Five essentials of fat extraction

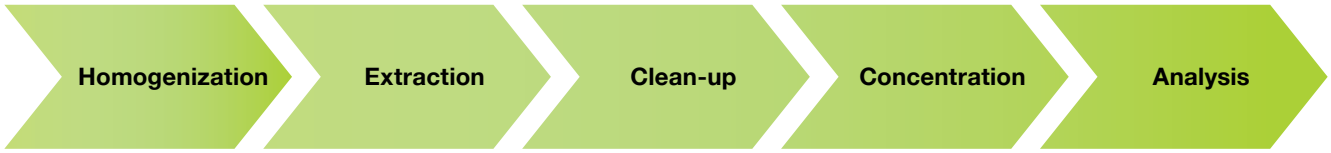
And how they make your process quick and compliant

Introduction

Extraction is one of the oldest separation techniques. Records indicate that civilizations going as far as back as the Babylonians and ancient Egyptians used extraction processes to prepare perfumes and salves. From the 19th century onwards, increasingly innovative and automated instruments, solvents and materials helped develop an ever-growing list of extraction applications. In fact, Soxhlet extraction, one of the most widely used extraction methods was developed in the 19th century, precisely in in 1879, by Franz von Soxhlet[1].

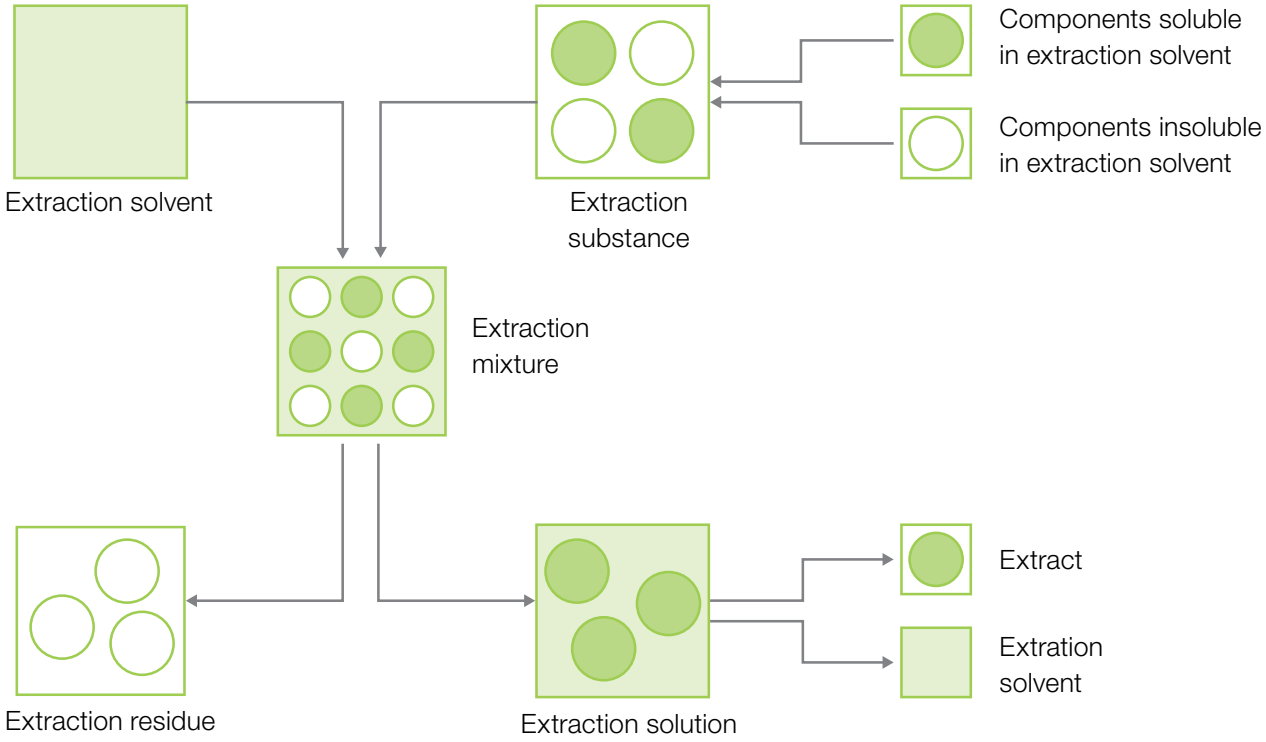
Image courtesy of Flickr[2]

The modern extraction process is part of a larger analysis workflow, which involves a combination of different steps depending on the sample to be assessed. One of the most common procedures for analysis of solid samples consists of the following steps:



The fat extraction principle

The fat extraction principle is illustrated with the figure below using fat extraction from sausage as an example[3].



The extraction substance "sausage" is a mixture of meat components and fat enclosed in these components. The homogenized substance is mixed with a solvent (extraction solvent). This extraction mixture is then separated and insoluble components remain as residue. If this procedure is repeated several times, the proportion of fat in the solvent increases. Pure fat is then obtained by distilling the solvent portion out of the mixture.

The method described above is used when soluble components are to be separated from insoluble solids. By using an extraction apparatus, the amount of solvent required and the time of extraction can be reduced to a fraction.

Factors that influence the extraction process

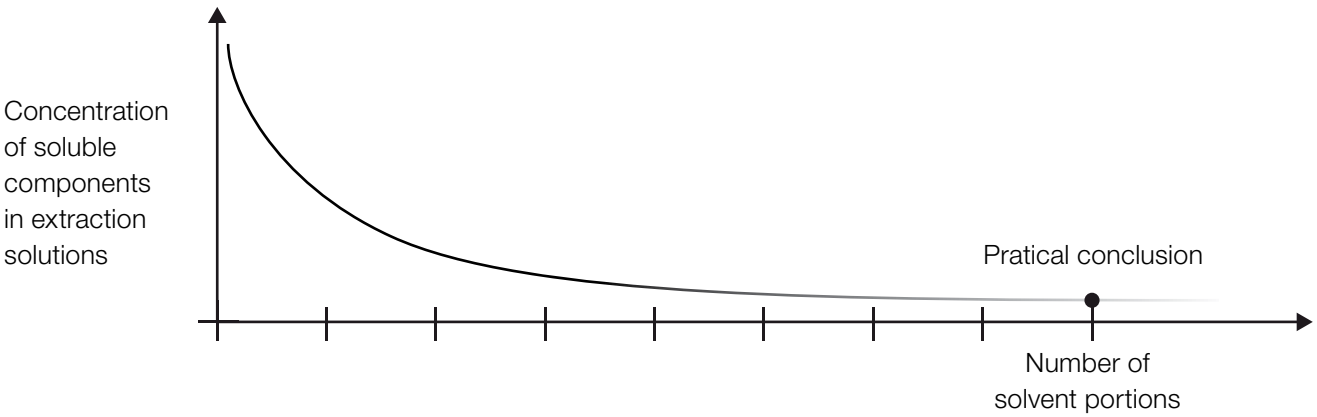
Importantly, the extraction solvent must always be inert with regards to the extraction substance. Many parameters influence the recoveries and the speed of the extraction. The most important points to consider when optimizing the extraction process are listed in the table below.

Factors that influence the recovery rate of the extraction	Factors that influence speed of extraction
Solubility of the components to be extracted in the selected extraction solvent (must be of similar polarity)	The particle size of the extraction substance
The thoroughness of the mixing of extraction substance with the extraction solvent	The degree to which extraction substance and extraction solvent are mixed
Size and number of extraction solvent portions (number of siphonings in the case of Soxhlet or drop rate of solvent in other methods)	Temperature (rule of thumb is that speed of reaction doubles as temperature for every temperature increase of 10 °C)
Nature of sample (enclosed fat, moisture, size of sample, surface, homogeneity of sample)	

One of the best strategies to ensure you are using optimal extraction parameters is to apply standard methods. These processes contain validated and recognized extraction methods and parameters, which help to increase the reliability of your extraction workflow.

How to determine the endpoint of the extraction process

During the extraction process, the concentration of soluble components in the extraction substance decreases steadily until it reaches a point where continuing the extraction is of no further value. This point is called the practical conclusion[3].

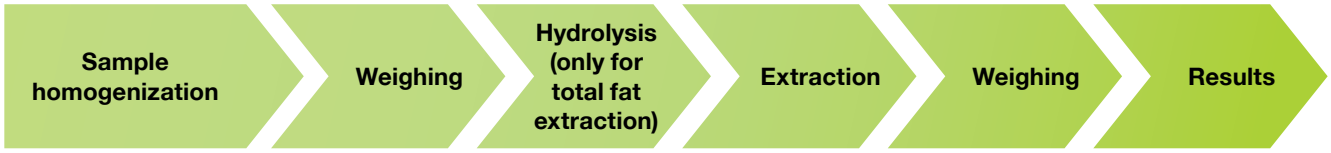


The amount of extraction solvent (number of siphonings) required for the extraction to reach the point of practical conclusion mostly depends on the solubility of the substance being extracted. Although in many cases, determining the practical conclusion is a matter of experience, there are approaches that can help assess if the point has been reached. One of the most common ways to determine the end of the extraction is to verify the extraction method by using reference material with known fat content.

1. Why hydrolysis is needed for reliable and compliant fat determination

There are two main types of fat extraction, total and crude fat extraction. Crude fat extraction is a direct extraction that is limited to determine only free fat. Total fat extraction is used to detect free fat and fat enclosed by other components of the sample matrix. Total fat extraction is the required method for compliancy with many industry standard methods.

Total fat extraction differs from crude fat extraction mainly in the addition of a hydrolysis step at the beginning of the process.



In general, a large amount of fat is encapsulated in the sample matrix. For example, in baked goods made with gelatinized starch, fat is often mechanically enclosed by other components, such as carbohydrates to form glycolipids and protein substances to form lipoproteins. In dairy products like milk, cream and cheese, surface tension forces cause colloidal components made out of protein to surround the fat droplets. In yeast and eggs, certain fat components are bound to other components chemically or by adsorption, forming phosphatide-protein complexes.

To determine the concentration of lipids within the lipoproteins and glycolipids, the bonds that hold lipid and non-lipid components together must be broken prior to solvent extraction. Hydrolysis is then used to release these bound lipids into extractable forms. Additionally, during hydrolysis, proteins are hydrolyzed and plant cell walls are broken down. Physically enclosed fats are also released and made accessible to the solvent during extraction.

Main advantages of hydrolysis:
- Efficient breakage of matrix structures enclosing the fat fraction of food and feed samples
- Assures conformity with official regulations for the declaration of total fat content
- Increased reproducibility thanks to the standardized and exhaustive procedure
- Ability to process liquid, moist and dry samples without the need to dry or mix samples with sand/sodium sulfate prior to extraction

Hydrolysis is a necessary method requirement to comply with industry norms and regulations. Reference methods, such as the ISO 8262-1 Weibull-Berntrop gravimetric method, require acid hydrolysis of fatty acids bound to glycerides, sterol esters, glycol and phospholipids. Hydrolysis disrupts cell walls and breaks up lipid-protein bonds, as well as fat emulsions to improve precision, productivity, reproducibility and applicability.

Did you know?

Both manual and semi-automated hydrolysis techniques are possible. Semi-automated hydrolysis is often preferred because it is faster and safer due to reduced user exposure to hydrochloric acid.

2. How finding the right extraction method improves speed, cost and reproducibility

Three of the most widely used extraction methods for fat determination include Soxhlet extraction, hot extraction and continuous extraction.

A Soxhlet extraction method is used to determine the content of soluble compounds from dry solid samples. During the process, the solvent in the flask is heated, the vapor passes the extraction chamber through a side tube and into the condenser, condenses and the solvent drops into the sample in the extraction chamber until a siphon point is reached. The extract flows back into the flask. With each extraction cycle, the sample is extracted with freshly distilled solvent at low temperature.

Classical hot extraction is a process according to the Randall method. The sample is placed in boiling solvent. The solvent is evaporated, flows to a condenser, condenses and the solvent drops back into the sample. In comparison to Soxhlet extraction, there is no sample-extract separation.

In the classical Twisselmann extraction, the solvent is placed in a heated flask and the sample is placed in an extraction thimble in the extraction chamber. In comparison to Soxhlet extraction the vapor does not pass through a side tube to the condenser, but passes directly into the extraction thimble to the condenser. The vapor condenses and the solvent drops into the sample.

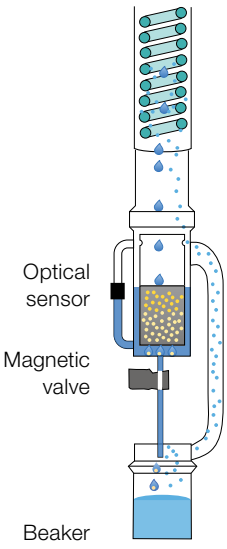
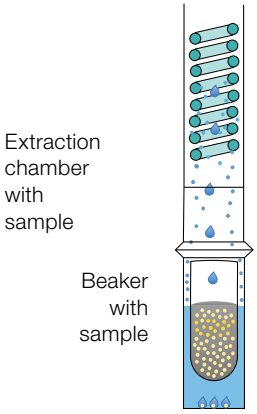
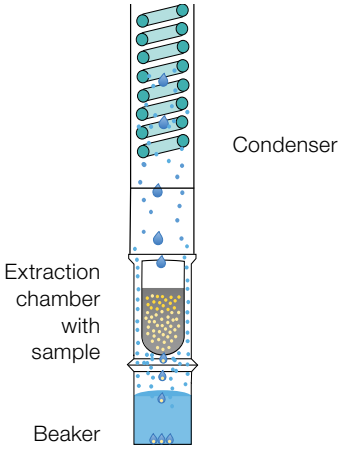
The extraction methods are similar but offer distinct advantages. For example, Soxhlet is the most robust and recognized method, but hot extraction offers reduced costs due to lower solvent consumption and faster extraction processes. Continuous extraction offers high efficiency and accelerated analyte-solvent exchange due to higher sample temperature than Soxhlet. Continuous and Soxhlet extraction are easier to set up as methods and tend to produce more reproducible results than hot extraction.

A general overview of the three methods is displayed in the table below[5].

A visual representation of how the methods work can also be found in a brief video.

Did you know?

BUCHI is the only extraction solutions provider to offer all three methods in one instrument thanks to an innovative interchangeable glass assembly design. This feature offers unprecedented flexibility in switching methods to fit your demands for any particular sample without needing several instruments that would otherwise overcrowd precious lab space.

	Soxhlet extraction E-500 SOX	**Hot extraction** E-500 HE	**Continuous extraction** E-500 ECE
Method and apparatus			
Method characteristics	· Separation of sample and extract · Freshly distilled solvent for each extraction cycle · Very gentle process at low temperature	· No sample-extract separation · Elevated temperature of sample leads to increased exchange · High extraction efficiency	· Separation of sample and extract · High extraction efficiency · Two-fold interaction between sample and solvent (vapors up/ condensate down)
Extraction time (incl. extraction, rinse and dry)	1.5 h	40 min	1 h
Solvent consumption (per position)	100 mL	50 mL	70 mL
Sample temperature			
Compliance / Reference methods	ISO / AOAC / EPA	ISO / AOAC / EPA	ISO / LFGB
Method programming	Simple method selection	Method development required	Simple method selection
Reproducibility (RSD)	Excellent	Good	Very good

Fat determination by extraction

3. How the right instrument set-up can improve the efficiency of your extraction

There are numerous instruments available for performing extraction for fat determination purposes. The type of instrument and extraction method you choose largely depends on your sample type, solvent use and the need of compliance that your process requires among other factors.

Certainly, having the right instrument at hand can make all the difference in achieving cost-efficient and effective results that satisfy your individual needs.

Use the decision tree below to easily find the instrument that best fits your sample and application.

Fat Extraction → Fat (gravimetric) either **total fat** or **crude fat**

Do you choose a solvent from the list?
- Petroleum ether
- Diethyl ether
- n-Hexane
- Chloroform

No → Talk to expert: application@buchi.com or visit the decision tree: www.buchi.com/extraction-decision-tree

Yes → Does your food sample require a **hydrolysis** prior to extraction?

Yes → Manual

Yes → semiautomated HydrolEx H-506

No → Sample type

Non-processed food
- Raw Material
- Natural Products

→ Low RSD («1%)
Fat < 5%
Fat < extractable matter

Processed Food sample → Fat > 5% mostly free fat
Fat ≈ extractable matter

Extraction SOX: Soxhlet
Extraction HE: Hot Extraction
Extraction ECE: Economic Continous Extraction (Twisselmann)

See standard and regulations



Application	Soxhlet Extraction	Hot Extraction	Extraction ECE
Chocolate	AOAC 963.15 AOAC 920.75 ISO 23275-1:2006		LFGB §64
Dairy	ISO 8262-1		LFGB §64
Bakery, cereal, nut	AOAC 945.16 AOAC 948.22	ISO 11085:2008 AOAC 2003.05	LFGB §64
Meat	ISO 1443:1973	AOAC 991.36 ISO 1444:1996	LFGB §64
Oilseed	ISO 734-1	ISO 734-2	ISO 659 ISO 734-1 ISO 734-2

Sample type

Processed food

No

Non-processed food
- Raw Material
- Natural Products

Bulky residue after **hydrolysis** and/or **fat < 5%**?

Yes

See standard and regulations

Yes

Compliant to any method?

No

Extraction ECE
- Costs are key
- Solvent consumption (70 mL)
- Convenience is important

Yes

Extraction HE
- Speed is important
- Very low solvent consumption (50 mL)
- Correspondance to third party automated extraction systems

No

Yes

Extraction SOX
- High reproducibility (RSD «1%))
- High analytical safety
- Very gentle process at low temperature

Yes

4. How competent troubleshooting generates reliable results

Quickly resolving any issues arising during the extraction process can help minimize downtimes and increase the reproducibility of the final data. Below are a few common pitfalls in fat extraction and suggestions on how to resolve any issues that threaten the integrity of the extraction process.

Issue to be resolved	Possible cause
Fat content is too high	Drying of extract was not sufficient
	Oxidation of fat due to excessively high temperature during drying step
	Celite® was washed out during extraction
	Impurities in solvent
Fat content is too low	Loss of sample during hydrolysis step
	Incomplete extraction
	Presence of water
	Differences in beaker temperature
Variation is too large	Sample weight is too small
	Inhomogeneous sample
	Low fat content
	Incorrect parameter set-up
Accumulation of solvent on top of the sample	Insufficient drying of sample after hydrolysis
	Solvent evaporation is too fast.
	Incorrect amount of sand on top of Celite® layer
	Compacted Celite® layers
	Frit is blocked

Did you know?

If you find yourself facing a tough challenge and you are stuck for answers, you are welcome to contact the BUCHI extraction specialists. With over 30 years of experience in fat extraction, we'd be happy to assist you in moving your project forward and achieving the best possible recoveries from your sample. Please get in touch via e-mail.

Corrective measure
Dry to a constant weight
Reduce the duration of the drying step, use the SmartDrying function
Dry the extract at lower temperatures and reduced pressure in a vacuum oven
Loose the pulp carefully before drying the hydrolyzed sample
Use new solvent or freshly distilled solvent
Wash the hydrolysis vessels with several aliquots of water so that the sample is transferred quantitatively into the glass sample tube
Adjust the temperature of the water (40-60°C). Water that is too hot can result in loss of fat, water that is too cold cannot dissolve remaining sample sufficiently for a complete sample transfer
Set the level sensor to the top end of the sample to assure complete immersion in solvent
Choose appropriate extraction times and cycles
Avoid accumulation of solvent on top of the sample
Water must be thoroughly removed, as it repels lipophilic solvents and hinders the extraction process. Careful dry the sample before extraction.
Make sure to use the same settings for drying (time and temperature) and cooling (time) of the beaker when weighing prior to and following the extraction
If sample is very inhomogeneous, increase sample weight
Homogenize your sample by using a powerful blender, such as Mixer B-400 or by using mortar and pestle
The sample weight can be increased up to 10 g
Setting the correct number of cycles is essential for Soxhlet extraction, whereas setting extraction time matters most for hot and continuous extraction
Ensure the glass sample tube with the sample is dried sufficiently in microwave or drying oven
Ensure that the right solvent from the library is selected and adjust heating level setting depending on altitude and ambient temperature.
Use enough sand on top of the Celite® layer. Amounts that are too low can result in solvent accumulation.
Mix the Celite® layers carefully and thoroughly using a spatula prior to drying
Rinse frit thoroughly to remove any remaining sand and Celite® prior to cleaning in dishwasher. If the frit cannot be unblocked, the glass sample tube must be replaced

5. How to find the right extraction parameters to make fat determination easier and faster

Fat determination is one of the most widely used analysis in the dairy industry. Parameters established in literature or for a certain instrument are a good starting point for initial method development. Here, simple and reliable procedure for fat determination in milk powder and yogurt using continuous extraction, hot extraction and Soxhlet are presented.

Method

Equipment: HydrolEx H-506, FatExtractor E-500 Soxhlet

Sample: milk powder LUV No. 17-4b with a certified fat content of 24.27 g/100 g (+/-0.542 g/100 g),
Yoghurt muva-jo-1422 with a certified fat content of 3.76 g/100 g (+/-0.13 g/100 g).

Process: the sample was mixed with quartz sand in a glass sample tube and Celite®545 was added on top. The samples were weighted and hydrolyzed with hydrochloric acid on the H-506 for 30 min. the hydrolysate was transferred and the vessels washed with warm water until a neutral pH was reached. The glass sample tubes were dried and cooled down, then another layer of quartz sand was added to the sample tube. The extraction was performed using the E-500 with the parameters specified in Table 1.

Table 1. Parameters for extraction with fat extractor E-500 ECE, HE and SOX

Method Parameters	Continuous Extraction	Hot Extraction	Soxhlet
Solvent	Petroleum ether / Hexane / Diethyl ether / Chloroform	Petroleum ether / Hexane / Diethyl ether / Chloroform	Petroleum ether / Hexane / Diethyl ether / Chloroform
Extraction step	60 min (heating level 5-8)*	5 min (heating level 4-8)*	20 cycles (heating level 5-9)*
Rinse step		30 min (heating level 5-8)*	5 min (heating level 5-9)*
Drain		3	
Drying step	6–12 min (heating level 4-7)*	3 min (heating level 3-5)*	10-13 min (heating level 5-7)*
Solvent volume	70 mL	50 mL	100 mL

* Heating level proposed by the system depending on the selected solvent
The samples were extracted in triplicate. The extracts were dried to a constant weight the total fat content was determined gravimetrically.

Results

The results are summarized in Table 2. The data corresponded well to certified values of the reference materials. The results show low relative standard deviations (rsd).

Table 2. Determined fat content in dairy products, fat in g/100 g (relative standard deviation in brackets), n=3

	Continuous Extraction		Hot Extraction		Soxhlet	
Solvent	Milk powder	Yogurt	Milk powder	Yogurt	Milk powder	Yogurt
Petroleum ether	24.72 (1.06)	3.68 (0.94)	24.26 (0.33)	3.66 (0.33)	24.35 (0.26)	3.80 (1.07)
Hexane	24.43 (0.03)	3.70 (0.72)	24.27 (0.24)	3.64 (1.42)	24.45 (0.28)	3.70 (0.75)
Diethyl ether	24.36 (0.44)	3.74 (0.56)	24.39 (0.35)	3.77 (0.55)	24.50 (0.22)	3.75 (0.26)
Chloroform	24.52 (0.25)	3.75 (1.31)	24.68 (0.28)	3.73 (0.36)	24.70 (0.41)	3.81 (1.29)

Conclusion

The determination of fat in different dairy products using the HydrolEx H-506 and the FatExtractor E-500 provides reliable and reproducible results. These results correspond well to the labelled values, with low relative standard deviations.

Did you know?

Application notes for fat extraction of all types of samples ranging from dairy products to chocolate could be found on the BUCHI Application Finder. Follow the QR code to see what parameters could be ideal for your own fat extraction process.

Outlook – what other methods exist for fat analysis?

Besides solvent extraction, which remains the standard in fat determination, other methods are available for fat analysis. The table below compares fat extraction to another common method for fat determination, NIR analysis.

Area of application	Fat «Extraction»	ProxiMate™ «NIR»
R&D	+++	+
Production	+	+++
Goods inspection	+	+++
Quality control / labeling	+++	++
Characterictics		
Range of applications	+	+++
Variation in sample types	+++	++
Automated throughput	++	+
Speed of analysis	+	+++
Compliance [1]	+++	+
Detection of adulterants	+	+++
Unattended operation	++	+
No contact with chemicals	+	+++
Ingress protection rating	+ (IP 20)	+++ (IP 65)
Low initial costs	+++	+
Low running costs	++	+++
Eco-friendly	++	+++
Technical Data		
Throughput in 9 h [3]	~ 36 samples	400+ samples
Analysis time	~ 90 min/6 samples	~ 15 s/sample
Max. sample amount	10 g	395 cm
Limit of detection (LOD)	0.1%	0.1%

[1] With respect to application regulations such as AOAC, ISO, DIN etc.

[2] Initial costs of the Kjeldahl products are very much depending on the level of automation

[3] Depending on sample composition, packaging material. No shift work assumed.

\+ applicable

++ more applicable

+++ most applicable

Did you know?

BUCHI's FatExtractor E-500 offers reduced time-to-result and unprecedent sample throughput per day. The Soxhlet process as performed by the FatExtractor E-500 is the reference method for NIR calibrations.

References

1. Soxhlet, F. (1879). “Die gewichtsanalytische Bestimmung des Milchfettes”. Dingler’s Polytechnisches Journal 232: 461–465.

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5. BUCHI Labortechnik. AG Classical Extraction Methods: available upon request.

6. BUCHI Labortechnik. AG (2017). Guidebook to Proximate Analysis: https://www.buchi.com/en/guidebook-to-proximate-analysis-by-buchi

Further reading

1. Application note 348/2019 Fat determination in dairy products, BUCHI Labortechnik AG. Available for download: https://www.buchi.com/sites/default/files/AN_348_2019_Fat_determination_in_dairy_products.pdf

2. Application note 349/2019 Fat determination in dairy products, BUCHI Labortechnik AG. Available for download: https://www.buchi.com/sites/default/files/AN_349_2019_Fat_determination_in_dairy_products.pdf

3. Application note 350/2019 Fat determination in dairy products, BUCHI Labortechnik AG. Available for download: https://www.buchi.com/sites/default/files/AN_350_2019_Fat_determination_in_dairy_products.pdf

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