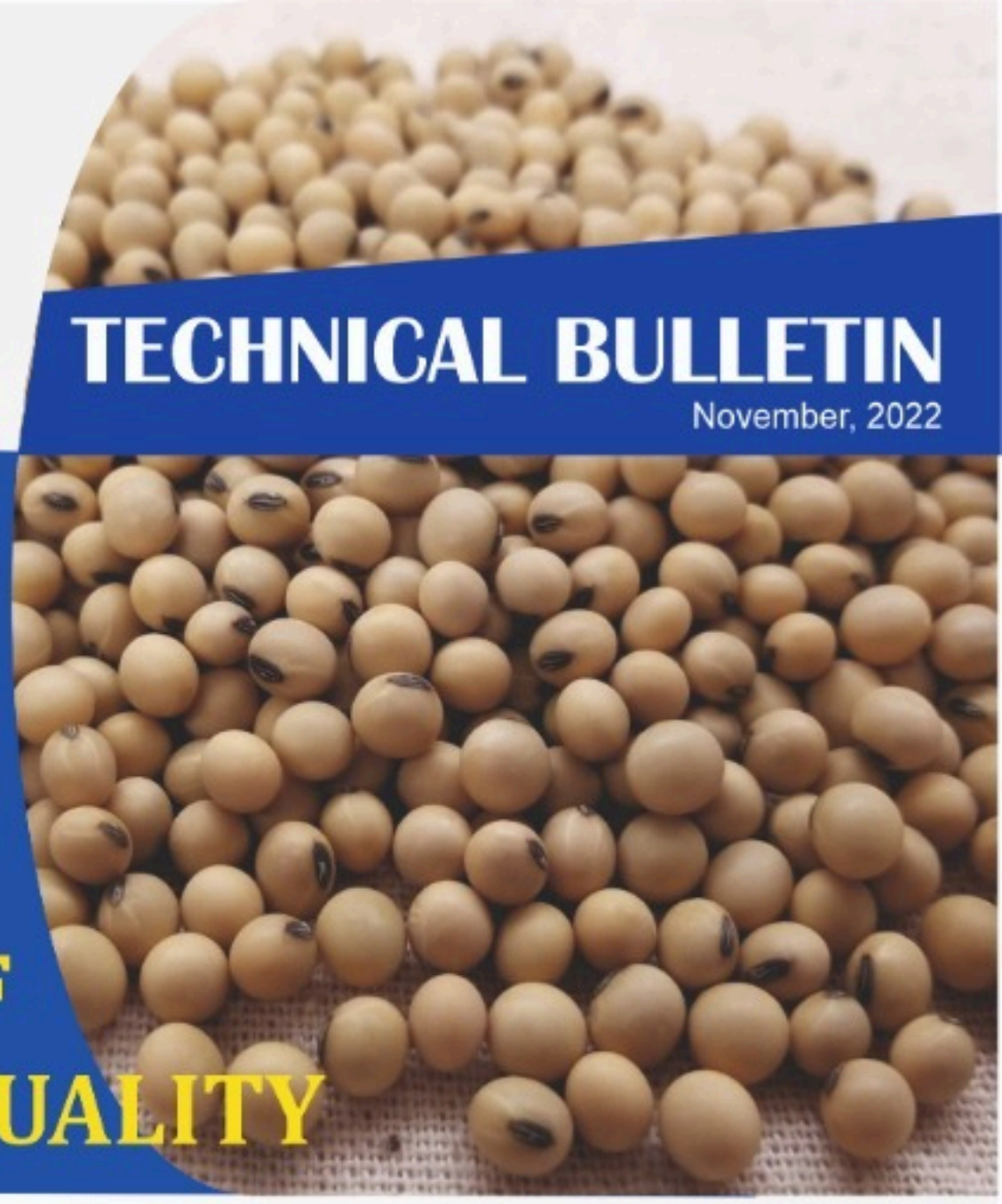
OPTIMA POULTRY PVT. LTD.

TECHNICAL BULLETIN

November, 2022



WHAT COMES FIRST ? QUANTITY OR QUALITY !

THE TRUE PICTURE OF SOYABEAN PROTEIN QUALITY

Protein quality of the soybean meal is the result of amino acid present in the meal and the portion in the bio available form to the animals. While intending to utilize the same for monogastric animals, it has to be properly heat processed so as to minimize the anti nutritional effect and thereby increasing the digestibility of amino acids present in the meal. At the same time as known to everyone, it should not be over processed which will decrease the concentration and digestibility of amino acid present in the meal. It is due to the Maillard reaction which binds free amino acids to free carbonyl groups. The resultant end product will not be bio available for the animal and poultry. Various methods are available to determine the protein quality of soybean meal for monogastric animals including poultry.

1. Urease Index
2. KOH protein solubility
3. Protein Dispersibility Index (PDI)
4. Nitrogen solubility index
5. Absorbance at 420 nm

1. Urease Index

Among these, Urease index **(AOCS, 2011)** is the most common and frequently used as an indicator to evaluate the quality of soybean subjected to heat processing. The method requires a pH meter and water bath. The method determines the residual urease activity of soybean meal as an indirect indicator of the quantum of anti nutritional factors such as trypsin inhibitor. If the soybean meal received the optimum required heat treatment, the trypsin inhibitor present in the soybean meal will be destroyed along with urease. Because, the heat treatment will minimize both the enzymes, such as, trypsin inhibitor and urease.

The laboratory method for the estimation of urease index measures the increase in pH as a result of release of ammonia. As ammonia is alkaline, while liberated into the media due to the breakdown of urea by the residual urease present after heat processing in the soybean meal will increase the pH. Various protocols are used to find out the endpoint in different ways. In the American Oil Chemists Society (AOCS, 2011) method, the endpoint is determined based on the increase in pH points in the medium where the reaction occurs. Another well known method is European Union (EU) method, the endpoint indicates the amount of acid required to maintain the constant static pH. Results of these two methods differ slightly. But it is negligible. Indicator method will provide qualitative information for on-site verification.

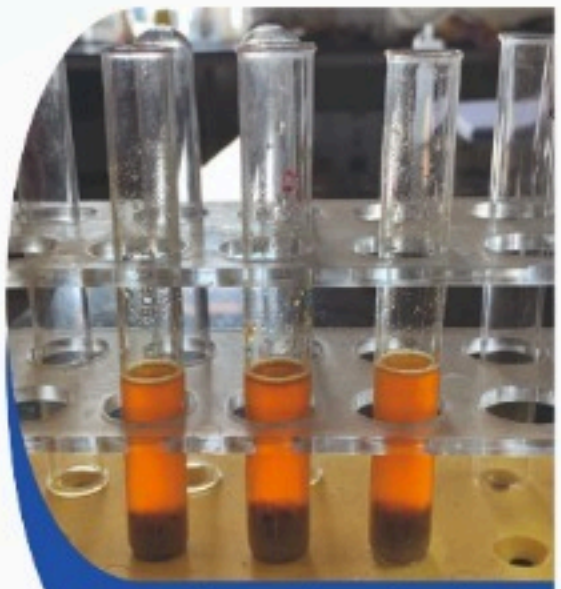
a) Indicator method (Bromothymol blue) - Qualitative

Interpretation is made based on the development of light green (cooked adequate), dark blue (under cooked) or yellow (over cooked) colour on visual examination of soybean meal

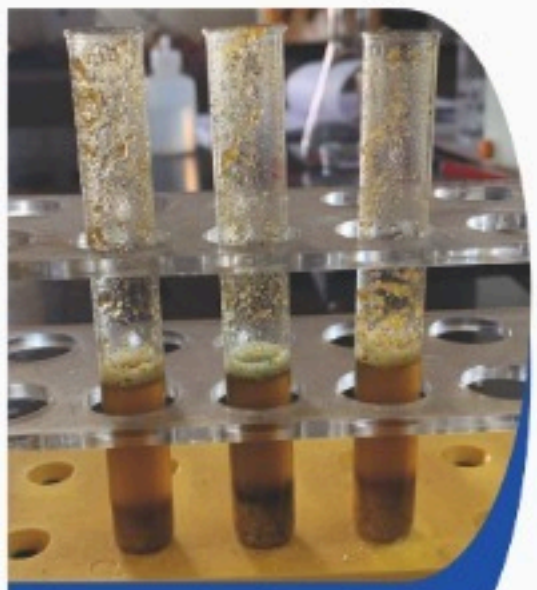
when treated with urea containing Bromothymol blue solution.

- 1g of fine grounded sample of soya in a test tube
- Add 10ml of bromothymol solution and shake well
- Set aside for 10 minutes and observe the colour change

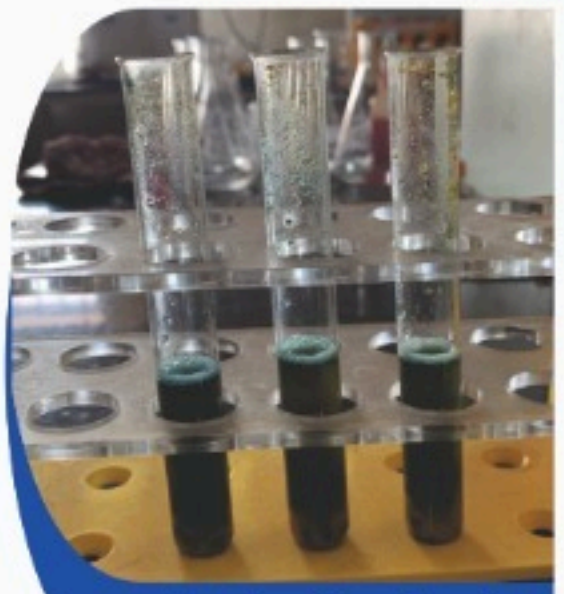
Fresh Solution



Over Cooked



Properly Cooked



Under Cooked

b) Indicator method (Urea -phenol red) - Qualitative

Interpretation is based on the intensity of development of red colour spots on visual examination of soybean meal when treated with Urea-Phenol solution.

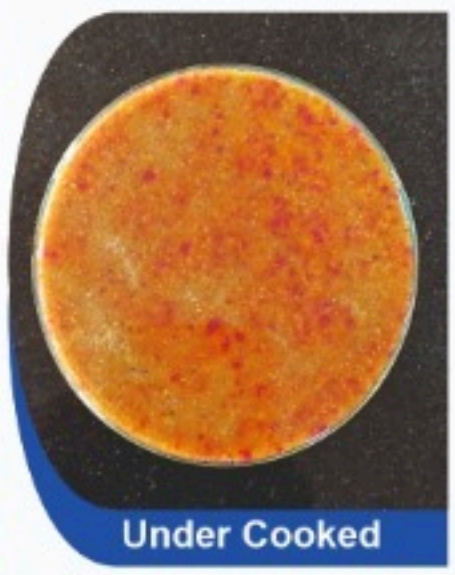
- Spread soybean meal uniformly
- Wet soybean meal using Urea – Phenol red solution
- After 5 minutes observe red colour spots developing on soybean meal

Fresh Solution

Over Cooked

Properly Cooked

Properly Cooked



Under Cooked

Table 1. Visual examination of soybean meal treated with Urea-Phenol Red solution

OBSERVATION	UREASE ACTIVITY	RANGE OF UREASE (APPROX.)	ASSESSMENT
• No visible red colour	Inactive	0.00	Over cooked
• Few scattered red particle	Slightly active	0.05-0.10	Properly cooked
• Approximately 25% or more red particles	Moderately active	0.10-0.20	Properly cooked
• Approximately 50% or more red particles	Very active	>0.20	Under cooked

c)pH difference method- Quantitative

- Take 0.2g of sample in two separate test tubes
- Add 20ml of Phosphate buffer / urea buffer in each tube and shake well
- Incubate 30min in water bath (30°C), filter

the content and check the pH

Optimum pH increase is considered to be between 0.05 and 0.20. Urease activity in excess of 0.20 is suggestive of under processing. Usually, all the overheated samples will give the urease activity less than 0.05. But it does not imply that all the samples with urease index below 0.05 have been overheated.

All the above urease tests are useful to determine whether soybean meal has been sufficiently heated to de-activate the trypsin inhibitor. **But, for the product which received an excessive heat, it is not a reliable method.**

2. KOH Protein solubility

This method determines the quantum / proportion of protein that is solubilized in potassium hydroxide solution. It is inversely correlated with the degree of heat treatment. Protein solubility values will be around 90% for the raw / unprocessed soybean meal. So it is not sensitive to differentiate the level of heat processing. But the soybean meal which undergoes over-processing will have lesser protein solubility values based on the heat treatment. **So, KOH protein solubility test is most useful for detecting excessive or over processed soybean meal from the rightly processed one.**

- Place 1.5 g of sample in 75 ml of 0.2% KOH solution and stir at 8500 rpm for 20 min
- 50 ml is taken, immediately centrifuged at 2500 rpm for 15 min
- Take aliquot 10 ml to determine nitrogen in liquid fraction by Kjeldahl method
- Results are expressed as percentage of original nitrogen content of the sample
- Lee and Garlich, 1992 and Araba and Dale, 1990 have correlated the solubility values with production performance of poultry and swine and found that there was a clear decline in performance with solubility below 72%. Adequately well processed soybean product should have a protein solubility values in the range of 75-84%.

3. Protein Dispersibility Index (PDI)

The Protein Dispersibility Index (PDI) and Nitrogen Solubility Index (NSI) both measures protein solubility in water. These two methods differ in stirring speed and vigor at which the sample is processed during estimation.

- 20 g of soybean with 300 ml deionised distilled water
- Blend at 8500 rpm for 20 min
- Centrifuge (1000 rpm for 10 min) / filter and measure the nitrogen content in liquid fraction by Kjeldahl method
- Results are expressed as percentage of original nitrogen content of the sample

This method is a good indicator for the adequately heat processed and under processed soybean meal.

The PDI values recommended by the National Soybean Processors Association is 15-30%. The results from PDI may be combined with the values of urease index and KOH protein solubility to judge the soybean meal quality.

PDI test is comparatively more recent and is utilized as a complement for other tests. The PDI method is recommended as the best method to differentiate between samples of good quality soybean meals. This test is having greater consistency in results while heating the soy flakes. Batal *et al.* (2000) recommended the PDI values below 45% based on the practical experiences.

4. Nitrogen solubility Index (NSI)

- Place 5 g of sample in 200 ml distilled water
- Stir at 120 rpm for 120 min
- Centrifuge (1500 rpm for 10 min) / filter and measure the nitrogen content in liquid fraction by Kjeldahl method
- Results are expressed as percentage of original nitrogen content of the sample

5. Absorbance at 420 nm

Aqueous extracts of heated protein supplements will be subjected to measurement of absorbance at 420nm. It will give the information on the possible damage from excessive heat through the formation of undesirable components due to Maillard reaction.

- The supernatant (if centrifuged) or the liquid fraction (if filtered) from the PDI technique is diluted 80 times.
- Filter through 0.2 µm pore size filter.
- Read the absorbance of the clear filtrate at 420 nm with a spectrophotometer. (Adapted from Dudley-Cash, 1999)

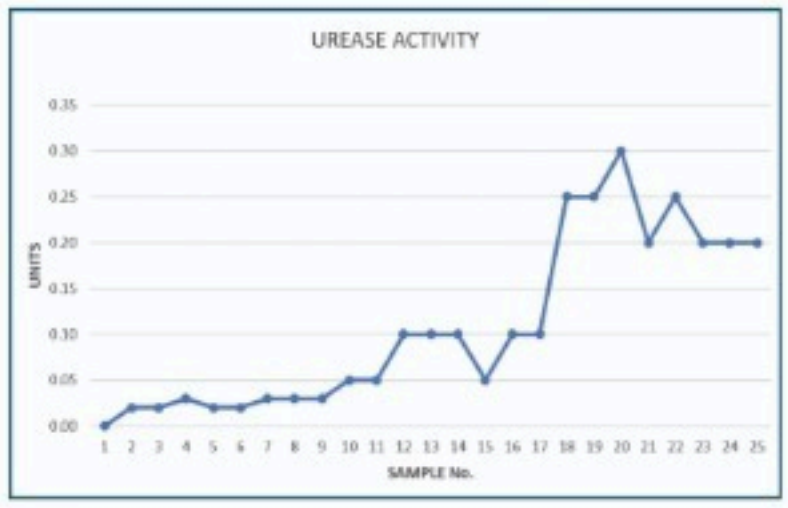


Table 2. Crude protein, Urease activity, KOH protein solubility index and Protein dispersibility index values of 24 samples of SOYA DOC analysed at Optima Poultry Pvt Ltd

Sample No.	CP (%)	UREASE ACTIVITY	PSI (%)	PDI (%)
1	44.78	0.02	66.70	10.27
2	44.64	0.02	71.60	08.52
3	45.01	0.03	70.38	10.35
4	42.34	0.02	68.78	07.35
5	44.17	0.02	68.15	06.20
6	45.06	0.03	63.66	05.80
7	47.48	0.03	69.36	12.81
8	42.67	0.03	70.49	10.07
9	47.75	0.05	78.15	15.10
10	45.10	0.05	76.88	15.41
11	44.69	0.10	77.51	15.39
12	44.33	0.10	83.54	15.88
13	44.52	0.10	84.60	16.87
14	45.61	0.05	77.19	16.09
15	45.08	0.10	81.14	14.98
16	45.01	0.10	80.78	15.83
17	43.75	0.25	79.36	12.15
18	41.28	0.25	77.20	11.20
19	46.29	0.30	76.30	10.20
20	44.70	0.20	81.31	12.75
21	46.08	0.25	81.24	13.28
22	45.07	0.20	76.95	10.85
23	46.12	0.20	75.18	12.30
24	46.60	0.20	76.04	12.73

Over toasted Adequately toasted Under toasted

- Question may arises whether to select soya DOC with

  (1) CP of 45% and higher Protein solubility index (83%) OR
  (2) CP of 46% and lower Protein solubility index (78%)
  (3) CP of 45% and higher protein dispersibility index (15-30%) OR
  (4) CP of 46% and lower protein dispersibility index (<15%)

- The relationship between Crude protein, Protein solubility index (PSI) and Protein dispersibility index (PDI) need to be established not only with more number of laboratory analyses but also with animal trials
- Direct correlation between protein quality and animal performance were already established through various feeding trial.
- Adequately processed soya DOC has to be selected based on HIGHER PROTEIN SOLUBILITY INDEX / PROTEIN DISPERSIBILITY INDEX/BOTH

Based on the above information, current recommendations for soybean meal are with PDI values between 15 and 30%, KOH solubilities between 73 and 85% and urease index of 0.20 pH unit change or below. These meals fits into the above bracket may be considered as definitely high quality without under, or over processing. All the laboratory tests may give slightly different results. It is depending on the particle size of the sample subjected for analysis, temperature of the solution, centrifugation speed and duration. As the particle size decreases, the protein solubility indexes will increase (Parsonset al 1991, Whitle and Araba, 1992). So, it is recommended to have the sample With uniform particle size / mesh size for the determination.

POINTS TO PONDER

- Overheated soybean meal will have low urease activity, low protein solubility index and low PDI values. To detect the extent of overcooking, values of Protein solubility index (PSI) and Protein dispersibility index (PDI) may be utilized.
- Urease activity is not a reliable method for screening overheated samples. In such cases conclusion can be drawn based on Protein solubility index and Protein dispersibility index.
- Adequately processed soybean meal is having Protein solubility index value of 73-85% and protein dispersibility index of 15-30%. Protein solubility is reduced below 73% in over heated soybean meal

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