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Effect of Combined Pressure and Temperature on Soybean Lipoxygenase. 2. Modeling Inactivation Kinetics under Static and Dynamic Conditions

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In previous research, kinetics for irreversible inactivation of soybean lipoxygenase (LOX) by combined pressure and temperature have been thoroughly studied in the pressure range 0.1−650 MPa at temperatures varying from 10 to 64 °C (Ludikhuyze et al. *J. Agric. Food Chem.* **1998**, *46*, 4074−4080.). In the present paper a mathematical model describing the variation of the inactivation rate constant of soybean LOX as a function of pressure and temperature was finalized, starting from the Eyring equation. In the latter equation, the temperature-dependent parameters (k_{refP}, V_a) were replaced by mathematical expressions. Subsequently, the proposed model structure was verified to predict likewise the extent of inactivation under variable pressure and temperature conditions, provided the kinetic parameters were re-estimated. Finally, it was observed that multiple application of high-pressure enhanced the inactivation of soybean LOX, the effect becoming more pronounced at low temperature and when the amount of cycles increased.

Keywords: *Soybean lipoxygenase; pressure−temperature inactivation kinetics; modeling; static; dynamic; cycling*

INTRODUCTION

In the past decade, it has become clear that high pressure may offer major advantages to the food preservation and processing industry (Mertens, 1995; Barbosa-Canovas et al., 1997). Next to inactivation of microorganisms and spoilage enzymes (Styles et al., 1991; Seyderhelm et al., 1996; Yen and Lin, 1996), promising results have been obtained with respect to application of high pressure in food processing, for example, gelation of food proteins (Richwin et al., 1992; Ohshima et al., 1993) and improving digestibility of proteins and tenderization of meat products (Bouton et al., 1977; Ohmori et al., 1991). Nevertheless, the industrial breakthrough of high-pressure technology, like that of other novel technologies, can merely be forced by the development of a scientific basis to assess its impact on food safety and quality aspects. Such quantitative assessment is indispensable to fulfill legislative safety requirements as well as to respond to the current quality demand of consumers. Systematic kinetic studies of food-relevant aspects, such as microorganisms, enzymes, quality attributes, and structural properties, are the basis of such an engineering approach. Because it is generally believed that the safest and most economically feasible use of high pressure is in combination processes, especially with moderately elevated temperature (Gould and Sale, 1970; Knorr, 1993; Smelt and Van Wely, 1993), a third dimension next to time and temperature, namely pressure, is introduced as compared to traditional thermal processing.

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The work presented in this paper is focused on developing a mathematical model to describe the pressure−temperature inactivation of soybean lipoxygenase (LOX), which can serve as a basis for the development of a concept and methodology for impact assessment of pressure processes. The most straightforward approach to develop such a model is to perform isobaric−isothermal treatments because these allow separating the contributions of pressure and temperature to changes in the inactivation rate constant. Nevertheless, in the context of developing a concept to assess the impact of industrial pressure−temperature processes, the predictive power of the developed models when applied to inactivation data obtained under variable pressure−temperature conditions has to be verified. Indeed, industrial application of high pressure is very unlikely to be isobaric and isothermal. Building up of pressure involves temperature changes as a function of time due to adiabatic heating. Moreover, when large-volume industrial vessels are considered, a nonuniform temperature distribution inside the vessel may be observed, involving temperature gradients as a function of position.

In addition, some attention has been paid to the effect of pressure cycling on food-quality-related enzymes because possible, additional inactivation effects would offer major advantages to the food industry in designing processes at lower pressure, thereby reducing the overall cost of the process and, consequently, rendering it more economically feasible. Hitherto it has seemed very difficult to foresee whether multicycle or pulsated pressure treatment will gain interest in food preservation and processing because contradictory results regarding the effect of pressure cycling on inactivation of both microorganisms and enzymes have been reported in the literature (Curl and Jansen, 1950a,b; Sale et al.,

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1970; Butz et al., 1990; Aleman et al., 1996; Ludikhuyze et al., 1997b).

MATERIALS AND METHODS

Enzyme and Activity Measurement. Lipoxygenase type IB from soybean (Sigma, product L7395) was dissolved in a 0.01 M Tris-HCl buffer at pH 9 at a concentration of 0.4 mg/mL. Residual activity was determined spectrophotometrically, using linoleic acid (40 mM) solubilized with Tween 20 as substrate. The reaction was carried out at 25 °C. The absorption at 234 nm was recorded as a function of reaction time (3 min), and the activity was determined from the linear portion of the curve (Ludikhuyze et al., 1998).

Isothermal and Isobaric−Isothermal Treatment. Isothermal treatments were performed in a thermostatic water bath at constant temperature. Isobaric−isothermal treatments were performed in multivessel high-pressure equipment (HPIU-10000, 95/1994, Resato). This equipment, consisting of eight individual thermostatic vessels that are connected to the central pressure circuit, was especially designed to perform kinetic experiments (Ludikhuyze et al., 1997a, 1998). A pressure intensifier is used to build up high pressure (up to 1000 MPa). After both thermal and pressure treatments, samples were transferred to a water bath at 25 °C and stored for 0.5 h (Ludikhuyze et al., 1998).

Variable Pressure−Temperature Treatment. Nonisobaric−nonisothermal treatments were performed in a laboratory pilot scale, warm isostatic press (SO, 5-7442-0, Engineered Pressure Systems International). This equipment is characterized by a vessel volume of 590 mL (l = 300 mm, d = 50 mm) and filled with a commercial pressure transferring liquid (TR10, Resato). The equipment is connected to a cryostat and enables high pressure (up to 600 MPa) to be combined with temperatures between −30 and 100 °C. One external temperature sensor and one internal temperature sensor allow measurement of the temperature of the thermostatic mantle and of the load, respectively. Four more thermocouples are provided to measure temperature at different positions inside the vessel. They were enclosed in dummy samples filled with water, to take heat transfer through the microtubes into account, and positioned at four levels in the vessel (4, 10, 16, and 22 cm from the bottom of the vessel). Next to these temperature sensors, one pressure sensor served to measure isostatic pressure inside the vessel. After connection of all sensors to a datalogger (Cobra 7-10, Mess und System Technik GmbH), pressure and temperature were recorded at regular time intervals (10 s) during the entire treatment. The samples were contained in flexible microtubes (0.25 mL, Elkay) and fixed next to the dummy samples at four positions in the vessel. After treatment, the samples were stored for 0.5 h at 25 °C before activity measurement (Ludikhuyze et al., 1998).

Data Analysis. *Modeling Isobaric−Isothermal Inactivation Kinetics.* Kinetic parameters describing isobaric−isothermal inactivation of LOX were previously calculated using a two-step linear regression approach (Ludikhuyze et al., 1998). This individual two-step linear regression approach has the advantage that it is used by many authors reporting on kinetics of food and pharmaceutical product attributes, allowing comparison of the obtained results with literature data. Moreover, this approach enabled k values to be calculated in pressure−temperature areas where the Arrhenius equation is not valid.

In this paper it is endeavored to fit a mathematical model describing the combined pressure and temperature dependence of the inactivation rate constant to the entire data set. For this purpose, k values have to be linked to their corresponding pressure−temperature profile, requiring a global analysis to be performed. A nonlinear regression procedure (SAS Institute, 1982) was applied to evaluate the proposed models and to determine the kinetic parameters. As opposed to linear regression, in which least-squares estimates can be written explicitly in terms of the data and calculated herefrom, nonlinear regression involves an iterative numerical procedure to determine the kinetic parameters corresponding to the minimal sum of squares. Initial values for this iterative procedure were taken from the results of the individual regression approach obtained at reference temperature and pressure. In general, the regression coefficient measures how well the nonlinear model fits the data. Because this statistical parameter is largely dependent on the model structure and on the number of observations and parameters, the corrected r^2 (eq 1) was defined to compare different models on their ability to fit data sets, possibly with a different number of observations.

$$\text{corrected } r^2 = 1 - [(j - i)(1 - r^2)/(j - p)] \quad (1)$$

Evaluation of the Proposed Kinetic Model under Variable Pressure−Temperature Conditions. On the basis of previous research regarding validation of thermal inactivation kinetics of enzymes under variable temperature conditions (De Cordt, 1994; Van Loey, 1996), the course of enzyme inactivation was assumed to remain unchanged under variable pressure and temperature, that is, dynamic, conditions. Hypothesizing first-order inactivation kinetics, the integral effect of an inactivation process under variable conditions, where the inactivation rate constant is no longer constant as a function of time, can be described by

$$\ln(A/A_0) = -\int_0^t k \, dt \quad (2)$$

Implementation of a kinetic model expressing the combined pressure−temperature dependence of the inactivation rate constant under static conditions then yields eq 3, which can be used to check the ability of the proposed kinetic model and its concomitant parameters to predict inactivation under dynamic conditions.

$$\ln(A/A_0) = -\int_0^t k_{f(P,T)} \, dt \quad (3)$$

After treatment, activity retentions were measured spectrophotometrically, whereas the corresponding, recorded pressure−temperature−time profiles were integrated numerically according to Simpson's rule (Carnahan et al., 1969).

By applying a nonlinear regression procedure (SAS Institute, 1982) to fit eq 3 on the data obtained under variable conditions, the proposed kinetic model could be evaluated under dynamic conditions, and the kinetic parameters obtained were compared to those from isobaric−isothermal experiments. The problem of finding good initial estimates as starting values for the iterative procedure was solved by performing a grid search in a fixed range of values comprising the estimates obtained from static experiments. The accuracy of the proposed kinetic models and concomitant parameters was again evaluated by the corrected correlation coefficient (eq 1), thereby eliminating the influence of model structure, number of observations, and number of parameters on the accuracy of the fit.

RESULTS AND DISCUSSION

Modeling Isobaric−Isothermal Inactivation Kinetics. In previous research, kinetic models have been finalized to describe the combined effect of pressure and temperature on the inactivation of *Bacillus subtilis* α-amylase (BSA) and avocado polyphenol oxidase (PPO) (Ludikhuyze et al., 1997a; Weemaes et al., 1998). However, as expected from the different shape of the pressure−temperature kinetic diagram of LOX compared to BSA and PPO, data on isobaric−isothermal inactivation of LOX could not be fitted by either of the former models. Unlike for BSA and PPO, temperature dependence of the inactivation rate constants of LOX could not be described by the Arrhenius equation over the entire temperature range. Therefore, development of another kinetic model was attempted. Hence, the

Table 1. Kinetic Parameter Estimates for Temperature Dependence of V_a, Temperature Dependence of ln k_{refP}, and Pressure–Temperature Dependence of the Logarithm of the Inactivation Rate Constant for LOX, 0.4 mg/mL in 0.01 M Tris-HCl at pH 9

	estimated value		
parameter	eq 5	eq 6	eq 7
a_1	-12.2 ± 1.5[a]		-15.6 ± 1.4
b_1	$(6.11 \pm 0.46) \times 10^{-2}$		$(7.1 \pm 0.28) \times 10^{-2}$
a_2		$(3.09 \pm 0.09) \times 10^{-3}$	$(2.66 \pm 0.27) \times 10^{-3}$
b_2		$(-1.83 \pm 0.08) \times 10^{-1}$	$(-1.39 \pm 0.18) \times 10^{-1}$
c_2		-2.42 ± 0.18	-3.12 ± 0.28
corr r^2	0.970	0.997	0.991

[a] Standard error.

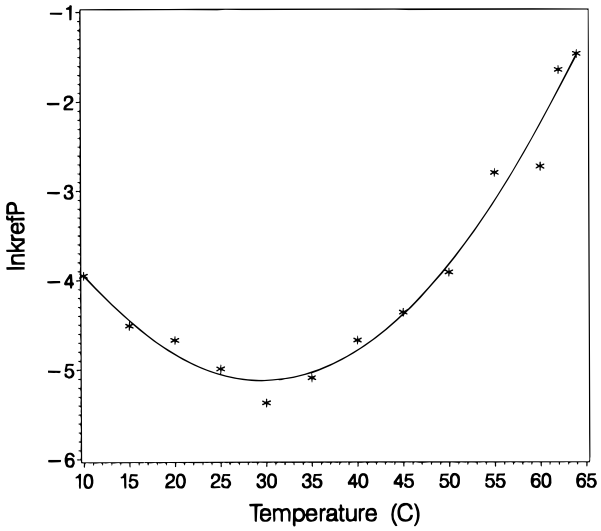

Figure 1. Temperature dependence of ln k_{refP} (P_{ref} = 500 MPa).

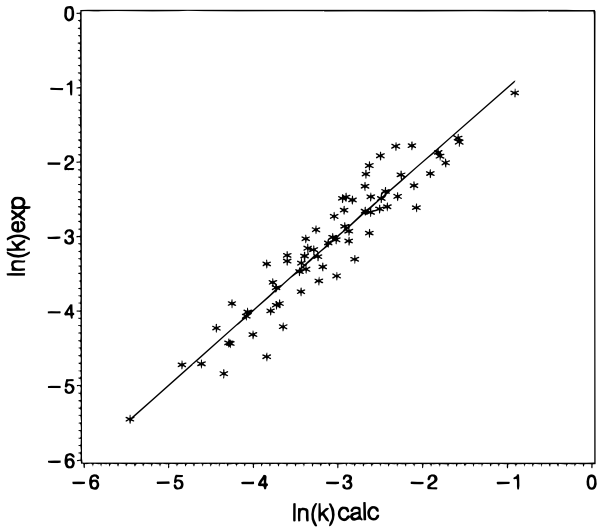

Figure 2. Correlation between the experimental k values for isobaric–isothermal inactivation of soybean LOX (0.4 mg/mL in 0.01 M Tris-HCl at pH 9) and the k values calculated according to eq 7 and concomitant parameters.

Eyring equation (eq 4), which was valid over the entire temperature domain, was used as starting point. In this equation, k_{refP} and V_a are temperature-dependent parameters. Mathematical expressions reflecting the temperature dependence of the latter parameters were fit on the data. Temperature dependence of the activation volume and inactivation rate constant at reference pressure of 500 MPa (Figure 1) could be described by eqs 5 and 6, respectively. Parameter estimates for a_1, b_1, a_2, b_2, and c_2 are shown in Table 1.

$$\ln k = \ln k_{refP} - \left[\frac{V_a}{R(T + 273)}(P - P_{ref})\right] \quad (4)$$

$$V_a = a_1 T \exp(-b_1 T) \quad (5)$$

$$\ln k_{refP} = a_2 T^2 + b_2 T + c_2 \quad (6)$$

Implementation of these mathematical expressions into the Eyring equation (eq 4) allows transformation to eq 7. Finally, it was tried whether the latter equation is able to predict isobaric–isothermal inactivation of LOX, 0.4 mg/mL in 0.01 M Tris-HCl at pH 9. Parameter estimates are presented in Table 1, and the correctness of the proposed model and the best fit of parameters is shown in Figure 2.

$$\ln k = (a_2 T^2 + b_2 T + c_2) - \left[\frac{a_1 T \exp(-b_1 T)}{R(T + 273)}(P - P_{ref})\right] \quad (7)$$

On the basis of the satisfactory correlation (Figure 2) and sufficient accuracy of the model and its attendant parameters (Table 2), it is concluded that the proposed model can adequately describe isobaric–isothermal inactivation of LOX under the specified conditions.

Evaluation of the Proposed Kinetic Model under Variable Pressure and Temperature Conditions. In a subsequent step, eq 7 and its concomitant parameters were used to fit inactivation data of soybean LOX, obtained under variable pressure and temperature conditions. For this purpose, eq 7 was implemented into eq 2, thereby yielding the appropriate kinetic model.

$$\ln\left(\frac{A}{A_0}\right) = -\int_0^t \exp(a_2 T^2 + b_2 T + c_2) \exp\left(\frac{-a_1 T \exp(-b_1 T)}{R(T + 273)}(P - P_{ref})\right) \quad (8)$$

Hereto, experiments under variable pressure and temperature conditions were carried out in the pressure range 350–525 MPa at temperatures varying from 10 to 40 °C, including both single-cycle and multicycle processes. Experimental activity retentions were then equated with activity retentions calculated according to eq 8, taking into account the recorded pressure–temperature–time profiles. When parameter estimates obtained from isobaric–isothermal inactivation experiments (Table 1) were used for the calculation of the activity retention, strong divergences between experimental and calculated values were noted. Moreover, the

Table 2. Kinetic Parameters To Describe Pressure−Temperature Inactivation of LOX, 0.4 mg/mL in 0.01 M Tris-HCl at pH 9, in the Temperature Range 10−40 °C, According to Eq 9[a]

parameter	isobaric−isothermal conditions	variable pressure and temperature conditions
V_{aT} (kJ/mol)	-56.3 ± 2.7[b]	-83.8 ± 7.1
a_2	$(2.14 \pm 0.33) \times 10^{-3}$	$(3.4 \pm 1.1) \times 10^{-3}$
b_2	$(-1.38 \pm 0.21) \times 10^{-1}$	$(-2.41 \pm 0.59) \times 10^{-1}$
c_2	-2.69 ± 0.28	$(1.04 \pm 0.17) \times 10^{-1}$
corr r^2	0.991	0.894

[a] Reference pressure was set at 500 MPa. [b] Standard error.

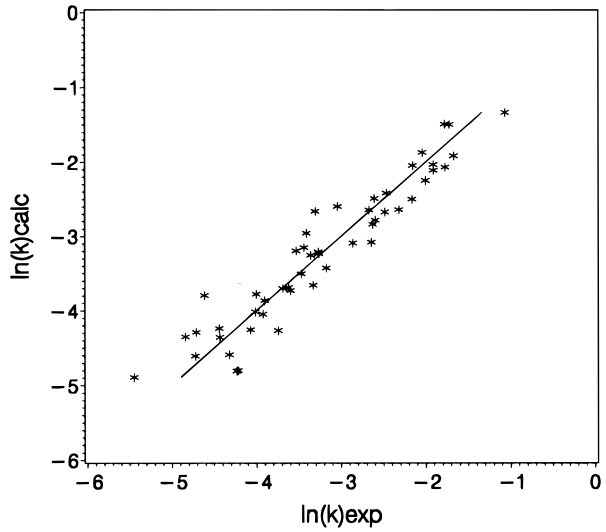

Figure 3. Correlation between the experimental activity retentions after isobaric−isothermal inactivation of LOX (0.4 mg/mL in 0.01 M Tris-HCl at pH 9) in the temperature range 10−40 °C and those calculated according to eq 9.

nonlinear regression procedure, applied to re-estimate the parameters from data obtained under variable conditions, could not converge to a minimal sum of squares and revealed the existence of some insignificant parameters. This can be explained from an experimental point of view. Indeed, dynamic experiments merely covered the temperature range 10−40 °C, in which the value of the activation volume seemed to be rather constant (Ludikhuyze et al., 1998). Therefore, the proposed model was simplified, assuming the V_a value to be constant, thereby yielding eq 9. The latter equation was tried to fit both static and dynamic inactivation data of LOX in the temperature range considered (10−40 °C).

$$\ln\left(\frac{A}{A_0}\right) = -\int_0^t \exp(a_2T^2 + b_2T + c_2) \exp\left(\frac{-V_{aT}}{R(T+273)}(P - P_{ref})\right) \quad (9)$$

First, the ability of the proposed model structure to predict isobaric−isothermal inactivation in the temperature range 10−40 °C was verified. Kinetic parameters together with standard deviations and corrected correlation coefficient are presented in Table 2. The high corrected r^2 and the satisfactory correlation between experimental and calculated activity retentions (Figure 3) ascertained the validity of the model in the temperature range considered. Percentage error on the predicted values varied between 0 and 20%. However, when parameter estimates obtained from static experiments were used to calculate the activity retention after inactivation under variable pressure and temperature conditions, a large and systematic overestimation of the activity retention was notified, pointing to an underestimation of the inactivation rate constant (Figure 4). For this reason re-estimation of the kinetic parameters from data obtained under dynamic conditions was evaluated (Table 2). Whereas upon re-estimation V_{aT}, a_2 and b_2 remained on the same order of magnitude, the value of c_2 was drastically changed. However, the low value for the corrected r^2 (0.894), as well as the large percentage error on the predicted values (35% of the data points are characterized by an error >50%), may put these results into perspective. Nevertheless, considerable improvement in predicting the activity retentions after inactivation under variable pressure and temperature conditions was observed when using the re-estimated parameters (Figure 4).

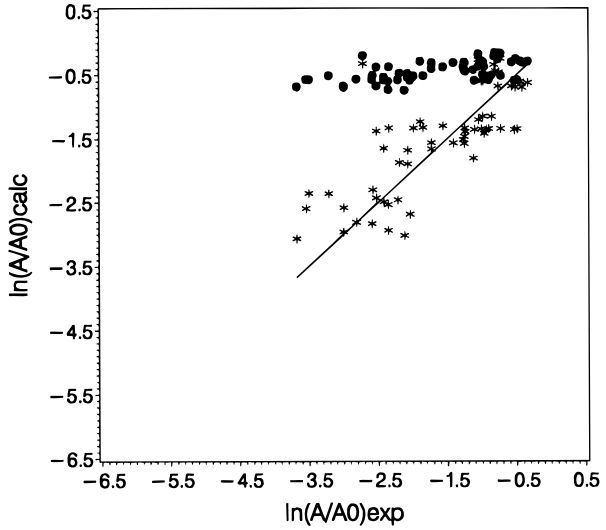

Figure 4. Improvement in fitting data for inactivation of LOX (0.4 mg/mL in 0.01 M Tris-HCl at pH 9) under dynamic conditions when using parameters estimated from dynamic (*) instead of static (●) conditions.

Several explanations can be postulated for this inability to estimate a set of kinetic parameters that is applicable for different experimental conditions. From an experimental point of view, calibration problems and variations in response time (time constant) of different thermocouples should be taken into account. As soybean LOX is known to be very heat sensitive, this problem should not be overlooked. From a mechanistic point of view, the history of the enzyme system prior to pressure−temperature treatment, which is different in static or dynamic experiments, may influence the inactivation mechanisms and hence the kinetics. Indeed, reference activity refers to a sample that has undergone a variable pressure−temperature profile in the case of static experiments (Ludikhuyze et al., 1998), whereas for dynamic experiments the reference sample is an untreated, native enzyme solution. A third possible explanation arises from a modeling point of view, because the kinetic model and corresponding parameters estimated from a static data set seem to be deficient in describing adequately a data set obtained under variable pressure−temperature conditions, thereby questioning the correctness of the parameters. Further research on these topics is needed to establish predictive modeling in design, evaluation, and optimization of high-pressure processing.

Table 3. Influence of Multicycling on the Inactivation of LOX, 0.4 mg/mL in 0.01 M Tris-HCl at pH 9

P/*T* treatment	total time (min)	cycling time (min)	activity retention
350 MPa, 40 °C	40	1 × 40	0.709
350 MPa, 40 °C	40	2 × 20	0.543
350 MPa, 40 °C	40	4 × 10	0.341
400 MPa, 35 °C	40	1 × 40	0.513
400 MPa, 35 °C	40	2 × 20	0.457
400 MPa, 35 °C	40	4 × 10	0.324
400 MPa, 40 °C	40	1 × 40	0.445
400 MPa, 40 °C	40	2 × 20	0.337
400 MPa, 40 °C	40	4 × 10	0.300
450 MPa, 25 °C	60	1 × 60	0.424
450 MPa, 25 °C	60	2 × 30	0.374
450 MPa, 35 °C	40	1 × 40	0.481
450 MPa, 35 °C	40	2 × 20	0.431
475 MPa, 10 °C	40	1 × 40	0.652
475 MPa, 10 °C	40	2 × 20	0.061
475 MPa, 15 °C	60	1 × 60	0.327
475 MPa, 15 °C	60	2 × 30	0.139
475 MPa, 20 °C	60	1 × 60	0.645
475 MPa, 20 °C	60	2 × 30	0.100
475 MPa, 25 °C	60	1 × 60	0.668
475 MPa, 25 °C	60	2 × 30	0.459
475 MPa, 25 °C	60	4 × 15	0.160
475 MPa, 30 °C	60	1 × 60	0.645
475 MPa, 30 °C	60	2 × 30	0.407
475 MPa, 30 °C	60	4 × 15	0.373
500 MPa, 10 °C	15	1 × 15	0.430
500 MPa, 10 °C	15	2 × 7.5	0.156
500 MPa, 10 °C	15	3 × 5	0.097
500 MPa, 15 °C	30	1 × 30	0.367
500 MPa, 15 °C	30	2 × 15	0.058
500 MPa, 15 °C	30	3 × 10	0.018
525 MPa, 20 °C	30	1 × 30	0.055
525 MPa, 20 °C	30	2 × 15	0.025
525 MPa, 25 °C	20	1 × 20	0.099
525 MPa, 25 °C	20	2 × 10	0.110
525 MPa, 30 °C	80	1 × 80	0.165
525 MPa, 30°c	80	2 × 40	0.127
525 MPa, 30 °C	80	4 × 20	0.062

Influence of Pressure Cycling on the Inactivation of Soybean LOX. The influence of multiple application of pressure on the inactivation of soybean LOX, 0,4 mg/mL in 0.01 M Tris-HCl at pH 9, was investigated by performing various experiments at the same pressure and temperature and for the same total treatment time but with various numbers of cycles. The latter experiments were carried out in the pressure range 350−525 MPa and the temperature range 10−40 °C (Table 3).

From Table 3 it can be seen that multiple application of pressure exerted an additional inactivation effect on LOX. This additional effect seemed to be most pronounced at low temperature. During low-temperature processes, the temperature inside the vessel drops below zero upon depressurization, which may be the reason for the enhanced inactivation effect. In addition, pressure inactivation of LOX has yet been shown to be more pronounced at low than at room temperature. Similar results have been obtained in previous research regarding the effect of pressure cycling on the inactivation of BSA (Ludikhuyze et al., 1997b). Crelier et al. (1995), on the other hand, contradicted the promising potentials of pressure cycling. They stated that pressure cycling exerted no additional inactivation effect on pectinmethylesterase (PME) of tomatoes up to pressures as high as 800 MPa. Irwe and Olsson (1994) stated that multiple application of pressure did not enhance the inactivation of orange PME at low pressure, whereas increased inactivation due to pressure cycling was noticed at pressure >600 MPa.

Moreover, augmenting the number of cycles seemed to further increase the extent of LOX inactivation. These results are somewhat conflicting with the ones obtained for BSA (Ludikhuyze et al., 1997b), which may be explained as follows. It has been shown that the temperature overshoot (due to adiabatic heating) accompanying each pressure cycle declines with increasing number of cycles. Along with depressurization, the temperature drops to a value lower than the starting value, causing the temperature reached when pressure is built up a second time to be lower. This phenomenon was assumed to be the reason why augmenting the number of cycles did not involve additional inactivation of BSA. However, because LOX is far more thermosensitive, this declining temperature overshoot may still be sufficient to account for the continued decrease in activity retention when cycling becomes more extensive.

Conclusions. By performing isobaric−isothermal inactivation experiments, the final step in the data analysis, that is, the formulation of an appropriate kinetic model describing the combined effect of pressure and temperature on the inactivation rate constant, can largely be simplified. In the case of LOX inactivation, a kinetic model was developed using the Eyring equation, which was valid in the entire pressure range studied, as starting point, and the attendant parameters were estimated. Subsequently, when the proposed kinetic model and attendant parameters were evaluated on their ability to predict activity retention after inactivation under variable conditions, kinetic parameters were noted to be changed. Next to deficiency of the proposed model structure, some other possible reasons were postulated both from mechanistic and from experimental points of view. Therefore, further research has to focus on refining the kinetic model, thereby ensuring its predictive power whatever the experimental conditions.

Furthermore, multiple application of high pressure seemed to enhance the extent of soybean LOX inactivation. Activity retentions after multicycle treatments were lower as compared to those after a single-cycle process for the same total treatment time, the effect becoming more pronounced at low temperature and when the number of cycles is increased. The latter effect was attributed to the high thermal sensitivity of soybean LOX.

ABBREVIATIONS USED

a_1, model parameter; a_2, model parameter; A, activity at time t; A_0, activity at time zero; b_1, model parameter; b_2, model parameter; BSA, *Bacillus subtilis* α-amylase; c_2, model parameter; i, 0 or 1 depending on the model structure; j, number of observations; k, inactivation rate constant (min^{-1}); k_{refP}, inactivation rate constant at reference pressure (min^{-1}); $k_{f(P,T)}$, mathematical expression of k as a function of pressure and temperature; LOX, lipoxygenase; p, number of parameters; P, pressure (MPa); P_{ref}, reference pressure (500 MPa); PME, pectinmethylesterase; PPO,polyphenoloxidase; R, universal gas constant (8.314 J/mol·K); T, temperature (°C); t, time (min); Tris, tris(hydroxymethyl)aminomethane; V_a, activation volume (cm^3/mol).

LITERATURE CITED

Aleman, G. D.; Ting, E. Y.; Mordre, S. C.; Hawes, A. C.; Walker, M.; Farkas, D. F.; Torres, D. A. Pulsed high-pressure treatments for pasteurisation of pineapple juice. *J. Food Sci.* **1996**, *61* (2), 388−390.

Barbosa-Canovas, G. V.; Pothakamury, U. R.; Palou, E.; Swanson, B. G. High-pressure food processing. In *Nonthermal Preservation of Foods*; Dekker: New York, 1997; pp 9−52.

Bouton, P. E.; Ford, A. L.; Harris, P. V.; Macfarlane, J. J.; O'Shea, J. M. Pressure-heat treatment of post-rigor muscle: effects on tenderness. *J. Food Sci.* **1977**, *42*, 132−135.

Butz, P. V.; Ries, J.; Traugott, U.; Weber, H.; Ludwig, H. Hochdruckinactivierung von Bakterien and Bakterien-sporen. *Pharm. Ind.* **1990**, *52* (4), 487−491.

Carnahan, B.; Luther, H. A.; Wilkes, J. O. Numerical Integration. In *Applied Numerical Methods*; Wiley: New York, 1969; pp 69−140.

Crelier, S.; Tâché, M.-C.; Renken, A.; Raetz, E. High pressure for the inactivation of enzymes in food products. Abstract of poster presentation, 9th World Congress on Food Science and Technology, Budapest, Hungary, July 31−Aug 4, 1995.

Curl, L.; Jansen, E. F. Effect of high pressure on trypsin and chymotrypsin. *J. Biol. Chem.* **1950a**, *184*, 45−54.

Curl, L.; Jansen, E. F. Effect of high pressure on pepsin and chymotrypsinogen. *J. Biol. Chem.* **1950b**, *185*, 713−723.

De Cordt, S. Feasibility of development of protein-based time−temperature-integrators for heat process evaluation. Ph.D. Dissertation 254, Katholieke Universiteit Leuven, Faculty of Agricultural and Applied Biological Sciences, Belgium, 1994.

Gould, G. W.; Sale, J. H. Initiation of germination of bacterial spores by hydrostatic pressure. *J. Gen. Microbiol.* **1970**, *60*, 335−346.

Irwe, S.; Olsson, I. Reduction of pectinersterase activity in orange juice by high-pressure treatment. In *Minimal Processing of Foods and Process Optimization: An Interface*; Singh, P. R., Oliveira, F. A. R., Eds.; CRC Press: Boca Raton, FL, 1994; pp 35−42.

Knorr, D. Effects of high hydrostatic pressure on food safety and quality. *Food Technol.* **1993**, *47* (6), 156−161.

Ludikhuyze, L. R.; Van den Broeck, I.; Weemaes, C. A.; Herremans, C. H.; Van Impe, J. F.; Hendrickx, M. E.; Tobback, P. P. Kinetics for isobaric−isothermal inactivation of *Bacillus subtilis* α-amylase. *Biotechnol. Prog.* **1997a**, *13*, 532−538.

Ludikhuyze, L. R.; Van den Broeck, I.; Weemaes, C. A.; Hendrickx, M. E.; Tobback, P. P. Kinetic parameters for pressure−temperature inactivation of *Bacillus subtilis* α-amylase under dynamic conditions. *Biotechnol. Prog.* **1997b**, *13*, 617−623.

Ludikhuyze, L. R.; Indrawati; Van den Broeck, I.; Weemaes, C. A.; Hendrickx, M. E. Effect of combined pressure and temperature on soybean lipoxygenase. 1. Influence of extrinsic and intrinsic factors on isobaric−isothermal inactivation kinetics. *J. Agric. Food Chem.* **1998**, *46*, 4074−4080.

Mertens, B. Hydrostatic pressure treatment of food: equipment and procesing. In *New Methods of Food Preservation*; Gould, G. W., Ed.; Blackie Academic and Professional: Glasgow, 1995; pp 135−158.

Ohmori, T.; Shigehisa, T.; Taji, S.; Hayashi, R. Effect of high pressure on the protease activities in meat. *Biol. Chem.* **1991**, *55* (2), 357−361.

Ohshima, T.; Ushio, H.; Koizumi, C. High-pressure processing of fish and fish products. *Trends Food Sci. Technol.* **1993**, *4*, 370−375.

Richwin, A.; Roosch, A.; Teichgräber, D.; Knorr, D. Effect of combined pressure and temperature on the functionality of egg-white proteins. *Eur. Food Sci.* **1992**, *43* (7/8), 27−31.

Sale, A. J. H.; Gould, G. W.; Hamilton, W. A. Inactivation of bacterial spores by hydrostatic pressure. *J. Gen. Microbiol.* **1970**, *60*, 323−334.

SAS Institute Inc. *SAS User's Guide: Statistics*; SAS Institute Inc.: Cary, NC, 1982.

Seyderhelm, I.; Bogulawski, S.; Michaelis, G.; Knorr, D. Pressure-induced inactivation of selected food enzymes. *J. Food Sci.* **1996**, *61* (2), 308−310.

Smelt, J. P. P. M.; Van Wely, E. J. M. Conservering van voedingsmiddelen met ultrahoge druk. *Voedingsmiddelentechnologie* **1993**, *14/15*, 11−13.

Styles, M. F.; Hoover, D. G.; Farkas, D. F. Response of *Listeria monocytogenes* and *Vibrio parahaemolyticus* to high hydrostatic pressure. *J. Food Sci.* **1991**, *56* (5), 1404−1407.

Van Loey, A. Enzymic time temperature integrators for the quantification of thermal processes in terms of food safety. Ph.D. Dissertation 319, Katholieke Universiteit Leuven, Faculty of Agricultural and Applied Biological Sciences, Belgium, 1996.

Weemaes, C.; Ludikhuyze, L.; Van den Broeck, I.; Hendrickx, M. Kinetics of combined pressure-temperature inactivation of avocado polyphenol oxidase. *Biotechnol. Bioeng.* **1998**, in press.

Yen, G.-C.; Lin, H.-T. Comparison of high-pressure treatment and thermal pasteurization on the quality and shelf life of guava puree. *Int. J. Food Sci. Technol.* **1996**, *31*, 205−213.

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