CEM CORPORATION

APPLICATION NOTES

RAPID FAMEs ANALYSIS

INTRODUCTION

The determination of the total amount of saturated & unsaturated fat is traditionally carried out by a procedure known as FAMEs (Fatty acid methyl ester analysis). Fat is traditionally extracted from samples using a cold solvent extraction into solvents such as DCM or other petroleum based solvents. Extracts are then dried and derivatized to form fatty acid methyl esters, using toxic reagents such as boron trifluoride. This process is both lengthy and hazardous.

Microwave assisted FAMEs replicates the AOAC method, but in a much safer, cleaner, and more efficient way. It creates exactly the same methyl esters that are ultimately quantified by Gas Chromatography. The microwave method can be carried out with virtually any sample type, including the following: meats, starchy carbohydrates, salads and vegetables, liquid milk, cheese, and all other dairy products. Unlike the traditional AOAC method, all food types can be analyzed by one simple microwave method, without making accommodations for different food types, such as cheese or encapsulated omega 3 fortified foods. A few select samples were prepared using this microwave method, with the results below. All samples were ready for analysis by GC in under one hour.

METHOD

1. Weigh out homogeneous food samples consisting of 1-2 drops of oil, or 1g of wet sample, or 0.5g of powder into a PFA MARSXpress vessel. For samples with a high fat content (greater than 50%), only 0.5g of the sample should be used so it does not saturate the gas chromatography column. The weights do not need to be recorded, unless the fatty acids are quantified against an internal standard.
2. Add 10ml of Reagent A to each vessel along with a 10mm magnetic stirrer and cap the vessel. Place the vessels into the turntable.
3. Heat the samples in a MARS 6 microwave, using a ramp to temperature method. Ramp to over 5 minutes to 90˚C and hold for 10 minutes. Stirring within the MARS 6 is essential. For 40 samples, typically 1500W is required. Remove the vessels from the Kevlar sleeves and add to the vessel stand. Submerse in cold water in the vessel stand for at least 5 minutes with the water above the sample height, to reduce the temperature to around 30˚C or lower.
4. Open the vessels and add 15 ml of Reagent B.
5. Microwave using a ramp to temperature method, ramp to over 5 minutes to 120˚C and hold for 6 minutes. Cool the vessels to room temperature or chilled (ice water can be used) C4-C6 analytes are extremely volatile at this point.
6. Add 10 ml of Reagent C. Place the MARSXpress vessel plug over the end of the vessel. While holding it there, invert the vessel and bring it vertical again (do not shake). Add Reagent D until the pentane layer reaches the bottom of the screw thread on the vessel. Take the top layer supernatant (a few milliliters) of the pentane layer into a glass vial containing a small amount of Reagent E. It is important to carry out all of these steps for each sample completely before moving onto the next sample and uncapping.

7. The samples will not be a clear solution (i.e. a typical acid digestion) and could be various different colors. The supernatant should be a clear solution, but if particles are floating in this solution, gently tap the air bubbles with the pipette and they will fall into the rest of the solution, leaving a clear supernatant.
8. Run the sample on a Gas Chromatography system, as per the normal procedure and quantify by peak area.
9. The fatty acids can be expressed as a percentage of the total fat measured on a CEM SMART Trac or quantified directly against an internal standard.

INSTRUMENT REQUIREMENTS

CEM MARS 6 microwave
512195 - Xpress temperature control
512185 - Reagent stirring option
567025 - Extraction option
162810 - 10ml magnetic stirrer bars
191525 - Magnetic vessel holder

PREPARATION OF REAGENTS

Reagent A - is a KOH solution in Methanol.

Reagent B - is a preparation of methylation solution.

Reagent C - is a pentane (HPLC grade) or similar solvent.

Reagent D - is made up of saturated aqueous sodium chloride.

Reagent E - is an anhydrous sodium sulfate mixture.

INTERNAL STANDARDS

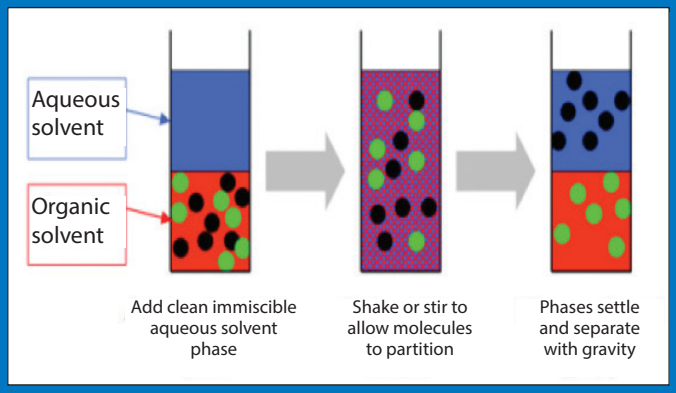
Internal standards can be used to confirm retention times and to compare their mass spectra in the GC. However, the quantity of the individual fatty acids is usually calculated by dividing up the total fat by the peak areas of the fatty acids, as determined by FAMEs. The amount of 1mg of C23:0 ME is added to each vessel, if internal standard spiking is required. A standard such as Supelco 37 FAME mix is suitable for the initial GC set up.

MICROWAVE PROCESS SUMMARY

Sample Preparation and Microwave Digestion
30 Minutes

One Extraction and Derivatization
15 Minutes

Separate Methyl Esters in Liquid/Liquid Extraction
15 Minutes



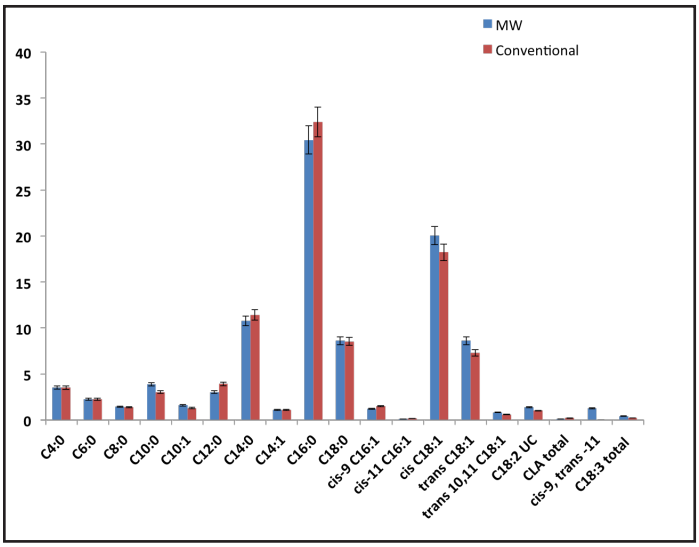
The Samples are Ready for the GC in Less than **1 Hour**

RESULTS ANALYSIS

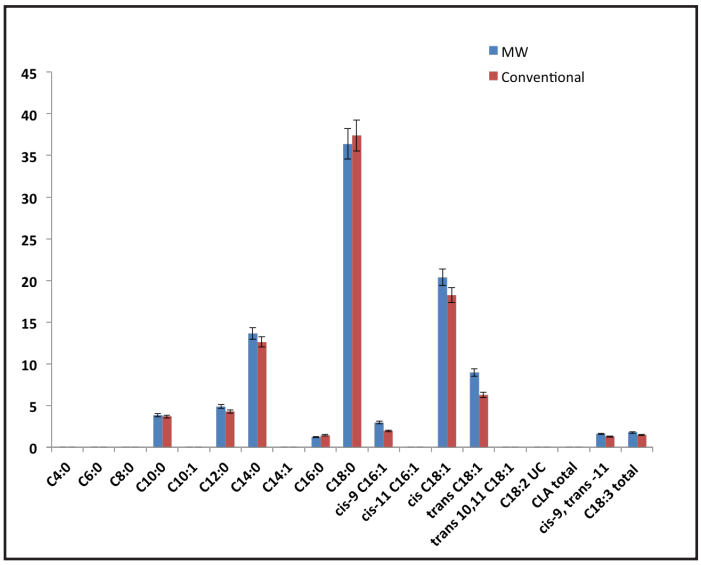
Lasagne

Fatty Acid	Conventional	MW assisted	Δ
C8:0	0.5	0.4	-0.1
C10:0	0.4	0.3	-0.1
C12:0	1.8	1.7	-0.1
C14:0	5.2	5.5	0.3
C16:0	26.5	27.4	0.9
C18:0	15.4	15.2	-0.2
cis-9 C16:1	1.2	1.25	0.05
cis-11 C16:1	0.1	0.2	0.1
cis C18:1	28.5	29.5	1
trans C18:1	4.5	3.3	-1.2
C18:2	14.25	12.42	-1.83
C18:3	2.3	2.4	0.1
C20:4	0.3	0.2	-0.1
C20:5	0.2	0.1	-0.1
C22.6	0.5	0.2	-0.3

Full Fat Milk



Infant Formula



CONCLUSION

The CEM Microwave Digestion FAMEs Method uses a universal method for all samples, yielding better results in minutes, rather than hours. This includes a complete recovery of long-chain fatty acids and encapsulated omega-3-fortified fatty acids that are not recovered by traditional AOAC methods. The results were obtained using no harsh reagents, no glassware, 4-fold less reagents, and no fume hood.

CEM Corporation
United States
Tel: (800) 726-3331 [USA & Canada]
e-mail: info@cem.com
www.cem.com

FRANCE
CEMμWave S.A.S.
Tel: (33-1) 69 35 57 80
e-mail: info.fr@cem.com
www.cemfrance.fr

GERMANY
CEM GmbH
Tel: (49) 2842-9644-0
e-mail: info@cem.de
www.cem.de

IRELAND
CEM Technology (Ireland) Ltd
Tel +353 (0)1 885 1752
Email: info.ireland@cem.com
www.cemmicrowave.co.uk

ITALY
CEM S.R.L.
Tel: (39) 35-896224
e-mail: info.srl@cem.com
www.cemmicroonde.it

JAPAN
CEM Japan K.K.
Tel: +03-5368-2507
e-mail: info@cemjapan.jp
www.cemjapan.jp

UNITED KINGDOM
CEM Microwave Technology Ltd.
Tel: (44) 1280-822873
e-mail: info.uk@cem.com
www.cemmicrowave.co.uk

APPLICATION NOTES