Kinetics of Peroxidase Deactivation in Blanching of Corn on the Cob

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A kinetic model was formulated for peroxidase deactivation in blanching of corn on the cob. A multifraction first-order model was fitted to deactivation data by using a nonlinear least-squares approach wherein data after different heating times at different temperatures are fitted to the model simultaneously. Results indicated existence of two enzymatic fractions, each deactivated by first order, exhibiting an Arrhenius temperature dependence: a thermolabile (E_a = 17.9 kcal/mol; $k_{100°C}$ = 8.9 min^{-1}) comprising 93% and a thermostabile (E_a = 21 kcal/mol; $k_{100°C}$ = 0.23 min^{-1}) comprising 7% of activity. A new approach was developed to correct kinetic data of a heterogeneous biological system for the heating come-up time, based on the temperature gradients in the sample, the assumed kinetic model, and the initial concentration distribution. Correction factors obtained by an iterative computation are multiplied by the raw kinetic data activity–concentration term to yield accurate kinetic parameters. Reactivation of peroxidase after partial deactivation was observed. High initial temperature dependent regeneration rates were measured, but the final extent of regeneration was independent of storage temperatures. A linear relationship was obtained between regeneration and residual activity after deactivation.

Deterioration of commerical frozen corn on the cob is attributed mainly to residual enzymatic activity. In such a large product, complete enzyme inactivation by the conventional hot water (or steam) blanching is not achieved due to relatively short exposure time dictated by organoleptic (texture, sweetness) considerations.

High correlation between the off-flavor development in frozen vegetables during storage and the residual peroxidase activity (Guyer and Holmquist, 1954; Labbee and Esselen, 1954; Nebesky et al., 1951), along with its thermal stability (Scott, 1975; Saunders et al., 1964) and regeneration ability (Guyer and Holmquist, 1954; Esselen and Anderson, 1956; Vetter et al., 1958; Joffe and Ball, 1962; Lu and Whitaker 1974) has made this enzyme useful as an index for the degree of blanching (Tressler et al., 1968).

The kinetics of the thermal deactivation of the soluble fraction of peroxidase has been reported (Yamamoto et al., 1961; Vetter et al., 1958; Joffe and Ball, 1962; Lu and Whitaker, 1974; Svenson, 1977; Lee and Hammes, 1979). These studies, having been carried out on the soluble fractions of the enzyme, have not taken into account the unextractable cell-bound fractions of the enzyme (De Jong, 1976; Gardner et al., 1969). Similarly, the phenomenon of peroxidase regeneration (Lu and Whitaker, 1974) has been studied only on the soluble fraction rather than in the total plant tissues, in vivo.

The main difficulty in determining the nonextractable cell-bound fraction of the enzyme stemmed from the lack of a satisfactory quantitative analytical method. Recently Naveh et al. (1981) developed a chemiluminescent method, overcoming the above difficulty. Determination of enzymatic activity in the whole tissue of corn on the cob is possible when employing this method.

The objective of this study was to obtain kinetic data and a mathematical model of thermal deactivation of peroxidase in whole corn on the cob.

EXPERIMENTAL SECTION

Materials. *Corn.* Jubilee sweet corn ears, harvested at optimum maturity with approximately 73% moisture content, of 14-cm length after trimming and with a maximum diameter of 5 ± 0.3 cm were used. Constant temperatures of 70, 80, 90, and 100 °C were maintained to ±0.2 °C of the presence value in a 65 i.d. × 20 cm water bath equipped with two 1500-W thermomix units controlled by a Brown Melsungen contact thermometer.

Methods. Three corn ears were cut into 0.4 ± 0.1 cm thick disks. A lot (30–40 g each) of randomly chosen disks were placed in a 0.5-in. mesh wire 50 i.d. × 2 cm cylindrical container and were immersed in a thermostatically controlled (±0.2 °C) temperature water bath. After being heated for a predetermined time, the lot was thrown into 10-fold weight of ice-cold distilled water and immediately blended (3 min) in a Waring blender. The procedure was repeated for several exposure times (0.5–30 min) for each temperature (70, 80, 90, and 100 °C). The blended samples were taken for peroxidase determination by the chemiluminescent method described by Naveh et al. (1981).

Peroxidase regeneration studies were conducted by heating 200-g lots of corn ear disks for different times and temperatures, in the same manner as described above. Immediately after being withdrawn, the heated lots were immersed in ice-cold water for 1 min and then placed in storage at 4 and 25 °C cabinets. Samples of 30–40 g were withdrawn periodically, blended in 10-fold distilled water, and taken for peroxidase determination.

Correction for Come-Up Time. Temperature distribution in the corn disks during the come-up time was calculated by using the series solution of transient conduction heating in an infinite slab. The thermal conductivity was determined by the Cenco-Fitch method (Bennet et al., 1963); the specific heat was calculated according to composition (Pflug et al., 1965), and the density was determined by double weighing (in air and in water). The value of the thermal diffusivity was calculated to be 4.2 × 10^{-3} ft^2/h.

Data processing was carried out on the Technion IBM 370/168 by using the nonlinear least-squares data fitting program ANALYZER (Wolberg, 1967).

RESULTS AND DISCUSSION

The semilogarithmic plot of a typical residual peroxidase activity vs. time at constant temperatures (Figure 1) markedly deviates from the straight line characteristic of a first-order reaction. This is not surprising, considering the reported data about the existence of two or more peroxidase fractions, each having markedly different thermal stability (Svenson, 1977; Yamamoto et al., 1961). Therefore the kinetic model was formulated taking into account a mixture of several enzyme fractions, each

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Table I. Kinetic Parameters for Deactivation of Peroxidase in Sweet Corn on the Cob

enzymatic fraction	A_0, %	Arrhenius coeff E_a, cal/mol	Arrhenius coeff σ_{Ea}, cal/mol	Arrhenius coeff $k_{100°C}$, min^{-1}	Arrhenius coeff σ_k, min^{-1}	Z model coeff Z, °C	Z model coeff $D_{100°C}$, s	Q_{10} (60–100 °C)
I	93	17 900	2000	8.9	2	39.3	15.5	1.9
II	7	21 000	2900	0.23	0.016	33	600	2.2

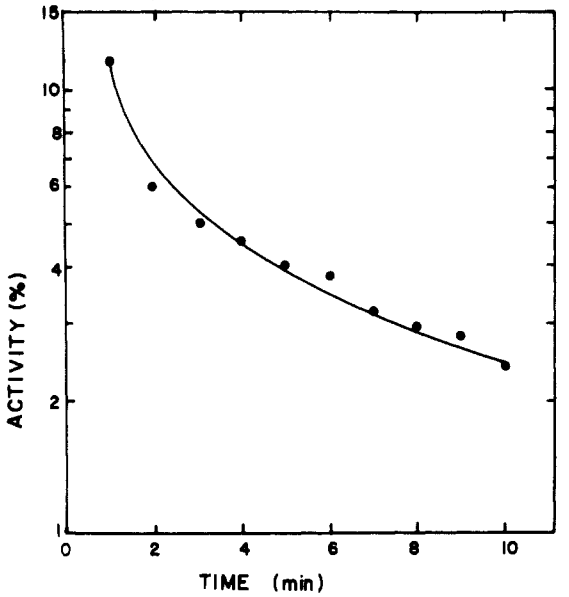

Figure 1. Residual peroxidase activity in 0.35 cm thick corn disks after different heating times at 90 °C.

deactivated in a first-order manner but with different rate constants, i.e., different k's and E_a's. The model reads

$$A = \sum_{i=1}^{n} A_{0,i} \exp\left\{-tk_{i,ref} \exp\left[\frac{E_{a,i}}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right]\right\} \quad (1)$$

where A is the total residual activity, A_0 is the initial activity, t is time, E_a is the energy of activation, R is the gas constant, T is product temperature, $k_{i,ref}$ is the rate constant of the ith fraction at a reference temperature T_{ref}, and the value of $A_{0,n}$ can be calculated from

$$A_{0,n} = A_0 - \sum_{i=1}^{n-1} A_{0,i} \quad (2)$$

Correction for Come-Up Time. A correction for come-up time should be applied to the raw data if accurate kinetic parameters are to be established. This is especially true at short heating times where significant temperature gradients exist. The correction for come-up time in a single enzyme fraction that follows a simple first-order reaction can be done by a shift in time base. This correction gives the equivalent (isothermal) time in which the same effect is obtained as in a sample heated to the given constant bath temperature and can be calculated by integrating the rate of reaction over time and location throughout the lag period. In this method, the enzymatic activity is as measured, but the actual time (t_{actual}) is replaced by the effective time ($t_{effective}$). The effective time is the actual time from and to which the actual come-up time (CUT) and the equivalent come-up time have been subtracted and added, respectively (eq 3).

$$t_{effective} = t_{actual} - (CUT)_{actual} + (CUT)_{equivalent} \quad (3)$$

However, in a case of two or more enzyme fractions, each having different thermal deactivation constants, the time shift correction method is not longer convenient and practical. This is due to the fact that each enzyme fraction will have a different equivalent come-up time. Therefore an alternative approach has been taken. Using this approach the time scale is the actual time, and the corrections are applied to the enzymatic activity by multiplying each of the fractions activity by a correction factor, f.

The correction factor, f, is a dimensionless number that is the ratio between the enzymatic activity calculated for zero come-up time and the enzymatic activity calculated by considering the temperature gradients in the sample during its come-up time. For a given medium temperature, sample geometry and thermal properties, the correction factor, f, is a function of the kinetic parameters, namely, the rate constant and the energy of activation, as well as the initial concentration distribution of each of the enzyme fractions along the direction of the heat flux.

Computation of the Correction Factor, f. The analytical procedure for the computation of the correction factor, f, is based upon dividing the corn disk into $2n$ layers (each having a thickness of x). Time–temperature history of the come-up time for each layer was computed according to Pflug et al. (1965):

$$\frac{T_1 - T}{T_1 - T_0} = \sum_{i=1}^{\infty} \frac{2 \sin \beta_i}{\beta_i + \sin \beta_i \cos \beta_i} \cos\left(\beta_i \frac{x}{a}\right) \exp\left(\frac{-\beta_i^2 \alpha t}{a^2}\right) \quad (4)$$

where

$$\beta_i = \pi/2 + i\pi$$

T_1 is the temperature of the heating medium, T_0 is the initial temperature, a is the half-thickness of the corn disk, and α is the thermal diffusivity. On the basis of the time–temperature data, the extent of enzyme deactivation at each layer during the come-up time was calculated by integrating the kinetic equation. For each enzyme fraction the integral reads

$$\ln\left(\frac{A_i}{A_{0,i}}\right) = \int_0^t k_{0,i} \exp\left(\frac{-E_{a,i}}{RT}\right) dt \quad (5)$$

where T is obtained from the calculated time–temperature history (eq 4).

The extent of the total enzyme deactivation in the sample can be obtained by the summation of the extent of inactivation for all fractions at all layers. By use of the same kinetic data, the extent of enzyme deactivation is calculated for the same time period, t, by assuming instantaneous heating. The ratio between the latter and former values constitutes the f value. Multiplying actual kinetic data with this f value yields the equivalent deactivation rates as for a body completely reaching the medium temperature instantaneously.

As f is dependent on kinetic parameters that are calculated from the nonlinear least-squares curve fitting of experimental data (uncorrected for CUT), an iterative procedure was developed for obtaining corrected kinetic data and parameters. This procedure is delineated in Figure 2.

The calculated f values based on the kinetic parameters (Table I) obtained in this study for different corn on the

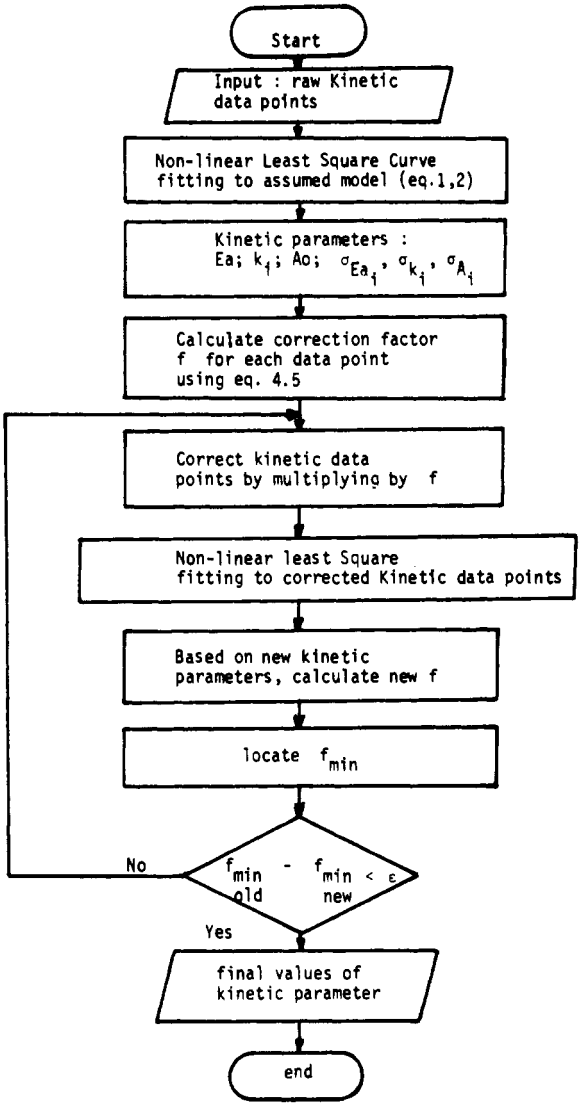

Figure 2. Algorithm for calculation of correction factor for come-up time.

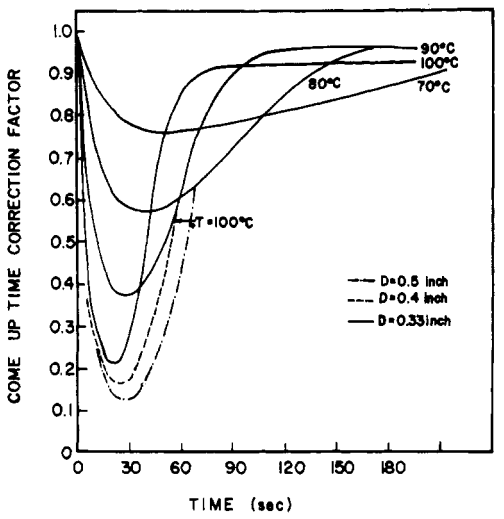

Figure 3. Come-up time correction factor for peroxidase activity for different medium temperature and disk thickness (D).

cob disk thickness, treated at different temperatures, are summarized in Figure 3. The figure shows that the correction factor reaches a minimum, depending on disk thickness and medium temperature, after approximately 30 s in this system. The maximal values, which are 1 (i.e., no correction needed), are approached at zero time and after relatively long times.

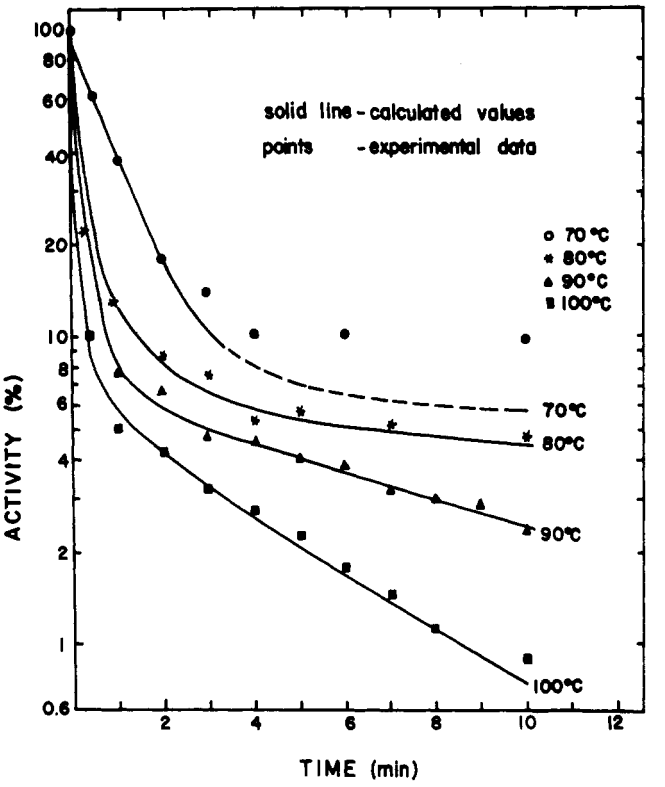

Figure 4. Deactivation of peroxidase as a function of thermal process at different temperature.

Peroxidase Fractions. The goodness of fit obtained between the model described by eq 1 with two terms only and corrected kinetic data (Figure 4) was satisfactory, thus indicating that this model is sufficiently accurate for practical blanching calculation. These results suggest that the system can be practically described by assuming two enzyme fractions with vastly different thermostability (Table I). From A_0 values obtained by the curve fitting the thermostable fraction comprised 7% of the enzymatic activity and a thermolabile fraction the residual 93%.

Although direct biochemical proof of these peroxidase fractions has not yet been obtained, the information in the literature, as already mentioned above, seems to support the existence of more than one enzyme fraction. For example, McCune (1960) has reported six isoenzymes of peroxidase in the husks alone. Yamamoto et al. (1961) indicated the presence of two fractions differing in their thermostability. It is interesting to note that despite large differences in experimental techniques and in data analysis, the results (Yamamoto et al., 1961) of the 5% thermostable fraction ($D_{95°C}$ = 10 min, Z = 12 °C) and a 95% thermolabile one ($D_{95°C}$ = 10 s, Z = 48 °C) compare well to the data in Table I, except for the Z value of the thermostable fraction. The difference is probably due to the fact that Yamamoto et al. have worked on whole kernel only and analyzed the soluble enzyme fraction that was extracted after the blanching. Any insolubilization of the enzyme that may have occurred during the heat treatment would therefore appear as enzyme deactivation, thus decreasing the Z value.

Lee and Hammes (1979) have argued that the nonlinearity obtained in semilogarithmic inactivation curves is due to slow heat penetration during heating rather than to the presence of several isoenzymes. However, this work, taking into account temperature gradients, contradicts their findings.

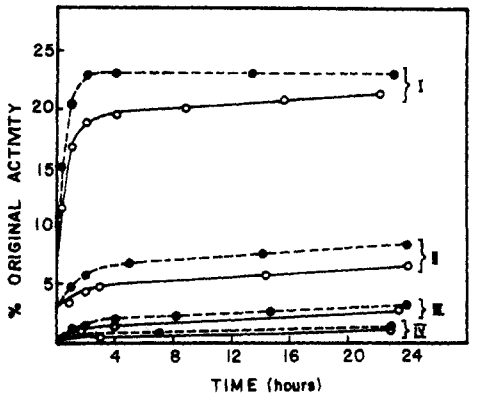

Figure 5. Peroxidase reactivation during storage at 4 °C (—) and at 25 °C (---) after heat treatments (at 100 °C: for I, 1 min; for II, 3 min; for III, 10 min; for IV, 20 min).

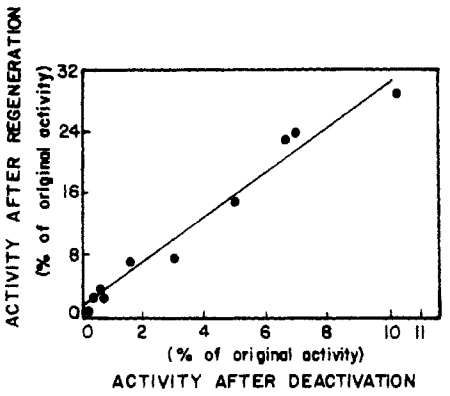

Figure 6. Effect of extent of thermal deactivation on the reaction of peroxidase.

Reactivation of Peroxide. Figure 5 summarizes regeneration of peroxidase in corn on the cob tissue during storage at 4 and 25 °C after different heat treatments at 100 °C. Regeneration of part of the enzymatic activity is evident from the figure. The extent of the regenerated activity is leveled off after about 3 h following the heat treatment. The level of the regained activity was higher at 25 °C than that at 4 °C. However, the difference in activity between those storage temperatures diminishes after 3 days.

This final value will depend on the intensity of heat treatment. A linear relationship ($r = 0.98$) was obtained between residual peroxidase activity immediately after heat deactivation and 95% of the maximal reactivation level (Figure 6). The linear equation reads

$$A_r = 1.1 + 2.95A_d \quad (6)$$

for $A_d \leq 30$ where A_r is 95% of the final value and A_d is the residual activity, both expressed as percent of the initial activity.

The regeneration phenomenon has been reported by several workers. Lu and Whitaker (1974) have reported high initial reaction rates of peroxidase, 10 min after deactivation. Joffe and Ball (1962), on the other hand, observed a lag period of 20 h before the reactivation started.

Due to the high initial rates, about 70% of peroxidase regeneration, under industrial conditions, will occur before the product is frozen. Moreover, as storage periods of corn on the cob are much longer than the expected regeneration times, it seems that this phenomenon is practically unpreventable. However, the large slope, of about 3, in eq 6 implied that more drastic heat processing will not only increase deactivation but also markedly decrease the extent of regeneration.

In conclusion, a kinetic model of peroxidase inactivation during blanching of corn on the cob has been formulated. Equations 1 and 6, together with the heat penetration data and the initial enzyme distribution in the corn on the cob, can be used to predict the residual enzymatic activity in the blanched frozen corn on the cob during processing and storage.

LITERATURE CITED

Bennet, A. H.; Chase, W. G.; Cubbedge, R. *ASHRAE J.* **1962**, *4*, 9.
De Jong, D. W. *J. Histochem. Cytochem.* **1976**, *15*, 335.
Esselen, W. B.; Anderson, E. E. *Food Res.* **1956**, *21*, 322.
Gardner, H. W.; Inglett, G. E.; Anderson, R. A. *Cereal Chem.* **1969**, *46*, 626.
Guyer, R. B.; Holmquist, J. W. *Food Technol. (Chicago)* **1954**, *8*, 547.
Joffe, M. F.; Ball, O. C. *J. Food Sci.* **1962**, *27*, 587.
Labbee, M. D.; Esselen, W. B. *Food Technol. (Chicago)* **1954**, *8*, 50.
Lee, Y. C.; Hammes, J. K. *J. Food Sci.* **1979**, *44*, 785.
Lu, A. T.; Whitaker, J. R. *J. Food Sci.* **1974**, *39*, 1173.
McCune, D. C. *Ann. N.Y. Acad. Sci.* **1960**, *94*, 724.
Naveh, D.; Mizrahi, S.; Kopelman, I. J. *J. Agric. Food Chem.* **1981**, *29*, 36.
Nebesky, E. A.; Esselsn, W. B.; Fellers, C. P. *Food Technol. (Chicago)* **1951**, *5*, 110.
Pflug, I. J.; Blaisdell, J. L.; Kopelman, I. J. *ASHRAE Trans.* **1965**, *71*, Part I.
Saunders, B. C.; Holmes, A. G.; Stark, B. P. "Peroxidase"; Butterworths: London, 1964.
Scott, D. In "Enzymes in Food Processing", 2nd ed.; Reed, G., Ed.; Academic Press: New York, 1975.
Svenson, S. G. In "Physical, Chemical and Biological Changes in Food Caused by Thermal Processing"; Hoyem, T.; Kvale, O., Eds.; Applied Science Publishers, Ltd.: London, 1977; Chapter 12.
Tressler, K. D.; Van Arsdel, B. W.; Copley, M. J. In "The Freezing Preservation of Foods"; Avi Publishing Co., Inc.: Westport, CT, 1968; Vol. 2.
Vetter, J. L.; Nelson, A. I.; Steinberg, M. P. *J. Food Technol.* **1958**, *12*, 244.
Wolberg, J. "Prediction Analysis"; Van Nostrand: Toronto, Canada, 1967.
Yamamoto, H. Y.; Steinberg, M. P.; Nelson, A. I. *J. Food Sci.* **1961**, *27*, 113.

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