

WHITEPAPER

Precise protein content: Chitin analysis for insect-based animal feed

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Whitepaper by CG

We have been a developer and producer of analytical systems and basic products for laboratory work since 1846. The efficient use and analytical accuracy of our systems in your laboratory are our top priority. That is why we accompany you in your daily laboratory routine even after you have purchased a Gerhardt apparatus. The transfer and continuous expansion of our specialist knowledge are of utmost importance to us.

Insects as the protein source of the future

Sustainable protein sources are becoming increasingly important and are bringing new trends to our diets. Insects are considered a promising alternative to conventional protein sources. Regulation (EU) 2015/2283 officially classifies them as 'novel food', which qualifies them for use in food. They can already be found in protein powders, muesli bars, pasta and baked goods in supermarkets.

But it is not only in the food industry that the issue of sustainability is becoming increasingly important. Insect-based proteins are also being used more and more frequently in the production of feed for poultry, pigs, aquaculture and pets. This is because insects require significantly less water and land to produce than traditional protein sources such as soy or fishmeal. They also cause lower CO₂ emissions, which makes them attractive not only ecologically but also economically.



Abbildung 1: Insekten als Nahrungsmittel

In addition to ecological and economic aspects, however, compliance with national and international norms and guidelines is a top priority for the food and feed industry. After all, it is a matter of the welfare of animals and end consumers. This means that various parameters must be analysed and declared transparently, such as fibre, fat and protein content. This requires precise analytics – especially when it comes to insects.

Unlike plant-based protein sources, insects contain bioactive compounds such as the polysaccharide chitin. And chitin, in turn, contains nitrogen.

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In classic protein determination, the nitrogen contained in chitin is recorded as crude protein, which increases its total value. However, since the nitrogen from chitin cannot be processed by humans or animals, it should be considered separately.

Chitin: The disruptive factor in protein analysis

Put simply, a polysaccharide is a chain of many sugar molecules. The sugar molecule that forms the basic building block of chitin is called N-acetylglucosamine. This is a sugar that is additionally linked to a nitrogen group. The molecules are closely linked to each other, forming stable and resistant fibres. This resistance is an important characteristic in nature for insects, crustaceans and fungi.

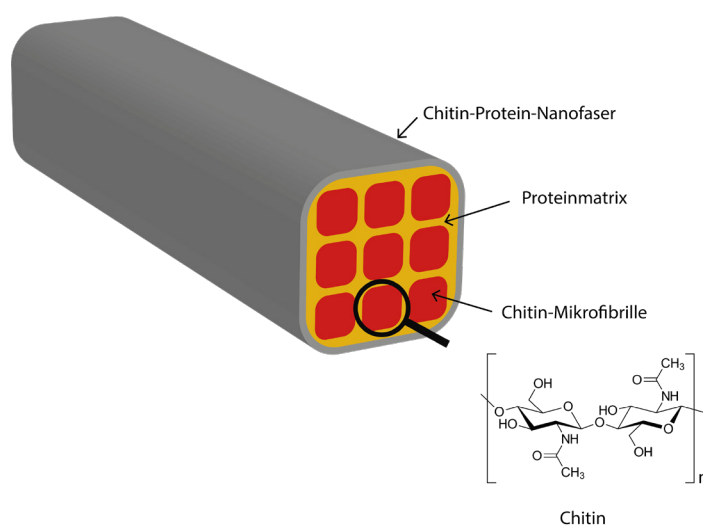


Abbildung 2: Aufbau Chitin

However, when it comes to consuming these products, this resistance is a disadvantage. This is because the enzymes present in the digestive tract of humans and animals cannot degrade the nitrogen groups bound to N-acetylglucosamine. They remain chemically bound and are simply excreted again without having served as protein or nutrients.

The physiological protein content of insects is therefore typically overestimated. And not insignificantly: with a nitrogen content of around 6.89%, chitin plays a decisive role in protein analysis.

This is because chitin is not considered separately in classic protein determination methods, such as the Kjeldahl method. The analysis records the total nitrogen content of a sample and uses this to calculate the crude protein content. The nitrogen content of chitin is automatically included in the calculation.

From an economic and nutritional point of view, this result is misleading, as chitin is not a usable protein. And in the feed industry in particular, where every gram of protein influences pricing, chitin leads to an artificial increase in protein content – and thus to higher costs without any actual added value.

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It is therefore necessary to rethink the method: the chitin content of insects in animal feed should be considered separately and measured accordingly, so that the nitrogen content from the chitin can be subtracted from the crude protein content after analytics.

Although methods for determining chitin do exist – such as basic chromatographic detection of monomers after hydrolysis or conversion to chitosan – they are usually complex, expensive or unsuitable for routine laboratory use. To make matters worse, these types of methods often use reagents that are harmful to the environment and health of laboratory staff.

C. Gerhardt and the International Research Association for Feed Technology (IFF) have therefore joined forces to tackle this challenge and have developed a more cost-effective and practical method for analysing the chitin content in insect-based feed.

An innovative method for determining chitin

There are many established techniques and methods in feed analysis. The goal of C. Gerhardt and the IFF was therefore to integrate chitin determination as easily as possible into existing feed analysis. Cost efficiency and reliability were also important factors. For this reason, the method developed relies on classic chemical procedures that have already been tried and tested:

- + the crude fibre determination according to Weender and
- + the nitrogen determination according to Kjeldahl.

The key innovation that complements these two methods in chitin analysis is deproteinisation by means of alkaline hydrolysis:

To this end, the samples are first transferred to filter bags with a highly specific mesh size, known as FibreBags. FibreBag technology enables consistent and reproducible filtration. In the FIBRE THERM analytical system, the samples are then boiled using a sodium hydroxide (NaOH) solution to remove the protein. The samples are then thoroughly rinsed with water and dried.

Once hydrolysis is complete, the non-chitinous nitrogen compounds – mainly proteins – are largely removed from the sample.

The remaining nitrogen content in the sample residue is determined by a Kjeldahl protein determination, which can be carried out using the semi- or fully automated systems of the KJELDATHERM and VAPODEST series from C. Gerhardt. The Kjeldahl method is typically divided into four main steps:

1. Sample weighing
2. Digestion of samples with sulphuric acid
3. Distillation of the digestion solution with steam
4. Titration of the distillate and calculation of results

„Validating an analytical method is a crucial step in demonstrating its precision, reliability, and suitability for use in the laboratory.“

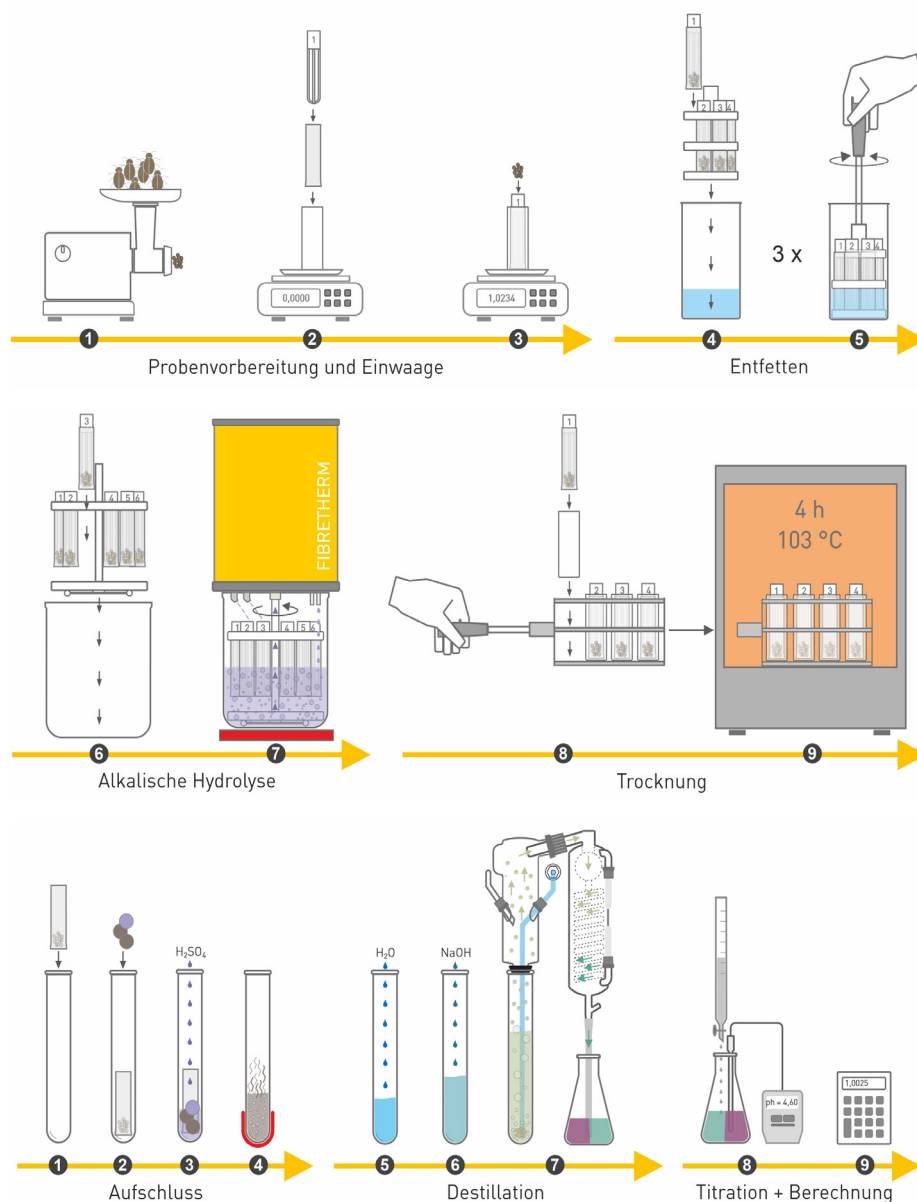


Abbildung 3: Analytischer Ablauf der Chitinbestimmung

The well-known nitrogen-chitin factor of 6.89% is used to determine the chitin content of the sample, which is then subtracted from the crude protein content. This gives the 'true' protein content.

Methodology: Alkaline hydrolysis and Kjeldahl analytics in detail

Validating an analytical method is a crucial step in demonstrating its precision, reliability, and suitability for use in the laboratory. This is the only way to guarantee reproducible results – especially with complex sample matrices such as insect-based animal feed.

As part of the validation of the new method, developed in cooperation between the IFF and C. Gerhardt, four common insect species were examined:

- + Black soldier fly (*Hermetia illucens*)
- + Mealworm beetle (*Tenebrio molitor*)
- + Cricket (*Acheta domesticus*)
- + Silk moth (*Bombyx mori*)

The mealworm beetle was analysed in all its different stages of development (larva, pupa, imago). Conventional pet food served as a reference. The method was tested on over 300 individual samples with a defined chitin content between 1.15 g and 92.25 g per 100 g sample. The sample matrices included whole insects, protein meals and animal feed.

Infrared spectroscopy was performed to ensure that pure chitin remained after deproteinisation. This analysis confirmed that no other nitrogen-containing compounds were present in the samples. This ruled out the possibility that the results were distorted by contaminants or incomplete separation.

Validation was carried out in accordance with the guidelines of the Federal Environment Agency and included extensive statistical evaluations. The results are summarised in the following tables:

Table 1: Recovery & standard deviation (SD) of chitin in various concentrations.

Chitin content [g/100 g]	n ¹	Min. - Max. [g/100 g]	Mean ± SD [g/100 g]	Recovery rate [%]	Nitrogen ± SD [g/100 g]
0,00	15	0,49 - 0,99	0,82 ± 0,13	below the determination limit	0,07 ± 0,02
1,15	8	1,49 - 1,68	1,59 ± 0,18	138,3 ± 0,2	0,09 ± 0,05
2,30	12	2,32 - 2,66	2,48 ± 0,42	107,8 ± 0,2	0,17 ± 0,01
4,61	22	4,27 - 4,87	4,56 ± 0,15	98,9 ± 0,1	0,32 ± 0,04
9,22	12	8,63 - 9,53	9,15 ± 0,21	99,3 ± 0,1	0,63 ± 0,01
92,25	27	88,79 - 93,00	90,67 ± 1,01	98,3 ± 0,1	6,29 ± 0,13

„The validation results confirm that the new method for determining chitin provides reliable and accurate data. It is therefore ideal for routine use in feed analysis.“

Table 1 shows the recovery rate for samples with known chitin content. The values range between 98% and 107%, which means that the actual chitin content of 100% was almost completely recovered in the samples – a clear indication of the precision of the method.

Table 2: Reproducibility & standard deviation (SD) of chitin in different sample matrices.

Sample	n	Mean value chitin content [g/100 g]	SD [g/100 g]	Relative SD [%]
Dried mealworms	90	6,4	0,2	3,2
Mealworm protein meal	118	9,7	0,1	1,4
Chitin	58	91,6	0,8	0,9
Reproducibility 4	12	9,1	0,2	2,3
Reproducibility 3	22	4,6	0,1	2,3
Reproducibility 2	12	2,6	0,1	3,9

Table 2 shows the reproducibility of the results. When the same sample is analysed multiple times, the results vary only slightly, with a standard deviation of less than 2%. These minor deviations once again demonstrate the high precision and also show the good reproducibility of the results in routine use.

The limit of quantification for samples containing chitin is approximately 2 g of chitin per 100 g of sample. Below this limit value, the background noise of the nitrogen analysis affects the accuracy of the results, which are then no longer reliable. In this case, it is recommended to increase the sample weighing, as this increases the absolute amount of chitin and thus also the amount of measurable nitrogen.

The validation results confirm that the new method for chitin determination provides reliable and accurate data. It is therefore ideal for routine use in feed analysis and enables a correct assessment of the usable protein content.

„Eine genaue Bestimmung des Chitin-gehaltes ermöglicht nicht nur eine korrekte Nährwertangabe, sondern trägt auch zu Tiergesundheit, Nachhaltigkeit und Wirtschaftlichkeit bei.“

Practical test: Chitin in commercial insect products and compound feed

The method developed for determining chitin content was validated in practice on commercial insect products and compound feed. The analyses revealed significant differences in chitin content: while wet food for dogs contained only 1.2% chitin, the proportion in dried larval skins was up to 42.8%.

This observation is particularly relevant for the animal feed industry. This is because in animal feed with an insect content of more than 20%, the chitin content is often more than 2%. This may seem like a minor deviation, but in an industry that relies on precise nutrient composition, it has a significant impact:

1. Animal welfare and product quality

Only healthy animals can produce high-quality food. A balanced diet promotes the animals' tolerance and performance.

2. Sustainability and economic efficiency

Specifically tailored animal feed is utilised more efficiently by animals. This reduces environmentally harmful emissions and conserves resources. At the same time, optimised feed conversion reduces production costs.

3. Transparency and pricing

Correct declaration of the usable protein content prevents overvaluation. Farmers and manufacturers can produce more cost-effectively and sustainably.

The results of the practical test emphasise that the chitin content in insect-based animal feed must not be neglected. Accurate determination not only enables correct nutritional information to be provided, but also contributes to animal health, sustainability and economic efficiency. The method developed offers a practical and reliable solution for the modern animal feed industry.

Advantages of the new method

The validation of the new method for determining chitin content developed by C. Gerhardt and IFF not only proves its analytical reliability, but also underlines its strategic importance for the modern food and feed industry.

This is because insects are establishing themselves as a sustainable source of protein not only in the feed industry, but also in food production. Whether in protein powders, snacks or functional food, the use of insect-based raw materials is steadily increasing. At the same time, chitin is not only found in insects, but also in fungi and crustaceans. This is increasing the demand for reliable analysis methods that enable the correct declaration of the usable protein content.

„The new method developed by C. Gerhardt and the IFF is not only technically mature, but also practical for everyday laboratory use – a decisive advantage in an industry that depends on efficiency, accuracy and cost-effectiveness.“

The new method developed by C. Gerhardt and the IFF meets this need: it is not only technically mature, but also practical for everyday laboratory use – a decisive advantage in an industry that depends on efficiency, accuracy and cost-effectiveness.

The new chitin analysis method stands out clearly from existing methods. While classic methods are often associated with high labour costs, high expenses or limited sample compatibility, the new solution offers the following advantages:

1. Minimal effort required

Unlike complex chromatographic methods (e.g. LC-MS or TLC), the new method does not require any complex sample preparation or special equipment. Instead, it uses established laboratory equipment such as FIBRE THERM and KJEL DATHERM, which are already in use in many laboratories. This means it can be integrated into existing workflows without additional investment.

2. Economic efficiency

While chitosan staining (TLC) is inexpensive but not very accurate, the new method offers optimal value for money. It does not require expensive reagents or special equipment, yet still delivers highly accurate results – a decisive advantage for routine laboratories and quality control.

3. High sample compatibility

Another key advantage is the broad applicability of the method. While traditional methods often have limited compatibility (e.g. LC-MS with complex sample preparation), the new chitin analytics are suitable for various sample matrices – from whole insects to protein meals and mixed feed. This makes it a universal solution for modern analytical methods.

Tabelle 3: Vergleich der analytischen Methoden zur Chitinbestimmung.

Methode	Arbeitsaufwand	Preis	Probenkompatibilität
Klassische Hydrolyse + LC- MS	hoch	teuer	eingeschränkt
Chitosan-Färbung (TLC)	mittel	gering	aufwendig in der Probenaufarbeitung
Neue Methode von C. Gerhardt und IFF	niedrig	mittel	hoch

Literature

[1] Article "Chitin Analysis in Insect-Based Feed Ingredients and Mixed Feed: Development of a Cost-Effective and Practical Method" by Dr. Patrick Sudwischer, Björn Krüger, Prof. Dr. Werner Sitzmann and Prof. Dr. Michael Hellwig
(Link: <https://www.iff-braunschweig.de/wp-content/uploads/2025/01/SD-567-Chitin-Analysis-in-Insect%E2%80%90Based-Feed-Ingredients-and-Mixed-Feed-.pdf>, 09.10.2025)

[2] Wellmitz, J., and M. Gluschke. 2005. Leitlinie zur Methodenvalidierung. Umweltbundesamt Texte. Nr. 01/05. ISSN 0722-186X.

Conclusion

The successful validation of the new method for determining chitin content represents a milestone in modern feed analysis – especially when dealing with alternative protein sources such as insects. With its high precision and reproducibility, the method delivers reliable results, even in complex sample matrices. A key advantage is that it requires little effort and standard laboratory equipment, making it ideal for routine use.

In addition, the new method enables manufacturers to accurately carry out the determination of the actual usable protein content. This allows:

1. Feed compositions to be optimised in a targeted manner – for a needs-based and compatible diet for the animals.
2. Nutritional information to be made transparent – for greater confidence among farmers and consumers.
3. Increase economic efficiency – through correct pricing and reduced feed waste.

A win-win situation for animal welfare and economic efficiency. Precise chitin analysis contributes significantly to improving animal welfare – because only a balanced and correctly declared feed ensures the health and performance of the animals. At the same time, operators benefit from greater economic efficiency as they can conserve resources and optimise costs.