INNOVATIVE METHOD OF MICROWAVE ASSISTED EXTRACTION OF MOSH AND MOAH IN FOOD

Mineral oil hydrocarbons are a growing concern pollutant in food quality control. Microwave-assisted solvent extraction is a well-established sample preparation technique and offers a reliable and efficient approach to their extraction. The Milestone ETHOS X approach for the simultaneous saponification and extraction allows laboratories to reduce the analytical costs and improve the overall productivity.

INTRODUCTION

In recent years, the assessment and quantification of the presence of mineral oil hydrocarbons, both aliphatic (MOSH) and aromatic (MOAH), has become a growing requirement for food quality control laboratories around the world. [1]

While MOAH, a class of compounds similar but not identical in structure to PAHs, are established carcinogens and therefore should not be present in food, MOSH are a broad class of hydrocarbons, present in both linear and branched forms, whose toxicology is not yet fully understood and therefore no definitive regulatory limits have been established.

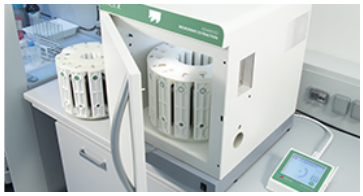
Since the first analytical method developed in 2009 by Biedermann and Grobb [2], many efforts have been made to address the occurrence of contamination in different matrices, in particular by the European Commission [3], and to develop analytical methods.

Following the direction given by the JRC guidance [4], all laboratories are expected to follow the performance criteria, but new developments are constantly being added to the method.

Particularly for samples with a high fat content, the fatty acid chain can cause some analytical problems due to rapid overloading of the LC column. To avoid this, sample preparation for this analysis requires a tedious purification step by silica or alternatively by saponification of the fatty acids. [5] [6] [7]

In the present work we have developed a simple, accurate and faster method to perform the saponification in the sample preparation of contaminated food samples. The evaluation of MOSH and MOAH content was performed on different food matrices ranging from cereal products to fish and vegetable oils.

The Microwave Assisted Saponification (MAS) method used the ETHOS X together with the SR-15 eT rotor. The rotor is equipped with segments of closed vessel technology, which allows it to operate at high temperature and pressure.



EXPERIMENTAL

EQUIPMENT

- Milestone ETHOS X [8] with easyTEMP
- SR-15 easyTEMP extraction rotor

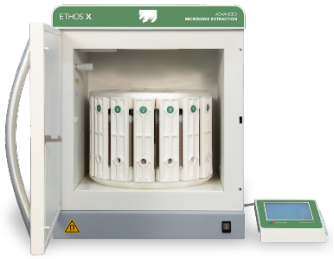


Figure 1 – Milestone's ETHOS X with SR-15 eT rotor

STANDARD AND REAGENTS

Potassium hydroxide (KOH), Methanol and n-hexane were used for saponification. All chemicals and solvent used are analytical grade and were purchased from Sigma-Aldrich (Milan, Italy). For the test of recoveries, a printing ink solvent (containing 91% of MOSH and 9% of MOAH in the n-C14–C20 range) was provided by a producer.

The working internal standard (IS) solution, was purchased from Restek (Milan, Italy) and consisted of: 5-a-cholestane (Cho) and perylene (Per) at 0.6 mg/mL, 1,3,5-tri-tert-butylbenzene (TBB), n-undecane, cyclohexyl cyclohexane (CyCy), pentyl benzene (5B), 1-methyl naphthalene (1MN), 2-methyl naphthalene (2MN) at 0.30 mg/mL, and n-C13 at 0.15mg/mL in toluene.

SAMPLES

All products were purchased in Italian local market. The bakery (cereals) products tested were dry and egg pasta, biscuits, bread and cake. Different kinds of packaging for the same product were tested with variable lifetime.

Different kinds of fish with different preservation treatments (fresh, frozen, smoked) and different kinds of packaging were tested.

Lastly, a group of 5 different virgin olive oils were extracted.

MICROWAVE-ASSISTED SAPONIFICATION

Sample to be saponified was weighted (5 g) directly inside the SR-15 eT extraction vessel and 5 µL of IS was added, followed by 10 ml of saturated KOH in methanol and finally 10 mL of n-hexane. Magnetic stirring bars were added to each vessel and the SR-15 eT segment was properly closed.

The following temperature method was employed, under a set constant stirring of 80 %:

Step	Time (min)	Power (W)	Temperature (°C)
1	5	up to 1600	120
2	15	up to 1600	120

Table -1 Microwave saponification program

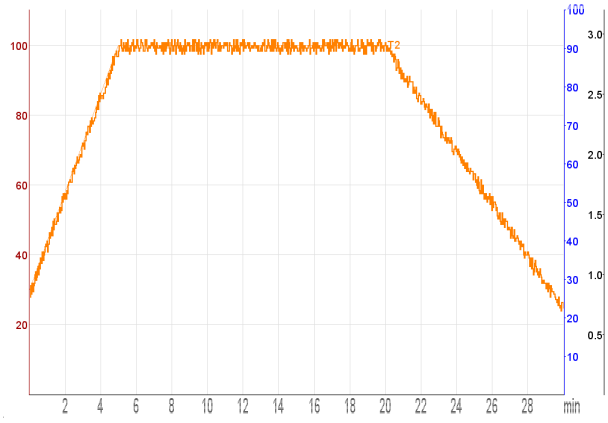
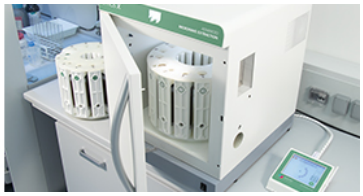


Figure 2 – Microwave run profile

At the end of the program, after cooling to room temperature, the SR-15 eT vessel was opened.

The dry pasta and bread samples did not require any sample post treatment before LC–GC injection. For all the other samples a washing step was performed, adding 40 mL of deionized water inside the vessel followed by 2 mL of methanol, letting the solvent flow along the walls of the extraction cell. The vessel was let to rest for 30 minutes at –20°C. 5 mL of the organic phase of n-hexane was collected and concentrated to 1 mL for following LC-GC analyses.



ANALITICAL CONDITIONS

On-line LC-GC-FID analysis was performed on a LC-GC. FID detector (sampling frequency of 50 Hz) and solvent vapor exit were heated at 360 and 140 °C, respectively.

The sample was eluted at 300 mL/min using a gradient starting with hexane (0.1 min hold) and reaching 30% of dichloromethane after 0.5 min. The GC run oven ramp consists of constant 20° C/min from 60 to 350 °C with hydrogen as carrier gas. The MOSH area was determined by the integration of the whole hump of largely unresolved peaks. All sharp peaks standing on the top of the MOAH "hump" were subtracted from the total area. The quantification was determined on the IS.

RESULTS AND DISCUSSION

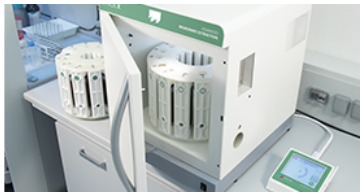
The method accuracy was assessed with recovery tests performed on 4 different bakery foodstuffs plus one fish sample spiked with known amount of printing ink solvent. Blank repetitions were performed to quantify the MOSH and MOAH contamination already present. For pasta and cake two levels of concentration were tested. The results are reported in the following table:

Sample	MOSH			MOAH		
	Added concentration (mg/Kg)	Recoveries (%)	RSD (%)	Added concentration (mg/Kg)	Recoveries (%)	RSD (%)
Biscuit	15	90	8.5	1.5	97	3.8
Bread	15	94	9.3	1.5	89	7.9
Dry pasta	5-15	94-103	6.7-4.9	0.5-1.5	92-108	7.7–4.3
Cake	5-15	93-101	6.2-7.2	0.5-1.5	95	8.7-5.6
Tuna	1.0	103	4.4	0.3	99	4.9

Table 2- Performance of the method, recoveries on spiked samples

After the recoveries studies the tests of contamination evaluation were performed. MOSH and MOAH are divided in groups according to the length of chain groups classification (C10-C16; C16-C25; C25-35; C>35) The results are divided for bakery, fish products and extra-virgin olive oils:

Sample	Packaging material	MOSH (mg/kg)				MOAH (mg/kg)			
		C10-C16	C16-C25	C25-C35	C >35	C10-C16	C16-C25	C25-C35	C >35
Semolina pasta	Virgin paperboard	0.1	0.5	0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	Recycled paperboard	<0.1	4.0	0.3	< 0.1	0.1	0.8	< 0.1	< 0.1
Egg pasta	Virgin paperboard	0.4	1.1	0.5	< 0.1	< 0.1	0.2	0.1	< 0.1
	Recycled paperboard	2.1	21.3	2.6	< 0.1	0.3	2.9	0.4	< 0.1



Biscuits	Aluminum	0.1	0.5	0.5	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	Plastic/ Recycled paperboard	1.8	2.4	6.3	< 0.1	0.1	0.3	< 0.1	< 0.1
Bread	Plastic	0.3	0.9	0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	Recycled paperboard	1.5	9.7	2.9	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Cakes	Plastic	0.1	0.4	1.0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	Plastic/ Recycled paperboard	2.4	9.8	7.8	< 0.1	0.1	0.3	0.1	< 0.1

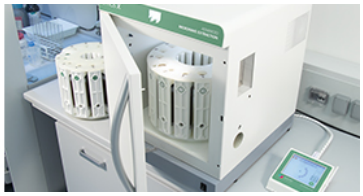
Table 3 – Level of MOSH and MOAH contamination detected in bakery products with different packaging materials.

Sample	MOSH (mg/kg)				MOAH (mg/kg)			
	C10-C16	C16-C25	C25-C35	C >35	C10-C16	C16-C25	C25-C35	C >35
EVO 1	1.6	4.0	4.7	1.2	< 0.1	< 0.1	< 0.1	< 0.1
EVO 2	2.4	4.6	7.7	3.5	< 0.1	< 0.1	< 0.1	< 0.1
EVO 3	2.8	5.0	1.6	0.8	< 0.1	< 0.1	< 0.1	< 0.1
EVO 4	3.9	4.1	6.3	3.3	< 0.1	< 0.1	< 0.1	< 0.1
EVO 5	4.3	11.9	15.1	6.7	< 0.1	< 0.1	< 0.1	< 0.1

Table 4 – Level of MOSH and MOAH contamination detected in 5 different extra-virgin olive oils.

Sample	Packaging material	MOSH (mg/kg)				MOAH (mg/kg)			
		C10-C16	C16-C25	C25-C35	C >35	C10-C16	C16-C25	C25-C35	C >35
Smoked salmon	Recycled paperboard / Al / Plastic	0.3	1.5	1.4	1.1	0.1	0.6	0.2	1.0
Smoked salmon	Plastic (PET)	< 0.1	0.2	0.3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Fresh flounder	None	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Frozen cod	Plastic	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Smoked swordfish	Plastic / aluminum	< 0.1	1.2	1.4	0.4	< 0.1	< 0.1	< 0.1	< 0.1
Frozen tuna	Plastic (LDPE)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Fresh tuna	Plastic (PET)	< 0.1	0.2	2.4	1.3	< 0.1	< 0.1	< 0.1	< 0.1

Table 5 – Level of MOSH and MOAH contamination detected in fish samples with different conservation techniques and packaging materials.



The results obtained permit to make the following observations:

- The recoveries study show that the method is efficient in extracting all the contamination present in the sample.
- Results on bakery products demonstrate the general hypothesis that food with higher fat content register higher MOSH and MOAH migration from the package and the material of the package determines the contamination level. As expected, recycled paperboard is generally the worst packaging material due to residual mineral oil from ink and processing oils.
- MOAH presence is registered only in the bakery products packaged in recycled paperboard containers.

CONCLUSION

Microwave Assisted Saponification (MAS) presents clear advantages over the traditional methods of extraction such as Soxhlet, sonication or wrist-shaking at room temperature.
In fact,the use of microwave as a heating source allows to significantly reduce the time of saponification (20 mins) and to have a simultaneous separation of the MOSH and MOAH fraction in the organic phase from the saponified fatty acids in the water phase, which can create analytical trouble in LC injections.
All this observation proves the ETHOS X as an effective and innovative system for the quantification of MOSH MOAH in different type of food matrixes.

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