Laboratory Procedures for Assessing Quality of Soybean Meal

Rodica Caprita, Adrian Caprita, Gheorghe Ilia, Iuliana Cretescu, and Vasile Octavian Simulescu

***Abstract*—Protein quality of soybean meal (SBM) is linked to both the reduction of anti-nutritional factors (ANFs), and the optimization of protein digestibility. Inadequate heating fails to completely destroy the ANFs. Excessive heating reduces the availability of lysine via the Maillard reaction and possibly, to a lesser extent, other amino acids, and decreases the protein nutritive quality. The objective of the study was the analysis of some chemical indices for determining SBM quality, and their correlation with biophysical indices. The experimental data revealed that urease index (UI) is useful to determine if the soybean meal has been heated enough to reduce ANFs, but it is not useful to determine excessive heat treatment. KOH solubility is a good index for determining over processing, but it is not a sensitive index for monitoring under processing of soybean meal. Combing protein dispersibility index (PDI) and UI could be useful to monitor soybean quality. The investigated biophysical indices are positively correlated with PDI: r = 0.9477 ($p<0.01$) for refractive index, and r=0.9406 ($p<0.01$) for dynamic viscosity. The biophysical methods have the advantage to be very rapid and nonpolluting since they don't use chemical reagents.**

***Index Terms*—soybean meal, urease index, protein solubility, refractive index, dynamic viscosity.**

I. Introduction

Today soybeans (*Glycine max*) are grown primarily for the production of vegetable oil for human consumption but, as a by-product, soybean meal (SBM) is becoming increasingly important. Soybean is the only vegetable food that contains complete protein, meaning all of the eight amino acids needed for human and animal health are present. Among plant protein sources, SBM has a high level of tryptophan and the highest digestible lysine content which complements the lysine deficiency of cereal grains used in animal feeds.

The anti-nutritional factors (ANFs) in soybean are often associated with the low acceptance of soybean products as they also inhibit protein digestibility. These mainly consist of heat labile (trypsin inhibitors, lectins, goitrogens, phytates) and heat stable (oligosaccharides) factors. In order for the nutritional value of soybean meal to be maximised, these anti-nutritional factors need to be inactivated or minimised [1]-[3].

Most SMB is produced today by the solvent extraction process whereby the soybeans are cracked, heated and flaked before the oil is extracted with the solvent hexane. Once the oil has been removed, the flakes are toasted and ground into meal. During this production process, temperature is critical in order to deactivate the anti-nutritional factors naturally present in raw soybeans. The challenge in SBM processing is to apply the optimum amount of heat to produce the most nutritious product.

While mild heating (~ 90^{o}C) improves the nutritional value of SBM by denaturing the proteins and exposing new sites for enzymatic hydrolysis as well as inactivating heat labile ANFs, excessive heating reduces the availability of lysine (via the Maillard reaction) and possibly, to a lesser extent, other amino acids [4], [5], lowering the nutritive value of SBM. Both insufficient and over heating result in poor quality SBM. Therefore, feed manufacturers require methods to distinguish adequately processed SBM from under or over processed meals.

An important and frequently observed effect of food processing is the reduction of protein nutritive quality. The denaturation of the protein and reduction in amino acid availability by cross-linking, racemization, degradation and formation of complexes with sugar may result in loss of digestibility [4], [6], [7]. Therefore, when attempting to estimate protein quality, one of the first factors that must be evaluated is its digestibility [8], [9].

Protein quality of SBM depends on two parameters: the reduction of anti-nutritional factors and the optimization of protein digestibility [10]. Direct analysis of both specifications is difficult in routine operations. It is therefore replaced with indirect tests such as urease index (UI), Protein Dispersibility Index (PDI), Nitrogen Solubility Index (NSI) and KOH protein solubility (PS).

SBM processors and their customers in the animal feed industry need reliable, rapid and cost-efficient methods to control the quality of their soybean meal.

Therefore, the goal of this research was to compare some chemical and biophysical laboratory procedures for assessing SBM quality, and to establish the most suitable for evaluating under and over processing.

Manuscript received July 24, 2010.
This work was supported by CNCSIS –UEFISCSU, project number 1054/2009 PNII – IDEI code 894/2008 and by project number 1055/2009 PNII – IDEI code 898/2008.

Rodica Caprita is with the Department of Exact Sciences, Banat University of Agricultural Sciences and Veterinary Medicine Timisoara, Calea Aradului 119, 300645 Timisoara, Romania (corresponding author, phone: 0040256277298; fax: 0040256200296; e-mail: rodi.caprita@ gmail.com).
Adrian Caprita is with the Department of Chemistry, Banat University of Agricultural Sciences and Veterinary Medicine Timisoara, Calea Aradului 119, 300645 Timisoara, Romania (e-mail: adi.caprita@ gmail.com).
Gheorghe Ilia is with the Institute of Chemistry Timisoara of Romanian Academy, Bd. Mihai Viteazul 24, 300223 Timisoara, Romania (e-mail: gheilia@yahoo.com).
Iuliana Cretescu is with the Department of Exact Sciences, Banat University of Agricultural Sciences and Veterinary Medicine Timisoara, Calea Aradului 119, 300645 Timisoara, Romania (e-mail: iuliana.cretescu@ gmail.com).
Vasile Octavian Simulescu is with the Institute of Chemistry Timisoara of Romanian Academy, Bd. Mihai Viteazul 24, 300223 Timisoara, Romania (e-mail: gianisimulescu@yahoo.com).

ISBN: 978-988-18210-0-3
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)

II. Experimental

Reagents

All chemicals used were of analytical reagent grade. Doubly distilled deionized water was used for all experiments.

Phosphate buffer solution (0.05 M phosphate buffer):

3.403 g of monobasic potassium phosphate (Merck) were dissolved in approximately 100 mL of distilled water. 4.355 g of dibasic potassium phosphate (Merck) were dissolved in approximately 100 mL of distilled water. The two solutions were combined and diluted to 1000 mL. Solutions were mixed using stir bar and stir plate.

Buffered urea solution:

15 g of urea (Merck) were dissolved in 500 mL of the phosphate buffer solution. 5 mL of toluene (Merck) were added to serve as a preservative and to prevent mold formation. Solutions were mixed using stir bar and stir plate.

Biuret reagent:

1.50 g of copper (II) sulfate (Merck) were dissolved in 250 mL of distilled water. 4.5g of sodium potassium tartrate (Merck) and 2.5 g of potassium iodide (Merck) were added. After solids have dissolved, 50 mL of 6.0 M NaOH (Merck) were added and diluted to a total volume of 500 mL with distilled water.

Apparatus

Spectrophotometric measurements were performed using a PerkinElmer UV/VIS-Lambda35 spectrophotometer. A Consort C861apparatus was used for pH measurement. Stirring was carried out by a Barnstead SP135930-33 magnetic stirrer. Heating at 120ºC was performed in an air forced oven Froilabo AC60. Samples were centrifuged with a Hettich 320R centrifuge. Crude protein of SBM was determined using a Kjeltec Foss Analyser Unit. Refractive indices were measured with a refractometer Krüss DR301-95. Dynamic viscosity was measured using a cone/plate viscometer Brookfield Model DVIII Cone CP-40. A Kern ABJ 220-4M balance was also used.

Procedures

Raw and toasted commercial SBM were used for the experiment. The samples were ground to pass the 200 μ sieve.

SBM samples were heated in a forced air oven at 120ºC for varying periods of time: 5, 10, 15, 20, 25 and 30 minutes.

Crude protein of SBM was determined by the Kjeldahl method [11].

The urease assay is based on the pH increase from ammonia released from urea by residual urease enzyme in SBM [12]. The urease test was conducted as following: 10 cm^3 buffered urea solution (pH=7.0) was added to 0.200 g finely ground SBM (test sample), and 10 cm^3 phosphate buffered solution was added to 0.200 g of the same sample (blank sample). The two solutions were incubated at 30ºC for 30 minutes under stirring. In the presence of significant urease activity the pH of the test solution increases due to the release of ammonia from urea. After incubation, the pH of the solutions are determined rapidly and the difference between pH of test and pH of blank is calculated as an index of urease activity.

The protein solubility was determined according to the procedure of Araba and Dale [8]. The KOH protein solubility test is based on the solubility of soybean proteins in a dilute solution of potassium hydroxide. The procedure involves incubation of 1.5 g sample with 75 mL 0.2% KOH (wt/vol; 0.036 N*)* solution for 20 min at room temperature using a magnetic stirrer. Following this incubation, the sample is centrifuged for 5 minutes at 6,000 rpm and the supernatant is analyzed for the protein concentration by the biuret method. The solubility of the protein, expressed as a percentage, was calculated by dividing the protein content of the KOH-extracted solution by the protein content of the original soybean sample (43.7%).

The Protein Dispersibility Index assay is also based on the solubility of soybean protein [13]. For this test the solvent is water. The PDI method uses ten minutes of high speed mixing in distilled water at 8,500 rpm [14].

Nitrogen Solubility Index uses a slow stirring technique. Nitrogen is extracted from the ground flour in distilled water. The sample is stirred at room temperature for 120 minutes (120 rpm).

The alkaline extract was measured for the refractive index (RI) and the dynamic viscosity (DV).

III. Results and Discussion

Soybeans and soybean meals contain urease, an enzyme that hydrolyzes urea to produce carbon dioxide and ammonia. The production of ammonia causes the pH of a solution to increase. The destruction of urease by heating is highly correlated with the destruction of trypsin inhibitors and other anti-nutritional factors. The primary purpose of the urease assay is to determine if soybean meal has been sufficiently heated to destroy most of the anti-nutritional factors. Urease index values of 0.05 to 0.2 pH rise are considered for properly processed soybean meal [15]. Values above 0.2 indicated under-heating and values below 0.05 indicated over-heating.

Fig. 1 shows the variation of UI when toasted (UI1) and raw (UI2) SBM were placed in an oven at 120ºC for up to 30 minutes; analysis were accomplished every 5 minutes. UI1 decreased from 0.03 to zero after 10 minutes of heating. UI2 was initially high and in the first increment of heating did not change much (from 2 to 1.8). After 5 minutes of heating, UI2 decreased rapidly (dropped to 0.02 at 10 minutes), so that zero value was reached at 15 minutes of heating. Additional heating could not have any effect on UI. Therefore, UI is useful to determine if SBM has been heated enough to reduce the anti-nutritional factors, but it is not very useful for determining if SBM has been over-processed.

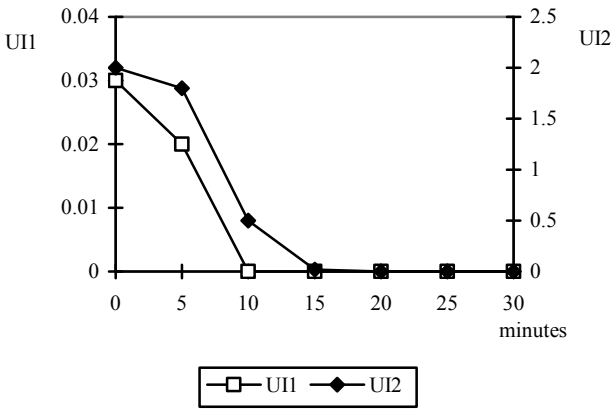

Fig. 1. Effect of heating time on UI

ISBN: 978-988-18210-0-3
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)

The simplest criterion used for the characterization of proteins is their solubility in various media. As in all legumes, the bulk of soybean proteins are globulins, characterized by their solubility in salt solutions. The solubility of soybean proteins in water is strongly affected by the pH. Close to 80% of the protein in raw seeds or unheated meal can be extracted at neutral or alkaline pH.

The effect of over processing on protein solubility of raw SBM was investigated. Fig. 2 shows the dynamic of PS, PDI and NSI when heating 30 minutes at 120ºC.

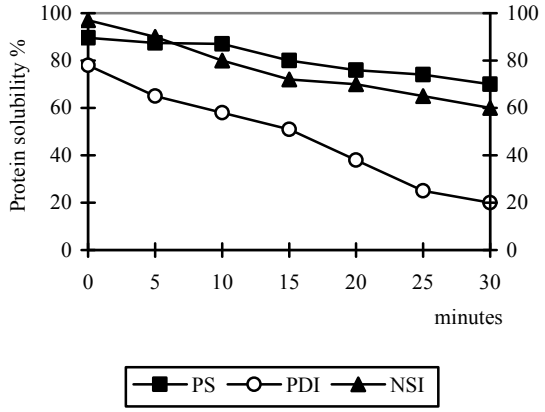

Fig. 2. Effect of heating time on PS, PDI and NSI of raw SBM

PS variation with the heating time was not linear. PS decreased very little up to 10 minutes of heating (from 89.65% to 87%) and after 30 minutes it reached 65.20%. PS is not a sensitive index for monitoring under processing of SBM and appears to be a better indicator for over processing. PDI and NSI decreased incrementally from 78% to 20% and from 97% to 60%, respectively, when heating 0 to 30 minutes. The experimental data suggest that PDI is more sensitive than UI, PS and even than NSI for determining the optimum amount of heat processing of SBM. PDI was reduced by approximately half when raw SBM was heated 20 minutes, while NSI decreased in the same time period only to 72% of the initial value.

PDI is also the simplest measurement tool of the chemical methods. Combing PDI and UI could be useful to monitor soybean quality. SBM containing low UI (0.3 or below) and high PDI (40 to 45%) may indicate that the sample is definitely high quality because it has been adequately heat processed, but not over processed.

Since the size and concentration of protein molecules influence the refractive index and the dynamic viscosity of solutions, the experiment had in view to observe the correlation between protein solubility and these biophysical parameters. If proteins are dissolved in alkaline aqueous solutions the change in the refractive index of the solvent is directly proportional to the concentration of the dissolved protein. The dynamic viscosity change is usually directly related to the size of protein molecules being often a linear function of the concentration.

The effect of over processing on toasted SBM was investigated, and following analyses were performed: PS, PDI, RI and DV. Samples were placed in an oven at 120ºC for up to 30 minutes; analysis was accomplished every 5 minutes. The experimental data are presented in Table 1.

Table 1. Effect of heating time on PS, PDI, RI and DV of toasted SBM

Heating time (minutes)	PS (%)	PDI (%)	RI	DV (cP)
0	87.40	79	1.3380	1.086
5	78.20	65	1.3378	1.085
10	71.20	62	1.3372	1.080
15	69.95	51	1.3370	1.078
20	59.95	38	1.3340	1.077
25	58.19	25	1.3338	1.063
30	53.20	20	1.3335	1.058

The refractive index and the dynamic viscosity of the dilute alkaline extract were determined at 25ºC. Heating soy proteins above 80°C causes dissociation of their quaternary structures, denatures their subunits, and promotes the formation of protein aggregates via electrostatic, hydrophobic and disulfide interchange mechanisms. These changes in protein structure result in RI and DV variations.

Both the refractive index and the dynamic viscosity of protein solutions are linear functions of the protein concentration. The biophysical indices are highly and positively correlated with PDI: $r = 0.9477$ ($p<0.01$) for refractive index (Fig. 3) and $r = 0.9406$ ($p<0.01$) for dynamic viscosity (Fig. 4).

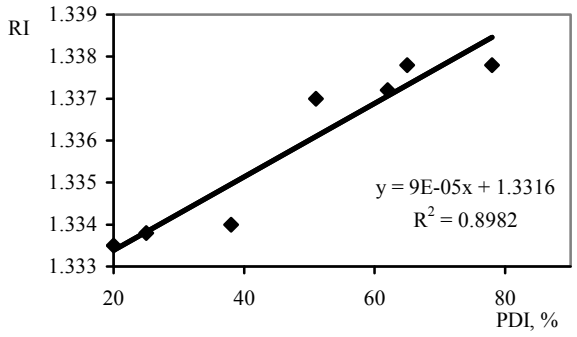

Fig. 3. Correlation between PDI and RI

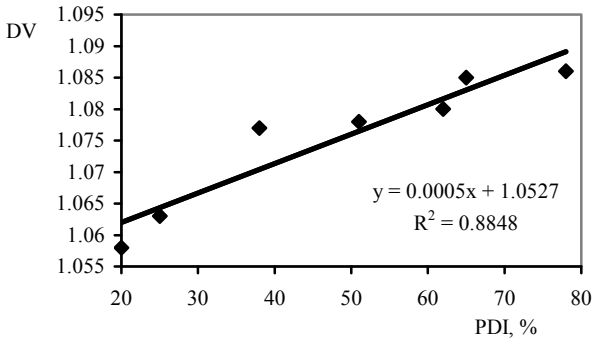

Fig. 4. Correlation between PDI and DV

Determination of biophysical parameters instead of chemical indices has two great advantages: the methods are nonpolluting since they don't use chemical substances, and the methods are very rapid.

ISBN: 978-988-18210-0-3
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)

IV. CONCLUSION

UI is useful to determine if the soybean meal has been heated enough to reduce the anti-nutritional factors, but it is not useful to determine excessive heat treatment.

KOH solubility is a good index for determining over processing of soybean meal, but it is not a sensitive index for monitoring under processing of soybean meal.

Determination of PDI is the best method of evaluating soybean for both under heating and over heating.

The investigated biophysical indices are positively correlated with PDI: $r = 0.9477$ ($p<0.01$) for refractive index and $r=0.9406$ ($p<0.01$) for dynamic viscosity. Determination of biophysical parameters instead of chemical indices has two great advantages: the methods are nonpolluting since they don't use chemical substances, and the methods are very rapid.

REFERENCES

[1] I. E. Liener, "Removal by processing of naturally occurring toxicants and antinutrients", in *Chemistry and World Food Supplies: The New Frontier*, L. W. Schemilt, Ed. Oxford, UK: Pergamon Press, 1983, pp. 453-463.

[2] I. E. Liener. (1994). Implications of antinutritional components in soybean foods. *Crit. Rev. Food Sci. Nutr. 34(1)*. pp. 31-67.

[3] K. Liu, "Chemistry and nutritional value of soybean components", in *Soybeans, Chemistry, Technology and Utilisation*, K. Liu, Ed. New York, USA: Chapman and Hall, 1997, pp. 415-418.

[4] F. R. Del Valle. (1981). Nutritional qualities of soya protein as affected by processing. *J. Am. Oil Chem. Soc. 58(3)*. pp. 419-429.

[5] A. Skrede, and A. Krogdahl. (1985). Heat affects nutritional characteristics of soybean meal and excretion of proteinases in mink and chicks. *Nutr. Repts. Int. 32(2)*. pp. 479-489.

[6] M. Friedman. (1996). Food browning and its prevention, an overview. *J. Agric. Food Chem. 44(3)*. pp. 631-653.

[7] R. Caprita, and A. Caprita. (2007). Effect of heat treatment on soybean protein solubility. *Scientific Papers Animal Sciences and Biotechnologies*. *40(1)* pp. 398-401.

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

[9] A. Caprita, and R. Caprita. (2008). *In vitro* chemical procedures to estimate amino acid digestibility in soybean. *Journal of Agroalimentary Processes and Technologies. XIV(1)*. pp. 106-108.

[10] C. M. Parsons, K. Hashimoto, K. J. Wedeking, and D. H. Baker. (1991). Soybean protein solubility in potassium hydroxide: an *in vitro* test of *in vivo* protein quality. *J. Anim. Sci. 69(7)*. pp. 2918-2924.

[11] AOAC Method 988.05. "Protein (crude) in animal feed, $CuSO_4$/ TiO_2 mixed catalyst Kjeldahl method", in *AOAC Official Methods of Analysis*, Arlington, Virginia, USA: Association of Official Analytical Chemists, 1990.

[12] American Oil Chemists Society. 1980a. Urease Activity. Official Method Ba 9-58. Champagne, IL, USA: American Oil Chemists Society.

[13] American Oil Chemists Society. 1980b. Protein Dispersibility Index. Official Method Ba 10-65. Champagne, IL, USA: American Oil Chemists Society.

[14] A. B. Batal, M. V. Douglas, A. E. Engram, and C. M. Parsons. (2000). Protein dispersibility index as an indicator of adequately processed soybean meal. *Poultry Sci. 79(11)*. pp. 1592-1596.

[15] W. A. Dudley-Cash. (1999). Methods for determining quality of soybean meal protein. *Feedstuffs. 71(1)*. pp. 10-11.

ISBN: 978-988-18210-0-3
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)