Received: 8 August 2022 | Revised: 14 October 2022 | Accepted: 1 November 2022

DOI: 10.1002/lipd.12364

ORIGINAL ARTICLE

AOCS WILEY

Geometrical and positional isomers of unsaturated furan fatty acids in food

Franziska Müller | **Tim Hammerschick** | **Walter Vetter**

Institute of Food Chemistry (170b), University of Hohenheim, Stuttgart, Germany

Correspondence
Walter Vetter, Institute of Food Chemistry (170b), University of Hohenheim, D-70599 Stuttgart, Germany.
Email: walter.vetter@uni-hohenheim.de

Abstract

Furan fatty acids (FuFA) are important antioxidants found in low concentrations in many types of food. In addition to conventional FuFA which normally feature saturated carboxyalkyl and alkyl chains, a few previous studies indicated the FuFA co-occurrence of low shares of unsaturated furan fatty acids (uFuFA). For their detailed analysis, the potential uFuFA were enriched by centrifugal partition chromatography (CPC) or countercurrent chromatography (CCC) followed by silver ion chromatography from a 4,7,10,13,16,19-docosahexaenoic acid ethyl ester oil, a 5,8,11,14,17-eicosapentaenoic acid ethyl ester oil and a latex glove extract. Subsequent gas chromatography with mass spectrometry (GC/MS) analysis enabled the detection of 16 individual uFuFA isomers with a double bond in conjugation with the central furan moiety. In either case, four instead of two uFuFA isomers previously reported in food, respectively, were detected by GC/MS. These isomers showed characteristic elution and abundance patterns in GC/MS chromatograms which indicated the presence of two pairs of *cis*/*trans*-isomers (geometrical isomers).

KEYWORDS
antioxidant, centrifugal partition chromatography, countercurrent chromatography, GC/MS, silver ion chromatography, unsaturated furan fatty acid

INTRODUCTION

Furan fatty acids (FuFA) are valued food constituents due to their effective radical scavenging and antioxidant properties (Ishii et al., 1989; Lauvai et al., 2019; Lemke et al., 2014; Masuchi Buscato et al., 2020; Okada et al., 1990, 1996; Spiteller, 2005). These minor fatty acids are biosynthesized by plants (Batna & Spiteller, 1991, 1993; Mawlong et al., 2016; Scheinkönig & Spiteller, 1991, 1993), algae (Batna et al., 1993; Spiteller, 2005) and bacteria (Lemke et al., 2014; Lemke et al., 2020; Shirasaka et al., 1997; Spiteller, 2005). Via feed, FuFA also enter marine and terrestrial animals, and relatively high amounts were detected in fish and dairy products (Spiteller, 2005; Vetter et al., 2016; Wendlinger & Vetter, 2014).

Structurally, FuFA are carrying the eponymous furan moiety embedded in the fatty acid acyl chain. Specifically, the typical odd numbered carboxyalkyl

Abbreviations: 11:1D3, 11-(3,4-dimethyl-5-propylfuran-2-yl)undec-10-enoic acid; 11:1D5, 11-(3,4-dimethyl-5-pentylfuran-2-yl)undec-10-enoic acid; 11:1M5, 11-(3-methyl-5-pentylfuran-2-yl)undec-10-enoic acid; 11D3, 11-(3,4-dimethyl-5-propylfuran-2-yl)undecanoic acid; 11D3:1, 11-(3,4-dimethyl-5-prop-1-enyl-furan-2-yl)undecanoic acid; 11D5, 11-(3,4-dimethyl-5-pentylfuran-2-yl) undecanoic acid; 11D5:1, 11-(3,4-dimethyl-5-pent-1-enyl-furan-2-yl)undecanoic acid; 11M5, 11-(3-methyl-5-pentylfuran-2-yl)undecanoic acid; 11M5:1, 11-(3-methyl-5-pent-1-enyl-furan-2-yl)undecanoic acid; 13D3, 13-(3,4-dimethyl-5-propylfuran-2-yl)tridecanoic acid; 13M3, 13-(3-methyl-5-propylfuran-2-yl) tridecanoic acid; 9:1M5, 9-(3-methyl-5-pentylfuran-2-yl)non-8-enoic acid; 9D5, 9-(3,4-dimethyl-5-pentylfuran-2-yl)nonanoic acid; 9M5:1, 9-(3-methyl-5-pent-1-enyl-furan-2-yl)nonanoic acid; Ag-fraction, silver ion chromatography fraction; CCC, countercurrent chromatography; CPC, centrifugal partition chromatography; D-FuFA, β-/β′-dimethylated furan fatty acid; DHA, 4,7,10,13,16,19-docosahexaenoic acid (22:6 *n*-3); DPA*n*-3, 7,10,13,16,19-docosapentaenoic acid (22:5 *n*-3); DPA*n*-6, 4,7,10,13,16-docosapentaenoic acid (22:5 *n*-6); EE, ethyl ester; EPA, 5,8,11,14,17-eicosapentaenoic acid (20:5 *n*-3); FAME, fatty acid methyl ester; FuFA, furan fatty acid; GC/MS, gas chromatography with mass spectrometry; IS, internal standard; ME, methyl ester; M-FuFA, β-monomethylated furan fatty acid; PUFA, polyunsaturated fatty acid; SIM, selected ion monitoring; uFuFA, unsaturated furan fatty acid.

chain in the α-position of the furan moiety usually consists of 7–13 carbon atoms, while most commonly propyl and pentyl chains are attached in the α′-position (Spiteller, 2005; Wendlinger & Vetter, 2014).

Additional methyl groups on the furan moiety allow a distinction between (i) the family of β-/β′-dimethylated FuFA (D-FuFA) and (ii) β-monomethylated FuFA (M-FuFA) (Müller et al., 2020; Spiteller, 2005). Next to these conventional FuFA, a few papers reported on the occurrence of FuFA with an additional double bond (unsaturated FuFA, in the following uFuFA), which is most likely located in conjugation with the furan moiety (Boselli et al., 2000; Kirres & Vetter, 2018; Pacetti et al., 2010; Schödel & Spiteller, 1987; Uchida et al., 2018; Wendlinger et al., 2016). Namely, uFuFA were very scarcely detected (∼5% of the papers on FuFA mentioned in the review article by Xu et al. (2017)).

Overall, very little is known about uFuFA in terms of their occurrence and the factors influencing their formation. Semiquantitative analyses indicated uFuFA amounts of 9–240 μg/100 g fresh weight in vegetables, grass or herbs (Kirres & Vetter, 2018), and 45–175 μg/100 g in extra virgin olive oil (Boselli et al., 2000). Further, uFuFA were detected in beef liver homogenisate (Schödel & Spiteller, 1987) and in the steryl ester fraction of hepatopancreas of crayfish (*Procambarus clarkii*, 0.28% of total fatty acids in this lipid fraction [Ishii et al., 1988]). These trace amounts, the chemical lability of FuFA (Masuchi Buscato et al., 2020; Vetter & Wendlinger, 2013; Wakimoto et al., 2011), and the lack of commercially available reference standards are severely restricting progress in research on uFuFA. Yet, a common feature of all reports on uFuFA in food was that they were co-occurring with structurally related and more abundant D-FuFA (Boselli et al., 2000; Kirres & Vetter, 2018; Pacetti et al., 2010; Uchida et al., 2018; Wendlinger et al., 2016). Accordingly, uFuFA could be more commonly occurring in food than currently anticipated.

In this study we aimed to get more insights into the presence and variety of food-relevant uFuFA in three potential sources. For this purpose, an initial enrichment step was performed with countercurrent chromatography (CCC) and the related centrifugal partition chromatography (CPC) technique. CCC and CPC are all-liquid based instrumental, semi-preparative chromatographic techniques which take advantage of a biphasic solvent system whereof one phase is being used as mobile and the other as stationary phase (Friesen et al., 2015; Ito, 2005). CCC and CPC are well suited for the isolation of natural products (Friesen et al., 2015; Skalicka-Woźniak & Garrard, 2014) and have also been used before for the enrichment of minor lipid compounds (Berthod et al., 2009; Hammann et al., 2015; Hammerschick et al., 2020). In the latter case, samples fractionated by CCC or CPC can be subsequently analyzed thoroughly, for example, by gas chromatography with mass spectrometry (GC/MS) (Hammerschick et al., 2020; Schröder & Vetter, 2013). Using this approach, several minor unsaturated tocopherols such as tocomonoenols and tocodienols were detected in a saponified pumpkin oil by prior CCC separation (Kröpfl et al., 2022). Without the CCC separation, these substances would have been overlooked in the sample. This concept was implemented in our study on uFuFA. Namely, three samples rich in FuFA were fractionated and then subjected to silver ion chromatography for further enrichment of uFuFA.

MATERIALS AND METHODS

Nomenclature

Individual FuFA were abbreviated using the short number-letter-number code proposed by Vetter et al. (2012) which is based on (i) the number of carbon atoms in the carboxyalkyl chain, (ii) a letter for the furan moiety including its substituents in β/β′-positions (D for D-FuFA or M for M-FuFA), and (iii) the number of carbon atoms in the alkyl residue. Hence, 11-(3,4-dimethyl-5-propylfuran-2-yl)-undecanoic acid is denoted 11D3. Within this system, the related uFuFA is denoted as 11:1D3 if the double bond is located in the carboxyalkyl chain or as 11D3:1 if it is located in the alkyl chain (Wendlinger et al., 2016).

Chemicals

Acetonitrile (≥99.5%, for analysis *p.a.*) and diethyl ether (≥99.5%, for analysis *p.a.*; low in peroxide; stabilized with ∼7 ppm 2,6-di-*tert*-butyl-4-methylphenol (BHT)) were ordered from Bernd Kraft (Duisburg, Germany). Methanol, ethanol and *n*-hexane (all HPLC grade) were purchased from Th. Geyer (Renningen, Germany). Concentrated sulfuric acid (96%, for analysis *p.a.*) and concentrated hydrochloric acid (32%) were from Carl Roth (Karlsruhe, Germany). Sodium chloride (≥98.5%), sodium sulphate (≥99%), silver nitrate (≥99.5%, for analysis *p.a.*), and silica gel 60 were ordered from Sigma-Aldrich (Steinheim, Germany). Ultrapure water was generated in-house using a PURELAB Classic system (Elga, Celle, Germany).

Analytical standards

9-(3-methyl-5-pentylfuran-2-yl)-nonanoic acid ethyl ester (9M5-EE, purity ≥98%) was extracted from latex gloves, ethylated and purified according to Müller et al. (2020). 11-(3,4-dimethyl-5-pentylfuran-2-yl)-undecanoic acid ethyl ester (11D5-EE, purity ≥99%) was isolated

from a 4,7,10,13,16,19-docosahexaenoic acid ethyl ester oil (DHA-EE oil) which was generated from fish oil according to Müller et al. (2018). Myristic acid (14:0, purity >98%) was obtained from Fluka (Steinheim, Germany), ethylated and used as internal standard (IS) (Wendlinger et al., 2014). A Supelco 37 component fatty acid methyl ester (FAME) mix was purchased from Sigma-Aldrich (Steinheim, Germany).

Samples

Powder free disposable latex gloves were ordered from VWR (Darmstadt, Germany). Two enriched ω-3 fish oils gently gained from fish oil, more specifically a DHA-EE oil and a 5,8,11,14,17-eicosapentaenoic acid ethyl ester oil (EPA-EE oil) were kindly provided by KD Pharma (Bexbach, Germany). Both samples were generated with a comparable technology than those mentioned by Müller et al. (2018) and Gottstein et al. (2019) but from different batches of fish. Hence, the presence of FuFA was expectable but amounts were unknown.

Fractionation of the DHA-EE oil using centrifugal partition chromatography (CPC)

Fractionation of the DHA-EE oil was carried out with CPC due to its higher sample capacity compared to CCC. Specifically, 3.7 g DHA-EE oil was fractionated with a CPC 250 PRO instrument (Gilson, Middleton, WI, USA) equipped with a Gilson FC 204 fraction collector (Gilson, Middleton, WI, USA) and a Gilson AP-MOD-250 pump (Gilson, Middleton, USA) described in detail elsewhere (Hammerschick & Vetter, 2022). The CPC instrument was operated in ascending mode (upper phase mobile). The solvent system *n*-hexane/acetonitrile (1:1, v/v) was prepared by mixing the two solvents in a 2.5 l separation funnel. After shaking vigorously and equilibrating for 1 h, the phases were separated and degassed in an ultrasonic bath (Müller et al., 2018). The lower phase (stationary phase) was pumped into the 250 ml CPC rotor at 100 ml/min and a rotor speed of 500 rpm. Subsequently, the rotor speed was raised to 1600 rpm and the upper (mobile) phase was filled into the CPC system with 5 ml/min. After equilibration of the system ($S_f = 84\%$, 39 ml displacement of stationary phase), the DHA-EE oil sample, diluted in upper (mobile) phase (total volume 20 ml), was injected into the CPC system. Twenty-five 5 ml fractions were taken from 50–175 ml (fractions CPC_1-CPC_{25}). The flash 10 diode array detector (DAD; Ecom, Praha, Czech Republic) was set at $\lambda = 230$ nm and monitored the effluent throughout the run. Each fraction was transferred into a pre-weighted vial, evaporated with a gentle stream of nitrogen (38°C), re-dissolved in 1 ml *n*-hexane and aliquots were analyzed by GC/MS after suitable dilution (volume 1 ml) and the addition of 4 µg 14:0-EE as IS. Selected fractions were methylated and fractionated by silver ion chromatography (see below).

Fractionation of the EPA-EE oil using countercurrent chromatography (CCC)

The lower purity of EPA-EE oil compared to the DHA-EE oil prompted us to fractionate it with CCC rather than CPC. An AECS QuikPrep MK 8 device (AECS, London, UK) was used with a periphery described by Hammann et al. (2015). The solvent system *n*-hexane/acetonitrile (1:1, v/v) was used in tail-to-head mode with the temperature being kept at 22°C by an external cooling device (Arrifana, Blaupunkt, Heidenheim, Germany) (Müller et al., 2018). Coil 2 of bobbin 1 and coil 3 of bobbin 2 (total volume of 236 ml) were used for the separation and filled with stationary (lower) phase at 10 ml/min. After the flow rate was decreased to 2 ml/min, the rotation speed was set to the maximum value of 870 rpm. The upper (mobile) phase was pumped into the coils at 2 ml/min and the retention of the stationary phase was determined ($S_f = 88\%$, 29 ml displacement of stationary phase). EPA-EE oil (1.1 g, dissolved in 9 ml upper phase) was injected into the CCC system and the effluent was monitored at $\lambda = 230$ nm as already described for the CPC separation. Forty 4 ml fractions (fractions CCC_1-CCC_{40}) were sampled after 46 ml by means of a Gilson 203 B fraction collector (Middleton, WI, USA) and processed as outlined in the CPC section.

Methylation of CPC and CCC fractions

Two or three CPC ($CPC_{10} + CPC_{11} + CPC_{12}$ and $CPC_{13} + CPC_{14}$; each ~30 mg) and CCC fractions ($CCC_8 + CCC_9$; $CCC_{10} + CCC_{11}$; $CCC_{12} + CCC_{13}$; $CCC_{14} + CCC_{15}$) were combined (Figure 1), respectively, in amber glass test tubes and the resulting six samples were evaporated to dryness under a gentle stream of nitrogen (38°C). Then, 1% sulfuric acid in methanol was added for transesterification (80°C; 2 h) according to Müller et al. (2020). After cooling on ice, 2 ml saturated sodium chloride and 2 ml demineralized water were added. FAME were extracted with 2 ml *n*-hexane (Müller et al., 2020) and the methylated fractions $CPC_{10\text{-}12}$-ME and $CPC_{13\text{-}14}$-ME were combined to give one sample of $CPC_{10\text{-}14}$-ME. Afterwards the FAME extracts were subjected to silver ion chromatography (see below).

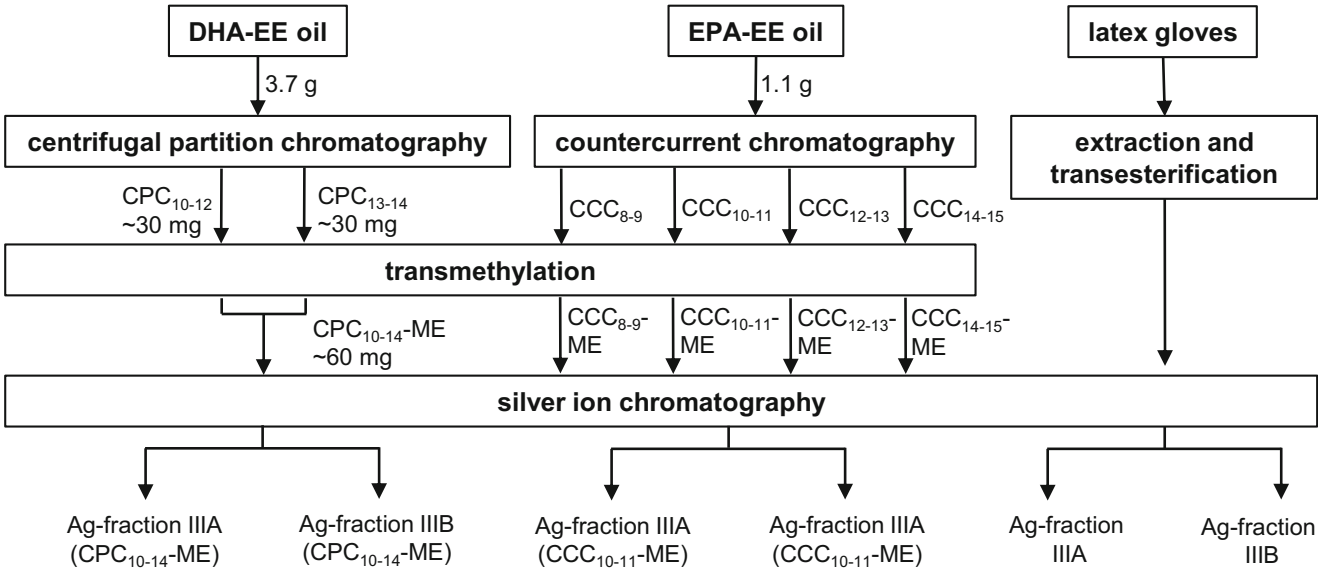

FIGURE 1 Flow chart of the enrichment and modifications of uFuFA from different sources, that is, 4,7,10,13,16,19-docosahexaenoic acid ethyl ester oil (DHA-EE oil), 5,8,11,14,17-eicosapentaenoic acid ethyl ester oil (EPA-EE oil), and latex gloves: After centrifugal partition chromatography (CPC) and countercurrent chromatography (CCC) the fractions (CPC_x or CCC_x) were converted in their methyl esters (ME). Silver ion chromatography fraction III was divided in two subfractions (Ag-fraction IIIA and Ag-fraction IIIB)

Extraction of FuFA from disposable latex gloves

Disposable latex gloves were extracted and prepared according to Müller et al. (2017) with slight modifications. In brief, about 60 g of disposable latex gloves were cut to small pieces and extracted with 900 ml *n*-hexane for 15 min in an ultrasonic bath (Müller et al., 2017). After filtration and solvent removal, the lipid extract was transesterified with 150 ml of 1% sulfuric acid in methanol (2 h, under reflux). Subsequently, 100 ml demineralized water and 100 ml saturated sodium chloride solution were added and FAME were extracted three times with 100 ml *n*-hexane (Müller et al., 2017). The organic phase was dried over sodium sulphate, filtered, rotary evaporated (38°C, 300 mbar) to ~5 ml and applied to silver ion chromatography.

Enrichment of uFuFA via silver ion chromatography

The stationary phase, silica gel with 20% $AgNO_3$ (w/w), was prepared according to Müller et al. (2020), except for the use of iron free silica gel (Buxbaum, 1967). A glass column (1 cm inner diameter) was packed with 5 g iron free silver ion silica gel deactivated with 1% ultrapure water (Müller et al., 2017). After conditioning the column with 10 ml of eluent of silver ion chromatography fraction I (Ag-fraction I; *n*-hexane/diethyl ether, 95.5:0.5, v/v), the FAME solution was applied and fractionated according to Müller et al. (2017) with some modifications as follows: silver ion chromatography fraction I (Ag-fraction I, *n*-hexane/diethyl ether 99.5:0.5, v/v, 70 ml), then 70 ml Ag-fraction II (*n*-hexane/diethyl ether, 97:3, v/v), next 25 ml Ag-fraction IIIA (*n*-hexane/diethyl ether, 80:20, v/v), and finally Ag-fraction IIIB (*n*-hexane/diethyl ether, 80:20, v/v, 25 ml). The volume of each fraction was reduced by rotary evaporation, the fractions were transferred into a 1.5 ml amber glass vial, and carefully evaporated to dryness with a stream of nitrogen (38°C). The residues were weighed and taken up in 1 ml *n*-hexane.

Gas chromatography coupled with electron ionization mass spectrometry (GC/MS)

GC/MS measurements of saturated FuFA-ME were performed with an HP 6890 GC plus/5973N MSD/6890 ALS system (Agilent, Waldbronn, Germany) equipped with an Rtx-2330 capillary column (90% biscyanopropyl, 10% cyanopropylphenyl polysiloxane; 60 m × 0.25 μm internal diameter × 0.1 μm film thickness; Restek Bellefonte, PA, USA) as reported by Müller et al. (2020). For the analysis of uFuFA, the GC oven temperature was programmed for 1 min at 60°C, then 13°C/min to 150°C, 3°C/min to 240°C and 20°C/min to 250°C (hold time 7 min). GC/MS spectra were recorded in full scan mode (*m*/*z* 50–550) after a solvent delay of 7 min. Further GC/MS analysis of uFuFA-ME were performed in the selected ion monitoring (SIM) mode: *m*/*z* 88.1 and *m*/*z* 101.1 (14:0-EE, IS), *m*/*z* 121.1 and *m*/*z* 135.1 (McLafferty-like ions), *m*/*z* 149.2, *m*/*z* 163.2, *m*/*z* 177.2, *m*/*z* 191.2, and *m*/*z* 205.2 (base peaks), as well as *m*/*z* 306.2, *m*/*z* 320.2, *m*/*z* 334.2, *m*/*z*

348.3, and *m*/*z* 362.4 (molecular ions, M^+) were measured throughout the run after a solvent delay of 7 min. GC/MS-SIM analysis of FuFA-EE was performed with a slightly modified method in which *m*/*z* 306.2 was substituted with *m*/*z* 376.4. All ions were measured to the first decimal place (Supplementary Table S1), but nominal masses will be reported below for better readability.

The share of uFuFA was estimated from peak area of the base peak (e.g., *m*/*z* 177 or *m*/*z* 205 in the case of unsaturated 11D5-EE (u11D5-EE) isomers) in the GC/MS-SIM chromatogram based on the peak area (*m*/*z* 88) of the IS 14:0-EE considering the GC/MS-SIM response factor of 11D5-EE (*m*/*z* 179) relative to 14:0-EE.

RESULTS

Detection of uFuFA in CPC fractions of the DHA-EE oil (∼96% DHA)

According to GC/MS analysis, CPC fractions of CPC_2-CPC_{15} of the DHA-EE oil contained the main FuFA 11D5-EE (maximum amount in $CPC_8 + CPC_9$; total amount ∼3.3% of DHA-EE oil). 11D5-EE eluted slightly prior to DHA-EE (22:6*n*-3-EE) (Supplementary Figure S1). Further ethyl esters of low abundance were 13D3-EE, 4,7,10,13,16-docosapentaenoic acid ethyl ester (DPA*n*-6-EE; 22:5*n*-6-EE), 7,10,13,16,19-docosapentaenoic acid ethyl ester (DPA*n*-3-EE; 22:5*n*-3-EE), 7,10,13,16-docosatetraenoic acid ethyl ester (22:4*n*-6-EE) and 10,13,16,19-docosatetraenoic acid ethyl ester (22:4*n*-3-EE) (Supplementary Figure S1). Application of GC/MS-SIM for saturated FuFA-ME/EE (Müller et al., 2020) enabled the detection of five additional FuFA-EE (i.e., 9D5-EE, 11D3-EE, 11M5-EE, 13M3-EE, and 13D3-EE) in CPC fraction CPC_3-CPC_{16} (Supplementary Figures S1–S2a). A similar GC/MS-SIM method was developed for uFuFA-ME/EE with 18–22 carbon atoms (calculated for free uFuFA) and a double bond position in conjugation to the furan moiety. The corresponding SIM values were derived from structural consideration and literature data (Jandke et al., 1988; Kirres & Vetter, 2018; Wendlinger et al., 2016). These covered (i) five molecular ions (2 u below those of the corresponding saturated FuFA), (ii) five base peaks, and (iii) two McLafferty-like ions which indicated the methylation degree of the furan moiety (section Materials and Methods). GC/MS-SIM screening of all CPC fractions produced strong evidence for the presence of low amounts of u11D5-EE isomers with a double bond in carboxyalkyl or alkyl chain in conjugation with the furan moiety mainly in fractions CPC_6-CPC_{15} (maximum amount CPC_{11}; Supplementary Figure S2b). As expected, u11D5-EE eluted slightly later from the CPC system than the saturated 11D5-EE (CPC_2-CPC_{14}, maximum amount $CPC_8 + CPC_9$) due to their slightly higher polarity resulting from an additional double bond. The total share of u11D5-EE was estimated at ∼0.008% of the DHA-EE oil or ∼0.23% of 11D5-EE, respectively. Due to the high sample capacity of the CPC system, this share still corresponded with ∼0.3 mg uFuFA in fractions CPC_6-CPC_{15} (much higher amounts of 11D5-EE, 22:5*n*-3-EE, 22:5*n*-6-EE, and further minor fatty acids ethyl ester aggravated the detection of uFuFA-EE in fractions $CPC_{>15}$ [Supplementary Figure S1-S2]). The calculated total sample weight in uFuFA containing CPC fractions ($\sum CPC_6$-CPC_{15}) was 0.12 g (Supplementary Figure S3). Accordingly, CPC enabled the enrichment of uFuFA by a factor of ∼30 (0.12 g vs. 3.7 g sample injected). Further enrichment of uFuFA from FuFA and PUFA was performed with silver ion chromatography, where the elution is mainly directed by the number of double bonds in a fatty acid (Dobson et al., 1995; Svetlana Momchilova, 2003). For this purpose, fractions CPC_{10}-CPC_{14} (total amount 62.4 mg) were selected based on favorable amounts of u11D5-EE versus PUFA-EE and 11D5-EE. These five CPC fractions were pooled, converted into methyl esters and subjected to silver ion chromatography (Figure 1). This method targeted saturated FuFA-ME in Ag-fraction II (*n*-hexane/diethyl ether, 97:3, v/v) (Müller et al., 2017). To ensure the complete elution of the much more abundant saturated FuFA-ME, the volume of Ag-fraction II was increased from 50 to 70 ml. Then, the elution of uFuFA-ME was continued with a considerably more polar eluent (Ag-fraction III: *n*-hexane/diethyl ether, 80:20, v/v) according to Kirres and Vetter (2018). Under these conditions, 18:2*n*-6-ME and 18:3*n*-3-ME were also eluted in this fraction (Müller et al., 2017), while methyl esters with more double bonds as those present in the sample (Supplementary Figure S1–S2) were assumed to remain on the column. Nevertheless, Ag-fraction III was split into two 25 ml-subfractions (named Ag-fraction IIIA and Ag-fraction IIIB) in order to obtain uFuFA fractions as pure as possible. GC/MS analysis of Ag-fraction IIIA from CPC_{10}-CPC_{14}-ME not only verified the successful performance of the silver ion chromatography but also enabled the detection of four u11D5-ME isomers (Figure 2a). Namely, all four peaks featured the molecular ion at *m*/*z* 362 (which is 2 u below the one of 11D5-EE, Figure 2b). Second, the $[M\text{-}31]^+$ fragment ion at *m*/*z* 331 (formed by α-cleavage; elimination of a methoxy radical) verified that (i) all four peaks originated from uFuFA-ME and (ii) the transmethylation was performed quantitatively (Figure 2c, d). Third, the characteristic fragment ion of the furan moiety of unsaturated D-FuFA-ME, which is formed by a McLafferty-like rearrangement including a hydrogen transfer to the oxygen atom, was shifted to *m*/*z* 135 (which is 12 u higher than in saturated D-FuFA-ME) (Boselli et al., 2000; Wendlinger et al., 2016). This shift produced first evidence that the additional double bond of the uFuFA-ME was in conjugation with the furan moiety (Figure 2b–d). Fourth, the characteristic base peak of FuFA-ME, that is, at *m*/*z* 179 in the case of 11D5-ME, was shifted to two

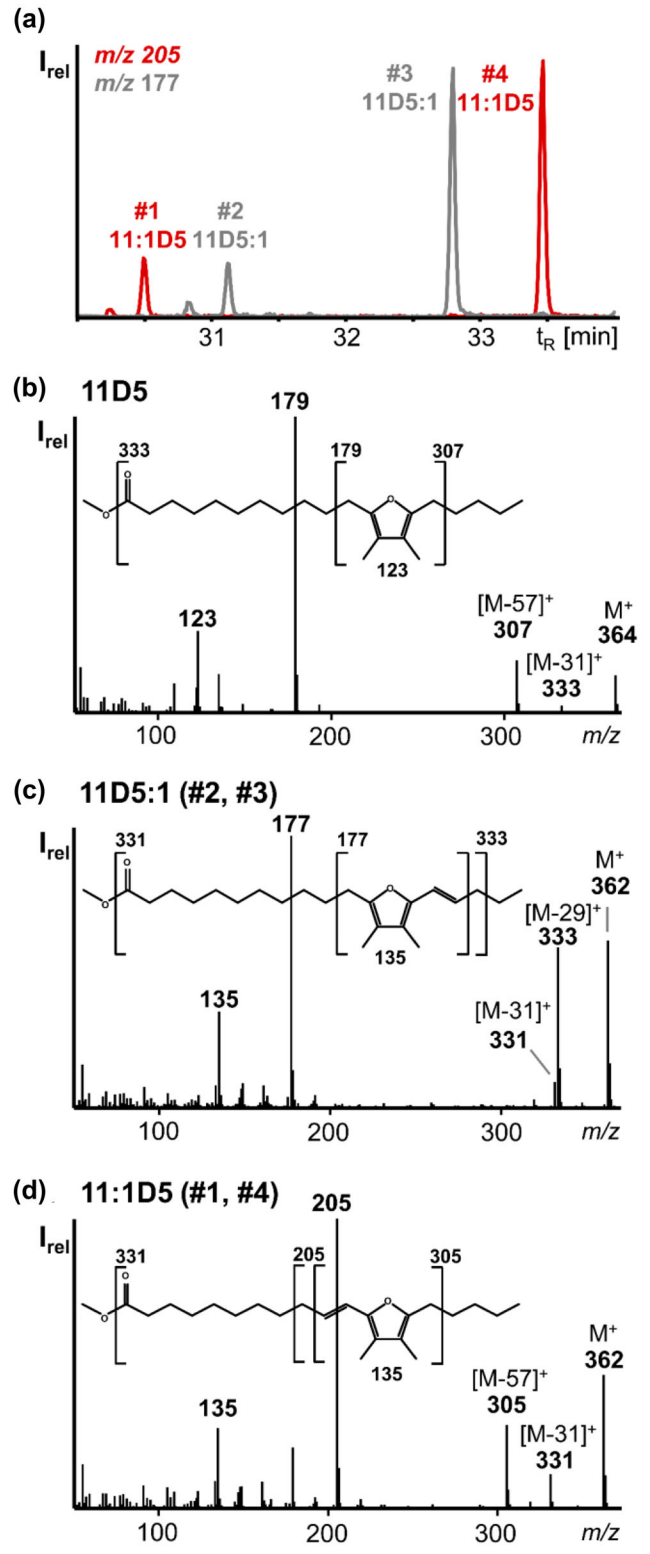

FIGURE 2 GC/MS chromatogram (*m*/*z* 177 and *m*/*z* 205) of fraction Ag-IIIA of $CPC_{10\text{-}14}$-ME (a), and mass spectra, molecular structure with marked fragmentations of 11D5-ME (b), 11D5:1-ME (peak #2 and peak #3 [c]) and 11:1D5-ME (peak #1 and peak #4 [d])

different *m*/*z* values in the GC/MS spectra of uFuFA-ME (Figure 2b–d). The base peak emerges from the cleavage in the carboxyalkyl chain between the first and the second carbon atom relative to the furan moiety (Figure 2b). Accordingly, this fragment ion allows one to determine the number of methyl substituents in the furan ring along with the length of the alkyl chain (here: two methyl groups and pentyl chain). In the case of uFuFA, information can be derived from the base peak about the position of the double bond.

Remarkably, the GC retention times of the four u11D5-ME isomers spread over a comparably wide range of 3.0 min (3.1–6.1 min after 11D5-ME). In addition, the two last eluting isomers were much higher abundant than the earlier eluting u11D5-ME isomers (Figure 2a, Supplementary Table S1). Also, the GC/MS spectra of isomers #1 and #4 (t_R 30.50 min and t_R 33.46 min, Figure 2a, red ion trace [dark gray in black and white print]) as well as isomers #2 and #3 (t_R 31.12 min and t_R 32.80 min, Figure 2a, gray ion trace [light gray in black and white print]), respectively, were virtually identical (Supplementary Table S1). Isomers #2 and #3 showed the base peak at *m*/*z* 177 (i.e., 2 u below 11D5-ME, Figure 3). Accordingly, these two isomers featured the additional double bond in the alkyl chain (Figure 2c and 3a). In addition, the fragment ion at *m*/*z* 333 ($[M\text{-}29]^+$, 26 u higher than in 11D5-ME), which is formed by allylic cleavage in the pentenyl residue (Figures 2c and 3b), confirmed the presence of two 11D5:1-ME isomers with the double bond in conjugation with the furan moiety.

By contrast, the base peak of isomers #1 and #4 was shifted to *m*/*z* 205. The mass difference between *m*/*z* 177 (isomers #2 and #3, t_R 31.12 min and t_R 32.80 min) and *m*/*z* 205 of 26 u indicated an additional "—CH═CH—" unit in the carboxyalkyl chain in conjugation with furan moiety compared to 11D5-ME (Figures 2b, d and 3a). Conjugation of the double bond with the furan ring was confirmed by the presence of *m*/*z* 305 ($[M\text{-}57]^+$), formed by allylic cleavage in the saturated pentyl chain (Figure 2d). The $[M\text{-}57]^+$ fragment ion is characteristic for FuFA with a pentyl residue (and this ion was thus also found shifted by 2 u to *m*/*z* 307 in the case of 11D5-ME, Figures 2b and 3b). Given this similarity, two pairs of isomers can only differ in the geometry of the double bond, that is, they must be *cis*-and *trans*-isomers, respectively.

Unexpectedly, silver ion chromatography Ag-fraction IIIB of sample $CPC_{10\text{-}14}$-ME (Figure 1) additionally featured four (even lower abundant) u11M5-ME isomers. Their presence agreed with the occurrence of 11M5 in the DHA-EE oil (Supplementary Figure S2). Similar to u11D5-ME isomers, the GC/MS chromatogram comprised two central isomers with a pentenyl chain (11M5:1-ME isomers, Figure 4a, orange ion trace [light gray in black and white print]) which were framed by two isomers with a double bond in the carboxyalkyl residue (11:1M5-ME isomers, Figure 4a, blue ion trace

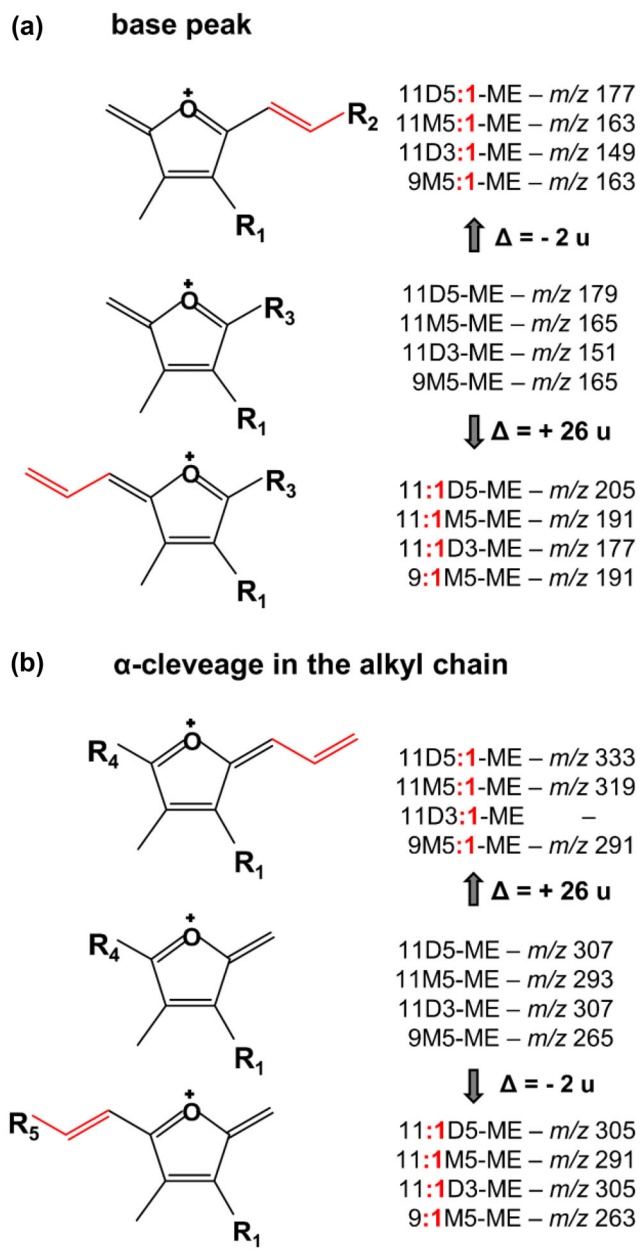

FIGURE 3 Structures of fragment ions of saturated FuFA-ME (central part) and uFuFA-ME with an additional double bond in the alkyl chain (top) and the carboxyalkyl chain (bottom) of (a) the base peak and (b) the fragment ion formed by α-cleavage in the alkyl/alkylene chain. With: R_1 = H for M-FuFA or R_1 = —CH_3 for D-FuFA; R_2 = —$(CH_2)_3$ for 11:1D5/11:1M5/9:1D5/11D5/11 M5/9M5-ME and R_2 = H for 11:1D3/11D3-ME; R_3 = —$(CH_2)_5$ for 11:1D5/11:1M5/9:1M5/11D5/11M5/9M5-ME and R_3 = —$(CH_2)_3$ for 11:1D3/11D3-ME; R_4 = —$(CH_2)_{10}$—$COOCH_3$ for 11D5:1/11M5:1/11D3:1/11D5/11M5/11D3-ME and R_4 = —$(CH_2)_8$—$COOCH_3$ for 9M5/9M5:1-ME R_5 = —$(CH_2)_8$—$COOCH_3$ for 11:1D5/11:1M5/11:1D3-ME and R_5 = —$(CH_2)_6$—$COOCH_3$ for 9:1M5-ME

[dark gray in black and white print]). All four isomers showed three characteristic fragment ions with the corresponding shifts (Figures 3 and 4c, e, Supplementary Tables S1-S3). Also, the elution range of u11M5-ME isomers and u11D5-ME isomers was similar (3.2 vs. 3.0 min). However, u13D3 isomers of 13D3, which was also present in the sample, could not be detected in CPC fractions.

Detection of uFuFA in CCC fractions of the EPA-EE oil

CCC fractionation of the EPA-EE oil and subsequent GC/MS analysis verified the presence of 9D5-EE, 11D3-EE, 11D5-EE, 9M5-EE and traces of 9D3-EE, 11M5-EE und 13D3-EE in fractions CCC_4-CCC_{17} (highest amount of 9D5-EE/11D3-EE in fraction CCC_8 and CCC_9). Since uFuFA could not be directly detected by GC/MS-SIM in any CCC fraction, four pools of two consecutive CCC fractions which potentially contained uFuFA (fractions $CCC_8 + CCC_9$; $CCC_{10} + CCC_{11}$; $CCC_{12} + CCC_{13}$; $CCC_{14} + CCC_{15}$) were created, respectively. After transmethylation and silver ion fractionation (Figure 1), Ag-fraction IIIA of CCC_{10+11}-ME enabled the detection of four u11D3-ME isomers (Figure 4b, d, f) whose GC/MS data resembled the features elaborated for u11D5-ME and u11M5-ME isomers (i.e., t_R profile, abundance ratio of fragment ions; Figures 3 and 4b, d, f). Yet, two peculiarities were observed in the GC/MS spectra of the two 11D3:1-ME isomers. First, the McLafferty-like ion of unsaturated D-FuFA at *m*/*z* 135 was only of very low abundance (<2% relative intensity). Second, the fragment formed by allyl cleavage within the propenyl chain — expected at *m*/*z* 333 (here: $[M\text{-}H]^+$) — was not detected at all (Figures 3b and 4e; Supplementary Table S1). However, these features were in line with Wendlinger et al. (2016) who detected one 11D3:1 isomer in fish. Despite the presence of 9D5-ME in the EPA-EE oil, u9D5-ME isomers were not detectable in the EPA-EE oil. This could be due to the lower abundance of 9D5 (~14% of 11D3) in the EPA-EE oil.

Enrichment and peak identification of u9M5 isomers from disposable latex gloves

Due to high amounts of 9M5 in latex (Hasma & Subramaniam, 1978; Liengprayoon et al., 2011) and latex gloves (Müller et al., 2017), an enrichment step via CCC or CPC was not necessary for this matrix (Figure 1). Instead, a transmethylated extract from disposable latex gloves was directly subjected to silver ion chromatography. Subsequent GC/MS analysis enabled the detection of four u9M5-ME isomers in Ag-fraction IIIB (and a smaller share in Ag-fraction IIIA). Again, all GC characteristics (elution pattern, abundance ratio) and MS characteristics ($[M]^+$ *m*/*z* 320, $[M\text{-}31]^+$ at *m*/*z*

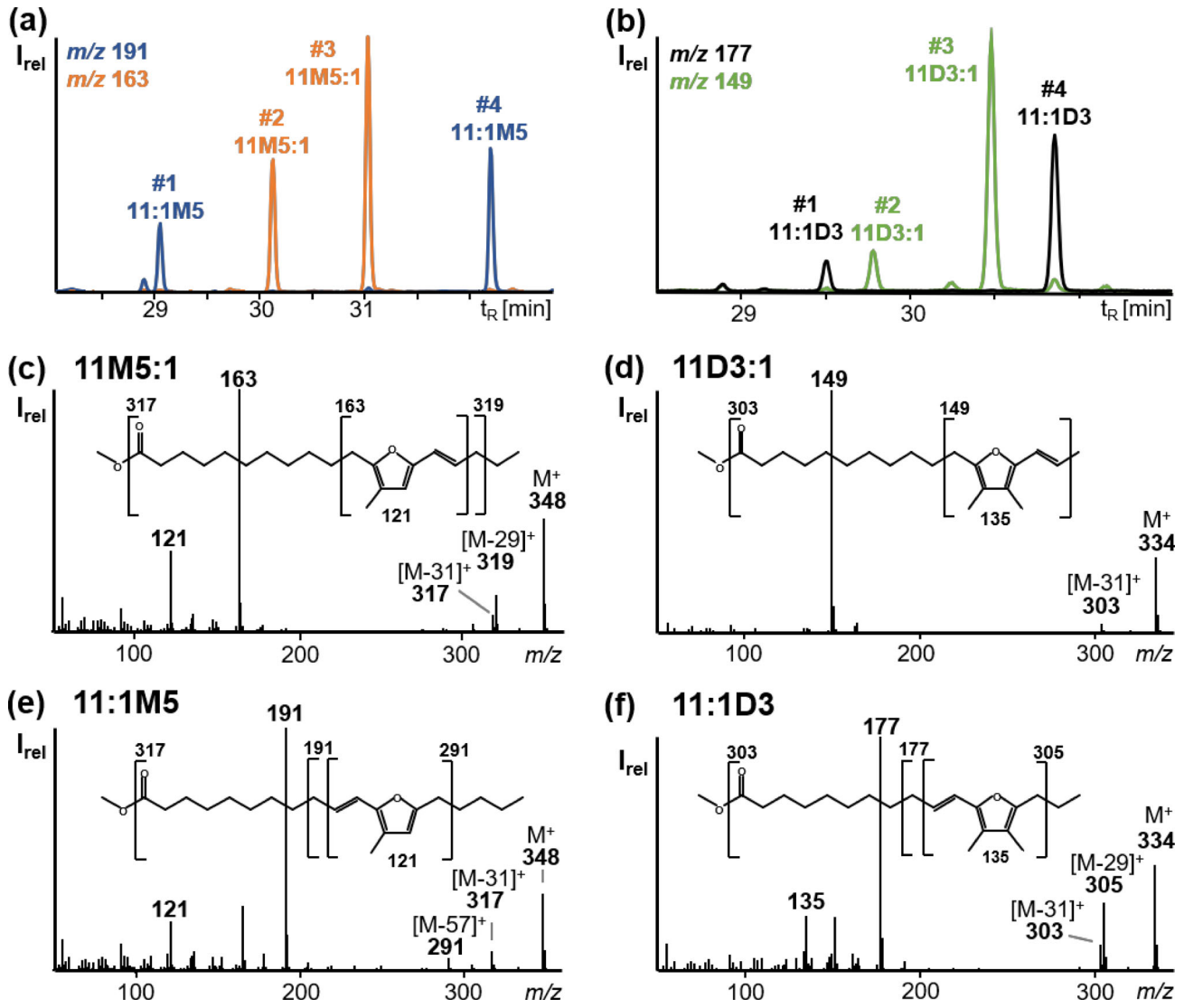

FIGURE 4 GC/MS chromatograms of the total ion current of (a) *m/z* 191 and *m/z* 163 of fraction $CPC_{10\text{-}14}$-ME-IIIB and (b) *m/z* 177 and *m/z* 149 of fraction $CCC_{10\text{-}11}$-ME-IIIA and mass spectra and molecular structure with M^+ and characteristic fragment ions of (c) 11M5:1-ME, (e) 11:1M5-ME, (d) 11D3:1-ME, and (f) 11:1D3-ME

289, and *m/z* 121 in all four u9M5-ME isomers; $[M\text{-}57]^+$ at *m/z* 263, *m/z* 191 for 9:1M5-ME vs. $[M\text{-}29]^+$ at *m/z* 291 and *m/z* 163 for 9M5:1-ME; Figure 3, Supplementary Tables S1-S3) agreed well with those described for other uFuFA-ME isomers.

DISCUSSION

Enrichment of uFuFA from natural sources by semi-preparative CPC and CCC and/or silver ion chromatography provided sufficient amounts that enabled the GC/MS analysis and verification of 16 uFuFA isomers in two fish oil fractions and a latex glove extract. Also, the slightly higher polarity of uFuFA in comparison to FuFA allowed us to predict their elution range in CCC and CPC. However, silver ion chromatography proved to be indispensable for the enrichment of uFuFA. Namely, this step enabled the separation of the more abundant saturated FuFA (i.e., 11D5-ME, 11D3-ME, 9D5-ME, and 11M5-ME) from the target compounds (uFuFA-ME isomers). Furthermore, the division of Ag-fraction III into two subfractions (Ag-fraction IIIA and Ag-fraction IIIB) enabled a partial separation between uD-FuFA and uM-FUFA (Supplementary Figure S4). Namely, u11D5-ME isomers (84% in Ag-fraction IIIA; 16% in Ag-fraction IIIB) eluted prior to u11M5-ME isomers (14% in Ag-fraction IIIA; 86% in Ag-fraction IIIB). These findings were comparable to saturated FuFA, since D-FuFA also eluted slightly earlier than M-FuFA. Noteworthily, the additional double bond enhanced the difference between the elution differences of D- and M-FuFA-ME.

Most importantly, the combination of the separation techniques allowed the investigation of very low abundant uFuFA. For example, four u11D5-ME isomers could be detected by GC/MS in the enriched DHA-EE oil with only 0.23% of the amount of 11D5-ME (3.3% of the DHA-EE oil).

GC/MS full scan spectra of 11:1D5-ME, 11D5:1-ME, 11:1D3-ME, and 11D3:1-ME (Supplementary Table S1) were in accordance with literature data (Boselli et al., 2000; Wendlinger et al., 2016). Yet, it is worth noting that all previous papers dealing with u11D5 or u11D3 isomers in food only reported one isomer of 11:1D5-ME and 11D5:1-ME, or 11:1D3-ME and 11D3:1-ME, respectively (Boselli et al., 2000; Ishii et al., 1988; Kirres & Vetter, 2018; Pacetti et al., 2010; Uchida et al., 2018; Wendlinger et al., 2016). However, re-examination of GC/MS data of Kirres and Vetter (2018), stored in our laboratory, unequivocally verified the presence of four unsaturated 11D5-ME isomers as well (Supplementary Figure S5). Apparently, and most likely a matter of their low abundance, only the high abundant isomers could be previously unequivocally assigned to uFuFA. Similarly, Pacetti et al. (2010) detected traces of one 11:1D5 and one 11D5:1 isomer in the same extract as 11D5 and Uchida et al. (2018) described only one u11D5 isomer (11:1D5) using HPLC/ESI-Q-TOF-MS which co-eluted with 13-hydroxy,13-(3,4-dimethyl,5-pentylfuran-2-yl)-tridecanoic acid on the HPLC column. Hence, the semipreparative approach of the present study was crucial for the discovery and assignment of the full array of four isomers. Once detected and assigned, subsequent GC/MS-SIM analysis of CPC fractions (without silver ion chromatography, as EE) verified that the four u11D5-isomers were natural constituents of the DHA-EE oil, which was intended for human nutrition and no artifacts produced during the transesterification procedure or silver ion chromatography.

Still, it is remarkable, that the GC retention times of the two pairs of *cis*/*trans*-isomers of uFuFA differed by 1.68 min (t_R 31.12 min and t_R 32.80 min, Figure 2, gray ion trace [light gray in black and white print]) and even 2.96 min (t_R 30.50 min and t_R 33.46 min, Figure 2a, red ion trace [dark gray in black and white print]), respectively for u11D5 isomers. Compared to that, GC retention times of conventional *trans*-fatty acids such as elaidic acid-ME (18:1*tr*-ME) differed from the corresponding *cis*-isomer oleic acid (18:1-ME) only by $\Delta t_R \sim 0.17$ min on a polar Rtx-2330 column (determined under the same chromatographic conditions). Also, the distance between 18:1*tr*-ME or 18:1-ME and 18:0-ME ($\Delta t_R \sim 0.43$ min and $\Delta t_R \sim 0.60$ min, respectively) was smaller compared to Δt_R 3.00 min between 11D5-ME and the first eluting 11:1D5-ME isomer. Similar retention time differences were observed for u11M5-ME and u9M5-ME isomers (Supplementary Figure S6). This pointed to distinct geometric reasons and a particular impact of the furan moiety on the conjugated double bond (spatially or electronically) leading to a unique elution pattern of uFuFA (Figure 2a and Supplementary Figure S6). Since, the retention time differences for u11D3 were smaller (Δt_R 1.4 min), indicated that the length of the alkyl chain had a strong impact on the interaction with the stationary GC phase (Supplementary Figure S6).

Up to now, four uFuFA isomers were only observed in two laboratory experiments with for 9D5 (Ishii et al., 1988; Jandke et al., 1988). Jandke et al. (1988) incubated isolated 9D5 with linoleic acid, lipoxidase 1 and ethandiol and studied the transformation products formed via the intermediate dioxoene (10,13-dioxo-11,12-dimethyl-octadec-11-enoic acid methyl ester) compounds. In this context, Jandke et al. (1988) not only observed four u9D5 isomers, but also reported their elution order to be (*Z*)-9:1D5-ME < (*Z*)-9D5:1-ME < (*E*)-9D5:1-ME < (*E*)-9:1D5-ME on an OV-101 capillary column without adding supportive spectroscopic data. Due to the lack of verifiable spectroscopic data, we could not assign the configuration of the geometric isomers to the peaks in GC/MS chromatograms.

Altogether, the methods used in the present study enabled the detection of 16 uFUFA-ME isomers of which only four had been reported before in literature. This set also included unsaturated M-FuFA which had not been previously mentioned in the literature. The GC/MS characterization of the novel uFuFA isomers indicated double bonds exclusively in conjugation on both sides of the furan moiety (positional isomers) and *cis*/*trans*-configuration (geometrical isomers). Yet, the origins and role of uFuFA in the sample remain a mystery. Jandke et al. (1988), Ishii et al. (1988), and Schödel and Spiteller (1987) suggested that uFuFA are obtained from dioxoenes which in turn are oxidation products of saturated FuFA, but the conditions of the formation of uFuFA in food products remained unclear.

However low abundant lipophilic antioxidants with double bonds in the side chains are not unusual in food. Also in the case of tocochromanols, such minor compound (i.e., α-, β- and γ-tocomonoenols and α- and γ-tocodienols) were detected after CCC fractionation in pumpkin seed oil (Kröpfl et al., 2022). Despite the relatively low concentrations, bioactivity and biofunctionality of these minor compounds cannot be excluded at this point. Hence, more research should be conducted in this field of uFuFA, including the unequivocal determination of *cis*/*trans* configuration in the case of geometrical uFuFA isomers.

AUTHOR CONTRIBUTIONS

Franziska Müller performed the analyses, evaluated the data, and wrote the original draft. Tim Hammerschick performed the CCC and CPC experiment. Walter Vetter conceived and supervised the research, provided resources, wrote, reviewed and edited the

manuscript. All authors contributed to and approved the final draft of the manuscript.

ACKNOWLEDGMENT

The authors thank the KD Pharma Group (Bexbach, Germany) for providing the enriched DHA-EE and EPA-EE oils. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

No human or animal subjects were used in this research.

ORCID

Franziska Müller https://orcid.org/0000-0002-1618-5586
Tim Hammerschick https://orcid.org/0000-0002-1478-6062
Walter Vetter https://orcid.org/0000-0002-5592-4265

REFERENCES

Batna A, Scheinkönig J, Spiteller G. The occurrence of furan fatty acids in *Isochrysis* sp. and *Phaeodactylum tricornutum*. BBA-Lipid Lipid Met. 1993;1166:171–6. https://doi.org/10.1016/0005-2760(93)90093-O

Batna A, Spiteller G. Herkunft des Sauerstoffatoms im Furanring von F-Säuren. Liebigs Ann Chem. 1991;1991:861–3. https://doi.org/10.1002/jlac.1991199101148

Batna A, Spiteller G. Biosynthesis of furan fatty acids in *Saccharum* species is Ca^{2+}-dependent. Phytochemistry. 1993;32:311–5. https://doi.org/10.1016/S0031-9422(00)94987-2

Berthod A, Ruiz-Angel MJ, Carda-Broch S. Countercurrent chromatography: people and applications. J Chromatogr A. 2009;1216:4206–17. https://doi.org/10.1016/j.chroma.2008.10.071

Boselli E, Grob K, Lercker G. Determination of furan fatty acids in extra virgin olive oil. J Agric Food Chem. 2000;48:2868–73. https://doi.org/10.1021/jf990857j

Buxbaum LH. Über die Oxydation von Cyclohexanol mit molekularem Sauerstoff. Liebigs Ann Chem. 1967;706:81–9. https://doi.org/10.1002/jlac.19677060109

Dobson G, Christie WW, Nikolova-Damyanova B. Silver ion chromatography of lipids and fatty acids. J Chromatogr B. 1995;671:197–222. https://doi.org/10.1016/0378-4347(95)00157-E

Friesen JB, McAlpine JB, Chen S-N, Pauli GF. Countercurrent separation of natural products: an update. J Nat Prod. 2015;78:1765–96. https://doi.org/10.1021/np501065h

Gottstein V, Müller M, Günther J, Kuballa T, Vetter W. Direct ^{1}H NMR quantitation of valuable furan fatty acids in fish oils and fish oil fractions. J Agric Food Chem. 2019;67:11788–95. https://doi.org/10.1021/acs.jafc.9b04711

Hammann S, Englert M, Müller M, Vetter W. Accelerated separation of GC-amenable lipid classes in plant oils by countercurrent chromatography in the co-current mode. Anal Bioanal Chem. 2015;407:9019–28. https://doi.org/10.1007/s00216-015-9068-5

Hammerschick T, Vetter W. Online hyphenation of centrifugal partition chromatography with countercurrent chromatography (CPC-CCC) and its application to the separation of saturated alkylresorcinols. Anal Bioanal Chem. 2022;414:5043–51. https://doi.org/10.1007/s00216-022-04136-x

Hammerschick T, Wagner T, Vetter W. Countercurrent chromatographic fractionation followed by gas chromatography/mass spectrometry identification of alkylresorcinols in rye. Anal Bioanal Chem. 2020;412:8417–30. https://doi.org/10.1007/s00216-020-02980-3

Hasma H, Subramaniam A. The occurrence of a furanoid fatty acid in *Hevea brasiliensis* latex. Lipids. 1978;13:905–7. https://doi.org/10.1007/BF02533847

Ishii K, Okajima H, Okada Y, Watanabe H. Effects of phosphatidylcholines containing furan fatty acid on oxidation in multilamellar liposomes. Chem Pharm Bull. 1989;37:1396–8. https://doi.org/10.1248/cpb.37.1396

Ishii K, Okajlma H, Koyamatsu T, Okada Y, Watanabe H. The composition of furan fatty acids in the crayfish. Lipids. 1988;23:818. https://doi.org/10.1007/BF02536228

Ito Y. Golden rules and pitfalls in selecting optimum conditions for high-speed counter-current chromatography. J Chromatogr A. 2005;1065:145–68. https://doi.org/10.1016/j.chroma.2004.12.044

Jandke J, Schmidt J, Spiteller G. Über das Verhalten von F-Säuren bei Oxidation mit Lipoxydase in Anwesenheit von SH-haltigen Verbindungen. Liebigs Ann Chem. 1988;1988:29–34. https://doi.org/10.1002/jlac.198819880107

Kirres C, Vetter W. Furan fatty acid content and homologue patterns of fresh green matrices. J Food Compos Anal. 2018;67:63–9. https://doi.org/10.1016/j.jfca.2018.01.001

Kröpfl A, Nemetz NJ, Goncalves Peca A, Vetter W. Countercurrent chromatography isolation of 11′-γ-tocomonoenol from pumpkin seed oil with detection of novel minor tocochromanols. J Am Oil Chem Soc. 2022;99:15–26. https://doi.org/10.1002/aocs.12559

Lauvai J, Becker A-K, Lehnert K, Schumacher M, Hieronimus B, Vetter W, et al. The furan fatty acid 9M5 acts as a partial ligand to peroxisome proliferator-activated receptor gamma and enhances adipogenesis in 3T3-L1 preadipocytes. Lipids. 2019;54:277–88. https://doi.org/10.1002/lipd.12152

Lemke RAS, Olson SM, Morse K, Karlen SD, Higbee A, Beebe ET, et al. A bacterial biosynthetic pathway for methylated furan fatty acids. J Biol Chem. 2020;295:9786–801. https://doi.org/10.1074/jbc.RA120.013697

Lemke RAS, Peterson AC, Ziegelhoffer EC, Westphall MS, Tjellström H, Coon JJ, et al. Synthesis and scavenging role of furan fatty acids. Proc Natl Acad Sci USA. 2014;111:E3450–7. https://doi.org/10.1073/pnas.1405520111

Liengprayoon S, Sriroth K, Dubreucq E, Vaysse L. Glycolipid composition of *Hevea brasiliensis* latex. Phytochemistry. 2011;72:1902–13. https://doi.org/10.1016/j.phytochem.2011.04.023

Masuchi Buscato MH, Müller F, Vetter W, Weiss J, Salminen H. Furan fatty acids in enriched ω-3 fish oil: oxidation kinetics with and without added monomethyl furan fatty acid as potential natural antioxidant. Food Chem. 2020;327:127087. https://doi.org/10.1016/j.foodchem.2020.127087

Mawlong I, Sujith Kumar MS, Singh D. Furan fatty acids: their role in plant systems. Phytochem Rev. 2016;15:121–7. https://doi.org/10.1007/s11101-014-9388-7

Müller F, Hogg M, Vetter W. Valuable furan fatty acids in soybeans and soy products. Eur Food Res Technol. 2020;246:1383–92. https://doi.org/10.1007/s00217-020-03497-w

Müller M, Hogg M, Ulms K, Vetter W. Concentrations, stability, and isolation of the furan fatty acid 9-(3-Methyl-5-pentylfuran-2-yl)-nonanoic acid from disposable latex gloves. J Agric Food Chem. 2017;65:7919–25. https://doi.org/10.1021/acs.jafc.7b02444

Müller M, Wasmer K, Vetter W. Multiple injection mode with or without repeated sample injections: strategies to enhance productivity in countercurrent chromatography. J Chromatogr A. 2018;1556:88–96. https://doi.org/10.1016/j.chroma.2018.04.069

Okada Y, Kaneko M, Okajima H. Hydroxyl radical scavenging activity of naturally occurring furan fatty acids. Biol Pharm Bull. 1996;19:1607–10. https://doi.org/10.1248/bpb.19.1607

Okada Y, Okajima H, Konishi H, Terauchi M, Ishii K, Liu I-M, et al. Antioxidant effect of naturally occurring furan fatty acids on oxidation of linoleic acid in aqueous dispersion. J Am Oil Chem Soc. 1990;67:858–62. https://doi.org/10.1007/BF02540506

Pacetti D, Alberti F, Boselli E, Frega NG. Characterisation of furan fatty acids in Adriatic fish. Food Chem. 2010;122:209–15. https://doi.org/10.1016/j.foodchem.2010.02.059

Scheinkönig J, Spiteller G. Herkunft der Methylsubstituenten in F-Säuren. Liebigs Ann Chem. 1991;1991:451–3. https://doi.org/10.1002/jlac.199119910182

Scheinkönig J, Spiteller G. F-Säure-haltige Phospholipidmoleküle in Zuckerrohrzellen. Liebigs Ann Chem. 1993;1993:121–4. https://doi.org/10.1002/jlac.199319930122

Schödel R, Spiteller G. Über das Vorkommen von F-Säuren in Rinderleber und deren enzymatischen Abbau bei Gewebeverletzung. Liebigs Ann Chem. 1987;1987:459–62. https://doi.org/10.1002/jlac.198719870354

Schröder M, Vetter W. Detection of 430 fatty acid methyl esters from a transesterified butter sample. J Am Oil Chem Soc. 2013;90:771–90. https://doi.org/10.1007/s11746-013-2218-z

Shirasaka N, Nishi K, Shimizu S. Biosynthesis of furan fatty acids (F-acids) by a marine bacterium *Shewanella putrefaciens*. BBA-Lipid Lipid Met. 1997;1346:253–60. https://doi.org/10.1016/S0005-2760(97)00042-8

Skalicka-Woźniak K, Garrard I. Counter-current chromatography for the separation of terpenoids: a comprehensive review with respect to the solvent systems employed. Phytochem Rev. 2014;13:547–72. https://doi.org/10.1007/s11101-014-9348-2

Spiteller G. Furan fatty acids: occurrence, synthesis, and reactions. Are furan fatty acids responsible for the cardioprotective effects of a fish diet? Lipids. 2005;40:755–71. https://doi.org/10.1007/s11745-005-1438-5

Svetlana Momchilova BN-D. Stationary phases for silver ion chromatography of lipids: preparation and properties. J Sep Sci. 2003;2003:261–70. https://doi.org/10.1002/jssc.200390032

Uchida H, Itabashi Y, Watanabe R, Matsushima R, Oikawa H, Suzuki T, et al. Detection and identification of furan fatty acids from fish lipids by high-performance liquid chromatography coupled to electrospray ionization quadrupole time-of-flight mass spectrometry. Food Chem. 2018;252:84–91. https://doi.org/10.1016/j.foodchem.2018.01.044

Vetter W, Laure S, Wendlinger C, Mattes A, Smith AWT, Knight DW. Determination of furan fatty acids in food samples. J Am Oil Chem Soc. 2012;89:1501–8. https://doi.org/10.1007/s11746-012-2038-6

Vetter W, Ulms K, Wendlinger C, van Rijn J. Novel non-methylated furan fatty acids in fish from a zero discharge aquaculture system. NFS J. 2016;2:8–14. https://doi.org/10.1016/j.nfs.2015.11.001

Vetter W, Wendlinger C. Furan fatty acids—valuable minor fatty acids in food. Lipid Technol. 2013;25:7–10. https://doi.org/10.1002/lite.201300247

Wakimoto T, Kondo H, Nii H, Kimura K, Egami Y, Oka Y, et al. Furan fatty acid as an anti-inflammatory component from the green-lipped mussel Perna canaliculus. Proc Natl Acad Sci USA. 2011;108:17533–7. https://doi.org/10.1073/pnas.1110577108

Wendlinger C, Hammann S, Vetter W. Various concentrations of erucic acid in mustard oil and mustard. Food Chem. 2014;153:393–7. https://doi.org/10.1016/j.foodchem.2013.12.073

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;9:459–68. https://doi.org/10.1007/s12161-015-0211-x

Wendlinger C, Vetter W. High concentrations of furan fatty acids in organic butter samples from the German market. J Agric Food Chem. 2014;62:8740–4. https://doi.org/10.1021/jf502975b

Xu L, Sinclair AJ, Faiza M, Li D, Han X, Yin H, et al. Furan fatty acids—beneficial or harmful to health? Prog Lipid Res. 2017;68:119–37. https://doi.org/10.1016/j.plipres.2017.10.002

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Müller F, Hammerschick T, Vetter W. Geometrical and positional isomers of unsaturated furan fatty acids in food. Lipids. 2023;58(2):69–79. https://doi.org/10.1002/lipd.12364