POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2004, Vol. 13/54, No 1, pp. 35–39

BROMOCRESOLE PURPLE INDEX IN ESTIMATING THE INFLUENCE OF MICROWAVE PROCESSING ON TRYPSIN INHIBITOR ACTIVITY OF SOYBEANS

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Key words: soy, microwave heating, trypsin inhibitor activity, bromocresole purple index, regression equation, sensitivity

The possibility of using bromocresole purple index (BCPI) for estimating trypsin inhibitor activity (TIA) in samples of soybeans processed by microwaves (with a radiation of 2 450 MHz working frequency) was tested. Ten intensity options of bean micronization were prepared and each of the three levels of radiant power (350, 500 and 650 W) had three processing times (60, 120 and 180 s) respectively, and one sample remained untreated. Seed micronization caused: the TIA decrease from 48.3 TUI/$mg_{d.m.}$ (for raw sample) to 3.78 TUI/$mg_{d.m.}$ (for heated – 180 s at 650 W power) and the BCPI increase from 22.63 mg/$g_{d.m.}$ (for raw sample) up to 50.05 mg/$g_{d.m.}$ (for heated – 180 s at 650 W power). Due to the significant interdependence of the results obtained with the use of BCPI and TIA methods (coefficient of correlation r=- 0.99; fitting coefficient R^2=97.63; trend line TIA=-1.65XBCPI+89.98 to experimental data points), as well as to the high sensitivity (χ=0.30), detectability (δ=273.96 s), distinguishability (ρ = 97.22%) and low time-consumption (η = 1.5 h) the BCPI test was proved useful for a rapid estimation of trypsin inhibitor activity of micronized soybeans.

Symbols and abbreviations used in the study

β – maximum relative error of the method, **BCPI** – bromocresole purple index, χ – sensitivity of the method, **CV** coefficient of variation, δ(**s**) - detectable minimum of the method, η(**h**) – time-consumption for the analyses, **r** – coefficient of correlation, **MD** – mean, **MTN** – the mass of thousand beans, **NIR** – the lowest significant difference of Tukey, **SD** – standard deviation, **TIA** – trypsin inhibitor activity, **TUI** – trypsin inhibitor activity unit, ρ(**%**) – distinguishability of the samples, ω – detectability of the method.

INTRODUCTION

Trypsin inhibitor activity (TIA) is one of the most significant anti-nutritive factors occurring in soybeans, and one of the basic traits for estimating the beans in respect of their nutritional use.

A safe value for using the beans as a component of a diet or feed is that not exceeding 5 trypsin inhibitor activity units per 1 mg of sample dry mass (TUI/$mg_{d.m.}$) [Full-fat Soya 1994].

Experimental observations confirming the presence of some substances inhibiting the protein proteolysis in soybean seeds were first recorded in the 1930s [Read & Haas, 1938 – after Dipietro & Liener, 1989]. Later, intensive studies led to the isolation of two general forms of soybean antiproteolytic activities, *i.e.* Kunitz Soya Trypsin Inhibitor (KSTI) [Kunitz, 1947 – after Dipietro & Liener, 1989] as well as Bowman Birk Inhibitor (BBI) [Birk, 1961]. A detailed description of their structure and properties is presented in Liener and Kakade's review [1980].

An analytical evaluation of total trypsin inhibitory activity (TIA) is based on the estimation of a part of trypsin activity that is blocked by a buffered extract containing KSTI and BBI achieved from seed sample and takes place under conditions ensuring the maximum activity of this enzyme. Methods applied for TIA evaluation can be divided into those where standardized protein (*e.g.* casein) is used as a trypsin substrate [PN-90/R64816], and those where a synthetic substrate is used (BAPA, *i.e.* N-α-benzoylo--DL-arginino-p-nitroanilide) [Kakade *et al.*, 1969, 1974]. As a result of the reaction between trypsin and BAPA, yellow p-nitroanilin is formed (the absorbance measured at a wavelength of 410 nm is proportional to its concentration).

Good result repeatability, along with the reagent property stability is the advantage of the method with BAPA substrate (in contrast to the case in method according to PN-90/R64816).

The drawbacks include the high labour and time expenditures and expensive analytical procedures involved. Therefore, application of the BAPA method for quality control on technological lines of soybean seed with microwave processing as a heating method is troublesome and often produces losses of raw material and elevated costs. Thus, there is a need to replace or modify the evaluation of antitrypsin activity in order to improve the quality control of the products obtained by microwave processing of soybeans.

The examination aimed at assessing the usefulness of the bromocresole purple index (BCPI) as a method of determining the influence of microwave processing on trypsin inhibitor activity in soybeans.

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MATERIALS AND METHODS

Tests were carried out on Progres variety soybeans whose average size was (MTN – 170.05 g). Some of these soybeans were isolated and left unprocessed, they were called "raw" beans. The dry mass of raw beans averaged 92.00%. This contained on average 36.22% of total protein, 20.65% of raw fat and 3.24% of raw ashes. The moisture, the mass of thousand beans, the content of protein, fat and ash were determined according to Polish standards (making three independent replications for every measurement). The remaining beans were divided into 50 g samples and subjected to heat processing. Microwave processing was carried out in a Whirlpool Vip 20 oven, applying radiation of 2450 MHz frequency and, autoclaving was done in a vertical laboratory stabilizer of the ASVE type.

The samples of non-ground soybeans were placed in 250 mL glass measuring cylinders and placed centrally in the heating chamber of the device. Nine variants of seed micronization were prepared, in which three processing times (60, 120 and 180 s) corresponded to each of three radiation power levels (350, 500 and 650 W).

Autoclaving was performed for 7200 s applying water vapour pressure corresponding to the processing temperature of 121°C. After thermal processing, the beans were cooled at room temperature.

Tests for trypsin inhibitior activity (TIA) [Kakade *et al.*, 1974] and bromocresole purple index (BCPI) [Szmigielski, 1999; 2002] were carried out for crude and every thermally--processed sample (in five independent replications).

The essence of the BCPI method is the measurement of the amount of acid-base indicator (5',5"-dibromo-3',3"--dimethylphenolsulphophtaleine – the so-called bromocresole purple) that is bound by 1 g of properly ground seeds exposed to a working solution containing that active substance.

A working solution at 0.13 mg/mL concentration (applied for BCPI determinations) was prepared in a measuring flask (1 L capacity) by dissolving 130 mg of bromocresole purple (Merck 1992/93 No 3025) in 40 mL of 0.1 mol/L NaOH, adjusting to 1 liter with 0.1 mol/L HCl and stirring to reach a homogenous concentration in the whole volume.

Seed samples were ground and sieved through a 0.2-mm mesh and 100-mg aliquots were placed in conical flasks (100 mL capacity), 50 mL of bromocresole purple solution was added (0.13 mg/mL concentration) and stirred for 30 min using a magnetic stirrer. The solution was then centrifuged for 5 min at 44.8xg and 1 mL of an extract was placed in a tube containing 20 mL of 0.02 mol/L NaOH.

The solution was stirred and after 10 min absorbance was measured at 589 nm wavelength against distilled water as a reference (A_b – equation 1).

The absorbance of the working solution was measured analogously (A_0 – equation 1), but instead of an extract (after centrifuging) 1 mL of working solution (0.13 mg/mL concentration) was taken.

The amount of adsorbed active substance (bromocresole purple) was calculated using Equation 1 as the difference between its quantity in the solution before and after contact with the ground seed sample:

$$BCPI = (A_0 - A_b) \times C \times V / A_0 \times M \quad (1)$$

where: BCPI – amount of absorbed dye ($mg/g_{d.m}$); A_0 – absorbance of the mixture achieved due to mixing 1 mL of working solution with 20 mL of 0.02 mol/L NaOH; A_b – absorbance of the mixture achieved due to mixing 1 mL of extract (after centrifuging) with 20 mL of 0.02 mol/L NaOH; C – dye solution concentration (mg/mL) (0.13 mg/mL); V – volume of dye solution added to the sample (mL) (50 mL); M – weight of the sample subjected to tests (g), (0.1 g).

The results obtained for the raw sample and the autoclaved sample provided a basis for calculating the sensitivity and detectability of the TIA and BCPI methods. Their sensitivity (χ) was determined by the absolute value of the directional coefficient for linear regression equation (the value of the test in the function of bean heating), based on the results for raw beans and those autoclaved for 120 min at a temperature of 121°C [Gawęcki & Wagner, 1982; Szmigielski *et al.*, 2001]. The method's detectability (ω) was expressed by the detectable minimum (δ) and calculated as the shortest time of thermal treatment necessary to change the measured value by a number equal to the maximum relative error of determination [Szmigielski *et al.*, 2001]. The maximum relative error of determination was calculated with the use of the differential calculus method [Sielanko & Sowa, 1994], with the maximum result of determination as the basis for calculations [Szmigielski *et al.*, 2001].

In order to calculate β, the parameters being measured were described using function equations (TIA – according to the procedure from PN-90/R64816, BCPI – according to equation 1). The variables being directly measured were identified in these equations and their maximum errors resulting from the class of devices were estimated.

Following this, according to standard procedure [Sielanko & Sowa, 1994], partial derivatives from the function (TIA, BCPI) for every variable measured were determined, and differentials of variables were replaced with estimated maximum errors in equations and were finally summed up. The sum was divided by the mean value achieved for the maximum result of every measurement obtaining β.

The samples' distinguishability (ρ) was evaluated for the samples that had undergone microwave processing, defined as the significance of the differences between the values of the tests for the samples, and expressed as the percent of the significant relations in reference to all the tested ones. The significance of the differences for determination results was established by analysing the variances (with a 5% level of significance) and marking the lowest Tukey differences – NIR [Oktaba, 1986].

On the basis of the results for crude seeds and all samples subjected to microwave processing, the correlation coefficient between TIA and BCPI traits as well as single-factor regression coefficients were calculated (TIA=f(BCPI)) [Oktaba, 1986].

RESULTS AND DISCUSSION

The study described in this paper used samples subjected to micronization under conditions of radiation emission of up to 2340 J/g energy, which favours the comparison of TIA and BCPI methods in a wide range of heating inten-

sity (from crude to excessively heated). According to the opinions of Rackis *et al.* [1986] and Rajko *et al.* [1997], reduction of about 95% of trypsin inhibitor activity (TIA) by crude soybean seeds requires the absorption of energy of not less than 1670 J/g. However, a study of Petres *et al.* [1990] on rats showed that the achievement of optimum nutritional value of soybean seed protein requires a 79–87% reduction of the initial TIA level.

In studies on soybean milk, Hackler *et al.* [1965] and Kwok *et al.* [1993] considered 90% of initial trypsin inhibitory activity as optimum to ensure the maximum protein nutritional value.

Microwave processing allows significant alterations in trypsin inhibitor activity of soybeans (Table 1). The difference between the result extremes (obtained for the raw sample and the sample exposed to 650 W – radiation for 180 s) is 44.52 TUI/$mg_{d.m.}$, which is almost 12 times higher than the lowest result.

The changes of TIA were to a great extent proportional to the intensity of heating, which contributed to the very high sample distinguishability (ρ=100%). A drawback of marking with this method is its high time-consumption and low precision.

The fall in the precision of TIA determination is particularly visible for samples with high heating intensity (Tables 1 and 3). Szmigielski and Matyka [2002] drew similar conclusions when testing the TIA of autoclaved bean seeds and analogous results were achieved by Petres *et al.* [1990] who studied micronized soybean seeds.

The value of bromocresole purple index for the examined soybean samples was significantly altered (Table 2), which was caused by the micronization of the beans. The BCPI increase (between the raw sample and the sample exposed to 650 W radiation for 180 s) was 35.48 mg/$g_{d.m.}$, which represents 150% of the lowest result. BCPI changes turned out to be to a great extent proportional to the thermal processing intensity of the beans, which distinguished 35 of the 36 compared pairs of results (ρ=97.22%, Table 3). The advantage of BCPI (unlike with TIA) is its low time-consumption and high precision of marking of both high and low heating intensity samples.

Moreover, the two methods differ (in favour for BCPI) in respect to the maximum relative error (β_{BCPI}=2.32%, and β_{TIA}=7.63%) and the detectable minimum (δ_{BCPI}=273.96, and δ_{TIA}=612.02 s), whereas the value of sensitivity was similar for BCPI and TIA (Table 3). Similar results were obtained in earlier experiments carried out by Szmigielski

TABLE 3. The criteria of comparing the traits (BCPI and TIA) for soybean samples.

The criteria of comparing traits	BCPI	TIA
Time-consumption (η) (h)	1.5	4.5
Sensitivity (χ)	0.30	0.36
Maximum relative error of determination (β) (%)	2.32	7.63
Precision of determination (CV)	0.12–1.76	0.28–28.41
Distinguishability (ρ) (%)	97.22	100
Detectability (ω) (δ)	273.96	612.02
Interdependence of the results (r)	-0.99	

TABLE 1. Trypsin inhibitor activity (TIA) for soybean seed samples (TUI/$mg_{d.m.}$).

Thermal processing	Parameters of thermal processing			
	Radiation power (W)	Thermal processing time (s)		
		60	120	180
Micronization	350	43.02 ± 0.40 (0.93)	39.26 ± 0.87 (2.21)	33.36 ± 0.42 (1.25)
	500	35.41 ± 0.82 (2.32)	25.80 ± 0.53 (2.06)	17.28 ± 0.31 (1.80)
	650	28.20 ± 0.08 (0.28)	15.38 ± 0.42 (2.74)	3.78 ±1.07 (28.41)
Autoclaving			4.89 ± 0.53 (10.84)	
Control			48.30 ± 0.94 (1.95)	

$NIR_{micronization}$=1.78

TABLE 2. Bromocresole purple index (BCPI) for soybean seed samples (mg/$g_{d.m.}$).

Thermal processing	Parameters of thermal processing			
	Radiation power (W)	Thermal processing time (s)		
		60	120	180
Micronization	350	26.78 ± 0.21 (0.78)	31.46 ± 0.43 (1.38)	34.69 ± 0.16 (0.45)
	500	32.62 ± 0.15 (0.46)	39.25 ± 0.28 (0.71)	44.90 ± 0.22 (0.49)
	650	38.08 ± 0.12 (0.32)	45.58 ± 0.41 (0.89)	50.05 ± 0.06 (0.12)
Autoclaving			58.11 ± 1.02 (1.76)	
Control			22.63 ± 0.37 (1.63)	

$NIR_{micronization}$=0.73

et al. [2001] on Progres variety soybeans subjected to autoclaving at a temperature of 121°C for times ranging from 10 to 120 min.

High interdependence of TIA and BPCI methods, measured by the coefficient of correlation (r=-0.99; Table 3), indicates a possibility of mutual replaceability of these traits for micronized soybeans and enables recalculation of the obtained results.

A high correlation coefficient r was also obtained by Masłowski *et al.* [2001] when examining samples of soybeans autoclaved at a temperature of 121°C.

What is particularly vital, is the possibility of replacing the laborious, time-consuming and expensive TIA test with the simple, quick and cheap BCPI (Table 3). The highly--matching coefficient (R^2=97.63) of the trend line (TIA= -1.65×BCPI+89.98) to experimental data confirms (Figure 1) the credibility of the result transformation for the tested samples.

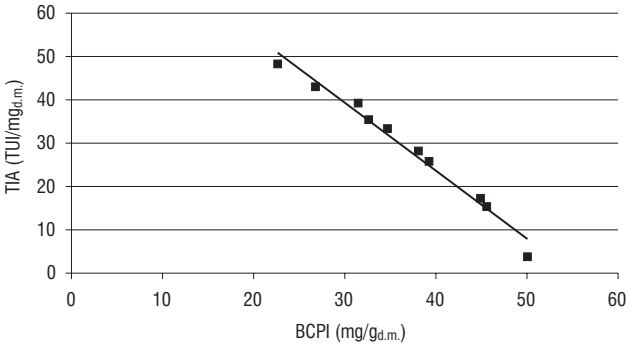

FIGURE 1. Trypsin inhibitor activity (TIA) of micronized soybean seeds as a function of bromocresole purple index (BCPI)/TIA= =- 1.65xBCPI + 89.98, R^2 = 97.63.

CONCLUSIONS

1. Bromocresole purple index (BCPI) is useful in evaluating the micronization intensity and trypsin inhibitor activity of soybeans.

2. Due to the high interdependence of the results obtained by BCPI and TIA methods, as well to the high sensitivity, detectability, distinguishability and low time--consumption, the BCPI test turned out to be helpful in the quick evaluation of trypsin inhibitor activity of micronized soybeans.

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Received February 2003. Revision received February and accepted August 2003.

ZASTOSOWANIE WSKAŹNIKA PURPURY BROMOKREZOLOWEJ DO OCENY WPŁYWU OBRÓBKI MIKROFALOWEJ NA AKTYWNOŚĆ ANTYTRYPSYNOWĄ NASION SOI

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Sprawdzono możliwość zastosowania wskaźnika purpury bromokrezolowej (BCPI) do oceny aktywności inhibitora trypsyny (TIA) dla prób nasion soi poddanych obróbce mikrofalowej (promieniowaniem o częstotliwości roboczej 2450 MHz). Przygotowano dziesięć wariantów intensywności mikronizacji nasion, w których każdemu z trzech poziomów mocy promieniowania (350, 500 i 650 W) odpowiadają trzy czasy obróbki (60, 120 i 180 s), zaś jedną z prób pozostawiono bez obróbki termicznej. Mikronizacja nasion spowodowała obniżenie TIA (tab. 1): z 48,3 TUI/$mg_{s.m.}$ (dla próby surowej) do 3,78 (dla próby ogrzewanej przez 180 s przy mocy 650W), oraz wzrost BCPI (tab. 2) z 22,63 mg/$g_{s.m.}$ (dla próby surowej) do 50,05 mg/$g_{s.m}$. (dla próby ogrzewanej przez 180 s przy mocy 650 W). Ze względu na dużą współzależność wyników uzyskanych metodami BCPI i TIA (współczynnik korelacji r=-0,99; współczynnik dopasowania R^2=97,63 linii trendu TIA = -1,65xBCPI + 89,98 do danych doświadczalnych), a także dużą czułość (χ=0,30), wykrywalność (δ=273,96s), rozróżnialność (ρ=97,22%) oraz małą czasochłonność (η=1,5h), test BCPI okazał się przydatny do szybkiej oceny aktywności antytrypsynowej mikronizowanych nasion soi.