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Quantitative Determination of Peroxidase in Sweet Corn by Chemiluminescence

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A chemiluminescent method is described for the quantitative determination of peroxidase activity in corn. This method is based on measuring the the light emitted in the oxidation of purporogallin by the peroxidase in the presence of hydrogen peroxide. The photons of the emitted light are counted by a scintillation counter between the 19th and 25th s of the reaction. A linear relationship was established between photon counts and enzymatic activity. The standard error of the means was found to be in the range of 1.0–2.5% of the reading. The method is free from any turbidity intereference, does not require cleanup, and is capable of measuring directly the bound and the soluble fractions of the enzyme.

Peroxidase activity is commonly used as an index for the extent of enzyme deactivation in blanching of vegetables. The development of off-flavor during storage of frozen vegetables has been repeatedly correlated to peroxidase activity (Svensson, 1977; Baardeth, 1978). A fast quantitative method for the determination of this activity is therefore of high practical importance.

Currently, peroxidase activity is determined colorimetrically by enzymatically oxidzed H donors such as *o*-dianisidine ("Worthington Engzyme Manual", 1972), guaiacol (Wise and Morrison, 1971), pyrogallol (Marshall and Chism, 1979), and *o*-phenylenediamine (Vetter et al., 1958). The relatively low sensitivity of these methods requires a proportional increase in sample size for adequate color development. This in turn increases the amount of leached turbid materials which obviously interferes with a direct colorimetric reading. A cleanup procedure is required to overcome this type of interference, especially pronounced in starchy vegetables such as corn. In sweet corn kernels, e.g., an alcoholic precipitation and centrifugation are employed (Vetter et al., 1958) to clarify the sample. There are evidences suggesting the existence of a soluble and a cell wall bound peroxidase fraction (Gordon and Alldridge, 1971; Haard, 1973; Henry, 1975; Yung and Northcoate, 1975). The existence of a bound enzyme fraction casts doubts on the accuracy of peroxidase determination in any system where suspended material is removed.

A possible approach to the determination of peroxidase activity in a blended (and turbid) whole sample may be presented by a chemiluminescence technique. The chemiluminescence determination of peroxidase-mediated oxidation of substrates such as pyrogallol (Nilsson, 1964) and luminol (Maehly, 1955) has been studied. Recently, Halmann et al. (1979) have investigated the chemiluminescence peroxidation of phenolic derivatives such as pyrogallol, resorcinol, phloroglucinol, and purpurogallin. The oxidation mechanism of the latter by H_2O_2 has been studied by Collier (1966).

The purpose of this work was to develop a chemiluminescent technique for the fast and sensitive determination of peroxidase activity directly on the whole blended sample of sweet corn.

EXPERIMENTAL SECTION

Recommended Procedure. Approximately 20 g of corn ear slices (or any other corn tissue) is blended with 10 parts of distilled water for 3 min in a Waring blender. One milliliter of the blended sample is introduced into a scintillation vial containing 2 mL of freshly prepared (every 4 h) purpurogallin solution. The purpurogallin solution is prepared by dissolving 0.02% purpurogallin (Sigma Chemical Co.) in cold 1:10 methanol to 0.18 M, pH 6.5 potassium phosphate buffer. The purpurogallin solution is kept in ice water.

The reaction is started by injecting 1 mL of H_2O_2 (0.12 M in the same phosphate buffer) into the scintillation vial already containing the sample and the purpurogallin so-

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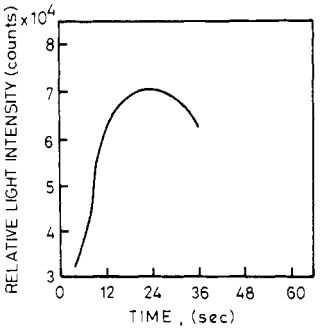

Figure 1. Light intensity time response of the chemiluminescent peroxidase test.

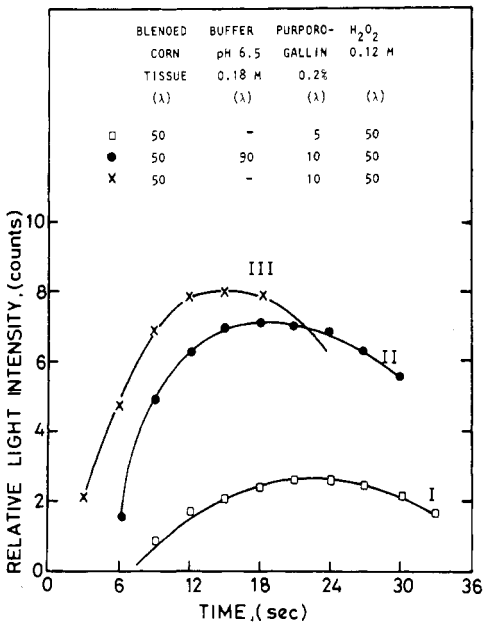

Figure 2. Light intensity time response in various test conditions.

lution. The vial is immediately shaken and inserted into the scintillation counter exactly 7 s after commencing the injection of the H_2O_2. The counter (Packard Tri-Carb 2002 ^3H setting at 0.1 mm, Packard Instrument Co., Downers Grove, IL) required 12 s to lower the sample to reading position and to start counting. The above schedule ensured the start of reading at the 19th s of the reaction. The counter reading is then taken at the 25th s and thus it was proportional to the integral of the light emitted between the 19th and 25th s, which was found to correlate best with peroxidase activity (see Results and Discussion).

Bound Peroxidase. Fresh corn ear slices were blended for 3 min in a Waring blender in 1:10 0.18 M pH 6.5 phosphate–citrate buffer (Vetter et al., 1958). The samples were centrifugated at 5000 rpm (~4000*g*) (M.S.E. Minor) for 10 min. After the samples were decanted the peroxidase activity was determined by the chemiluminescence method on both the supernatant and the percipitate. The latter was resuspended in the same buffer, and the procedure of centrifugation, separation, counting, and resuspension was repeated 6 times. The peroxidase activity in the precipitate was also determined in a semiquantitative manner (time until color is observed) by the guaiacol method (Joslyn, 1949).

RESULTS AND DISCUSSION

Light Emitting Time Response. The light emitted time response of the chemiluminescent peroxidase test was monitored on a 760 luminescenic Biometer (Du Pont Instruments, Newtown, CT), and a typical curve is shown in Figure 1. By use of this instrument, the chemiluminescent activity was followed for samples of various ratios of buffer, purporagallin stock solution, and *p*-phenylenediamine (Figure 2).

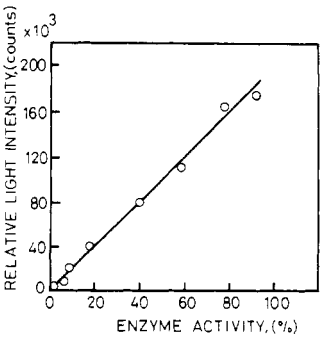

Figure 3. Calibration curve for various dilution ratios.

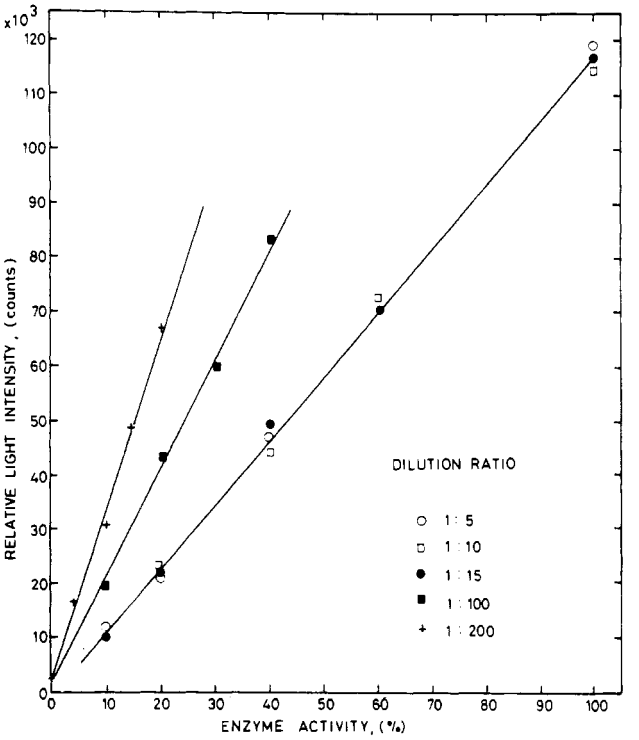

Figure 4. Calibration curves for various levels of sample dilution.

Figure 2 indicates that the light intensity and the width of the peak depend upon the reaction conditions. Curve II presents both relatively high light intensity and a flat peak. It was therefore selected as suitable working conditions for a chemiluminescent procedure based on the usage of a scintillation counter. The favorable time range for such procedure is between the 19th and the 25th s, since flatness of the curve in this range allows for maximal light counting and minimal error due to possible deviation in reading time.

Calibration Curve. A linear relationship was obtained when the light emission reading (according to recommended procedure) was plotted against different enzyme concentrations in corn tissue (Figure 3). The different enzyme concentrations were obtained by mixing homogenate of cooked (1 h at 100 °C) and fresh corn slices. The fresh corn tissue (100% enzyme activity) gave ~150 000 counts; the cooked tissue (0% enzyme activity) gave 100 counts, indicating a low background reading. In accordance with the work of Halmann et al. (1979), a linear relationship was also found between the light emitted and peroxidase concentration in the clear model enzyme solution, where one peroxidase (H.R.P. Sigma) international unit emits 338 cpm. The effect of turbidity as a probable

Table I. Peroxidase Activity in a Sequential Enzyme Extraction of Corn Tissue

extraction stage	chemiluminescence, cpm		guaiacol[a]
	precipitate	supernatant	precipitate
1	72 000	78 000	7
2	47 000	24 000	15
3	22 000	260	60
4	20 000	150	70
5	15 000	400	70
6	20 000	250	80

[a] Time (in seconds) until color appears.

source of interefence was evaluated by determining the calibration curves for various levels of sample dilution (Figure 4). The data indicate that the same calibration curve is obtained for a dilution factor in range of at least up to 1:15. At that range of dilution, the extent of light absorbed by the turbidity reaches a constant level, independent of the amount of suspended material. It is therefore suggested that working conditions should be within this range. A 10:1 dilution ratio was selected for this study.

Bound Enzyme. Data summarized in Table I show that part of the peroxidase activity in corn tissue is bound to the precipitate and is not extracted by the citrate–phosphate buffer. This buffer is used for peroxidase determination by colorimeteric techniques (Vetter et al., (1958).

Obviously, this technique is limited to soluble enzymes. On the other hand, the chemiluminescent method reacts with both the soluble and bound enzymes (Figure 1) and thus can be used to determine the total activity.

An overall peroxidase activity mass balance indicates that 30–50% of the enzyme activity in fresh corn ear can be attributed to the bound fraction. A similar pattern was also obtained when 0.18 M potassium phosphate buffer was used. It seems that low ionic strength buffer, commonly used in peroxidase assays, is unable to solublize all the enzyme bound to the cell wall.

Sampling. Unheated ear corn were sliced into 1.5 cm thick disks, and peroxidase activity was determined in each of the blended samples. All the blended disk samples showed similar enzyme activity, thus indicating a uniform enzyme distribution along the fresh corn cob. However, once the corn ear is being blanched, the residual enzyme activity is no longer uniformly distributed. A typical activity distribution for three samples heated for 10 min at 100 °C is shown in Figure 5. This pattern is expected considering the different radial heating rates due to changes in thickness along the main axes and the end effects. The sampling procedures should therefore take into account the activity distribution. The above suggests that unless all the ear is taken as one sample, data can be accurately interpreted only when the enzyme activity distribution is known. Obviously, the middle section, the slowest heating zone, represents the most sensitive indicator for the degree of blanching.

Reproducibility. Reproducibility was tested on 10 disks of a fresh corn (sample 1) and on 10 replicates taken from each of four different homogenates (samples 2–5).

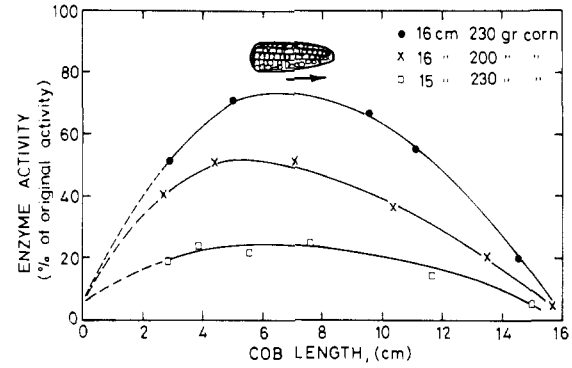

Figure 5. Enzyme distribution along the corn ear from butt to tip (after 10-min boiling in water).

Table II. Reproducibility of the Enzyme Activity Determination

sample	av reading, counts	SEM[a]	
		counts	% of reading
1	150 800	1330	0.88
2	68 400	1700	2.5
3	46 250	860	1.8
4	32 000	750	2.3
5	30 300	560	1.8

[a] SEM, standard error of the mean.

The average enzyme activity and the standard error of the mean are given in Table II. Considering the nonhomogeneous nature of the corn on the cob, a 1.0–2.5% standard error of the mean is very satisfactory.

In conclusion, the proposed method was found to be free from turbidity interference and thus capable of measuring directly the bound and the soluble fractions of the peroxidase in corn on the cob. Further investigations, using this method, on other vegetables are being carried out.

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