

## **Title: Plant-based foods Compliant Fat and Protein Determination**

### **ABSTRACT**

Growing concerns about the climate and animal welfare together with wider health awareness are causing consumers to opt increasingly for plant-based diets. As a result of this trend, plant-based meat alternatives are gaining more attention; they are advertised as a greener alternative to traditional animal meat and meat products based on emissions-intensive livestock farming. Five commercially available plant-based foods of various protein sources and preparational procedures were analyzed in triplicate with BUCHI's automated Kjeldahl and Soxhlet Extraction instruments for protein and fat, respectively. The content of these nutrients is an important marker of product quality and a legal requirement for nutrition labelling.

Overall, the results for all plant-based foods were in close agreement with the labelled nutrient content and showed low relative standard deviation. For the fat determination the acid hydrolysis prior to the Soxhlet extraction is beneficial as it yields about 1% higher fat content in average. Moreover, the results for the Kjeldahl protein determination in triplicates showed good repeatability with a relative standard deviation of 2% or less.

The BUCHI standard applications work smoothly for the plant-based foods and the samples did not require special treatment.

### **INTRODUCTION**

Novel food products such as plant-based burgers and other meat substitutes have become very popular. Not only individuals with a vegan or vegetarian diet appreciate these products, but also consumers with concern about the sustainability and health. Although products are vegan, they do not have the negative connotation of abstinence and rigid diets but are trendy products for a contemporary lifestyle [1].

A meat-substitute or meat analogue approximates certain qualities (primarily texture, flavour and appearance) or chemical characteristics of a specific meat. The texture of meat is imitated by processing pea, soy or wheat proteins, red beet root juice or plant heme for the colour [2]. A special focus is placed on the proteins. The protein ingredients are the most important components for product identity and product differentiation. The role of fats and oils in meat analogue formulations is to contribute to the juiciness, tenderness, mouthfeel, and flavour release of the product [3].

In the presented paper, fat and protein content of a wide variety of plant-based food samples were determined. The content of proteins and fat are important for controlling the production process as well as for quality control of the final product. Nutritional labelling is a legal requirement and of interest to many health-conscious consumers.

The samples were chosen in a way to cover different protein sources (soy, wheat, pea) and various ways in which the proteins were processed (dried, extruded, etc.).



Figure 1: Plant-based food samples used for this study prior to homogenization: soy schnitzel; fish sticks based on wheat; boiled sausage based on tofu and wheat protein; steak based on soy and wheat; and cold cuts based on pea and soy protein (from top left to bottom right)

## FAT DETERMINATION WITH FATEXTRACTOR E-500

Fat content of foods is usually determined by solvent extraction according to different extraction methods. Other methods such as NIR or NMR can also be used. The choice for a method depends on the purpose of the analysis (e.g., official nutrition labeling or rapid quality control), the daily sample load and the need for compliance with official standard methods.

Crude fat or free fat is determined by pure solvent extraction. When the total fat content is determined, an acid hydrolysis prior to the solvent extraction is carried out. The acid hydrolysis releases all the encapsulated or bound fat from the sample matrix.

Soxhlet extraction is the most widely used method for fat determination. It is a very robust and easy method and is demanded by many official standard methods. Fat determination according to the Weibull-Stoldt method includes acid hydrolysis and Soxhlet extraction.



Figure 2: HydrolEx H-506 in combination with the FatExtractor E-500 Soxhlet for compliant and fast solvent extraction of fat according to the Weibull-Stoldt method

The FatExtractor E-500 is designed for quick and compliant fat extraction according to Soxhlet, Randall or Twisselmann. The HydrolExH-506 offers a smooth and safe process with convenient system handling for acid hydrolysis as a sample preparation step prior to fat extraction for total fat determination.

The total fat content of the samples as well as the free fat content (without prior acid hydrolysis) were determined, the results are shown in Table 1 and Figure 3.

|                | Free fat<br>[g/100g] | Total fat<br>[g/100g] |
|----------------|----------------------|-----------------------|
| Soy schnitzel  | 0.10 (10.2%)         | 1.66 (4.45%)          |
| Boiled sausage | 15.8 (0.64%)         | 16.8 (0.59%)          |
| Steak          | 10.7 (0.92%)         | 11.8 (0.77%)          |
| Fish sticks    | 12.9 (1.67%)         | 14.1 (0.89%)          |
| Cold cuts      | 11.2 (0.30%)         | 12.1 (0.64%)          |

Table 1: Free fat and total fat determined in plant-based foods. (n=3, RSDs in brackets) The samples were extracted with petroleum ether 40-60°C using Soxhlet extraction for 20 cycles. For total fat, the samples were hydrolyzed with 4M HCl for 30 min prior to extraction

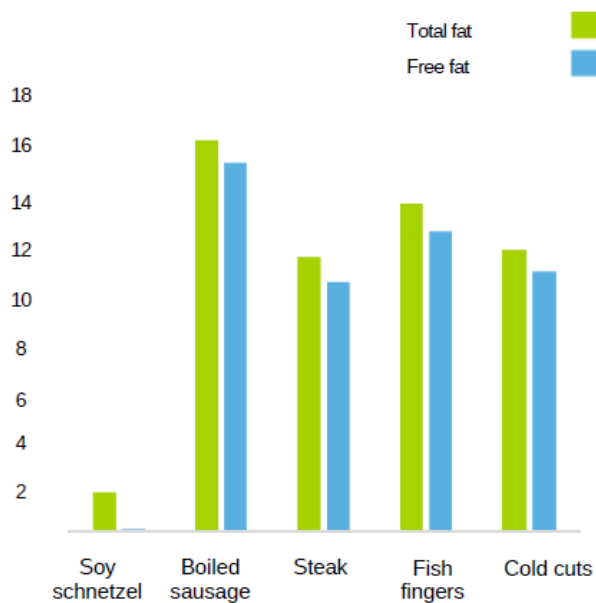


Figure 3: Mean values of the determined fat contents for total and free fat of five plant-based food samples. The error bars indicate the standard deviation (n=3)

All determined fat contents were higher, when the samples were hydrolyzed before the fat extraction. This preparation step helps to release bound fats from the matrix and is advisable for plant-based foods.

## PROTEIN DETERMINATION WITH KJELMASTER K-375

The three step Kjeldahl protein determination was established at the end of the 19th century by Johan Kjeldahl at the Carlsberg Breweries in Copenhagen, Denmark.

Despite its age the method is widely used as the reference according to most official norms worldwide. The method consists of three main steps which include digestion to convert organic nitrogen to inorganic ammonium sulfate, distillation preceded by alkalization to separate ammonia from the digest, and titration for quantification of the ammonia. Due to the automated process the method is safer, more application-friendly with an impact on chemical savings.

The BUCHI KjelDigester K-449 along with the KjelMaster K-375 adjacent to the KjelSampler K-377 is designed for automated, high-sample throughput and a potentiometric or colorimetric titration for compliance to the broadest range of official methods.



Figure 4: KjelDigester K-449 connected to the Scrubber K-415 for neutralization of acidic fumes for increased longevity of the instruments and environmental protection

The BUCHI standard method was applied [5] according to the freely available Kjeldahl Optimizer App [6]. Overall the results (Table 2) for the protein content are in close accordance with the labelled values with only minimal deviations. The consistency of the results (RSD < 2%) is exceptionally good.



Figure 5: KjelMaster K-375 in combination with the KjelSampler K-377 for highest sample throughput

|                | <b>Labelled content</b><br>[g/100g] | <b>Determined content</b><br>[g/100g] |
|----------------|-------------------------------------|---------------------------------------|
| Soy schnitzel  | 49                                  | 47.4 (0.04 %)                         |
| Boiled sausage | 17                                  | 16.4 (0.11 %)                         |
| Steak          | 20                                  | 19.7 (0.95 %)                         |
| Fishsticks     | 12.6                                | 12.8 (0.44 %)                         |
| Cold cuts      | 6.5                                 | 6.9 (1.32 %)                          |

Table 2: Comparison between labelled and determined protein content (n=3, RSDs in brackets)

## CONCLUSION

The fat and protein contents of plant-based foods were determined with BUCHI's automated fat extraction and Kjeldahl solutions. The determined values corresponded well to the expected values with low variation. The standard application parameters could be used and no specific adaptation for these types of samples was necessary.

## REFERENCES

- [1] Rützler, H. 2019. Article from Food Report 2019, <https://www.zukunftsinstitut.de/artikel/food/plant-based-food-der-neue-spin-bei-ersatzprodukten/>
- [2] Wikipedia, definition of meat analogue, as seen on 10.03.2020. [https://en.wikipedia.org/wiki/Meat\\_analogue](https://en.wikipedia.org/wiki/Meat_analogue)
- [3] Bohrer, B. 2019. An investigation of the formulation and nutritional composition of modern meat analogue products. Food Science and Human Wellness. 8 (320-329)
- [4] Application note 387/2020. Determination of total fat content in plant-based meat substitutes.  
Available for download at: <https://www.buchi.com/en/node/10871>
- [5] Application note 395/2020. Protein determination in plant-based meat alternatives.  
Available for download at: <https://www.buchi.com/en/node/11642>
- [6] Kjeldahl Optimizer App Available for download at: <https://www.buchi.com/en/service-support/scientific-mobile-apps/kjeldahl-optimizer>