

1 **Update on the microwave-assisted saponification**
2 **conditions for mineral oil hydrocarbons determination**
3 **in fats and oils**

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27 1 Abstract

28 The analysis of mineral oil saturated (MOSH) and aromatic (MOAH)
29 hydrocarbons in edible fats and oils typically includes a saponification followed by
30 liquid–liquid extraction. The official ISO 20122:2024 method has shown limitations,
31 particularly due to inconsistent recoveries of MOAH internal standards (ISs), which
32 compromise quantitative accuracy. A previously developed microwave-assisted
33 saponification/extraction (MASE) method improved IS recovery consistency through
34 optimized solvent composition but proved insufficient to fully saponify certain oils (e.g.,
35 extra virgin olive oil, hydrogenated fats) under its original conditions (60 °C, 30 min).

36 In this study, saponification parameters were reoptimized and validated by
37 gravimetric control of residual fat and evaluation of ISs ratios, which are ideally 1.00.
38 The final conditions (120 °C, 20 min) ensured complete saponification across a wide
39 range of fats and oils with varying compositions and melting points. ISs ratios were
40 consistently closer to 1.00 than with the ISO method, although matrix-dependent
41 deviations persisted. These effects correlated with the fatty acid profiles of the oils,
42 which affects the properties of the soaps formed during saponification and, in turn, the
43 partition of MOAH ISs during extraction.

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45 **Keywords:** mineral oil aromatic hydrocarbons; fats and oils; sample preparation;
46 microwave-assisted extraction; saponification

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53 *Table S1: Data used to generate Figure 1*

Summary of the data	Average					<i>(Value 1 - value 2)/2</i>				
	60°C - 30 min ISO	60°C - 30 min	90°C - 30 min	120°C - 30 min	120°C - 20 min	60°C - 30 min ISO	60°C - 30 min	90°C - 30 min	120°C - 30 min	120°C - 20 min
Fully hydrogenated rapeseed oil	52%	39%	1%	1%		2.8%	2.2%	0.5%	0.3%	
Hydrogenated palm kernel olein	2%	11%	1%	3%	0%	0.1%	1.6%	0.7%	1.3%	0.2%
Refined palm oil	2%	18%	1%	1%		1.1%	4.5%	0.0%	0.5%	
Crude palm oil	6%	9%	2%	1%		2.2%	1.0%	0.0%	0.1%	
Coconut oil	3%	7%	1%	6%	1%	0.0%	1.9%	0.1%	3.3%	0.1%
Rice bran oil	3%	11%	6%	3%		0.0%	1.0%	0.1%	0.4%	
Extra virgin olive oil	2%	14%	2%	1%	2%	0.3%	0.5%	0.9%	0.3%	0.8%

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64 *Table S2: Data used to generate Figure 3B*

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Sample	Solvent/reagents conditions	Heating mode	Temperature (°C)	Time (min)	Oil mass (g)	Flask (g)	Flask + residue (g)	Residue (g)	Residue (%)	Average (n=2)	(Value 1 - value 2)/2 (as alternative to SDEV since n = 2)
EVOO	ref 5	Microwave	60	20	1.0278	24.975	25.3472	0.3722	36%	31%	5%
EVOO	ref 5	Microwave	60	20	1.1124	25.4618	25.7544	0.2926	26%		
EVOO	ref 5	Microwave	75	30	1.1122	23.9626	24.1065	0.1439	13%	9%	4%
EVOO	ref 5	Microwave	75	30	1.0623	25.2351	25.29	0.0549	5%		
EVOO	ref 5	Microwave	75	10	0.9842	25.2487	25.4166	0.1679	17%	13%	4%
EVOO	ref 5	Microwave	75	10	1.1797	25.4847	25.5916	0.1069	9%		
EVOO	ref 5	Microwave	90	20	1.111	24.8135	24.8601	0.0466	4%	4%	0%
EVOO	ref 5	Microwave	90	20	1.0206	25.6923	25.7283	0.036	4%		
EVOO	ref 5	Microwave	105	30	1.1328	24.7758	24.8313	0.0555	5%	3%	2%
EVOO	ref 5	Microwave	105	30	0.9905	25.1443	25.1525	0.0082	1%		
EVOO	ref 5	Microwave	105	10	1.0506	27.1743	27.1822	0.0079	1%	1%	0%
EVOO	ref 5	Microwave	105	10	1.0197	25.239	25.252	0.013	1%		
EVOO	ref 5	Microwave	120	20	1.06	28.8422	28.853	0.0108	1%	2%	1%
EVOO	ref 5	Microwave	120	20	1.0313	29.2833	29.3212	0.0379	4%		

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71 Table S3: Data used to generate Figure 3C

		EVOO1	EVOO2	EVOO3	EVOO4	EVOO5	EVOO6	EVOO7	EVOO8	EVOO9	EVOO10	EVOO11	EVOO12	EVOO13	EVOO14
	T (°C)	60	60	75	75	75	75	90	90	105	105	105	105	120	120
	Time (min)	20	20	10	10	30	30	20	20	10	10	30	30	20	20
	Mass (g)	1.1264	1.1341	1.0128	1.052	1.1854	1.0711	1.1022	1.0462	1.0319	1.0732	1.0304	1.0948	1.0947	1.0946
PEAK AREA GC×GC-FID	5B	27535.5	24782.4	31703.3	31077.9	27104.3	38708	37256.4	29052.8	42594.2	24851.2	38942.3	37874.8	33074.6	28265.4
	1MN	27290.5	24866	31501.4	29745.4	29257.6	38712.7	35042.7	28847.7	39789.8	23687.6	37945.7	35991	31265.6	27420.6
	2MN	29272.7	26330.1	33443	31928.1	31209.1	40755	36798.1	30809	41415.8	25488.2	39824.5	38110.3	32954	29066.4
	TBB	30664.3	28038.7	35548	34010.6	33362.2	44987.3	40658.7	35718.6	46515.7	27771.6	42693.8	41723.4	36296.9	30656
	Per	50164.2	44085	55549.5	53263	57516.4	70447.7	59657.2	49774.5	64925.8	36805.2	62370.9	56840.7	48310.4	43892.9
IS RATIO	5B/2MN	0.94	0.94	0.95	0.97	0.87	0.95	1.01	0.94	1.03	0.98	0.98	0.99	1.00	0.97
	1MN/2MN	0.93	0.94	0.94	0.93	0.94	0.95	0.95	0.94	0.96	0.93	0.95	0.94	0.95	0.94
	2MN/2MN	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	TBB/2MN	1.05	1.06	1.06	1.07	1.07	1.10	1.10	1.16	1.12	1.09	1.07	1.09	1.10	1.05
	(Per/2)/2MN	0.86	0.84	0.83	0.83	0.92	0.86	0.81	0.81	0.78	0.72	0.78	0.75	0.73	0.76
AVERAGE & SDEV		AVERAGE	(Value 1 - value 2)/2	AVERAGE	(Value 1 - value 2)/2	AVERAGE	(Value 1 - value 2)/2	AVERAGE	(Value 1 - value 2)/2	AVERAGE	(Value 1 - value 2)/2	AVERAGE	(Value 1 - value 2)/2	AVERAGE	(Value 1 - value 2)/2
	5B/2MN	0.94	0.00	0.96	0.01	0.91	0.04	0.98	0.03	1.00	0.03	0.99	0.01	0.99	0.02
	1MN/2MN	0.94	0.01	0.94	0.01	0.94	0.01	0.94	0.01	0.95	0.02	0.95	0.00	0.95	0.00
	2MN/2MN	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
	TBB/2MN	1.06	0.01	1.06	0.00	1.09	0.02	1.13	0.03	1.11	0.02	1.08	0.01	1.08	0.02
(Per/2)/2MN	0.85	0.01	0.83	0.00	0.89	0.03	0.81	0.00	0.75	0.03	0.76	0.02	0.74	0.01	

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78 Table S4: Data used to generate Figure 3D

Results after making sure that the emulsion at the interface was fully broken

	EVOO 1 (60°C, 30 min)	EVOO 2 (60°C, 30 min)	EVOO 3 (60°C, 20 min)	EVOO4 (60°C, 20 min)	EVOO 5 (90°C, 20 min)	EVOO 6 (90°C, 20 min)	EVOO 7 (120°C, 20 min)	EVOO 8 (120°C, 20 min)
5B	3952.5	11249.5	7214.2	25257.3	15137.4	11566	19355.2	13922.6
TBB	10607.3	13199.6	9759.6	26525	16623.3	12622.2	20652	15477
2MN	9255.6	11546.4	8565.9	24526.4	15537.8	11382.6	18439	13632.8
1MN	9667.6	11728	8688.7	25192.9	15499.7	11691.4	19170.4	14057.1
Per	17466.9	21637.3	18293.6	46052	27038.8	20173.4	33100.6	25279.7
5B/2MN	0.43	0.97	0.84	1.03	0.97	1.02	1.05	1.02
TBB/2MN	1.15	1.14	1.14	1.08	1.07	1.11	1.12	1.14
2MN/2MN	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1MN/2MN	1.04	1.02	1.01	1.03	1.00	1.03	1.04	1.03
(Per/2)/2MN	0.94	0.94	1.07	0.94	0.87	0.89	0.90	0.93

	AVERAGE				(Value 1 - value 2)/2 (as alternative to SDEV since n = 2)			
	60°C, 30 min	60°C, 20 min	90°C, 20 min	120°C, 20 min	60°C, 30 min	60°C, 20 min	90°C, 20 min	120°C, 20 min
5B/2MN	0.70	0.94	1.00	1.04	0.27	0.09	0.02	0.01
1MN/2MN	1.03	1.02	1.01	1.04	0.12	0.12	0.06	0.08
TBB/2MN	1.14	1.11	1.09	1.13	0.14	0.11	0.09	0.13
(Per/2)/2MN	0.94	1.00	0.88	0.91	0.10	0.01	0.12	0.13

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