Peroxidase and Lipoxygenase Influence on Stability of Polyunsaturated Fatty Acids in Sweet Corn (Zea mays L.) during Frozen Storage

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ABSTRACT

The effect of blanching treatments and packaging materials on lipoxygenase (LOX) and peroxidase (POD) activity and fatty acid stability of two cultivars of sweet corn (Jubilee and GH-2684) were evaluated during 9 mo storage at -20°C. Complete inactivation of LOX and POD was obtained with 9 and 15 min of steam blanching, respectively. Relative fatty acid content revealed no change in fatty acid composition during storage. Control of degradation of polyunsaturated fatty acids (PUFA) did not depend on oxygen permeability of different packaging materials. Blanching had little effect on PUFA degradation after 9 mo storage.

Key Words: sweet corn, peroxidase, lipoxygenase, polyunsaturated, fatty acids

INTRODUCTION

LIPID OXIDATION is a major cause of food spoilage because it leads to development of off-flavors and odors in edible oils and fat-containing foods (Nawar, 1985). The oxidative deterioration of food lipids involves primarily autoxidation by free radical chain reaction, photo-oxidation (Frankel, 1980; Nawar, 1985) and enzymatic oxidation catalyzed by lipoxygenases (Siedow, 1991; Galliard and Chan, 1980; Hildebrand, 1989). The primary initial products of lipid oxidation are hydroperoxides which decompose into a wide range of carbonyl compounds, hydrocarbons, aldehydes, ketones and other materials causing rancidity (Frankel, 1991).

Freezing is used to maintain product quality over long storage and results in a slower rate of most deteriorative reactions such as senescence, enzymatic decay, chemical decay and microbial growth (Labuza, 1982). However, freezing does not prevent off-flavor development, color and texture deterioration in frozen vegetables because enzyme systems may remain active even at sub-zero temperatures. Blanching, before freezing, is used to inactivate enzymes, reduce microorganisms and increase digestibility of some products. It also improves color and flavor of vegetables as a result of removal of gases. Adverse effects of blanching are permanent modification of cellular structure, solubilization and/or destruction of some nutrients and vitamins, and conversion of green chlorophylls to yellow green phytochlorins (Bald, 1991; Katsboxakis, 1984).

To minimize thermally induced textural changes or nutrient leaching, it has been suggested that blanching be optimized for each product. This requires determining the heat treatment needed to inactivate enzymes responsible for deleterious changes during freezing and frozen storage (Reid, 1990; Williams et al., 1986).

POD is widely used to indicate adequate blanching because it is highly resistant to heat inactivation, is present in most vegetables and fruits. It is detected by a sensitive and simple colorimetric test (Richardson and Hyslop, 1985). Correlations have been reported between off-flavor development and POD activity in frozen sweet corn on the cob (Lee and Hammes, 1979). POD, like most heme proteins, catalyzes the nonenzymatic, peroxidative degradation of unsaturated fatty acids yielding volatile and flavorful carbonyl compounds that may contribute to oxidized flavor (Richardson and Hyslop, 1985). However, some studies have shown that POD is not directly involved in quality deterioration of frozen unblanched vegetables and its complete inactivation may result in overblanching (Williams et al., 1986). The measure of LOX activity has been suggested as an indicator of adequacy of blanching for several vegetables (Axelrod et al., 1981; Sheu and Chen, 1991; Chen and Hwang, 1988; Williams et al., 1986; Chen and Whitaker, 1986; Wagenknecht and Lee, 1958) including sweet corn (Garrote et al., 1985; Wagenknecht, 1959). It is closely related to destruction of essential fatty acids, off-flavor development and pigment degradation.

Our objective was to evaluate the relationship between LOX and POD activities with polyunsaturated fatty acid stability in two cultivars of sweet corn on the cob (Jubilee and GH-2684). They were subjected to different blanching treatments and stored using different packaging materials for 9 mo at -20°C.

MATERIALS & METHODS

Plant material

Fresh sweet corn (Zea mays L.) of Jubilee and GH-2684 (Rogers NK seed Co, Research Center) cultivars were obtained from the Oregon State University Vegetable Experimental Station. Both were harvested on the same day, using the percent moisture content of kernels as an index of maturity.

Processing of samples

Sweet corn (~1000 ears) of each variety were harvested and immediately transported to the Oregon State University Vegetable Experimental Station. Both were harvested on the same day, using the percent moisture content of kernels as an index of maturity. After blanching, the sweet corn ears were cooled immediately in water (9°C) for 25 min, drained and frozen on trays in a blast freezer at -35°C for 2 hr. The frozen ears from each treatment group were cut at each end to provide ears of ~14 cm length. Three ears were used for zero time analysis and the remaining ears were divided into four subgroups of nine ears each and packed using polyethylene, Cryovac E-Bag (E bags), Cryovac Barrier Bag (B bags) (Cryovac Division, W.R. Grace & Co., Duncan, SC) or no packing material (control). Cryovac E and Cryovac B bags have moisture permeabilities of 0.1 g/100 cm²/24 hr at 100% relative humidity and 35°C. They differed in oxygen permeability (40 mL/100 cm²/24 hr at 23°C and 1 atm for E-bags and 0.3-0.4 mL/100 cm²/24 hr at 23°C and 1 atm for B-bags, Deak et al., 1987). Ears were placed three to a pack for each packing material. The polyethylene bags were hand sealed and the Cryovac B and E bags were heat sealed using a Verwachting vacuum sealer. The ears were stored for 9 mo and analyses were performed at 0, 3, 6 and 9 mo.

Moisture analysis

The moisture content was determined using the microwave method of Beckwar et al. (1977). Analyses were performed before processing and after 9 mo frozen storage at -20°C.
The moisture content on fresh corn was determined from samples composed of kernels pooled from six separate ears of corn. A total of 18 ears were used for analysis (triplicate analysis). Moisture analyses were done at 9 mo on samples prepared from kernels pooled from three separate ears of corn packed under specific packaging materials. Nine ears of corn were used to prepare three samples, with two of the samples being analyzed.

Sample preparation

Whole kernels of sweet corn (100 g) were frozen in liquid nitrogen and blotted using a Waring Blender at 100 rpm for 1 min. In order to keep the sample frozen for efficient blending, liquid nitrogen was added every 15 sec. A fine powder was obtained which was stored in a cold container at -20°C until analyzed.

Enzyme assays

Enzyme extraction. The powdered corn (2 g) was placed in a centrifuge tube and 20 mL of 0.1M Tris-HCl buffer pH 8.0 were added. The mixture was homogenized using a tissuemizer at 50 rpm for 3 min. After homogenization, the probe was rinsed with 1 mL of 0.1M Tris-HCl buffer pH 8.0. The homogenate was centrifuged using a Sorvall RC5 superspeed refrigerated centrifuge in an SS 34 rotor at 12,000 rpm (17210 x g) for 1 hr at 4°C. The supernatant was removed, avoiding the floating top layer, and used for activity measurements.

Lipoxygenase activity. Lipoxygenase (linolate:oxygen oxidoreductase, EC 1.13.11.1) was assayed by the method of Chen and Whittaker (1986), based on absorption at 234 nm of the conjugated dienes formed when linoleic acid (used as substrate) was oxidized in the presence of LOX. The substrate solution was prepared by mixing 157.2 µL linoleic acid, 157.2 µL Tween 20 and 10 mL distilled water, clarified by adding 1 mL of 1.0N NaOH and diluted with 0.1M sodium phosphate buffer pH 7.0 to a final volume of 200 mL. Prior to assay, the substrate solution was transferred to an amber container, aerated with oxygen for 10 min and allowed to stand in a shaking water bath (American YB-521) at 25°C for 10 min.

For the assay, 150 µL of enzyme extract was added to 2.85 mL of linoleic acid substrate solution in a quartz cuvette and mixed. The LOX activity was measured from the change in absorbance at 234 nm over time using a Shimadzu 160U UV-VIS spectrophotometer and the temperature was controlled at 25°C. One unit of enzyme activity was defined as that amount of protein that produced a change of 1 unit in absorbance/min.

Peroxidase activity. Peroxidase was assayed by the method of Sheu and Chen (1991), based on the increase in absorbance at 420 nm resulting from oxidation of guaiacol in the presence of hydrogen peroxide. The substrate solution was prepared by mixing 558 µL guaiacol and 194.4 µL of 30% hydrogen peroxide; the solution was then diluted to 100 mL with 0.2M sodium phosphate buffer pH 6.0 to give a concentration of 0.05M guaiacol and 0.02M hydrogen peroxide. The reaction vessel was measured from initial increase in absorbance at 420 nm over time with a Shimadzu 160U UV-VIS recording spectrophotometer and the temperature was controlled at 25°C. One unit of enzyme activity was defined as that amount of protein that produced a change of one unit in absorbance/min.

Protein

Purified was assayed using the method of Lowry et al. (1951) by measuring absorbance at 700 nm. Bovine serum albumin was used as standard.

Fatty acids analysis

Corn lipids were extracted following the procedures proposed by Bligh and Dyer (1958). Fatty acids were analyzed by the fatty acyl methyl esters (FAME) method (Schindelbeck, 1977). The lipid extract was transesterified by heating in 4% methanolic H2SO4 solution at 80-90°C for 90 min. A partition of hexane and water was used to extract the methyl esters. The hexane phase was collected and evaporated under nitrogen and the dried sample was taken up into 0.5 mL isooctane, flushed with nitrogen and stored at -80°C until analyzed by gas chromatography.

Analysis of methyl esters was performed on a Hewlett Packard 5890 Gas Chromatograph equipped with FID, 3393A Integrator and 9122 Dual Disc Drive. A 30m x 0.25 mm i.d. fused silica Supelco 2330 capillary column was used for analysis of all nitrogen powered corn fatty acid methyl esters. Conditions were injection port and detector 230°C, the column was run isothermally at 175°C for 10 min and increased to 210°C at 5°C/min with a final hold time of 10 min. The carrier gas was helium at 1 mL/min. Fatty acid methyl esters were identified by comparing to authentic standards Nu Check 20A (Nu Check Prep) and corn oil fatty acids.

Determination of peroxide value

The peroxide value was determined using the method of Schmedes and Holmer (1989). The method is based on the oxidation of Fe(II) to Fe(III) by peroxides; Fe(III) forms a violet complex with thiocyanate and this complex is quantitated spectrophotometrically. For calculation of peroxide value, the absorbance of the sample and lipid blank were converted to mg Fe(II)/5 mL solution using standard curve values and the net value in terms of mg of Fe(III)/5 mL of solvent was calculated by the difference between values obtained for the sample and blank.

The peroxide value was expressed in terms of meq oxygen/kg lipid (Stine et al., 1954).

Statistical analysis

The experiment was conducted using a split-plot design. The split occurred at the blanching stage, with variety and blanching as whole plot and packaging material and storage time as sub-plot. The data were evaluated using Statistical Analysis System (SAS Institute, Inc. software). Data were further evaluated by a regression analysis using the stepwise procedure (Ramsey and Schafer, 1992). The regression analyses were performed using the Statistical Graphics System (Stat-Graphs software).

RESULTS & DISCUSSION

Lipoxygenase activity

There was substantial evidence that the initial level of LOX activity in the unblanched whole kernels varied between the cultivars (2-sided p-value < 0.0001, step backward regression analysis). The Jubilee showed an initial mean LOX activity of 0.143 ± 0.032 units/mg protein, 32.4% higher than the mean LOX activity of the GH-2684 with a level of 0.105 ± 0.017 units/mg protein. It has been reported that LOX activity varied among cultivars, different organs within the same plant (Garrote et al., 1985; Vick and Zimmerman, 1976), and development stages (Kermasha and Metche, 1987). The regression analysis indicated that LOX activity decreased during the 9 mo of frozen storage in both unblanched sweet corn cultivars, even after accounting for the effects of different cultivars (2 sided p-value < 0.0001). Steam blanching at 100°C for 9 min completely inactivated LOX activity in both cultivars. Blanching for 15 min had no further effect on LOX activity (Table 1).

Peroxidase activity

No significant difference in initial POD activities were found in kernels of any unblanched sweet corn (Table 1). An average activity of 1.082 ± 0.039 and 1.075 ± 0.051 units/mg protein was determined for Jubilee and GH 2684 respectively. Regression analysis indicated (2 sided p-value < 0.0001) that the POD activity of unblanched samples decreased as a quadratic function of frozen storage time. The POD activity decreased sharply in the first 3 mo with a tendency to stabilize during later frozen storage. Both cultivars showed similar changes during 9 mos frozen storage (Table 1).

After 9 min of blanching some residual POD activity was present. A regression analysis for the 9 min blanched samples over storage time showed that the POD activity had a tendency to decrease faster in the GH 2684 than in the Jubilee (2 sided p-value < 0.0001), suggesting the presence of POD isozymes that were more stable to low temperatures in the Jubilee. Complete inactivation of POD activity was obtained when the corn was blanched for 15 min.

The data showed a decrease in activity of LOX and POD during frozen storage. According to Richardson and Hyslop...
Table 1—Lipoxygenase (LOX) and Peroxidase (POD) activity in whole kernels of Jubilee and GH-2684 corn during 9 mo storage at -20°C

<table>
<thead>
<tr>
<th>Blanching time (min)</th>
<th>Storage time (months)</th>
<th>LOX activity (change in O.D. at 234 nm/min/mg protein)</th>
<th>POD activity (change in O.D. at 420 nm/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1.047 (0.019)</td>
<td>0.443 (0.024)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.180 (0.016)</td>
<td>0.034 (0.034)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.180 (0.016)</td>
<td>0.034 (0.034)</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0.180 (0.016)</td>
<td>0.034 (0.034)</td>
</tr>
<tr>
<td>Jubilee</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.143 (0.018)</td>
<td>0.148 (0.018)</td>
<td>0.148 (0.018)</td>
</tr>
<tr>
<td>9</td>
<td>N.A.</td>
<td>0.165 (0.017)</td>
<td>N.A.</td>
</tr>
<tr>
<td>15</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>GH-2684</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.108 (0.017)</td>
<td>0.102 (0.014)</td>
<td>0.094 (0.010)</td>
</tr>
<tr>
<td>9</td>
<td>N.A.</td>
<td>0.102 (0.014)</td>
<td>0.094 (0.010)</td>
</tr>
<tr>
<td>15</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

aAverages over packaging materials; no significant differences observed among them (Z-sided p-value > 0.05). (1): Standard deviation.
b N.A.: no activity detected.

Table 2—Relative percent linoleic and linolenic acid in whole kernels of the Jubilee and GH-2684 corn during 9 mo storage at -20°C

<table>
<thead>
<tr>
<th>Blanching time (min)</th>
<th>Storage time (months)</th>
<th>Relative percent linoleic acid content (%)</th>
<th>Relative percent linolenic acid content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>44.73 (2.57)</td>
<td>1.82 (0.25)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>45.87 (2.57)</td>
<td>1.97 (0.25)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>45.87 (2.57)</td>
<td>1.97 (0.25)</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>45.87 (2.57)</td>
<td>1.97 (0.25)</td>
</tr>
<tr>
<td>Jubilee</td>
<td></td>
<td>44.73 (2.57)</td>
<td>1.82 (0.25)</td>
</tr>
<tr>
<td>0</td>
<td>45.76 (1.43)</td>
<td>2.01 (0.25)</td>
<td>1.91 (0.25)</td>
</tr>
<tr>
<td>9</td>
<td>45.76 (1.43)</td>
<td>1.88 (0.25)</td>
<td>1.87 (0.25)</td>
</tr>
<tr>
<td>15</td>
<td>45.76 (1.43)</td>
<td>1.98 (0.25)</td>
<td>1.99 (0.25)</td>
</tr>
<tr>
<td>GH-2684</td>
<td></td>
<td>46.77 (1.43)</td>
<td>1.98 (0.25)</td>
</tr>
<tr>
<td>0</td>
<td>45.88 (1.43)</td>
<td>1.79 (0.25)</td>
<td>1.82 (0.25)</td>
</tr>
<tr>
<td>9</td>
<td>45.88 (1.43)</td>
<td>1.82 (0.25)</td>
<td>1.82 (0.25)</td>
</tr>
<tr>
<td>15</td>
<td>45.88 (1.43)</td>
<td>1.81 (0.25)</td>
<td>1.82 (0.25)</td>
</tr>
</tbody>
</table>

aAverages over different packaging materials; no statistical differences observed among them (2-sided p-value > 0.05). (1): Standard deviation.

(1985) the decrease in enzyme activity during freezing and/or frozen storage may be due to a change in stability of enzyme conformers, increased intra-enzymic hydrogen bonding or decreased accessibility of enzyme to substrate. It could also be due to increased hydrogen bonding between water and either substrate or enzyme active site, formation of enzyme polymers, changes in mechanism, shifts in pH or increases in viscosity.

Fig. 1—Change in peroxide value, (meq O₂/kg lipid), in whole kernels of Jubilee and GH-2684 corn during sixth and ninth months of storage at -20°C. Averages over different packaging materials; no statistical differences observed among them (2-sided p-value > 0.05).

Fatty acid composition

Although the corn evaluated had different genotypes, no significant difference in initial fatty acid content was observed. The major fatty acids were palmitic (14.93 ± 1.94%), stearic (2.79 ± 1.00%), oleic (31.54 ± 2.87%), linoleic (46.87 ± 5.88%), and linolenic (1.89 ± 0.36%) acids. There was no statistical difference in relative percent linoleic acid between the cultivars. Different blanching treatments had no effect on relative percent linoleic acid content, suggesting that enzymatic oxidation of lin-
oleic acid did not occur during frozen storage. There was statistical evidence (2-sided p-value < 0.001) that the percent linoleic acid content changed during storage. After 3 mo frozen storage a small decrease was observed in relative percent of linoleic acid, which tended to stabilize later (Table 2). This suggested possible autooxidation of the fatty acid due to a self-catalytic free radical mechanism.

There was significant evidence that the mean percent linolenic acid content varied during frozen storage (2-sided p-value < 0.0001). The relative percent linolenic acid content in the whole kernels decreased during frozen storage (Table 2). Regression analysis indicated that the mean % linolenic acid content was dependent on blanching treatment (2-sided p-value < 0.0001). Unblanched samples decreased at a faster rate than blanched samples in both cultivars, suggesting enzymatic oxidation of linolenic acid during storage. Poca et al. (1990) characterized a corn lipoxygenase isozyme (L1) with high affinity for α-linolenic acid leading to the formation of 13-hydroperoxides. In addition, autoxidation by free radical mechanisms was suggested by the decrease in relative percent linolenic acid of blanched samples during frozen storage, especially at 15 min where both enzymes were inactivated.

**Peroxide value**

The peroxide value was related to cultivar (2-sided p-value < 0.01) and blanching treatment (2-sided p-value < 0.01). Changes in peroxide value during storage were different in the two cultivars (2-sided p-value < 0.02). The peroxide value of unblanched Jubilee increased (Fig. 1) an average of 50% from the sixth to the ninth month of storage. The Jubilee, 9 min blanched, sample showed some increase in peroxide value but no increases were observed in the Jubilee, 15 min blanched, sample nor any GH 2684 samples.

The highest peroxide value obtained was 0.5 meq O2/kg lipid in the unblanched Jubilee sample after 9 mo storage. The peroxide value is a good guide for quality of a lipid; freshly refined fats should have peroxide values < 1 meq O2/kg (Rossell, 1989). All peroxide values we found were much less than that.

Packaging materials showed no effect on control of polyunsaturated fatty acid degradation. The low rates of lipid degradation in all samples during 9 mo frozen storage suggested that degradation was not related to oxygen concentration around the corn.

**Moisture**

The initial moisture content in the sweet corn was, on average, 72.5%. After 9 mo frozen storage, the different packaging materials used were related to the control of moisture loss from corn kernels (2-sided p-value < 0.01)(Fig. 2). Ears stored in Cryovac B and E bags showed the best moisture retention (72.2% final moisture), followed by polyethylene bags (71.4%). Those stored without packaging materials showed severe dehydralation (70.1%).

**SUMMARY**

Packaging materials did not effect on controlling PUFA degradation or enzymatic activity; however, they had an important effect on preventing moisture loss. Blanching of ears of corn reduced LOX and POD activities but had little effect on PUFA degradation during 9 mo storage at -20°C.

**REFERENCES**


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