

## Kinetic parameters for thermal inactivation of cut green beans lipoxygenase calculated using unsteady-state methods

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**Summary** Kinetic parameters describing lipoxygenase inactivation during heating of cut green beans were determined using two unsteady-state procedures. The model used an analytical solution for heat conduction in a finite cylinder to predict time–temperature profiles, and a trial and error and a non-linear regression of experimental lipoxygenase retentions to estimate kinetic parameters. Thermal diffusivity and convective heat transfer coefficient were determined experimentally, but thermal conductivity was estimated. Mean values obtained, with standard deviations between parenthesis, were  $k_{76^{\circ}\text{C}} = 27.2 (9.4) \text{ s}^{-1}$ ;  $k_{82^{\circ}\text{C}} = 92.9 (7.5) \text{ s}^{-1}$ ;  $k_{88^{\circ}\text{C}} = 212.1 (52.7) \text{ s}^{-1}$ ;  $k_{94^{\circ}\text{C}} = 407.8 (56.7) \text{ s}^{-1}$ ;  $E_a = 160.7 (8.1) \text{ KJ mol}^{-1}$  using the trial and error procedure;  $k_{85^{\circ}\text{C}} = 150 (26.3) \text{ s}^{-1}$  and  $E_a = 164 (4.7) \text{ KJ mol}^{-1}$  using the non-linear regression method. Predicted and observed lipoxygenase retentions showed good agreement.

**Keywords** Blanching index, enzymatic activity, frozen vegetables, heat transfer, heating treatment.

### Introduction

Most raw vegetables can be stored for only a short time even at  $-20^{\circ}\text{C}$ . This is because of the changes in texture, colour, flavour and nutritional quality that occur as a result of the action of several enzymes that are still active after harvest (Williams *et al.*, 1986). The blanching process involves exposing plant tissue to some form of heat, usually steam or hot water, for a prescribed time at a specified temperature. Blanching is the primary pre-freezing means of inactivating undesirable enzymes present in the vegetable (Barrett & Theerakulkait, 1995). The temperature–time combination used in the blanching process will generally be determined by the thermal stability of enzymes involved in mediating quality deterioration of the processed product (Svensson &

Eriksson, 1974b). Of the enzymes naturally occurring in vegetables, peroxidase has been found to be a useful indicator of deterioration of frozen, stored vegetables. Although peroxidase has served well as an indicator, the use of this enzyme has been questioned because heat-resistant peroxidase (Ling & Lund, 1978; Adams, 1978) isoenzymes are also present in most frozen vegetables, and peroxidase has not been shown to be directly responsible for quality deterioration during frozen storage (Lee *et al.*, 1988). Williams *et al.* (1986) evaluated the sensory character of blanched vegetable purees to which isolated enzymes had been added and found that lipoxygenase was the enzyme most active in aroma deterioration in English green peas and green beans. Lipoxygenase is widely distributed in vegetables and evidence is mounting to support its involvement in off-flavour development and colour loss (Barrett & Theerakulkait, 1995). Lipoxygenase in sweet corn germ is important in off-odour formation, particularly

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in production of odours described as painty and stale/oxidized; peroxidase presence in sweet-corn germ does not appear to affect off-odour (Theerakulkait *et al.*, 1995). These authors suggest that analysis of lipoxygenase activity, rather than peroxidase, may be a more appropriate index of blanching adequacy.

In order to optimize the blanching process of vegetables it is essential to establish a kinetic model of the inactivation of the indicator enzyme. Isothermal and dynamic thermal approaches have been used to determine kinetic parameters (Lenz & Lund, 1980). In steady-state procedures the thermal lag (heat up or cool down) is insignificant compared with overall processing time and the inactivation reaction is considered to occur at constant temperature. The data required to establish a kinetic model are concentration of the degraded enzyme and heating time at constant temperature. In unsteady-state procedures the inactivation reaction occurs at a variable temperature and the data required are concentration of the degraded enzyme and the temperature profile of the sample during the heating-cooling process (Rodrigo *et al.*, 1998). The unsteady-state procedure is more flexible and can be applied to uniform and non-uniform heating situations and generally a food medium, rather than an aqueous buffer solution, is always used for determining kinetic parameters (Welt *et al.*, 1997). Svensson & Eriksson (1974b) studied the thermal inactivation of lipoxygenase in peas, but using kinetic parameters previously determined in pea press juice (Svensson & Eriksson, 1974a). Reported  $E_a$  values of lipoxygenase are the following: 479 KJ mol<sup>-1</sup> for pea press juice (Svensson & Eriksson, 1974a), 198.8 KJ mol<sup>-1</sup> for asparagus lipoxygenase in CMC purified preparation (Ganthavorn *et al.*, 1991), 102.9 KJ mol<sup>-1</sup> for purified lipoxygenase of immature English peas (Chen & Whitaker, 1986). Naveh *et al.* (1982) and Luna *et al.* (1986) studied the thermal destruction of peroxidase in the blanching of corn-on-the-cob, considering the corn-cob as a finite homogeneous cylinder. There is no information about kinetic parameters of thermal inactivation of lipoxygenase in foods using the unsteady-state procedure.

The objective of our work was to test the hypothesis that one of two unsteady-state methods

would describe the kinetic parameters of thermal inactivation of green bean lipoxygenase optimally.

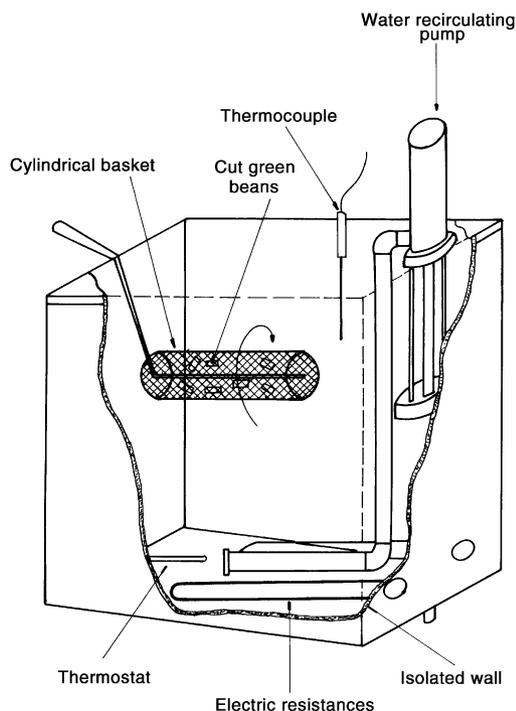
## Materials and methods

### Green beans

Green beans, variety Blue Lake, were obtained from a field close to Santa Fe City (Argentina); harvest was controlled by a member of the working group; maturity was determined by size and diameter and the weight of seeds to pods had to be below 10% (Namesny, 1999); once in the laboratory green beans were sized (those with diameters below 8 mm or above 9.5 mm were discarded) and cut to a length of 20 mm; cut green beans had to be clearly cylindrical in shape and full of pulp. From each lot to be thermally treated a sample was obtained to determine green bean density; the sample was weighed in air, and then its volume determined measuring the displacement of a established volume of water; the equivalent diameter of the green bean of the lot was obtained as  $D = 2 \times (\text{weight of the lot} / 3.1416 \times \text{density} \times \text{number of cut green beans})^{0.5}$ . The percentage content of water, carbohydrates, lipids, proteins and ashes of the cut green beans were also determined (AOAC, 1984).

### Thermal treatment

For thermal treatment of cut green beans a stainless steel bath with a capacity of 40 dm<sup>3</sup> was used (see Fig. 1). The water bath was heated by electric resistances and a thermostatic device controlled its temperature; bath temperature was measured with an Ellab type T probe connected to a Leeds and Northrup potentiometer and the bath was continuously agitated with a circulating pump. Cut green beans (approximately 40 g, corresponding to 36–41 pieces), with initial temperatures between 22.1 and 27.8 °C, were placed in a cylindrical basket made of stainless steel mesh (no. 8), which was immersed in the water bath, rotating as a consequence of water agitation. Water bath temperatures and heating times used were 76 °C (15, 15 and 25 s), 82 °C (15, 15 and 20 s), 88 °C (10, 15 and 20 s) and 94 °C (15, 20 and 20 s); rotational speeds of the cylindrical basket were the following: 38, 42, 47 and 54 r.p.m. at 76, 82, 88 and 94 °C, respectively; no modification of water bath temperature was detected when the green



**Figure 1** Sketch of heating equipment.

beans were immersed in it. Once the thermal treatment finished, the operator took the cylindrical basket from the water bath, extracting the shaft with the lid attached to it with one hand (this operation was instantaneous); meanwhile, the other hand poured out the opened basket, dropping the green beans into a liquid nitrogen bath, where they were frozen in a very short time. Then each lot was packed in a pouch of high-density polyethylene, stored at  $-20\text{ }^{\circ}\text{C}$ , and lipoxygenase activity determined during the following week. For each combination of time-temperature, a sample of fresh cut green beans was also frozen with liquid nitrogen, and used to determine initial lipoxygenase activity.

*Experimental determination of convective heat transfer coefficient between water and cut green beans surface*

To determine  $h$  (Califano, 1981; Califano & Calvelo, 1983), approximately 40 g of cut green beans and an acrylic cylinder ( $D = 10\text{ mm}$ ,  $L = 20\text{ mm}$ ) were placed in the cylindrical basket. A type K thermocouple was inserted into the centre of the

acrylic cylinder and connected to a Leeds and Northrup potentiometer. The cylindrical basket was immersed in the water bath and the experimental temperature profile of the acrylic cylinder centre was obtained (ten tests were performed); bath temperatures ranged between  $64$  and  $95\text{ }^{\circ}\text{C}$  and the heating times between  $0$  and  $240\text{ s}$ ; the temperature was measured at intervals of  $10\text{ s}$ . The water bath temperature was measured using an Ellab type T probe connected to a Leeds and Northrup recorder; at each temperature the rotational speed of the cylindrical basket was measured and a white mark was painted at the lid of the basket recording the number of revolutions that the basket rotated in  $1\text{ min}$ . Experimental centre temperatures were compared to theoretical temperatures by varying the convective heat transfer coefficient between  $200$  and  $1500\text{ W m}^{-2}\text{ }^{\circ}\text{C}^{-1}$ , and determining the value of  $h$  that minimized the variance. Theoretical centre temperatures were obtained using the analytical solution of the heat conduction problem for a finite cylinder, with boundary condition of the third kind (Luikov, 1968) using 36 terms; acrylic thermal conductivity and thermal diffusivity used were  $0.208\text{ W m}^{-1}\text{ }^{\circ}\text{C}^{-1}$  and  $1.201 \times 10^{-7}\text{ m}^2\text{ s}^{-1}$ , respectively (Califano, 1981). The experimental heat transfer coefficients determined were used to obtain the following equation of variation of  $h$  with water bath temperature ( $T$ ) and rotational speed of the cylindrical basket ( $N$ ) ( $r^2 = 0.9999$ ):

$$h^2 = 2.9508 \times 10^6 - 8.8602 \times 10^8 \ln(TN)/TN \quad (1)$$

*Green bean thermal conductivity estimation*

The equation of Maxwell-Eucken was used to estimate the thermal conductivity of green beans (Calvelo, 1984); it was considered that the food is a binary system (a matrix of dried solids in which water spheres are dispersed); the thermal conductivity of green beans solids was estimated from the weighted average of the component fractions and the thermal conductivities of each component. Thermal conductivities for each basic component, according to the known composition of green beans, as well as water densities and thermal conductivities as a function of temperature, were obtained from Heldman

(1992). The estimated values for green beans thermal conductivities as a function of green bean temperature were fitted with this equation ( $r^2=0.9975$ ):

$$\lambda = 0.4318 + 0.05117 T^{0.312} \quad (2)$$

#### *Experimental determination of cut green bean thermal diffusivity*

The same methodology as described for the determination of  $h$  was followed. The difference was that now the thermocouple was inserted in the centre of a cut green bean and that the acrylic cylinder was not included in the system. The experimental temperature profiles of the cut green bean centre (six determinations) were obtained for temperatures between 73 and 93 °C and heating times between 0 and 180 s. Experimental centre temperatures were compared to theoretical temperatures, varying the green bean thermal diffusivity between 1.30 and  $1.70 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$ , and determining the value of  $\alpha$  that minimized the variance.  $Biot_R$  and  $Biot_Z$  were calculated using the values of  $h$  obtained from equation 1, and green bean thermal conductivities from equation 2 at a mean green bean temperature between  $T_0$  and  $T_a$ . Analytical solutions used were the same as described previously. The following expression was obtained for the variation of  $\alpha$  with water bath temperature ( $r^2=0.9999$ ):

$$\alpha^{0.5} = 3.65 \times 10^{-4} + 3.386 \times 10^{-7} T \quad (3)$$

#### *Experimental determination of lipoxygenase activity*

A modified method of that of Surrey (1964) was used. A sample of 4–5 g was used for lipoxygenase activity determination in frozen fresh green beans and 34–40 g were used for thermal treated frozen cut green beans.

#### *Enzyme extract preparation*

The frozen fresh or thermal treated green beans were blended in a Waring blender with enough distilled water at 1 °C to reach a volume of 300 mL during 2.5 min. The homogenized mixture was filtered immediately through a stainless steel mesh (opening of 0.7 mm) and the enzymatic extract was immediately used.

#### *Substrate preparation*

Tween 20 (0.125 mL) was dissolved in 2.5 mL borate buffer (0.05 M, pH=9); 0.125 mL of linoleic acid and 0.35 mL of NaOH (1 N) were mixed until a translucent mixture was obtained; finally 22.5 mL of borate buffer (pH=9) was added and made up to 50 mL with distilled water. The pH was adjusted to 6.3 with HCl (2 N and 0.1 N).

#### *Reaction development*

The substrate solution (27 mL) at 25 °C had  $O_2$  passed through it with continuous mixing; 3 mL of the enzyme extract was added. At 20 s, 1 mL of the reacting mixture was transferred to a centrifuge tube containing 2 mL of absolute ethanol (blank tube, zero time) and shaken. At 1, 2, 3, 4 and 5 min 1 mL of the reacting mixture was transferred to five centrifuge tubes, also containing 2 mL of absolute ethanol (Surrey, 1964) and the mixtures were shaken; 7 mL of 60% ethanol was added to each of the centrifuge tubes and the mixtures were homogenized. The tubes were centrifuged for 10 min (at 1200 g). The clear centrifuged samples were transferred to quartz cuvettes and read at 234 nm in a Spectronic spectrophotometer (model Genesys, Milton Roy Company, Rochester, USA). From the graph of absorbance vs. time (the reaction is of zero order) the slope of the line was obtained. Results were expressed as units of LPO activity  $\text{g}^{-1} = \Delta \text{absorbance unit min}^{-1} \text{ g}^{-1} \text{ sample}$ . Retentions (%) were calculated as (lipoxygenase activity of thermal treated frozen sample/lipoxygenase activity of frozen fresh sample)  $\times 100$ .

#### **Kinetic models**

Two unsteady-state procedures were used to estimate kinetic parameters of lipoxygenase inactivation: (1) the trial and error procedure developed by Lenz & Lund (1977a, b, 1980) and applied by Luna (1986) and Luna *et al.* (1986); and (2) the unsteady method used by Rodrigo *et al.* (1998).

By procedure (a) a set of kinetic data (rate constants vs. temperatures) must be assumed; using this model an average enzyme activity retention in the whole volume of the cut green bean is calculated as:

$$(x_{\text{average}})_{\text{calc.}} = \frac{\int_V x dV}{\int_V dV} \quad (4)$$

this integral was evaluated by using the Gauss numerical integration, with 18 points; each of these points was calculated by using a first-order inactivation reaction of the labile lipoxygenase, as:

$$x = \exp \left\{ -k_{\text{ref.}} \left\{ \int_0^{t_h} \exp \left[ -\frac{E_a}{R_g} \left( \frac{1}{T_{(t,r,z)}} - \frac{1}{T_{\text{ref.}}} \right) \right] dt \right\} \right\} \quad (5)$$

where  $T_{(t,r,z)}$  was obtained from the solution of the unsteady heat conduction in a finite cylinder; the boundary condition used for the heating step was of the third kind and cooling was considered to have a negligible effect on lipoxygenase inactivation. Gutschmidt (1968), working with strawberries and green beans, determined freezing rates by immersion in liquid nitrogen at a rate of 5–10 cm min<sup>-1</sup>. The tests revealed that the freezing rate of cut green beans was 5–6 cm min<sup>-1</sup>, so lipoxygenase inactivation during cooling might be considered not significant. Convective heat transfer coefficient and thermal conductivity were used to calculate  $Biot_R$  and  $Biot_Z$  and thermal diffusivity to calculate  $Fo_R$  and  $Fo_Z$ . They were estimated using developed equations 1, 2 and 3, respectively. One hundred terms were used in the analytical solution. Equation 5 was numerically solved using Simpson's method; the time intervals used varied from 0.5 to 1 s.

When  $(x_{\text{average}})_{\text{calc.}}$  was obtained a new value for the rate constant could be calculated by:

$$k_{i+1} = \ln(x_{\text{average}})_{\text{calc.}} \cdot k_i / \ln(x_{\text{average}})_{\text{exp.}} \quad (6)$$

These new rate constants were used to obtain by regression, a new  $E_a$ ; calculations were repeated in successive iterations until the calculated retentions were equal or proximate to the experimental retentions; finally at each temperature three constants were obtained which were averaged; the three  $E_a$  obtained were also averaged, obtaining the standard deviations. For iterations the following subsets were used, (a): 76 °C–15 s, 82 °C–15 s, 88 °C–15 s and 94 °C–15 s; (b): 76 °C–25 s, 82 °C–15 s, 88 °C–20 s, 94 °C–20 s; (c): 76 °C–15 s, 82 °C–20 s, 88 °C–10 s, 94 °C–20 s.

By using procedure (2) the rate constant at reference temperature (mean value of the four

temperatures used) and  $E_a$  were estimated by minimizing the following expression:

$$\sum_1^{12} [(x_{\text{average}})_{\text{exp.}} - (x_{\text{average}})_{\text{calc.}}]^2 \quad (7)$$

by non-linear regression;  $(x_{\text{average}})_{\text{calc.}}$  were estimated as was described in procedure (1). The experimental error in the parameters estimated were obtained by minimizing equation (7) 12 times, each time using only 11 of 12 sets of data.

Computer programs were developed in Lotus 123 version 5 (1994) in an IBM Aptiva PC. For some applications Table Curve software was used.

## Results and discussion

Average chemical composition of green beans was: water = 91.75%; carbohydrates = 5.99%; proteins = 1.53%; lipids = 0.22%; ashes = 0.51%. The densities of green beans ranged from 902 to 988 kg m<sup>3</sup>; meanwhile calculated equivalent diameters ranged from 8.26 to 9.21 mm.

Table 1 shows the average convective heat transfer coefficient as a function of water bath temperature and rotational speed of the cylindrical basket. The range of  $h$  for hot water processing is between 280 and 1700 watts m<sup>2</sup> °C<sup>-1</sup> (Ling *et al.*, 1974). When water is heated between 65 and 121 °C,  $h$  is between 830 and 1175 watts m<sup>2</sup> °C<sup>-1</sup> (Holdsworth, 1997). Anderson (1994), studying the modelling of potato blanching, used values for  $h$  of 350 watts m<sup>2</sup> °C<sup>-1</sup>, as determined by Califano & Calvelo (1983) for free convection heating, and 750 watts m<sup>2</sup> °C<sup>-1</sup>, as determined by Lamberg & Hallström (1986) when forced convection heating prevailed. The convective heat transfer coefficients

**Table 1** Average convective heat transfer coefficient (water-cut green bean surface) as a function of water bath temperature and rotational speed of cylindrical basket

Average bath Temperature, °C	Average rotational speed, r.p.m.	Average convective heat transfer coefficient <sup>a</sup> , watts m <sup>2</sup> °C <sup>-1</sup>
75.45	38	701 <sup>a</sup>
85	45	1018 <sup>b</sup>
94.85	54	1215 <sup>c</sup>

<sup>a</sup> Means in the same column with different letters are significantly different ( $P < 0.05$ ).

determined in this work are within the range of values presented by other authors.

Average thermal diffusivities obtained were the following:  $1.525 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$  (25–73.9 °C),  $1.555 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$  (25–85.2 °C) and  $1.575 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$  (25–92.65 °C), the first and the third being significantly different ( $P < 0.05$ ). These values are similar to those compiled by Holdsworth (1997) for pea purée,  $1.54\text{--}1.59 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$ , potato purée,  $1.39\text{--}1.46 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$  (at 60–100 °C) and tomato pulp,  $1.46\text{--}1.50 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$  (at 28 °C) and those determined by Lund *et al.* (1972) for carrots,  $1.56 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$  (in the range 20–100 °C) and Garrote *et al.* (2000) for potatoes,  $1.62 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$  (in the range 20–150 °C).

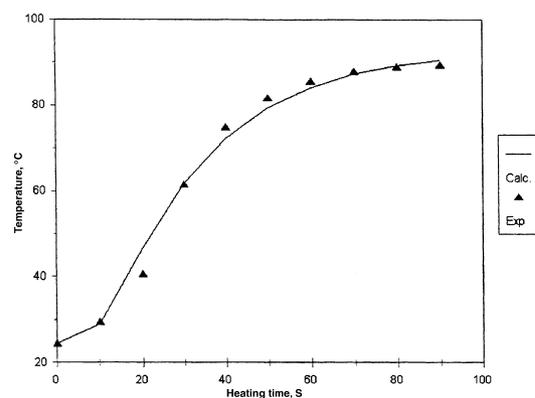
Table 2 shows the estimated values for green beans thermal conductivities as a function of green bean temperature. Sweat (1974) has experimentally determined the thermal conductivity of carrot (0.605 watts  $\text{m}^{-1} \text{ °C}^{-1}$ ), beet (0.601 watts  $\text{m}^{-1} \text{ °C}^{-1}$ ) and turnip (0.563 watts  $\text{m}^{-1} \text{ °C}^{-1}$ ), these have water content (90%) similar to the green beans used in this investigation. Potatoes (80% water) were used by Lamberg & Hallström (1986) to obtain a value of 0.684 watts  $\text{m}^{-1} \text{ °C}^{-1}$  for  $\lambda$ . These values are similar to those estimated in this work.

In Fig. 2 experimental and theoretical temperature profiles during green bean heating are shown. Good agreement was found and thus the model could predict the temperature inside the cut green bean reasonably well; the 'heat up' period represented all the process time, justifying the use of the unsteady procedure.

Table 3 shows the experimental retention (%) of cut green bean lipoxygenase activity as a function

**Table 2** Estimated cut green bean thermal conductivity as a function of temperature

Green bean temperature, °C	Thermal conductivity, watts $\text{m}^{-1} \text{ °C}^{-1}$
20	0.5619
30	0.5797
40	0.5931
50	0.6067
60	0.6145
70	0.6254
80	0.6325
90	0.6375
100	0.6493



**Figure 2** Experimental and calculated temperature profiles of cut green bean centre during heating.  $R = 0.0042 \text{ m}$ ;  $L = 0.02 \text{ m}$ ;  $T_a = 93^\circ\text{C}$ ;  $T_0 = 24.5^\circ\text{C}$ ;  $h = 1,210 \text{ W m}^{-2} \text{ °C}$ ;  $\alpha = 1.575 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$ ;  $\lambda = 0.615 \text{ W m}^{-1} \text{ °C}$ .

of different combinations of time and temperature; letters (a), (b) and (c) show the order in which the combinations were used to make iterations of procedure (1); for example, the subset (c) includes the following temperatures, times and retentions: 76 °C–15 s–30.91%; 82 °C–20 s–10.12%; 88 °C–10 s–29.63%; 94 °C–20 s–1.92%.

In order to determine the kinetic parameters of lipoxygenase inactivation by procedure (1) it was necessary to assume starting values for  $k_{85^\circ\text{C}}$  and  $E_a$ . As no information was available for green beans, the set of values determined for lipoxygenase inactivation in pea press juice,  $k_{85^\circ\text{C}} = 5.1 \text{ s}^{-1}$

**Table 3** Experimental lipoxygenase retention as function of time-temperature heat treatment

Temperature, °C	Time, s	Retention, %
76	15 (a)	40.08
	15 (c)	30.91
	25 (b)	18.77
82	15 (a)	21.32
	15 (b)	21.86
	20 (c)	10.12
88	10 (c)	29.63
	15 (a)	14.47
	20 (b)	7.68
94	15 (a)	16.62
	20 (b)	1.50
	20 (c)	1.92

(a), (b) and (c) are subsets used for iterations in procedure (1).

and  $E_a = 479 \text{ KJ mol}^{-1}$  were chosen (Svensson & Eriksson, 1974a). Preliminary testing using these values allowed us to find the best set of definitive starting values,  $k_{85^\circ\text{C}} = 148 \text{ s}^{-1}$  and  $E_a = 154.8 \text{ KJ mol}^{-1}$ .

For subset (a) the number of iterations were five, meanwhile for subsets (b) and (c) only three were necessary. Table 4 shows the values of  $k_{\text{ref}}$  at four temperatures and the corresponding  $E_a$  obtained; mean values and standard deviations are also shown.

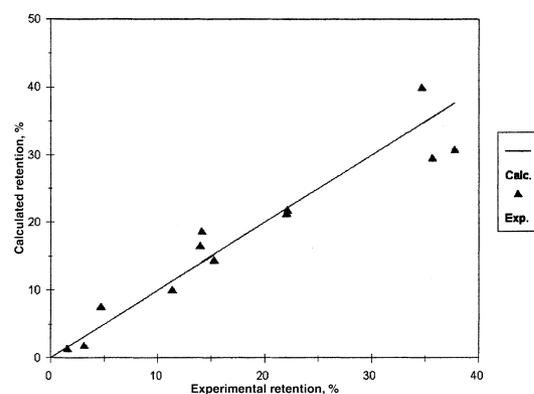
The  $k_{\text{ref}}$  and  $E_a$  that minimized eqn (7) using procedure (2) were the following:  $k_{85^\circ\text{C}} = 150 \text{ s}^{-1}$  and  $E_a = 164 \text{ KJ mol}^{-1}$ ; standard deviations obtained were  $26.3 \text{ s}^{-1}$  and  $4.7 \text{ KJ mol}^{-1}$ , respectively. The variability of the kinetic parameters may be attributed to different causes: cut green beans are not perfect finite cylinders; they are heterogeneous materials, possibly with small seeds having different lipoxygenase activity compared to the pulp; processing times to inactivate lipoxygenase were short, so errors could arise more easily.

The  $E_a$  determined for green beans were much lower than that obtained for pea lipoxygenase ( $479 \text{ KJ mol}^{-1}$ ) by Svensson & Eriksson (1974a); this means that green bean lipoxygenase is less sensitive to temperature changes than pea lipoxygenase. If we compare the inactivation rate constants for both lipoxygenases, at  $82^\circ\text{C}$   $k_{\text{pea}} = 1.32 \text{ s}^{-1}$  is lower than  $k_{\text{green bean}} = 92.9 \text{ s}^{-1}$ , but at  $100^\circ\text{C}$ ,  $k_{\text{pea}} = 3295 \text{ s}^{-1}$  is greater than  $k_{\text{green bean}} = 1094 \text{ s}^{-1}$ . It is also interesting to compare the thermal resistance of lipoxygenase and peroxidase, the latter being used as an index of appropriate blanching; Lund *et al.* (1972) determined a  $k_{100^\circ\text{C}} = 1.4 \text{ s}^{-1}$  and  $E_a = 167.4 \text{ KJ mol}^{-1}$  for carrot labile peroxidase. It was observed that this  $E_a$  is very similar to that obtained for green bean lipoxygenase, but the rate constant is much lower; the estimated temperature at which the rate constant of green bean lipoxygenase equals the

value of  $k_{100^\circ\text{C}} = 1.4 \text{ s}^{-1}$  for peroxidase is  $57.5^\circ\text{C}$ , revealing that lipoxygenase is much less resistant to heating treatment than labile peroxidase. The residual sum of squares (RSQ) between experimental retention values and those predicted by using rate constants and  $E_a$  obtained by procedure (1) was 154.2 (see Fig. 3), meanwhile when  $k_{85^\circ\text{C}}$  and  $E_a$  obtained by procedure (2) were used the RSQ was 197.3. Although kinetic parameters obtained by both procedures gave satisfactory results, average  $E_a$  and rate constants obtained by procedure (1) predicted lipoxygenase retentions closer to experimental values, probably because at each heating temperature the corresponding rate constants were used as reference, while with procedure (2) only  $k_{85^\circ\text{C}}$  was used as reference.

## Conclusions

Both unsteady-state methods which were tested to determine kinetic parameters of green bean lipoxygenase inactivation during blanching treatment gave similar results. Predicted and observed lipoxygenase retentions showed good agreement. The



**Figure 3** Comparison of  $(x_{\text{average}})_{\text{calc}}$  with  $(x_{\text{average}})_{\text{exp}}$  using kinetic parameters obtained from the unsteady-state procedure (1).

**Table 4** Kinetic parameters of cut green bean lipoxygenase inactivation determined by procedure (1)

Subset	$k_{76^\circ\text{C}}, \text{s}^{-1}$	$k_{82^\circ\text{C}}, \text{s}^{-1}$	$k_{88^\circ\text{C}}, \text{s}^{-1}$	$k_{94^\circ\text{C}}, \text{s}^{-1}$	$E_a, \text{KJ mol}^{-1}$
a	20.8	101.5	237.1	345.8	165.2
b	22.7	89.2	151.5	420.5	164.8
c	38.0	88.1	247.7	457.0	152.2
Mean value (standard deviation)	27.2 (9.4)	92.9 (7.5)	212.1 (52.7)	407.8 (56.7)	160.7(8.1)

$E_a$  and  $k$  values obtained proved that green bean lipoxygenase might be inactivated easily at temperatures used normally in a blanching process. The kinetic parameters that were determined and the appropriate mathematical model could be used to optimize and improve thermal treatment of green beans using lipoxygenase as the index of blanching adequacy.

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### Nomenclature

$Biot_R$	Biot number, radial direction, $h R/\lambda$
$Biot_Z$	Biot number, axial direction, $h Z/\lambda$
$D$	Cut green bean diameter, m
$E_a$	Activation energy, KJ/mol
$FO_R$	Fourier number, radial direction, $\alpha t/R^2$
$FO_Z$	Fourier number, axial direction, $\alpha t/Z^2$
$h$	Convective heat transfer coefficient, watts $m^2 \text{ } ^\circ C^{-1}$
$k_{ref.}$	Reaction rate constant at the reference temperature, $s^{-1}$
$(k_{ref.})_i$	Reaction rate constant at the reference temperature, for iteration $i$ , $s^{-1}$
$(k_{ref.})_{i+1}$	Reaction rate constant at the reference temperature, for iteration $i+1$ , $s^{-1}$
$L$	Cut green bean height, m
$N$	Rotational speed, r.p.m.
$r$	Radial co-ordinate, m
$R$	Cut green bean radius, m
$R_g$	Universal gas constant, $8.31439 \text{ J mol}^{-1} \text{ K}^{-1}$
$t$	Time, s
$t_h$	Heating time, s
$T_a$	Heating medium temperature, $^\circ C$
$T_0$	Initial temperature, $^\circ C$
$T_{ref.}$	Reference temperature, K
$T_{(t,r,z)}$	Temperature as a function of $t$ , $r$ and $z$ , K
$V$	Volume, $m^3$

$x$	Retention of lipoxygenase activity at a point
$(x_{average})_{calc.}$	Calculated average retention of lipoxygenase activity
$(x_{average})_{exp.}$	Experimental average retention of lipoxygenase activity
$z$	Axial co-ordinate, m
$Z$	$L/2$ , m
$\alpha$	Thermal diffusivity, $m^2 \text{ s}^{-1}$
$\lambda$	Thermal conductivity, watts $m^{-1} \text{ } ^\circ C^{-1}$

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