APPLICATION OF BROMOCRESOLE PURPLE INDEX (BCPI) FOR EVALUATION OF CONTENTS OF AVAILABLE LYSINE FROM MICRO-VAVE-HEATED SOYBEAN SEEDS

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Abstract. Usefulness of analytical method (bromocresole purple index – BCPI) as a new and fast way for available lysine (LA) content determination in micro-waved soybean seeds was tested. Due to a great interdependence of LA and BCPI determinations, which was confirmed by high correlation and determination coefficients values ($r = 0.78$ and R^2) for proposed regression equations LA = f(BCPI), the BCPI method appeared to be useful for the description of available lysine (LA) content changes in micro-waved soybean seeds and soybean meal.

Key words: soybean seeds, protein, micro-waving, available lysine, bromocresole purple index, regression equation

INTRODUCTION

Content of available lysine (LA) is one of the indicators of protein biological value in foodstuff and fodder. It is commonly known that improper (excessively intensive) heating of raw material contributes to its unfavorable chemical transformations [Arnoldi 2001] resulting in general protein solubility and availability decrease along with the availability decrease of some amino acids (namely lysine) particularly sensitive to heating.

Those processes intensify when uncontrolled changes of industrial process parameters occur (e.g. during micro-waving of soybean seeds), which may be associated with great increment of temperature during thermal processing. Therefore, maintaining required quality of soybean products under conditions when their heating is realized due to microwaves, needs to apply sensitive, precise and quick evaluation methods adapted to the specificity of the production process as well as simple conditions in the industrial laboratory where product quality assessment is performed.

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Up-to-date applied analytical methods, in which efficiency of soybean seeds (or soybean meal) heating was evaluated through classical determinations such as available lysine (LA) [Ramírez-Jiménez et al. 2004], trypsin inhibitor activity (TIA) [Szmigielski 2004] or urease activity (UA) [PN-ISO 5506: 2002], do not fulfill modern on-line quality monitoring during microwave heating of raw material due to high costs and procedure complexity.

The aim of the study was to test the usefulness of new analytical method (bromocresole purple index – BCPI) for evaluation of available lysine (LA) contents in microwaved soybean seeds.

MATERIAL AND METHODS

Among the studied soybean seeds (Polish variety Progres – lot I and II as well as Polan), samples that remained at their native non-heated from, were separated – raw seeds. Raw seeds were characterized by: moisture content 93.34% – Progres I, 92.32% – Progres II, and 93.92% – Polan; their dry matter contained: 36.23%, 34.16%, and 33.50% of total protein, 21.28%, 20.63%, and 22.14% of crude fat, and 5.62%, 5.45%, and 5.07% of total ash. Determinations of moisture content, total proteins, crude fat, and total ash were made in accordance to recommended Polish Norms (making three independent replications of every determination and calculating mean values).

Remaining seeds (for each of three lots) formed 9 samples (50 g each); they were transferred into glass beakers (250 cm^3 capacity), then every beaker was separately placed in the geometrical center of working area of microwave oven (Whearpool – Vip 20) and heated under conditions of one of 9 intensity variants. Each of three radiation intensity levels (350, 500, or 650 W) corresponded to three exposure times (60, 120, or 180 s). All 10 soybean seed heating variants (9 microwave heated plus raw sample) were subjected to determinations for available lysine (LA – recalculated onto 100 g protein in seed dry matter) and bromocresole purple indicator (BCPI) by making three independent analyses for every trait (LA, BCPI) of every sample in each of three lots.

Available lysine (LA) was determined by means of HPLC technique according to Ramírez-Jiménez et al. [2004] with own modifications of HPLC conditions. The method consisted in forming color complex of ε-dinitrophenyl-lysine (ε-DNP-lysine) in reaction with dinitrofluorobenzene (DNFB), sample hydrolysis, and purifying from DNFB using ethyl ether. ε-DNP-lysine was determined applying HPLC system equipped with UV-VIS detector. Chromatographic conditions were as follows: column – ODS Hypersil, 200 × 4.6 mm, mobile phase – methanol:water (52:48), flow rate – 1ml/min, detector – UV-VIS Spectroflow 773, detection wavelength – 358 nm.

Determinations of BCPI were performed according to Szmigielski [2004], and results were recalculated onto protein weight unit in seed dry matter.

Experimental data achieved from analyses (LA and BCPI) were statistically processed (calculating mean and standard deviation values) and hypothesis on traits interdependence was verified (calculating correlation coefficients – r between particular result series) [Oktaba 1986]. On a base of statistically confirmed credibility of the hypothesis (high correlation coefficient values – r), results were subjected to mathematical approximating procedure resulting in a series of hypothetical functions of a form

LA = f(BCPI), helpfulness of which (in a description of real experimental data) was verified by means of calculating determination coefficients (R^2) [Oktaba 1986].

Sample discrimination (ρ) was defined as the difference significance between mean values for every trait determined (LA and BCPI) for every sample tested, and expressed (separately for each of traits) as a per cent of significant relations referring to the total of verified relations [Szmigielski 2004, Szmigielski and Matyka 2004]. Difference significance for test results was defined by variance analysis (at 5% of significance level) and determining the least significant differences using Tukey's test (LSD) [Oktaba 1986]. Determination precision (π) was defined (separately for each of mean values from tests) by means of calculating the percentage shares of standard deviations (SD) in mean value of every determined trait (LA, BCPI) and for every tested sample. Determination time-consumption (τ) was defined as a time necessary to perform one replication for a single seed sample.

RESULTS AND DISCUSSION

Tested soybean seeds were characterized by similar available lysine contents (differences between tested seeds appeared to be in 100% insignificant – Table 1). Microwave heating induced changes of soybean protein properties. A tendency to decrease available lysine level along with the process intensity (i.e. longer exposure and higher radiation intensity) was observed. The highest losses of available lysine (14.82% in relation to raw Progres I, 9.90% to raw Progres II, and 10.26% to raw Polan) were recorded for samples heated with radiation of 650 W power for 180 s (Table 1). Similar tendency of decreasing the available lysine along with sample heating intensity was observed in earlier studies [Gujska and Khan 2002, Žilić et al. 2006].

Differences of available lysine contents between soybean seeds heated under the same radiation intensity and exposure conditions appeared to be, for majority of variants, insignificant (Table 1), which indicates the versatility of LA trait in testing soybean seeds.

Statistically insignificant differences of LA levels (recorded for raw seeds as compared to slightly heated ones – e.g. 350 W for 60, 120, or 180 s as well as 500 W for 60 s) result probably from negligible changes of available lysine contents, and in part, from medium precision of LA determination (Tables 1 and 5). LA determination precision (π_{LA}) was similar to the results achieved by Ferrer et al. [2003] (result scatter more than 2%) and it was several times lower as compared to determinations made by means of BCPI method (π_{BCPI}; Tables 2 and 5), which no doubt contributed to differences of properties of majority tested samples ($\rho_{BCPI} = 97.78\%$ – Table 4 as compared to $\rho_{LA} = 31.11\%$ – Table 5). BCPI determinations made by Szmigielski [2004] were characterized by similarly high precision. Results were also similar, when those achieved by Szmigielski [2004] are recalculated onto protein content in seed dry matter (analogously as in present study), which indirectly indicates the mechanism of bromocresole purple sorption (5',5"-dibromo,3',3"-dimethylphenolosulfophthalein) on a surface (i.e. chemisorption with participation of protein amino acid moieties).

Additional virtue of BCPI result recalculation (in reference to protein weight unit in seed dry matter) is the possibility to compare determinations for samples with wide spectrum of chemical compositions (e.g. full-fat seeds with post-extraction soybean meal).

Table 1. Available lysine (LA) contents micro-waved soybean seed samples Progres I, Progres II and Polan, g·100 g protein in DM^{-1}

Thermal processing	Parameters of thermal process				
	radiation power, W	seeds	time of thermal processing, s		
			60	120	180
Micro-waving	350	Progres I	6.09 ± 0.26 (4.29)	6.15 ± 0.17 (2.76)	6.09 ± 0.11 (1.81)
		Progres II	6.07 ± 0.11 (1.81)	6.03 ± 0.17 (2.82)	5.96 ± 0.25 (4.19)
		Polan	6.01 ± 0.19 (3.16)	5.97 ± 0.10 (1.68)	6.01 ± 0.11 (1.83)
	500	Progres I	6.03 ± 0.15 (2.49)	5.98 ± 0.18 (3.01)	6.04 ± 0.29 (4.80)
		Progres II	5.99 ± 0.14 (2.34)	5.98 ± 0.12 (2.01)	5.90 ± 0.12 (2.03)
		Polan	6.01 ± 0.12 (2.00)	6.01 ± 0.23 (3.83)	5.97 ± 0.11 (1.84)
	650	Progres I	5.75 ± 0.26 (4.52)	5.46 ± 0.16 (2.93)	5.17 ± 0.09 (1.74)
		Progres II	5.90 ± 0.09 (1.53)	5.86 ± 0.12 (2.05)	5.46 ± 0.10 (1.83)
		Polan	5.96 ± 0.15 (2.52)	5.99 ± 0.09 (1.50)	5.46 ± 0.16 (2.93)
Control (raw seeds)	Progres I			6.07 ± 0.13 (2.14)	
	Progres II			6.06 ± 0.15 (2.48)	
	Polan			6.02 ± 0.25 (4.15)	

Variability coefficients [%] in round brackets ().

Similarly as for determination of available lysine content (LA), differences of BCPI values between soybean seeds samples (heated at the same radiation intensities and exposure times), for most of heating variants, appeared to be insignificant (Table 2), which indicated the versatility of BCPI method as the way to evaluate the soybean protein.

Due to specific perspective of BCPI and LA determinations application (i.e. assessment of processed products realized in industrial laboratory), determination time-consumption is also important; for LA method it amounts to $\tau_{LA} = 26.0$ h, as opposite to BCPI test ($\tau_{BCPI} = 1.5$ h – Table 5).

Statistical processing of experimental data achieved from BCPI and LA methods indicates their great interdependence (high correlation coefficients r = from 0.63 to 0.86 –

Table 2. Bromocresole purple index (BCPI) of micro-waved soybean seed samples Progres I, Progres II and Polan, mg·g of protein in DM^{-1}

Thermal processing	Parameters of thermal processing				
	radiation power, W	seeds	time of thermal processing, s		
			60	120	180
Micro-waving	350	Progres I	74.00 ± 0.49 (0.66)	85.30 ± 0.57 (0.67)	96.70 ± 0.44 (0.46)
		Progres II	73.93 ± 0.45 (0.61)	85.45 ± 0.65 (0.76)	96.77 ± 0.38 (0.39)
		Polan	74.17 ± 0.25 (0.34)	85.40 ± 0.56 (0.66)	96.83 ± 0.35 (0.36)
	500	Progres I	89.70 ± 0.82 (0.91)	108.00 ± 0.64 (0.59)	125.00 ± 0.74 (0.59)
		Progres II	89.67 ± 0.84 (0.94)	108.09 ± 0.36 (0.33)	125.37 ± 0.42 (0.34)
		Polan	89.53 ± 0.57 (0.63)	108.03 ± 0.25 (0.23)	124.90 ± 0.87 (0.70)
	650	Progres I	105.75 ± 0.38 (0.36)	126.00 ± 0.41 (0.33)	138.00 ± 0.57 (0.41)
		Progres II	105.60 ± 0.58 (0.61)	125.87 ± 0.85 (0.68)	138.11 ± 0.34 (0.25)
		Polan	105.53 ± 0.57 (0.54)	125.80 ± 0.82 (0.65)	138.23 ± 0.31 (0.22)
Control (raw seeds)	Progres I			62.70 ± 0.30 (0.48)	
	Progres II			62.57 ± 0.31 (0.50)	
	Polan			63.23 ± 0.38 (0.60)	

Variability coefficients [%] in round brackets ().

Table 3) when testing soybean seeds heated in microwaves and it justifies these results approximation using hypothetical mathematical curves – regression equations of LA = f(BCPI) type. High values of fitting coefficients (R^2) for selected equations of that type points out to their significant credibility in describing the changes of lysine availability for microwave heated soybean seeds (Table 3). Similar traits changes range for LA and BCPI, similarity of regression equations course, as well as identity of the area the curves are localized on plot indicate the possibility to apply those data to work out generalized curves LA = f(BCPI) on a base of a set experimental data (containing experimental points for all tested soybean seeds – Table 4).

Table 3. Available lysine (LA) contents in micro-waved soybean seeds (Progres I, Progres II and Polan) as a function of bromocresole purple index (BCPI)

Correlation coefficient (r)	Type of equation		Equation	Determination coefficient (R^2) %
0.86			Progres I	
	Polynomial	II°	$LA = -0.0003(BCPI)^2 + 0.0425(BCPI) + 4.4664$	93.58
	Linear		$LA = -0.0116(BCPI) + 7.0551$	73.77
	Exponential		$LA = 7.2113e^{-0.002(BCPI)}$	72.47
	Logarithmic		$LA = -1.0476Ln(BCPI) + 10.69$	64.54
	Power		$LA = 13.599(BCPI)^{-0.1829}$	63.16
0.82			Progres II	
	Polynomial	II°	$LA = -0.0001(BCPI)^2 + 0.0194(BCPI) + 5.3113$	81.54
	Linear		$LA = -0.006(BCPI) + 6.5234$	66.56
	Exponential		$LA = 6.5646e^{-0.001(BCPI)}$	65.18
	Logarithmic		$LA = -0.5422Ln(BCPI) + 8.4094$	59.12
	Power		$LA = 9.0728(BCPI)^{-0.0931}$	57.69
0.63			Polan	
	Polynomial	II°	$LA = -0.0002(BCPI)^2 + 0.0301(BCPI) + 4.7369$	68.94
	Linear		$LA = -0.0045(BCPI) + 6.3961$	40.16
	Exponential		$LA = 6.4268e^{-0.0008(BCPI)}$	39.68
	Logarithmic		$LA = -0.3949Ln(BCPI) + 7.7535$	32.91
	Power		$LA = 8.1319(BCPI)^{-0.0685}$	32.46

LA = f(BCPI of protein in DM), LA – dependent variable, BCPI – independent variable.

Table 4. Available lysine (LA) contents in micro-waved soybean seeds as a function of bromocresole purple index (BCPI)

Correlation coefficient (r)	Type of equation		Equation	Determination coefficient (R^2) %
0.78	Polynomial	II°	$LA = -0.0015(BCPI)^2 + 0.0264 (BCPI) + 5.9834$	83.40
		III°	$LA = -0.0002(BCPI)^3 + 0.0057(BCPI)^2 - 0.0639(BCPI) + 6.2357$	95.49
	Linear		$LA = -0.0206(BCPI) + 6.234$	62.99
	Exponential		$LA = 6.247e^{-0.0036(BCPI)}$	61.40
	Logarithmic		$LA = -0.1684Ln(BCPI) + 6.3341$	39.36
	Power		$LA = 6.3535(BCPI)^{-0.029}$	37.86

LA = f(BCPI of protein in DM), LA – dependent variable, BCPI – independent variable.

Table 5. Time-consumption, discrimination and precision of analytical methods applied for evaluation of soybean seed heating efficiency

Parameter compared	Name of analytical method	
	BCPI_{B.S.M.} mg·g protein in DM^{-1}	LA g·100 g protein in DM^{-1}
τ, h	1.5	26.0
ρ, %	97.78	31.11
π (Cv), %	0.33-0.91	1.77-4.77

CONCLUSIONS

1. Bromocresole purple index (BCPI) appeared to be useful in describing the changes of available lysine contents (LA – determined by means of HPLC technique).

2. Differences between tested soybean varieties referring to available lysine contents (LA) and BCPI level appeared to be insignificant for majority of microwave heating variants, which indicates the versatility of these traits in soybean seeds evaluation.

3. Due to a high precision and discrimination of determinations made by means of BCPI as well as low time-consumption and great interdependence of LA and BCPI traits (high correlation coefficient r = 0.78), BCPI method appeared to be useful for fast, routine determinations of available lysine contents (LA) in microwave heated soybean seeds or soybean products (realized under industrial laboratory conditions).

Shortcuts used in text

BCPI – bromocresole purple index ρ – sample discrimination
CV – variability coefficient R^2 – determination coefficient
LA – available lysine τ – determination time-consumption
π – determination precision TIA – trypsin inhibitor activity
r – correlation coefficient UA – urease activity

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ZASTOSOWANIE WSKAŹNIKA PURPURY BROMOKREZOLOWEJ (BCPI) DO OCENY ZAWARTOŚCI LIZYNY PRZYSWAJALNEJ W OGRZEWANYCH MIKROFALOWO NASIONACH SOI

Streszczenie. Sprawdzono przydatność nowej metody analitycznej (wskaźnika purpury bromokrezolowej – BCPI) jako nowego, szybkiego sposobu określania zawartości lizyny przyswajalnej (LA) w ogrzewanych mikrofalowo próbach nasion soi. Ze względu na dużą współzależność wyników oznaczeń (LA i BCPI), potwierdzoną poprzez uzyskanie dużego współczynnika korelacji (r = 0,78) oraz wysoką wartość współczynników determinacji (R^2) dla zaproponowanych równań regresji LA = f(BCPI), metoda wskaźnika purpury bromokrezolowej (BCPI) okazała się przydatna do opisu zmian zawartości lizyny przyswajalnej (LA) w ogrzewanych mikrofalowo nasionach soi i śrucie sojowej.

Słowa kluczowe: nasiona soi, białko, ogrzewanie mikrofalowe, lizyna przyswajalna, wskaźnik purpury bromokrezolowej, równanie regresji