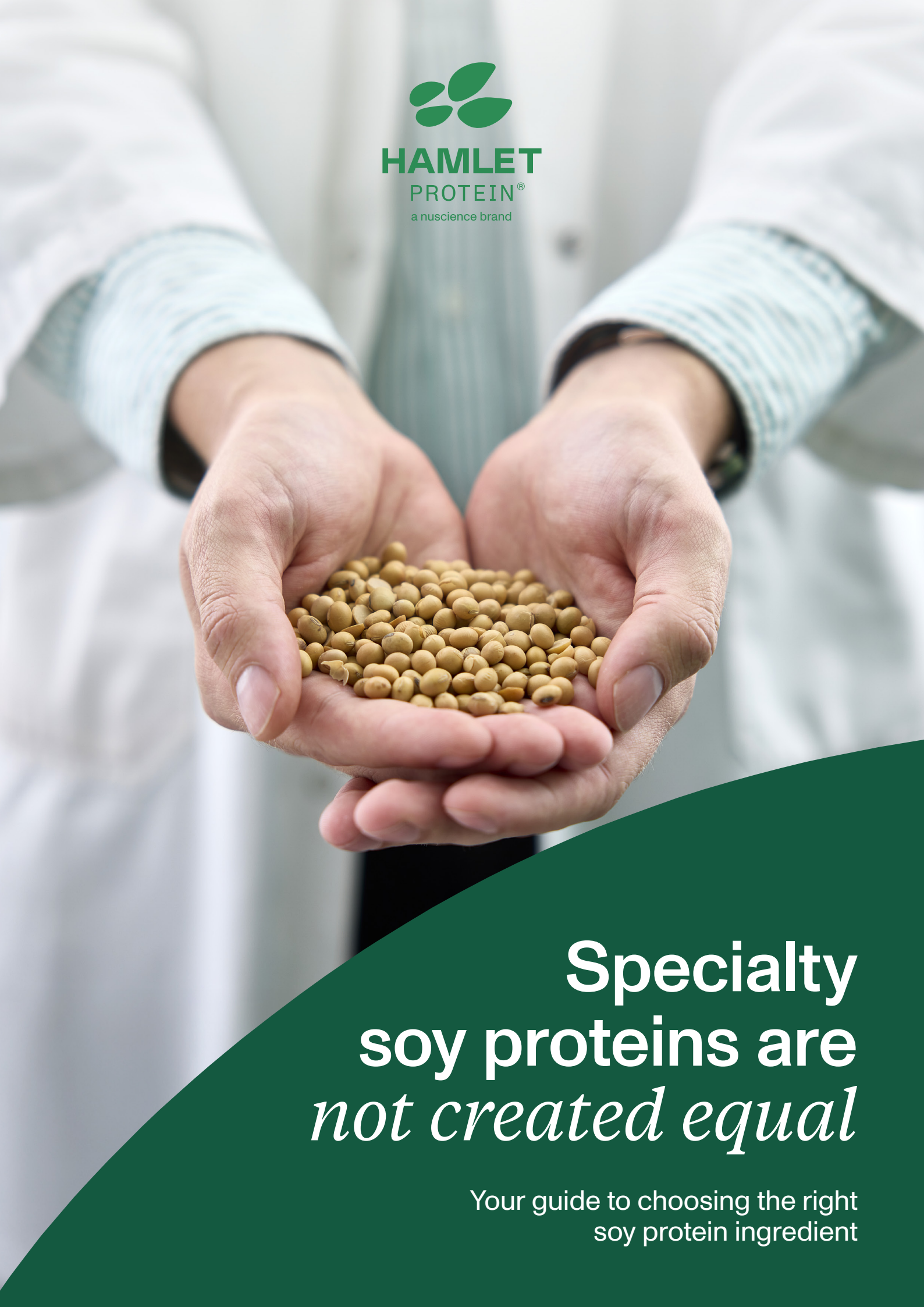
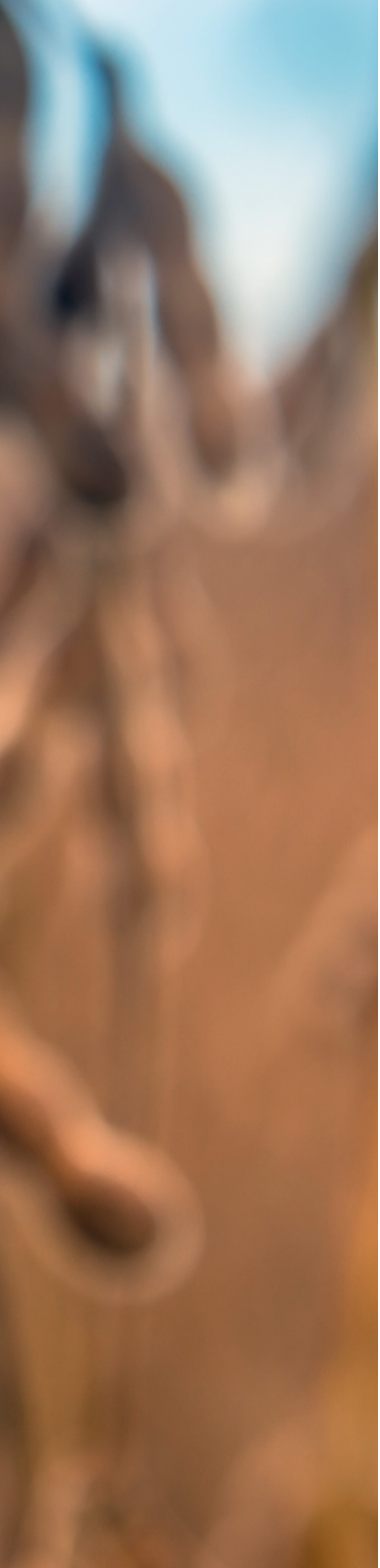


HAMLET
PROTEIN®
a nuscience brand

Specialty soy proteins are *not created equal*

Your guide to choosing the right soy protein ingredient





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Summary: How to evaluate soy-based protein products

Your guide to assessing soy protein quality for animal nutrition.



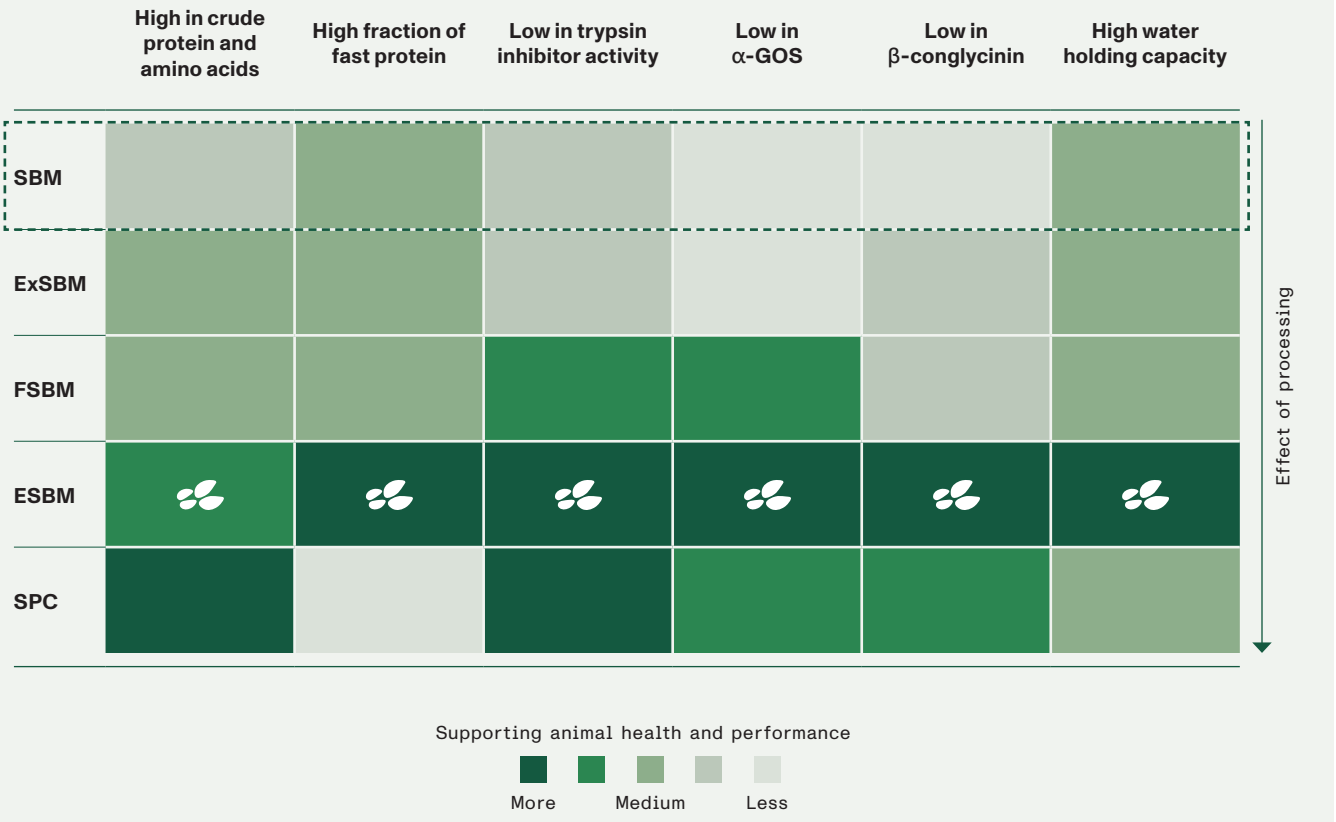
Processing technique matters as it directly impacts essential quality parameters for protein ingredients, and thus animal performance. It is very important to understand the principles of each technology to know how it affects the final product.

This guide will provide you with a technical description of the most commonly used technologies for SBM processing, and we will share data from our large, and continuously growing database to help build the connection between technology and product properties.

Table 1 summarizes data from coming sections and serves as your guide for evaluating the quality of different SBM-based products. The guide lists important parameters to consider when choosing the right soy protein solution to meet the needs of young or sensitive animals. By reading from top to bottom, you can easily check how each processing technique affects the different quality parameters compared to SBM and relative to one another.

The unique processing technique of Hamlet Protein leaves no room for gambling with the health and performance of young or sensitive animals.

Table 1: Summary of the effects of each processing technique on parameters critical for young or sensitive animals. The ranking of crude protein, fast protein fraction, trypsin inhibitor activity, α-GOS, β-conglycinin and water holding capacity is based on data presented in Figure 4, 5, 6 and 7 with focus on both mean values and variation.



Your guide for selecting the *optimal soy protein* for animal feed

Soy-based proteins are well-known ingredients in animal nutrition due to their content and availability of important nutrients. Soy is recognized as one of the few plant-based protein sources that contain all essential amino acids necessary for animal growth and development in significant and balanced amounts.



The type of soy protein product you choose does matter



Farmers and feed producers have a wide range of soy-based products available for animal feed. Each type of product is developed to enhance the nutritional or functional value of the original soybean. However, the different processing techniques used to obtain these products lead to significant differences in protein quality, anti-nutritional factors content and physical properties. These differences have a substantial impact on animal performance and gut health, which is critical for young animals and subsequent life performance.

The most suitable soy product for young or sensitive animals

The type of soy protein product you choose does matter. Even though many products originate from soybeans, their distinct processing techniques lead to different compositions and digestibilities, causing different responses in the digestive system of animals. When you feed young or sensitive animals, you must take extra precautions. The digestive system of young animals is not fully mature, which limits their ability to efficiently absorb and utilize certain nutrients. In some cases, inappropriate feed can harm their developing digestive system affecting performance and gut health negatively. Therefore, it is important to understand the underlying principles of different processing techniques and how they impact the properties of soy protein products.

Important quality parameters to guide your choice

The quality of a protein ingredient is not only defined by protein and lysine content, but also by digestibility and biological utilization. Soybeans contain several compounds that negatively affect the utilization of amino acids in the complete diet and compromise gut health in young animals. We refer to these compounds as anti-nutritional factors (ANFs) of which the primary ones include antigenic proteins like β-conglycinin, oligosaccharides such as stachyose and raffinose, and trypsin inhibitors **(see text box on page 8 for further description)**.

Minimizing the level of ANFs in soy protein ingredients is crucial for improving health, welfare, and life performance of young animals.

Another important parameter is how fast protein is hydrolyzed and absorbed. The fraction of fast protein in the diet is directly related to the utilization of amino acids and excretion of nitrogen[1]. Not all proteins are equally available; some proteins are rapidly degraded and absorbed, and the animal can fully utilize them, whereas other proteins are slowly degraded in the last part of the intestine, where harmful bacteria can utilize them. Protein kinetics refer to the rate and extent at which proteins are hydrolyzed and absorbed, and it is vital for evaluating how proteins behave in the digestive system of animals. It is important to have sufficient fast proteins to meet the animal's growth and health requirements.



Anti-nutritional *factors*

Trypsin inhibitors

Trypsin inhibitor activity (TIA) originates from native proteins in soybeans, which interfere with protein digestion by blocking endogenous proteases such as trypsin and chymotrypsin. In young animals, the natural secretion of these digestive enzymes is insufficient to overcome the effects of TIA. As a result, undigested protein reaches the hindgut, where it becomes a substrate for pathogenic fermentation. This fermentation process produces toxins that damage the gut lining, compromise gut health, and ultimately lead to reduced animal performance. TIA can be reduced by heat or proteolytic activity.

Oligosaccharides

For oligosaccharides, the focus is on raffinose and stachyose, also referred to as α-galacto-oligosaccharides (α-GOS). These molecules are sucrose-based with one or two galactose units bound in the structure. Stachyose and raffinose are indigestible in animals due to the absence of galactosidase enzymes, which means that the α-GOS will pass to the large intestine to be fermented by bacteria. This passage and unfavorable fermentation leads to abdominal discomfort, flatulence, diarrhea, and lowers energy utilization. These molecules cannot be removed by heat, and additional techniques such as extraction, fermentation, or enzyme treatment must be applied to reduce those to tolerable levels.

β-conglycinin

In total, around 65% of the raw soybean protein is made up of β-conglycinin and glycinin, which are the main storage proteins of soybean. A subunit of β-conglycinin causes an allergic response in the gut epithelia of young animals. In a mature animal gut, β-conglycinin is broken down by trypsin and pancreatic proteases. The allergic response initiated in young animals leads to oxidative stress, inflammation, and increased gut permeability also known as “leaky gut” syndrome. Heat treatment can only reduce the content of allergenic β-conglycinin to some extent but additional techniques are needed to deactivate it completely.

Processing technique impacts *soy protein quality*

When it comes to specialty soy proteins, the processing technique plays a crucial role for the level of ANFs and the fraction of fast protein in the final product. Different processing techniques yield different results, reinforcing the fact that not all specialty soy proteins are equal.

This guide will explore the advantages and challenges of the main categories of processed soybean meal used commercially:

1. Soybean meal (SBM)
2. Extruded soybean meal (ExSBM)
3. Fermented soybean meal (FSBM)
4. Enzyme-treated soybean meal (ESBM)
5. Extracted soy protein concentrates (SPC)

You will discover that each processing technique impacts reduction of ANFs differently. In addition to minimizing ANF content, the processing technique influences protein quality, the fraction of fast protein, and functional properties such as water holding capacity.



1. SBM | 2. ExSBM | 3. FSBM | 4. ESBM | 5. SPC

Data driven: More than 500 analyses conducted every year

At the dedicated laboratories of Hamlet Protein, more than 500 analyses are conducted each year to evaluate and compare commercial soy products, and the processing techniques used to produce them. Additionally, around 13,000 product tests are performed annually in our two on-site laboratories to monitor and control our processes, ensuring high-quality and consistency across all batches.

A key element of our quality control is the ability to analyze all three major ANFs in-house, along with other critical parameters such as protein content, moisture, ash, iron, and water holding capacity. To further ensure product safety, we also carry out chemical and microbiological analyses through accredited external laboratories including salmonella, mycotoxins and heavy metals.

By combining our in-house analytical data with findings from international studies and feeding trials, we have a comprehensive understanding of which soy products are most suitable for various applications. This knowledge is essential for anyone involved in formulating young animal feed.

The laboratory of Hamlet Protein

- 2 on-site labs (Findlay, USA and Horsens, DK)
- 5 lab technicians
- +15,000 total analyses annually
  - +13,000 internally
  - +2,000 externally
- ≈14,000 QC analyses annually
- Extensive database on commercial soy products





The importance of soy in animal nutrition

Soy has long been a cornerstone of animal nutrition due to its high nutrient content and broad availability.



Everything starts with soybeans

Soybeans *(Glycine max)* provide an excellent balance of essential amino acids with high levels of lysine, which is often limited in many cereal grains. The digestibility and favorable amino acid profile make soy proteins suitable for feeding monogastric animals as well as ruminants.

Soybeans are the most grown oilseed worldwide, accounting for more than 60% of the global oilseed production **(see Figure 1)**. They are a major component of global agriculture due to their dual-purpose value: crude soybean oil (SBO) for consumption and industrial use, and SBM for animal feed. Soybeans are also valued for their ability to fix atmospheric nitrogen, which reduces the need for synthetic fertilizers and contributes to soil fertility.

Global soybean production is dominated by a few key countries: Brazil, United States, Argentina, China and India, where Brazil, the United States, and Argentina account for more than 80% of the world's soybean supply[2]. Production of soybeans in China and India is mostly for domestic use.

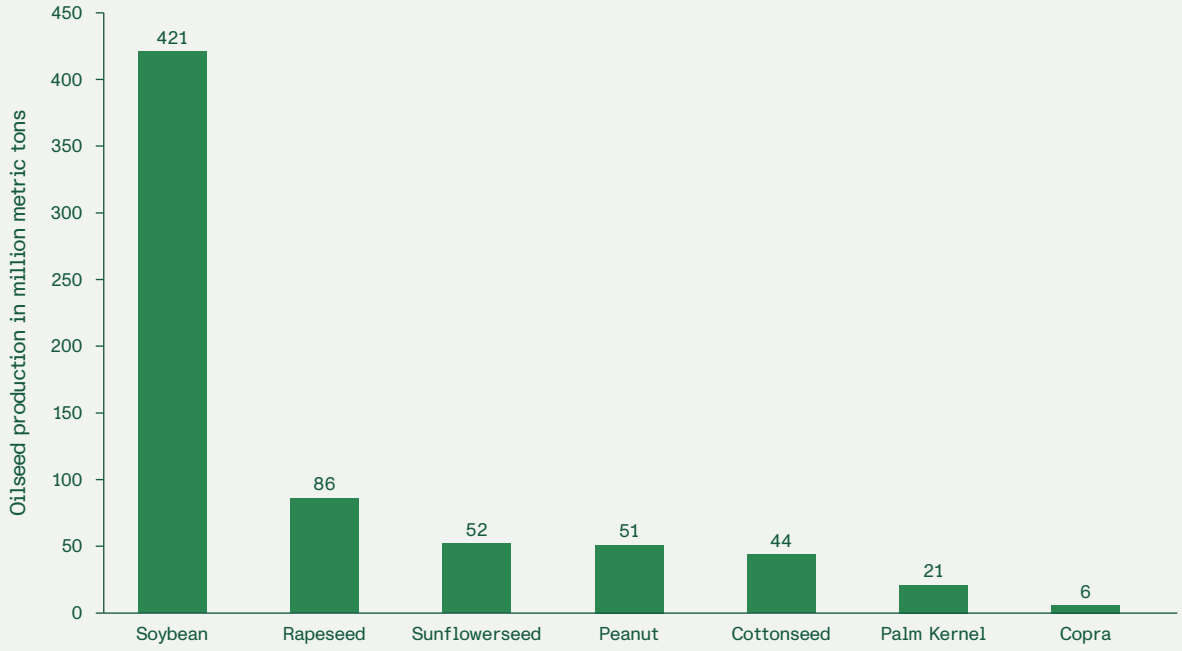


Figure 1 Yearly soybean production vs other oilseed proteins.
Source: USDA database (Mar2024/2025).



Examples of soybean meal (SBM).

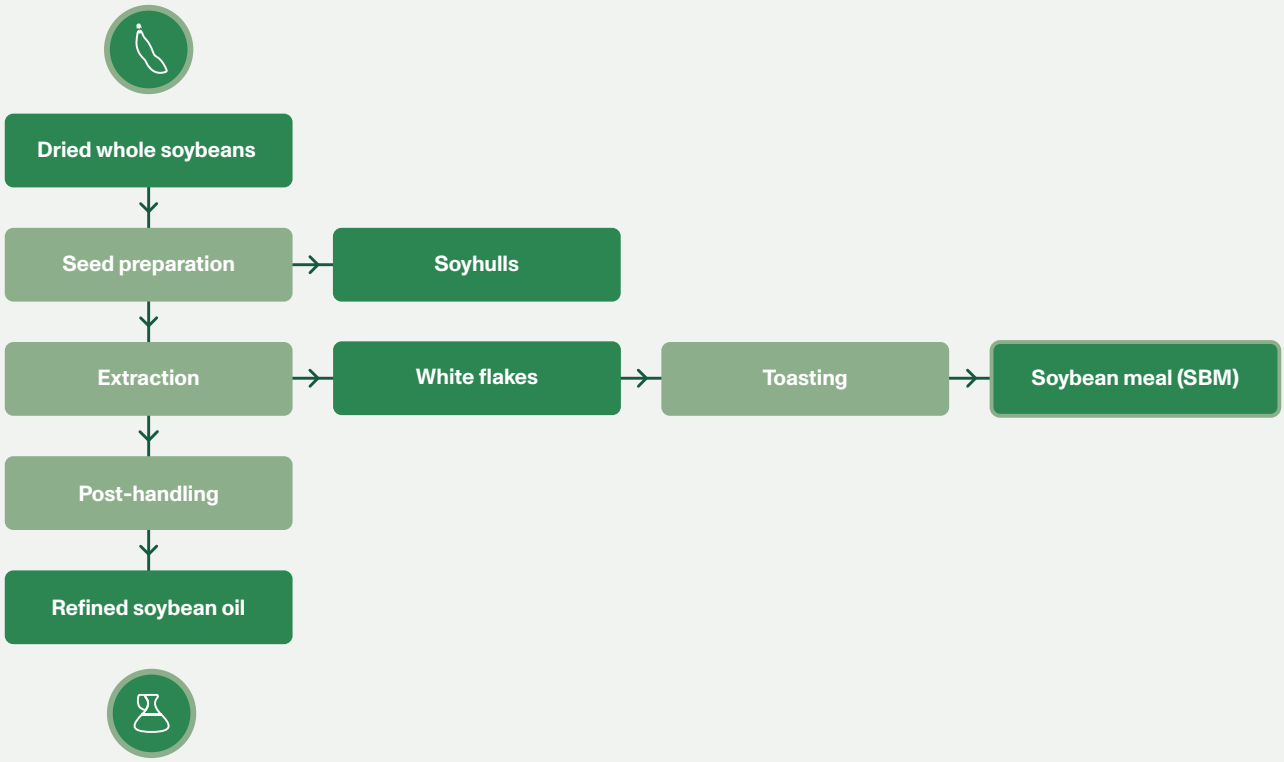


Figure 2 Simplified production flow of dehulled and solvent-extracted soybean meal; a valuable byproduct from soybean oil production.

A valuable byproduct from soybean oil production

Soybeans contain around 20% oil, and SBM is an important byproduct of SBO production **(see Figure 2)**. The production process begins with dried soybeans, which serve as the raw material for both SBO and SBM. The beans are cleaned, foreign particles such as stones, dust and sticks are removed. After cleaning, the soybeans are cracked and passed through a dehulling system of rollers and aspirators to separate the hulls from the inner part of the beans.

The hulls are fiber-rich and low in protein, therefore this dehulling step is critical for the protein content in the final meal. Some hulls may be partially added back to the meal to adjust protein and fiber levels depending on the target meal type **(see Table 2)**. After cracking and dehulling, most beans are slightly heated to ≈60-75°C (140-167°F) and moisturized through conditioning to soften and prepare the beans for processing.

After conditioning, the soybeans are rolled into thin flakes using flaking rollers to increase surface area and enhance oil extraction efficiency. Flakes are treated with hexane, a solvent that dissolves and extracts oil from the flakes. The solvent mixture is separated into crude SBO and defatted flakes, the oil is then refined to yield the end-product of SBO.

Defatted flakes are referred to as white flakes. The proteins of white flakes are highly soluble but not very digestible due to high levels of trypsin inhibitors. To produce SBM, white flakes are toasted and dried to obtain a typical moisture content of 10-12%. The main reason for the extra heating step is to improve digestibility by reducing the level of trypsin inhibitors[3,4,5,6].

Implications for young animal nutrition

While SBM crushers can adjust protein content to some extent by controlling the inclusion of hulls, they have limited control over other critical quality parameters. According to industry reports and data from soy organizations, the nutritional quality of SBM varies significantly across regions and even between production batches[7,8,9,10]. These fluctuations are observed not only in crude protein and amino acid profiles, but also in the level of ANFs.

Among the most pressing concerns in feed formulation is the wide variability in ANF content, especially since detailed data on specific ANFs is rarely available to nutritionists and feed formulators.

While SBM typically does not cause major digestive issues in older animals, young animals with immature digestive systems are more sensitive to ANFs, which can lead to digestive disturbances, reduced feed efficiency, and impaired growth.

The toasting step in soybean meal production is primarily designed to inactivate part of the trypsin inhibitors, but this process is only partially effective against trypsin inhibitors and ineffective against other major anti-nutritional compounds, such as the antigenic protein β-conglycinin and α-GOS (stachyose and raffinose). Because these compounds are more heat-stable or only partially affected by toasting, additional processing methods are required to reduce their levels effectively to produce specialized soy products suitable for young or sensitive animals.

Table 2: Overview of different soybean meal types based on crude protein and fiber content.

Type of Soybean Meal	Crude protein (%)	Fiber (%)	Short description
High-Pro Meal	47-49%	≈3-4%	Made from fully dehulled soybeans with no hulls reintroduced.
Mid-Pro Meal	44-46%	≈4-6%	Some hulls are added back after dehulling to balance fiber and protein.
Low-Pro Meal	41-43%	>6%	Made from non-dehulled beans or have a higher hull inclusion rate.

High-Pro SBMs are preferred in poultry and pig diets due to the protein content and digestibility. Low-Pro SBM is not commonly fed to monogastrics.

How processing techniques differ and *why it matters*

The full nutritional potential of SBM is limited by the presence of ANFs. To address these limitations and to tailor SBM for specific animal needs, a range of advanced processing technologies have been developed.



Soybean



SBM



Hamlet Protein

Soy processing techniques aim to improve digestibility and nutrient availability by selectively removing or inactivating undesirable components while preserving or enhancing beneficial properties. The most widely applied technologies include extrusion, fermentation, enzyme treatment, and extraction. **Figure 3** shows a schematic overview of how SBM can be converted into different soy products with protein content increasing from left to right.

Soy protein isolates (≈90% protein) are not included in the figure below as they are not commonly used in standard commercial livestock feed due to high processing costs[11].

Milling

SBM can be converted into milled or micronized SBM through an additional milling step. This process reduces particle size and improves uniformity, which significantly increases the surface area of the material. Micronization is an ultra-fine grinding process aiming for particle sizes <100µm (often 1-50µm), which involves higher energy input and specialized equipment. Ultra-fine grinding will make the color of SBM appear whiter.

From a feed manufacturing perspective, modifying the physical characteristics of SBM through fine grinding offers several functional advantages. Finely milled SBM improves mixing uniformity, minimizes ingredient segregation, and enhances pellet durability[12].

Importantly, milling does not alter the chemical composition of SBM; it is a physical process only. While some heat is generated during milling due to friction, the temperature is not sufficient to significantly reduce heat-sensitive ANFs to tolerable levels. Therefore, standard mechanical milling should not be considered a substitute for thermal processing when targeting ANF inactivation[13,14].

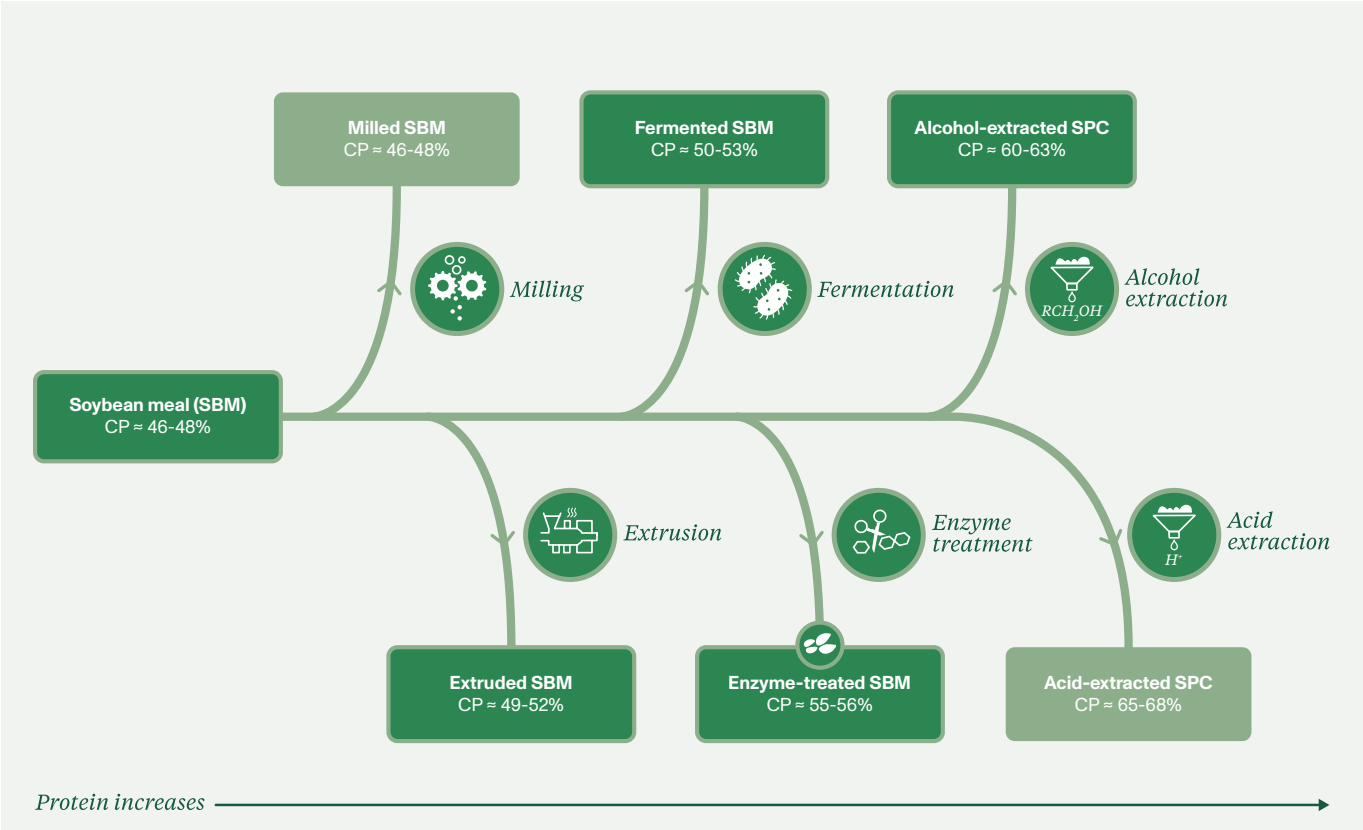


Figure 3 Overview of different processing techniques with soybean meal as starting point. Protein content of the products increases from left to right. SBM, extruded SBM, fermented SBM, enzyme-treated SBM and alcohol-extracted SPC will be described and supported with data in this guide, which explains why they are represented in the same green color.

Extrusion of soybean meal

One commonly applied technique to SBM is extrusion. It is essential to differentiate between extruded soybeans and extruded SBM (ExSBM). Extrusion of soybeans can be used to produce SBM instead of solvent-extraction, but 99% of SBM in the US is produced by solvent-extraction due to the efficiency of oil removal[15].

Extrusion is a high-intensity process that subjects the material to extreme temperatures (typically 100–160°C / 212–320°F), pressure and mechanical shear.

While the original principle of extrusion dates back to the 18th century for metals[16], its adaptation for food and animal feed is more modern.

Production of ExSBM often begins with conditioning, where SBM is treated with steam and heat to soften the material and prepare it for extrusion. The conditioned meal is then fed into an extruder, the central component of the process. The extruder consists of a barrel housing one or more rotating screws, which compress, mix, and heat the material as it moves through the barrel. The combination of heat and mechanical treatment explains why extrusion often is referred to as (hydro)-thermal mechanical treatment. Hydro is used if steam is applied.

Extrusion of soy is known to facilitate starch gelatinization and protein denaturation. While the starch content of SBM is low, the effect on protein is important to keep in mind when extrusion is applied in production of feed ingredients. Excessive heat or shear reduces the availability of essential amino acids, especially lysine, via Maillard reactions[17]. This reaction forms poorly digestible protein aggregates via cross-link interactions.

In terms of ANF reduction, extrusion only affects heat-sensitive compounds like trypsin inhibitors and to some extent, β-conglycinin. Here, it really comes down to balancing heat treatment to achieve a significant reduction in heat-sensitive ANFs without overheating, thereby negatively affecting protein availability.

The moisture content is typically reduced during extrusion, resulting in slightly higher crude protein content on an "as-fed" basis compared to conventional SBM. However, the protein content on a dry matter basis remains similar to unprocessed SBM.

The key differences between ExSBM and conventional SBM lie in slightly lower trypsin inhibitor and β-conglycinin contents, and reduced moisture due to the thermal processing.



Examples of extruded SBM products.

Fermentation of soybean meal

The second technique often applied to SBM is fermentation. Worldwide, fermented food has been part of the traditional human diet for thousands of years. Around the turn of the millennium, fermented products derived from SBM began to emerge in the Asian feed market. Today, FSBM is also produced in Europe and the US, and represents a commonly known category of processed SBM.

While food has often been fermented for preservation and taste reasons, fermented products for feed focus on reducing anti-nutritional factors and gut health issues, especially for young animals.

FSBM is produced by inoculating SBM with water and a microbial culture, utilizing that microorganisms will start modifying components of SBM.

The fermentation takes place in tanks or in bins. Unlike most other forms of soy protein production, fermentation is typically produced in individual batches instead of continuous production. After fermentation, the product is dried by heat treatment; some producers use this step to inactivate microorganisms, whereas others keep the living microorganisms, potentially affecting the stability of the final product.

The fermentation conditions and resulting product depend on the specific strain of microorganism used for the process. The most commonly known fermentation is the use of lactic acid bacteria that convert sugars into lactic acid. As bacteria – or other microbes – act differently on the soy substrate, products within this category will turn out with different nutritional values.

The pH of a fermented soy product serves as an indicator of the primary reaction during fermentation. Low pH values (5.0 and below) indicate lactic acid bacteria fermentation, whereas products based on fermentation with *Bacilli* will be in the range of 6.0 to 7.5. The applied strain of bacteria also affects protein concentration, namely *Bacilli* tend to increase the protein more than lactic acid bacteria[18].

One of the downsides associated with fermented soy products is that the technique offers limited control during production, which can lead to high batch-to-batch variation. Visual color differences are a first indication of variations in chemical and nutritional properties, and should lead to quality checks. When using microorganisms, it is important to pay attention to lysine levels, as some microorganisms may consume and thereby reduce the lysine content of the product[19].

Fermentation has the potential to affect all ANFs as the production design comprises both a fermentation step and a subsequent heat treatment. If the water needed for microbial activity is removed by drying, high temperatures may be required, which increases the risk of Maillard reaction. The exact effect depends on the strain used and the amount of heat applied and may even vary from batch to batch.

The key difference between FSBM and conventional SBM depends on the strain of microorganism used; fermentation as a processing technique generally does not reduce the inherent variability of SBM.



Examples of fermented SBM products.

Enzyme treatment of soybean meal

ESBM first went into production in the 1990s, where a continuous flow process was developed specifically to reduce all major ANFs in soy without compromising protein bioavailability. Hamlet Protein established and still dominates this category with two production sites, one in the US and one in Europe.

The core of the patented process of Hamlet Protein is a bioconversion where enzymatic reactions take place. The process is based on continuous flow, where SBM, water and enzymes are precisely mixed and processed under controlled and optimal conditions. After enzymatic treatment, a gentle sterilization process takes place to deactivate the enzymes and ensure the stability of the product. The sterilization process significantly reduces heat-sensitive ANFs to consistently low levels, while the subsequent drying process effectively dries the product with focus on preserving protein quality.

What is unique about this process is the ability to produce consistent products regardless of variations within the raw material.

The quality of refined soy products often depends on the quality of the incoming SBM. Since ANF content varies a lot between SBM batches, the ANF content will vary in the final soy product as well. The Hamlet Protein process eliminates this variance by carefully monitoring SBM quality and adjusting the production parameters accordingly. It also improves protein accessibility and modifies physical properties to enhance overall functionality.



Hamlet Protein

The key advantage of ESBM lies in the controlled processing environment. Enzymatic reactions take place in reactors at optimal temperatures with sufficient retention time and customized conditions to utilize the full potential of the enzymes contrary to enzyme-enriched products **(se text box)**.

Knowing the exact quality of every single batch of raw material coming in, the controlled enzymatic treatment does not only effectively reduce ANFs but also standardizes a naturally variable raw material.

It is crucial to distinguish between enzyme-treated and enzyme-enriched soy products

In enzyme-treated products, enzymatic reactions occur during the manufacturing process. The enzymes are active only during production and are no longer functional in the final product; they have already performed their intended role. In contrast, enzyme-enriched products simply contain added enzymes that must remain active and perform their function within the animal after ingestion.

In enzyme-enriched products, the effect of the enzyme depends on variables such as feed production conditions and the internal environment of the animal, both of which are far more difficult to control.

Enzyme-treated SBM is predigested for improved nutrient availability, while enzyme-enriched SBM products remain undigested at the time of feeding.

Extraction of soybean meal

Extraction of SBM became commercially relevant between 1960 and 1970 representing one of the earliest efforts to improve the quality of conventional SBM. The soy product of extraction is often referred to as soy protein concentrate (SPC) and was traditionally produced by water-alcohol extraction of soy white flakes[20]. A new generation of SPC has emerged over the last decade, being produced based on SBM instead of white flakes.

Traditionally SPC were produced in continuation of the oil extraction of soybeans, where the solid fraction from the oil extraction was subjected to a further extraction process using water and alcohol, typically 1/3 water and 2/3 ethanol.

The aim is to solubilize simple sugars and oligosaccharides by removing all soluble carbohydrates and, at the same time, avoid extracting the proteins.

Alcohol denatures proteins and forms aggregates, making the proteins largely insoluble in the liquid phase of extraction. A positive side effect is the denaturation of antigenic proteins, a negative side effect is low protein solubility resulting in reduced accessibility by gastrointestinal enzymes. The SPC is heated and dried after extraction, ending with a protein content above 60%.

Standard SPC produced on white flakes has a low residual content of ANFs. The only issue is that the oligosaccharides are not completely removed during the extraction process, as not all of them may be present in a soluble form. This is especially true for stachyose.

When SPC is produced from SBM, the process is separated from soybean oil extraction. While this simplifies production, it induces protein issues. During the toasting step used to produce SBM, additional heat is applied, which causes proteins and carbohydrates to form indigestible complexes. These complexes reduce the efficiency of the subsequent extraction process and negatively affect the nutritional quality of the final product.

To provide a complete picture of SPC, it is important to mention a few related products that are produced using a slightly different method and exhibit distinct quality characteristics. These variants are processed through acid extraction, where the pH of the extraction liquid plays a critical role. The solubility of proteins is lowest at the isoelectric point, which for soy proteins is around 4.5. At more acidic or alkaline pH levels, soy proteins tend to solubilize into the liquid phase, which can be utilized to separate and extract the soy proteins. This method effectively removes oligosaccharides and sugars but without the same denaturation of antigenic proteins. Heat treatment is still required to reduce the content of trypsin inhibitors[20, 21, 22].

In summary, the extraction process for SPC can influence all three major ANFs depending on the choice of solvent and the subsequent heat treatment. However, the solvent used can also negatively impact protein solubility, thus potentially reducing overall protein accessibility by the animal in the final product. A key difference between conventional SBM and solvent-extracted SPC is the high protein content.



Examples of extracted SPC products.

Comparing quality parameters across processing techniques: *Soybean meal as baseline*

With the main processing technologies now outlined, we can examine the value each of them offers and how various quality parameters are influenced by the chosen processing technique.





Soy protein quality cannot be accurately evaluated using a single index such as KOH solubility or Protein Dispersibility Index (PDI). These tests were originally developed to assess processing conditions in solvent-extracted SBM, where high values indicate under-processing and low values indicate over-processing[23, 24]. While both aim to indirectly reflect levels of heat-sensitive ANFs and protein availability, they are limited in scope. Their applicability is confined to the specific processing method of solvent extracted SBM, as different processes alter protein properties in distinct ways. Instead, we prioritize biologically relevant parameters that more directly reflect the nutritional impact of the protein ingredient in animals.

All data presented in this section originates from Hamlet Protein's internal database, with analyses performed either in our in-house laboratories or in collaboration with trusted academic institutions. Protein content, TIA, α-GOS, β-conglycinin, and water holding capacity were analyzed at the laboratories of Hamlet Protein. References and descriptions of analysis methods are available upon request. Protein kinetics properties were assessed by Aarhus University using a modified pH-stat method[25, 26]. Fast proteins are calculated as the fraction of protein hydrolyzed within the first 30 minutes of the pH-stat analysis[27].

Crude protein

When evaluating protein ingredients, the crude protein content is the first parameter to evaluate.

As seen in **Figure 4**, the order of categories supports the overview presented in **Figure 3**. SBM varies in crude protein content, and the data shown covers all three SBM categories; Low-, Mid- and High-Pro.

Extrusion slightly increases protein content compared to High-Pro SBM as moisture is being removed during the heat treatment.

Using **fermentation**, protein levels vary significantly depending on the microbial strains used and their efficiency in preserving or concentrating protein. Typically, the protein content of FSBM is comparable to or slightly higher than that of High-Pro SBM, covering a similar range as extruded products.

Enzyme treatment generally shows a reasonably high protein content: It is higher than conventional High-Pro SBM but lower than SPC. Importantly, the protein content is consistent with a variance of no more than ± 1.5% according to specifications.

The highest protein content is found when using **extraction**. This technology is the most effective at concentrating SBM protein, as it removes several soluble components, leaving behind a protein-rich fraction. However, this also means that a significant portion of the raw material is discarded, making it essential to find value-added uses for the resulting side streams to ensure the sustainability and economic viability of the process.

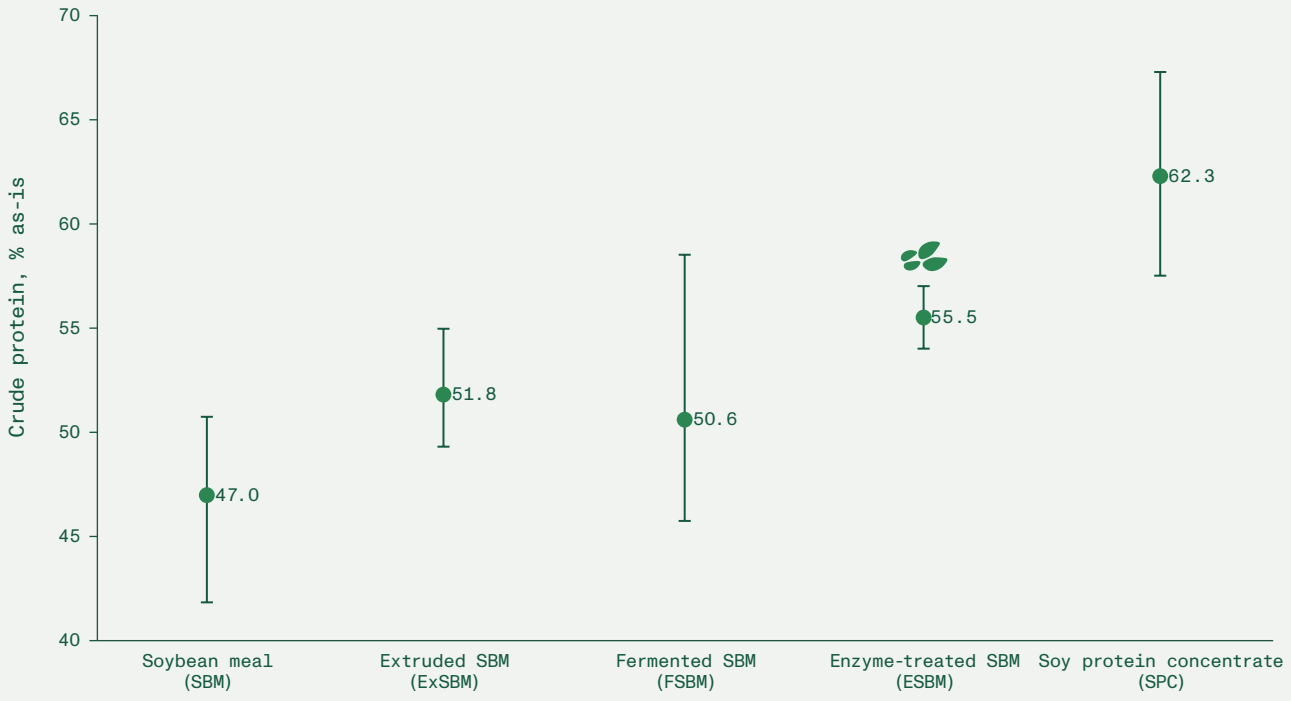


Figure 4 Crude protein content in available commercial soy protein products (+300 samples), all based on SBM or further processed SBM using extrusion (ExSBM), fermentation (FSBM), enzyme treatment (ESBM), and alcohol extraction (SPC). Green dots represent average values, while error bars reflect minimum and maximum values.

Fast protein

When evaluating protein quality, it is not sufficient to rely solely on standardized ileal digestibility (SID) values. The fraction of fast protein refers to protein absorbed within the first 30 minutes of digestion and is crucial for evaluating protein utilization.

The term fast protein reflects not only digestibility at a certain point, but also the accessibility of dietary enzymes and the kinetics of protein breakdown.

Figure 5 compares the fraction of fast protein across the five different categories of SBM-based products. In SBM, approximately 72% of the protein fraction is classified as fast. **Extrusion** does not alter this percentage, while **fermentation** slightly reduces it, likely due to structural modifications induced by microbial metabolism. In contrast, **enzyme treatment** shows a significant increase in the fraction of fast protein with a value of 83%. This improvement is attributed to multiple factors: intact protein solubility, increased content of shorter peptides, high water holding capacity, and consistently low levels of ANFs, all of which improve enzyme access and protein hydrolysis.

On the other hand, **alcohol extraction** exhibits a significant reduction in the fraction of fast protein compared to conventional SBM. This is due to the denaturing effect of alcohol (e.g., ethanol) used during extraction. Alcohol disrupts hydrogen bonds and hydrophobic interactions that maintain protein structure, leading to aggregation and reduced solubility[28]. As a result, large protein complexes are generated, which are less accessible to digestive enzymes. While alcohol extraction is effective in concentrating protein, it does so at the cost of reducing protein solubility and enzyme accessibility.

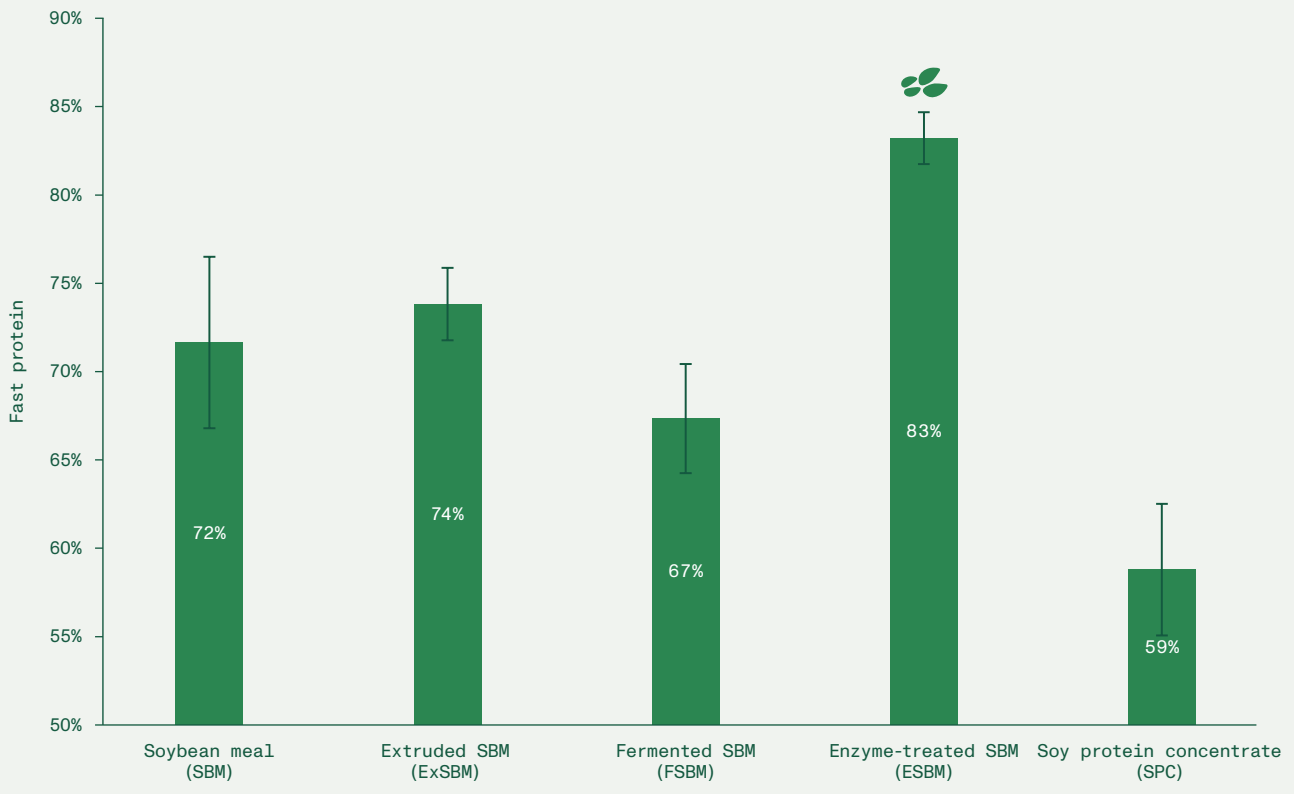


Figure 5 Overview of fast protein levels in different SBM based products (n=26) covering conventional SBM, ExSBM, FSBM, ESBM and alcohol-extracted SPC. The level of fast protein is determined by the pH-stat method conducted at Aarhus University. Error bars represent standard deviation.

Anti-nutritional factors

Processing technique and process control play a critical role in determining both the content and consistency of anti-nutritional factors (ANFs), which in turn have a significant impact on feed formulation and animal performance.

Figure 6 shows how the content and consistency of all three major ANFs are affected by processing technique.

Starting with SBM, **Figure 6** shows high variability in all three major ANFs: TIA, α-GOS and β-conglycinin. Notably, similar levels of variability are observed within the category of High-Pro SBM (data available on request), which indicates that the variability shown in **Figure 6** is not driven by differences across Low-, Mid-, and High-Pro categories, but exists within each category as well.

When comparing ExSBM to conventional SBM, the average levels of ANFs seem slightly reduced, but the variation remains high. The modest decrease in TIA and β-conglycinin can be attributed to the heat applied during extrusion. However, **extrusion** does not target or reduce α-GOS, meaning that the slightly lower level of α-GOS seen in **Figure 6** is caused by differences in the raw material itself. In fact, extrusion can lead to higher levels of α-GOS compared to the original raw material due to concentration caused by water removal. Overall, extrusion does not eliminate the inherent variability found in conventional SBM. While it can slightly reduce heat-sensitive ANFs, its ability to standardize or significantly improve ANF consistency is limited, especially when protein quality preservation is also a priority.

Contrary to extrusion, **fermentation** can reduce the content of α-GOS, as most microorganisms are capable of consuming or converting the sugars naturally present in soy. FSBM also shows a lower level of TIA compared to SBM, which may result from either severe heat treatment or from microbial degradation. Some strains produce proteolytic enzymes that can break down proteins, including TIA. For β-conglycinin, extremely high variation is observed within the category of FSBM. This inconsistency may stem from both the effects of heat treatment and the variable capacity of microorganisms to modify or degrade

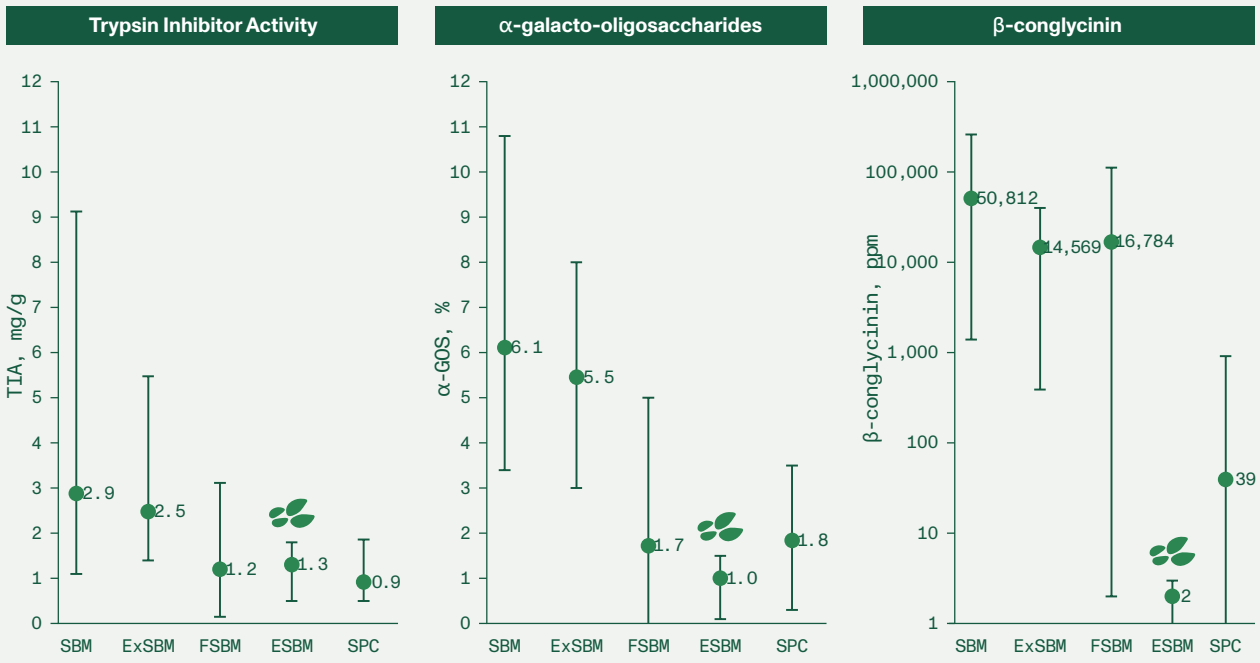


Figure 6 Overview of ANF levels in different SBM processed products covering conventional SBM, ExSBM, FSBM, ESBM and alcohol-extracted SPC (n=300). Trypsin inhibitor activity is determined by modified AOCS Ba 12-75 method, α-GOS (sum of stachyose and raffinose) is determined by Thin-Layer-Chromatography, and β-conglycinin is determined by direct double antibody sandwich Enzyme-Linked Immunosorbent Assay (ELISA). All analyses are conducted in the laboratory of Hamlet Protein. Green dots show average values, while error bars reflect minimum and maximal values.

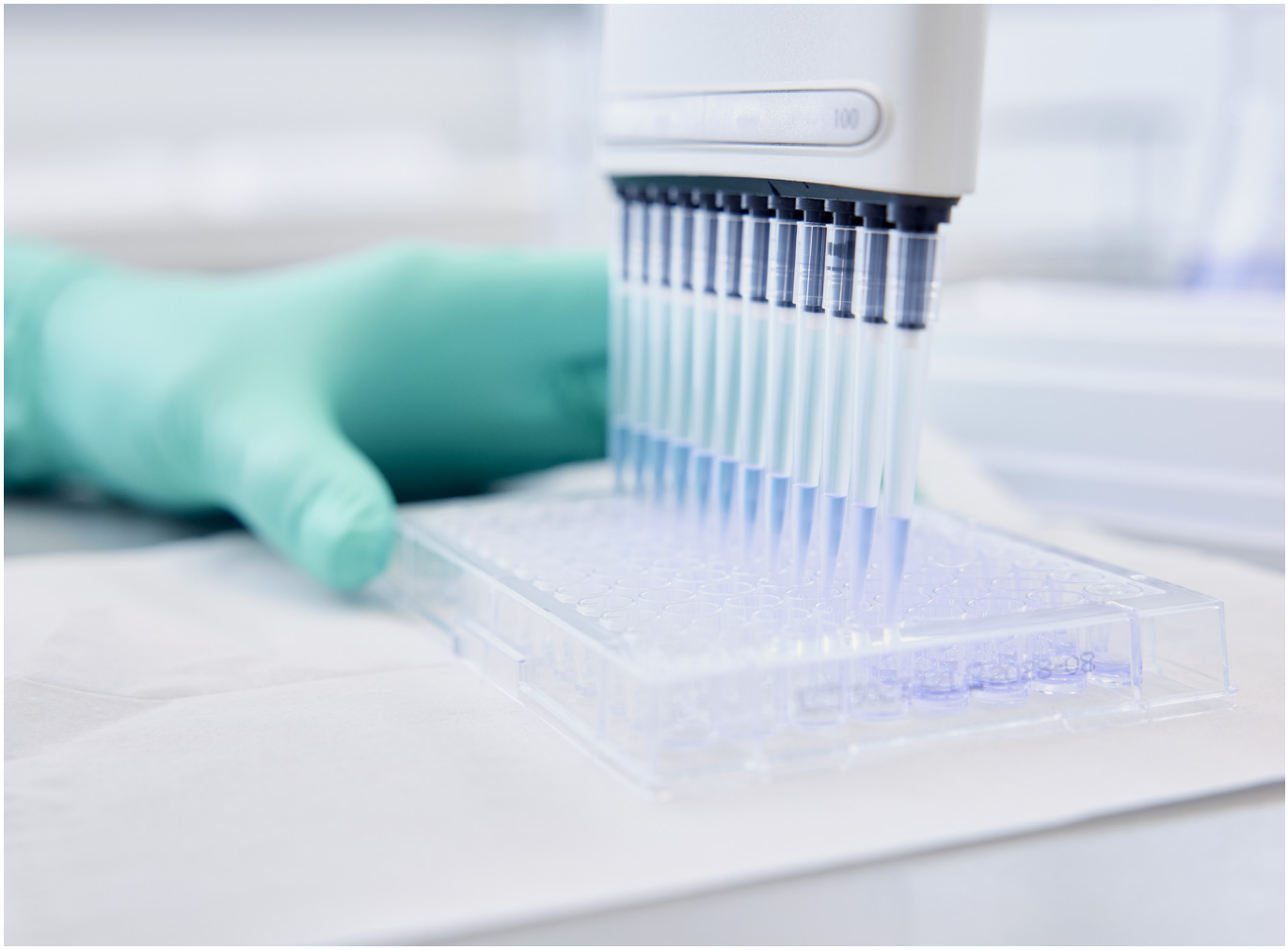
specific soy proteins. In fact, FSBM products tend to show significant variability across all ANFs, with β-conglycinin being particularly inconsistent. This high degree of variation is likely due to a combination of different factors, including differences in the SBM, variability in microbial strains and their enzymatic activities, and limited process control, all of which contribute to batch-to-batch inconsistencies.

Enzyme treatment effectively reduces all three ANFs, and proper control of raw materials and process conditions ensures consistency. TIA levels are reduced to an average of 1.3 mg/g, comparable to what is achieved by fermentation and extraction processes. For α-GOS, ESBM exhibits the lowest average content with consistently low levels across batches, which further reinforces the effectiveness of this approach. The same is seen for β-conglycinin which is reduced to a consistently low level of 2 ppm, and it demonstrates the ability to eliminate the variability observed in conventional SBM.

For **alcohol-extracted SPC**, the TIA level is low, and data also shows good consistency across products and batches. α-GOS is reduced significantly compared to conventional SBM, but the average level is higher than ESBM and similar to FSBM. Compared to fermentation, the variability is lower for SPC, recognizing higher consistency for chemical treatment compared to microbial reactions. The higher average level seen for α-GOS compared to ESBM may be because not all oligosaccharides are fully soluble in the solvent used for extraction.

When looking at β-conglycinin results, extraction can significantly reduce the average levels compared to SBM, as alcohol chemically denatures and disrupts the structure and folding of native proteins. However, the variability and average value are not as low as for ESBM.

When evaluating ANF levels in soy products, it is important to consider all three compounds to ensure healthy and effective growth of young animals.



Water holding capacity

Physical properties are also relevant when evaluating protein ingredients as they affect feed production and efficiency. Here we focus on water holding capacity, which is the ability of a product to retain water without binding it chemically.

Feeds with higher water holding capacity slow down gut transit time and aid nutrient absorption as the retained water can improve the texture of the digesta and more easily allow enzymatic digestion.

Different factors contribute to the water holding capacity of a product with particle size and the content and structure of proteins and carbohydrates being the most important ones. These factors are affected by processing techniques, meaning that processing of SBM also modifies how the product interacts with water. From **Figure 7** it is seen that enzyme treatment is the only technology that significantly improves the water holding capacity compared to conventional SBM.

This unique feature of products from Hamlet Protein can easily be visualized.

Scan the QR code to see.

If you want to try it out yourself, our water holding capacity kit is available upon request.

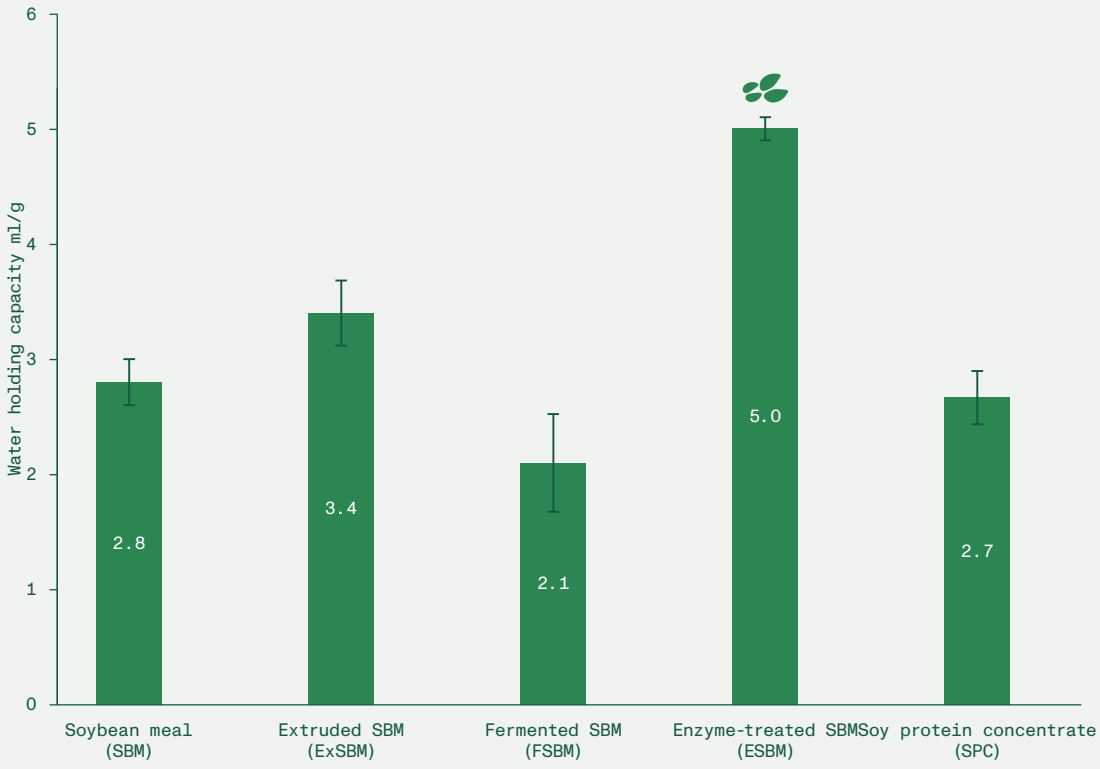


Figure 7 Overview of water holding capacity (n=18) in different SBM processed products covering conventional SBM, ExSBM, FSBM, ESBM and alcohol-extracted SPC. Water holding capacity is measured as the amount (ml) of water held by the product (g). The analyses are conducted in the laboratory of Hamlet Protein. Error bars represent standard deviation.







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