Inactivation Kinetics Study of the Kunitz Soybean Trypsin Inhibitor and the Bowman−Birk Inhibitor

Rob van den Hout,* Marieke Pouw, Harry Gruppen, and Klaas van't Riet

Department of Food Science, Food and Bioprocess Engineering Group, Wageningen Agricultural University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

The inactivation of trypsin inhibitors (TIs) in soy flour exhibits a two-phase inactivation behavior. It is sometimes assumed that this behavior is caused by a difference in the heat stabilities of the Kunitz soybean trypsin inhibitor (KSTI) and the Bowman−Birk inhibitor (BBI). Kinetics studies with KSTI and BBI in soy flour showed that this two-phase inactivation behavior of TIs could not be explained by the difference in the heat stabilities of KSTI and BBI. Inactivation of KSTI and BBI in an aqueous solution and in a starch matrix followed a first-order reaction. KSTI and BBI in a starch matrix with added cysteine showed a two-phase inactivation behavior. The existence of thiols in soy flour seems to be responsible for the two-phase inactivation of TIs in soy flour. It is suggested that TIs in soy flour are inactivated by sulfhydryl−disulfide interchange during the first inactivation phase and by heat during the second phase.

Keywords: *Kunitz soybean trypsin inhibitor; Bowman−Birk inhibitor; inactivation mechanism; thiols*

INTRODUCTION

Soybeans are generally heat processed to inactivate their 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 behavior for TIs was observed in soy flour (Van den Hout et al., 1997). Such a two-phase inactivation behavior has also been found in *Phaseolus vulgaris* beans (Roa et al., 1989; Van der Poel, 1990). A possible explanation of this two-phase inactivation behavior 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 on the basis of their stability in aqueous solutions (Birk, 1961; Obara and Watanabe, 1971). Rouhana et al. (1996) found that both KSTI and BBI followed a first-order reaction in soy milk 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 as BBI by comparing the inactivation rate of TIs in a commercial soy flour and in 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 behavior 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 soy flour and in 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 they did not relate their experiments with a two-phase inactivation behavior of TIs. The results from the literature concerning the difference in the heat stabilities of KSTI and BBI in soy flour are conflicting.

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

In this study it was investigated if the two-phase inactivation behavior of TIs in soy flour can be explained by a difference in the heat stabilities of KSTI and BBI. Additional experiments were performed to examine the inactivation kinetics of KSTI and BBI in a KSTI-free soy flour, an aqueous solution, and a starch matrix. As Friedman et al. (1982, 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 behavior of TIs in this study.

MATERIALS 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 of dry sample (ds)]$^{-1}$. Anhydrotrypsin−agarose was obtained by PanVera (TAK 7302, Madison, WI). The KSTI-free isoline (L81-4590) was grown by Illinois Foundation Seeds Inc. (Champaign, IL) 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).

* Author to whom correspondence should be addressed [telephone +031-(0)317 482884; fax +031-(0)317 482237].

S0021-8561(97)00506-2 CCC: $15.00
Published on Web 01/19/1998

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 moisturized by adding water dropwise to the flour in a cooled (15 °C) blender to the desired moisture content. The soy flour was stored for 5−7 days at 4 °C to equilibrate.

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 of mg KSTI or BBI/mL of water) was added dropwise to 5 g of starch with optionally added 100 mg of cysteine. The starch was stored for 5−7 days at 4 °C to equilibrate.

Inactivation Experiments. Inactivation experiments were performed with steel cells in a stirred oil bath according to the method of 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.

Determination of the Moisture Content. The moisture content was measured according to an AOAC method (AOAC, 1990).

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 25 mL of 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 the 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, catalytically 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 dialyzed against distilled water and freeze-dried. Two hundred microliters of a ~1 mg of TIs mL^{-1} solution was applied to a FPLC system with a Superdex 30 column (HiLoad 16/60 Pharmacia) using a 0.1 M sodium phosphate buffer (pH 6.9) containing 0.1 M sodium sulfate as eluent. The absorbance of the effluent was measured at 280 nm. The error of the measured peak area (the difference between measured and mean value, divided 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 commercially 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 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 in soy flour.

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

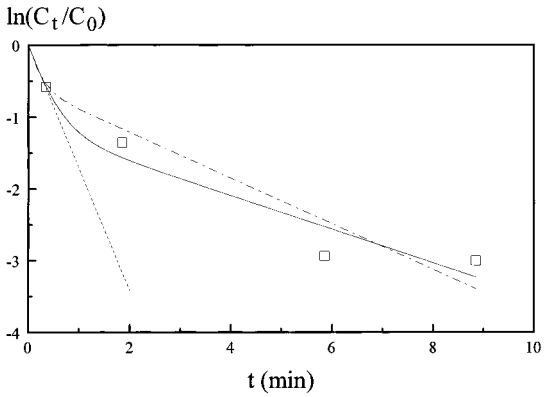

Figure 1. Inactivation of TIs in soy flour at 119 °C and 0.23 g (g ds)$^{-1}$ as measured by anhydrotrypsin−agarose chromatography (□). Equation 1 is used to fit the experimental data from Table 1: TIA (—), $KSTI_1$ + $KSTI_2$ (− -) and BBI (- - -).

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)$^{-1}$]

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

[a] Commercial KSTI and BBI and heat-treated soy flour samples; time in minutes. [b] Activity percentages of $KSTI_1$, $KSTI_2$, and BBI of total TIA.

Estimation of the Kinetics Parameters. The two-phase inactivation behavior of TIs is described with the following equation (Van den Hout et al., 1997):

$$TIA_t/TIA_0 = A\, e^{-\lambda_1 t} + (1 - A)\, e^{-\lambda_2 t} \quad (1)$$

If the two-phase inactivation behavior 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 eq 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 eq 1 to the experimental data using the NLIN procedure of the SAS (SAS Institute Inc., 1988). For a first-order reaction kinetics, only parameter λ_1 was estimated (A = 1 in eq 1). When the inactivation experiment is started, the temperature of the samples increases until 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 subtracting the time needed for 95% temperature equilibration (69 s).

RESULTS AND DISCUSSION

Figure 1 shows the typical two-phase inactivation behavior 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). Using this information, the results of this work measured by anhydrotrypsin−agarose chromatography can be related to the results of our previous

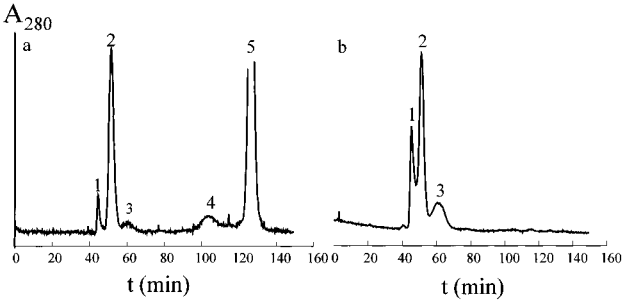

Figure 2. GPC analyses of heat-treated soy flour (a, t =1.5 min) and commercially available KSTI (b).

work measured by trypsin−agarose chromatography (Van den Hout et al., 1997).

A typical example of a GPC elution profile of a soy sample is shown in Figure 2a. Commercially available KSTI and BBI were analyzed 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,b; 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 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.

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 behavior and is responsible for the two-phase inactivation behavior of TIs in soy flour. The results show that the two-phase inactivation behavior of TIs cannot be explained by the difference in the heat stabilities 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 eq 1 and the inactivation of BBI with a first-order reaction kinetics (A = 1 in eq 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).

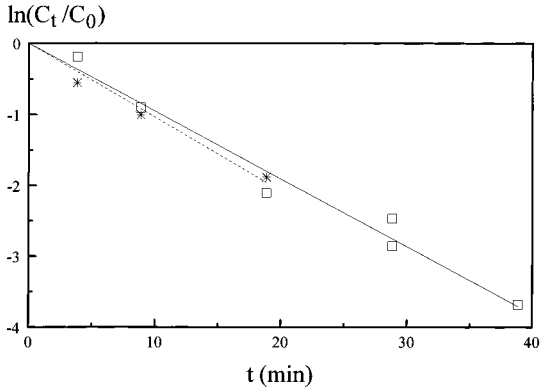

Figure 3. Measured and estimated inactivation of BBI in KSTI-free soy flour at 119 °C and 0.09 g (g ds)$^{-1}$ (□), and at 104 °C and 0.17 g (g ds)$^{-1}$ (*).

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.

Since the two-phase inactivation behavior of TIs in soy flour cannot be explained by the difference in the heat stabilities of KSTI and BBI, the inactivation kinetics of isolated KSTI and BBI was studied. It was examined if the typical two-phase inactivation behavior 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 behavior of TIs in soy flour cannot be explained by a similar two-phase inactivation behavior 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).

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-phase inactivation behavior 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

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 ($\times 10^4$ s^{-1})	λ_2 ($\times 10^4$ s^{-1})
TI[a]	soy flour	104	0.09	0.9	0.2
TI[a]	soy flour	104	0.17	14	2.8
TI[a]	soy flour	104	0.25	155	7.6
TI[a]	soy flour	119	0.09	11	2.0
BBI[b,c]	KSTI-free soy flour	104	0.09	2.1	
BBI[b]	KSTI-free soy flour	104	0.17	17 (4.6)[d]	
BBI[b]	KSTI-free soy flour	119	0.09	16 (1.5)	
KSTI[b]	buffer	104		2.5 (0.2)	
BBI[b]	buffer	104		2.5 (0.3)	
KSTI[b]	starch	104	0.26	12 (2.2)	
BBI[b]	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)

[a] Rate constants are predicted by the inactivation kinetics model of TIs in soy flour presented by Van den Hout et al. (1997). [b] Fitted with first-order reaction kinetics ($A = 1$ in eq 1). [c] Inactivation rate constant was estimated with only two datum points. [d] The estimated 95% confidence intervals of the rate constants λ_1 and λ_2 are given in parentheses.

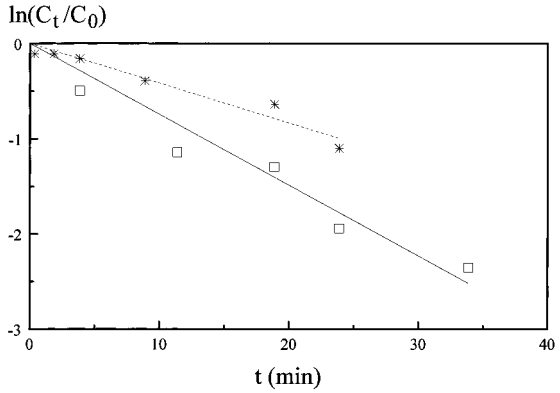

Figure 4. Measured and estimated inactivation of KSTI [□, mc = 0.26 g (g ds)$^{-1}$] and BBI [*, mc = 0.24 g (g ds)$^{-1}$] in starch at 104 °C.

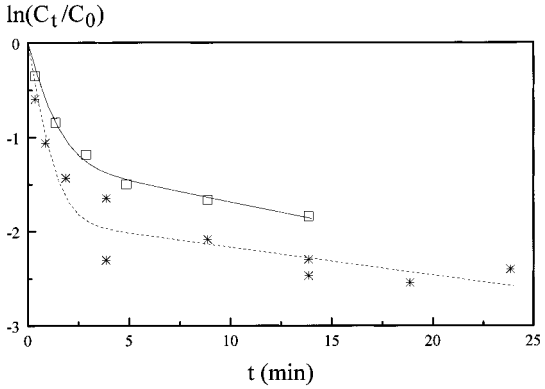

Figure 5. Measured and estimated inactivation of KSTI [□, 0.26 g (g ds)$^{-1}$] and BBI [*, 0.24 g (g ds)$^{-1}$] in starch with added cysteine at 104 °C.

temperature as during the kinetics experiments with soy flour in our previous study (Van den Hout et al., 1997).

Commercially 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 (Table 2) but are in the same order as the predicted rate constant λ_2 of TIs in soy flour. 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.

The inactivation of commercially available KSTI and BBI in a matrix other than soy flour but at the same temperature and moisture content follows first-order reactions. These first-order reactions cannot explain the two-phase inactivation behavior of TIs in soy flour. Possibly the composition of the soy flour matrix is important for the inactivation behavior of both TI groups. Friedman et al. (1982, 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 behavior of KSTI and BBI.

The inactivation rate of both KSTI and BBI increased when cysteine was added to the starch matrix (Figure 5; Table 2). Both KSTI and BBI exhibit a two-phase inactivation behavior. These experiments show that the existence of thiols in soy flour is most probably the cause of the two-phase inactivation behavior of TIs in soy flour. The observed two-phase inactivation behavior 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 behavior of BBI in starch with added cysteine can probably be explained by the two-phase inactivation behavior 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).

On the basis of 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 sulfhydryl−disulfide interchange during the first inactivation phase. In the second phase SH groups are no longer available for a sulfhydryl−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 and to λ_2 of TIs in soy flour.

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, SH groups are no longer available for reaction with TIs, and the TIs will inactivate by heat only and with a lower inactivation rate.

CONCLUSIONS

The two-phase inactivation behavior of TIs in soy flour could not be explained by the difference in the heat stabilities of KSTI and BBI. KSTI and BBI in commercial soy flour inactivated simultaneously 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 behavior of TIs in soy flour could not be explained by the inactivation behavior 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 behavior for both TI groups. The existence of thiols seems to be responsible for the two-phase inactivation behavior of TIs in soy flour. We suggest that the TIs are inactivated by sulfhydryl−disulfide interchange during the first inactivation phase and by heat during the second phase.

ABBREVIATIONS USED

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)$^{-1}$]; λ, time constant (s^{-1}); 0, at t = 0; 1, 2, number of inactivation phase; t, at $t = t$.

ACKNOWLEDGMENT

We acknowledge J. de Groot, M. Houben G. Meerdink, and V. Vascenovsky for their contributions to this work. The KSTI-free isoline was kindly provided by M. Hessing (TNO Nutrition and Food Research, The Netherlands).

LITERATURE CITED

AOAC. Association of Official Analytical Chemists. *Official Methods of Analysis*; Heldrich, K., Eds.; AOAC: Arlington, VA, 1990, 777 pp.

Birk, Y. Purification and some properties of a highly active inhibitor of trypsin and α-chymotrypsin from soybeans. *Biochim. Biophys. Acta* **1961**, *54*, 378−381.

DiPietro, C. M.; Liener, I. E. Heat inactivation of the Kunitz and Bowman−Birk soybean protease inhibitors. *J. Agric. Food Chem.* **1989a**, *37*, 39−44.

DiPietro, C. M.; Liener, I. E. Soybean protease inhibitors in foods. *J. Food Sci.* **1989b**, *54*, 606−609.

Friedman, M.; Grosjean, O. K.; Zahnley, J. C. Inactivation of soybean trypsin inhibitors by thiols. *J. Sci. Food Agric.* **1982**, *33*, 165−172.

Friedman, M.; Gumbmann, M. R.; Grosjean, O. K. Nutritional improvement of soy flour. *J. Nutr.* **1984**, *114*, 2241−2246.

Friedman, M.; Brandon, D. L.; Bates, A. H.; Hymowitz, T. Comparison of a commercial soybean cultivar and an isoline lacking the Kunitz trypsin inhibitor: composition, nutritional value, and effects of heating. *J. Agric. Food Chem.* **1991**, *39*, 327−335.

Ishii, S.; Yokosawa, H.; Kumazaki, T.; Nakumura, I. Immobilized anhydrotrypsin as a specific affinity absorbent for tryptic peptides. *Methods Enzymol.* **1983**, *91*, 378−383.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.

Liener, I. E.; Tomlinson, S. Heat inactivation of protease inhibitors in a isoline lacking the Kunitz trypisin inhibitor. *J. Food Sci.* **1981**, *46*, 1354−1356.

Multon, J. L.; Guilbot, A. Water activity in relation to the thermal inactivation of enzymatic proteins. In *Water Relations of Foods*; Duckworth, R. B., Eds.; Academic Press: London, U.K., 1975.

Obara. T.; Watanabe. Y. Heterogeneity of soybean trypsin inhibitors II. Heat inactivation. *Cereal Chem.* **1971**, *48*, 523−527.

Roa, V.; DeStefano, M. V.; Perez, C. R.; Barreiri, J. A. Kinetics of thermal inactivation of protease (trypsin and chymotrypsin) inhibitors in black bean (*Phaseolus vulgaris*) flours. *J. Food Eng.* **1989**, *9*, 35−46.

Roozen, J. P.; De Groot, J. Analysis of trypsin inhibitors and lectines in white kidney beans (*Phaseolus vulgaris*, va. Processor) in combined method. *J. Assoc. Off. Anal. Chem.* **1991**, *74* (6), 940−943.

Rouhana, A.; Adler-Nissen, J.; Cogan, U.; Frøkiær, H. Heat inactivation kinetics of trypsin inhibitors during high temperature short time processing of soymilk. *J. Food Sci.* **1996**, *61*, 265−269.

SAS Institute Inc. *SAS Introductory Guide for Personal Computers*, release 6.03 ed.; SAS Institute: Cary, NC, 1988.

Van den Hout, R.; Meerdink, G.; Van't Riet, K. Modelling of the inactivation kinetics of trypsin inhibitors in soy flour. *J. Sci. Food Agric.* **1997**, submitted for publication.

Van der Poel. A. F. B. Effects of processing on bean (*Phaseolus vulgaris* L.) protein quality. Ph.D. Thesis, Wageningen Agricultural University, The Netherlands, 1990.

Van Zuilichem, D. J.; Van der Poel, A. F. B.; Stolp, W. Different HTST inactivation methods for ANFs and the description of inactivation kinetics for soya. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds*; Van der Poel, A. F. B., Huisman, J., Saini, H. S., Eds.; Wagingen Pers: Wageningen, The Netherlands, 1993.

Received for review June 9, 1997. Revised manuscript received September 2, 1997. Accepted September 5, 1997.[⊗] Financial support of TNO Nutrition and Food Research (Zeist, The Netherlands) is gratefully acknowledged.

JF970506+

[⊗] Abstract published in *Advance ACS Abstracts*, October 15, 1997.