Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 30, pp 78-83

BrJAC

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Total Fat Determination in any Matrix by Simultaneous, Robust and Comprehensive Hydrolysis and Extraction Microwave Approach

This report was extracted from the Milestone Application Report: Total Fat Determination in Food and Feed samples

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.

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).

Solvents and 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.

Table 1. ETHOS X microwave hydrolysis and extraction program

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

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.

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. 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.

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

		Reference values		ETHOS X results	
Sample	**ID**	**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 3. ETHOS X total fat method – Data on quality control samples (n=12)

		Reference values		ETHOS X results	
Sample	**ID**	**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 4. ETHOS X total fat method – Data on samples purchased at grocery store (n=12)

	Reference values	ETHOS X results	
Sample	**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

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.

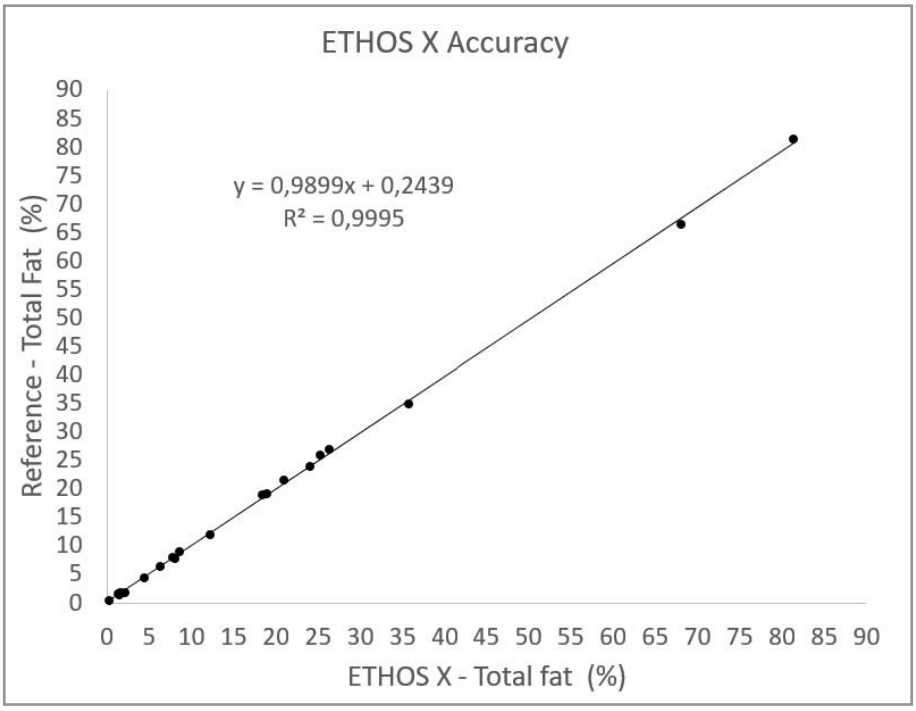

Figure 2. Milestone's ETHOS X accuracy evaluation (data source: Tables 2-4).

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.

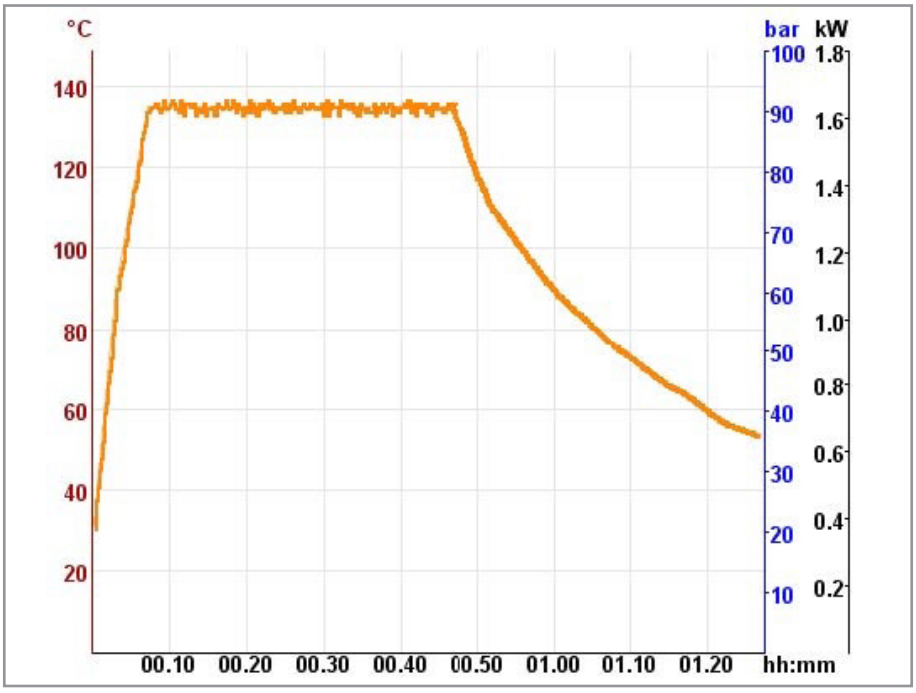

Figure 3. Milestone's ETHOS X run profile.

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 unmatchable 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/microwave-extraction/ethos-x-for-fat-determination

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