



## Application Review No. 010/2022

### Free or Total Fat- the Hydrolysis in Review

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## 1. Abstract

Fat determination is of great importance for the industry to control the quality of their raw materials, intermediates and final products. The labelling of fat and other nutrients on food products is mandatory in most countries. Furthermore, extracted fat is also used for further analysis such as the determination of fat-soluble contaminants or for fat quality parameters, eg. free fatty acids, saponification- or acid value.

There are two main types of fat extraction, total and crude fat extraction. Free fat extraction is a direct extraction that is limited to determine only free fat. Total fat extraction covers both free fat and fat enclosed by other components of the sample matrix.

This application review provides an overview of the two types of fat – free and total fat – with their corresponding determination methods and a discussion when the acid hydrolysis is necessary or recommended.

## 2. Background Information - Extraction

The gravimetric determination of the fat content is performed by extraction with a non-polar solvent. An extraction is a separation process between two phases, most commonly are either solid-liquid or liquid-liquid extractions. Hereby, the target substance(s) gets separated from the so-called matrix. An everyday example can be found in making tea or coffee, where water-soluble compounds get extracted from the matrix (tea leaves or ground coffee), forming the extract.

The Soxhlet extraction, named after Franz von Soxhlet in 1879, is hereby one of the most widely used extraction methods for fat determination. An illustration is shown in Picture 1, whereas a visual presentation can be found on [here](#).

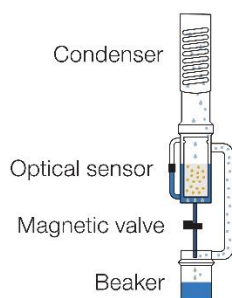


Figure 1: Soxhlet extraction assembly with extraction chamber containing the sample. Visual representation of the [FatExtractor E-500 SOX](#), a fully automated true Soxhlet extraction unit.

## 3. General Method Overview- Free vs. Total Fat Content

### Free fat

The free fat, sometimes also referred to as crude fat, content is determined by direct extraction of the sample. It is applicable to samples where fat was added to the food/feed as ingredient – e.g., sausages, where lean meat is mixed with fat or by processing eg. frying the product.

Sometimes, the free fat is determined from samples where none of the before mentioned criterias apply. This can be because free fat determination

- corresponds to the industry standard
- or reference standard methods describe the free fat determination.

For the extraction with a non-polar solvent, the sample must be dry. It is therefore necessary to dry the sample or to mix it with a drying agent prior to extraction.

## Total fat

For total fat determination, the matrix of the sample must be broken up prior to extraction, because the fat is being enclosed in a cell- or protein structure. This applies to all matrices being the result of growth (plant- or animal origin). The break-up of the matrix can be achieved by performing an acid hydrolysis using hydrochloric acid. The sample can be liquid, moist or dry.



Figure 2: Workflow from sample to result for free and for total fat determination. For free fat determination, the acid hydrolysis is skipped.

The determined free – and total fat contents of a series of certified reference materials (CRM) and commercial food samples are compared in Tables 3 - 4. Please refer to BUCHI application note for the detailed procedure (see links in chapter 6 “Application examples”)

Table 1: Free and total fat content of various samples, compared with the certified value. Values in red are outside of the specified range.

| Sample         | Certified range % | Free Fat % (rsd) | Total Fat % (rsd) |
|----------------|-------------------|------------------|-------------------|
| Milk Powder    | 26.54 - 27.04     | 21.91 (0.05%)    | 26.55 (0.49%)     |
| Chocolate      | 30.58 - 31.30     | 30.42 (0.56%)    | 30.86 (0.18%)     |
| Meat (Sausage) | 21.29 - 22.52     | 21.64 (0.14%)    | 21.95 (0.24%)     |
| Soy beans      | 19.27 - 21.11     | 20.71 (0.17%)    | 23.09 (1.12%)     |
| Cookie         | 27.159 - 27.781   | 27.07 (0.39%)    | 27.53 (0.62%)     |
| Turkey Feed    | 4.35-5.19         | 4.15 (0.76%)     | 5.13 (0.53%)      |

Determining a fat content in the given range of a CRM validates the method and equipment. While most measured samples were perfectly within the total fat range and therefore require the hydrolysis, exceptions like the sausage give the end user the possibility to validate either or both fat determinations. For soybeans, the standard method used for the reference value is a free fat determination. Although the total fat content being higher, is therefore outside of the range.

Table 2: Free and total fat content of various commercial samples, compared with the labelled value.

|                        | Labelled value % | Free Fat % (rsd) | Total Fat % (rsd) |
|------------------------|------------------|------------------|-------------------|
| Hazelnuts              | 61               | 49.97 (0.51%)    | 61.26 (0.31%)     |
| Snack crackers         | 13               | 10.64 (1.72%)    | 11.83 (0.52%)     |
| Snack pretzels         | 7.9              | 6.85 (0.65%)     | 8.03 (0.76%)      |
| Soy schnitzel          | 2.3              | 0.10 (10.2%)     | 1.66 (4.45%)      |
| Boiled sausage (vegan) | 17               | 15.83 (0.64%)    | 16.77 (0.59%)     |
| Vegan steak            | 10.8             | 10.70 (0.92%)    | 11.79 (0.77%)     |
| Vegan fish fingers     | 9.3              | 12.88 (1.67%)    | 14.07 (0.89%)     |
| Vegan Lyoner           | 12               | 11.15 (0.30%)    | 12.07 (0.64%)     |

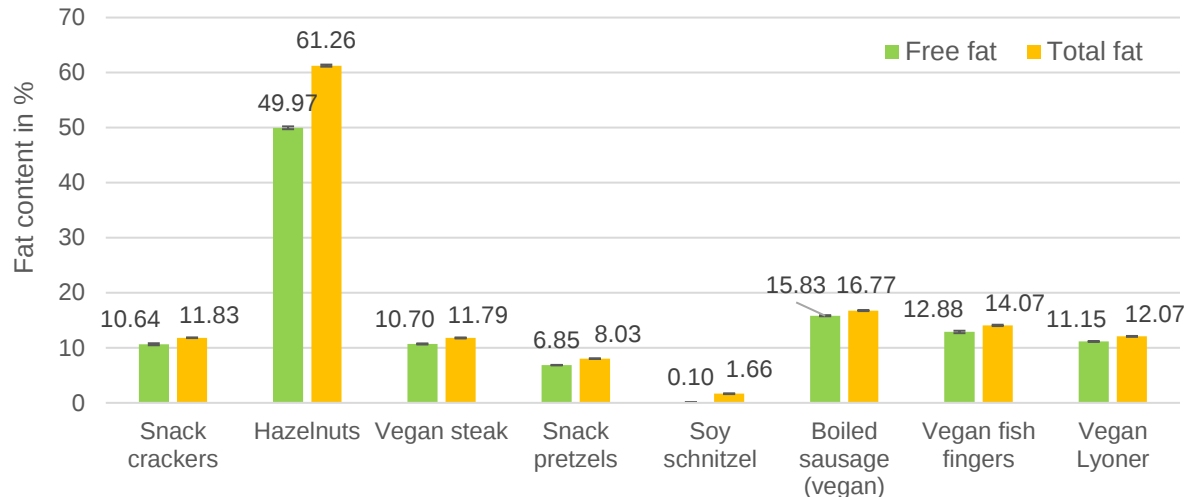


Figure 3: Free fat and total fat determined in various commercial samples. All the total fat values were higher than the free fat values.

#### 4. Key Parameters to Consider – Hydrolysis - Is it Worth the Trouble?

Main advantages of direct extraction/ free fat determination:

- Faster process
- No investment in hydrolysis equipment
- Less chemicals
- No changes in fat quality, fat can be used for further analysis of fat parameters or for contaminants

Main advantages of hydrolysis/ total fat determination:

- Highest fat recovery thanks to efficient breakage of sample matrix
- Conformity with official regulations for the declaration of total fat content
- Increased reproducibility due to the standardized and exhaustive procedure
- Ability to process liquid, moist and dry samples without the need to dry or mix samples with drying / dispersing agent prior to extraction
- Same extraction method for all sample types. The acid hydrolysis leaves the pure fat easily accessible for extraction, independent of the original sample matrix.

The time saving advantages of the free fat determination are undeniable. However, nutrition facts usually cover the total fat content, making the hydrolysis an essential tool across the food industry. The hydrolysis is also universal to all samples, enabling the fat determination of liquid samples with a liquid-solid extraction such as Soxhlet, Hot extraction or economic continuous extraction.

## 5. Additional Tips & Tricks to Increase Fat Recovery

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- 8 M HCl solution can be used for hydrolysis to improve the performance. The boiling duration is hereby set to 15 min instead of 30 min.
- For free fat determination, the extraction of a voluminous or a sticky sample can be improved by adding more sand/celite/diatomaceous earth to increase the surface area of the sample.

High relative standard deviations indicate incomplete hydrolysis or extraction, in this case please check the detailed procedure, for example found in application note 380/2019 or 366/2019

## 6. Application Examples

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The detailed procedure for free or total fat determination are described in various [application notes](#), available on BUCHI website.

Application note 380/2019 - [Total fat determination in meat products](#).

Application note 366/2019 – [Crude and total fat determination in a feed sample by Soxhlet extraction](#).

Application note 387/2020 – [Determination of total fat in plant-based meat alternatives](#).

Application note 402/2020- [Determination of fat in avocado](#)

## 7. References

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Booklet "[Five essentials on fat extraction](#)"