



# ETHOS X APPLICATION REPORTS

Microwave Total Fat Determination in Milk and Milk Products



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# Introduction

Lipids are among the major components of food of plant and animal origin. The term lipids usually includes a broad category of compounds that have some common properties and compositional similarities. Lipids are materials that are insoluble in water, but soluble in selected organic solvents such as benzene, chloroform, diethyl ether, hexane, cyclohexane and methanol. Lipids in nature are associated with other molecules via (1) weak intermolecular forces, for example, interaction of several lipid molecules with proteins; (2) electrostatic and hydrogen bonding, mainly between lipids and proteins; and (3) covalent bonding among lipids, carbohydrates and proteins (Di Casimir C. Akoh, 2008). To determine the concentrations of these components it is therefore necessary to break the bonds which hold the lipid and non-lipid components together prior to solvent extraction. Acid or alkali hydrolysis are commonly used and required to release covalently and ionically bound lipids to proteins and carbohydrates as well as to break emulsified fats and turn them into easily extractable forms. Once treated with alkali or acids, lipids can be then separated from proteins, carbohydrates and water in the tissues exploiting their wide range of relative hydrophobicity using specific types of solvents.

The reference extraction processes for the gravimetric determination of fatty acids in milk and milk products are Weibull-Stoldt as universal method, Roese-Gottlieb for milk, milk powders, cream and buttermilk, Weibull-Berntrop and Schmid-Bondzynski-Ratzlaff for cheese. These classic extraction techniques differentiate for either an alkaline or acid hydrolysis prior extraction and for the type of solvents used during the extraction step. One aspect that all these reference methods has in common is that they are labor-intensive, require long extraction times as well as high consumption of solvents. Alternative gravimetric processes, which are able to reduce the working steps of these methods, are therefore highly requested.

## PRINCIPLE OF OPERATION (SEE THE APPLICATION REPORT FOR DETAILED PROCEDURE)

Approximately 3g of sample are weighted and placed in each extraction vessel of a 12-position medium pressure rotor. 10 mL of diluted acid (25%  $H_2SO_4$ ) and 25 mL of solvent (Cyclohexan) are then added in the vessels. After having placed the 12-position medium pressure rotor inside the microwave cavity, acid hydrolysis coupled with extraction take place in parallel at 120°C for 40 minutes. After cooling down the solvent/water (acid) phases for 15 minutes, 10 mL of the upper solvent fraction, containing the extracted lipids, are pipetted out and weighted in previously tared aluminum cups to proceed with the evaporation conducted in the new Evaporation system Combi-VAC supplied by Milestone in the package. After a fast and efficient evaporation of the solvent, the aluminum cups are weighted again and thanks to an easy-to-use software, the lipid content is automatically determined. The present method can be then eventually adjusted in term of type and quantity of acid and solvent used depending on the type of dairy product to be assessed.

## WHY TO CHOOSE THE MICROWAVE ETHOS X SYSTEM FOR TOTAL LIPID EVALUATION?

The new Milestone Ethos X microwave system in combination with the 12 position evaporation rotor RAR-12 enables to revise the nearly 120 years old Weibull-Stoldt reference method in an innovative, easy to operate way, assuring at the same time results of high analysis accuracy and quality, fully comparable with the reference one. The big advantages of the newly Microwave Extraction System ETHOS X are the following:

### › **Low solvent consumption and working time saving**

The acid hydrolysis is coupled with the microwave extraction working in parallel. This allows to reduce drastically the working time needed (just 40 minutes) as well as costs related to a traditional two-step process where huge amounts of solvent are consumed.

### › High throughput

The new Milestone Ethos X microwave system allows to work up to 12 samples with a capacity of 100mL vial per run.

### › High extraction performances with high accuracy results

The new Milestone Ethos X microwave system with the new SR 12-position medium pressure rotor permits to work above the boiling point of all selected organic solvents used for fatty acids extraction up to 30bar. This ensures the 100% extraction of the total fatty acid fraction, with comparable results obtained through reference methods (Weibull-Stoldt, Schmid-Bondzynski-Ratzlaff and Roese-Gottlieb).

### › Easy to operate

The new Milestone Ethos X microwave system is equipped with an easy to use software that automatically calculates gravimetrically the final fatty acids weigh starting from the original sample weigh. Any unintended deviations from the method and inaccuracies in the process are ruled out.

### › Single and unique method for milk and milk products

The new Milestone Ethos X microwave system has been specifically developed for milk and milk products. It is not an adjusted technology for extraction of fatty acids.

## VALIDATION OF THE MICROWAVE EXTRACTION METHOD OF FATTY ACIDS FROM MILK PRODUCTS

To validate the microwave extraction method of total fatty acid content in milk products with the new ETHOS X system, 6 different milk products have been selected, whose total fatty acids amount had been previously determined following the related reference methods. The comparability and the reproducibility of the results have been determined by a standard deviation on a set of 10 data values. The hydrolysis and extractions in parallel of all samples have been conducted using a combination of Sulfuric Acid/Cyclohexane and Hydrochloric Acid/Petroleum Ether. The difference in the lipid contents between the reference methods and the newly ETHOS X microwave extraction method are very close to the official standard acceptable values (Comparability). Even the repeatability shows results within the official repeatability limits or nevertheless significantly close. The lower limit content of fatty acids measurable by the instrument amounts to 0.5g whereas the higher was established at 15g. The evidence of this range of work has been proved at least for the lower measurable limit through an evaluation using a low-fat milk 0.03% fat content.

Product	Reference Method*	Reference value [%]	MW Sulfuric acid/ Cyclohexane [%]	MW Hydrochloric Acid/Petroleum Ether [%]	MW Repeatability	MW Comparability [%]
Soft Cheese	WS	27.04	27.85	27.91	0.25	0.71
Lemon Yoghurt	WS	3.20	3.48	3.29	0.07	0.19
Chester	SBR	33.97	34.90	34.51	0.11	0.99
Camembert	SBR	22.17	22.70	21.79	0.10	0.36
Milk	RG	1.50	1.57	1.54	0.11	0.14
Powdered Yoghurt	RG	28.93	29.42	28.88	0.15	0.37

\* WS= Weibull-Stoldt, SBR= Schmid-Bondzynski-Ratzlaff, RG= Roese-Gottlieb, trace (<0.1%).

## CONCLUSIONS

The new Milestone ETHOS X for Total Fat Determination in Milk and Milk Products coupled with the 12-position evaporation rotor RAR-12 permits to achieve the same results accuracy of the conventional fat determination methods (Weibull-Stoldt, Schmid-Bondzynski-Ratzlaff, Roesse-Gottlieb). At the same time, it permits to reduce drastically the extraction time, the working time as well as the solvent consumption of the conventional approaches. The new Milestone ETHOS X represents the best solution for a precise and reproducible total fat determination in milk and milk products at highest throughput and lowest initial investment.

## REFERENCES

- Bakri, R. (2014). Untersuchungen zur mikrowellenunterstützten gravimetrischen Fettbestimmung in Milch und Milchprodukten im Vergleich zur Referenzanalytik. Hochschule Hannover.
- Di Casimir C. Akoh, D. B. (2008). Food Lipids: Chemistry, Nutrition, and Biotechnology, Third Edition. CRC Press.

# Application Report

## EXPERIMENTAL DISCUSSION

The purposes of this application report is to evaluate the quality of the results obtained with Milestone system in terms of both accuracy and repeatability. Two type of samples have been tested:

- IRMM-BCR-380R : whole milk powder (CRM) certified reference material
- Semi-skimmed milk

## INSTRUMENTATION

- ETHOS X microwave system
- SR-12 extraction rotor
- RAR-12 evaporation rotor
- Combi-VAC vacuum system
- Analytical balance

## ANALYTICAL PROCEDURE

As mentioned above, the analytical procedure is gravimetric and is made up of two main steps:

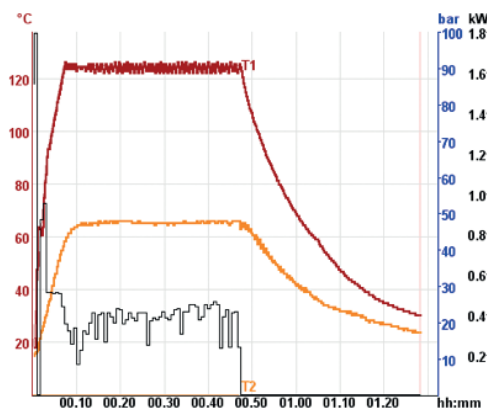
- Hydrolysis/Solvent extraction
- Solvent evaporation

The connection with an analytical balance and a fully dedicated software allow to directly record the weights and give immediate results of the total fat content. Here are the conditions used for the tests:

Test	Sample	Sample amount	Hydrolysis reagent
1	BCR-380R	10 mL of H <sub>2</sub> SO <sub>4</sub> 25%	25 mL of Cyclohexane
2	Semi-skimmed milk	4 mL of H <sub>2</sub> SO <sub>4</sub> 25%	25 mL of Cyclohexane

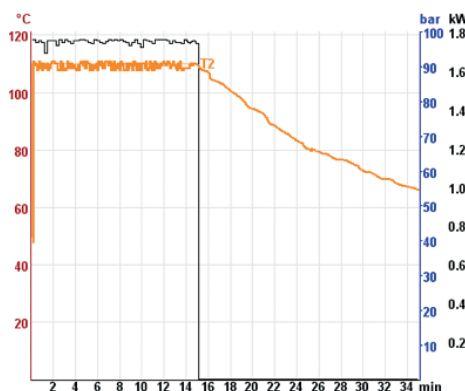
### Hydrolysis/Extraction method

Step	Time	Power	Temp
1	3 min	1800 W	90°C
2	4 min	1800 W	125°C
3	40 min	1800 W	125°C



### Evaporation method

Step	Time	Power	Temp
1	1 sec	800 W	110°C
2	15 min	800 W	110°C



## RESULTS

### Test 1

Sample: BCR 380R (Whole milk powder) Certified reference material

Certified total fat content (g/100g) : 26,95 ± 0,16

Test	Sample	Sample [g]	Fat content [%]	Mean [%]	Cert. value [%]	Recovery [%]	Std.dev. [%]
1	BCR380R (1)	1,0652	26,26	26,34	26,95	97,73	0,066
2	BCR380R (2)	1,0339	26,37				
3	BCR380R (3)	1,0633	26,39				

### Test 2

Sample: Semi-skimmed milk

Test	Sample	Sample [g]	Fat content [%]	Mean [%]	Std dev. [%]
1	Semi-skimmed milk (1)	15,1530	1,33	1,35	0,011
2	Semi-skimmed milk (2)	14,7136	1,34		
3	Semi-skimmed milk (3)	15,0047	1,35		
4	Semi-skimmed milk (4)	15,3652	1,36		
5	Semi-skimmed milk (5)	15,6557	1,35		

## CONCLUSIONS

The data reported in this technical note show very good accuracy and repeatability and demonstrate the high reliability of the method. The quality of the results combined with time saving, high throughput and the lowest solvent consumption make the new Milestone Ethos X as the best solution for Total Fat Determination.