Precision of laboratory methods based on protein solubility in quality control of heat treated feedstuffs

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Abstract

Some feedstuffs used as raw materials in feed industry contain anti-nutritional factors that negatively influence their quality. One of them is soybean, which is, prior to oil extraction, referred to as full-fat soybean (FFSB). Anti-nutritional factors in raw FFSB can be destroyed by moderate heating, but both over- and under-heat processing limits the availability of soybean amino acids. Among laboratory procedures that are available for assessing the degree of FFSB heat treatment, two methods – protein dispersibility index (PDI) and protein solubility in potassium hydroxide (PSKOH) – are based on protein solubility, which is claimed to be the most reliable indicator of the degree of FFSB heat treatment. This paper presents the results of an inter-laboratory study conducted to establish precision of the PDI and PSKOH methods by determining their reproducibility limits. Five samples of FFSB were heat-treated at temperatures between 110 and 164 °C and analysed by six laboratories for PDI and PSKOH. Established reproducibility limit for PDI method of 8.87 index units found in this study appeared to be too wide, indicating a low precision of this method. PSKOH method produced a very good reproducibility limit of 8.56% and could be recommended as the preferred method for FFSB quality control in feed laboratories.

Keywords: full-fat soybeans; heat treatment; protein solubility.

Available online at the Journal website: http://www.ache.org.rs/HI/

PROFESSIONAL PAPER

UDC 633.34:543.384:543.6

Hem. Ind. **66** (1) 53–57 (2012)

doi: 10.2298/HEMIND110623054P

Some feedstuffs used as raw materials in feed industry contain compounds with anti-nutritional factors (ANFs) [1] that negatively influence their quality. One of those feedstuffs is soybean, one of the most important oilseed crop because of its highly valuable proteins and oils, which makes it good alternative to animal proteins and oils. Soybean prior to oil extraction is referred to as full-fat soybean (FFSB). Processing of the raw soybeans by means of heat destroys ANFs [2,3], but both over- and under-heat processing limits the availability of soybean amino acids [4]. Therefore, if full potential of FFSB as a feedstuff is to be realized, reliable analytical procedures need to be available to the feed industry for quality control of the adequacy of FFSB heat treatment.

There are a number of laboratory methods that can be used to estimate the degree of FFSB heat treatment [5]. Two of them, protein dispersibility index (PDI) and protein solubility in potassium hydroxide (PSKOH), are based on protein solubility, which was claimed to indicate soybean meal quality better than other methods [6].

It has been clearly illustrated [7,8] that the results of analysis of the same sample of FFSB obtained by currently available analytical techniques vary widely, causing uncertainty and confusion among soybean processors, feed manufacturers and end-users. The aim of this study was to determine the reproducibility limits of the PDI and PSKOH methods and make recommendation for their use in laboratory practice.

MATERIAL AND METHODS

Raw soybean was processed by dry extrusion at five temperatures: 110, 127, 140, 151 and 164 °C and analysed in duplicate by 6 laboratories for the PDI and PSKOH.

Protein dispersibility index (PDI) was determined according to the AOCS method [9]. Protein solubility in potassium hydroxide (PSKOH) was determined according to the method of Arabe and Dale [10] as modified by Palić [11].

Both methods were subjected to an inter-laboratory study, which was conducted according to the AOCS Collaborative Study Procedures [12]. Reproducibility limit for both methods was determined according to the AOCS procedure [13].

RESULTS

Values of PDI and PSKOH in FFSB samples treated at five different temperatures, and analysed by six laboratories are shown in Tables 1 and 2.

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Paper received: 23 June, 2011
Paper accepted: 29 July, 2011

Table 1. Values of PDI (index units)in heat treated FFSB samples (SD – standard deviation)

Lab no.	*t* / °C				
	110	127	140	151	164
1	38.21	25.77	9.67	7.25	6.21
	36.66	22.10	10.55	7.82	6.75
2	37.26	21.32	9.07	7.77	6.74
	36.15	21.46	9.27	7.30	6.09
3	47.63	26.39	8.91	7.27	4.09
	47.18	28.20	8.36	6.80	4.23
4	44.72	31.10	10.87	3.96	1.62
	48.42	30.10	8.51	4.57	2.43
5	45.50	19.83	9.55	8.45	8.66
	47.62	18.17	9.50	8.26	8.38
6	46.24	27.79	9.12	8.89	7.78
	45.61	28.58	8.92	8.23	8.10
SD	4.88	6.37	0.75	1.50	2.34

Table 2. Values of PSKOH (%) in heat treated FFSB samples (SD – standard deviation)

Lab no.	*t* / °C				
	110	127	140	151	164
1	89.74	84.7	74.68	68.27	57.84
	89.85	85.35	74.77	68.01	59.48
2	87.1	88.05	70.94	66.18	56.7
	87.42	88.2	71.65	66.53	57.34
3	93.57	86.57	75.4	72.47	61.62
	95.45	85.15	76.71	73.9	60.57
4	95.7	88.63	80.05	69.66	62.69
	95.88	87.73	78.02	71.99	63.97
5	89.72	87.01	67.37	64.83	59.94
	87.37	87.35	68.3	64.14	61.18
6	85.76	87.12	76.64	71.21	65.08
	83.54	88.96	76.64	71.8	65.55
SD	3.61	1.39	4.24	3.10	2.94

The influence of temperature of processing on PDI and PSKOH values is shown in Figures 1 and 2. The high correlation (R^2 = 0.94) has been established between PSKOH values and treatment temperature, whereas for PDI the correlation was lower (R^2 = 0.88).

The precision of a method is defined [13] by its reproducibility limit (*R*), which is the absolute difference between two single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment and it should not be greater than the value for *R* as calculated from the formulas in Tables 3 and 4.

DISCUSSION

It has been reported [14–16] that the temperatures for adequately treated soybean are in the range from 135 to 145 °C. In the previous studies, Palić and Coetzee [17] established that the range of values corresponding to these temperatures, *i.e.*, to adequately heat processed FFSB, were 8.50–10.30 index units for PDI and 67–77% for PSKOH.

With regard to that, reproducibility limit for PDI method of 8.87 index units (Table 3) found in this inter-laboratory study appears to be too wide, indicating a low precision of this method. On the other hand, PSKOH method produced very good reproducibility limit of 8.56%, taking into account the range (67–77%) for adequately processed soybean.

There is inconsistency in reports regarding comparison of laboratory methods for assessing the degree of FFSB processing. While Betal *et al*. [18] found that the PDI was better than the PSKOH method, Parson *et al*. [19] concluded that the PSKOH was a good indicator of FFSB protein quality. It should be noted that those

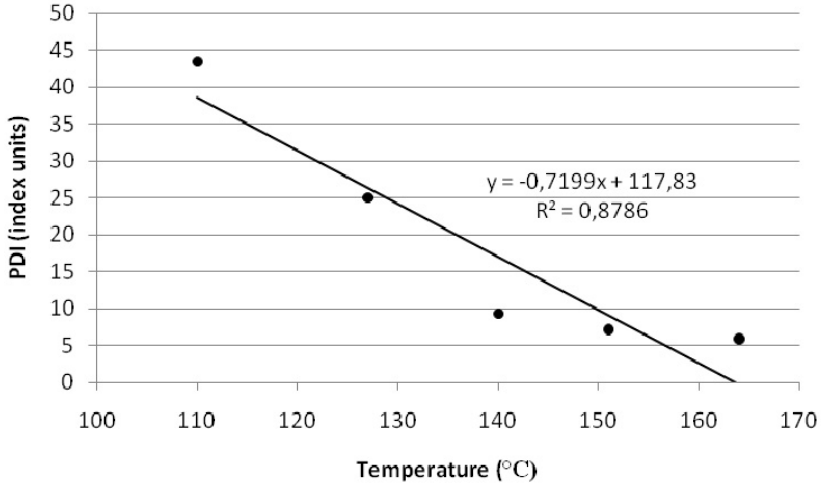

Figure 1. The influence of heat treatment temperature on PDI values (average of 6 laboratories).

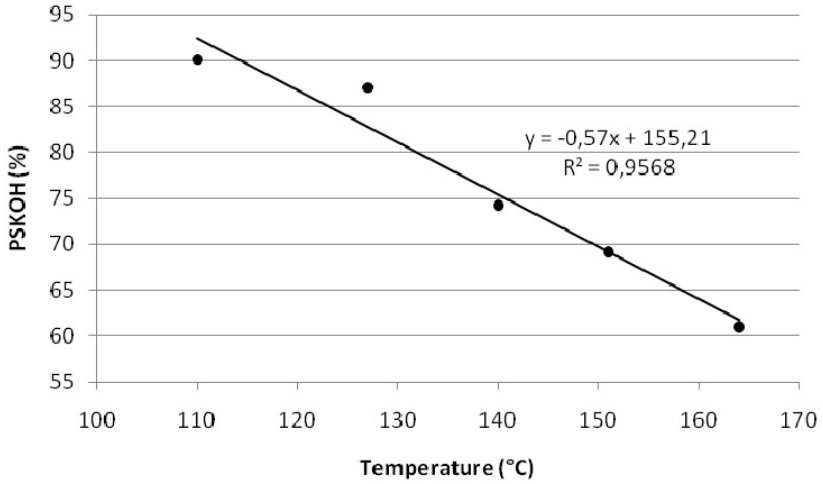

Figure 2. The influence of heat treatment temperature on PSKOH values (average of 6 laboratories).

Table 3. Precision parameters of PDI method

Parameter	*t* / °C					Average value
	110	127	140	151	164	
Number of laboratories	6	6	6	6	6	–
Number of laboratories eliminated as outliers	0	0	0	0	0	–
PDI values (index units), average for 6 laboratories	43.43	25.07	9.36	7.18	5.92	–
Reproducibility standard deviation (s_R), index units	4.883	6.371	0.746	1.504	2.338	3.168
Reproducibility limit ($R = 2.8s_R$), index units	13.67	17.84	2.09	4.21	6.55	8.87

Table 4. Precision parameters of PSKOH method

Parameter	*t* / °C					Average value
	110	127	140	151	164	
Number of laboratories	6	6	6	6	6	–
Number of laboratories eliminated as outliers	0	0	0	0	0	–
PDI values (index units), average for 6 laboratories	90.09	87.07	74.26	69.17	60.99	–
Reproducibility standard deviation (s_R), %	3.617	1.391	4.245	3.099	2.938	3.058
Reproducibility limit ($R = 2.8s_R$), index units	10.13	3.89	11.89	8.68	8.23	8.56

were the results of single-laboratory studies and that reports of inter-laboratory studies on PDI and PSKOH could not be found in accessible literature.

CONCLUSION

When precision is considered, the protein solubility in potassium hydroxide (PSKOH) method could be recommended as the preferred method for FFSB quality control in feed laboratories. Under conditions of this inter-laboratory study, the absolute difference between two single PSKOH results of the analysis of the same FFSB sample obtained in different laboratories should not exceed the reproducibility limit of 8.56%.

Acknowledgement

This study has been supported by the Protein Research Foundation of South Africa and the Ministry of Science of Republic of Serbia through the project III46012.

REFERENCES

[1] R.G. Maclachlan, Soybeans: Antinutrients – What? Why?, in Proceedings Workshop on Processing of Full Fat Soya, Irene, South Africa, 1998, pp. 10–12.

[2] P. Vohra, F.H. Kratzer, Evaluation of soybean meal determines adequacy of heat treatment, Feedstuffs **25** (1991) 22–26.

[3] N. Ruiz, F. De Belalcazar, G.J. Diaz, Quality control parameters for commercial full-fat soybeans processed by two different methods and fed to broilers, J. Appl. Poult. Res. **13** (2004) 443–450.

[4] C.M. Parsons, Assessment of nutritional quality of soy products for animals. In: Soy in Animal Nutrition, J.K. Drackley, (Ed.), Federation of Animal Science Societies, U.S.A., 2000, pp. 90–105.

[5] S. Monari, Fullfat Soya Handbook, 2nd ed., American Soybean Association, Brussels, Belgium, 1996, pp. 22–28.

[6] W.A. Dudley-Cash, PDI may better indicate soybean meal quality than other indices, Feedstuffs **73** (2001) 10–23.

[7] H. Davies, Processing of full-fat soybeans, in Proceedings Workshop on the processing of full fat soya, Irene, South Africa, 1998, pp. 21–23.

[8] D. Palić, J. Lević, S. Sredanović, O. Djuragić, Quality control of full-fat soybeans using urease activity: critical assessment of the method, Acta Periodica Technologica **39** (2008) 47–53.

[9] AOCS Official Methods, Sampling and Analysis of Oilseed By-products, Protein Dispersibility Index, AOAC Official Method Ba, 1997, pp. 11-–65.

[10] M. Araba, N.M. Dale, Evaluation of protein solubility as an indicator of over processing of soybean meal, Poultry Sci. **69** (1990) 76–83.

[11] D. Palić, Quality control of processed full-fat soybeans using protein solubility in KOH: Critical review and modification of the method, in Proceedings of 11th International Feed Technology Symposium, Vrnjačka Banja, Serbia, 2005, pp. 106–110.

[12] AOCS Official Methods, Collaborative Study Procedures, AOCS Procedure M 4-86, 1997.

[13] AOCS Official Methods, Determination of precision of analytical methods, AOCS Procedure M 1-92, 1997.

[14] A.I. Nelson, W.B. Wijeratne, S.W. Weh, T.M. Wei, L.S. Wei, Dry extrusion as an aid to mechanical expelling of oil from soybeans, J. Am. Oil Chem. Soc. **64** (1987) 1341–1347.

[15] Y. Zhang, C.M. Parsons, K.E. Weingartner, W.B. Wijeratne, Effect of extrusion and expelling on the nutritional quality of conventional and Kunitz trypsin inhibitor-free soybeans, Poultry Sci. **72** (1993) 2299–2308.

[16] D. Palić, F.K. Siebrits, S.E. Coetzee, Determining the optimum temperature for dry extrusion of full fat soybeans, S. Afr. J. Anim. Sci. **39** (Suppl. 1) (2009) 69–72.

[17] D. Palić, S.E. Coetzee, Protocol for using protein solubility as an indicator of full-fat soybean heat treatment, Acta Periodica Technologica **40** (2009) 71–77.

[18] A.B. Batal, M.W. Douglas, A.E. Engram, C.M. Parsons, Protein Dispersibility Index as an Indicator of Adequately Processed Soybean Meal, Poultry Sci. **79** (2000) 1592–1596.

[19] C.M. Parsons, K. Hashimoto, K.J. Wedeking, D.H. Baker, Soybean protein solubility in potassium hydroxide: an *in vitro* test of *in vivo* protein quality, J. Anim. Sci. **69** (1991) 2918–2924.

IZVOD

PRECIZNOST LABORATORIJSKIH METODA ZASNOVANIH NA RASTVORLJIVOSTI PROTEINA, KOJE SE KORISTE ZA KONTROLU KVALITETA TERMIČKI OBRAĐENIH HRANIVA

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(Stručni rad)

Neka od hraniva koja se koriste kao sirovine u industriji hrane za životinje sadrže anti-nutritivne faktore, koji negativno utiču na njihov kvalitet. Jedno od tih hraniva je punomasna soja (FFSB). Anti-nutritivni faktori sirove FFSB, kao što su tripsin inhibitor i ureaza, mogu se neutralisati termičkim tretmanom. Nedovoljno ili suviše termički obrađena soja će limitirati nivo amino kiselina dostupnih životinji. Postoje brojne laboratorijske metode koje se mogu koristiti za procenu adekvatnosti termičke obrade FFSB. Dve metode, indeks disperzije proteina (PDI) i rastvorljivost proteina u kalijum hidroksidu (PSKOH), su zasnovane na rastvorljivosti proteina, za koju se tvrdi da je jedan od najboljih pokazatelja za ocenu stepena termičke obrade, a time i za kontrolu kvaliteta termički obrađene FFSB. U ovom radu su predstavljeni rezultati međulaboratorijskog istraživanja sprovedenog da bi se utvrdila preciznost PDI i PSKOH metoda. Kao parametar preciznosti je određivana reproduktivnost (R) koja predstavlja apsolutnu razliku izmedju dva pojedinačna rezultata analize dobijenih korišćenjem iste metode na identičnom uzorku, u različitim laboratorijama, i od stane različitih analitičara. Pet uzoraka FFSB je termički obrađeno suvim ekstrudiranjem na temperaturama 110, 127, 140, 151 i 164 °C i analizirano u šest laboratorija na PDI i PSKOH. Za PDI metodu je utvrđena reproduktivnost R = 8,87 indeks jedinica, što ukazuje na nedovoljnu preciznost ove metode. PDKOH metoda je pokazala vrlo dobru reproduktivnost od R = 8,56 % uzimajući u obzir opseg (67–77%) koji karakteriše adekvatno termički obradjenu FFSB, te joj, sa stanovišta preciznosti, treba dati prednost u kontroli kvaliteta termički obrađene punomasne soje.

Ključne reči: Punomasna soja • Termička obrada • Rastvorljivost proteina