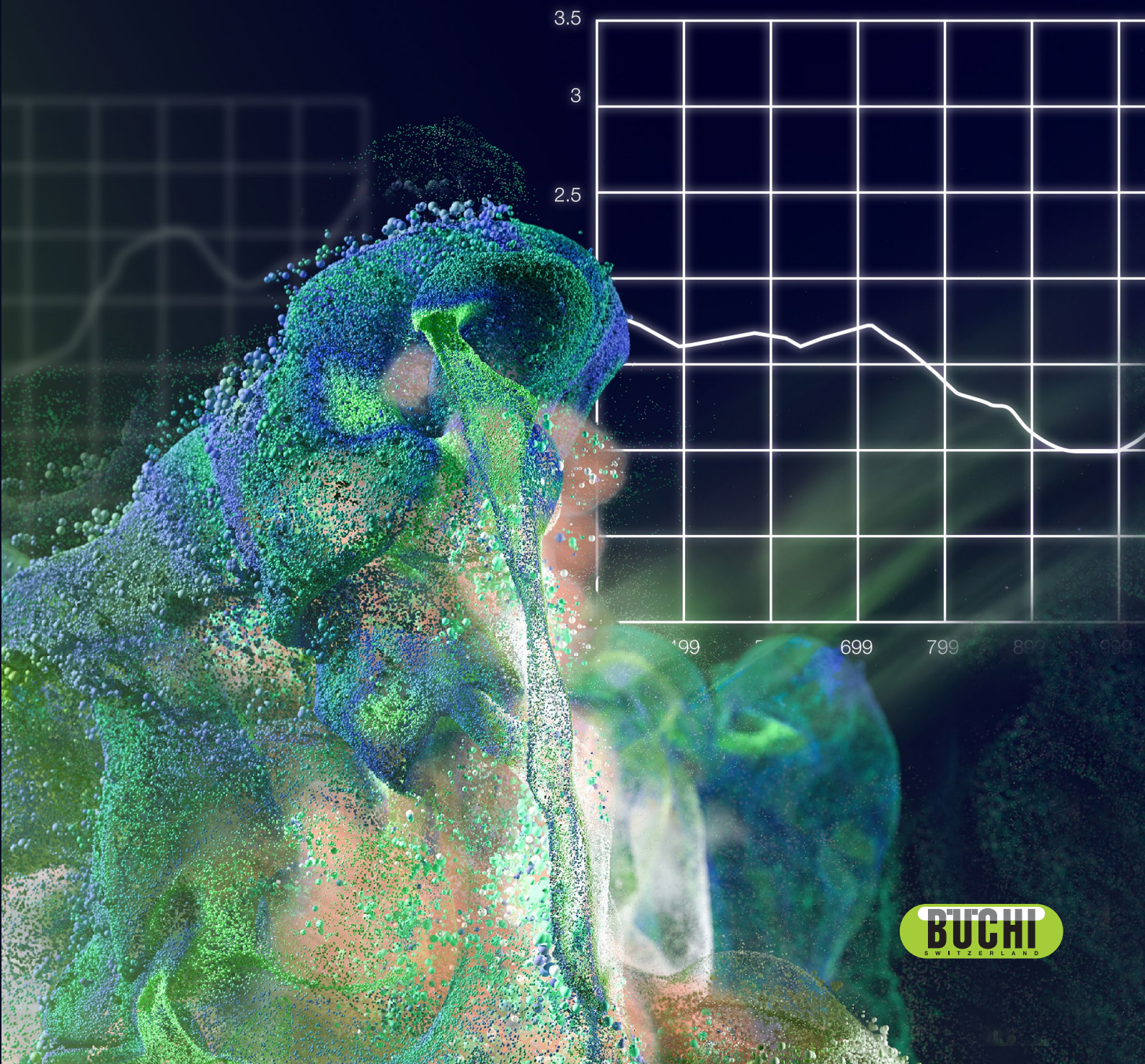
Reference Methods Guide

Using proximate analysis as the basis for NIR calibrations



BÜCHI
SWITZERLAND

Introduction

This guide focuses on protein and fat determination, a topic of critical commercial concern to food and pet food manufacturing companies to ensure their products abide by the appropriate laws and legal declaration requirements. Official reference methods will be discussed, along with their use in creating NIR calibrations that can streamline production processes and ultimately cut costs in food and pet food industries.

Nutritional analysis began back in 1861, and the methods have continuously been developed, modified, and improved over the years. The method for the quantitative analysis of macronutrients in food and pet food is called proximate analysis. To meet industry standards and remain competitive, food-manufacturing companies must utilize reliable analytical techniques to ensure compliance; this has led to the creation of reference methods. There are reference methods for determining a whole range of values from samples; however, this guide will specifically describe the reference methods used by different food and pet food industry sectors for protein and fat determination. Once having understood how to apply reference methods properly, this guide aims to describe how the obtained reference values become the foundation for building NIR calibrations. NIR analysis can then be used to streamline the entire process of protein and fat determination and get results in seconds. Other values, such as moisture content, can also be determined rapidly and simultaneously using NIR technology. Refer to each subsection for a more comprehensive list of analytes covered by NIR spectroscopy.

The primary reference method for protein determination is the tried and tested Kjeldahl method. This method involves using digestion to release nitrogen from the protein. The nitrogen is converted into ammonia, which is then titrated to determine the total nitrogen content. The protein content is determined by using a sample-specific empirical protein factor (conversion factor). Multiplying the total nitrogen content by this conversion factor accurately depicts protein content. The Kjeldahl method is time-consuming, involving many steps; however, it is a reliable and accepted reference method for accurate protein determination.

Several reference methods are used for fat determination for various samples. The most long-standing and official method used is the Weibull-Stoldt method, which involves acidic hydrolysis followed by Soxhlet extraction. The food industry has established several accepted standards based on this method, though other methods exist that can offer some advantages. Economic Continuous Extraction (ECE) is faster than Soxhlet, though fewer officially accepted standards exist. Hot Extraction is the fastest and cheapest of the three methods mentioned, and it requires less solvent. Like with ECE, though, there are fewer official methods. The requirement of performing hydrolysis prior to extraction when using methods other than Soxhlet is of particular importance. The hydrolysis step is essential as it releases fat from the matrix, making it available for extraction; this greatly impacts the results and accuracy of the analysis, more so than the solvent used or extraction method. For these reasons, specific standards governing the food and pet food industry define the method to be followed for specific sample types. The pet food industry is often more flexible and likely to use methods other than Weibull Stoldt, as the hydrolysis step is only sometimes required. There are other methods than those mentioned in this guide, such as alkaline hydrolysis followed by liquid-liquid extraction (Schmid-Bondzynski-Ratzlaff) for cheese.

Plant-Based Meat

Plant-based foods, such as meat alternatives, are becoming increasingly popular due to growing health and environmental concerns. There are many plant-based alternatives to other animal products, such as cheese, milk, yogurt, butter, and more. The meat alternative market is experiencing rapid growth, and the role of proteins and fats in food production is crucial.

Find out more

Meat

Fats and proteins are the two main macronutrients in meat, and their nutritional value is of high importance to consumer health. The measurement of fat and protein content, including connective tissue critical, and helps the food and pet food industry with product development. An accurate analysis of fat and protein also ensures compliance with regulations.

Find out more

Dairy

Fat and protein determination are of particular importance to producers of dairy products. Fat and protein content significantly influence the final product in several ways. The industry must carefully balance these factors to ensure their produce remains fresh and functional over time.

Find out more

Bakery

This guide will look at protein determination in gluten and starch samples and fat determination in cookie samples. Proteins and fats are essential to the quality and texture of baked goods. Proteins, especially gluten – as the name suggests – act as the glue that gives baked goods their structure. Fats provide flavor and prolong the shelf life of baked goods.

Find out more

Plant-Based Meat
Protein determination: Vegan Steak

The role of proteins in meat alternatives is of critical importance as they are used to replicate the taste and texture of real meat. The nutritional value is also of particular interest as the proteins come from plant-based sources instead of traditional sources of animal origin.

Procedure:
The Kjeldahl method is an easy and reliable method to determine protein in plant-based meats. Each sample is homogenized by grinding, depending on the matrix. The sample is then digested using the KjelDigester K-449. Steam distillation and boric acid titration are performed with the KjelMaster K-375 and KjelSampler K-377.

Parameters	Settings
Digestion time	120 min
Digestion temperature	420 °C
Distillation time	240 s
Titration type	Boric acid
Sensor type	Potentiometric

Results:
Highlights from the results of this experiment are shown below. For a range of results for several other plant-based alternatives, click the link and download a copy of the full Application Note that describes the experiment in detail.

Sample	Labeled protein content [g / 100 g]	Ø determined protein content [%]	RSD [%]
Vegan steak	20	19.68	0.95

Plant-Based Meat
Fat determination: Vegan Steak

Fat plays an important role in plant-based meats as they are used for flavor and to replicate the juiciness of actual meat. Compliant fat determination is equally necessary for labeling and quality control purposes.

Procedure:
The method used for fat determination is the Weibull-Stoldt method. Total fat determination requires a mandatory hydrolysis step to release the matrix's chemically bound and naturally encased fat. The samples are first hydrolyzed with the HydrolEx H-506. The fat must then be extracted with a suitable solvent, according to Soxhlet. Extraction was performed with the FatExtractor E-500 Soxhlet. Afterward, the extract is dried to a constant weight, and the total fat content is determined gravimetrically.

Method parameters	
Hydrolysis	30 min with hydrochloric acid
Extraction	
Solvent	Petroleum ether
Extraction step	20 cycles (heating level 6)
Rinse step	5 min (heating level 6)
Drying step	SmartDrying
Solvent volume	100 mL

Results:
Highlights from the results of this experiment are shown below. For a range of results for several other plant-based alternatives, click the link and download a copy of the full Application Note that describes the experiment in detail.

Sample	Labeled fat content [g / 100 g]	Ø determined fat content [g / 100 g]	RSD [%]
Vegan steak	10.8	11.8	0.77

NIR spectroscopy covers analytes such a moisture, fat, protein, carbohydrates, pH, fibre, ash, and color.

Meat
Protein determination: Sausage

Protein in meat is of particular interest to athletes and bodybuilders due to the role protein plays in muscle development and repair. For people with diabetes, protein helps regulate their blood sugar easing their daily lives.

Procedure:
For the analysis of meat, the samples are digested with the SpeedDigester K-439 using a combination of Kjeldahl Tablets Titanium and H_2O_2. The H_2O_2-assisted digestion protocol can reduce the digestion time by half. Steam distillation followed by boric acid titration is then performed using the MultiKjel together with the Metrohm Eco Titrator.

Parameters	Settings
Digestion time	135 min
Digestion temperature	420 °C
Distillation time	180 s
Titration type	Boric acid
Sensor type	Potentiometric

Parameter	After standard Kjeldahl digestion
Distillation Time	180 s
Titration Type	Boric Acid Titration
Sensor type	Potentiometric (pH)

Results:
Highlights from the results of the standard experiment are shown below. For the results of the accelerated recovery using H_2O_2 as a catalyst, click the link and download a copy of the full Application Note that describes the experiment in detail.

Sample	Labeled protein content [g / 100g]	Ø determined protein content [%]	RSD [%]
Cooked ham	13.677	13.845	0.56

Meat
Fat determination: Sausage

For labeling requirements, total fat content must be determined by laboratory analysis and accurately declared. Like with protein, fat content is also concerning for consumers that like to stay fit and healthy.

Procedure:
Economic Continuous Extraction (ECE), also called Twisselmann, is a variation of the traditional Soxhlet extraction method that aims to increase efficiency and reduce the cost of fat extraction. First, the sample is hydrolyzed with the HydrolEx H-506. Then the ECE is performed on the FatExtractor E-500; this process involves the sample being kept in the hot solvent vapor while being efficiently rinsed with freshly distilled solvent. The total fat content is determined gravimetrically once the extract has been dried to a constant weight.

Method parameters	
Hydrolysis	30 min with hydrochloric acid
Extraction	
Solvent	Petroleum ether
Extraction step	60 min (heating level 5)
Drying	SmartDrying
Solvent volume	70 mL

Results:
Highlights from the results of the experiment are shown below. Click the link and download a copy of the full Application Note that describes the experiment in detail.

Sample	Labeled fat content [g / 100 g]	Ø determined fat content [g / 100 g]	RSD [%]
Cooked sausage, LVU No. 16 – 01j	27.46	27.86	0.25

NIR spectroscopy covers analytes such a moisture, fat, protein, CTP, BEFFE, salt, and ash.

Dairy
Protein determination: Powdered milk

The protein content of dairy products affects their stability in many ways. When proteins denature, they exhibit several characteristics, such as a loss of solubility leading to coagulation. Protein content can cause changes in the texture and appearance of dairy products, impacting the quality and storage stability.

Procedure:
For the analysis of milk powder, samples are digested with a combination of Kjeldahl Tablets Titanium and H_2O_2 using the SpeedDigester K-439. A steam distillation protocol followed by boric acid titration is then performed with the MultiKjel coupled with the Metrohm Eco Titrator; this results in an easy and fast analysis, and the H_2O_2-assisted digestion can reduce the digestion time by half.

Parameters	Settings
Distillation Time	180 s
Digestion temperature	490 °C
Titration Type	Boric Acid Titration
Sensor type	Potentiometric (pH)

Results:
The results of the experiment are shown below. Click the link and download a copy of the full Application Note that describes the experiment in detail.

Sample	Labeled protein content [g / 100 g]	Ø Determined protein content [g / 100 g]	RSD [%]
Milk powder	20.54	20.57	0.20

Dairy
Fat determination: Powdered milk

Like protein, fat also significantly impacts dairy production. Fat affects the taste and texture of dairy products. Dairy products, however, are prone to rancidification, which is the autoxidation or hydrolysis of fats and oils when exposed to light, air, moisture, or bacterial action. When the fat in dairy products is broken down, free fatty acids are produced and, in time, cause the milk to turn rancid. To prevent this process and increase shelf life, spray drying is used to remove moisture. The high heat used creates an environment that is hostile to bacteria and enzymes, ultimately leading to their inactivation.

Procedure:
The fat determination of milk powder involves hydrolysis with hydrochloric acid in the HydrolEx H-506 and a Hot Extraction using the FatExtractor E-500. Hydrolysis is a mandatory step for milk powders to obtain accurate results. Hot Extraction is the fastest and cheapest method mentioned so far. It requires less solvent than the other extraction methods. The process is completed by gravimetric determination of fat content after the extract has been dried to a constant weight.

Method parameters	
Hydrolysis	30 min with hydrochloric acid
Extraction	
Solvent	Petroleum ether
Extraction step	5 min (heating level 4)
Rinse step	30 min (heating level 5)
Drying step	3 min (heating level 3)
Solvent volume	50 mL

Results:
Highlights of the results of the experiment are shown below. For further results for several dairy products, click the link and download a copy of the full Application Note that describes the experiment in detail.

Sample	Labeled fat content [g / 100 g]	Ø determined fat content [g / 100 g]	RSD [%]
Milk powder, LVU No. 17 – 4b	24.27	24.35	0.26

NIR spectroscopy covers analytes such a moisture, fat, protein, lactose, pH, salt, and sugar.

Bakery
Protein determination: Gluten and starch

Gluten is a structural protein found in certain cereal grains and is used as a binding and stabilizing agent to give baked goods a particular structure and texture. Starch is used as a thickener by the food and pet food industry; it is also an essential source of energy.

Procedure:
The samples are analyzed for their protein content according to the Kjeldahl method. The Kjeldahl method is used to determine organic nitrogen by digesting the sample using the KjelDigester K-449. This is followed by the distillation and the boric acid titration that is performed with the KjelMaster System K-375 / K-376.

Parameters	Settings
Digestion time	125 min
Digestion temperature	420 °C
Distillation time	180 s
Titration type	Boric acid
Sensor type	Potentiometric

Results:
Highlights from the results of the Kjeldahl analysis for gluten and starch are shown below. Click the link and download a copy of the full Application Note that describes the experiment in detail.

Sample	Ø Determined nitrogen content [g / 100 g]	Ø Determined protein content [g / 100 g]	RSD [%]
Gluten	9.498	59.336	0.338
Starch	0.046	0.289	0.619

Bakery
Fat determination: Cookies

The role of fat in baked goods is of particular importance to texture and shelf life, not to mention its influence on taste. Although a number of methods are used for fat determination, none are as thorough as the Weibull-Stoldt method, which combines hydrolysis and Soxhlet extraction.

Procedure:
The method used for fat determination, the Weibull-Stoldt method, involves the hydrolyzation of the sample with the HydrolEx H-506. The subsequent Soxhlet extraction is performed with the FatExtractor E-500. The extract is then dried to a constant weight before the total fat is determined gravimetrically.

Method parameters	
Hydrolysis	30 min with hydrochloric acid
Extraction	
Solvent	Petroleum ether
Extraction step	20 cycles (heating level 5)
Rinse step	5 min (heating level 5)
Drying	SmartDrying
Solvent volume	100 mL

Results:
Highlights from the results of the experiment are shown below. Click the link and download a copy of the full Application Note that describes the experiment in detail.

Sample	Labeled fat content [g / 100 g]	Ø determined fat content [g / 100 g]	RSD [%]
Cookie, LVU No. 17 – 11	27.47	27.57	0.16

NIR spectroscopy covers analytes such a moisture, fat, protein, gluten, starch, sugar, color, salt, and baking absorption.

Conclusion

All the reference methods described give accurate, reliable, and reproducible results; they comply with the official methods required by law for the determination of protein and fat. Nevertheless, their main pitfall is the time taken to go through the steps of each procedure. It is not practical for many food and pet food processing plants to continually perform such time-consuming procedures for quantitative and qualitative identification of sample composition. Thankfully the accurate results produced by these reference methods also form the basis of reliable NIR calibrations.

NIR spectroscopy is an effective technology that provides fast and cost-effective analysis of fat, protein, and moisture content, to name only a few parameters. NIR instruments can be installed at-line or on-line at critical stages of the process to give immediate analytical results or in the lab for quality control. NIR spectroscopy can considerably streamline a workflow and cut costs significantly. To speed up the implementation of NIR even more, pre-calibrated applications that already contain a large pool of samples with reference values can be used out of the box. The combined expertise of NIR and reference methods is important when it comes to calibration development. To learn more about NIR and take advantage of the benefits of the technology, check out our comprehensive courses and training sessions.

Follow us on:

@büchi-labortechnik-ag

@buchilabequipment

@buchilabequipment

@buchilabequipment

@buchilabeqpt

@buchilabequipment

www.shallot-holmes.com

BÜCHI Labortechnik AG Meierseggstrasse 40 Switzerland – 9230 Flawil
T+41 71 394 63 63 marketing@buchi.com www.buchi.com