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Inactivation of Soybean Trypsin Inhibitors and Lipoxygenase by High-Pressure Processing

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Trypsin inhibitors (TIA), one of the antinutritional factors of soy milk, are usually inactivated by heat treatment. In the current study, high-pressure processing (HPP) was evaluated as an alternative for the inactivation of TIA in soy milk. Moreover, the effect of HPP on lipoxygenase (LOX) in whole soybeans and soy milk was studied. For complete LOX inactivation either very high pressures (800 MPa) or a combined temperature/pressure treatment (60 °C/600 MPa) was needed. Pressure inactivation of TIA was possible only in combination with elevated temperatures. For TIA inactivation, three process parameters, temperature, time, and pressure, were optimized using experimental design and response surface methodology. A 90% TIA inactivation with treatment times of <2 min can be reached at temperatures between 77 and 90 °C and pressures between 750 and 525 MPa.

KEYWORDS: High-pressure processing; soy; trypsin inhibitors; lipoxygenase; inactivation; experimental design

INTRODUCTION

Soy milk is a traditional Asian food product, which is more and more consumed in Western countries as well. The increasing consumption of soy milk is probably related to the consumer's interest in the positive health aspects of soy protein products. In 1999, the FDA approved the use of a soy protein health claim in the United States stating that consumption of 25 g of soy protein a day as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease (*1*).

Besides these favorable features, soybeans are known to contain antinutritional factors such as trypsin inhibitors, lectin, and phytate. The presence of trypsin inhibitors in animal feed has been associated with growth suppression (*2*, *3*) and pancreatic hypertrophy (*2*, *4*, *5*). On the other hand, trypsin inhibitors have been reported to have an anticarcinogenic effect (*6*). Although the nutritional effects of trypsin inhibitors in humans are not fully clear, reduction of the level of trypsin inhibitors by, for example, heat or fractionation, is generally applied to soy products (*5*).

Soybeans contain two types of trypsin inhibitors, the Kunitz trypsin inhibitor (KTI) and the Bowman−Birk inhibitor (BBI). Both inhibitors are rather heat stable, which is caused by the presence of several disulfide bridges, two in the KTI and seven in the BBI (*7*, *8*). As soybean trypsin inhibitors are rather heat stable, long treatment times, for example, 30 min at 100 °C or 22 min at 110 °C (*9*), are required to reach 90% inactivation. These long treatment times may also affect other properties of soy milk such as taste due to browning reactions, protein quality as a result of amino acid reactions (*8*), and vitamin content (*10*). To reduce treatment times and heat damage, soy milk can be heat treated by ultrahigh-temperature treatment (UHT). Kwok and co-workers found that treatment at 143 °C for 62 s is enough to reduce TIA by 90% (*11*). This treatment retains thiamin, one of the vitamins in soy milk, at 90% and minimizes sensory deterioration (*12*).

During the past few decades alternative food preservation methods have been developed, inspired by the desire to produce more fresh-like products that meet the microbial safety status of pasteurized or sterilized foods. One of the techniques that have been introduced is high-pressure processing (HPP). HPP can be applied for the inactivation of microorganisms, for the inactivation of enzymes, and for the unfolding of proteins (*13*, *14*). As protein unfolding is promoted by HPP, whereas nutritional and sensorial factors are mostly preserved, HPP might be a suitable technique to inactivate trypsin inhibitors in soy milk. Trypsin inhibitors might be rather stable to high pressure at room temperature due to the conformational stability of the proteins caused by the presence of disulfide bonds. The stabilizing effect of disulfide bonds has been suggested to be responsible for the relative high-pressure stability of BSA (*15*) and of α-lactalbumin compared to *β*-lactoglobulin (*16*−*18*). Denaturation of α-lactalbumin by pressure was increased if temperatures were increased to 50−60 °C or higher (*16*, *18*).

Besides the inactivation of trypsin inhibitors, the inactivation of lipoxygenase (LOX), an enzyme causing lipid oxidation resulting in the formation of off-flavors, is important for soy milk quality. Inactivation of LOX by high-pressure processing

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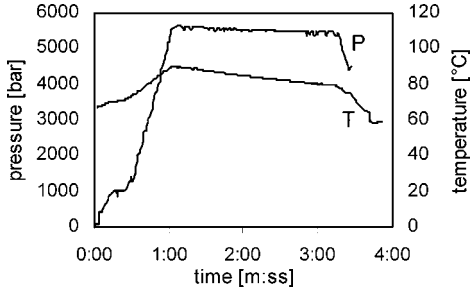

Figure 1. Pressure and temperature profiles in a typical adiabatic high-pressure experiment. The temperature profile is measured outside the product container.

has been studied extensively by Ludickhuyze and Indrawati (*19*−*21*). The kinetic data on soybean LOX inactivation obtained from a model system described by Indrawati (*21*) were used in the present study to estimate the conditions needed to inactivate LOX in whole soybeans and in soy milk.

The aim of the present research was to explore the possibility of inactivating soybean trypsin inhibitors by high-pressure treatment, which can potentially result in a better quality soy milk. High-pressure treatments at room temperature and moderate pressure (500 MPa) were performed to investigate the possibility of inactivating trypsin inhibitors at relatively mild conditions. Moreover, more severe conditions, that is, higher pressure or elevated temperature, were tested as well, because soybean LOX is rather pressure stable at ambient temperatures (*21*) and, as outlined above, the presence of disulfide bridges in trypsin inhibitors might cause pressure stability at moderate HPP conditions.

MATERIALS AND METHODS

Linoleic acid and Kunitz trypsin inhibitor were obtained from Sigma Aldrich Chemie (Zwijndrecht, The Netherlands); sodium benzoylarginine-4-nitroanilide hydrochloride (BAPA) and bovine trypsin were from Merck (Darmstadt, Germany).

First, explorative experiments were performed to estimate the potential of high-pressure processing in the inactivation of trypsin inhibitors. Second, high-pressure conditions were optimized with a response surface methodology.

Initial High-Pressure Experiments. *Preparation of Samples.* Dehulled soybeans (Brazilian soybeans, obtained from Nutrilab BV, Royal Schouten Group, The Netherlands) were soaked overnight in demineralized water at 5 °C (w/v = 1:5). Some soaked beans were removed and stored for high-pressure treatment. The remaining soybeans were ground in a blender with the soaking water to soy milk. The pulp was removed by filtration over a nylon cloth (Sefar Nitex 1 002 m 03-5/1). Soaked soybeans and filtered soy milk were kept refrigerated until processing.

High-Pressure Process. The high-pressure process is an adiabatic process, which implies that adiabatic heating occurs in the vessel during pressurization as described previously (*22*). During the holding phase the temperature of the pressure medium decreases due to the temperature difference between the vessel wall and the pressure medium (**Figure 1**). To minimize the temperature decrease in the product, a product container was used. On the basis of a heat conduction model, the temperature decrease in the product inside the product container can be calculated (*22*). In the setup used, the temperature loss of the product is maximally 2 °C during a process time of 2 min.

During pressure buildup and pressure release, inactivation of microorganisms and denaturation of proteins may occur, in addition to the inactivation during the period of maximum pressure (adiabatic phase). The "inactivating" effect of these pressure increase and decrease phases can be accounted for by treating a reference sample with a holding time of 0 min, in which the adiabatic phase is omitted.

Table 1. Process Parameters Used in the Experimental Design

	parameter level				
process parameter	$-\alpha$	−1	0	+1	$+\alpha$
pressure (MPa)	365	440	550	660	735
temperature (°C)	40	50	65	80	90
time (min)	0.16	0.5	1	1.5	1.84

In the initial high-pressure tests, samples were high-pressure treated in a Resato high-pressure apparatus (volume = 150 mL, maximum pressure = 1000 MPa, developed by Resato, Roden, The Netherlands). The pressure medium used was glycol. Pressure buildup time was 15 MPa/s. To insulate the pressure chamber, an insulator was placed in the reaction vessel. Soy milk samples (1 mL) or soaked soybeans (eight beans/sample) were packed in PE/PA pouches, air was removed, and pouches were sealed. Samples were treated at 20 °C and 500 MPa with holding times of 0, 1, and 2 min; at 20 °C and 800 MPa for 2 min; and at 60 °C and 600 MPa for 0, 1, and 2 min. After pressure treatment, samples as well as reference (nontreated) samples were frozen in liquid nitrogen and stored at −80 °C. For treatments at 60 °C, samples and liner were immersed for 2 min in a water bath of 60 °C before the sample was pressure treated. A reference sample was heated at 60 °C at atmospheric pressure for 8 min (period equivalent to the longest pressure treatment).

Optimization Experiments. *Preparation of Soy Milk.* Dehulled dry soybeans were processed to soy milk by grinding the beans in hot water and subsequent filtration to remove remaining pulp. The hot soy milk was further heated to 100 °C in a heat exchange unit, keeping the milk at 100 °C for 2 min for pasteurization. The milk was stored refrigerated before high-pressure processing.

High-Pressure Treatment. For practical reasons, soy milk samples were high-pressure treated in a high-pressure apparatus different from that used in the initial experiments. Both apparatuses work under adiabatic conditions, but the chamber volumes differ. For the optimization experiments the Resato high-pressure apparatus with a volume of 180 mL and a maximum pressure of 1000 MPa was used (Resato, Roden, The Netherlands). The pressure buildup rate was 15 MPa/s. To insulate the pressure chamber, an insulator was placed in the reaction vessel. Soy milk samples were sealed in pouches as described for the initial experiments. Prior to high-pressure treatment, samples were heated to the treatment temperature in a water bath for a standard time of 2 min at atmospheric pressure. Directly after high-pressure treatment, samples as well as nontreated references were frozen in liquid nitrogen and stored at −80 °C until analysis.

Optimization of High-Pressure Treatment. With the data obtained from the initial experiments, high-pressure treatment to inactivate trypsin inhibitors was optimized using a central composite rotatable design (CCRD) (*23*) with three process parameters: temperature, pressure, and holding time. For each variable five levels were defined, coded $-\alpha$, −1, 0, 1, and α (**Table 1**). The CCRD consisted of 17 experiments: 3 center samples (all parameters set at level 0), 8 cube samples (all combinations of levels −1 and +1), and 6 star samples (two parameters at level 0 and one parameter at $-\alpha$ or $+\alpha$). The center samples were repeated to estimate reproducibility.

Lipoxygenase Activity. LOX activity was measured in untreated and high-pressure-treated soaked beans and soy milk. To extract LOX from whole soaked beans, beans were ground in a mortar with cold water (4 °C) with a water/original dry beans ratio of 5:1 and subsequently filtered over a nylon cloth. Filtrates of ground soybeans were centrifuged for 10 min at 11000*g* and 4 °C. Soy milk samples were thawed and centrifuged at the same conditions. Supernatants were used for enzyme activity test.

Lipoxygenase activity was determined using a continuous spectrophotometric method based on the enzymatic oxidation of linoleic acid to the corresponding hydroperoxide. Linoleic acid solution (0.032 M) in 0.05 M borate buffer, pH 9, containing 1% (v/v) Tween 20 was used as substrate solution. The oxygen in the substrate solution was removed by flushing with nitrogen. Linoleic acid emulsion (final concentration = 0.04 M) was added to 0.2 M air-saturated borate buffer, pH 9, of 25 °C. The reaction was initiated by adding LOX solution,

and the increase in absorbance at 234 nm was monitored using a spectrophotometer (Perkin-Elmer Lambda 16 UV−vis). The LOX activity in treated samples was expressed as residual activity relative to the untreated sample (soaked beans or soy milk for the bean and milk samples, respectively).

Trypsin Inhibitory Activity Assay. To extract TIA from soaked beans, 2.2 g of soaked beans was crushed with a mortar in a solution of 0.015 M NaOH and 0.5 M NaCl. Extraction solvent was added to the pulp to a total volume of 25 mL. Extract was stirred for 2 h at room temperature. For extraction of TIA from the milk, samples were diluted 5× in the same NaOH/NaCl solution and stirred for 2 h. Extracts from both soaked beans and milk were subsequently centrifuged (30000*g*, 20 °C, 20 min). Supernatants were used for activity assay. The trypsin inhibitor activity assay is based on the hydrolysis of BAPA by trypsin adjusted for use in microtiter plates. If trypsin is active, a yellow color is formed due to the liberation of nitroanilide. As a reference, inhibition by Kunitz soybean trypsin inhibitor (KSTI) was measured. Samples and trypsin (0.08 mg/mL) were diluted in Tris buffer (0.02 M Tris, 0.02 M $CaCl_2$, pH 8.2). In a microtiter plate 50 μL of sample or KSTI standard and 50 μL of trypsin solution were pipetted. After a mixing time of 10 min, 50 μL of BAPA (4 mg/mL) was added, and the increase in absorbance at 405 nm in time was followed for at least 10 min. The reaction rate was calculated over the linear part and expressed as absorption increase per minute. A calibration curve based on the KSTI standard was used to express trypsin inhibitor activity as milligrams of KSTI per milligram of product. Trypsin inhibitory activity in samples was expressed as residual activity compared to the untreated sample (soaked beans or soy milk for the bean and milk samples, respectively, and pasteurized soy milk for the optimization experiments).

Statistical Analysis. Population standard deviation (deviation from the mean over all samples) and standard deviation (SD) over center samples were calculated according to eq 1. For calculation of the

$$\mathrm{SD} = \sqrt{\frac{\sum (x_i - \bar{x})}{m}} \qquad (1)$$

population SD, m is the total number of experiments and for calculation of the SD of the center samples $m = p - 1$, where p is the number of center samples.

Regression analysis of the data obtained in the optimization experiments was performed using The Unscrambler (Camo, Camo ASA, Oslo, Norway). Response surface analysis was based on multiple linear regression taking into account the main, quadratic, and interaction effects (eq 2). Nonsignificant parameters were subsequently removed

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i<j=2}^{3} b_{ij} X_i X_j + e \qquad (2)$$

from the model.

To estimate the weight of the various parameters, the regression was performed with standardized variables [centered and weighted (1/SD)].

RESULTS AND DISCUSSION

Initial Experiments. To estimate the pressure stability of LOX and trypsin inhibitors in whole soybeans and in soy milk, some initial experiments at relatively mild high-pressure conditions, at 500 MPa and room temperature, were performed. Moreover, the effect of a further increase of pressure to 800 MPa was studied, as well as the effect of an increase of treatment temperature and a slightly higher pressure (60 °C and 600 MPa).

Inactivation of LOX. Initial LOX activity in soy milk was ∼50% lower than that in soaked beans. LOX is possibly inactivated during the processing of the soaked beans to soy milk in the blender or remains in the residue after the filtration step. The residual activity of the treated beans and milk was expressed relative to the activity of the untreated soaked beans or soy milk, respectively.

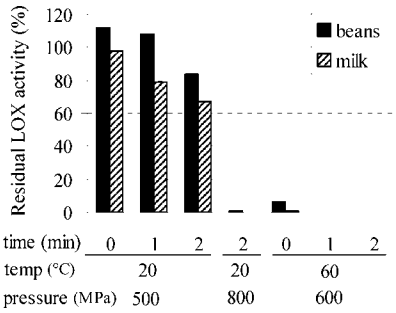

Figure 2. Inactivation of lipoxygenase in whole soaked soybeans and in soy milk after high-pressure treatment.

Pressure treatment at 500 MPa and ambient initial temperature resulted in partial inactivation of LOX for the treatment times tested (**Figure 2**). In whole soybeans, LOX inactivation was observed only after 2 min of holding time, whereas in soy milk LOX activity decreased already after a holding time of only 1 min. Moreover, remaining activity in beans was higher compared to the remaining activity in treated soy milk. Increase of the pressure to 800 MPa resulted in complete LOX inactivation after a treatment time of 2 min in both soaked beans and soy milk (**Figure 2**). High-pressure treatment at 600 MPa with an initial temperature of 60 °C also resulted in complete inactivation of LOX. LOX inactivates extremely rapidly under these conditions; a holding time of 0 min, which means that the sample is immediately depressurized after the desired pressure has been reached, resulted in almost complete LOX inactivation (**Figure 2**). The initial temperature of 60 °C was not responsible for this inactivation, because heating of soybeans and soy milk at 60 °C for 8 min (comparable to the total process time) at atmospheric pressure caused only a 5% inactivation of LOX (data not shown). The additional inactivation during the high-pressure treatment can be (partly) explained by the temperature increase caused by the pressure increase (**Figure 1**). In the isobaric−adiabatic high-pressure setup used in the present study, a pressure increase of 100 MPa will result in a temperature increase of the product of ∼4 °C (*22*). Consequently, maximum product temperature during pressurization to 600 MPa was ∼84 °C. Inactivation of soybean LOX by a range of pressure temperature combinations has been studied by Indrawati (*24*). If these data are extrapolated to higher temperatures, the *D* value of LOX inactivation at 84 °C and 600 MPa is predicted to be 1.1 min. The total pressure cycle in our experiments with a 0 min holding time takes ∼1 min. The temperature increase and decrease between 60 and 84 °C during this treatment probably resulted in inactivation of the enzyme.

According to the same literature data, the *D* value for LOX inactivation at 500 MPa and ambient temperature, with a maximum temperature during treatment of 40 °C, is 131 min. This means that on the basis of these data no significant inactivation of LOX for the holding times tested in our experiments was expected. The treatment at 800 MPa (with a maximum temperature of ∼52 °C) was expected to result in ∼50% inactivation of LOX. For both treatments, measured inactivation was considerably higher than expected on the basis of the model of Indrawati. The kinetic model was based on model studies with purified LOX in Tris buffer, pH 9, whereas soy milk made with water has a neutral pH. It has been reported that LOX inactivation depends on pH. Ludikhuyze and co-workers reported increased pressure stability of LOX with increased pH (from pH 6.6 to 9) (*25*), which could explain the higher inactivation measured in the present study compared to inactivation in the model solutions. However, Tangwongchai

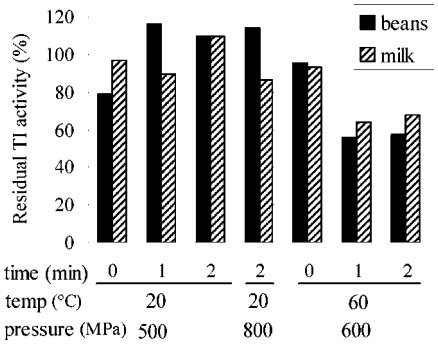

Figure 3. Inactivation of trypsin inhibitor in whole soaked soybeans and in soy milk by high-pressure treatment.

and co-workers measured decreased pressure stability with a pH increase from 7 to 9 (*26*). Matrix differences between intact soybeans and soy milk could possibly explain the lower LOX inactivation in whole soybeans compared to soy milk found in the present study. However, in intact green beans LOX inactivation appeared to be more extensive than in green bean juice (*21*). In conclusion, these results show that a model system is very suitable to obtain an indication about enzyme inactivation at various temperature−pressure combinations, but for application in a real food system additional experiments are needed.

Inactivation of Trypsin Inhibitors. The samples prepared in the initial high-pressure experiments were also analyzed for their trypsin inhibitory activity. The high-pressure treatments at ambient initial temperature resulted in minor or no loss of TIA (**Figure 3**). High-pressure treatment with a start temperature of 60 °C resulted in ±40% decrease of TIA in both soybeans and soy milk. These results demonstrate that for inactivation of TIA high-pressure treatment should be performed at elevated temperature. For α-lactalbumin, which like trypsin inhibitors is relatively rich in disulfide bonds, also a combined pressure temperature treatment was needed to obtain protein denaturation (*16*, *18*).

In the above-described preliminary experiments both soaked soybeans and soy milk were tested. As both milk and beans show similar trends for inactivation of LOX and TIA, both could be used as material for high-pressure treatment. However, if high-pressure treatment would be applied in practice, it would preferably be used for treatment of the final soy milk product, because in that case inactivation of TIA and microbial pasteurization or sterilization can be combined in one treatment. Therefore, succeeding high-pressure experiments were performed with soy milk. To prevent degradation of fatty acids by LOX during the production process, soybeans were ground in the presence of hot water. In the soy milk prepared for these experiments no residual LOX activity was present (results not shown).

Optimization of High-Pressure Process for TI Inactivation. To find the optimal conditions for inactivation of trypsin inhibitors in soy milk, that is, the mildest temperature and pressure needed to obtain sufficient (90%) inactivation, the main process variables, that is, pressure, temperature, and treatment time, were varied. The three variables were varied according to a central composite design. The average initial temperature was set at 65 °C, because the previous experiments showed that elevated temperatures are needed to reach TI inactivation. Treatment times were varied between 0 and 1.84 min, with an average of 1 min. Longer treatment times might result in significant temperature loss due to cooling on the outer side of the sample. The analysis of the trypsin inhibitory activity in the pressure-treated samples showed that within the set of process conditions tested, the residual TIA varied between 11 and 107%. Most inactivation was reached in the experiments with the highest initial temperature, indicating that temperature is an important factor in the inactivation of TIA. The average value of the center samples was comparable to the average value over all samples (**Table 2**), which indicates that the model experiments are well distributed around the middle. The center samples are the only repeated samples and give an indication about the reproducibility of the treatment. The standard deviation over all samples (population SD) shows the variation obtained by using different process parameters. A high population SD relative to the SD of the center samples indicates that differences between samples are probably due to differences in process conditions and not due to coincidence. As shown in **Table 2**, the SD of center samples is very low, showing good reproducibility, and the population SD is considerably higher, indicating significant effects of process parameters.

Table 2. Summarized Statistics for Trypsin Inhibitory Activity of High-Pressure-Treated Soy Milk Using Process Parameters According to the Central Composite Design

	samples	
	all	center
range (% inhibition)	11–107	52–56
average	56	55
standard deviation	32[a]	2.2[b]

[a] Population standard deviation. [b] Standard deviation over repeated center samples.

Table 3. Linear Regression Model of Combined High-Pressure Temperature Inactivation of Soybean Trypsin Inhibitors[a]

	all factors		significant factors	
	b coefficients	*p* value	*b* coefficients	*p* value
intercept	55.35	0.000	55.70	0.000
pressure (*P*, MPa)	–11.78	0.002	–12.20	0.000
temperature (*T*, °C)	–28.52	0.000	–28.39	0.000
time (*t*, min)	–9.90	0.005	–9.99	0.001
$P \times T$	–2.63	0.444		
$P \times t$	3.56	0.309		
$T \times t$	–0.41	0.896		
P^2	0.35	0.880		
T^2	2.12	0.435		
t^2	–1.89	0.484		
R^2	0.962		0.944	
	SS	df	SS	df
residual	683	7	1023	13
pure error	9.3	2	9.3	2
lack of fit	674	5	1014	11
F value	28.9		19.8	
p value	0.034		0.049	

[a] Model based on standardized variables.

Data for trypsin inhibition were analyzed by multiple linear regression. For regression analysis standardized variables were used to facilitate the interpretation of the effect of each process variable. The regression analysis showed that quadratic and interactive effects were not significant (**Table 3**). Only the main factors were significant. Therefore, all quadratic and interaction effects were removed from the model, and a new model with only main effects was calculated. The model has a good correlation coefficient ($R^2 = 0.94$) and a nonsignificant lack of fit.

The equation describing the inactivation of trypsin inhibitors (eq 3) demonstrates that the temperature parameter has the

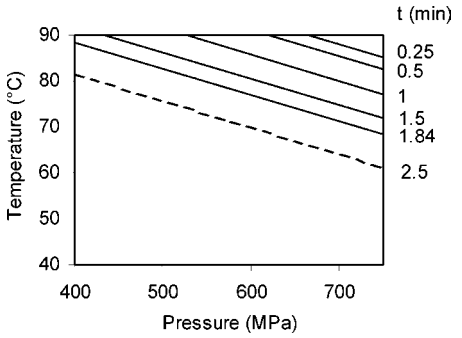

Figure 4. High-pressure process parameters resulting in 90% inactivation of TIA in soy milk: (broken line) extrapolated data; (solid lines) interpolated data. Temperatures are initial temperatures.

highest *b* coefficient. Because the parameters were standardized, which means that the average and standard deviation of the variables are all the same, it can be concluded that temperature is the most important factor in TIA inactivation:

$$Y = 56 - 12P - 28T_{ini} - 10t \quad (3)$$

where Y = residual TIA and T_{ini} = initial temperature.

During pressure treatment, the temperature in the vessel will increase due to adiabatic heating. This temperature increase will contribute to the inactivation of the trypsin inhibitors. In the above-described regression analysis, the initial temperature of the soy milk was used as temperature variable. This means that the pressure effect is partly caused by a temperature effect. To distinguish this temperature effect from the pressure parameter, a new model was made using the maximum temperature reached during the pressure treatment (T_{max}) as process parameter.

The regression equation with T_{max} (eq 4) shows that if T_{max} is taken as temperature parameter, the magnitude of the pressure effect decreases. This confirms that an important part of the pressure effect is related to the temperature increase caused by the pressure increase:

$$Y = 56 - 4P - 30T_{max} - 9.7t \quad (4)$$

where T_{max} = maximum temperature of pressure treatment.

From this regression analysis it can be concluded that the benefit of high-pressure treatment in TIA inactivation is twofold: a direct pressure effect and an indirect effect by the fast temperature increase due to adiabatic heating.

Using the above-presented equations, combinations of temperature and pressure settings needed to inactivate TIA by 90% can be calculated. In **Figure 4**, pressure−temperature combinations for several holding times are plotted. As only the main effects appeared to be significant (eq 4), the TIA inactivation is a linear function of pressure, temperature, and time, thus resulting in linear lines. In the experimental design the maximum holding time was 1.84 min, so for predictions of TIA inactivation with longer holding times the model has to be extrapolated, which might give less accurate predictions. Therefore, the line of 2.5 min is depicted as a broken line. The graph shows that initial temperatures of ≥65 °C are needed to reach 90% TIA inactivation.

Reported conditions for heat inactivation of TIA are, for example, 30 min at 100 °C or 22 min at 110 °C (*9*). For UHT inactivation, 143 °C and 62 s are needed to reach 90% TIA inactivation, whereas for temperatures lower than ∼136 °C, 90% inactivation within 90 s of treatment time (the maximum treatment time tested) was not possible (*11*). Conversely, the maximum temperature reached during our high-pressure experiments was 110 °C (initial temperature = 90 °C) and 90% inactivation within 90 s (1.5 min) was possible (**Figure 4**). For 90% inactivation within a holding time of 1 min, initial temperatures between 77 and 90 °C and pressures between 750 and 525 MPa are needed. Hence, high-pressure treatment results in TIA inactivation at lower temperatures or shorter treatment times than a conventional heat treatment. These milder conditions could possibly contribute to a better conservation of other components in soy milk such as vitamins, lysine, and taste. Studies concerning these compounds by Kwok and co-workers showed that at 90 °C these compounds are stable during long treatment times (∼50 min) (*12*). Moreover, from high-pressure studies it is known that low molecular weight compounds are relatively unaffected by pressure (*27*). Therefore, high-pressure conditions needed to inactivate TIA might result in minimal negative effects on components of soy milk such as vitamin and lysine content and taste. Further research would be needed to confirm this.

Microbial inactivation was not measured in these experiments because pasteurized milk was used in the high-pressure treatment for practical reasons. However, it is to be expected that vegetative microorganisms in soy milk will be inactivated under the conditions needed for TIA inactivation, resulting in a pasteurized product (*28*).

The present study demonstrates that high-pressure treatment in combination with elevated temperatures can be used as an alternative to ultrahigh-temperature treatment to inactivate trypsin inhibitors present in soy milk. For 90% inactivation of TIA, initial temperatures of at least 65 °C are needed if holding times of <166 s are used. Possibly, temperature can be reduced if treatment times are prolonged. To further compare the high-pressure treatment with commonly used UHT treatment, the effect on taste, vitamin content, and protein quality should be studied.

ABBREVIATIONS USED

HPP, high-pressure processing; TIA, trypsin inhibitor activity; LOX, lipoxygenase; BAPA, sodium benzoylarginine-4-nitroanilide hydrochloride; K(S)TI Kunitz (soybean) trypsin inhibitor; BBI, Bowman−Birk inhibitor; UHT, ultrahigh temperature; SD, standard deviation.

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