Technology

Pak. J. Sci. Ind. Res. 2003 ***46*** *(5) 389 - 394*

Retention of Nutritional Quality of Soybean during Extrusion Cooking

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(Received October 30, 2001; accepted March 24, 2003)

Trypsin inhibitor (TI) is one of the major anti-nutritional components of soybean and must be inactivated before its protein content can be safely and efficiently utilized for food and feed purposes. However, retention of the protein quality is also a prime consideration while inactivating TI. This research was conducted to study the effect of extrusion process conditions (temperature, screw speed and moisture content) on trypsin inhibitor activity (TIA) and nitrogen solubility index (NSI) and to develop a model for prediction of TI inactivation during extrusion cooking based on its reaction kinetics. A laboratory size single screw extruder was used for extrusion cooking of full-fat soybean implementing a (4x4x4)x2 full factorial design. TIA was measured using a standard procedure and NSI by AACC procedure. The reaction rate constant for loss of TIA was calculated based on its activation energy from literature and experimental TIA data. The statistical models correlating product temperature with operating conditions and activation energy were combined with mathematical equations for predicting TIA during the cooking process. TIA and NSI of the soybean (William 82 variety) were found to be 47.0 TIU mg^{-1} and 78% respectively. Trypsin inhibitor inactivation ranged from 90% of that of raw soybean at low screw speed (75 rpm) and high barrel temperature (170°C) (LSHT) to 50% for higher screw speed (150 rpm) and low barrel temperature (140°C) (HSLT). Reduction in NSI for similar extrusion conditions ranged from 95% at LSHT to 50% at HSLT of that of raw soybeans. Variations between predicted and measured TIA values were less than 1% for the given conditions. Results indicated that reduction in TIA and NSI occurred mainly in the compression and metering sections of the extruder and that they paralleled each other, thereby making it difficult to retain high NSI while inactivating TI. However, the efficiency of extrusion cooking for TI inactivation has been proved. The model can be used for determining optimum conditions for extrusion cooking of soybean for food and feed purposes.

Key words: Soybean, Trypsin inhibition, Extrusion cooking, Nitrogen solubility index.

Introduction

Soybean, by virtue of its large fraction of high quality protein, is appearing as the most promising source for satisfying the world's protein requirements. In raw form, it contains approximately 35-50% protein, 12-25% fat, 33-35% carbohydrate and 5% ash (Smith 1989). However, soybean also contains anti-nutritional factors such as trypsin-inhibitor (TI) that limit protein digestibility, inhibit growth, and cause pancreatic hypetrophy and flatulence (Reddy and Pierson 1994). TI, a protein by itself, reacts with the trypsin (protein digesting enzyme) and reduces its protein digesting capability besides causing other problems. TI, therefore, must be inactivated before the protein content can be safely utilized for food and feed purposes. Macrae *et al* (1993) suggested that inactivation of TI in different legumes is a function of temperature, heating duration, particle size and moisture content. The capability of the extrusion cooking process in providing a lively control over these variables has resulted in its evolution as a low-cost and effective method for cooking soybean.

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The high temperatures encountered by soybean during extrusion cooking, although for a short time, not only denatures TI but also other proteins resulting in reduced nutritional and functional properties. Very limited studies are available on modeling of TI inactivation during extrusion cooking. The present study aimed at optimizing the process conditions for extrusion cooking of soybeans on one hand and development of a model for prediction of TI inactivation during the process is based on TI reaction kinetics and temperature history, on the other. Development of such a model will help a great deal in predicting quality of processed soybean as well as in design of extruders for this purpose.

Materials and Methods

The extruder. The laboratory size single screw extruder (C.W. Brabender Instrument, S. Hackensack, NJ) used in these experiments had a length to diameter (L/D) ratio of 25:1 and compression ratio of 5:1. The extruder had four heating zones (three for the barrel and one for the die) that could be independently heated to set temperatures. Cooling and temperature control was accomplished by compressed air flow

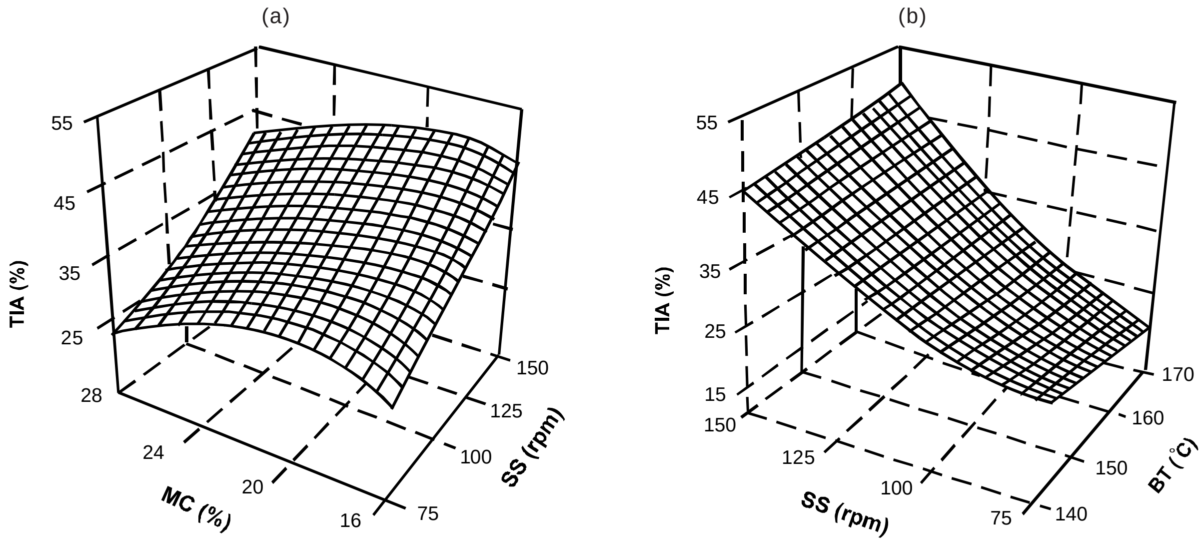

Fig 1. Effect of **(a)** Moisture Content (MC) and Screw Speed (SS) and **(b)** Screw Speed (SS) and Barrel Temperature (BT), on Trypsin-Inhibitor Activity (TIA).

Table 1
Experimental plan

Parameters	A	B	C	D
Moisture content (%db)	16	20	24	28
Temperature (°C)	140	150	160	170
Screw speed (rpm)	75	100	125	150

through jackets surrounding the barrel. Barrel temperatures in the feed and compression sections were set at 60°C and 100°C respectively and maintained constant throughout these experiments. Temperatures of the metering section and die varied according to the experimental plan (Table 1). A long (11cm) tubular tapering die with 1.10 cm and 0.48cm entrance and exit diameters respectively, was used on the extruder.

Product (Soybean) temperature inside the extruder was measured with thermocouples attached at the end of feed and compression sections and at the die. Pressure at the die was measured with a pressure transducer (Dynasco, Model TPT 432A-3M-6/18) in the same port along with thermocouple. The temperature and pressure data were recorded through an IMB personal computer in every six seconds during each extrusion run.

Experimental design. A 4x4x4 full factorial design (Table 1) was employed in duplicate with screw speed, moisture content and barrel temperature (metering zone & die) as the control variables. As mentioned earlier, barrel temperature in the feed and compression sections were kept constant for all the experiments. Statistical models for prediction of TIA and NSI were developed using non-linear regression. TI inactivation along the screw channel based on temperature history was computed by a program developed for this purpose (Khan 1996).

Soybean preparation for extrusion cooking. The soybean (William 82 1994 Missouri crop), purchased from Missouri Seed Foundation (Columbia MO) were cracked and de-hulled using a Fitz Mill (Model No.DASO6, The Fitzpatrick Co. Elmhurst, IL) such that each seed was broken into 3 or 4 pieces. Moisture content of the cracked soybean was measured using a moisture balance (CSC Scientific Co. Inc.). The quantity of water needed for adjusting moisture content to the desired level (Table 1) was calculated using the following moisture balance equation:

$$Wm = \frac{Ws.(DMC-OMC)}{1 - DMC}$$

Where

Wm: Quantity of Additional Water Required (kg)
Ws: Total Weight of Soybean (22.73 kg per Batch)
DMC: Desired Moisture Content (Table 1)
OMC: Original or Measured Moisture Content

Moisture content of each batch was adjusted to the desired level of 24 h before extrusion cooking and stored in moisture proof plastic containers. The material was thoroughly mixed (Leland Food "Double Action Mixer", Model L-100DA) immediately before the extrusion run. Samples collected during these runs were stored at - 4°C for analysis.

Chemical analysis. The frozen extrudate samples were freeze-dried (Labconco Corporation, Kansas City, MO),

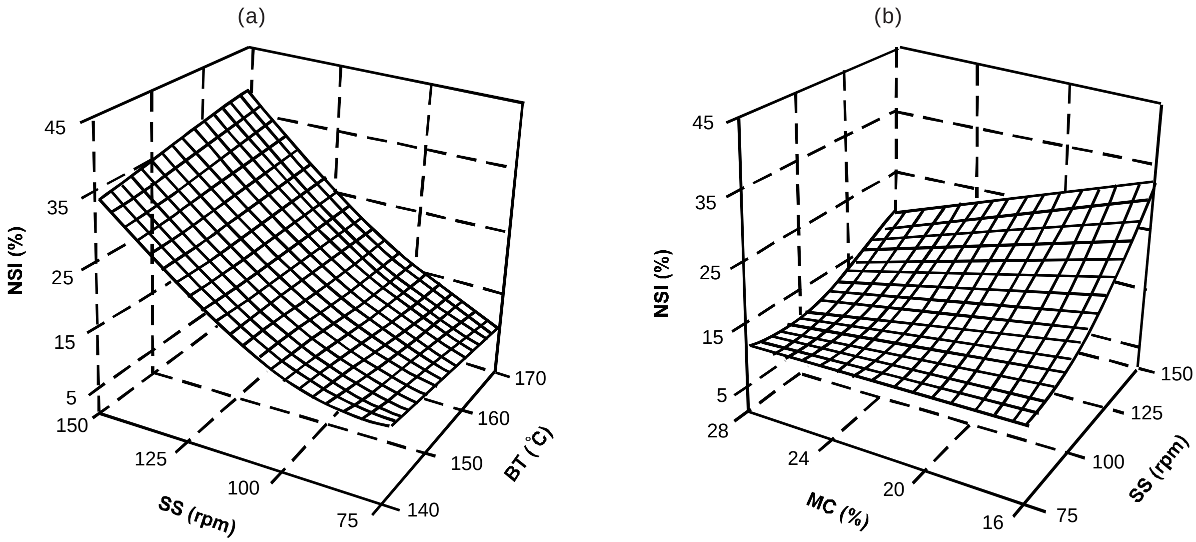

Fig 2. Effect of **(a)** Screw Speed (SS) and Barrel Temperature (BT), and **(b)** Moisture Content (MC) and Screw Speed (SS), on Trypsin-Inhibitor Activity (TIA).

ground and de-fatted by solvent extraction. TIA of soybean samples was determined using a slight modification of the standard procedure (AACC 1995a) suggested by Hamerstrand *et al* (1981). NSI was determined by using a standard procedure (AACC 1995b).

Results and Discussion

TIA and NSI of raw soybean were 47.0 TIU mg^{-1} and 78% respectively. Maximum (85%) and minimum (45%) TI inactivation were observed at screw speeds of 75 and 150 rpm respectively (Fig 1a) indicating an inverse relationship. The lower screw speed allowed for a longer residence time at high temperatures and proved more effective for TI inactivation. The effect of barrel temperature and moisture content, although statistically significant (Table 2) was unexpectedly less pronounced. The small changes in TIA with barrel temperature (Fig 1b) occured because of the small changes in product's temperature history inside the extruder (Khan 1996). TIA varied nonlinearly over a small range with moisture content. Although it has been shown previously (Macrae *et al* 1993) that increasing moisture content caused increased reduction in TIA. It seems that moisture content was too high and therefore, did not show any difference in effectiveness in the given range. A study of lower range of moisture content would be valuable. These results indicated that screw speed or in other words residence time and shear had the most important effect on TIA for a given temperature history.

Nitrogen solubility index (NSI) was more sensitive to extrusion operating conditions than TIA (Fig 2a). However, the trend for changes in NSI was similar to that of TIA except for moisture content (Fig 2b). Maximum reduction in NSI (95%) was observed at 28% moisture content, 170°C barrel temperature and 75 rpm screw speed while minimum reduction (60%) was observed at 16% moisture content, 170°C and 150 rpm (Table 3). The effect of moisture is more significant at 150 rpm as compared to 75 rpm. For example, at 170°C and 150 rpm, NSI decreased from 38% at 16% moisture content to 18% at 28% moisture content. While for the same barrel temperature and 75 rpm, NSI remained constant with increasing moisture content, thus suggesting that the effect of moisture is enhanced by shearing at high screw speed.

Table 2

Model for trypsin inhibitor activity, $R^2 = 0.54$

Parameter	Estimate	Pr > F
Intercept	87.719	0.0349
MC	4.845	0.0045
BT	-7.76×10^{-1}	0.0008
SS	-1.05	0.0056
BT x SS	6.47×10^{-3}	0.0011
MC^2	-1.16×10^{-1}	0.0026
SS^2	-1.78×10^{-3}	0.0703

MC: Moisture Content (%), BT: Barrel Temperature (°C), SS: Screw Speed (rpm).

Selection of extrusion conditions for cooking of soybean will mainly depend on the purpose of the end product. If the soybean or soy protein is intended for direct consumption as food or feed without any further heat treatment, conditions giving lowest TIA should be used. However, if the soybean protein is intended for imparting functional properties, conditions giving high NSI should be used.

Table 3
Model for nitrogen solubility index, $R^2 = 0.75$

Parameter	Estimate	Pr > F
Intercept	87.474	0.0001
BT	-6.13×10^{-1}	0.0001
SS	-8.99×10^{-1}	0.0001
MC x BT	-8.61×10^{-3}	0.0001
BT x SS	6.14×10^{-3}	0.0001
MC x BT x SS	-1.18×10^{-4}	0.0001
SS^2	2.59×10^{-3}	0.0001

MC: Moisture Content (%), BT: Barrel Temperature (˚C), SS: Screw Speed (rpm).

Reaction kinetics of loss of trypsin inhibitor (TI). The data for reaction rate constant (k) for inactivation of TI (Table 4) was taken from literature (Savage *et al* 1995, Rouhana *et al* 1996). The two data sets covered consecutive ranges of temperature and were combined to cover a broader temperature range from 90 to 150˚C. Although this data was not obtained under extrusion conditions, it covers the temperature range usually encountered during extrusion cooking and can therefore be used for estimating the reaction rates in this temperature range.

Rouhana *et al* (1996) showed that 60% of the TIA was caused by Kunitz trypsin inhibitor (KTI) and 40% by the Bowman-Birk inhibitor (BBI). Reaction rate constant (k) in Table 4 is the sum of k_{KTI} times 0.6 and k_{BBI} times 0.4. The data was fitted to the Arrhenius equation:

$$k = k_O e^{\frac{-E_a}{RT}}$$

E_a is activation energy, R is gas constant (8.314 Joule.g^{-1} mol^1. K^{-1}) and T is absolute temperature (˚K). A linear regression of 1n(k) vs T^{-1} had an R^2 of 0.98 and resulted in the following equation:

$$k = 1924160.e^{-7513.53\frac{1}{T}}$$

Table 4
Reaction rate constant data

Temperature (˚C)	k_{KTI} (S^{-1})	K_{BBI} (S^{-1})	K (S^{-1})	
90			0.00185	Savage *et al* 1995
95			0.00255	″
100			0.00283	″
110	0.01156	0.00237	0.00788	Rouhana *et al* 1996
120	0.01428	0.00569	0.01084	″
130	0.01803	0.01158	0.01545	″
140	0.02049	0.02204	0.02111	″
150	0.02372	0.05386	0.03578	″

The above mentioned relationship covered the temperature range from 90 to 150˚C, while inactivation of TI starts at 30˚C (Kunitz 1948). The reaction rate was constant for the entire range of temperature inside the extruder was derived by using the given activation energy.

Residence time for given extrusion conditions was determined using the equation of continuity. The mass flow rate through the extruder is given by:

$$Q_m\left(\frac{kg}{s}\right) = \rho\left(\frac{kg}{m^3}\right).A\,(m^2).V\left(\frac{m}{s}\right)$$

or

$$V\left(\frac{m}{s}\right) = \frac{Q_m\left(\frac{kg}{s}\right)}{\rho\left(\frac{kg}{m^3}\right).A(m^2)}$$

Q_m: mass flow rate (kg s^{-1}) measured experimentally
ρ : 1300 kg m^{-3} (Density of Soybean), measured experimentally
A1: 5.43×10^{-5} m^2 (Cross-Sectional Area of the Screw Channel in the Feed Section)
A2: 3.72×10^{-5} m^2 (cross-Sectional Area of the Screw Channel in the Compression Section)
A3: 1.86×10^{-5} m^2 (Cross-Sectional Area of the Screw Channel in the Metering Section)

Table 5
Predicted TIA (TIU/mg) values using the kinetics and experimental data for 20% moisture content

Screw speed (rpm)	k_o (140˚C)	k_o (150˚C)	Pred.TIA (140˚C)	Pred.TIA (150˚C)	Actual TIA (140˚C)	Actual TIA (150˚C)
75	11941590	10641590	12.56	9.59	12.45	9.51
100	12641590	9341590	17.36	18.05	17.33	18.02
125	15841590	13941590	17.78	16.33	17.67	16.24
150	16841590	12341590	21.04	22.67	20.95	22.61

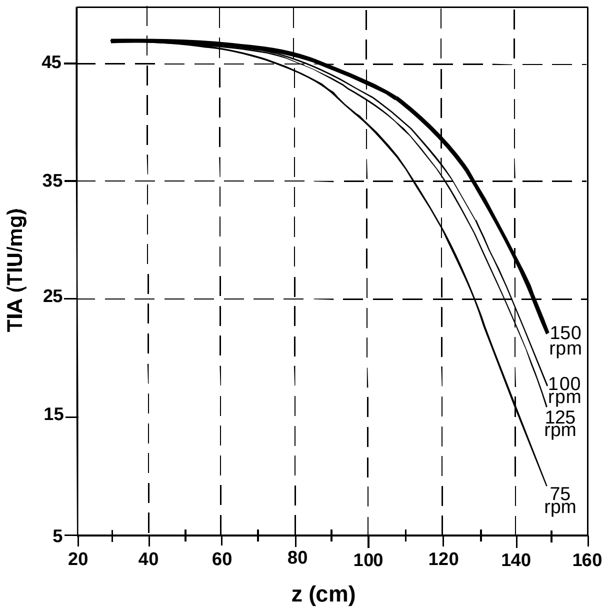

Fig 3. Inactivation of Trypsin Inhibitor (TI) along the Screw Channel (z) at 20% Moisture Content and 150˚C Barrel Temperature and 75,100, 125 and 150 rpm Screw speeds.

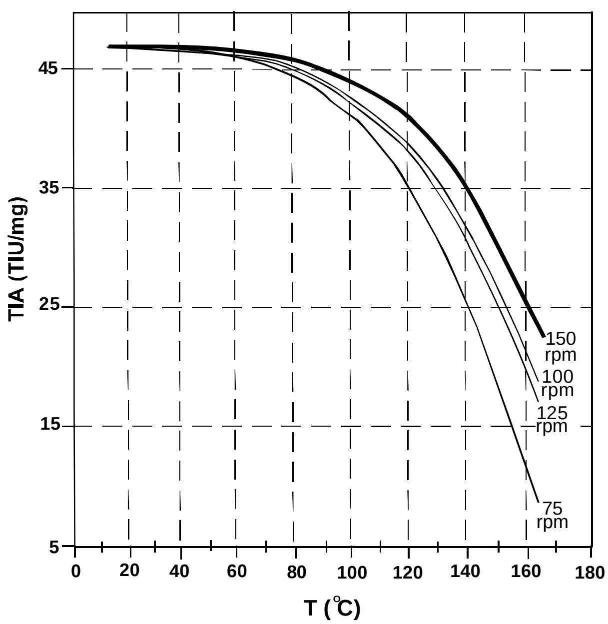

Fig 4. Inactivation of Trypsin Inhibitor (TI) with increasing Soybean Temperature (T) at 20% Moisture content, 150˚C Barrel Temperature and 75,100,125 and 150 rpm Screw speeds.

The velocity V is assumed constant in a given section of the extruder, although it changed from the feed section to the compression section and from compression section to the metering section because of changes in the cross-sectional area. The velocity in a given section is also:

$$V\left(\frac{m}{s}\right) = \frac{Dz(m)}{t(s)}$$

The entire screw channel (164 cm) (Khan 1996) was divided into small steps (Dz) 0.1 cm each. Since Dz was fixed and V already known, the residence time t over a small distance could be calculated.

Beginning at Z1 (distance from feed hopper in down channel direction) equal to zero, product temperature was calculated using the experimental temperature profile. If the temperature was less than 30˚C, Z1 was incremented by 0.1 until the product temperature reached to 30˚C. If Z1 was less than 95 cm (feed section) between 95 and 127 cm (compression section) or between 127 and 164 cm (metering section), corresponding cross sectional area of the screw channel was used to determine the residence time over the 0.1 cm length in the section. The activation energy was assumed constant and a value for K_o was initially used as a guess. The reduction in TIA was calculated step by step incrementing Z1 by 0.1 until Z1 reached 164 cm. Then the residual TIA was compared with experimental TIA for that condition. If the two values were not close, K_o was incremented or decremented depending on whether calculated TIA was larger or smaller than the experimental value.

The K_o and experimental and predicted values of TIA for 20% moisture content and 140 and 150˚C barrel temperatures and 75, 100, 125 and 150 rpm screw speeds are shown in Table 5. A computer program was written for these computations by using Matlab (1992).

The k value for any given condition can then be predicted using the Arrhenius equation and covers the range from 30 to 140 or 150˚C depending on the product exit temperature.

It can be seen from Table 5 that the k_o and k for 140˚C barrel temperature increased with increasing screw speed. Since k is independent of residence time, this data suggested that k increased with increasing shear rate that results in smaller particles and thus instant heating. TI inactivation also depends on particle size (Macrae *et al* 1993). Thus it can be concluded that TIA is also affected by shear rate along with moisture content, temperature and residence time.

The reduction in TIA of soybeans as they pass along the screw channel for 20% moisture content, 140˚C barrel temperature and 75, 100, 125 and 150 rpm screw speeds is shown in Fig 3. As shown TI inactivation started in the feed section at a lower rate and most of the inactivation occurred in the compression and metering sections at higher temperatures.

Reduction in TIA with rising temperature during extrusion cooking has the same trend as it had with channel length (Fig 4). This relationship comes from the fact that soybean temperature increased linearly with distance along the screw channel. Figs. 3 and 4 suggested that TI inactivation started in the feed section. However, most of the inactivation occurred in the compression and metering sections. It can be concluded that the chemical changes in soybean occurs in the compression and metering sections and that any model describing the process only for metering section will not be adequate. Also, it is noteworthy, that the temperature history was not affected so much by the barrel temperature as would be expected.

Conclusion

TIA and NSI of the soybean were mainly affected by screw speed. Lower screw speeds resulted in higher TI inactivation and larger reduction in NSI. Since TI inactivation and reduction in NSI paralleled each other, selection of suitable parameters for extrusion cooking of soybean will depend on whether complete TI inactivation or higher NSI is more desirable. The reaction rate constant for TI inactivation sharply increased in the compression and metering sections, thereby showing that most of the inactivation occurred in these sections. These studies also revealed that TIA is affected by shear rate along with the other variables. The degree of TI inactivation and NSI reduction can be estimated using the given models within the range of the experimental conditions.

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