



ETHOS™ X INFOPACK

TOTAL FAT DETERMINATION

Thank you for your interest in Milestone's ETHOS X Microwave Fat determination in food and feed. This Infopack will help you learn how the ETHOS X can enhance your lab's efficiency in analyzing total fat in food and feed samples.

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ETHOS™ X

Microwave Fat Determination
in Food and Feed



APPLICABLE TO ANY MATRIX



45 SAMPLES A DAY



LOWER COST PER SAMPLE



COMPLETE FAT DETERMINATION



SAFE AND COMPACT

FAT DETERMINATION: ONE APPROACH FOR ANY SAMPLE

Fat determination in food and feed matrices is a routine analysis for several testing and food processor labs, as it is part of the labelling process and of the QC testing. Nowadays, the process still relies on outdated and matrix-dependent methods which use obsolete and time-consuming technologies. The ETHOS X offers a new approach to gravimetric fat determination, moving toward a more modern, greener and more efficient approach to total fat, Fatty Acid Methyl Ester (FAME) and free fat determinations in food and feed matrices.



| FAT DETERMINATION BY MICROWAVE

The ETHOS X applies the well-known selectivity of microwave heating and the advantages of closed vessel technology to fat determination. Its configuration and methods have been specifically built to address lab requirements in terms of throughput, ease of use and universal method approach.

STREAMLINE YOUR FAT DETERMINATION

Conventional total fat determination methods, such as Soxhlet and automated Soxhlet, go through a multi-step process, that includes long hydrolysis and extraction procedures, while secondary methods are matrix-dependent and require frequent calibrations. The ETHOS X allows to process any food and feed matrix with a single approach, directly impacting the lab workflow. In addition to the commonly performed total fat determination, the ETHOS X makes FAME and free fat feasible within a single platform.

I TOTAL FAT DETERMINATION

The process begins with the weighing of the sample and the reagents into the SR-15 closed vessels rotor (Picture 1). The process benefits from the microwave heating, the closed-vessel technology and powerful stirring, so that hydrolysis and extraction take place simultaneously and efficiently. The easyTEMP, contactless temperature sensor, controls the entire process ensuring great reproducibility and high safety. Once this step is completed, an aliquot of solvent with the fat is transferred and weighed into the RAR-15 disposable cup (Picture 2) for the evaporation step. The weights are automatically transferred to the easyCONTROL user interface software, which guides the operator throughout the procedure, providing total fat calculation, statistics, and full traceability of the complete process (Picture 3). The determination of total fat in 15 samples is completed in less than three hours.



1 - Weighing step on the hydrolysis/ extraction vessel



2 - Evaporation step



3 - Data processing

MICROWAVE EVAPORATION

The RAR-15 enables the simultaneous evaporation of 15 samples using microwave energy and a dedicated vacuum system. The solvent that contains the extracted fat is placed into a unique evaporation rotor with disposable aluminum caps. The process runs under vacuum, available with a recondensation module too, thus ensure the highest level of safety and not expose the operator to solvent vapors.



ETHOS X with RAR-15 evaporation rotor and vacuum pump

ONE METHOD FITS ALL

The ETHOS X is not matrix-dependent, so it uses a single method and setup for virtually any food such as milk and dairy products, meat, bakery product and chocolate as well as any feed sample. This approach leads also to a lighter accreditation procedure, as it is carried out on several samples using a single technique. Therefore, the ETHOS X method expedites the lab workflow and simplifies the total fat determination.

Standard and QC material	Reference values (%)	ETHOS X (%)	RSD (%)
Wheat Flour (ERM-BC382)	1.39 ± 0.17	1.41	0.20
Lyophilized Pork muscle (ERM-BB384)	8.99 ± 0.2	8.63	0.21
Condensed Milk (TET036RM)	0.33 ± 0.07	0.29	0.06
Dairy feed (BCR-708)	6.5 ± 0.8	6.32	0.28
Butter (quality control material)	81.37 (80.83 – 81.91)	81.38	1.28
Chocolate (quality control material)	34.85 (33.52 – 36.17)	35.74	0.67

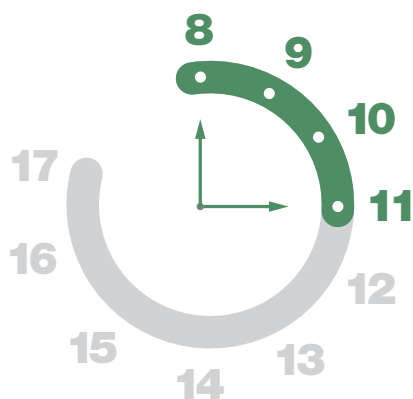
Total fat determination on several CRMs and Quality control materials

IMPROVED TURNAROUND TIME & PRODUCTIVITY

In QC and food testing laboratories a fast delivery of results and high throughput are key factors for total fat determination. Even modern automated Soxhlet techniques require several hours in order to deliver results affecting the competitiveness of the lab too. The ETHOS X processes 15 samples simultaneously, and as many as 45 samples in a single working day, using one rotor and 75 samples with two rotors. Its specific method combines the hydrolysis and extraction into a single step, thus having a direct impact on the lab's turnaround time and throughput.

ETHOS X

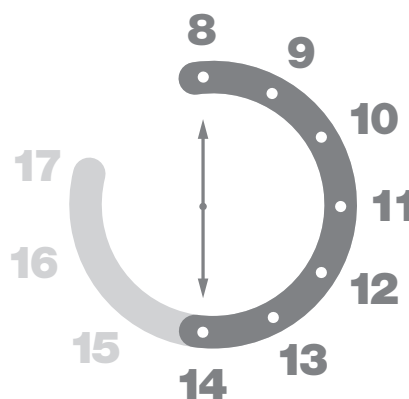
15 SAMPLES



IN 3 HOURS

Automated Soxhlet

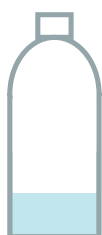
6 SAMPLES



IN 6 HOURS

LOWER COST PER SAMPLE

Automated Soxhlet systems are based on the outdated Soxhlet technology which uses open vessels and large flasks. This approach requires elevated acid and solvent volumes for the hydrolysis and extraction steps. The ETHOS X uses closed vessels technology to increase the temperature and improve extraction efficiency, reducing the reagents volume too. The unique setup offers a reagent and waste reduction of up to 60%. In combination with the higher productivity and compelling initial investment, it provides a lower cost per sample.



ETHOS X

35 mL of reagents per sample



Automated Soxhlet

125 mL of reagents per sample

EFFICIENCY & PRODUCTIVITY IN TOTAL FAT DETERMINATION

COMPLETE FAT DETERMINATION

In addition to total fat determination, Fatty Acid Methyl Ester (FAME) analysis is a common analysis as it determines saturated and unsaturated fats. The flexibility of the ETHOS X configuration and its dedicated procedures enables to perform FAME and free fat determinations of food samples, performing the complete fatty acids profile within a single platform.

	Saturated fat	Monounsaturated	Polyunsaturated
LIVER PATÈ (IFIP 1704)			
Reference value	14.40	15.40	6.40
ETHOS X (%)	16.29	14.53	6.18
LEGUME SOUP			
Reference value	0.27	0.37	0.98
ETHOS X (%)	0.30	0.39	0.94
SUNFLOWER OIL			
Reference value	10.82	30.34	58.85
ETHOS X (%)	10.88	30.57	58.55

Complete fat profiles on three samples using ETHOS X methods

HIGHER SAFETY & LOWER FOOTPRINT

The traditional approach requires two main systems, one for hydrolysis and one for extraction, offering limited productivity and occupying valuable space in the lab or in the fume hood. The ETHOS X setup allows to carry out all the steps within a single platform while improving productivity, it is not installed in the fume hood and saves over 50% of the lab space. Its dedicated exhaust system combined with up-to-date features, ensures the highest operator safety level throughout the entire process.

WIZARD PROCESS

The easyCONTROL software, installed on the user interface, fully controls all the parameters of the process, and collects all the data. When connected to a balance, it records and associates every weighing step to the sample, providing great traceability and guiding the operator throughout the entire process. The easyCONTROL software acquires the information needed to support the analysis report, such as automatic calculations and sample statistics, and additionally offers full integration with LIMS.

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Established in 1988, Milestone is headquartered in Italy and has offices in Germany, Switzerland, the United States, China, Japan and Korea. We operate worldwide through a network

of over 100 exclusive distributors, all providing our customers premium application and service support. Milestone's mission is to help chemists by offering them the most advanced instrumentation for sample preparation and direct mercury analysis in the world. Our industry-leading technology, in combination with fast, responsive service and applications support, allows Milestone to support our goal of providing you the highest return on investment possible.

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Microwave Digestion
System



ultraWAVE

The Game Changer in
Microwave Digestion



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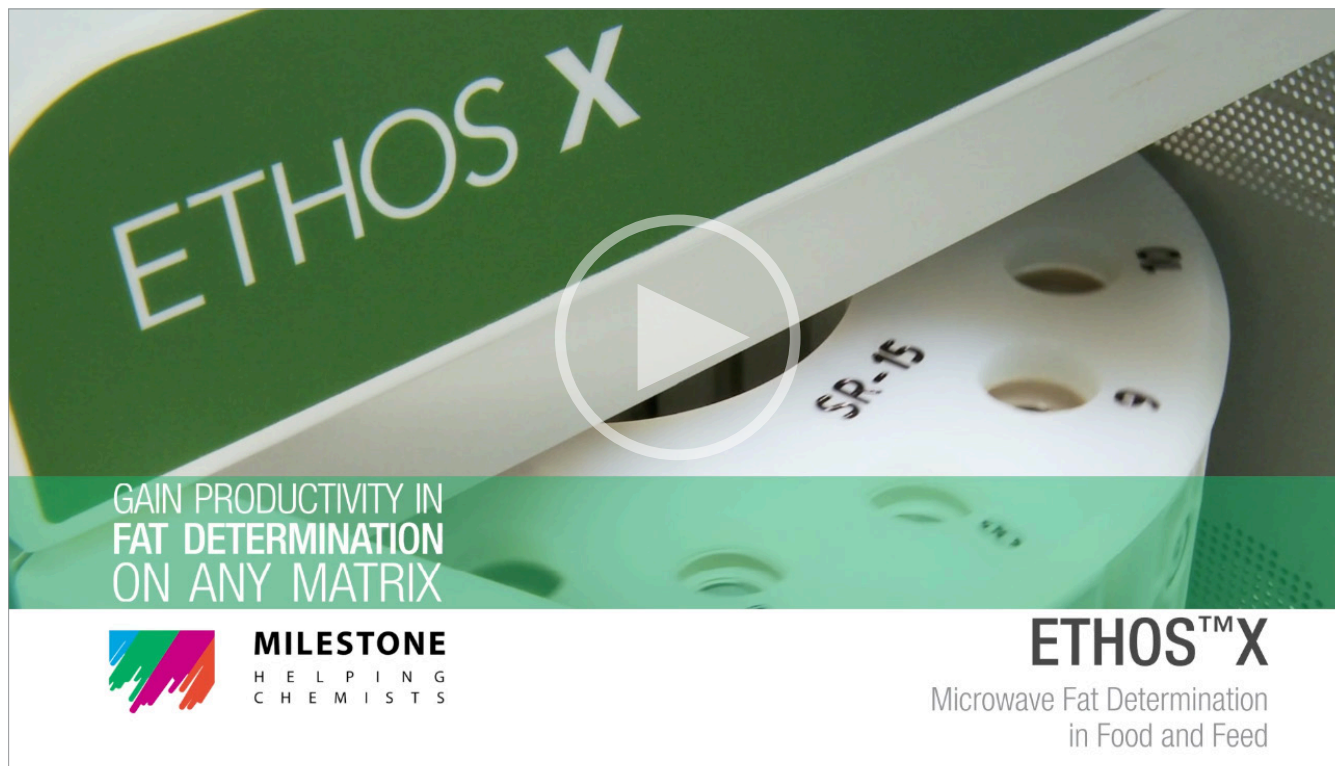
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ETHOS X VIDEO



GAIN PRODUCTIVITY IN
FAT DETERMINATION
ON ANY MATRIX

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ETHOS™ X
Microwave Fat Determination
in Food and Feed

The ETHOS X method for total fat determination is applicable to any matrix; enables to process 45 sample a day, ensure lower cost per sample, offers complete fat determination and it is safe and compact.

→ **see the ETHOS X Video**



IMPROVING TURNAROUND TIME AND PRODUCTIVITY IN TOTAL FAT DETERMINATION

CUSTOMER



Eurofins Scientific is the recognized leader in food and pharmaceutical product testing. They also specialize in environmental laboratory services and are one of the global market leaders in agrosience, genomics and central laboratory services. Eurofins has over 15,000 staff in more than 190 laboratories across 36 countries that use state-of-the-art analytical techniques to support clients' quality and safety standards.

CHALLENGE

At Eurofins Scientific in Nantes, France, the laboratory needed to reduce analysis time on over 200 samples received per day in order to produce results faster for their clients. The current sequential technology was a time consuming technique and not able to provide reproducible results on samples with very low fat content.

SOLUTION

The lab purchased seven Milestone's ETHOS X microwave extraction systems as an alternative to sequential and Soxhlet techniques. The ETHOS X performs total fat determination on food samples in a fraction of the time with minimum solvent usage.

BACKGROUND

Eurofins offers high quality assurance and control services such as testing, certification, inspection, audits, technical services and regulations for manufacturers, brand owners, distributors and retailers throughout the world.

Since throughput and fast delivery of results is a key point for the Eurofins group, they have already in use a Milestone ultraWAVE digestion system for sample preparation for trace metal analysis.

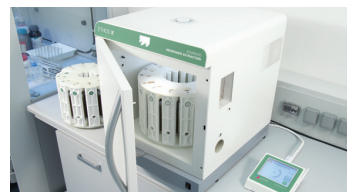
CHALLENGE

Eurofins needed to determine the **total fat content in over 200 samples per day** on several food matrices such as meat, sausage, cheese, cookies etc.

Prior to the implementation of the ETHOS X method, they were using two different techniques:

1. Firstly, a sequential approach technique based on hydrolysis followed by solvent extraction that employs an "inverted gravimetric method" where fat content is determined by weight loss of the sample instead of by weight of the extract as normally calculated. The process involved the use of HCl and took approximately 3 days to complete. The main challenge was to reduce the time to obtain results as it was too long for a laboratory with a very high throughput and productivity per day. The process took approximately 3 days to complete. Moreover, the process required high volumes of reagents

LAB PROFILE EXTRACTION | FAT DETERMINATION



to remove fat from the sample matrix, resulting in very high running costs and corrosion of the unit.

2. Secondly, traditional Soxhlet approach was used for samples with low fat content. This approach was time consuming and required a large volume of solvent thus exposing laboratory technicians to possible health risk.

THE ETHOS X IMPLEMENTATION

Milestone product specialists presented the ETHOS X for total fat determination to Eurofins lab in Nantes (France) and Mr. Regis Guerin, Production Unit Manager Nutritional Labelling, saw the potential of this new approach, so they invested in one system. After a couple of months of testing and method optimization on several CRM materials, they purchased four more instruments.

Today they have installed **five (5) ETHOS X units with ten (10) rotors** to perform total fat determination on a wide suite of food samples. Eurofins recognized in the ETHOS X a perfect solution for their needs since the new method boost their total fat determination to 300 samples in a single working day.

An additional benefit of the ETHOS X process is the ability to connect a balance to the instrument itself, so that its operating software automatically calculates the fat content and provides all the statistics associated to the analysis of the samples. This new method has allowed Eurofins to replace all the current methods of total fat determination and to **standardize the use of a single method** in on most of their samples.

Moreover, Eurofins had to use the outdated Soxhlet method, when larger sample mass was required, as the newer sequential systems were not suitable for that.

“The first reason we chose ETHOS X was that we need to have faster results. Sequential technology required us 3 days for delivery of the results. Moreover, with that technology, we are not able to provide reproducible results on samples with very low fat content since the sample amount was limited.”

– Mr. Guerin, Production Unit Manager, Eurofins Nantes Lab



The ETHOS X matches these requirements too, enabling total fat determination even on large sample amounts. For over two years, **Eurofins has validated and implemented the ETHOS X method for a wide array of samples**, replacing the other time-consuming procedures.

This approach result into a better turnaround time for their customers and a lower cost per sample, through a lower initial investment and higher throughput.

“The advantages of ETHOS X compared to sequential technology are the lower initial investment, the universal method for all food samples and with no unit corrosion.”

– Mr. Guerin, Production Unit Manager, Eurofins Nantes Lab

FUTURE PLANS

They have chosen ETHOS X over conventional extraction techniques for its advantages in terms of effectiveness, reproducibility and reduced solvent consumption. They are planning to further expand the ETHOS X capabilities for other fat determinations that are still done with soxhlet or other techniques.

ABOUT MILESTONE

With over 50 patents and more than 20,000 instruments installed in laboratories around the world, Milestone has been widely recognized as the global leader in metals prep technology for the past 30 years. Committed to providing safe, reliable and flexible platforms to enhance your lab's productivity, customers worldwide look to Milestone for their metals digestion, organic extractions, mercury analysis and clean chemistry processing needs.

APPLICATION REPORT

Total Fat Determination in Food and Feed samples



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The determination of total fat content in food and feed samples is a common task for food industry quality control labs and for third party contract labs. The classical procedures use different methods based on the matrix, antique technology and approaches. Analytical laboratories have to manage several methods in order to cover the demand while increasing the overall analysis costs. The Milestone ETHOS X used for total fat determination enables a simultaneous hydrolysis and extraction and can be applied on all food matrices, as it is not matrix dependent. With the ETHOS X, the fat analysis costs and turnaround time are strongly reduced. The ETHOS X enables to perform the total fat determination in just a few hours.

INTRODUCTION

The Nutrition Facts Label is nowadays fundamental for packaged foods and drinks. The U.S. Food and Drug Administration (FDA) has recently updated the information required on nutrition facts labels in order to better inform consumers on quality and nutritional parameters of products.¹

Fat content is by far one of the most important parameters. The determination of total fat, saturated fat, monounsaturated fat and *trans* fat content in food samples is necessary to comply with the food labeling requirements. In particular, total fat content plays a pivotal role for several reasons. The evaluation of total fat content allows to properly dose other expensive ingredients, to comply with

nutritional labeling regulations, to produce healthy and quality food with a low fat content, and to select the right process conditions according to the lipid content. All these considerations elevate total fat value to an important parameter for Economic, Legal, Health, Quality and Process evaluations. Fat determination is of interest also for the feed industry since its value allows to set the quality and price of feed products.

Several determination protocols are available for the analysis of total fat content in food and feed samples; most of them are selective for specific food classes and cannot be applied to others. This leads to the application of several determination protocols depending on the food stuff that has to be analyzed.

APPLICATION REPORT

ETHOS X - Total Fat Determination in Food and Feed samples



Moreover, most of the conventional protocols involve an elevated use of organic solvents and long processing times since they are often based on hydrolysis with inorganic acids, followed by the Soxhlet extraction with organic solvents. This leads to considerable costs due to a high solvent consumption and a long turnaround time. Microwave energy sources have been widely applied both in elemental and molecular sample preparation and have been tested in this application report for the total fat determination. In particular, an innovative total fat determination protocol was developed enabling the simultaneous hydrolysis and extraction process within a sole step. This approach aims to provide a unique method for the total fat determination of all food and feed samples by dramatically reducing both the overall analysis time and the solvent consumption. This method allows analytical laboratories to deliver total fat analysis in a few hours reducing costs and solvent wastes.

EXPERIMENTAL

EQUIPMENT

- Milestone ETHOS X
- SR-15 eT extraction rotor
- RAR-15 evaporation rotor
- Aluminum caps
- Vacuum system with condensation module
- Analytical balance (with direct interface to ETHOS X terminal)



Figure 1 – Milestone’s ETHOS X with SR-15 extraction rotor (left) and RAR-15 evaporation rotor (right).

SOLVENT SAND REAGENTS

Solvents and reagents were purchased by Sigma Aldrich. Sulphuric acid (25%) and Cyclohexane ACS reagent grade were used.

SAMPLES

For this study, certified reference materials (BCR and ERM), quality control samples and labeled food stuff (purchased at the grocery store) were used (Tables 2-4). Regarding commercial food stuff, the samples should be homogenized before the weighing step in order to get a representative aliquot of sample. The sample has been used as it is, avoiding any drying step.

PROCEDURE

Approximately from 1 to 3 g of sample was directly weighed into the SR-15eT extraction vessels; 10 mL of sulphuric acid (25%) and 25 mL of cyclohexane were subsequently added, recording its final mass. Magnetic stirring bars were added to each vessel. The SR-15eT was properly assembled. The microwave program is reported in Table 1.

STEP	TIME	T	POWER	Stirrer
1	00:03:00	90°C	1400w	80%
2	00:04:00	135°C	1400W	80%
3	00:40:00	135°C	1400W	80%
Cooling				

Table 1 – ETHOS X microwave hydrolysis and extraction program

At the end of the program, the SR-15 eT vessels were opened and the aliquots of the organic phase were transferred into aluminum caps and then weighed. After a fast solvent evaporation, using the RAR-15 evaporation rotor, the aluminum caps were newly weighed. The ETHOS X easyCONTROL software tracks and records all the steps and weights necessary for the calculation. Total fat values are delivered at the end of the run thanks to the capabilities of the easyCONTROL software and the direct interface with the analytical balance. The data can be saved with the possibility to generate customized reports.

APPLICATION REPORT

ETHOS X - Total Fat Determination in Food and Feed samples



RESULTS AND DISCUSSION

In this study, the total fat content of several food samples was analyzed by applying the ETHOS X simultaneous hydrolysis and extraction method.

Despite the availability of several sequential total fat determination methods, the ETHOS X method enhances total fat determination by simultaneously performing hydrolysis and extraction during the same heating run with up to 15 matrices.

Sample	ID	Reference values		ETHOS X results	
		Total Fat (%)	Uncertainty (%)	Total Fat (%)	RSD (%)
Whole milk powder	BCR-380R	26.95	± 0.16	26.3	0.23
Wheat Flour	ERM-BC382	1.39	± 0.17	1.41	0.2
Lyophilized Pork muscle	ERM-BB384	8.99	± 0.2	8.63	0.21
Condensed Milk	TET036RM	0.33	± 0.07	0.29	0.06
Dairy feed	BCR-708	6.5	± 0.8	6.32	0.28

Table 2 – ETHOS X total fat method – Data on CRM materials (n=12).

Sample	ID	Reference values		ETHOS X results	
		Total Fat (%)	Acceptability [Range for z ≤2] (%)	Total Fat (%)	RSD (%)
Porridge Oats	T2477QC	7.82	7.36 – 8.28	8.03	0.187
Butter	T25160QC	81.37	80.83 – 81.91	81.38	1.28
Fish Paste	T25163QC	4.43	3.77 – 5.10	4.4	0.07
Chocolate	T25166QC	34.85	33.52 – 36.17	35.74	0.67
Fat Spread	T14190QC	66.47	64.8 - 68.1	68.0	0.48

Table 3 – ETHOS X total fat method – Data on quality control samples (n=12).

Sample	Reference values		ETHOS X results	
	Total Fat (%)		Total Fat (%)	RSD (%)
Taralli	19.1		18.95	0.19
Cookies	19		18.42	0.1
Wurstel	26		25.2	0.28
Skimmed Milk	1.6		1.35	0.01
Pudding	1.9		1.55	0.073
Semolina	1.9		2.17	0.05
Cooked ham	12		12.14	0.37
Raw Sausage	24		23.94	1.4
Condensed Milk	8		7.82	0.06
Cream	21.5		20.94	0.1

Table 4 – ETHOS X total fat method – Data on samples purchased at grocery store (n=12).

Moreover, thanks to the RAR-15 evaporation rotor, the ETHOS X microwave extraction platform also extends its capacity to the evaporation step. For this purpose, a mix of certified reference materials (CRM), quality control samples and foodstuff locally purchased at grocery stores were used. Several food samples were tested, ranging from cookies, dairy, meat, sausages and even feed samples, among others. Tables 2 to 4 report all the samples tested with the total fat results and the relative standard deviations.

The samples were selected to explore a wide range of total fat content, from condensed milk (0.33%) to butter (81.37%) samples. For all the ranges tested, the measured total fat content was always in the acceptance range of the certified materials, with very high reproducibility proven by very low relative standard deviations even when working on 12 repetitions. Figure 2 summarizes the accuracy of the ETHOS X method in all the tested ranges in relation to the reference values of each sample.

APPLICATION REPORT

ETHOS X - Total Fat Determination in Food and Feed samples

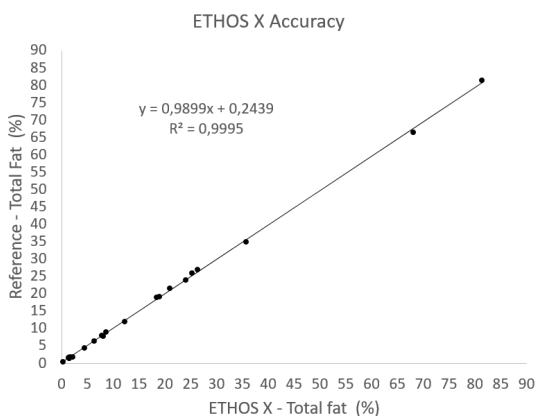


Figure 2 – Milestone’s ETHOS X accuracy evaluation (data source: tables 2-4)

High reproducibility and data traceability are ensured thanks to the easyTEMP sensor, which controls the temperature and, therefore, the reaction conditions in all the positions of the SR-15 rotor.

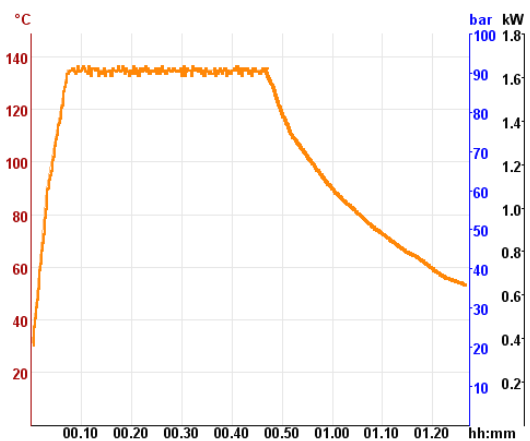


Figure 3 – Milestone's ETHOS X run profile

WORKING RANGE

The working range of the ETHOS X during the total fat determination method depends on the sample mass and on the balance capability, being thus calculated from the minimum mass able to be weighed by the analytical balance. Furthermore, the higher the sample mass, the lower the value of the detectable fat content. A typical working range, using an analytical balance, varies from 0.1 to 100% of total fat content, depending on the sample mass.

CONCLUSION

The ETHOS X method for total fat determination proved to be precise and accurate on a wide working range from 0.1 to 100 % of total fat content. Thanks to the performance of the ETHOS X, and to the innovative simultaneous hydrolysis and extraction capability during the same run, this method allows an unmatched turnaround time, a low solvent consumption and a streamlined workflow. Moreover, the 15 positions of both the SR and RAR rotors ensure the highest throughput available in the market. The total fat residue may be further exploited, after gravimetric evaluation, for the fatty acid methyl ester (FAME) analysis. See dedicated application reports.

The ETHOS X with its unique features fully addresses the needs of food laboratories in terms of productivity, ease of use, running costs, and turnaround time.

REFERENCES

- 1- <https://www.fda.gov/food/food-labeling-nutrition>
- 2- <https://www.milestonesrl.com/products/micro-wave-extraction/ethos-x-for-fat-determination>

ABOUT MILESTONE

At Milestone we help chemists by providing the most innovative technology for metals analysis, direct mercury analysis and the application of microwave technology to extraction, ashing and synthesis. Since 1988, Milestone has helped chemists in their work to enhance food, pharmaceutical and consumer product safety, and to improve our world by controlling pollutants in the environment.