Application Note: 51873

Protein, Fat and Moisture Analyses of Fresh Fishmeal with an Antaris II FT-NIR Analyzer

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Key Words

- Antaris
- Fishmeal
- FT-NIR
- Kjeldahl Method
- Soxhlet Extractor

Introduction

This study demonstrates the validity of replacing several analytical techniques used to analyze fishmeal with a single measurement using Fourier Transform-Near Infrared (FT-NIR) spectroscopy.

Fishmeal is used worldwide as a feedstock for agricultural industries including food for economically important fish species such as salmon and catfish, as well as shrimp and carp. It is ideally suited as the most appropriate aqua feed source of protein and growth factors for these species. Fishmeal is produced from various fish and fish byproducts that are cooked, pressed and dried. The pressed cakes are then ground and homogenized to a final product that has the consistency of ground wheat.

Due to the variable nature of the raw material, the final product requires careful analysis to ensure it is consistent and meets appropriate quality standards. The quality of the product is determined after the material is dried. The quality of the final product can be controlled during the process based on the results of critical chemical analysis. Typically, the following three parameters are measured: protein, fat content, and moisture. Each of these components has historically required fairly complex, labor intensive, expensive and sometimes dangerous laboratory analytical techniques. The protein content is normally measured using Kjeldahl analysis and the fat content is determined using Soxhlet extraction methods. The moisture content can be determined by loss on drying methods or by Karl Fischer titration. The nature of these analytical techniques require samples to be removed from the process line and brought to a laboratory, where a technician may spend several hours performing the analysis. Each of these methods is difficult to implement in a process environment, whereas real-time on-line results will improve efficiency and produce less loss due to poor quality product. A brief description of the traditional analytical techniques is provided below.

The Reference Methods

The Kjeldahl Method

The Kjeldahl method is the standard method for determining protein content. The principle of the method is as follows: all the Nitrogen is converted to NH_4 by digestion with concentrated H_2SO_4 and an inorganic salt catalyst at high temperature. Then, it is made alkaline with NaOH and the ammonia steam is distilled into a solution of boric acid that contains an indicator (methyl orange). The distilled ammonia is then titrated against 0.1 m (HCl).

The sample preparation needs to be done carefully since food is a heterogeneous mixture of chemicals, some of which are difficult to isolate and measure. Several samples must be taken randomly or systematically to increase the accuracy.

The method is highly reliable compared to other methods, and it is used to calibrate physical and automatic methods. The nitrogen content of a particular protein mixture is seldom known precisely; and the methods of determining nitrogen are wrought with difficulties. Although interference from other non-protein nitrogen is generally small, it is a disadvantage.

Soxhlet Extraction

The Soxhlet extraction determines the total fat analysis. The analysis is extremely time-consuming, especially when solvent pre-extraction before hydrolysis is required. It was originally designed for the extraction of a lipid from a solid material. However, a Soxhlet extractor is not limited to the extraction of lipids.

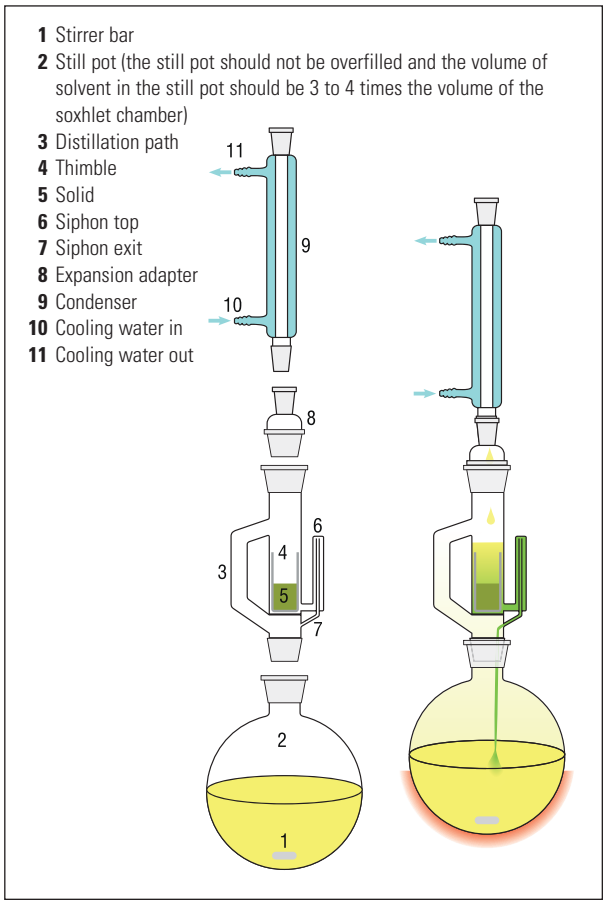

Figure 1: A schematic representation of a Soxhlet extractor

Typically, Soxhlet extraction (Figure 1) is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent, then a simple filtration can be used to separate the compound from the insoluble substance. The solvent is heated to reflux. The solvent vapor travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles, the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

Traditional chemical techniques are very difficult to handle and produce a lot of chemical waste. In addition, they do not quickly determine all the necessary parameters required. In this application note, it is shown how the Thermo Scientific Antaris FT-NIR analyzer achieves rapid, accurate analysis where a traditional chemical takes too much time and is dangerous for the people in the lab.

Methods and Results

The needed samples were taken directly from the production process and analyzed on the Antaris™ II Method Development Sampling (MDS) system with a sample cup spinner and a petri dish under standard laboratory conditions (Figure 2).

Figure 2: Antaris II with sample cup spinner

Due to the fact that production samples were used, it was possible to include the natural variation of the process. There was variation in season, which has an influence on the fat amount and the area where the fish were caught. In total, 247 standards were used over time to build the calibration. Different results were achieved. Pressing the sample into the petri dish was found to improve the results.

Spectroscopic Collection Parameters

Spectroscopic range	9000 to 4000 cm^{-1}
Resolution	16 cm^{-1}
Number of scans	32
Collection time	12 seconds

Table 1: The spectra were collected with the following parameters. (Figure 3 shows a representative spectrum.)

After the development of the methods on the Antaris II MDS system, the process was continued on a Thermo Scientific Antaris MX FT-NIR process analyzer.

Calibration Development

The calibration was developed with the software package Thermo Scientific TQ Analyst. All the spectra were converted to the first derivative with a Norris smoothing of length = 3 and a gap = 3 (see Figure 4 for the standards with this pretreatment). The PLS is the most suitable method for this calibration. The whole spectral range is used for the calibration. Calibration Plots for two representative components (protein and water) are shown in Figures 5 and 6. These graphically demonstrate the agreement between the primary method of analysis and the NIR method. Other parameters showed similar plots.

Table 2 shows the different parameters developed from calibration.

	Factors	Corr. Coefficient	RMSEC	RMSECV	Range
Protein	10	0.96	0.57	0.712	68.7-72.3
Fat	9	0.984	0.27	0.31	8.3-10.5
NH_3	10	0.97	0.012	0.015	0.12-0.25
Ash	8	0.935	0.605	0.74	12-15.67
Water	7	0.985	0.242	0.32	5.6-9.3

Table 2: Results achieved for the different components

Conclusion

The Antaris II FT-NIR analyzer equipped with a sample cup spinner can accurately measure moisture and other compounds like fat and protein. The fit of the calibration method ($R > 0.935$) was proven to be excellent across the wide range of compounds to be measured.

Using the Antaris II allows for rapid collection of spectra and analysis in real-time. The Antaris also precludes the need for other expensive lab equipment and solvents and eliminates chemical exposure of laboratory personnel. The accuracy, precision, linearity and speed of analysis by FT-NIR allows definitive determination of the components in the fish meal samples. Additionally, the chemometric analysis is easily transferable from the lab-based Antaris II system to the Antaris MX production system.

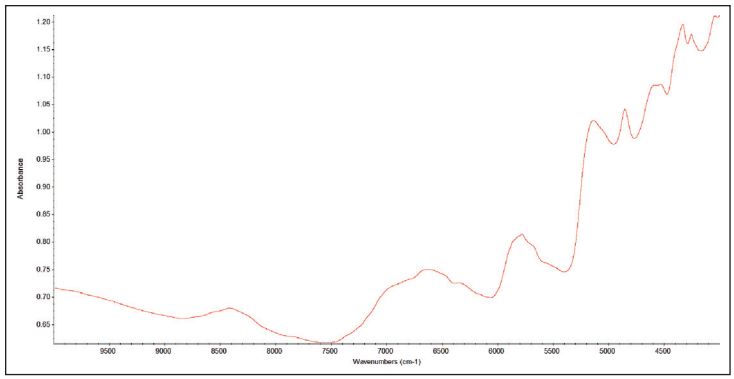

Figure 3: Representative spectra of a fishmeal sample

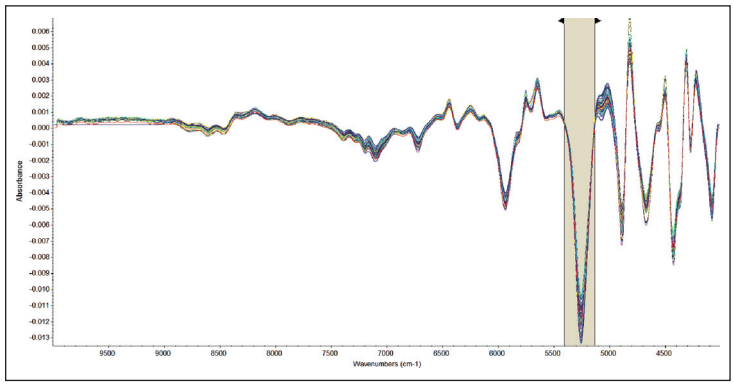

Figure 4: First derivative spectra of the standards used to build the calibration. The water peak is clearly marked.

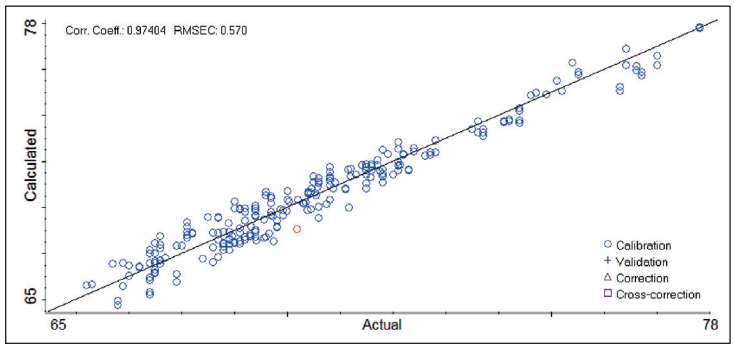

Figure 5: Protein calibration

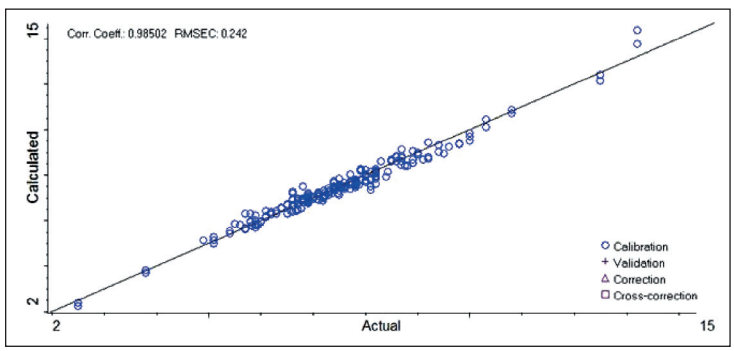

Figure 6: Water calibration

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