Designed Experiments for Reducing Antinutritive Agents in Soybean by Microwave Energy

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To increase biological benefit and ease of digestion and decrease antinutrient compounds in legumes, traditional procedures, heating or blanching, are generally used. The use of microwave energy is more efficient than the traditional heating treatment. The characteristic feature of microwave heating is that it ensures homogeneous operation in the whole volume of substance, great penetrating depth, and selective absorption. The effect of microwave energy for reducing enzyme activity in whole soybeans has been investigated. This application has appeared in the literature; however, the optimal physical parameters were not researched. All of the experiments were performed with Labotron 500 vacuumable microwave equipment. The experiments investigated here were carefully designed to find the optimum conditions for the treatment. The paper gives the results and a description of the statistical methods with which the evaluation was more effective and informative even though fewer measurements were required. These laboratory-scale results are easily extendible to factory-scale as well.

Keywords: *Soybean; urease and trypsin inhibitor activity; microwave heating; operational parameters; experimental design*

INTRODUCTION

Increasing the nutritional quality of soybean (digestibility, biological utilization) can be accomplished by several processes, e.g., heat treatment (boiling, steaming), spun soy fiber, extrusion, grinding, fermentation, germination, dry roasting, hydrolysis, and homogenization (Valle, 1981). In addition, with the heat treatment the antinutritive constituents may be decreased as well. Horii and Miyazaki (1973) studied destruction of trypsin inhibitor in soybeans by dry heat treatment at 100 °C for 5 h and by steam-cooking at 120 °C for 1 h. The trypsin inhibitor activity (TIA) reduced to 80% and to 0.4% of the initial value, respectively. Collins and Beaty (1980) held soybeans in boiling water for 0, 1, 2, 3, 6, and 9 min to inactivate trypsin. They found that heating for 3 min reduced activity by 90% and for 9 min by 96%.

Manorama and Sarojini (1982) used five different heat treatments: (i) puffing in sand at 250 °C for 3 min; (ii) roasting in a pan for 3, 6, 9, and 12 min at 85−95 °C; (iii) soaking in water for 20 h and boiling for 10, 20, 30, 40, and 60 min; (iv) boiling in water for 10, 20, 30, 40, and 60 min; and (v) soaking in water for 20 h and steaming under 15 lb/in.^{2} (approximately 1 bar) pressure in a pressure cooker for 5, 10, 15, 20, and 30 min. These treatments were examined for their TIA. Treatment v was found to be the best, followed by treatments iv, i, and ii, for inactivation of TIA.

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The most important thermolabile antinutritive constituents are trypsin and chymotrypsin inhibitors, lectins, and urease. The trypsin and chymotrypsin inhibitors form inactive complexes in the ilium, and they inhibit the proteolytic action of the pancreatic enzyme trypsin. More information about trypsin inhibitors can be found in the literature (Clark et al., 1970; Liener and Kakade, 1980; Liener, 1981; Rackis et al., 1985; Liener, 1994). Several TIA determination methods have appeared in the literature (Smith et al.,1980; Hamerstrand et al., 1981; Charpentier and Lemmel, 1981; Della-Gatta et al., 1988; Raspi et al., 1990; Stauffer, 1990), too. The standard method is cited under Materials and Methods.

Dielectric heating of soybean has been studied by a number of researchers (Pour et al., 1981; Petres et al., 1990; Kovacs et al., 1991). According to Rackis et al. (1986), a minimum energy absorbed of 1200 J/g would be sufficient to inactivate the total amount of urease enzyme and an energy of 1670 J/g was required to destroy over 95% of TIA in case of soybean.

Several papers gave accounts of the efficiency of microwave heating (Pour et al., 1981; Hafez et al., 1983; Rodda et al., 1984; Nelson, 1985; Esaka et al., 1986; Sakla et al., 1988; Szabó and Dörnyei, 1988; Yoshida and Kajimoto, 1988; Szabó, 1989; Snyder et al., 1991; Sakac et al., 1996). The use of microwave energy is more efficient than the traditional heating treatment. The characteristic feature of microwave heat is that it ensures homogeneous operation in the whole volume of substance, great penetrating depth, and selective absorption.

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The purposes of this work were to develop experimental design strategies to decrease antinutritive constituents by microwave heating. The experiments investigated here were carefully designed to find the optimum conditions for the treatment.

MATERIALS AND METHODS

The soybeans (Boly 44) used to investigate the effect of the microwave heating had 10.34% moisture, 19.06% crude fat, TIA = 100.97 TIU/mg in dry matter, and urease activity = 7.52 mg of mg N/(g min) at 25 °C.

Urease activity was determined according to International Standard 5506 (International Standards Organization, 1978). The TIA was measured with the method introduced by Kakade et al. (1974)—in the United States the standard method is AACC Method 22-90, which is essentially the cited Kakade method—with modification by Petres and Kárpáti (1981) (Hungarian Standard MSZ 21175−1988, 1989). The modification was fairly small; Petres and Kárpáti (1981) suggested using defatted soybean for measuring. There is a slight differentiation in the concentration of the trypsin solution used, and they used the least-squares method (LSM) to fit the spectroscopic data instead of graphical procedure.

Accurate adjustment of the resoaking rate was a critical point. The industrial and laboratory practice needs quick effectuation, so we chose a rather simple, but effective, process. A calculated quantity of water was added to a calculated quantity of soybean:

$$m_{\text{water}} = m_{\text{total}} \frac{w\%_{\text{target}} - w\%_{\text{initial}}}{100 - w\%_{\text{initial}}} \quad (1)$$

m_{water} is the quantity of water added to air-dried soybean, m_{total} is the quantity of conditioned soybean ($m_{\text{total}} = m_{\text{water}} + m_{\text{air-dried soybean}}$), $w\%_{\text{initial}}$ is the moisture content of the air-dried soybean, $w\%_{\text{target}}$ is the moisture content described by the experimental design.

The quantity of soybean was 100 g for all samples at a thickness of 10 cm. The condition of soybean was executed during 12 and 24 h. A longer conditioning period was not possible since the seeds germinated.

All experiments was performed with Labotron 500 vacuumable microwave equipment. The effective power of microwaves (ν = 2450 MHz) was 275 W.

RESULTS AND DISCUSSION

Background of Microwave Heating. Microwave heating includes conductive as well as dielectric heating, and the governing equation is the energy equation with a heat generation term:

$$\frac{\partial t}{\partial \tau} = a\nabla^2 t + \frac{\phi_V}{\rho c_p} \quad (2)$$

ϕ_V is the rate of volumetric heat generation at a location and it can be formulated by (Goldblith, 1967)

$$\phi_V = CfE_{\text{eff}}^2(\kappa'' tg\delta) \quad (3)$$

Equation 1 can be solved using the alternating direction implicit (ADI) finite difference method only under simplifying conditions (Datta and Hu, 1992). Komolprasert and Ofoli (1989) suggested dimensional analysis to solve the problem for practice. The practical function g(.) among physical parameters given in Table 1 is

$$\tau = g(t_0, t - t_0, c_p, \lambda, \rho, w, \kappa'', f, P, R, y, x, H, p - p_0) \quad (4)$$

Table 1. Physical Parameters in Equation 3

property	notation	unit	dimension
processing time	τ	s	T
local temperature	t	K	θ
initial temperature	t_0	K	θ
specific heat capacity	c_p	J·kg^{-1}·K^{-1}	L^{2}T$^{-2}\theta^{-1}$
thermal conductivity	λ	W·m^{-1}·K^{-1}	MLT$^{-3}\theta^{-1}$
density	ρ	kg·m^{-3}	ML^{-3}
resoaking rate	w	kg·kg^{-1}	MM^{-1}
relative dielectric loss	κ''		l
frequency of incident radiation	f	Hz	T^{-1}
microwave power	P	W	ML^{2}T^{-3}
radius	R	m	L
distance from the surface	y	m	L
radial distance	x	m	L
typical size of the sample	H	m	L
vacuum	p_0	Pa	MT^{-2}L^{-1}

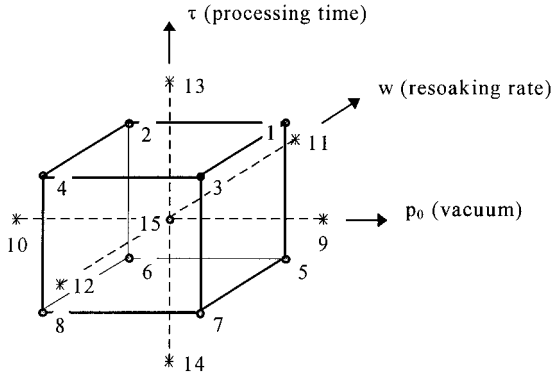

Figure 1. Arrangement of the complete factorial design (points 1−8) and central composite design (points 1−15).

Experimental Designs. According to the previous technological experiments, we decided to study the interactive effect of 3 i.e., p_0 vacuum in the cavity resonator, w, resoaking rate of the soybean, and τ, processing time, of the 15 properties listed in Table 1, because other properties either were constants (e.g., initial temperature, frequency of incident radiation, typical size of the sample) or were not controllable by us (e.g., local temperature, thermal conductivity, relative dielectric loss).

Our aim was to reduce the antinutritive agents, i.e., the urease and trypsin inhibitor (TI) activity in soybean, so the levels of urease and TI activity were chosen as the optimization parameters, which are the functions $f_1(.)$ and $f_2(.)$ of the three factors:

$$y_{\text{urease act.}} = f_1(p_0, w, \tau) \quad (5)$$

$$y_{\text{TIA}} = f_2(p_0, w, \tau) \quad (6)$$

The first series of the experiments was due to reduce only urease activity, because Caskey and Knapp (1944) have pointed out that heat destruction of urease is positively correlated with the decrease in trypsin inhibitor content.

We created a design for fitting a second-order model using *central composite design* with the spreadsheet program Microsoft Excel v 5.0a (1994). The central composite design is constructed as a complete 2^3 factorial layout, 2 × 3 axial or star points along the coordinate axes, and a point at the design center. The arrangement of that design can be seen in Figure 1 (point 1−8 are for complete 2^3 factorial design, points 9−14 are for axial points, and point 15 is the central point). The levels of the factors and the results of the experiments are in Table 2.

Table 2. Results Obtained by Central Composite Design (Urease Activity)

sample	vacuum (p_0) (hPa)	resoaking rate (w) (%)	processing time (τ) (min)	urease act. ($y_{\text{urease act}}$) [mg of N/(g min)]
1	858	35.6	3.7	3.74
2	172	35.6	3.7	7.51
3	858	14.4	3.7	7.00
4	172	14.4	3.7	6.95
5	858	35.6	1.3	7.99
6	172	35.6	1.3	6.67
7	858	14.4	1.3	6.48
8	172	14.4	1.3	6.33
9	1000	25	2.5	5.68
10	30	25	2.5	6.74
11	515	40	2.5	5.62
12	515	10	2.5	7.10
13	515	15	4.2	4.90
14	515	25	0.8	7.30
15	515	25	2.5	6.58

Table 3. Results Obtained by Complete Composite Design (Urease Activity)

sample	vacuum (p_0) (hPa)	resoaking rate (w) (%)	processing time (τ) (min)	urease act. ($y_{\text{urease act.}}$) [mg of N/(g min)]
1	1000	28.3	4.1	0.098
2	716	28.3	4.1	0.520
3	1000	22.7	4.1	0.098
4	716	22.7	4.1	1.039
5	1000	28.3	3.3	0.571
6	716	28.3	3.3	1.901
7	1000	22.7	3.3	1.529
8	716	22.7	3.3	1.604

Table 4. Results Obtained by Gradient Design (Urease Activity)

sample	vacuum (hPa)	resoaking rate (%)	time (min)	urease activity [mg of N/(g min)]	TIA (y_{TIA}) (TIU/mg)
1	887	25.8	3.81	0.409	5.82
2	916	26.0	3.92	0.229	8.88
3	946	26.3	4.04	0.180	6.05
4	975	26.5	4.16	0.204	7.67
5	1004	26.8	4.27	0.230	5.46

According to the measurements eq 5 can be approximated by the following model

$$y_{\text{urease act.}} = 5.80 - 0.00315p_0 + 0.000327p_0w + 0.00225p_0\tau + 0.0226w\tau - 0.000201p_0w\tau \quad (7)$$

calculated by the linear least-squares application (LIN.ILL) of Microsoft Excel v 5.0a (1994).

After that a first-order *complete factorial design* was created around the optimum calculated by eq 7 (see the arrangement in Figure 1 again and the results in Table 3).

The response function can be written statistically by the following first-order function:

$$y_{\text{urease act.}} = 0.920 - 0.346p_0 - 0.148w - 0.481\tau \quad (8)$$

According to eq 8 we have created the *gradient design*, the final step to find the optimum. For the gradient design, the levels of urease and TI activities were measured as well. See the results in Table 4.

As can be seen in Figure 2, the urease activity has the unquestionable minimum in the gradient design, but this is not the case according to the TIA. The TIA, however, is always less than the tolerance limit of 10 TIU/mg, so the treated soybean can be used as a basic material according to the Hungarian food specifications as well.

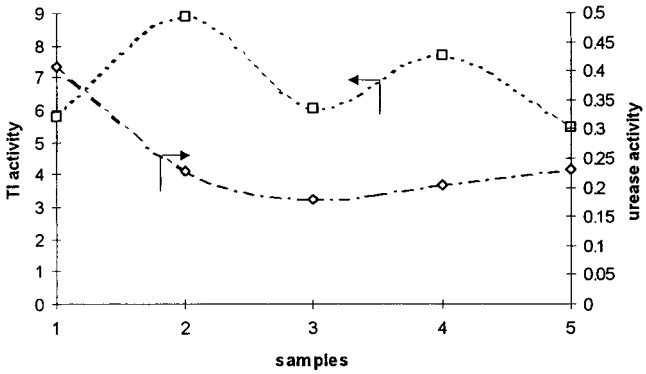

Figure 2. Curves of the gradient design for urease activity (the TI activities were also determined and are shown).

Table 5. Results Obtained by Central Composite Design (TIA)

sample	vacuum (p_0) (hPa)	resoaking rate (w) (%)	processing time (τ) (min)	TIA (y_{TIA}) (TIU/mg)
1	961	28.6	14.0	9.29
2	561	28.6	14.0	8.72
3	961	15.4	14.0	8.48
4	561	15.4	14.0	9.29
5	961	28.6	5.0	4.87
6	561	28.6	5.0	9.53
7	961	15.4	5.0	10.35
8	561	15.4	5.0	7.76
9	1004	22.0	9.5	5.43
10	518	22.0	9.5	8.37
11	761	30.0	9.5	10.88
12	761	14.0	9.5	9.48
13	761	22.0	15.0	10.48
14	761	22.0	4.0	10.21
15	761	22.0	9.5	9.46

Table 6. Results Obtained by Complete Factorial Design

sample	vacuum (p_0) (hPa)	resoaking rate (w) (%)	processing time (τ) (min)	TIA (y_{TIA}) (TIU/mg)
1	1004	35.0	11.0	13.20
2	920	35.0	11.0	9.79
3	1004	25.0	11.0	11.08
4	920	25.0	11.0	10.32
5	1004	35.0	7.0	11.99
6	920	35.0	7.0	7.28
7	1004	25.0	7.0	7.15
8	920	25.0	7.0	6.58

As a continuation of our work, TIA was chosen as the optimization parameter according to eq 6. We created again a design for fitting a second-order model using the *central composite design*. The arrangement of that design can be seen in Figure 1, and the results of the design are in Table 5.

According to the measurements eq 6 can be approximated by the following model:

$$y_{\text{TIA}} = 9.61 - 0.54p_0 - 0.16w + 0.33\tau - 0.73p_0w + 0.23p_0\tau + 0.49w\tau - 1.87p_0^2 + 0.35w^2 + 0.46\tau^2 \quad (9)$$

After that a first-order *complete factorial design* was created (see the arrangement in Figure 1 and the results in Table 6).

The response function can be written statistically by the following first-order function:

$$y_{\text{TIA}} = 9.67 + 1.18p_0 + 0.89w + 1.42\tau \quad (10)$$

According to eq 10 we could create the *gradient design*, the final step to find the optimum. See the results in Table 7.

Table 7. Results Obtained by Gradient Design

sample	vacuum (p_0) (hPa)	resoaking rate (w) (%) designed	resoaking rate (w) (%) realized	processing time (τ) (min)	TIA (y_{TIA}) (TIU/mg)
1	962	30.0	30.3	9.00	5.21
2	951	29.0	30.0	8.37	6.65
3	940	28.0	29.5	7.74	6.85
4	929	27.0	28.6	7.10	4.32
5	918	26.0	24.3	6.47	6.10
6	907	25.0	24.5	5.84	6.48

For the optimization of the process of reducing urease and trypsin inhibitor activity in soybean, we chose the method of steepest ascent via second-order and first-order designs (Box et al., 1978; Mason et al., 1989; Kemény and Deák, 1990; Davies, 1993) rather than the simplex method, because the measurements and the analyses required too much time.

From the central composite design we evaluated the range about the minimum of the second-order function and created the first-order complete factorial design.

Statistically examining the results obtained by the factorial design, we can establish that eq 10 fit very well to the data. The correlation coefficient equals 0.898, and the two-sided F-test at both 90% and 98% confidence levels is not contradictory to the linear hypothesis:

$$F\text{-ratio} = 5.54 < F_{90\%}(3,4) = 6.94 < F_{98\%}(3,4) = 16.7$$

The final step was the gradient design based on the eq 10 oriented from the center of the factorial design. From these results we can give the optimal ranges of the three factors:

$$\text{vacuum (hPa):} \quad 918.0 < p_0 < 940.0$$

$$\text{resoaking rate (\%):} \quad 24.3 < w < 29.5$$

$$\text{processing time (min):} \quad 6.5 < t < 7.7$$

These data are in agreement with our previous results for which the optimization parameter was the urease activity, but the processing time became longer, because inactivation of the trypsin inhibitor requires more absorbed heat energy than does inactivation of the urease:

$$\text{vacuum (hPa):} \quad 916.0 < p_0 < 1004.0$$

$$\text{resoaking rate (\%):} \quad 26.0 < w < 26.8$$

$$\text{processing time (min):} \quad 3.92 < t < 4.27$$

CONCLUSIONS

As is mentioned earlier, several investigations on reducing antinutritive constituents by microwave heating have been presented in the literature. None of them, however, dealt with optimization of the operational parameters. To the best of our knowledge, this paper is the first on that theme.

Our interesting results show that near the optimum the correlation between urease and TI activities vanishes (see Figure 2), so the TIA should be chosen as the optimization parameter for the optimization.

In such a condition, the optimum ranges of the parameters are 918.0−940.0 hPa for vacuum, 24.3−29.5% for resoaking rate, and 6.5−7.7 min for processing time.

The advantages of microwave heating process to decrease TIA in the light of our investigations are the equal heat treatment, the quick process, and the simple equipment. The quality of treated soybean was commercially suitable. A disadvantage may be the resoaking process; however, the required level is easily attainable with the suggested simple moistening procedure using eq 1.

We intend to expand our results to factory circumstances, because the laboratory-scale experimental design methodology is easily extendible to factory scale as well.

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