

Modelling of the inactivation kinetics of the trypsin inhibitors in soy flour

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Abstract: The inactivation kinetics of trypsin inhibitors (TIs) in soy flour was measured over a large range of temperatures (80–134°C) and moisture contents (0.08–0.52 g (g ds)⁻¹). The inactivation of TIs showed a two-phase inactivation behaviour. The influence of moisture content on the inactivation rate of TIs was large at moisture contents < 0.30 g (g ds)⁻¹. Six different inactivation kinetics models were used to describe the decrease of the trypsin inhibitor activity at constant moisture content. The models were compared statistically using a corrected Akaike information criterion. The most parsimonious models at moisture contents ≤ 0.30 g (g ds)⁻¹ were the model with two first-order reactions each for a different TI group, and the model with an irreversible inactivation of a native TI to a partially active intermediate TI, followed by a denaturation step. The *n*th order reaction model was favoured at moisture contents ≥ 0.40 g (g ds)⁻¹. The kinetics parameters of the model with two first-order reactions were modelled as a function of moisture content. The overall inactivation model described well the experimental inactivation data of TIs.

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Keywords: soy flour; trypsin inhibitor activity; kinetics; statistics; modelling

INTRODUCTION

Soybeans are a good source of proteins for animals and humans. However, a number of antinutritional factors (ANFs) are present in soybeans, of which the trypsin inhibitors (TIs) are generally considered as the most important. Two major groups of TIs can be distinguished: the Kunitz soybean trypsin inhibitor (KSTI) and the Bowman–Birk inhibitor (BBI). The TIs are heat labile and can be inactivated by a heat treatment such as steaming and extrusion cooking.¹

A kinetics model for the trypsin inhibitor activity (*TIA*) is useful in combination with a process model to design or optimise the heat treatment of soybeans. Inactivation kinetics of TIs has been investigated by several authors in different kinds of beans at different temperatures and moisture contents. DiPietro and Liener² observed a first-order reaction for *TIA* in defatted soy flour (75 ≤ *T* ≤ 95°C). Buerra *et al*³ found a first-order reaction for *TIA* in *Phaseolus vulgaris* (70 ≤ *T* ≤ 100°C). A two-phase inactivation for TIs in *Phaseolus vulgaris* (90 ≤ *T* ≤ 118°C) and soybeans (102 ≤ *T* ≤ 137°C) was observed by Roa *et al*⁴ and Van Zuilichem *et al*,⁵ respectively. This two-phase inactivation behaviour for TIs was also found during steaming of *Phaseolus vulgaris* beans.⁶ A possible explanation of this two-phase inactivation

behaviour is a difference in heat stability of KSTI and BBI. BBI generally has been considered to be more heat stable than KSTI, based on their stability in aqueous solutions.^{7,8} Rouhana *et al*⁹ found that both KSTI and BBI followed a first-order reaction in soymilk and that BBI was more heat stable than KSTI at temperatures below 137°C. Other literature reported that KSTI is equally¹⁰ or more heat stable^{2,11} than BBI in soy flour. Sanderson *et al*¹² suggested that thermal denaturation of KSTI in a potassium phosphate buffer is not a simple two-state process and that significant levels of at least one intermediate form must accumulate during denaturation.

The inactivation rate constant of TIs in soy flour as measured by DiPietro and Liener² was very dependent on moisture content at 95°C (5 ≤ *mc* ≤ 15%). Buerra *et al*³ found that the inactivation rate constant of TIs in *Phaseolus vulgaris* beans was maximal at 0.30 g (g ds)⁻¹. However, the differences in the reaction rate constants were small above 0.30 g (g ds)⁻¹. The inactivation rate constant of TIs in *Phaseolus vulgaris* of the first inactivation phase⁴ showed a maximum at 0.26 g (g ds)⁻¹. The inactivation rate constant of the second phase increased with increasing moisture content (0.10–0.47 g (g ds)⁻¹).

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Roa *et al*⁴ modelled the two-phase inactivation behaviour of TI in *Phaseolus vulgaris* with a discontinuous equation of two successive first-order reactions. Since the discontinuity of this equation was not defined, this kinetic model cannot be combined with process models.

There is clearly no agreement on the inactivation kinetics of TIs. Different authors have presented different models and different explanations about the underlying mechanism. A predictive model to describe the inactivation of TIs in soy flour at different temperatures and moisture contents has not yet been presented.

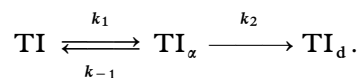
The first aim of this study was to set up an overall kinetics model to describe the inactivation of TIs in soy flour as a function of temperature and moisture content. The second aim was to compare mechanistically different kinetics models for their ability to describe the experimental data. Experimental inactivation data were measured within a large range of temperatures, moisture contents and residence times. When an inactivation experiment is started, the temperature of the sample increases until the equilibrium temperature has been reached. The temperature of the samples during the experiments must be known to estimate the kinetics parameters. A heat transfer model to predict the mean temperature of the sample during an inactivation experiment is incorporated within the estimation procedure of the kinetics parameters. Six kinetics models were fitted to the experimental data at constant moisture content and were compared statistically. The kinetics parameters of the most parsimonious kinetics model were modelled as a function of moisture content.

THEORY

Inactivation kinetics models

Different kinetics models have been used in the literature to describe the denaturation of proteins. In Table 1 six different inactivation kinetics models for TIs are presented. Model 1 describes a single first-order reaction. Model 2 describes the inactivation of two TI groups, eg KSTI and BBI. These two groups inactivate each with a different first-order reaction rate constant. Parameter *A* is the fraction of one of the two groups of TIs in the unprocessed sample.

Henley and Sadana¹³ have presented a model for a series-type of enzyme deactivation. The initial enzyme alters the TIs, by a first-order reversible reaction, to a partially active form which changes again by a first-order reaction to a completely inactive state. Assuming that only three forms of TIs exists – native TI, a partially active form TI_α and a complete denaturated form TI_d – the inactivation of TIs is as follows (model 3):



Parameter *α* is the activity fraction of TI_α. It is not *a priori* known if *α* is dependent on temperature or moisture content. Two simplified alternative readings of this model are also used to describe the inactivation of TIs. One reading describes a first-order irreversible reaction to a partially active intermediate TI_α followed by a first-order denaturation (*k*₋₁ = 0, model 4). This model was used by Henley and

Model	Integrated equation (constant <i>T</i> and <i>mc</i>)	Kinetics parameters (constant <i>mc</i>)
1	$\frac{C_t}{C_0} = e^{-kt}$	<i>E</i> _a , <i>k</i> _r
2	$\frac{C_t}{C_0} = A e^{-k_1t} + (1 - A)e^{-k_2t}$	<i>E</i> _{a1} , <i>E</i> _{a2} , <i>k</i> _{r1} , <i>k</i> _{r2} , <i>A</i>
3 ^a	$\frac{dC_{t,TI}}{dt} = -k_1 C_{t,TI} + k_{-1} C_{t,TI_{\alpha}}$ $\frac{dC_{t,TI_{\alpha}}}{dt} = k_1 C_{t,TI} - (k_{-1} + k_2) C_{t,TI_{\alpha}}$ $C_t = C_{t,TI} + \alpha C_{t,TI_{\alpha}}$	<i>E</i> _{a1} , <i>E</i> _{a-1} , <i>E</i> _{a2} , <i>k</i> _{r1} , <i>k</i> _{r-1} , <i>k</i> _{r2} , <i>α</i>
4	$\frac{C_t}{C_0} = \beta e^{-k_1t} + (1 - \beta)e^{-k_2t}$ $\left(\beta = 1 + \frac{\alpha k_1}{k_2 - k_1} \right)$	<i>E</i> _{a1} , <i>E</i> _{a2} , <i>k</i> _{r1} , <i>k</i> _{r2} , <i>α</i>
5 ^a	Same as model 3, except <i>α</i> = 0	<i>E</i> _{a1} , <i>E</i> _{a-1} , <i>E</i> _{a2} , <i>k</i> _{r1} , <i>k</i> _{r-1} , <i>k</i> _{r2}
6	$\ln(C_t) = \frac{\ln((n - 1)kt + C_0^{1-n})}{1 - n}$	<i>E</i> _a , <i>k</i> _{r1} , <i>n</i>

Model 1: first-order reaction; model 2: model with two TI groups; model 3: reversible reaction to a partially active intermediate TI_α followed by a denaturation step; model 4: irreversible reaction to a partially active intermediate TI_α followed by a denaturation step; model 5: reversible reaction to an inactive intermediate TI_{α=0} followed by a denaturation step and model 6: *n*th-order reaction
^a Integration of these equations is not possible

Table 1. Inactivation kinetics models and their parameters

Sadana¹³ to fit deactivation data of several enzymes. The other reading describes the inactivation of TIs by a first-order reversible reaction to an inactive intermediate $TI_{\alpha=0}$ followed again by a denaturation step ($\alpha = 0$, model 5). The n th-order reaction model is used as model 6. Such a model was used to describe the aggregation of β -lactoglobulin.¹⁴ Since no inactivation mechanism is given here, the n th-order reaction model for TIA is used for its predictive value only.

Different TI groups are assumed to inactivate with equal reaction rate constants for all models, except the model with two TI groups. Notice that the model with two TI groups (model 2) and the model with irreversible reaction to a partially active TI_{α} (model 4) are mathematically identical at constant temperature and moisture content (Table 1).

Reaction rate constants are assumed to be dependent on temperature following the Arrhenius equation. In order to diminish the correlation between the activation energy and the pre-exponential factor, the pre-exponential factors (k_r) were estimated at a reference temperature (T_r):

$$\ln(k) = \ln(k_r) - \frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_r} \right). \quad (1)$$

A reference temperature of 383 K was chosen. The kinetics parameters of Table 1 were estimated using the experimental data at different temperatures and at constant moisture content.

Comparison of the kinetics models

When models with different numbers of parameters are compared, the residual sum of squares (RSS) does not give enough information to discriminate between these models. The minimum corrected Akaike information criterion (AIC_c) produces a ranking of parsimonious models when the number of experimental data is small, or when the number of fitted parameters is a moderate to large fraction of the number of data.¹⁵

$$AIC_c = m \ln(S_w^2) + \frac{m(m+p)}{(m-p-2)} \quad (2)$$

in which:

$$S_w^2 = \frac{RSS}{m} \quad (3)$$

$$RSS = \sum_{i=1}^m \left(\frac{C(i) - \hat{C}(i)}{(C(i) + \hat{C}(i))/2} \right)^2 \quad (4)$$

MATERIAL AND METHODS

Materials

Defatted, untoasted soy flakes (protein dispersibility index = 80) from Cargill (Amsterdam, The Netherlands) were used. The initial moisture content

of the flour was $0.08 \text{ g (g ds)}^{-1}$. The TIA of the untreated flour was $23.3 \text{ mg (g ds)}^{-1}$. KSTI was obtained from Merck (art no 24020).

Conditioning of the soy flakes

The soy flakes were milled on a Retsch mill with a 0.2 mm sieve. The flour was moistened by adding water dropwise to the flour in a cooled blender. The flour was stored for 5 to 7 days at 4°C to equilibrate. The moisture content was measured according to AOAC.¹⁶

Inactivation experiments

A steel cell (1 mm steel thickness) was used for the inactivation experiments. The inner chamber of the cell (2 mm height and 7 cm diameter) was filled with 5 g soy flour and placed in a stirred oil or water bath. In a number of experiments the temperature in the middle of the cell was measured with a thermocouple. Experiments were performed at moisture contents of 0.08, 0.13, 0.23, 0.30 and $0.40 \text{ g (g ds)}^{-1}$, each at temperatures of 90, 104, 119 and 134°C , and at $0.52 \text{ g (g ds)}^{-1}$ at 80, 85, 90 and 104°C . After the heat treatment, the cell was immediately transferred to an ice-water bath. The flour was dried at 35°C for 24 h before the determination of TIA .

Trypsin inhibitor activity assay

TIA in soy flour was measured with trypsin-agarose chromatography as described by Roozen and De Groot¹⁷ with minor modifications. This assay was chosen because only the activity of the protein-like TIs is determined by this method. The samples were extracted with a 25 ml 0.015 M NaOH solution containing 0.5 M NaCl . The extraction solution was applied to the column. The column was subsequently washed with a 0.02 M Tris-HCl buffer (pH 8.0, 0.5 M NaCl) and a NaOAc buffer (pH 5.2, 0.5 M NaCl). TIs were eluted with a glycine-HCl buffer (pH 3.0, 0.5 M NaCl). The protein concentration in the effluent was measured using a modified Lowry method,¹⁷ using KSTI as a standard. The error (the difference between measured and mean value, divided by the mean value) of the analysis was 5%.

Physical properties of the soy flour

Heat conductivity of the flour was measured with a needle probe at 60°C at different moisture contents according to Pantaloni *et al.*¹⁸ Measured values were 0.135, 0.160 and $0.226 \text{ Wm}^{-1} \text{ }^\circ\text{C}^{-1}$ at 0.25, 0.32 and $0.41 \text{ g (g ds)}^{-1}$, respectively. The specific heat of soy flour was taken from Wallapapan *et al.*¹⁹

Estimation of the kinetics parameters

The characteristic inactivation time τ_r (defined as the time to attain 37% residual TIA) was in the same order of magnitude as the time needed for 99% temperature equilibration within the inactivation cell for several inactivation experiments. Therefore, a heat transfer model was incorporated within the estima-

tion procedure of the kinetics parameters. The soy flour in the cell was considered to be an infinite plate placed into a medium with a constant temperature assuming internal and external heat transfer resistance.²⁰ External heat transfer coefficients were estimated by fitting the heat transfer model to the measured temperatures in the cell. The coefficients were used to predict the mean temperature of the flour in the cell as a function of time during the inactivation experiments. The differential equations of the kinetics models were combined with the heat transfer model. Parameter α (models 3 and 4) was assumed to be independent of temperature. The total data set of TIA values was used to estimate the kinetics parameters of the overall kinetics model (Table 4). Estimation of the parameters was performed using the NLIN-procedure of SAS.²¹

RESULTS AND DISCUSSION

Figure 1 shows that the inactivation of TIs in soy flour does not follow first-order kinetics. A two-phase inactivation behaviour of TIs is observed. This two-phase inactivation behaviour is more pronounced at temperatures $\geq 104^\circ\text{C}$. This observation agrees with the findings of Roa *et al.*⁴ for TIs in *Phaseolus vulgaris*. If the inactivation kinetics of TIs consists of different reaction steps, differences in activation energies can result in a pseudo single first-order reaction at temperatures lower than $\sim 100^\circ\text{C}$ and a two-phase inactivation at temperatures higher than $\sim 100^\circ\text{C}$.

Figure 2 shows a large increase of the inactivation rate of TIs with increasing moisture content at low moisture contents ($< 0.3 \text{ g (g ds)}^{-1}$). The influence of moisture content on the inactivation rate is smaller at high moisture contents ($> 0.3 \text{ g (g ds)}^{-1}$): no difference in inactivation rate is observed between 0.40 and $0.52 \text{ g (g ds)}^{-1}$ at 104°C . The observation of the moisture content dependency of the inactivation rate

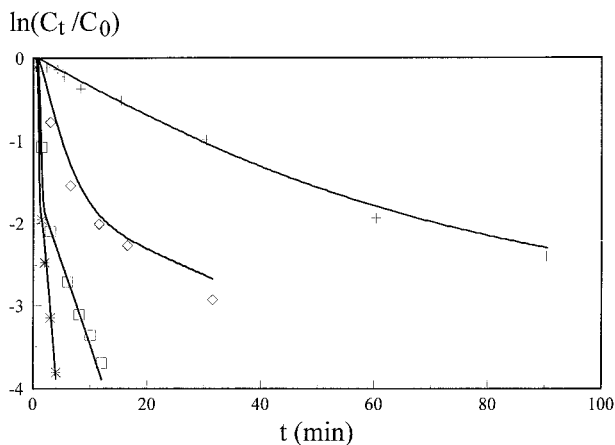


Figure 1. Measured and estimated (—) inactivation of TIs in soy flour ($mc = 0.23 \text{ g (g ds)}^{-1}$). Temperatures are: (+), 90°C ; (\diamond), 104°C ; (\square), 119°C ; (*) 134°C . Estimations were performed with the overall kinetics model (Table 4).

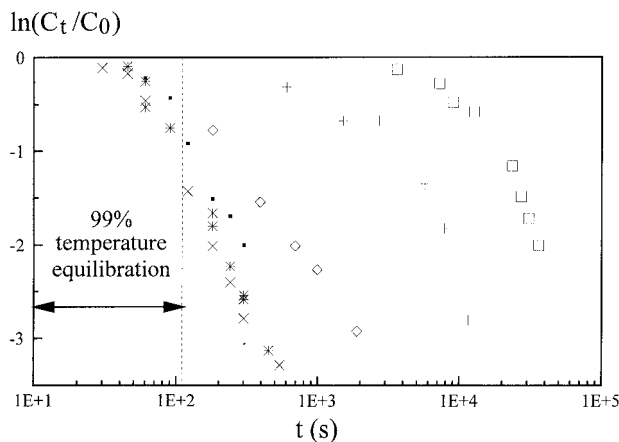


Figure 2. Influence of the moisture content on the inactivation of TIs in soy flour ($T = 104^\circ\text{C}$). Moisture contents are: (\square), 0.08 ; (+), 0.13 ; (\diamond) 0.23 ; (\blacksquare), 0.30 ; (\times), 0.40 ; (*), $0.52 \text{ g (g ds)}^{-1}$.

is in general agreement with the findings of Buerra *et al.*³ and Roa *et al.*⁴

The estimated time for 99% temperature equilibration is about 110 s. Figure 2 shows that the inactivation of TIs has already started in this heating-up period in several experiments. For example, the characteristic inactivation time τ_i at $0.40 \text{ g (g ds)}^{-1}$ and 104°C is lower than the time needed for 99% temperature equilibration. This makes the need of a heat transfer model to be applied obvious, yet this means that the accuracy of the heat transfer model plays an important role in the estimation of the kinetics parameters at very low τ_i values.

The results of the estimation of the kinetics parameters of the six inactivation kinetics models and the corresponding AIC_c values (eqn (2)) are given in Table 2 and 3, respectively. As expected from the experimental data in Fig 1, the deviation s_w of the first-order reaction model (model 1) is much higher than the deviation of the other models for all moisture contents. Also the estimated AIC_c values for the first-order reaction model are higher than the values for the other models. The first-order reaction model is clearly unfavourable compared to the other five models.

At moisture content $\leq 0.30 \text{ g (g ds)}^{-1}$ the model with the two TI groups (model 2) is the most parsimonious model compared to the other five models. The differences between the AIC_c values of the model with the two TI groups and the values of the irreversible model to a partially active TI_α (model 4) are small. The estimated kinetics parameters are almost equal for both models and A almost equals $(1 - \alpha)$. These two models are almost mathematically identical (Table 1) because $k_1 \gg k_2$ and $A \cong (1 - \alpha)$. Consequently, the AIC_c values of these two models will be almost equal. Since these models cannot be distinguished by comparing the AIC_c values, the estimated values of the parameters A and α are examined. Parameters A and α have a different physical meaning. Figure 3 shows that the estimated 95%

	Moisture content (g(g ds) ⁻¹)					
	0.08	0.13	0.23	0.30	0.40	0.52
Model 1						
$E_a \times 10^{-3}$	195	165	110	122	103	153
s_w	29	37	62	53	70	49
Model 2						
$E_{a1} \times 10^{-3}$	200	221	204	222	247	221
$E_{a2} \times 10^{-3}$	208	148	134	130	117	180
A	0.832	0.672	0.833	0.764	0.840	0.785
s_w	9	14	8	11	21	12
Model 3						
$E_{a1} \times 10^{-3}$	204	209	196	216	164	222
$E_{a-1} \times 10^{-3}$	241	226	182	207	39	667
$E_{a2} \times 10^{-3}$	314	143	124	103	51	207
α	0.000	0.000	0.113	0.000	0.105	0.171
s_w	9	13	8	11	15	11
Model 4						
$E_{a1} \times 10^{-3}$	200	215	199	221	249	210
$E_{a2} \times 10^{-3}$	253	146	129	124	113	178
α	0.073	0.311	0.166	0.229	0.154	0.203
s_w	9	15	8	11	21	12
Model 5						
$E_{a1} \times 10^{-3}$	204	209	195	218	230	211
$E_{a-1} \times 10^{-3}$	246	227	194	210	185	214
$E_{a2} \times 10^{-3}$	334	143	126	104	67	176
s_w	9	13	8	11	17	12
Model 6						
$E_a \times 10^{-3}$	209	184	157	157	199	184
n	1.48	1.79	2.04	2.53	2.80	2.20
s_w	11	17	13	13	17	11

Table 2. Kinetics parameters (E_a 's, A , α and n) and deviation s_w of the inactivation kinetics models at different moisture contents

confidential intervals of parameter A overlap each other and that no trend in the values of A with moisture content is to be seen. The same can be concluded for α . The conclusion that the estimated parameter A is independent of moisture content agrees with its physical meaning. Parameter α should not be independent of moisture content *per se* from a mechanistic point of view. Both models can be correct with respect to these results.

The n th-order reaction model (model 6) has the lowest AIC_c values of the six models at 0.40 and

0.52 g (g ds)⁻¹. However, the values of this model are higher at moisture contents ≤ 0.30 g (g ds)⁻¹ than the values of the model with two TI groups and the irreversible model with a partially active TI $_{\alpha}$. The estimated reaction order n is dependent on moisture content (Table 2) and the estimated 95% confidence intervals of n do not overlap each other. Considering that the model with two TI groups and the irreversible model with a partially active TI $_{\alpha}$ are most parsimonious at $mc \leq 0.30$ g (g ds)⁻¹, and that the

Table 3. AIC_c values of the inactivation kinetics models at different moisture contents

Model	p	Moisture content (g(g ds) ⁻¹)					
		0.08	0.13	0.23	0.30	0.40	0.52
1	2	-41	-16	8	1	16	-9
2	5	-93	-43	-77	-52	-45	-84
3	7	-83	-39	-69	-45	-52	-87
4	5	-90	-42	-77	-52	-46	-84
5	6	-87	-44	-72	-50	-48	-79
6	3	-84	-37	-56	-51	-58	-91
m		32	23	25	23	31	35

The lowest AIC_c values are given in bold

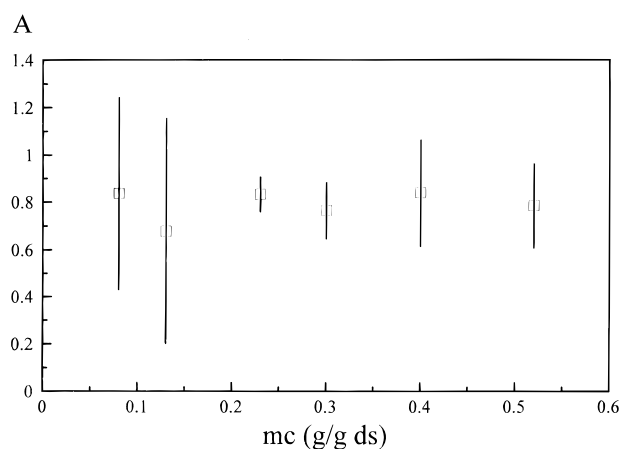


Figure 3. Influence of moisture content on the estimated parameter A (model 2) and its 95% confidence intervals.

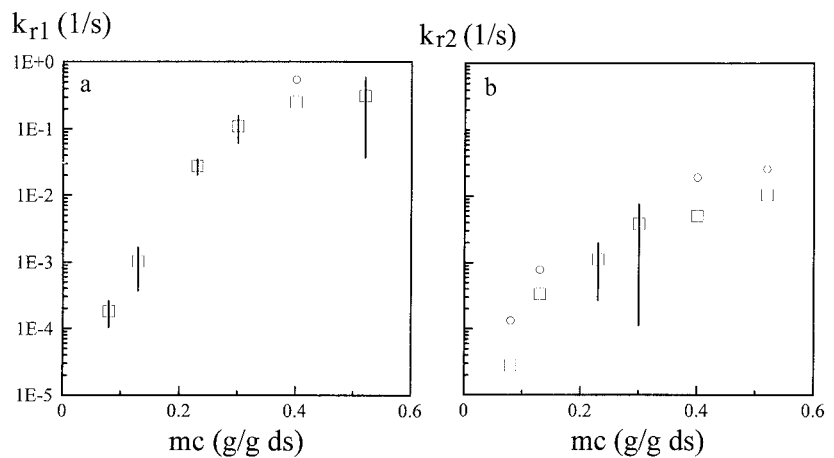


Figure 4. Influence of the moisture content on the estimated pre-exponential factors (a) k_{r1} and (b) k_{r2} and their 95% confidence intervals. In some cases only the upper 95% confidence interval is shown (○) because the estimated lower interval was negative.

n th-order reaction model is most parsimonious at $mc \geq 0.40 \text{ g (g ds)}^{-1}$, the mechanism of TI inactivation might be dependent on moisture content.

The model with two TI groups (model 2) and the irreversible model to a partially active TI_α (model 4) can be considered more parsimonious than the reversible model with inactive $\text{TI}_{\alpha=0}$ (model 5), yet the difference between these models is small.

The AIC_c values of the reversible model with partially active TI_α (model 3) are in 4 of 6 cases higher than the values of its two readings (model 4 and 5). The lower AIC_c value for the reversible model with partially active TI_α at $0.40 \text{ g (g ds)}^{-1}$ can possibly be explained by the experimental conditions. It was mentioned previously that the accuracy of the heat transfer model plays an important role in the estimation of the kinetics parameters at very low τ_r (eg at $mc = 0.40 \text{ g (g ds)}^{-1}$ and $T = 134^\circ\text{C}$). This might also explain the low activation energy E_{a-1} for this model at $0.40 \text{ g (g ds)}^{-1}$ (Table 2).

The estimated activity fraction α of the intermediate TI_α of this model (3) was zero at moisture contents of 0.08, 0.13 and $0.30 \text{ g (g ds)}^{-1}$. The estimated 95% confidence intervals of α at the other moisture contents overlapped zero. These results indicate that model 3 is equal to the reversible model with inactive $\text{TI}_{\alpha=0}$ (model 5).

Both the introduction of a partially active intermediate TI_α in model 4 and the introduction of the reversibility of the reaction in model 5 are sufficient to describe the two-phase inactivation behaviour of TIs. This might explain that the combination of the partially active intermediate TI_α and the reversibility in one model does not statistically give better fits. It is therefore concluded that this model is worse than its two readings.

The model with two TI groups and the irreversible model with partially active TI_α are parsimonious models that describe the inactivation of TIs well. For engineering purposes, both models are suitable to describe the inactivation of TIs. The model with two TI groups was chosen to develop an overall inactivation kinetics model of TIs.

It was previously concluded that parameter A is independent of moisture content. Figures 4 and 5 show the estimated pre-exponential factors and activation energies with their estimated 95% confidence intervals at different moisture contents. The logarithm of the pre-exponential factors k_{r1} and k_{r2} were assumed to be dependent on moisture content following a logistic-like equation. The 95% confidence intervals of the activation energy E_{a1} overlap each other and the estimated values of E_{a1} do not show a trend with moisture content. E_{a1} is therefore

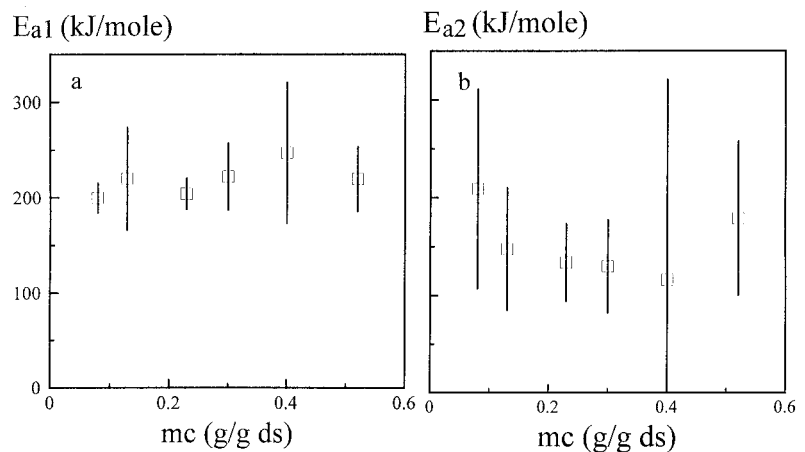


Figure 5. Influence of the moisture content on the estimated activation energies (a) E_{a1} and (b) E_{a2} and their 95% confidence intervals.

Table 4. Kinetics parameters of the overall inactivation kinetics model (model 2 from Table 2 is used)

	k_{r1}	$E_{a1} \times 10^{-3}$	k_{r2}	$E_{a2} \times 10^{-3}$	A
a	-11.0	205	-5.95	49.2	0.827
b	9.61		3.62	253	
c	2.31		-0.382	0.893	
d	-15.2				

$$k_{r1} = a + b/(1 + e^{dmc+c}); k_{r2} = a + bmc + c/mc; E_{a2} = a + bmc + c/mc^2; E_{a1} \text{ and } A \text{ are independent of moisture content}$$

assumed to be independent of moisture content. Also, the 95% confidence intervals of E_{a2} overlap each other. The estimated values of E_{a2} show, however, a hyperbolic-like trend with moisture contents. Different overall kinetics models with E_{a2} dependent on and independent of moisture content were therefore investigated. The overall model that gave the best fit is shown in Table 4. This overall model results in a deviation s_w of 14.5%. This deviation is low compared to the average deviation of the sum of fits of the inactivation data at constant moisture content (12.5%, Table 2). Figure 1 shows the estimated inactivation of TIs by the overall kinetics model at a moisture content of $0.23 \text{ g (g ds)}^{-1}$.

CONCLUSIONS

The inactivation of TIs in soy flour showed a two-phase inactivation behaviour. The inactivation rate of TIs increased with increasing moisture content. The rate was less dependent on moisture content at moisture content $> 0.30 \text{ g (g ds)}^{-1}$. Consequently, an increase in moisture content above $0.30 \text{ g (g ds)}^{-1}$ during processing is useless from a kinetics point of view. The most parsimonious kinetics models at moisture contents $\leq 0.30 \text{ g (g ds)}^{-1}$ were a model assuming two first-order reactions for two TI groups, and a model with an irreversible inactivation of a native TI to a partially active intermediate TI, followed by a denaturation step. The n th-order reaction model showed the best results at moisture content $\geq 0.40 \text{ g (g ds)}^{-1}$. An overall kinetics model was developed that described the inactivation of TIs within a large range of temperatures and moisture contents. This model can be used in combination with models for processes such as steaming and extrusion cooking to design and optimise the heat treatment.

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NOMENCLATURE

a	Fitting parameter ($\text{J mol}^{-1}, \text{s}^{-1}$)
A	Activity fraction of one of the two TI groups at $t = 0$
AIC_c	Corrected Akaike information criterion
b	Fitting parameter ($\text{s}^{-1}, (\text{g ds}) \text{s}^{-1} \text{g}^{-1}, \text{J (g ds)} \text{g}^{-1} \text{mol}^{-1}$)
c	Fitting parameter ($-, \text{g s}^{-1} (\text{g ds})^{-1}, \text{J g}^2 \text{mol}^{-1} (\text{g ds})^{-2}$)
$C(i)$	Measured trypsin inhibitor activity (mg (g ds)^{-1})
$\hat{C}(i)$	Estimated trypsin inhibitor activity (mg (g ds)^{-1})
d	Fitting parameter ($(\text{g ds}) \text{s}^{-1} \text{g}^{-1}$)
E_a	Activation energy (J mol^{-1})
k	Reaction rate constant ($(\text{mg (g ds)}^{-1})^{1-n} \text{s}^{-1}$)
k_r	Pre-exponential factor at reference temperature T_r , ($(\text{mg (g ds)}^{-1})^{1-n} \text{s}^{-1}$)
m	Sample size
mc	Moisture content (g (g ds)^{-1})
n	Reaction order
p	Number of parameters
R	Gas constant ($\text{J K}^{-1} \text{mol}^{-1}$)
RSS	Residual sum of squares
s_w	$\sum \frac{ C(i) - \hat{C}(i) }{C(i)/m} \times 100$ (%)
S_w^2	Weighted variance
T	Temperature (K)
T_r	Reference temperature (K)
TI	trypsin inhibitor
TIA	trypsin inhibitor activity (mg (g ds)^{-1})
t	time (s)
<i>Greek:</i>	
α	Fraction of activity of TI_α
β	Constant
τ_r	Characteristic inactivation time (s)
<i>Subscript:</i>	
0	At time $t = 0$
1, -1, 2	Reaction step
d	Completely inactivated
t	At time t
α	Intermediate partially active TI

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