Blanch Time and Cultivar Effects on Quality of Frozen and Stored Corn and Broccoli


ABSTRACT: Three corn and 2 broccoli cultivars were steam blanched for various times and evaluated for residual enzyme activity. Lipoxigenase was inactivated in 4 min in supertlement corn, while sweet corn required a 6-min blanch; peroxidase was inactivated in 8 min. Inactivation of broccoli lipoxigenase, peroxidase, and cystine lyase was achieved in 90 s. Blanched samples were stored 9 mo at −18 °C, then analyzed for color, texture, hexanal, free fatty acids, and sugars. Firmness increased significantly with blanching in both commodities, then declined. Short blanch treatments targeting lipoxigenase inactivation positively affected color and texture of both corn and broccoli. Changes in current industry practices are recommended.

Key words: corn, broccoli, enzymes, blanching, frozen storage

Introduction

Blanching vegetables is a critical step prior to freezing. However, the severity of the process should be limited in order to maintain color, texture, flavor, and nutritional quality. Peroxidase (POD) is used commercially as an index of blanching adequacy; however, recent work (Theerakulkait and others 1995; Lim and others 1989; Williams and others 1986) has shown that other enzymes, such as lipoxigenase (LPO), may be better correlated to quality changes. Theerakulkait and others (1995) found that LPO in sweet corn catalyzed off-odor formation, and blanch treatments targeting inactivation of this enzyme enhanced desirable characteristics, such as sweetness and corn flavor. Addition of POD extracts to corn homogenates did not result in a significant difference in sensory quality. Lim and others (1989) found that cystine lyase (CL) is the principal enzyme responsible for off-aroma production in broccoli, and Ramirez and Whitaker (1998) determined that cystine lyase is more thermolabile than POD. There is little published evidence of correlation between residual POD activity and undesirable quality in frozen vegetables.

The choice of LPO as a blanching indicator in many vegetables would result in significantly shorter blanch times, therefore improving nutritional and sensory quality, decreasing energy and water use, and allowing for more efficient processing production rates. Barrett and Theerakulkait (1995) found that LPO inactivation in supertlement corn at 93 °C was accomplished in 6 to 9 min while POD inactivation under the same conditions required 18 to 20 min. Inactivation of POD and LPO at 93 °C in green beans required times of 2.0 and 0.5 min, respectively.

Cultivars may vary widely in initial enzyme activity and quality. Barrett and Theerakulkait (1995) noted that 2 cultivars of green beans had significantly different initial enzyme activities. Therefore, target blanch times varied by cultivar. Azanza and others (1994) found significant differences between inbred supertlement lines and commercial supertlement and sweet corn cultivars for sweet corn aroma, texture, and flavor. These authors determined that sweetness was strongly correlated with sweet corn flavor, sugar, and sucrose content, while grassy flavor and aroma were logarithmically correlated with dimethyl sulfide (DMS) concentration. DMS is a product of the thermal degradation of S-methylmethionine (Bills and Keenan 1968), produced during the cooking process and associated with overall “corn-like” aroma (Flora and Wiley 1974).

Loss of green color is a primary factor limiting the shelf life of fresh broccoli (Shewfelt and others 1984). Klein (1992) reported that color stability could be improved by blanching. The heating medium and process conditions that were used significantly affected sensory properties of blanched broccoli. Brewer and others (1995) found that broccoli blanched using microwave, boiling water, or steam varied significantly for sensory flavor, color, texture, and overall acceptability, along with instrumental chroma and ascorbic acid content.

The objectives of this study were to determine blanch times required to inactivate critical enzymes (POD, LPO, and CL) in corn and broccoli and to evaluate the effect of cultivar and blanch time on color, texture, and flavor components in the frozen, stored product.

Results

Enzyme activity

Enzyme activity decreased with longer blanching time in both corn-on-the-cob and broccoli, regardless of cultivar (Tables 1 and 3). Analysis of variance (ANOVA) revealed significant differences among corn cultivars (Table 2) for LPO (p < 0.01) and POD (p < 0.001), as well as among blanching times (Table 1) for both LPO and POD (p < 0.001) activity.

In a comparison of mean activity levels for the 3 corn cultivars (Table 1), LPO activities in corn that was blanched for 4, 6, and 8 min were not significantly different. Therefore, it might be concluded that the 4 min blanch was sufficient to inactivate LPO. Corn POD was more heat resistant than LPO, and complete POD

Table 1—Cultivar means for enzyme activity, color and firmness of three blanched corn-on-the-cob cultivars

<table>
<thead>
<tr>
<th>Analysis/time</th>
<th>0 min</th>
<th>2 min</th>
<th>4 min</th>
<th>6 min</th>
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<tbody>
<tr>
<td>Lipoxigenase</td>
<td>3.37  a</td>
<td>1.17  b</td>
<td>0.25  c</td>
<td>0 c</td>
<td>0 c</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>6.44  a</td>
<td>6.26  b</td>
<td>1.90  c</td>
<td>0.73  cd</td>
<td>0 d</td>
</tr>
<tr>
<td>Chroma</td>
<td>38.84 b</td>
<td>50.0 a</td>
<td>48.61 a</td>
<td>50.0 a</td>
<td>49.42 a</td>
</tr>
<tr>
<td>L* Value</td>
<td>67.80 a</td>
<td>64.40 b</td>
<td>64.57 b</td>
<td>63.27 b</td>
<td>63.68 b</td>
</tr>
<tr>
<td>Hue</td>
<td>88.50 c</td>
<td>90.40 b</td>
<td>90.50 b</td>
<td>90.20 b</td>
<td>92.00 a</td>
</tr>
<tr>
<td>Hexanal (ppb)</td>
<td>0.38 a</td>
<td>0.28 b</td>
<td>0.29 b</td>
<td>0.32 ab</td>
<td>0.30 ab</td>
</tr>
<tr>
<td>Firmness</td>
<td>129.1b</td>
<td>163 ab</td>
<td>184 a</td>
<td>199 a</td>
<td>171 ab</td>
</tr>
</tbody>
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Same letter in same row indicates no significant difference.

Table 2—Cultivar means for enzyme activity, color and firmness of three blanched broccoli cultivars

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inactivation required an 8 min blanch. Therefore, use of POD as
the target enzyme for blanching adequacy of corn would result in
a requirement for twice the process time (8 min in contrast to 4
min) than if LPO were the target enzyme.

Cultivar played an important role in enzyme activity. The sweet corn cultivars A and B had significantly higher activity of
both LPO and POD than the supersweet cultivar C (Table 2). A
comparison of mean blanch times showed that there was no sig-
nificant difference in LPO activity of cultivars A and B. However,
cultivar B, followed by A and C, demonstrated significantly high-
er POD activity.

LPO activity, which declines with increasing blanch time, may
result in the formation of short chain alcohols (for example, hexa-
nal) that confer grassy flavor and aroma (Azanza and others
1994). Earlier work by our group (Theerakulkait and Barrett
1995) illustrated that LPO activity relates more to corn quality
changes during frozen storage and is inactivated in shorter times
than POD.

Broccoli blanch times showed significant differences for the
following enzyme activities (Table 3): floret LPO (p < 0.001), flo-
ret POD (p < 0.01), and floret CL (p < 0.01), stalk LPO (p < 0.001),
stalk POD (p < 0.001), and stalk CL (p < 0.001). Broccoli cultivar
was a significant factor only in the activity of stalk LPO (p < 0.05),
and the other enzymes showed no significant cultivar effect (Ta-
ble 4).

Activity of POD, LPO, and CL in broccoli florets declined signif-
icantly between the 0- and 45-s blanch (Table 3). Residual activi-
ty of all 3 enzymes was detectable in samples of both florets and
stalks exposed to 45 s blanching. However, comparing means of
both cultivars showed the only significant activity was that of flo-
ret LPO in the 90 s blanch treatment (Table 3). Trends for enzyme
inactivation in floret in contrast to stalk material were similar;
LPO activity was essentially the same in florets and stalks follow-
ing a 45-s blanch. CL activity was initially higher in the floret ma-
terial than stalk material of unblanched samples. The 45-s blanch,
however, resulted in a significant reduction in both floret and stalk,
with slightly higher residual activity in the stalk. CL ac-
tivity in the stalks was significantly lower following the 90-s
blanch than after the 45-s treatment. Ramirez and Whitaker
(1998) blanched broccoli for 2 min at 90, 80, and 63 °C and found
total inactivation of CL after 30 s at either 80 or 90 °C and after 3
min at 63 °C.

POD activity was initially similar in both stalk and florets; how-
ever, the 45-s blanch left more residual activity in the stalks
than the florets. It may be that slower heat penetration into stalk
material in contrast to florets is responsible for the presence of
residual activity. Again, comparing means of all blanch times (Ta-
ble 4) demonstrated LPO activity in cultivar A broccoli stalks was
significantly higher than that in cultivar B.

The broccoli results are interesting because in this case,
LPO inactivation in the floret material actually required a
longer blanch than POD inactivation. Wu and Whittaker (1986)
found that broccoli did not have significant amounts of LPO, but
they did not evaluate time required for inactivation. These authors found that CL was less stable than POD and li-
pase at 70 and 80 °C. However, the presence of more than 1
isozyme of CL may have allowed for residual activity. Lin and
others (1989) found that CL was the enzyme most responsible
for off-flavor formation in blanched broccoli homogenates.
Results of the present study indicate that both CL and POD
became inactive in a relatively short time. Further investiga-
tion is warranted to determine whether broccoli LPO activity is
a catalyst for off-flavor development in broccoli. If LPO is the
enzyme most responsible for frozen storage quality changes, a
blanch targeting POD inactivation may not be adequate to in-
activate LPO.

Hexanal and dimethyl sulfide

None of the blanched corn samples contained detectable
amounts of dimethyl sulfide (DMS). However, hexanal was
present. Hexanal content (p < 0.05) was significantly different
among corn cultivars, and a comparison of mean blanch times
(Table 2) shows that cultivar C was significantly higher in hexa-
nal concentration than A or B. The unblanched sample was sig-
nificantly higher in hexanal content (Table 1) than those
blanched 2 or 4 min. However, the hexanal content increases
again in the 6 and 8 min blanched samples.

Other investigators have found that blanching vegetables re-
sults in a rapid loss of volatiles and a detectable change in aroma.
Azanza and others (1994) reported low levels of both DMS and
hexanal in commercial corn samples and attributed results to
volatile loss during thermal processing. Shamaila and others
(1996) determined that water blanching carrots for 60 s resulted
in at least a 50% loss in most volatiles.

There was no detectable DMS in either unblanched broccoli
florets or those blanched for 45 s; however, it was detectable at
longer blanch times (Table 3). The effect of cultivar was not
found to be significant in production of DMS. Usually considered
a defect, the presence of sulfur compounds is often responsible
for “off-aromas” in foods and beverages (Goniak and Noble
1987). Published thresholds for DMS in beer include ranges from
21 to 68 µg/L, while 25 µg/L is the established threshold in wine,
both of which are much higher than the amount detected in this
study.

There was a significant difference between broccoli blanch
times in terms of hexanal content (p < 0.01). Comparison of
means of the 2 cultivars (Table 3) indicates that hexanal concen-
tration decreased significantly when blanching broccoli for as little
as 45 s and remained constant up to 180 s of blanching.

| Table 2—Blanch time means for enzyme activity, color, firmness and hexanal content for three corn-on-the-cob cultivars |
|-----------------|-----------|-----------|-----------|-----------|-----------|
| Variety/        | Hexanal   | LPO       | POD       | Chroma    | L* value  |
| Analysis        | 0 sec     | 45 sec    | 90 sec    | 135 sec   | 180 sec   |
| DMS (ppb)       | 0 b       | 0.05 b    | 0.11 ab   | 0.39 a    | 0.17 b    |
| Hexanal (ppb)   | 0.37 a    | 0.25 b    | 0.27 b    | 0.27 b    | 0.28 b    |
| LPO floret      | 2.37 a    | 0.24 b    | 0.04 c    | 0 c       | 0 c       |
| POD floret      | 43.61 a   | 0.99 b    | 0 b       | 0 b       | 0 b       |
| CL floret       | 2.58 a    | 0.03 b    | 0 b       | 0 b       | 0 b       |
| LPO stalk       | 2.28 a    | 0.20 b    | 0 b       | 0 b       | 0 b       |
| POD stalk       | 41.33 a   | 4.80 b    | 0 b       | 0 b       | 0 b       |
| CL stalk        | 0.43 a    | 0.15 b    | 0 c       | 0 c       | 0 c       |
| Chroma          | 18.32 c   | 27.95 a   | 22.00 c   | 24.11 a   | 21.55 c   |
| L* Value        | 39.24 a   | 32.28 b   | 30.47 c   | 29.97 c   | 29.11 c   |
| Firmness        | 163 c     | 230 b     | 301 a     | 277 a     | 158 c     |

Same letter in same row indicates no significant difference.

| Table 3—Cultivar means for enzyme activity, color, firmness and fla-
vor volatiles of two blanched broccoli cultivars |
<table>
<thead>
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<tbody>
<tr>
<td>Variety/</td>
<td>LPO stalk</td>
<td>Chroma</td>
<td>L* value</td>
<td></td>
</tr>
<tr>
<td>Analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.63 a</td>
<td>20.41 b</td>
<td>30.87 b</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.36 b</td>
<td>25.16 a</td>
<td>33.56 a</td>
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| Table 4—Blanch time means for enzyme activity, color, firmness and fla-
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<table>
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<tr>
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Sugars and free fatty acids
Statistical analysis of data for sugar and free fatty acid content is limited, because evaluations of only 1 of the 2 process replicates occurred. Of the fatty acid and sugar moieties evaluated in blanched and frozen sweet corn, only sucrose content varied significantly (p < 0.05) with blanch time. It is noteworthy that when comparing means of the 3 cultivars, sucrose content increased from 1.08% in the unblanched cobs to 5.90% in the 4 min blanched cobs; there was no difference between sucrose content in samples blanched for 4, 6, or 8 min (data not shown). There was no significant difference between corn cultivars for free fatty acids or sucrose. The total mono- and disaccharide content of the blanched samples of the cultivar C (supersweet) ranged from 7% to 9%, while B (sweet) averaged 2.7% to 3.7% and A (sweet) ranged from 4% to 8.8%.

Evaluations of one process replicate of the broccoli samples found that there were significant differences between cultivars in dextrose content (p < 0.01) and blanch times produced significant differences in linoleic acid (C18:2) concentration (p < 0.01). None of the other free fatty acids or sugars analyzed showed differences as an effect of either cultivar or blanch time (data not shown). Cultivar A had significantly higher concentrations of the monosaccharides fructose and dextrose, and of total mono- and disaccharides, which may contribute to observed differences in sensory flavor. A 45-s blanch treatment significantly reduced both linoleic acid and dextrose concentration to a level that resulted in no significant differences in linoleic acid (C18:2) concentration (p > 0.05) with blanch time. It is noteworthy that when comparing means of the 3 cultivars, sucrose content varied significantly (p < 0.05) with blanch time. It is noteworthy that when comparing means of the 3 cultivars, sucrose content varied significantly (p < 0.05) with blanch time. Therefore, blanch treatments of 90 s or longer result in a darker green color that is significantly different from the lighter color of the unblanched and 45-s blanched samples. Cultivar B had higher L* lightness values (lighter color) than cultivar A (Table 4).

Changes in the green color of vegetables following harvest have been associated with LPO activity and oxidation of unsaturated fatty acids; indeed the bleaching of either chlorophyll or carotenoids is often used as an indicator of LPO activity (Buckle and Edwards 1970). Thermal treatments are also known to result in degradation of chlorophyll and conversion to pheophytins, which are gray-green in color. It is interesting that Shewfelt and others (1984) found the greatest color change during fresh broccoli storage was indicated by the hue angle, but, in this study, chroma and L* lightness value changes were more significant.

Texture
There was a significant difference (p < 0.1) in firmness of the 3 cultivars of blanched corn, and a difference (p < 0.5) between blanch times. Textural changes as a result of blanching differed by cultivar (Table 2). Comparing the means of the 3 corn cultivars (Table 1) showed that firmness increased with blanch time up to 6 min and then declined. Application of heat is known to promote the gelatinization of starch in corn, and this results in a firmer textured product. A comparison of the 3 cultivars (Table 2) revealed that B and C were significantly firmer than A.

The 2 broccoli cultivars differed significantly in firmness (p < 0.1), and blanch time was also a factor that affected texture (p < 0.5). Firmness increased in both cultivars (Table 3) until either the 90- or 135-s blanch and then declined. Table 4 indicates that the firmness of the cultivar B was greater at all blanch times. Significant increases in the firmness of both corn and broccoli products took place with increased blanching to an optimum time and then declined. Unblanched samples of either commodity may have experienced a decline in textural integrity during frozen storage due to the activity of polygalacturonase, which catalyzes softening, or mechanical damage to the cell walls resulting from the freezing process.

Heat-induced activation of endogenous pectin esterase (PE) might explain textural changes in broccoli. PE hydrolyzes methyl groups from pectin chains and increases availability of carboxyl groups that may bind calcium and thereby improve firmness (Bartolome and Hoff 1972). Bourne (1989) and Stanley and others (1995) have reported that low temperature blanching and holding vegetables at 60 to 65 °C results in significantly firmer products. While this study did not employ low temperature blanching, the longer blanch treatments allowed for more time during temperature come-up at the temperatures critical to PE activation.

Principal Component Analysis of instrumental measurements
Corn. Instrumental analysis determined that the 3 corn cultivars were significantly different in every instrumental attribute measured (Table 2). Therefore, Principal Component Analysis (PCA) took into consideration each cultivar separately. PC factor 1 explains 57.7% of the variance and relates to color, LPO, and POD enzyme activity; the second PC (20.6%) relates to firmness (Fig. 1).

Unblanched corn samples from all 3 cultivars (A0, B0, and C0) were higher in enzyme activity, hexanal, and L* lightness value than blanched samples. Blanched samples of cultivar A were clustered in the bottom left quadrant of the PCA diagram and were characterized by high chroma; hue, L* lightness value, low
Blanched samples of cultivar C were clustered in the center of the diagram. Corn blanched for 4 min were significantly firmer than those blanched for 2, 6 and 8 min. Cultivar C samples blanched for 8 min (C8) were higher in chroma and hue (saturated orange color) than those of the shorter blanch treatments and had the least enzyme activity. Cultivar B still had high POD activity following the 2-min blanch (B2). The top left side of Fig. 1 contains a cluster of the cultivar B, the firmest of the 3 cultivars. This cultivar had a very low L* lightness value, indicating that it is quite dark in color.

**Broccoli**. Broccoli enzyme activities were all related (Fig. 2) and the correlation between them was r > 0.87. In addition, activity of all the enzymes except CLs correlated with hexanal concentration (r > 0.87). The 1st factor in the PCA diagram relates to enzyme activity, which explains 78.6% of the variance; the 2nd factor relates to firmness and color (chroma and L* lightness value), which explain 12.4% of the variance.

Unblanched samples of both cultivars (A0 and B0) appear opposite all the blanched samples on the right-hand side, near the vectors corresponding to floret and stalk enzyme activity. Unblanched cultivar A broccoli was higher in PODf, CLf, and hexanal and lowest in chroma and firmness than B0. Unblanched cultivar B had higher LPOf, LPOs, PODs, and CLs activity and had the highest L* lightness value (lightness). Firmness (Factor 2) was highest at the top of the PC and decreased towards the bottom; therefore, unblanched cultivar B was a bit firmer than A.

Figure 2 illustrates that the primary effect of blanching time was on broccoli firmness and chroma (saturation or vividness). The relationship between enzyme activity and blanch time is obvious in this PCA. Both cultivars of broccoli blanched for 45 s still retained some residual enzyme activity, while samples blanched longer had none. After 90 s of blanching, enzyme activity was undetectable and firmness and chroma changes predominated.

Broccoli firmness increased with blanch time to 90 or 135 s, depending on the cultivar, and then decreased. Chroma or saturation generally increased with blanch time. There is a clear separation of all the blanched cultivar A and B samples.

**Conclusions**

There were significant differences among the 3 corn cultivars evaluated. These cultivars also behaved differently as a function of blanching time. Corn POD inactivation in all 3 cultivars required an 8-min blanch, while LPO inactivation required a 6-min blanch in cultivars A and B and a 4-min blanch in C. Reducing blanch time is desirable from a sensory standpoint, because longer blanch treatments generally reduce firmness and fresh corn attributes and increase cooked flavors. Sucrose content increased with blanch times up to 4 min and, then, was relatively constant. After 9 mo of storage at 0 °F (−18 °C), cultivar C required the shortest blanch, was the firmest, and showed the least number of undesirable attributes. Instrumental texture analysis of the means of the 3 cultivars found that firmness increased with blanching up to 6 min and then declined.

The 2 broccoli cultivars responded to blanching time increases in a similar fashion, which resulted primarily in textural changes. Broccoli firmness increased with blanching up to 90 to 135 s and then decreased. Complete inactivation of broccoli enzymes required a 90-s blanch. Dimethyl sulfide concentration increased significantly between 90 and 135 s, which presents an argument for maintaining broccoli blanch times at 90 s to avoid off-flavor production.

Overall, short blanch treatments positively affected both broccoli and corn color, and longer blanch times resulted in undesirable effects on color. Blanch times for both corn and broccoli may be significantly reduced from current industry practices. Reduced blanch times would benefit the industry by decreasing energy costs, water use, and clean-up cost and would result in a more desirable product.

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**Fig. 1**—Principal component analysis of the matrix of mean instrumental ratings across samples for 3 corn cultivars. Sample identification is by cultivar (A, B and C) and blanch time (0, 2, 4, 6 and 8 min).

**Fig. 2**—Principal component analysis of the matrix of mean instrumental ratings across samples for 2 broccoli cultivars. Sample identification is by cultivar (A and B) and blanch time (45, 90, 135 and 180 sec).
Materials and Methods

Materials

Three cultivars of sweet corn-on-the-cob (Zea mays), designated A (sugary or su), B (sugary or su) and C (supersweet or su sh2) were harvested at 77%, 76%, and 78% moisture, respectively, in Darien, Wis. (U.S.A.), water-cooled, and shipped on ice overnight to the University of California (Davis, Calif., U.S.A.). Two cultivars of broccoli (Brassica oleracea), designated A and B, were harvested in Watsonville, Calif. (U.S.A.), and shipped on ice the same day to the University of California (Davis). Samples were sorted, cleaned, and washed in the Pilot Plant of the Dept. of Food Science and Technology. Two 2-in segments were obtained from the central portion of each corn ear using an electric saw before processing. Broccoli samples consisted of both floret material and approximately 1 in (2.54 cm) of stalk just below the floret. Some enzyme analyses were carried out on the floret and stalk material separately, and all other analyses were conducted on the floret with 1 in of stalk.

Corn-on-the-cob blanching was carried out in a pilot steam blancher (Key Technology, Walla Walla, Wash., U.S.A.) at 100 °C for 0, 2, 4, 6, and 8 min, while times for blanching broccoli samples were 0, 45, 90, 135, and 180 s. Two process replicates were run for each blanch time treatment for both commodities and results were averaged. Fatty acid and sugar analysis were carried out on one process replicate, other analyses were done on both replicates. Following blanching, all samples were cooled in ice-water for 3 min, drained, and frozen on metal trays in a blast freezer (−44 °C) until internal temperature reached 0 °F (−18 °C). Samples were stored in sealed polyethylene freezer bags at 0 °F (−18 °C). A subsample was analyzed immediately for enzyme activity and the remainder stored for 9 mo before quality evaluation.

Sigma Chemical Company (St. Louis, Mo., U.S.A.) provided linoleic acid, Tween-20, polyvinyl-polypyrrolidone (PVPP), guaiacol, H₂O₂, CuSO₄·5H₂O, and crystallized bovine serum albumin. All experiments used deionized, distilled water, and chemicals of reagent grade.

Enzymes

Immediately after blanching and cooling, corn and broccoli samples were analyzed for residual enzyme activity. Frozen, stored samples were not evaluated for enzyme activity. LPO and POD activity were determined in corn-on-the-cob kernels and LPO, POD, and CL were evaluated in broccoli florets and stalks.

Corn lipoxygenase

Extraction of corn LPO was carried out according to the method of Theerakulkait and Barrett (1995), with slight modifications. Acetone powder (2 g) was homogenized in a ratio of 1:10 (w/v) with 0.2 M Tris-HCl (pH 8, 4 °C) for 3 min using a Polytron homogenizer at 15,000 rpm. The extract was centrifuged for 1 h at 34,540 × g (4 °C), and the supernatant was kept on ice until analyzed.

LPO activity was assayed using the method of Chen and Whitaker (1986), with modifications as described in Theerakulkait and Barrett (1995). The initial rate of conjugated diene formation was measured from the linear change in absorbance at 234 nm. Equipment used for the assay was a double beam UV-VIS scanning spectrophotometer (Model UV-2102 PC, Shimadzu, Japan) and a 1-cm path length quartz cuvette. The definition used for 1 unit of enzyme activity was the amount of enzyme that produced a change in absorbance of 1.0/min/g acetone powder at 234 nm, under the assay conditions, defined one unit of enzyme activity.

Broccoli lipoxygenase

Extraction of LPO from broccoli does not require acetone powder preparation because of the relatively low content of lipid and starch. The extraction buffer consisted of 1L of phosphate (0.05 M K2HPO4)-citric acid (0.05 M)-NaCl buffer (0.86 M) adjusted to pH 6.4 with 2.5 M KOH. Twenty grams of broccoli florets and 2 g of PVPP were homogenized with 40 mL of cold (4 °C) extraction buffer in a ratio of 1:0.12 w/w/v. After blending for 40 s, the homogenate was filtered through 2 layers of cheesecloth; the volume was measured, and then the homogenate was centrifuged (4 °C) at 27,000 × g for 30 min. The supernatant was filtered through Whatman #1 paper and kept on ice until analyzed. Broccoli LPO was assayed using the method of Chen and Whitaker (1986) as described above.

Corn peroxidase

POD extraction also utilized preparation of acetone powders as described in Theerakulkait and Barrett (1995). POD assay followed the procedure of Chen and Whitaker (1986). Preparation of the substrate solution required mixing 0.05 M guaiacol, 0.2 M H₂O₂, 0.2 M sodium phosphate buffer (pH 6.5) and water in a ratio of 1:1:1.7 v/v/v/v. Three mL of this substrate solution were added to 25 μL of enzyme extract or to 25μL of a 1:10 dilution of the extract. The reaction was monitored for 3 min at 420 nm. Equipment used for the assay was a double beam spectrophotometer (UV-VIS scanning spectrophotometer, model UV-2102 PC, Shimadzu, Japan) and a 1-cm path length quartz cuvette. The definition used for 1 unit of enzyme activity was the amount of enzyme that produced a change in absorbance of 1.0/min/g acetone powder at 420 nm, under the assay conditions.

Broccoli peroxidase

The same POD extraction buffer utilized for corn was used for broccoli. Twenty g of broccoli florets and 2 g of PVPP (polyvinylpyrrolidone) were homogenized with 40 mL of cold (4 °C) extraction buffer in a ratio of 1:0.12 w/w/v. After blending for 1 min, the homogenate was filtered through 2 layers of cheesecloth; the volume was measured and then the solution was centrifuged (4 °C) at 27,000 × g for 30 min. The supernatant was filtered through Whatman #1 paper and kept on ice until analyzed. The substrate solution was prepared in the same way as described above and the POD assay was conducted in the same manner as corn. The definition used for 1 unit of enzyme activity was the amount of enzyme that produced a change in absorbance of 1.0/min/g acetone powder at 420 nm, under the assay conditions.

Broccoli cystine lyase

CL in broccoli was extracted and assayed according to the method of Ramirez and Whitaker (1998). Twenty g of broccoli (either florets or stalks) were homogenized for 1 min at maximum speed in 40 mL of cold extraction buffer containing 0.86 M NaCl and 2 g PVPP. The homogenate was strained through 2 layers of cheesecloth, centrifuged at 27,000 × g and filtered. All operations were carried out at 4 °C. The standard reaction mixture contained the following constituents in a volume of 1 mL: 150 μmol Bicine (pH 8.4), 0.025 μmol PALP, 12 μmol L-cystine and 10–100 μg enzyme extract. After incubating the mixture at 30 °C for 10 min, the addition of 1 mL 10% TCA terminated the reaction. After centrifugation to remove precipitated protein, an aliquot of the supernatant was assayed for pyruvate colorimetrically by the formation of dinitrophenylhydrazone (Friedemann and Haugen 1943). Activity was expressed in terms of μmol of pyruvate per minute.
Hexanal and dimethyl sulfide

Flavor volatiles were determined in frozen, stored corn and broccoli samples according to the method of Sucan and Russell (1997). A 5-g portion of broccoli or corn was placed into a 20 mL headspace vial (Alltech, Deerfield, Ill., U.S.A.). The vial was sealed with a 19-mm Teflon-faced septum (Alltech, Deerfield, Ill., U.S.A.) and a 20-mm aluminum seal (Alltech, Deerfield, Ill., U.S.A.). The sample equilibrated in a temperature controlled water bath at 35 °C for 20 min. The samples were oscillating at 150 rotations per minute during equilibration. A 2-mL portion of the headspace was drawn into a 5-mL gas tight syringe (Hamilton Co., Reno, Nev., U.S.A.) for gas chromatographic analysis. Before injection, the sample was cryofocused by placing a portion of the column in liquid nitrogen. The 2-mL headspace sample was inserted through the injector septum and injected at a rate of 1 mL/min. After the injection, the sample was cryofocused for an additional 3 min.

Samples were analyzed using a gas chromatograph (GC) (Hewlett Packard HP 5890, Avondale, Pa., U.S.A.) equipped with a flame ionization detector (FID). The injector temperature was constant at 200 °C and that of the detector at 300 °C. The GC oven temperature was held at 40 °C for 5 min, increased to 170 °C at a rate of 7 °C/min, and then held at 170 °C for 3 min. Hydrogen was the carrier gas for the FID. Equipment used to perform separation was a DB-WAX open tubular fused silica column, 30 m × 0.25 mm inside dia, 0.25-mm film thickness (J&W Scientific, Folsom, Calif., U.S.A.). A Hewlett Packard HP 3392A integrator accomplished peak integration.

Hexanal and dimethyl sulfide reference standards (Aldrich, Milwaukee, Wis., U.S.A.) were diluted in appropriate solvents of water and carbon tetrachloride, respectively. Serial dilutions were made and subjected to GC analysis. Hexanal standards were analyzed as described above and DMS standards were subjected to direct injections. Standard curves for detector and integrator responses were then used to normalize the integrator responses to DMS and hexanal peaks in the chromatograms to the standard curve concentrations.

Free fatty acids

Only 1 of the 2 process replicates of each treatment was analyzed for fatty acid content. Free fatty acids were analyzed according to the method of Deeth and others (1983). One to 2 grams of sample were weighed and combined with 5-mL internal standard (C17), 0.2 ml H2SO4, and 2.5-g anhydrous sodium sulfate and then capped and mixed for 30 min using a vortex mixer and a sonic bath. Five ml of hexane was added, and the mixture was shaken and then centrifuged if necessary to separate aqueous and organic components. One g of activated carbon was added to an extraction column (Alltech, Deerfield, Ill., U.S.A.), and the organic layer was added and pulled through with gentle vacuum. The column was washed with 2.5-mL portions of hexane/ethyl ether, and the eluent was discarded. The alumina was dried by drawing a gentle vacuum for a few minutes and then was placed into a gas chromatography vial. One ml of 6% formic acid in diisopropyl ether was added; the cap was crimped and swirled then allowed to settle. Two microliters were injected into a gas chromatograph (Hewlett Packard Model 5890 Series II, Avondale, Pa., U.S.A.), which increased from 60 °C to 149 °C at a rate of 9 °C/min. Injector temperature was held at 121 °C and detector temperature at 138 °C. Free fatty acid (FFA) standards were prepared for C4-C18, C18:1, C18:2 and C18:3 by mixing 50 to 70 mg of the fatty acid with 95 mg C17 (internal standard or IS) and 4 g of formic acid, then diluting to 100 ml with diisopropyl ether. Calculation of free fatty acid concentration were as follows:

\[
\text{mg/ml FFA (std)} = \frac{(\frac{\text{Area FFA (std)}}{\text{Area IS (std)}} \times \text{Area FFA (sample)} \times \text{Area IS (sample)}) \times 1000}{\text{sample wt (g)}}
\]

Sugars

Only 1 of the 2 process replicates of each treatment was analyzed for content of fructose, dextrose, sucrose, maltoose, and lactose. Seven grams of sample were mixed with 10 ml of isopropyl alcohol, stirred for 5 min, diluted to 100 ml with deionized water, filtered, and injected into a high performance liquid chromatograph (HPLC, Varian Instruments, Palo Alto, Calif., U.S.A.). A Rainin (Woburn, Mass., U.S.A.) Dynamax NH2 (8 μm) column was used with a 70% acetonitrile/30% water mobile phase and a flow rate of 1.5 ml/min. Standard solutions of varying saccharide levels were prepared and used for the standard curve. Peak heights were evaluated and used for determination of sugars.

Color

Instrumental color measurements were made on samples frozen and stored at 0 °F (−18 °C) for 9 mo. Hue angle (tan−1 b/a), chroma (light to gray), and L* lightness value (white to black or light to dark) measurements were taken with a Minolta CR-200 Chroma Meter, calibrated with the white plate and either the green standard tile (broccoli) or orange standard tile (corn). Frozen samples were thawed before measurement and evaluated at 23 °C. From each cob segment 3 readings were taken, and 3 cob segments were evaluated per sample. All treatments took place in duplicate; therefore, average corn hue values represent 18 readings. Five readings were obtained from broccoli florets, and treatments were duplicated therefore average hue values represent 10 readings.

Texture

Firmness of frozen, stored samples was evaluated after 9 mo using a Texture Technologies T.A.XT2 (Menlo Park, Calif., U.S.A.) instrument equipped with a Kramer shear cell. Two hundred gram representative samples were thawed and drained for 1 min and then weighed. The sample was loaded into a metal shear cell with plexiglass walls and leveled, and a 5-blade probe was attached to the press ram. The probe was placed near the surface of the sample before beginning each test to avoid false triggers. The test was run a 1 mm/sec and 90% strain. Both peak force and area under the curve were recorded. Three or more replicate samples were evaluated and averages were calculated.

Data analysis

Two-way analysis of variance (ANOVA) were carried out with blanch times, cultivars and replications and their 2-way interactions as sources of variation. Because cultivars behaved differently as a function of blanch time, they were evaluated independently by ANOVA. Principal Component Analysis (PCA) of the mean quality attributes across samples were calculated to determine the relationship between quality attributes, cultivars and blanch times.
Quality Evaluation of Corn and Broccoli

References

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