Phenolic Autoxidation Is Responsible for Color Degradation in Processed Carrot Puree

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Strained carrots were thermally processed with reduced oxygen pretreatments and exposed to elevated storage temperatures to accelerate physicochemical changes (40 °C for 4 weeks). Strained carrot pretreatments prior to processing included a nitrogen sparge (N_2), blanch/frozen with nitrogen sparge (BFN_2), oxygen sparge (O_2), and a control (C) that received no pretreatment. Changes in color, total soluble phenolics, total carotenoids, phenolic acid molecular weight, sugars, and pH were monitored during storage. Greater losses of color, total soluble phenolics, and total carotenoids occurred in control and O_2-sparged samples as compared to N_2-sparged and BFN_2 samples. Molecular weight of phenolic acids was lower in nitrogen-sparged samples than control and oxygen-sparged samples. Phenolic polymerization due to autoxidation was responsible for color loss in processed strained carrots. Processing treatments that reduce residual oxygen may result in better color retention after processing and during storage. Determining the mechanism(s) and magnitude of these reactions are important for devising strategies to prevent quality loss in strained carrots.

Keywords: *Strained carrots; phenolic acids; autoxidation; color; carotenoids*

INTRODUCTION

Brown color formation in processed strained carrots is perceived as a quality defect, and methods to preserve fresh carrot color are important for improving consumer acceptance. Maceration of carrot roots followed by heating in holding tanks causes oxidative changes that result in brown color and possible off-flavor formation. Processing in enclosed systems resulted in better flavor scores for pears that were attributed to fast inactivation of enzymes and essence capturing (Leonard et al., 1976). Numerous browning reactions in food systems have been attributed to changes in pH (Cilliers and Singleton, 1990; Yeo and Shibamoto, 1991) and time and temperature of processing (Friedman and Molnar-Perl, 1990; Reyes et al., 1982). Alkali treatment of cooked, grated carrots resulted in product darkening as shown by decreased lightness values (Archana et al., 1994). Reaction rates for Maillard and phenolic nonenzymatic browning (NEB) are greatly influenced by temperature and pH. Thermal processing of low acid foods, involving elevated temperatures for extended periods of time, impacts the overall quality of carrots (Simon, 1985). Maillard browning reactions in strained carrots have been proposed but not conclusively proven. Maillard browning was postulated to contribute to color, nutritional, and flavor losses in retorted strained carrots (Luh et al., 1969), and studies reporting loss of reducing sugars and amino acids in processed carrots were consistent with this type of browning (Howard et al., 1996; Toribio and Lozano, 1986). However, reported losses of amino acids in many food systems can result from complexation with *o*-quinones, which are intermediates in phenolic acid oxidation (Cheftel et al., 1985). Since Maillard reactions do not occur extensively below pH 6.0 (retail carrot puree pH 5.0−5.5) or at commercial storage temperatures, other sources of browning may be significant.

Phenolic polymerization reactions due to autoxidation can result in brown-colored pigments that are detrimental to processed food quality. Browning rates of caffeic acid solutions are pH dependent with yellow pigments formed initially, followed by brown pigments as pH increases (Cilliers and Singleton, 1990). The limiting factor for phenolic autoxidation is the presence and concentration of the phenolate ion, which declines with decreasing pH (Cilliers and Singleton, 1989). The phenolate ion is a highly reactive species that catalyzes oxidative reactions in phenolic acid systems. Acidified phenolic solutions will generally remain colorless unless polymerization occurs directly from molecular oxygen present in the system. Therefore, oxygen exclusion coupled with product pH is important for controlling phenolic polymerization reactions (Bucheli and Robinson, 1994). Major phenols in carrots include chlorogenic, caffeic, and *p*-hydroxybenzoic acids along with numerous cinnamic acid derivatives (Babic et al., 1993). Acting as a phytoalexin, phenolic acids may increase up to 7-fold in carrot peel due to abiotic stress during postharvest handling and storage (Sarker and Phan, 1979). Excluding oxygen in low and medium acid food systems may prevent condensation of phenolic acids, resulting in greater color retention. This study was undertaken to explore the contribution of phenolic NEB reactions to color degradation of processed strained carrots.

MATERIALS AND METHODS

Materials and Processing. Carrots (*Daucus carota* L.) were purchased from a local market and stored at 4 °C until processed. Roots were washed, hand-peeled, cut into 2-cm

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pieces, steam blanched for 30 min, and cooled in an ice water bath. Roots were then combined with 75% water (carrot weight after blanching) and blended into a puree using a kitchen-scale blender. All blends were combined to create a homogeneous pooled sample. Treatments applied to subsamples included (1) a vacuum/nitrogen sparge (N_2) where the puree was deaerated for 10 min in a 4-L sidearm flask under a vacuum pressure of 635 mmHg and sparged for 20 min with nitrogen gas, and the jar headspace was flushed with nitrogen prior to capping; (2) an oxygen sparge (O_2) where the sample was sparged for 20 min, and the jar headspace was flushed with oxygen prior to capping; and (3) a blanch/frozen with nitrogen sparge (BFN_2) treatment where peeled whole carrots were steam blanched, cooled in ice water, packaged in polyethylene pouches, and frozen at −20 °C. After being thawed to room temperature, carrots were blended to a puree and treated in a manner similar to the N_2-sparged samples. A control (C) was processed directly from the pooled puree with no pretreatment. For all treatments, carrot puree was hand-filled into 11-oz glass jars, heated to 60 °C, and thermally processed at 121 °C for 30 min in a rotary retort (Stock Pilot-Rotor 900, Stock America Inc., Milwaukee, WI) at 19 rpm (F_o > 6). An unprocessed puree sample was analyzed to determine physicochemical changes during processing. Treated samples were stored 4 weeks at 40 °C and analyzed 0, 1, 2, 3, and 4 weeks after processing for physicochemical attributes.

In a separate experiment, carrot puree was thermally processed, as described above, to simulate a naturally high occurrence of phenolic acids or carrots that had experienced stress-induced synthesis of phenolic acids. Two treatments, a control and a phenolic spike, were applied to monitor the rate of phenolic oxidation and subsequent physicochemical changes during storage. Phenolics spiked into raw carrot puree included equal amounts of gentisic, *p*-hydroxybenzoic, *m*-coumaric, *o*-coumaric, and caffeic acids (~1250 mg/kg total). Carrots were thermally processed and monitored weekly against a nonspiked control for 4 weeks at 40 °C.

To chemically simulate strained carrot puree, a model solution containing 3 g/100 g sucrose, 0.5 g/100 g glucose, 0.5 g/100 g fructose, 50 mg/L caffeic acid, 50 mg/L syringic acid, 100 mg/L cysteine and citric/malic acids (1:1 mixture for pH adjustment) was heated for 2 h at 100 °C to simulate residence time of carrots in batch and hold tanks in a commercial setting. Variations in model solution pH ranged from 5 to 7.0. Phenolic browning was differentiated from Maillard browning by the exclusion of cysteine in identical models and calculating the difference in absorbance at 420 nm.

Physicochemical Analysis. Color, total soluble phenolics, total carotenoids, molecular weight of phenolic acids, sugars, and pH were measured in processed strained carrots. Carrot pH was measured using a Corning model 125 pH meter. Brown color formation in model systems was measured at 420 nm (Garcia et al., 1992). Tristimulus color was measured using a Hunter Labscan model 5100 colorimeter. Hunter a^* and b^* values were converted to hue (arctan b^*/a^*) and chroma $(a^{*2} + b^{*2})^{0.5}$ values. Total water-soluble phenolics were extracted in deionized water (5 g/50 mL) and measured using the Folin−Ciocalteau assay as described by Swain and Hillis (1959).

Sugars were extracted in water (5 g/50 mL) using a Tekmar model TP 18 tissuemizer (Cincinnati, OH). After filtering through Miracloth (Calbiochem, San Diego, CA), the isolate was passed through a prep-column containing 1 g of 200−400 mesh Bio-Rex 5 anion-exchange resin (Bio-Rad, Richmond, CA) to remove organic acids. Samples were filtered through a 0.45-μm filter and injected into a Spectra Physics model P100 HPLC equipped with a 300 × 7.8 mm Aminex HPX-87C (Bio-Rad, Richmond, CA) carbohydrate column heated at 85 °C. Mobile phase consisting of 100% Milli-Q water, run isocratically at 0.8 mL/min, was monitored with a Shodex model RI-71 refractive index detector. Sucrose, glucose, and fructose were quantified using external standards.

Total carotenoids were extracted (2 g/25 mL) with a solution containing acetone/ethanol (1:1) with 200 mg/L BHT added. Samples were extracted in the dark, filtered through Whatman No. 4 filter paper, and washed until the residue was colorless. Samples were adjusted to 100 mL, and the absorbance was measured using a Hewlett-Packard 8452A diode array spectrophotometer. Total carotenoids were calculated according to Gross (1991) using the equation $(AV \times 10^6)/(A^{1\%} \times 100G)$, where A is the absorbance at 470 nm, V is the total volume of extract, $A^{1\%}$ is the extinction coefficient for a mixture of solvents arbitrarily set at 2500, and G is the sample weight in grams.

Molecular weight determination of soluble phenolic compounds was performed at 4 °C using Sepharose CL-6B agarose resin (Sigma Chemical Co., St. Louis, MO) packed in a glass column (4.5 cm × 100 cm). Soluble phenolic acid isolates were obtained by centrifuging strained carrots until supernatant could be passed through a 0.45-μm filter. Deionized water carried 3 mL of strained carrot isolate through the column, and fractions were collected every 125 drops using a drop counter. Dextran standards were used to determine molecular weight distributions from the column and fractions analyzed for total carbohydrate (DuBois et al., 1956) by mixing 1 mL from each fraction with 1 mL of 5% phenol and 5 mL of concentrated H_2SO_4 in a test tube. Tubes were heated for 10 min at 80 °C and cooled, and the absorbance was read at 490 nm. Fractions from the carrot isolate were measured at 280 nm (Litridou et al., 1996), and the data were reported as total soluble phenolics.

Statistical Analysis. Three jars from the four treatments were analyzed in triplicate at each storage time. Chemical data were analyzed by analysis of variance (SAS Institute, Inc., 1985), and mean separation was conducted using Duncan's multiple range test ($P < 0.05$). Stepwise linear regression analysis was performed to predict carrot color from chemical data.

RESULTS AND DISCUSSION

Chemical Analysis. Browning reactions in processed strained carrots were primarily related to phenolic acid condensation with a minor contribution from carotenoid oxidation. Changes in color, pH, total soluble phenolics, carotenoids, and sucrose were significantly affected by storage time and treatment ($P < 0.05$). A decline in soluble phenolic content was consistent with an increased level of oxygen incorporated into the samples (Figure 1A). Since no enzyme activity remains in thermally processed products, oxidation of phenolic acids occurred via autoxidation reactions. Levels of water-soluble phenolics declined throughout the study with the greatest losses occurring during thermal processing. Thermal processing accounted for 58.5% of total phenolic acids lost in the study for the control, 27.2% in N_2-sparged, 27.8% in BFN_2, and 51.4% in O_2-sparged samples. Losses indicate that unit operations that exclude oxygen prior to thermal processing are critical for maintaining strained carrot color. Phenolic acids, as monitored by the Folin−Ciocalteu assay, were polymerized enough to become water-insoluble since the assay will detect any soluble reducing compounds including some conjugated phenols and reducing sugars. The decline in pH was greatest after thermal processing (0.9 unit), which can be attributed to a reduction in particle size and subsequent release of organic acids or additional unknown reactions (Figure 1B). The pH continued to decline during storage at elevated temperature and reached its lowest value of 5.27 in O_2-sparged samples. Decreased pH is detrimental to overall carrot flavor, and additional work is needed to understand the mechanism responsible for the decline in thermally processed products.

Greater carotenoid retention was observed in N_2-sparged and BFN_2 treatments as compared to O_2-sparged and control samples (Figure 1C). Carotenoid

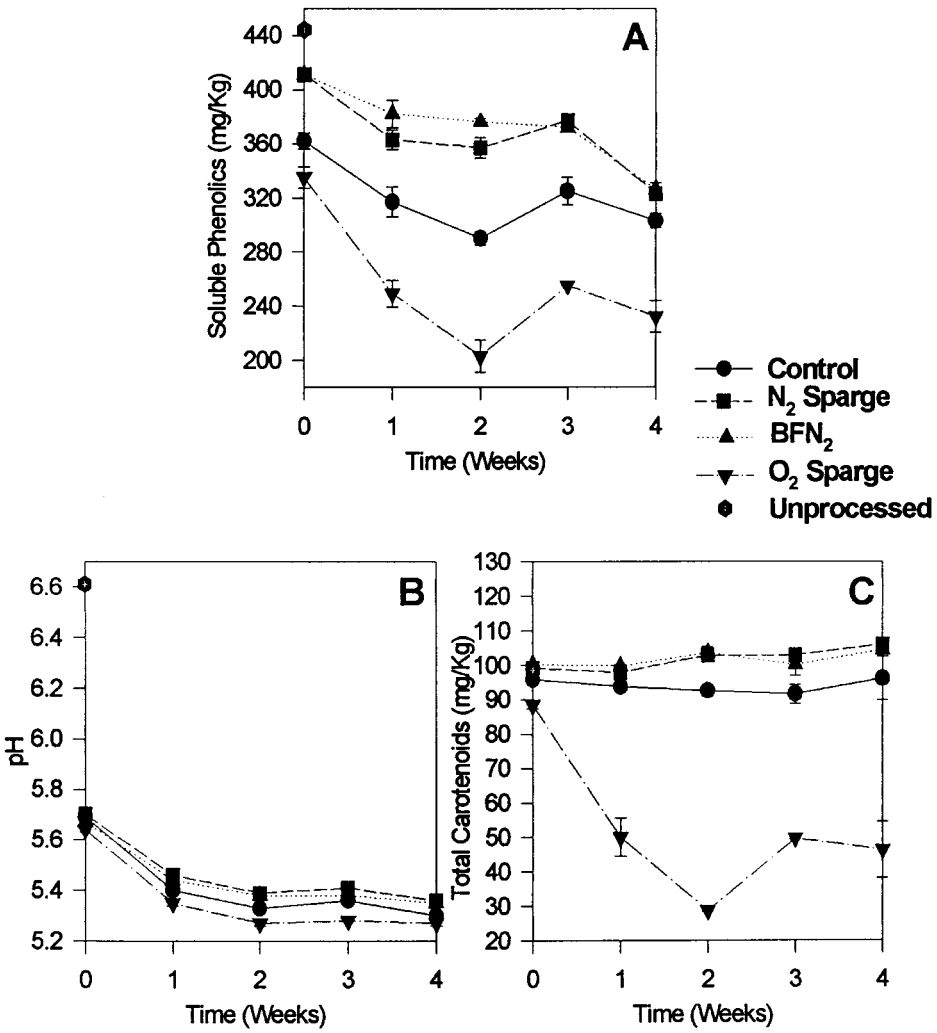

Figure 1. Effects of pretreatment on (A) total soluble phenolics, (B) pH, and (C) total carotenoids in strained carrots. Treatments include no pretreatment (control), nitrogen sparge (N_2 sparge), blanched/frozen with nitrogen sparge (BFN_2), and oxygen sparge (O_2 sparge). Unprocessed puree demonstrates changes during thermal processing. Bars represent standard error of the mean.

concentrations declined expectedly in control (6.4%) and O_2-sparged (40.6%) as compared to N_2 treatments. Greater retention in N_2-treated samples demonstrated that O_2 exclusion from strained carrots was beneficial for carotenoid and color retention. Hue angle values (Figure 2A) significantly increased after thermal processing in O_2-sparged samples and then declined throughout the study, which may be due to the formation of colored pigments from NEB phenolic polymerization reactions. Nitrogen sparging treatments resulted in lower hue angles after thermal processing and declined at a slightly slower rate over time as compared to control and O_2-sparged samples. An elevated hue angle in O_2-sparged and control samples was indicative of initial oxidation products (yellow pigments) of phenolic acids that were prevented from polymerizing in N_2-treated samples. Therefore, a high hue angle that declined over time was indicative of product darkening as monomer units polymerized into brown pigments.

Declines in pH, soluble phenolics, and total carotenoids all correlated with decreased chroma values (r^2 = 0.49, 0.90, and 0.70, respectively) in strained carrots (Figure 2B). Nonpolar and polymerized phenolic acids may have affinity for the lipid fraction in carrots, which contain carotenoids, directly lowering chroma values or upon their subsequent oxidation. This action would serve to dull the vivid orange color found in non-oxidized purees. Linear regression analysis demonstrated that hue angle and chroma were significantly affected by the levels of soluble phenolics and puree pH ($R^2 = 0.91$ and 0.83, respectively), which demonstrates the importance of strict oxygen exclusion prior to thermal processing.

The inability to completely reverse color formed after oxygen sparging of phenolic acid model systems has been demonstrated in our lab; however, visible color formed by alkali addition can be reversed if the puree is acidified within a short period of time. Similar colored pigments formed in a caffeic acid model system above pH 5.60 and were attributed to quinone formation during oxidation (Fulcrand at al., 1994). Strained carrots that were held in long-term storage (>2 yr) exhibited improved color ($L^* = +1.59$) when acidified, indicating that irreversible pigments from Maillard browning were not the sole source of pigmentation formed. A similar increase in lightness due to acidification was demonstrated by Archana et al. (1994) that was attributed to changes in carotenoid isomers present. Change in pigmentation was also noticed in pH-altered sweet potato puree where normal color returned as the sample pH was adjusted back to its original value (Ice et al., 1980). Lightness (L^*) values in strained carrots declined continually throughout the shelf life study with the largest decline occurring during processing (Figure 2C). High L^* values indicate a lighter color while

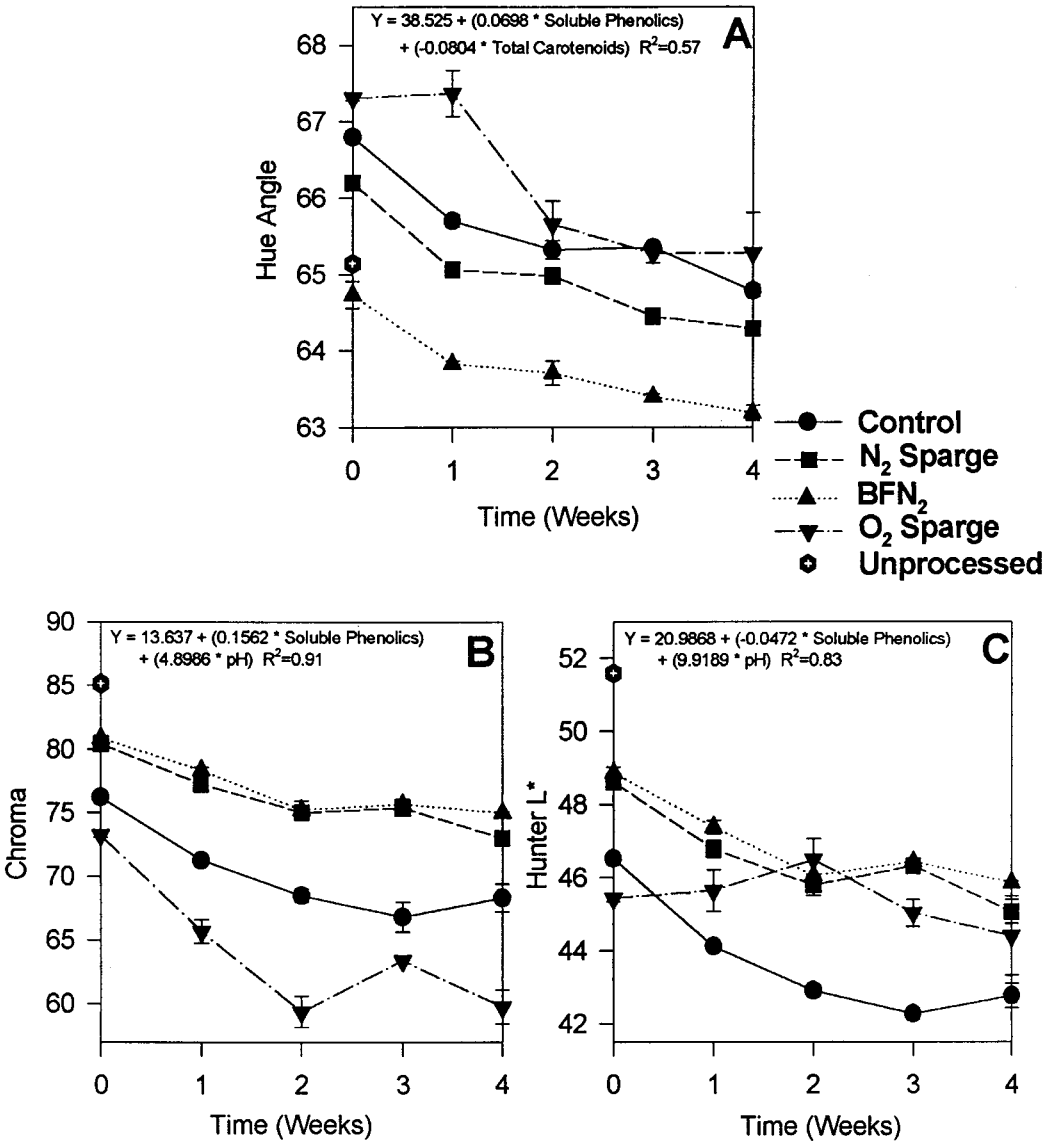

Figure 2. Effects of pretreatment on (A) hue angle, (B) chroma, and (C) Hunter L^* of strained carrots. Treatments include no pretreatment (control), nitrogen sparge (N_2 sparge), blanched/frozen with nitrogen sparge (BFN_2), and oxygen sparge (O_2 sparge). Unprocessed puree demonstrates changes during thermal processing. Bars represent standard error of the mean.

decreased chroma and hue values correspond to darker colors (Yang and Yang, 1987). Based on lightness values of unprocessed strained carrots, control samples declined 9.8% after processing as compared to 5.8% and 5.3% losses for N_2 and BFN_2 samples, respectively. During storage, O_2-sparged samples slowly bleached and were almost completely white from the headspace to the center of the product, resulting in elevated lightness values. The presence of oxygen likely initiated extensive phenolic and lipid oxidation, resulting in the formation of reactive peroxides responsible for carotenoid losses ($R^2 = 0.57$). However, the regression model for lightness was not greatly improved when the O_2 treatment was removed from the model ($R^2 = 0.64$). Compared to control and O_2-sparged samples, N_2 and BFN_2 treatments resulted in higher lightness values, demonstrating a relationship with the degree of oxidation.

Molecular weight (MW) determination of water-soluble phenolics was performed on samples isolated from day 0 (immediately after thermal processing) and after 4 weeks storage (Figures 3 and 4, respectively). Molecular weight fractions near the maximum absorbance at 280 nm were quantified by the Folin−Ciocalteu assay and corresponded to the retention of low molecular weight phenolic compounds (Table 1). Nitrogen and BFN_2 treatments had higher amounts of low molecular weight phenolics (252 and 253 mg/kg, respectively) than control and O_2-sparged samples (223 and 207 mg/kg, respectively) after processing, indicating that phenolic polymerization was reduced with oxygen exclusion. Molecular weight determination of phenolic acids served to further identify their role in product darkening and is apparently related to phenolic polymerization reactions resulting in the loss of these compounds.

Sucrose concentrations were slightly higher in BFN_2 samples than control, O_2-sparged, and N_2-sparged samples (data not shown). This result may be attributed to the freeze−thaw cycle that resulted in cellular disruption and greater liberation of sugars. Yan (1989) also found that processing frozen carrots into puree resulted in higher Brix levels than puree obtained from nonfrozen carrots. Glucose and fructose concentrations were unaffected by processing treatments, indicating that reducing sugars were not consumed in Maillard reactions. In contrast, Howard et al. (1996) reported a

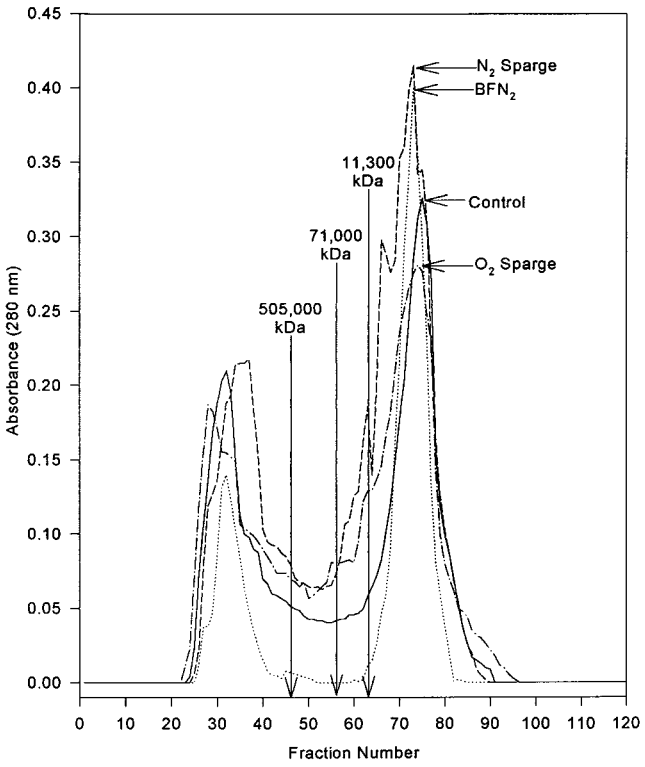

Figure 3. Effect of pretreatment on molecular weight distribution (kDa) of phenolic acids in strained carrots immediately after thermal processing. Treatments include no pretreatment (control), nitrogen sparge (N_2 sparge), blanched/frozen with nitrogen sparge (BFN_2), and oxygen sparge (O_2 sparge).

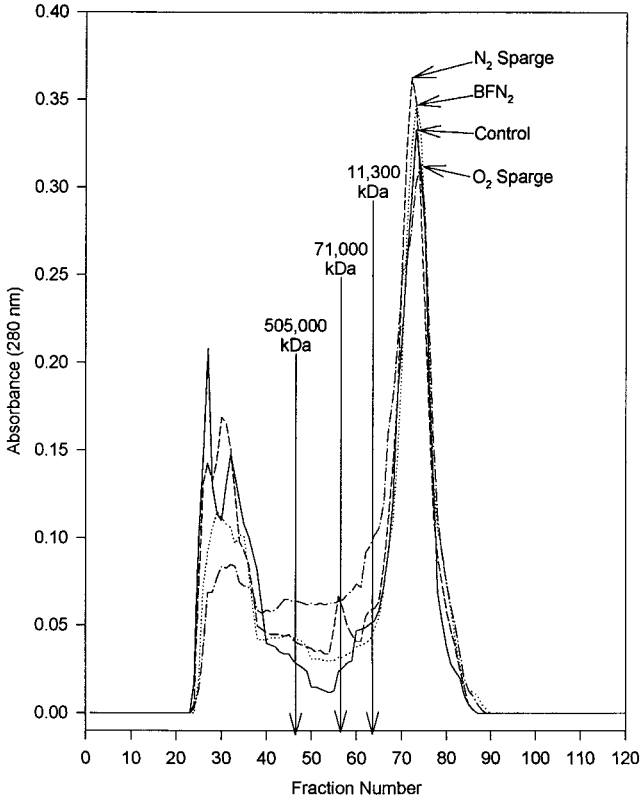

Figure 4. Effect of pretreatment on molecular weight distribution (kDa) of phenolic acids in strained carrots after 4 weeks storage at 40 °C. Treatments include no pretreatment (control), nitrogen sparge (N_2 sparge), blanched/frozen with nitrogen sparge (BFN_2), and oxygen sparge (O_2 sparge).

loss of reducing sugars in carrot puree, which were hypothesized to be consumed in Maillard browning reactions. Organic acids remained unchanged throughout the study and were unaffected by processing treatments (data not shown).

Strained carrot samples spiked with phenolic acids followed similar trends for color degradation as described in the accelerated shelf life study (data not shown). Soluble phenolic changes in control and spiked samples declined at a constant rate with significant losses observed after processing (9.4% and 4.2%, respectively; Figure 5A). Strained carrot pH demonstrated a consistent rate of decline, but spiked samples exhibited a lower pH (5.28) due to acidity imparted by the phenolics as compared to control samples (pH 5.82; Figure 5B). This acidification effect served to lower the initial decline in pH after processing for the spiked samples (0.08 unit) as compared to the control (0.74 unit). This small decline in pH after processing for spiked samples demonstrated that either oxidative reactions or a change in the buffering ability of the puree resulted in a pH decline during processing. Since oxidation rates are generally pH dependent, the rate of pH decline was slower for spiked samples as compared to the control during the first week of storage. It was hypothesized that increasing phenolic acid concentration would result in more rapid losses of soluble phenolics, resulting in a faster decline in pH, and would result in rapid discoloration during storage. However, spiked samples resulted in a 16.6% loss of phenolic acids as compared to a 31.8% loss in nonspiked samples. Results indicate that the reaction rate of physicochemical changes would likely be constant under similar storage conditions regardless of phenolic concentration, but a lower puree pH would serve to slow oxidative reactions affecting strained carrot color. On the basis of percentage loss, the effect of phenolic acid concentration and pH decline was similar between spiked and control samples. Individual phenolic acids have been shown to oxidize at different rates (Garcia et al., 1992); therefore, concentration and identity of these compounds after processing and storage may impact strained carrot color.

Table 1. Concentration of Water-Soluble Phenolics (mg/kg) in Strained Carrots after Thermal Processing (Day 0) and after 4 Weeks of Storage (Week 4) in an Accelerated Shelf Life Held at 40 °C for 4 Weeks[a]

treatment	day 0 (mg/kg)	week 4 (mg/kg
control	223	188
N_2	252	200
BFN_2	253	202
O_2	207	145

[a] A molecular weight column was used to separate phenolic acid fractions (λ_{max} = 280 nm) and quantification was conducted by the Folin−Ciocalteu assay.

Model System. Carrot model solutions were prepared to verify the major role of phenolic oxidation in color production and to determine if Maillard browning was a potential source of pigmentation during commercial processing applications. After heating each model system, solution pH was adjusted to original levels and color was monitored at 420 nm. Color formation due to Maillard browning was determined by subtracting the absorbance due to phenolic acid condensation (no cysteine added) from the total color formed (with cysteine) and expressed on a percentage basis (Table 2). Phenolic acid condensation was responsible for the yellow and brown pigments formed, with

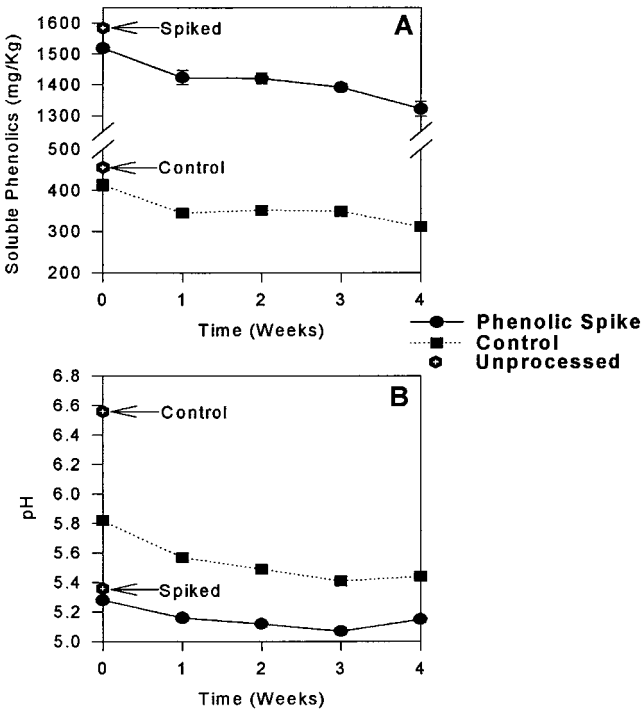

Figure 5. Effects of pretreatment on (A) total soluble phenolics and (B) pH in strained carrots stored 4 weeks at 40 °C. Treatments included a nonspiked control and a phenolic acid spike (~1250 mg/kg). Unprocessed puree demonstrates changes during thermal processing. Bars represent standard error of the mean.

Table 2. Contribution (% of Total Color Formation) of Phenolic Condensation and Maillard Browning at 420 nm to Total Color Formation in a Strained Carrot Model Solution[a]

	total browning	
initial pH	phenolic browning (%)	Maillard browning (%)
7.00	54.9	45.1
6.50	68.3	31.7
6.00	97.5	2.2
5.50	88.0	12.0
5.00	100	0

[a] Phenolic browning calculated as color formed without cysteine minus color formed with cysteine. Model solution contained 3% sucrose, 0.5% glucose, 0.5% fructose, 50 mg/L caffeic acid, 50 mg/L syringic acid, 100 mg/L cysteine, and citric/malic acid (1:1) adjusted to pH 5−7. Solutions were heated for 2 h at 212 °F.

little contribution by Maillard browning. More pigmentation was formed at higher pH values as reported by numerous investigators (Cerrutti et al., 1985; Yeo and Shibamoto, 1991; Friedman and Molnar-Perl, 1990) which were attributable to both phenolic acid condensation and Maillard browning. Results indicated that color formation due to Maillard reactions would develop at a slow rate at the pH range of strained carrots with the majority of pigmentation (>88%) resulting from phenolic condensation.

CONCLUSIONS

Processors should avoid unit operations that allow for excessive heat and oxygen incorporation into macerated fruit and vegetable products due to phenolic autoxidation potential. Utilization of nitrogen sparge techniques immediately after maceration followed by headspace flushing with nitrogen prior to capping should improve overall color retention in strained carrots. Heat in combination with dissolved oxygen is detrimental to strained carrot color, and attempts should be made to reduce oxygen concentration in the system to prevent phenolic autoxidation and carotene bleaching.

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