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EFFECT OF AUTOCLAVING IN SEEDS PROCESSING ON THE ACTIVITY OF NEUROTOXINS OF CHICKLING VETCH DETERMINED WITH THE USE OF BROMOCRESOLE PURPLE INDEX (BCPI)

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Key words: chickling vetch, seeds, autoclaving, neurotoxin, bromocresole purple index (BCPI), regression equation

A study was undertaken to examine the usability of a new analytical method, referred to as bromocresole purple index (BCPI), for the evaluation of neurotoxins activity in autoclaved seeds of chickling vetch (determined as converted into β-N-oxalyl-L alanine - BOAA).

The experimental material were seeds of chickling vetch of Polish cultivar Krab, autoclaved under conditions of one of the ten intensity variants of that process. In the process, three periods of seeds exposure (10, 20 or 30 min) correspond to three level of thermal treatment (112°C, 121°C or 134°C). One sample was left untreated (*i.e.* without heating). Each of the mentioned samples was analysed with the BCPI and BOAA methods. A statistical analysis of the experimental results indicates that the BCPI and BOAA methods are co-dependent in the examination of autoclaved chickling vetch seeds (r=86.34%), and that both the parameters may be linked with hypothetical mathematical functions TIA = f(BOAA). The usability of those functions in the analysis was confirmed statistically by the obtained high coefficients of fit of their equations to the actual experimental data (R^2 ranging from 83.79% in a linear equation to 94.09% in a polynomial quartic equation).

ABBREVIATIONS AND NOTATIONS

BCPI – bromocresole purple index; $BCPI_{B.S.M.}$ – bromocresole purple index (converted into g of protein in dry matter); BOAA – neurotoxins activity; CV – variability coefficient; χ – test sensitivity; TKM – thousand kernel mass; NIR – the least significant difference; π – precision of assays; r – coefficient of correlation; R^2 – coefficient of fit; ρ – distinguishability; SD – standard deviation; τ- time-consumption of assays.

INTRODUCTION

Various forms and methods of heating constitute the oldest and, simultaneously, the simplest means of preparing chickling vetch seeds for consumption. The contribute to the suppression of the activity of antinutrients in seeds and increase bioavailability of their dietary constituents [Grela *et al.*, 1997; Rotter *et al.*, 1990].

The effect of different forms of heating on the dynamics of changes in the activity of various antinutritional compounds and other quality indicators in grain legume seeds as well as the modeling of those changes (with consideration given to some number of selected parameters) have long been the key issue addressed in investigations carried out in the subject of agricultural and food processing [Petres & Czukor, 1989; Petres *et al.*, 1990; Rayko *et al.*, 1997].

However in the description of objects those studies, of necessity, use a finite number of factors affecting properties of a sample, and hence are not universal since their results may be applied in the description of changes in properties of a product under conditions of industrial processing only to a limited extent (due to variety and complexity of factors of such processes as *e.g.* extrusion, granulation or expansion). A universal and potentially applicable under conditions of industrial processing seems to be only a mathematical formula (function or algorithm) which by combining the level of selected properties of processed seeds (*e.g.* activity of antinutrients) with other, independent factors will enable direct read out or calculation of a value constituting a basis for the qualitative assessment of those seeds (irrespective of the method of thermal treatment used in the processing).

The study was aimed at determining usability of a new analytical method, referred to as bromocresole purple index (BCPI) in the evaluation of the activity of neurotoxins (BOAA) in autoclaved seeds of chickling vetch as well as at elaborating a mathematical formula for Reading out the level of BOAA activity based on the calculated BCPI value.

MATERIAL AND METHODS

The experimental material were seeds of chickling vetch of a Polish cultivar Krab with the thousand kernel mass (TKM) reaching 180.40 g (containing on average: 87.91% of dry matter as well as 26.9% of total protein and 1.32% of crude fat on dry matter basis). The thousand kernel mass, contents of total protein, crude fat and dry matter were determined according

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Polish Standards [PN/R-65950:1994, PN-ISO 6496:2002, PN–75/A–04018, PN–ISO 6492:2005] in three independent replications each.

Samples of seeds (25 g) were transferred to 250-mL glass vessels and fixed (separately, independently of one another) in a geometric centre of an ASVE laboratory autoclave, then each sample was autoclaved under conditions of one of the intensity variants of that process, in which three periods of seeds exposure (10, 20 or 30 min) correspond to each of the three temperatures of processing (112°C, 121°C or 134°C). One sample was left untreated (without autoclaving).

Each of the above-described samples was determined (in three independent replications) for: the activity of neurotoxins (BOAA) according to Briggs *et al.* [1983] and bromocresole purple index (BCPI) according to Szmigielski & Matyka [2004].

The essence of the BCPI method is measurement of the content of dye (5', 5"-dibromo, 3', 3"–dimethyl–phenylsulfophthalein – commonly called bromocresole purple) that is bound by gram of comminuted seeds subjected to analyses. A solution of the dye with a concentration of 0.13 mg/mL was prepared by solving 130 mg of bromocresole purple in 40 mL of a sodium base (NaOH) solution with a concentration of 0.1 mol/L and supplementing with a solution of hydrochloric acid (HCl) with a concentration of 0.1 mol/L to a volume of 1 L.

Samples of seeds used in the experiment were ground, sieved through a screen with mesh side of 0.2 mm, and then 100-mg weighed portions were treated with 50 mL of a bromocresole purple solution (concentration of 0.13 g/mL), stirred for 30 min, and centrifuged for 5 min (at 3000 rad/min). Next, 1 mL of a clear extract was transferred to a test tube with 20 mL of NaOH solution with a concentration of 0.02 mol/L and after 10 min absorbance was measured at a wavelength of 589 nm on a Spekol EK 1 photoelectric spectrophotometer (Karl Zeiss Jena) against distilled water as a reference material.

Absorbance of a working solution was measured analogously, yet 1 mL of a bromocresole purple (concentration of 0.13 mg/mL) was taken instead of the extract.

Sorption of dye on the surface of samples was calculated according to the formula:

$$S = (E_o - E_b)'C'V/ E_o{}^* m$$

where: S – quantity of dye adsorbed ($mg/g_{d.m.}$), (numerically equal to BCPI value); E_o – absorbance of a working solution; E_b – absorbance of extract; C – concentration of a dye solution (0.13 mg/mL); V – volume of a dye solution (mL); and m – sample mass (g).

The value of BCPI is numerically equal to the quantity of bromocresole purple adsorbed by a 1-g sample of ground seeds ($mg/g_{d.m.}$) or is converted into 1 g of protein in dry matter of seeds ($mg/g_{\text{d.m. protein}}$).

Results of those analyses (BCPI and BOAA) were elaborated with methods of mathematical statistics, their correlation coefficient (r) was calculated, which enabled verification of a hypothesis on the correlation of those analyses [Oktaba, 1986]. Once the coefficient appeared to be high, a number of hypothetical functions in the form of BOAA = f(BCPI) were worked out that made it possible to combine and reciprocal re-calculation of results of those assays.

Reliability of those hypothetical mathematical formulae was evaluated by calculating coefficient of determination (R^2) of the proposed functions to the actual experimental data [Oktawa, 1986].

Distinguishability of samples (ρ) was determined as the significance of differences between mean values of each trait examined (BOAA and BCPI) for each analysed sample and expressed (separately for each of those traits) as a per cent of significant relations in respect of all tested ones [Szmigielski, 2004]. The significance of differences between results obtained was estimated by means of an analysis of variance (at a significance level of 5%) and by determining the lowest significant differences of Tukey's - NIR [Oktaba, 1986].

Precision of assays (π) carried out with the methods of BCPI and BOAA was determined by calculating the percentage of standard deviations (SD) on the mean value of each trait for each sample examined.

RESULTS AND DISCUSSION

Seeds of chickling vetch of a Polish cultivar Krab were characterised by a relatively low activity of neurotoxins (2.51 $mg/g_{d.m.}$, Table 1) and in this respect were similar to results obtained by Desphande & Campbell [1992] for Canadian cultivars, by Briggs *et al.* [1983] as well as by Szmigielski & Matyka [2005], and reaching levels typical of crops cultivated in the temperate climate zone (*i.e.*, *ca.* 50% of the maximum activity of neurotoxins in seeds of chickling vetch [Aletor *et al.*, 1994]). It is worth emphasizing that clinical disease symptoms referred to as lathyrism are induced by monotonous diet con-

TABLE 1. Activity of neurotoxins (BOAA), (mg/gd.m.) in autoclaved samples of chickling vetch cv. Krab.

Thermal treatment	Parameters of thermal treatment			
	Temperature (°C)	Exposure time (s)		
		600	1200	1800
Autoclaving	112	2.05 ± 0.09 (4.39)	1.87 ± 0.06 (3.21)	1.35 ± 0.08 (5.92)
	121	1.67 ± 0.08 (4.79)	1.53 ± 0.07 (4.58)	1.20 ± 0.09 (7.50)
	134	1.38 ± 0.09 (6.52)	1.16 ± 0.10 (8.62)	1.05 ± 0.15 (14.30)
Control	2.51 ± 0.07 (2.79)			

values in brackets represent coefficients of variability of the results obtained

TABLE 2. Bromocresole purple index (BCPI) of autoclaved seeds of chickling vetch cv. Krab ($mg/g_{protein\ in\ d.m.}$).

Thermal treatment	Parameters of thermal treatment			
	Temperature (°C)	Exposure time (s)		
		600	1200	1800
Autoclaving	112	54.85 ± 2.46 (4.48)	63.88 ± 2.75 (4.30)	70.83 ± 1.98 (2.80)
	121	82.73 ± 2.88 (3.48)	87.98 ± 1.90 (2.16)	93.40 ± 3.64 (3.89)
	134	91.94 ± 3.06 (3.33)	94.86 ± 4.21 (4.44)	98.44 ± 3.83 (3.89)
Control	49.60 ± 3.38 (6.81)			

values in brackets represent coefficients of variability of the results obtained

TABLE 3. Activity of neutrotoxins (BOAA) as a function of bormocresole purple index BCPI for autoclaved seeds of chickling vetch cv. Krab.

Dependent variable	Independent variable	Correlation coefficient (r)	Type of equation		Equation	Determination coefficient (R^2) (%)
BOAA	BCPI	86.34	Polynomial	II°	$BOAA = 0.0141(BCPI)^2 - 0.293(BCPI) + 2.646$	89.38
				III°	$BOAA = - 0.0048(BCPI)^3 + 0.0931(BCPI)^2 – 0.6575(BCPI) + 3.057$	93.16
				IV°	$BOAA = 0.001(BCPI)^4 - 0.0274(BCPI)^3 + 0.2587(BCPI)^2 - 1.1101(BCPI) + 3.41$	94.09
			Logarithmic		$BOAA = -0.5989 Ln(BCPI) + 2.48$	92.52
			Power		$BOAA = 2.6028(BCPI)^{-0.335}$	89.36
			Exponential		$BOAA = 2.4336 e^{-0.0853 (BCPI)}$	87.94
			Linear		$BOAA = - 0.138(BCPI) + 2.336$	83.79

sisting of food products that contain at least ca. 0.1% BOAA [Roy & Narasinga Rao, 1968 after Quareshi *et al.*, 1977].

The autoclaving of seeds contributed to a considerable decrease in the activity of neurotoxins. Seeds of chickling vetch subjected to autoclaving under conditions of the highest intensity of that process (*i.e.* at 134°C for 30 min) were characterised by neurotoxins activity at a level of *ca*. 40% of their initial level (*i.e.* in reference to raw seeds), yet the obtained level of the activity of that antinutritional compound could be acknowledged as safe – preventing the risk of lathyrism incidence [Roy & Narasinga Rao, 1968 after Quareshi *et al.*, 1977].

It should be noticed, however, that in the reduction of neurotoxins activity in chickling vetch of key significance is, probably, obtaining an appropriate level of processing temperature (and not its time span), since a similar reductive effect of *ca*. 55% of the initial activity of neurotoxins was reported by Rotter *et al.* [1990] already after autoclaving for 120 min at a temperature of 121°C. In turn, short merely 3-min microwave heating (at an increasing temperature exceeding 150°C at the end of the process) evoked a reduction in the activity of that antinutrient to as little as *ca*. 30% of its initial level [Szmigielski *et al.*, 2005].

Those results point to a considerable efficiency of HTST type technological treatments (high temperature short time) in reducing the activity of neurotoxins in chickling vetch, though they require confirmation in further research programmes.

A typical traits of assays carried out with the BOAA method is diminished precision of determinations along with an increase in the intensity of the autoclaving process (Table 1), which undoubtedly contributed to decreased distinguishability

TABLE 4. Time-consumption, distinguishability and precision of analytical methods used in the evaluation of autoclaving efficiency of chickling vetch seeds.

Compared parameter	Analytical method	
	$BCPI_{P.D.M.}$ ($mg/g_{protein\ in\ d.m.}$)	BOAA ($mg/g_{d.m.}$)
τ (h)	1.5	12.0
ρ (%)	73.33	73.33
π (Cv), (%)	2.16–6.81	2.79–14.30

of the samples (most of all in analyses of seeds subjected to intensive heating – Table 1 and 4). Similar findings were also reported by Szmigielski *et al.* [2005] in analyses of chickling vetch seeds subjected to microwave heating with various intensity.

The value of the bromocresole purple index (BCPI) odetermined for raw seeds of chickling vetch fluctuated at a level similar to that reported in literature [Szmigielski *et al.*, 2005; Szmigielski & Matyka, 2004 (after conversion into mass unit of protein in dry matter of seeds)]. The autoclaving process of seeds contributed to a significant increase in their BCPI values, namely samples heated under conditions of the highest process intensity, *i.e.* at 134°C for 30 min, were characterised by nearly two and a half higher BCPI values than the raw seeds (Table 2). An upward tendency of BCPI values along with increasing intensity of thermal treatment was also observed in all available reports describing such analyses [Szmigielski *et al.*, 2005; Szmigielski & Matyka, 2004].

In contrast to results of BOAA analyses, precision of BCPI assays remained at a similar level (not exceeding *ca.* 7%, Table 2), which has inevitably contributed to greater distinguishability of the samples (r_{BCPI} = 73.33%). The similarity of results obtained with the BCPI method occurs, most of all, as a result of incidental similarity of properties of the autoclaved seeds, yet the phenomenon of results concurrence occurs seldom in some interval of heating intensity, *e.g.* in the BOAA method – in the case of seeds subjected to intensive heating (Table 1 and 2).

It should be noted, however, that dispersion of results in the reported study (autoclaving) was considerably lower as compared to that obtained by Szmigielski *et al.* [2005] who applied microwave heating of samples. This may point to a higher stability of properties of the autoclaved samples in contrast to those microwave-treated, yet it needs confirmation in further research on that matter.

Both traits examined (BOAA, BCPI) appeared to be very highly correlated (Table 3), which indicates a high correlation of results of those assays for autoclaved seeds of chickling vetch and finds confirmation in earlier investigations of Szmigielski *et al.* [2005] carried out on microwave-heated samples.

High determination coefficients of hypothetical equations of regression in the form BOAA = f(BCPI), (Table 3) enabled relatively reliable evaluation of neurotoxins activity in chickling vetch (BOAA) based on determinations carried out with the BCPI method and substitution of time- and labour consuming analyses (BOAA) with a rapid, simple and sensitive test (BCPI), (Table 4). A detailed analysis of the forms of those regression equations (Table 3) indicates their high affinity to similar functional correlations referring to analogous equations postulated by Szmigielski *et al.* [2005] for microwave-heated samples of chickling vetch.

CONCLUSIONS

The results obtained in the study point to universality of the introduced regression equations, *i.e.* to the possibility of their application in any thermal treatment of chickling vetch seeds, also to that in which due to a variety of factors affecting the sample (*e.g.* extrusion, granulation, expansion) and limited calculation capacities of computers used, multifactorial, complicated equations of regression considering only a few such factors affecting properties of processed seeds prove unreliable. Confirmation of those results, however, need undertaking extensive research in that field.

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WPŁYW AUTOKLAWOWANIA, W PROCESIE PRZETWÓRSTWA NASION, NA AKTYWNOŚĆ NEUROTOKSYN LĘDŹWIANU SIEWNEGO OZNACZANYCH Z ZASTOSOWANIEM WSKAŹNIKA PURPURY BROMOKREZOLOWEJ (BCPI)

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Sprawdzono przydatność nowej metody analitycznej, nazwanej wskaźnikiem purpury bromokrezolowej (BCPI), jako nowego sposobu oceny aktywności neurotoksyn w autoklawowanych nasionach lędźwianu siewnego (oznaczanych w przeliczeniu na β-N-oxalyl-L alaninę - BOAA).

Obiektem badań były nasiona lędźwianu siewnego polskiej odmiany Krab autoklawowane w warunkach jednego z dziesięciu wariantów intensywności tego procesu, w których każdemu z trzech poziomów temperatury obróbki (112°C, 121°C lub 134°C) odpowiadają trzy czasy ekspozycji nasion (10, 20, lub 30 minut), zaś jedną z prób pozostawiono w pierwotnej formie (tj. bez ogrzewania). Każdą z wymienionych prób poddano badaniom metodą BCPI oraz BOAA. Statystyczne opracowanie danych doświadczalnych wskazuje, że metody BCPI i BOAA są współzależne w badaniu autoklawowanych nasion lędźwianu siewnego (współczynnik korelacji – r wynosi 86,34%), a ponadto, że obie te cechy można powiązać hipotetycznymi funkcjami matematycznymi TIA = f(BOAA). Przydatność tych funkcji w analizie potwierdzono statystycznie, uzyskując wysokie współczynniki dopasowania ich równań do rzeczywistych danych doświadczalnych (R^2 = od 83,79% - równanie liniowe do 94,09% - równanie wielomianowe IV^{o}).