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THE EFFECT OF AUTOCLAVING ON SOLUBLE PROTEIN COMPOSITION AND TRYPSIN INHIBITOR ACTIVITY OF CRACKED SOYBEANS

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The effects of autoclaving conditions (heating for 5, 10 and 15 minutes at 0.5 bars over pressure) and oil-extracting temperatures (40°C, 60°C) on protein content, composition, and inhibitor activity of cracked soybeans were investigated. The results obtained indicated that oil-extracting method and heat treatment had significant influence on soluble protein content and composition. Raw soybean samples defatted at lower temperature had better solubility (535.42±2.10 mg/g) than those obtained by the Soxhlet procedure (345.53±2.80). The same results were obtained for nitrogen solubility index. Autoclaving combined with two oil-extraction methods decreased protein solubility to 180.32±1.50 - 245.41±1.41 mg/g, while the dominant component of heat treated flours was 11S fraction. High content of glycinin fraction (44.59-41.10%) implies the possible use of treated samples in food industry. Residual activity of treated samples was 43.40-84.26%. Kunitz inhibitor (KTI) was responsible for residual inhibitor activity.

KEYWORDS: Soy protein; soy flour; autoclaving; oil extraction; trypsin inhibitor

INTRODUCTION

Soybeans provide a source of low-cost protein which has excellent qualities in terms of nutritional and functional properties (1). Soybeans are also a rich source of biologically active components. Biologically active components may have beneficial but also adverse effect in diets. The possible beneficial effects of soy containing diets include soy-induced lowering of cholesterol, anticarcenogenic effect of Bowman-Birk inhibitors (BBI), protective effects against obesity, diabetes, irritants of the digestive tract, bone and kidney di-

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seases, whereas the latter include poor digestability and allergy to soy proteins (2). Thus, their content and their activity must be balanced in soy-based products.

Soy flour is usually manufactured as full-fat and defatted, enzyme-active or toasted. Defatted flour is prepared by different extracting methods, mostly with hexane. This method reduces oil content from 20 to below 1% (3). Residual lipid components are lipo-proteins, lipo-carbohydrates and lecithin gums (4). To reduce trypsin inhibitor (TI) and lectins activity, different modes of thermal treatments are in use. Beside their inactivation, thermal treatmetns at temperatures over 70°C causes the change of protein solubility, their content and composition. Knowing the effects of processing treatments (oil-extracting, heating and drying methods) of soy protein flours is important in the industry in evaluating other properties, in order to screen them for potential applications. The aim of this work was to investigate the effect of autoclaving at different temperatures during oil-extracting of soy flour proteins and trypsin-inhibitor activity.

EXPERIMENTAL

Soybean seeds of "Hodgson" var. were cracked into six to eight pieces and dehulled. 100-g portions of cracked soybeans (moisture 6.70%; protein 39.05%, oil 20.10%) were treated in laboratory autoclave at 96°C (0.5 bars overpressure) for 5, 10 and 15 minutes, in one layer. After this treatment moisture content was 7.88, 7.97 and 8.02%, respectively. These samples were ground to flour consistency (80% of flour passed through 80-mesh screen). 10-g portions of each thermally treated sample, as well as raw soy flour, were defatted according to the Soxhlet method at 60˚C and immersed procedures at 40°C with *n*-hexane. Immersed procedure included stirring of *n*-hexane-flour dispersion (on heating magnetic stirrer; flour: *n*-hexane, ratio w/V, 1:20) for 90 minutes at 40°C. Soy flour was separated on glass filter at reduced pressure, and washed three times with 10 ml of *n*-hexane. According to the Soxhlet procedure soy flour was defatted during 6 hours at 60°C. Residual hexane was evaporated in fume hood at room temperature overnight. As a control, we prepared soy flour from initial (non-treated) cracked beans defatted according to the same procedure. All samples were analyzed in triplicate.

Soluble proteins were extracted with Tris-HCl pH 8.0 and determined according to method of Lowry et al. (5). Nitrogen solubility index (NSI) was determined according to standard A.O.C.S. method (6), while total protein content was determined according to the Kjeldahl method (7).

Trypsin-inhibitor activity was assayed and quantitated as described by Liu and Markakis (8). The nature of residual activity and the change of soluble soy flour protein composition were detected by PA-gel electrophoresis according to Davis and Ornstein (9) and densitometric analysis of destained gel. Kunitz and Bowman-Birk inhibitors (Sigma, USA) were used as standards. Electrophoresis was performed on 5% stacking and 7% resolving gel. Electrophoresis unit LKB-2001-100 was used in conjunction with power supplies LKB-Macrodrive 5 and LKB-Multitemp as a cooling unit (LKB, Sweden). Destaining unit (LKB, Sweden). Destained gel was scanned on a PC-scanner (12000 SP, Mustek, Germany). Densitometric analysis of the scanned gels was performed using SigmaGel for Windows software (Jandal Sci. Co, USA).

RESULTS AND DISCUSSION

The effect of treatments used for soy flour preparation on soluble protein content is shown Tables 1, 2 and 3. Soluble protein content of raw soy flour defatted according to the procedures used was significantly different ($p<0.05$) (Table 1). Soluble protein content and NSI-values of raw soy flour defatted at lower temperature were higher than those obtained by the Soxhlet procedure. The average soluble protein content of the former samples was by 35.46% higher than those obtained by the Soxhlet procedure. Similar results were obtained for NSI values of these samples. The NSI value of raw flour extracted at 60°C was 70.00±0.05, while the NSI value of flour extracted by immersed procedure was 86.42 ±0.04 (Table 1). This could be due to the longer treatment (6 hours) at higher extracting temperature (60°C). Thus, our results indicated that extracting method must be considered as a factor of soy protein product processing.

Table 1. Soluble protein content of raw and defatted samples*

	Raw soy bean				
	**total protein (%)	sol. prot. mg/g***	NSI	oil (%)	moisture (%)
	39.05±0.03	327.52±2.71	89.20±0.03	20.10±0.01	6.7
Lipid extraction	**Thermally untreated sample**				
Sohxlet procedure	53.13±0.20	345.53±2.80 a	70.0±0.05	1.42±0.02	7.62
Immersion procedure	47.43±0.22	535.42±2.10 h	86.42±0.04	1.80±0.03	8.67

*mean of three measurements expressed on dry matter

**content of total nitrogen × 6.25

***soluble protein content of Tris-HCl extrats expressed as mg sol. prot. per g of dray sample

NSI - nitrogen solubility index

Means with different letters are significantly different at $p< 0.05$

Thermal treatment combined with oil-extracting treatment reduces protein solubility. Very strong positive correlation (r=0.98, r=0.99) between the NSI values and soluble protein content was found. Depending on the duration of autoclaving and oil extracting method soluble protein content decreased from 327.52 ± 2.71 to 197.35±1.82 – 192.36±2.42 mg/g (samples extracted by the Soxhlet method) and 245.41±1.41 – 180.32±1.50 mg/g (samples extracted at 40˚C) (Table 3). Autoclaving had strongest influence on soluble protein content (Table 2). After 5 min of autoclaving the soluble protein contents were reduced to 250.30±1.71, while the NSI values decreased to 40.70 ± 0.03 of the initial values**.**

According to our results, oil-extracting methods caused a further, but minor, reduction of soluble protein content in Tris-HCl extracts, except for the case of 5 min-treated samples extracted by Soxhlet procedure (Table 3). On the other hand, the differences be-

tween samples autoclaved for 10 and 15 minutes and extracted at higher temperatures was not significant ($p<0.05$). In the case of 10 min-autoclaved samples extracted at lower temperature, similar effect was observed.

Longer treatment caused a minor decrease of soluble protein content; after 15 minutes soluble protein content was 198.0 ± 1.01 mg/g.

Table 2. Soluble protein content of autoclaved samples*

Thermally treted samples				
treatment (min)	sol. prot. mg/g**	NSI	moisture (%)	total protein(%)
5	250.30±1.71	40.70±0.03	7.88	39.37±0.03
10	200.0±2.01	39.70±0.02	7.97	39.07±0.02
15	198.0±1.01	37.39±0.02	8.02	38.99±0.03

* mean of three measuremements expressed on dry matter
** soluble protein content of Tris-HCl extrats expressed as mg sol. prot. per g of dray sample
NSI - nitrogen solubility index

Table 3. Total and soluble protein content of autoclaved samples extracted according to two different procedures*

Lipid extraction	treatment (min)	**total protein (%)	sol. prot. mg/g***	NSI	moisture (%)	oil (%)
Soxhlet procedure	5	51.87±0.02	197,35±0.82 b,i	37.58±0.05	6.1	1.40±0.01
	10	50.25±0.04	193,00±1.13 c,k,j	37.5±0.03	5.6	1.41±0.01
	15	44.37±0.01	192,36±2.42 d,k	30.71±0.05	5.3	1.40±0.02
Immersion procedure	5	45.31±0.07	245,41±1.41 e	34.51±0.01	9.23	1.81±0.04
	10	42.94±0.06	194,57±2.20 f,i,j	34.49±0.03	11.5	1.80±0.03
	15	41,38±0,10	180,32±1.50 g	30.39±0.02	11.51	1.79±0.04

* mean of three measurements expressed on dry matter
** content of total nitrogen × 6,25
*** soluble protein content of Tris-HCl extrats expressed as mg sol. prot. per g of dray sample
NSI - nitrogen solubility index
Means with different letters are significantly different at $p< 0.05$

As shown in Table 3, second group of samples (extracted at lower temperatures) had lower total protein content. Due to the dissipation of non-protein nitrogen in low-temperature oil-extracting method, these samples had less total nitrogen content, and consequently low total protein content. Furthermore, the NSI values of these samples were lower than of the samples extracted according to the Soxhlet procedure. In contrast to this, their content in Tris-HCl extracts was higher. This could be a result of the total protein content decrease, as well as the different degree of protein alteration caused by the treatments. Namely, soybean proteins are liable to aggregation at neutral pH used for nitrogen solubility index determination. Consequently, it seems that thermal treatment combined with low-temperature oil-extracting method (40°C) produced lower denaturation degree of proteins that are insoluble at a neutral pH. On the other hand, at a higher pH (in Tris-HCl extracts) these fractions become soluble.

In spite of the registered decrease, treated samples had relatively good solubility. According to the obtained NSI (all values were in the range of 20-60, Table 3), the autoclaved flour could be characterized as a modestly toasted flour.

Soluble protein composition of Tris-extract of treated samples were determined by PAGE (Figure 1) and densitometric analysis of destained gels (Table 4).

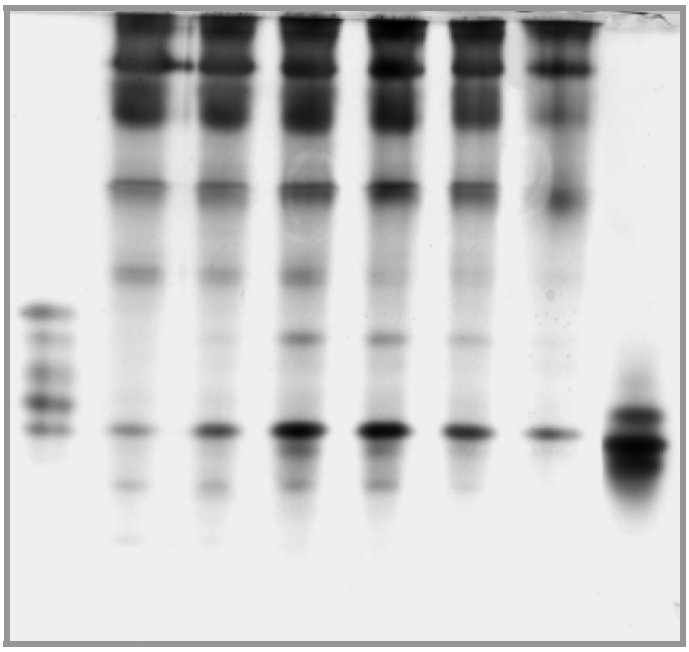

Fig. 1. PAGE-analysis of soluble soybean proteins. Samples autoclaved for (2)-5 minutes, (3)-10 minutes, (4)-15 minutes and extracted by Soxhlet method. Samples autoclaved for (5)-5 minutes, (6)-10 minutes, (7)-15 minutes and extracted by immersed procedure. Trypsin inhibitor standard: KTI-(1), BBI-(8)

Unexpectedly, the soluble protein composition of treated samples was quite different (Table 4). Treated samples extracted at lower temperature were characterized with low

content of the 7S fraction (20.46-29.22%). According to our results, the most susceptible was γ-conglycinin; the content of this protein decreased to 3.42-8.91%. In opposite, due to the high content of γ-conglycinin (20.04-29.04%), the content of 7S fraction of the autoclaved samples extracted at higher temperatures was 39.02-48.89 %. The differences in the γ-conglycinin content could be explained by the different type of the alterations that occurred during autoclaving and oil-extracting methods. It is possible that autoclaving combined with oil-extracting method at 60°C yielded soluble degradation products of other proteins (such as 15S fraction and β-conglycinin) with the same relative mobility as γ-conglycinin.

According to our results, glycinin exhibited high thermal stability. The content of this protein was 41.10-44.59%. Due to the decrease of 7S fraction, glycinin become the dominant soluble protein of treated samples (except for the sample treated for 15 min and extracted at 60°C). Higher thermal stability of glycinin was a result of compact quaternary structure stabilized via disulfide, electrostatic and hydrophobic interactions (10). Glycinin fraction was detected as monomer and dimmer form. Monomer form of treated samples was dominant (24.35-26.96%) while the dimmer form represented 19.13-15.53% of soluble proteins.

Table 4. Content of souble flour protein composition (%)*

Fraction		**Lipid extraction**						**Non-treated fluor**	
		Soxhlet-method					**immersion procedure**		
		autoclaved samples (min.)						Soxhlet	immerzion
		5	10	15	5	10	15		
7S-fraction		39.02	40.91	48.89	29.22	28.03	20.46	47.38	45.76
11S-fraction		42.06	44.59	41.36	44.46	41.35	41.1	38.12	43.58
β-conglycinin		16.33	20.87	19.85	20.31	18.63	17.04	23.85	24.57
γ-conglycinin		22.69	20.04	29.04	8.91	9.4	3.42	23.53	21.19
glycinin	dimer	15.53	19.13	17.01	17.5	15.6	15.59	16.03	18.15
	monomer	26.53	25.46	24.35	26.96	25.75	25.51	22.09	25.43
KTI		6.39	4.33	4.12	10.09	11.93	11.98	5.3	4.76
BBI		/	/	/	/	/	/	0.35	0.62

* means of triplicate measurements in % , expressed on dry metter
KTI - Kunitz inhibitor ; BBI - Bowman - Birk inhibitors
7S - fraction is β - conglycinin + γ - conglycinin
11S - fraction (glycinin)

The possible use of heat-treated flour as functional food ingredient is determined by the dominant protein ratio. Treated samples had higher glycinin (41.10-44.59%) than β-conglycinin (16.33-20.87%) content. Glycinin fraction formed stronger gels due to the higher water holding capacity (WHC). Also, glycinin has higher content of hydrophilic groups (11) and formed more stable emulsions. Thus, it is possible that treated samples could be applicable as gelling and emulsifying ingredients.

Thermal treatment based on autoclaving at lower temperature (96°C) for 5 to 15 min had no satisfactory effect on the reduction of TI activity. Depending on treatment conditions, the average residual activity of treated samples was 43.40-84.30% (Table 5). Commercially heated meals retain up to 20% of the residual TI activity with no adverse effect in nutrition (12, 13). It is known that the level of residual TI-activity of soy flour is affected by several factors such as treatment conditions (treatment mode, temperature level, heating time) (13), the content of moisture (4, 14), pH conditions and the presence of reducing agents (15, 16). It is well known that dry heat has no significant reducing effect on TI-activity, even at 121°C. In the opposite, treatments with steam at over pressure (0.5-2.0 bar) (17-19) and steam jet cooking (20, 21) were more effective. Further, microwave roasting at a frequency of 2450 MHz for only 2.0 minutes reduced inhibitor-activity to 13.33% (22).

Treated flour had significantly different ($p<0.05$) residual trypsin inhibitor activity (Table 5). The TI-activity of autoclaved samples extracted at higher temperature (60°C) was too high (80.51-75.52%), while the TI activity of the samples extracted at lower temperature was 84.30±0.08 – 43.40±0.12. As we mentioned, these samples were treated under the same conditions except for the way of oil-extracting method. Further, as shown in Table 3 the moisture content of these samples was different. Fulmer (4) showed that the decrease in TI activity correlated with cooking time; at 0% moisture TI was quite resistant to denaturation, but at 12% TI activity decreased rapidly. It is possible that the high content of moisture (11.51%) has a synergistic effect on TI activity reduction.

Table 5. Trypsin inhibitor activity of raw and treated soy flour (%)

Lipid extraction	Treatment (min)			Raw soybean
	5	10	15	
Soxhlet method	80.51±0.20	76.67±0.15	75.52±0.20	100
Immersion method	84.30±0.08	71.08±0.05	43.40±0.12	100

Our results indicate that KTI is responsible for residual activity of treated flour. The same results were reported for live steam treatment of cracked soybean by Vucelic-Radovic et al. (23). In the opposite, both types of inhibitors were responsible for residual activity of microwave-treated soybeans (22). According to densitometric analysis KTI represents 4.12-6.39 % and 10.09-11.98% of soluble proteins of treated samples (Table 4). Relatively high content of these inhibitors suggests that the part of them exist as soluble, but inactive form. On the other hand, the increase of KTI content in extracts of samples defatted at 40°C is a result of rapid decrease of the 7S fraction content.

CONCLUSION

Oil-extracting method of cracked raw soybean has a significant influence on soluble protein content. Autoclaving process followed by different oil-extracting methods caused significant decrease of soluble protein content and NSI values. Very strong positive correlation (r=0.98, r=0.99) between the NSI values and soluble protein content was found. Major storage proteins showed different stability during thermal treatment followed by oil-extracting method at a lower temperature (40°C). The change of soluble protein composition of these samples was induced by a decrease of the 7S protein fraction (especially of γ-conglycinin). Thermal treatment used in these experiments had no satisfactory effect on TI activity reduction.

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УТИЦАЈ ТРЕТМАНА ЛОМЉЕНОГ СОЈИНОГ ЗРНА У АУТОКЛАВУ НА САДРЖАЈ И САСТАВ РАСТВОРЉИВИХ ПРОТЕИНА И ТРИПСИН ИНХИБИТОРСКУ АКТИВНОСТ

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Испитиван је утицај термичке обраде ломљеног зрна у аутоклаву при надпритиску од 0.5 бара и температури од 96°C у току 5, 10 и 15 минута, као и ефекат екстракције уља при температурама од 40 и 60°C на садржај и састав растворљивих протеина и трипсин инхбиторску активност. Добијени резултати указују да ови третмани имају значајан утицај на садржај и састав растворљивих протеина. Нетретирани узорци одмашћени при нижим температурама карактеришу се већим садр-

жајем растворљивих протеина од узорака одмашћених при 60°C. Третман у аутоклаву комбинован са два различита поступка екстракције уља редукује садржај протеина растворљивих у пуферу pH 8.0 до 180.32±1.50 -245.41±1.41 mg/g. Резервни протеини соје испољили су различиту стабилност током обраде. Најосетљивијим су се показале компоненте 7S фракције. Резидуална инхибиторска активност третираних узорака је 43.40-84.26%, при чему је КТИ окарактерисан као носилац те активности.

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