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PROTEIN DISPERSIBILITY INDEX AND PROTEIN SOLUBILITY IN POTASSIUM HYDROXID AS INDICATORS OF THE DEGREE OF FULL-FAT SOYBEAN HEAT TREATMENT

INDEKS DISPERZIJE BJELANČEVINA I RASTVORLJIVOST BJELANČEVINA U KALIJEVOM HIDROKSIDU KAO INDIKATORI STUPNJA TERMIČKE OBRADE PUNOMASNE SOJE

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SUMMARY

Soybean prior to oil extraction is referred to as full-fat soybean (FFSB). Raw FFSB contains anti-nutritional factors (ANFs), which can be destroyed by moderate heating. Both over- and under- heat processing will limit the level of amino acids available to the animal. There is a number of laboratory methods that can be used to estimate the degree of FFSB heat treatment. Two of them, Protein dispersibility index (PDI) and Protein solubility in potassium hydroxide (PSKOH), are based on protein solubility, which was 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 determine the repeatability limits of the PDI and PSKOH methods. Five samples of FFSB were processed by dry extrusion at temperatures between 110 °C and 164 °C and sent to 8 laboratories for PDI determination and to 9 laboratories for PSKOH analysis. The repeatability limit for the PDI method was found to be 2.31 index units, whereas for the PSKOH method the obtained repeatability limit was 3.77%. Both methods generated acceptable repeatability limits. Due to relatively expansive special blender required for the PDI analysis, which is not always available to laboratories, the preference in routine, every-day analysis should be given to the PSKOH method.

Key words: full-fat soybean, heat treatment, protein dispersibility index, protein solubility in potassium hydroxide, repeatability limit

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INTRODUCTION

Soybean prior to oil extraction is referred to as full-fat soybean (FFSB). Soybean is by far the most important oilseed crop in the world. As an animal feed, it is used as a high protein source (Wiseman, 1986). Raw FFSB contains anti-nutritional factors (ANFs) (Maclachlan, 1998), which limits its use in diets for monogastric animals.

A number of authors (Holmes, 1987; Ruiz et al., 2004; Zarkadas and Wiseman, 2005) showed that ANFs can be destroyed by moderate heating, which leads to denaturation of tertiary and quaternary protein structures, allowing more effective penetration of digestion enzymes and increases digestibility of soybean protein in non-ruminants.

The problem relating to the availability of the amino acids in the heat treated soybeans arises due to the fact that only an optimum level of heat treatment will produce maximal availability of the amino acids to the animal. Under-processing of the FFSB limits amino acid availability due to only partial destruction of the anti-nutrional factors (ANFs). Over-processing, on the other hand, decreases amino acid availability as a result of the Maillard reaction that occurs between aldehyde groups of sugar and free amino groups of amino acids (mostly epsilon-group of lysine) (Monary, 1996). The main objective of heat processing of FFSB is to achieve an optimum balance between degradation of ANFs and maintenance of amino acid availability.

There are a number of laboratory methods that can be used to estimate the adequacy of FFSB heat treatment. Commonly used methods for assessing the processed FFSB quality are those for determination of urease activity index (UAI), trypsin inhibitor activity (TIA), nitrogen solubility index (NSI), protein dispersibility index (PDI) and protein solubility in potassium hydroxide (PSKOH).

In a critical assessment of methods, Palic et al. (2007) concluded that protein solubility was the most reliable indicator for FFSB quality control and that therefore NSI, PDI and PSKOH would be the preferred methods. Since Batal *et al.* (2000) reported that the PDI displayed the most constant response to heating of FFSB, the preference in this study has been given to PDI over the NSI method.

It has been clearly illustrated (Davies, 1998; Palic, 2004) that the results of analysis of the same sample of FFSB obtained by currently available analytical techniques vary widely, causing uncertainty and confusion among full-fat soybean processors, feed manufacturers and end-users. The aim of this study was to determine the repeatability limits of the PDI and PSKOH methods and make recommendation for their use in practice.

METHODS AND MATERIAL

Raw soybeans, with moisture of 10-11%, were processed by dry extrusion, using industrial „Insta-Pro 2000R“ single screw extruder at 5 temperatures: 110, 127, 136, 145 and 164°C, with the processing time ranging between 30 and 40 seconds.

Protein dispersibility index (PDI) was determined according to the AOCS method (1997a). A “Hamilton Beach” Commercial Blender, model G936, (constructed according to the requirements of the AOCS method, 1997a) was used by all laboratories. Protein solubility in potassium hydroxide (PSKOH) was determined according to the method of Arabe and Dale (1990) as modified by Palic (2005). Both methods were subjected to an inter-laboratory study, which was conducted according to the AOCS Collaborative Study Procedures (1997). Five dry extruded FFSB samples were analysed in duplicate by 8 laboratories for the PDI and by 9 laboratories for the PSKOH. Repeatability limit (absolute difference between two single results of analysis of the same sample obtained in one laboratory) for both methods was determined according to the AOCS procedure (1997b).

RESULTS AND DISCUSSION

Results of the PDI determination in FFSB samples obtained by 8 laboratoris are shown in Table 1.

Results of the PSKOH determination in FFSB samples processed at different temperatures, obtained by 9 laboratoris are shown in Table 2.

Table 1. Results obtained by 8 laboratories on PDI determination (in duplicate) in FFSB samples processed by dry extrusion at different temperatures

Tablica 1. Rezultati dobiveni u 8 laboratorija pri određivanju PDI (u duplikatu) u uzorcima punomasne soje obrađenim suhim ekstrudiranjem na različitim temperaturama

Lab number Broj laboratorija	PDI (index units) - PDI (indeks jedinica)				
	110 ºC	127 ºC	136 ºC	145 ºC	164 ºC
1	38.21	25.77	11.62	8.42	6.21
	36.66	22.10	9.22	8.89	6.75
2	37.26	21.32	10.34	8.74	6.74
	36.15	21.46	10.01	8.81	6.09
3	47.63	26.39	9.64	8.79	4.09
	47.18	28.20	10.01	9.39	4.23
4	44.72	31.10	14.69	9.10	1.62
	48.42	30.10	10.64	8.86	2.43
5	45.5	19.83	11.88	10.08	8.66
	47.62	18.17	12.54	9.90	8.38
6	46.24	27.79	10.17	9.02	7.78
	45.61	28.58	10.18	8.70	8.10
7	41.80	25.40	10.10	8.60	7.20
	41.90	25.25	10.70	8.10	7.10
8	35.86	11.84	6.15	5.01	4.66
	36.80	12.25	6.81	5.42	5.39

Table 2. Results obtained by 9 laboratories on the PSKOH determination (in duplicates) in FFSB samples processed by dry extrusion at different temperatures

Tablica 2. Rezultati dobiveni u 9 laboratorija pri odredjivanju PSKOH (u duplikatu) u uzorcima punomasne soje obrađene na različitim temperaturama

Lab number Broj laboratorija	PSKOH (%)				
	110 ºC	127 ºC	136 ºC	145 ºC	164 ºC
1	89.74	84.70	77.50	67.04	57.84
	89.85	85.35	76.77	67.41	59.48
2	87.10	88.05	76.91	65.84	56.70
	87.42	88.20	76.19	66.49	57.34
3	93.57	86.57	77.70	69.63	61.62
	95.45	85.15	78.23	71.25	60.57
4	95.70	88.63	75.12	68.39	62.69
	95.88	87.73	78.40	67.44	63.97

Nastavak tablice na sljedećoj stranici

Nastavak tablice s prethodne stranice

Lab number Broj laboratorija	PSKOH (%)				
	110 °C	127 °C	136 °C	145 °C	164 °C
5	89.72	87.01	71.45	58.14	51.94
	87.37	87.35	74.13	57.22	50.18
6	85.76	87.12	76.13	72.41	65.08
	83.54	88.96	75.90	70.77	65.55
7	88.42	82.31	74.64	65.36	61.23
	88.71	82.35	73.73	65.36	60.01
8	94.18	92.48	77.70	70.63	68.61
	96.23	88.31	78.40	66.14	70.00
9	90.45	88.38	79.47	70.78	64.54
	88.93	84.99	78.74	68.23	61.46

Repeatability limit (r) is defined (AOCS Official Methods, 1997b) as the absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time which should not be greater than the value for r as calcualted from the formula in Tables 3 and 4.

Parameters for repeatability calculation of the PDI and PSKOH methods are shown in Tables 3 and 4.

Table 3. Repetability limit of the PDI method
Tablica 3. Granica ponovljivosti za PDI metodu

Parameter - Parametar	Sample - Uzorak					Average
	110 °C	127 °C	136 °C	145 °C	164 °C	Prosjek
Number of laboratories - Broj laboratorija	8	8	8	8	8	
Number of laboratories retained after eliminating outliers Broj laboratorija poslije elimininranja outliers	8	8	8	8	8	
PDI values (index units), average for 8 laboratories Prosjek PDI vrijednosti (indeks jedinice) za 8 laboratorija	42,35	23,47	10,30	8,49	5,96	
Repeatability standard deviation (s_r) Standardna devijacija ponovljivosti (s_r)	1,207	1,155	1,216	0,273	0,363	0,753
Repeatability relative standard deviation (RSD_r) Relativna standardna devijacija (RSD_r)	2,850	4,920	11,808	3,221	6,094	4,944
Repeatability limit (r) [$r = 2,8 \times s_r$] , index units Granica ponovljivosti (r) [$r = 2,8 \times s_r$], indeks jedinice	3,380	3,433	3,403	1,902	0,966	2,31

Table 4. Repetability limit of the PSKOH method
Tablica 4. Limit repetabilnosti za PSKOH metodu

Parameter - Parametar	Sample - Uzorak					Average
	110 °C	127 °C	136 °C	145 °C	164 °C	Prosjek
Number of laboratories - Broj laboratorija	9	9	9	9	9	
Number of laboratories retained after eliminating outliers Broj laboratorija poslije eliminiranja outliers	9	9	9	9	9	
PSKOH values (%), average for 9 laboratories Prosjek PSKOH vrijednosti (%) za 9 laboratorija	90,45	86,87	76,51	67,14	61,05	
Repeatability standard deviation (s_r) Standardna devijacija ponovljivosti (s_r)	1,073	1,407	1,085	1,380	1,107	1,242
Repeatability relative standard deviation (RSD_r) Relativna standardna devijacija (RSD_r)	1,187	1,620	1,418	2,056	1,814	1,621
Repeatability limit (r) [$r = 2,8 \times s_r$] , index units Granica ponovljivosti (r) [$r = 2,8 \times s_r$] , (%)	3,205	3,941	3,437	3,864	3,100	3,77

Repeatability limits for the PDI and PSKOH methods obtained in this study were 2.31 index units and 3.77% respectively. This means that, when the PDI method is used the absolute difference between two single results of analysis of the same sample obtained in one laboratory, should not exceed 2.31 index units. On the other hand, for the PSKOH method, the absolute difference between two single results of analysis of the same sample obtained in one laboratory, should not be greater than 3.77%.

CONCLUSIONS

Both the PDI and PSKOH methods generated acceptable repeatability limits. Absolute difference between two single results of analysis of the same FFSB sample in one laboratory using the PDI method should not exceed 2.31 index units. The PSKOH method also produced good repeatability limit of 3.77%. Due to relatively expensive special blender required for the PDI analysis, which is not always available to laboratories, the preferance in routine, every-day analysis should be given to the PSKOH method.

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SAŽETAK

Zrno soje prije ekstrakcije ulja naziva se punomasnom sojom. Sirova punomasna soja sadrži anti-nutritivne faktore, koji se mogu ukloniti termičkom obradom. Međutim, i nedovoljna i prekomjerna termička obrada dovodi do smanjivanja razine amino kiselina pristupačnih životinji. Postoji veći broj laboratorijskih metoda koje se koriste za utvrđivanje stupnja termičke obrade punomasne soje. Dvije od ovih metoda, indeks disperzije bjelančevina (PDI) i rastvrorljivost bjelančevina u kalijevom hidroksidu (PSKOH), temelje se na rastvorljivosti, bjelančevina za koje je utvrdjeno da su jedan od najboljih indikatora razine termičke obrade punomasne soje.

U ovom radu su prikazani rezultati inter-laboratorijskog ispitivanja u cilju odredjivanja ponovljivosti PDI i PSKOH metoda. Pet uzoraka sirove punomasne soje je termički obrađeno suhim ekstrudiranjem na temperaturama između 110 °C i 164 °C i analizirano u 8 laboratorija na PDI, a u 9 laboratorija na PSKOH. Za PDI metodu dobijena je ponovljivost od 2,31 indeks jedinica, dok je ponovljivost za PSKOH metodu bila 3,77%, te je za obje ispitivane metode dobivena prihvatljiva ponovljivost. Kako je za izvođenje PDI metode neophodan specijalni blender, koji nije uvijek dostupan laboratorijima, preporuka je da se u rutinskoj analizi kvaliteta obrađene punomasne soje koristi PSKOH metoda.

Ključne riječi: punomasna soja, termička obrada, indeks disperzije bjelančevina, rastvorljivost bjelančevina u kalijevom hidroksidu, granica ponovljivosti.