Modelling of heat treatment of soy

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Stellingen

1. Het verschil in hittestabiliteit van de twee belangrijkste groepen trypsineremmers, de Kunitz- en de Bowman-Birk-remmer, is niet, zoals soms wordt gesteld, de verklaring voor het twee-fasen-inactiveringsgedrag van de trypsineremmers in soja. Thiolen lijken een belangrijke invloed te hebben op het inactiveringsgedrag van de trypsineremmers.

   Dit proefschrift, Hoofdstuk 3.

2. Het verdampen en elders weer condenseren van water tijdens de enkelschroefs-extrusie van biomaterialen krijgt bij het modelleren van de warmte-overdracht in een enkelschroefsextruder te weinig aandacht.

3. De productkwaliteit van sojabonen kan bij stomen beter geoptimaliseerd worden door verandering van het initiële vochtgehalte van de bonen dan, zoals vaak wordt aangenomen, door aanpassing van de stoomtemperatuur.

   Dit proefschrift, Hoofdstuk 5.

4. Bij onderzoek naar het efficiënter gebruiken van de voedingswaarde van sojabonen moet, behalve naar de optimalisatie van individuele processen in de keten sojaboon-veevoeder-vlees, ook naar alternatieve voedingsketens gekeken worden waarbij sojabonen rechtstreeks in levensmiddelen worden verwerkt.

5. Het milieu-argument bij het stimuleren van het thuiswerken is 's winters zeer dubieus, gezien het hogere energieverbruik door de verwarming van de eigen woning.

6. De toenemende mobiliteit van de reiziger heeft een groter effect op de totale milieubelasting dan de keuze voor een bepaald vervoersmiddel (auto, trein of vliegtuig) die de reiziger voor zijn/haar vervoer maakt.

7. Sommige technieken om levensmiddelen langer houdbaar te maken, zoals het verlagen van de zuurgraad en het toevoegen van suikers, zijn helaas slecht voor de houdbaarheid van de tanden.

8. Er zou bij de technische opleidingen meer aandacht besteed moeten worden aan de geschiedenis van de techniek en de samenhang hiervan met ontwikkelingen in de samenleving.

9. Talen en cultuurstudies zouden in een dienstverlenend land als Nederland meer aandacht moeten krijgen tijdens de opleidingen, juist in een tijd van globalisering.

10. De complexiteit van de plots van 'filmspotjes' in reclameblokken benadert inmiddels die van vele reguliere speelfilms.

Stellingen behorende bij het proefschrift "Modelling of heat treatment of soy".

Rob van den Hout
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Contents

1. Introduction

General introduction

Soybeans are the dominant oilseed in the world market. During a normal production year, soybeans make up about one-half of all oilseeds produced world-wide. The total world production of soybeans accounts for almost 134 million metric tons (forecast for 1996/1997), and is expected to continue to increase as long as the world population increases and people continue to upgrade their diet to include more fats, oils and livestock-based proteins. The United States (47%), Brazil (20%), China (10%) and Argentina (10%) are the dominant sources for soybeans (Mielke, 1996). Soybeans are popular because of their high oil (20%) and protein content (39%). The protein content in defatted soybean meal is about 48% compared to 25-30% for other beans (Belitz and Grosch, 1985). Soybean oil is used for baking-, frying-, salad- and cooking oil and margarine. Soybean meal is used extensively in high-protein commercial feeds for poultry and swine, and to a lesser extend for beef cattle. A small amount of soybean meal is used for human foods.

Consumption of raw seeds has negative effects on the growth and health of human beings and animals. These negative effects are caused by antinutritional factors (ANFs). Several reviews have been published on the ANFs subject (Liener, 1994, Huisman, 1990 and Rackis *et al*, 1986). ANFs in plants and seeds often act as biopesticides, protecting the seed against attacks of moulds, bacteria, insects and birds. The main classes of ANFs in soybeans are the protease inhibitors, lectines, flatulence factors and allergens.

The trypsin inhibitors (TIs) are generally regarded as the main ANFs in soybeans. TIs cause an inhibition in the growth of chicks, rats and mice, a depression in the digestibility of the proteins in the diet, and an enlargement of the pancreas (Liener, 1994 and Huisman, 1990). The TIs can be divided in two main groups: the Kunitz soybean trypsin inhibitor (KSTI) and the Bowman-Birk inhibitor (BBI). KSTI has a molecular weight of about 20,000 g mol^{-1}, two disulphide bridges, and inhibits primarily trypsin. BBI has a molecular weight of 6000 to 10,000 g mol^{-1}, a high proportion of disulphide bridges, and the capability of inhibiting trypsin as well as chymotrypsin at independent sites (Liener, 1994). It is noteworthy that, when an average TI content in soybeans of 20 mg g^{-1} is assumed, the annual world production of TIs from soybeans is about 2.7 million metric tons!

TIs and some of the other ANFs are heat labile and are generally inactivated by atmospheric steaming. Other heat treatments like pressurised steaming, extrusion cooking and expander cooking are also used. Over-processing reduces the availablilty of the proteins. When the process cost for steaming is about 16 Dutch guilders per ton beans (Melcion and Van der Poel, 1993), the annual cost for steaming the world production of soybeans is estimated to be 2 billion Dutch guilders.

The residual feed quality of the beans after a heat treatment can be measured *in vivo* or *in vitro* (Figure 1). *In vivo* experiments are costly and time consuming. Therefore, the adequacy of a heat treatment on the feed quality is mostly evaluated by *in vitro* measurements.

process parameters		bean quality parameter (*in vitro*)		animal performance (*in vivo*)
temperature	↔	TIA	↔	feed conversion
moisture content		UA		faecal digestibility
residence time		NSI		ileal digestibility
bean size		PDI		
shear		available lysine		

Fig. 1. Connection between process parameters, bean quality parameters, and animal performance.

The most used *in vitro* analyses are trypsin inhibitor activity (TIA), urease activity (UA), nitrogen solubility index (NSI), protein dispersibility index (PDI), and available lysine. TIA and UA are considered to indicate under-processing, NSI and PDI under- and over-processing (Dale *et al*, 1990), and available lysine over-processing. In this thesis residual TIA levels were followed to evaluate the effect of the heat treatment on ANFs in soybean. The reduction of the protein availability was followed by determining residual NSI and available lysine levels.

The heat treatments of soybeans are usually considered as 'black boxes'. Process design and control are usually based on experience and 'trial-and-error'. Sometimes a small number of plant experiments are performed to get a quick insight in the significance of a process parameter (e.g. residence time) on the product quality. The number of experiments to be performed, however, increases when the number of process parameters increases (e.g. process temperature, initial moisture content, and residence time). This involves a substantial number of expensive experimental runs. Moreover, optimisation and design of the process are difficult to perform.

An alternative is the 'white box' approach. In the 'white box' approach, *process models* (e.g. heat and mass transfer during steaming) and *reaction kinetics models* (e.g. decrease of TIA and NSI) using *physical properties* of the feed (e.g. water and heat diffusion coefficient, and heat conductivity) are combined to predict the change of feed quality during the heat treatment. In this approach experiments are necessary to determine the kinetics and physical parameters and to validate the overall model. However, most of these experiments are performed on a less expensive lab-scale. The combined kinetics and process model is validated with only a few additional plant experiments. Once the model is validated, predictions can be performed with none or only very few experiments.

A brief state of the art of the inactivation kinetics of TIs and the influence of steaming and extrusion cooking on the inactivation of TIs will be discussed in the next paragraphs. Next, the aim and the outline of this thesis will be presented.

Inactivation kinetics of TIs

Kinetics model

The inactivation kinetics of TIs in beans was investigated by several authors at different temperatures and moisture contents. DiPietro and Liener (1989) observed a first order reaction for TIA in defatted soy flour ($75 \leq T \leq 95$ °C). Buerra *et al* (1984) found a first order reaction for TIA in Phaseolus Vulgaris beans ($70 \leq T \leq 100$ °C). A two-phase inactivation behaviour for TIs in Phaseolus Vulgaris beans ($90 \leq T \leq 118$ °C) and soybeans ($102 \leq T \leq 137$ °C) was observed by Roa *et al* (1989) and Van Zuilichem *et al* (1993), respectively. This two-phase inactivation behaviour for TIs was also found during steaming of Phaseolus Vulgaris ($102 \leq T \leq 136$ °C; Van der Poel, 1990).

The inactivation rate constant of TIs in soy flour measured by DiPietro and Liener (1989) was strongly dependent on moisture content at 95 °C ($5 \leq mc \leq 15\%$). Buerra *et al* (1984) found that the inactivation rate constant of TIs in Phaseolus Vulgaris was maximal at 0.30 g (g ds)$^{-1}$. The inactivation rate contstant of the first inactivation phase of TIs in Phaseolus Vulgaris (Roa *et al*, 1989) showed a maximum at 0.26 g (g ds)$^{-1}$. The rate constant of the second phase increased with increasing moisture content (0.10-0.47 g (g ds)$^{-1}$).

Roa *et al* (1989) modelled the two-phase inactivation behaviour of TIs in Phaseolus Vulgaris with a discontinous equation of two successive first order reactions. Since the discontinuity of this equation was not defined, this kinetics model can not be combined with process models.

In concluding, no experimental inactivation data of TIs in soy flour within a large range of process conditions of temperature and moisture content are available in literature. A continous inactivation kinetics model to predict TIA in soy flour as a function of temperature and moisture content was not presented.

Inactivation mechanism

A two-phase inactivation behaviour for TIs in soybeans and Phaseolus Vulgaris beans was found by several authors (Roa *et al*, 1989, Van der Poel, 1990 and Van Zuilichem *et al*, 1993). Different explanations for this phenomenon were presented. One explanation is a difference in heat stability of the two major TI groups: KSTI and BBI. BBI generally has been considered to be more heat stable than KSTI, because of the heat stability of BBI in aqueous solutions (Birk, 1961 and Obara and Watanabe, 1971). Rouhana *et al* (1996) found that BBI in soymilk was more heat stable than KSTI at temperatures below 137 °C. Other literature reported that KSTI is equal (Liener and Tomlinson, 1981) or more heat stable (DiPietro and Liener, 1989 and Friedman *et al*, 1991) than BBI in soy flour. The results in literature about the heat stability of KSTI and BBI are clearly conflicting.

Roa *et al* (1989) and Van Zuilichem *et al* (1993) referred to Multon and Guilbot (1975) to explain the two-phase inactivation behaviour of TIs in beans. Multon and Guilbot (1975) explained the two-phase inactivation behaviour of ribonuclease in wheat grains by the catalytic role of water during the inactivation. The role of water in the two-phase inactivation behaviour of TIs in soybeans was not investigated and is therefore not known.

Heat processing

Steaming

Steaming (toasting) is mostly applied in the animal feed industry to inactivate the ANFs. Rackis (1974) and Smith *et al* (1964) studied the effect of atmospheric steaming on the nutritional value of soya meal. Jansen *et al* (1985) examined the effect of steaming at about 95 °C on the residual TIA, protein solubility and UA in soybeans. With pressurised steaming an additional degree of freedom, the steam temperature, is introduced. The use of pressurised steaming results in shorter residence times, so called HTST (High Temperature Short Time) processes. Additionally, pressurised steaming might result in a change in availability of essential amino-acids. The effect of autoclaving on the residual TIA and NSI in soya meal and feed-weight gain ratio of chickens was investigated by Dale *et al* (1990). The effect of pressurised steaming on residual TIA, PDI, UA and ileal digestibility of soybeans was studied experimentally by Qin *et al* (1996).

Although experimental data are available, no model to predict the feed quality during steaming is presented in these studies.

Extrusion cooking

The effect of extrusion cooking on the extrudate characteristics is widely described in literature. Heat is transferred by convection through the barrel and generated by viscous dissipation due to shear stresses. Proteins like TIs are denaturated by the high temperature of the soy during extrusion cooking. Several authors studied the effect of temperature, feed moisture content, and screw speed on extrudate characteristics such as TIA and available lysine (Hendrix *et al*, 1994 and Mustakas *et al*, 1970). Petres *et al*

(1993) and Aguilera and Kosikowski (1976) used response surface analysis to study the effect of these process parameters on TIA.

Contrary to steaming, shear forces are involved during extrusion cooking. Mechanical degradation during plastic polymer extrusion is related to molecular siccion induced by the application of stresses. This results in a reduction of the molecular weight of the plastic polymers (Rauwendaal, 1994). Little is known about the effect of shear forces on the denaturation of proteins during extrusion cooking. Shear can cause physical deformation of proteins and therefore possibly has an additional denaturation effect on proteins. Marsman *et al* (1995) studied the influence of shear forces on some chemical, physical and physiological factors of toasted soybean meal during extrusion cooking. They found no correlation between different shear levels and animal performance. If TIs in soy flour are inactivated by the deformation of the TI molecules, this will result in an extra process parameter to optimise the heat treatments where shear is involved, such as extrusion- and expansion cooking.

It is unknown if the deformation of proteins during extrusion cooking has any effect on the denaturation of proteins or on the inactivation of TIs particularly.

Aim of this thesis

The first aim of this thesis is to model the influence of steaming on the product quality of soybeans. The 'white-box' approach has been worked out in Figure 2. The inactivation kinetics of TIs in soy flour is measured and modelled under a large range of temperatures and moisture contents. A process model for steaming is set up and combined with the kinetics models for TIA and NSI to predict the residual TIA and NSI levels in the steamed soybeans. The combined model is used to optimise the feed quality of soybeans using TIA and NSI as quality parameters.

Second aim is to investigate the mechanism of TI inactivation in soy flour. The inactivation kinetics of KSTI and BBI is studied.

Third aim is to investigate the influence of shear forces on the inactivation of TIs in soy flour during extrusion cooking. The influence is examined by performing theoretical calculations and extrusion cooking experiments.

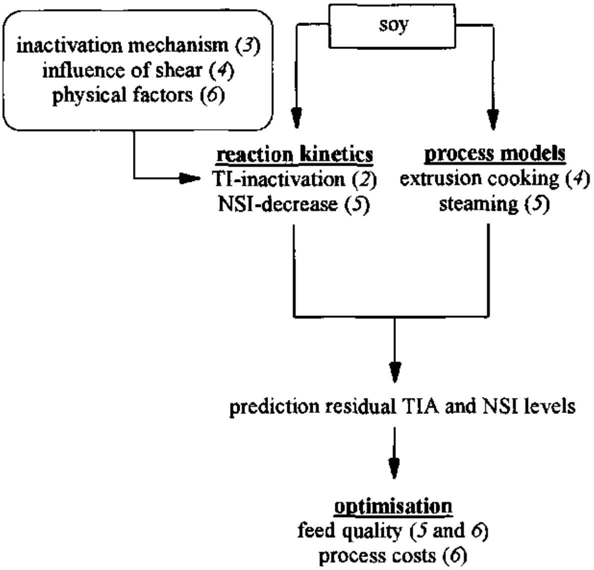

Fig. 2. Outline of the thesis. Chapter numbers are between brackets.

Outline of this thesis

Figure 2 shows an overview of the topics that are covered in this thesis. The inactivation kinetics of TIs is measured and modelled in Chapter 2. The inactivation mechanism of TIs in soy flour is studied in Chapter 3. In Chapter 4, the influence of extrusion shear forces on the inactivation of TIs in soy flour is studied theoretically and experimentally. In Chapter 5, the steaming process is modelled. The kinetics of NSI-decrease in soy flour is determined in separate experiments. The decrease of TIA and NSI in soybeans during steaming is predicted and compared with experimental data. In Chapter 5 and 6, the feed quality of soybeans during steaming is optimised using TIA and NSI as quality parameters. Furthermore, some additional aspects on the inactivation kinetics of TIs will be discussed in Chapter 6.

Symbols

ANF	antinutritional factors
BBI	Bowman-Birk inhibitor
KSTI	Kunitz soybean trypsin inhibitor
NSI	nitrogen solubility index
PDI	protein dispersibility index
TI	trypsin inhibitor
TIA	trypsin inhibitor activity
UA	urease activity

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

Abstract

The inactivation kinetics of tryspin inhibitors (TIs) in soy flour was measured within 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 the 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 criterium. 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 nth order reaction model was favored 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 the experimental inactivation data of TIs well.

This chapter has been submitted as:

Rob van den Hout, Gerrit Meerdink, Klaas van 't Riet. Modelling of the inactivation kinetics of the trypsin inhibitors in soy flour.

Introduction

Soybeans are a good source of proteins for animals and human beings. However, a number of antinutritional factors (ANFs) are present in soybeans, of which the trypsin inhibitors (TIs) are generally considered as the most important ANFs. Two major groups of the 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 (Liener, 1994).

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 Liener (1989) observed a first order reaction for TIA in defatted soy flour ($75 \leq T \leq 95$ °C). Buerra *et al* (1984) found a first order reaction for TIA in Phaseolus Vulgaris beans ($70 \leq T \leq 100$ °C). A two-phase inactivation for TIs in Phaseolus Vulgaris beans ($90 \leq T \leq 118$ °C) and soybeans ($102 \leq T \leq 137$ °C) was observed by Roa *et al* (1989) and Van Zuilichem *et al* (1993), respectively. This two-phase inactivation behaviour for TIs was also found during steaming of Phaseolus Vulgaris beans (Van der Poel, 1990). 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 (Birk, 1961 and Obara and Watanabe, 1971). Rouhana *et al* (1996) 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 equally (Liener and Tomlinson, 1981) or more heat stable (DiPietro and Liener, 1989 and Friedman *et al*, 1991) than BBI in soy flour. Sanderson *et al* (1982) 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 Liener (1989) was very dependent on moisture content at 95 °C ($5 \leq mc \leq 15\%$). Buerra *et al* (1984) 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 phase (Roa *et al*, 1989) 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 $(g\ ds)^{-1}$).

Roa *et al* (1989) modelled the two-phase inactivation behaviour of TI in Phaseolus Vulgaris with a discontinous equation of two successive first order reactions. Since the discontinuity of this equation was not defined, this kinetics model can not be combined with process models.

There is clearly no agreement on the inactivation kinetics of TIs. Different authors 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 was not presented yet.

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. Second aim was to compare mechanistically different kinetics models in 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 incorperated 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 are used in 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, e.g. 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.

Table 1. Inactivation kinetics models and their parameters

model	integrated equation (constant T and mc)	kinetics parameters (constant mc)
1	$\frac{C_t}{C_0} = e^{-k \cdot t}$	E_a, k_r
2	$\frac{C_t}{C_0} = A \cdot e^{-k_1 \cdot t} + (1 - A) \cdot e^{-k_2 \cdot t}$	E_{a1}, E_{a2}, k_{r1}, k_{r2}, A
3 1)	$\frac{dC_{t,TI}}{dt} = -k_1 \cdot C_{t,TI} + k_{-1} \cdot C_{t,TI_\alpha}$ $\frac{dC_{t,TI_\alpha}}{dt} = k_1 \cdot C_{t,TI} - (k_{-1} + k_2) \cdot C_{t,TI_\alpha}$ $C_t = C_{t,TI} + \alpha \cdot C_{t,TI_\alpha}$	E_{a1}, E_{a-1}, E_{a2}, k_{r1}, k_{r-1}, k_{r2}, α
4	$\frac{C_t}{C_0} = \beta \cdot e^{-k_1 \cdot t} + (1 - \beta) \cdot e^{-k_2 \cdot t}$; $\left(\beta = 1 + \frac{\alpha \cdot k_1}{k_2 - k_1}\right)$	E_{a1}, E_{a2}, k_{r1}, k_{r2}, α
5 1)	same as model 3, except $\alpha=0$	E_{a1}, E_{a-1}, E_{a2}, k_{r1}, k_{r-1}, k_{r2}
6	$\ln(C_t) = \frac{\ln\left((n-1) \cdot k \cdot t + C_0^{1-n}\right)}{1-n}$	E_a, k_{r1}, n

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 a inactive intermediate $TI_{\alpha=0}$ followed by a denaturation step and model 6: nth order reaction.

1): integration of these equations is not possible.

Henley and Sadana (1984) have presented a model for a series-type of enzyme deactivation. The initial enzyme alters by a first order reversible reaction to a partially active form which changes again by a first order reaction to a complete 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):

$$TI \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} TI_\alpha \xrightarrow{k_2} TI_d$$

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 Sadana (1984) 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 (α=0, model 5). The nth order reaction model is used as model 6. Such a model was used to describe the aggregation of β-lactoglobulin (Roefs, 1994). Since no inactivation mechanism is given here, the nth 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 mathematical 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):

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

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 (Hurvich and Tsai, 1989):

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

in which:

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

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

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 g (g ds)$^{-1}$. The TIA of the untreated flour was 23.3 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 moisterised by adding water dropwise to the flour in a cooled blender. The flour was stored for 5 till 7 days at 4 °C to equilibriate. The moisture content was measured according to AOAC (1990).

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 g (g ds)$^{-1}$, each at temperatures of 90, 104, 119 and 134 °C, and at 0.52 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 hours 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 Groot (1991) 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 (Roozen and De Groot, 1991), using KSTI as a standard. The error (the difference between measured and mean value, devided by the mean value) of the analysis was 5%.

Physical properties of the soy flour

Heat conductivity of the flour was measured according to Pantaloni *et al* (1977) with a needle probe at 60 °C and at different moisture contents. Measured values were 0.135, 0.160 and 0.226 $Wm^{-1}{}^{\circ}C^{-1}$ at 0.25, 0.32 and 0.41 g $(g\ ds)^{-1}$, respectively. The specific heat of soy flour was taken from Wallapapan *et al* (1984).

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 estimation 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 (Luikov, 1968). 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 on 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 (SAS Institute Inc., 1988).

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 ≥104 °C. This observation agrees with the findings of Roa *et al* (1989) 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 ~100 °C and a two-phase inactivation at temperatures higher than ~100 °C.

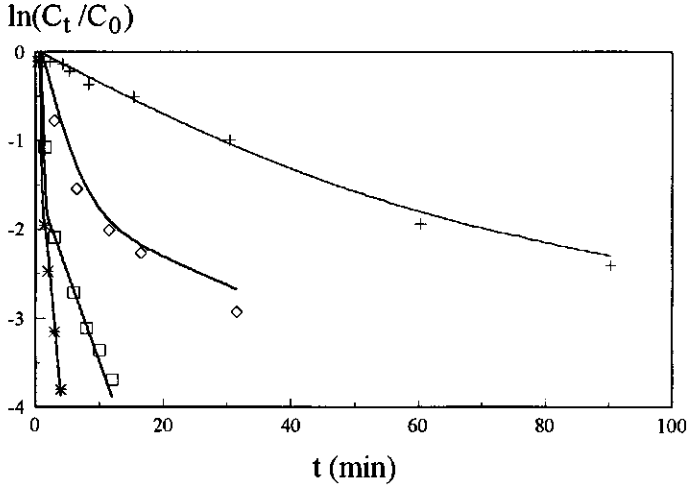

Fig 1. Measured and estimated (—) inactivation of TIs in soy flour (mc=0.23 g (g ds)$^{-1}$). Temperatures are: 90 (+), 104 (◊), 119 (□) and 134 °C (*). Estimations were performed with the overall kinetics model (Table 4).

Figure 2 shows a large increase of the inactivation rate of TIs with increasing moisture content at low moisture contents (<0.3 g (g ds)$^{-1}$). The influence of the moisture content on the inactivation rate is smaller at high moisture contents (>0.3 g (g ds)$^{-1}$): no difference in inactivation rate is observed between 0.40 and 0.52 g (g ds)$^{-1}$ at 104 °C. The observation of the moisture content dependency of the inactivation rate is in general agreement with the findings of Buerra *et al* (1984) and Roa *et al* (1989).

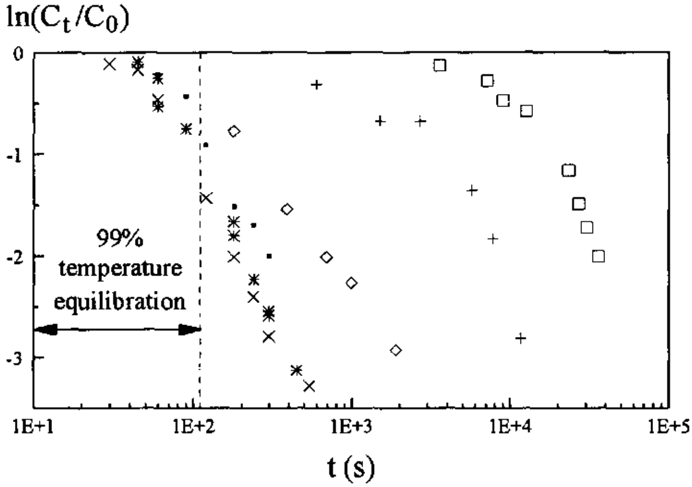

Fig 2. Influence of the moisture content on the inactivation of TIs in soy flour (T=104 °C). Moisture contents are: 0.08 (□), 0.13 (+), 0.23 (◇), 0.30 (▪), 0.40 (×) and 0.52 g (g ds)$^{-1}$ (∗).

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_r at 0.40 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 applied obvious. Yet, this involves that the accuracy of the heat transfer model plays an important role in the estimation of the kinetics parameters at very low τ_r values.

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

	moisture content (g (g ds)$^{-1}$)					
	0.08	0.13	0.23	0.30	0.40	0.52
model 1						
$E_a\cdot10^{-3}$	195	165	110	122	103	153
s_w	29	37	62	53	70	49
model 2						
$E_{a1}\cdot10^{-3}$	200	221	204	222	247	221
$E_{a2}\cdot10^{-3}$	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						
$E_{a1}\cdot10^{-3}$	204	209	196	216	164	222
$E_{a-1}\cdot10^{-3}$	241	226	182	207	39	667
$E_{a2}\cdot10^{-3}$	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						
$E_{a1}\cdot10^{-3}$	200	215	199	221	249	210
$E_{a2}\cdot10^{-3}$	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						
$E_{a1}\cdot10^{-3}$	204	209	195	218	230	211
$E_{a-1}\cdot10^{-3}$	246	227	194	210	185	214
$E_{a2}\cdot10^{-3}$	334	143	126	104	67	176
s_w	9	13	8	11	17	12
model 6						
$E_a\cdot10^{-3}$	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

The results of the estimation of the kinetics parameters of the six inactivation kinetics models and the corresponding AIC_c values (Equation 2) are given in Table 2 and 3, respectively. As expected from the experimental data in Figure 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.

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

		moisture content (g (g ds)$^{-1}$)					
model	p	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 bold.

At moisture contents ≤0.30 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-α). 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 can not 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%-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 on moisture content agrees with its physical meaning. Parameter α should not be independent on moisture content per sé from a mechanistic point of view. Both models can be correct with respect to these results.

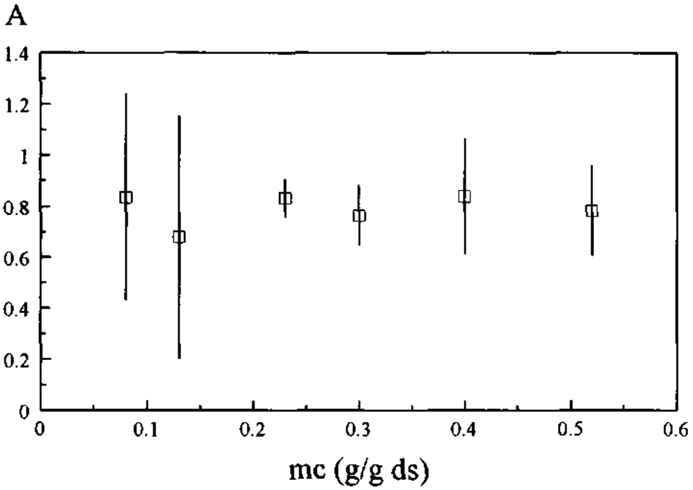

Fig 3. Influence of moisture content on the estimated parameter A (□, model 2) and its 95% confidence intervals.

The nth 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 at moisure contents ≤0.30 g (g ds)$^{-1}$ are higher 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% confidental 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≤0.30 g (g ds)$^{-1}$, and that the nth order reaction model is most parsimonious at mc≥0.40 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 $TI_{\alpha=0}$ (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 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 (e.g. at mc=0.40 g (g ds)$^{-1}$ and T=134 °C). This might also explain the low activation energy $E_{a,1}$ for this model at 0.40 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 g (g ds)$^{-1}$. The estimated 95% confidential intervals of α at the other moisture contents overlapped zero. These results indicate that model 3 is equal to the reversible model with inactive $TI_{\alpha=0}$ (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 reversibilty 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 decribe 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 on moisture content. Figures 4 and 5 show the estimated pre-exponential factors and activation energies with their estimated 95%-confidential intervals at different moisture contents. The logaritm 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%-confidential 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 assumed to be independent on moisture content. Also the 95%-confidential intervals of E_{a2} overlap each other. However, the estimated values of E_{a2} show a hyperbolic-like trend with moisture content.

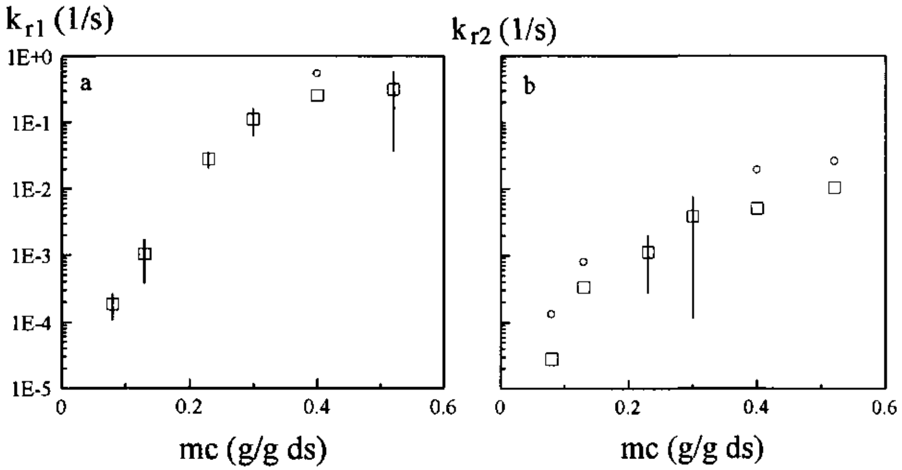

Fig 4. Influence of the moisture content on the estimated pre-exponential factors k_{r1} **(a)** and k_{r2} **(b)** and their 95% confidence intervals. In some cases only the upper 95% confidence interval is shown (o) because the estimated lower interval was negative.

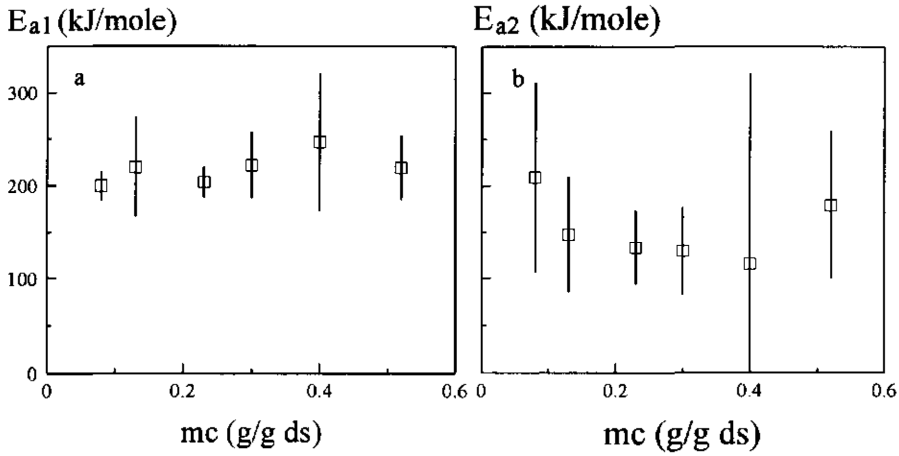

Fig 5. Influence of the moisture content on the estimated activation energies E_{a1} **(a)** and E_{a2} **(b)** and their 95% confidence intervals.

Therefore, different overall kinetics models with E_{a2} dependent and independent on moisture content were 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 g (g ds)$^{-1}$.

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

	k_{r1}	$E_{a1}\cdot 10^{-3}$	k_{r2}	$E_{a2}\cdot 10^{-3}$	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				

$k_{r1} = a + \frac{b}{1+e^{d\cdot mc+c}}$; $k_{r2} = a + b\cdot mc + \frac{c}{mc}$; $E_{a2} = a + b\cdot mc + \frac{c}{mc^2}$; E_{a1} and A are independent on moisture content.

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 towards higher moisture contents (>0.30 g (g ds)$^{-1}$). Consequently, an increase in moisture content above 0.30 g (g ds)$^{-1}$ during processing is useless from a kinetics point of view. The most parsimonious kinetics models at moisture contents ≤0.30 g (g ds)$^{-1}$ were a model assuming two first order reactions for two TI groups, and a model with a irreversible inactivation of a native TI to a partially active intermediate TI, followed by a denaturation step. The nth order reaction model showed the best results at high moisture contents (≥0.40 g (g ds)$^{-1}$). An overall kinetics model was developped 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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Symbols

a	fitting parameter	(J mol^{-1}, s^{-1})
A	activity fraction of one of the two TI groups at t=0	(-)
AIC$_c$	corrected Akaike information criterion	(-)
b	fitting parameter	(s^{-1}, (g ds) s^{-1} g^{-1}, J (g ds) g^{-1} mol^{-1})
c	fitting parameter	(-, g s^{-1} (g ds)$^{-1}$, J g^2 mol^{-1} (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	((g ds) s^{-1} g^{-1})
E$_a$	activation energy	(J·mol^{-1})
k	reaction rate constant	((mg·(g ds)$^{-1}$)$^{1-n}$s^{-1})
k$_r$	pre-exponential factor at reference temperature T$_r$	((mg·(g ds)$^{-1}$)$^{1-n}$s^{-1})
m	sample size	(-)
mc	moisture content	(g (g ds)$^{-1}$)
n	reaction order	(-)
p	number of parameters	(-)
R	gas constant	(J·K^{-1}·mol^{-1})
RSS	residual sum of squares	(-)
s$_w$	$\dfrac{\sum \dfrac{\lvert C(i)-\hat{C}(i)\rvert}{C(i)}}{m} * 100$	(%)
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)

Greek

α	fraction of activity of TI_α	(-)
β	constant	(-)
τ_r	characteristic inactivation time	(s)

Subscripts

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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3. Inactivation kinetics study of the Kunitz soybean trypsin inhibitor and the Bowman-Birk inhibitor

Abstract

The inactivation of trypsin inhibitors (TIs) in soy flour exhibits a two-phase inactivation behaviour. It is sometimes assumed that a difference in heat stability of the Kunitz soybean trypsin inhibitor (KSTI) and the Bowman-Birk inhibitor (BBI) is the reason for this inactivation behaviour. Kinetics studies were performed with KSTI and BBI in soy flour. These studies showed that this two-phase inactivation behaviour of TIs could not be explained by the difference in heat stability of KSTI and BBI. KSTI and BBI in commercial soy flour inactivated simultaneously during the first inactivation phase. BBI probably inactivates with a first order reaction with a rate constant comparable to the rate constant of the first phase of TIs in soy flour. The residual activity of TIs in the second phase was caused by KSTI.

Inactivation of KSTI and BBI in an aqueous solution (T=104 °C) and in a starch matrix ($mc \cong 0.25$ g (g ds)$^{-1}$ and T=104 °C) followed a first order reaction. KSTI and BBI in a starch matrix with added cysteine ($mc \cong 0.25$ g (g ds)$^{-1}$ and T=104 °C) showed a two-phase inactivation behaviour. The existence of thiols in soy flour seems to be responsible for the two-phase inactivation of TIs in soy flour. We suggest that TIs in soy flour are inactivated by sulphydryl-disulfide interchange during the first inactivation phase, and by heat during the second phase.

This chapter has been submitted as:

Rob van den Hout, Marieke Pouw, Harry Gruppen, Klaas van 't Riet. Inactivation kinetics study of the Kunitz soybean trypsin inhibitor and the Bowman-Birk inhibitor.

Introduction

Soybeans are generally heat processed to inactivate the antinutritional factors (ANFs). Trypsin inhibitors (TIs) are generally considered to be the main ANFs in soybeans. TIs can be divided in two main groups: the Kunitz soybean trypsin inhibitor (KSTI) and the Bowman-Birk inhibitor (BBI). In kinetics studies a two-phase inactivation behaviour for TIs was observed in soy flour (Van den Hout *et al,* 1997). Such a two-phase inactivation behaviour has also been found in Phaseolus Vulgaris beans (Roa *et al*, 1989 and Van der Poel, 1990).

A possible explanation of this two-phase inactivation behaviour is that the two TI groups inactivate with a different first order reaction rate constant each. BBI generally has been considered to be more heat stable than KSTI, based on their stability in aqueous solutions (Birk, 1961 and Obara and Watanabe, 1971). Rouhana *et al* (1996) 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. Liener and Tomlinson (1981) concluded that KSTI is equally heat stable to BBI by comparing the inactivation rate of TIs in a commercial and a KSTI-free soy flour. DiPietro and Liener (1989a) found that KSTI in soy flour was more heat stable than BBI. However, they did not observe the expected two-phase inactivation behaviour upon TIs. This might be explained by the fact that the authors did not measure at low residual TIA levels, and, therefore, performed their kinetics study in the first inactivation phase of TIs. Moreover, the inactivation rate constant of BBI was only 1.4 times higher than the rate constant of KSTI (T=95 °C and mc=15%). Friedman *et al* (1991) determined the residual activities of KSTI and BBI in a commercial and a KSTI-free soy flour after autoclaving. They concluded that KSTI was more heat stable than BBI. However, they did not study the inactivation kinetics of KSTI and BBI, and did not relate their experiments with a two-phase inactivation behaviour of TIs.

Van Zuilichem *et al* (1993) and Roa *et al* (1989) referred to Multon and Guilbot (1975) to explain the two-phase inactivation behaviour of TIs in soybeans and Phaseolus Vulgaris beans, respectively. Multon and Guilbot (1975) explained the two-phase inactivation behaviour of ribonuclease in wheat grains by the catalytic role of water during inactivation.

The results from literature concerning the difference in heat stability of KSTI and BBI in soy flour are conflicting. In this study it was investigated if the two-phase inactivation behaviour of TIs in soy flour can be explained by a difference in heat stability of KSTI and BBI. Additional experiments were performed to examine the inactivation kinetics of BBI (and

KSTI) in a KSTI-free soy flour, an aqueous solution and a starch matrix. As Friedman *et al* (1982 and 1984) showed that the addition of thiols increases the inactivation rate of TIs in an aqueous medium, it was investigated how thiols influence the inactivation behaviour of TIs in this study.

Material and methods

Materials

Defatted, untoasted soy flakes from Cargill (Amsterdam, The Netherlands) were used. These flakes are from the same batch as used for the inactivation kinetics experiments in previous research (Van den Hout *et al,* 1997). The trypsin inhibitor activity (TIA) of the untreated flour was 23.3 mg (g ds)$^{-1}$. Anhydrotrypsin-agarose was obtained by PanVera (TAK 7302, Madison, WI, USA). The KSTI-free isoline (L81-4590) was grown by Illinois Foundation Seeds Inc. (Champaign, IL, USA) and obtained via TNO Nutrition and Food Research (Zeist, The Netherlands). The initial TIA of the isoline was 10.9 mg (g ds)$^{-1}$. Potato starch was supplied by AVEBE (Perfectamyl D-6, Veendam, The Netherlands), KSTI by Merck (art. no. 24020), BBI by Sigma (art. no. T-9777) and L-cysteine by BDH Chemicals (art. no. 37218).

Conditioning of the samples

TIs in soy flour. The KSTI-free soybeans were milled using a Retsch mill with a 1 mm sieve. The obtained full fat soy grits were extracted with hexane at room temperature. The defatted soy grits and the soy flakes (Cargill) were milled using a 0.2 mm sieve. The flour was moisterised by adding water dropwise to the flour in a cooled (15 °C) blender to the desired moisture content. The soy flour was stored for 5 till 7 days at 4 °C to equilibriate.

KSTI/BBI in buffer. A solution of 1 mg ml^{-1} KSTI or BBI in 0.1 M Tris buffer (pH 8.0) was prepared.

KSTI/BBI in starch. A 0.75 ml TI-solution (6.7 mg KSTI or BBI/ml water) was added dropwise to 5 gram starch with optionally added 100 mg cysteine. The starch was stored for 5 till 7 days at 4 °C to equilibriate.

Determination of the moisture content

The moisture content was measured according to AOAC (1990).

Inactivation experiments

Inactivation experiments were performed with steel cells in a stirred oil bath according to Van den Hout *et al* (1997). The experimental conditions of the heat treatments of the soy flour, the KSTI-free soy flour, the buffer and the starch matrix (with and without cysteine added) are listed in Table 2.

TIA in soy flour

Combined KSTI/BBI activity. TIA in soy flour was measured with trypsin-agarose chromatography as described by Roozen and De Groot (1991) with minor modifications (Van den Hout *et al*, 1997). 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 (Roozen and De Groot, 1991), using KSTI as standard. In case of the KSTI-free soy flour, BBI was used as standard.

Individual KSTI/BBI activity. The individual activity of KSTI and BBI in soy flour was measured by combining affinity chromatography with gel permeation chromatography (GPC). Anhydrotrypsin-agarose was used for affinity chromatography because anhydrotrypsin also binds TI but is, in contrast to trypsin, catalytic inert (Ishii, 1983). Samples of the untreated and heat treated soy flour were eluted on an anhydrotrypsin-agarose column using the same procedure as described for the trypsin-agarose column. The eluate from the column was dialysed against distilled water and freeze dried. 200 μl of a ~1 mg TIs ml^{-1} solution was applied to a FPLC system with a Superdex 30 column (HiLoad 16/60 Pharmacia) using a 0.1 M sodiumphosphate buffer (pH 6.9) containing 0.1 M sodiumsulphate as eluens. The absorbance of the effluent was measured at 280 nm. The error of the measured peak area (the difference between measured and mean value, devided by the mean value) was 18%. Absorbances at 280 nm (A_{280}) of KSTI$_1$, KSTI$_2$ and BBI were fitted using the peak areas of the GPC analyses of the commercial available KSTI and BBI, assuming the A_{280} of KSTI$_1$ and KSTI$_2$ to be equal (Table 1).

TIA in buffer and starch

KSTI/BBI in buffer. The sample was diluted with a 0.02 M Tris-HCl-buffer (pH 8.0, 0.5 M NaCl) and applied to a trypsin-agarose column. The further procedure of the analysis was similar to the analysis of combined KSTI/BBI activity in soy flour.

KSTI/BBI in starch. TIs in starch were extracted with a 0.02 M Tris-HCl buffer (pH 8.0, 0.5 M NaCl) instead of a 0.015 M NaOH 0.5 M NaCl solution. No glycine was added after centrifugation of the extraction solution. The supernatant was applied to a trypsin-agarose column. The further procedure of the analysis was similar to the analysis of combined KSTI/BBI activity in soy flour.

Electrophoresis

SDS-PAGE was performed using a Phastsystem seperation unit (Pharmacia) essentially according to Laemmli (1970). Phastgels (8-25%, Pharmacia) were used. The gels were stained with Coomassie Brilliant Blue according to the instructions of the manufacturer.

Estimation of the kinetics parameters

The two-phase inactivation behaviour of TIs is described with the following equation (Van den Hout *et al,* 1997):

$$\frac{\mathrm{TIA}_t}{\mathrm{TIA}_0} = A \cdot e^{-\lambda_1 \cdot t} + (1 - A) \cdot e^{-\lambda_2 \cdot t} \qquad (1)$$

If the two-phase inactivation behaviour of TIs in soy flour can be explained by a difference in heat stability of the two TI groups (KSTI and BBI), each of them inactivating with a first order reaction, then λ_1 and λ_2 in Equation (1) are the inactivation rate constants of the two TI groups, and parameter A is the fraction of one of the two TI groups in the unprocessed sample. The kinetics parameters λ_1, λ_2 and A were estimated by fitting Equation (1) to the experimental data using the NLIN-procedure of SAS (SAS Institute Inc., 1988). For a first order reaction kinetics, only parameter λ_1 was estimated (A=1 in Equation (1)). When the inactivation experiment is started, the temperature of the samples increases untill the equilibrium temperature has been reached. These heating-up effects play a role in the estimation of the kinetics parameters. Therefore, the measured time of the experiments was corrected by substracting the time needed for 95% temperature equilibration (69 s).

Results and discussion

Figure 1 shows the typical two-phase inactivation behaviour of TIs in soy flour. The average deviation between the residual TIA levels measured by anhydrotrypsin-agarose chromatography and the levels measured by trypsin-agarose chromatography was 12 % (Table 1). In concluding, the results of this work measured by anhydrotrypsin-agarose chromatography can be related to the results of our previous work measured by trypsin-agarose chromatography (Van den Hout *et al*, 1997).

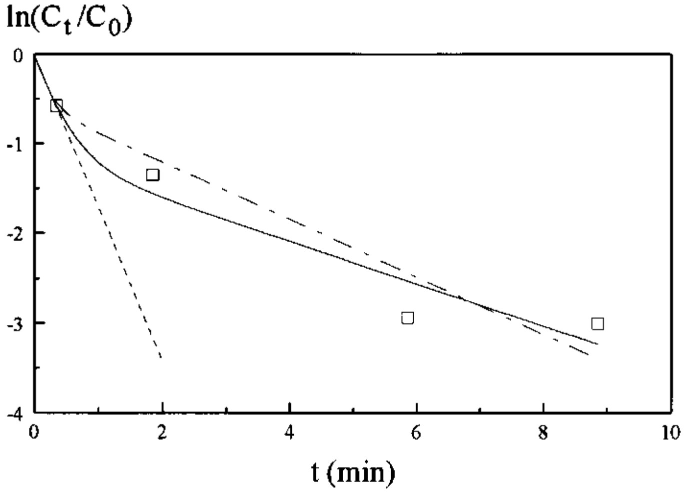

Fig. 1. Inactivation of TIs in soy flour at 119 °C and 0.23 g (g ds)$^{-1}$ as measured by anhydrotrypsin-agarose chromatography (☐). Equation (1) is used to fit the experimental data from Table 1: TIA (—), $KSTI_1$+$KSTI_2$ (— -) and BBI (- - -).

A typical example of a GPC elution profile of a soy sample is shown in Figure 2a. Commercially available KSTI and BBI were analysed with GPC to identify the peaks. Figure 2b and Table 1 show that commercially available KSTI contains residual levels of BBI and vice versa. Three peaks in commercially available KSTI and BBI, and in the soy samples have the same elution times (Figure 2a and b, and Table 1). The first peak at 45 min is probably an aggregate of KSTI (encoded $KSTI_1$) as SDS-PAGE analysis showed that this peak consists of molecules with a molecular weight of approximately 20,000 (no further results shown). The peaks at 51 min and 62 min had molecular weights of approximately 20,000 and 8,000, and were identified by SDS-PAGE analysis as KSTI (encoded $KSTI_2$) and BBI, respectively (Table 1). A fourth small peak in the soy sample at 103 min contains molecules with a low molecular weight, and could not be identified. The fifth peak was eluted at the included volume and represents residual buffer components.

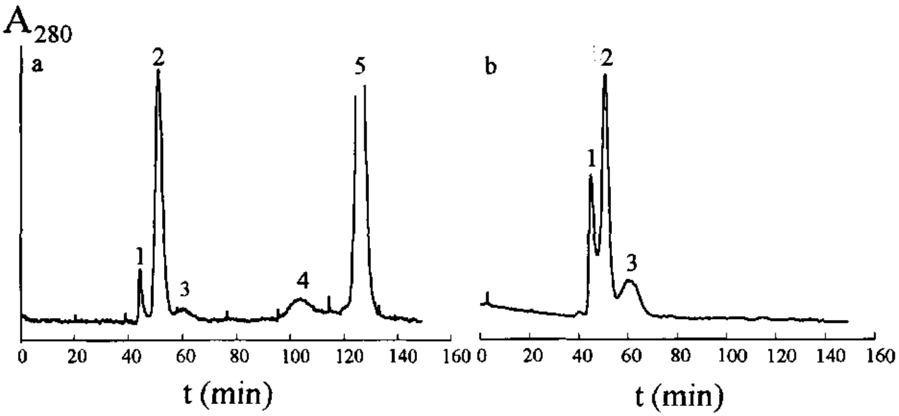

Fig. 2. GPC analyses of heat treated soy flour (**a**, t =1.5 min) and commercially available KSTI (**b**).

BBI contributes for 21% of the total activity of TIs in native soy flour (Table 1). This initial percentage of BBI agrees with the values of 22% and 30% given by DiPietro and Liener (1989b), and Friedman *et al* (1991), respectively. The activity percentages of $KSTI_1$, $KSTI_2$ and BBI after a heat treatment of the flour of 1.5 min are almost equal to the activity percentages in the native sample (Table 1). The inactivation rate of BBI during the first inactivation phase seems to be approximately equal to the rate of KSTI. This result agrees with the observation by DiPietro and Liener (1989a) that the inactivation rate constant of BBI in soy flour is only 1.4 times higher than the rate constant of KSTI (T=95 °C and mc=15%). The activity percentage of BBI of total TIA in the heat treated flour has (almost) decreased to zero in the second inactivation phase (3, 7 and 10 min, Table 1). The residual activity of TIs in the second inactivation phase is caused by the residual activity of KSTI. The inactivation of KSTI shows a two-phase inactivation behaviour and is responsible for the two-phase inactivation behaviour of TIs in soy flour. The results show that the two-phase inactivation behaviour of TIs can not be explained by the difference in heat stability of KSTI and BBI. The conclusion that BBI in soy flour is overall more heat labile than KSTI is in line with the results of Friedman *et al* (1991).

Figure 1 shows the estimated inactivation of KSTI and BBI in soy flour. The inactivation of KSTI was described with Equation (1), and the inactivation of BBI with a first order reaction kinetics (A=1 in Equation 1). It is noted that the reaction rate constants of KSTI and BBI used in Figure 1 could not be estimated accurately because of the small number of data (Table 1).

Table 1. TIA values and activity percentages of $KSTI_1$, $KSTI_2$ and BBI of total TIA in commercial KSTI and BBI, and in (heat treated) soy samples (T=119 °C and mc=0.23 g (g ds)$^{-1}$).

sample, time[1]	TIA trypsin-agarose mg (g ds)$^{-1}$	TIA anhydrotrypsin-agarose mg (g ds)$^{-1}$	$KSTI_1$ (%)[2]	$KSTI_2$ (%)[2]	BBI (%)[2]
com. KSTI			21	49	30
com. BBI			4	14	82
soy flour, 0	23.3	22.6	6	73	21
soy flour, 1.5	15.5	12.7	9	73	18
soy flour, 3	5.72	5.85	6	90	3
soy flour, 7	1.69	1.20	11	89	0
soy flour, 10	1.15	1.13	2	98	0

[1]: commercial KSTI and BBI, and heat treated soy flour samples; time in minutes. [2]: activity percentages of $KSTI_1$, $KSTI_2$ and BBI of total TIA.

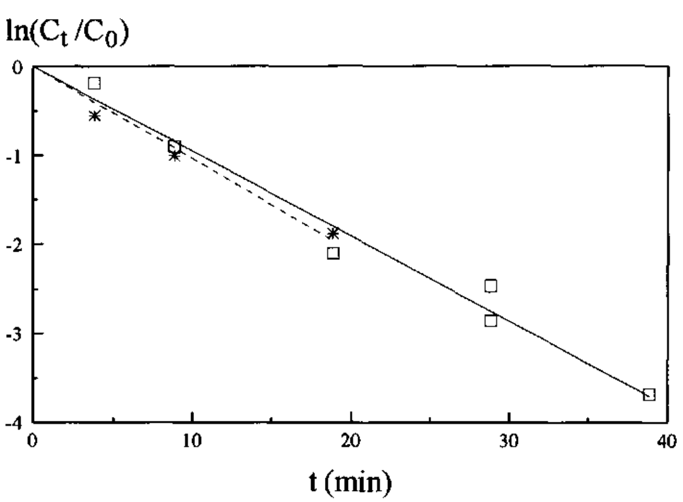

Fig. 3. Measured and estimated inactivation of BBI in KSTI-free soy flour at 119 °C and 0.09 g (g ds)$^{-1}$ (□), and at 104 °C and 0.17 g (g ds)$^{-1}$ (*).

The results of the kinetics study of KSTI and BBI in commercial soy flour were compared with a kinetics study of BBI in a KSTI-free soy flour. Figure 3 shows that the inactivation of BBI in a KSTI-free soy flour follows a single first order reaction at 104 °C (0.17 g (g ds)$^{-1}$), and at 119 °C (0.09 g (g ds)$^{-1}$). Table 2 shows that the inactivation rate constant of BBI in a KSTI-free soy flour is almost equal to the rate constant λ_1 of TIs in soy flour at the same temperature and moisture content. It was shown previously that the inactivation rate of BBI in soy flour was almost equal to the inactivation rate of TIs in soy flour in the first inactivation phase. As a result, the inactivation rate of BBI in a KSTI-free soy flour is probably equal to the rate of BBI in commercial soy flour. This corresponds with the experimental results of Friedman *et al* (1991). Consequently, BBI in commercial soy flour probably inactivates with a first order reaction with a rate constant equal to the rate constant λ_1 of TIs in soy flour.

Table 2. Inactivation rate constants of TIs, KSTI and BBI in different matrices and at different conditions.

component	matrix	T (°C)	mc (g (g ds)$^{-1}$	λ_1 ($\cdot 10^4$ s^{-1})	λ_2 ($\cdot 10^4$ s^{-1})
TI$^{1)}$	soy flour	104	0.09	0.9	0.2
TI$^{1)}$	soy flour	104	0.17	14	2.8
TI$^{1)}$	soy flour	104	0.25	155	7.6
TI$^{1)}$	soy flour	119	0.09	11	2.0
BBI$^{2), 3)}$	KSTI-free soy flour	104	0.09	2.1	-
BBI$^{2)}$	KSTI-free soy flour	104	0.17	17 (4.6)	-
BBI$^{2)}$	KSTI-free soy flour	119	0.09	16 (1.5)	-
KSTI$^{2)}$	buffer	104	-	2.5 (0.2)	-
BBI$^{2)}$	buffer	104	-	2.5 (0.3)	-
KSTI$^{2)}$	starch	104	0.26	12 (2.2)	-
BBI$^{2)}$	starch	104	0.24	6.9 (1.3)	-
KSTI	starch+cysteine	104	0.26	180 (127)	7.3 (15)
BBI	starch+cysteine	104	0.24	260 (95)	4.9 (10)

$^{1)}$: rate constants are predicted by the inactivation kinetics model of TIs in soy flour presented by Van den Hout *et al* (1997). $^{2)}$: fitted with first order reaction kinetics (A=1 in Equation (1)). $^{3)}$: inactivation rate constant was estimated with only two datum-points. The estimated 95% confidence intervals of the rate constants λ_1 and λ_2 are given between brackets.

Since the two-phase inactivation behaviour of TIs in soy flour can not be explained by the difference in heat stability of KSTI and BBI, the inactivation kinetics of isolated KSTI and BBI was studied. It was examined if the typical two-phase inactivation behaviour is also observed for KSTI or BBI in an aqueous solution.

Both KSTI and BBI in a buffer inactivated with a first order reaction (Table 2). Consequently, the two-phase inactivation behaviour of TIs in soy flour can not be explained by a similar two-phase inactivation behaviour of isolated KSTI or BBI. The inactivation rate constant of KSTI in buffer is equal to the rate constant of BBI (Table 2). DiPietro and Liener (1989a) found that BBI was more heat stable than KSTI in buffer at 100 °C and pH 7. The rate constants of KSTI and BBI in the buffer are lower than the rate constants λ_1 and λ_2 of TIs in soy flour with a high moisture content of 0.25 g (g ds)$^{-1}$ (T=104 °C, Table 2). A higher heat stability of KSTI and BBI in a buffer compared to soy flour was also found by DiPietro and Liener (1989a).

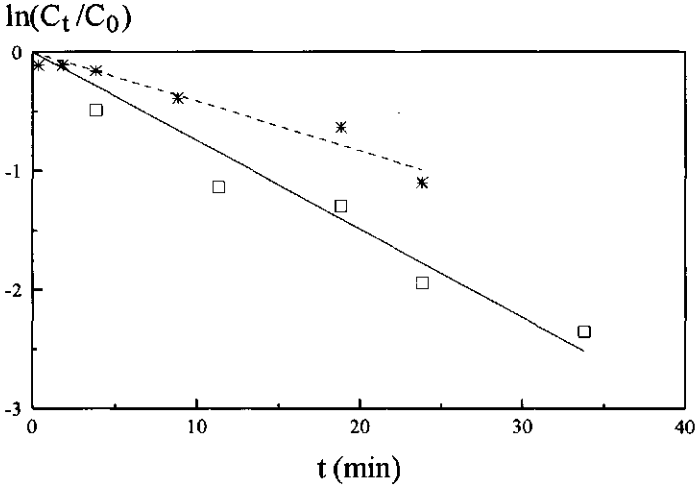

Fig. 4. Measured and estimated inactivation of KSTI (□, mc=0.26 g (g ds)$^{-1}$) and BBI (*, mc=0.24 g (g ds)$^{-1}$) in starch at 104 °C.

The moisture content and the matrix during the inactivation of TIs in the buffer and in the soy flour are different. It was examined if the inactivation of isolated KSTI or BBI at low moisture condition, exemplified with a starch matrix, exhibits a two-pase inactivation behaviour like TIs in soy flour, or a first order reaction like KSTI and BBI in an aqueous solution. The experiments were performed at the same moisture content and temperature as during the kinetics experiments with soy flour in our previous study (Van den Hout, 1997).

Commercial available KSTI in starch follows a single first order reaction (Figure 4). Also BBI in starch follows a single first order reaction, although no experimental data at low residual activities of BBI were measured. The inactivation rate constant of KSTI in starch is higher than the rate constant of BBI (Table 2). The difference can be explained by the difference in moisture content (0.02 mg (g ds)$^{-1}$) using the inactivation kinetics model of TIs in soy flour (Van den Hout *et al*, 1997). The rate constants of KSTI and BBI in starch are lower than the predicted rate constant λ_1 of TIs in soy flour at 0.25 g (g ds)$^{-1}$ and 104 °C, but are in the same order as the predicted rate constant λ_2 of TIs in soy flour (Table 2). DiPietro and Liener (1989a) found that the rate constants of purified KSTI and BBI added to autoclaved soy flour were lower than the rate constants of KSTI and BBI *in situ*.

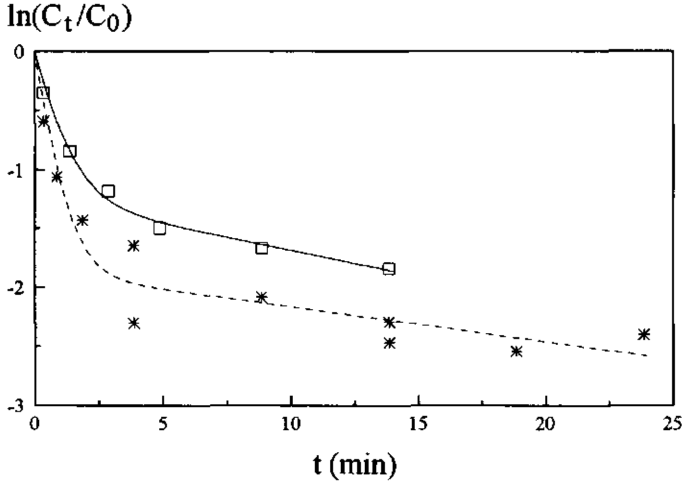

Fig. 5. Measured and estimated inactivation of KSTI (□, 0.26 g (g ds)$^{-1}$) and BBI (*, 0.24 g (g ds)$^{-1}$) in starch with added cysteine at 104 °C.

The inactivation of commercial available KSTI and BBI in a different matrix than soy flour but at the same temperature and moisture content follow first order reactions. These first order reactions can not explain the two-phase inactivation behaviour of TIs in soy flour. Possibly the composition of the soy flour matrix is important for the inactivation behaviour of both TI groups. Friedman *et al* (1982 and 1984) showed that the addition of thiols increases the inactivation rate of TIs in an aqueous medium. It was investigated how thiols have an effect on the inactivation behaviour of KSTI and BBI.

The inactivation rate of both KSTI and BBI increases when cysteine was added to the starch matrix (Figure 5 and Table 2). Both KSTI and BBI exhibit a two-phase inactivation behaviour. These experiments show that the existence of thiols in soy flour is most probably the cause of the two-phase inactivation behaviour of TIs in soy flour. The observed two-phase inactivation behaviour of BBI in starch with added cysteine is in contradiction with the observed first order reaction of BBI in the KSTI-free isoline. The inactivation behaviour of BBI in starch with added cysteine can probably be explained by the two-phase inactivation behaviour of the KSTI fraction that is present in the commercially available BBI used for these experiments (Table 1).

The reaction rate constants λ_1 and λ_2 of BBI in starch with added cysteine are equal to the rate constants λ_1 and λ_2 of KSTI, respectively, considering the estimated confidence intervals of the kinetics parameters (Table 2). The reaction rate constants λ_1 of KSTI and BBI in starch with cysteine added are almost equal to the rate constant λ_1 of TIs in soy flour under the same conditions. The rate constants of the second phase λ_2 of KSTI and BBI in starch with added cysteine are equal to the first order rate constants λ_1 of KSTI and BBI in starch without cysteine, and to λ_2 of TIs in soy flour under the same conditions (Table 2).

Based on the results of the inactivation kinetics study of KSTI and BBI, the following hypothesis was postulated. The inactivation of KSTI and BBI in soy flour follows a pseudo first order reaction rate by sulphydryl-disulfide interchange during the first inactivation phase. In the second phase no SH-groups are available any more for a sulphydryl-disulfide reaction with KSTI. Friedman *et al* (1984) showed that the cysteine sulfhydryl (SH) content in soy flour after a heat treatment (T=45, 65 or 75 °C, mc≅3.6 g $(g\ ds)^{-1}$ and t=1 h) was less than the content in native soy flour. This implies that the number of SH-groups is indeed reduced during the heat tratment. In the second phase KSTI is solely inactivated by heat. This is in line with the observation that the rate constant of the second phase λ_2 of KSTI in soy flour is equal to the first order rate constant λ_1 of KSTI in starch without cysteine.

The observation of DiPietro and Liener (1989a) that the rate constants of purified KSTI and BBI added to autoclaved soy flour were lower than the rate constants of KSTI and BBI *in situ* is not in contradiction with this hypothesis. The available SH-groups in the flour have reacted during the autoclaving. When the purified TIs are added to the autoclaved soy flour, no SH-groups are availabile any more for the reaction with TIs, and the TIs will inactivate by heat only and with a lower inactivation rate.

Conclusions

The two-phase inactivation behaviour of TIs in soy flour could not be explained by the difference in heat stability of KSTI and BBI. KSTI and BBI in commercial soy flour inactivated simultaniously during the first inactivation phase. The residual activity of TIs in the second phase was caused by KSTI. BBI in commercial soy flour probably inactivates with a first order reaction with a rate constant comparable to the rate constant of the first phase of TIs in soy flour. The two-phase inactivation behaviour of TIs in soy flour could not be explained by the inactivation behaviour of isolated KSTI or BBI in a buffer or a starch matrix. Inactivation experiments with KSTI and BBI in a starch matrix with added cysteine showed a two-phase inactivation behaviour for both TI groups. The existence of thiols seems to be responsible for the two-phase inactivation behaviour of TIs in soy flour. We suggest that TIs are inactivated by sulphydryl-disulfide interchange during the first inactivation phase, and by heat during the second phase.

Acknowledgements

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Symbols

A	fitting parameter	(-)
A_{280}	absorbance at 280 nm	(-)
C	trypsin inhibitor activity of TI, KSTI or BBI	$(mg\ (g\ ds)^{-1})$
mc	moisture content	$(g\ (g\ ds)^{-1})$
t	time	(s)
T	temperature	(°C)
TIA	trypsin inhibitor activity	$(mg\ (g\ ds)^{-1})$

Greek

λ	time constant	(s^{-1})

Subscripts

0	at t=0
1,2	number of inactivation phase
t	at t=t

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4. Influence of extrusion shear forces on the inactivation of trypsin inhibitors in soy flour

Abstract

The effect of deformation of the trypsin inhibitor (TI) molecules due to shear forces on the inactivation of TIs in soy flour during extrusion cooking was studied. The strain of the TI molecules and the reduction of the activation energy for TI inactivation due to shear stresses were calculated at different positions in the melt section in an extruder. The strains are probably maximal in the order of 10-20%. The strain calculations indicate that it can not be excluded that shear is a factor in the inactivation of TIs. A maximal decrease of the activation energy of $7 \cdot 10^2$ J mol^{-1} was calculated, which is more than two orders of magnitude lower than the activation energies for TI inactivation. Single screw extrusion experiments were performed to examine the effect of shear forces experimentally. Different die configurations were used to impose different shear rates to soy flour The decrease of the trypsin inhibitor activity (TIA) due to heat inactivation during extrusion cooking was calculated by combining the axial temperature profile and the residence time distribution of soy with the inactivation kinetics model of TIs. The measured residual TIA values of the extrudates could be predicted properly by heat inactivation. There was no indication that shear forces were involved in the inactivation of TIs.

This chapter has been submitted as:

Rob van den Hout, Jan Jonkers, Ton van Vliet, Dick J. van Zuilichem, Klaas van 't Riet. Influence of extrusion shear forces on the inactivation of trypsin inhibitors in soy flour.

Introduction

Soybeans are used as a resource of proteins and energy for animals and humans beings. However, a number of antinutritional factors (ANFs) are present in soybeans. The trypsin inhibitors (TIs) are generally considered to be the main ANFs in soybeans. In order to benefit the nutritional value of soybeans, heat treatments such as steaming and extrusion cooking are generally used. During extrusion cooking, heat is generated by viscous dissipation due to shear forces, and is transferred by convection through the barrel. The effects of temperature, feed moisture content, and screw speed on the trypsin inhibitor activity (TIA) in soy during extrusion cooking have been investigated by several authors (Hendrix *et al*, 1994, Petres *et al*, 1993 and Mustakas *et al*, 1970).

Shear forces also cause physical deformation of proteins and therefore possibly have an additional denaturation effect on TIs. Denaturation of proteins due to shear forces can be caused by changes in the secondary or tertiary structure, or by fracture of the backbone. Breaking of the non-covalent interactions between groups in the protein molecule is probably sufficient to denaturate the protein. Fracture of plastic polymers on molecular scale has been treated by Zhurkov (1966) using the kinetics theory of fracture. In this approach, the activation energy to cause bond rupture is believed to be reduced by the application of stress. Marsman *et al* (1995) investigated the influence of shear forces on some chemical, physical and physiological parameters of toasted soybean meal during extrusion cooking. They mainly examined the correlation between different shear levels and animal performance on the one hand, and the correlation between *in vitro* measurements (TIA, protein dispersibility index and nitrogen solubility index) and *in vivo* measurements on the other hand.

The aim of this study was to investigate the influence of extrusion shear forces on the inactivation of TIs in soy flour. The influence was investigated theoretically by calculating (i) the strains of the TI molecules, and (ii) the reduction of the activation energy of TI inactivation, due to the application of stress at different positions in the melt section in an extruder. In order to investigate the influence of shear forces on TIA experimentally, extrusion cooking experiments were performed. The residence time distribution (RTD) of soy in the extruder was measured and modelled. The inactivation kinetics of TIs in soy flour was measured and modelled separately. The decrease of TIA due to heat inactivation during extrusion cooking was calculated by combining the axial temperature profile and the RTD of soy with the inactivation kinetics model of TIs. The predicted TIA levels were compared with the measured values of the extrudates.

Theory

Calculation of the strain

Assuming that the deformation is uniform, the strain (relative deformation) ε of a molecule due to the exerted stress can be calculated using Hooke's law:

$$\varepsilon = \frac{\sigma}{E} \tag{1}$$

in which σ is the tensile stress exerted and E is the Young's modulus.

A shear flow will exert tensile and compression stresses on a particle dispersed in it. The tensile stress is approximately equal to:

$$\sigma = \mu \cdot \dot{\gamma} \tag{2}$$

The shear rate $\dot{\gamma}$ at different positions in the extruder can be calculated by the next equations (Rauwendaal, 1994). The shear rate at the wall of the die can be calculated with:

$$\dot{\gamma}_w = \frac{3m+1}{4m} \cdot \left(\frac{32Q_v}{\pi \cdot d^3} \right) \tag{3}$$

The shear rate in the screw channel and in the flight clearance is calculated with:

$$\dot{\gamma} = \frac{\pi \cdot N \cdot D}{Y} \tag{4}$$

where Y is height of the screw channel (H) or flight clearance (δ).

The soy melt is assumed to behave like a power-law fluid (Morgan, 1979 and Remsen and Clark, 1978). It is not known which strain is needed to inactivate the TIs. However, a rough estimation can be made. Thermal movement of a protein will already result in some deformation of the molecule. Therefore, a strain of a few per cents will not cause any inactivation of a TI molecule. On the other hand, if the molecule is deformed in such way that it has obtained the dimensions of a freely rotating protein chain, it has most likely lost its activity. To achieve these dimensions from its native state, a strain of roughly 200% is needed (see appendix). Rather arbitrarily we assumed that a strain of a TI molecule of 10 to 40% is needed to inactivate the protein.

Reduction of the activation energy

The activation energy for the inactivation of a TI molecule is reduced by an amount equal to the work done in moving a segment of a molecule with a cross-sectional area A over a distance x with respect to the other parts of the molecule:

$$\Delta E_a = N_a \sigma A x \tag{5}$$

It is difficult to calculate x. If it is assumed that x can be approximated by $\varepsilon \cdot d_0$ (= $= \frac{\sigma d_0}{E}$) and $A = \frac{\pi}{4} d_0^2$, Equations (5) becomes:

$$\Delta E_a = \frac{N_a \sigma^2 \frac{\pi}{4} d_0^3}{E} \tag{6}$$

It is assumed that the pre-exponential factor will not change by the application of stress.

Inactivation kinetics of TIs

The inactivation kinetics of TIs in soy flour was described by the following equation (Van den Hout *et al*, 1997):

$$\frac{TIA_t}{TIA_0} = \alpha \cdot e^{-k_1 \cdot t} + (1-\alpha) \cdot e^{-k_2 \cdot t} \tag{7}$$

The inactivation constants k_1 and k_2 are assumed to be dependent on temperature following the Arrhenius equation.

Estimation of the length of the melt section

The extruder length is built up by a solid conveying section and a melt section. The length of the melt section L_{ms} in the screw channel is calculated with Equations 8 and 9 (Rauwendaal, 1994):

$$L_{ms} = \frac{P}{g_z} \tag{8}$$

The pressure gradient g_Z is calculated from the equation for the volumetric throughput of a power law fluid:

$$g_z = \frac{(\frac{4+m}{10}) \cdot pWHv_{bz} - Q_v}{(\frac{1}{1+2m}) \cdot \frac{pWH^3}{4\mu}} \tag{9}$$

Melted soy flows both in the screw channel and in the die head. Therefore, the volume of the die head that contains melted soy was determined and recalculated in an equivalent axial screw length. This length was added to L_{ms} resulting in a corrected length for the melt section (L'_{ms}).

Residence time distribution

The RTD of soy in the extruder is described by a model consisting of a plug flow with a residence time t_p for the solid conveying section, and a cascade of *n* perfect mixers with a mean residence time t_{ms} for the melt section (Van Zuilichem *et al*, 1973). When L_{ms} is known, the length of the solid conveying section L_{scs} can be calculated. t_p is calculated from the measured degree of fill of the soy in the channel of the solid conveying section, the mass flow rate Q_m, and L_{scs}. The parameters *n* and t_{ms} are calculated using the experimental data.

Material and methods

Conditioning of the soy flour

Untoasted and defatted soy flour (protein dispersibility index=80) was provided by Cargill (Amsterdam, The Netherlands). TIA of the initial flour was 30.0 mg (g ds)$^{-1}$. The flour was mixed with water to a moisture content of 0.40 g (g ds)$^{-1}$ in a 20 l Günther Papenmeier mixer. The flour was stored for 1 night at 4 °C to equilibrate. The moisture content was measured according to AOAC (1990).

Physical properties of soy

The TIs can be divided in two main groups: the Kunitz soybean trypsin inhibitor (KSTI) and the Bowman-Birk inhibitor (BBI). The Young's moduli of KSTI and BBI were assumed to be comparable with the moduli of other soy proteins. Data of Young's moduli of soy proteins at low moisture contents (0.40 g (g ds)$^{-1}$) and high temperatures (70-120 °C) are absent in literature. Baird (1981) found a dependency for the storage shear modulus G' on protein concentration of soy isolate C. Interpretation of his results gives a power-law relation: $G' \sim C^{2.8}$. Combining this result with measurements of the shear moduli of soybean protein gels (Kleef, 1986) results in an estimation of the Young's modulus E of 10^6 N m^{-2}. This value of 10^6 N m^{-2} is probably at the lower side. Moduli of several other solid foods (apples, bananas, peaches, pears and potatoes) are higher (10^6 up to $3 \cdot 10^7$ N m^{-2}, Rao and Skinner, 1986). KSTI has 181 amino acid residues. A number of bonds n_b of 543 was calculated from this value. The diameter of KSTI is assumed to be $3.5 \cdot 10^{-9}$ m (Wolf, 1977). A value for the length of the bonds l_b of $1.3 \cdot 10^{-10}$ m was used. The length of chain elements l'_b for KSTI is not presented in literature. As a first approximation, the length l'_b of $2.5 \cdot 10^{-9}$ m as observed for β-casein was used for completely denaturated KSTI. An average viscosity of soy in the screw channel, clearance and die was calculated for each extrusion experiment by using the mean residence time of the flour in the melt section, the axial temperature profile, and the relation for the viscosity given by Morgan (1979). The flow index m was assumed to be 0.26 (Morgan, 1979). The density of the soy melt was calculated by dividing the weighted amount of an extrudate sample by the volume of this sample for every experiment. The average density was 1167 kg m^{-3}. No expansion of the extrudates was observed.

Determination of the kinetics parameters

Inactivation of TIs in the soy flour was measured and modelled at a moisture content of 0.40 g (g ds)$^{-1}$ according to Van den Hout *et al* (1997). The kinetics parameters (E_{a1}, E_{a2}, k_{r1}, k_{r2} and α) were estimated using the experimental inactivation data. The pre-exponential factors k_{r1} and k_{r2} were estimated at a reference temperature T_r (Van den Hout *et al*, 1997).

Trypsin inhibitor activity assay

Samples were milled on a Retsch mill with a 0.2 mm sieve. TIA was measured with an affinity chromatography method as described by Roozen and De Groot (1991) with a few modifications (Van den Hout *et al*, 1997). Extrudate samples were analysed in duplo. The error of the analysis (the difference between measured and mean value, devided by the mean value) was 5%.

Extrusion cooking experiments

Extrusion cooking experiments were performed with an Almex Battenfeld single screw extruder (Figure 1). The barrel length to inner diameter ratio (L/D) was 12. The compression ratio of the screw was 1.15. Different die diameters were used to impose different shear rates on soy. The heating elements at the barrel of the extruder were turned off to avoid excessive heating of the soy. One extrusion cooking experiment was performed with a compression ratio of 4 to impose a higher shear rate on soy in the melt section. This experiment was performed without a die head to avoid excessive high temperatures. The axial temperature profile was measured at the barrel wall, and inside the screw by inserting a lance with thermocouples into the (hollow) screw. The measured temperature profile of the lance was fitted with a polynomial-like equation. The temperature of the product in the die was measured with a thermocouple. A slow cooling of the extrudate samples possibly results in an additional decrease of TIA. The decrease of temperature in the centre of the extrudates was measured as a function of time with a thermocouple. The pressure before the die was measured with a Kistler piezo-quartz crystal pressure probe (type 601H). Experiments were performed at a screw speed of 150 rpm. The mass flow was measured at the die and corrected for the loss of evaporated water. Extrudate samples were collected and dried at 35 °C. The degree of fill of the screw channel was measured after a sudden stop by collecting and weighing soy samples from several screw pockets after pulling out the screw.

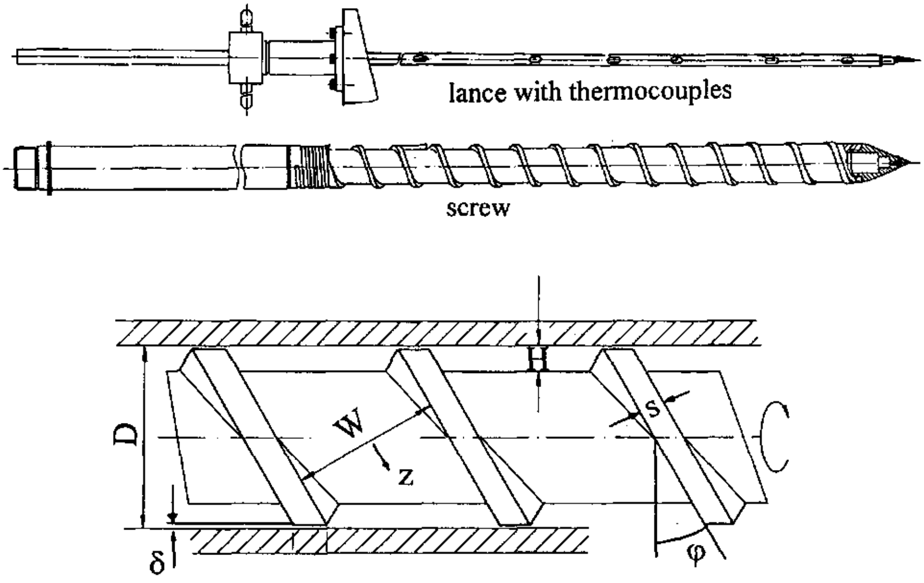

Fig. 1. Configuration of the extruder ($D=4.88\cdot10^{-2}$ m, $W=2.70\cdot10^{-2}$ m, $H=4.18\cdot10^{-3}$ m, $s=4.19\cdot10^{-3}$ m, $\delta=8.5\cdot10^{-4}$ m and $\varphi=13.2°$).

RTD measurements

RTD measurements were performed according to Peng *et al* (1994). A weighted amount of Congo red (about 100 mg) was added into the feed port of the extruder. Samples were collected, dried at 35 °C and milled with a 1 mm sieve. A standard curve was prepared by mixing solutions with different amounts of Congo red with grounded extrudates using a blender. These samples were dried again at 35 °C and milled with a 1 mm sieve. The colour values of the samples and the standard curve were measured with a colorimeter (Tricolor LFM3 Colorimeter). The colour value 'a' devided by 'L' (DIN 6174, CIE-LAB) was used to estimate the concentration of Congo red.

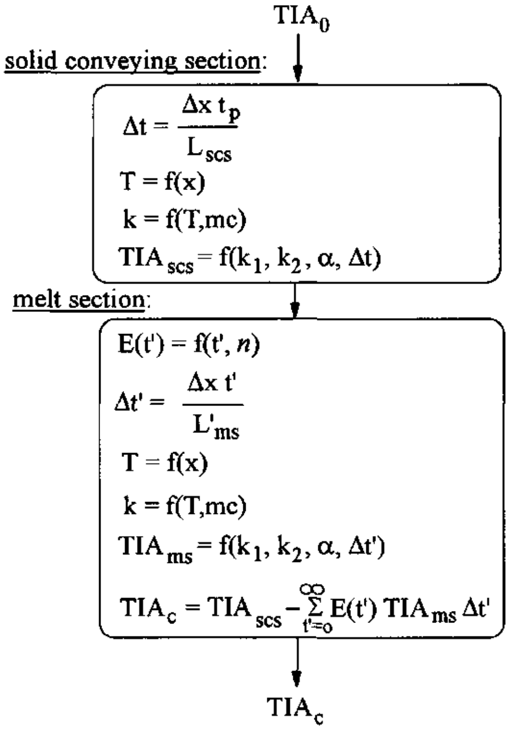

Fig. 2. Calculation procedure of TIA during extrusion cooking.

Calculation procedure of TIA in the extrudates

The decrease of TIA due to heat inactivation was estimated by combining the axial temperature profile inside the screw and the RTD of the flour during extrusion cooking with the inactivation kinetics model of TIs. Figure 2 presents a scheme of the calculation procedure of the inactivation of TIs during extrusion cooking.

Results and discussion

Strains of the TI molecules were calculated using Equations 1, 2, 3 and 4. The calculated shear rates in the clearance and at the die wall are higher than the rate in the screw channel, as expected (Table 1). Yet, the calculated strains in the screw channel, clearance and at the die wall are comparable due to the dependency of the viscosity on the shear rate. The calculated strains are around 15% at the three locations in the extruder. It should be noted that these calculations only intend to estimate orders of magnitude of the strain in the TI molecules. Considering that the Young's modulus used in this study is probably at the lower side, the calculated strains will be at the higher side. Although the calculated strains are not very high, breaking of non-covalent interactions between groups in the protein molecule and therefore inactivation of TIs due to shear can not be excluded.

A maximal decrease of the activation energy of 0.7 kJ mol^{-1} was calculated (see Table 1). This value is more than two orders of magnitude lower than the activation energies for TI inactivation (E_{a1}=200 kJ mol^{-1} and E_{a2}=158 kJ mol^{-1}). According to this theory, shear will not be an important factor in the inactivation of TIs.

Table 1. Calculated shear rate, viscosity, strain and decrease in activation energy at different positions in the extruder (d=6 mm).

position	$\dot{\gamma}$ (s^{-1})	μ (Pa s)	ε (-)	ΔE_a (kJ mol^{-1})
channel	92	864	0.08	0.1
channel[1)]	368	417	0.15	0.5
clearance	451	277	0.13	0.3
die wall	330	550	0.18	0.7

[1)]:extrusion experiment with a compression ratio of 4 and no die head. Q_m is listed in Table 2.

The inactivation kinetics of TIs in soy flour was determined. Equation (7) describes the measured data well (s_w =13%, Figure 3).

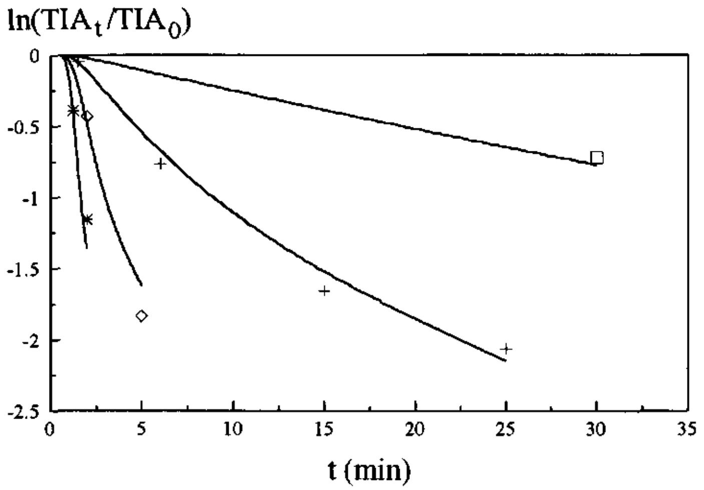

Fig. 3. Measured and estimated (—) inactivation of TIs at 0.40 g (g ds)$^{-1}$. Temperatures are: 80 °C (□), 90 °C (+), 99 °C (◊) and 109 °C (∗). Estimated kinetics parameters are: $E_{a1}=2.00\cdot10^5$ J mol^{-1}, $k_{r1}=1.42\cdot10^{-1}$ s^{-1}, $E_{a2}=1.58\cdot10^5$ J mol^{-1}, $k_{r2}=1.48\cdot10^{-2}$ s^{-1} and $\alpha=0.583$ ($T_r=110$ °C).

Table 2. Measured pressure P and mass flow Q_m, and calculated parameters.

d/l (mm/mm)	P (10^5 N m^{-2})	Q_m (g s^{-1})	L_{ms} (10^{-2} m)	t_p (s)	t_{tot} (s)	n (-)
7/3.5	22	4.92	1.8	9.4	24.1	7
7/7	22	4.48	1.7	10.2	30.1	5
6.5/6.5	24	4.70	1.9	9.7	29.1	8
6/6	24	4.77	2.0	9.6	27.9$^{1)}$	6$^{1)}$
5/5	27	4.72	2.2	9.6	25.6	3

$^{1)}$: parameters are linear interpolated from experiments with a d/l of 6.5/6.5 and 5/5.

The measured die pressure P increases little with decreasing die diameter (Table 2). This is explained by the high entrance pressure drop of the die. Calculations showed that the pressure drop in the die itself ranges from $1.5\cdot10^5$ (l=3.5 mm) to $7.9\cdot10^5$ N m^{-2} (l=5 mm).

This shows that the entrance pressure drop of the die was probably around $20 \cdot 10^5$ N m^{-2}. The calculated L_{ms} is almost constant at $2 \cdot 10^{-2}$ m because the die pressures and mass flow (Table 2) are almost constant for different experiments. The equivalent axial screw length, that was calculated from the volume of the soy melt in the die head, is $8.1 \cdot 10^{-2}$ m. This means that the volume of the soy melt in the channel is relatively small compared to the volume of the melt in the die head. An average L'_{ms} of $10 \cdot 10^{-2}$ m was used for further calculations.

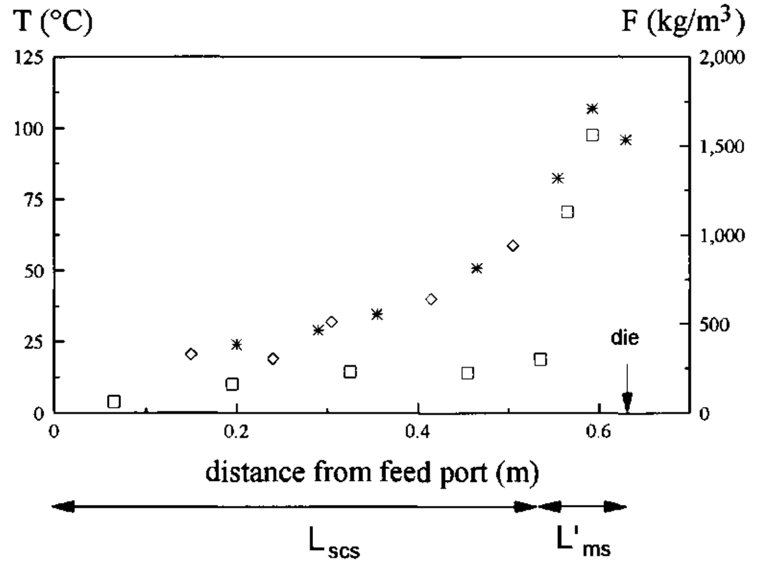

Fig. 4. Axial temperature profile in the screw (*) and barrel (◇), and the degree of fill (□) of soy in the channel along the extruder (l=6.5 mm and d=6.5 mm).

Figure 4 gives an example of the axial temperature profiles and the degree of fill F of the soy in the screw channel. In the solid conveying section, the temperature increased due to condensation of evaporated water from the melt section. At the end of this section, the temperature of the soy was about 70 °C that is considered to be the melting temperature of soy (Morgan, 1979 and Remsen and Clark, 1978). The temperature profiles of the barrel were in agreement with the profiles of the lance. The calculated values for L_{scs} and

L'_{ms} are in line with the sharp rise of the degree of fill in the screw channel (Figure 4). The temperature increased rapidly from about 70 °C to about 110 °C in the melt section due to viscous dissipation only. The decrease of temperature in the die is due to evaporation of water from the soy.

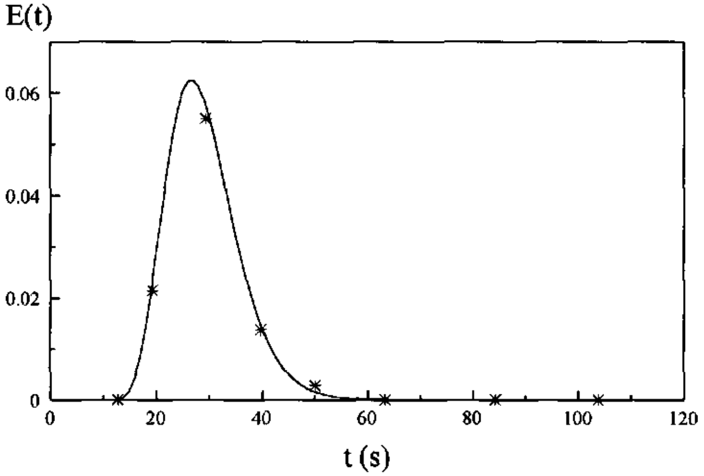

Fig. 5. Measured (*) and estimated (—) RTD of the tracer in soy (l=6.5 mm and d=6.5 mm).

The breakthrough of Congo red was observed between 8 and 15 s in different experiments (Figure 5). This observation is in line with the calculated plug flow residence time t_p of 10 s (Table 2). This calculated value was used in the modelling of the RTD of soy in the extruder. The estimated number of perfect mixers *n* ranged from 3 to 8. The total residence time t_{tot} ($=t_p+t_{ms}$) ranged from 24 to 30 seconds (Table 2). The estimated residence times in the melt section t_{ms} are in the same order as the t_{ms} values that were calculated by dividing the volume of the melt in the extruder by the volumetric flow rate. The calculated total amount of Congo red in the extrudate samples (average value is 70 mg) is not in agreement with the added amount of Congo red (~100 mg). The reason for this difference is probably that the samples of the standard curve were prepared with a blender and not with the extruder. Perhaps, this results in a different inclusion of the

Congo red particles in the soy flour. It was assumed that the relative decrease in the calculated amount of Congo red was the same for all collected extrudate samples.

Table 3 shows that the measured TIA values of the extrudates are almost equal to the calculated values. An increase in shear rate did not change these results (Table 3). The results indicate that the inactivation of TIs during extrusion cooking can be explained by heat inactivation only.

The reliability of the calculated TIA values is dependent on the accuracy of the estimated process parameters. The effect of a deviation in the measured temperature profile and RTD on the calculated residual TIA level was studied. A decrease in the axial temperature profile with 5 °C results in an average increase of the predicted TIA values of 4 mg $(g\ ds)^{-1}$. A decrease in t_{ms} with 5 s results in an average increase of the predicted TIA values of 2 mg $(g\ ds)^{-1}$. If the RTD of the soy in the melt section is considered to consist out of 2 perfect mixers for all experiments, an average increase of the predicted TIA values of 0.4 mg $(g\ ds)^{-1}$ was calculated. If cooling down of the extrudates is taken into account, an additional decrease of 0.6 mg $(g\ ds)^{-1}$ was calculated. This sensitivity analysis shows that, considering the possible deviations in the measured temperature profile and RTD, the inactivation of TIs can be explained properly by heat inactivation. This involves that TIs in soy flour are not inactivated by the deformation of the TI molecules during extrusion cooking.

Table 3. Calculated and measured TIA values in the extrudates.

die d/l (mm)	$\dot{\gamma}_w$ (s^{-1})	T_{high} (°C)	TIA_c (mg $(g\ ds)^{-1}$)	TIA_m (mg $(g\ ds)^{-1}$)
7/7	189	106	22	20
7/3.5	189	107	23	22
6.5/6.5	236	107	21	20
6/6	304	112	17	17
no$^{1)}$	-	83	30	28

$^{1)}$:extrusion experiment with a compression ratio of 4 and no die head, T_{high}: highest measured temperature. TIA_0=30.0 mg $(g\ ds)^{-1}$.

The feed quality of the extruded product is dependent on the residual levels of ANFs in the feed. The feed quality is also dependent on the bio-availability of the amino-acids. Possibly the shear forces have some influence on the denaturation of other soy proteins than TIs. This can have consequences for the feed quality of the extruded product. Equation (6) shows that the decrease of the activation energy is strongly dependent on the molecule diameter. The molecular weight of KSTI is 20,000 g mol^{-1}. The 7S and 11S storage proteins have an average molecular weight of approximately 156,000 and 348,000 g mol^{-1}, respectively. The denaturation of a fraction of the storage proteins by shear forces may therefore be a factor of importance.

Conclusions

Calculated strains of TI molecules due to exerted shear force at different positions in the extruder are at most in the order of 10-20%. A maximal decrease of the activation energy for TI inactivation of $7 \cdot 10^2$ J mol^{-1} was calculated. This value is more than two orders of magnitude lower than the activation energies for TI inactivation. These calculations suggest that some effect of shear forces on the inactivation of TIs can not be excluded, although this is not very likely. Results from extrusion experiments showed that the residual TIA levels predicted by heat inactivation was approximately equal to the measured residual TIA in the extrudates. The inactivation of TIs during extrusion cooking is caused by heat inactivation. The deformation of the TI molecules is not a relevant factor in the inactivation of TI during extrusion cooking.

Acknowledgements

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Appendix

The average radius of gyration S for a freely rotating polymer chain is (Young, 1983):

$$S = \frac{R}{\sqrt{6}} = \frac{1}{\sqrt{6}} \cdot l_b \cdot \sqrt{n_b} \tag{A1}$$

where l_b and n_b are the length and number of bonds, respectively. Since the angle of the bonds is limited and a protein is more rigid than a plastic polymer due to side-chains, S is larger in practice:

$$S = \frac{R}{\sqrt{6}} = \frac{1}{\sqrt{6}} \cdot l'_b \cdot \sqrt{n'_b} \tag{A2}$$

where $l'_b \cdot n'_b = l_b \cdot n_b$. l'_b and n'_b are the length of a statistical chain length and the number of statistical chain elements for proteins, respectively (Flory, 1953). The calculated radius of gyration for a freely rotating chain of TI is about $5.4 \cdot 10^{-9}$ m. The strain to achieve this radius is 210%.

Symbols

A	cross-sectional area	(m^2)
C	protein concentration	(g ds (g tot)$^{-1}$)
d	die diameter	(m)
d_0	molecule diameter	(m)
D	extruder diameter	(m)
E	Young's modulus	(N m^{-2})
E(t)	exit age distribution	(-)
E_a	activation energy	(J mol^{-1})
ΔE_a	decrease of activation energy	(J mol^{-1})
F	degree of fill in screw channel	(kg m^{-3})
g	pressure gradient	(N m^{-3})
G'	storage shear modulus	(N m^{-2})

H	channel height	(m)
k	inactivation rate constant	(s^{-1})
k_r	pre-exponential factor at reference temperature	(s^{-1})
l	die length	(m)
l_b	bond length	(m)
l'_b	length of chain elements that can be considered as effectively freely rotating with respect to each other	(m)
L	extruder length	(m)
L'	corrected axial extruder length	(m)
m	flow index of power law fluid	(-)
mc	moisture content	(g (g ds)$^{-1}$)
n	number of perfect mixers	(-)
n_b	number of bonds	(-)
n'_b	number of chain elements that can be considered as effectively freely rotating with respect to each other	(-)
n_s	sample size	(-)
N	screw speed	(s^{-1})
N_a	Avogadro constant	(mol^{-1})
p	number of channels	(-)
P	die pressure	($N\ m^{-2}$)
Q_m	mass flow rate	($kg\ s^{-1}$)
Q_v	volumetric flow rate	($m^3\ s^{-1}$)
R	root mean square displacement length	(m)
s	flight width	(m)
s_w	$\dfrac{\sum \dfrac{\lvert TIA_m - TIA_e \rvert}{TIA_m}}{n_s}$	(-)
S	root mean square radius of gyration	(m)
t	time	(s)
t'	residence time in melt section	(s)
t_{ms}	mean residence time in melt section	(s)
t_p	mean residence time in solid conveying section	(s)

t_{tot}	mean residence time in extruder	(s)
T	temperature	(°C)
T_r	reference temperature	(°C)
TIA	trypsin inhibitor activity	(mg (g ds)$^{-1}$)
v_b	barrel velocity	(m s^{-1})
W	channel width	(m)
x	distance	(m)
Y	H or δ	(m)

Greek

α	fitting parameter	(-)
δ	clearance height	(m)
ε	strain	(-)
$\dot{\gamma}$	shear rate	(s^{-1})
φ	angle screw channel	(°)
σ	shear stress	(N m^{-2})
μ	viscosity	(N s m^{-2})

Subscripts

0	at t=0
1,2	number of inactivation phase
c	calculated
e	estimated
m	measured
ms	melt section
scs	solid conveying section
t	at t=t
w	die wall
z	z-direction

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5. Modelling of the product quality of soybeans during steaming

Abstract

The decrease of the trypsin inhibitor activity (TIA) and the nitrogen solubility index (NSI) in soybeans during (pressurised) steaming was determined. A process model for steaming was set-up. Separate experiments were performed to estimate the thermal diffusivity and water diffusion coefficients in soybeans. The kinetics of NSI decrease was determined in separate experiments and modelled with a n^{th} order reaction. The process model for steaming was combined with the kinetics models of TIA and NSI to predict the influence of steaming on the residual TIA and NSI levels in the soybeans. The combined models were validated with the presented experimental data and data from literature. The possibility for optimisation of the product quality of the soybeans during steaming was investigated by performing model simulations. The simulations indicate that the steaming process can be optimised using TIA and NSI as quality parameters. Initial moisture content rather than steam temperature can be used to optimise the process.

This chapter has been submitted as:

Rob van den Hout, Gerrit Meerdink, Willem Stolp, Klaas van 't Riet. Modelling of the product quality of soybeans during steaming.

Introduction

The nutritional value of raw soybeans is reduced by the presence of antinutritional factors (ANFs). Trypsin inhibitors (TIs) are generally considered to be the most important ANFs in soybeans. Atmospheric steaming (toasting) of soybeans is commonly used in the animal feed industry to reduce the ANFs. The heat treatment also decreases the bio-availability of essential amino acids. Therefore over-processing should be avoided. The effect of atmospheric steaming on the nutritional value of soybean meal has been investigated by several authors (Rackis, 1974 and Smith *et al*, 1964). Jansen *et al* (1985) studied the effect of steaming at circa 95 °C on the residual trypsin inhibitor activity (TIA), protein solubility and urease activity (UA) in soybeans.

The only degree of freedom in adjusting the process conditions during atmospheric steaming is the residence time. With pressurised steaming an additional degree of freedom, the steam temperature, is introduced. The use of pressurised steaming results in a shorter residence time, so called HTST (High Temperature Short Time) processing. Additionally pressurised steaming might result in a change in the availability of the essential amino-acids. Qin (1996) studied the effect of steam temperature and residence time on residual TIA, protein dispersibility index (PDI) and available lysine of soybeans. Yin *et al* (1993) studied the influence of autoclaving (T=125 °C) on residual TIA, UA and available lysine in soybeans.

Although experimental data are presented in literature, a model to predict the quality of the soybeans during steaming is not available. Such a model can be used to optimise and design the heat treatment.

The aim of this study was to model the influence of steaming on the feed quality of soybeans. Residual levels of TIA and nitrogen solubility index (NSI) were used as quality parameters. NSI was used to monitor the protein availability. A process model for (pressurised) steaming was developed and combined with the kinetics models for TIA and NSI. Physical and kinetics parameters were measured in separate experiments. The possibility for optimisation on feed quality during steaming was investigated by performing model simulations.

Theory

The heat and mass transfer equations were used (i) to determine the physical parameters (thermal diffusivity of and water diffusion coefficients in soybeans), and (ii) to describe the steaming process. The boundary conditions are dependent on the actual process.

Determination of the physical parameters

The thermal diffusivity coefficients were determined by measuring the change in temperature of a bean in saturated steam. The heat transfer is internal limited because of the high Biot number due to the high heat transfer coefficient of the condensing steam. The water diffusion coefficients were determined by soaking beans in water. The mass transfer during soaking is internal limited.

Steaming process

A horizontal vessel is pressurised by steam and (un)loaded with beans by two rotary valves (Figure 2). When a bean enters the toaster the (pressurised) steam condenses on the bean. The temperature of the bean increases and the condensate is absorbed by the bean. The heat transfer in the bean during the condensation process is internal limited because of the high Biot number due to the high heat transfer coefficient of the condensing steam. The mass transfer in the beans during the condensation process is assumed to be internal and external limited. When the temperature of the beans has reached the steam temperature, the condensation of steam on the beans stops. The mass transfer in the beans remains internal and external limited due to absorption of condense droplets.

In order to model the heat and mass transfer in a soybean the following assumptions were made: (i) the thermal diffusivity coefficient and the water diffusion coefficient are independent on moisture content, (ii) the heat and water diffusion only takes place in radial direction, (iii) the soybeans are spherical, (iv) the effect of volume change of the soybeans due to absorption of water is neglected, (v) the influence of (bio)chemical reactions like protein denaturation and Maillard reactions on the physical properties of soybeans are neglected, and (vi) the water diffusion coefficient is constant in the soaking and steaming experiments, because the characteristic time for heat transfer is much lower than the characteristic time for water transfer.

Mass transfer

The diffusion equation for a sphere is:

$$\frac{\partial C}{\partial t} = \frac{1}{r^2} \cdot \frac{\partial}{\partial r}\left(r^2 D \frac{\partial C}{\partial r}\right) \tag{1}$$

The following initial and boundary conditions were applied:

$t=0$: $0 \leq r \leq R$ $\quad C=C_0$; **(1A)**

$t>0$: $r=0$ $\quad \frac{\partial C}{\partial r} = 0$ **(1B)**

Boundary conditions at $t>0$ and $r=R$ are:

for soaking: $C_s=C_e$ (1C)

for steaming (see appendix): $-D \cdot \frac{\partial C}{\partial r} = \frac{V \rho C_p \frac{d\overline{T}}{dt}}{A \cdot H_{v,T_e}} + k_m (C_e - C_s)$ (1D)

Heat transfer

An analytical solution of the heat diffusion equation (Luikov, 1968) was used.

Reaction kinetics of TIs and NSI

The inactivation kinetics of TIs in soybeans was described by the following equation (Van den Hout *et al*, 1997).

$$\frac{TIA_t}{TIA_0} = \alpha \cdot e^{-k_1 \cdot t} + (1 - \alpha) \cdot e^{-k_2 \cdot t} \tag{2}$$

The temperature dependency of the inactivation rate constants was described by the Arrhenius equation. The activation energies and pre-exponential factors were assumed to be dependent on moisture content according to Van den Hout *et al* (1997).

A n^{th} order reaction kinetics model was used to describe the decrease of NSI. The temperature dependency of the reaction rate constant was described by the Arrhenius equation. The pre-exponential factor was assumed to be dependent on moisture content. The activation energy and the reaction order were assumed to be independent on moisture content.

Both kinetics models have no mechanistic background. The reaction order *n* has no mechanistic meaning.

Material and methods

Properties of the soybeans

Soybeans from Mervo Products (Hengelo, The Netherlands) were used. The beans that were used for the determination of the thermal diffusivity coefficient and the water diffusion coefficient, were sieved. Beans that remained on a 5.6 mm pore sieve and went through a 6.2 mm sieve were selected. The volume of the beans was calculated from the measured dimensions of the beans, assuming the beans to be an ellipsoid. This procedure was checked by pyknometer measurements. The volume was used to calculate an equivalent radius.

Analyses

Moisture content of soybeans and grits. The steamed soybeans were carefully dried with a tissue to remove the water layer between the cotyledon and seed coat. The moisture content of the beans was determined by drying the beans at 130 °C for 15 h. The average error of the analysis (the difference between measured and mean value, devided by the mean value) was 2%. The moisture content of the soy grits was measured according to AOAC (1990).

Trypsin inhibitor activity. The oil in the (processed) soy grits was extracted with hexane at room temperature. The defatted soy grits were milled on a Retsch mill with a 0.2 mm sieve. TIA was measured with an affinity chromatography method as described by Roozen and De Groot (1991) with minor modifications (Van den Hout *et al*, 1997).

Nitrogen solubility index. NSI was determined by the method described by Dale *et al* (1987). NSI was calculated as the percentage between the nitrogen content in the supernatant after extraction, and the total nitrogen content of the material. The determination of total nitrogen content and the nitrogen content in the supernatant were performed by a semi-automated micro-Kjeldahl method (Roozen and Van Boxtel, 1979). The average error of the analysis was 4%.

Determination of the thermal diffusivity coefficient

The thermal diffusivity coefficient of soybeans was determined by following the heating-up of a bean in a tube under steaming conditions (Figure 1a). A stainless steel tube with a lid with an insulated thermocouple, and two valves was used. The steam was supplied by an autoclave. The temperature in the centre of the bean was recorded. The experiment was started by opening valve 1 between the tube and the autoclave. The steam velocity was adjusted by valve 2. The steam velocity had no influence on the rate of the heat transfer process as checked in separate experiments. Experiments were performed at different steam temperatures (104, 105, 110, 118, 123 and 130 °C). The deviation of the steam temperature during the experiments was 1.3 °C.

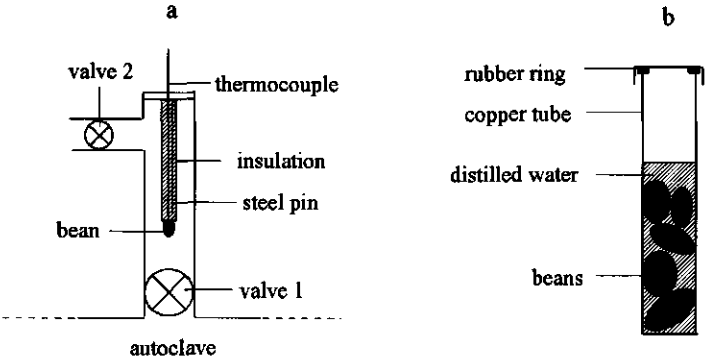

Fig. 1. Experimental set-up used for the determination of the thermal diffusivity coefficients (**a**) and the water diffusion coefficients at temperatures higher than 100 °C (**b**).

Determination of the water diffusion coefficient

The water diffusion coefficient of soybeans was determined by following the water absorption during soaking of the beans in water. Water absorption experiments at temperatures ≤100 °C were performed in beakers with distilled water, placed in a water bath. Water adsorption experiments at temperatures >100 °C were performed in pre-heated copper cylindrical tubes, placed in a stirred oil bath (Figure 1b). The temperature of the water was registered with a thermocouple in the centre in one of the tubes. The time for 95% temperature equilibrium of the water in the tubes was about 1.5 minutes. The moisture absorption was not influenced by the rate of heating-up of the water in the tubes as determined in separate experiments.

Determination of the inactivation kinetics of TIs

Soybeans were milled on a Retsch mill with a 1 mm sieve. Inactivation experiments with the full fatt soy grits were performed at 109 and 119 °C (mc=0.12 g (g ds)$^{-1}$) as described by Van den Hout *et al* (1997).

Determination of the kinetics of NSI decrease

Experiments were performed with untoasted defatted soy flour. Residual NSI levels were determined in the samples used in earlier research (Van den Hout *et al*, 1997). The influence of temperature was studied at 0.30 g (g ds)$^{-1}$ (T=89, 104, 119 and 134 °C). The influence of moisture content was examined at 104 °C (mc=0.08, 0.13, 0.23 and 0.30 g (g ds)$^{-1}$). To obtain more experimental data, NSI levels in additional samples with a heat treatment at 0.13 (119 °C), at 0.23 g (g ds)$^{-1}$ (134 °C), and at 0.40 g (g ds)$^{-1}$ (80, 90, 100 and 110 °C), and different residence times were determined. The experiments at 0.40 g (g ds)$^{-1}$ were performed with a different batch of untoasted defatted soy flour (Cargill, The Netherlands). The NSI of the unheated soy flour was 92% and 90% for the two batches, respectively.

Steaming experiments

Steaming experiments were performed with a pilot plant-scale pressurised toaster with a maximum capacity of 225 kg h^{-1} (Van der Poel, 1990, Figure 2). The steam in the toaster was saturated. The toaster was loaded every experiment with 1 kg soybeans. It was assumed that the beans flash water when they leave the toaster. The measured moisture content of the beans after steaming was therefore corrected by adding the calculated amount of vaporised water needed to cool the beans from steam temperature to 100 °C ($\Delta mc = 2.04 \cdot 10^{-3}$, $1.02 \cdot 10^{-2}$ and $2.04 \cdot 10^{-2}$ g (g ds)$^{-1}$ at 102, 110 and 120 °C, respectively).

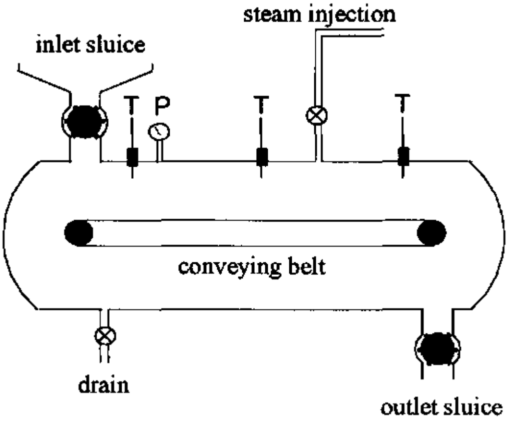

Fig. 2. Pressurised toaster.

Estimation of the physical and kinetics parameters

The thermal diffusivity coefficient was fitted to the experimental data using the analytical solution of the heat diffusion equation. The mass transfer problem was solved numerically using a finite difference method (Crank, 1975). In order to estimate the water diffusion coefficient and equilibrium moisture content, Equation (1) with boundary conditions (1A), (1B) and (1C) was fitted to the experimental soaking data. In order to estimate the external mass transfer coefficients, Equation (1) with boundary conditions (1A), (1B) and (1D) was fitted to the experimental steaming data. The kinetics parameters were fitted to the experimental data using the kinetics equations. The physical and kinetics parameters were estimated using the NLIN procedure of SAS (SAS, 1988).

Prediction of the residual TIA and NSI levels

The temperature and moisture profiles in the bean during steaming were predicted using the analytical solution for heat transfer and Equation (1) with boundary conditions (1A), (1B) and (1D), respectively. This process model was combined with the kinetics models of TIA and NSI to predict the residual TIA and NSI levels of the steamed beans. It was assumed that moisture only existed in the fat free phase of the beans. The moisture content used in the kinetics model of TIA and NSI was therefore based on fat free samples (g (g ds ff)$^{-1}$). The fat phase fraction in the soybeans was taken from the Soya Bluebook (1994). Experimental data showed that the inactivation rate of TIs was almost independent on moisture content at moisture contents higher than 0.3 g (g ds)$^{-1}$ (Van den Hout *et al*, 1997). Experimental data indicated that the reaction rate of NSI at 0.30 g (g ds)$^{-1}$ was almost equal to the rate at 0.40 g (g ds)$^{-1}$. In order to avoid computation errors due to extrapolation of the kinetics parameters, the inactivation rate constants for TIA and NSI above 0.52 g (g ds)$^{-1}$ were assumed to be equal to the inactivation constants at 0.52 g (g ds)$^{-1}$. The data used for the steaming simulations are listed in Table 1.

Prediction of data from literature

Qin (1996) studied the effect of steaming on TIA in soybeans at different steam temperatures (102-136 °C). The author used three different soybean batches. These batches were different in origin, initial TIA level and initial moisture content: (i) Argentina, TIA_0=23.4 mg (g ds)$^{-1}$ and mc_0=0.119 g (g ds)$^{-1}$, (ii) Argentina, TIA_0=17.0 mg (g ds)$^{-1}$ and mc_0=0.116 g (g ds)$^{-1}$, and (iii) China, TIA_0=22.3 mg (g ds)$^{-1}$ and mc_0=0.083 g (g ds)$^{-1}$. The residual TIA levels in the steamed beans were predicted using the combined kinetics and process models. The data listed in Table 1 were used, except for the initial TIA levels and initial moisture contents. Because of the inaccuracy of the TIA analysis below TIA levels of 1.0 mg (g ds)$^{-1}$, experimental TIA data from Qin (1996) below 1.0 mg (g ds)$^{-1}$ were not used.

Results and discussion

Determination of the physical and kinetics parameters

Thermal diffusivity coefficient

Figure 3 shows typical heating-up curves of soybeans. The temperature measurements in the centre of the bean show some deviation between experiments with different beans. This is probably due to the different shapes of the beans. Figure 3 shows that the steam temperature does not influence the heat diffusion process. The estimated thermal diffusivity coefficients showed no trend with temperature and the estimated 95%

confidence intervals partially overlapped each other (data not shown). The coefficient was therefore assumed to be independent on steam temperature. When the equation for heat transfer was fitted to all experimental data, an average thermal diffusivity coefficient of $1.26 \cdot 10^{-7}$ m^2 s^{-1} was estimated (Figure 3). The deviation s_w between measured and estimated temperatures was reasonable low (6.1% and 2.8% for θ_c>0.63).

It is not possible to compare the estimated coefficient with values from literature because no data for beans are available at these high temperatures. Wallapapan *et al* (1984) reported a value of about $8 \cdot 10^{-8}$ m^2 s^{-1} for defatted soy meal under extrusion conditions (ρ=1200 kg m^{-3}, T=130 °C and mc=0.15 g (g ds)$^{-1}$).

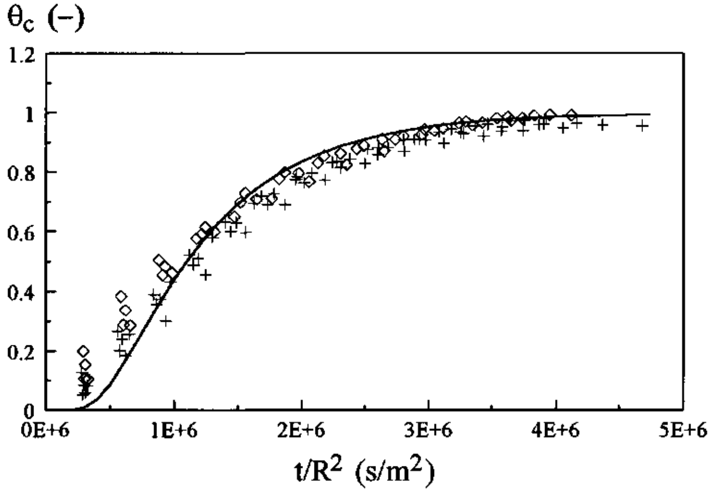

Fig. 3. Measured and estimated (—) increase of temperature in the centre of the bean at 104 °C (+) and 130 °C (◊). Data: see Table 1.

Water diffusion coefficient

The experimental data of the soaking experiments are shown in Figure 4. A bend in the moisture absorption curves at 15-25 min at temperatures ≥100 °C was observed. These bends are probably due to protein denaturation. Model simulations showed that the NSI level of beans in water under the same conditions decreases rapidly as a result of protein denaturation. The temperature dependency of the water diffusion coefficients was described with the Arrhenius equation. An activation energy of $3.48 \cdot 10^4$ J mol^{-1} was estimated.

The water diffusion coefficient of $1.2 \cdot 10^{-10}$ m^{2}s^{-1} (40 °C and 0.14 g (g ds)$^{-1}$) given by Hsu (1983a) corresponds well with our estimated value of $9.2 \cdot 10^{-11}$ m^{2}s^{-1} at 40 °C. The apparent diffusivity of approximately $5.5 \cdot 10^{-12}$ m^{2}s^{-1} (30 °C and 0.16 g (g ds)$^{-1}$) found by Saravacos (1969) is lower than our predicted value of $4.4 \cdot 10^{-11}$ m^{2}s^{-1} at 30 °C.

The activation energy of $5.1 \cdot 10^4$ J mol^{-1} of Hsu (1983a) at 0.14 g (g ds)$^{-1}$ is higher than the value found in this study, $3.48 \cdot 10^4$ J mol^{-1}, but is overlapped by our estimated 95% confidence interval (± $2.2 \cdot 10^4$ J mol^{-1}).

The average value for the equilibrium moisture contents that is overlapped by all estimated 95% confidence intervals, was 1.66 g (g ds)$^{-1}$. This value is in agreement with the value of 1.43 g (g ds)$^{-1}$ presented by Hsu (1983b). The estimated equilibrium moisture content was assumed to be independent on temperature.

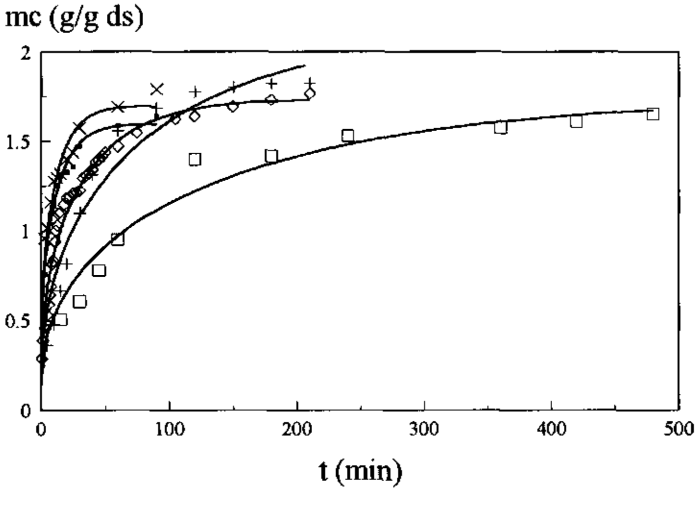

Fig. 4. Measured and estimated (—) moisture absorption during soaking at different temperatures (□ :40, +:70, ◊:100, ▪:110 and ×:120 °C).

Kinetics parameters of TIs

The inactivation of TIs in full fat soy grits was predicted with the inactivation kinetics model of TIs in defatted soy flour as developed by Van den Hout *et al* (1997). The predicted values agreed with the measured TIA values in the full fat soy grits (s_w=12%). These results show that the inactivation kinetics model for TIs in defatted soy flour can be used to describe the inactivation kinetics of TIs in full fat soybeans.

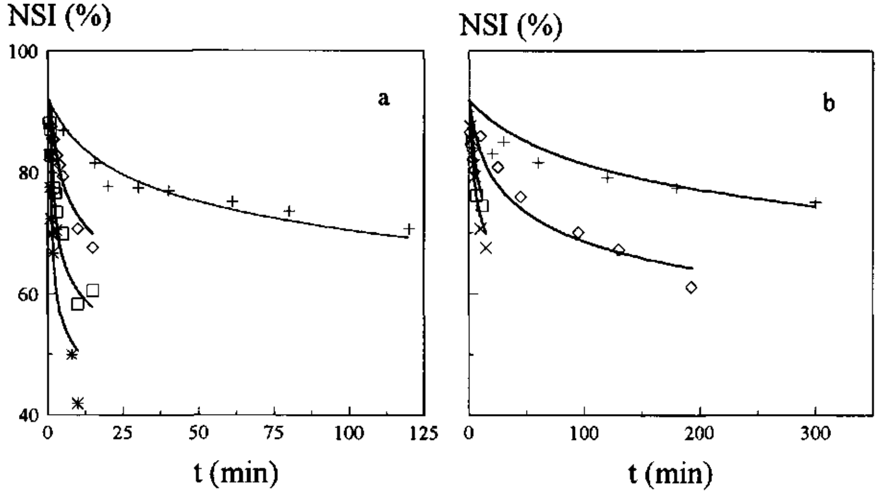

Fig. 5. Measured and estimated (—) decrease of NSI in defatted soy flour at 0.30 g (g ds)$^{-1}$ and 90 (+), 104 (◇), 119 (□) and 134 °C (*) **(a)**, and at 104 °C and 0.08 (+), 0.13 (◇), 0.23 (□) and 0.30 (×) g (g ds)$^{-1}$ **(b)**. Data: see Table 1.

Kinetics parameters of NSI

The estimated 95% confidence intervals of the parameters *n* and E_a were large and partially overlapped each other. An overall kinetics model to predict the NSI decrease as a function of temperature and moisture content was set up assuming *n* and E_a to be independent on moisture content. The estimated reaction order *n* was 10.6. Pilosof *et al* (1981) found a first order reaction kinetics for nitrogen solubility loss in Phaseolus Vulgaris beans at lower temperatures (60-90 °C). The nth order reaction model described the experimental NSI data with a deviation s_w of 4% (Figure 5a and b). This value is equal to the error of the NSI analysis.

Steaming model

The moisture content of the beans increased rapidly during the first minute of steaming due to condensation of steam on the beans (Figure 6). After the condensation has stopped, a constant increase of the moisture content takes place. This increase is probably due to the absorption of condense droplets present in the saturated steam, or condense droplets dripping from the colder wall of the toaster. This explanation is in line with the observed increase of the rate of water absorption when the valve to drain the condensed water was opened, or when the layer thickness of beans on the conveyor belt was decreased.

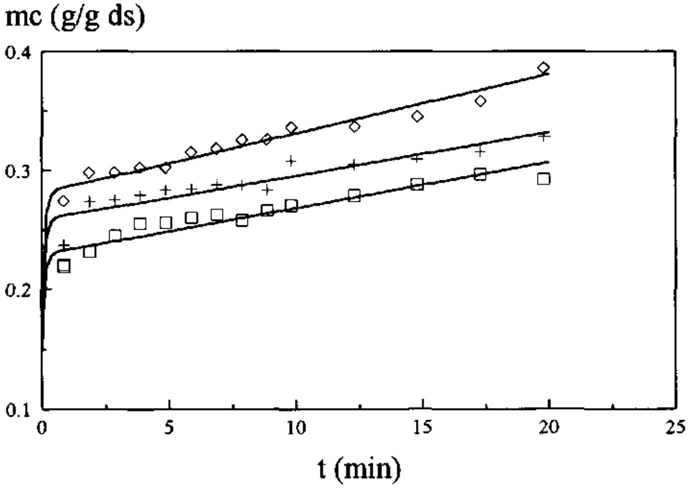

Fig. 6. Measured and estimated (—) moisture absorption of soybeans during steaming at 102 (□), 110 (+) and 120 °C (◊). Data: see Table 1 (K_m=0.262, 0.199 and 0.224 at 102, 110 and 120 °C, respectively).

The moisture content at the inflexion point at 110 and 120 °C is higher than is expected on basis of the predicted condensation. The steam in the sluices is more saturated at higher steam temperature. This results in an extra absorption of condensed steam droplets by the beans in the sluices. This extra amount of absorbed droplets was included in the diffusion model (Figure 6). The dimensionless external mass transfer coefficient K_m could be assumed constant.

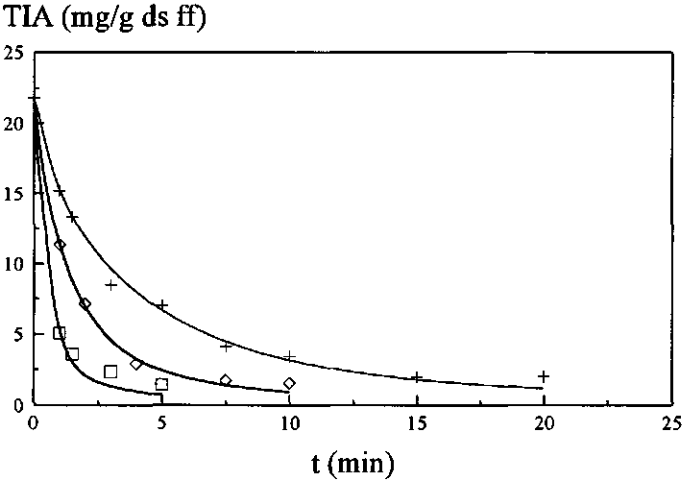

Fig. 7. Measured and predicted (—) TIA values of soybeans steamed at 102 (+), 110 (◊) and 120 °C (□). Data: see Table 1 (K_m=0.224).

Figure 7 shows that the combined kinetics model of TIA and the process model for steaming described the measured TIA values in the steamed beans well. The deviation s_w between measured and predicted TIA values was 16%. For a soybean meal used in feeds, a maximal TIA level between 2 and 5 mg g^{-1} is often recommended (Huisman, 1990). The experimental results show that the residence time required to attain a residual TIA level of 3.5 mg (g ds ff)$^{-1}$ is approximately 10 and 1.5 minutes at 102 and 120 °C, respectively (Figure 7).

The scattered experimental steaming data of residual NSI levels (Figure 8) indicate some difficulties with the method of NSI analysis of the steamed beans. The trend of the measured NSI decrease can nevertheless be described by the combined process model and kinetics model of NSI. The combined model is suitable to perform steaming simulations to predict trends in residual NSI levels.

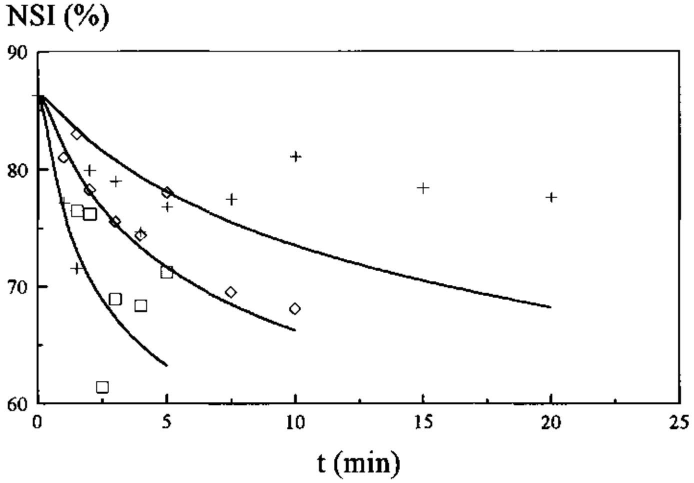

Fig. 8. Measured and predicted (—) NSI values of soybeans steamed at 102 (+), 110 (◇) and 120 °C (□). Data: see Table 1 (K_m=0.224).

The combined process and kinetics model was used to predict the experimental TIA data of Qin (1996). The predicted TIA values were in agreement with the measured TIA values in the steamed beans: the average deviation s_w for the different soybean batches was 31%. In concluding, the combined kinetics and process model is able to predict experimental data from literature as well.

The time needed for drying of the beans to an appropriate moisture content for storage is dependent on the residual moisture content and the residual moisture profile of the steamed beans. Predicted residual moisture profiles in a bean after steaming at different steam temperatures were compared. The moisture and TIA profiles in a steamed soybean with an average residual TIA level of 3.5 mg (g ds ff)$^{-1}$ are shown in figure 9. The difference in steam temperature results in different residual profiles. The moisture profile in the bean steamed at 100 °C is more flat than the profile at 120 °C. The TIA profile at 100 °C is almost equal to the profile at 120 °C. These two beans probably need a different drying step.

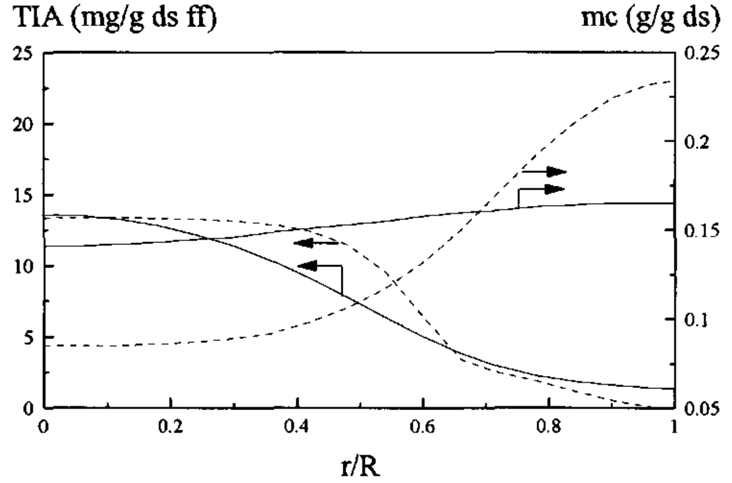

Fig. 9. Predicted residual TIA and moisture profiles of soybeans with residual TIA levels of 3.5 mg (g ds)$^{-1}$ steamed at 100 (—) and at 120 °C (- - -). Data: see Table 1 (K_m=0, mc_0=0.08 g (g ds)$^{-1}$).

Optimisation of the product quality

The nutritional value of soybeans depends on both the ANF level and the availability of essential amino-acids. Model simulations were performed to investigate if it is possible to optimise the product quality. TIA and NSI were used as quality parameters. The effect of steam temperature and initial moisture content of the beans on product quality was examined. It was investigated if it is possible to attain different NSI levels in soybeans with the same residual TIA level (3.5 mg (g ds ff)$^{-1}$). Increasing the initial moisture content increased the residual NSI levels in the steamed soybeans (Figure 10a). Increasing the steam temperature resulted in almost simular residual NSI levels in the steamed beans (Figure 10b). These simulations indicate that the steaming process can be optimised better by changing the initial moisture content than the steam temperature.

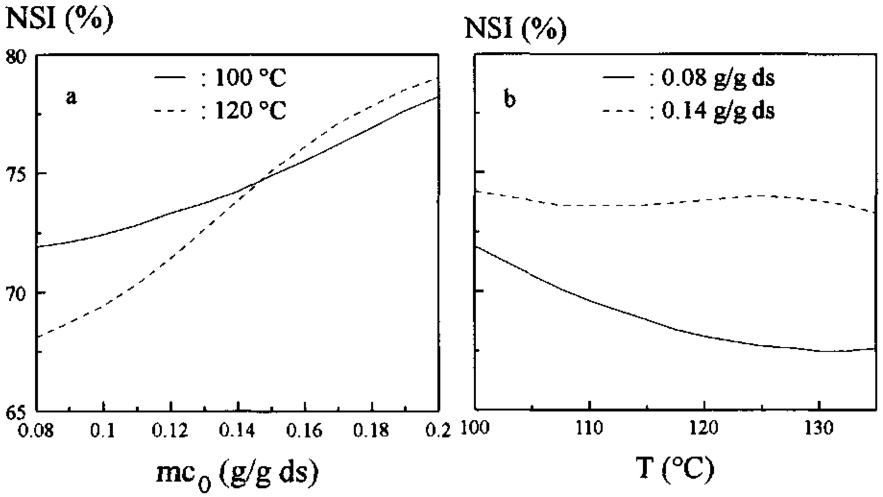

Fig. 10. Predicted influence of initial moisture content (**a**) and steam temperature (**b**) on the residual NSI level of steamed soybeans. The predicted residual TIA level in the beans is 3.5 mg $(g\ ds)^{-1}$). Data: see Table 1 (K_m=0).

Conclusions

The TIA levels predicted with the combined process model for steaming and kinetics model of TIA were in good agreement with the measured TIA values in steamed soybeans. The model was able to predict experimental TIA data from literature. The combined model was able to describe the trend of the measured decrease of NSI. Model simulations indicate that the steaming process can be optimised better on initial moisture content of the soybeans than on steam temperature using TIA and NSI as quality parameters.

Table 1. Data used for steaming predictions

initial moisture content	0.14 g (g ds)$^{-1}$
initial TIA level	21.8 mg (g ds ff)$^{-1}$
initial NSI level	87%
equivalent bean radius	$3.15 \cdot 10^{-3}$ m
density	1230 kg m^{-3}
thermal diffusivity coefficient	$1.26 \cdot 10^{-6}$ m^{2}s^{-1}
water diffusion coefficient	$E_a = 34.8 \cdot 10^3$ J mol^{-1}, $D_\infty = 4.44 \cdot 10^{-5}$ m^2 s^{-1}
equilibrium moisture content	1.66 g (g ds)$^{-1}$
kinetics model of TIA	see Van den Hout *et al* (1997)
kinetics model of NSI $\ln(k_\infty) = a + \frac{b}{mc}$	n=10.61, $E_a = 1.56 \cdot 10^5$ J mol^{-1}, a=1.10 and b=$-4.07 \cdot 10^{-1}$ g (g ds ff)$^{-1}$
dimensionless mass transfer coefficient	0.224 (model validation) 0 (simulations)

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Appendix

Determination of the boundary condition at r=R and t>0 during steaming.
The moisture absorption of the beans during steaming is caused by two external fluxes: (i) a flux of condensing steam, and (ii) a flux of condensed steam droplets.
The flux of condensing steam on the bean is assumed to be proportional to the rate of the increase of the mean bean temperature. The flux of the condensed steam droplets is described by introducing an external mass transfer coefficient k_m.

flux of water diffusion at the surface in the bean:	$-D \cdot \frac{\partial C}{\partial r}$
flux of condensing steam:	$\frac{V\rho C_p \frac{d\overline{T}}{dt}}{A \cdot H_{v,T_e}}$
flux of condensed steam droplets:	$k_m(C_e - C_s)$

Symbols

a	fitting parameter	(-)
A	bean surface area	(m^2)
b	fitting parameter	(g (g ds ff)$^{-1}$)
C	water concentration in bean	(kg m^{-3})
C_p	specific heat	(J kg^{-1} K^{-1})
D	water diffusion coefficient	(m^2 s^{-1})
D_∞	pre-exponential factor	(m^2 s^{-1})
E_a	activation energy	(J mol^{-1})
$H_{v,Te}$	condensation enthalpie at T=T_e	(J kg^{-1})
k	reaction rate	(s^{-1})
k_∞	pre-exponential factor	($\%^{1-n}s^{-1}$)
k_m	external mass transfer coefficient	(m s^{-1})
K_m	dimensionless mass transfer coefficient: $k_m \cdot R/D$	(-)
mc	moisture content	(g (g ds)$^{-1}$) or (g (g ds ff)$^{-1}$)
n	reaction order	(-)

n_s	sample size	(-)
NSI	nitrogen solubility index	(%)
P	pressure	(N m^{-2})
r	distance	(m)
R	sphere radius	(m)
s_w	$\frac{\sum \frac{\lvert X(i) - \hat{X}(i) \rvert}{X(i)}}{n_s}$	(-)
t	time	(s)
T	temperature	(°C)
$\bar{T}$:	mean bean temperature	(°C)
TIA	trypsin inhibitor activity	(mg (g ds ff)$^{-1}$)
V	bean volume	(m^3)
X(i)	measured value of T, mc, TIA or NSI	
$\hat{X}$	estimated or predicted value of T, mc, TIA or NSI	

Greek

α	fitting parameter	(-)
θ	dimensionless temperature: $(T-T_0)/(T_e-T_0)$	(-)
ρ	density	(kg m^{-3})

Subscripts

0	at time t=0
1,2	number of inactivation phase
c	centre
e	equilibrium
s	surface
t	at time t

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6. General discussion

Introduction

Kinetics models of the trypsin inhibitor activity (TIA) and the nitrogen solubility index (NSI) in soy flour and a process model for steaming have been developed. These models have been combined to predict the influence of steaming on the residual TIA and NSI levels in soybeans. Kinetics studies of the two main TI groups, the Kunitz soybean trypsin inhibitor (KSTI) and the Bowman-Birk inhibitor (BBI), have been performed to study the inactivation mechanism of trypsin inhibitors (TIs). The influence of extrusion shear forces on the inactivation of TIs in soy flour has been investigated.

In this chapter most aspects that have been discussed in the previous chapters will be reviewed. Furthermore, additional results will be considered. The usefulness of the parameter values of the inactivation kinetics model of TIs (Chapter 2) for different soy batches will be studied. The correlation between the water activity and glass transition temperature of soy flour, respectively, and the inactivation rate constants of TIs will be examined. The influence of the deformation of the storage protein molecules on protein denaturation during extrusion cooking will be reported. Simulations will be performed to study the influence of bean size and initial TIA level in the soybeans on the feed quality during steaming (toasting). The influence of the most important process conditions on the costs for steaming will be investigated.

A. Kinetics studies

The inactivation kinetics of TIs in defatted soy flour was measured and modelled in Chapter 2. The inactivation of TIs in soy flour exhibited a two-phase inactivation behaviour. A statistical criterion was used for ranking six different kinetics models. Two kinetics models proved to be parsimonious models: (i) two first order reactions of two different TI groups, e.g. KSTI and BBI, and (ii) the irreversible inactivation of a native TI to a partially active intermediate TI, followed by an irreversibel denaturation step. The first kinetics model was used to set-up an overall inactivation kinetics model for TI. The mechanistic explanation of the two-phase inactivation behaviour of TIs was further examined in Chapter 3. It was shown that this inactivation behaviour cannot be explained by the difference in heat stability of the two main TI groups: KSTI and BBI. The existence of thiols seems to be responsible for the two-phase inactivation behaviour of TIs. The mechanistic explanation of the second parsimonious model of Chapter 2 was not validated.

The kinetics model of TIs in combination with a process model is a powerful tool to optimise a heat process. The kinetics model was combined with a process model for extrusion cooking in order to investigate the influence of extrusion shear forces on the inactivation of TIs in soy flour (Chapter 4). A combined kinetics model and process model for steaming was able to predict the residual TIA levels in steamed soybeans (Chapter 5).

A.1 Kinetics parameters

The inactivation kinetics model of TIs (model 2 in Table 1 of Chapter 2) was applicable for other soy batches (Chapters 4 and 5). In Chapter 4 the parameter values of the kinetics model were fitted again to the experimental TIA data. The parameter values of the kinetics model may be different for different soy batches. In this chapter it will be investigated if the kinetics parameter values from Chapter 2 can be used to describe the experimental inactivation data of different soy batches.

The difference in heat stability of the two TI groups, KSTI and BBI, could not explain the inactivation behaviour of TIs (Chapter 3). Therefore, parameter A of the inactivation kinetics model does not reflect the fraction of the activity of the heat labile TI group of total TIA in the native soy flour. The mechanistic background of parameter A is not known. In Chapter 2 it was shown that the parameter A was independent on moisture content. In this chapter the dependency of parameter A on the initial TIA level in beans of different origin will be investigated.

A.2 Interruption of the heat treatment

From a process engineering point of view it is important to know (i) if the inactivation of TIs in soy is a reversible process, and (ii) if an interruption of the heat treatment has any effect on the inactivation behaviour of TIs when the heat treatment is continued. The reversibility of TIs and the effect of an interruption of the heat treatment will be investigated.

A.3 Water activity and glass transition temperature

Rates of deterioration and microbial growth are often related to water activity. The moisture content has a large influence on the inactivation rate of TIs (Chapter 2). In this chapter the degree of correlation between the pre-exponential factors k_{r1} and k_{r2} (model 2, Chapter 2) and the water activity of soy flour will be investigated.

Molecular mobility is an important temperature-dependent factor that may influence the rates of deterioration in food (Roos, 1995). The existence of a correlation between the inactivation rate constants of TIs and the glass transition temperatures of soy flour will be investigated.

B. Influence of shear forces on NSI

In Chapter 4 it was shown that the deformation of TI molecules in soy flour during extrusion cooking does not inactivate TIs. Little is known about the effect of extrusion shear forces on the denaturation of storage proteins in soy flour. Soy proteins contain approximately 34% 7S proteins (β- and γ-conglycinin) and 42% 11S proteins (glycinin). These groups have an average molecular weight of 156,000 and 348,000 g mol^{-1}, respectively. These molecular weights are an order of magnitude larger than the values of the TI molecules (8,000 and 20,000 g mol^{-1} for BBI and KSTI, respectively). The reduction of the activation energy is proportional to the molecular diameter, d_0, to the third power (Equation 6 in Chapter 4). Perhaps the storage proteins are denaturated by deformation of proteins during extrusion cooking.

In this chapter the influence of extrusion shear forces on the denaturation of soy proteins was determined. NSI is assumed to be a good indicator to follow the denaturation of the soy proteins during a heat treatment. The reduction of the activation energy for NSI by the application of stress will be calculated. The effect of shear forces on the decrease of NSI will be determined experimentally using the same procedure as in Chapter 4.

C. Steaming

A process model for (pressurised) steaming was set up in Chapter 5. Residual TIA and NSI levels in the steamed soybeans were predicted by combining the kinetics models for TIA and NSI with the process model. These predictions were compared with presented experimental data and data from literature (Chapter 5). Process conditions (e.g. steam temperature and residence time) and product properties of soybeans (e.g. initial moisture content, initial TIA level and particle size) can be used to optimise the steaming process. Simulations were performed to study the possibilities for optimisation of the product quality (Chapter 5). These simulations indicated that the product quality can be optimised using TIA and NSI as quality parameters. Initial moisture content rather than steam temperature should be used to optimise the process.

C.1 Prediction of residual TIA in Phaseolus Vulgaris beans

In this chapter it will be investigated if the inactivation of TIs in Phaseolus Vulgaris beans during steaming can be predicted using the kinetics model of TIA in soy flour and the process model for steaming.

C.2 Influence of process parameters on the product quality

Besides TIA and NSI, the available lysine level is often used in the animal feed industry to determine the quality of heat treated beans. Available lysine is considered to indicate over-processing of the beans. The influence of the residence time and the steam temperature on the residual FDNB reactive lysine in steamed soybeans will be reported in this chapter.

Batches of soy vary in kernel size (whole beans, cracked beans, flakes, etc.). Initial TIA levels in soybeans of different origin may vary between approximately 10 and 30 mg g^{-1} (Qin, 1996). In this chapter the influence of bean size and initial TIA level on the residual NSI levels in steamed soybeans will be investigated by performing model simulations.

C.3 Influence of process parameters on the process costs

Process costs play an important role in the optimisation of the steaming process. The influence of the process conditions and product properties on the costs of steaming and the subsequent drying of soybeans will be estimated qualitatively in this chapter.

Material and methods

A. Kinetics studies

Kinetics parameters. Parameter value A was estimated using our experimental data (Chapter 2, 4 and 5), and data from literature of steaming experiments of soybeans (Qin, 1996) and Phaseolus Vulgaris beans (Van der Poel, 1990). The parameter value was fitted by the NLIN-procedure of SAS (SAS Institute Inc., 1988) using the kinetics equation of model 2 (Table 1 in Chapter 2).

Reversibility. Untoasted defatted soy flour was moisturised and heated in steel cells as described in Chapter 2. The heat treatment was performed during 3 min at 0.23 g (g ds)$^{-1}$ and 119 °C. The samples were transferred to petri dishes. The petri dishes were closed with parafilm and stored at 25 °C. Residual TIA levels were measured after 0, 1, 2, 3, 5 and 7 days. The method for TIA determination was described in Chapter 2.

Interruption of the heat treatment. Untoasted defatted soy flour was heated at 119 °C and 0.21 g (g ds)$^{-1}$. The heat treatment was stopped at the inflexion point of the inactivation curve of TIs (t=110 s). The samples were cooled in ice-water and kept at room temperature for 30 min, or at 4 °C for 24 h. After the interruption period, the samples were heated again at 119 °C and different residence times. The method for TIA determination was described in Chapter 2. The heat transfer model is incorporated in the simulation procedure of TI inactivation after the interruption.

Sorption isotherms. Adsorption isotherms of untoasted defatted soy flour, defatted KSTI-free soy flour and full fat soy grits were measured with the gravimetric method at 25 °C. In order to examine the influence of a heat treatment on the isotherm of soy flour, an adsorption isotherm of heat treated soy flour (T=134 °C, mc=0.23 g (g ds)$^{-1}$, t=8.5 h) was measured. The initial moisture content of the flours was approximately 0.08 g (g ds)$^{-1}$. The following salt solutions were used: LiCl (a_w=0.11), $MgCl_2$ (0.33), $Mg(NO_3)_2$ (0.53), NaCl (0.75), KCl (0.84) and $BaCl_2$ (0.90). The sorption isotherm of the untoasted defatted soy flour was described with the Gugenheim Andersen and De Boer (GAB) equation (Equation 4.23 in Van den Berg, 1981).

Glass transition temperature. The thermal analyses of the untoasted defatted soy flour were performed with a Modulated DSC at TNO Nutrition and Food Research (Zeist, The Netherlands).

B. **Influence of shear forces on NSI**

Reduction of the activation energy of NSI. The decrease of the activation energy ΔE_a of NSI was calculated using equations 1 to 6 from Chapter 4. β-Conglycinin appears as a flat disk with a diameter of ca. 8.4 nm and a thickness of ca. 3.3 nm (Yamauchi *et al*, 1991). The native glycinin molecule appears as a disk with a diameter of 11 nm and a thickness of 7.5 nm (Badley *et al*, 1975). An equivalent sphere radius of 7 and 11 nm was calculated for β-conglycinin and glycinin, respectively.

Kinetics model of NSI decrease. Residual NSI levels were determined in the same samples as used for the inactivation kinetics model for TIs (Chapter 4). The method for NSI determination was described in Chapter 5. The decrease of NSI was described with a n^{th} order reaction kinetics. The rate constant was assumed to be dependent on temperature following the Arrhenius equation. The heat transfer model is incorporated in the estimation procedure of the kinetics parameters. The kinetics parameters were estimated using the NLIN-procedure of SAS (SAS Institute Inc., 1988).

Extrusion cooking experiments. Residual NSI levels in the extruded samples (Chapter 4) were calculated and measured as described in Chapter 5. The data of Table 2 in Chapter 4 were used to calculate residual NSI levels in the extruded samples.

C. **Steaming**

Prediction of residual TIA in Phaseolus Vulgaris beans. TIA data of steaming experiments were taken from Van der Poel (1990). A similar batch of Phaseolus Vulgaris beans as used by Van der Poel (1990) was prepared. This batch consisted of about 60% white and 40% red beans. About 30% of the beans were broken. The average volume of the beans was calculated from the measured dimensions of the white and red beans assuming the beans to be ellipsoid. The average volume was used to calculate an equivalent sphere radius. It was assumed that the moisture was only present in the fat free phase of the beans. The moisture content used in the kinetics model of TIs was therefore based on fat free samples (g (g ds ff)$^{-1}$). The oil phase content of Phaseolus Vulgaris beans was taken from Van der Poel (1990). Because of the inaccuracy of the TIA analysis below TIA levels of 0.75 mg (g ds ff)$^{-1}$, experimental data <0.75 mg (g ds ff)$^{-1}$ were not used.

Product quality. Total and available lysine levels were determined in the steamed soybeans from Chapter 5. The determination of total lysine and available lysine was performed by S/G Nutrilab b.v. (Giessen, The Netherlands), and was based on the method described by Mason and Bench-Anderson (1980) and ISO 5510 (1985), respectively. Total lysine content was measured in 4 samples. The average value was 28.1 mg (g ds ff)$^{-1}$. The available lysine in the untreated soybeans was 22.9 mg (g ds ff)$^{-1}$. The error (the difference between measured and mean value, devided by the mean value) of the total and available lysine analysis was 4 and 2%, respectively.

The physical data used for steaming simulations are listed in Table 1 of Chapter 5. The external mass transfer coefficient k_m was assumed to be zero during the simulations. The influence of initial TIA levels on the feed quality was examined assuming parameter A to be constant.

Process costs. Relevant data from Chapter 5 and this chapter were used.

Results and discussion

A. Kinetics studies

A.1 Kinetics parameters

The residual TIA levels in the heat treated samples of the different soy batches from Chapter 4 and 5 can be predicted reasonable well with the parameter values of the inactivation kinetics model of TIs of Chapter 2 (Table 1). The deviation s_w between the measured and predicted TIA levels (26 and 12%, respectively) is in the same order of magnitude as the deviation s_w of the kinetics model of Chapter 2 (14%). The inactivation rate constants of TIs in soy flour presented by DiPietro and Liener (1989) can be predicted well with our inactivation kinetics model (Table 1). The combined kinetics and process model was able to predict experimental data of TIs in steamed soybeans of different origin (Chapter 5). These results strongly indicate that the parameter values of the inactivation kinetics model of TIs of Chapter 2 can be used for soybeans of different origin.

Table 1. Deviation s_w (%) of TIA levels and rate constants when the kinetics parameters (A, E_a's and k_r's) were fitted to the experimental data, and when the parameter values of Chapter 2 were used to describe the data.

experimental data used	s_w (parameters were fitted)	s_w (parameters from Chapter 2)
TIA in Chapter 2	14[1]	-
TIA in Chapter 4	12	26
TIA in Chapter 5	-	12
k in DiPietro (1989)[2]	-	25

[1]: deviation s_w of overall kinetics model. [2]: the experimental conditions used by DiPietro and Liener (1989) were 5.3% (95 °C), 7.7% (95 °C) and 14.7% (75, 85 and 95 °C). The moisture content used by DiPietro and Liener (1989) was assumed to be based on total basis.

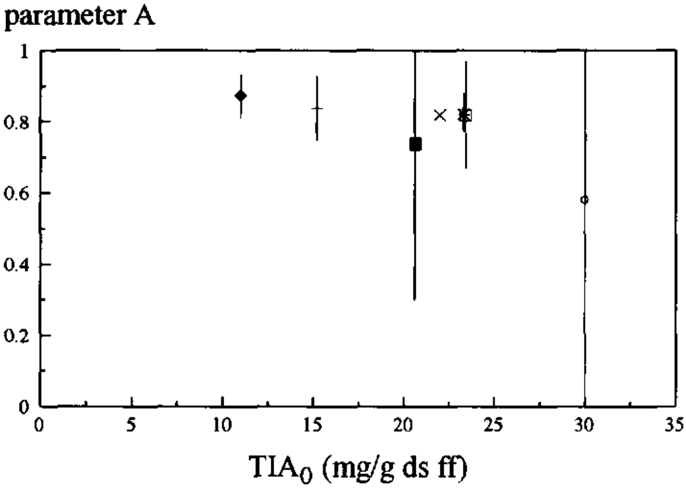

Fig. 1. Estimated parameter A with 95% confidential intervals as a function of initial TIA level in Phaseolus Vulgaris beans (♦, Van der Poel, 1990), and in different soy batches: Argentinian soybeans (+, Qin, 1996), Chinese soybeans (■, Qin, 1996), full fat soy grits (×, kinetics model of Chapter 2 was used to describe experimental data of Chapter 5), Argentinian soybeans (*, Qin, 1996), defatted soy flour (□, Chapter 2) and defatted soy flour (o, Chapter 4).

The dependency of the estimated parameter A on the initial TIA level in different soy batches is shown in Figure 1. The figure suggests that parameter A is independent on initial TIA level, and also on the origin of the soy batch.

The estimated parameter A for Phaseolus Vulgaris beans is equal to the values for soy, considering the 95% confidence intervals. In Chapter 3 it was shown that the inactivation rate constants of KSTI and BBI in a starch matrix containing cysteine were almost equal to the rate constants of TIs in soy flour. The inactivation of TIs in Phaseolus Vulgaris beans during steaming can be predicted reasonable well with the combined kinetics model for TIs in soy flour and the process model for steaming (Section C in this chapter). These results indicate that the inactivation kinetics model for TIs in soy flour can be used to describe the inactivation of TIs in different matrices containing thiols.

A.2 Interruption of the heat treatment

Figure 2 shows that the inactivation of TIs in the heat treated soy flour is not reversible during at least 7 days.

The experimental data in Figure 2 indicate that the interruption of the heat treatment does not influence the inactivation behaviour of TIs. Two simulations with the inactivation kinetics model of TIs of Chapter 2 were performed: (i) the inactivation is continued after the interruption and (ii) the inactivation starts a two-phase inactivation again. The predicted inactivation is small when the heat treatment was started again, because the calculated temperature has not reached 95% temperature equilibration yet. The simulations show that the inactivation of TIs is continued in the same way as it would without interruption, and does not start a two-phase inactivation again.

These conclusions concerning the reversibility and interruption are important in case the soybeans are subjected to a series of heat treatments.

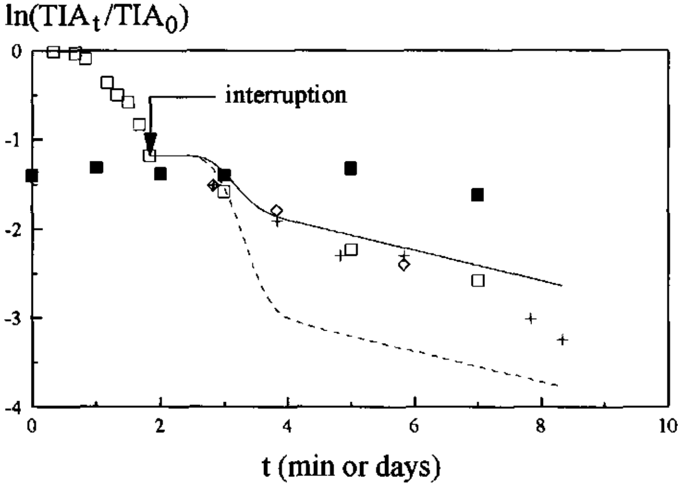

Fig. 2. Residual TIA level in soy flour (■) during 7 days at 0.23 g (g ds)$^{-1}$ and 25 °C after a heat treatment. Residual TIA level in soy flour (in min) after a heat treatment without an interruption (□), with an interruption after 110 s during 30 min at room temperature (+), and with an interruption after 110 s during 24 h at 4 °C (◇). The inactivation of TIs after the interruption is predicted assuming the inactivation (i) to continue (—), and (ii) to start a two-phase inactivation again (- -).

A.3 Water activity and glass transition temperature

The isotherm of the native soy flour is described with the GAB equation (Figure 3). The sorption isotherm of the full fat soy grits and the KSTI-free soy flour resembles, as expected, the isotherm of native soy flour when the moisture content is based on fat free basis (g (g ds ff)$^{-1}$). Figure 3 shows that the effect of protein denaturation on the adsorption isotherm of soy flour is small.

The correlation between the logarithm of the pre-exponential factors k_{r1} and k_{r2} of the kinetics model of TIs (T_r=110 °C), and the water activity of soy flour at 25 °C is shown in Figure 4. The water activity was predicted with the GAB equation. The figure suggests a linear relation between the logarithm of k_{r1} and k_{r2}, and the water activity of soy flour.

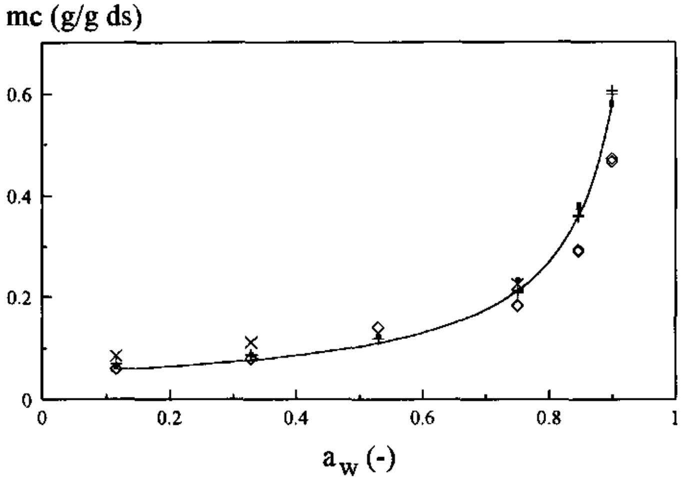

Fig. 3. Sorption isotherms of native defatted soy flour (+), severe heat treated defatted soy flour (◊), full fat soy grits (•, mc in g (g ds ff)$^{-1}$), and defatted KSTI-free soy flour (×) at 25 °C. Estimated parameters of the GAB equation: W_1=5.1·10^{-2} g (g ds)$^{-1}$, C_g=1.4·10^9 and K=1.02.

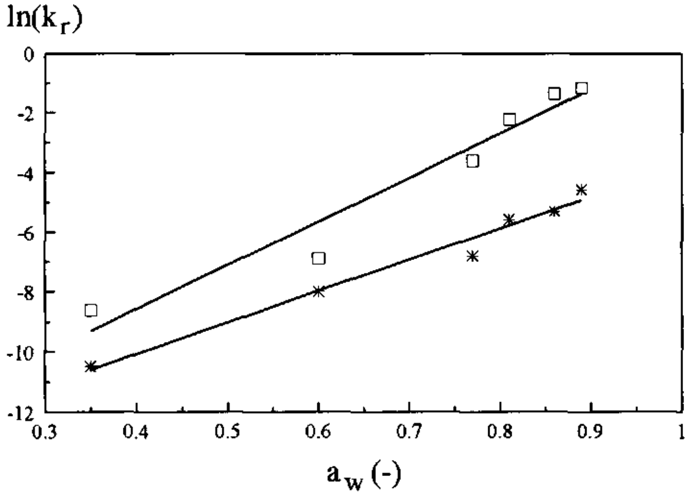

Fig. 4. Correlation between the estimated pre-exponential factors k_{r1} (□) and k_{r2} (*) of TIs in soy flour (T_r=110 °C), and the water activity of native soy flour at 25 °C.

Figure 5 shows that the glass transition temperature T_g of soy flour is very dependent on moisture content. This observation agrees with the studies of other food systems by Roos and Karel (1991). The measured glass transition temperatures of the flour are at least 30 °C lower than the temperatures at which the inactivation experiments of Chapter 2 were performed. In concluding, the glass transition in soy flour will probably not influence the inactivation kinetics of TIs.

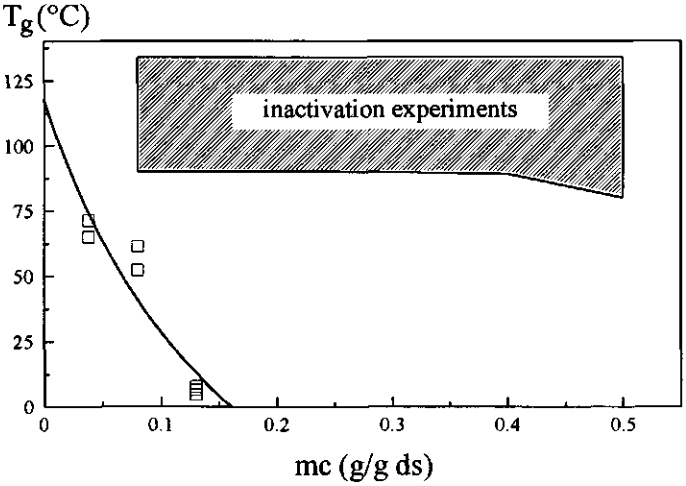

Fig. 5. Measured glass transition temperatures (□) of defatted soy flour at different moisture contents. The shaded area reproduces the conditions of the inactivation experiments (Chapter 2). An empirical equation from Roos and Karel (1991, equation 1) was used to fit the experimental data (—, estimated parameters: k=5.4 and $T_{g,soy}$=118 °C).

B. **Influence of shear forces on NSI**

Reduction of the activation energy of NSI

The calculated decrease of the activation energy ΔE_a for the 7S and 11S proteins due to deformation of the proteins in the screw channel, the flight clearance, and at the die wall are listed in Table 2. These values only intend to present orders of magnitude of the reduction of activation energy of NSI. The calculated ΔE_a's for the storage proteins are high enough to result in an additional decrease of NSI due to extrusion shear forces.

Table 2. Calculated decrease of the activation energy ΔE_a for the 7S and 11S proteins at different positions in the extruder.

position	ΔE_a (kJ mol^{-1})	
	7S	11S
channel	1	4
clearance	3	10
die wall[1]	5	21

[1]: die configuration used for calculation: d=6 mm and l=6 mm.

Extrusion cooking experiments

The n^th order reaction kinetics model describes the decrease of NSI in soy flour with a deviation s_w of 1% (Figure 6).

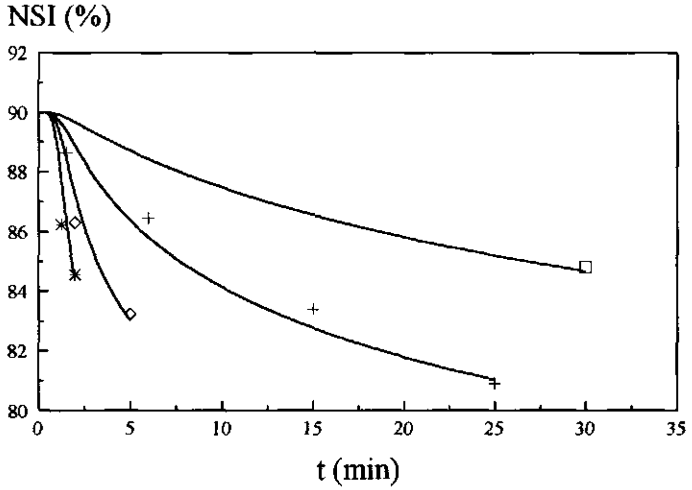

Fig. 6. Measured and predicted (—) decrease of NSI in soy flour at 0.40 g (g ds)$^{-1}$. The temperatures are: 80 (□), 90 (+), 99 (◊) and 109 °C (∗). The estimated parameters are: E_a=1.45·10^5 J mol^{-1}, k_r=2.80·10^{-46} %$^{1-n}$·s^{-1} and n=23.07.

Table 3 shows that the predicted decrease of NSI due to heat is very small. The measured NSI values in the extrudates are systematically lower than the calculated NSI values, except for the extrusion experiment performed with no die head. The differences between the calculated and measured NSI values are only little larger than the relative deviation of the NSI analyses (4%). It was studied if these differences could be explained by a deviation in the measured temperature profile of the soy during extrusion cooking. Calculations showed that an increase of the axial temperature profile of 25 to 35 °C is necessary to predict the measured NSI values in Table 3. This increase of temperature is higher than the expected experimental error. The results indicate that the measured decrease of NSI can not be predicted by heat inactivation only.

The calculated NSI value for the extrusion experiment performed without a die head was equal to the measured value. One explanation is that the decrease of NSI is caused by the shear forces in the die. A synergetic effect of shear and temperature can be another explanation, since the measured temperature profile in the screw channel for the experiment without a die head is lower than the profile for the other extrusion experiments. This synergetic effect was also predicted when the decrease of the activation energies ΔE_a for the 7S and 11S proteins from Table 2 were subtracted from the activation energy of NSI decrease to calculate NSI value in the extrudates.

Table 3. Calculated and measured NSI values in the extrudates.

die d/l (mm/mm)	$\dot{\gamma}_w$ (s^{-1})	T_{high} (°C)	NSI_c (%)	NSI_m (%)
7/7	189	106	89	83
7/3.5	189	107	89	83
6.5/6.5	236	107	88	82
6/6	304	112	88	77
no[1)]	-	83	90	90

[1)]: extrusion cooking experiment with compression ration of 4 and no die head. NSI_0=90%. T_{high}: highest measured temperature.

In concluding, theoretical calculations indicate that shear can cause an additional denaturation of the storage proteins during extrusion cooking. Extrusion cooking experiments showed that the decrease of NSI can not be explained by heat inactivation only. The observed difference might be due to the deviation in the NSI analysis. However, it is also quite possible that the difference is caused by additional denaturation as a result of the deformation of proteins during extrusion cooking.

C. Steaming

C.1 Prediction of residual TIA in Phaseolus Vulgaris beans

The combined steaming model and kinetics model of TIs in soy flour were used to predict the inactivation of TIs of Phaseolus Vulgaris beans during steaming. Figure 7 shows that the inactivation can be predicted reasonable well (s_w=28%). This is remarkable, since the composition of Phaseolus Vulgaris beans is different from the composition of soybeans (Belitz and Grosch, 1995).

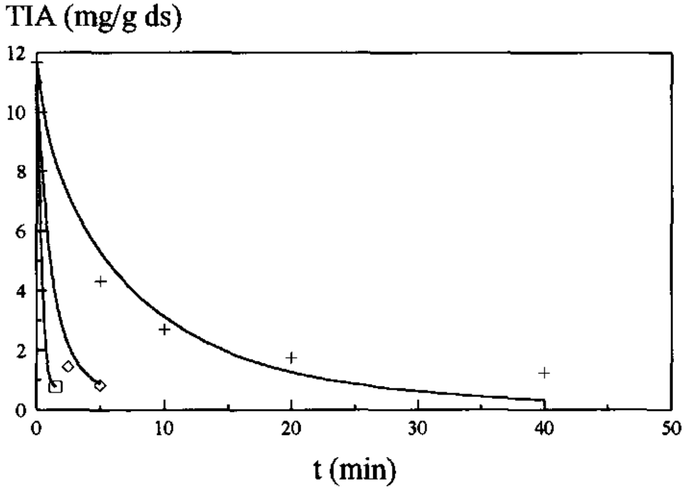

Fig. 7. Measured and predicted (—) residual TIA levels of steamed Phaseolus Vulgaris beans at 102 (+), 119 (◇) and 136 °C (□). Experimental data were taken from Van der Poel (1990).

C.2 Influence of process parameters on the product quality

The FDNB reactive lysine levels in the steamed soybeans showed no decrease with the prolongation of the residence time, except perhaps at 120 °C and 4 min (Table 4). Soybeans with a residual TIA level of 1.5-3.5 mg (g ds ff)$^{-1}$ after steaming at different steam temperatures have almost equal FDNB reactive lysine levels. This result agrees with the findings that the predicted residual NSI value in steamed soybeans with a residual TIA level of 3.5 mg (g ds ff)$^{-1}$ is almost independent on steam temperature (Chapter 5). Yin *et al* (1993) autoclaved two different batches of soybeans at 125 °C. One batch showed a significant decrease of FDNB reactive lysine after 5 minutes, the other batch did not (residual TIA $\cong$ 2.2 mg g^{-1}). Qin (1996) measured the FDNB reactive lysine in soybeans of different origin (mc_0=0.083 and 0.116 g (g ds)$^{-1}$, respectively) after steaming at different steam temperatures (100, 118 and 136 °C) and residence times. Although their measured data are not very regular, they found no decrease in residual FDNB reactive lysine in steamed soybeans with a residual TIA level of 1-2 mg (g ds ff)$^{-1}$.

Table 4. Residual FDNB reactive lysine and TIA levels in soybeans steamed at different temperatures and residence times.

	102 °C		110 °C		120 °C	
time[1]	lysine[2]	TIA[3]	lysine	TIA	lysine	TIA
1	25.0	15.2		11.4		5.1
1.5		13.3			25.4	3.6
2	25.0		25.9	7.1	25.8	
2.5					25.8	
3	24.9	8.5				2.4
4			25.0	2.9	24.7	
5	25.4	7.0	25.9			1.4
7.5	25.8	4.1	25.5	1.7		
10	25.7	3.4		1.6		
15	25.9	2.0	25.9			
20	25.4	2.0				

[1]: residence time of the beans in the toaster in min; [2]: FDNB reactive lysine in mg (g ds ff)$^{-1}$; [3]: TIA in mg (g ds ff)$^{-1}$. The FDNB reactive lysine in the untreated beans was 22.9 mg (g ds ff)$^{-1}$.

In concluding, changing the steam temperature results in approximate similar NSI and FDNB reactive lysine levels in steamed soybeans with the same residual TIA level of 3.5 mg (g ds ff)$^{-1}$. The High Temperature Short Time (HTST) property of pressurised steaming does not provide a tool in optimising the soybean feed quality using *in vitro* measurements as feed quality parameters. An increase in initial moisture content results in higher predicted NSI levels in steamed beans with the constant residual TIA levels (Chapter 5). Considering our results and results from Qin (1996), no change in FDNB reactive lysine level is predicted in soybeans steamed at different initial moisture contents.

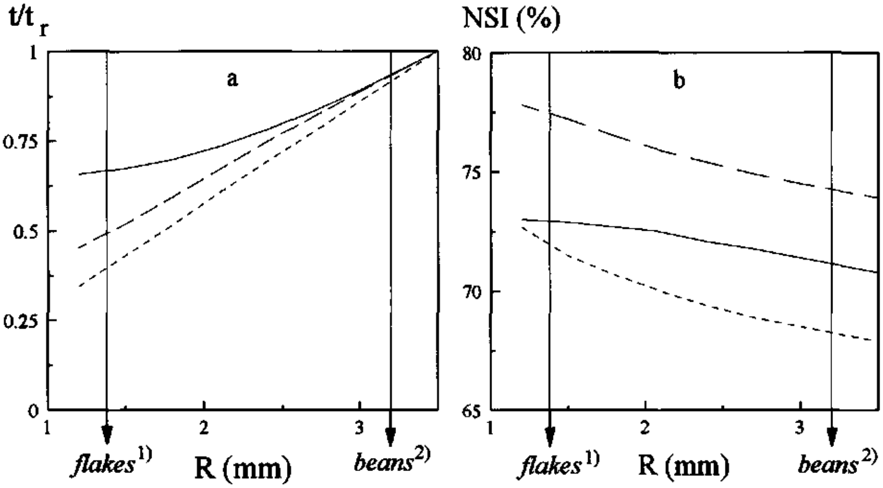

Fig. 8. Predicted influence of the bean size on the residence time needed to achieve a residual TIA level of 3.5 mg (g ds ff)$^{-1}$ **(a)**, and on the residual NSI level in the soybeans **(b)**. Conditions are 100 °C and 0.08 g (g ds)$^{-1}$ (—, t_r=2456 s), 100 °C and 0.14 g (g ds)$^{-1}$ (— —, t_r=842 s), and 120 °C and 0.08 g (g ds)$^{-1}$ (- - -, t_r=342 s). 1): value was calculated from the average thickness of flakes. 2): value was taken from Chapter 5. t_r= t at R=3.5 mm. Data: see Table 1 in Chapter 5.

Model simulations were performed to study the influence of bean size and initial TIA level on the residual NSI level in steamed soybeans. Figure 8a shows that the particle size has a large influence on the residence time needed to reach a residual TIA level of

3.5 mg (g ds ff)$^{-1}$ in the steamed soybeans. An increase in particle size results in a decrease of the Fourier number for moisture transport and in a less flat moisture profile in the bean. The moisture content near the centre of the bean will be lower in larger beans. The influence of a change in moisture content on the reaction rate of TIA will be higher in the centre of the bean than in the outside of the bean (Chapter 2). The overall inactivation rate of TIs will therefore be smaller in the beans with larger bean size. Consequently, beans larger in size need a longer residence time to achieve a residual TIA level of 3.5 mg (g ds ff)$^{-1}$.

The predicted residual NSI decreases with increasing bean size (Figure 8b). As was explained previously, the residual moisture content near the centre of the bean decreases with increasing bean size at constant residence time. The ratio of inactivation rate constants of TIs and the constant of NSI increases with increasing moisture content. Consequently, the residual NSI will decrease with increasing bean size. The influence of kernel size on the predicted residual NSI value, however, is small (Figure 8b).

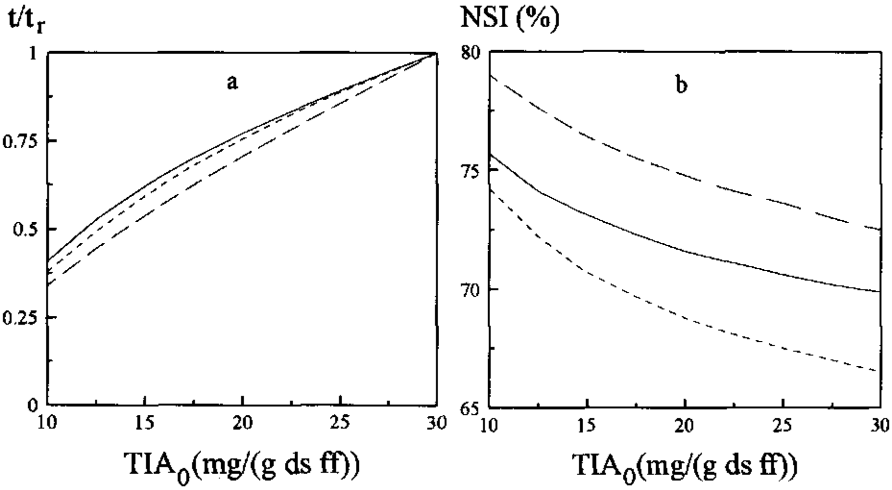

Fig. 9. Predicted influence of the initial TIA value on the residence time needed to achieve a residual TIA level of 3.5 mg (g ds ff)$^{-1}$ (**a**), and on the residual NSI value in the soybeans (**b**). Conditions are 100 °C and 0.08 g (g ds)$^{-1}$ (—, t_r=2780 s), 100 °C and 0.14 g (g ds)$^{-1}$ (— —, t_r=1022 s), and 120 °C and 0.08 g (g ds)$^{-1}$ (- - -, t_r=383 s). t_r= t at TIA_0=30 mg (g ds ff)$^{-1}$. Data: see Table 1 in Chapter 5.

Figure 9a shows that the predicted residence time increases, as expected, with increasing initial TIA value in the soybeans. Consequently, the predicted residual NSI value will decrease with increasing initial TIA value (Figure 9b). However, the predicted influence of initial TIA value in soybeans on the residual NSI is small.

Summarising, the steaming simulations indicate that the product quality of the soybeans could be optimised using initial moisture content, bean size or initial TIA. Changing the bean size and the initial TIA, however, have their practical limitations, and the predicted influence of these parameters on the product quality is small. The predicted product quality of the soybeans is almost independent on the steam temperature (Table 5).

Table 5. Influence of important process parameters on the soybean product quality and the process costs. TIA and NSI are used as feed quality parameters.

	T	mc_0	R	TIA_0
product quality	—	↗	↘	↘
steaming costs	↘	↘	↗	↗
drying costs	—	↗	—	—

C.3 Influence of process parameters on the process costs

The average costs for steaming and drying of soybeans were estimated to be around Dfl 13 per ton beans (T=102 °C and mc_0=0.08 g (g ds)$^{-1}$). The total process costs are divided over 60% for steaming and 40% for drying. The steaming costs are equally divided over steam requirement and toasting equipment.

Table 5 gives an overview of the qualitative influence of the most important process conditions and product properties on the costs for steaming and drying.

Firstly, the influences of these process parameters on the costs for steaming are discussed. The steaming costs can be divided in the cost for steam requirement and cost for toasting equipment. Increasing the steam temperature will, of course, increase the costs for the amount of steam needed. The costs for the toasting equipment are dependent on the dimensions of the equipment, and, consequently, the residence time of the beans in the toaster. The residence time is decreased by increasing the initial moisture content or steam temperature, and decreasing the bean size or initial TIA level. Consequently, these changes will decrease the costs of the toasting equipment. Preliminary calculations showed that the decrease of toasting equipment costs with increasing steam temperature is larger that the increase of steam requirement costs.

Secondly, the influences of the process parameters on the drying costs are discussed. A drying step is needed after steaming. Generally a moisture content of 0.15 g $(g\ ds)^{-1}$ is accepted for storing and handling of soybeans. Storage of up to one year is feasible at this level.

The costs for drying are dependent on the residual moisture content and moisture profile in the beans after steaming. The residual moisture content of the beans after steaming is, of course, dependent on the initial moisture content of the beans before steaming. The beans flash water when they leave the pressurised toaster. Consequently, the residual moisture content is independent on the steam temperature. The residual moisture content is independent on bean size and initial TIA level.

The residual moisture profile of the beans after steaming is dependent on the residence time of the beans in the toaster. When the residence time increases the moisture will penetrate more deeply in the bean and the moisture content near the centre of the bean will increase. Consequently, a longer residence time of the beans in the dryer is needed to dry the beans to the desired moisture content. An increase in drying costs is expected when bean size and initial TIA level are increased, and the steam temperature is decreased. The influence of different residual moisture profiles in the beans on the drying costs is difficult to quantify. However, its contribution to the total process costs will probably be small and is therefore not listed in Table 5.

It is difficult to predict quantitatively the influence of the process parameters on the process costs. Preliminary calculations showed that the influence of the process conditions and product properties discussed above on the net process costs seems to be relatively small. The steam temperature and to a lesser extend the initial moisture content seem to have the largest influence on the total process costs compared to initial TIA and bean size.

Concluding remarks

The inactivation kinetics of TIs in defatted soy flour was measured and modelled under a large range of temperatures and moisture contents. The inactivation of TIs showed a two-phase inactivation behaviour. The parameter values of the kinetics model can be used for different soybean batches.

The two-phase inactivation behaviour can not be explained by the difference in heat stability of the two main TI groups: KSTI and BBI. The existence of thiols seems to be responsible for the two-phase inactivation behaviour of TIs. The role of thiols on the inactivation mechanism of TIs is an interesting subject for more in depth biochemical research.

Theoretical calculations showed that it can not be excluded that shear forces are a factor in the inactivation of TIs during extrusion cooking. The inactivation of TIs in soy flour during extrusion cooking can be explained properly by heat. There is no indication that TIs were inactivated by the deformation of the TI molecules due to extrusion shear forces. Theoretical calculations and experimental results indicated that the decrease of NSI is influenced by deformation of proteins during extrusion cooking. If shear has an influence on the denaturation of soy proteins, but not on the inactivation of the most important ANFs, the TIs, this can have consequences for the feed quality of the extruded product. More precise experimental data are needed to study the effect of shear on NSI more comprehensively.

A process model for steaming was set up and combined with the kinetics models of TIs and NSI in soy flour. The combined kinetics and process models were used to predict residual TIA and NSI levels in the steamed soybeans. Simulations using these models indicate that the steaming process can be optimised using TIA and NSI as quality parameters. Initial moisture content and bean size rather than steam temperature should be used to optimise the process. Soybeans with a residual TIA level of 1.5-3.5 mg (g ds ff)$^{-1}$ do not show a decrease in FDNB reactive lysine at different steam temperatures.

More research is needed to verify the conclusions concerning the influence of initial moisture content, bean size and steam temperature on the feed quality by performing *in vivo* experiments.

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Symbols

a_w	water activity	(-)
A	fitting parameter	(-)
d	die diameter	(m)
d_0	molecule diameter	(m)
E_a	activation energy	$(J\cdot mol^{-1})$
k	inactivation rate	(s^{-1})
k_m	external mass transfer coefficient	$(m\ s^{-1})$
k_r	pre-exponential factor at reference temperature T_r	$(\%^{1-n}\cdot s^{-1})$
l	die length	(m)
mc	moisture content	$(g\ (g\ ds)^{-1})$ or $(g\ (g\ ds\ ff)^{-1})$
n	reaction order	(-)
n_s	sample size	(-)
NSI	nitrogen solubility index	(%)
R	beans radius	(m)
s_w	$\dfrac{\sum \dfrac{\lvert X(i) - \hat{X}(i) \rvert}{X(i)}}{n_s}$	(-)
t	time	(s)

T	temperature	(°C, K)
T_g	glass transistion temperature	(°C)
TIA	trypsin inhibitor activity	(mg (g ds ff)$^{-1}$)
X(i)	measured value of TIA, NSI or k	
$\hat{X}(i)$	estimated value of TIA, NSI or k	
Greek		
$\dot{\gamma}$	shear rate	(s^{-1})
Subscripts		
0	at t=0	
1,2	number of inactivation phase	
c	calculated	
m	measured	
t	at t=t	
r	reference	
w	die wall	

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Summary

Soybeans are used as raw material for human nutrition and animal feed because of their high nutritional value. Consumption of raw beans has negative effects on the growth and health of human beings and animals. These effects are caused by antinutritional factors (ANFs). Trypsin inhibitors (TIs) are generally considered as the most important ANFs in soybeans. TIs can be divided in two main groups: the Kunitz soybean trypsin inhibitor (KSTI) and the Bowman-Birk inhibitor (BBI). TIs and some of the other ANFs are heat labile and are inactivated by heat treatments such as steaming (toasting) and extrusion cooking. Optimal design and optimisation of the heat treatment are necessary because over-processing reduces the protein availability to the animals. In practice, design and optimisation are based on experience and 'trial-and-error'. In some studies 'black-box' models are presented. No explicit models were developed in previous studies to predict the influence of a heat treatment on the feed quality of soybeans.

The aim of this research is to develop 'white-box' models to predict the influence of a heat treatment on the product quality of soybeans. Kinetics and process models are developed to predict the change of the product quality during the process. Particular attention is paid to the mechanistic background of the inactivation of TIs in soy flour, and to the influence of shear forces on the inactivation of TIs during extrusion cooking.

In order to develop an inactivation kinetics model, the inactivation of TIs in soy flour is measured within a large range of temperatures and moisture contents. The inactivation of TIs exhibits a two-phase inactivation behaviour. Six different mechanistic kinetics models are used to describe the experimental data. These models are compared statistically. Two parsimonious models are able to describe the two-phase inactivation behaviour of TIs well with a minimal number of kinetics parameters. One model describes the difference in heat stability of two TI groups, e.g. KSTI and BBI. The second model describes the irreversible inactivation of a native TI to a partially active intermediate TI, followed by the denaturation to a complete inactive form of TI.

The hypothesis that the two-phase inactivation behaviour of TIs is caused by a difference in heat stability of two TI groups is further examined. The activity of KSTI and BBI is determined in different heat treated soy samples. The results show that the two-phase inactivation behaviour of TIs cannot be explained by the difference in heat stability of

KSTI and BBI. Additional experiments show that the addition of a thiol (cysteine) resulted in a two-phase inactivation behaviour of KSTI and BBI, respectively, in starch. We suggest that TIs in soy flour inactivate by sulphydryl-disulfide interchange during the first inactivation phase, and by heat during the second phase.

During extrusion cooking, TIs in soy flour are inactivated by heat and possibly also by the deformation of the TI-molecules due to the shear forces. First, the theoretical influence of these shear forces on the inactivation of TIs is examined. The calculations show that some influence of shear forces on the inactivation of TIs can not be excluded. Furthermore, single screw extrusion experiments are performed to examine to effect of shear experimentally. The decrease of TIA due to heat inactivation during extrusion cooking is calculated by combining the extrusion conditions (temperature profile and residence time distribution) with the inactivation kinetics model of TIs. The results show that the measured residual trypsin inhibitor activity (TIA) values of the extrudates can be predicted properly by only heat inactivation. There is no indication that shear forces are involved in the inactivation of TIs during extrusion cooking.

Atmospheric steaming (toasting) is the most used heat treatment of soybeans and flakes. A process model is developed to describe the temperature and moisture profiles in the beans during steaming. In order to evaluate the effect of steaming on the protein availability, the kinetics of nitrogen solubility index (NSI) change is measured and modelled. The kinetics models of TIA and NSI are combined with the process model for steaming to predict TIA and NSI levels in the steamed soybeans. The model predictions are validated with experimental data. The possibility for the optimisation of the product quality of soybeans during steaming is investigated by performing simulations. These simulations indicate that the steaming process can be optimised using TIA and NSI as quality parameters. Initial moisture content rather than steam temperature should be used to optimise the process.

Samenvatting

Sojabonen worden vanwege hun hoge nutritionele waarde veel gebruikt als grondstof in de humane voeding en veevoer. De consumptie van rauwe sojabonen heeft een negatief effect op de groei en de gezondheid van mens en dier. Dit effect wordt veroorzaakt door antinutritionele factoren (ANF's). De trypsine remmers (TI's) worden over het algemeen als de belangrijkste ANF's in sojabonen gezien. TI's kunnen worden onderverdeeld in twee belangrijke groepen: de Kunitz sojaboon trypsine remmer (KSTI) en de Bowman-Birk remmer (BBI). De TI's en sommige andere ANF's zijn hittelabiel en kunnen bij de bereiding van veevoer met behulp van een hittebehandeling, zoals stomen of extrusie, geïnactiveerd worden. Optimaal ontwerp en optimalisatie van de hittebehandeling zijn nodig omdat de beschikbaarheid van eiwitten door oververhitting afneemt. In de praktijk zijn procesontwerp en -optimalisatie gebaseerd op ervaring en 'trial-and-error'. In sommige studies worden 'black-box' modellen gepresenteerd, maar er zijn geen expliciete modellen ontwikkeld om de invloed van een hittebehandeling op de voederkwaliteit van sojabonen te voorspellen.

Het doel van dit onderzoek is om 'white-box' modellen te ontwikkelen om de invloed van een hittebehandeling op de produktkwaliteit van sojabonen te beschrijven. Er zijn kinetiek- en procesmodellen ontwikkeld om de verandering van de produktkwaliteit tijdens de procesbehandeling te voorspellen. Er is speciale aandacht besteed aan de mechanistische achtergrond van de inactivering van de TI's in sojabloem en de invloed van afschuifkrachten op de inactivering van de TI's tijdens extrusie.

Om een inactiveringskinetiek model voor de TI's te ontwikkelen, is de inactivering van de TI's in sojabloem gemeten binnen een groot traject van temperaturen en vochtgehalten. De inactivering van de TI's vertoont een twee-fasen inactiveringsgedrag. Er zijn zes mechanistisch verschillende kinetiekmodellen gebruikt om de experimentele data te beschrijven. De modellen zijn met behulp van de statistiek met elkaar vergeleken. Twee spaarzame modellen zijn in staat het twee-fasen inactiveringsgedrag van de TI's met weinig kinetiekparameters te beschrijven. Eén model beschrijft het verschil in hittestabiliteit van twee TI-groepen, bijvoorbeeld KSTI en BBI. Het tweede model beschrijft de inactivering van een natieve TI naar een gedeeltelijk actieve intermediaire TI, die vervolgens inactiveert naar een compleet inactieve vorm.

De hypothese dat het twee-fasen inactiveringsgedrag van de TI's wordt veroorzaakt door een verschil in hittestabiliteit van twee TI-groepen is verder onderzocht. De activiteit van KSTI en BBI is in verschillende hittebehandelde sojamonsters gemeten. De resulaten laten zien dat het inactiveringsgedrag van de TI's niet verklaard kan worden door het verschil in hittestabiliteit van KSTI en BBI. Aanvullende experimenten laten zien dat de toevoeging van thiolen (cysteine) in een twee-fasen inactivering van KSTI en BBI in zetmeel resulteert. Een hypothese is voorgedragen dat de TI's in sojabloem inactiveren door middel van waterstof-disulfide interactie tijdens de eerste inactiveringsfase en door middel van hitte tijdens de tweede fase.

Tijdens extrusie worden de TI's in sojabloem geïnactiveerd door hitte, maar mogelijk ook door de vervorming van de TI-molekulen door afschuifkrachten. De theoretische invloed van deze afschuifkrachten op de inactivering van de TI's is berekend. De berekeningen laten zien dat enige inactivering van de TI's door de vervorming niet uitgesloten kan worden. Enkelschroefsextrusie experimenten zijn uitgevoerd om het effect van de afschuiving experimenteel te bepalen. De afname van de TI's ten gevolge van hitte-inactivering werd berekend door de procesomstandigheden tijdens extrusie (temperatuurprofiel en verblijftijdsspreiding) te combineren met het inactiveringskinetiekmodel van de TI's. De resultaten laten zien dat de gemeten afname van de trypsine remmende activiteit (TIA) in de extrudaten goed voorspeld kan worden door hitte-inactivering. Er is geen indicatie dat afschuiving een belangrijke rol speelt bij de inactivering van de TI's tijdens extrusie.

Atmosferisch stomen is de meest gebruikte hittebehandeling van sojabonen en -flakes. Er is een procesmodel opgesteld om de temperatuur- en vochtprofielen in de bonen tijdens het stomen te beschrijven. Om het effect van stomen op de eiwitbeschikbaarheid te evalueren, is de kinetiek van stikstofoplosbaarheidsindex (NSI) gemeten en gemodelleerd. De kinetiekmodellen van TIA en NSI zijn gecombineerd met het procesmodel voor stomen om de TIA- en NSI-gehalten in gestoomde sojabonen te voorspellen. De modelvoorspellingen zijn gevalideerd met experimentele waarden. De mogelijkheid voor optimalisatie van de produktkwaliteit van de sojabonen is met behulp van simulaties bestudeerd. Deze simulaties geven aan dat het stoomproces kan worden optimaliseerd bij het gebruik van TIA en NSI als kwaliteitsparameters. Het proces kan beter geoptimaliseerd worden door het beginvochtgehalte van de sojabonen te veranderen dan door de stoomtemperatuur te veranderen.

Nawoord

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Curriculum vitae

Rob van den Hout werd op 19 april 1966 geboren te Tilburg. Hij behaalde in 1985 het VWO diploma aan het Maurickcollege te Vught. In datzelfde jaar begon hij zijn studie Scheikundige Technologie aan de Technische Universiteit te Eindhoven. Zijn stage deed hij bij Flour Daniel te Haarlem. Eind 1992 ronde hij zijn studie af met een afstudeervak op het gebied van de membraanfiltratie bij de vakgroep Chemische Technologie. Vanaf 1992 was hij werkzaam als Assistent in Opleiding bij de sectie Proceskunde van de Landbouwuniversiteit te Wageningen en verrichte hij het onderzoek dat leidde tot dit proefschrift.