Amino Acid Composition, Urease Activity and Trypsin Inhibitor Activity after Toasting of Soybean in Thick and Thin Layer

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Summary

The objective of this study was to determine amino acid content, urease activity and trypsin inhibitor activity in soybean grain for polygastric animals' feed after toasting with the aim to introduce thick layer in toasting technology. Hence, soybean was toasted both in thick and thin layer at 130 °C during 10 minutes. In order to properly monitor the technological process of soybean thermal processing, it was necessary to study crude protein content, urease activity, trypsin inhibitor activity and amino acid composition of soybean in natural and toasted samples. Results demonstrate that protein content in soybean toasted in thick and thin layer was found to be slightly increased while urease activity was reduced in relation to non-treated sample. Study also established a significant reduction of trypsin inhibitor activity after toasting, at higher extent in thin layer toasting. Amino acid content of soybean was slightly increased in relation to natural sample, as well as difference between amino acid content in samples toasted in thick and thin layers.

Key words

soybean, toasting, thick layer, thin layer

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Received: November 3, 2008 | Accepted: February 24, 2009

ACKNOWLEDGEMENTS
This research was supported by the Ministry of Science, Education and Sports of Republic of Croatia through project 178-1780703-0698.

Introduction

For many years, soy (*Glycine max* L.) is considered as one of five most significant plants in the world, primarily because of its exceptionally high nutritional values. The nutritional value of soybean lies in the fact that it is rich in a good balance of proteins, amino acids and essential lipids, which confer a good nutritional value (Haddad and Allaf, 2007). Soybean grains contain highly valuable proteins (39-41%) and oil (18-21%). As the main source of vegetable proteins on the world market, soybean is a standard against which other protein feeds are compared. Soybean amino acid content is similar to that of protein concentrates of animal origin and contains about ten essential amino acids which are significant for human and animal nutrition.

Most legume grains have excellent nutritional value in terms of protein, calories, vitamins and minerals. It is, however, important to note that, despite their promising nutritional significance, legumes have been found to contain some inherent antinutritional factors, which limit their nutritive value by exerting certain deleterious effects. Namely, these ingredients block digestion enzymes, causing metabolic disorders and diseases or impairing growth in animals fed with crude soy and may eventually lead to the animal's death (Umoren et al., 2005).

Consequently, soybean grain contains antinutritional factors, such as trypsin inhibitors, which are a serious obstacle to the use of untreated soybeans (Haddad and Allaf, 2007). Trypsin inhibitors account for 30–50% of the growth inhibition effect and almost all of the hypertrophic response of the pancreas of animals fed with raw soybean meal (Bau et al., 2001). The activity towards trypsin disappears with acid and alkali denaturation, as well as with heating (Liener, 1980). It can be said that reduction of trypsin inhibitor activity by 90-95% in relation to crude soybean indicates that thermal processing is satisfactory. Furthermore, indicator of successful soybean thermal processing is urease, which is thermally unstable enzyme, so its heat-induced activity decline indicates that the processing is efficient in relation to crude soybean (Sanderson et al., 1982). The advantage of soybeans compared to other grain legumes is that their antinutritional factors are heat sensitive (Bau et al., 2001).

During feed manufacturing, different processes can alter the physico-chemical properties of feedstuffs, thereby affecting their digestive behaviour (Goelema et al., 1999). The use of moderate heat treatment causes partial denaturation of proteins and generally has a beneficial effect on nutritional value; by facilitating enzyme access it makes proteins more digestible (Haddad and Allaf, 2007). Hence, thermal processing has a positive effect on nutritional value of soybean, but its usage is primarily aimed at inactivating antinutritional substances in crude soybean, which should not cause any damages on proteins (Monari, 1990).

In recent years, with the outbreak of BSE (bovine spongiform encephalopathy or mad cow disease), there is growing demand for using soybean in animal feed and very often soybean is thermally processed by toasting. Toasting is a process in which soybean is exposed to dry heating reducing the initial moisture content with soybean grain temperature in the range from 110-165 °C. According to investigation conducted by Krička et al. (2003) on soybean toasting at temperatures 125 °C, 130 °C and 135 °C for 10- and 15-minute periods, it was determined that soybean has to be toasted at minimum temperature of 125 °C for duration of 15 minutes for monogastric animals and at 130 °C for 10 minutes for polygastric animals, as to keep the level of trypsin inhibitor and urease in compliance with the recommendation of the European Feed Manufacturer's Federation, FEFAC.

The objective of this study was to determine the amino acid content, urease activity and trypsin inhibitor activity in soybean grain for polygastric animals' feed after thermoplastic process of toasting. The aim was to introduce thick layer in the toasting technology in order to increase toaster capacity, but providing that the quality of toasted soybean remains unchanged.

Material and methods

Material

Regionally cultivated soybean, cultivar Iva, was planted and harvested in 2006 by the Faculty of Agriculture, University of Zagreb. The cultivar Iva belongs to the "0" maturity group, namely has an increased protein concentration.

Toasting device

The toaster (Seting-inženjering d.o.o., Delnice) used for these studies consisted of a casing with the door and with inserted perforated board of dimension 800 x 800 mm. The toaster was filled through the horizontal door. The deep layer could be maximally 100 mm high. The soybean which was filled in the toaster had to be clean and equally ripe. The heated air was drawn out from the toaster by axial cable of the fan. In the toaster itself, three PT 1000 probes were built in for measuring air temperature at the entrance and exit from the toaster. The temperatures of soybean in the air current were also measured. Air temperature regulation was automatic or manual. The maximum temperature on the entry thermometer was 145 °C, and on the exit thermometer the temperature should be 125 °C.

Soybean toasting

The research was conducted on soybean toasted in thick (150 mm) and thin layer (30 mm). The overall sample mass was 72 kg, namely 14.4 kg per batch. Toasting was carried out at average temperature of 130 °C for the period of 10 minutes, from the time when desired temperature was reached. The air temperatures were measured at entrance and exit of the toaster, and soybean grain temperature was measured in the upper and lower areas of the layer. After thermal processing, soybean grains were cooled down to room temperature.

Soybean analyses

In order to properly monitor the technological process of soybean thermal processing, it was necessary to study nutri-

Table 1. Air temperature and soybean grain temperature in thick and thin layer

Thick layer	Air, t (°C)		Kernel, θ (°C)		Thin layer	Air, t (°C)		Kernel, θ (°C)
	Entrance	Exit	Upper layer	Lower layer		Entrance	Exit	
Mean average	136.70	102.38	130.22	125.22	Mean average	132.83	125.00	130.77
min.	132.00	99.00	128.00	117.47	min.	130.00	119.00	127.00
max.	140.00	106.00	133.00	129.01	max.	136.00	128.00	133.00
S.D.	2.57	2.88	3.73	4.24	S.D.	2.23	2.96	1.99

tive properties of soybean, and amino acid content before and after toasting. Under scrutiny were the shares of crude proteins, urease (mgN/g/min), trypsin inhibitor activation (mg TI/g and J/g) and amino acid content of soybean in natural samples and in samples toasted in thick and thin layer.

Crude proteins (Nx6.25) were determined by the Kjeldahl method (ISO 1871:1975), breaking down soybeans using sulphuric acid with mercury as a catalyst. The urease activity in crude and thermally processed soybean was determined in a buffered urea solution (ISO 5506:1988). Trypsin inhibitor activation in crude and thermally processed soybean was determined by means of Kakade, Simons and Liener method (1974). This method was used to determine total and residue inhibitor, by measuring product of the hydrolysis of caseine used here as a substrate at 28 °C.

The amino acid content in proteins in crude and thermally processed soybean was determined after protein hydrolysis. Total amino acids were determined according to methods described by Cavallarin et al. (2005), via acid hydrolysis. The water content in soybean sample, i.e., dry matter content, was determined by drying in the drier at 105 °C for at least three hours until constant mass was obtained (Lewandowski et al., 1997).

Statistical analysis

All measurements were carried out in triplicates. Variance analysis was performed following the General Linear Model (GLM) of SAS. The least significant differences (LSD) among mean values were calculated at $\alpha<0.05$ confidence level (SAS, 2001).

Results and discussion

Air temperatures and soybean grain temperatures were determined during the toasting process. The obtained results are presented in Table 1.

Table 1 shows that air temperature difference between entrance and exit is greater in thick layer than in thin layer. It indicates that air in thick layer is used more efficiently; hence, better performance of the process in energy terms is obtained. However, in thick layer toasting, the difference in layer thickness determines the difference in sample temperature of approximately Δθ 5°C, as well.

Table 2 shows the mean values of crude proteins, urease, and trypsin inhibitor activity in non-treated sample, and sample toasted in thick and thin layer at temperature of 130°C for a period of 10 min.

Soybean grain composition is affected by genotype, location, and year effects (Bhardwaj et al., 1999; Cober et al., 1997; Maestri et al., 1998). However, the relative contribution of each of these factors varies with grain component

Table 2. Nutritional value of soybean toasted in thick layer

Sample		Crude proteins (g/100 g)	Urease (mg N/g/min)	TIA (mg TI/g)	TIA (J/g)
Natural sample		35.13	4.52	23.44	44.54
Thick layer	upper layer	38.47	0.18	3.39	6.44
	middle layer	38.53	0.21	3.39	6.44
	lower layer	38.50	0.26	3.40	6.46
Thin layer		38.96	0.08	1.53	2.91

evaluated, soybean type and geographical area (by different climate and different soil). Protein, which is an essential nutrient, affects many metabolic processes that are directly and indirectly related to tissue growth (Meziani et al., 1999). Table 2 shows that in non-treated sample crude protein content was determined to be 35.13 g/100 g. After thick- or thin layer toasting, crude protein content was slightly increased, but this content was equal in samples toasted in either layer, which corresponds to literature data (Umoren et al., 2005). Table 2 also shows that urease enzyme activity in crude sample as an indicator of crude soybean thermal processing efficiency was 4.52 mg N/g/min, but it was reduced in the thermal processing. Indeed, it was determined that in thick layer toasting the urease activity decreased 20.5 times, while in thin layer toasting it decreased as much as 55 times. This was in accordance with the literature that says that duration of thermal processing is known to reduce or inhibit urease activity in soybean products (McNaughton and Reece, 1980). The trypsin inhibitor activity in crude sample was 23.44 mg TI/g, i.e., 44.542 J/g, while in toasted samples it was significantly reduced according to the previous data (Canibe and Eggum, 1997). In samples toasted in thick layer, the extent of trypsin inhibitor activity reduction was below that in the samples toasted in thin layer (3.39 mg TI/g in relation to 1.53 mg TI/g and 6.44 J/g in relation to 2.91 J/g).

Comparing to most of vegetable protein sources, soybean has well-balanced amino acid content, with methionine as the only limiting amino acid (Storebakken et al., 2000). It also has a relatively steady composition of amino acids (Porter

Table 3. Amino acid composition in natural sample and in soy toasted in thick and thin layer

Amino acid	Natural sample	Thick layer	Thin layer	p value
Aspartic acid	3.88b ±0.04	3.94b ±0.03	4.14a ±0.03	<0.0001
Threonin	1.31c ±0.01	1.49a ±0.02	1.44b ±0.03	< 0.0001
Serine	1.73b ±0.03	1.86a ± 0.02	1.84ab ±0.09	0.0641
Glutamic acid	6.41c ±0.03	7.22a ± 0.01	6.89b ± 0.03	< 0.0001
Proline	1.42c ±0.02	1.91a ± 0.03	1.81b ± 0.02	< 0.0001
Glycine	1.52b ± 0.02	1.62a ± 0.02	1.64a ± 0.03	0.0002
Alanine	1.53c ± 0.02	1.73a ± 0.07	1.65b ± 0.02	< 0.0001
Cistine	0.60b ±0.02	0.51c ± 0.02	0.69a ± 0.02	< 0.0001
Valine	1.61c ±0.01	1.68b ± 0.01	1.85a ± 0.02	< 0.0001
Methionine	0.47b ± 0.01	0.50a ± 0.02	0.49ab ± 0.01	0.0723
Isoleucine	1.60ab ± 0.02	1.58b ± 0.04	1.63a ± 0.02	0.0114
Leucine	2.56c ± 0.01	2.67b ± 0.02	2.78a ± 0.01	< 0.0001
Thyrosine	1.18b ± 0.03	1.21a ± 0.01	1.22a ± 0.02	< 0.0213
Phenylalanine	1.70c ± 0.03	1.77b ±0.02	1.87a ± 0.02	< 0.0001
Lisine	2.10a ± 0.01	2.06a ±0.01	2.35a ± 0.47	0.4155
Hystidine	0.97b ± 0.01	1.02 a ± 0.04	1.05a ± 0.01	0.0012
Arginine	2.47b ± 0.04	2.51a ± 0.01	2.49ab ± 0.02	0.0156
Tryptophan	0.46a ± 0.02	0.42b ± 0.01	0.42b ± 0.01	0.0025

and Jones, 2003). Therefore, the change in amino acid content of soybean (depending on layer thickness in toasting as thermal processing) was determined in order to confirm or eliminate the potential application of thick layer toasting due to the mentioned difference in sample temperature depending on the layer and layer thickness. Table 3 gives the mean values of specific amino acids in natural sample and in soybean toasted both, in thick and thin layers.

According to the USDA *National Nutrient Database for Standard Reference* (2004), the average values of amino acids content (g/100 g) in crude soybean are as follows: Arg 2.831, Hys 0.984, Leu 2.972, Ile 1.770, Lys 2.429, Cys 0.588, Phe 1.905, Tyr 1.380, Thr 1.585, Trp 0.530, Val 1.821, Asn 4.589, Ser 1.687, Gln 7.068, Pro 2.135, Gly 1.687, Ala 1.719 and Met 0.492 with protein share of 36.49 g/100 g. According to the same source, the share of amino acids in dry toasted soybean are: Arg 3.071, Hys 1.068, Leu 3.223, Ile 1.920, Lys 2.634, Cys 0.638, Phe 2.066, Tyr 1.497, Thr 1.719, Trp 0.575, Val 1.976, Asn 4.977, Ser 2.294, Gln 7.667, Pro 2.315, Gly 1.830, Ala 1.865 and Met 0.534 with increased protein share of 39.58 g/100 g.

From Table 3 it is evident that the obtained values generally correspond to the data found in the literature so far (Refstie et al., 1999). The data on amino acid content of soybean showed that there was no significant difference in nutritional values of soybean toasted in thin or thick layer, but there was no discernible change in amino acid content in bean after thermal processing in relation to amino acid content in crude proteins. Therefore, toasting did not affect the amino acid composition, nor any Lys damage was detected (Canibe and Eggum, 1997). The results make it evident that Gln was excessive in relation to other amino acids (7.217% Gln/% of total proteins for thick layer and 6.890% Gln/% of total proteins for the thin layer). By its share in total proteins after bean toasting, Gln was followed by Asn (3.940% Asn/% of total proteins for the thick layer and 4.139% Asn/% of total proteins for the thin layer), Leu (2.670% Leu/% of total proteins for the thick layer and 2.780% Leu/% of total proteins for the thin layer) and Arg (2.513% Arg/% of total proteins for the thick layer and 2.493% Arg/% of total proteins for the thin layer). Met was present in lesser amount either in crude proteins or in proteins of toasted bean (0.503% Met/% of total proteins for the thick layer and 0.493% Met/% of total proteins for the thin layer), which is, though, the characteristic of the soybean proteins. However, it should be noted that unlike some findings quoted in the literature (Refstie et al., 1999), Lys, and especially Arg, were found in higher quantities (2.057% Lys/% of total proteins and 2.513% Arg/% of total proteins for the thick layer; 2.353% Lys/% of total proteins and 2.493% Arg/% of total proteins for the thin layer).

Conclusion

In this study, protein content in soybean was found to be increased with toasting, but it was not affected by the layer thickness. Thus, in terms of protein content it is recommended to apply a thick layer. Regardless of a wide difference in the extent of reduction of urease activity between thick and thin layer toasting, the urease activity was reduced in thick layer toasting enough to make it fit for polygastric animals' feed. Since the urease activity reduction in toasting in either layer thickness meets the set requirements, it is recommended to apply thick layer in toasting because in that case toaster is used more efficiently. Furthermore, the study established a significant reduction of trypsin inhibitor activity after thermal processing by toasting, at higher extent in thin layer (by 93.5%) than in thick layer toasting (by 86%). The trypsin inhibitor activity in samples of toasted soybean in thin layer fully complies with the FEFAC criteria, while in samples toasted in thick layer it is marginal. Amino acid content of soybean was slightly increased in relation to natural sample, as well as the difference between amino acid content in samples toasted in thick and thin layers. Given the FEFAC recommendations to monitor the urease activity for the purpose of polygastric animals feed, it can be concluded that the soybean toasted at 130 °C for 10 minutes in thick layer meets these requirements.

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