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Effect of Selected Thermal Processes on the Stability of Reactive Lysine in Domestic Cultivars of Common Bean (*Phasoleus Vulgaris*)

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Samples of 3 domestic cultivars of bean seeds (Warta, Raba and Narew) were used to determine the content of dry matter, protein, fat, ash, amino acids and reactive lysine. Essential amino acid index (EAAI) and limiting amino acid index (CS) were calculated as well. The samples were subjected to autoclaving or heating in a dryer for 10, 20, 30 or 40 min at the temperature of 121°C. Next, the content of dry matter and reactive lysine in the samples were determined. It was observed that processing led to an increase in the content of dry matter in the seeds and to a decrease in the content of reactive lysine. The losses of reactive lysine were similar in both processes, amounting to 11–20%, regarding the content of the component in dry matter. Statistically significant changes were noted after 30 or 40 min of processing.

INTRODUCTION

Dry seeds of legumes provide a valuable source of plant protein for their contain significant quantities of protein and additionally lysine. They are consumed after processing with the use of traditional methods, mainly cooking, or in highly processed forms, as extrudates, protein isolates, flour added to bread, pasta, supplements in milk products, *etc*. Such products are obtained with the help of thermal processes occurring in the aqueous environment or "in a dry run", with different parameters of the processes. Processing the seeds results in deactivating toxic substances and those inhibiting the adequate use of nutrients, as well as in some changes in the structure, facilitating the access of proteolytic enzymes [Mubarak, 2005; Bieżanowska-Kopeć *et al.*, 2006]. However, the drastic conditions of such processes pose a risk of lowering the quality of protein through reducing its solubility and digestibility. Lower digestibility results mainly from the losses of reactive lysine [Gujska & Khan, 2002]. At higher temperatures, ε-amino groups in lysine may react with reducing sugars (Maillard reactions) [Naranjo *et al*., 1998]. Fragments of the peptide chain in which lysine reacted through its ε-amino groups with other compounds (starches or products of fat oxygenation) proved to be resistant to the activity of proteolytic enzymes. In this aspect, the assessment of the stability of reactive lysine during thermal processing seems to be of vital importance.

The aim of the study was to determine the effect of the time of autoclaving or dry heating on the content of reactive lysine in the seeds of domestic bean cultivars.

MATERIALS AND METHODS

The seeds of three domestic cultivars of bean seeds, Warta (2001), Raba (2002) and Narew (2000) cultivated in 2005 by IHAR in Radzikow near Warsaw (the year given in brackets indicates their registration in the Polish National List of Cultivars) were used in the study. After removing contamination, the samples were stored in sealed glass containers at room temperature.

Humidity, total protein, crude fat and ash were determined in the samples, according to AOAC [1990]. Amino acids were determined with the help of ion exchange chromatography on the Beckman automatic amino acid analyzer, model 119Cl. Prior to the analysis, the samples were subject to acid hydrolysis at the presence of 6 mol/L HCl at 105°C for 24 h. Sulphur amino acids were determined after their oxidation. Tryptophan was analysed in compliance with the AOAC [1990] method.

The essential amino acid index (EAAI) and limiting amino acid index (CS) were calculated on the basis of the formulae postulated by Oser [1951] and Block & Mitchell [1946]. The model was provided by the amino acid composition of the egg according to FAO/WHO [1965]. Reactive lysine was determined with the use of the HPLC technique according to Ramírez-Jiménez *et al.* [2004]. The principle of the method consists in creating a colored ε-DNP-lysine complex in a reac-

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tion with dinitrofluorobenzene (DNFB) and next hydrolyzing the sample and determining the content of ε-DNP-lysine on a liquid chromatograph with the UV VIS – Spectroflow 773 detector. All analyses were performed in three replications.

For every cultivar of bean seed eight samples were prepared, each of 500 g. Four of them were autoclaved for 10, 20, 30 or 40 min at the temperature of 121°C and the pressure of 0.25 MPa. Autoclaving was performed in a vertical steam sterilizer of the ASVE type, manufactured by SPM in Warsaw. The remaining samples of each seed cultivar were dry-heated at the temperature of 121°C for 10, 20, 30 or 40 min in a laboratory drier. The range of the listed processing periods included typical options used in the industrial processing of bean seeds [Shi *et al.*, 2009]. Immediately after processing, the samples were cooled through spilling their thin layers on metal trays, until they reached the temperature of the environment (*ca.* 20°C), and next their humidity was determined. The content of reactive lysine was determined in all the samples in three replications.

Mean values of reactive lysine in bean seeds were compared by means of the variance analysis, while the significance of the differences between the mean values was verified with the Tukey test, at $p \leq 0.05$. Calculations were performed using the Statistica 8.0 program.

RESULTS AND DISCUSSION

The content of protein in the analyzed cultivars of bean ranged from 21.14% in dry matter (Raba) to 23.56% in dry matter (Warta), which was lower than the values quoted by Korus *et al.* [2005] for five new cultivars of common bean but similar to those presented by Winiarska-Mieczan & Koczmara [2006] and Rodríguez *et al.* [2008] (Table 1). The content of fat in the studied bean cultivars was within fluctuation limits accepted for domestic cultivars [Korus *et al.*, 2005].

The results provided by the determination of the amino acid composition revealed the most significant differences regarding the content of lysine, arginine and tyrosine in particular cultivars of bean. The contribution of these amino acids was the highest in the seeds of the cultivar Warta and amounted to 17.3 g/kg of lysine and arginine in dry matter and to 7.2 g/kg of tyrosine in dry matter, while in seeds of Raba cultivar the contents of these amino acid were the lowest, *i.e.* 13.7 of lysine, 12.9 of arginine and 5.1 g/kg of tyrosine in dry matter. The content of lysine in the cultivar Warta seeds was higher than the values reported by Gujska & Khan [2002] or Rodríguez *et al.* [2008], with the lowest content of reactive lysine in the total lysine, amounting to 73%. Reactive lysine found in the seeds of the cultivars Narew and Warta was at the level of, respectively, 80 and 84% of total lysine.

TABLE 1. Chemical composition of bean seeds.

Specification	Warta	Raba	Narew
Dry matter (g/kg)	879	880	876
Total protein (g/kg dry matter)	235	211	224
Crude fat (g/kg dry matter)	15.4	19.3	16.9
Ash (g/kg dry matter)	37.6	40.2	43.6
Lysine (g/kg dry matter)	17.3	13.7	14.8
Methionine (g/kg dry matter)	3.5	2.8	2.7
Cystine (g/kg dry matter)	2.5	2.4	2.4
Threonine (g/kg dry matter)	10.7	10.2	9.6
Arginine (g/kg dry matter)	17.3	12.9	14.8
Tyrosine (g/kg dry matter)	7.2	5.1	5.6
Histidine (g/kg dry matter)	8.1	6.5	6.6
Reactive lysine (g/kg dry matter)	12.7	11.5	11.9
EAAI	74.1	73.7	70.5
CS (%)	40.6	40.6	35.9
Limiting amino acid	Met+Cys	Met+Cys	Met+Cys

TABLE 2. Dry matter content after processing and cooling bean seeds (g/kg).

Processing time (min)	Warta	Raba	Narew
0	879	880	876
Autoclaving			
10	900	897	892
20	902	896	892
30	897	893	892
40	896	900	899
Drying			
10	915	916	919
20	925	921	924
30	924	925	930
40	936	937	935

The content of methionine, cystine and threonine in the analysed bean seeds was similar to the results presented by Gujska & Khan [2002], while the content of tyrosine and histidine was lower than the values quoted by these authors. The varied amino acid composition of the seeds representing particular cultivars was reflected in the values of EAAI which ranged from 70.5 (Narew) to 74.1 (Warta). The limiting amino acids were methionine and cystine, while the degree to which they limited the value of protein ranged from 35.9 (Narew) to 40.6% (Warta and Raba).

The content of mineral elements in the studied material was within the limits of 3.76 (Warta) to 4.35% (Narew) of dry matter, which was similar to the values presented by Korus *et al.* [2005] and Krupa & Soral-Śmietana [2005].

The initial content of dry matter in the seeds of all the cultivars was similar and amounted to *ca.* 880 g/kg (Table 2). The type of processing as well as the time of seed exposition was found to affect the changes in dry matter content. Autoclaving led to its slight increase, by maximum *ca.* 2%. As expected, changes following the drying process were more significant and were intensifying along with the prolonged time of exposure, reaching *ca.* 6%.

The autoclaving process led to reducing the content of reactive lysine along with prolonging the processing time. The exposition of seeds for 10 or 20 min did not evoke any statistically significant changes in any of the studied cultivars

TABLE 3. Reactive lysine content after processing bean seeds (g/kg dry matter).

Processing time (min)	Warta		Raba		Narew	
	$\bar{x}$±SD	Retention (%)	$\bar{x}$±SD	Retention (%)	$\bar{x}$±SD	Retention (%)
			Autoclaving			
0	12.7^{a}±0.80	100	11.5^{a}±0.15	100	11.9^{a}±0.36	100
10	12.5^{a}±0.45	98	11.5^{a}±0.71	100	12.1^{a}±0.64	100
20	12.6^{a}±0.95	99	10.8^{ab}±0.35	94	11.0^{ab}±0.50	92
30	11.8^{ab}±0.21	93	10.1^{bc}±0.17	88	10.6^{b}±0.32	89
40	10.9^{b}±0.44	86	9.2^{c}±0.22	80	10.4^{b}±0.30	87
			Drying			
0	12.7^{a}±0.80	100	11.5^{a}±0.15	100	11.9^{a}±0.36	100
10	12.6^{a}±0.35	99	11.6^{a}±0.32	100	11.6^{a}±0.42	97
20	12.6^{a}±0.66	99	11.6^{a}±0.23	100	11.3^{ab}±0.31	95
30	11.5^{ab}±0.31	90	11.5^{a}±0.32	100	10.6^{b}±0.31	89
40	11.2^{b}±0.25	88	10.3^{b}±0.35	89	10.5^{b}±0.26	88

^{a,b,c} – mean values differ in columns at $p \leq 0.05$.

(Table 3). Significant losses were observed after 30 min in the seeds of the cultivars Raba and Narew – with reactive lysine content decreasing by, respectively, 12% and 11%. The final retention of reactive lysine in the autoclaved seeds of the Warta and the Narew beans was similar and amounted to, respectively, 86% and 87%, whereas in the cultivar Raba it accounted for 80% of the initial content.

In dry-heated bean seeds a statistically significant decrease in the content of reactive lysine (up to 89% of the initial content) was noted after 30 min of the process in the case of the cultivar Narew (Table 3). In the other cultivars, statistically significant differences were observed first after 40 min of heating. The final losses of reactive lysine were from 11% for the cultivar Raba to 12% for the cultivars Warta and Narew.

The results obtained revealed that autoclaving or drying the seeds of the studied cultivars of bean at the temperature of 121°C was safe for maintaining the level of reactive lysine over the time up to 30 min. Rocha *et al.* [2002], while assessing the effects of heating bean seed albumins, did not observe any drop in the content of reactive lysine, even at a higher temperature (135°C) and during a longer time (60 minutes) of processing. In the case of soybean seeds, Žilić *et al.* [2006] noted that autoclaving and extrusion performed in a closed system of increased humidity led to smaller changes in the content of reactive lysine, compared to micronization and microwave processing. Toasting (at the temperature of 135°C, for 10 min), at increased humidity resulted in 7–16% degradation of the discussed component [Qin *et al.*, 1998]. In the authors' earlier studies, the parameters of extruding seeds and false flax oil cake (temperature of 165°C, with natural humidity) proved to be neutral for lysine reactivity [Jaśkiewicz *et al.*, 2006]. Higher humidity (20–25%) at the temperature of 133 or 156°C led to the losses of reactive lysine in bean seeds, amounting to 14.4–24.7% [Gujska & Khan, 2002]. The lack of significant differences in the losses of reactive lysine during autoclaving or heating in a drier in the present study may have been caused by the fact that the processes were carried out in standardized conditions of time and temperature.

CONCLUSIONS

1. Drying or autoclaving bean seeds did not have any effect on the content of reactive lysine when the processes lasted for 10 or 20 min.
2. The 30-min processing resulted in a significant reduction in the content of reactive lysine (in reference to raw seeds) in the case of autoclaved seeds of the cultivars Raba and Narew, as well as the dried seeds of the cultivar Narew.
3. Prolonging the processes up to 40 min resulted in 11–20%. losses of reactive lysine in all the studied samples.

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