Supplementary Material for

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Geometrical and positional isomers of unsaturated furan fatty acids in food

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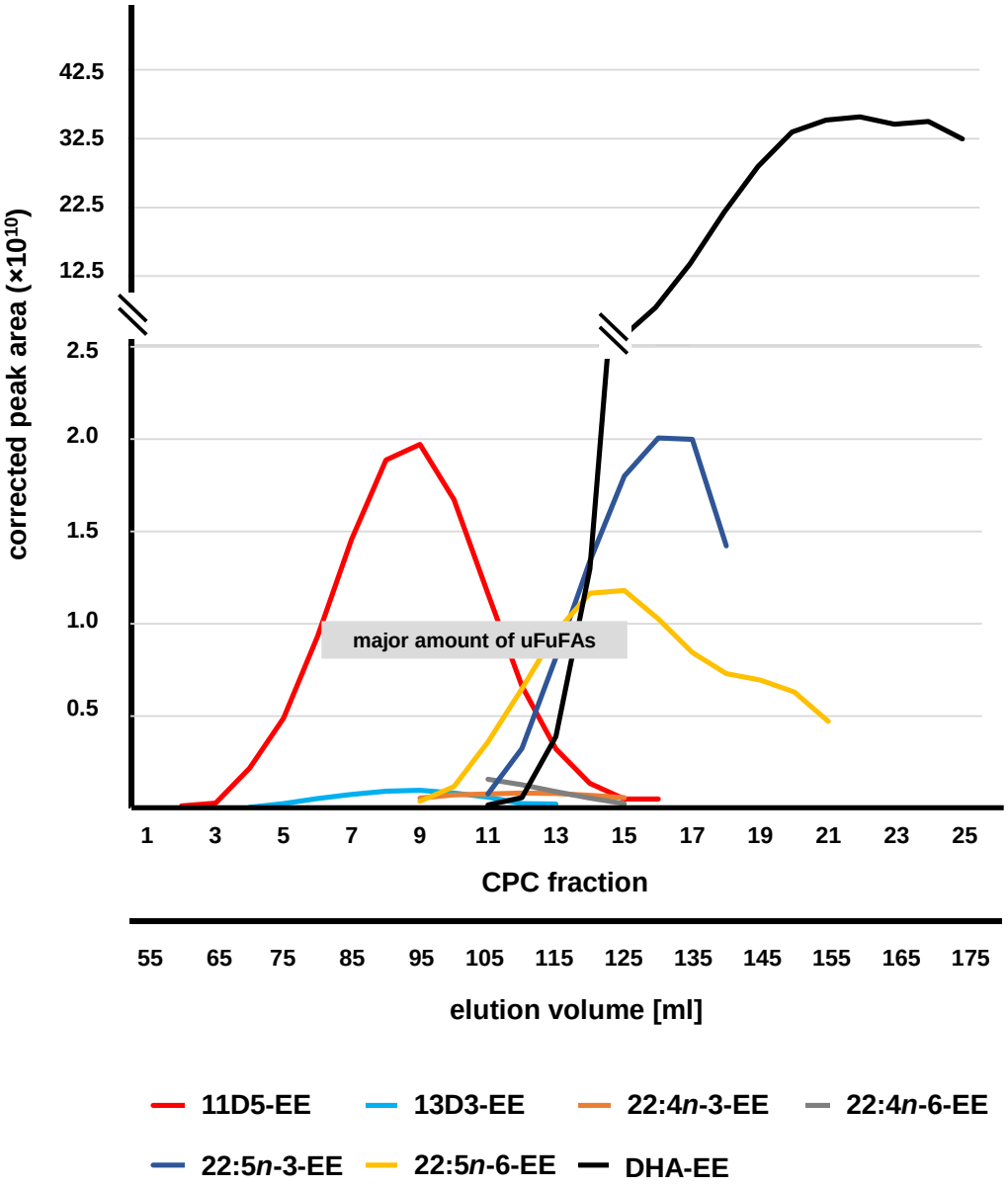

FIGURE S1. CPC elution profile of major fatty acid ethyl esters in a DHA-EE oil. The elution range of the majority of unsaturated FuFA is additionally marked with a grey bar. Elution profiles were generated from peak area in GC/MS chromatograms (full scan mode) of individual CPC fractions, corrected via the internal standard 14:0-EE, and finally by a weighted 9-point smoothing according to Müller et al. (2019).

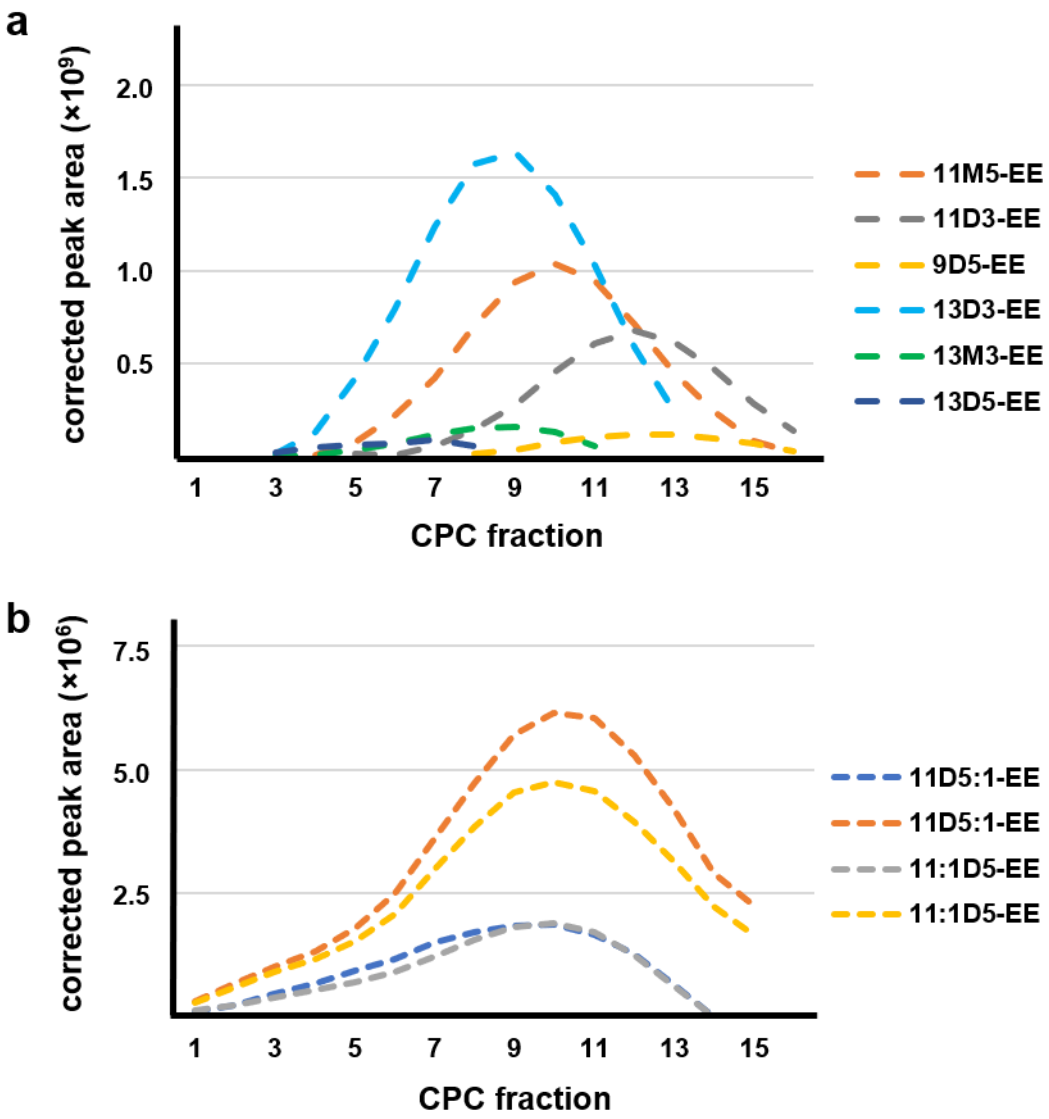

FIGURE S2. CPC elution profile (excerpts fractions CPC_1-CPC_{16}) showing the saturated FuFA except of 11D5-EE (**a**) and the four unsaturated 11D5-EE (u11D5-EE) isomers (**b**). The peak areas were recorded with different GC/MS-SIM methods for FuFA and unsaturated FuFA. The peak area of FuFA-EE (**a**) and u11D5-EE (**b**) were corrected via the internal standard 14:0-EE, smoothed (weighted 9-point smoothing according to Müller et al. (2019)), and plotted against the number of CPC fraction (CPC_1-CPC_{16}).

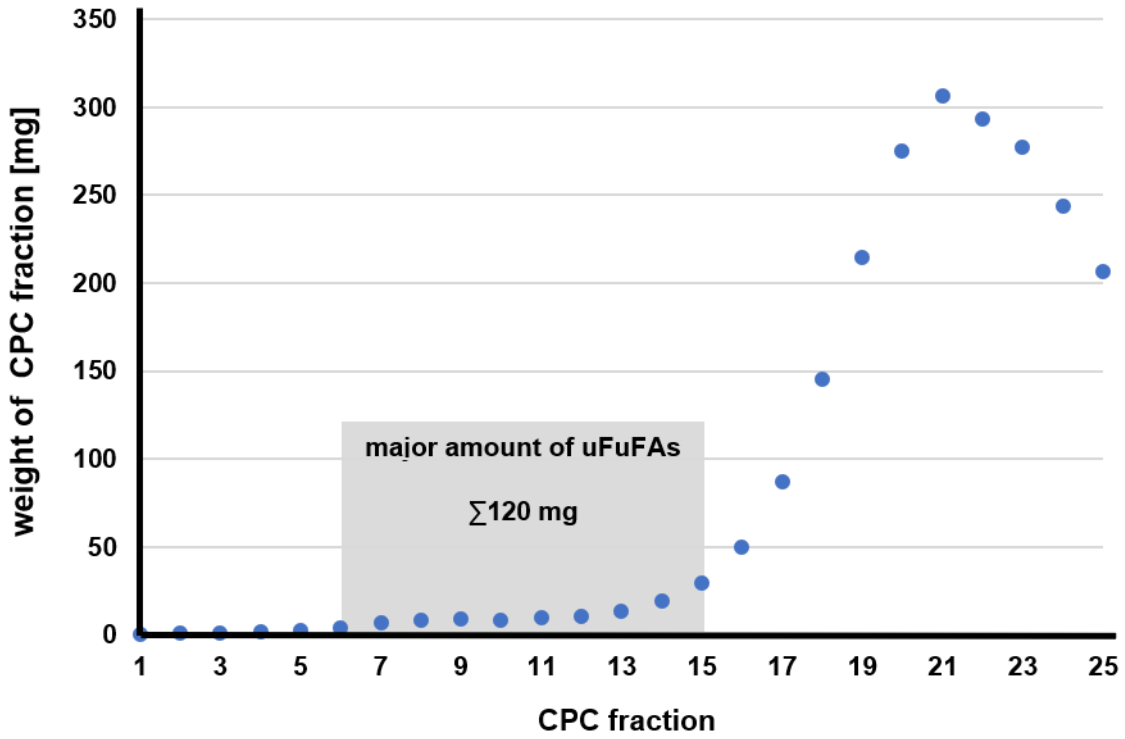

FIGURE S3. Smoothed mass elution profile of the CPC separation of a DHA-EE oil with marked elution region, where the majority of the unsaturated FuFA emitted. The total amount of these CPC-fractions ($\sum CPC_6$-CPC_{15}) was 120 mg. The masses per fraction were determined gravimetrically, the data was smoothed (weighted 9-point smoothing according to Müller et al. (2019)), and plotted against the number of CPC fraction (CPC_1-CPC_{25}).

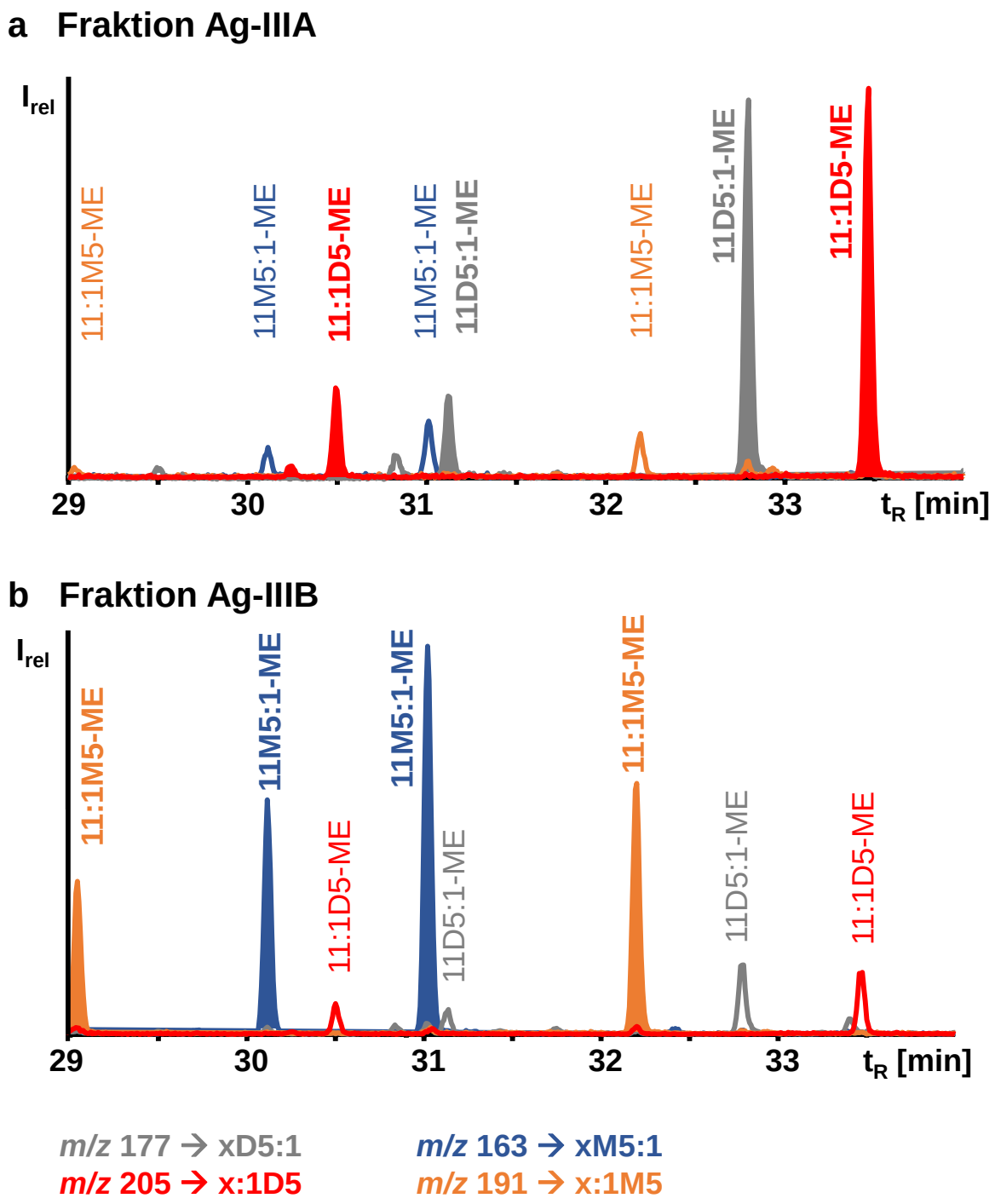

FIGURE S4. GC/MS-SIM chromatograms (excerpts) of silver ion fraction Ag-IIIA (**a**) and silver ion fraction Ag-IIIB (**b**) obtained from $CPC_{10\text{-}14}$-ME of an enriched and transesterified DHA-EE oil. The characteristic ions tracks for unsaturated xD5-FuFA (*m/z* 177, *m/z* 205) and unsaturated xM5-FuFA (*m/z* 163, *m/z* 191) are shown. The unsaturated 11D5-ME isomers eluted mainly in fraction Ag-IIIA (**a**) before the unsaturated 11M5-ME isomers, which eluted in fraction Ag-IIIB (**b**).

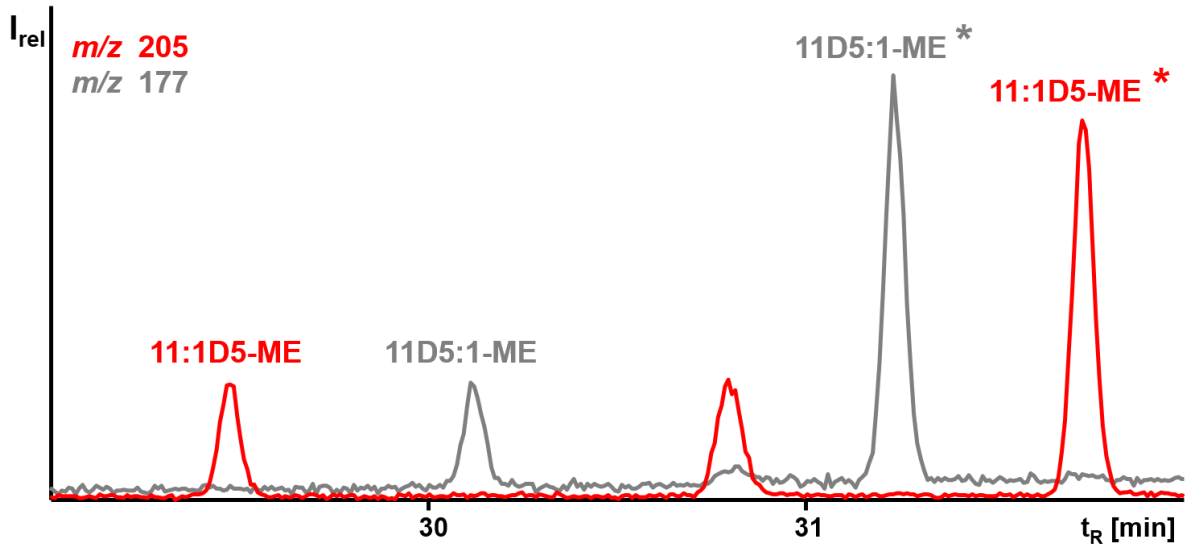

FIGURE S5. Re-examination of a GC/MS-SIM chromatogram of uFuFA as methyl esters in a spinach sample in silver ion fraction III originally recorded by Kirres and Vetter (2018). Only the later eluting 11D5:1-ME and 11:1D5-ME isomers marked with an asterisk (*) were initially reported by Kirres and Vetter (2018).

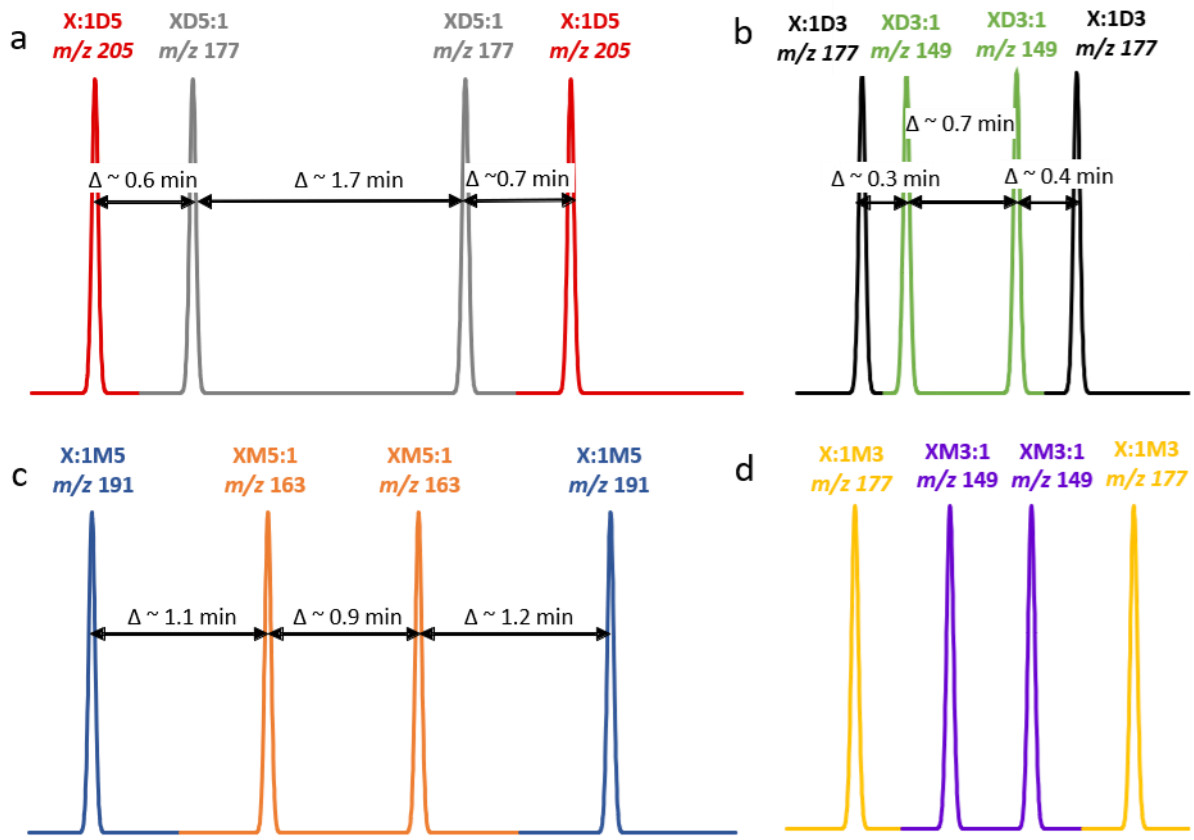

FIGURE S6. Elution pattern of (**a**) unsaturated XD5-ME (determined via unsaturated 11D5-ME isomers), (**b**) XD3-ME (determined via unsaturated 11D3-ME isomers), (**c**) XM5-ME (mean of unsaturated 11M5-ME and unsaturated 9M5-ME, and (**d**) the presumed elution order of XM3-ME (distances not determined due to the absence of unsaturated XM3-ME isomers in the investigated samples) on a polar Rtx-2330 column. For the individual isomers, the characteristic *m/z* of base peak were given. The underlying data (retention times differences) were derived from GC/MS chromatograms. The peak areas were set equal for all isomers for better readability and did not reflect the actual isomer ratio in the samples.

Tables

TABLE S1. Abbreviations and GC/MS data (relative retention time, M^+ and important fragment ions) of uFuFA-MEs and their relative abundances in full scan spectra

	Abbr.[a]	t_R rel[b]	furan moiety[c]	base peak	$[M]^+$	α-cleavage[d]	$[M-31]^+$ [e]
9-(3-Methyl-5-pentylfuran-2-yl)-non-8-enoic acid-ME	9:1M5 A	0.93	121.1 (14.9%)	191.1 (100%)	320.2 (28.2%)	263.2 (1.1%)	289.2 (7.3%)
	9:1M5 B	1.01	121.1 (14.2%)	191.1 (100%)	320.2 (30.9%)	263.2 (3.9%)	289.2 (7.3%)
9-(3-Methyl-5-pent-1-enylfuran-2-yl)-nonanoic acid-ME	9M5:1 A	0.97	121.1 (35.9%)	163.1 (100%)	320.2 (40.7%)	291.2 (18.2%)	289.2 (7.4%)
	9M5:1 B	1.05	121.1 (33.2%)	163.1 (100%)	320.2 (44.1%)	291.2 (18.3%)	289.2 (8.7%)
11-(3-Methyl-5-pentylfuran-2-yl)-undec10-enoic acid-ME	11:1M5 A	1.06	121.1 (23.7%)	191.1 (100%)	348.3 (29.0%)	291.2 (4.7%)	317.3 (8.8%)
	11:1M5 B	1.17	121.1 (19.8%)	191.1 (100%)	348.3 (31.3%)	291.2 (5.0%)	317.3 (7.8%)
11-(3-Methyl-5-pent-1-enylfuran-2-yl)-undecanoic acid-ME	11M5:1A	1.10	121.1 (35.4%)	163.1 (100%)	348.3 (41.9%)	319.2 (14.2%)	317.3 (6.8%)
	11M5:1 B	1.13	121.1 (33.2%)	163.1 (100%)	348.3 (46.5%)	319.2 (15.1%)	317.3 (6.9%)
11-(3.4-Dimethyl-5-pentylfuran-2-yl)-undec-10-enoic acid-ME	11:1D5 A	1.11	135.1 (31.2%)	205.2 (100%)	362.3 (42.6%)	305.2 (28.7%)	331.3 (8.8%)
	11:1D5 B	1.22	135.1 (28.0%)	205.2 (100%)	362.3 (46.4%)	305.2 (29.1%)	331.3 (12.1%)
11-(3.4-Dimethyl-5-pent-1-enyl-furan-2-yl)-un-decanoic acid-ME	11D5:1 A	1.13	135.1 (40.7%)	177.1 (100%)	362.3 (71.8%)	333.2 (59.2%)	331.3 (9.3%)
	11D5:1 B	1.20	135.1 (35.4%)	177.1 (100%)	362.3 (61.1%)	333.2 (59.1%)	331.3 (8.9%)
11-(3.4-Dimethyl-5-propylfuran-2-yl)-undec-10-enoic acid-ME	11:1D3 A	1.08	135.1 (34.3%)	177.1 (100%)	334.2 (63.0%)	305.2 (65.0%)	303.2 (10.0%)
	11:1D3 B	1.12	135.1 (23.1%)	177.1 (100%)	334.2 (44.9%)	305.2 (29.0%)	303.2 (11.1%)
11-(3.4-Dimethyl-5-prop-1-enyl-furan-2-yl)-un-decanoic acid-ME	11D3:1 A	1.09	135.1 (1.4%)	149.1 (100%)	334.2 (29.2%)	333.2 (0.8%)	303.2 (4.5%)
	11D3:1 B	1.11	135.1 (1.8%)	149.1 (100%)	334.2 (31.1%)	333.2 (0.0%)	303.2 (3.6%)

[a] abbreviation according to Wendlinger et al. (2016). A indicates the first eluting isomer, B the second eluting one; [b] retention time relative to 11D5-ME; [c] furan moiety identification ion according to Wendlinger *et al.* (2016); [d] characteristic fragment for methyl ester; [e] α-cleavage of the methoxy group

TABLE S2. Important mass spectral data for the identification of uFuFA: *m/*z of the base peaks and *m/*z of the furan moiety identification fragment of possible uFuFA isomers according to Kirres and Vetter (2018). The most common occurring uFuFA are marked in bold.

base peak (*m/z*)	205		191		177		163		149	
furan moiety identification ion (*m/z*)	121	135	121	135	121	135	121	135	121	135
corresponding F-acid	X:1M6 XM8:1	**X:1D5** XD7:1	**X:1M5** XM7:1	X:1D4 XD6:1	X:1M4 XM6:1	**X:1D3** **XD5:1**	**X:1M3** **XM5:1**	X:1D2 XD4:1	X:1M2 XM4:1	X:1D1 **XD3:1**

TABLE S3. Important mass spectral data for the identification of uFuFA: *m/*z of the molecular ions (M^+) of possible uFuFA-ME isomers according to Kirres and Vetter (2018) including positional isomers having the double bond in the alkylen or carboxyalkylen chain.

molecular ion (m/z)	320	334	348	362	376	390
uFuFA isomers with carboxy-alkenyl chain	8:1D5 9:1M5 10:1D3 11:1M3	9:1D5 10:1M5 11:1D3 12:1M3	10:1D5 11:1M5 12:1D3 13:1M3	11:1D5 12:1M5 13:1D3 14:1M3	12:1D5 13:1M5 14:1D3 15:1M3	13:1D5 14:1M5 15:1D3 16:1M3
uFuFA isomers with alkenyl chain	8D5:1 9M5:1 10D3:1 11M3:1	9D5:1 10M5:1 11D3:1 12M3:1	10D5:1 11M5:1 12D3:1 13M3:1	11D5:1 12M5:1 13D3:1 14M3:1	12D5:1 13M5:1 14D3:1 15M3:1	13D5:1 14M5:1 15D3:1 16M3:1

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Wendlinger C, Hammann S, Vetter W. Detailed study of furan fatty acids in total lipids and the cholesteryl ester fraction of fish liver. Food Anal. Methods. 2016;9:459–468. doi:10.1007/s12161-015-0211-x