Rapid Methods for Lipoxygenase Assay in Sweet Corn

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ABSTRACT

Potential methods for rapid assay of sweet corn lipoxygenase (LPO) were evaluated and compared by visual inspection. Both model system (partially purified) and vegetable (LPO extract) system studies were carried out. Model system studies indicated that LPO had maximum activity at pH 6.0 and 2.0 mM linoleic acid. Methylene blue bleaching (MBB), carotene bleaching (CB), and potassium iodide-starch (KI-S) methods were evaluated both spectrophotometrically and visually. Both MBB and KI-S methods were effective for the model system. For the vegetable LPO extract systems (sweet corn and green beans), the MBB method indicated positive results for both, the CB method was not effective for either, and the KI-S method was very effective for green beans but not for sweet corn.

Key Words: lipoxygenase, sweet corn, rapid assay, methylene blue

INTRODUCTION

BECAUSE PEROXIDASE (POD) is considered one of the most stable enzymes in vegetables, its thermal destruction has been used as an indicator of blanching adequacy. The ubiquity of POD and the availability of a simple colorimetric assay for evaluation of its activity also make it a preferred indicator for vegetable blanching (Joslyn, 1949; Morris, 1958; Schwimmer, 1981).

Some studies have reported low correlations between residual POD activity and frozen food keeping quality (Bottcher, 1975; Chen et al., 1987; Lim et al., 1989) and that it may not be necessary to completely inactivate POD (Bottcher, 1975). Such inactivation might result in overblanching which could lead to reduced quality (e.g. color, flavor, texture, and nutrient) and economic losses (Schwimmer, 1981; Williams et al., 1986; Lim et al., 1989; Velasco et al., 1989; Reid, 1990; Barrett and Theerakulkait, 1995).

Research studies have indicated that lipoxygenase (linoleate: oxygen oxidoreductase, EC 1.13.11.12 or LPO) should be considered as an enzyme indicator alternative to POD. LPO causes off-flavor in many foods including sweet corn (Lee, 1981; Schwimmer, 1981; Williams et al., 1986; Chen et al., 1987; Halpin and Lee, 1987; Chen and Hwang, 1988; Ganthavorn and Powers, 1989; Velasco et al., 1989; Sheu and Chen, 1991; Barrett and Theerakulkait, 1995; Theerakulkait and Barrett, 1995). Being less heat stable than POD, this enzyme requires shorter heat treatment, hence its inactivation should result in minimum quality deterioration and economic loss.

The greatest limitation to use of LPO as an indicator is that a rapid assay is not currently used in the frozen food industry. Williams et al. (1986) developed a rapid semi-quantitative assay for LPO activity based on a potassium iodide-starch method. It required daily preparation of reagents and was applicable to many fruits and vegetables but limited in the case of commodities containing carotenoids. Preferential oxidation of carotenes over iodide may be responsible for this limitation (Whitaker, 1995).

Relatively simple, rapid visual and spectrophotometric assays have been developed by soybean breeders for determination of LPO isozyme activity (Kikuchi and Kitamura, 1987; Hammond et al., 1992; Suda et al., 1995; Toyosaki, 1992). The standard laboratory assay for LPO is the spectrophotometric method wherein an increase in absorbance at 234 nm due to conjugated diene formation is monitored.

All available assays, whether ''rapid'' or laboratory have deficiencies for various reasons. Some require use of a spectrophotometer; others require relatively long analysis times; low sensitivity is a problem with others and some use solvents that may be unsafe or unpleasant in a food processing environment. Many involve complicated procedures and the qualitative nature of some methods requires further development to make them at least semi-quantitative.

Most of the ''rapid'' assays were developed for use with soybeans and it is necessary to determine whether they are also applicable to sweet corn and other vegetables. Our objective was to evaluate and compare three potential methods for ''rapid'' assay of lipoxygenase in sweet corn which could be carried out in a processing environment.

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MATERIALS & METHODS

Materials

Sweet corn served as the source of LPO for model system (which utilized partially purified LPO) and a corn LPO extract since these were carried out at different times of the year, the cultivars and growing regions were not the same. For the model system, sweet corn (*Zea mays* L. cv. Zenith) was harvested by hand (on a separate day for each blanch treatment) at the proper stage of maturity (average 73.24% moisture) from the University of California, Davis, Vegetable Crops Field Station. For the LPO extract system, 'Supersweet' corn from Florida was obtained from a local wholesaler and green beans were purchased from a local retailer.

Linoleic acid, Tween-20, methylene blue, dithiothreitol, β-carotene, potassium iodide, and soluble starch were obtained from Sigma Chemical Company (St. Louis, MO). Other chemicals used were of reagent grade and in all experiments, deionized distilled water was used.

Model system

Sample preparation. Freshly harvested sweet corn was transported to the Dept. of Food Science & Technology, immediately dehusked, cleaned, and damaged or defective cobs removed. The corn was immediately brought to the laboratory where preparations were made for moisture determination, steam blanching and acetone powder preparation. Cob ends were trimmed and one or two 7.6 cm corn-on-the-cob sections were obtained. Corn was blanched at 100°C using a pilot Key Technology (Walla Walla, WA) steam blancher for 0, 60, 180, 300, and 420 sec. After blanching, samples were immediately cooled in ice-water for 3 min, drained and frozen on metal trays in a blast freezer (−44°C) for 2 hr. Samples were then stored in sealed freezer bags at −20°C.

Lipoxygenase extraction and partial purification. Acetone powder was prepared (Theerakulkait and Barrett, 1995, with slight modifications) by gently pushing frozen whole kernels by hand from the cob and blending in liquid nitrogen to a fine powder using a stainless steel blender. The powder was transferred to a beaker and homogenized (Brinkman Polytron homogenizer, Model PT3000, Kinematica AG, Brinkman Instruments Inc.) in cold acetone (−20°C) at a ratio of 1:20 (w/v) at 15,000 rpm for 2 min. The slurry was poured through Whatman #1 filter paper into a Buchner funnel. The residue was homogenized with 10 volumes of cold acetone for 1 min and filtered. This step was repeated until the supernatant was colorless. The final residue was homogenized with 5 volumes of cold acetone, filtered, vacuum dried overnight at room temp, ground using a mortar and pestle, and stored at −20°C until analyzed.

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

Lipoxygenase activity, optimum pH and substrate concentration. LPO activity was assayed using the method described by Chen and Whitaker (1986). The 0.01 M substrate stock mixture contained 157.2 µL linoleic acid, 157.2 µL Tween-20, and 10 mL water. Mixing was carried out by filling and ejecting the solution in a Pasteur pipet several times while avoiding air bubbles. The solution was clarified by adding 1.0 mL 1.0 N NaOH and made to 50 mL with water. Prior to the assay, the substrate stock solution was diluted to 200 mL with 0.2M sodium phosphate buffer (pH 7.0) to give a final concentration of 2.5 mM linoleic acid. The solution was then flushed with pure O_2 for 2 min and allowed to equilibrate at 25°C in a water bath for 10 min before use. All glassware used in the preparation of substrate solution has been wrapped with aluminum foil to exclude light.

For the LPO activity assay, 0.3 mL enzyme extract was added to 2.7 mL substrate solution and mixed well. The initial rate of conjugated diene formation was measured from the linear change in absorbance at 234 nm. A double beam spectrophotometer (UV-VIS scanning spectrophotometer, model UV-2102 PC, Shimadzu) and a 1-cm path length quartz cuvette were used in the assay. One unit of enzyme activity was defined as that amount of enzyme that produced a change in absorbance of 1.0/min at 234 nm, under the assay conditions. Activity was expressed in terms of g acetone powder (AP). This procedure was used for determination of optimum pH and substrate concentration for LPO activity by varying the buffer system (pH 3.0–9.0) and the final linoleic acid concentration (0.50–2.25 mM), respectively.

Vegetable LPO extract systems

Sweet corn and green beans were blanched for 0, 60, or 180 sec at 100°C prior to assay. Sweet corn kernels were cut as close to the cob as possible and green beans were cut into 3.8 cm long pieces following blanching. Cut vegetables (25g) were suspended in 50 mL water and homogenized for 3 min in a blender. The slurry was filtered through 4 layers of cheesecloth and the filtrate was used as the vegetable LPO extract for visual assays. A control which was used as a color comparison was prepared by boiling the LPO extract for 10 min.

Spectrophotometric assay comparison

The assays of interest were compared to the standard LPO assay using a model system. This was the same method used for determining LPO activity (see above) except that a concentration of 1.5 mM linoleic acid was used. This substrate concentration was also used for the other assays. A control was prepared by boiling the LPO extract for 10 min. All analyses were carried out in triplicate.

Methylene blue bleaching (MBB) method. The MBB method of Suda et al. (1995) was used with slight modifications. The following were mixed in a cuvette: 1.2 mL 0.2M sodium phosphate buffer (pH 7.0), 0.3 mL 0.1 mM methylene blue, 0.3 mL 0.2M dithiothreitol in 0.2M sodium phosphate buffer, pH 7.0 (prepared fresh before the assay), 0.3 mL acetone, 0.45 mL 0.01M linoleic acid substrate and 0.45 mL LPO extract with a total reaction mixture volume of 3.0 mL. The reaction was initiated by addition of the extract and then the absorbance at 660 nm was measured for 15 min at 25°C using a double beam spectrophotometer.

Carotene bleaching (CB) method. The CB procedure described by Suda et al. (1995) was followed with some modifications. The reaction mixture contained 1.5 mL 0.2M sodium phosphate buffer (pH 7.0), 0.3 mL β-carotene at 50% saturation in acetone, 0.45 mL 0.01M linoleic acid substrate and 0.75 mL LPO extract with a total volume of 3.0 mL. To obtain a clear solution, the β-carotene solution was mixed quickly with all other components except the enzyme extract. The reaction was initiated by addition of the extract and then the absorbance at 452 nm was measured for 10 min at 25°C.

The β-carotene solution was prepared by dissolving 10.0 mg of β-carotene in 10.0 mL acetone. The solution was stirred vigorously several times and centrifuged for 10 min at 26,890 × *g* at 4°C. The orange-colored supernatant was diluted with the same volume of acetone to give 50% saturation with respect to β-carotene and stored in a brown vial at 4°C. Fresh β-carotene solution was prepared daily as needed.

Potassium iodide-starch (KI-S) method A slightly modified version of the KI-S method developed by Williams et al. (1986) was used. LPO extract (0.10 mL) and 0.45 mL 0.01M linoleic acid substrate were mixed in a cuvette and incubated for 4 min at room temp (23°C). At the end of 4 min, 0.01 mL starch solution, 0.2 mL potassium iodide solution (prepared fresh) and 2.25 mL 15% acetic acid solution were added and the solution was mixed well. At exactly 4 min after adding the last three components, the absorbance at 470 nm was measured spectrophotometrically.

The starch solution was prepared by suspending 1.0g of soluble starch in 100 mL water and heating in a water bath until the suspension cleared. The solution was cooled before use. Saturated KI solution was prepared by dissolving 1.5g of KI in 1.0 mL water, warming slightly. On cooling, some KI precipitated. Starch and KI solutions were prepared fresh daily. Acetic acid solution (15% v/v) was prepared by diluting 15.0 mL glacial acetic acid to 100 mL with water.

Visual assay comparison

Semiquantitative visual assays were conducted for both model and vegetable LPO extract systems to determine if the methods were effective with both in the presence of potential interfering components. Sweet corn and green beans were used in the LPO extract system to compare methods in carotenoid-containing and/or non-carotenoid-containing vegetables. In both systems, 1.0 mL LPO extract was used.

MBB method. A visual procedure for the MBB method was modified from Suda et al. (1995). The dye-substrate was prepared by first weighing 154.25 mg of dithiothreitol and transferring it to a 125 mL Erlenmeyer flask. The following additional components were then added to the flask: 25.0 mL 0.2M sodium phosphate buffer (pH 7.0), 5.0 mL 0.1 mM methylene blue, 5.0 mL acetone, and 9.0 mL 0.01M linoleic acid substrate. After adding all components, the mixture was swirled. This dye-substrate was adequate for 20 samples. The assay was carried out by mixing the LPO extract and 2.0 mL dye-substrate in a test tube. A positive test was indicated by bleaching of the blue color and scores of 4+, 3+, 2+, 1+, and − were subjectively assigned by the researchers, depending on the speed at which the color change occurred (4+ = within 1 min, 3+ = 1 to 3 min, 2+ = 3 to 5 min, 1+ = 5 to 10 min, and − = no change after 10 min).

CB method. The method developed by Suda et al. (1995) was used in this assay with slight modifications. Sufficient carotene substrate for 20 samples was prepared by mixing 25.0 mL 0.2M sodium phosphate buffer (pH 7.0), 9.0 mL 0.01M linoleic acid substrate, and 5.0 mL water and adding these to a 125 mL Erlenmeyer flask containing 5.0 mL β-carotene at 50% saturation in acetone. The mixture was immediately shaken vigorously. The assay was conducted by putting the LPO extract in a test tube and then adding 2.0 mL carotene-substrate. The test was considered positive for the presence of LPO if the yellow-orange color bleached. The same scores as used in the MBB method were assigned.

KI-S method. The procedure described by Williams et al. (1986) was followed with slight modifications. LPO extract and 0.47 mL 10 mM linoleic acid substrate were incubated for 5 min in a test tube. Starch solution (0.10 mL), 0.30 mL freshly prepared 73% (w/v) KI solution, and 1.28 mL 15% acetic acid solution were then added. A positive test was indicated by development of a purplish or brownish color, and results were scored in the same way as for the MBB and CB tests.

Statistical analysis

The data were analyzed using the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA, 1989). When analysis of variance revealed a significant effect ($p \leq 0.05$), data means were compared with a Duncan's Multiple Range test.

RESULTS & DISCUSSION

Optimum pH for activity

Most plant lipoxygenases have optimum activity in the range of pH 5.5–7.5. The activity profile for sweet corn LPO across the pH range used in this study was developed (Fig. 1). The optimum pH was 6.0 and very low activity was detected below pH 4.0 or above pH 8.0. Although the highest LPO activity was at pH 6.0, the subsequent substrate optimum study was evaluated at pH 6.6 and 7.0. This pH range was used because most assays under consideration operated best there and LPO activity was still relatively high (~50% of maximum activity, 44.05 unit/g AP/min) in that pH range. In addition, substrate solution prepared at pH 6.0 was slightly turbid and caused interference

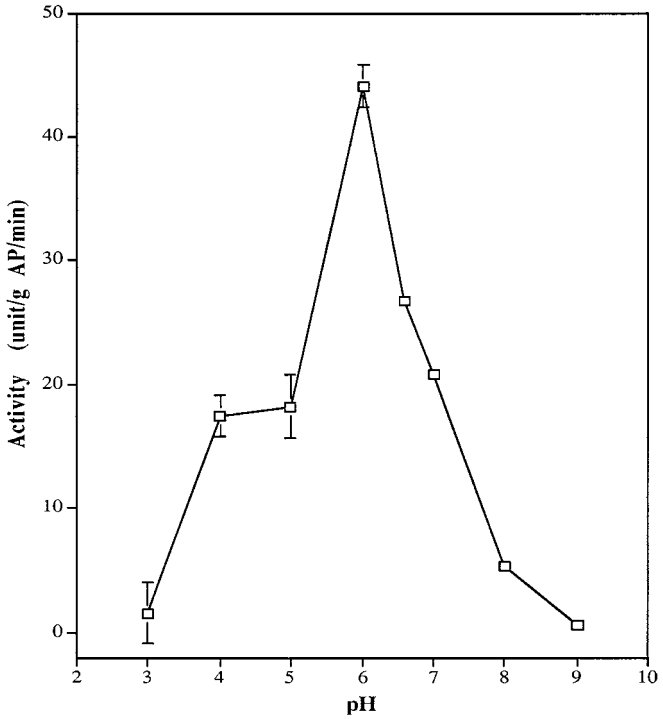

Fig. 1—Sweet corn lipoxygenase activity as related to pH. (Means of two replications. Buffer systems—citrate phosphate, pH 3.0–5.0; sodium phosphate, pH 6.0–7.0; Tris-HCl, pH 8.0–9.0).

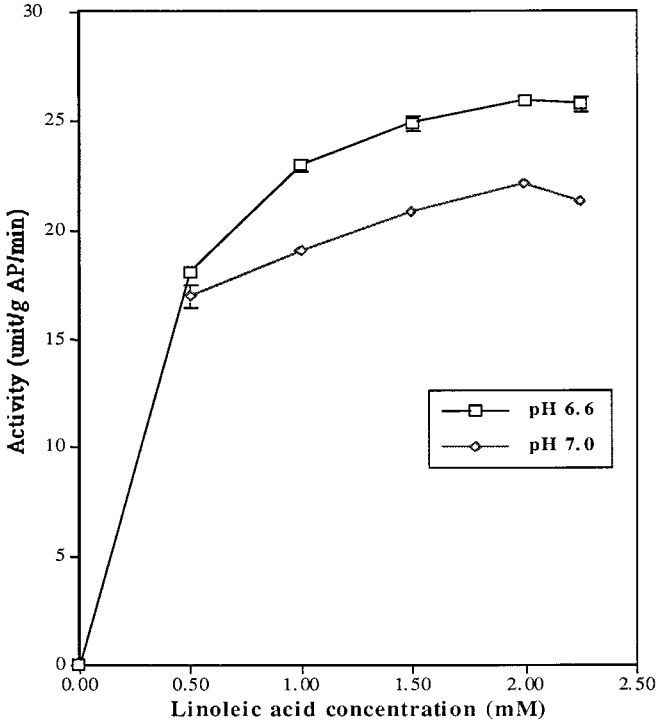

Fig. 2—Sweet corn lipoxygenase as related to substrate concentrations. (Means of two replications).

with the assay, while the solution at pH 6.6–7.0 was much clearer.

The optimum pH we obtained was comparable to reported values on corn. Theerakulkait and Barrett (1995) found the optimum pH for LPO from sweet corn germs was 6.0–7.0. Gardner (1988) reported a value between pH 6.0 and 7.2 for the LPO of mature maize germs. A broader range of optimum pH (6.0–8.2) was found by Poca et al. (1990) for LPO isozyme-1 of mature maize seeds. Somewhat higher optimum pH values have also been reported: pH 6.8–7.0 for the embryo LPO of inbred yellow dent corn (Belefant and Fong, 1991) and pH 7.0–9.0 for LPO isozyme-2 of mature maize seed (Poca et al., 1990).

Optimum substrate concentration for activity

An important feature of any LPO substrate is the presence of a *cis, cis*-1,4-pentadiene structure, commonly found in polyunsaturated fatty acids and derivatives. The naturally occurring, nutritionally essential fatty acid, linoleic acid, is one of the most common LPO substrates in plants. In our study, the activity of LPO from sweet corn acetone powder was determined at pH 6.6 and 7.0 using different linoleic acid concentrations (0.50, 1.00, 1.50, 2.00, and 2.25 mM).

At both pH 6.6 and 7.0, the lowest and highest activities were obtained at 0.50 and 2.00 mM linoleic acid, respectively (Fig. 2). At pH 6.6, there was no difference ($p \geq 0.05$) found in LPO activity using substrate concentrations of 1.50, 2.00, and 2.25 mM. When the study was carried out at pH 7.0, activity at 2.00 mM linoleic acid was higher than at 1.50 mM but not significantly different from 2.25 mM. In addition, activities at 1.50 mM and 2.25 mM were not different from each other ($p \geq 0.05$).

A linoleic acid concentration of 1.50 mM was used for subsequent comparisons of methods because it approximated the most common substrate concentration used for all methods. This concentration was also much higher than the estimated K_m which was 0.30 mM (at activity = 0.5 maximum activity, Fig. 2).

During the substrate optimum study, we observed that solutions prepared at pH 7.0 were more clear than those prepared at pH 6.6. This could be attributed to the insolubility of the substrate at lower pH. Even with addition of Tween 20, solutions at pH 6.6 and lower remained slightly turbid. Similar observations were reported by Chen and Whitaker (1986) who obtained slightly turbid solutions at pH 6.5 and lower. For this reason, pH 7.0 was used for comparison of all methods.

Spectrophotometric assay comparison (model system)

A commonly used assay for LPO activity is the spectrophotometric method, based on following the increase in absorbance at 234 nm arising from enzyme-catalyzed production of conjugated diene products. This method is considered relatively simple, rapid, sensitive, specific, and accurate (Holman, 1955; Grossman and Zakut, 1979; Galliard and Chan, 1980). It was chosen as the standard LPO assay to which other ''rapid'' methods involving couple oxidation, were compared.

The percent original activity remaining, as measured spectrophotometrically, in acetone powder LPO extract from sweet corn blanched at 100°C for 0, 60, 180, 300, and 420 sec was compared (Fig. 3). As expected, an inverse relationship was observed between percent remaining activity and blanching time.

Methylene blue bleaching method

The percentage of original MBB activity remaining in the samples (Fig. 3) decreased with blanching time. Toyosaki (1992) proposed a mechanism for the MBB reactions. Methylene blue was hypothesized to be bleached when the hydroperoxide was produced by H removal. Suda et al. (1995) also proposed that a redox reaction was involved in the bleaching reaction, because methylene blue is a redox indicator.

Carotene bleaching method

Based on absorbance readings, values from the CB assay were only about 1/6 those observed using the MBB assay, and some samples (e.g. those blanched 420 sec) did not show any carotene bleaching. Values were somewhat erratic (Fig. 3), with some

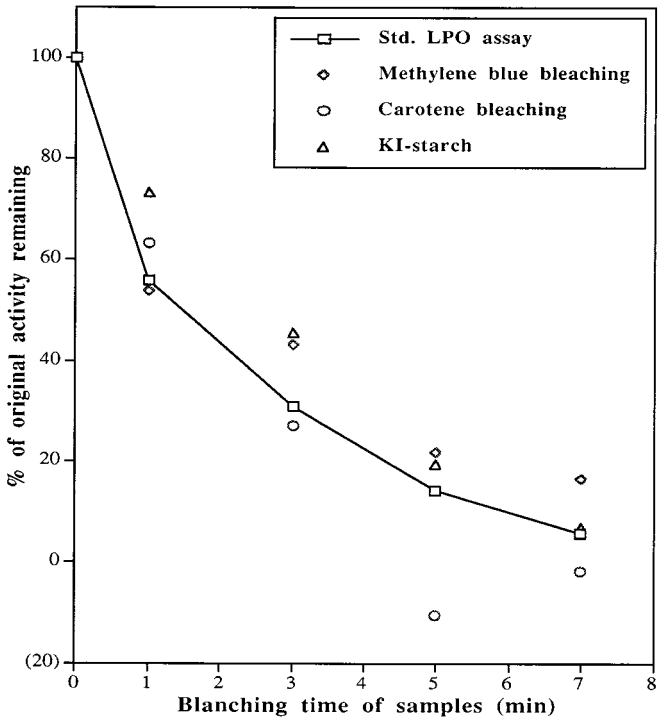

Fig. 3—Comparison of different spectrophotometric assays for rate of LPO loss.

Table 1—LPO activity detection in blanched sweet corn using visual assays (Model System)[a]

Blanching time (sec)	Methylene blue bleaching	Carotene bleaching	Potassium iodide-starch
0	4+[b]	—	4+
60	3+	—	3+
180	2+	—	—
300	—	—	—
420	—	—	—

[a] Acetone powder preparations were used.
[b] Change in color: 4+ within 1 min; 3+ between 1 to 3 min; 2+ between 3 to 5 min; — no change in color.

Table 2—LPO activity detection in blanched vegetable LPO extracts using visual assays (Vegetable LPO extract System)

Blanching time (sec)	Methylene blue bleaching	Carotene bleaching	Potassium iodide-starch
Sweet Corn			
0	3+[a]	—	—
60	2+	—	—
180	1+	—	—
Green Beans			
0	3+	—	3+
60	2+	—	—
180	—	—	—

[a] Change in color: 3+ within 3 min; 2+ between 3 to 5 min; 1+ between 5 to 10 min; — no change in color.

samples blanched for longer times having higher remaining activity than others blanched for shorter times. The β-carotene used in this method is very susceptible to oxidation. This assay measures a secondary reaction with an empirical relation to the primary reaction. A linear relationship between enzyme concentration and carotene loss exists over a very narrow range (Grossman and Zakut, 1979). Also, any errors associated with calculations may have been maximized by the small changes in absorbance. The CB method did correlate well with the standard LPO assay.

Potassium iodide-starch method

As with the standard LPO assay, as blanching time increased the remaining activity decreased (Fig. 3). Values from the KI-S method were similar to those from the standard LPO assay and the MBB method. The mechanisms of the KI-S method have been reported (Williams et al., 1986).

Visual assay (model system)

The spectrophotometric method is an effective, reliable measure of LPO activity, but it is not practical to use in a food processing plant. In order to use this enzyme as a blanching indicator, development of a simple, rapid visual assay would be more practical. In our study, visual colorimetric adaptations of the MB, CB (Suda et al., 1995) and KI-S (Williams et al., 1986) methods were compared. A positive test for MB and CB methods is the disappearance or bleaching of the blue or yellow colors. In the KI-S method, LPO activity is indicated by development of a purplish or brownish color.

Detection of LPO activity in the acetone powder samples by the different visual assays was compared (Table 1). Using the MBB method, the unblanched and 60 sec blanched samples scored 4+ and 3+, respectively. The change in color for the 180 sec blanched sample occurred somewhat later (3 to 5 min), indicating lower LPO activity. The 300 and 420 sec blanched samples both gave negative results, indicating not enough LPO to detect visually.

Spectrophotometric determination in the model system determined LPO activity in samples blanched 0, 60, 180 and 300 sec were 20.00, 11.15, 6.17 and 2.80 units/g AP/min, respectively. Using the MBB method, a visible color reaction was observed in the 180 sec blanched sample (6.17 units/g AP/min) but not in the 300 sec blanched sample (2.80 units/g AP/min). Because blanch times between 180 and 300 sec were not studied the exact number of units of LPO activity required to visualize a color change for this method could not be determined. The limit of detection for this procedure appears to be between 2.80 and 6.17 units/g AP/min.

Use of the CB method did not result in bleaching the yellow color, even in unblanched samples (20.00 units/g AP/min) after 10 min. Slight bleaching was shown by spectrophotometer but the color change in this method was not detectable visually. This method was not sensitive enough to be applicable.

Relatively fast color development was observed in unblanched and 60 sec blanched samples using the KI-S method. However, the method did not detect LPO activity in samples blanched for 180, 300, and 420 sec. It detected LPO activity through visible color changes in the 60 sec blanched sample which contained 11.15 units/g AP/min. The limit of detection for the method therefore was between 6.17 (180 sec blanched sample) and 11.15 units/g AP/min. Williams et al. (1986) detected LPO activity in a several unblanched fruits and vegetables using the method, but they did not detect activity in carotenoid-containing commodities. The KI-S method worked with our model system samples because in preparing acetone powder from sweet corn, most carotenoids had been removed.

Both the MBB and KI-S methods worked well with the model system, giving visible colorimetric reactions in samples containing 6.17 and 11.15 units/g AP/min, respectively. The MBB method was, however, more sensitive. The CB method did not show visible color change, even in unblanched samples with 20.00 units/g AP/min activity.

Visual comparison (vegetable LPO extracts)

The three methods were also tested with vegetable LPO extracts. The vegetables were sweet corn (carotenoid-containing) and green beans (non-carotenoid-containing). Both vegetables were steam blanched for 0, 60, and 180 sec (Table 2). These times were used because with the model system, positive results for visual assays had been found only for samples blanched 180 sec.

The MBB method was effective with sweet corn and the blue dye-substrate mixture added to yellow LPO extract gave a blue-

green color in both control and blanched samples. After differing times, the blue color in the blanched samples started to disappear and the original yellow color of the LPO extract was eventually restored, while the control remained blue-green. This was taken as a positive test for this assay. Similar results were reported by Toyosaki (1992) and Suda et al. (1995). All samples gave positive results for the MBB method.

In green beans, addition of the methylene blue dye-substrate to control and blanched samples led to development of a very dark green color. When the blue color started to disappear, the dark green color began to lighten. A positive test, therefore, was indicated by the change from dark green to light green. LPO activity was detected in the samples blanched for 0 and 60 sec and was not detected in the 180 sec blanched sample.

As with the model system, the CB method was not sensitive enough to detect LPO activity in corn or green bean LPO extracts. No LPO activity was detected in any unblanched or blanched sweet corn samples. This was consistent with results obtained by Williams et al. (1986) for carotenoid-containing vegetables. As reported by Williams et al. (1986), the KI-S method was effective with green beans, and unblanched samples developed a purplish or brownish color but no LPO activity was detected in samples blanched 60 or 180 sec.

Results indicated that the MBB method was suitable for both carotenoid-containing and non-carotenoid-containing vegetables. In addition, it was more sensitive than the KI-S method. For sweet corn, the MBB method would be best because the color change (blue green to yellow) was very distinct and it was relatively easy to distinguish between positive and negative results.

The MBB method was easier to perform than the KI-S method because there were fewer solutions to prepare the day of analysis. Most reagents could be prepared and stored beforehand. With development both methods may be made simpler and faster.

Results suggest the MBB method may be a rapid assay to determine LPO activity in sweet corn. Additional investigation is needed to determine whether this assay may be applied for other vegetables. Processors will need to adapt the method for their facility and equipment, e.g. depending on blancher bed depth, heat penetration, temperature of blanching, and other operational parameters. The LPO activity of corn varies with cultivar, time from harvest to process, and other factors. It may be helpful to evaluate incoming loads for LPO activity prior to blanching. The frozen food industry is encouraged to use lipoxygenase, rather than peroxidase, as an indicator of adequate blanching in vegetables. This could provide time and energy savings as well as improvements in product quality.

CONCLUSIONS

BOTH THE SPECTROPHOTOMETRIC MBB and KI-S methods correlated well with standard LPO assay. Visual comparisons showed both methods were reliable with a model system, but MBB was more sensitive. In vegetable LPO extract systems, the MBB method indicated positive results for both sweet corn and green beans whereas the CB was not reliable. The KI-S method was effective for green beans but not for sweet corn. The MBB method was applicable to both carotenoid-containing and non-carotenoid-containing vegetables. It was also more sensitive than the KI-S method. The MBB method was easier and faster than the KI-S method because most reagents could be prepared in advance. Therefore, the MBB method is recommended for determination of LPO activity in sweet corn, and potentially other vegetables.

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