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Article

Soybean Meal Protein Extraction and Comparative Analysis with Soy Protein Isolate

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Abstract: This study delves into the extraction and compositional analysis of soybean meal protein isolates (SMPI) compared to soy protein isolates (SPI), focusing on their functional and structural properties, digestibility, amino acid composition, and the impact of various proteases on antinutritional factors. By leveraging alkaline (0.25 % of NH₄OH) extraction and isoelectric precipitation, SMPI was efficiently obtained. Compositional characteristics revealed that SMPI has high moisture, protein, and ash, but lower fat, and carbohydrate content compared to SPI. Alcalase, Neutrase and Pepsin hydrolysis indicated lower digestibility of SMPI than SPI. Fourier-transform infrared spectroscopy and sodium dodecyl sulfate-polyacrylamide gel electrophoresis provided similar molecular structure and protein molecular weight distribution. This comprehensive analysis underscores the valorization of soybean meal, promoting its use beyond conventional animal feed towards nutritional supplements and bio-based products, thereby contributing to environmental sustainability and the enhancement of global food systems.

Keywords: soybean meal; protein isolates; extraction; enzymatic hydrolysis; characterization

1. Introduction

Soybean (*Glycine max*) remains one of the most valuable agricultural commodities in the world [1]. Although soybeans can be processed into a wide variety of foods such as tofu, soy milk, soy flour, and soy nut butter, more than 80% of soybeans produced in the United States are crushed into edible oil [2]. Soybean oil, primarily harnessed to produce renewable diesel, constitutes approximately 20% of the weight of a bushel of soybeans (or ~12 lbs./1.55 gal); the remaining is soybean meal (SM), a by-product of soybean processing [3]. With a growing number of soybean processing facilities across the U.S., the supply of SM is on the rise, making it an accessible resource for a diverse range of applications, including animal feed, feedstock for organic fertilizer, nutritional supplements, and bio-based products. Moreover, the protein content in SM is regarded as high-quality protein due to its abundance of essential amino acids such as lysine, methionine, and tryptophan [4]. Effective management and valuation of SM can significantly impact protein availability [5]. Similarly, efficient valorization of SM could improve resource efficiency, reduce adverse environmental effects, and offer an avenue to extract economic value from waste streams [6]. Due to its versatility and high protein content, SM plays a crucial role in food processing [7] and is a valued ingredient in a wide range of food products, including bakery goods and meat substitutes [8]. SM is equally a notable alternative to animal proteins in plant-based diets, making it a necessary part of diets for vegetarians and vegans [9].

Protein is the most vital component of SM, making up about 47% to 49% of its total composition. This highlights the significance of SM as a bioresource in a wide variety of applications that require high quantities of protein, particularly animal feed formulations. Other resourceful components in SM include essential amino acids, dietary fiber, sugars, and starch [10]. These components vary in concentration among samples due to factors such as geographic location of soybean production, processing methods of soybean, soybean variety processed amongst others [11]. For instance, U.S.

SM is known to have a more consistent and higher digestibility, better protein quality (based off essential amino acid levels), and lower fiber than SM from Argentina, India, and Brazil, whereas the oil content of SM from South American countries was higher than in U.S. SM [12]. Several studies have investigated the nutritional profile of SM, revealing that it contains high levels of lysine and limited levels of methionine and cystine [13,14,15].

Efforts to enhance the nutritional value of SM to improve the digestibility of its amino acids by reducing anti-nutritional factors (ANFs) include enzyme addition and fermentation [16-18]. Moreover, fermentation has shown promise in increasing SM's concentration of easily digestible proteins and essential amino acids [19]. The research iteration to fully harness SM has mostly focused on reducing ANFs, characterizing and enhancing its nutritional profile at the detriment of other untapped fundamental properties and applicability of SM. While its use in livestock nutrition is undisputed, SM's broad applicability suffers from insufficient exploration of its functional and structural properties. This gap restricts the utilization of SM within the conventional bound of animal feed, neglecting prospective value as human dietary supplements, organic fertilizers, and environment-friendly bio-based products. To utilize SM protein (SMP) for biochemical production, [20] used a reusable solvent to extract SMP. Progressively, SM-extracted protein could be fermented to produce sizeable quantities of biological ammonia [21]. Herein, we evaluated SMP's functional and structural properties, focusing on its digestibility, proximate composition, functional group, and biomolecular structures. Specifically, this study delved into the extraction of SMP, hydrolysis of SMP using various proteases, activities of SMP antinutritional factors such as trypsin inhibitor, proximate analysis, and FTIR characterization. Collectively, these properties can provide useful insights into ways to valorize SMP for economic value creation, environmental sustainability, and improvement of global food systems.

2. Materials and Methods

2.1. Materials

The pilot plant unit, agricultural and biosystem department, North Dakota State University, provided the soybeans used for this study. The defatted soybean meal and whole soybean grains were finely ground before use. All chemicals and reagents used for the study are of analytical grade. Novozymes supplied the enzymes used. A mini-protean precast gel and protein standard were purchased from Bio-Rad. The Pilot plant laboratory supplied other laboratory tools and apparatus.

2.2. Extraction of Soybean Meal Protein

The soluble matter was extracted from soybean meal by dissolving the meal in an aqueous alkaline solution in a 1:10 sample-to-solvent ratio with different concentrations of NH_4OH . The range of solvent concentration variables measured is 0, 0.25, 0.5, 0.75 and 1% NH_4OH . 2.5g of soybean meal powder was dispersed in 25 ml of each solvent concentration. The dissolving solution was placed in a water bath at 52.5°C and 130 rpm for 12 hours [22]. The soluble fraction was obtained by centrifuging each sample solution at 1500 rpm for 10 minutes, followed by decantation. Further analysis was carried out on the supernatant using an isoelectric point to extract soybean meal protein. With 1N HCl, the pH of the supernatant was adjusted to between 4.5 and 5.0 [23], and the mixture was then allowed to precipitate overnight. The residue was rinsed with distilled water and oven-dried for 12 hours at 60°C using a binder ED-56 woven dryer. All extraction experiments were carried out in triplicate. Further analysis was done using SMPI obtained from 0.5% NH_4OH concentrations under the same procedures. Figure 1 shows a graphical illustration of the alkaline extraction of soybean meal protein isolates.

The total dry matter yield was determined using Equation 1 as a percentage of the ratio of total dry matter after extraction TDM_o to the total dry matter in the initial suspension TDM_s (Gerzhova et al., 2015).

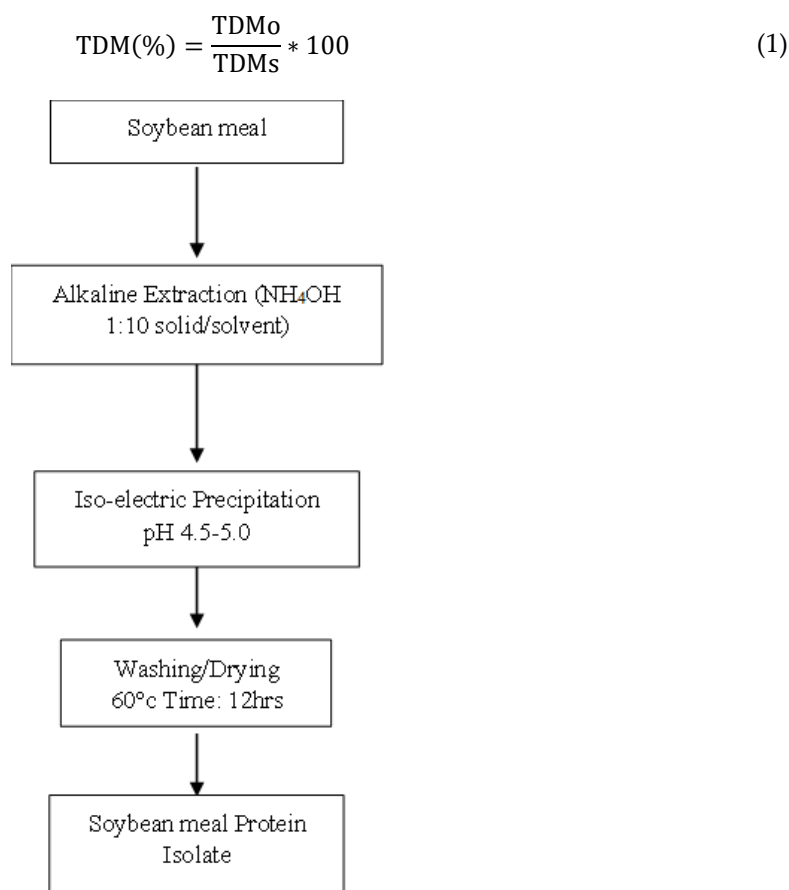


Figure 1. Flowchart Of Protein Isolates Production.

2.3. Proximate Analysis

2.3.1. Moisture Content

Moisture contents were determined according to AOAC 930.15 [24]. Briefly, 1g of sample was weighed in a crucible and oven-dried at 105°C for 24 hrs. The weight of the empty crucible was recorded before samples were added for drying. The percentage of moisture content was determined by Equation 2.

$$\%Mc = \frac{Mw}{Sw} * 100 \quad (2)$$

MW= Sw-(Dc-Ec)

Where Mw = moisture weight

Sw= sample weight

Ec= empty crucible

Dc= dried sample and crucible weight

2.3.2. Ash Content

Determination of the total ash content of the samples was performed by incineration using Thermos Fisher Scientific Thermolyne Benchtop TM 1100 °C Muffle Furnace (Vernon Hills, IL 60061, USA). A 2g of each sample was weighed in a crucible and placed in the muffle furnace at 575 °C for 24 hrs. Samples were allowed to cool in a desiccator, and the Total ash was calculated using Equation 3.

$$\%ash = \frac{M2 - M1}{Ms} * 100 \quad (3)$$

M_2 = Weight of ash

M_1 =Weight of crucible

M_s .= Weight of sample

2.3.3. Fat Content

To determine the total fat content, 2.00 g of the homogenized sample was subjected to continuous lipid extraction for 5 hours in a Soxhlet extractor using petroleum ether as solvent [25].

2.3.4. Total protein

Total protein was determined by the Dumas method using a Thermos scientific flash elemental Analyzer. 2-3mg of sample was combusted at high temperature in an oxygen atmosphere. Via subsequent oxidation and reduction tubes, nitrogen is quantitatively converted to N_2 measured by a thermal conductivity detector [25]. The total protein is calculated by using a conversion factor of 6.25 [26].

2.3.5. Total Carbohydrate

The arithmetic difference method was used to estimate the samples' total carbohydrate content [27]. Using the equation below, the proximate composition values of other parameters were subtracted from 100 to estimate the amount of available carbohydrates.

$$\% \text{Carbohydrate} = 100 - (\% \text{moisture} + \% \text{Fat} + \% \text{protein} + \% \text{ash}) \quad (4)$$

2.4. Enzymatic Hydrolysis of Soybean Meal Protein Isolates

Soybean meal protein hydrolysate was prepared according to the method described by [28]. Three different protease enzymes, namely Alcalase, Neutrase, and Pepsin, were used for the experiment. About 5g of each sample of protein isolate was dissolved in PBS in a 1:10 solid-to-solvent ratio. pH of the suspension was then adjusted to optimal pH for each enzyme using 1M NaOH. Suspension was allowed to stabilize for 2 hours in a water bath at 40°C. Subsequently, 5% enzyme concentration was added to the mixture, and the reaction was continued by placing it in a water bath for 24 hours at 40°C and 100 rpm. The reaction was halted by heating at 90°C for 10 minutes. The resulting mixture was centrifuged at 4000 rpm for 10 minutes to obtain the hydrolysate.

2.4.1. Amine Quantification

Total amines present in the hydrolysate were quantified using a similar method described by [22]. The 2,4,6-Trinitrobenzene Sulfonic Acid (TNBS) assay method was employed for estimating the total amount of amine present in the sample. 0.25ml of TNBS (0.01% w/v) was added to 0.5ml of each sample and the mixture was vortexed. Afterwards, the solution was incubated in a water bath for 2 hours at 37 °C. To each sample, 0.125 ml of 1 N HCl and 0.25 ml (about 0.01 oz) of 10% SDS were added to stop the reaction. The resultant solution's absorbance was measured at a wavelength of 335 nm. Prior to the runoff of the experiment, glycine standard (20 µg/ml) was prepared by dissolving 0.2 g of glycine in 10 ml of distilled water and then diluted as needed. In 0.1 M sodium bicarbonate reaction buffer (pH 8.5), soy samples were dissolved. The standard curve generated was used in calculating the concentration of extracted amine in each sample.

2.5. FTIR

Using a Fourier-transform infrared (FTIR) apparatus coupled with an attenuated total reflectance (ATR) accessor (Thermos Scientific Nicolet 8700), dried SMP and SPI were analyzed on a diamond plate at a resolution of 4cm⁻¹. Spectra were collected at frequency region 4000-600cm⁻¹ and 64scan was recorded for each sample.

2.6. GEL ELECTROPHORESIS

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on Bio-Rad Mini-protean precast gel using Bio-Rad Precision Plus Protein Standards (10–250 kDa) as molecular markers according to the method of Laemmli 1970. All samples were prepared by dilution with an equal volume of Laemmli sample buffer. The gels were run in mini-protean electrophoresis cells at 150V for 40 minutes or until the dye reached the bottom of the gel and then stained with Coomassie blue dye R-250.

3. Results and Discussion

3.1. Extractability of Soybean Meal Protein

The rigid and complex structure of plant cell walls promotes the lodging of plant protein within the matrix of cell walls, making it less accessible for extraction. Previous research has indicated that alkaline conditions are more effective than neutral or acidic conditions for extraction of plant protein [29] because they facilitate the breaking down of cell wall structures in plant materials, resulting in the release of protein trapped within the cells [30]. The alkaline condition uses severe pH to break down hydrogen, amide, and disulfide bonds [31]. However, extraction at a higher pH can disintegrate the structure of lysine and cysteine amino acids and affect protein digestibility. This observation highlights the efficiency of alkaline conditions, particularly NH_4OH , in breaking down plant cell walls to improve plant protein extractability[20].

In this study, extraction of soybean meal protein isolates was performed using ammonium hydroxide as the sole agent for extraction of protein, followed by further precipitation at their isoelectric points. It was observed that at 0% NH_4OH concentration, the protein yield was significantly low. A drop in protein yield was observed when concentrations of NH_4OH increased from 0.25% to 0.5%; however, a marginal increase in yield occurred as concentrations of solvent increased from 0.5 to 1% solvent concentration. Figure 2 shows the highest protein yield, 24.1%, obtained at a 1% NH_4OH concentration, but not significantly different from other concentrations. Therefore, 0.25% NH_4OH is suitable for SMP extraction.

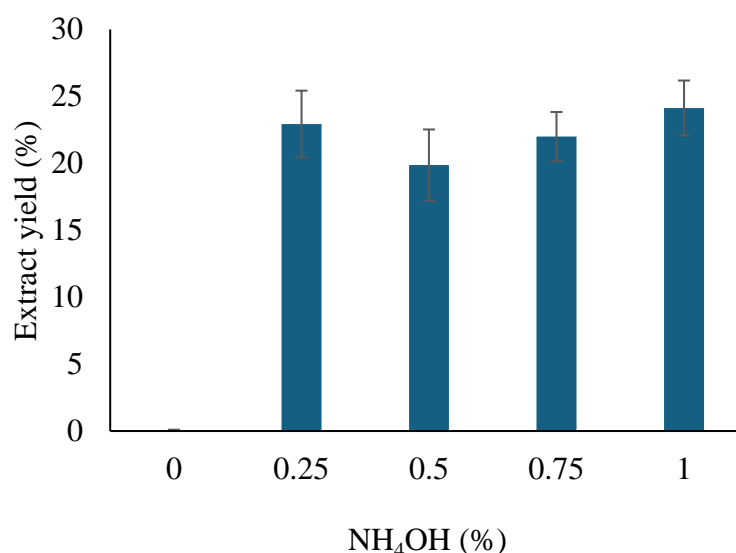


Figure 2. Effect of different concentrations of alkaline solvent on soybean meal protein extractability.

The observed difference in protein yield, as shown in Figure 2, can be attributed to a change in the pH [32]. Protein molecules tend to become negatively charged at pH above the isoelectric point, leading to repulsive force between like charges; likewise, solution pH below the isoelectric point causes protein molecules to gain positive charges. Furthermore, an increase in alkaline concentration

beyond this point can result in the denaturation of protein [33]. Extremely high alkaline concentrations have the potential to cause permanent structural changes in proteins, which can result in precipitation or aggregation and eventually lower the percentage of soluble, functional protein synthesized [34].

3.2. Proximate Composition

Table 1 shows the proximate composition of soybean meal and protein isolates obtained through alkaline extraction and isoelectric precipitation. The results reveal that SMPI contains 14% moisture, 2.12% ash, 0.02% fat, 68% protein, and 16% carbohydrates, while SPI contains 11% moisture, 1.66% ash, 8.67% fat, 57% protein, and 22% carbohydrates.

Table 1. Proximate Composition of Soybean meal and Soybean Protein.

Parameters	SMPI (%)	SPI (%)
Moisture content	14 ± 0.58	11 ± 1.00
Ash	2.12 ± 2.47	1.66 ± 0.28
Fat	0.02 ± 0.00	8.67± 0.01
Protein	68 ± 4.50	56.8 ± 0.80
Carbohydrate	15.86 ± 3.01	21.87 ± 0.34

SMPI has a higher moisture content than SPI. This disparity may be attributed to various factors, including inconsistencies in the oil extraction process, a less intensive drying protocol to preserve protein functionality, and soybean meal’s porous nature, which allows it to retain more moisture than soybeans [35]. Conversely, SPI’s lower moisture content may be attributed to the hydrophobic nature of lipids present in soybean protein isolates.

A high contrast in protein content is evident between SMPI (68±4.5%) and SPI (56.8±0.8%). The substantial variability observed in SMPI, as indicated by the standard error, may be due to the impact of processing conditions on soybean protein isolates. During oil extraction, a large amount of fat is removed, resulting in more protein concentrate products. [36] indicated that the protein content of soybean meal isolates falls within the range of 61%–91%, while [37] reported a higher value of more than 90% SMPI dried-weight protein content.

Furthermore, SPI displays a slightly lower ash content than SMPI. However, the standard error for the ash content of SMPI (± 2.47) is significantly larger, indicating higher variability in the data. These differences could be due to the incorporation of non-meal materials, such as minerals, during processing. Ash content measures mineral content [38] in the sample, and the lower ash content in SPI suggests a higher protein purity.

SPI had a higher fat content than SMPI. The increased fat content may be attributed to processing methods or SPI’s inherent composition. Lipids can impact overall moisture content by repelling water, potentially resulting in lower moisture levels in protein isolates [22].

The carbohydrate content analysis reveals that SMPI had a lower carbohydrate composition of 15.86%, while SPI had a higher carbohydrate content of 21.87%. A significant factor that may be responsible for the observed difference is the thermal treatment processes involved in soybean meal production, such as drying or roasting, which leads to the degradation of carbohydrates [39]. As a result, the carbohydrate content in soybean meal may be further reduced compared to the raw soybean material. This observed disparity in carbohydrate concentrations between the two isolates signifies a substantial difference in their respective nutritional profiles.

3.3. Digestibility of Soybean Meal Protein by Different Industrial Proteases

Protein digestibility is the proportion of proteins that can be hydrolyzed by digestive enzymes with the potential to be absorbed and utilized as amino acids or other nitrogen compounds [40]. There is an ever-increasing demand for plant-based protein for human consumption and easily hydrolyzed protein feedstock for bio-based product manufacturing. Although soybeans are the largest source of

plant protein, the constituent proteins have low digestibility primarily due to antinutritional factors (ANFs), which are protease inhibitors. However, ANFs can be inactivated by different soybean processing methods, especially heat treatment [41]. The heat treatment involved in the processing of soybeans into soybean meal (SM) is known to significantly reduce the levels of ANFs present in raw soybeans [42]. Albeit the known benefits of heat treatment in soybean protein digestibility, the relationship between thermal processing and SM protein digestibility is not linear. While the inactivation of ANFs is beneficial and could increase protein digestibility, excessive heat can reduce the availability of some essential amino acids, potentially generating new types of ANFs [43]. Moreover, there is a lack of studies to validate that heat treatment alone will simultaneously increase the digestibility of SM protein.

To investigate the digestibility of SM protein, we performed *in-vitro* hydrolysis of SMPI with three known proteases — Alcalase, Neutrase, and Pepsin using SPI as control. Interestingly, the results show that the digestibility of soybean protein, expressed as the degree of hydrolysis (DH), was significantly higher than that of SM protein, with pepsin having the highest hydrolytic effects of the three enzymes (Figure 3). This result is in contrast with the findings that protein digestibility of soybean protein increases with various processing methods, including those involved in the oil extraction process that generates SM [44-46]. The joint effect of most processing methods, especially heat treatment of soybean is the reduction of ANFs [44]. All processing methods are reported to reduce protease inhibitors (ANFs) such as trypsin, hemagglutinins, and chymotrypsin inhibitors [47,48].

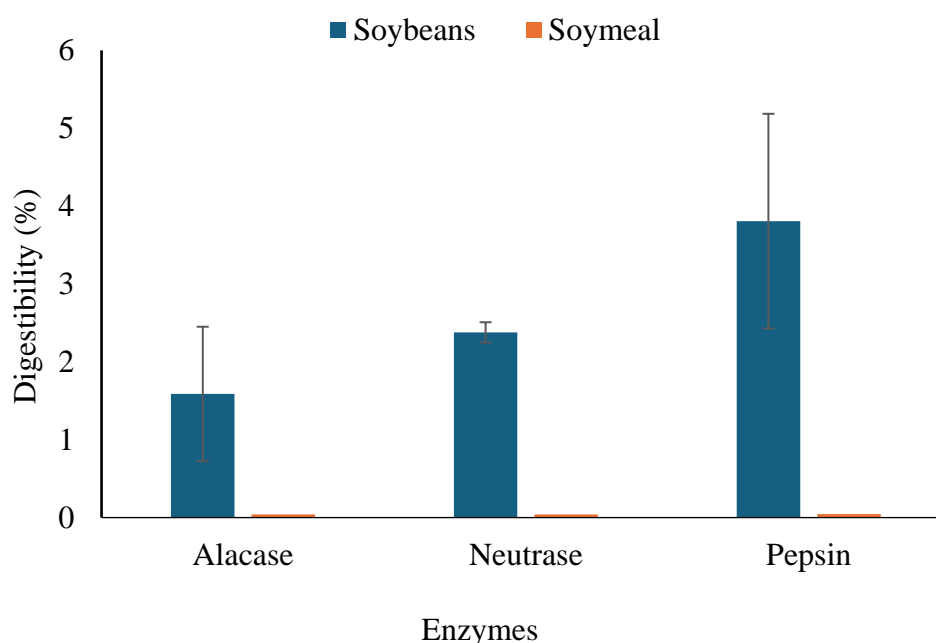


Figure 3. Digestibility of soybean protein and soybean meal protein.

Elimination of protease inhibitors in soybean protein may not increase digestibility of SM protein. It should be noted that some inhibitors such as Bowman-Birk inhibitor (BBI) are relatively heat stable due to their multiple disulfide bridges that block the activity of proteases at independent binding sites [49]. Heat-stable inhibitors like BBI would be present in SM produced from processing that involved less soybean heating (like in solvent extraction), which is usually the case as a compromise to keep the protein quality intact while thermally destroying protease inhibitors. Although the chronic consumption of SM protein with residual levels of heat-stable protease inhibitors will unlikely have any negative health impacts on humans and animals, it could have some pharmacological effects [50]. Similarly, the pretreatment of soybean biomass before oil extraction, leading to SM production and the subsequent protein isolation from SM, can influence protein digestibility.

Processing methods such as soaking, boiling, and fermentation can also modify the protein structure, making it more accessible for enzymatic hydrolysis [44]. Fermentation of soybean protein specifically is known to enhance protein digestibility and increase the levels of indispensable amino acids [51,52]. In contrast, the processing steps for producing SM, which primarily focus on oil extraction, may not always optimize conditions for improving SM protein digestibility, as the product of interest is the oil rather than the meal. The resulting SM may contain less digestible proteins than soybean protein due to these alterations in protein structure and the loss of components that may aid in digestibility. It should be noted, however, that soybean protein products intended for human consumption often undergo more controlled processing methods designed to maximize nutritional value and digestibility. These methods may include specific treatments to reduce heat-stable protease inhibitors and enhance the bioavailability of the constituent amino acids.

3.4. FTIR Spectroscopy

The FTIR spectrum of soybean and soybean meal, recorded in the range of 3915 to 600 cm^{-1} as shown in Figure 4 provides insights into their molecular structures and functional groups. The presence of OH peak at 3505 cm^{-1} in soybean meal suggests the presence of hydroxyl groups, commonly found in carbohydrates such as cellulose and sugars [53]. Additionally, peaks observed at 3125 cm^{-1} and 1625 cm^{-1} suggest the presence of C-H and C=O stretching vibrations, respectively. These findings align with the FTIR composition of soybean, which are known to contain significant amounts of carbohydrates and lipids [20,22]. However, the FTIR analysis of SPI reveals a distinct set of peaks indicative of its composition. Notably, a distinct peak of NH observed at the broad absorption band of 3039 cm^{-1} , suggests the presence of amino acids symmetric stretching vibrations [54]. The distinct peak at 2924 cm^{-1} indicates the presence of C-H stretching vibrations associated with lipids, which are typically present in higher concentrations in soybean meal compared to whole soybeans [55]. Furthermore, peaks at 1743 cm^{-1} and 1626 cm^{-1} suggest the presence of C=O stretching vibrations and amide groups, respectively, further confirming the presence of proteins.

Despite these differences, both soybean and soybean meal FTIR spectra share common functional groups. PO₂- at 1235 cm^{-1} indicates phospholipids, essential components of cell membranes in both soybean and soybean meal [56,57]. The presence of methyl (CH₃) groups at 1395 cm^{-1} suggests the presence of lipids, consistent with the composition of both samples. Additionally, the presence of CO-O-C at 1158 cm^{-1} indicates the presence of ester linkages, which are commonly found in triglycerides [58], further supporting the lipid content in both samples.

Based on these FTIR results, it was observed that soybean comprises a mixture of carbohydrates, lipids, and proteins, with a relatively higher proportion of carbohydrates and lipids compared to soybean meal. Soybean meal, however, seems enriched in proteins and lipids, with a lower proportion of carbohydrates. The presence of amide groups in soybean meal suggests a higher concentration of proteins, likely present in the form of residual meal after oil extraction from soybeans. Also, distinct NH peaks in SMPI indicate a higher protein concentration than SPI. The FTIR results provide valuable insights into the structural composition of both soybean and soybean meal, which can further aid their characterization and utilization in various applications.

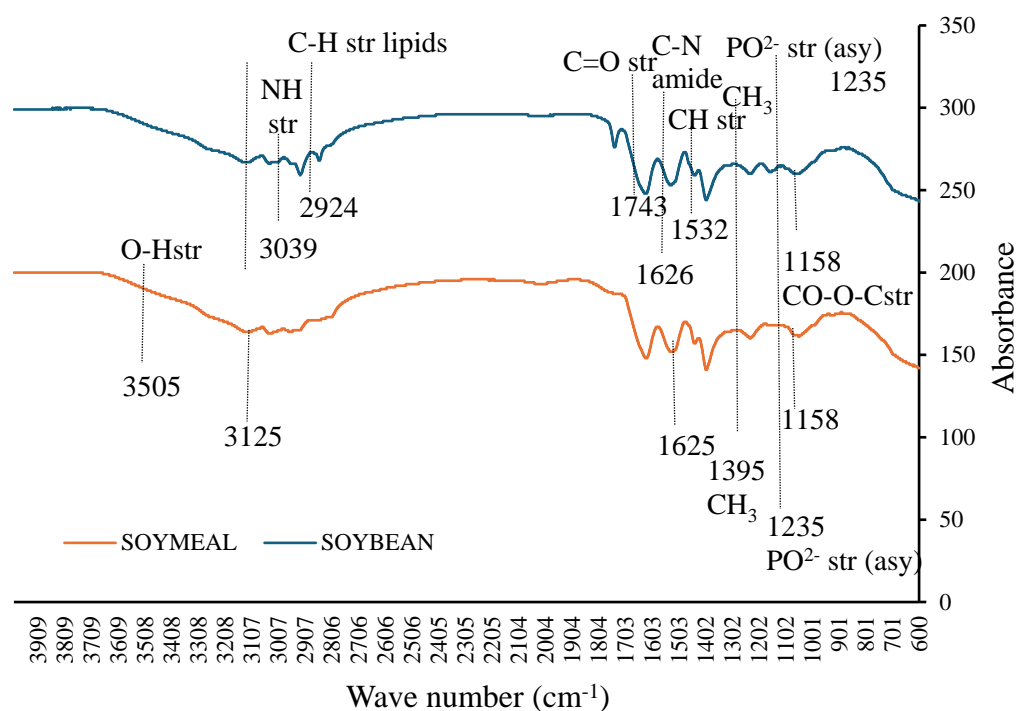


Figure 4. FTIR Spectroscopy of soybean and soybean meal.

3.5. SDS-PAGE Banding Pattern of Soybean and Soybean Meal Protein Isolates

The heat treatment of soybean during oil extraction could affect the quality of the resulting meal, especially the meal protein's nutritional value. Excessive heat during the extraction process can reduce the availability of specific amino acids and compromise the meal's protein structure [44]. To gain insights into the protein molecular weight changes that might have occurred during soybean oil extraction, SPI and SMPI were profiled using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Similar banding patterns were observed for both SPI and SM using two protein concentrations (50 mg/ml and 25 mg/ml). Figure 5 (Panel A) shows the banding pattern of 50 mg/ml of SM and SPI protein isolates in lane 2 and lane 3, respectively, while Panel B shows the corresponding 25 mg/ml samples. In the 50 mg/ml samples, protein molecular weight ranges from 250 kD to 20 kD (Panel A); whereas the range is from 150 kD to about 35 kD in the 25 mg/ml isolates (Panel B). The appearance of visible and undegraded bands in both concentrations of SMPI revealed that the meal protein has not been structurally affected. Similarly, the banding pattern of the 50 mg/ml SPI showed that the soybean used in this study was undegraded and contained the major globular proteins usually found in soybeans (glycinin and β -conglycinin). Our results showed that these globular proteins are not visible at low protein concentrations, as demonstrated by their absence in the 25 mg/ml sample. When up to 5 g/L SPI concentrations were used, both globular proteins were visible, having molecular weights between about 95 kD and 20 kD [59]. Thus, given that the samples used in this study were undegraded, the similar banding patterns observed for both isolates at different concentrations demonstrated that heat treatment during soybean oil extraction has a limited impact on the protein profile of the resulting meal.

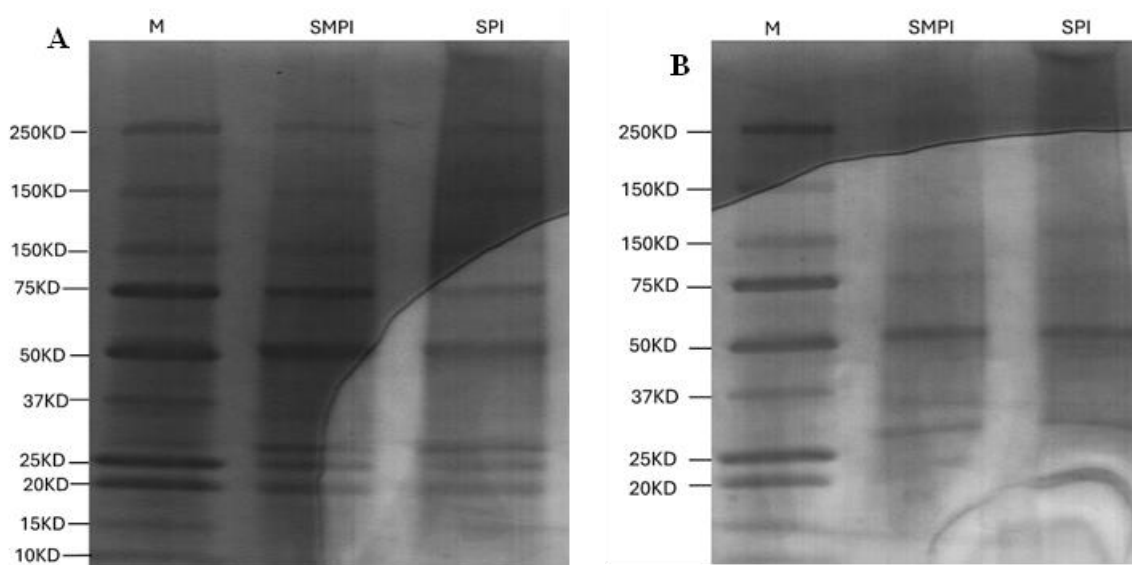


Figure 5. Molecular weight distribution at different concentrations (A: 50 mg/mL and B: 25 mg/mL) of soybean meal protein isolate (SMPI) and soybean protein isolate (SPI). M is the protein ladder.

4. Conclusion

Soybeans and its by-product soybean meal are rich sources of protein for animal feed. They can also be utilized to supplement human protein requirement and as feedstock for biochemicals production. The heat treatment involved in soybean processing during oil extraction is hypothesized to impact the protein composition of the resulting soybean meal. Through multiple analytical parameters, including proximate composition, digestibility, FTIR spectroscopy, and SDS-PAGE banding patterns, this study is a comparative analysis of the protein profile of soybean meal protein isolates (SMPI) and soybean protein isolate (SPI). Low concentrations of ammonium hydroxide (NH_4OH) ranging from 0.25–1% was shown to extract about 25% protein from soybean meal (SM). Contrary to previous opinions that heat treatment would increase the protein digestibility of soybean meal protein, this study established that SPI had higher digestibility than its meal. Relative to unprocessed soybean protein, proximate analyses showed that soybean meal is more protein dense. However, the observation that both isolates of soybean and soybean meal proteins had similar molecular structure and gel banding pattern revealed that heat treatment of soybeans during processing has limited impact on the protein quality of soybean meal. These findings provide insights into the utilization of soybean meal protein towards improving global food systems and environmental sustainability. Future studies should explore the optimization of extraction methods and the digestibility of soybean meal protein to broaden its application scope as a resource in the food and various industrial sectors.

References

1. Ruiz, N., Parsons, C. M., Stein, H. H., Coon, C. N., Eys, J., & Miles, R. D. A review: 100 years of soybean meal. ADM (2020).
2. "USDA ERS–Oil Crops Sector at a Glance." Accessed February 22, 2024. <https://www.ers.usda.gov/topics/crops/soybeans-and-oil-crops/oil-crops-sector-at-a-glance/>
3. Challenges Ahead For Soybean-Based Renewable Fuels Demand | Biodiesel Magazine." Accessed February 22, 2024. <https://biodieselmagazine.com/articles/challenges-ahead-for-soybean-based-renewable-fuels-demand-2518696>
4. Sá, Amanda Gomes Almeida, João Borges Laurindo, Yara Maria Franco Moreno, and Bruno Augusto Mattar Carciofi. "Influence of Emerging Technologies on the Utilization of Plant Proteins." *Frontiers in Nutrition* 9 (2022). <https://www.frontiersin.org/articles/10.3389/fnut.2022.809058>

5. Gerliani, Natela, Riadh Hammami, and Mohammed Aïder. "Extraction of Protein and Carbohydrates from Soybean Meal Using Acidic and Alkaline Solutions Produced by Electro-Activation." *Food Science & Nutrition* 8, no. 2 (2020): 1125–38. <https://doi.org/10.1002/fsn3.1399>.
6. Colletti, Alessandro, Andrea Attrovio, Luisa Boffa, Stefano Mantegna, and Giancarlo Cravotto. "Valorisation of By-Products from Soybean (Glycine Max (L.) Merr.) Processing." *Molecules* 25, no. 9 (January 2020): 2129. <https://doi.org/10.3390/molecules25092129>
7. Scott, E., Peter, F., et al., 2007. Biomass in the manufacture of industrial products—the use of proteins and amino acids. *Appl. Microbiol. Biotechnol.* 75, 751–762
8. Singh, Preeti, R. Kumar, S. N. Sabapathy, and A. S. Bawa. "Functional and Edible Uses of Soy Protein Products." *Comprehensive Reviews in Food Science and Food Safety* 7, no. 1 (2008): 14–28. <https://doi.org/10.1111/j.1541-4337.2007.00025.x>.
9. Detzel, Andreas, Martina Krüger, Mirjam Busch, Irene Blanco-Gutiérrez, Consuelo Varela, Rhys Manners, Jürgen Bez, and Emanuele Zannini. "Life Cycle Assessment of Animal-Based Foods and Plant-Based Protein-Rich Alternatives: An Environmental Perspective." *Journal of the Science of Food and Agriculture* 102, no. 12 (2022): 5098–5110. <https://doi.org/10.1002/jsfa.11417>.
10. Grieshop, Christine M., Charles T. Kadzere, Gina M. Clapper, Elizabeth A. Flickinger, Laura L. Bauer, Rodney L. Frazier, and George C. Fahey. "Chemical and Nutritional Characteristics of United States Soybeans and Soybean Meals." *Journal of Agricultural and Food Chemistry* 51, no. 26 (December 1, 2003): 7684–91. <https://doi.org/10.1021/jf034690c>.
11. Karr-Lilienthal, L. K., Grieshop, C. M., Spears, J. K., & Fahey, G. C. (2005). Amino Acid, Carbohydrate, and Fat Composition of Soybean Meals Prepared at 55 Commercial U.S. Soybean Processing Plants. *Journal of Agricultural and Food Chemistry*, 53(6), 2146–2150. <https://doi.org/10.1021/jf048385i>
12. Thakur, M., & Hurburgh, C. R. (2007). Quality of US Soybean Meal Compared to the Quality of Soybean Meal from Other Origins. *Journal of the American Oil Chemists' Society*, 84(9), 835–843. <https://doi.org/10.1007/s11746-007-1107-8>
13. Stein, Hans H., Larry L. Berger, James K. Drackley, George C. Fahey, David C. Hernot, and Carl M. Parsons. "18–Nutritional Properties and Feeding Values of Soybeans and Their Coproducts." In *Soybeans*, edited by Lawrence A. Johnson, Pamela J. White, and Richard Galloway, 613–60. AOCS Press, 2008. <https://doi.org/10.1016/B978-1-893997-64-6.50021-4>
14. Sriperm, N., G. M. Pesti, and P. B. Tillman. "The Distribution of Crude Protein and Amino Acid Content in Maize Grain and Soybean Meal." *Animal Feed Science and Technology* 159, no. 3 (August 11, 2010): 131–37. <https://doi.org/10.1016/j.anifeedsci.2010.05.009>
15. Banaszkiwicz, T. (2011). Nutritional Value of Soybean Meal. In H. El-Shemy (Ed.), *Soybean and Nutrition*. InTech. <https://doi.org/10.5772/23306>
16. Feng, J., X. Liu, Z. R. Xu, Y. Z. Wang, and J. X. Liu. "Effects of Fermented Soybean Meal on Digestive Enzyme Activities and Intestinal Morphology in Broilers." *Poultry Science* 86, no. 6 (June 1, 2007): 1149–54. <https://doi.org/10.1093/ps/86.6.1149>.
17. Li, Hao, Jie Yin, Xi He, Zhiqing Li, Bie Tan, Qian Jiang, Jiashun Chen, and Xiaokang Ma. "Enzyme-Treated Soybean Meal Replacing Extruded Full-Fat Soybean Affects Nitrogen Digestibility, Cecal Fermentation Characteristics and Bacterial Community of Newly Weaned Piglets." *Frontiers in Veterinary Science* 8 (2021). <https://www.frontiersin.org/articles/10.3389/fvets.2021.639039>.
18. Abdel-Raheem, Sherief M., El Said Yehia Mohammed, Rania Elsaid Mahmoud, Mahmoud Fathy El Gamal, Hend S. Nada, Waleed Rizk El-Ghareeb, Mohamed Marzok, et al. "Double-Fermented Soybean Meal Totally Replaces Soybean Meal in Broiler Rations with Favorable Impact on Performance, Digestibility, Amino Acids Transporters and Meat Nutritional Value." *Animals: An Open Access Journal from MDPI* 13, no. 6 (March 11, 2023): 1030.
19. Sukhikh, Stanislav, Olga Kalashnikova, Svetlana Ivanova, Alexander Prosekov, Olesia Krol, Olga Kriger, Nataly Fedovskikh, and Olga Babich. "Evaluating the Influence of Microbial Fermentation on the Nutritional Value of Soybean Meal." *Fermentation* 8, no. 9 (September 2022): 458. <https://doi.org/10.3390/fermentation8090458>
20. Bello, Ibrahim, Adewale Adeniyi, Taofeek Mukaila, and Ademola Hammed. "Developing and Modelling of Sustainable Protein Extraction Using Ammonium Hydroxide—A Recoverable and Reusable Solvent." *Food and Bioproducts Processing* 140 (August 1, 2023): 16–28.
21. Adeniyi, Adewale, Ibrahim Bello, Taofeek Mukaila, Ewumbua Monono, and Ademola Hammed. "Enzyme-Driven Bioprocessing for Enhanced Bio-Ammonia Production from Soybean Meal Protein Isolate." *Biomass Conversion and Biorefinery*, September 15, 2023.
22. Bello, I., Adeniyi, A., Mukaila, T., Hammed, A. (2023). Optimization of Soybean Protein Extraction with Ammonium Hydroxide (NH₄OH) Using Response Surface Methodology. *Foods*, 12(7), 1515.
23. Verfaillie, Diete, Frederik Janssen, Geert Van Royen, and Arno G. B. Wouters. "A Systematic Study of the Impact of the Isoelectric Precipitation Process on the Physical Properties and Protein Composition of Soy

- Protein Isolates." *Food Research International* 163 (January 1, 2023): 112177. <https://doi.org/10.1016/j.foodres.2022.112177>.
24. Beard, E. Gregory. (2001). Moisture Measurement. Manufacturing Confectioner, 73.
 25. Official Methods of Analysis of AOAC INTERNATIONAL. Oxford University Press, 2023. <https://doi.org/10.1093/9780197610145.001.0001>.
 26. He, Z., Zhang, H., Olk, D. C., Shankle, M., Way, T. R., & Tewolde, H. (2014). Protein and fiber profiles of cottonseed from upland cotton with different fertilizations. *Modern Applied Science*, 8(4), 97.
 27. Conto, Leilane Costa de, Marco Antonio Lefevre Gagnani, Diogo Maus, Hiria Cristina Ifanger Ambiel, Ming Chih Chiu, Renato Grimaldi, and Lireny Aparecida Guaraldo Gonçalves. "Characterization of Crude Watermelon Seed Oil by Two Different Extractions Methods." *Journal of the American Oil Chemists' Society* 88, no. 11 (2011): 1709–14. <https://doi.org/10.1007/s11746-011-1850-8>.
 28. Hunsakul, K., Laokuldilok, T., Sakdatorn, V., Klangpetch, W., Brennan, C. S., & Utama-ang, N. (2022). Optimization of enzymatic hydrolysis by alcalase and flavourzyme to enhance the antioxidant properties of jasmine rice bran protein hydrolysate. *Scientific Reports*, 12(1), Article 1. <https://doi.org/10.1038/s41598-022-16821-z>
 29. Tömösközi, S., Lásztity, R., Haraszi, R., & Baticz, O. (2001). Isolation and study of the functional properties of pea proteins. *Food / Nahrung*, 45(6), 399–401. [https://doi.org/10.1002/1521-3803\(20011001\)45:6<399::AID-FOOD399>3.0.CO;2-0](https://doi.org/10.1002/1521-3803(20011001)45:6<399::AID-FOOD399>3.0.CO;2-0)
 30. Hadidi, M., Aghababaei, F., & McClements, D. J. (2023). Enhanced alkaline extraction techniques for isolating and modifying plant-based proteins. *Food Hydrocolloids*, 145, 109132. <https://doi.org/10.1016/j.foodhyd.2023.109132>
 31. Contreras, María del Mar, Antonio Lama-Muñoz, José Manuel Gutiérrez-Pérez, Francisco Espínola, Manuel Moya, and Eulogio Castro. "Protein Extraction from Agri-Food Residues for Integration in Biorefinery: Potential Techniques and Current Status." *Bioresource Technology* 280 (May 1, 2019): 459–77. <https://doi.org/10.1016/j.biortech.2019.02.040>.
 32. Sari, Y. W., Mulder, W. J., Sanders, J. P. M., & Bruins, M. E. (2015). Towards plant protein refinery: Review on protein extraction using alkali and potential enzymatic assistance. *Biotechnology Journal*, 10(8), 1138–1157. <https://doi.org/10.1002/biot.201400569>
 33. Deleu, Lomme J, Marlies A Lambrecht, Julie Van De Vondel, and Jan A Delcour. "The Impact of Alkaline Conditions on Storage Proteins of Cereals and Pseudo-Cereals." *Current Opinion in Food Science* 25 (February 2019): 98–103. <https://doi.org/10.1016/j.cofs.2019.02.017>.
 34. Hou, Furong, Wenhui Ding, Wenjuan Qu, Ayobami Olayemi Oladejo, Feng Xiong, Weiwei Zhang, Ronghai He, and Haile Ma. "Alkali Solution Extraction of Rice Residue Protein Isolates: Influence of Alkali Concentration on Protein Functional, Structural Properties and Lysinoalanine Formation." *Food Chemistry* 218 (March 1, 2017): 207–15. <https://doi.org/10.1016/j.foodchem.2016.09.064>.
 35. Zhang, Yukun, Manabu Ishikawa, Shunsuke Koshio, Saichiro Yokoyama, Serge Dossou, Weilong Wang, Xiaoxiao Zhang, et al. "Optimization of Soybean Meal Fermentation for Aqua-Feed with *Bacillus Subtilis* Natto Using the Response Surface Methodology." *Fermentation* 7, no. 4 (December 2021): 306. <https://doi.org/10.3390/fermentation7040306>.
 36. Gorissen, Stefan H. M., Julie J. R. Crombag, Joan M. G. Senden, W. A. Huub Waterval, Jörgen Bierau, Lex B. Verdijk, and Luc J. C. van Loon. "Protein Content and Amino Acid Composition of Commercially Available Plant-Based Protein Isolates." *Amino Acids* 50, no. 12 (December 1, 2018): 1685–95. <https://doi.org/10.1007/s00726-018-2640-5>.
 37. Russin, Ted A., Yves Arcand, and Joyce I. Boye. "Particle Size Effect on Soy Protein Isolate Extraction." *Journal of Food Processing and Preservation* 31, no. 3 (2007): 308–19. <https://doi.org/10.1111/j.1745-4549.2007.00127.x>.
 38. Etiosa, Ogbemudia and Chika, Nnadozie and Benedicta, Anuge (2018) Mineral and Proximate Composition of Soya Bean. *Asian Journal of Physical and Chemical Sciences*, 4 (3). pp. 1-6. ISSN 24567779
 39. Sun, X. F., F. Xu, R. C. Sun, P. Fowler, and M. S. Baird. "Characteristics of Degraded Cellulose Obtained from Steam-Exploded Wheat Straw." *Carbohydrate Research* 340, no. 1 (January 17, 2005): 97–106. <https://doi.org/10.1016/j.carres.2004.10.022>.
 40. Boye, Joyce, Ramani Wijesinha-Bettoni, and Barbara Burlingame. "Protein Quality Evaluation Twenty Years after the Introduction of the Protein Digestibility Corrected Amino Acid Score Method." *British Journal of Nutrition* 108, no. S2 (August 2012): S183–211. <https://doi.org/10.1017/S0007114512002309>.
 41. Ketnawa, Sunantha, and Yukiharu Ogawa. "In Vitro Protein Digestibility and Biochemical Characteristics of Soaked, Boiled and Fermented Soybeans." *Scientific Reports* 11, no. 1 (July 9, 2021): 14257. <https://doi.org/10.1038/s41598-021-93451-x>.
 42. Liener, I. E. "Factors Affecting the Nutritional Quality of Soya Products." *Journal of the American Oil Chemists' Society* 58, no. 3 (March 1, 1981): 406–15. <https://doi.org/10.1007/BF02582390>.

43. Delgado-Andrade, Cristina. "Maillard Reaction Products: Some Considerations on Their Health Effects." *Clinical Chemistry and Laboratory Medicine* 52, no. 1 (January 1, 2014): 53–60. <https://doi.org/10.1515/cclm-2012-0823>.
44. Kohli, Vinny, and Siddhartha Singha. "Protein Digestibility of Soybean: How Processing Affects Seed Structure, Protein and Non-Protein Components." *Discover Food* 4, no. 1 (February 15, 2024): 7. <https://doi.org/10.1007/s44187-024-00076-w>.
45. El-Adawy, T. A., E. H. Rahma, A. A. El-Bedawy, and T. Y. Sobihah. "Effect of Soaking Process on Nutritional Quality and Protein Solubility of Some Legume Seeds." *Food / Nahrung* 44, no. 5 (2000): 339–43. [https://doi.org/10.1002/1521-3803\(20001001\)44:5<339::AID-FOOD339>3.0.CO;2-T](https://doi.org/10.1002/1521-3803(20001001)44:5<339::AID-FOOD339>3.0.CO;2-T).
46. Kathuria, Deepika, Anju K. Dhiman, Surekha Attri, and Manish Kumar. "Effect of Processing Method on Quality Characteristics of Harit Soybean (Glycine Max): In Vitro Protein Digestibility, Hplc, Ftir Analysis." *Nutrition & Food Science* 52, no. 4 (January 1, 2021): 684–97. <https://doi.org/10.1108/NFS-07-2021-0212>.
47. Shi, Lan, Kaiwen Mu, Susan D. Arntfield, and Michael T. Nickerson. "Changes in Levels of Enzyme Inhibitors during Soaking and Cooking for Pulses Available in Canada." *Journal of Food Science and Technology* 54, no. 4 (March 1, 2017): 1014–22. <https://doi.org/10.1007/s13197-017-2519-6>.
48. Kayembe, N. C., and C. Jansen van Rensburg. "Germination as a Processing Technique for Soybeans in Small-Scale Farming." *South African Journal of Animal Science* 43, no. 2 (June 24, 2013): 167–73. <https://doi.org/10.4314/sajas.v43i2.7>.
49. Xu, Zhicun, Yeming Chen, Caimeng Zhang, Xiangzhen Kong, and Yufei Hua. "The Heat-Induced Protein Aggregate Correlated with Trypsin Inhibitor Inactivation in Soymilk Processing." *Journal of Agricultural and Food Chemistry* 60, no. 32 (August 15, 2012): 8012–19. <https://doi.org/10.1021/jf3021249>.
50. Gilani, G Sarwar, Kevin A Cockell, and Estatira Sepehr. "Effects of Antinutritional Factors on Protein Digestibility and Amino Acid Availability in Foods." *Journal of AOAC INTERNATIONAL* 88, no. 3 (May 1, 2005): 967–87. <https://doi.org/10.1093/jaoac/88.3.967>.
51. Zahir, Mostafa, Vincenzo Fogliano, and Edoardo Capuano. "Effect of Soybean Processing on Cell Wall Porosity and Protein Digestibility." *Food & Function* 11, no. 1 (2020): 285–96. <https://doi.org/10.1039/C9FO02167A>.
52. Nkhata, Smith G., Emmanuel Ayua, Elijah H. Kamau, and Jean-Bosco Shingiro. "Fermentation and Germination Improve Nutritional Value of Cereals and Legumes through Activation of Endogenous Enzymes." *Food Science & Nutrition* 6, no. 8 (2018): 2446–58. <https://doi.org/10.1002/fsn3.846>.
53. Wolkers, Willem F., Ann E. Oliver, Fern Tablin, and John H. Crowe. "A Fourier-Transform Infrared Spectroscopy Study of Sugar Glasses." *Carbohydrate Research* 339, no. 6 (April 28, 2004): 1077–85. <https://doi.org/10.1016/j.carres.2004.01.016>.
54. Priyadarshini, K. M., Chandramohan, A., Babu, G. A., Ramasamy, P. (2014). Synthesis, crystal growth, spectral, optical, thermal and dielectric studies of dichloro (4-hydroxy-L-proline) cadmium (II) single crystals. *Optik*, 125(3), 1390–1395. Retrieved from https://www.sciencedirect.com/science/article/pii/S0030402613011637?casa_token=elMaUyLKqScAAAAA:6aKj-f78eHj8aAdtNKMV3oAdOJgU-w6vSf04KJgAnppyRWVW3XwrKHAZq0583w7o7TXnKuxg
55. Hoffmann, D. H. (2019). Assessment of soy cake protein quality for broiler feeding through infrared spectroscopy calibration validated by in-vitro feed analyses and in-vivo feeding studies (PhD Thesis). Technische Universität München. Retrieved from <https://mediatum.ub.tum.de/1468716>
56. Dixit, A. K., Antony, J., Sharma, N. K., Tiwari, R. K. (2011). 12. Soybean constituents and their functional benefits. *Research Signpost*, 37(661), 2. Retrieved from https://www.researchgate.net/profile/Navin-Sharma-3/publication/304524926_Soybean_constituents_and_their_functional_benefits/links/5772319308ae6219474b1a20/Soybean-constituents-and-their-functional-benefits.pdf
57. Tocher, D. R., Bendiksen, E. Å., Campbell, P. J., Bell, J. G. (2008). The role of phospholipids in nutrition and metabolism of teleost fish. *Aquaculture*, 280(1–4), 21–34. Retrieved from https://www.sciencedirect.com/science/article/pii/S0044848608003384?casa_token=XenQEUx_ziIAAAAA:UrKnUgR--9fn2xeSyYWpNR4CUOf5DcZRds5xohGYd_v0q-0SqrXcIL-wv3GVI0EQIAWX-eQg
58. Brimas, E., Raudonis, R., Kareiva, A. (2022). Analytical methods used for the characterisation of specific features of biological tissues related with obesity: A review. *Chemija*, 33(4). Retrieved from <https://www.lmaleidykla.lt/ojs/index.php/chemija/article/view/4809>
59. Chen, Nannan, Mouming Zhao, Christophe Chassenieux, and Taco Nicolai. "Data on the Characterization of Native Soy Globulin by SDS-Page, Light Scattering and Titration." *Data in Brief* 9 (October 25, 2016): 749–52. <https://doi.org/10.1016/j.dib.2016.10.016>.

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