

SUPPLEMENTARY MATERIAL

LC–MS metabolomic profiling of five types of unrefined, cold-pressed seed oils to identify markers to determine oil authenticity and to test for oil adulteration

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Table S1. Preparation of binary oil mixtures

OIL	5%	10%	30%	50%	70%	90%
Black seed	25 µl	50 µl	150 µl	250 µl	350 µl	450 µl
Evening Primrose						
Hemp						
Milk Thistle						
Pumpkin						
Sunflower	475 µl	450 µl	350 µl	250 µl	150 µl	50 µl
Rapeseed						
Sesame						

Table S2. Differences in the intensity of the oil markers in products from different producers and from different production batches, expressed as relative standard deviation of the peak areas of the extracted ion chromatograms (EIC).

Oil	No	Observe <i>m/z</i>	tR [min]	RSD [%]
Black seed	M1	166.1226	2.6	36.48
	M2	328.1551	4.2	90.14
	M3	342.1710	5.1	64.15
	M4	313.1802	7.3	49.58
	M5	238.0901	7.9	90.70
	M6	131.0862	9.7	47.15
	M7	385.2376	11.4	84.36
	M8	404.2641	13.1	128.73
	M9	677.3231	19.1	78.66
	M10	635.3118	20.9	94.15
Evening Primrose	M1	469.3317	10.9	29.31
	M2	455.3161	11.8	30.38
	M3	409.2207	14.4	72.38
	M4	492.3174	16.5	78.06
	M5	634.4167	20.9	90.87
	M6	720.4547	21.1	92.00
	M7	577.39	22.9	25.71
	M8	429.3741	24.2	25.27
Hemp seeds	M1	375.2178	13.3	59.37
	M2	369.1343	14.4	45.96
	M3	437.1965	18.3	48.36
	M4	341.2123	18.6	55.57
	M5	315.2329	19.3	49.80
	M6	498.3586	22.6	57.13
	M7	460.4163	28.2	65.57
Milk Thistle	M1	145.0764	2.1	61.21
	M2	483.1296	8.8	67.97
	M3	233.1545	11.6	68.34
	M4	239.2013	12.1	103.14
Pumpkin seed	M1	269.1904	8.7	54.06
	M2	181.1231	17.9	63.41
	M3	358.2389	18.4	28.39
	M4	578.4219	19.6	14.72
	M5	697.4592	22.1	66.81
	M6	682.4482	25.1	29.66
	M7	578.5152	28.6	50.18

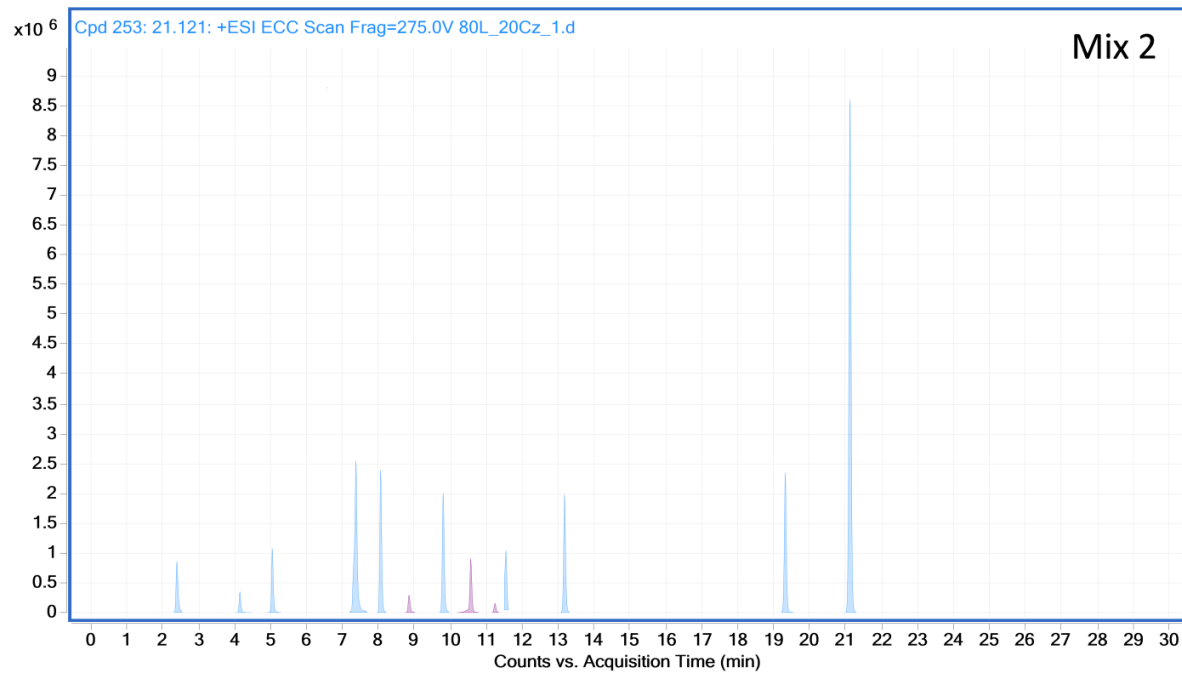
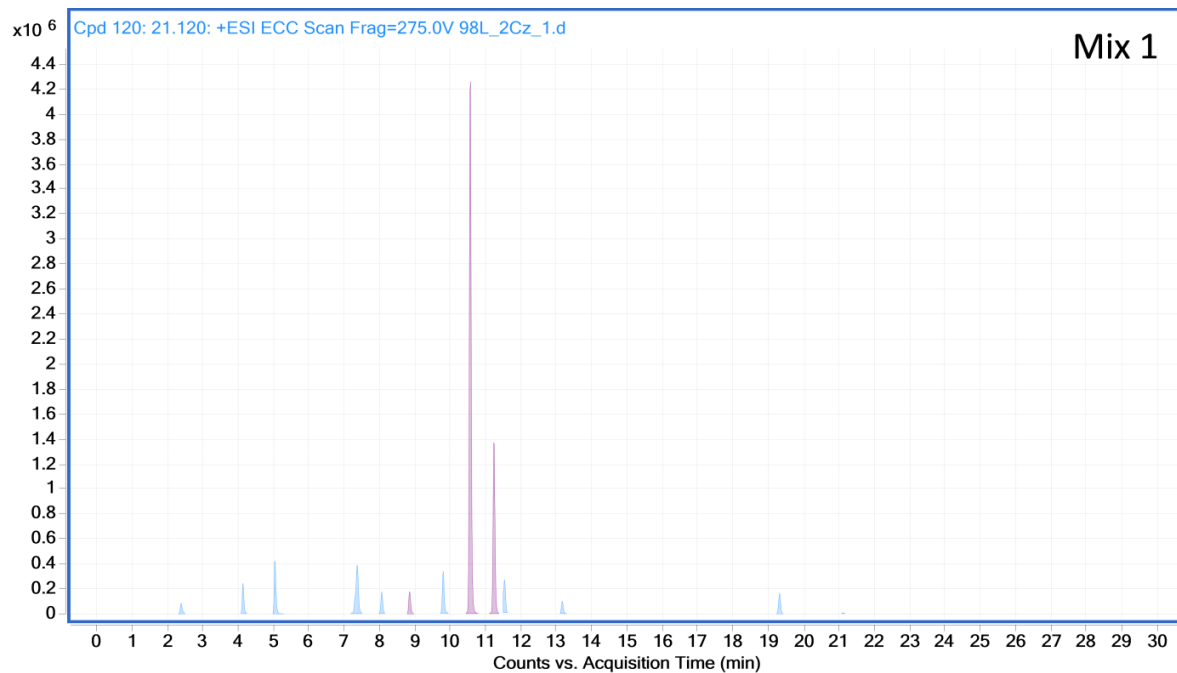
Table S3. LOD for sunflower, rapeseed and sesame specific metabolomic markers.

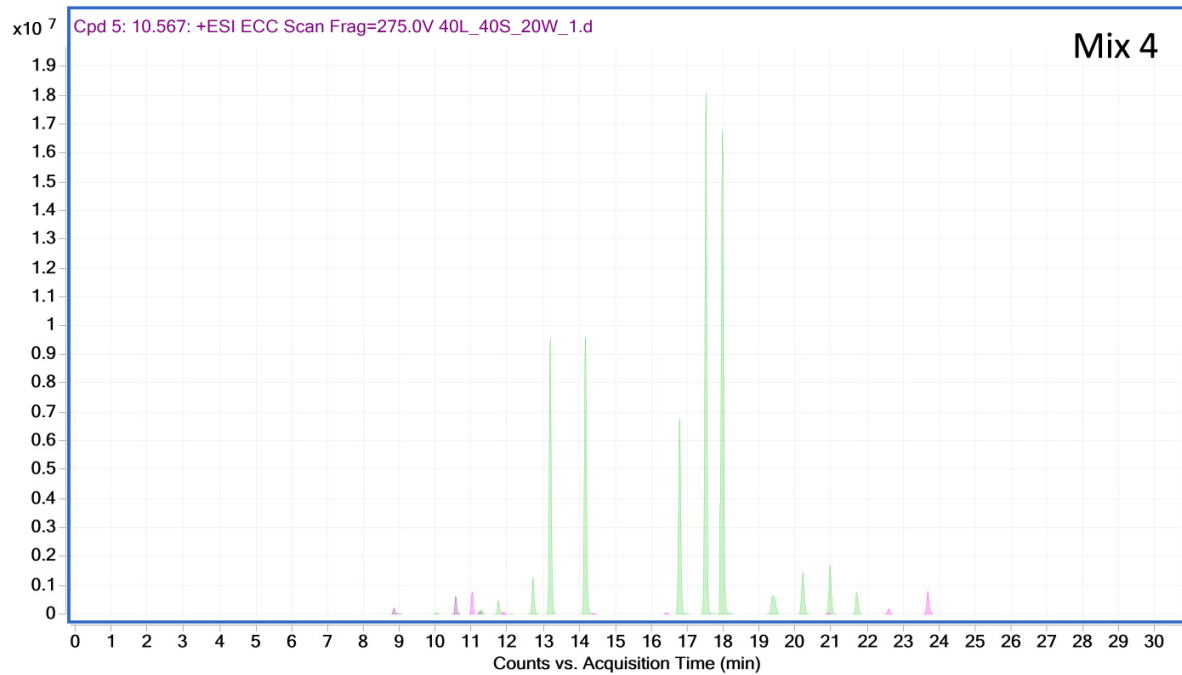
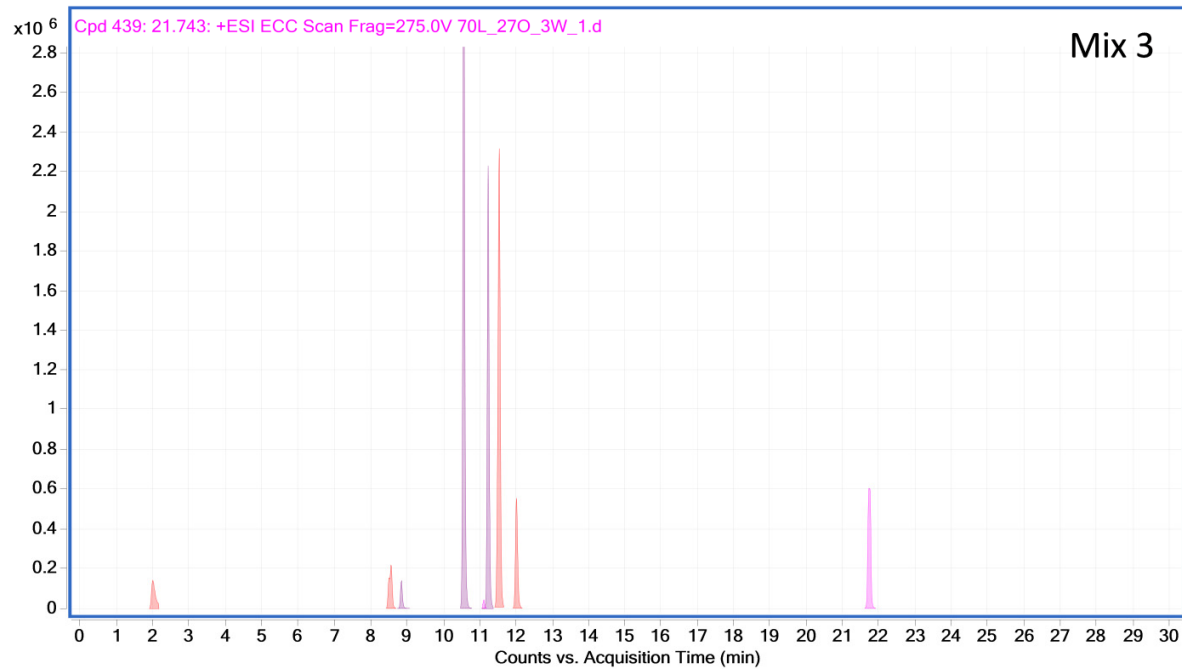
OIL				Black seed	Hemp	Evening Primrose	Milk Thistle	Pumpkin seed
				LOD	LOD	LOD	LOD	LOD
Sunflower	1	231.1386	10.03 ± 0.18	25.00	3.49	*	25.00	*
	2	217.0865	11.31 ± 0.25	2.91	0.38	8.11	12.00	3.49
	3	345.0975	11.77 ± 0.25	14.29	*	18.75	*	*
	4	203.1076	12.76 ± 0.24	3.30	0.40	0.71	2.40	0.90
	5	301.2172	13.15 ± 0.2	14.29	16.67	15.79	12.00	13.04
	6	392.2444	14.14 ± 0.26	0.52	0.42	1.60	2.83	1.35
	7	420.2758	16.76 ± 0.3	9.09	0.28	0.66	3.57	4.48
	8	432.2758	17.47 ± 0.28	6.52	0.52	1.60	2.83	2.04
	9	434.2913	17.98 ± 0.33	4.69	0.20	4.92	2.59	2.13
	10	576.3394	19.44 ± 0.26	*	*	*	21.43	25.00
	11	590.3552	20.23 ± 0.31	14.29	1.96	1.62	3.80	2.63
	12	604.3699	20.97 ± 0.3	8.33	2.73	5.77	4.11	5.66
	13	618.3865	21.74 ± 0.26	*	0.80	7.14	8.33	8.33
Rapeseed	14	162.0557	6.01 ± 0.28	1.29	1.23	1.66	2.17	4.55
	15	225.1488	6.44 ± 0.16	10.00	10.71	10.71	9.38	17.65
	16	288.1237	10.05 ± 0.13	1.26	0.48	5.45	3.41	5.36
	17	467.3510	20.42 ± 0.3	*	1.09	3.37	9.09	13.04
	18	483.3824	22.01 ± 0.18	*	14.29	5.08	*	*
	19	379.3365	24.26 ± 0.34	*	0.67	16.67	*	14.29
	20	413.3429	24.81 ± 0.34	0.68	0.15	1.68	4.35	3.30
	21	773.5184	27.95 ± 0.7	*	0.72	6.82	13.04	20.00
Sesame	22	373.1286	8.71 ± 0.15	5.66	3.49	12.00	13.64	8.57
	23	371.1136	10.6 ± 0.19	0.15	0.12	0.59	1.52	0.55
	24	299.2592	12.88 ± 0.07	0.62	0.85	1.38	4.69	2.83
	25	337.1082	13.66 ± 0.22	0.23	0.11	0.63	1.01	3.09
	26	173.0604	14.53 ± 0.23	0.30	0.11	3.03	1.25	2.63

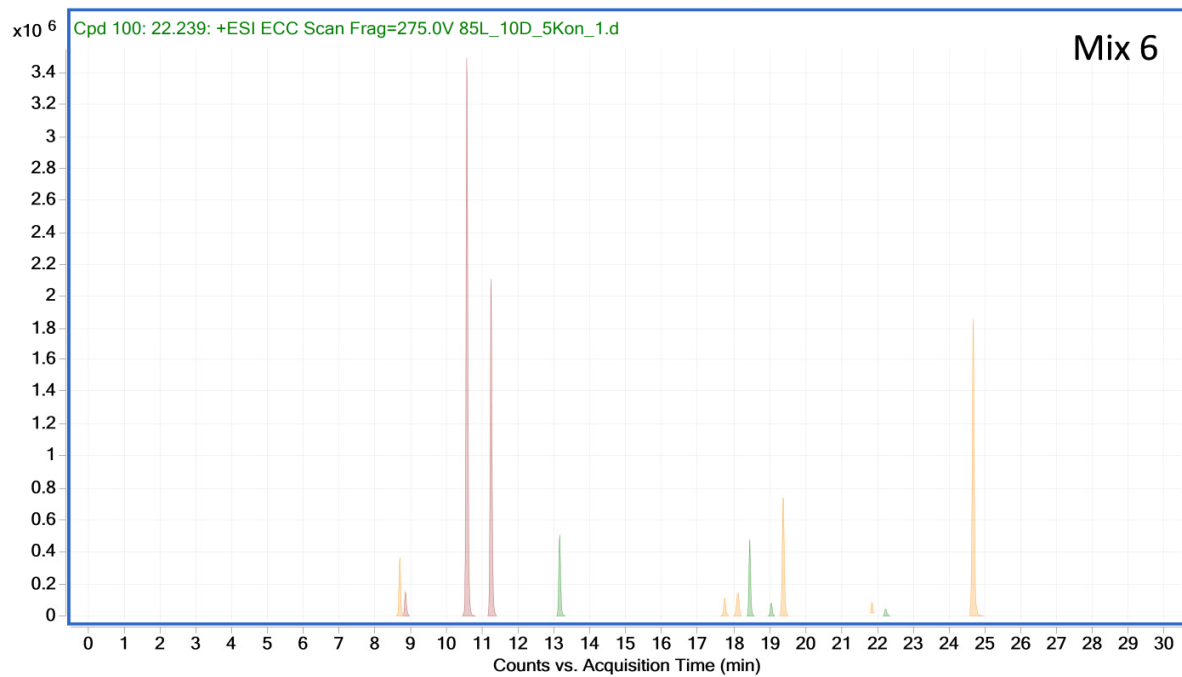
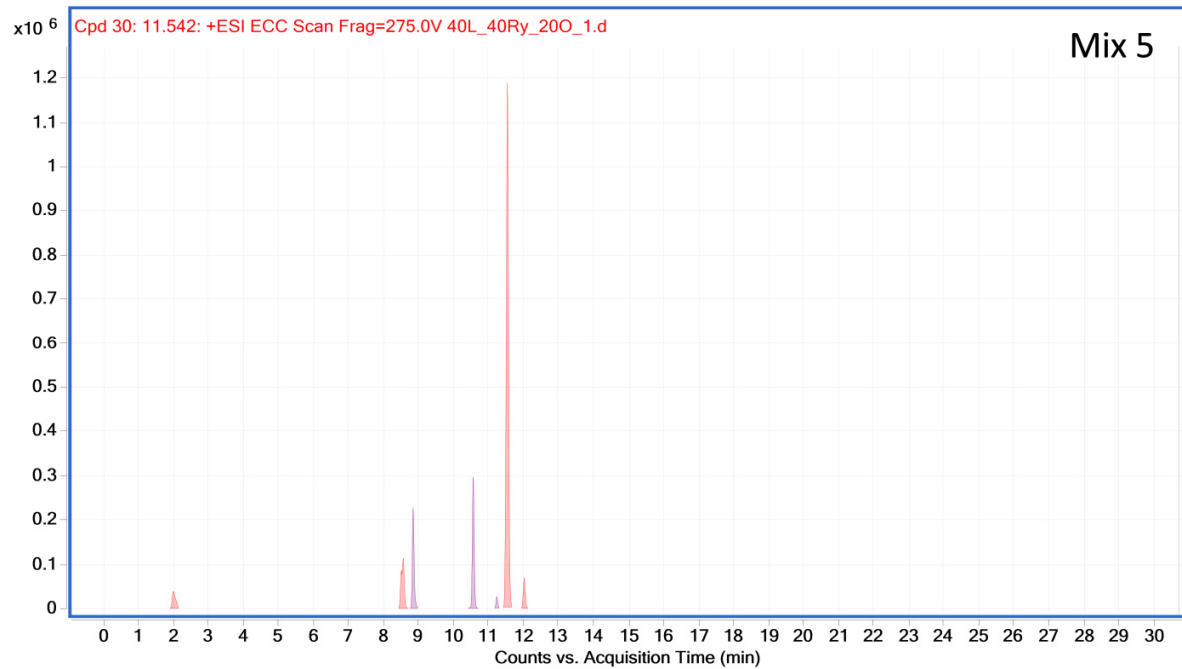
LOD - Limit of detection; asterix (*) - value no calculated; metabolomic markers of sunflower, rapeseed and sesame oil were as reported previously by Sumara et al. 2023

Table S4. Composition of the commercial unrefined, cold- pressed oil blends products

Name	No.	Oil composition			
Oil for children	Mix 1	98% Flax seed	2% Black seed		
Oil for the heart	Mix 2	80% Flax seed	20% Black seed		
Oil for women	Mix 3	70% Flax seed	27% Milk thistle	3% Evening primrose	
Oil for mothers	Mix 4	40% Flax seed	40% Sunflower	20% Evening primrose	
Oil for elderly	Mix 5	40% Flax seed	40% Camelina	20% Milk Thistle	
Oil for men	Mix 6	85% Flax seed	10% Pumpkin seed	5% Hemp	
Immuno oil	Mix 7	87% Camelina	5% Evening primrose	4% Pumpkin seed	4% Black seed







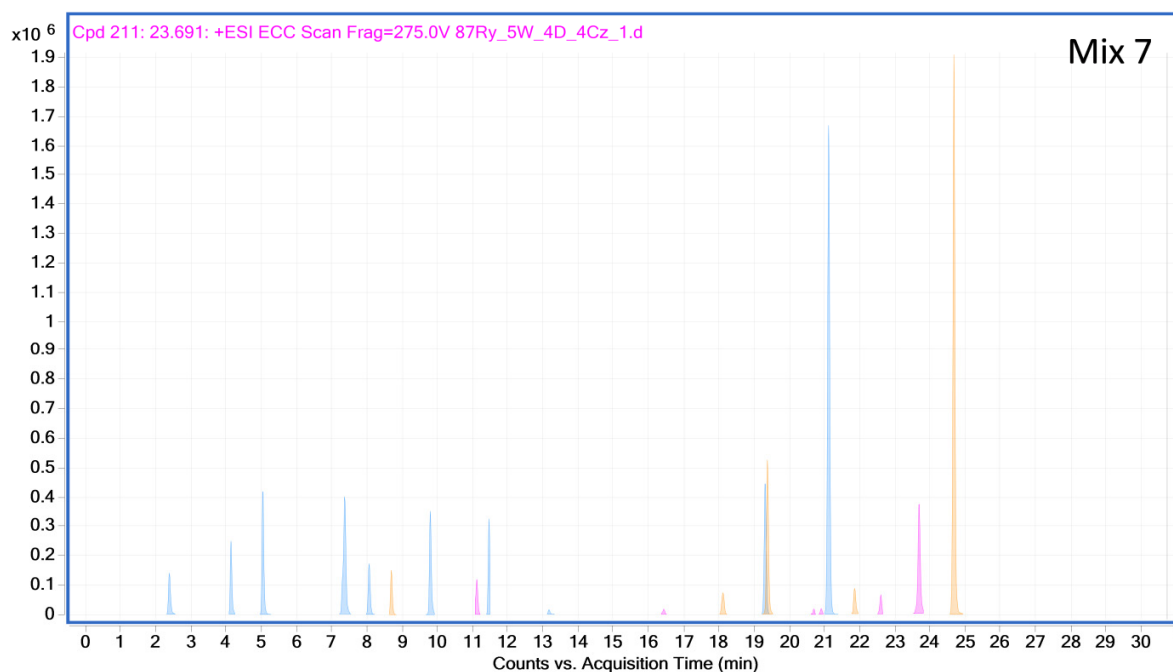


Figure S1. LC–QTOF extracted ion chromatograms of oil metabolite markers in 7 commercial unrefined, cold-pressed vegetable oils blends, of two-, three- and four-component (Mix 1- Mix 7). Flax seed oil markers - purple, milk thistle oil markers - red, evening primrose oil markers - pink, sunflower oil markers - light green, black seed oil markers- blue, pumpkin seed oil markers- orange, hemp oil markers- green. All details for oil specific markers are presented in Fig. S1 are given in Table 1 and Table S3