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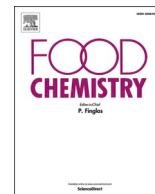
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Analysis of glycerol bound ω -oxo-fatty acids as ω -dioxane-FAME-derivatives[☆]

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ABSTRACT

ω -oxo-fatty acids, also known as aldehydic fatty acids, are major products of fatty acid oxidation and pose potential health risks. When bound to glycerol, ω -oxo-fatty acids (core aldehydes) can be ingested with food. Challenges in GC-MS quantification include the absence of an appropriate internal standard. Additionally, substantial analyte losses during sample preparation, caused by the high volatility of short-chain compounds, alter their pattern based on molecular weight. In this study, among various tested derivatization methods, the formation of ω -dioxane derivatives demonstrated improved recovery rates after three evaporation cycles. For methyl 7-oxo-heptanoate, recovery increased from 43 % to 88 %, while recovery rates for different chain lengths and a novel synthesized internal standard improved from a range of 43 %–76 % to 87 %–92 %. Additionally, ω -dioxane derivatives displayed favorable GC-MS behavior, enabling clear identification and increased sensitivity. Finally, ω -oxo-fatty acids were quantified as their ω -dioxane-derivatives in thermally treated sunflower and rapeseed oil.

1. Introduction

Fats and oils are important macronutrients as they provide about 35 % of the calorie uptake in an average Western diet (Deutsche Gesellschaft für Ernährung, 2015). They primarily consist of glycerol-bound fatty acids (FA) of different lengths and degrees of saturation. In addition to their caloric value, some polyunsaturated FAs such as eicosa-pentaenoic acid or docosahexaenoic acid are essential dietary components. They serve as precursors for various messenger substances and are an important part of cell membranes in the human body (Calder, 2015; Husted & Bouzinova, 2016).

When exposed to oxygen, particularly polyunsaturated FAs can

easily undergo lipid oxidation. This can occur, for example, when fats and oils used in food preparation are stored for extended periods, especially under sunlight and without refrigeration. However, the most severe conditions for lipid oxidation occur during food frying, especially deep frying. While the food is heated in hot oil for only a few minutes, the oil itself is possibly maintained at temperatures between 140 °C and 180 °C for several hours. During and after frying, large amounts of the frying oil (and consequently formed lipid oxidation products) can be absorbed into the food (Smith et al., 1985). Alongside the loss of valuable FAs, hydroperoxy FAs are formed, which quickly decompose to a wide range of secondary lipid oxidation products. The β -scission of an alkoxy radical, generated from the initial hydroperoxides, can result in

Abbreviations: RI_{FAME}, retention index of FAME; FA, fatty acid; FAME, fatty acid methyl ester; GC-MS, gas chromatography mass spectrometry; IS-ME, internal standard after transesterification (methyl 3-((1R*,2S*)-2-(3-oxopropyl)cyclopropyl)propanoate); IS-EE, internal standard before transesterification (ethyl 3-((1R*,2S*)-2-(3-oxopropyl)cyclopropyl)propanoate); LOD, limit of detection; LOQ, limit of quantification; S/N, signal to noise ratio; SPE, solid phase extraction; X-A, ω -oxo-fatty acid methyl ester with chain length X; X-A', derivatized ω -oxo-fatty acid methyl ester with chain length X; X-D, di fatty acid methyl ester with chain length X; X-K, keto fatty acid methyl ester with chain length X; X-K', derivatized keto fatty acid methyl ester with chain length X.

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cleavage at two distinct fracture sites. Cleavage at the C—C bond closer to the carboxyl end of the fatty acid can produce free short-chain aldehydes. Some of these volatile aldehydes, such as (E,E)-2,4-decadienal, exhibit strong aroma activity and contribute to the deep-fried, fatty odor of the oil. (Warner et al., 2001; Xu et al., 2022). Upon β -scission cleaving the C—C bond towards the methyl end of the FA, glycerol-bound short-chain ω -oxo-FAs (also referred to as aldehydic FA or core aldehydes) can be formed (Berdeaux et al., 2012). Bound to the glycerol backbone of the triacylglycerol (TG), the ω -oxo-FAs are not volatile and are ingested with the oil. Kamal-Eldin et al. identified the bound ω -oxo-FAs to be the most abundant of the short-chain glycerol bound oxidation products formed through hydroperoxide breakdown (Kamal-Eldin et al., 1997).

Likewise to other FA oxidation products, (Esterbauer, 1993) ω -oxo-FAs show toxic properties. They are predicted to be highly reactive (Sottero et al., 2018), e.g. as they interact with amino acids (Störmer et al., 2023). Studies showed that 9-oxo-9:0-FA affects hepatic metabolism (Kanazawa & Ashida, 1991; Minamoto et al., 1988) and could induce hepatic lipid peroxidation (Minamoto et al., 1985). In order to investigate the potential impact of ω -oxo-FAs on the human body, more data on the occurrence and quantity of ω -oxo-FAMES in food are required.

Various methods have already been employed to analyze ω -oxo compounds. Long chain fatty aldehydes were analyzed as dimethyl acetal derivatives by GC (Berdyshev, 2011). Oxidized triacylglycerols were isolated as dinitrophenylhydrazone derivatives by thin-layer chromatography and determined using LC-MS (Sjövali et al., 2002). For simpler datasets compared to analysis as TGs, the core aldehydes were analyzed as fatty acid methyl esters (FAME) using GC. Berdeaux et al. evaluated various methods for the conversion to FAMES, of which the methods in an alkaline medium - especially with sodium methanolate - were particularly suitable (Berdeaux, Marquez-Ruiz, & Dobarganes, 1999). This method was then used to identify many different saturated ω -oxo-FAMES - with 9-oxo-9:0-FAME (9-A) as the most abundant homologue - and also unsaturated ω -oxo-FAMES in heated 18:1-ME and 18:2-ME model systems (Berdeaux et al., 2012). Other matrices analyzed included used and unused deep-frying oil (Kamal-Eldin et al., 1997) and butter (Schröder & Vetter, 2013). The contents of some saturated ω -oxo-FAMES such as 7-oxo-7:0-ME (7-A), 8-oxo-8:0-ME (8-A) or 9-A were also already quantified in thermally treated 18:1-ME and 18:2-ME. Medium chain length saturated FAMES were used as internal standard (13:0-ME and 15:0-ME) and 9-A for external calibration (Berdeaux et al., 2002). This method was also used to quantify 9-A in roasted peanuts and thus investigate the interactions with amino acids (Störmer et al., 2022; Störmer et al., 2023).

A key issue in the analysis of ω -oxo-FAs is their volatility in the sample preparation after transesterification to ω -oxo-FAMES. Due to the β -scission, the portion remaining on the ester exhibits a significantly reduced molecular weight and, consequently, a markedly lower boiling point than its precursors, regular FAs. So far, saturated FAMES of short to medium chain length have been used as internal standards. However, these do not exhibit the same extraction properties and volatility as the ω -oxo-FAMES, which likely leads to different recovery rates in a multi-step sample preparation protocol. In addition to the volatility of ω -oxo-FAMES themselves, another challenge is the relationship between their volatility and chain length. The correlation can lead to either underestimation or overestimation of different ω -oxo-FAMES.

In our study, we developed a derivatization method for ω -oxo-FAMES to decrease their volatility and minimize the differences in volatility between individual ω -oxo-FAMES as well as the internal standard. As a new class-specific internal standard, a novel synthesized cyclopropyl modified ω -oxo FA ester (with a chain length matching the most relevant ω -oxo-FAMES) was used to enable the most accurate quantification of saturated ω -oxo-FAMES. The applicability of this method was demonstrated by the analysis of ω -oxo-FAMES in heat-treated sunflower and rapeseed oil.

2. Material and methods

2.1. Chemicals

Sodium methanolate (5 M in methanol), *tert*-butyl methyl ether (MTBE, $\geq 99.8\%$), dextro-martin-periodinane (97%), 1,6-hexanediol (99.0%), 1,2-octanediol (98%), *n*-hexane (HPLC grade), anhydrous sodium sulfite ($\geq 99.99\%$), 16-hexadecanolid (97%) and Ag-Ion-SPE cartridges were purchased from Sigma Aldrich (Steinheim, Germany). 1,3-propanediol ($\geq 98\%$), ethylene glycol (99%), sulfuric acid (95–97%), and ethyl acetate (HPLC grade) were purchased from Carl Roth (Karlruhe, Germany). 3-methyl-1,3-butandiol (98%), dichloromethane (HPLC grade), and glycerol monostearate were purchased from Thermo Fisher Scientific (Dreieich, Germany), methanol (HPLC grade) from Alfa Aesar (Heysham, England), NaHCO_3 (99%) from Merck Millipore (Darmstadt, Germany), silica gel 60; 0.04–0.063 mm from Macherey Nagel (Düren, Germany) and sodium chloride (99%) from Häberle (Lonsee-Ettlenschieß, Germany). Ozone was generated with an Ozone Generator BMT 802 N (Berlin, Germany).

2.2. Devices

For GC–MS analysis, an 8860 GC with a 7693 autosampler and a 5977B mass spectrometer (Agilent Technologies, Santa Clara, California) were used. Samples (1 μL) were injected using a split/splitless injector in splitless mode at 300 °C onto a HP 5 ms-UI column (30 m \times 0.25 mm; 0.25 μm ; Agilent Technologies (Santa Clara, California)). Helium (purity 5.0) was used as a carrier gas at a constant flow rate of 1.0 mL/min. The temperature program of the oven started at 50 °C and was heated up at a rate of 10 K/min to 300 °C and held for 5 min. The temperatures of the ion source and transfer line were set to 250 °C and 260 °C, respectively and electron ionization at 70 eV was used. Full scan data were recorded from m/z 50 to 650. This device was used for the identification and characterization of ω -oxo-FAMES and derivatization products and to test the effect of evaporation on the recovery rates of the ω -oxo-FAMES.

For the other GC–MS analysis, a Trace GC Ultra with an AS 3000 autosampler and a DSQ II mass spectrometer (Thermo Scientific) was used. Samples (1 μL) were injected using a split/splitless injector in splitless mode at 300 °C onto a DB5-MS column (30 m \times 0.25 mm; 0.25 μm) Agilent Technologies (Santa Clara, California)). Helium (purity 5.0) was used as a carrier gas at a constant flow rate of 1.0 mL/min. The temperature program of the oven started at 50 °C (held for 2 min) and was heated up at a rate of 5 K/min to 180 °C, then with 30 K/min to 300 °C (held for 5 min). The temperatures of the ion source and transfer line were set to 250 °C and 300 °C, respectively and electron ionization at 70 eV was used. Quantification of ω -oxo- and ω -dioxane-FAMES was performed in SIM-Mode monitoring m/z 74, 87, 115, 129, 140, 143, 157, 215, 229, 241, 243 and 257 (dwell time 50 ms). To support the identification of the FAMES every sample was measured additionally in full-scan using a mass range of m/z 50–650. This device was used for method validation and quantification of the ω -oxo-FAMES.

2.3. Methods

2.3.1. Preparation of standard substances

2.3.1.1. Preparation of methyl 16-oxohexadecanoate. 16-hexadecanolid was converted to methyl 16-hydroxyhexadecanoate as described previously by Christie (Christie, 1994) and Berdeaux et al. (Berdeaux, Márquez-Ruiz, & Dobarganes, 1999). To 1 g of the lactone, 2 mL of 5 M NaOMe in methanol and 5 mL MTBE were added to a test tube, sealed, and heated for 30 min at 50 °C. The resulting gel was dissolved with 100 mL of a mixture of MeOH/MTBE 1/1 (v/v). After adjusting the solution to a neutral pH using acetic acid, 50 mL of water were added, the

aqueous phase was extracted with 50 mL of MTBE three times and the combined organic phases extracted with a saturated NaCl-solution and water twice. The organic phase was dried over anhydrous Na₂SO₄ and the solvent was evaporated using a gentle stream of nitrogen. The identity of the product was confirmed by ¹H and ¹³C NMR spectroscopy as well as GC-MS after silylation.

The oxidation of 200 mg (0.5 mmol) methyl 16-hydroxyhexadecanoate with 3.5 eq of Dess-Martin-periodinane (1.75 mmol) and 800 mg of NaHCO₃ in 20 mL of DCM was performed following the protocol of Sønderkov et al. (Sønderkov et al., 2020). The suspension was stirred in a round bottom flask for 8 h at room temperature, after which the reaction was stopped by adding 20 mL of a saturated Na₂SO₃-solution. The aqueous phase was extracted three times with 100 mL of DCM and the combined organic phases were washed with 100 mL saturated NaCl-solution and 100 mL water twice. After drying the solution over anhydrous Na₂SO₄, the solvent was evaporated. The product was isolated by solid phase extraction (SPE) using activated silica gel and *n*-hexane/ethyl acetate 95/5 (v/v) as eluent. The identity of the product was confirmed by ¹H and ¹³C NMR spectroscopy (Supporting Information) as well as GC-MS.

2.3.1.2. Preparation of ethyl 3-((1*R,2*S**)-2-(3-oxopropyl)cyclopropyl)propanoate (8-oxo-4,5-methylene-8:0-EE (IS-EE)).** A two-step synthesis was performed: First, the intermediate bicyclo-[6.1.0]non-4-ene was prepared following the procedure reported by Cope et al. (Cope et al., 1962) from 1,5-cyclooctadiene (5 mL scale) via a cyclopropanation procedure. The crude reaction mixture containing unreacted starting material and bicyclo-[6.1.0]non-4-ene at an almost 1:1 ratio was submitted to the following ozonolysis procedure: 4.1 g of the crude reaction mixture (14.7 mmol) in 40 mL of DCM/EtOH 5/1 (v/v) and 2.0 g of anhydrous sodium bicarbonate were kept at -80 °C under an ozone stream until the solution turned pale blue. The ozone addition was stopped and nitrogen passed through the solution until the blue color was discharged, and then the cold bath was removed. The solution was filtered into a 500 mL, round-bottomed flask and 40 mL of toluene were added. The volume was reduced to approximately 40 mL by rotary evaporation. After dilution with 50 mL of DCM, the flask was cooled to 0 °C and 7.01 g of triethylamine (0.069 mol) and 14.16 g of acetic anhydride (0.138 mol) were added, and the solution was stirred under a nitrogen atmosphere for 15 min. The ice bath was removed and stirring was continued for 4 h. The solution was washed with 75 mL portions of aqueous 0.1 N HCl, aqueous 10 % NaOH, and saturated NaCl-solution. The organic layer was dried over anhydrous magnesium sulfate and filtered, and the solvent was removed by rotary evaporation. Flash chromatography failed to remove the remaining cyclooctadiene ozonolysis by-product, which had similar chromatographic properties to the desired compound, necessitating further purification using silver ion chromatography. A normal phase cartridge modified with silver ions (750 mg filling (Supelco)) was conditioned with three column volumes of DCM/*n*-hexane 9/1 (v/v) and 45 mg of the sample, dissolved in 0.5 mL of the same solvent mixture, was then transferred to the column. The product was eluted with three column volumes of the same DCM/*n*-hexane mixture. The identity of the product was confirmed by ¹H and ¹³C NMR spectroscopy (Supporting Information) as well as GC-MS.

2.3.1.3. Comment on the use and labeling of the internal standard (IS-EE). The internal standard 8-oxo-4,5-methylene-8:0-EE (IS-EE) was added to the samples. During transesterification, it converts to its methyl ester derivative, 8-oxo-4,5-methylene-8:0-ME (IS-ME), to account for potential incomplete transesterification yields of the fatty acids. Thus, IS-EE was added to the sample and IS-ME was analyzed. After acetalization to the respective dioxane, the converted internal standard was labeled as IS-ME.

2.3.2. Conversion of methyl 16-oxohexadecanoate to the respective acetal

For the conversion to the acetal the protocol of Piasecki (Piasecki, 1992) was adapted for the use with reduced reaction quantity and a reaction set-up in a vial. Approximately 50 µg of methyl 16-oxohexadecanoate were transferred to a vial, 200 µL of the respective diol with 0.1 % sulfuric acid (w%) added, and the sealed vial was heated at 70 °C for 2 h. The derivatives 15-(1,3-dioxolan-2-yl)-15:0-ME, 15-(1,3-dioxan-2-yl)-15:0-ME, 15-(4,4-dimethyl-1,3-dioxan-2-yl)-15:0-ME, 15-(4-heptyl-1,3-dioxolan-2-yl)15:0-ME were prepared using the respective diols ethylene glycol, 1,3-propane diol, 3-methyl-1,3-butane diol and 1,2-octane diol. In addition, 1,6-hexane diol was also tested for its suitability, but no conversion was observed, probably because of the steric instability of the product. A liquid-liquid extraction was performed using water and *n*-hexane. The organic layer was diluted and measured by GC-MS.

2.3.3. Quantification of ω-oxo-FAMES (Fig. 1)

2.3.3.1. Final method: quantification of ω-oxo-FAMES as ω-dioxane-derivatives. Sunflower oil and rapeseed oil (1 g each) were heated for a period of 20 h at 180 °C in an open vial. Subsequently, 5 mg of the heat-treated oils were weighed into separate vials, 5 µg of IS-EE were added and the solvent was evaporated. The following sample preparation is illustrated in Fig. 1. For the derivatization, 5 mg of glycerol monostearate, 200 µL of 1,3-propane diol with 0.1 % sulfuric acid (w%), and 50 µL CHCl₃ were added. The vials were flushed with argon, sealed, immersed in an ultra-sonic bath for 5 min and stirred for 2 h at 70 °C. The addition of glycerol monostearate and CHCl₃ and ultra-sonic treatment were required due to the poor solubility of the TGs and consequently a reduced conversion rate during acetal formation. Argon flushing was used to mitigate possible further oxidation during acetal formation. After the reaction, the lipids were transferred to a tube, 1 mL of deionized water was added and extracted three times with 1 mL of *n*-hexane. The combined organic phases were evaporated using a gentle stream of nitrogen. The sample was transesterified with 2 mL of 0.5 M NaOME in methanol and 2 mL MTBE. The tube was sealed and heated for 30 min at 50 °C. After the sample cooled down, 150 µL of acetic acid, 0.5 mL of saturated NaCl-solution and 2 mL of deionized water were added and the lipids were extracted twice with MTBE. The organic layer was washed with 2 mL deionized water and dried over anhydrous

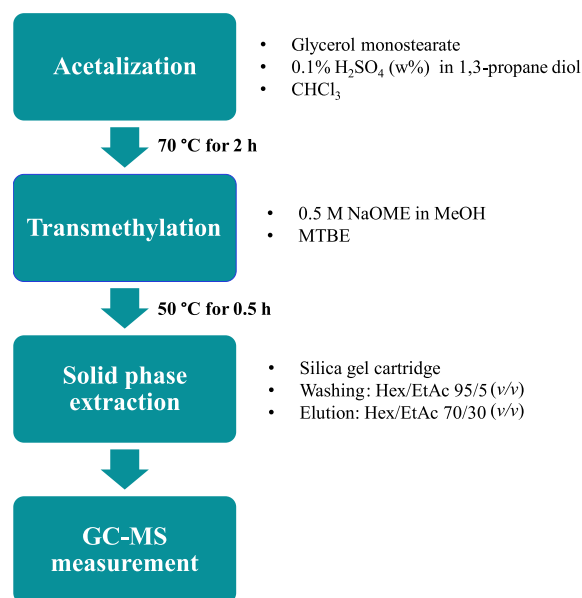


Fig. 1. Flow chart of the final method for the quantification of ω-oxo-FAMES as ω-dioxane-derivatives.

Na_2SO_4 . The solvent was evaporated using a gentle nitrogen stream. For the SPE, a glass cartridge of 0.6 cm diameter was filled with 0.7 g activated silica gel and conditioned with *n*-hexane/ethyl acetate 95/5 (v/v). The sample was dissolved in the starting eluent and transferred to the cartridge. Three cartridge volumes of *n*-hexane/ethyl acetate 90/10 (v/v) were used to elute other FAMES and the fraction with the ω -dioxane-FAMES were eluted using of *n*-hexane/ethyl acetate 70/30 (v/v). After the SPE, the solvent was evaporated and the residue was redissolved in 1 mL of *n*-hexane. The samples were measured by GC–MS in SIM-mode and the ω -oxo-FAs quantified by a one-point calibration with the internal standard. The samples were prepared and measured as triplicates. The conversion rates of the ω -dioxane-FAMES with remaining respective ω -oxo-FAMES > limit of quantification (LOQ) (for 8-A', 9-A' and 10-A') were calculated, averaging 93 %. The limits of detection (LOD) and LOQ were determined by signal-to-noise-ratio (S/N) calculations, with thresholds set at S/N: 3 and 10, respectively. The LOD/LOQ for 5-A', 6-A', 7-A', 8-A', 9-A' and 10-A' were 0.2 ng/mL/0.7 ng/mL; 0.2 ng/mL/0.7 ng/mL; 0.2 ng/mL/0.7 ng/mL; 0.6 ng/mL/2.0 ng/mL; 1.0 ng/mL/3.3 ng/mL and 1.6 ng/mL/5.3 ng/mL respectively.

2.3.3.2. Quantification of ω -oxo-FAMES without derivatization. Samples (5 mg) of the heated oils described above were weighed into separate tubes, IS-EE was added and the solvent evaporated. The following transesterification and extraction, as well as the SPE (with the entire sample) were pursued as described in the paragraph above. After the SPE, the solvent was evaporated and the residue was redissolved in 1 mL of *n*-hexane. The samples were measured by GC–MS in SIM-mode and the ω -oxo-FAs quantified by a one-point calibration with the internal standards. The samples were prepared and measured as triplicates. LOD/LOQ were 1.2 ng/mL/4.0 ng/mL; 5.3 ng/mL/17.7 ng/mL; 3.3 ng/mL/11.2 ng/mL and 7.8 ng/mL/26.0 ng/mL for 7-A, 8-A, 9-A and 10-A respectively.

2.4. Method validation of the final method: quantification of ω -oxo-FAMES as ω -dioxane-derivatives

2.4.1. Effect of derivatization of ω -oxo-FAMES on volatility

Sunflower oil (1 g) was heated for 12 h at 180 °C in an open vial and an aliquot of 60 mg was subsequently transesterified and the SPE performed as described in Section 2.3.3. The solvent was evaporated with a gentle stream of nitrogen and the sample was redissolved in 300 μL *n*-hexane.

Aliquots of 50 μL of the solution containing the ω -oxo-FAMES and 2.5 μg of IS-ME were added to six vials and for three vials the derivatization to the respective dioxanes was performed as described in Section 2.3.3. To the other three vials, 200 μL of 1,3 propanediol were added without the acid catalyst. For all samples, a liquid-liquid extraction using water and *n*-hexane was performed and 5 μg of 22:0-ME as non-volatile internal standard were added. A volume of 1.5 mL *n*-hexane was added to every vial and the solvent was evaporated with a gentle, continuous nitrogen stream at 40 °C until all solvent disappeared and the vial reached a slightly warm temperature. The samples were measured before; after one, two and three evaporation cycles. The samples were compared by calculating the ratio of area (ω -oxo-FAME or ω -dioxane-FAME) to the area of (22:0-ME) before and after the evaporation cycles. The experiment was performed in triplicates.

2.4.2. Performing the derivatization in matrix

Aliquots of 0.05 μg , 0.1 μg , 0.5 μg , 1 μg , 5 μg , 10 μg , 50 μg and 100 μg of methyl 16-oxohexadecanoate were added to 5 mg of 16:0-ME as matrix resulting in concentrations between 0.001 % and 2 % of the analyte in the matrix. The derivatization to the respective ω -dioxane-FAME was performed as described in Section 2.3.3. The yield of the reaction was controlled by GC–MS in SIM-mode. For 0.001 % to 0.02 % the signal of the educt was <LOD, resulting in a yield of ≥ 70 %. For the

experiments with ≥ 0.02 % analyte content, the remaining educt could be quantified and yields of the acetal were ≥ 73 % for each concentration and 87 % average yield. The experiment was performed in triplicate.

2.4.3. Linearity

Approximately 50 mg of heated sunflower oil was derivatized, transesterified and the ω -dioxane-FAME fraction was enriched by SPE as described above. The resulting solution was concentrated and subsequently diluted, resulting in concentration levels of 5 %, 10 %, 25 %, 50 %, 100 %, 200 % and 400 % of ω -dioxane-FAMES compared to the regular sample preparation with the oxidized oil. An aliquot of the IS-ME was added, resulting in a constant concentration of 5 $\mu\text{g}/\text{mL}$ in the sample. The minimum and the maximum contents of the individual ω -dioxane-FAMES for the different concentration levels was between 3.8 ng/mL for 5-A' and 40 $\mu\text{g}/\text{mL}$ for 9-A'. The ratio of the area of the respective analytes and the internal standard was plotted against the concentration level and R^2 was > 0.998 for all examined analytes (5-A', 6-A', 7-A', 8-A', 9-A', 10-A') showing adequate linearity for a large concentration range. The experiment was performed in triplicates.

3. Results and discussion

3.1. Derivatization of ω -oxo-FAMES to ω -acetal-FAMES

To develop a derivatization method which decreases the volatility of short-chain ω -oxo-FAMES, acetalization was tested, a method which was often used in organic chemistry to protect aldehydes during synthesis. Several 1,3-diols and 1,2-diols forming dioxanes and dioxolanes were used and the products analyzed. The changes in volatility of the four derivatives were estimated by GC–MS analysis using a nonpolar column (HP-5 ms-ui). The retention times of the products were compared to the educt and a range of saturated FAMES. The corresponding retention index of FAME (RI_{FAME}) was calculated by a linear regression (Fig. 2 and Table 1 in supporting information) for the educt and all products.

The first dioxolane, 15-(1,3-dioxolan-2-yl)-15:0-ME (product 1), showed the smallest decrease on the volatility but still increased the RI_{FAME} by 3 carbon atoms compared to the underivatized 16-oxo-16:0-ME (molecule 0). The other dioxolane, 15-(4-heptyl-1,3-dioxolan-2-yl)-15:0-ME (product 4), showed the strongest decrease of volatility with a RI_{FAME} increase of 7 additional carbons. However, two diastereomers were formed due to two stereo centers at the dioxolane group of the molecule, complicating the determination of the ω -oxo-FAMES. The dioxanes 15-(1,3-dioxan-2-yl)-15:0-ME (product 2) and 15-(4,4-dimethyl-1,3-dioxan-2-yl)-15:0-ME (product 3) showed a similar RI_{FAME} of approximately 22 and 22.5. Since branched carbon chains are more volatile, the difference in the volatility of both derivatives is marginal, even though product 3 contains two additional methyl groups.

The GC–MS spectra of both dioxanes differed in complexity and abundance of their base ion (Fig. 3). While product 3 showed two highly abundant fragment ions detected at m/z 69 and m/z 115, a low abundant $[\text{M}-\text{H}]^+$ as well as a high number of medium abundant fragments, the fragmentation pattern of product 2 was significantly simpler. In fact, product 2 showed a very abundant base ion of m/z 87, which probably mostly derived from the α -cleavage of the dioxane (Fig. 3). The fragments $[\text{M}-\text{H}]^+$ and $[\text{M}-31]^+$ were also visible in low abundances in the spectrum of product 2 and enabled the differentiation and identification of different ω -oxo-FAMES. The high proportion of m/z 87 in the fragmentation and the visibility of $[\text{M}-\text{H}]^+$ and $[\text{M}-31]^+$ is well suited for a SIM-method, which could potentially allow the analysis of low concentrations of ω -oxo-FAMES in complex mixtures even without prior enrichment. Considering (1) the observed decrease in volatility and (2) favorable GC–MS properties together with (3) the good yields in derivatization and (4) the easy removal of the reactant 1,3-propanediol by liquid-liquid-extraction, this derivative was chosen for further experiments for the analysis of ω -oxo-FAMES.

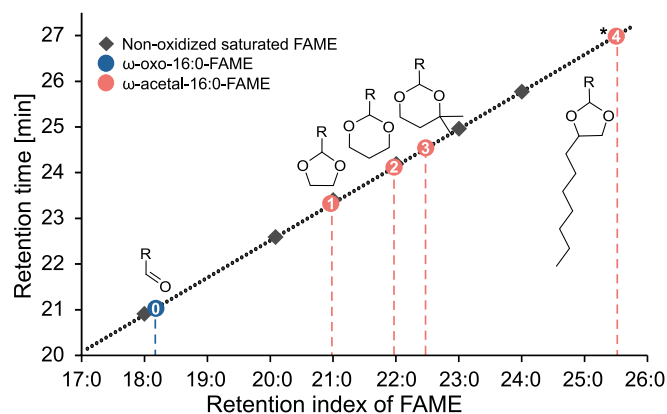


Fig. 2. Retention times and retention indices of non-oxidized saturated FAMES (grey diamonds, 16-oxo-16:0-ME (molecule 0) and the respective acetal derivatives (product 1–4). The retention index of the FAME was calculated by linear regression of the retention time and chain length of the non-oxidized FAMES ($R^2 > 0.998$). *Corresponding retention index for product 4 was extrapolated.

3.2. GC–MS properties of ω -dioxane-derivatives

Heated sunflower oil was transesterified and ω -oxo-FAMES were collected in one fraction of a SPE, which was analyzed using GC–MS. Saturated ω -oxo-FAMES with different chain lengths were found (Fig. 4, top). The most abundant peak was 9-A, which is formed through a cleavage of the 9-hydroperoxide, one of the major products formed by lipid oxidation from oleic acid and especially linoleic acid (Berdeaux et al., 2012), the unsaturated FAs with the highest concentrations in sunflower oil (Kamal-Eldin et al., 1997). The internal standard IS-ME and 9-A coeluted under the applied conditions in the underivatized fraction as they have the same number of carbons. Another abundant substance was 8-A, followed by 10-oxo-10:0-ME (10-A) and finally 7-A in significantly lower concentrations. Other oxo-FAME species like keto-FAMES (8-oxo-9:0-ME (9-K), 9-oxo-10:0-ME (10-K), 10-oxo-11:0-ME (11-K) or di-FAMES (6:0-diME (6-D), 7:0-diME (7-D), 8:0-diME (8-D), 9:0-diME (9-D)) were identified as well (Fig. 4, top).

After derivatization, all previously identified ω -oxo-FAMES (X-A) were retrieved as their respective ω -dioxane-FAMES (X-A). The retention times shifted by around 4.5 min corresponding to an average increase of RI_{FAME} of 3.6 carbons, implying a significantly reduced volatility of the FAMES. The retention time of 8-A e.g. shifted from a similar retention

time as 10:0-ME (bp of ~ 224 °C (Yuan et al., 2005)) to a retention time between 13:0-ME and 14:0-ME (bp of ~ 295 °C (Yuan et al., 2005)) for 7-dioxane-7:0-ME (8-A). As another effect of derivatization 9-A' and IS-ME' could be successfully baseline-separated in contrast to their reactants. Furthermore, the keto-FAMES were derivatized as well and showed a significant retention time shift similar to the ω -oxo-FAMES, while diacids did not show any change (Fig. 4, bottom).

The GC–MS spectra of 9-A, 10-K and their respective derivatization products 9-A' and 10-K' are shown in Fig. 5. Prior to derivatization, the high abundant fragment ions (m/z 74 and m/z 87) of the ω -oxo-FAME 9-A resembled those of saturated FAMES, but more specific fragment ions for oxo-modified FAMES such as m/z 111 ($[M-75]^+$), m/z 143 ($[M-43]^+$) and m/z 155 ($[M-31]^+$) enabled a distinct identification. In the GC–MS spectrum of 10-K, the base ion was found at m/z 83, which was also the base ion as for all other keto-FAMES found in this study. Some other characteristic fragment ions such as m/z 111 ($[M-89]^+$) and m/z 143 ($[M-57]^+$) were shared with 9-A since the oxo-group is in the same position and cleavage takes place at the same bonds. Besides the different base ion and a different retention time, the $[M-31]^+$ fragment ion (m/z 169) was 14 amu heavier for 10-K and the $[M-15]^+$ (m/z 185) fragment ion was visible with low intensity.

After derivatization, distinctly clearer spectra with an intensive base ion for both 9-A' and 8-(2-methyl-1,3-dioxan-2-yl)-9:0-ME (10-K') were obtained. The base ions were most likely formed through the cleavage of the dioxolane or methyl-dioxolane moiety resulting in a group-specific base ion of m/z 87 and m/z 101, respectively. This simplified the distinction of derivatized ω -oxo-FAMES and keto-FAMES tremendously and enabled a more selective and sensitive analysis of those lipid oxidation products when measuring in SIM-Mode. Unambiguous identification of different dioxane-FAMES was enabled by the $[M-1]^+$ and $[M-31]^+$ (for 9-A' m/z 243 and m/z 213) and for the methyl-dioxane-FAMES by the $[M-15]^+$ and $[M-31]^+$ fragment ions, respectively (for 10-K' m/z 243 and m/z 227).

3.3. Effect of the derivatization of ω -oxo-fatty acids on their recovery rate

During the sample preparation, evaporation can take place at multiple steps, e.g., to remove the solvent of the extraction after the transesterification, SPE or when adjusting concentrations before instrumental analysis. In order to observe the effects regarding the recovery rate of ω -oxo-FAMES and ω -dioxane-FAMES their contents were determined before and after three evaporation cycles (More details in Section 2.4.).

The recovery rates for the ω -oxo-FAMES 8-A, 9-A, 10-A and the IS-ME were determined in triplicates; the concentration of 7-A could not be

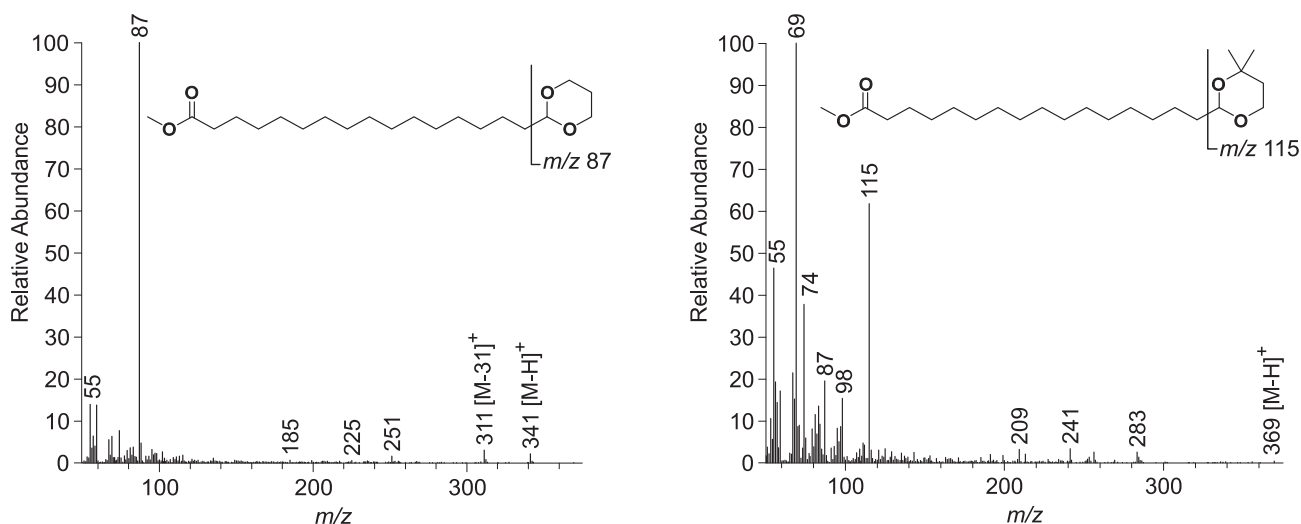


Fig. 3. GC-EI-MS spectra of 15-(1,3-dioxan-2-yl)-15:0-ME (product 2) on the left, and 15-(4,4-dimethyl-1,3-dioxan-2-yl)-15:0-ME (product 3) on the right.

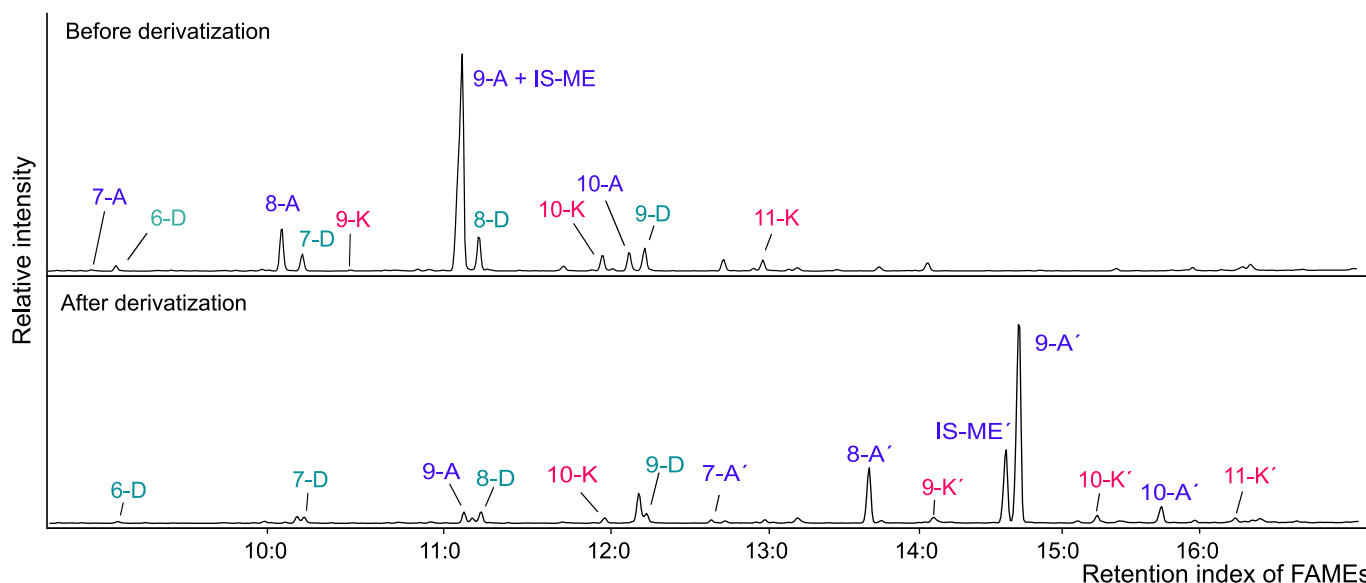


Fig. 4. GC-MS-chromatograms of heat-treated sunflower oil after transesterification and solid phase extraction. The chromatogram at the top shows the untreated SPE-fraction containing saturated ω -oxo-FAMES (X-A), keto-FAMES (X-K) and di-FAMES (X-D) with different chain lengths (respective number X), as well as the internal standard (IS-ME) coeluting with 9-oxo-9:0-ME (9-A). The chromatogram at the bottom shows the same fraction after derivatization to the respective dioxanes, marked with an apostrophe after the name of the educt (e.g. 9-A'). The x-axis displays the retention indices of different non-oxidized saturated FAMES.

reliably determined, since its concentrations were below LOQ. Even though recovery rates of all the ω -oxo-FAMES decreased with every evaporation cycle, there were large differences between the single substances. After three evaporation cycles, only 43 % of the original concentration of 8-A were detected, while 9-A and IS-ME showed higher recovery rates of 63 % and 64 %, respectively (Figs. 6 and 7). The highest recovery rate after 3 cycles of evaporations was observed for 10-A (76 %), which corresponded to almost the double recovery rate compared to 8-A. Moreover, 8-A showed significantly higher variances of the individual samples in the triplicates than the heavier analytes. The absolute alteration in pattern not only depends on the individual volatilities of the various ω -oxo-FAs but also on factors such as the number and duration of evaporation cycles, set temperature, and sample quantity. Consequently, it is not possible to predict with certainty how much of the individual ω -oxo-FAMES will be lost during sample preparation.

For accurate quantification of ω -oxo-FAMES, using an internal standard with similar properties to the analytes is crucial, but previously there was no suitable commercial internal standard available. The synthesized IS-ME showed suitable properties in the combination of its ω -oxo-function and a medium chain length comparable to the analytes and was therefore considerably more favorable than internal standards without the ω -oxo-function or widely deviating chain length. Nevertheless, the recovery rate was highly correlated with the chain length of the ω -oxo-FAMES (Fig. 6 and 7), so that this one particular IS-ME would be only suitable to properly quantify 9-A, while 8-A would get underestimated and 10-A overestimated.

Every single dioxane product showed a higher recovery rate compared to their respective educts and also all other ω -oxo-FAMES. In fact, the lowest recovery rate of 87 % was found for 6-dioxane-6:0-ME (7-A). It is noteworthy that 7-A' could only be quantified as dioxane derivative, owing to the higher abundant base ion (m/z 87) compared to its precursor 7-A. Comparing the recovery rates of product to educt, 8-A' showed the highest increase with a recovery rate of 88 % compared to 8-A (43 %). Despite a slight remaining trend of decreased volatility with a higher molecular weight, all ω -dioxane-FAMES showed a similar recovery rate. Again, 8-dioxane-8:0-ME (9-A) and the derivatized internal standard showed almost identical values, but the recovery rates for all products were in a range of 87 %–92 %.

Among the smaller loss of the ω -dioxane-FAMES in general, it is even

more important, that the pattern of the analytes with a different chain length did not change, since these individual losses could not be covered by using only one internal standard with a specific chain length. Prior to derivatization, the internal standard therefore would have been only suitable to quantify 9-A; after derivatization also ω -dioxane-FAMES with a different chain length would have been covered properly regarding the losses due to their volatility.

3.4. Quantification of bound ω -oxo-fatty acids in heat treated edible oils

The optimized derivatization method was used for the quantitative determination of ω -oxo-fatty acids in heat-treated sunflower oil and compared with a determination without prior derivatization to the respective dioxanes (Fig. 8, left). Without derivatization, saturated ω -oxo-FAMES with a chain length of 7 to 10 carbons could be quantified in highly varying concentrations. For the dioxane-derivatized aldehydes, additionally the ω -oxo-FAs with a chain length of 5 and 6 could be identified and quantified caused by a higher sensitivity for the dioxane-derivatives as a result of the more favorable fragmentation pattern and mitigated losses from their high volatility.

For both methods 9-A was the most abundant analyte with appr. 1.7 $\mu\text{g}/\text{mg}$ oil. The contents were in a similar scale, but a factor of two lower than the findings of Berdeaux et al. (3.13 mg/g) in methyl linoleate heated (180 °C for 15 h) (Berdeaux et al., 2002), which might be caused by the lower content of polyunsaturated fatty acids in the sunflower oil compared to the model solution. This prominence of 9-A can be attributed to the high levels of oleic and linoleic acid in sunflower oil (Gunstone, 2013), as their first double bond is located at position 9. During lipid oxidation, hydrogen is likely to get abstracted of the position next to a one or preferably two double bonds. After a shift of the double bond, the hydroperoxide formed at position 9 would be the most likely to react further to form a saturated ω -oxo-FA, which explains the high contents of 9-A compared to the other ω -oxo-FAMES. Other ω -oxo-FAs occurred in lower amounts, indicating a pattern where, starting from 9-A, their levels decreased with a decreasing number of carbon atoms. Compound 5-A was the ω -oxo-FA with lowest contents of 0.011 $\mu\text{g}/\text{mg}$ of all of the quantified analytes, corresponding to about 0.6 % of the amount of 9-A.

The ω -oxo-FAs were present in similar concentration ranges with and

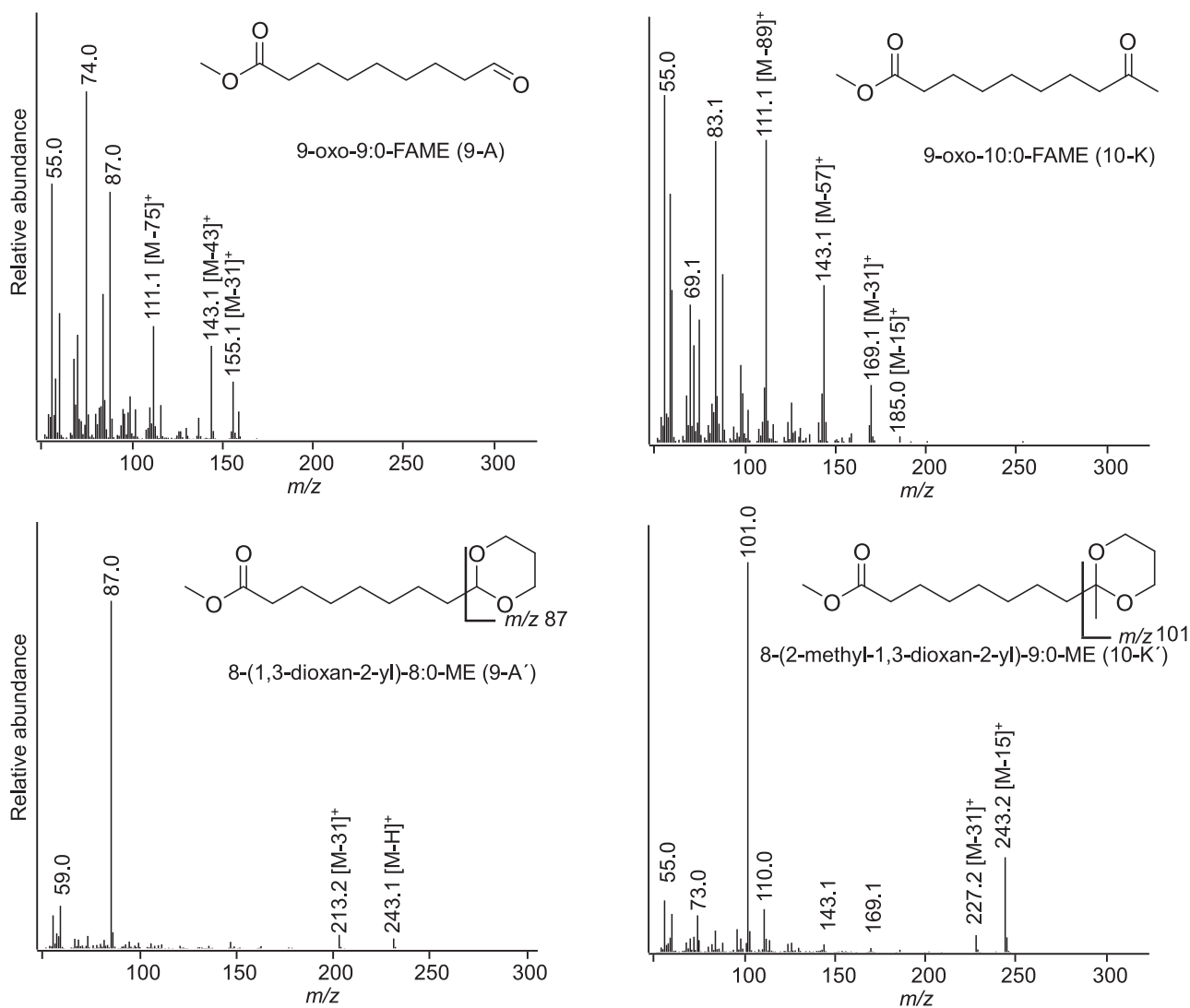


Fig. 5. GC-MS - spectra of 9-oxo-9:0-FAME (9-A) on the top left, 9-oxo-10:0-FAME (10-K) on the top right and the respective acetals 8-(1,3-dioxan-2-yl)-8:0-ME (9-A) on the bottom left and 8-(2-methyl-1,3-dioxan-2-yl)-9:0-ME (10-K) on the bottom right after derivatization.

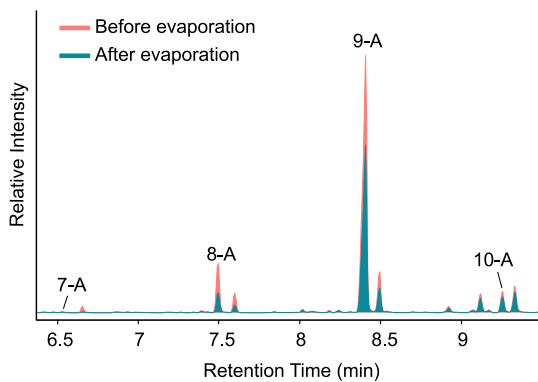


Fig. 6. (top): Overlaid GC-MS chromatograms of SPE-fractions of a heat treated, transesterified sunflower oil (180 °C for 12 h) containing ω -oxo-FAMES (A) with different chain lengths (respective number). The chromatograms of the sample before and after three evaporation cycles are shown in pink and turquoise.

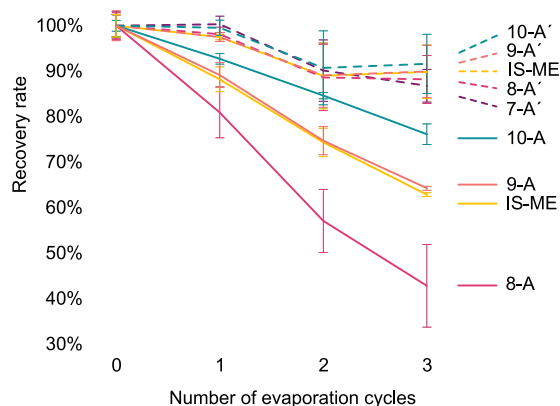


Fig. 7. (bottom): Recovery rates of ω -oxo-FAMES (continuous lines) and ω -dioxane-FAMES (dashed lines) before and after one; two and three cycles of adding solvent and evaporating. The respective products before and after derivatization show lines in the same color. 7-oxo-7:0-FAME (7-A) is not displayed, since its concentrations were below LOQ.

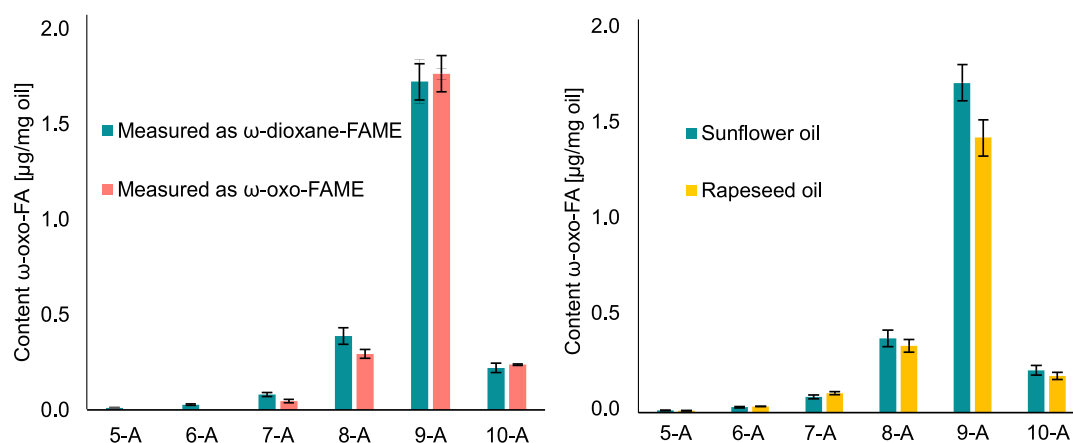


Fig. 8. Contents of ω -oxo-FAs in heat treated (180 °C, 20 h) sunflower oil measured as ω -dioxane-FAMES and ω -oxo-FAMES (left) and contents of ω -oxo-FAs in heat treated sunflower oil and rapeseed oil (180 °C, 20 h) measured as ω -dioxane-FAMES (right).

without prior derivatization to the respective dioxanes, however trends for the single analytes could be observed. The contents of 9-A matched very well with only 1 % deviation from each other and no significant difference. This behavior aligns with the results of Section 3.3, as both 9-A and the internal standard have nearly the same molecular weight and therefore showed similar properties regarding the losses during the sample preparation caused by evaporation. The contents of the more volatile 7-A and 8-A showed significant decreases of 43 % and 25 %, respectively, compared to their derivatized analogs. 10-A showed slightly higher but no significant increased contents compared to the derivatized analyte. These results confirm observations of Section 3.3, showing that the pattern of short chain ω -oxo-FAMES was altered during the sample preparation caused by different volatilities correlating to the molecular weight.

The formation of ω -oxo-FAs in heat treated sunflower and rape seed oil was compared (Fig. 8 right), as both are commonly used for deep frying processes and show a FA distribution with different proportions of the unsaturated FAs oleic and linoleic acid. The content pattern of ω -oxo-FAs was similar overall for both oils. The used sunflower oil exhibited slightly but significantly higher levels of ω -oxo-FAs, possibly due to its higher linoleic acid content of 63 % compared to 18 %. The increased susceptibility of linoleic acid to oxidation likely resulted in elevated ω -oxo-FA levels, a pattern previously observed for 8-A and 9-A (Berdeaux et al., 2002 & Velasco et al., 2005). Similar to Velasco et al. who compared heat treated sunflower and olive oil, our study found that the 8-A to 9-A ratio was higher in rape seed oil compared to sunflower oil due to its higher oleic acid (69 % vs. 29 %, respectively) and lower linoleic acid content (18 % and 63 %), resulting in increased formation of hydroperoxide at position 8 compared to the hydroperoxide at position 9 (Velasco et al., 2005). Velasco et al. found similar levels of 9-A in both laboratory-heated oils (1.27 mg/g) and used restaurant frying oils (0.61 to 1.61 mg/g). Since our laboratory conditions mirrored theirs, it is likely that our aldehydic fatty acid levels are realistic and comparable to those in restaurant frying oils. However, precise determination still needs to be conducted (Velasco et al., 2005).

4. Conclusions

In this study an innovative approach for the determination of ω -oxo-FAs by GC-MS was developed to enhance the recovery rates and reduce the pattern change during the sample preparation.

The ω -oxo-FAMES, that were transesterified for subsequent analysis by GC showed significant losses in the sample workup, especially during solvent evaporation. Using a novel synthesized internal standard with a similar molecular structure these losses could be mapped very precisely for the most abundant ω -oxo-FA (9-A) with the same chain length.

The decrease in recovery of ω -oxo-FAMES was correlated with the chain length, which in turn did not only affect the recovery of ω -oxo-FAMES themselves but also altered the relative distribution of individual ω -oxo-FAMES. Of the different derivatizations tested to reduce the volatility of the ω -oxo-FAMES, the ω -dioxane-derivative formed by reaction with 1,3 propane diol exhibited the best combined results in terms of reduced volatility (+4 RI_{FAME} -carbons), easy extraction and favorable GC-MS fragmentation behavior. The recovery rate of the derivatized analytes after three evaporation cycles was increased significantly and the recovery rates of the analytes with different chain lengths and the internal standard clearly converged. This method can be used to identify and quantify saturated core-bound aldehydes after transesterification and enrichment using SPE, within the parameters established during validation. In direct comparison of the quantification, a significantly higher content of the shorter ω -oxo-FAs (7-A and 8-A) was detected for the sample with derivatization to the respective ω -dioxane-FAME, confirming the previous observations. Furthermore, two additional short ω -dioxane-FAMES (5-A and 6-A) could be quantified in rapeseed and sunflower oil only after derivatization due to higher recovery rates and higher sensitivity caused by the favorable fragmentation pattern.

To date, there is little research on ω -oxo-FAs in food, despite the known harmful properties. We now offer a reliable and sensitive quantification method to observe the contents of ω -oxo-FAs in different foods and the factors that accelerate or inhibit their formation. In the future, sample preparation possibly might be simplified by determining the ω -oxo-FAs without prior SPE due to the increased sensitivity.

CRediT authorship contribution statement

Anne Zartmann: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jean-Marie Galano:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Conceptualization. **Simon Hammann:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2024.141223>.

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