JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2002,** *50,* 997−1001 **997**

Improved Amperometric Method for the Rapid and Quantitative Measurement of Lipoxygenase Activity in Vegetable Tissue Crude Homogenates

JOSÉ I. REYES-DE-CORCUERA AND RALPH P. CAVALIERI*

Department of Biological Systems Engineering, Washington State University, Pullman, Washington 99164-6120

JOSEPH R. POWERS

Department of Food Science and Human Nutrition, Washington State University, Pullman, Washington 99164

An improved amperometric method for rapid (2 min) quantitative determination of lipoxygenase (LOX) activity in vegetable tissue crude homogenates is presented. Measured LOX activity was linear ($R^2 > 0.99$) throughout the entire activity range for green bean and for corn below 70% activity. The resolution was 0.4% or 1.11 μmol L^{-1} s^{-1} of oxygen. The limit of detection was 3.43 μmol L^{-1} s^{-1} of oxygen. The amperometric method was improved by encapsulating linoleic acid (LA) in β-cyclodextrin (CD) resulting in a stable substrate−buffer solution at a pH below 8.0. Ethanol and Tween 20 were not effective in solubilizing high LA concentrations required by the assay. A prototype benchtop instrument with the potential for use in an industrial environment is also presented.

KEYWORDS: Lipoxygenase assay; enzyme activity; rapid assay; blanching indicator

INTRODUCTION

Lipoxygenase (LOX) has been identified as the enzyme responsible for the development of off-flavors in green pea (*1*), green bean (*2*), soybean (*3*), and corn (*4*) during frozen storage. However, to determine the extent of blanching, peroxidase and catalase are the most commonly assayed enzymes in the frozen vegetable industry. This is largely due to the lack of a rapid and quantitative method for measuring LOX activity. There are three main assays for LOX activity: spectrophotometric, coupled oxidation, and amperometric determination of dissolved oxygen consumption. The first two require extraction and partial purification of the enzyme and a spectrophotometer in order to make them quantitative (*5*). Until recently, only the amperometric assay had been successfully used in vegetable tissue crude homogenates (*5*, *6*). A quantitative colorimetric assay using crude tissue homogenates was demonstrated (*7*) but requires relatively long incubation times (10−20 min). This method, however, is better suited for the rapid determination of relative levels of activity. Other work has been done to develop a rapid assay for LOX (*1*, *6*, *8*, *9*). However, only one rapid and quantitative method has been developed and applied to green bean tissue crude homogenates (*6*). This method was based on the amperometric assay (*5*). While effective, Zhang's prototype (*6*) had two main limitations that made it unsuitable for use in an industrial environment. First, because of the instability of the substrate−buffer solution at a pH below 8.0 (*10*), the substrate and buffer needed to be added separately. Second, its automated system required many parts and occupied a relatively large benchtop surface. Substrate buffer instability is due to the low solubility of linoleic acid (LA) at pH < 8.0. The use of ethanol increased the solubility of LA (*5*), and its effects on the activity of wheat germ LOX were studied (*11*). Tween 20 inhibited soybean LOX-2 activity at pH 9.0 and low LA concentrations (30 μM), whereas at high LA concentrations (110 μM), the addition of Tween 20 resulted in increasing the rate of reaction (*12*). Similar results were found for wheat germ LOX (*11*). The effects of adding cyclodextrins (CD) to encapsulate LA on the activity of LOX-1 and -2 from the legume Bengal gram and LOX-2 from potato were studied (*13*, *14*). All of the studies mentioned above were carried out in purified systems; therefore, it is necessary to carry out similar experiments in tissue crude homogenates to explore the possibility of improving the amperometric assay. Even though amperometric techniques have been used for over 30 years, a systematic and rapid protocol for data acquisition and analysis and a simple apparatus with the potential for use in industry have not been demonstrated.

The objectives of this study are to improve the chemistry of the LOX amperometric assay and to fabricate a prototype benchtop device integrated to a data acquisition system and a user-friendly program, with the potential for use in an industrial environment.

* Author to whom correspondence should be addressed. Tel: 509-335-4563. Fax: 509-335-6751. E-mail: cavalieri@wsu.edu.

10.1021/jf0109423 CCC: $22.00
Published on Web 01/25/2002

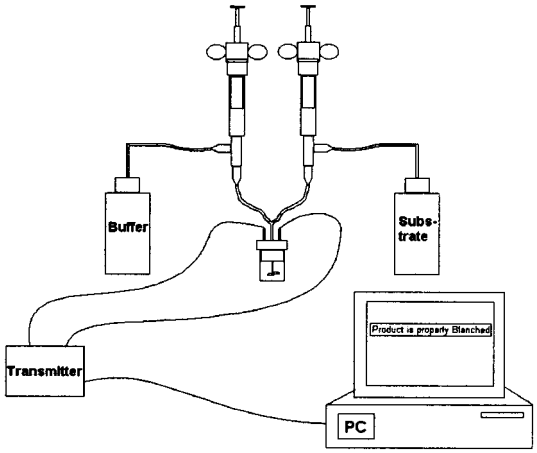

Figure 1. Schematic representation of benchtop device.

MATERIALS AND METHODS

Materials. *Reagents.* Fresh green beans and corn were purchased in the local market. LA (99%), Trizma-Base (Tris[hydroxymethyl]amino methane), β-CD (cyclopentamylose), and Tween 20 (polyoxyethylene-sorbitan monolaureate) were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium hydroxide pellets and hydrochloric acid (36.5−38.0%) were purchased from J. T. Baker (Phillipsburg, NJ). Ethanol was purchased from Fisher Scientific (Pittsburgh, PA). Deionized water was used for all tests and solution preparations.

Hardware. The system, depicted in **Figure 1**, consists of two 500 mL low-density polypropylene reagent bottles, two Micromatic 10 mL pipettor syringes (Popper & Sons, Inc., New Hyde Park, NY), and a reaction cell in whose cap a dissolved oxygen Clark type electrode model 730 and a temperature probe (AD 590 transducer) (Diamond General, Ann Arbor, MI) are mounted. The reaction vessel is a 20 mL (nominal volume) beaker. The probes are connected to the Diamond General Microsensor II (Diamond General, Ann Arbor, MI), which continuously measures temperature and dissolved oxygen concentration. A mechanical stirrer is mounted on the cap. Data are collected by the computer at a specified rate through a DynaRes 8 A/D board (12 bits, 8 channels, 1700 Hz) and T71 terminal panel (IOtech/Strawberry Tree, Cleveland, OH). A personal computer with an Intel processor 80386 was used. Vegetable tissue "crude homogenates" were prepared with a commercial laboratory blender model 31 BL 91 (Waring, Torrington, CT). A pH meter model 420A and a pH combination electrode model 91-57BN (ORION Research, Inc., Beverly, MA) were used for pH measurements.

Software. QuickLog software version 2.4.1 (IOtech/Strawberry Tree, Cleveland, OH) was used for data collection. The analysis of variance (ANOVA) was carried out using Minitab (Minitab Inc., State College, PA).

Methods. *General.* Enzyme activity is reported as percent activity. The definition of 100% enzyme activity was arbitrarily selected and is not constant among experiments. Inherent variation of active enzyme content among samples of fresh vegetables exists because samples were purchased at different times and were stored for different periods of time at 4 °C. This variation results in different enzyme activities even when all of the rest of the experimental conditions were held constant. To compensate for this variation, when required, control tests were run immediately before the experiment. Enzyme activity was determined as the rate of oxygen depletion in μmol L^{-1} s^{-1}. The rate of oxygen depletion was calculated using a computer program that determined the linear section of the plot of dissolved oxygen concentration vs time and calculated the slope. Because of variability in LOX activity among vegetable samples, enzyme activity is reported as a percentage. For each experiment and in each plot, the definition of 100% enzyme activity and its equivalent in absolute units (μmol L^{-1} s^{-1}) of oxygen were specified. Once inserted in the system, the total volume of the reaction cell was 22 mL, and 5 g of vegetable tissue crude homogenate was used in all experiments. Vegetable tissue crude homogenates were prepared by blending 100 g of vegetable in 50 mL of water for 1 min at 18 000 rpm. LA 2.54 mM stock solution was prepared as described elsewhere (*6*) and stored at 4 °C in an amber flask until needed. Tris-HCl buffer, pH 6.4, $I = 0.123$ was prepared as described elsewhere (*6*). Unless indicated otherwise, all tests were triplicated.

For each of the following sections, LOX activity determinations were carried out in a completely randomized design. One-way ANOVA was performed as well as multiple comparisons or comparisons to a control as indicated in the Results and Discussion for each section.

Characterization of the Instrument. Green beans or corn were water-blanched at 80 °C for 6 min to completely inactivate LOX. Immediately after the vegetables were blanched, they were cooled in ice water and macerated in the blender. For each vegetable, fresh product was added to blanched product to make aliquots with different concentrations of active enzyme. The linearity, sensitivity, and limit of detection were determined. LA concentration in the reaction cell was 0.38 g L^{-1} unless indicated otherwise.

Organic Cosolvent. Ethanol (15 mL) was added to a 35 mL aliquot of pH 6.5 LA−Tris-HCl mixture stored for 24 h until the cloudiness was visually similar to the cloudiness of the mixture stored at pH 8.0. The latter was used as a control for visual comparison of solutions' cloudiness because the pH 8.0 LA−Tris-HCl mixture was stable and showed higher LOX activity than the pH 9.0 mixture. Two additional levels of ethanol were used, 7.5 and 30 mL. To compensate for dilution effects, water was added to the two solutions to which less ethanol was added. To an aliquot of pH 7.0 LA−Tris-HCl mixture stored for 24 h, 5, 7.5, and 10 mL of ethanol were added, and dilution effects were compensated as before. The mean LOX activity for fresh green bean at pH 6.5 and pH 7.0 using fresh LA−Tris-HCl solution without ethanol was defined as 100% activity and was equal to 5.7 μmol L^{-1} s^{-1} and 6.4 μmol L^{-1} s^{-1} of oxygen per gram of vegetable, respectively.

Surfactant. Three different amounts of Tween 20 were added to 1 g L^{-1} LA stock solutions at pH 6.4 and pH 9.0. The mean LOX activity for fresh green bean at pH 6.5 using fresh LA−Tris-HCl solution without Tween 20 was defined as 100% activity and is equal to 2.17 μmol L^{-1} s^{-1} of oxygen per gram of vegetable.

Surfactant and β-CD. Tween 20 (0.25 mL) was added to a 1 g L^{-1} LA stock solution in order to obtain a concentration of 0.005% v/v of the surfactant in the reaction mixture. A 1:1 mol LA−CD solution was prepared by dissolving 2.2 g of CD in 400 mL of deionized water. To this solution, 500 mg of LA was added, and the system was agitated with a magnetic stirrer until a visually uniform dispersion of phases was observed. Deionized water was added to make 500 mL of solution. Substrate−buffer solutions were prepared by mixing 250 mL of each of the previous solutions with 250 mL of Tris-HCl buffer at pH 7.5. Mixtures were stored at room temperature and wrapped in aluminum foil to protect the reagent from light that might initiate autoxidation. The remaining buffer and substrate solutions were stored at 4 °C. Substrate solution containers were wrapped in aluminum foil. Because of LOX activity variation among fresh green bean samples and to variations of LOX activity of crude homogenates with respect to storage time, it was necessary to use control tests. As controls, enzyme activity was determined in triplicate for fresh mixtures with fresh crude homogenates at times 8 and 24 h. LOX activity was determined for stored mixtures at times 0, 8, and 24 h. The mean LOX activity for fresh green bean at pH 7.5 using fresh LA−Tris-HCl solution without CD was defined as 100% activity and is equal to 8.45 μmol L^{-1} s^{-1} of oxygen per gram of vegetable.

Effect of LA−CD Concentration on LOX Activity. Diluted stock solutions with concentrations 0.1, 0.2, 0.3, 0.4, 0.5, and 1.0 g L^{-1} LA were prepared from the 1:1 LA−CD solution of the previous experiment. In this experiment, enzyme activities were normalized to 100% of the average of the maximum obtained activity using CD−LA−Tris-HCl solution and LA−Tris-HCl without CD.

RESULTS AND DISCUSSION

Characterization of the Instrument. To determine the linearity of the instrument, the effect of enzyme concentration on the instrument's response was determined. **Figure 2** shows the effect of fresh vegetable proportion on enzyme activity. For

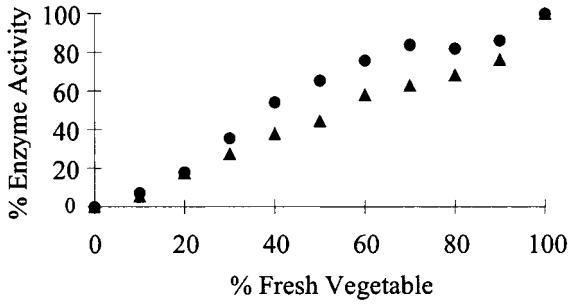

Figure 2. Measured enzyme activity at different active enzyme concentrations for green bean (▲) and corn (●). A 100% activity corresponds to 12.9 and 2.3 μmol L^{-1} s^{-1} of oxygen, respectively, using 1 g L^{-1} LA stock solution. The percentage of fresh vegetable is reported with respect to 5 g of tissue homogenate added to the reaction cell.

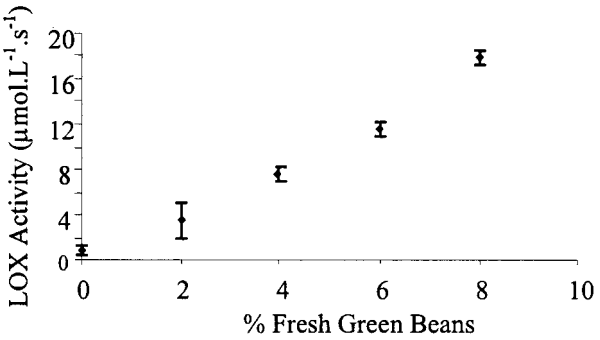

Figure 3. Measured enzyme activity at low active enzyme concentrations. The percentage of fresh vegetable is reported with respect to 5 g of tissue homogenate added to the reaction cell. Error bars represent ± one standard deviation.

green bean, the sensor's response was linear (R^2 > 0.99) throughout all of the measured range. For corn, correlation coefficients greater or equal to 0.99 were obtained in the range of 0−70% of active enzyme. Deviations from the expected activity in this figure are due not only to instrument operation (reagent addition, cell filling, oxygen electrode, and data acquisition system) but also to sample preparation and variability of enzyme activity in fresh samples. Deviations at high enzyme concentrations are mainly due to the electrode's response delay and can be avoided by diluting samples with high LOX activity. Finally, for blanching assessment, most LOX must be inactivated, and low or null enzyme activity is expected. Therefore, deviations at high active enzyme concentration become irrelevant. One-way ANOVA with $\alpha = 0.05$ gave a p value of 0.00 at low active enzyme concentrations (0−8% fresh green bean). In that range, the resolution of the instrument calculated as the confidence intervals with $\alpha = 0.05$ was 0.4% or 1.11 μmol L^{-1} s^{-1} of oxygen. Tukey's multiple comparison confidence intervals show that all of the measured levels of fresh vegetable are different from each other in that interval. The family error rate is 0.05. Therefore, as shown in **Figure 3**, our sensing device's limit of detection was 3.43 μmol L^{-1} s^{-1} of oxygen for green bean.

Organic Cosolvent. The addition of ethanol at different concentrations resulted in clearer solutions, caused by the solubilization of LA molecules. However, as shown in **Figure 4**, activity was significantly lower than for fresh mixtures at pH 6.5 and pH 7.0 (100% activity). The best results were for ethanol concentrations in the reaction cell from 0.78 to 1.17 mol L^{-1}; however, LOX activity was approximately 30% of the activity with fresh substrate at pH 6.5. Note that mixtures stored at pH 8.0 were stable and had about 40% LOX activity.

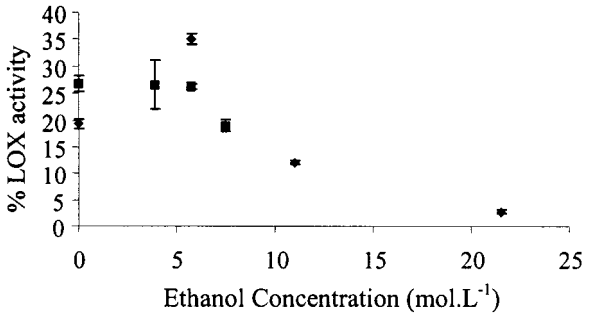

Figure 4. Effect of ethanol addition to solutions stored for 24 h at pH 6.5 (◆) and pH 7.0 (■). A 100% LOX activity corresponds to the activity of fresh mixtures at pH 6.5 or 7.0 (5.7 and 6.4 μmol L^{-1} s^{-1} of oxygen, respectively, using 5 g of vegetable homogenate). Error bars represent ± one standard deviation.

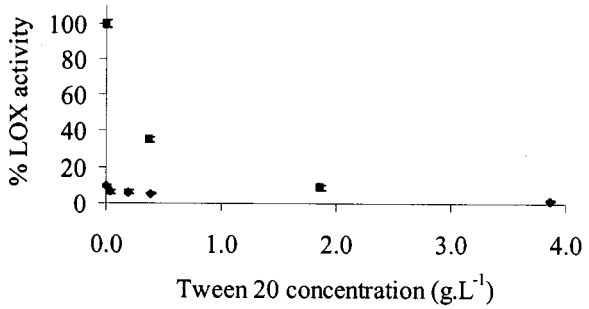

Figure 5. Effect of Tween 20 concentration on green bean LOX activity at pH 6.4 (■) and pH 9.0 (♦). A 100% activity corresponds to the mean LOX activity for fresh green bean at pH 6.5 using fresh LA–Tris-HCl solution without Tween 20 and is equal to 2.17 μmol L^{-1} s^{-1} of oxygen per gram of vegetable. Error bars represent ± one standard deviation.

Solubilization of LA was not accompanied by a large increase of the reaction rate. Furthermore, the addition of large amounts of ethanol inhibited LOX activity. Apparently, while ethanol solubilized LA, it also inhibited the enzymatic reaction. It was reported (*11*) that at a LA concentration of 350 μM an increase of ethanol concentration from 25 to 375 mM resulted in a decrease of wheat LOX activity and increasing LA concentration exacerbated ethanol's inhibitory effect. In our experiment, the LA concentration was 830 μM.

While the maximum concentration of ethanol suggested for LOX activity measurements was 0.03% (*5*), in our experiment, the required ethanol concentration to obtain a slightly cloudy solution was 11.7% or 2.7 M because of the relatively high concentration of LA. The optimum ethanol concentration for wheat LOX was 0.17 M (*11*). Therefore, the addition of ethanol was discarded as a possible way of stabilizing LA−buffer solution for determining LOX activity in green bean.

Surfactant. The effects of Tween 20 concentration on green bean LOX activity at pH 6.4 and pH 9 are depicted in **Figure 5**. At pH 6.4, the observed inhibition is similar with results found for soybean LOX-1 at pH 9 (*15*). At pH 9.0 in 1 g L^{-1} LA solution, the LOX-catalyzed reaction was inhibited by the presence of Tween 20 in the concentration range of 0.04−0.38 g L^{-1}. This inhibitory effect may be due to the entrapment of free LA molecules within the surfactant micelles or the formation of combined micelles, resulting in an overall decrease in the concentration of free LA. This inhibition is contrary to the activation effect that was observed for soybean LOX-2 using low concentrations of surfactant and a relatively high LA concentration (*15*). However, in that case, purified enzyme and a different surfactant were used. In our case, the presence of

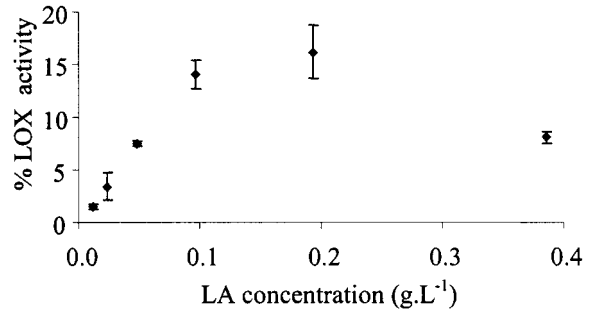

Figure 6. Effect of LA concentration on green bean LOX activity at pH 9.0. A 100% activity corresponds to the mean LOX activity for fresh green beans at pH 6.5 using fresh LA–Tris-HCl solution without Tween 20 and is equal to 2.17 μmol L^{-1} s^{-1} of oxygen per gram of vegetable. Error bars represent ± one standard deviation.

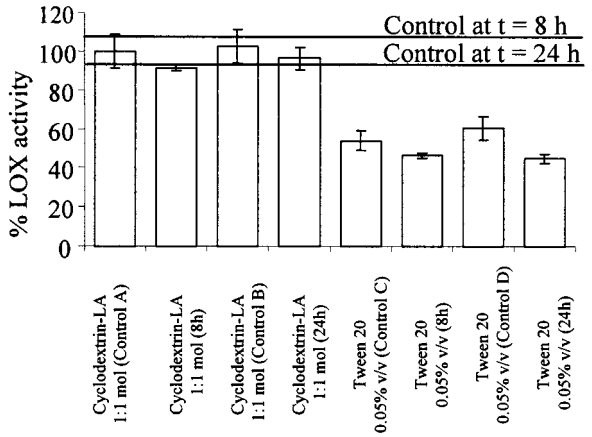

Figure 7. Effect of Tween 20 and β-CD on green bean LOX activity during substrate–buffer solutions storage. A 100% activity corresponds to the mean LOX activity for fresh green bean at pH 7.5 using fresh LA–Tris-HCl solution without CD and is equal to 8.45 μmol L^{-1} s^{-1} of oxygen per gram of vegetable. Error bars represent ± one standard deviation.

vegetable tissue may cause the association of surfactant molecules at the interfaces between the reagent solution and the vegetable solids.

Figure 6 shows the effect of LA concentration on green bean activity at pH 9.0. Because the optimum pH for green bean LOX is around 6.5, the activity at pH 9 was lower than at pH 6.5. The increased LA solubility at pH 9 did not compensate for the low enzyme activity, and substrate inhibition became significant at LA concentrations in stock solution greater than 0.2 g L^{-1}; that is, at concentrations greater than 7.75×10^{-4} M in the reaction cell. Note that this value is similar to the linoleate calculated critical micelle concentration (5.01×10^{-4} M), suggesting that micelle formation could be involved in the inhibition of LOX. However, assuming that micellar linoleate is inert, micelle formation alone would be responsible for the leveling off of the rate of reaction only and not for the decrease in the rate of reaction that was observed at linoleate concentration in the reaction cell between 0.2 and 0.4 g L^{-1}. In addition, the inhibitory effect can be observed at pH 9.0 and not at pH 6.4 or 7.0 probably because at lower pH leveling off of the rate of reaction is due to the self-association of LA in solution and not to an enzymatic substrate inhibition effect.

CD. Figure 7 shows the effect of storage time of substrate−buffer mixtures that contain LA with or without CD on fresh green bean LOX activity. ANOVA on LOX activity of reaction mixtures containing Tween 20 or CD with respect to their

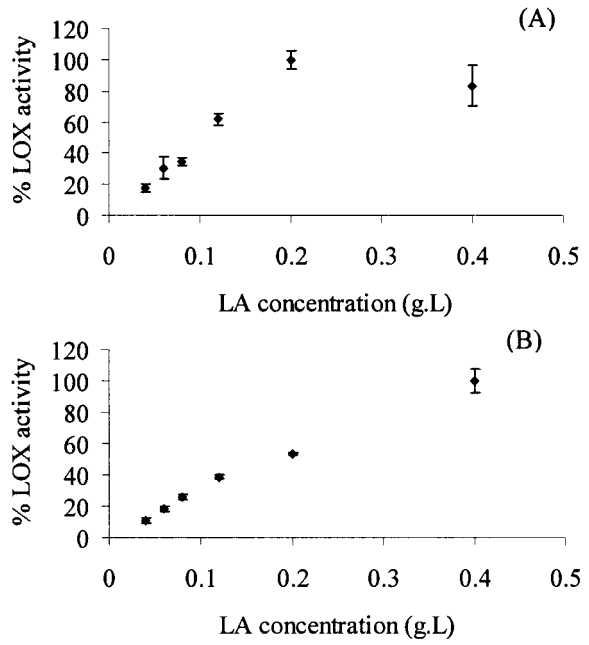

Figure 8. Effect of LA concentration on green beans LOX activity for mixtures containing (A) 1:1 mol LA:CD and (B) without CD at pH 7.0. A 100% activity is the average of the maximum activity obtained using CD–LA–Tris-HCl buffer or LA–Tris-HCl corresponding to 2.2 and 1.0 μmol L^{-1} s^{-1} of oxygen per gram of vegetable, respectively. Error bars represent ± one standard deviation.

controls was carried out. In the case of the solution containing Tween 20, one-way ANOVA with $\alpha = 0.05$ gave a p value of 0.009 indicating that there are significant differences among means. Dunnet's comparison test with respect to control C (containing Tween 20) showed that there was no significant difference in LOX activity between samples at time 0 and samples after 8 h, but there were significant differences between time 0 (control D containing Tween 20) and time 24 h. Therefore, the substrate−buffer mixture containing Tween 20 is stable for 8 h but not for 24 h. For the mixtures with CD, there were no significant differences among means for LOX activity (p value of 0.285). Therefore, the substrate−buffer with CD was stable for at least 24 h. **Figure 8** also shows that Tween 20 inhibits the rate of reaction by about 50% with respect to control solutions containing LA and Tris-HCl only (horizontal lines), whereas CD−LA solutions did not show significant differences with respect to the controls. In agreement with our results, the substrate−buffer mixture (1.34×10^{-4} M LA in phosphate-citrate) containing 0.004% Tween 20 has been reported to be unstable (*10*). CD complexes of LA and arachidonic acid were studied using nuclear magnetic resonance technique (*14*). For LA, the carboxylic moiety, carbons 1−9 and at least part of the 9−10 double bond were buried in the CD cavity. This observation is contrary to what is expected because the CD cavity is hydrophobic (*16*). However, assuming that LOX binds LA in a "head-to-tail" orientation (*17*), the observed regioselective formation of 13-hydroperoxide supports the previous observation (*14*). The observed rate of reaction is probably the result of competing effects. The rate may be reduced due to steric hindrance caused by CD. Conversely, by burying the carboxyl arm in the CD cavity, LA self-association is minimized, resulting in an increased rate of reaction.

Effect of LA−CD Concentration on LOX Activity. Figure 8A,**B** shows the means of percent LOX activity for different

concentrations of LA with and without CD for two different green bean tissue crude homogenates. The results in each case were normalized to give 100% activity for the highest activity. As expected, a decrease in activity was observed at higher free LA concentrations than in the case of CD−LA solutions at pH 7. This result confirms that at low pH the leveling off of the rate of reaction at LA concentrations higher than 0.2 g L^{-1} is due to the low solubility of LA and not to effects of enzyme kinetics. Similar results are reported for soybean LOX at pH 7.5 (*13*).

In this study, the amperometric determination of LOX activity was improved by integrating a user-friendly compact system with an improved assay. The use of CD stabilized the substrate−buffer mixture and resulted in a 2-fold larger response as compared to Tween 20-stabilized substrate−buffer mixtures. The use of CD to solubilize LA has the potential to increase green bean LOX activity and therefore improve the sensitivity of the assay when using higher concentrations of LA than those used in this study. The prototype instrument presented here measured quantitatively and rapidly LOX activity in vegetable tissue crude homogenates and has the potential to be used in industrial quality assurance laboratories for the assessment of the extent of blanching of some vegetables.

LITERATURE CITED

(1) Williams, D. C.; Lim, M. H.; Chen, A. O.; Pangborn, R. M.; Whitaker, J. R. Blanching of vegetables for freezing- which indicator enzyme to choose. *Food Technol.* **1986**, *40*, 130−140.

(2) Velasco, P. J.; Lim, M. H.; Pangborn, R. M.; Whitaker, J. R. Enzymes responsible for off-flavor and off-aroma in blanched and frozen vegetables. *Biotechnol. Appl. Biochem.* **1989**, *11*, 118−127.

(3) Sheu, S. C.; Chen, A. O. Lipoxygenase as blanching indicator for frozen vegetable soybeans. *J. Food Sci.* **1991**, *56*, 448−451.

(4) Barrett, D. M.; Theerakulkait, C. Quality indicators in blanched, frozen vegetables. *Food Technol.* **1995**, *49*, 62−65.

(5) Grossman, S.; Zakut, R. Determination of the activity of lipoxygenase (lipoxidase). In *Methods of Biochemical Analysis*; Glick, D., Ed.; Wiley and Sons: New York, 1979.

(6) Zhang, Q.; Cavalieri, R. P.; Powers, J. R.; Wu, J. Measurement of lipoxygenase activity in homogenized green bean tissue. *J. Food Sci.* **1991**, *56*, 719−721, 742.

(7) Anthon, G. E.; Barrett, D. M. Colorimetric method for the determination of lipoxygenase activity. *J. Agric. Food Chem.* **2001**, *49*, 32−37.

(8) Villafuerte-Romero, M.; Barrett, D. M. Rapid methods for lipoxygenase assay in sweet corn. *J. Food Sci.* **1997**, *62*, 696−700.

(9) Suda, I.; Hajika, M.; Nishiba, Y.; Furuta, S.; Igita, K. Simple and rapid method for the selective detection of individual lipoxygenase isozymes in soybean seeds. *J. Agric. Food Chem.* **1995**, *43*, 742−747.

(10) Adams, J. B.; Ongley, M. H. The behaviour of green bean lipoxygenase on chromatography and isoelectric focusing. *Food Chem.* **1989**, *31*, 57−71.

(11) Bhirud, P. R.; Sosulski, F. W.; Sosulski, K. Optimizing assay and extraction of lipoxygenase in wheat germ. *J. Food Sci.* **1993**, *58*, 1090−1094.

(12) Shilstra, M. J.; Veldnik, G. A.; Vliegenthart, J. F. G. The dioxygenation rate in lipoxygenase catalysis is determined by the amount of iron (III) lipoxygenase in solution. *Biochemistry* **1994**, *33*, 3974−3979.

(13) Jyothirmayi, N.; Ramadoss, C. Soybean lipoxygenase catalyzed oxygenation of unsaturated fatty acid encapsulated in cyclodextrin. *Biochim. Biophys. Acta* **1991**, *1083*, 193−200.

(14) Jyothirmayi, N.; Ramadoss, C.; Divakar, S. Nuclear magnetic resonance studies of cyclodextrin complexes of linoleic acid and arachidonic acid. *J. Agric. Food Chem.* **1991**, *39*, 2123−2127.

(15) Schilstra, M. J.; Veldnik, G. A.; Vliegenthart, J. F. G. Effect of nonionic detergents on lipoxygenase catalysis. *Lipids* **1994**, *29*, 225−231.

(16) Rao, K. R. New trends in biocatalysis in the presence of cyclodextrins. *Pure Appl. Chem.* **1992**, *64*, 1141−1145.

(17) Hatanaka, A. The fresh green odor emitted by plants. *Food Rev. Int.* **1996**, *12*, 303−350.

Received for review July 20, 2001. Revised manuscript received November 21, 2001. Accepted December 4, 2001. The authors are grateful for the financial support of J.I.R.-D.-C. provided by CONACyT (National Council for Science and Technology, Mexico), Fulbright, and for the financial support of the WSU IMPACT center.

JF0109423