European Journal of Lipid Science and Technology

WILEY-VCH

European Journal of
Lipid Science and Technology

SHORT COMMUNICATION OPEN ACCESS

Enzymatically Formed Fatty Acid Hydroperoxides Determined Through GC-MS Analysis of Enantiomeric Excess of Hydroxy Fatty Acids After Reduction and Ibuprofen Derivatization

Lisa Hotz[1] | Anne Zartmann[1,2] | Isabelle Noack[2] | Luca J. Drees[3] | Meret K. Kuschow[3] | Markus R. Heinrich[3,4] | Hans-Gerd Janssen[5,6] | Simon Hammann[1,2,4]

[1]Food Chemistry, Department of Chemistry and Pharmacy, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany | [2]Department of Food Chemistry and Analytical Chemistry (170a), Institute of Food Chemistry, University of Hohenheim, Stuttgart, Germany | [3]Pharmaceutical Chemistry, Department of Chemistry and Pharmacy, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany | [4]FAU NeW—Research Center New Bioactive Compounds, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany | [5]Department of Agrotechnology and Food Sciences, Laboratory of Organic Chemistry, Wageningen University, Wageningen, The Netherlands | [6]Unilever Foods Innovation Centre, Wageningen, The Netherlands

Correspondence: Simon Hammann (simon.hammann@uni-hohenheim.de)

Received: 27 March 2024 | **Revised:** 27 November 2024 | **Accepted:** 18 December 2024

Funding: This study was supported by Rudolf und Henriette Schmidt-Burkhardt Stiftung, Universitätsbund Erlangen-Nürnberg, Josef-Schormüller-Gedächtnisstiftung.

Keywords: enantiomeric excess | gas chromatography | hydroperoxide | ibuprofen | lipid oxidation

ABSTRACT

Unsaturated fatty acids are susceptible to lipid oxidation through autoxidation, photooxygenation or enzymatical oxidation. A characteristic feature of enzyme-catalyzed oxidation is the high regio- and stereospecificity of the formed fatty acid hydroperoxides. In this study, we present a method to quantify enzymatic lipid oxidation through reducing hydroperoxy fatty acid methyl esters to hydroxy fatty acid methyl esters and derivatizing them with enantiopure (S)-ibuprofen, allowing the resolution of the enantiomer pairs as diastereomers via achiral GC-MS. After application to enantiopure reference fatty acids, the approach was applied to autoxidation products of linoleic acid, and the expected racemic mixtures of the 9- and 13-hydroperoxide derived hydroxy fatty acids were detected. On the other hand, when linoleic acid was oxidized using soybean lipoxygenase, clear enantiomeric excess of the (13S) enantiomer could be detected, proving the applicability of this method to detect enzymatic oxidation through enantiomeric excess.

1 | Introduction

Lipids comprise a diverse group of compounds with vastly different structures and physico-chemical properties. In food, they are important nutrients, act as emulsifiers, and contribute to a pleasant mouthfeel and flavor of the food [1]. From a quantitative standpoint, fatty acids—usually bound in triacylglycerols—are the most important lipid species in food. Among these, polyunsaturated fatty acids are particularly valuable, as they can be essential food components for humans and are precursors for, for example, eicosanoids and other hormones [2].

Abbreviations: DCC, dicyclohexylcarbodiimide; DCM, dichlormethane; DMAP, 4-(dimethylamino)-pyridine; ee, enantiomeric excess; FAME, fatty acid methyl ester; HP, hydroperoxide.

SUMMARY

• Lipid oxidation widely affects the quality and safety of food. Understanding and mitigating lipid oxidation is therefore of utmost importance to avoid food spoilage and, consequently, food waste and ensure the absence of lipid oxidation products with adverse effects on human health.

• The analysis of the hydroperoxides formed through lipid oxidation allows the deduction of the principal mechanisms of lipid oxidation, which is required to guide mitigation strategies.

• Our approach does not require expensive (NMR) equipment nor specialized (chiral) chromatography columns and can therefore be easily implemented in laboratories to determine the enantiomeric excess of hydroperoxides and thus the extent of enzymatic oxidation.

• Furthermore, the principle of using ibuprofen as a chiral derivatization agent with good chromatographic properties could be used in different fields where enantioselective information is required, such as flavor or pharmaceutical analysis.

However, unsaturated fatty acids are inherently susceptible to lipid oxidation, either through autoxidation, photooxygenation or action of lipoxygenases [3]. During lipid oxidation, these valuable food components are lost, while newly formed oxidation products can impair the flavor and taste of the food and have adverse health effects [4]. Therefore, a lot of attention is put on devising appropriate measures to suppress or at least delay lipid oxidation in food. Particularly in complex food products (such as vegan meat imitations based on plant protein) this is highly challenging since lipid oxidation could be caused by a number of different ingredients or processing steps. Selecting appropriate mitigation measures requires knowledge about the route (or routes) of lipid oxidation active in a given food item and the respective reaction pathways: While autoxidation involves different radical species and initially forms peroxyl species, during photooxygenation and enzymatic oxidation the hydroperoxides (HP) are formed directly without free radical reactions. Hence, an addition of radical scavengers would only be an effective countermeasure for autoxidation, while photooxygenation and enzymatic oxidation would be largely unaffected [3].

Autoxidation, photooxygenation and enzymatic oxidation are known to lead to different patterns of fatty acid hydroperoxide positional isomers, which can be used for identification of the principle pathway of oxidation [5, 6]. For example, autoxidation of linoleic acid almost exclusively leads to the formation of (conjugated) 9- and 13-hydroperoxides as main products, while during photooxygenation additionally the non-conjugated 10- and 12-hydroperoxides are formed (Figure 1). Finally, enzymatic oxidation with soybean lipoxygenase exclusively forms the 13-hydroperoxide [5, 7]. However, using these differences analytically can be exceedingly difficult when several mechanisms are active at the same time, resulting in superimposed patterns. As an alternative, the stereoselectivity of the reaction can be investigated, specifically to reveal enzymatic oxidation, as hydroperoxide formation in this oxidation route is not only highly selective for the position, but also for the stereochemistry of the

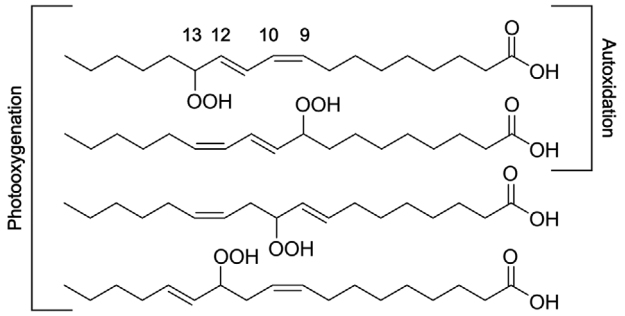

FIGURE 1 | Structures of the 13-, 9-,10-, and 12-hydroperoxides formed from linoleic acid. The principal products formed during autoxidation and photooxygenation, respectively, are marked with brackets.

formed hydroperoxide [7, 8]. Autoxidation and photooxygenation lead to racemic hydroperoxides, while enzymatic oxidation tends to lead to high selectivity for either the R or the S enantiomer Several techniques for determining the stereochemistry of lipid hydroperoxides have been described [9–12], with chromatography being the most readily applicable method. Yet, this usually requires specific chiral chromatographic conditions and is therefore difficult to implement in standard analytical protocols.

Here, we present a new approach to quantify the relative contribution of enzymatic lipid oxidation through analysis of the enantiomeric excess of hydroperoxides formed from unsaturated fatty acids using GC-MS. Our approach is based on reduction and methylation of unsaturated fatty acid hydroperoxides and the derivatization of the resulting saturated hydroxy fatty acid methyl esters using enantiopure (S)-ibuprofen, adapting a method previously published by Cryle et al. [13]. This leads to diastereomer pairs that can be separated under standard, achiral GC conditions, and the relative contribution of each diastereomer can be used for calculation of potential enantiomeric excess if enzymatic oxidation had occurred. The applicability of the method for determination of enantiomeric excess is demonstrated by the analysis of hydroxy fatty acid standards and linoleic acid samples subjected to autoxidation and enzymatic oxidation leading to racemic and enantio-enriched hydroperoxides.

2 | Materials and Methods

2.1 | Chemicals

Dichloromethane, chloroform and *n*-hexane (all HPLC grade) were from Fisher Chemical (Waltham, MA, USA). Dioxane (anhydrous, 99.8%), pyridine (99.8%), dicyclohexylcarbodiimide (DCC, 99%) and 4-(dimethylamino)-pyridine (DMAP, 99%), linoleic acid (99%), type 1-B soybean lipoxidase (activity >50 000 units/mg), (S)-ibuprofen (99%) and rhodium-tris-(triphenylphosphine)-chloride (Wilkinson's catalyst) were supplied from Sigma-Aldrich (Steinheim, Germany). Authentic standards of 3-hydroxy stearic acid, 9-hydroxy stearic acid, and 12-hydroxy stearic acid (all >95%) were obtained from Cayman Chemicals (Ann Arbor, MA, USA) and ricinoleic acid methyl ester (12R-OH-18:1Δ9-ME) was isolated from a transesterified castor oil. Ethyl acetate was from Carl Roth (Karlsruhe, Germany) and the Silica gel 60 (0.04–0.063 mm) was from Macherey-Nagel

(Düren, Germany). Deuterium gas (99.8% D) was supplied by Linde (Unterschleissheim, Germany). Racemic ibuprofen was isolated and purified from pharmaceutical products within the FAU expired drug initiative (see Supporting Information)

2.2 | Esterification of Fatty Acids

Fatty acid and castor oil samples were converted to fatty acid methyl esters (FAME) by acid-catalyzed esterification using 1% sulfuric acid in methanol as described previously [14].

2.3 | Autoxidation of Linoleic Acid Methyl Ester and Enzymatic Oxidation of Linoleic Acid

For autoxidation, neat linoleic acid methyl ester (80 mg, prepared from linoleic acid as described above) was weighed into an amber glass vial and covered loosely with aluminum foil. The vial was stirred at 40°C for 72 h and the contents were afterward subjected to deuteration. For enzymatic oxidation, linoleic acid (5 mg in 1 mL ethanol) was dissolved in 9 mL borate buffer (50 mM, pH 10) and 4 mg of soybean lipoxygenase was added [15]. The mixture was stirred at room temperature for 1 h before the solution was acidified to pH 4 using 1 M HCl solution, the fatty acids were extracted using chloroform and subjected to deuteration.

2.4 | Deuteration of Fatty Acids and Fatty Acid Methyl Esters

The previously autoxidized linoleic acid methyl ester or enzymatically oxidized linoleic acid was redissolved in *n*-hexane/DCM (80:20, 1 mL) and transferred to a two-necked round bottom flask where the solvents were evaporated using a stream of nitrogen. Afterward, dioxane (2 mL) and the Wilkinson's catalyst (7 mg; 2 mg for enzymatically oxidized linoleic acid) were added. A balloon full of deuterium gas was used to generate a deuterium atmosphere, and the flask volume was flushed two times with deuterium by opening the valve and carefully raising the glass stopper. Maintaining a deuterium atmosphere, the apparatus was heated to 60°C in a sand bath and stirred for 2 h [16].

The color of the solution turned from orange to dark brown. After 2 h, the solvent was evaporated using a gentle stream of nitrogen and the residue was redissolved in *n*-hexane/DCM (80:20, 2 mL). In case of enzymatic oxidation of linoleic acid, the sample was subjected to esterification as described above. Both samples were then subjected to solid phase extraction. A 0.5 cm i.d. glass column filled with activated silica (0.5 g) was conditioned with *n*-hexane (6 mL). After addition of the solvent, unoxidized fatty acid methyl esters were eluted with *n*-hexane/ethyl acetate (95:5, 6 mL) before saturated hydroxy fatty acid methyl esters were collected using *n*-hexane/ethyl acetate (80:20, 6 mL).

The fractions were collected in screw-cap culture tubes, transferred into pre-weighed vials and the solvents were evaporated using a gentle stream of nitrogen. The residue was redissolved in *n*-hexane (1 mL), diluted to 20 µg/mL and derivatized for the GC-MS measurement.

2.5 | Preparation of Fatty Acid Methyl Ester O-Ibuprofen Esters

Ibuprofen (9.80 mg, 47.5 µmol), hydroxy fatty acid methyl ester (10 mg, ca. 30 µmol or less), DMAP (80 µL, 125 mg/mL in DCM, 81.9 µmol) and DCC (175 µL, 113 mg/mL in DCM, 72.7 µmol) and DCM (1 mL) were added to a screw-cap culture tube following a *Steglich* esterification procedure previously described by Lísa et al. [17]. Subsequently, the mixture was shaken for 22 h at room temperature and a white solid precipitated. Afterward, the solvent was evaporated, and the residue was redissolved in *n*-hexane and purified by solid phase extraction. A 0.5 cm i.d. glass column filled with activated silica (0.5 g) was conditioned with *n*-hexane (6 mL) and the ibuprofen esters were eluted with *n*-hexane/ethyl acetate (95:5, 6 mL).

A scheme showing the complete sample preparation workflow can be found in Figure S1.

2.6 | GC-MS Analysis of Fatty Acid Methyl Ester O-Ibuprofen Esters

GC-MS analyses were performed using an Agilent 8860 GC coupled to a 5977B mass spectrometer and 7393A autosampler. The samples (1 µL) were injected onto a VF-23 ms column (60 m × 0.25 mm; 0.25 µm; Agilent Technologies; Santa Clara, CA, USA) using a split/splitless injector held at 260°C in splitless mode. Helium (purity 5.0) was used as the carrier gas at a constant flow rate of 1.2 mL/min. The GC oven was programmed as follows: After 2 min at 50°C the temperature was increased to 260°C at a rate of 10°C/min and this final temperature was held for 20 min. The temperatures of the transfer line, ion source and quadrupole were set to 260°C, 230°C, and 150°C, respectively. Electron ionization at 70 eV was used and after a solvent delay of 5 min data were recorded in full scan mode from m/z 50 to 650.

Data acquisition and analysis were performed using Agilent Mass Hunter Workstation 10.0 and Qualitative Analysis 10.0, respectively. Fatty acid methyl ester O-ibuprofen esters were identified through their GC elution range and the base peak at m/z 161 as well as the fatty acid specific fragment ion formed through cleavage or elimination of the ibuprofen moiety in the GC-MS spectra (see below).

3 | Results and Discussion

3.1 | Analysis of Ibuprofen Esters of Hydroxy Fatty Acid Standards

The GC-MS analysis of the hydroxy FAME ibuprofen esters (Figure 2) could be achieved using a standard FAME analysis set-up using a 60 m highly polar column and a maximum operating temperature of 260°C. Analysis of ricinoleic acid methyl ester (FAME 12R-OH-18:1Δ9) esterified with (S)-ibuprofen yielded one principal peak with very minor contribution of a partly resolved second peak with the same mass spectrum (ca 1%), while the reaction with racemic ibuprofen led to two peaks of similar intensity (Figure 3a). This confirmed that the diastereomer pairs can be separated under our conditions and confirmed the earlier elution

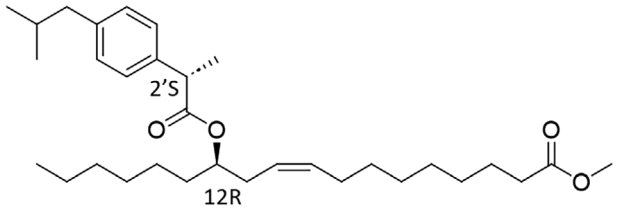

FIGURE 2 | Structure of FAME 12R-OH-18:1Δ9 (ricinoleic acid methyl ester) as O-(S)-ibuprofen ester.

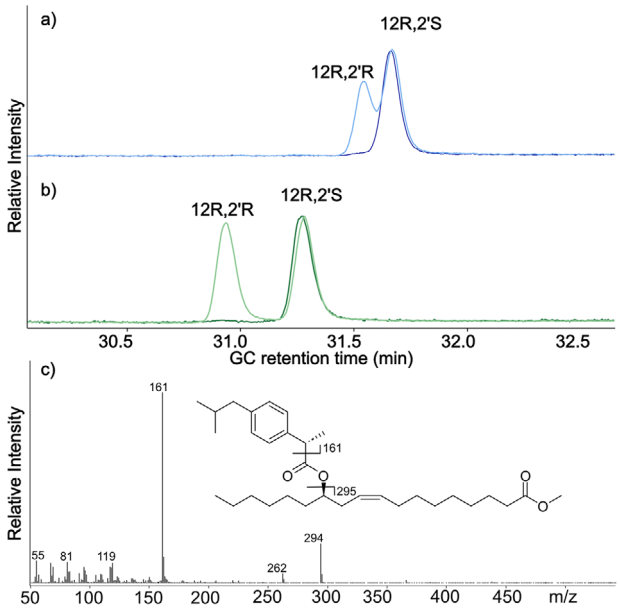

FIGURE 3 | Overlaid partial GC-MS total ion chromatograms of (a) ricinoleic acid methyl ester esterified with (S)-ibuprofen (dark blue) and racemic ibuprofen (light blue), (b) 12-OH-18:0-ME esterified with (S)-ibuprofen (dark green) and racemic ibuprofen (light green), and (c) GC-MS spectrum of the ricinoleic acid methyl ester ibuprofen ester. For nomenclature see Figure 2.

of the S/S (or the enantiomeric R/R) diastereomer compared to the R/S (S/R) diastereomer, as previously observed by Cryle et al. [13]. Based on this, the enantiomeric excess (ee) of ricinoleic acid was determined as 99%, confirming previous findings by Kula et al. [18]. Applying the same procedure to a commercial 12-OH-stearic acid standard showed the later eluting (R/S') diastereomer to be dominating with an ee of 99%, suggesting that the standard was in fact derived from the hydrogenation of ricinoleic acid which shows the same (R) configuration at the hydroxy group (Figure 3b). Noteworthy, the chromatographic separation of the saturated 12-OH-stearic acid methyl ester O-ibuprofen ester was significantly better compared to the corresponding ricinoleic acid methyl ester derivatives. The base peak of all GC-MS spectra of FAME O-ibuprofen esters was detected at *m/z* 161, likely resulting from an α-cleavage at the carboxyl group of ibuprofen. A further fragment ion in the GC-MS spectrum of the ricinoleic acid derivative, putatively formed through elimination of the ibuprofen moiety (*m/z* 294) to form a conjugated double bond, allows the deduction of the length and unsaturation of the fatty acid chain moiety, but not the position of the OH group (Figure 3c). Interestingly, the corresponding fragment ion was detected at *m/z* 297 for the O-ibuprofen ester of 12-OH-18:0-ME, suggesting a cleavage instead of an elimination reaction in absence of the double bond. Further fragment ions detected at *m/z* 206 and *m/z* 188 can be tentatively assigned as M^+ and $[M\text{-}18]^+$ (fragment) ions of ibuprofen, respectively. Additional analysis of O-(S-)-ibuprofen esters of authentic standards of 3-OH-18:0-ME and 9-OH-18:0-ME revealed that the GC-MS spectra of all three compounds were virtually identical and did not allow the assignment of hydroxy group position within the chain.

However, analysis of all three standards allowed to determine elution orders, with 3-OH-18:0-ME eluting before 9-OH-18:0-ME and finally 12-OH-18:0-ME (Figure S2). Noteworthy, when analyzing the standards on a low-polarity HP-5MS column, retention time differences were lower and co-elutions were observed. Furthermore, the resolution of diastereomer pairs was much lower. For 9-OH-18:0-ME, both diastereomers were baseline separated using a polar stationary phase, while only a shoulder on the peak and no valley was observed for the low-polarity stationary phase. It should be noted that the lower-polarity GC column was only 30 m long instead of 60 m. Still, based on our observations, the use of a high polarity stationary phase is recommended for the analysis of hydroxy-FAME O-ibuprofen esters.

3.2 | Analysis of Fatty Acid Hydroperoxides From Autoxidation and Enzymatic Oxidation After Reduction as Ibuprofen Esters by GC-MS

Initial attempts of reducing the linoleic acid methyl ester hydroperoxides generated through autoxidation to hydroxy fatty acids using $NaBH_4$ and subsequent generation of the ibuprofen esters failed due to instability of the derivatives and the formation of conjugated linolenic acid derivatives (data not shown). This was most likely due to the position of the hydroxy group allylic to two conjugated double bonds, which strongly favored an elimination of water (or the ibuprofen moiety) to the conjugated triene system. Therefore, the hydroperoxy fatty acids were reduced using deuterium gas, which formed more stable (deuterated) saturated hydroxy fatty acids.

During autoxidation of linoleic acid methyl ester, the formation of 9-HP and 13-HP as main products as racemic mixtures is expected. GC-MS analysis of the respective (S)-ibuprofen esters showed four equally abundant peaks, which confirmed this expectation (Figure 4). It could be observed that the 9-OH-FAME eluted before the 13-OH-FAME and that the separation of the ibuprofen esters was better for FAMEs with the OH group further toward the methyl end of the fatty acid, which had previously been noted by Cryle et al. [13].

For the enzymatic oxidation of linoleic acid, soybean lipoxygenase was used. This enzyme is known to form the (13S)-hydroperoxide as major product [8]. However, if the enzymatic oxidation is performed under atmospheric conditions, as it was done in our case, a simultaneous autoxidation of the substrate has to be expected [8, 19].

The GC-MS analysis of the racemic ibuprofen esters showed two pairs of peaks with the same intensity of the two peaks in each pair, respectively. These pairs could be identified as the 9-OH and 13-OH esters (Figure 5, top). Noteworthy, the

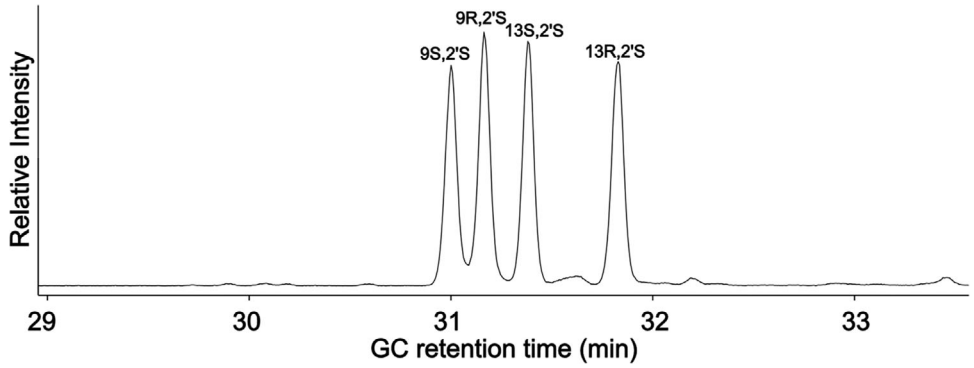

FIGURE 4 | Partial GC-MS total ion chromatogram of the (S)-ibuprofen derivatives of hydroxy FAMEs generated through autoxidation of linoleic acid methyl ester after reduction with deuterium gas.

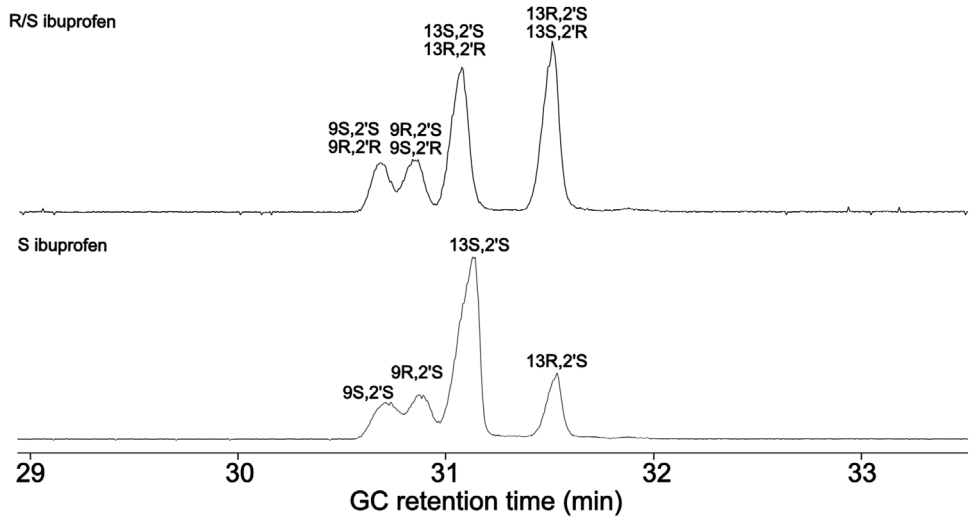

FIGURE 5 | GC-MS extracted ion chromatogram (*m/z* 301) of the hydroxy FAMEs generated through reaction of linoleic acid with soybean lipoxygenase, reduction with deuterium gas, methylation and derivatization with racemic (R/S)-ibuprofen (top) and (S)-ibuprofen (bottom). Slight differences in retention times compared to Figure 4 stem from routine column maintenance.

9-OH esters, most likely formed exclusively through autoxidation, were found at ca. 0% intensity of the 13-OH esters, which were most likely partly generated by enzymatic oxidation and autoxidation. This could be confirmed through GC-MS analysis of the (S)-ibuprofen esters, where the 9-OH-fatty acids were found as racemic mixture while for the 13-OH esters a dominance of the (enzymatically) formed 13S-OH (ca. 4:1) isomer over the 13R-isomer could be detected (Figure 5, bottom). Comparing the 9/13 as well as the 13S/13R ratio to calculated distributions suggested that enzymatic oxidation accounted for ca. 43% of the formed hydroperoxides while autoxidation contributed about 57% (Table 1). This demonstrates that this approach could be used to identify and quantify the contribution of enzymatic oxidation in samples, where lipid oxidation occurs through multiple mechanisms. Importantly, while the 9/13 ratio could be convoluted by concurrent autoxidation and photooxygenation, the enantiomeric excess of 13S over 13R could still be used for determination of the enzymatic oxidation.

4 | Conclusions

Ibuprofen esters of corresponding hydroxy fatty acids are an interesting tool in determining the enantiomeric excess of hydroperoxides formed during lipid oxidation and deduce the contribution of enzymatic oxidation to the formed hydroperoxides. The chromatographic resolution of the diastereomer pairs is very good even under standard GC conditions using highly polar stationary phases, which allows the convenient analysis without specialized set-ups. After this demonstration of the general applicability of the approach, the laboratory procedures should be simplified to allow its wider application. Furthermore, since in food most fatty acids are bound in triacylglycerols, the methylation of free fatty acids used here needs to be replaced by an appropriate transesterification procedure, either acid-catalyzed (as used here for methylation) or base-catalyzed. Our approach could complement existing methods and allow the selection of appropriate methods to mitigate lipid oxidation in food.

TABLE 1 | Calculated relative contributions of (9R)-, (9S)-, (13R)-, and (13S)-hydroperoxides as well as the sum of (9R/S)- and (13R/S)-hydroperoxides of linoleic acid in dependence of the percentage of enzymatically formed hydroperoxides* and experimentally found contributions of these isomers in one incubation of linoleic acid with soybean lipoxygenase.

Percentage of enzymatically formed hydroperoxides	Calculated relative contribution (%)					
	9R	9S	13R	13S	Sum 9R+9S	Sum 13R+13S
0	25	25	25	25	50	50
10	22.5	22.5	22.5	32.5	45	55
20	20	20	20	40	40	60
30	17.5	17.5	17.5	47.5	35	65
40	15	15	15	55	30	70
50	12.5	12.5	12.5	62.5	25	75
60	10	10	10	70	20	80
70	7.5	7.5	7.5	77.5	15	85
80	5	5	5	85	10	90
90	2.5	2.5	2.5	92.5	5	95
100	0	0	0	100	0	100
	Experimentally determined relative contribution (%)					
Soybean lipoxygenase (R/S)-ibuprofen	n/a	n/a	n/a	n/a	27	73
Soybean lipoxygenase (S)-ibuprofen	14	15	57	14	29	71

*Assuming the remaining hydroperoxides are formed through autoxidation at a ratio of 1:1:1:1 for (9R)-, (9S)-, (13R)-, and (13S)-hydroperoxides while enzymatic oxidation forms only (13S)-hydroperoxides.

Acknowledgments

The authors are grateful to Rudolf und Henriette Schmidt-Burkhardt Stiftung and Universitätsbund Erlangen-Nürnberg for financial support. S.H. is grateful to the Josef-Schormüller-Gedächtnisstiftung for financial support facilitating a research stay at H.G.J.'s group. The authors are grateful to Katja Lehnert and Vanessa Bauer (Department of Food Chemistry, University of Hohenheim) for assistance with additional GC-MS analyses.

Conflicts of Interest

Hans-Gerd Janssen is employed by Unilever, a multinational company active in foods and home and personal care products.

The authors declare there are no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Z. Tietel, S. Hammann, and S. W. Meckelmann, "An Overview of Food Lipids Toward Food Lipidomics," *Comprehensive Reviews in Food Science and Food Safety* 22 (2023): 4302–4354.

2. J. P. Schuchardt, I. Schneider, I. Willenberg, et al., "Increase of EPA-Derived Hydroxy, Epoxy and Dihydroxy Fatty Acid Levels in Human Plasma After a Single Dose of Long-Chain Omega-3 PUFA," *Prostaglandines and Other Lipid Mediators* 109 (2014): 23–31.

3. F. Shahidi and Y. Zhong, "Lipid Oxidation and Improving the Oxidative Stability," *Chemical Society Reviews* 39 (2010): 4067–4079.

4. S. A. Vieira, G. Zhang, and E. A. Decker, "Biological Implications of Lipid Oxidation Products," *Journal of the American Oil Chemists' Society* 94 (2017): 339–351.

5. D. B. Min and J. M. Boff, "Chemistry and Reaction of Singlet Oxygen in Foods," *Comprehensive Reviews in Food Science and Food Safety* 1 (2002): 58–72.

6. E. N. Frankel, "Chemistry of Free Radical and Singlet Oxidation of Lipids," *Progress in Lipid Research* 23 (1984): 197–221.

7. H. W. Gardner, "Lipoxygenase as a Versatile Biocatalyst," *Journal of the American Oil Chemists' Society* 73 (1996): 1347–1357.

8. H. W. Gardner, "Soybean Lipoxygenase-1 Enzymically Forms Both (9S)-and (13S)-Hydroperoxides from Linoleic Acid by a pH-Dependent Mechanism," *Biochimica Et Biophysica Acta (BBA)-Lipids and Lipid Metabolism* 1001 (1989): 274–281.

9. J. C. Andre and M. O. Funk, "Determination of Stereochemistry in the Fatty Acid Hydroperoxide Products of Lipoxygenase Catalysis," *Analytical Biochemistry* 158 (1986): 316–321.

10. C. P. A. van Os, M. Vente, and J. F. G. Vliegenthart, "A NMR Shift Method for Determination of the Enantiomeric Composition of Hydroperoxides Formed by Lipoxygenase," *Biochimica Et Biophysica Acta (BBA)-Lipids and Lipid Metabolism* 574 (1979): 103–111.

11. M. Cebo, X. Fu, M. Gawaz, M. Chatterjee, and M. Lämmerhofer, "Enantioselective Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry Method Based on Sub-2µm Particle Polysaccharide Column for Chiral Separation of Oxylipins and Its Application for the Analysis of Autoxidized Fatty Acids and Platelet Releasates," *Journal of Chromatography A* 1624 (2020): 461206.

12. H. Kühn, R. Wiesner, V. Z. Lankin, A. Nekrasov, L. Alder, and T. Schewe, "Analysis of the Stereochemistry of Lipoxygenase-Derived Hydroxypolyenoic Fatty Acids by Means of Chiral Phase High-Pressure Liquid Chromatography," *Analytical Biochemistry* 160 (1987): 24–34.

13. M. J. Cryle and J. J. d. Voss, "Facile Determination of the Absolute Stereochemistry of Hydroxy Fatty Acids by GC: Application to the Analysis of Fatty Acid Oxidation by a P450BM3 Mutant," *Tetrahedron: Asymmetry* 18 (2007): 547–551.

14. S. Schneider, S. Hammann, and H. Hayen, "Determination of Polar Lipids in Wheat and Oat by a Complementary Approach of Hydrophilic Interaction Liquid Chromatography and Reversed-Phase High-Performance Liquid Chromatography Hyphenated with High-Resolution Mass Spectrometry," *Journal of Agricultural and Food Chemistry* 71 (2023): 11263–11275.

15. E. P. Dobson, C. J. Barrow, J. A. Kralovec, and J. L. Adcock, "Controlled Formation of Mono- and Dihydroxy-Resolvins from EPA and DHA Using Soybean 15-lipoxygenase," *Journal of Lipid Research* 54 (2013): 1439–1447.

16. N. Tsevegsuren, W. W. Christie, and D. Lösel, "Tanacetum (Chrysanthemum) Corymbosum Seed Oil—A Rich Source of a Novel Conjugated Acetylenic Acid," *Lipids* 33 (1998): 723–727.

17. M. Lísa and M. Holčapek, "Characterization of Triacylglycerol Enantiomers Using Chiral HPLC/APCI-MS and Synthesis of Enantiomeric Triacylglycerols," *Analytical Chemistry* 85 (2013): 1852–1859.

18. J. Kula, R. Bonikowski, M. Szewczyk, and K. Ciolak, "Synthesis of (S)-Ricinoleic Acid and Its Methyl Ester With the Participation of Ionic Liquid," *Chemistry and Physics of Lipids* 183 (2014): 137–141.

19. P. Ludwig, H.-G. Holzhütter, A. Colosimo, M. C. Silvestrini, T. Schewe, and S. M. Rapoport, "A Kinetic Model for Lipoxygenases Based on Experimental Data With the Lipoxygenase of Reticulocytes," *European Journal of Biochemistry* 168 (1987): 325–337.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.