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Elimination of Trypsin Inhibitor Activity and Beany Flavor in Soy Milk by Consecutive Blanching and Ultrahigh-Temperature (UHT) Processing

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Soy foods contain significant health-promoting components but also may contain beany flavor and trypsin inhibitor activity (TIA), which can cause pancreatic disease if present at a high level. Thermal processing can inactivate TIA and lipoxygenase. Ultrahigh-temperature (UHT) processing is relatively new for manufacturing soy milk. Simultaneous elimination of TIA and soy odor by UHT processing for enhancing soy milk quality has not been reported. The objective was to determine TIA in soy milk processed by traditional, steam injection, blanching, and UHT methods and to compare the products with commercial soy milk products. Soybean was soaked and blanched at 70−85 °C for 30 s−7.5 min. The blanched beans were made into base soy milk. The hexanal content of the base soy milk was determined by gas chromatography to determine the best conditions for further thermal processing by indirect and direct UHT methods at 135−150 °C for 10−50 s using the Microthermics processor. Soy milk was also made from soaked soybeans by traditional batch cooking and steaming methods. Eighteen commercial products were selected from the supermarket. Residual TIA in soy milk processed by the traditional and steam injection to 100 °C for 20 min was approximately 13%. Blanching could inactivate 25−50% of TIAs of the raw soy milk. The blanch conditions of 80 °C and 2 min were selected for UHT processing because these conditions produced blanched soy milk without hexanal, indicating a complete heat inactivation of lipoxygenases. The TIA decreased with increased temperature and time of UHT heating. The maximal trypsin inhibitor inactivation was achieved by UHT direct and indirect methods with residual activities of approximately 10%. Some commercial soy milk products contained high TIAs. The results are important to the food industry and consumers. Kinetic analysis showed that heat inactivation (denaturation) of TIA, under the continuous processing conditions of the Microthermics processor, followed first-order reaction kinetics, and the activation energy of the inactivation was 34 kJ/mol.

KEYWORDS: Soy milk; trypsin inhibitor; thermal processing; kinetic analysis; UHT

INTRODUCTION

Soy food consumption in the world has increased due to its potential health benefits (*1*). Soy foods contain significant health-promoting bioactive components such as proteins and isoflavones but also contain undesirable beany flavor (*2, 3*) and trypsin inhibitor activity (TIA). It is well-known the consumption of the raw and inadequately cooked bean causes a decrease in protein digestibility and nutritive value and also causes pancreatic hypertrophy (*4*−*6*). The deleterious effect is due to trypsin inhibitors (TI) and lectins and the compact structure of the native forms of soybean major storage proteins, β-conglycinin and glycinin (*4*). When trypsin inhibitors are heat inactivated, lectins, lipoxygenases, and major storage proteins are also denatured. Trypsin inhibitors in soybeans consist of two types, namely, the Kunitz trypsin inhibitor and the Bowman−Birk inhibitor (*7*), which also is a chymotrypsin inhibitor and is the major form of TI in cooked soy milk (*8*). Instead of protein unfolding during heat denaturation, interchanges of disulfide linkages (cystine residues) between inhibitors and storage proteins such as glycinins and the degradation of cysteine/cystine have been hypothesized to be partly responsible for the inactivation of Bowman−Birk trypsin inhibitor (*7*).

Hackler et al. (*9*) reported that 4−10% residual trypsin inhibitory activity in soy milk gave the highest protein nutritive value for the heated soy milk. However, inactivating 100% trypsin inhibitor may cause overheating, which damages soy proteins by destroying lysine, tryptophan, and cysteine in the soy milk (*9*−*11*). Cysteine retention is very important to protein nutritive value because it is one of the two sulfur amino acids that are the limiting essential amino acids in soy foods. Cysteine destruction also has been related to off-flavor (*11*). Research

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discoveries from the past two decades have shown purified Bowman−Birk inhibitor has protective effect against oral and other cancers (*12*−*15*). However, it is not known if the low concentrations that are present in heated soy foods can have any beneficial effect in humans (*14, 15*). Bowman−Birk inhibitor can stimulate the secretion of trypsin, chymotrypsin, and carboxypeptidases and cause an increase in cholescystokinin in humans (*16*). Therefore, caution must be taken in the interpretation of the long-term impact to frequent users of high levels of Bowman−Birk inhibitors.

Kwok and others (*17*−*19*) investigated the effect of indirect ultrahigh-temperature (UHT) heating on soy milk trypsin inhibitors by placing soy milk in small-diameter stainless tubes, which were heated in an oil bath at selected temperatures. They reported that heating at 143 °C for approximately 60 s can inactivate TI to approximately 10% of the original raw soy milk. Under some conditions, UHT may achieve commercial sterility, but the heat may not achieve adequate inactivation of trypsin inhibitors in the final products as some commercial products have been found to be much higher than 10% TI. In a study (*20*) conducted in Hong Kong, several UHT-processed commercial soy milk products contained very high trypsin inhibitor activities (TIAs) (20−47% of the TI of raw beans). Liener (*21*) expressed a concern of that the consumption of trypsin inhibitor residues in the range of 5−20% of the raw bean may have a negative effect on human health. Ensuring a low trypsin inhibitory activity is important to health, particularly for infants or young children who cannot drink cow's milk and rely on soy formula or soy milk as the primary source of protein for growth. The long-term consumption of residual trypsin inhibitors also may affect the health of frequent soy-consuming vegetarians. It is important that trypsin inhibitory activity be considered in designing thermal processes to produce the lowest beany off-flavor intensity and to retain the highest nutritional profiles.

Although the kinetic analysis of trypsin inhibitor inactivation using indirect UHT heating and hydrothermal steam fusion has been reported (*8, 18, 19, 22, 23*), their studies have not used a continuous vacuum process such as that used in the direct steam injection process by the Microthermics DIP processor and have not used a pretreatment to reduce odor production of the soy milk. UHT is a relatively new processing method for processing soy milk in modern soy milk manufacturing companies. The small pilot-scale of the Microthermics DIP processor mimics modern commercial UHT processes for soy milk processing. The effect of UHT conditions using the Microthermics processor on TI and odor elimination has not been studied. Another factor affecting TIA is material differences due to variety and storage. Proto is a high-protein cultivar, and IA2032 is a lipoxygenase-null variety, and they are excellent materials for soy milk manufacturing. However, the characteristics of TIAs in the soy milk made from these varieties have not been studied. The objective was to determine TIA in soy milk processed by traditional indirect and steam injection and to determine the effect of soybean blanching and UHT processing methods using the Microthermics DIP processor on the inactivation of trypsin inhibitors.

MATERIALS AND METHODS

Soybean Materials. Soybeans (*Glycine max*) of the variety of Proto (harvested in 2005 and 2006) were obtained from Sinner Brothers and Breshnahan (Casselton, ND). IA2032, a lipoxygenase-null variety (harvested in 2005), was obtained from Stonebridge Ltd. (Cedar Falls, IA). The chemical reagents obtained for TI and odor analysis were obtained from Sigma-Aldrich Co. (St. Louis, MO).

Soy Milk Processed by Traditional and Steam Injection Batch Processes. Whole Proto (2005) and IA2032 soybeans were soaked in tap water at room temperature for 15 h. The soaked beans were drained, rinsed, and ground with tap water using a bean/water ratio of 1:9 (w/w). In the traditional (atmospheric pressure) batch cooking treatment, the soybeans were ground for 3 min at high speed using a Hamilton Beach blender (model 585-1, Peabody, MA). The soy slurry was filtered through a muslin cloth to separate the insoluble residues from the soy milk. The raw soy milk (1 L) in a small pot was heated within a larger pot, which contained boiling water on a stove, which was set at the highest heat level, to approximately 90 °C, and then the small soy milk pot was switched to the hot stove surface to heat to 100 °C and held at this temperature with stirring to prevent foaming for up to 30 min. It took approximately 8 min of heating to bring the temperature to boiling. Soy milk (approximately 30 mL) was sampled at 0, 3, 6, 9, 12, 20, and 30 min after boiling. Immediately after sampling at each time interval, the soy milk in a small beaker was cooled in an ice bath. The soy milk was freeze-dried and analyzed for TIA.

For the direct steam injection treatment, soy milk (2 L) was produced by a continuous grinder equipped with an autocentrifugal separator with 120-mesh size screen (Chang-Sheng Machinery Co., Taoyuan, Taiwan). The soy milk from the continuous grinder was immediately injected with live steam at about 45 psi to boiling and held for 20 min at boiling. It took approximately 15 s for soy milk to reach boiling. Soy milk was sampled at 0, 3, 6, 9, 12, and 20 min after boiling. Immediately after sampling at each time interval, the soy milk in a small beaker was cooled in an ice bath. The soy milk was freeze-dried and analyzed for TIA.

Soy Milk Processed by Blanching and UHT Methods. The following processing methods were carried out.

A. Selected Literature UHT Methods

(1) Processing Conditions of 143 °C for 60 s Reported by Kwok and Others (17−*19).* Approximately 2 kg of Proto (2005) and IA2032 soybeans was soaked for 15 h at room temperature. The soaked soybeans were drained and ground in water with a 9:1 water-to-bean ratio using an automatic centrifugal grinder (Chang-Seng Machinery, Taiwan). The soy milk was processed using the Microthermics UHT/HTST DIP processor (Raleigh, NC) by two methods: the indirect and the direct steam injection−vacuum cooling methods. The Microthermics processor heated the soy milk in two stages. The first stage was preheating, which was set at 110 °C. The flow rate was set at 1 L/min. The second stage was heated by either heat exchanger in the indirect mode or by direct steam injection in the direct mode to 143 °C. The heated soy milk was pumped through a well-insulated holding tube (60 s) so that the processing could be continued in a continuous manner. The tube length was constructed in a way to allow the soy milk to flow through with a desired time at the flow rate of 1 mL/min. In addition to the built-in insulation, which covered the tubes, the entire holding tube coil set was further covered with a heavy blanket to maintain the holding temperature. In the indirect mode, the soy milk coming out of the holding tube was cooled by a tubular heat exchanger using cold tap water. In the direct steam injection mode, a vacuum cooling chamber was used to remove the water condensed from the steam injected and to remove odor. The temperature of the product at the vacuum chamber was set at 110 °C to maintain the same solid content as that of the raw soy milk. After vacuum cooling, the product was further cooled by circulating tap water in a tubular heat exchanger. The product at the exit was approximately 23 °C.

(2) Commercial UHT Settings Reported by Prabhakaran and Perera (24). The two-stage processing method was reported to be a common commercial practice in Singapore. The first stage was 120 °C for a longer period of time (80 s) to inactivate 80% TI, and the second stage was 140 °C for a short period of time (4−6 s) to take advantage of the high power of sterilization before aseptic packaging. Therefore, a two-stage process using our Microthermics processor was carried out to mimic this commercial practice to test its effect on TI inactivation. Proto and IA2032 were processed into raw soy milk using the methods described in the traditional cooking methods as described above. The raw soy milk was then processed at 120 °C for 80 s, cooled to the room temperature, and followed by direct and indirect UHT heating at 140 °C for 4 s (with online preheating at 110 °C). The soy milk obtained was freeze-dried and analyzed for TIA.

B. Blanching of Soaked Soybeans followed by UHT Inactivation of TI

(1) Blanching Experiments. For minimizing soy odor generation during soy milk manufacturing, experiments were carried out to inactivate the lipoxygenase activities of Proto soybean because it contained high lipoxygenase activity (*2*). Soybeans (Proto of the 2006 crop) were soaked for 15 h at the room temperature (~22 °C). The soaked soybeans were rinsed with tap water and immersed for a period of time ranging from 0.5 to 10 min in a large tank of hot water (140 L), which was maintained manually from 70 to 85 °C using a live steam injector. The heated soybeans were cooled in a tank containing cold water (10−15 °C) immediately after heating. The cooled soybeans were made into soy milk using the automatic soy milk machine as described above for traditional steam injection method. The soy milk obtained was analyzed for mass yield (grams of soy milk produced per 100 g of soybean), protein content, and percent protein recovery (extraction rate) as compared to the raw soy milk made from unblanched soaked soybeans. The TIA and hexanal in the soy milk were analyzed. The lipoxygenase activity of the soy milk with the lowest hexanal content was also analyzed to ensure the enzyme was totally inactivated by the heating conditions.

(2) Direct UHT Processing of the Soy Milk Made from Blanched Soybeans. One kilogram of Proto soybeans (2006 crop) was soaked at room temperature for 15 h to rehydrate and then blanched in 140 L of hot water at 80 °C for 2 min. The heated beans were cooled in a tank of cold tap water (140 L). The cooled blanched soybeans were processed into soy milk and preheated at 110 °C using the Microthermics DIP processor, which then heated the soy milk with a continuous flow at 135, 140, 145, and 150 °C for 10−50 s, respectively, using the direct steam injection mode, which was equipped with a vacuum chamber for removing water and soy odor. The reason for cooling the soybeans immediately after blanching was to control the heat remaining in the whole soybeans between processing steps because our laboratory was not equipped with a continuous transport system between blancher and grinder for extraction of soy milk from the whole blanched beans and between extracted soy milk from the exit of the grinder and the UHT heat processor. Such continuous integrated transport/heating systems exist in modern commercial practice to eliminate the effect of transit time.

Lethality (F_0) was calculated according to the equation $F_0 = 10^{(T-121)/Z}$ for comparing the heat power for inactivating bacteria spores or TI. When compared to the inactivation of bacterial spores PA 3679 (*Clostridium sporogenes*) in nonacidic foods such as soy milk, a Z value of 10 was applied (*20*). When testing the heat power for the inactivation of TI, a Z value of 28 as proposed by Kwok and others (*17*) was applied.

Chemical Analyses. *(1). Trypsin Inhibitor Activity Analysis.* The TIA assay of Kakade and others (*26*) was used. The TIA was expressed as TIU per gram of soy milk on a dry basis. TIA also was expressed as milligrams of trypsin inhibitor equivalent by dividing the TIU by 1900 because each milligram of trypsin inhibitor produced 1900 TI units (*27*)

(2). Soy Odor Using Hexanal as a Marker. The solid phase microextraction (SPME) method was used to extract the volatile compounds in the soy milk. The extracted odors were analyzed by gas chromatography. The details of external and internal standards and SPME and GC conditions were according to those of Yuan and Chang (*2, 3*). Results were expressed as parts per million (μg/mL of soy milk).

(3). Lipoxygenase Activity. The lipoxygenase activity of the soy milk was analyzed according to the method of Anthon and Barrett (*28*).

(4). Protein Content. Protein in soy milk made from blanched Proto soybean and raw soybean was determined by using the Kjeldhal method according to method 988.05 of the AOAC International (*29*) using a protein conversion factor of 6.25.

Kinetic Analysis of Trypsin Inhibitor Activity. Kinetic analysis of residual trypsin inhibitor activity in soy milk processed by direct UHT processing method as described in the above experiments was carried out according to the combined model of the two trypsin inhibitors in soy milk: Kunitz soybean trypsin inhibitor (KSTI) and Bowman−Birk inhibitor (BBI) as reported by Rouhana and others (*8*). Such a mixed activity model was also used by Kwok and others (*19*) in their mathematical analysis of TI inactivation.

In general, heat denaturation (inactivation) of the native (active) trypsin inhibitors can be described as follows.

$$N \text{ (native TI)} \xrightarrow{\text{inactivation rate constant, } k} \text{denatured TI} \quad (1)$$

If heat denaturation of TI follows the first-order reaction, the velocity of the reaction can be expressed as

$$\text{velocity of denaturation} = kN \quad (2)$$

Therefore, the disappearance of TI activity at a given time (t) can be expressed in the equation

$$-\mathrm{d}N/\mathrm{d}t = kN \quad (3)$$

where N is the TIA. Integration from time 0 to time t shows

$$-\int \mathrm{d}N/N = \int k\, \mathrm{d}t \quad (4)$$

$$\ln N_t/N_0 = -kt \quad (5)$$

where N_t and N_0 are the TI activities at times t and 0, respectively.

$$\ln N_t = -kt + \ln N_0 \quad (6)$$

The inactivation rate constant (k) at each specific temperature from 135 to 150 °C, respectively, was calculated by plotting the natural log (ln) of residual TIA versus time (s).

The ln k values at each temperature of inactivation were plotted against (1/T, reciprocal of absolute temperature, K) according to the Arrhenius equation of

$$\ln k = \ln k_0 - E_a/RT \quad (7)$$

The slope of this equation was $-E_a/R$, where R is the gas constant, -8.314 J mol^{-1} K^{-1}. Therefore, the E_a was equal to the slope of the negative line multiplying by the R value (*8*).

Statistical Analysis. Soy milk production was completed in duplicate, and each sample at each processing method−time was analyzed twice. The data were subjected to analysis of variance using the SAS 9.1 package (*30*). Significant differences among treatments were analyzed using Duncan's multiple-range test with a probability level of <0.05.

RESULTS AND DISCUSSION

Trypsin Inhibitor Activity As Affected by Traditional and Steam Injection Methods. Table 1 shows the trypsin inhibitor activities in two food soybean cultivars as affected by the traditional and steam injection methods (atmospheric heating conditions) for up to 20−30 min. The raw soy milk made from IA2032 possessed a similar TIA as compared to that made from Proto soybean. When the soy milk was heated from raw to boiling at 100 °C by the traditional cooking method, the residual TIA ranged from 55 to 66%, which was similar to that (57%) reported by Miyagi and others (*31*). Steam injection seemed to have a higher power in inactivating TI in Proto soy milk than in IA2032 soy milk. After boiling by traditional cooking or steam injection for 9 min, >20% of the original TIAs remained. When soy milk was boiled at 100 °C for 20 min, 82−87% of the TIAs were inactivated, whereas Miyagi and others (*31*) reported 95% trypsin inhibitors were inactivated (5% residual activity) under the same boiling temperature and time. Boiling soy milk by the traditional cooking method at 100 °C for 30 min was necessary to reduce TIAs to about 10% or less. This heat requirement was greater than the 10 min at 100 °C reported by Miyagi and others (*31*), similar to that (29 min at 99 °C) observed by Johnson and others (*22*), but was less than that (60 min at 99 °C) reported by Wallace and others (*32*). The differences between our study and others were possibly due to

Table 1. Trypsin Inhibitor Activity As Affected by Traditional and Steam Injection Methods under Atmospheric Pressure for Various Time Periods[a]

heating time (min)	TIU/g		mg of TI/g		residue %	
	Proto	IA2032	Proto	IA2032	Proto	IA2032
			Traditional Method (100 °C)			
raw	66475 (4067)	72750 (4031)	35A (2.1)	38.3A (2.1)	100 (0)	100 (0)
0	44085 (1069)	39966 (1409)	23.2B (0.6)	21.1B (0.7)	66.5 (5.7)	55.1 (5.0)
3	26699 (296)	22358 (969)	14.1C (0.2)	11.8C (0.5)	40.3 (2.1)	30.8 (0.4)
6	18876 (232)	19721 (1298)	10.0D (0.1)	10.4DC (0.7)	28.5 (2.1)	27.1 (0.3)
9	13604 (180)	16849 (366)	7.2E (0.1)	8.9DE (0.2)	20.5 (1.6)	23.2 (1.8)
12	11141 (475)	14597 (450)	5.9EF (0.3)	7.7E (0.2)	16.8 (1.7)	20.1 (0.5)
20	8868 (100)	9908 (436)	4.7Fb (0.1)	5.3Fc (0.2)	13.4 (0.6	13.6 (0.1)
30	5130 (137)	7766 (22)	2.7G (0.1)	4.1F (0)	7.7 (0.3)	10.7 (0.6)
			Steam Injection (100 °C)			
raw	65200 (1131)	63195 (391)	34.3A (0.6)	33.3A (0.2)	100 (0)	100 (0)
0	26962 (171)	38914 (395)	14.2B (0.1)	20.5B (0.2)	41.4 (0.5)	61.6 (0.2)
3	19231 (98)	27169 (12)	10.2C (0.1)	14.3C (0)	29.5 (0.4)	43.0 (0.3)
6	15821 (496)	22244 (132)	8.3D (0.3)	11.8D (0.1)	24.3 (1.2)	35.2 (0)
9	13348 (40)	18787 (26)	7.0E (0)	9.9E (0)	20.5 (0.4)	29.8 (0.2)
12	12900 (0)	17228 (221)	6.8E (0)	9.1F (0.1)	19.8 (0.4)	27.3 (0.5)
20	8429 (123)	11701 (171)	4.5Fb (0.1)	6.2Gb (0.1)	12.9 (0.4)	18.2 (0)
			UHT (143 °C, 60 s)			
raw	65200 (1131)	63195 (391)	34.3A (0.6)	33.3A (0.2)	100 (0)	100 (0)
direct	13513 (207)	12737 (740)	7.2Ba (0.1)	6.7Ba (0.4)	20.7 (0)	20.2 (0.2)
indirect	13648 (200)	10322 (563)	7.1Ba (0.1)	5.4Cc (0.3)	21.0 (0.6)	16.3 (1.0)

[a] Data are means of two replicates with standard deviations given in parentheses. Means with different capital letters A−G in the same column indicate significant differences among different heating times of mg of TI/g at $p < 0.05$. Means with different lower case letters a−d among four processing methods (traditional method 20 min, steam injection 20 min, direct and indirect UHT at 143 °C, 60 s) are significant at $p < 0.05$.

Table 2. Trypsin Inhibitor Activities in Soy Milk Made from Proto and IA2032 by Selected UHT Processing Methods[a]

heating time (min)	F_0 value	TIU/g		mg of TI/g		residue %	
		Proto 05	IA2032	Proto 05	IA2032	Proto 05	IA2032
UHT Indirect							
raw		65200 (1131)	63195 (391)	34.3A (0.6)	33.3A (0.2)	100 (0)	100 (0)
143 °C, 60 s	6.11	13648 (200)	10322 (563)	7.1C (0.1)	5.4D (0.3)	21.0 (0.6)	16.3 (1.0)
120 °C, 80 s	1.23	16892 (701)	17493 (1025)	8.9B (0.4)	9.2B (0.5)	25.9 (1.1)	27.7 (1.6)
140 °C, 4 s	0.32	15352 (566)	15347 (630)	8.1B (0.3)	8.1C (0.3)	23.5 (0.9)	24.3 (0.9)
120 °C, 80 s + 140 °C, 4 s	1.55	13107 (771)	15261 (72)	6.9C (0.4)	8.0C (0)	20.1 (1.2)	24.1 (0.1)
UHT Direct							
raw		65200 (1131)	63195 (391)	34.3A (0.6)	33.3A (0.2)	100 (0)	100 (0)
143 °C, 60 s	6.11	13513 (207)	12737 (740)	7.1B (0.1)	6.7B (0.4)	20.7 (0)	20.2 (0.2)
120 °C, 80 s	1.23	12558 (233)	11871 (378)	6.6C (0.2)	6.2C (0.2)	19.3 (0.3)	18.8 (0.5)
120 °C, 80 s + 140 °C, 4 s	1.55	9839 (774)	9312 (52)	5.2D (0.4)	4.9D (0.3)	15.1 (0.5)	14.7 (0.6)

[a] Data are means of two replicates with standard deviations given in parentheses. Means with different capital letters A−D in the same column indicate significant differences among different heating treatments within each of the direct or indirect methods at $p < 0.05$. Lethality (F_0) was calculated according to the equation $F_0 = 10^{(T-121)/Z}$, where Z is 28 °C for inactivating TI in soy milk.

differences in soybean materials and cooking practice (water to bean ratio, different cooking utensil, and heating rates). The inactivation curves (not shown) of the two atmospheric heating methods did not fit the first-order reaction kinetics because ln residual activities versus time was not a linear line.

Trypsin Inhibitor Activity As Affected by Selected UHT Methods. Kwok and others (*18, 19*) reported that indirect batch UHT heating conditions (143 °C for 60 s in a capillary tube immersed in an oil bath) could reduce the TIAs to <10% of the original activity. We decided to analyze the TIA in the soy milk heated by similar conditions (143 °C for 60 s using a holding tube that was made to be 60 s length at a 1 L/min flow rate) to confirm if that was true for our soy milk heated by a continuous Microthermics processor. We also compared these processing conditions to that practiced in commercial settings (120 °C for 80 s + 140 °C for 4 s). In addition to this process that integrated these two temperature−time procedures, we also tested the characteristics of heat inactivation by 120 °C for 80 s and 140 °C for 4 s, separately, to test the TI inactivation at each respective process. **Table 2** shows the results of TI inactivation by these selected UHT processing methods. The results indicated that 16−21% of the TI remained after indirect UHT heating at 143 °C for 60 s, whereas approximately 20% remained after direct UHT heating. The discrepancies in TI inactivation by similar processing temperature−time combinations between our results and that of Kwok and others (*17*) might be due to differences in processing methods and soybean materials. It is interesting to note that 120 °C for 80 s produced a similar TI inactivation effect as the 140 °C for 4 s process. Integrating these two processes did not increase much TI inactivation, particularly with no improvement for IA2032 soy milk in the indirect heating mode.

When lethality (F_0) values (**Table 2**) among these selected processing conditions were compared using the $Z = 28$ °C as reported by Kwok and others (*17*), we found that the differences between residual TI among these processes did not behave according to the calcuated lethality power manner because F_0 was 6.1 for the 143 °C for 60 s conditions and 0.32 for the 140 °C for 4 s conditions (the ratio was about 19 or 1900%), but the residual TI did not change that much (only 2−8% differences). Therefore, the mathematical model published by Kwok and others (*17*) using a batch heating system could not be

Table 3. Blanching Effect on Proto Soy Milk Yield and Protein Recovery[a]

temperature (°C)	time (min)	soy milk yield (g/100 g of beans)	protein (%)	protein recovery (%)
raw		806.0 (4.24)	3.53 (0.01)	100
70	5	823.5 (10.61)	3.25 (0.02)	94
70	7.5	820.5 (2.12)	3.24 (0.09)	93
70	10	829.5 (0.71)	3.13 (0.16)	91
75	2	823.5 (0.71)	3.26 (0.09)	94
75	3	824.5 (0.71)	3.19 (0.01)	92
75	4	832.5 (9.19)	3.16 (0.02)	92
80	0.5	816.5 (4.95)	3.27 (0)	94
80	1	792.0 (9.9)	3.26 (0.05)	91
80	2	800.0 (5.66)	3.18 (0.01)	89
80	3	790.5 (10.61)	3.16 (0.07)	88
85	0.5	801.5 (4.95)	3.19 (0.07)	90
85	1	800.5 (4.95)	3.25 (0)	91
85	1.5	775.5 (34.65)	3.13 (0.13)	85

[a] Data are means of two replicates with standard deviations given in parentheses.

applied to our laboratory conditions. This is interesting and implies that the kinetics of TI inactivation must be developed for each respective industrial processing condition, because processing variables such as grinding method, solid content, pretreatment method, machinery design, and final heating method differed for various soy milk manufacturers.

For this reason, we conducted further experiments to test the kinetics of TI inactivation in conjunction with our intent to improve soy flavor by first inactivating lipoxygenases in soybeans with blanching prior to the UHT processing.

As compared to the UHT direct and indirect heating at 143 °C for 60 s, traditional atmospheric cooking and steam injection at 100 °C for 9 min was required to produce similar TI inactivation for Proto soy milk, whereas it took 20 min at 100 °C for IA2032 soy milk to produce a similar effect (**Table 1**). It should be noted that the lethality at 100 °C for 9−20 min was minimal as compared to that at 143 °C for 60 s.

Soybean materials may contain different proportions of Kunitz and Bowman−Birk inhibitors and processing methods, including water-to-bean ratio, which could affect the extraction and heat stability of two different types of trypsin inhibitors in soybeans (*7, 8*). Heating could cause not only denaturation of proteins due to change in protein secondary and tertiary structures but also may cause interchanges of disulfide bonds between different proteins (*7*) to cause aggregations. In addition, under severe conditions, heating soy milk may degrade amino acids such as cysteine (*10, 11*) and lysine (*9*), indicating potential Maillard reactions between lysine and reducing sugars. Unlike purified TI in water experimental model system, trypsin inhibitors in natural complex soy milk food systems may interact with other proteins such as glycinins and reducing sugars during thermal processing. Such interactions may affect TI activities. A recent report suggested that UHT processing at 142 °C for 4−6 s could eliminate the ability of soy protein to decrease LDL-cholesterol in humans (*33*). The authors attributed the changes in cholesterol-lowering effect to protein structural alterations by ultraheat treatment.

Effect of Blanching. The results of blanching whole soybeans on soy milk yield and protein extraction in terms of percent protein in soy milk and total protein recovery in soy milk are presented in **Table 3**. Soy milk yield was not largely affected due to a constant water-to-bean ratio used for extraction. There was a trend within each temperature that protein recovery was slightly decreased with the increase in heating time.

The effects of blanching on TI inactivation and hexanal generation in soy milk are presented in **Table 4**. The residual TI in the soy milk prepared from blanched soybeans ranged from 51%

Table 4. Trypsin Inhibitor Activity and Hexanal in Soy Milk Made from Blanched Soybeans

blanching conditions	TIU/g	TIA/g of protein	TI residue (% of raw soy milk)	hexanal (ppm)
raw	112392 A	213004	100	3.48 (0.20)
70 °C 5 min	76007 CD	161835	67.6	0.515 (0.09)
70 °C 7.5 min	67757 EF	138441	60.3	0.44 (0.02)
70 °C 10 min	57005 G	121658	50.7	0.28 (0.10)
75 °C 2 min	72721 DE	155257	64.7	0.05 (0.01)
75 °C 3 min	65356 F	146691	58.2	0.09 (0.01)
75 °C 4 min	64772 F	139997	57.6	0.13 (0.05)
80 °C 30 s	84901 B	189014	75.5	0.76 (0.10)
80 °C 1 min	79351 BC	164299	70.6	0.04 (0.01)
80 °C 2 min	64111 F	134069	57.0	nd
80 °C 3 min	57304 G	118959	51.0	0.02 (0.00)
85 °C 30 s	84653 B	176471	75.3	0.54 (0.14)
85 °C 60 s	68608 EF	141226	61.0	0.04 (0.01)
85 °C 90 s	65309 F	137711	58.1	0.31 (0.03)

[a] Data are means of two replicates with standard deviations given in parentheses.

when heated at 80 °C for 3 min to approximately 75% when heated at 80 or 85 °C for 30 s. Please note that the TI in the raw soy milk made from Proto was higher than that presented in **Table 1**. This was because the Proto materials were from different years of harvest. The Proto soybeans used for **Tables 1** and **2** were harvested in 2005, whereas the Proto soybeans used for blanching experiments were from the 2006 crop. Earlier we also observed a very high TI in the raw Proto soy milk in our yuba research (*29*). Therefore, the year of production presented a seasonal effect on the soybean chemistry. The hexanal contents in the soy milk made from blanched soybeans under all conditions were all largely decreased, and no hexanal was detected in soy milk processed by 80 °C for 2 min, at which the protein recovery was 89%. The lipoxygenase test showed that the enzymes were inactivated under these conditions. Therefore, the conditions of 80 °C for 2 min were chosen for further UHT heating experiments due to the consideration for enhancing flavor in the soy milk products. Wilkens and others (*35*) reported soy milk produced by hot grinding of soybeans at approximately 80 °C or higher could lower soy odor concentrations in soy milk products. Endo and others (*36*) reported heating whole soybeans on hydroperoxide reduction in normal soybean. They reported that under some steaming and boiling conditions, soybeans could produce soy milk with similar levels of hydroperoxides as that prepared from lipoxygenase-null soybeans. Sensory evaluation showed that a dessert product prepared from soy milk that was made from heat-treated soybean gave lower beany flavor scores than that made from soybean without preheating treatment. The low hexanal content in soy milk made from blanched soybeans was consistent with the low-hydroperoxide findings by Endo and others (*36*).

Direct UHT Processing of the Soy Milk made from Blanched Soybeans. Table 5 shows the results of TI inactivation by direct UHT Processing of soy milk at 135−150 °C for 10−50 s, respectively. Similar to the trends reported in the literature, TI decreased with the increase in the time of heating at each temperature, and TI decreased when the temperature was increased. The only conditions that decreased the TI to about 10% of that in the raw soy milk was at 150 °C for 50 s, which gave an equivalent F_0 value of 661 for inactivation of PA3679. Johnson and others (*22*) reported a much higher heat requirement at 154 °C for 40 s (F_0 of 1330) for inactivating TI to <10% of the original TI in raw soy milk. We did not calculate F_0 for TI inactivation according to $Z = 28$ as reported by Kwok and others (*17*) because this Z value was specific to laboratory processing conditions and, therefore, could not be applied to our experimental conditions. As shown in **Table 5**, the behavior

Table 5. Trypsin Inhibitor Activity in Soy Milk Processed by Direct-UHT Processing Methods from Soybeans Blanched at 80 °C for 2 min[a]

heating conditions	F_0[b] (Z = 10 °C)	TIU/g	TIU/g of protein	residual TI (% of blanched soy milk)	residual TI (% of raw soy milk)
80 °C, 2 min		64111 A	134069	100.0	57.0
135 °C, 10 s	4.18	42366 B	28118	66.1	37.7
135 °C, 20 s	8.38	32483 DE	23261	50.7	28.9
135 °C, 30 s	12.54	27170 GFH	21353	42.4	24.2
135 °C, 40 s	16.72	26157 FGHI	19282	40.8	23.3
135 °C, 50 s	20.9	22719 KIJ	17179	35.4	20.2
140 °C, 10 s	13.23	37334 C	28424	58.2	33.2
140 °C, 20 s	26.47	30428 DEF	23956	47.5	27.1
140 °C, 30 s	39.71	24755 HIG	19682	38.6	22.0
140 °C, 40 s	52.92	23903 HIJ	17951	37.3	21.3
140 °C, 50 s	66.15	19376 KL	15506	30.2	17.2
145 °C, 10 s	41.9	33010 D	24104	51.5	29.4
145 °C, 20 s	83.8	28436 EFG	18404	44.4	25.3
145 °C, 30 s	125.7	21999 IJKL	16606	34.3	19.6
145 °C, 40 s	167.6	19828 JKL	15225	30.9	17.6
145 °C, 50 s	209.5	17772 L	13554	27.7	15.8
150 °C, 10 s	132.3	30443 DEF	22972	47.5	27.1
150 °C, 20 s	264.7	28563 EFG	22303	44.6	25.4
150 °C, 30 s	397.1	21979 IJKL	16129	34.3	19.6
150 °C, 40 s	529.5	19519 JKL	14645	30.4	17.4
150 °C, 50 s	661.5	12305 M	10228	19.2	10.9

[a] Data are means of two replicates. Means followed by different letters are significantly different at $p < 0.05$. [b] Lethality (F_0) was calculated according to the equation $F_0 = 10^{(T-121)/Z}$, where Z is 10 °C for inactivating bacterial spores PA 3679 in nonacidic foods.

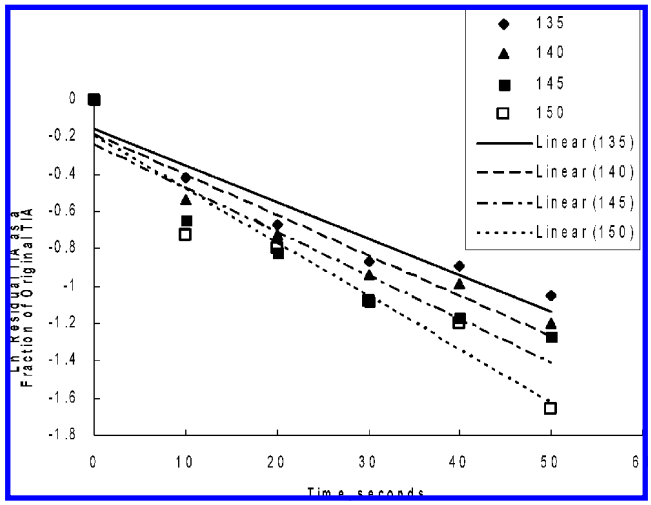

Figure 1. Thermal inactivation curves plotted using a combined inactivation model of two trypsin inhibitors in soy milk processed at 135−150 °C for 10−50 s.

of TI is much more resistant than bacterial spores because TI inactivation was much lower than bacterial inactivation.

Figure 1 shows the kinetics analysis of the results of TI inactivation by the UHT processing conditions as presented in **Table 5**. Linear lines of ln residual TI versus time had a negative slope with correlation coefficients ranging from 0.92 to 0.95, indicating a good fit of the first-order reactions. Similar to the report of Rouhana and others (*8*), the combination model of KSTI−BBI inhibitors worked well for these sets of results from the specific conditions of this study. However, Johnson and others (*22, 23*) reported a nonlinear kinetics for soy milk TI inactivation by hydrothermal heating of soy milk. They obtained a fast inactivation period, which was followed by a slow inactivation period. They attributed the second slow period of the TI inactivation to the Bowman−Birk inhibitors.

The first-order reaction constant k values for these lines obtained from **Figure 1** were plotted against $1/T$ (reverse of temperature in K scale), and the results are shown in **Figure 2**. Through linear regression ($r^2 = 0.95$, $P < 0.05$), the slope of the line was −4098 K. The activation energy E_a of 34 kJ/mol was obtained by multiplying this negative slope with −8.314 J mol^{-1} K^{-1}. Rouhana and others (*8*) applied the combination model for calculation of the E_a of TI inactivation in soy milk, and they obtained the −6670 K, which was equivalent to E_a of about 55 kJ/mol. On the basis of a separate KSTI and BBK model, Rouhana and others (*8*) reported that E_a for KSTI and BBK were 24 and 102 kJ/mol, respectively. On the other hand, Johnson and others (*23*) analyzed the TI inactivation of KSTI and BBK and reported 47 kJ/mol for KSTI and 20 kJ/mol for BBI. One major difference between the experiments of soy milk between Rouhana and others (*8*) and Johnson and others (*23*) was the ratios of water-to-bean materials. A water-to-bean ratio of 4:1 (w/w) was used by Johnson and others (*23*), whereas 10:1 was used by Rouhana and co-workers (*8*). The different ratios might have resulted in different extraction yields of KSTI and BBI into the soy milk. Moreover, solid and protein concentrations of soy milk might have a major impact on the heat resistance of these inhibitors. This is a plausible explanation because Dipietro and Liener (*7*) discovered that heating soy flour in situ, where the solid/protein concentration was much higher than in soy milk, resulted in a faster inactivation of BBI than KSTI. However, in a diluted soy extract in water such as soy milk, BBI was much more resistant than KSTI. A low water-to-bean ratio could result in high protein concentration to promote a faster rate of BBI inactivation. Dipietro and Liener (*7*) attributed this effect to interdisulfide bond exchanges between BBI and glycinin, a major storage protein rich in disulfide and sulfhydryl groups. When solid is high, the interactions between proteins such as inhibitors and other constituents also would be more complex to contribute to different behaviors of inhibitor inactivation as reported by various researchers.

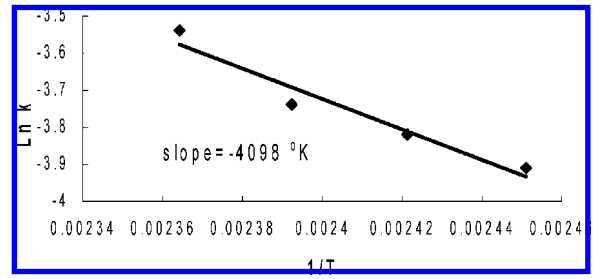

Figure 2. Arrhenius plot for the inactivation of trypsin inhibitors under ultrahigh-temperature conditions by direct Microthermics UHT processor.

Soy milk can be commercially sterilized ($F_0 \geq 3$) (*20*), aseptically packaged, and safely stored at ambient temperature even if 90% trypsin inhibitor activity is not inactivated. The refrigerated soy milk products that are packaged in milk cartons should also be heated to inactivate 90% of trypsin inhibitor to reduce the toxicity of trypsin inhibitors. However, some soy milk manufacturers have ignored this because the trypsin inhibitor levels in commercial products have been found to be much higher than this level. Guo and others (*20*) investigated the trypsin inhibitor activity of 10 commercial soy milk/tofu products on the market in Hong Kong and observed that unacceptably high TI activities (about 30−48% of the original TI) existed in most of the UHT-processed soy milk and in one 100 °C pasteurized soy milk. We also found that some commercial soy milk produced in the United States contained as high as 40000 TIU/g on a dry basis. A complete report of our findings on trypsin inhibitors in U.S. commercial soy milk products is forthcoming from our laboratory. The high TIA in food products is a concern for the health of frequent users of soy milk (*21*). However, if the Bowman−Birk inhibitor is clearly established

to have anticarcinogenic properties in humans and have no adverse physiological effect at a certain level, future soy milk processing research should be directed to selectively eliminate the Kunitz soybean trypsin inhibitor but retain the Bowman−Birk inhibitor as much as possible in the final soy milk products that have good flavor and nutritive values. Our research contributes to the understanding of the behavior of TI, soy odor, and protein extraction yield by heat processing and would be of interest to the food industry in designing thermal processes for producing low beany off-flavor and low TI activity without overheating, which can cause negative effects on soy nutritive values and potential health benefits.

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