recommended that storage of raw potato products be limited to about 2 weeks.

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APPENDIXES

APPENDIX A

LIST OF ANTIBROWNING TREATMENTS USED ON SLICED RAW POTATOES

TRT 1) 100 ppm potassium metabisulfite ($K_2S_2O_5$)
TRT 2) 2% L-ascorbic acid + 1% citric acid + 0.1% $CaCl_2$
TRT 3) 2% isoascorbic acid + 1% citric acid + 0.1% $CaCl_2$
TRT 4) 100 ppm EDTA
TRT 5) 2% L-ascorbic acid + EDTA + 1% citric acid + 0.1% $CaCl_2$
TRT 6) 2% isoascorbic acid + EDTA + 1% citric acid + 0.1% $CaCl_2$
TRT 7) 1% L-ascorbic acid + 1% isoascorbic acid + EDTA + 1% citric acid + 0.1% $CaCl_2$

APPENDIX B

THE MEAN ENDOGENOUS ASCORBIC ACID CONCENTRATION (mg/100 g) OF SLICED RAW POTATOES STORED AT 10°C[a]

	Day 1		Day 8		Day 15		Day 22	
Trt[b]	VO[c]	V	VO	V	VO	V	VO	V
1	.009	.009	.012	.013	.012	.011	.008	.009
2	.027	.029	.028	.033	.020	.023	.015	.020
3	.026	.024	.037	.025	.028	.030	.025	.029
4	.010	.009	.011	.011	.011	.010	.009	.008
5	.031	.035	.035	.030	.024	.026	.016	.020
6	.028	.046	.033	.036	.038	.029	.029	.030
7	.023	.037	.028	.031	.024	.029	.021	.021

[a] Means; n=2; aveaged over 2 blocks.
[b] VO= without vacuum pack; V=Vacuum pack.
[c] Treatments listed in Appendix A.

APPENDIX C

INFLUENCE OF SELECTED ANTIBROWNING AGENTS (T), PACKAGING METHODS (P) AND LENGTH OF STORAGE (D) ON SOME CHARACTERISTICS OF SLICED RAW POTATOES

F ratio

				Interactions				
	TRT	PKG	DAY	TP	TD	PD	TPD	MSE
Asc. A.	34.65[a]	3.11	13.37[b]	1.40	1.66	2.16	1.49	.00002
Suc	1.33	14.31[e]	9.57[b]	1.01	1.01	2.51	1.23	6216.4
Glu	0.79	1.89	4.08[c]	0.31	3.04[d]	0.37	1.25	11473.
Fru	0.60	0.05	7.68	1.09	0.77	0.29	1.79[f]	31671.
PPO Act.	1.43	2.75	17.04[b]	0.37	0.96	0.56	0.21	0.0066
Tot. Phenols	4.09[f]	0.00	17.60[b]	0.12	0.15	0.02	0.20	0.0016
Deg. Brown.	0,33	0.86	12.39[b]	0.05	0.18	0.11	0.04	0.1345
Hue Angle	2.13	0.88	4.98	0.69	1.00	2.74[g]	0.46	2003.9
Chroma	3.09	0.63	15.25[b]	0.09	0.11	0.13	0.06	24.078
Total Color Diff.	0.53	0.03	10.28[h]	0.02	0.05	0.04	0.03	230.46

[a]=p≤.0002
[b]=p≤.0001
[c]=p≤.012
[d]=p≤.001
[e]=p≤.0004
[f]=p≤.0553
[g]=p≤.0532
[h]=p≤.0003

APPENDIX D

THE MEANS OF ANTIBROWNING AGENTS, LENGTH OF STORAGE, AND PACKAGING METHOD ON TOTAL PHENOL(mg/100g), OF SLICED RAW POTATOES STORED AT 10°C*

TRT	TOTAL PHENOL							
	ATMOSPHERIC PACK				VACUUM PACK			
	DAY 1	DAY 8	DAY 15	DAY 22	DAY 1	DAY 8	DAY 15	DAY 22
1	0.038	0.066	0.113	0.091	0.029	0.121	0.093	0.083
2	0.026	0.071	0.093	0.095	0.034	0.075	0.108	0.125
3	0.024	0.072	0.101	0.101	0.029	0.070	0.111	0.076
4	0.033	0.081	0.121	0.083	0.033	0.080	0.106	0.088
5	0.034	0.102	0.106	0.088	0.032	0.079	0.105	0.099
6	0.032	0.055	0.094	0.096	0.029	0.060	0.097	0.073
7	0.026	0.073	0.106	0.025	0.025	0.060	0.101	0.097

*Means; n=2; averaged over 2 blocks.

APPENDIX E

THE MEANS OF ANTIBROWNING AGENTS, LENGTH OF STORAGE, AND PACKAGING METHOD ON PPO ACTIVITY(A_{475}), OF SLICED RAW POTATOES STORED AT 10°C*

TRT	PPO ACTIVITY							
	ATMOSPHERIC PACK				VACUUM PACK			
	DAY 1	DAY 8	DAY 15	DAY 22	DAY 1	DAY 8	DAY 15	DAY 22
1	.135	.140	.276	.234	.148	.224	.234	.183
2	.128	.263	.307	.254	.134	.193	.260	.241
3	.142	.190	.347	.356	.147	.155	.258	.231
4	.157	.160	.304	.264	.149	.184	.270	.232
5	.156	.183	.269	.428	.138	.152	.252	.416
6	.174	.178	.295	.229	.169	.187	.236	.283
7	.203	.227	.343	.311	.183	.185	.268	.226

*Means; n=2; averaged over 2 blocks.

APPENDIX F

THE MEANS OF ANTIBROWNING AGENTS, LENGTH OF STORAGE, AND PACKAGING METHOD ON DEGREE OF BROWNING, OF SLICED RAW POTATOES STORED AT 10°C*

	DEGREE OF BROWNING							
TRT	ATMOSPHERIC PACK				VACUUM PACK			
	DAY 1	DAY 8	DAY 15	DAY 22	DAY 1	DAY 8	DAY 15	DAY 22
1	.669	.378	.800	.400	.686	.378	.800	.400
2	.615	.272	.839	.419	.659	.272	.839	.419
3	.599	.263	.807	.444	.600	.263	.807	.444
4	.679	.264	.901	.467	.657	.264	.901	.467
5	.668	.224	.764	.660	.591	.224	.764	.660
6	.739	.256	.726	.471	.696	.256	.726	.471
7	.729	.262	.831	.390	.668	.262	.831	.390

*Means; n=2
Degree of Browning:A_{475} and L* were normalized to give
$[A_{475}]n = (A_{475}-A_{475min})/(A_{475max}-A_{475min})$and
$[L^*]n = (L^*-L^*max)/(L^*min-L^*max)$, respectively. They were then summed, $S = [A_{475}]n + [L^*]n$, and the degree of browning was expressed as the normalized sum
$[S]n = (S-Smin)/(Smax-Smin)$

APPENDIX G

THE MEANS PPO ACTIVITY, TOTAL PHENOLS, DEGREE OF BROWNING, HUE ANGLE, CHROMA AND TOTAL COLOR DIFFERENCE OF SLICED RAW POTATOES STORED AT 10 °C*

	DAY 1	DAY 8	DAY 15	DAY 22
PPO Activity (abs)	.15429[a]	.18693[a]	.27971[b]	.27732[b]
Total Phenols (mg/100g)	.03004[a]	.07579[b]	.10368[c]	.09164[bc]
Degree of Browning	.661[ab]	.299[a]	.873[c]	.494[a]
Hue Angle	-41.5666[a]	-42.2013[a]	-79.9798[b]	-42.9206[a]
Chroma	9.0879[b]	3.8907[a]	10.9504[b]	3.8828[a]
Total Color Difference	0[a]	23.0281[b]	7.0682[a]	22.9775[b]

*Means=28; averaged over 2 blocks.
[a-c]Means in the same row with the same superscripts are not significantly different ($p \leq 0.05$).

VITA

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Doctor of Philosophy

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