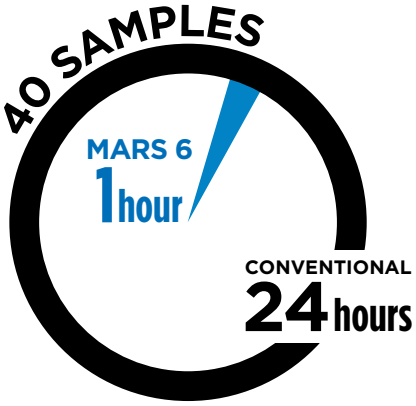
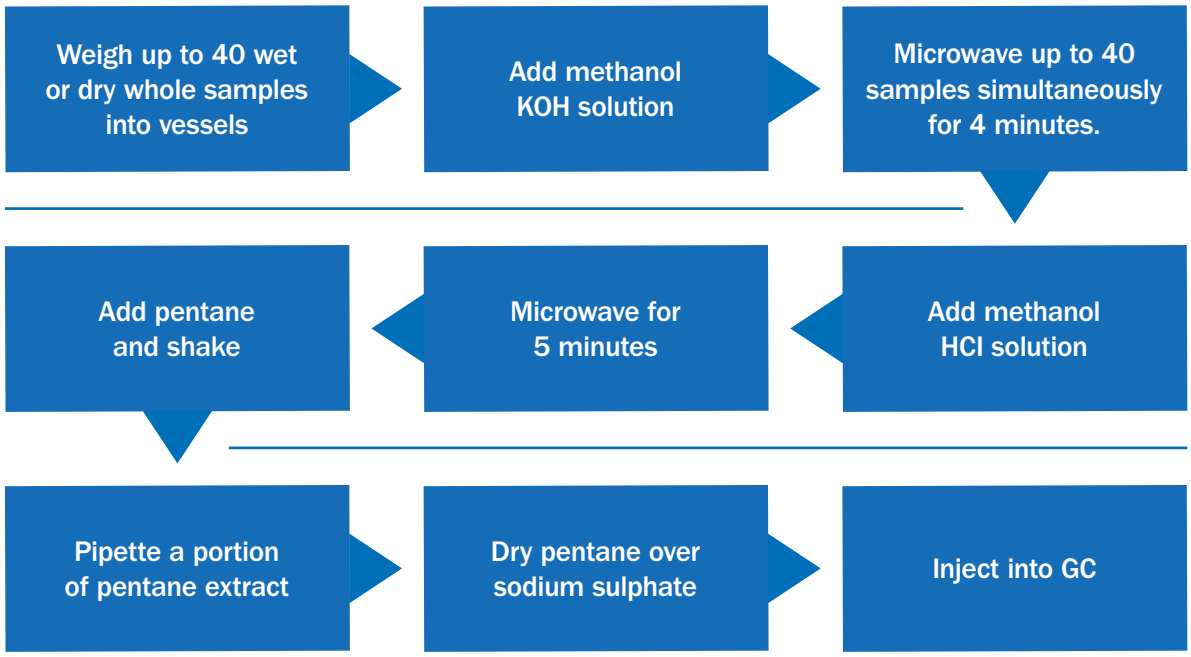
Fatty Acid Analysis

FAMEs — Fatty Acid Methyl Esters

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 FAME's replicates the AOAC method but in a much safer, cleaner and more efficient way. It creates exactly the same methyl esters that ultimately quantified by Gas Chromatography.

- Reduced analysis time
- Eliminates toxic BF3
- Complete extraction of both bound and free fatty acids
- Complete recovery of long-chain fatty acid and encapsulated omega-3 fatty acids





Sample Analysis

Microwave assisted FAME's 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. In fact, all food types can be analyzed by one simple microwave method, unlike the many different traditional methods that are required 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 1 hour.

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

Internal Standard (If required)

Internal standards can be used to confirm retention times and comparing 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.

Preparation of reagents

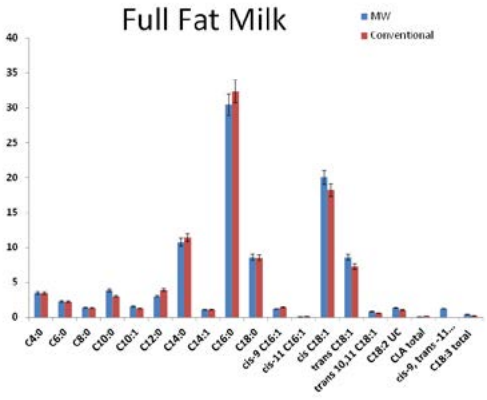
Reagent A – Reagent a is a KOH solution in Methanol.

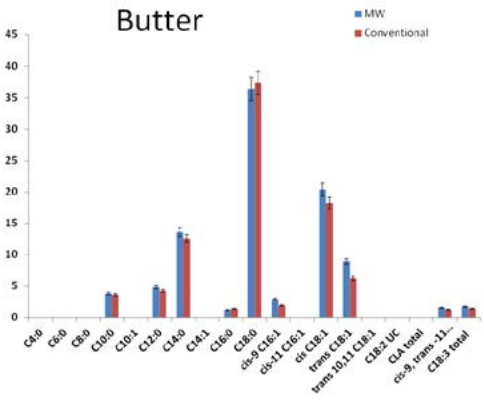
Reagent B – Reagent B is a preparation of methylation solution.

Reagent C – If the laboratories have an existing GC procedure, Reagent C is a pentane (HPLC grade) or similar solvent for FAMES (optimal if chilled).

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

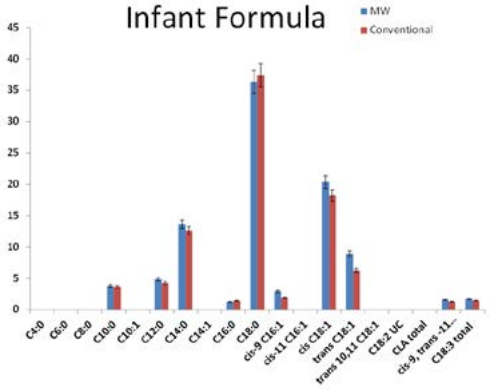
Reagent E – Reagent E is an anhydrous sodium sulfate mixture.





Meat samples– Sausages

Fatty Acid (wt %)	Conventional	MW assisted	Δ
C12:0	ND	ND	0
C14:0	1.38	1.37	-0.01
C16:0	23.43	23.43	0
cis-9 C16:1	1.87	1.69	-0.18
cis-11 C16:1	2.49	2.46	-0.03
C18:0	13.29	12.91	-0.38
C18:1n9	34.61	35.73	1.12
C18:2n6	16.92	15.28	-1.64
C18:3n6	0.15	0.17	0.02
C18:3n3	1.27	1.33	0.06
C20:0	0.15	0.15	0
C20:3n3	0.24	0.17	-0.07
C20:4n6	0.47	0.35	-0.12
C22:5n6	0.08	ND	0
C22:5n3	0.16	0.21	0.05
C22:6n3	0.08	0.09	0.01



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 90C 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 30C 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 120C and hold for 6 minutes. Then cool the vessels to room temperature or chilled (ice water can be used) C4-C6 analytes are extremely volatile at this point.
6. Add 10ml of Reagent C. Place the MARSXpress vessel plug over the end of the vessel and 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. This will minimize volatile losses.
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.

Results

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 AOAC methods. The results were obtained using no harsh reagents, no glassware, 4-fold less reagents and no fume hood.