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Evaluation of protein quality and heat processing adequacy of commercial soybean de-oiled cake using *in vitro* quality indicators

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Abstract

The present study was undertaken to evaluate the protein quality and processing adequacy of commercially available soybean de-oiled cake (Soya DOC) samples used in poultry feed manufacture. Thirty Soya DOC samples (approximately 500 g each) were collected from different feed manufacturers in Tamil Nadu and analysed for crude protein (CP), pH, urease activity, protein solubility index (PSI) and protein dispersibility index (PDI) following standard analytical procedures. The crude protein content ranged from 42.37 to 46.72%, pH from 6.45 to 6.80, urease activity from 0.02 to 0.30 ΔpH, PSI from 63.15 to 84.22% and PDI from 6.43 to 17.12%, indicating considerable variability in the processing quality of the samples. Based on the combined interpretation of urease activity, PSI and PDI, the samples were categorized as over-processed, adequately processed and under-processed. The findings revealed substantial variation in the quality of commercially available Soya DOC, reflecting differences in processing conditions among manufacturers. The study highlights the importance of routine quality evaluation using multiple processing indicators to ensure optimum protein quality and improve the nutritional value of soybean meal used in poultry feed formulation.

Keywords: Heat processing, protein dispersibility index, protein solubility index, soybean de-oiled cake, urease activity

Introduction

Poultry feed primarily comprises cereal grains as the principal source of energy and oilseed meals as the major source of dietary protein. Among the various plant protein ingredients, soybean de-oiled cake, commonly referred to as soybean meal, is the most preferred protein supplement in poultry diets owing to its high crude protein content, balanced amino acid composition and excellent digestibility. It is particularly rich in lysine and other essential amino acids, making it an ideal complement to cereal-based diets and an indispensable ingredient for supporting optimum growth, feed conversion efficiency and productive performance in poultry (Ravindran *et al.*, 2014) [14].

The nutritional value of soybean meal, however, is highly dependent on the processing conditions employed during oil extraction. Raw soybeans contain several anti-nutritional factors, including protease inhibitors, lectins, phytates, oligosaccharides, goitrogens and antivitamin, which interfere with nutrient digestion and utilization. Among these, the Kunitz trypsin inhibitor and Bowman-Birk inhibitor are the most important because they inhibit the digestive enzymes trypsin and chymotrypsin, thereby reducing protein digestion and amino acid absorption. Feeding inadequately processed soybean meal can result in poor feed efficiency, reduced growth performance and pancreatic hypertrophy in poultry (Lambo *et al.*, 2024) [10].

Appropriate heat treatment during soybean processing is therefore essential to inactivate these heat-labile anti-nutritional factors and improve protein utilization. However, the degree of heat treatment must be carefully controlled because both insufficient and excessive heating adversely affect protein quality. Under-processing leaves residual trypsin inhibitor activity, whereas over-processing causes protein denaturation and Maillard reactions between reducing sugars and amino acids, particularly lysine, thereby reducing amino acid availability and protein digestibility (Fontaine *et al.*, 2007) [9]. Consequently, maintaining optimum processing conditions is critical to preserve the nutritional quality of soybean meal intended for poultry feeding.

Assessment of soybean meal quality has therefore become an important component of feed quality assurance programmes. Several analytical parameters are routinely employed to evaluate the adequacy of heat processing and protein quality. Crude protein content provides an estimate of the protein concentration, while pH serves as an indicator of processing conditions. Urease activity is widely recognized as a sensitive indicator of residual anti-nutritional activity and inadequate heat treatment. Likewise, protein solubility and the protein dispersibility index (PDI) are reliable indicators of protein integrity and processing adequacy, helping to distinguish under-processed, optimally processed and over-processed soybean meals (Araba & Dale, 1990; AOCS, 2017) [5, 2]. Collectively, these quality indices provide valuable information on the suitability of soybean meal for incorporation into poultry feeds and help ensure consistency in feed formulation and animal performance.

Although soybean meal is extensively used by the poultry feed industry, variations in processing practices among feed manufacturers may influence its nutritional quality and protein availability. Periodic evaluation of commercially available soybean de-oiled cake is therefore necessary to verify processing adequacy and ensure that the ingredient meets acceptable quality standards for poultry nutrition.

In view of the importance of soybean meal quality in poultry feed formulation, the present study was undertaken to evaluate the protein quality of commercially available soybean de-oiled cake samples collected from different feed manufacturers in Tamil Nadu by determining crude protein content, pH, urease activity, protein solubility index and protein dispersibility index. The findings of this study are expected to provide useful information on the processing quality of soybean meal used by the poultry feed industry and its suitability as a protein source for poultry diets.

Materials and Methods

Thirty soybean de-oiled cake samples (approximately 500 g each) were collected from different poultry feed manufacturers across

Tamil Nadu, India. All samples were transported to the laboratory in clean polyethylene bags and stored under appropriate conditions until analysis. The crude protein content of the samples was determined according to AOAC Official Method 984.13 (AOAC, 2019) [4]. The pH of each sample was measured using a calibrated digital pH meter in a 1:5 (w/v) soybean meal–distilled water suspension after thorough mixing and equilibration, following AOCS Official Method Ba 7-58 (AOCS, 2017) [3]. Urease activity was determined according to the method described by Caskey and Knapp (1944) [8] and AOCS Official Method Ba 9-58 (AOCS, 2017a) [1]. The results were expressed as pH difference (Δ pH), which serves as an indicator of the adequacy of heat processing of soybean meal. Protein solubility was determined following the method described by Araba and Dale (1990) [5]. The protein solubility index was calculated and expressed as the percentage of soluble protein relative to the total crude protein content of the sample. The protein dispersibility index was determined according to AOCS Official Method Ba 10-65 (AOCS, 2017b) [2] and expressed as the percentage of dispersed protein relative to the total protein content.

Results and Discussion

The results of the present study (Table 1) demonstrated considerable variability in the chemical composition and processing quality indicators of soybean de-oiled cake collected from different feed manufacturers in Tamil Nadu. The crude protein content ranged from 42.37 to 46.72 %, pH from 6.45 to 6.80, urease activity from 0.02 to 0.30 Δ pH, protein solubility index from 63.15 to 84.22% and protein dispersibility index from 6.43 to 17.12%. The wide variation observed among the samples indicates differences in soybean quality and heat-processing conditions adopted during manufacturing, which directly influence the nutritional value of soybean meal. Similar variability among commercial soybean meal samples has been reported by Lopez *et al.* (2020) [11], emphasizing the importance of routine quality assessment before incorporation into poultry feeds.

Table 1: Crude protein, pH, urease activity, PSI and PDI of soya DOC

Sample No.	¹ CP %	pH	Urease activity	² PSI %	³ PDI %	Indications [#]
1	45.87	6.45	0.03	63.78	9.62	Over processed Urease activity 0.02 – 0.03 PSI 63.15 – 72.82 PDI 6.43 – 11.93
2	43.53	6.6	0.03	72.82	7.98	
3	44.11	6.5	0.03	71.46	7.82	
4	43.45	6.6	0.03	66.84	10.91	
5	45.03	6.65	0.02	69.23	8.43	
6	44.98	6.55	0.02	63.15	6.43	
7	46.72	6.45	0.03	68.45	11.93	
8	43.91	6.55	0.02	71.53	9.64	
9	45.26	6.48	0.03	70.63	10.53	
10	45.73	6.51	0.02	69.92	8.43	
11	46.45	6.7	0.06	80.85	13.99	Adequately processed Urease activity 0.05 – 0.10 PSI 76.98 – 84.22 PDI 13.95 – 17.12
12	45.94	6.6	0.07	76.98	14.13	
13	45.32	6.5	0.09	79.18	16.68	
14	45.86	6.45	0.08	82.75	16.24	
15	43.97	6.8	0.07	84.22	17.12	
16	45.28	6.75	0.06	79.36	15.67	
17	44.34	6.65	0.09	80.92	13.95	
18	44.15	6.6	0.08	78.24	14.52	
19	45.90	6.64	0.10	83.76	16.90	
20	43.65	6.57	0.09	84.10	15.86	
21	42.85	6.7	0.23	77.15	11.23	Under processed Urease activity 0.20 – 0.30 PSI 74.46 – 81.52 PDI 10.42 – 13.89
22	42.37	6.75	0.26	79.98	10.98	
23	45.49	6.8	0.28	75.86	10.42	
24	45.23	6.65	0.22	81.52	11.88	
25	45.66	6.75	0.26	80.57	13.89	
26	44.86	6.7	0.23	74.80	12.96	
27	44.38	6.8	0.28	74.46	10.71	
28	44.75	6.7	0.29	80.23	10.38	
29	45.01	6.65	0.28	79.63	13.01	
30	44.06	6.8	0.27	81.21	12.13	
Range	42.37-46.72	6.45-6.80	0.02-0.30	63.15-84.22	6.43-17.12	Wide variability

The crude protein content of all samples was within the acceptable range reported for commercial soybean meal used in poultry diets (Ravindran *et al.*, 2014) ^[14]. The observed variation may be attributed to differences in soybean cultivar, dehulling efficiency and oil extraction methods. Likewise, the pH values (6.45–6.80) were within the normal range reported for processed soybean meal and may serve as a supplementary quality indicator when interpreted together with urease activity, PSI and PDI (AOCS, 2017; Van Eys & Ruiz, 2021) ^[3, 17].

Urease activity, a widely accepted indicator of processing adequacy, showed marked variation among the samples. Eight samples exhibited very low urease activity (0.02–0.03 ΔpH), indicating excessive heat treatment, whereas eight samples recorded values between 0.20 and 0.30 ΔpH, suggesting inadequate processing and the possible presence of residual trypsin inhibitor activity. Samples with urease activity between 0.05 and 0.10 ΔpH were considered adequately processed. Wright (1981) ^[18] suggested that urease activity values between 0.05 and 0.20 ΔpH indicate satisfactory heat treatment, while higher values reflect insufficient processing. However, recent investigations have demonstrated that urease activity alone is insufficient to accurately assess soybean meal quality because excessive heating rapidly inactivates urease while simultaneously reducing amino acid digestibility and protein functionality. Therefore, protein solubility and trypsin inhibitor activity should be evaluated alongside urease activity for a comprehensive assessment of processing adequacy (Ruiz *et al.*, 2024; Parsons, 2024) ^[15, 13].

Protein solubility index ranged from 63.15 to 84.22%. Samples with PSI values below the recommended range (approximately 70–85%) were indicative of excessive heat treatment and protein denaturation, whereas values within the recommended range reflected adequate processing (Araba & Dale, 1990) ^[5]. Excessive heating reduces protein solubility through irreversible denaturation and Maillard reactions, thereby decreasing amino acid availability, particularly lysine (Fontaine *et al.*, 2007) ^[9]. Recent reviews have confirmed that optimum thermal processing improves protein digestibility through inactivation of anti-nutritional factors, whereas excessive heating promotes protein denaturation and Maillard reactions, thereby reducing amino acid availability and nutritional quality (Toomer *et al.*, 2024; Asghar *et al.*, 2024) ^[16, 6].

The PDI values varied from 6.43 to 17.12 %, further confirming differences in processing quality among the soybean meal samples. Lower PDI values (6.43–11.93%) indicated reduced

protein dispersibility associated with over-processing, whereas values between 13.95 and 17.12% suggested optimum heat treatment. Monary (1996) ^[12] recommended a PDI range of 15–28% for adequately processed soybean meal. Likewise, Batal *et al.* (2000) ^[7] demonstrated that PDI is a sensitive indicator of heat damage and when used together with urease activity and protein solubility, provides a more reliable assessment of soybean meal processing quality than any single parameter alone.

Based on the combined interpretation of urease activity, PSI and PDI following the recommendations of Van Eys and Ruiz (2021) ^[17], the soybean meal samples were categorized into over-processed (Samples 1–10), adequately processed (Samples 11–20) and under-processed (Samples 21–30) groups. The over-processed samples were characterized by very low urease activity together with reduced PSI and PDI values, indicating excessive heat exposure and possible loss of protein quality. In contrast, the adequately processed samples exhibited optimum values for all three quality indicators, while the under-processed samples showed elevated urease activity, suggesting incomplete inactivation of trypsin inhibitors.

To further illustrate the relationship between urease activity and protein solubility index, the analytical data were plotted as a scatter plot (Figure 2). The figure revealed three distinct clusters corresponding to the processing categories established from the combined interpretation of urease activity, PSI and PDI. Over-processed samples were clustered at lower urease activity (0.02–0.03 ΔpH) and lower PSI values (63.15–72.82%), reflecting excessive heat treatment that reduced protein solubility through protein denaturation. Adequately processed samples occupied the intermediate urease activity range (0.05–0.10 ΔpH) and exhibited the highest PSI values (76.78–84.22%), indicating sufficient heat treatment to effectively inactivate anti-nutritional factors while preserving protein quality. Under-processed samples were clearly separated by their higher urease activity (0.20–0.30 ΔpH), indicating inadequate thermal processing, although some overlap in PSI values with adequately processed samples was observed. This overlap demonstrates that protein solubility alone cannot reliably distinguish processing quality. Therefore, simultaneous interpretation of urease activity, PSI and PDI provides a more robust and reliable assessment of soybean meal processing adequacy than any single quality indicator. The distinct clustering observed in Figure 1 further validates the classification of commercial soybean de-oiled cake samples into over-processed, adequately processed and under-processed groups.

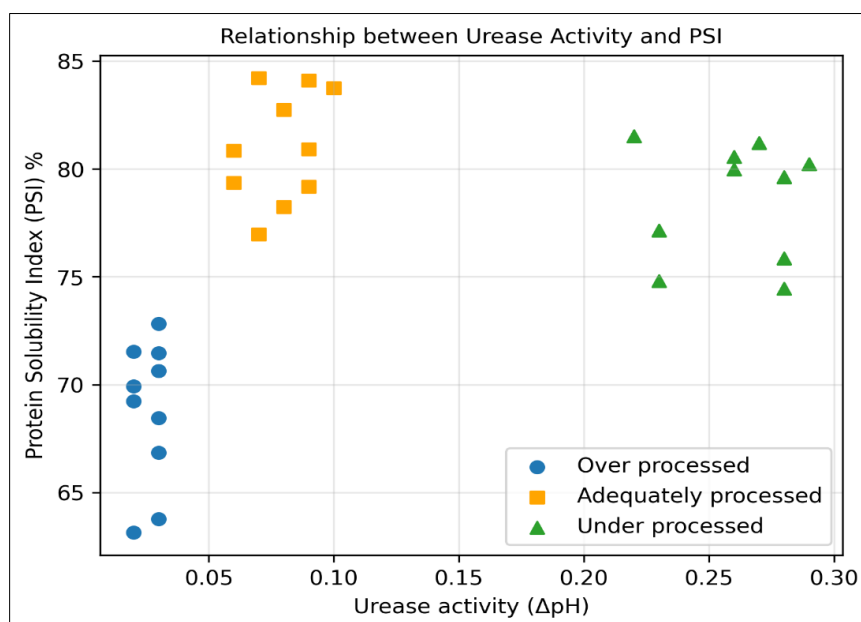


Fig 1: Relationship between urease activity (ΔpH) and protein solubility index (PSI) of commercial soybean de-oiled cake samples

Overall, the findings of the present study confirm that considerable variability exists in the processing quality of commercially available soybean de-oiled cake marketed in Tamil Nadu. The results highlight the importance of routine quality evaluation using a combination of crude protein, urease activity, PSI and PDI to accurately assess processing adequacy. Simultaneous interpretation of these parameters provides a comprehensive evaluation of soybean meal quality, helping to identify under- and over-processed products and ensuring optimum nutrient availability, feed efficiency and poultry performance.

Conclusion

The present investigation demonstrated that crude protein content alone is insufficient for accurately assessing the quality of soybean de-oiled cake, as it does not reflect the extent of heat processing or protein functionality. Comprehensive evaluation using *in vitro* quality indicators such as urease activity, protein solubility index and protein dispersibility index provides a more reliable assessment of processing adequacy and nutritional quality. The observed variability among commercial Soya DOC samples highlights the need for routine batch-wise quality evaluation before incorporation into poultry feeds. Such an approach would facilitate more accurate feed formulation, improve nutrient utilization, minimize the use of under- or over-processed soybean meal and ultimately enhance poultry health, growth performance and production efficiency.

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