



# Application Note

## No. 762/2021

### Fat determination of calcium soap

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FatExtractor E-500:

Total fat determination of calcium soap from palm fatty acid distillate (PFAD) using E-500 with simultaneous hydrolysis



## 1. Introduction

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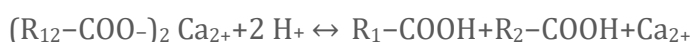
A simple, reliable and unique procedure for the determination of fat in calcium soap is introduced. Calcium soap has become a common supplement to feed since 1980 for high milkyielding dairy ruminants, such as cows, goats and sheeps, to increase the milk yield [1-3]. Ruminants acquire nutrients from plant-based food by their special digestive tract, including the rumen. The rumen is the first chamber digesting carbohydrates (cellulose, sugars etc.) and proteins but requires a pH ideally above 6. Fatty acids interrupt with those conditions, lowering the total energy intake [3]. Therefore, the calcium salt is formed, which is not digested in the first chamber but later when in contact with gastric acids [4].

In the presented application, the total fat content of two samples was determined. The hydrolysis is crucial for the total fat recovery. Therefore, a unique method that combines hydrolysis and extraction in one working step is introduced. The extraction with simultaneous hydrolysis was performed using the FatExtractor E-500 Soxhlet. The total fat content was determined gravimetrically. The fat recovery was within the expected value with low relative standard deviations.

## 2. Theory

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The samples consist of calcium salts of fatty acids, which are still highly hydrophobic yet insoluble in most hydrocarbons (e.g. n-hexane). The hydrolysis step includes the formation of fatty acids by protonation and removal of the calcium-ions according to the following chemical equation:



The formed fatty acids are highly soluble in hydrocarbons and can therefore be extracted and separated from the remaining sample matrix.

### 2.1 Optimizing to accomplish a complete reaction

Different parameters can affect the reaction rate. These include: contact time, temperature, solubility, chemical equilibrium.

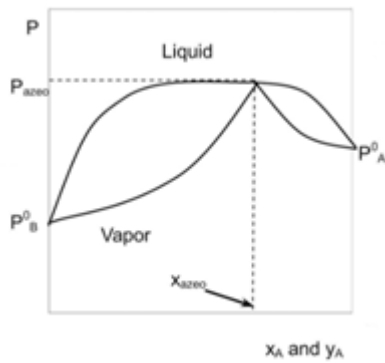
**Contact time and solubility:** Acid-base reactions are considered to be instantaneous upon contact of the two reactants. Nevertheless, performing a hydrolysis with aqueous acids is difficult due to the hydrophobic nature of the samples and the consequent poor contact.

**Chemical equilibrium:** The abovementioned chemical equation is reversible, therefore an equilibrium is established. Highly acidic compounds will preferably react with the calcium salts, but other parameters such as concentration, solubility and volatility may affect the equilibrium.

**Temperature:** It is widely assumed in the chemical industry that the reaction rate is doubled by increasing the temperature by 10 °C [5]. However, with the instantaneous nature of acid-base reactions, temperature will not affect the performance of the application.

### 2.2 Direct extraction using n-hexane:formic acid mixture

A formic acid (HCOOH) – hexane mixture was found to form a minimum azeotrope [6], allowing to directly hydrolyse and extract the sample.



At a certain composition  $x_{\text{azeo}}$ , the mixture reaches the maximum vapour pressure  $P_{\text{azeo}}$ . If the vapour pressure equals to the pressure exerted by the environment, the liquid starts to boil. Therefore, maximum vapour pressure results in the lowest boiling point. The maximum vapour pressure of n-hexane-formic acid was found to be 72:28 w% respectively [6]

Boiling point formic acid: 101 °C

Boiling point n-hexane: 69 °C

Boiling point minimum azeotrope: 61 °C

Figure 1: The figure shows the nature of minimum azeotrope.

### 2.3 How it works in Soxhlet

The beaker, containing the n-hexane:formic acid mixture, will be heated. The azeotrope will evaporate first. If one of the compounds is in excess, it will remain solely in the beaker after all azeotrope was built and evaporated. At the condenser, the azeotrope condenses and drops onto the sample.

Note: The azeotrope vapours are at a certain concentration, which do not mix well in the liquid state. A liquid-liquid system was observed during the extraction at the collector heading to the tank.

When the solvent gets in contact with the sample, the contained formic acid will react with the calcium salts, forming calcium formate and fatty acids. Unlike the calcium salts, the fatty acids are soluble in n-hexane and will therefore dissolve and be extracted down into the beakers.

## 3. Equipment

- FatExtractor E-500 Soxhlet, with I-300 Pro Interface and Analyte protection sensor
- Analytical balance (accuracy  $\pm 0.1$  mg)
- Drying oven / Vacuum drying oven
- Mixer B-400

## 4. Chemicals and Materials

### Chemicals:

- Quartz sand, particle size 0.3-0.9 mm (BUCHI, Order No. 037689)
- Formic acid 99%, pro analysi, ACS Reag. Ph Eur (Merck, Order No. 1.00264.0100)
- Hexane, puriss p.a. ACS Ph. Eur., (VWR, Order No. 32293)

For a safe handling please pay attention to all corresponding MSDS!

### Samples:

- Sample 1, calcium soap from palm fatty acid distillates (PFAD), expected fat content: 84%, sample consists of pellets. Therefore, it was mixed using the Mixer B-400. Furthermore, the mixed sample was sieved and only particles  $<0.2$  mm were used for all procedures due to the increased surface area of the sample
- Sample 2, calcium soap from palm fatty acid distillates (PFAD), expected fat content: 84%, sample consists of coarse powder. Therefore, it was grinded using a mortar and pestle to a fine powder.

## 5. Procedure

### 5.1 Preparation of the glass fibre thimbles

- 0.5 g homogeneous sample are placed in the glass fibre thimble
- 10 g quartz sand are added to the glass fibre thimble
- A spatula is used to stir the mixture well
- A piece of tissue is used to carefully wipe off the spatula
- The sample is covered with glass wool

### 5.2 Preparation of the solvent

n-Hexane and formic acid are not miscible and form 2 phases. The lower phase consists of mostly formic acid while the upper phase consists of mostly n-hexane. An initial mixture containing 900 mL n-hexane and 100 mL formic acid was prepared. After stirring thoroughly, 100-120 mL of the upper phase were transferred into the beaker (for example by using a separating funnel). 5 mL of the lower phase were transferred using a 5 mL volumetric pipette into the beaker.

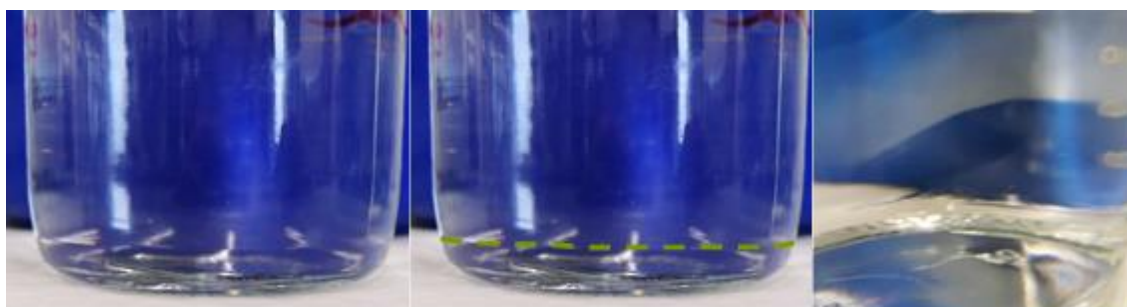


Figure 2: n-hexane and formic acid only mix to a certain extent. Therefore, the initial mixture using 900 mL n-hexane and 100 mL formic acid formed 2 phases. The lower phase consists of formic acid, whereas the upper phase consists of n-hexane.

After the extraction, the recovered solvent from the drying process can be reused in the next extraction. For this, add the recovered solvent to the initial n-hexane:HCOOH mixture. As long as there are 2 phase visible the concentration will be consistent, resulting in reproducible results.

### 5.3 Soxhlet extraction

The sample is contained in a glass fibre thimble placed in a glass sample tube with frit. A cellulose thimble is not suitable, as it would simply be partially hydrolyzed. The glass fibre thimble can also accumulate the solvent during extraction and, due to its weight, fall from the holder. To avoid this, the glass fibre thimble was placed in a glass sample tube with frit.

1. Place the glass sample tubes containing the sample into the extraction chamber and adjust the level sensor to the sample's height (see Figure 2).
2. Place the beakers on their corresponding heating plate.
3. Close the safety shield and lower the rack. Alternatively, fill in the solvent by the condensers after lowering the rack.
4. Activate the occupied positions, open the cooling water or switch on the connected chiller and start the extraction according to the parameters listed in Table 1.

Table 1: Parameters for the Soxhlet extraction with the FatExtractor E-500

Parameter	Value	Heating level
Solvent	n-Hexane:HCOOH mixture	
Extraction	360 min	8 <sup>1</sup>
SOX valve open time	Short	
Rinse	5 min	8 <sup>5</sup>
SmartDrying	On	-
Solvent volume [mL]	120	

<sup>1</sup> Heating level proposed by the system depending on the selected solvent.

## 5.4 Drying of the extract

Dry the beakers containing the extract in a drying oven at 102 °C until constant weight. Let the beakers cool down to ambient temperature for at least 1 h in a desiccator and record the weight. Make sure that the cooling down time of the beakers in the desiccator is the same before and after extraction. Differences in beakers temperature falsify the results.

## 5.5 Calculation

$$\% \text{ Fat} = \frac{m_{\text{Total}} - m_{\text{Beaker}}}{m_{\text{Sample}}} \cdot 100 \% \quad \rightarrow \quad (1) \quad \text{¶}$$

The results are calculated as percentage of the fat according to equation (1).

% Fat: Percentage of fat in the sample

$m_{\text{Total}}$ : Beaker + extract [g]

$m_{\text{Beaker}}$ : Empty beaker weight [g]

$m_{\text{Sample}}$ : Sample weight [g]

## 6. Results

All the calcium soap samples are analysed in triplicates. The determined fat content corresponds well with the expected values with low relative standard deviations (rsd). The results are shown in Table 2.

Table 2: Results for the total fat determination of all samples, determined with FatExtractor E-500 Soxhlet.

Sample	$m_{\text{sample}}$ [g]	$m_{\text{beaker}}$ [g]	$m_{\text{total}}$ [g]	Fat [%]	Mean value	Labelled
Sample 1 (powder from pellets)	0.5115	108.5695	108.9885	81.92	<b>82.67%</b> rsd : 0.80%	84%
	0.5136	111.644	112.0699	82.92		
	0.4961	111.0042	111.4168	83.17		
Sample 2 (fine powder from coarse powder)	0.4953	111.113	111.5362	85.44	<b>85.20%</b> rsd : 0.70%	84%
	0.5218	110.5061	110.9471	84.52		
	0.5387	108.595	109.0563	85.63		

## 7. Conclusion

The determination of total fat content in different calcium soap samples using the FatExtractor E-500 Soxhlet provides reliable and repeatable results. These results correspond well to the expected values with low relative standard deviations (rsd). The new method using formic acid and n-hexane was successfully applied. It is a time- and cost-saving method since the solvent can be reused in the next extraction.

## 8. References

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- [1] [http://www.lipico.com/processes\\_calcium-soap-production.html](http://www.lipico.com/processes_calcium-soap-production.html), 16.07.2021
- [2] Handojo L. et al., Calcium soap from palm fatty acid distillate for ruminant feed: Ca(OH)<sub>2</sub> as calcium source, MATEC Web of Conferences, 159, 2018.
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- [4] Sukhija P. S., Palmquist D. L., Dissociation of Calcium Soaps of Long-Chain Fatty Acids in Rumen Fluid, J Dairy Sci, 73, p. 1784-1787, 1990.
- [5] Connors, K., Chemical Kinetics: The Study of Reaction Rates in Solution, 3rd edition, p. 14
- [6] [Azeotrope tables - Wikipedia](#), 19.07.2021

Extraction Reports App  
Operation Manual of the FatExtractor E-500