Sweet Corn Germ Enzymes Affect Odor Formation

CHOCKCHAI THEERAKULKAIT, DIANE M. BARRETT, and MINA R. McDANIEL

ABSTRACT

Descriptive sensory analysis of a homogenate of frozen stored unblanched sweet corn indicated that mean overall intensity and most descriptors describing undesirable characteristics were higher than those from blanched corn. To investigate the involvement of corn germ enzymes in off-odor formation, crude enzyme and purified lipoxygenase (LPO) and peroxidase (POD) extracts were prepared and added to homogenates of blanched corn. Addition of the LPO extract increased "painty" and "stale/oxidized" off-odor descriptors and lowered "sweet" and "corn" descriptors. Evidence suggested that sweet corn germ peroxidase is not important in off-odor formation, in which case lipoxygenase may be more appropriate as a blanching indicator.

Key Words: sweet corn, corn germ, lipoxygenase, peroxidase, off-odors

INTRODUCTION

SWEET CORN OFF-FLAVOR and off-odor formation, which occur after harvesting and during frozen storage, result in quality deterioration (Smittle et al., 1972; Wagenknecht, 1959; Lee, 1981; Velasco et al., 1989). Off-odor formation in frozen stored raw or underblanched vegetables, including sweet corn, is hypothesized to be the result of enzymatic action (Joslyn, 1949; Wagenknecht, 1959; Lee, 1981; Williams et al., 1986; Ganthavorn and Powers, 1989; Velasco et al., 1989; Sheu and Chen, 1991).

Lipoxygenase (LPO) has most often been suggested as the cause of off-flavor development (Wagenknecht, 1959; Lee, 1981; Velasco et al., 1989), and its activity is particularly high in the fraction containing the germ (Wagenknecht, 1959; Lee, 1981). In contrast, there is no evidence that peroxidase (POD), long used as a blanching index for sweet corn and other vegetables prior to freezing, is directly associated with off-flavor or other quality deterioration (Morris, 1958; Burnette, 1977; Williams, et al., 1986; Lim et al., 1989; Sheu and Chen, 1991).

Velasco et al. (1989) separated POD, LPO and catalase from sweet corn kernels and evaluated catalysis of off-odor formation by those enzymes. They could not clarify which enzyme(s) was responsible. The involvement of LPO and other enzymes isolated specifically from sweet corn germ tissue has never been reported.

Our objectives were to investigate the effects of sweet corn germ enzymes, in particular LPO, on off-odor formation using descriptive sensory analysis. The odor profiles of homogenates prepared from blanched and unblanched frozen stored corn were studied and compared to those of homogenates to which enzyme extracts had been added.

MATERIALS & METHODS

Materials

Freshly harvested sweet corn (*Zea mays* L. var. Jubilee) was obtained from the National Frozen Food Co. (Albany, OR) and was immediately transported to the pilot plant of the Dept. of Food Science and Technology, Oregon State Univ. (Corvallis, OR). The fresh sweet corn was dehusked by hand, and randomly separated and processed into four lots:

Author Theerakulkait is with the Faculty of Agro-Industry, Kasetsart Univ., Bangkok 10900, Thailand. Author Barrett is with the Department of Food Science & Technology, Univ. of California, Davis, CA 95616-8598. Author McDaniel is with the Dept. of Food Science & Technology, Oregon State Univ., Corvallis, OR 97331-6602. Direct inquiries to Dr. D.M. Barrett.

(1) unblanched intact kernels: corn was frozen immediately in liquid nitrogen and intact kernels were removed from the cob by hand and stored at −35°C; (2) blanched intact kernels: corn on the cob was water blanched at 98°C for 30 min to ensure inactivation of enzymes, cooled in water and frozen immediately in liquid nitrogen. Intact kernels were then removed from the cob by hand and stored at −35°C until utilized; (3) unblanched corn on the cob frozen immediately in liquid nitrogen and stored at −23.3°C for up to 1.75 years for investigation of off-odor; and (4) remainder of the fresh corn temporarily stored at 4°C prior to germ separation, which was carried out over a period of 3 days.

Linoleic acid, ammonium sulfate, Tween-20 (polyoxoethylene-sorbitan monolaureate), and gel filtration media (Sephacryl S-300 HR) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent grade. Deionized distilled water was used in all purification experiments. A prepacked Fast Protein Liquid Chromatography (FPLC) column of Mono Q HR 5/5 (5 × 0.5 cm i.d.), and a prepacked disposable PD-10 (Sephadex G-25 M) column were obtained from Pharmacia-LKB (Uppsala, Sweden). The FPLC system (Pharmacia) was previously described by Theerakulkait and Barrett (1995a).

Reference standards for odor descriptors were linseed oil (Grumbacher, Artists Oil Medium by M. Grumbacher, Inc., New York, NY), corn tortilla mix (Quaker Masa Harina De Maiz, manufactured by the Quaker Oats Co., Chicago, IL), canned whole kernel corn (Golden sweet, family style, Del Monte brand, Del Monte Co., San Francisco, CA), dried straw and hay (Dept. of Animal Science, Oregon State University, Corvallis, OR), and fresh sweet corn and cabbage (purchased at local markets, Corvallis, OR). Reference standards for odor intensity were safflower oil (Saffola Quality Foods Inc., Los Angeles, CA), orange drink (Hi-C, Coca Cola Foods, Houston, TX), grape juice (Welch's, Concord, MA), and cinnamon bubble gum (Plen T-Pak Big Red, WM. Wrigley Jr. Co., Chicago, IL).

Enzyme preparation and purification

Rapid isolation of sweet corn germ. Rapid isolation of the germ fraction from sweet corn on the cob was carried out using a modification of the method of Fong and Smith (1985). The precooled fresh sweet corn was husked, inspected, and cut at the top and base of the kernels using a TUC cutter (The United Company, Westminster, MD). The kernels were gently crushed by hand using a rolling pin to release the intact germ and then sieved through three stacked screens with 6.3, 2.36, and 0.83 mm openings. The enriched germ fraction was collected and further isolated by suspending it in ≈35% (w/v) sucrose in a 50 mM sodium phosphate buffer pH 7. All floating tissues were collected and centrifuged in ≈30% (w/v) sucrose in 50 mM sodium phosphate buffer pH 7 at 2,000 × g (4°C) for 5 min to separate germ from the debris. The isolated germ fraction was visually inspected and sorted by hand from non-embryonic tissues before freezing in liquid nitrogen. The frozen isolated germ was stored at −80°C until used.

Preparation of crude enzyme extract. Isolated sweet corn germ was prepared as an acetone powder and extracted with 0.2 M Tris-HCl, pH 8.0 (4°C) as described by Theerakulkait and Barrett (1995b). The supernatant of the crude extract was lyophilized and stored at −23.3°C until needed, at which time it was dissolved in 0.2 M sodium phosphate buffer, pH 7.0, and centrifuged at 17,000 × g for 30 min (4°C). The supernatant was buffer exchanged with 50 mM phosphate buffer, pH 7.0, using a prepacked PD-10 gel filtration column. The extract was frozen in liquid nitrogen and stored at −23.3°C.

Purification of sweet corn germ LPO and POD. LPO in sweet corn germ was purified as described by Theerakulkait and Barrett (1995b). The germ was prepared as an acetone powder, extracted with 0.2M Tris-HCl, pH 8.0 (4°C), fractionated by 40–60% ammonium sulfate saturation, and purified by conventional column chromatography on Sephacryl S-300 HR and the FPLC on a Mono Q column. The pooled active LPO fraction was desalted and buffer exchanged with deionized distilled water using a prepacked PD-10 column, lyophilized, and stored in a desiccator at −23.3°C.

Table 1—Odor descriptors, definitions, reference standards and their preparation and amount used for serving[a] for descriptive sensory evaluation of sweet corn homogenate samples

Descriptors	Reference standards and preparations	Definitions
Overall odor	—	The overall odor impact (intensity) of all compounds perceived by nose.
Painty	Linseed oil: Used 15 mL linseed oil (Grumbacher Artists Oil Medium)	Odor quality associated with the deterioration of the oil fraction. It may be described as linseed oil, paint thinner, shoe polish.
Stale/oxidized	Wet masa harina: Prepared by mixing 1 cup corn tortilla mix (Quaker Masa Harina de Maiz) with 1/2 cup of hot water	Cardboard, old corn flour or the dusty/musty odor that does not include painty.
Cooked cabbage	Sliced cooked cabbage: Prepared by cooking 250 g sliced cabbage with 500 mL of spring water on gas stove at high (10) for 4 min and at low (2) for 30 min; used 10 mL liquid portion and 15 g cooked cabbage	All characteristic notes associated with odor of cooked cabbage, e.g., sour, cabbage, fermented
Straw/hay	Chopped straw and hay: Prepared by chopping the dried straw and hay in a length about 1 to 2 cm; used 3 g chopped straw and 3 g chopped hay	All characteristic notes associated with straw and hay
Corn	Cooked fresh cut sweet corn: Prepared by cooking 75 g fresh cut sweet corn with 5 mL spring water using microwave at full power for 1.5 min; used 30 g cooked cut corn	The characteristic note of "corn" associated with cooked sweet corn
Sweet	Liquid of canned whole kernel corn: Used 30 mL of liquid portion of canned whole corn kernels (Del Monte brand Golden Sweet, Family Style)	The characteristic note of "sweet" associated with canned sweet corn
Cobby/husky	Diced fresh corn cob and fresh corn husk: Prepared by dicing fresh corn husk (thickness about 0.5 cm) and fresh corn cob (thickness about 0.1 cm; used 15 g for diced cob and 8 g for diced husk	The characteristic note associated with diced fresh corn cob and husk

[a] Served in 250 mL clear wine glasses covered with tight fitting aluminum lids.
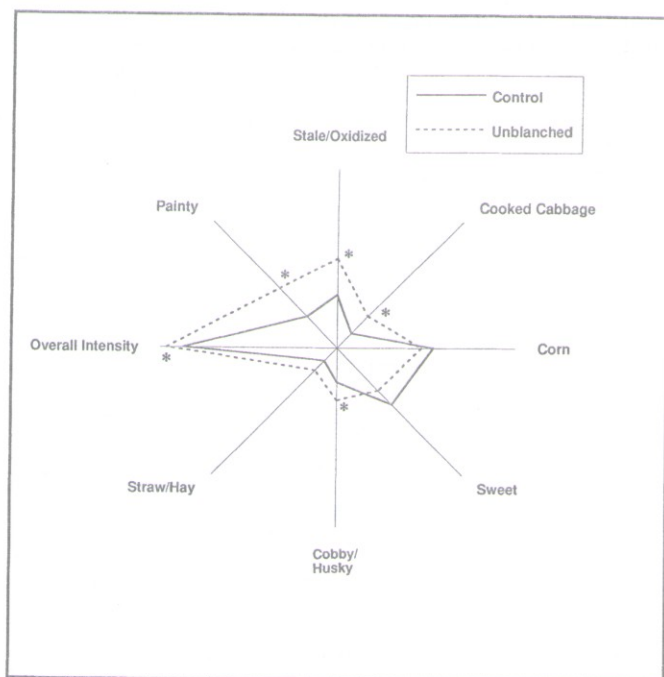

Fig. 1—Descriptive sensory profile for unblanched sweet corn homogenate samples compared with the control; the distance from the center is the mean value for that odor descriptor. Means designated with "*" are significantly different from the control at p < 0.05.

POD active fractions eluting from both the Sephacryl S-300 HR and the Mono Q columns were collected and those containing at least 30% of the POD activity in the most active fractions from the columns were pooled. The pooled POD fraction was then subjected to further purification by FPLC on a Mono Q column eluted with a linear NaCl gradient. The collected fractions were lyophilized and stored in the desiccator at −23.3°C until used. To prepare the POD for use, it was dissolved in 50 mM sodium phosphate buffer, pH 7.0, and buffer exchanged with phosphate buffer, pH 7.0, using a prepacked PD-10 column.

Enzyme activity assays. LPO activity was determined spectrophotometrically by monitoring the formation of conjugated dienes at 25°C (Theerakulkait and Barrett, 1995a). One unit of enzyme activity is defined as an increase in absorbance of 0.001 at 234 nm/min under assay conditions.

POD activity was determined spectrophotometrically at 470 nm (25°C) by a modification of the procedure of Flurkey and Jen (1978). The substrate solution was prepared by mixing 0.90 mL of guaiacol with ≈180 mL of 0.2 M sodium phosphate buffer, pH 6.0, for about 20 min, adding 0.02 mL of 30% hydrogen peroxide, and mixing thoroughly. The solution was then adjusted to 200 mL with 0.2 M sodium phosphate buffer, pH 6.0. One unit of enzyme activity was defined as an increase in absorbance of 0.001 at 470 nm/minute under assay conditions.

Protein determination. The protein elution profile was monitored for both conventional column chromatography and FPLC and protein in pooled active fractions was estimated by measuring absorbance at 280 nm. One unit of protein was defined as absorbance of 1.0 at 280 nm.

Sweet corn germ enzymes in off-odor formation

Panel selection and training. A seven-member panel (six females and one male) was selected based on interest, completion of training sessions, availability and consistent performance. Panel training included orientation and development of individual descriptors for the odor of sweet corn homogenate samples. Reference materials were provided to assist with terminology and standardization. Reference standards were anchored at point 3 (30 mL of safflower oil), point 7 (20 mL of orange drink), point 11 (20 mL of grape juice), and point 13 (1 stick of cinnamon bubble gum) and were presented in covered stem glasses. After sufficient training and discussion, the panel agreed on selection of specific odor descriptors. Training was continued until results from the panel and individual panelists were consistent. Final odor descriptors and definitions agreed on by panelists, and reference standards used for each odor descriptor are listed (Table 1).

Odor profile of homogenate of blanched and unblanched frozen stored corn: Sample preparation. Unblanched frozen corn on the cob stored at −23.3°C for 1.75 years and blanched (98°C, 30 min) frozen intact corn kernels stored at −35°C for 1.75 years were used. Samples were prepared by a modification of the procedure described by Velasco et al. (1989). Intact kernels were removed from unblanched, frozen cobs and homogenized in liquid nitrogen using a stainless steel Waring Blendor with the powerstat setting at 100. The liquid nitrogen powder (75g) was weighed into a 450 mL wide mouth freezer jar and allowed to stand at room temperature for about 30 min, after which 15 mL of 50 mM so-
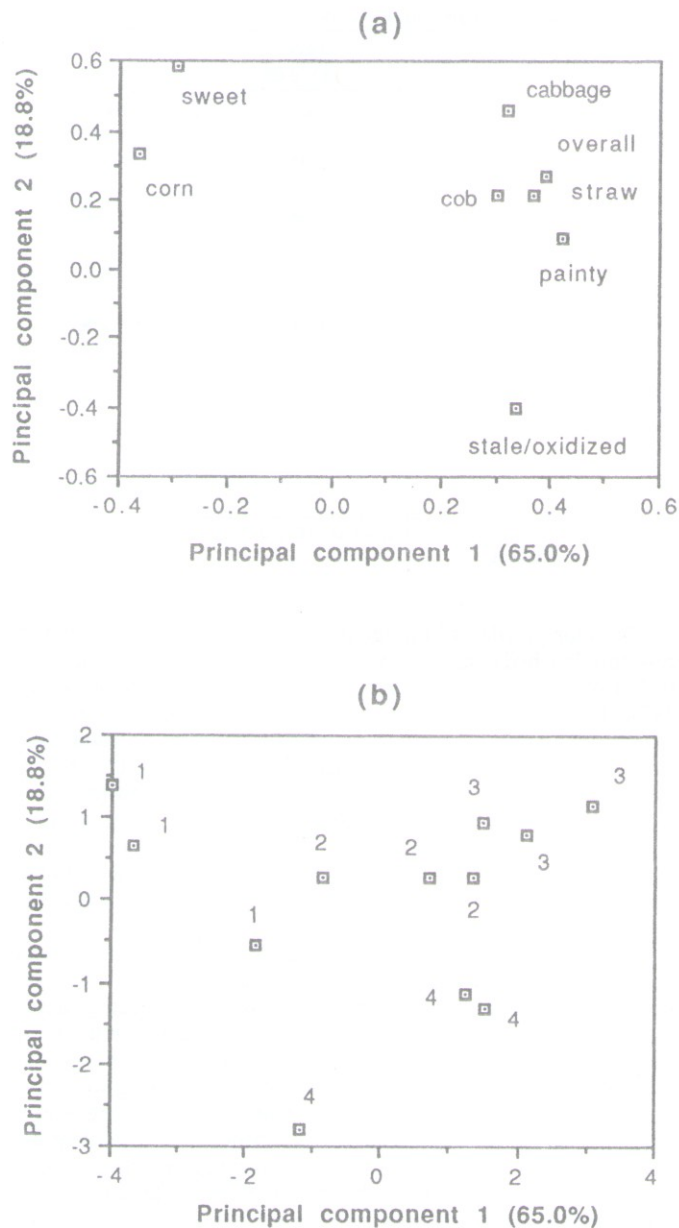
(a)

(b)

Fig. 2—(a) Loading of odor descriptors used for descriptive analysis, and (b) principal component analysis plot of odor intensity rating of odor descriptors for the samples of sweet corn homogenate (1=control, 2=unblanched, 3=added crude enzyme extract and 4=added LPO; each contains three replications) on PC1 vs PC2.
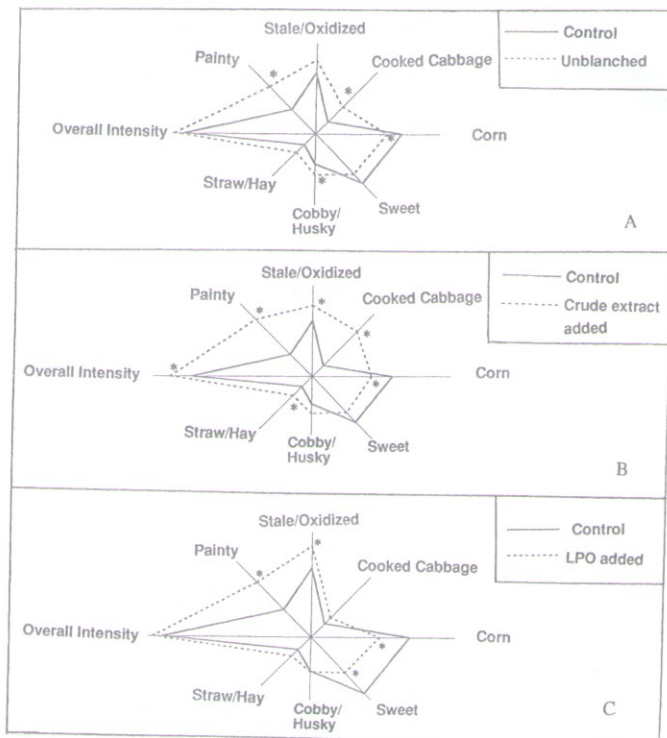

Fig. 3—Descriptive sensory profile for sweet corn homogenate samples (A=unblanched, B=added crude enzyme extract, and C=added LPO; respectively) compared with the control; the distance from the center is the mean value for that odor descriptor. Means designated with "*" are significantly different from the control at p < 0.05.

dium phosphate buffer, pH 7.0, were added. The control sample was prepared in the same manner, except the homogenate of blanched frozen corn kernel was used. Each sample was mixed thoroughly, covered with a watch glass, and heated at 93°C in a water bath for 30 min, stirring every 10 min. Each sample (8g) was weighed into 250 mL black, tulip-shaped stern glasses coded with three digit random numbers, capped with aluminum lids and sealed with parafilm.

Sample testing. Samples were cooled by leaving them at room temperature (22°C) for at least 30 min. A set of two samples was presented in random order to each panelist in individual booths. Panelists were asked to remove the cover, sniff the head space odor and rate the intensity of each odor descriptor. Panelists sniffed water after each sample to avoid minimize cross-over effects.

Each odor descriptor intensity was rated using a 16-point intensity scale, (0=none, 1=just detectable, 3=slight, 5=slight to moderate, 7=moderate, 9=moderate to large, 11=large, 13=large to extreme, and 15=extreme). Reference standards for each odor descriptor and for odor intensity were available for panelists to review in every session. The testing was performed in 3 individual sessions (3 replications).

Sensory profile with addition of purified LPO. Unblanched and blanched frozen intact sweet corn kernels stored at −35°C for 1.75 years were used. Samples included: (1) blanched corn homogenate with added buffer (control), (2) homogenate to which purified LPO was added, (3) homogenate to which crude enzyme extract was added, and (4) unblanched corn homogenate with buffer added. Each of the first three samples were prepared by adding either 50 mM sodium phosphate buffer, pH 7.0, purified LPO solution, or crude enzyme extract in the same phosphate buffer, respectively, to the blanched corn liquid nitrogen powder in a 1 to 5 (v/w) ratio. The unblanched sample was prepared in the same manner as the control. Total LPO activity in the unblanched sample and in the purified LPO and crude extract addition samples was about the same level (675,000 units), while that in the control was insignificant.

Each sample was mixed thoroughly and incubated in a slow speed shaking water bath (30°C) for 3 hr and stirred every 30 min. Samples were heated at 93°C in a water bath for 30 min and then analyzed in the same way, except a set of four (instead of two) samples were used.

Sensory profile with addition of purified POD. Investigation of the involvement of POD in off-odor formation was carried out in a similar manner to the LPO study, except that purified POD was added. Total POD activity in the purified POD and crude enzyme samples was ≈650,000 units.

Statistical analysis. A randomized complete block design was used in all experiments. The block corresponded to each of the seven panelists in each replication. All experiments provided three replications over the treatments using the same panelists. Assessments by panelists were analyzed per odor descriptor through three-way ANOVAs with panelist (P), replication (R) and treatment (T) as factors. The interactions for each descriptor were also tested for significance. SAS version 6 (SAS Institute, Inc., 1987) was used for statistical analysis. A mixed effect linear model was used with panelist and replication as random effects (Lundahl and McDaniel, 1988), while treatment was considered a fixed effect. For the model containing all 2- and 3-factor interactions, the F-statistic for testing treatments (F_t) was calculated according to Steele and Torrie (1980) by the following formula:

$$F_t = MS(T) + MS(P^*R^*T)$$

$$MS(R^*T) + MS(P^*T)$$

However, since the replication-by-treatment interactions (R*T) for all

Table 2—Mean ratings[z], standard deviations (SD), and LSD values for odor descriptors among the treatments for LPO study

Descriptors	Treatments				LSD (p ≤ 0.05)	Sig.
	Control	Unblanched	Crude extract	LPO		
Overall intensity	9.09[b] (1.22)	9.81[b] (1.29)	10.71[a] (1.35)	9.71[b] (1.68)	0.84	**
Painty	2.38[b] (1.99)	4.62[a] (3.54)	6.10[a] (2.81)	4.71[a] (3.20)	2.02	**
Stale/Oxidized	4.24[b] (1.81)	5.14[ab] (1.35)	5.38[a] (1.60)	5.62[a] (1.53)	1.06	p = .06
Cooked cabbage	1.19[c] (1.97)	2.67[b] (2.39)	4.86[a] (2.95)	1.71[bc] (1.95)	1.41	***
Straw/Hay	1.10[b] (1.67)	1.86[ab] (1.82)	2.05[a] (2.40)	1.67[ab] (1.83)	0.79	p = .09
Corn	6.24[a] (1.22)	5.14[b] (1.32)	4.62[b] (1.80)	4.33[b] (1.35)	0.95	**
Sweet	4.86[a] (1.88)	3.95[ab] (1.75)	3.86[ab] (1.77)	3.10[b] (1.48)	1.15	*
Cobby/Husky	2.10[b] (1.61)	2.86[a] (1.49)	2.81[ab] (1.94)	2.14[ab] (1.56)	0.79	p = .08

[z] Sixteen point intensity scale (0 = none, 15 = extreme).
[a-c] Means with the same letter, in the same row, are not significantly different at the 0.05 significance level by LSD.

descriptors in all experiments were not statistically significant, the appropriate F-statistic was simplified to:

$$F_t = \frac{MS(T)}{MS(P^*T)}$$

The mean square for panelist-by-treatment interaction (P*T) was used as the error term for the test for treatment effect. Comparisons of treatment means of each odor descriptor were conducted using Fisher's least significant difference test (p ≤ 0.05).

Data were also analyzed by principal component analysis (PCA) using SAS (SAS Institute, Inc., 1987). The PCA scores for each axis were analyzed by ANOVA and by Fisher's least significant difference test (p ≤ 0.05).

RESULTS & DISCUSSION

Purification of sweet corn germ LPO and POD

In order to study involvement of sweet corn germ enzymes in off-odor formation, a crude enzyme extract and purified LPO and POD extracts were prepared. The purification of LPO was 188 fold with 26.3% recovery. The majority of the POD eluted from the column later than LPO, indicating that the molecular size of the major POD isozymes in sweet corn germ was smaller than that of LPO. On the FPLC Mono Q column, the majority of POD eluted from the column before starting the NaCl gradient both during purification of LPO and POD, indicating that the majority of POD was in the basic form. The purification and recovery of POD in pooled fraction was 58 fold and 27.6%, respectively. The purification scheme for both enzymes was taken to this point because both POD and LPO were free of activity of the other enzyme.

Sweet corn germ enzymes in off-odor formation

Odor profile of homogenate of blanched and unblanched frozen stored corn. Prior to investigating the involvement of sweet corn germ enzymes in off-odor formation, the profile of a homogenate of blanched frozen intact corn (control) was evaluated. The odor profile of the control was described as slight to moderate "sweet" and "corn", just detectable to slight "cobby/husky", "painty", and "stale/oxidized", and just detectable "straw/hay", and "cooked cabbage" with moderate to large "overall odor intensity" (Fig. 1). The overall odor characteristics were relatively high in the "desirable" odors of sweet corn including "sweet" and "corn", and relatively low in "undesirable" or "off-odor" characteristics, including "stale/oxidized," "painty," "cobby/husky," "cooked cabbage" and "straw/hay" descriptors.

The odor profile of homogenate prepared from frozen stored raw (unblanched) corn on the cob was compared with the control using a typical descriptive sensory profile (Stone et al., 1974, Fig. 1). Univariate ANOVA on each descriptor showed significant treatment differences between unblanched and control samples for most of the off-odor descriptors except for "straw/hay" and "desirable" odor descriptors "sweet" and "corn." This implied that differences between samples were significantly detectable by the trained panel for most descriptors of off-odor characteristics. Replication effects were not significant for any descriptors except "painty," indicating good reproducibility of replicates.

When both treatment and panelist by treatment interactions are significant, it is important to examine whether panelist by treatment interaction influences conclusions regarding treatments. This was done by comparing the line graph plot between each panelist's ratings for each treatment to search for systematic inconsistencies among the panelists contributing to variation. For example, in the case of "stale/oxidized" there was a significant effect of both treatment and panelist-by-treatment interaction. It was found that most of the panelists responded similarly, suggesting that main treatment effect differences were important.

The mean odor intensity of the homogenate of unblanched sample was higher than the control in "overall intensity" and in most off-odor descriptors, while the mean intensity of "desirable" odor descriptors not different from the control. Unblanched corn on the cob developed off-odor during frozen storage as indicated by the off-odor characteristics of its homogenate. The development of off-flavor and off-odor in frozen raw (unblanched) or underblanched sweet corn stored in freezers for extended times has also been reported for cooked corn on the cob (Wagenknecht, 1959; Lee, 1981) or cooked whole kernels (McDaniel et al., 1988).

Sensory profile with addition of purified LPO. The effects of addition of crude enzyme extract and purified sweet corn germ LPO on off-odor formation were studied. Only the first two principal components (PC) were significant (Fig. 2) with 64.99 and 18.81% of total variation explained by PC1 and PC2, respectively. The loading of odor descriptors for PC1 and PC2 indicated that PC1 could be defined as a "desirable" vs. "undesirable" (off-odor) descriptor axis (Fig. 2a). The overall intensity and off-odor descriptors, especially "painty," negatively correlated with "desirable" odor descriptors, especially "corn." PC2 might be defined as "stale/oxidized" vs "sweet" and "cooked cabbage."

ANOVA tests indicated that there were significant differences between the mean PC1 and PC2 scores among samples, with
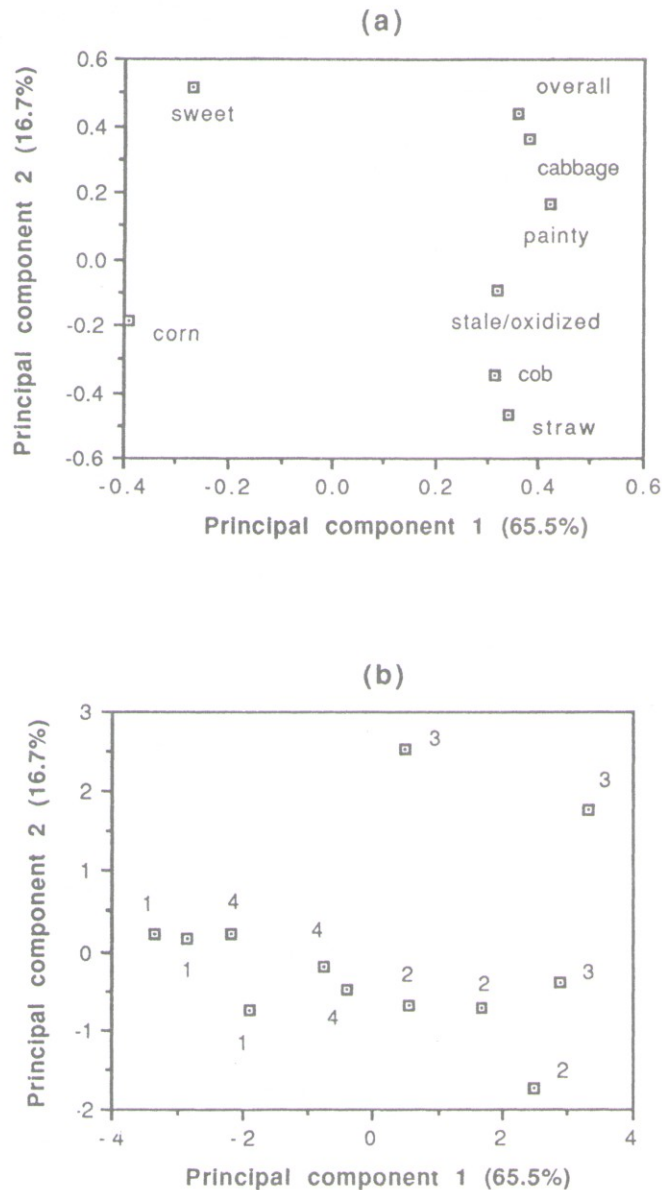
(a)

(b)

Fig. 4—(a) Loading of odor descriptors used for descriptive analysis, and (b) Principal component analysis plot of odor intensity rating of odor descriptors for the samples of sweet corn homogenate (1=control, 2=unblanched, 3=added crude enzyme extract and 4=added POD; each contains three replications) on PC1 vs PC2.

non-significant effects of replications indicatị ̣ good reproducibility. This was also observed from the grouping of 3 replications for each sample (Fig. 2b). The PCA plot of odor intensity ratings indicated that the samples could be classified into 3 groups (Fig. 2b): (1) control, unblanched and added crude extract (2 and 3), and added purified LPO (4). The mean PC1 score of the control was different from that of unblanched, added crude extract, and added purified LPO samples, which were not different from each other. The control was best described by "desirable" odor descriptors while the other samples were described by off-odor descriptors (Fig. 2a and b). However, the mean PC2 score of the sample with added purified LPO was different from those of unblanched and added crude extract. The sample with added purified LPO seemed to be best described by "stale/oxidized" and "painty" compared to the other samples (Fig. 2a and b).

The results of univariate ANOVA on each descriptor showed treatment differences among samples for "cooked cabbage," "overall intensity," "painty," "corn," and "sweet." A pan-

elist effect was significant for all odor descriptors; however, this was not unusual and reflects some panelists' usage of different portions of the intensity scale (Power, 1988). The replication effect was not significant for any odor descriptors, indicating good reproducibility. Panelist by treatment interaction effects were significant for "painty," "sweet," "corn" and "cobby/husky;" however, most of panelists responded similarly.

The mean values of odor intensity of most off-odor descriptors including "painty," "cooked cabbage," and "cobby/husky" of the unblanched sample were higher, while the descriptor "corn" was lower than that of the control. The odor profile of the unblanched sample incubated at 30°C for 3 hr seemed to be similar to that of the unblanched sample frozen stored at −23.3°C for 1.75 years (Fig. 1 and Fig. 3).

The crude enzyme extract sample was higher in intensity of most off-odor descriptors including "painty," "stale/oxidized," "cooked cabbage," "straw/hay" than that of the control, but the "corn" odor was lower (Fig. 3). This sample, like the unblanched one, showed a higher mean intensity of off-odor descriptors, and a lower intensity of "desirable" odor descriptors than the control. However, the mean intensity of descriptors "overall intensity" and "cooked cabbage" of the crude extract was also higher than that of the unblanched and added LPO samples (Table 2).

Addition of purified LPO to the homogenate resulted in an increase in off-odor descriptors "painty" and "stale/oxidized," and a decrease in "desirable" odor descriptors "sweet" and "corn" (Fig. 3). The decrease in "sweet" and "corn" odors may be due to masking by the increase in intensity of those off-odor descriptors. The formation of typical cooked "corn" odor is generally hypothesized to be heat activated, while raw sweet corn has very little odor. Dimethyl sulfide (DMS) is one of the principle low-boiling volatile compounds that contributes to cooked "corn" odor (Bills and Keenan, 1968; Williams and Nelson, 1973; Flora and Wiley, 1974; Dignan and Wiley, 1976; Wiley, 1985; Azanza et al., 1994).

Azanza et al (1994) found no association between DMS concentration and raw sweet corn odor, but found that grassy odor and flavor scores correlated with DMS concentration. They found that sweet corn samples with high DMS concentrations also had high concentrations of other volatiles which may contribute to grassy odor and flavor. LPO activity was not analyzed, therefore they could not determine whether enzyme-catalyzed production of hexanal and other short chain alcohols was responsible for grassy odor or flavor.

The observed increases in the off-odor descriptors "painty" and "stale/oxidized" in the added purified LPO and crude extract samples may be caused by LPO-catalyzed hydroxyperoxidation of polyunsaturated fatty acids and esters containing a cis, cis-1,4-pentadiene system. This reaction initially yields hydroperoxides which subsequently degrade to form a variety of secondary products, including aldehydes, alcohols, and ketones, which may result in off-odor formation (Eskin et al., 1977; MacLeod and Ames, 1988). Wagenknecht (1959) and Lee (1981) suggested that enzymes, particularly LPO, induced off-flavors in unblanched sweet corn.

Previous studies indicated that LPO was important in off-flavor and off-odor formation. McDaniel et al. (1988) reported that mean intensity of the descriptor "stale/oxidized" was higher in underblanched frozen stored corn than in commercially blanched frozen stored corn. Kalbrener et al. (1974) reported a "musty/stale" odor was a predominant descriptor of the linoleic hydroperoxide produced by soy LPO oxidation of linoleic acid. Other investigators (Ashraf and Synder, 1981; Johnsen et al., 1988; Civille and Dus, 1992; Mistry and Min, 1992) have reported "painty" and "stale/oxidized" off-odors in soy milk, peanut and vegetable oil, and other products in which LPO is present. Moreover, LPO was the key enzyme involved in off-odor formation in English green peas and green beans (Williams et al., 1986).

Table 3—Mean ratings[z], standard deviations (SD), LSD values for odor descriptors among the treatments for POD study

Descriptors	Treatments				LSD (p ≤ 0.05)	Sig.
	Control	Unblanched	Crude extract	POD		
Overall intensity	8.67[c]	9.76[ab]	10.57[a]	8.95[bc]	0.90	**
	(0.97)	(1.55)	(1.40)	(1.32)		
Painty	1.57[c]	4.43[ab]	5.57[a]	2.57[bc]	1.96	**
	(1.96)	(3.44)	(2.34)	(2.50)		
Stale/Oxidized	3.33[a]	4.33[a]	4.29[a]	4.29[a]	1.58	ns
	(2.08)	(1.96)	(2.55)	(2.35)		
Cooked cabbage	1.05[c]	2.90[b]	4.86[a]	1.48[bc]	1.69	***
	(1.66)	(2.66)	(2.65)	(1.94)		
Straw/Hay	1.05[b]	2.00[a]	1.52[ab]	1.29[b]	0.48	**
	(1.32)	(1.76)	(1.81)	(1.55)		
Corn	6.19[a]	4.90[b]	3.90[bc]	5.29[ab]	1.16	**
	(1.36)	(1.64)	(1.95)	(1.38)		
Sweet	4.71[a]	3.48[a]	4.05[a]	4.38[a]	1.43	ns
	(2.39)	(1.63)	(2.80)	(2.18)		
Cobby/Husky	2.33[a]	3.05[a]	2.62[a]	2.29[a]	1.04	ns
	(2.11)	(1.63)	(1.91)	(1.90)		

[z] Sixteen point intensity scale (0 = none, 15 = extreme).
[a-c] Means with the same letter, in the same row, are not significantly different at the 0.05 significance level.
*,**,*** refers to significance at p ≤ 0.05, 0.01 and 0.001, respectively by ANOVA F-test. ns = not-significant at p ≤ 0.05.

Although lipoxygenase appears to be a primary cause, other sweet corn germ enzymes may be involved in formation of other off-odor descriptors, particularly "cooked cabbage," which was higher in the sample with added crude extract than that with added purified LPO. Gardner (1970) reported that linoleate hydroperoxide isomerase was present in the germ of mature corn and that it catalyzed the production of hydroperoxide products in addition to LPO. Velasco et al. (1989) also hypothesized that LPO was important in off-odor development in sweet corn, however; they suggested that other enzymes, such as hydroperoxide isomerase and hydroperoxide lyase, may be important.

Sensory profile with addition of purified POD. In terms of POD involvement in off-odor formation, the first two PC explained most of the total variation, with 78.53 and 13.58% for PC1 and PC2, respectively. As with the LPO experiment, PC1 may be defined as a "desirable" and "off-odor" descriptor axis (Fig. 4a). However, mean PC scores among the samples were different only for PC1. The replication effect was not significant in either case indicating good repeatability.

Based on the LSD test of means in PC1, the samples could be classified into two groups: control and added purified POD samples, and unblanched and added crude extract samples. The mean PC1 score of the added purified POD sample was not different from that of the control, but was different from those of unblanched and added crude extract samples. However, the unblanched and added crude extract samples were not different from each other. The control and added purified POD samples were similar in odor profile and could be best described by "desirable" odor descriptors "sweet" and "corn", while the added crude extract and unblanched samples were best described by off-odor descriptors (Fig. 4a, b).

The results of univariate ANOVA on each descriptor showed treatment differences among samples for "cooked cabbage," "overall intensity," "painty," "straw and hay," and "corn." The experiment showed good reproducibility as indicated by the non-significant replication effect. The panelist by treatment interaction was significant for most descriptors except "straw/hay" and most of the panelists responded similarly. Mean intensity of most off-odor descriptors for the unblanched sample, including "painty," "cooked cabbage," and "straw/hay," were higher than the control, but "corn" odor was lower than that of the control (Table 3). This result was similar to that observed for the unblanched sample from the LPO study.

The mean values of intensity for the added crude enzyme extract sample were higher than the control in "overall intensity" and the off-odor descriptors "painty" and "cooked cabbage." The "corn" odor was lower than that of the control (Fig.
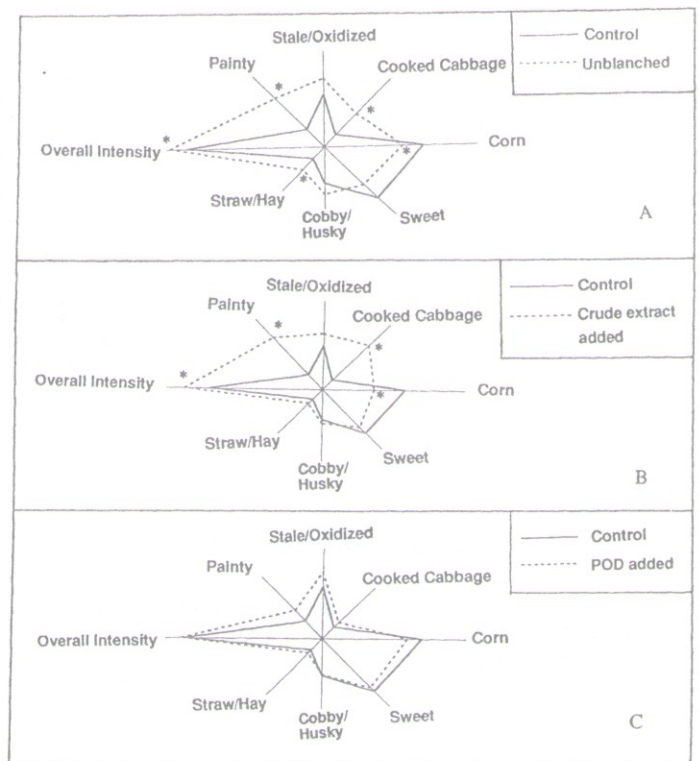

Fig. 5—Descriptive sensory profile for sweet corn homogenate samples (A=unblanched, B=added crude enzyme extract, and C=added POD; respectively) compared with the control; the distance from the center is the mean value for that odor descriptor. Means designated with "*" are significantly different from the control at p < 0.05.

5). The odor profile of the sample with added crude enzyme extract was similar to that of unblanched sample. The mean intensity of descriptors describing off-odor was higher than the control while that of descriptors describing "desirable" odor was lower than that of the control. However, the mean intensity of descriptor "cooked cabbage" was higher than that of the unblanched sample (Table 3).

The mean values for intensity of all odor descriptors for the added POD sample were not different from that of the control. However, adding crude extract, which contained the same total POD activity, resulted in increases in mean intensity of off-odor

descriptors ''painty'' and ''cooked cabbage'' and a decrease in mean intensity of desirable-odor descriptor ''corn.'' These results suggests that POD presence in sweet corn germ was not an important factor in off-odor formation, especially in the formation of ''painty'' and ''cooked cabbage'' odors. Other investigators (Williams et al., 1986; Lim et al., 1989; Velasco et al., 1989) have suggested that POD was not important in off-odor formation in plant tissues.

CONCLUSIONS

LPO IN SWEET CORN GERM is important in off-odor formation, particularly in production of odors described as ''painty'' and ''stale/oxidized.'' POD presence in sweet corn germ does not appear to affect off-odor. Other enzymes in the germ may also be involved in off-odor formation, especially the ''cooked cabbage'' odor. Results suggest that analysis of LPO activity, rather than POD, may be a more appropriate index of blanching adequacy.

REFERENCES

Ashraf, H-R.L. and Snyder, H.E. 1981. Influence of ethanolic soaking of soybeans on flavor and lipoxygenase activity of soymilk. J. Food Sci. 46: 1202–1204.

Azanza, F., Juvik, J.A., and Klein, B. 1994. Relationships between sensory quality attributes and kernel chemical composition of fresh-frozen sweet corn. J. Food Quality 17: 159–172.

Bills, D.D. and Keenan, T.W. 1968. Dimethyl sulfide and its precursor in sweet corn. J. Agric. Food Chem. 16: 643–645.

Burnette, F.S. 1977. Peroxidase and its relationship to food flavor and quality: a review. J. Food Sci. 42: 1–6.

Civille, G.V. and Dus, C.A. 1992. Sensory evaluation of lipid oxidation in foods. Ch. 15, *In Lipid Oxidation in Food*, A.J. St. Angelo (Ed.), p. 279–291. American Chemical Society, Washington, DC.

Dignan, D.M. and Wiley, R.C. 1976. DMS levels in the aroma of cooked frozen sweet corn as affected by cultivar, maturity, blanching and packaging. J. Food Sci. 41: 346–348.

Eskin, N.A.M., Grossman, S., and Pinsky, A. 1977. Biochemistry of lipoxygenase in relation to food quality. CRC Crit. Rev. Food Sci. Nutr. 9: 1–40.

Flora, L.F. and Wiley, R.C. 1972. Effect of various endosperm mutants on oil content and fatty acid composition of whole kernel corn (Zea mays L.). J. Am. Soc. Hortic. Sci. 97(5): 604–607.

Flora, L.F. and Wiley, R.C. 1974. Sweet corn aroma, chemical components and relative importance in the overall flavor response. J. Food Sci. 39: 770–773.

Flurkey, W.H. and Jen, J.J. 1978. Peroxidase and polyphenol oxidase activities in developing peaches. J. Food Sci. 43: 1826–1828, 1831.

Fong, F. and Smith, J.D., 1985. Rapid isolation and purification of immature zygotic embryos. Maize Gen. Coop. Newslett. 59: 30–31.

Ganthavorn, G. and Powers, J.R. 1989. Partial purification and characterization of Asparagus Lipoxygenase. J. Food Sci. 52: 371–73.

Gardner, H.W. 1970. Sequential enzymes of linoleic acid oxidation in corn germ: lipoxygenase and linoleate hydroperoxide isomerase. J. Lipid Res. 11: 311–321.

Gardner, H.W. and Inglett, G.E. 1971. Food products from corn germ: enzyme activity and oil stability. J. Food Sci. 36: 645–648.

Gibian, M.J. and Vandenberg, P. 1987. Product yield in oxygenation of linoleate by soybean lipoxygenase: the value of the molar extinction coefficient in spectrophotometric assay. Anal. Biochem. 163: 343–349.

Johnsen, P.B., Civille, G.V., Vercellotti, J.R., Senders, T.H., and Dus, C.A. 1988. Development of a lexicon for the description of peanut flavor. J. Sensory Studies. 3: 9–17.

Joslyn, M.A. 1949. Enzyme activity in frozen vegetable tissue. Adv. Enzymol. 9: 613–640.

Kalbrener, J.E., Warner, K., and Eldridge, A.C. 1974. Flavors derived from linoleic and linolenic acid hydroperoxides. Cereal Chem. 51: 406–416.

Lee, Y.C. 1981. Lipoxygenase and off-flavor development in some frozen foods. Korean J. Food Sci. Technol. 13: 53–56.

Lim, M.H., Velasco, P.J., Pangborn, R.M., and Whitaker, J.R. 1989. Enzymes involved in off-aroma formation in broccoli. Ch. 7, In *Quality Factors of Fruits and Vegetables*, J.J. Jen (Ed.), p. 72–83. American Chemical Society, Washington, DC.

Lundahl, D.S. and McDaniel, M.R. 1988. The panelist effect—fixed or random? J. Sensory Studies 3: 113–121. *Biomedical Approach*, R.G.D. Steele and J.H. Torrie (Ed.), p. 357–58. McGraw-Hill Book Co., Inc., New York, NY.

Stone, H., Sidel, J., Oliver, S., Woolsey, A., and Singleton, R.C. 1974. Sensory evaluation by quantitative descriptive analysis. Food Technol. 28: 24–34.

Theerakulkait, C. and Barrett, D.M. 1995a. Lipoxygenase in sweet corn germ: isolation and some physicochemical properties. J. Food Sci. 60: 1029–1033, 1040.

Theerakulkait, C. and Barrett, D.M. 1995b. Partial purification and characterization of sweet corn germ lipoxygenase. J. Food Biochem. 18: 355–372.

Velasco, P.J., Lim, M.H., Pangborn, R.M., and Whitaker, J.R. 1989. Enzymes responsible for off-flavor and off-aroma in blanched and frozen stored vegetables. Appl. Biochem. Biotechnol. 11: 118–127.

Wagenknecht, A.C. 1959. Lipoxidase activity and off-flavor in underblanched frozen corn-on-the-cob. Food Res. 24: 539–547.

Weber, E.J. 1978a. Corn lipids. Cereal Chem. 55(5): 572–584.

Weber, E.J. 1978b. The lipids of corn germ and endosperm. J. Am. Oil Chem. Soc. 56: 637–641.

Wiley, R.C. 1985. Sweet corn aroma: studies of its chemical components and influence on flavor. Ch. 13, In *Evaluation of Quality of Fruits and Vegetables*, H.E. Patee (Ed.), p. 349–366. AVI Publishing Co., Westport, CT.

Williams, D.C., Lim, M.H., Chen, A.O., Pangborn, R.M., and Whitaker, J.R. 1986. Blanching of vegetables for freezing: Which indicator enzyme to choose. Food Technol. 40(6): 130–140.

Williams, M.P. and Nelson, P.E. 1973. Effect of hybrids and processing on the diethyl sulfide potential of sweet corn. J. Food Sci. 37: 408–410.

Ms received 7/18/94; revised 3/8/95; accepted 4/1/95.