in melamine concentrations in the dosed canned beef of 1.17–1.27 ppm versus melamine in nondosed canned tissue of 1.02–1.11 ppm. The melamine in the canned beef tissues may be due to the migration of the melamine from the melamine–formaldehyde resin in the can lining.

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Registry No. Sulfamethazine, 57-68-1; chloramphenicol, 56-75-7; cyromazine, 66215-27-8; melamine, 108-78-1.

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Thermal Inactivation Kinetics of Potato Tuber Lipoxygenase

Kwan-Hwa Park,* Yeong-Myeong Kim, and Chang-Won Lee[1]

The thermal inactivation kinetics of two isozymes, crude and reconstituted enzyme preparations of potato tuber lipoxygenase, was investigated in solution. Kinetic parameters of thermal inactivation were determined graphically and by a least-squares method using nonlinear regression analysis. Each of the two isozymes followed first-order kinetics with different inactivation rate constants. However, the apparent inactivation kinetics of crude and reconstituted lipoxygenases exhibited second-order kinetics, and this could be explained by the presence of two isozymes having different heat resistances. Heat inactivation data (percent residual activity) of crude and reconstituted lipoxygenase could also be adequately calculated from the two isozymes' first-order inactivation rate constants and their enzyme reaction rate constants. The results suggested the possible utilization of parameters derived from the apparent second-order kinetics for predicting the extent of thermal inactivation of isozymic mixture of potato tuber lipoxygenase.

Lipoxygenase is an ubiquitous enzyme in plant kingdom (Pinsky et al., 1971). It acts upon free fatty acids in foodstuffs, destroying essential fatty acids such as linoleic, linolenic, and arachidonic acids, and produces mainly hexanal and pentanal. These volatile compounds are known to be a major cause of rancidity and off-flavor occurring in inadequately processed, stored foodstuffs, especially of plant origin (Grosch, 1972). The enzyme appears in most plants as multiple isozymes, some of which are more heat-resistant than the others of the same plant. Thus, incomplete inactivation of the enzyme during heat treatment of foodstuffs has been explained by the presence of heat-resistant isozymes (Chenchin and Yamamoto, 1973; Delincee et al., 1975; Ling and Lund, 1978; Naveh et al., 1982).

Kinetic studies of thermal inactivation have been carried out for lipoxygenases of various origin to rationalize the thermal-processing conditions of foodstuffs to inactivate the enzyme, thus preventing the eventual occurrence of rancidity and off-flavors during storage and distribution

Department of Food Science and Technology, College of Agriculture, Seoul National University, Suwon 440-744, Republic of Korea.

[1]Present address: Department of Microbiology, College of Natural Sciences, Gyeongsang National University, Chinju 660-300, Republic of Korea.

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Table I. Apparent Reaction Order and Reaction Rate Constants for Thermal Inactivation of Potato Lipoxygenase

	app reaction order[a]				reaction rate const					
temp, °C	F-I	F-II	mixture	crude enzyme	F-I, $s^{-1} \times 10^4$	F-II, $s^{-1} \times 10^4$	reconst enzyme, L/mol·s	crude enzyme, L/mol·s	E_a, kcal/mol F-I	E_a, kcal/mol F-II
50					1.4 ± 0.1	3.1 ± 0.2	17.4 ± 0.1	16.1 ± 0.1		
55	1.1 ± 0.03	1.0 ± 0.04	2.2 ± 0.4	2.1 ± 0.4	3.9 ± 0.4	5.9 ± 0.6	47.0 ± 1.0	56.5 ± 1.3	40.8 ± 2.4	46.5 ± 4.9
60					8.6 ± 0.3	15.0 ± 0.7	146.3 ± 1.0	185.7 ± 1.0		
65					28.9 ± 2.9	97.2 ± 40.9	486.8 ± 1.0	766.4 ± 1.0		

[a] Mean value computed in the temperature range 50–65 °C.

(Borhan and Snyder, 1979; Christopher et al., 1970; Park, 1976; Svensson and Eriksson, 1972a,b). Usually, such studies are complicated by the presence of isozymes with different heat resistances. Ling and Lund (1978) used successfully each isozyme's first-order rate constants of heat inactivation in an equation to fit their experimental data.

The present study describes the fitting of the kinetic data of thermal inactivation of isozymic mixtures (both crude extract and an enzyme preparation reconstituted from partially purified isozymes) to apparent second-order kinetics and compares it with the method of Ling and Lund (1978). It also describes the kinetics of thermal inactivation of partially purified, individual isozymes of potato tuber lipoxygenase.

EXPERIMENTAL SECTION

Potato Tuber Lipoxygenases. The two isozymes (F-I, F-II) of lipoxygenase were purified in this laboratory (Kim et al., 1987) from potato tubers (*Solanum tuberosum* cv. Dejima). The purification was comprised of the following steps: an ammonium sulfate fractionation between 30 and 60% saturation of clear supernatant (crude enzyme) obtained from tuber homogenates, CM-cellulose column chromatography, and DEAE-cellulose column chromatography. The two activity peaks appearing on the last step (F-I and F-II in eluting order) were concentrated separately, equilibrated with 50 mM sodium phosphate buffer, pH 6.8, and used for thermal inactivation studies. These preparations showed about 20- and 30-fold enrichment, respectively, for F-I and F-II in lipoxygenase specific activity compared with that of crude enzyme.

Thermal Inactivation of Lipoxygenase. The thermal inactivation procedure described by Park (1976) was followed with some modifications. Nine milliliters of 50 mM phosphate buffer, pH 6.8, was transferred to a 2.0 × 15 cm test tube with cap and equilibrated to a desired temperature of inactivation (50, 55, 60, 65 °C) in a water bath. Then, 1 mL of an enzyme solution in 50 mM buffer was added to the test tube, and the mixture was stirred continuously by a magnetic stirrer. Aliquots of 0.5 mL were taken at specified time intervals and transferred to and kept in an ice bath for later determination of residual enzymatic activity.

Determination of Enzyme Activity. Lipoxygenase activity was determined spectrophotometrically by measuring the increase in absorbance at 234 nm caused by the formation of conjugated diene hydroperoxide. The substrate stock solution of linoleate prepared by the modified method of Rackis et al. (1972) was stored at 4 °C up to 7 days.

The reaction mixture contained 25 μL of the substrate stock solution and 2.5 mL of 0.1 M phosphate buffer, pH 5.7. After thermal equilibration at 25 °C, the reaction was initiated by the addition of 0.1 mL of enzyme solution. The increase in the absorbance was followed by a recording spectrophotometer (Shimadzu UV 200). The enzyme activity was calculated from the slope of the linear portion of time course curves. One unit of lipoxygenase activity was defined as the amount of the enzyme causing an increase of 0.1 AU/min under the described conditions.

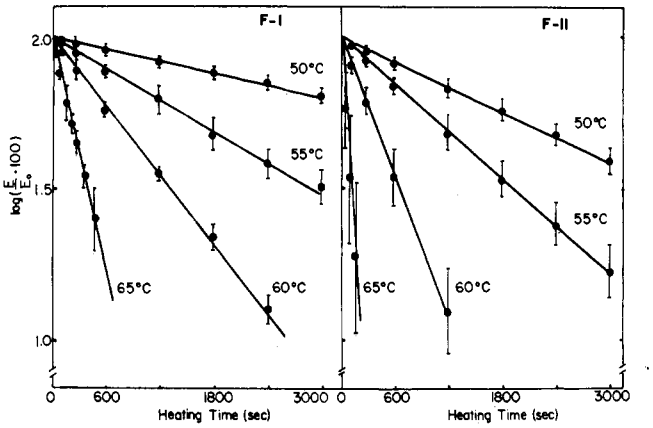

Figure 1. Thermal inactivation curves of F-I and F-II as a function of heating time and temperature. Key: E, residual enzyme activity after heating time t at a given temperature; E_0, enzyme activity at zero heating time. 95% confidence intervals are indicated by vertical bars.

Estimation of Kinetic Parameters for Thermal Inactivation Reaction. Apparent reaction order, n, was determined by Hill's method (Hill, 1977)

$$\text{reaction rate} = dc/dt = -k[E]^n \quad (1)$$

where [E] is the enzyme concentration or activity at time t and k is the reaction rate constant of the reaction. The fitting of heat inactivation data to eq 1 for the estimation of reaction order and rate constants was carried out with the nonlinear regression program of Duggleby (1981).

The theoretical residual activity of the enzyme after heat treatment was calculated by eq 2 (Ling and Lund, 1978),

$$\%\ \text{residual act.} = \frac{K_I E_{I0} e^{-k_I t} + K_{II} E_{II0} e^{-k_{II} t}}{K_I E_{I0} + K_{II} E_{II}} \quad (2)$$

where K_I and K_{II} are enzyme reaction rate constants for F-I and F-II, respectively; k_I and k_{II}, first-order rate constants of thermal inactivation for the respective isozymes; and E_{I0} and E_{II0}, concentrations of F-I and F-II at zero heating time, respectively.

RESULTS AND DISCUSSION

Thermal Inactivation of Individual Isozymes. When the logarithms of the residual activities of F-I and F-II were plotted against heating time, both isozymes showed linear inactivation kinetic curves (Figure 1) with apparent reaction orders close to 1.0 as determined from eq 1 by the least-squares method (Table I). Thus, the inactivation reaction of each isozyme followed first-order kinetics, F-I being more heat-resistant as indicated by its smaller inactivation rate constants (Table I). Such first-order kinetics was observed for the thermal inactivation

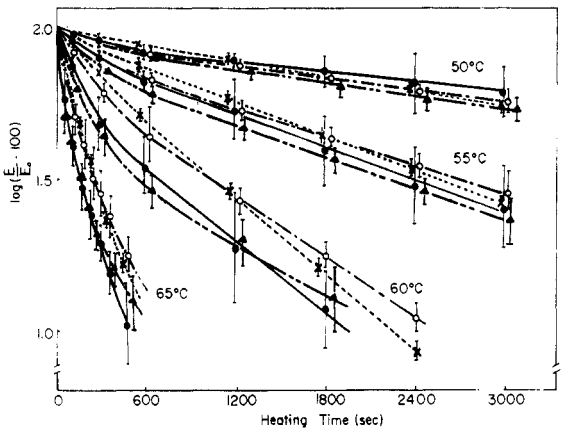

Figure 2. Thermal inactivation curves of crude and reconstituted enzyme solution as a function of heating time and temperature. For E and E_0, see legend for Figure 1. Symbols: ●, crude enzyme; ○, reconstituted enzyme; ×, calculated from first-order rate constants of each isozyme according to eq 2 of the text; ▲, calculated from the second-order parameters of crude enzyme. 95% confidence intervals are indicated by vertical bars.

reaction of most enzymes (Aylward and Haisman, 1969). Activation energies for thermal inactivation of each isozyme (F-I, F-II) were determined from the Arrhenius equation and were found to be 40.8 and 46.5 kcal/mol, respectively.

Thermal Inactivation of Crude and Reconstituted Enzymes. When the heat activation data of crude and reconstituted (F-I:F-II = 6:4, the activity composition ratio of crude enzyme) preparations were plotted semilogarithmically, both showed similar biphasic kinetic curves with an initial steeper slope (Figure 2). Similar results have been reported also for crude peroxidase (Clochard and Guern, 1973; Resende et al., 1969) and lipoxygenases (Borhan and Snyder, 1979; Kim et al., 1987; Park, 1976). Henley et al. (1985) showed that the enzyme was inactivated in a series-type process involving first-order steps and classified the inactivation curves into two categories.

It is notable that the thermal destruction behavior of crude enzyme could be faithfully mimicked by that of the reconstituted enzyme, especially at lower temperatures (Table I; Figure 2). The result enforces the belief that the non-first-order behavior of the crude enzyme is principally due to the presence of more than one lipoxygenase species with different heat resistances in the crude preparation. Especially, Yamamoto et al. (1962) interpreted the biphasic curves as resulting from a rapid inactivation of a less heat-resistant component followed by a progressive one of more heat-resistant isozymes. Our data might be accommodated by an analogous interpretation.

The kinetic model of a mixture with two isozyme fractions, each inactivated in a first-order kinetics but with different rate constants, is given by eq 3. From eq 3 the

$$dc/dt = -k_I[E_I] - k_{II}[E_{II}] \quad (3)$$

extent of the total enzyme inactivation in food systems can be obtained by the summation of inactivation of each isozyme. However, the determination of inactivation rate constants of each isozyme is no longer convenient and practical.

Fitting heat inactivation data of crude and reconstituted enzymes to eq 1 utilizing a nonlinear regression program gave reaction orders close to 2.0 for both enzyme preparations (Table I), and the goodness of fit was satisfactory. Thus, this indicated that second-order kinetics could be used to adequately describe a heat inactivation reaction of these lipoxygenases in a mixture composed of two isozymes with different first-order reaction rate constants. In fact, the theoretical curve calculated for crude enzyme assuming a second-order inactivation kinetics (i.e. treating the inactivation reaction as if a single species, rather than two, of lipoxygenase was being inactivated according to second-order kinetics) fits well with the curve obtained by eq 2 (Ling and Lund, 1978) which treats the inactivation reaction as the sum of two independent ones of each isozyme.

Thus, our results showed that the inactivation reaction of composite lipoxygenase solutions could be conveniently described by second-order kinetics. Similar treatments should be easily applied to any isozymic mixture of enzymes frequently encountered in foodstuffs.

Registry No. Lipoxygenase, 9029-60-1.

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Effect of Selected Canning Methods on Trypsin Inhibitor Activity, Sterilization Value, and Firmness of Canned Navy Beans

Chiun-Chuang R. Wang[1] and Sam K. C. Chang*

The effects of selected canning methods on trypsin inhibitor activity and the relationships among sterilization, firmness, and trypsin inhibitor activity were investigated. Retort-cooking at 115.6 and 121.1 °C for longer than 35 and 20 min, respectively, did not increase trypsin inhibitor inactivation. The presence of Ca^{2+}/EDTA in the brine had a pronounced effect in increasing inactivation of trypsin inhibitor activity. Retort-cooking of navy beans in brine containing EDTA and calcium chloride at 115.6 °C for 35 min or at 121.1 °C for 15 min was optimal in terms of trypsin inhibitor inactivation, sterilization value, and firmness of the canned product.

Dry beans (*Phaseolus vulgaris* L.) are generally recognized as a good source of food protein and dietary fiber. However, most of legume seeds contain antinutritional factors such as trypsin inhibitor (Liener and Kakade, 1980).

Protein quality is a function of essential amino acid content and bioavailability. In food legumes protease inhibitors are important (Liener, 1958). The most studied protease inhibitors are the inhibitors of trypsin and chymotrypsin, which play a key role in the digestion of proteins (Liener and Kakade, 1980).

Active trypsin inhibitors have a double-detrimental effect on the utilization of bean proteins because they inhibit trypsin or chymotrypsin and decrease protein digestibility and they contain relatively high content of cystine (Kakade et al., 1969). Approximately 40% of the growth depression as well as 40% of the pancreatic hypertrophic effect on rats can be attributed to trypsin inhibitors in the unheated soybeans (Kakade et al., 1973; Liener, 1976). Heat treatment methods for inactivating the protease inhibitors of legume seeds include boiling, dry roasting, dielectric heating, microwave radiation, and extrusion cooking (Liener, 1981).

Inactivation of trypsin inhibitors improves the nutritive value of food proteins as demonstrated with rats (Liener, 1979). Different protease inhibitors have different degrees of heat stability (Chang and Satterlee, 1982; Apostolatos, 1984). The Bowman–Birk proteinase inhibitor, which has a molecular weight of 7861 and contains seven disulfide cross-linkages, can inhibit both trypsin and chymotrypsin and is very heat-resistant (Odani and Ikenaka, 1973). The properties of a trypsin inhibitor purified from the seeds of *P. vulgaris* cv. Kintoki are similar to that of the Bowman–Birk proteinase inhibitor (Miyoshi et al., 1978). A trypsin inhibitor with a molecular weight of 10000 purified from kidney beans (*P. vulgaris*) is unaffected by heating (up to 90 °C) or by pepsin and by low pH value (pH 2) (Pusztai, 1968). Autoclaving (wet heat) at 120 °C for 20 min has been found to be more efficient than dry heating at 100 °C for 2 h for the inactivation of trypsin inhibitor activity (Tan and Wong, 1982). The protein efficiency ratio (PER) of navy beans can be improved by inactivating 80% of trypsin inhibitor activity (Kakade and Evans, 1965). Inactivation of trypsin inhibitor activity in crushed field bean flour follows first-order kinetics, and the rate of loss increases with increasing the moisture content from 2% to 55% (Buera et al., 1984).

Pressure cooking (121 °C) over 30 min decreased the nutritive value (decreased methionine, lysine, valine, and available lysine) of the black beans (Bressani et al., 1961). Autoclaving longer than 5 min has an adverse effect on the essential amino acids of navy beans (Kakade and Evans, 1965). Protein efficiency ratio decreases from 1.57 to 0.67 in the navy beans autoclaved from 5 to 60 min.

Cooking temperature and time have a significant effect on the firmness of canned pinto and light red kidney beans (Quast and da Silva, 1977; Wang et al., 1988). Light red kidney and pinto beans processed at 121 °C for 20 min are firmer than those processed at 115.6 °C for 45 min (Davis, 1976). Although trypsin inhibitor activity can be decreased by thermal processing, the relationships among sterilization value (F value), firmness, and trypsin inhibitor activity have not been reported.

The purposes of this study were (1) to investigate the effect of packing brine composition and retort-cooking methods on trypsin inhibitor activity in navy (Upland) beans and (2) to investigate the relationship between the textural quality and trypsin inhibitor activity after processing.

MATERIALS AND METHODS

Materials. Navy beans, *P. vulgaris* L., of the Upland cultivar were grown in a test plot of North Dakota State University in Fargo, ND. The beans were stored in plastic bags at 4 °C until use.

Department of Food and Nutrition, Agricultural Experiment Station, North Dakota State University, Fargo, North Dakota 58105.
[1] Present address: Department of Food and Nutrition, Kansas State University, Manhattan, KS 66502.

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