Glycerol-bound oxidized fatty acids: formation and occurrence in peanuts

Lars Störmer1 · Martin Globisch1 · Thomas Henle1

Received: 4 February 2022 / Revised: 8 April 2022 / Accepted: 9 April 2022 / Published online: 6 May 2022
© The Author(s) 2022

Abstract
For peanuts, roasted at 170 °C, the formation of selected glycerol-bound oxidized fatty acids (GOFAs), namely 9-oxononanoic acid (9-ONA), azelaic acid (AZA) and octanoic acid, was observed by GC-MS (EI). The content of octanoic acid as well as AZA increased with continuous roasting time (from 59 mg/kg peanut oil to 101 mg/kg peanut oil and from not detectable to 8 mg/kg peanut oil, respectively), whereas the content of 9-ONA initially decreased from 25 mg/kg peanut oil to 8 mg/kg peanut oil (20 min) and increased again up to 37 mg/kg peanut oil following roasting for 40 min. Due to its aldehyde function, 9-ONA could contribute to amino acid side chain modifications as a result of lipation, which could directly influence the functional properties of peanut proteins. Both 9-ONA and octanoic acid are potential markers of thermal processes. Furthermore, in model experiments using methyl linoleate and methyl oleate, up to 18 oxidized fatty acids could be identified as methyl esters, 9-ONA as well as octanoic acid as major components and a faster formation of GOFAs under roasting conditions (170 °C, 20 min). In addition, 9-ONA contributes to the formation of AZA and octanoic acid in both free and bound form as a result of oxidative subsequent reactions in presence of iron (III).

Keywords Peanut · Lipid peroxidation · Glycerol-bound oxidized fatty acids · Oxidative subsequent reactions · Lipation · Neo-lipoproteins

Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| 9-HPODE      | 9-Hydroperoxy-10E,12Z-octadecadienoic acid |
| 9,12-DiHPODE | 9,12-Dihydroperoxy-10E,13E-octadecadienoic acid |
| 10-HPODE     | 10-Hydroperoxy-8E,12Z-octadecadienoic acid |
| 10,13-DiHOPDE| 10,13-Dihydroperoxy-8E,11E-octadecadienoic acid |
| 9-ONA        | 9-Oxononanoic acid |
| AZA          | Azelaic acid |
| FOFAs        | Free oxidized fatty acids |
| GC-FID       | Gas chromatography coupled with a flame ionisation detector |
| GC-FTIR      | Gas chromatography coupled with a Fourier transform infrared spectrometric detector |
| GC-MS (EI)   | Gas chromatography coupled to a mass spectrometric detector after electron ionisation |
| GOFA         | Glycerol-bound oxidized fatty acids |
| HPLC-ESI-MS/MS | High-performance liquid chromatography coupled to a tandem mass spectrometric detector after electron spray ionisation |
| IV           | Iodine value |
| LA           | Linoleic acid |
| LPO          | Lipid peroxidation |
| MDA          | Malondialdehyde |
| Methyl 9-ONA | Methyl 9-oxononanoate |
| Methyl ODA   | Methyl 10-oxo-8E-decenoate |
| MP-lysine    | 2-Amino-6-(3-methylpyridin-1-ium-1-yl)hexanoic acid |
| OA           | Oleic acid |
| ODA          | 10-Oxo-8E-decenoic acid |
| POV          | Peroxide value |
| TBA          | 2-thiobarbituric acid |
| TBAV         | Thiobarbituric acid value |

* Lars Störmer
lars.stoermer1@tu-dresden.de

1 Chair of Food Chemistry, Technische Universität Dresden, 01062 Dresden, Germany
Introduction

Besides its high content of protein (21.0–36.4%) and fat (35.8–54.2%), peanuts are often used as an ingredient in food production [1, 2]. Along with milk, it is one of the eight main food allergen sources [3, 4]. Recent studies show that 1–2% of the world’s population is affected by peanut allergy, with an increase observed especially in children [4, 5]. Following roasting, which is carried out at temperatures of 150–170 °C, an increased immunoglobulin E-binding capacity of the proteins of roasted peanuts compared to raw peanuts was observed in the previous studies [6–8]. In contrast, Mondoulet et al. (2005) observed no increased immunoglobulin E-binding capacity following roasting [9]. Due to their high content of nucleophilic amino acids (arginine 10.6%, cysteine 3.8%, histidine 2.8%, and lysine 3.4%), it is assumed that nucleophilic side chains of protein-bound amino acids can be modified as a result of the roasting and roasting process, and that this might have an influence on the allergenic potential of peanuts [1]. Wellner et al. (2012) were able to show that a lysine modification of up to 30–40% occurred as a result of peanut roasting. However, only 10% of this modification could be explained by Maillard reactions and the formation of the glycation compounds pyrraline, Nε-fructoselysine and Nε-carboxymethyllysine [10]. Due to the high proportion of monounsaturated and polyunsaturated fatty acids of up to 85% of the total fat content, reactions with carbonyl compounds (secondary reaction products) formed during lipid peroxidation (LPO) could contribute to the observed lysine loss [2, 10]. Globisch et al. (2014) referred to these subsequent reactions of secondary reaction products of LPO with nucleophilic side chains of protein-bound amino acids as lipation [11]. In their studies, peanuts were roasted at 170 °C for 20 min as well as 40 min, and they could explain about 2–3% of the occurring lysine loss in roasted peanuts by the formation of the lipation products 2-pentylpyrrole lysine, which is formed from lysine and 4-hydroxy-2E-nonenal, as well as MP-lysine resulting from a reaction of lysine with acrolein, for example, quantitated by HPLC-ESI-MS/MS [12, 13]. As shown in Fig. 1, in addition to volatile compounds, glycerol-bound oxidized fatty acids (GOFAs, e.g., compound 4 in Fig. 1) can be formed during LPO in a mirror-image reaction [14]. Like the volatile components, these GOFAs are able to contribute to amino acid side chain modifications due to their electrophilic center. In model experiments, Berdeaux et al. (2012) identified 21 different GOFAs that, like the volatile components, could be classified in the functional classes of aldehydes, alkanes, primary alcohols, dicarboxylic acids, furans, and methyl ketones. For this purpose, methyl linoleate as well as methyl oleate were thermoxidized for 15 h at 180 °C simulating a frying process and subsequently measured by GC-MS and GC-FTIR for identification purposes [15]. Furthermore, Velasco et al. (2005) could quantitate short-chain glycerol-bound components in sunflower and olive oil after thermal treatment at 180 °C for 5, 10 h, and 15 h following a two-step methylation with diazomethane and sodium methoxide using GC-FID measurements. The focus was placed on the determination of methyl 8-oxocitrate and methyl 9-oxononanoate (methyl 9-ONA), as these represented the main compounds of glycerol-bound short-chain aldehyde fatty acids in the matrices investigated. For example, using the methyl ester of 9-ONA, it could be shown that their content increased with increasing heating time from 5 to 15 h in sunflower oil from 600 to 1270 mg/kg oil and olive oil from 500 to 900 mg/kg oil. The contents were explained by the significant formation of the major hydroperoxide 9-hydroperoxy-10E,12Z-octadecadienoic acid (9-HPODE) from the two unsaturated fatty acids linoleic (LA) and oleic acid (OA) [16]. Based on the current state of the literature concerning the formation and occurrence of GOFAs, it can be assumed that, similar to volatile components such as hexanal, 4-hydroxy-2E-nonenal and acrolein, GOFAs might have an increased potential for modifying nucleophilic side chains of protein-bound amino acids and, therefore, might also contribute to lysine modification in the course of peanut roasting [10]. As precursors, GOFAs could considerably influence protein properties such as allergenic potential, aggregation, and digestion resistance through the formation of neo-lipoproteins. Neo-lipoproteins represent the lipation products formed between nucleophilic side chains of amino acids and GOFAs. However, to the best of our knowledge, there are no literature reports about the formation and occurrence of GOFAs in raw and roasted peanuts.

The aim of this study, therefore, was to show that in addition to volatile components, such as hexanal, GOFAs also occur in peanuts as a result of the thermal process. Furthermore, the formation of GOFAs is induced by the roasting process and that they are precursors of amino acid side chain modifications due to their electrophilic center. For this purpose, phenomenological studies on the formation of secondary reaction products of LPO were provided by means of UV spectroscopic investigations, the determination of the LA and OA content by GC-FID, as well as selected fat indices in raw and roasted peanuts. Also, a GC-MS (EI) method based on Kamal-Eldin et al. (1997) was established for the identification of GOFAs in model systems, the detection of oxidative subsequent reactions of free oxidized fatty acids (FOFAs) and GOFAs as well as the semiquantitation of selected GOFAs in peanuts roasted at 170 °C. Native and roasted peanut oil was used as reference and methyl undecanoate as internal standard [17]. For the first time, a semiquantitation of 9-ONA, azelaic acid (AZA), and
octanoic acid in peanuts and peanut oil was possible by means of GC-MS (EI).

Materials and methods

Materials

Methyl stearate (99%), monohydrogen azelate (96%), oleic acid (99%), and trimethylsilyldiazomethane solution (2 M in n-hexane) were from Alfa Aesar (Haverhill, Massachusetts, USA). Potassium iodate was used by AppliChem (Darmstadt, Germany). Cyclohexane and glacial acetic acid were used by Carl Roth (Karlsruhe, Germany). Chloroform, ethyl acetate, potassium hydroxide, petroleum ether (boiling point 40–60 °C), and sulphuric acid (> 95%) were obtained from Fisher Scientific (Loughborough, UK). L-ascorbic acid and sodium thiosulphate pentahydrate were from Fluka (Buchs, Switzerland). 1-Butanol (99.5%), disodium hydrogen phosphate dihydrate (99%), magnesium sulphate (99%), sodium

Fig. 1 Postulated pathway for the formation of secondary reaction products of lipid peroxidation in a mirror-image reaction [14]. Formation of 9-hydroxy-12-oxo-10E-dodecenoic acid 4, hexanal 5, 9-oxononanoic acid 6, and 4-hydroxy-2E-nonenal due to degradation of 9,12-dihydroperoxy-10E,13E-octadecadienoic acid (9,12-DiHPODE) 2 and 10,13-dihydroperoxy-8E,11E-octadecadienoic acid (10,13-DiHPODE) 3 from glycerol-bound linoleic acid 1, for example. Detailed information is presented in the text. R₁ = –C₁₇H₃₃ (linoleic acid)
heated by the Rancimat 892 professional (Methrom, Herisau, Switzerland) under an oxygen flow of 20 L/h at 170 °C for 20 min and 40 min [15].

Iodine value

The iodine value was measured according to Kaufmann [18]. Therefore, about 0.2 g of the cold extracted peanut oil was weighed into a 250 mL Erlenmeyer flask, and mixed with 10 mL dichloromethane and 25 mL methanolic bromine solution (0.1 M), and the Erlenmeyer flask was closed and briefly inverted and left to stand for 30 min in the absence of light. Then, 15 mL potassium iodide solution (10%) was added to the mixture and titrated with a sodium thiosulphate solution (0.1 M) until yellow colouration. Finally, after adding a starch solution (1%), titration was carried out until colourless. In the same procedure, a blank was carried out without sample weighing.

Peroxide value

The peroxide value was measured according to Wheeler [18]. Therefore, about 1.0 g of the cold extracted peanut oil was weighed into a 250 mL Erlenmeyer flask, mixed with 30 mL of a glacial acetic acid/chloroform (3:2, v/v) mixture and 0.5 mL of a saturated potassium iodide solution, and sealed and shaken vigorously for exactly 60 s. Then, 30 mL of double-distilled water was added to the mixture and titrated with a sodium thiosulphate solution (0.01 M) until yellow colouration. Finally, a starch solution (1%) was added and titrated until colourless. In the same procedure, a blank was carried out without sample weighing.

Thiobarbituric acid value

About 0.2 g of the cold extracted peanut oil was weighed into a 10 mL graduated flask, dissolved in 1-butanol, followed by 0.5 mL hydrochloric acid (0.1 M), and finally made up with 1-butanol. Pipette 3.0 mL of the prepared sample solution into a screw test tube and add 3.0 mL of TBA solution (0.2% in 1-butanol). In the same way, a blank experiment consisting of 2.85 mL 1-butanol, 0.15 mL hydrochloric acid (0.1 M), and 3.0 mL TBA solution (0.2% in 1-butanol) was carried along. The test tube was closed, shaken well, and incubated for 2 h in a water bath preheated to 95 °C. Finally, the sample was cooled down. After cooling, the sample was measured at a wavelength of 532 nm on the Ultrospec 1000 photometer (Pharmacia-Biotech, Uppsala, Germany) against the blank as a reference. For the previously performed calibration, 44.3 μmol of 1,1,3,3-tetramethoxypropane was dissolved in 6.0 mL hydrochloric acid (0.1 M) and then heated in a water bath at 40 °C for 45 min to release the

Preparation of samples

In a ULM 500 drying oven (Memmert, Schwabach, Germany) pre-tempered to (170 ± 2) °C, 50 g of raw, unroasted peanuts with shell were distributed on an aluminium foil and roasted for 20 min or 40 min, respectively. After cooling at room temperature, the peanuts were shelled, the seed coats were removed, and the peanuts were ground at 5000 rpm for a few seconds using the Grindomix 100 food processor (Retsch, Haan, Germany) and stored in the freezer at −21 °C [12]. The fat content was analysed using the Weibull-Stoldt method [18]. For the cold extraction of the peanut oil, about 3.0 g of peanuts were weighed into a 50 mL Greiner tube, mixed with 30 mL diethyl ether, and placed in the overhead shaker REAX 2 (Heidolph, Schwabach) for 1.5 h at room temperature. After subsequent filtration (Whatman 595.5), the solvent was completely rotated off under vacuum at 40 °C and 600 mbar. 2.0 g of native peanut oil were heated by the rancimat 892 professional (Methrom, Herisau, Switzerland) under an oxygen flow of 20 L/h at 170 °C for 20 min and 40 min [15].

Iodine value

The iodine value was measured according to Kaufmann [18]. Therefore, about 0.2 g of the cold extracted peanut oil was weighed into a 250 mL Erlenmeyer flask, and mixed with 10 mL dichloromethane and 25 mL methanolic bromine solution (0.1 M), and the Erlenmeyer flask was closed and briefly inverted and left to stand for 30 min in the absence of light. Then, 15 mL potassium iodide solution (10%) was added to the mixture and titrated with a sodium thiosulphate solution (0.1 M) until yellow colouration. Finally, after adding a starch solution (1%), titration was carried out until colourless. In the same procedure, a blank was carried out without sample weighing.

Peroxide value

The peroxide value was measured according to Wheeler [18]. Therefore, about 1.0 g of the cold extracted peanut oil was weighed into a 250 mL Erlenmeyer flask, mixed with 30 mL of a glacial acetic acid/chloroform (3:2, v/v) mixture and 0.5 mL of a saturated potassium iodide solution, and sealed and shaken vigorously for exactly 60 s. Then, 30 mL of double-distilled water was added to the mixture and titrated with a sodium thiosulphate solution (0.01 M) until yellow colouration. Finally, a starch solution (1%) was added and titrated until colourless. In the same procedure, a blank was carried out without sample weighing.

Thiobarbituric acid value

About 0.2 g of the cold extracted peanut oil was weighed into a 10 mL graduated flask, dissolved in 1-butanol, followed by 0.5 mL hydrochloric acid (0.1 M), and finally made up with 1-butanol. Pipette 3.0 mL of the prepared sample solution into a screw test tube and add 3.0 mL of TBA solution (0.2% in 1-butanol). In the same way, a blank experiment consisting of 2.85 mL 1-butanol, 0.15 mL hydrochloric acid (0.1 M), and 3.0 mL TBA solution (0.2% in 1-butanol) was carried along. The test tube was closed, shaken well, and incubated for 2 h in a water bath preheated to 95 °C. Finally, the sample was cooled down. After cooling, the sample was measured at a wavelength of 532 nm on the Ultrospec 1000 photometer (Pharmacia-Biotech, Uppsala, Germany) against the blank as a reference. For the previously performed calibration, 44.3 μmol of 1,1,3,3-tetramethoxypropane was dissolved in 6.0 mL hydrochloric acid (0.1 M) and then heated in a water bath at 40 °C for 45 min to release the
malondialdehyde. After cooling down, the concentration of the stock solution was determined, and the calibration solutions with a concentration of 2 to 20 μM malondialdehyde were prepared and proceeded in the same way as for the sample solutions. Hydrochloric acid (0.1 M) was used as a blank [18].

**UV spectroscopy**

Exactly 100 mg of the cold extracted peanut oil was weighed into a 25 mL graduated flask and dissolved in n-hexane. As a reference, a solution with methyl stearate was prepared in the same way. The starting solutions had a concentration of 0.25%. After dilution of the reference and sample solutions to 0.01% and 0.1%, they were measured against n-hexane at 37 °C in the wavelength range of 210–350 nm using the Specord 50Plus spectrophotometer (Analytik Jena, Jena, Germany) [18].

**GC-FID quantitation of linoleic and oleic acid**

About 15 mg of the cold extracted peanut oil was weighed into a screw-top test tube, and mixed with 1.0 mL cyclohexane and 0.45 mL of a 3.5 mg/mL concentrated internal standard of methyl valerate and methyl heptadecanoate. After adding 0.5 mL of a methanolic potassium hydroxide solution (5%), the screw-top test tube was closed, and the mixture was shaken vigorously and incubated for 15 min in a water bath preheated to 60 °C with occasional shaking. After cooling down at room temperature, 1.5 mL sulphuric acid (2%) as well as 3.0 mL cyclohexane were pipetted in, shaken vigorously again and the sample was left to stand at 6 °C overnight. The quantitation of LA and OA content in peanuts was performed using the standard mix Supelco 37 Component FAME Mix (FAME: 2–4%) mixed with methyl valerate and methyl heptadecanoate. After confining with nitrophthalic acid, Phenomenex, Aschaffenburg, Germany). Nitrogen was used as carrier gas with a constant flow rate of 2.5 mL/min. The injection temperature was set at 250 °C and 1 μL of sample was injected using a pulsed split mode of 10:1 at a carrier flow rate of 75 mL/min, which was adjusted after 4 min to 15 mL/min with the split mode turned off. The starting temperature of the oven program was set to 60 °C with a hold time of 3 min. Subsequently, the temperature increased in a first step at a rate of 15 °C/min to 150 °C, which was continued at a rate of 3 °C/min to 200 °C. In a final step, the temperature was increased at a rate of 15 °C/min to 240 °C. Finally, a post-run was carried out for 10 min at 240 °C [18].

**Nuclear magnetic resonance spectroscopy (NMR)**

For 9-ONA, 10-oxo-8E-decenoic acid (ODA), methyl 9-ONA, and methyl 10-oxo-8E-decenoate (methyl ODA), the 1H-NMR spectra were recorded at 500.13 MHz and the 13C-NMR spectra at 125.75 MHz using the Avance III HDX NMR spectrometer (Bruker, Rheinstetten, Germany). Deuterochloroform (CDCl3) was used as solvent in each case. All chemical shifts were reported in parts per million (ppm) relative to the solvent signal as an internal standard. Correlation spectroscopy (COSY), nuclear Overhauser enhancement spectroscopy (NOESY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) were performed as 2D NMR experiments for 9-ONA, ODA, Methyl 9-ONA, as well as methyl ODA.

**Synthesis of 9-oxononanoic acid (9-ONA)**

In a 1000 mL two-neck round-bottom flask 8.0 g OA were dissolved in 360 mL dichloromethane. Ozonolysis was carried out on the laboratory ozoniser 300.5 (Erwin Sander, Uetze-Eltze, Germany) at – 78 °C using a dry ice-acetone mixture, a current of 0.45 A, and an oxygen flow of 20 normal litres/h for 30 min with constant stirring until the synthesis mixture showed a clear blue colour. Subsequently, the mixture was flushed with nitrogen for 20 min until decolourisation. After addition of 1.46 mL dimethyl sulphide, the mixture was left to stand overnight at room temperature with constant stirring. Then, the dichloromethane was rotated off under vacuum at 40 °C as well as 600 mbar and the residue was dissolved in 50 mL ethyl acetate. The ethyl acetate solution was washed three times with 50 mL of saturated sodium chloride solution each time. The ethyl acetate solution, which was then dried over magnesium sulphate, was concentrated again under vacuum at 40 °C and 200 mbar until the solvent was completely removed. The residue obtained from the synthesis was purified by column chromatography over silica gel 60. In the first purification step, 20% diethyl ether in n-hexane was used for the separation of impurities and for the elution of the 9-ONA n-hexane/ethyl acetate (1:1, v/v) [20]. A second purification step was used to pre-purify using 20% diethyl ether in n-hexane and finally eluted using 60% diethyl ether in hexane [21]. To identify the fractions containing the 9-ONA, they were analysed by thin-layer chromatography using 60% diethyl ether in n-hexane (1:3) followed by heating for 2 min at 100 °C in the ULM 500
Synthesis of 10-oxo-8E-decenolic acid (ODA)

25 mL dichloromethane was purged oxygen-free for 15 min under nitrogen flow before starting the synthesis. Subsequently, 504 mg of 8-nonenic acid, 1003 mg of acrolein dimethyl acetal, and 25.9 mg of Hoyveda-Grubbs-Catalyst M720 were weighed into a 50 mL three-neck flask. The synthesis mixture was then left to stir for 5 h at room temperature under a nitrogen atmosphere. After the addition of 25.9 mg of Hoyveda-Grubbs-Catalyst M720, the mixture was left to stir for another 20 h under the conditions described above. After incubation, the solvent was evaporated under a stream of nitrogen. For the release of the ODA, 10 mg of the 10,10-dimethoxy-8E-decenolic acid (ODA-DMA) were incubated in 10 mL hydrochloric acid (0.1 M) for 45 min in a water bath pre-tempered to 40 °C. Furthermore, the residue obtained from the synthesis was purified by column chromatography over silica gel 60 fraction by fraction using the eluent diethyl ether/n-pentane (50:50, v/v) mixed with 0.1% triethylamine. To identify the fractions containing the ODA, they were analysed by thin-layer chromatography using the eluent as mobile phase. The following detection was carried out using the spray reagent 0.01% 2,4-dinitrophenylhydrazine in methanol/hydrochloric acid (36%) 100:1 (v/v) followed by heating for 2 min at 100 °C in the ULM 500 drying oven (Memmert, Schwabach, Germany). Relevant fractions were combined, concentrated under vacuum at 40 °C and 200 mbar to completely remove the eluent and lyophilised. The purity was determined by GC-MS (EI) [22]. Methyl 9-ONA: 1H-NMR (500 MHz, CDCl3) δ / ppm 1.29–1.33 (m, 4H, J = 7.00 Hz, J = 7.67 Hz, H-6, H-7), 1.2–1.6 (m, 4H, J = 7.00 Hz, J = 7.40 Hz, J = 7.63 Hz, H-4), 2.3–2.4 (td, 2H, J = 6.89 Hz, J = 7.43 Hz, H-4), 2.4–2.5 (t, 2H, J = 7.37 Hz, H-9), 6.1–6.2 (dd, 1H, J = 10.32 Hz, J = 15.82 Hz, H-2), 6.8–7.0 (dt, 1H, J = 6.89 Hz, J = 15.82 Hz, H-3), 9.5 (d, 1H, J = 10.32 Hz, H-1). The compound was present as an ochre-coloured solid with a chromatographic purity of 65.1% (GC-MS). Yield = 300.4 mg (molar yield = 32.9%).

## Transmethylation

Approximately 100 mg of the cold extracted peanut oil was weighed into a 14 mL Greiner tube, mixed with 1.0 mL of the internal standard solution of 5 μg/mL methyl undecanoate in methyl tert-butyl ether, and homogenised until the sample was completely dissolved. Peanut oil dissolved in 1.0 mL of the internal standard solution containing
400 μg/mL methyl undecanoate in methyl tert-butyl ether was used as a reference. Then, 0.2 mL of a methanolic sodium methoxide solution (0.5 M) was added and shaken vigorously for 2 min at room temperature. After addition of 50 μL of sulphuric acid (1 M), the mixture was shaken vigorously for 10 s. Afterwards 1.5 mL double-distilled water was added and shaken vigorously for 10 s again. For the following phase separation, the mixture was centrifuged at 4500 × g for 10 min at room temperature using Centrifuge 5804 (Eppendorf, Hamburg, Germany). The removed organic phase was directly used for the measurement by GC-MS (EI). For the determination of the recovery rate, tritridecanoin, which was dissolved in 1.0 mL of the internal standard solution of 500 μg/mL methyl undecanoate in methyl tert-butyl ether, was carried along and transmethylated in the same procedure [23]. For verification of transmethylation with sodium methoxide, a recovery rate of (99.5 ± 0.9)% could be determined in a triple determination.

Model system 1 (for studies on reaction mechanisms)

75.0 mg of methyl linoleate or methyl oleate or 10.0 mg of 9-ONA or methyl 9-ONA were weighed into a 5 mL round-bottom flask, mixed with 1.0 mL phosphate buffer (0.1 M, pH 7.4), 27 μL of ferric chloride hexahydrate solution (5 mg/mL in double-distilled water) and 23.0 mg L-ascorbic acid, and finally, a stirring bar was added. The mixture was then incubated for 24 h at 37 °C. To stop the reaction, 1.0 mL of double-distilled water was added to 0.5 mL of the incubation mixture, extracted for a few seconds, and centrifuged at 10,000 × g for 5 min at room temperature. Then, 200 μL of n-hexane was added, extracted again for a few seconds, centrifuged at 10,000 × g for 3 min, and dried over magnesium sulphate. The removed organic phase was used directly for measurement by GC-MS (EI). The preparation of 9-ONA and methyl 9-ONA was measured against the corresponding untreated standard [24].

Model system 2 (simulating technological conditions)

Approximately 2.0 g of methyl linoleate and methyl oleate, respectively, were incubated using the rancimat 892 professional (Methrom, Herisau, Switzerland) under an oxygen flow of 20 L/h at 170 °C for 20 min. After incubation, 100.0 mg of the heated methyl linoleate or methyl oleate was mixed with 1.0 mL of methyl tert-butyl ether and used directly for measurement by GC-MS (EI) [15].

GC-MS (EI) analysis of peanuts, peanut oil and model systems

The semiquantitation of 9-ONA, AZA, as well as octanoic acid in peanuts and peanut oil as their corresponding methyl esters, the determination of the recovery rate, the qualitative detection of GOFAs in model experiments, as well as the detection of oxidative subsequent reactions of 9-ONA and methyl 9-ONA, respectively, were performed using the Agilent 7890A GC system consisting of a 7683 series injector with autosampler, a 5975C MS detector in positive EI mode, an HP-5MS capillary column (30.0 m × 0.25 mm ID, 0.25 μm film thickness), all from Agilent (Böblingen, Germany), and a Zebron ZB 5 ms-Guardian capillary column (deactivated, 5.0 m × 0.25 mm ID, Phenomenex, Aschaffenburg, Germany). Helium was used as the carrier gas with a constant flow rate of 1 mL/min. The injection temperature was set at 250 °C and 1 μL of sample was injected using a pulsed split mode. The split ratio for the peanut samples was 10:1 and for the peanut oil samples and model experiments 30:1. The auxiliary temperature was set to 250 °C, the ion source and quadrupole to 230 °C and 150 °C, respectively, and a solvent delay time of 4 min. The starting temperature of the oven program was set to 60 °C, which was held for 9 min, then increased to 90 °C at 30 °C/min and held for 2 min, and finally increased to 200 °C at a rate of 3 °C/min. This was followed by a post-run for 3 min at 275 °C. Only for the model experiments to detect oxidative subsequent reactions of 9-ONA and methyl 9-ONA, the starting temperature of the oven program was set to 40 °C, which was held for 9 min, then increased to 43 °C at a rate of 3 °C/min, and finally increased to 300 °C at a rate of 30 °C/min. This was followed by a post-run at 300 °C for 3 min. The mass spectrometer operated in electron impact mode at 70 eV in SCAN mode (mass window: m/z 30.0—300.0).

Statistical analysis

Results are given as means of duplicates ± standard deviations, except otherwise indicated. To test for significant differences, results of peanuts roasted at 170 °C for 20 min or 40 min, respectively, were compared with results obtained for raw peanuts using a two-sided two-sample t test with unequal variance. OriginPro Version 2019b (9.65) Graphing & Analysis by OriginLab (Northampton, USA) was used.

Results and discussion

Phenomenological studies to the formation of secondary reaction products of LPO in peanuts

As a result of roasting peanuts at 170 °C for 20 or 40 min, respectively, the iodine value (IV) decreased from
92.4 g I₂/100 g peanut oil to 90.6 g I₂/100 g peanut oil (significant, two-sample t test, two-sided, α = 0.05) and the peroxide value (POV) decreased from 0.95 meq O₂/kg peanut oil to 0.85 meq O₂/kg peanut oil (highly significant, two-sample t test, two-sided, α = 0.01) (Table 1). Similarly, the isoleic and conjugate bands recorded by UV spectroscopy decreased during peanut roasting (Fig. S1 in Supplementary Information). The commercial sample, on the other hand, had an IV of 96.7 g I₂/100 g peanut oil and a POV of 3.25 meq O₂/kg peanut oil, which was confirmed by the increased relative intensity of the isoleic and conjugate bands (Table 1 and Fig. S1 in Supplementary Information). At the same time, the thiobarbituric acid value (TBAV) slightly increased from 38.5 mg MDA/kg peanut oil to 42.4 mg MDA/kg peanut oil (not significant, two-sample t test, two-sided) as the roasting time progressed. The commercial sample had a similar TBAV of 43.3 mg MDA/kg peanut oil (Table 1). Minor changes, however, could be documented for the content of LA, which slightly decreased from 34.2 g/100 g peanut oil to 32.7 g/100 g peanut oil (not significant, two-sample t test, two-sided) as a result of peanut roasting at 170 °C (Table 1). Whereas, no significant changes for OA were observed (from 40.7 g/100 g peanut oil to 39.3 g/100 g peanut oil, Table 1). For the commercial sample, a content of LA of 38.3 g/100 g peanut oil and of OA ranging from 23.9 to 39.3 g/100 g peanut oil and for OA ranging from 53.5 to 52.6 g/100 g peanut oil, respectively [25]. The decrease of IV, POV as well as the content of LA with a simultaneous increase of TBAV with continuous roasting time suggests the initially beginning of the LPO. This is also confirmed by the decreasing relative intensities of the isoleic and conjugate bands. The isoleic band is directly related to the IV as well as the content of LA (polyunsaturated fatty acids) and the conjugate band to the POV (hydroperoxides). Induced by the roasting process, secondary reaction products of LPO (increasing TBAV) are formed with simultaneous degradation of monounsaturated and polyunsaturated fatty acids (decreasing IV, content of LA and relative intensity of the isoleic band) as well as hydroperoxides (decreasing POV and relative intensity of the conjugate band) due to the Hock rearrangement and cleavage as well as by the β-cleavage (Table 1 and Fig. S1 in Supplementary Information). Overall, these phenomenological investigations indicate the course of LPO with the formation of secondary reaction products during the roasting process of peanuts.

### Table 1

|        | IV*       | POV*       | TBAV*      | LA*       | OA*       |
|--------|-----------|------------|------------|-----------|-----------|
| raw    | 92.4 ± 0.1 | 0.95 ± 0.00 | 38.5 ± 0.1  | 34.2 ± 0.8 | 40.7 ± 1.0 |
| 20 min | 91.5 ± 0.00 | 0.90 ± 0.00 | 40.5 ± 0.6  | 32.8 ± 0.1 | 39.5 ± 0.1  |
| 40 min | 90.6 ± 0.20 | 0.85 ± 0.00 | 42.4 ± 1.0  | 32.7 ± 0.1 | 40.3 ± 0.1  |
| commercial | 96.7 ± 0.1 | 3.25 ± 0.01 | 43.3 ± 0.2  | 38.3 ± 0.4 | 46.0 ± 0.6  |

To check significance of the results, a two-sided two-sample t test was used (for explanation, see statistical analysis in Materials and methods)

*a*Not significantly, significantly (α = 0.05)*b* and highly significantly (α = 0.01)*c* different when compared to raw sample
Fig. 2 Identification of methyl heptanoate 13, methyl octanoate 8, 2E,AE-decadienal 9, methyl 8-oxooctanoate 10, methyl undecanoate 11, methyl 9-ONA 6, dimethyl azelate 14, and methyl 10-oxo-8E-decenoate 12 in A raw peanuts and B peanuts roasted at 170 °C for 40 min. GC-MS (EI) scan chromatograms from 10 to 35 min showing in consecutive numbering the formed methyl esters of oxidized fatty acids. Mass spectra of C methyl octanoate (m/z 158.1, retention time 13.6 min), D methyl 9-ONA (m/z 186.1, retention time 25.7 min), and E dimethyl azelate (m/z 216.1, retention time 30.2 min). Detailed information is presented in the text.

Fig. 3 Semiquantitation of selected GOFAs: 9-ONA, AZA, and octanoic acid by GC-MS (EI): A amounts in peanuts roasted at 170 °C compared with a commercial sample; B amounts in peanut oil heated at 170 °C.
(Fig. 3). The content of 9-ONA initially decreased during the roasting process from 25 mg/kg peanut oil or 12 mg/kg peanut (124 μmol/kg peanut oil or 62 μmol/kg peanut) to 8 mg/kg peanut oil or 4 mg/kg peanut (40 μmol/kg peanut oil or 20 μmol/kg peanut, respectively) after 20 min of roasting (significant, two-sample t test, two-sided, α = 0.05). With continued roasting time (40 min), however, there was an increase to 37 mg/kg peanut oil or 18 mg/kg peanut (184 μmol/kg peanut oil or 91 μmol/kg peanut, respectively) (highly significant, two-sample t test, two-sided, α = 0.01) (Fig. 3). In the commercial sample, 51 mg/kg peanut oil or 26 mg/kg peanut (255 μmol/kg peanut oil or 127 μmol/kg peanut, respectively) could be determined (Fig. 3). In contrast to the peanuts, a steady increase in the content of 9-ONA was observed in the peanut oil samples. Here, the content increased more markedly up to 807 mg/kg peanut oil (4029 μmol/kg peanut oil) (highly significant, two-sample t test, two-sided, α = 0.01) (Fig. 3). For AZA, a steady increase up to 8 mg/kg peanut oil or 4 mg/kg peanut (37 μmol/kg peanut oil or 18 μmol/kg peanut, respectively) could be determined (highly significant, two-sample t test, two-sided, α = 0.01) (Fig. 3). In the commercial sample, 6 mg/kg peanut oil or 3 mg/kg peanut (30 μmol/kg peanut oil or 15 μmol/kg peanut, respectively) could be determined (Fig. 3). The same trend was observed in the peanut oil samples, where the content increased up to 29 mg/kg peanut oil (147 μmol/kg peanut oil) (highly significant, two-sample t test, two-sided, α = 0.01) (Fig. 3). Similar to AZA, the content of octanoic acid in peanuts increased from 59 mg/kg peanut oil or 30 mg/kg peanut (295 μmol/kg peanut oil or 174 μmol/kg peanut, respectively) to 101 mg/kg peanut oil or 50 mg/kg peanut (502 μmol/kg peanut oil or 247 μmol/kg peanut, respectively) (highly significant, two-sample t test, two-sided, α = 0.01) (Fig. 3). In the commercial sample, 130 mg/kg peanut oil or 65 mg/kg peanut (650 μmol/kg peanut oil or 325 μmol/kg peanut, respectively) could be determined (Fig. 3). The same trend was observed in the peanut oil samples. Here, however, the content increased more significantly from 26 mg/kg peanut oil (131 μmol/kg peanut oil) to 843 mg/kg peanut oil (4210 μmol/kg peanut oil) (highly significant, two-sample t test, two-sided, α = 0.01) (Fig. 3). Generally, by comparing the amounts of 9-ONA, AZA, and octanoic acid in peanuts roasted at 170 °C with peanut oil heated at rancimat conditions at 170 °C and 20 L/h air, significantly lower contents in peanuts roasted at 170 °C could be documented (Fig. 3). A decisive cause for higher contents in peanut oil is heating at 170 °C under saturated oxygen conditions, which contributes to an increased formation of secondary reaction products of LPO. For the 9-ONA, an opposite trend between peanuts and peanut oil could be observed. Due to its reactive aldehyde group, 9-ONA in peanuts is able to modify nucleophilic side chains of amino acids, for example lysine, in the presence of proteins with the formation of neo-lipoproteins (glycerol-bound lipation products, Fig. 4). A roasting time of 20 min resulted in significant decrease in free 9-ONA, which is presumably associated with lipation of proteins. The increase in the content after a roasting time of 40 min can be explained on one hand by a neoformation within the LPO process and on the other hand by possible release of the protein-bound 9-ONA. The increasing contents of 9-ONA in peanut oil with continuous heating time at rancimat conditions are in agreement with the amounts determined by Velasco et al. (2005) in sunflower and olive oil by GC-FID. They observed an increase from 600 mg/kg oil to 1270 mg/kg oil in sunflower oil and from 500 mg/kg oil to 960 mg/kg oil in olive oil during heating simulating discontinuous frying from 5 to 15 h at 180 °C [16]. It could also be demonstrated that the 9-ONA in both peanuts and peanut oil can be considered as a major component of the reactive GOFAs, and represents a possible marker for thermal processing. On the other hand, the semiquantitatively determined contents of AZA and octanoic acid showed that the content of both peanuts and peanut oil increased as the heating time progressed. Due to its lack of an electrophilic center, octanoic acid cannot modify nucleophilic side chains of peanut proteins, which was evident from the continuously increasing content during the roasting process. The same applies to AZA, which is quite stable, in comparison to the corresponding aldehyde. Similar to the 9-ONA, octanoic acid is suitable as a marker for the part of the non-reactive GOFAs formed during thermal processing in both peanut oil and peanuts. For all selected GOFAs, the total content of unsaturated fatty acids with a double bond at the C9 atom in raw peanuts must be taken into account for the assessment of the semiquantitated contents, since the content of LA or OA, for example, is subject to fluctuations due to cultivation conditions, such as region or climatic conditions, or peanut variety [25]. The contents of 9-ONA, AZA, and octanoic acid of the standard roasting series determined by GC-MS (EI) at 170 °C were comparable to the contents of the commercial sample. Furthermore, the increasing amounts of the selected GOFAs with continuous roasting time confirm the results of the commercial sample. For model system 1, mild incubation conditions were used, namely 37 °C for 24 h, serving as prove of principle to study possible reaction mechanisms of the LPO process and the formation of GOFAs. On the other hand, for model
system 2, more severe thermal conditions (170 °C, 20 min) served to study the influence of the roasting process on the formation of GOFAs. Furthermore, to proof the influence of transition metals to oxidative subsequent reactions of GOFAs as well as free oxidized fatty acids (FOFAs), methyl 9-ONA, and 9-ONA were incubated at 37 °C for 24 h in the presence of iron (III). Generally, a total of 18 methyl esters of oxidized fatty acids could be identified for methyl linoleate and 10 for methyl oleate by means of GC-MS (EI) (Fig. 5 as well as Fig. S3 and Table S1 in Supplementary Information). In both applied model systems, a comparable product spectrum of GOFAs was observed for methyl linoleate as well as methyl oleate. The identification of the GOFAs was carried out in each case via their typical fragmentation patterns and the comparison of their mass spectra with the literature and a mass spectrometric database (Fig. 5 and Table S1 in Supplementary Information). In addition, the GOFAs methyl hexanoate, methyl heptanoate, methyl octanoate, methyl 9-ONA, methyl ODA, as well as monohydrogen azelate were verified by comparing with their respective standards. For the identified GOFAs, the source of formation, retention time, and key fragment ions are listed in Table S1 in Supplementary Information. The methyl 9,12-dioxo-10E-dodecenoate (compound 28, m/z 240.1, retention time 36.7 min) could only be identified for methyl linoleate in model system 1 in the presence of L-ascorbic acid and iron.

**Fig. 4** Postulated formation of neo-lipoproteins 16 due to reaction of glycerol-bound 9-ONA 6, for example formed by 9-HPODE 15 of linoleic acid 1, with nucleophilic side chains of proteins (Nu-Protein). Detailed information is presented in the text. $R_1 + R_2 = –C_{17}H_{31}$ (linoleic acid)
The same observation was made for the model systems of methyl oleate. Here, the following methyl esters of oxidized fatty acids could only be identified in the model system 2: methyl hexanoate, methyl 8-oxooctanoate, methyl 10-oxodecanoate, as well as monohydrogen azelate, chromatograms are not shown. The GOFAs identified in both model systems are in agreement with identified GOFAs in peanuts roasted at 170 °C (Fig. 2) as well as with the results of Berdeaux et al. (2012), who were able to identify 21 GOFAs in a similar technological model approach of methyl linoleate and methyl oleate using GC-MS as well as GC-FTIR [15]. In our experiments, we could show via comparing the total peak areas in both applied model systems that the formation of GOFAs under roasting conditions (170 °C, 20 min) is induced more quickly. Similar to the results of Velasco et al. (2005), octanoic acid was observed as the major component of the non-reactive GOFAs and 9-ONA as the major component of the reactive GOFAs on the basis of their relative peak areas in both model systems (Fig. 5 and Fig. S3 in Supplementary Information). This is also in accordance with our semiquantiitated amounts in peanuts roasted at 170 °C (Fig. 3). [16] In the literature reports, the high amounts of 9-ONA are explained by its formation from the hydroperoxides 9-hydroperoxy-10E,12Z-octadecadienoic acid (9-HPODE), 9,12-dihydroperoxy-10E,13E-octadecadienoic acid (9,12-DiHPODE), 10-hydroperoxy-8E,12Z-octadecadienoic acid (10-HPODE), and 10,13-dihydroperoxy-8E,11E-octadecadienoic acid (10,13-DiHOPDE) (Fig. 6) [16, 27]. In further investigations on the model substances methyl 9-ONA for GOFAs and 9-ONA for FOFAs, oxidative subsequent reactions were observed after incubation for 24 h at 37 °C in the presence of iron (III) by GC-MS (EI). For this purpose, the model preparations were measured against the corresponding standard.

![Figure 5](image-url)
and the relative proportion of the peak areas to the total peak area were determined. The relative amount of methyl 9-ONA decreased up to 53.8% with an increased formation of monohydrogen azelate of 51.0% and methyl octanoate of 2.8%. Similar results were observed for 9-ONA. Passi et al. (1993) already postulated that the 9-ONA can be further oxidized to AZA in the presence of transition metals [28]. In our experiments, it was possible to show that AZA, formed from 9-ONA, can oxidatively decarboxylate in the presence of iron (III) to form octanoic acid (Fig. 7), which partly explains the high amounts in peanuts roasted at 170 °C in addition to the β-cleavage of hydroperoxides (Fig. 3). Furthermore, it was possible to assess the influence of the roasting process on the increased formation of GOFAs by comparison of the used model systems with peanuts roasted at 170 °C.

**Conclusion**

For the first time, selected GOFAs, namely 9-ONA, AZA, and octanoic acid, were identified and semiquantitated in peanuts roasted at 170 °C. In addition, it was possible to demonstrate that a continuous roasting time induces an increased formation of GOFAs. These results confirm that GOFAs are simultaneously formed together with volatile compounds, such as hexanal or acrolein, during the LPO process. 9-ONA (reactive GOFAs) and octanoic acid (non-reactive GOFAs) represent markers for thermal processes such as the roasting. Furthermore, by comparing the amounts of 9-ONA, AZA, and octanoic acid in peanuts roasted at 170 °C with peanut oil heated at rancimat conditions at 170 °C and 20 L/h air, it was possible to show that GOFAs occurred in significantly lower contents in peanuts roasted at 170 °C. Our model studies also confirmed that 9-ONA and octanoic acid are major compounds of the LPO process and that the roasting process of peanuts induces an increased formation of GOFAs. In model experiments using methyl 9-ONA and 9-ONA, it was possible to demonstrate the occurrence of oxidative subsequent reactions for free as well as glycerol-bound oxidized fatty acids in the presence of iron (III), which partly explains the amounts of AZA and octanoic acid in peanuts roasted at 170 °C. Further investigations concerning the formation of further GOFAs as well as possible reactions of GOFAs with proteins in the course of lipation reactions are currently in progress.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00217-022-04030-x.

**Acknowledgements** We thank Dr. Kai Schwedtmann, Chair of Inorganic Molecular Chemistry, Technische Universität Dresden, for performing the NMR measurements.

**Author contributions** Conceptualization, LS, MG, and TH; investigations, LS; writing, LS, MG, and TH.

**Funding** Open Access funding enabled and organized by Projekt DEAL. This study was funded by the Bundesanstalt für Landwirtschaft und Ernährung, 281A301C18

**Declarations**

**Conflict of interest** All authors declare that they have no conflict of interest.
**Ethical statement** No studies with human or animal subjects are contain in this article.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

**References**

1. Hoffpaur CL (1953) Peanut composition – relation to processing and utilization. J Agric Food Chem 10(1):668–671. https://doi.org/10.1021/jf60010a004
2. Maguire LS, O’Sullivan SM, Galvin K, O’Connor TP, O’Brien PP (2000) Peanut allergy, allergen composition, and methods of reducing allergenicity: a review. Int J Food Sci Nutr 51(4):285–297. doi:10.1080/000527600100005366
3. Sampson HA (2001) Effects of cooking methods on peanut allergenicity. J Allergy Clin Immunol 107(6):1077–1081. https://doi.org/10.1067/ma1.2001.128802
4. Grundy J, Matthews S, Bateman B, Dean T, Arshad SH (2002) Peanut composition – relation to processing and utilization. J Agric Food Chem 10(1):668–671. doi:10.1021/jf60010a004
5. Zhou Y, Wang JS, Yang XJ, Lin DH, Gao YF, Su YJ, Zheng JJ (2013) Peanut allergy, allergen composition, and methods of reducing allergenicity: a review. Int J Food Sci 2013:1–8. https://doi.org/10.1155/2013/909140
6. Beyer K, Moreau E, Li XM, Bardina L, Bannon GA, Burks W, Sampson HA (2001) Effects of cooking methods on peanut allergenicity. J Allergy Clin Immunol 107(6):1077–1081. https://doi.org/10.1067/ma1.2001.115480
7. Chung SY, Champagne ET (2001) Association of end-product additives with increased iGE binding of roasted peanuts. J Agric Food Chem 49:3911–3916. https://doi.org/10.1021/jf0101180
8. Maleki SJ, Chung SY, Champagne ET, Raumann JP (2000) The effects of roasting on the allergenic properties of peanut proteins. J Allergy Clin Immunol 106(4):763–768. https://doi.org/10.1007/mai.2000.109620
9. Mondoulet L, Patey E, Drumare MF, Ah-Leung S, Scheinmann P, Willemot RM, Wal JM, Bernard H (2005) Influence of thermal processing on the allergenicity of peanut proteins. J Agric Food Chem 53:4547–4553. https://doi.org/10.1021/jf050091p
10. Wellner A, Nüßpickel L, Henle T (2012) Glycation compounds in peanuts. Eur Food Res Technol 234:423–429. https://doi.org/10.1007/s00217-011-1649-8
11. Globisch M, Schindler M, Kreßler J, Henle T (2014) Studies on the reaction of trans-2-heptenal with peanut proteins. J Agric Food Chem 62:8500–8507. https://doi.org/10.1021/jf502501f
12. Globisch M, Kaden D, Henle T (2015) 4-Hydroxy-2-nonenal (4-HNE) and its lipation product 2-pentylpyrrolyl lysine (2-PPL) in peanuts. J Agric Food Chem 63:5273–5278. https://doi.org/10.1021/acs.jafc.5b01502
13. Globisch M, Deuber M, Henle T (2016) Identification and quantification of the lipation product 2-amino-6-(3-methylpyridin-1-ium-1-yl)hexanoic acid (MP-Lysine) in peanuts. J Agric Food Chem 64:6605–6612. https://doi.org/10.1021/acs.jafc.6b03371
14. Sayre LM, Lin D, Yuan Q, Zhu X, Tang X (2006) Protein adducts generated from products of lipid peroxidation: focus on HNE and ONE. Drug Metab Rev 38:651–675. https://doi.org/10.1080/03602530600959508
15. Berdeaux O, Fontagné S, Sénon E, Velasco J, Sébédo JL, Dobarganes CA (2012) A detailed identification study on high-temperature degradation products of oleic and linoleic acid methyl ester by GC-MS and GC-FTIR. Chem Phys Lipid 165:338–347. https://doi.org/10.1016/j.chemphyslip.2012.02.004
16. Velasco J, Marmesat O, Berdeaux O, Márquez-Ruiz G, Dobarganes CA (2005) Quantitation of short-chain glycerol-bound compounds in thermoxidized and used frying oils. A monitoring study during thermoxidation of olive and sunflower oils. J Agric Food Chem 53:4006–4011. https://doi.org/10.1021/jf050050t
17. Kamal-Eldin A, Márquez-Ruiz G, Dobarganes C, Appelqvist LÅ (1997) Characterisation of aldehydic acids in used and unused frying oils. J Chromatogr A 776:245–254. https://doi.org/10.1016/S0021-9673(97)00355-5
18. Matsissk R, Steiner G, Fischer M (2014) In: Lebensmittelanalytik, 5th edn. Springer-Verlag. Berlin
19. Yun JM, Surh J (2012) Fatty acid composition as a predictor for he oxidation stability of korean vegetable oils with or without induced oxidative stress. Prev Nutr Food Sci 17:158–165. https://doi.org/10.3746/pnfs.2012.17.2.158
20. Woodcock SR, Mamitz AJV, Bruno P, Branchad BP (2006) Synthesis of nitroilipids: all four possible diastereomers of nitroilic acids: (E) and (Z), 9- and 10-nitro-octadec-9-enoic acids. Org Letters 18:1–33. https://doi.org/10.1021/ol0613463
21. Minamoto S, Kanazawa K, Ashida H, Natake M (1988) Effect of orally 9-oxononanoic acid on lipogenesis in rat liver. Biochem Biophys Acta 958:199–204. https://doi.org/10.1016/0005-2760(88)90177-4
22. Presser A, Hufner A (2004) Trimethylsilyldiazomethane – a mild and efficient reagent for the methylation of carboxylic acids and alcohols in natural products. Monatshefte für Chemie / Chemical Monthly 135:1015–1055. https://doi.org/10.1007/s00706-004-0188-4
23. Velasco J, Berdeaux O, Márquez-Ruiz G, Dobarganes MC (2002) Sensitive and accurate quantitation of monoepeoxy fatty acids in thermoxidized oils by gas – liquid chromatography. J Chromatogr A 982:145–152. https://doi.org/10.1016/S0021-9673(02)01481-4
24. Refsgaard HHF, Tsal L, Stedman ER (2000) Modification of proteins by polyunsaturated fatty acid peroxidation products. PNAS 97(2):611–616. https://doi.org/10.1073/pnas.97.2.611
25. Souci SW, Fachmann W, Kraut H (2008) In: Food Composition and Nutrition Tables, 7th edn. MedPharm Scientific Publishers, Stuttgart
26. Belitz H-D, Grosch W, Schieberle P (2012) In: Lehrbuch der Lebensmittelchemie, 6th edn. Springer-Verlag. Berlin
27. Spickett CM (2013) The lipid peroxidation product 4-hydroxy-2-nonenal: Advances in chemistry and analysis. Redox Biol 1:145–152. https://doi.org/10.1016/j.redox.2013.01.007
28. Passi S, Picardo M, De Luca C, Nazzaro-Porro M, Rossi L, Rotilio G (1994) Saturated dicarboxylic acids as products of unsaturated fatty acid oxidation: focus on HNE and ONE. Drug Metab Rev 38:651–675. https://doi.org/10.1080/03602530600959508
29. Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.