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EVALUATION OF THE INACTIVATION OF HEAT SENSITIVE ANTINUTRITIVE FACTORS IN FULLFAT SOYBEAN

PROCJENA NEAKTIVNOSTI PROTUHRANJIVIH ČIMBENIKA OSJETLJIVIH NA TOPLINU U PUNOMASNOJ SOJI

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SUMMARY

The regular quality control on the adequacy of heat treatment of fullfat soybeans requires the application of rapid chemical methods. In the present work the trypsin inhibitor activity test and the urease test were applied on fullfat soya samples that were cooked in a pressured steam (toasted) or extruded at different temperatures and speed rates. In the case of toasting both of the results of the laboratory examinations proved that the heating was adequate, while in the case of the extruded samples the two tests gave different results. In the case of certain temperature and time combinations the more rapid and less accurate urease test claimed that the heat treatment reached the aim, while the results of the trypsin inhibitor activity test showed that the level of the inhibitors was still high and the fullfat soya was underheated.

Key words: fullfat soybeans, dry extrusion, toasting, trypsin inhibitor activity, urease test

INTRODUCTION

Soybeans are the primary vegetable protein source in animal feed. Nowadays the use of soya without oil extraction, that is fullfat soybeans, has a great importance. Apart from its high protein content of unique biological value, its fat content contributes to the energy required for protein synthesis. It is suitable to formulate high-energy diets, thereby part of the cereals can be replaced. Fullfat soya contains antinutritive factors that reduce the digestibility and utilization of amino acids in nonruminants and immature ruminants. The effect of proteinaceous antinutritive compounds can be eliminated by heat treatments (*Monari,* 1996). The objectives of heating processes for fullfat soybeans are to maintain an optimum balance between degradation of antinutritive factors on the one hand and maintenance of bioavailability of essential amino acids on the other (*Qin et al.,* 1994; *Kaankuka et al.,* 1996). The best way to evaluate the adequacy of processing and the quality of the product is conducting biological tests. However, the cost, time requirement and complexity of biological tests mean that reliable laboratory procedures, of which trypsin inhibitor activity (TIA) determination perhaps the

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most appropriate still have a valuable role to play in quality control procedures (*Monary,* 1996). The urease test is an indirect method, based on the inactivation of urease by heat. Due to its rapidness, low skill and minimum amount of laboratory equipment requirements it is suitable for quality control of heating in the plant.

The current study was undertaken to investigate the influence of two sorts of heat treatments on the TIA and urease activity of fullfat soybeans and comparison of the results of the two tests are discussed.

MATERIALS AND METHODS

Pressurized steam cooking (toasting)

Fullfat soybeans were processed at the Bóly Stock Company (Bóly-Állomáspuszta, Hungary). Soybeans were cracked into 9-12 pieces then boiled in a KAHL HR-1600 hydrothermic reactor (toaster). In this stirrer autoclave soya was heated with pressurized steam at 120 °C for 30 minutes. After steam processing the product called "hydrothermic soya" were air-dried and cooled. In the manufacture of the other type of product called "hydrothermic soya grain" an additional step followed that was grinding in a hammer mill and both products were stored at -20 °C prior to laboratory analyses.

Extrusion

The extrusion experiment was carried out at the Budapest University of Technology and Economics, Department of Biochemistry and Food Technology. Fullfat soybeans (*Borostyán* sp.) were ground in a hammer grinder and the particle size distribution was determined. Extrusion was carried out using a Do-Corder DC 2001 type laboratory-scale Brabender machine which has been described in detail elsewhere (*Vargáné et al.* 2004). Extrusion trials with the full cross-classification of the applied nominal temperature and screw speed levels (Table 1) were repeated three times. From the two reported zone temperatures (T_1, T_2), one value was calculated (T) to characterize the effect of temperature. Minimum residence time was determined by introducing a small amount of dye into the feeding port and measuring the time required for the first colored extrudate to exit the die. Prior to sampling, the machine was allowed to equilibrate to the desired temperature, then the sample was collected and after cooling it was homogenized and sealed in polyethylene bags and stored at -20 °C until chemical analyses began. Control samples were taken from each batch and treated in the same way as extruded samples.

Table 1. Extrusion of fullfat soybean - Nominal temperature and screw speed levels

Tablica 1. Ekstrudiranje punomasne soje - Nominalna temperatura i razine brzine okreta

Temperature levels	T_1 (°C) 1. zone (barrel)	T_2 (°C) 2. zone (barrel)	Screw speed levels	Screw speed (rpm)
1	100	100	1	50
2	140	140	2	90
3	180	180	3	130
4	220	220	4	170

Chemical analyses

The trypsin inhibitor activity (TIA) of samples was determined according to the EN ISO 14902 standard. The method based on the measurement of activity decrease of trypsin in a model solution due to the inhibitors that were dissolved from the sample. An artificial substrate benzoyl-L-arginine-*p*-nitroanilide (L-BAPA) was added to the solution containing trypsin and the sample extract, and the quantity of the released *p*-nitroaniline was measured spectrometrically. The trypsin inhibitor content was expressed as mg trypsin inhibited per g of the sample. The acceptable level of TIA depends on the protein content of the material. The European Federation of Feed Manufacturers recommended the next upper TIA limits for fullfat soybeans (*Monary*, 1996):

% of protein in the feed	Trypsin inhibitor activity (mg/g)
50	5
40	4
30	3

The urease test was conducted as follows: 50 cm^3 phosphate buffer (0.07 M, pH = 7.5) was added to 1.000 g soybean grain (first solution), and 50 cm^3 buffered urea solution was added to 1.000 g of the same sample (second solution). The buffered urea solution consisted of 30 g urea in 1000 cm^3 phosphate buffer (0.07M, pH = 7.5). The two solutions were incubated at 35 °C for 30 minutes after stirring. In the presence of significant urease activity the pH of the second solution increased due to the release of ammonia from urea. After incubation the pH of the solutions had to be determined rapidly and the degree of heating was estimated basing on the pH difference between the first and the second solution.

Soybean product	pH difference
Raw or not heated	1.7 - 2.5
Under cooked	0.2 - 1.7
Well cooked	0 - 0.2

RESULTS AND DISCUSSION

The influence of pressurized steam cooking on the trypsin inhibitor activity and urease activity of fullfat soybean

The results of the heat treatment evaluating analyses can be seen in Table 2. The data clearly show that the activity of the trypsin inhibitors was reduced successfully below the required level for both of the products and the adequacy of the heat treatments was also verified with the results of the urease test. However, the pH difference was slightly higher in the case of hydrothermic soya product than that of hydrothermic soya grain. In any case, the differences in the size of the particles of the products and thus higher surface area of grain material was not important in view of toasting because grinding was carried out after steam cooking.

The theoretical and the measured properties of extrusion can be seen in Table 3. The adjusted screw speed levels and residence time values could substitute each other because the temperature did not exert a significant effect on the residence time due to minor changes in the viscosity of the material.

Table 2. The trypsin inhibitor activity (TIA) and urease activity of toasted fullfat soybean products (n=3)
Tablica 2. Aktivnost inhibitora tripsina (TIA) i aktivnost ureaze tostiranih proizvoda punomasne soje (n=3)

Chemical examination	Fullfat soya samples		
	Control	Hydrothermic soya grain	Hydrothermic soya
TIA (mg/g)	17.2 ± 0.5	1.1 ± 0.2	1.2 ± 0.3
Urease test (ΔpH)	1.5 ± 0.1	0.05 ± 0.02	0.14 ± 0.02

The influence of dry extrusion on the trypsin inhibitor activity and urease activity of fullfat soybean

Table 3. Nominal and measured properties of extrusion of fullfat soya
Tablica 3. Nominalne i izmjerene karakteristike ekstrudiranja punomasne soje

Levels	Nominal temperature (°C)	Measured temperature (°C) average ± s.d. (n=12)	Levels	Screw speed (s^{-1})	Residence time (s) average ± s.d. (n=12)	Throughput (kg/h) average ± s.d. (n=12)
1	100	101 ± 4	1	50	29 ± 0.2	1.6 ± 0.4
2	140	140 ± 3	2	90	17 ± 0.2	2.8 ± 0.8
3	180	180 ± 3	3	130	12 ± 0.8	4.1 ± 1.1
4	220	220 ± 3	4	170	10 ± 1.4	4.8 ± 1.4

Table 4. The trypsin inhibitor activity (TIA) and urease activity of fullfat soybean products extruded at different temperatures and different screw speed (residence time, n=3)

Tablica 4. Aktivnost inhibitora tripsina (TIA) i aktivnost ureaze u proizvodima punomasne soje ekstrudirane na različitim temperaturama i na različitim brzinama okreta (prisutno vrijeme, n=3)

Temperature (°C)	Screw speed (s^{-1})	TIA (mg/g)	Urease test (ΔpH)
100	50	17.0 ± 1.0	1.47 ± 0.10
100	90	16.6 ± 0.3	1.47 ± 0.06
100	130	16.8 ± 0.3	1.49 ± 0.08
100	170	16.6 ± 0.3	1.50 ± 0.09
140	50	16.4 ± 0.7	1.49 ± 0.08
140	90	16.7 ± 0.5	1.43 ± 0.05
140	130	16.4 ± 0.3	1.47 ± 0.07
140	170	16.1 ± 0.9	1.47 ± 0.09
180	50	11.4 ± 0.6	0.10 ± 0.08
180	90	13.4 ± 3.3	0.95 ± 0.08
180	130	15.8 ± 0.8	1.31 ± 0.09
180	170	15.5 ± 0.7	1.44 ± 0.06
220	50	5.0 ± 0.4	0.03 ± 0.01
220	90	9.2 ± 0.5	0.08 ± 0.05
220	130	12.4 ± 1.0	0.53 ± 0.32
220	170	14.0 ± 0.6	1.18 ± 0.16
Control		17,2 ± 0.5	1.53 ± 0.10

In samples extruded at low temperatures (100 °C and 140 °C) the level of TIA remained almost as high as in the controls and the effect of the lengthening of residence time was also negligible (Table 4).

Based on the result of the urease test of fullfat soybeans extruded at 180 °C for 29 s (50 s^{-1}) it can be claimed that the extent of the heat treatment is adequate, while the result of the TIA measurement clearly shows, that the activity of trypsin inhibitors barely decreased. Similar tendency can be seen in the case of samples extruded at 220 °C for 17 and 29 s (90 and 50 s^{-1}, respectively). In the latter case the TIA value almost dropped to the required level that is 4 mg inhibited trypsin/g sample in fullfat soybean samples with the protein content of 37%.

The authors are aware of the fact that the exact chemical characterization of a protein source in view of the adequacy of heat treatment requires more additional laboratory examinations. The aim of this work was solely to draw the attention to the urease test that is often used in plants as a quality control test may not in all the cases give reliable results compared to the more accurate TIA determination.

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SAŽETAK

Redovita kontrola kakvoće primjerenosti toplinskog tretiranja punomasne soje zahtijeva primjenu brzih kemijskih metoda. U ovom radu primijenjeni su test aktivnosti inhibitora tripsina i test ureaze na uzorcima punomasne soje kuhane u pari pod pritiskom (tostirane) ili ekstrudirane na različitim temperaturama i brzinama. U slučaju tostiranja oba rezultata laboratorijskih ispitivanja su dokazala da je grijanje bilo primjereno, dok su u slučaju ekstrudiranja uzoraka oba testa dala različite rezultate. U slučaju određene temperature i vremenske kombinacije brzi i manje točan test ureaze potvrdio je da je toplinsko tretiranje postiglo svrhu dok su rezultati testa aktivnosti inhibitora tripsina pokazali da je razina inhibitora još uvijek visoka, a punomasna soja nedovoljno zagrijana.

Ključne riječi: punomasna soja, suho ekstrudiranje, tostiranje, aktivnost inhibitora tripsina, test ureaze