Identification and Quantification of Oxidized Lipids in LC-MS Lipidomics Data

Christoph A. KRETTLER[a,1], Jürgen HARTLER[b] and Gerhard G. THALLINGER[a,1]
[a] *Institute of Computational Biotechnology, Graz University of Technology, Austria*
[b] *Department of Pharmacology, University of California San Diego, USA*

Abstract. Changes in lipid homeostasis can lead to a plethora of diseases, raising the importance of reliable identification and measurement of lipids enabled by bioinformatics tools. However, due to the enormous diversity of lipids, most contemporary tools cover only a marginal range of lipid classes. To reduce such a shortcoming, this work extends the lipid species covered by Lipid Data Analyzer (LDA) to galactolipids and oxidized lipids. Appropriate mass lists were generated for MS^1 identifications and the proprietary decision rule sets were extended for MS^2 identifications of the novel lipid classes. Furthermore, LDA was extended to enable identification of oxidatively modified fatty acyl chains. With these extensions, LDA can reliably identify the most important galactolipids as well as oxidatively modified versions of the 22 previously implemented lipid classes. Comparison with other up to date lipidomics tools show that LDA has a better coverage of the newly implemented lipid species. The extended version of LDA provides researchers with a powerful platform to elucidate diseases caused by perturbations in the oxidized lipidome. LDA is freely available from https://genome.tugraz.at/lda.

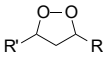
Keywords. bioinformatics, cheminformatics, galactolipids, lipidomics, mass spectrometry, oxidized lipids

1. Introduction

Being loosely defined as biomolecules that are soluble in nonpolar solvents [1], lipids form a diverse group of organic compounds, which are indispensable for living organisms. The aggregate of lipids in tissues, cells or organelles is defined as the lipidome. The structural diversity of lipids originates from various combinations of the two basic building blocks – ketoacyl groups and isoprene groups [2] – together with additional modifications. Based on these building blocks, lipids are categorized and classified. One new frontier is oxidative lipidomics, in which the lipids (particularly their fatty acyl chains) are modified under oxidative stress, by a process called lipid peroxidation. This process can be mediated either by free radicals or by enzymes, and results in a wide range of oxidized products that depend on the nature of the oxidant species [3,4]. The most prominent oxidation moieties are oxo, hydroxy and hydroperoxy modifications, but many other oxidations are possible (Table 1) [3].

[1] Corresponding Authors: Christoph A. Krettler, Gerhard G. Thallinger, Graz University of Technology, Petersgasse 14, 8010 Graz, Austria, E-Mail: c.krettler@gmail.com, gerhard.thallinger@tugraz.at

Table 1. Common oxidation moieties with their chemical structure and mass formula.

Prefix	Structure	Fatty Acyl (FA) Mass Formula	Prefix	Structure	Fatty Acyl (FA) Mass Formula
oxo		FA+O-H_2	hydroperoxy		FA+O_2
keto		FA+O-H_2	cyclopentane		FA-H_2
epoxy		FA+O-H_2	bromo		FA+Br-H
furan		FA+O-H_2	chloro		FA+Cl-H
hydroxy		FA+O	fluoro		FA+Fl-H
epidioxy		FA+O_2-H_2	iodo		FA+I-H
carboxy		FA+O_2-H_2	nitro		FA+NO_2-H

R: rest of the acyl chain, in case of esterified FA, esterified part; R': rest of the acyl chain, non-esterified carboxy part.

The biological role of oxidized lipids (Figure 1) in humans and other mammals depends on the location and nature of changes [5]. Roles in age-related and chronic diseases [6], atherosclerosis [7], and inflammation and immune response [8] have been reported. Mutagenic, carcinogenic and cytotoxic properties are thought to be connected to elevated levels of oxidized lipids [9]. In addition, the presence of oxidized lipids in biological membranes induces changes in physical properties such as fluidity, which can have an impact on the integrity of the membrane [5,10], causing apoptotic events [5,11]. As such, there is an imminent need for reliable methods for oxidized lipid identification and quantification.

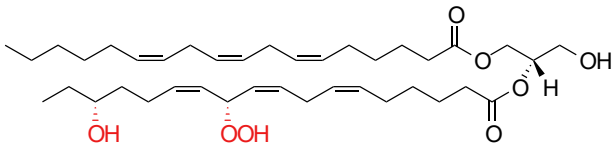


Figure 1. Structure of an oxidized lipid on the example of a diacylglycerol: DG(18:3/18:3[OH,OOH]); oxidation moieties are represented in red.

Breakthroughs in mass spectrometry (MS) technologies have led to an increase in mass accuracy and resolution as well as advances in ionization modalities, allowing contemporary lipidomics approaches to qualitatively and quantitatively analyze the entire complement of lipids in biological samples [12]. This facilitates investigation of structures, functions and interactions of lipids as well as dynamics of the entire lipidome.

Ferreri and Chatgilialoglu suggest that fatty acid analysis is already mature enough to translate test values into valid molecular diagnostics tools, but that the majority of oxidative stress tests available on the market use imprecise or non-optimized methodologies, while it would be more appropriate to include lipidomics approaches [13]. They further recommend educating health professionals on lipidomics to help them appreciate this approach, to enable investigation of lipid profiles of their patients and to use lipidomics to assist in deciding appropriate interventions in clinical practice. Thus,

the objective is to use lipidomics methods in clinical protocols and recognize its strengths towards an integrated vision for optimal health [13].

Lipid Data Analyzer (LDA) is a powerful tool using decision rule sets to enable automated and reliable annotation of lipid species and their molecular structures in high-throughput data from chromatography-coupled tandem mass spectrometry (LC-MS^2) [14]. LDA uses a targeted approach: It first scans the MS^1 spectra looking only for precursor ions previously defined in a mass list containing exact masses of the desired lipids. In a second step, the MS^2 fragmentation patterns of the precursor ions are evaluated. Lipid annotation is controlled by so-called decision rule sets and a mass list of possible fatty acyl (FA) constituents. In LDA, lipids are annotated based on the available mass spectrometric evidence. Thus, the level of structural information may vary. The lowest level of evidence is identification by precursor mass only. However, as lipids of completely different structures may have similar (isobars) or even exactly the same mass (isomers), this method may lead to misannotations, because no structural confirmation can be derived from the MS^1 identification. At this point, the fragmentation patterns of the MS^2 spectra help in deriving more elaborate structural details. Information about headgroup and constituent FA chains can be derived, and even determination of their positions on the backbone is possible for a large number of lipid classes.

2. Methods

2.1. Datasets

Dataset 1 - oxPC Standard: Raw data (a single file in Waters raw format, negative ion mode) was obtained from a study on oxidized phosphocholine (PC) standards [15], which was kindly provided by Maria Fedorova and Zhixu Ni. The investigated sample contained oxidized products of PC(16:0/18:1) and PC(16:0/18:2), verified manually. The sample was measured on an ACQUITY UPLC M-class (Waters Corp., Milford, MA, USA) using a reverse-phase column coupled online to a Synapt G2-Si mass spectrometer equipped with an ESI source (Waters Corporation, Milford, MA, USA) [15]. The dataset includes 3234 scans.

Dataset 2 - Wheat Seeds: The raw data (81 files in *mzXML* format, positive ion mode) originated from a study on oxidized wheat seeds [16] and was kindly provided by David Riewe. Analytes were separated by a 1290 UHPLC device (Agilent Technologies, Santa Clara, Ca, USA) using a C8 reverse-phase column and mass spectral analysis was conducted using a Bruker Maxis HD device upgraded with a Maxis II detector (Bruker Corp., Billerica, MA, USA) [16]. The scans per file range from about 1500 to 2000.

2.2. Tools

MSConvertGui 3.0.1899.0 (part of the ProteoWizard package [17]) was used for conversion of raw data to *ms2*, *mzXML* and *mzML* files. The mass list for Lipid Data Analyzer 2.8.0_nightly [14] was generated using R 3.3.3 [18] in RStudio 1.1.383 [19] with the packages XLConnect [20], janitor [21], plyr [22], pracma [23], and data.table [24]. LPPtiger (hotfix2019-version) [15] and LipidMatch 2.0.2 [25] were used for benchmarking of results. MZmine 2.23 [26] was used for generating a peak area list according to the LipidMatch manual, with the help of the MZmine batch file included in the LipidMatch directory.

2.3. Analysis Settings

Dataset 1 - oxPC Standard: For LDA analysis, the setting *Orbitrab_velos_pro_HCD, noIntensity* with *ms2PrecursorTolerance* set to 0.1 proved to be the most reliable option. For generating the *in-silico oxidation* in LPPtiger, all settings were used at their maximum option (*i.e. Oxidation-level: 3*, *Max modification sites: 8*, with *OAP*, *OCP*, *Lyso OAP* and *Lyso OCP* as well as *Prostanes* boxes checked). For the identification itself, default settings were used, except for *Overall score filter* which was set to >70%. In Lipid Match, *Retention Time Window* was set to 0.6, *ppm window* to 200, *Mass accuracy window* to 0.2 and *MS/MS Isolation Window* to 2.
Dataset 2 - Wheat Seeds: The setting *Orbitrab_velos_pro_HCD, noIntensity* proved to be the most reliable option for LDA analysis, with *ms2PrecursorTolerance* set to the standard value of 0.01.

3. Results

3.1. Mass List Generation

LDA utilizes Excel spreadsheets for defining the search space (*m/z* values) in MS^1 spectra. A valid mass list contains columns for name, double bonds and chemical formula as well as columns for each possible adduct of the lipid subclass, whereupon each subclass is defined in a separate worksheet. Optionally, a retention time can be defined in a separate column for individual or groups of molecules to limit the search space and subsequently minimize misannotations on the MS^1 level. LDA uses separate mass lists for positive and negative ion mode, respectively.

Table 2. Implemented lipid classes: Head group composition and adducts in positive and negative ion modes; values in the H column are added to the formula *C atoms*2 - double bonds*2* to derive the number of H atoms.

Lipid Class	Chains	C	H	O	P	N	S	Positive Adducts	Negative Adducts
MGMG	1	9	-2	9	0	0	0	$[M+NH_4]^+$, $[M+Na]^+$	$[M+C_2H_3O_2]^-$, $[M+HCO_2]^-$, $[M-H]^-$
DGMG	1	15	-4	14	0	0	0	$[M+NH_4]^+$, $[M+Na]^+$	$[M+C_2H_3O_2]^-$, $[M+HCO_2]^-$, $[M-H]^-$
SQMG	1	9	-2	11	0	0	1	$[M+NH_4]^+$, $[M-H+Na_2]^+$	$[M-H]^-$
MGDG	2	9	-4	10	0	0	0	$[M+NH_4]^+$, $[M+Na]^+$, $[M+Li]^+$	$[M+C_2H_3O_2]^-$, $[M+HCO_2]^-$, $[M-H]^-$
DGDG	2	15	-6	15	0	0	0	$[M+NH_4]^+$, $[M+Na]^+$, $[M+K]^+$, $[M+Li]^+$	$[M+C_2H_3O_2]^-$, $[M+HCO_2]^-$, $[M-H]^-$
TriGDG	2	21	-8	20	0	0	0	$[M+NH_4]^+$, $[M+Na]^+$	$[M+C_2H_3O_2]^-$, $[M+HCO_2]^-$, $[M-H]^-$
SQDG	2	9	-4	12	0	0	1	$[M+NH_4]^+$, $[M-H+Na_2]^+$	$[M-H]^-$
PC	2	8	0	8	1	1	0	$[M+H]^+$, $[M+Na]^+$	$[M+HCO_2]^-$, $[M-CH_3]^-$
PE	2	5	0	8	1	1	0	$[M+H]^+$, $[M+Na]^+$	$[M-H]^-$
PG	2	6	-1	10	0	0	0	$[M+H]^+$, $[M+Na]^+$	$[M-H]^-$
PI	2	9	-3	13	1	0	0	$[M+H]^+$, $[M+Na]^+$, $[M+NH_4]^+$	$[M-H]^-$
PS	2	6	-2	10	1	1	0	$[M+H]^+$	$[M-H]^-$
TG	3	3	-4	6	0	0	0	$[M+NH_4]^+$, $[M+Na]^+$	

MGMG: Monogalactosylmonoacylglycerol; DGMG: Digalactosylmonoacylglycerol; SQMG: Sulfo-quinovosylmonoacylglycerol; MGDG: Monogalactosyldiacylglycerol; DGDG: Digalactosyldiacylglycerol; TriGDG: Trigalactosyldiacylglycerol; SQDG: Sulfoquinovosyldiacylglycerol; TG: Triacylglycerol; PG: Phosphatidylglycerol; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PS: Phosphatidylserine; PI: Phosphatidylinositol.

In this project, an R script has been implemented that creates an appropriate target mass list for the new lipid subclasses: The head groups and possible adducts of the implemented classes (Table 2) can be defined in an Excel sheet and the script adds FA constituents covering a user-specified carbon atoms and double bonds range. Furthermore, the script adds oxidation moieties defined in another Excel sheet (Table 1) to the target lipids and to the mass list of possible constituent FAs. Atoms' masses were obtained from an appropriate paper on atomic weights [27].

3.2. Rule Sets

LDA uses plain text files for the specification of fragmentation rules. Each possible adduct defined in the mass list requires its own rule file, in order to facilitate an MS^n identification. The file comprises four parts - *GENERAL*, *HEAD*, *CHAINS* and *POSITION*. The section *GENERAL* lists the number of chains and other common properties of the particular lipid class. The sections *HEAD* and *CHAINS* are further divided in a *!FRAGMENTS* and a *!INTENSITIES* part. The *!FRAGMENTS* part lists the fragment ions used for the identification of the headgroup and of the fatty acyl chains, respectively. The *!INTENSITIES* part can be used to describe intensity relationships between fragments in one of the previous *!FRAGMENTS* sections. Each fragment and intensity equation can be set as mandatory (or not), in order for an identification to be made. Additionally, the option *other* is available to define fragments originating from isobaric or isomeric species. Using these *other* fragments, a differentiation between species producing similar fragments can be made, and consequently the number of misannotations can be reduced effectively. The last section *POSITION* can be used to define intensity relationships between chain fragments in order to identify the *sn*-position of a FA chain.

In this work, we assumed that the fragmentation spectra for oxidized lipids are very similar to those of unmodified lipids. Still, for the $[M\text{-}CH_3]^-$ adduct of oxPC, the positional rules were removed, as they proved unreliable. Up to now, we could accomplish the support of the most important galactolipid classes MGMG, MGDG, DGMG, DGDG, SQMG, and SQDG by an extension of the algorithmic concept without developing novel fragmentation rule sets.

3.3. Nomenclature for Oxidized Lipids in LDA

As of now, notation guidelines for oxidized lipids are scarce and incomplete. Hence, many different approaches are being used [16,28,29]. We settled on adding an *ox* prefix to the lipid abbreviation to mark oxidized classes. The modification itself is enclosed in square brackets and written directly after the chain it concerns. Multiple modifications of different types are jointly enclosed in square brackets and separated by a comma. For multiple modifications of the same type, their number is simply added in front of the modification. Accordingly, the lipid oxMGDG(16:2[O,OH]_18:4[4OH]) carries an additional oxygen atom and a hydroxy (OH) modification on its 16:2 chain, and four hydroxy modifications on its 18:4 chain.

3.4. Novel LDA Features

To allow LDA to identify fragments of oxidized lipids in MS^2 spectra, the list of considered fatty acyl chains (corresponding alkyl/1-alkenyl counterparts are included

automatically), the target mass list and LDA source code had to be extended. First, a new column *oxidation-state* was introduced in the fatty acyls mass list in which the respective chain modification is listed. In the target mass list, the same column was introduced to allow LDA to filter combinations of FAs that do not have the same sum of modifications as the parent molecule. To illustrate this point further, the lipid species oxMGDG(36:6[O,OH]) could represent either oxMGDG(18:3[O]_18:3[OH]) or oxMGDG(18:3[O,OH]_18:3), but not oxMGDG(18:3[O,OH]_18:3[O,OH]). The *ox* prefix is programmatically added to the lipid whenever the respective *oxidation-state* cell is not empty. Concerning the decision rules, the algorithm looks for the respective *ox* rule first (for example, *oxMGDG_NH4.frag.txt*); but will fall back to the standard rule (for example, *MGDG_NH4.frag.txt*) to guarantee backward compatibility (*i.e.* can be used with FA mass lists and target mass lists without the *oxidation-state* column).

3.5. Analysis of Datasets with LipidMatch, LPPtiger, and LDA

***Dataset 1* -** With the preexisting knowledge of the sample containing PC(16:0/18:1) and PC(16:0/18:2). The sample was analyzed using LDA as well as LPPtiger and LipidMatch. For LDA, a mass list was generated for oxidation products between PC(34:0) and PC(34:2) with up to two additional oxygen atoms and up to three hydroxy modifications. In the following analysis, LDA annotated 19 oxidized lipid species in dataset 1 (Table 3). After manual inspection of the MS^2 spectra, four identifications were considered to be false positives, as only fragments of low abundance were annotated and major fragments were left unannotated. LPPtiger and LipidMatch annotated eight and three lipid species, respectively, where one of the three LipidMatch identifications seems to be a false positive, as the *m/z* value doesn`t fit the molecule's mass properly.

Table 3. Lipid molecular species identified in dataset 1 by LDA, LPPtiger and LipidMatch; putative false positives are marked in gray; species' names adapted to LDA shorthand notation.

m/z	LDA	LPPtiger	LipidMatch	Adduct
744.5	PC(16:0/18:1)			
758.5	oxPC(16:0_18:2[OH])			
760.5	oxPC(16:0_18:1[OH])			
772.5	oxPC(16:0_18:1[2O])			
774.5	oxPC(16:0_18:2[2OH])	adduct not implemented	adduct not implemented	$[M-CH_3]^-$
776.5	oxPC(16:0_18:1[2OH])			
778.5	oxPC(16:0_18:0[2OH])			
790.5	oxPC(16:0_18:2[3OH])			
792.5	oxPC(16:0_18:1[3OH])			
802.5	PC(16:1_18:1)			
804.5	PC(16:0/18:1)	PC(16:0/18:1)		
818.5	oxPC(16:0/18:2[OH])	oxPC(16:0/18:1[O])	oxPC(16:0_18:2[OH])	
820.5	oxPC(16:0/18:1[OH])	oxPC(16:0/18:0[O])	oxPC(16:0_18:1[OH])	
822.6	oxPC(16:0_18:0[OH])			
832.5	oxPC(16:0/18:1[2O])	oxPC(16:0/18:1[2O])		$[M+HCO_2]^-$
834.5	oxPC(16:0/18:2[2OH])			
836.5		oxPC(16:0/18:0[O,OH])		
838.5	oxPC(16:0/18:0[2OH])	oxPC(16:0/18:0[2OH])		
850.5	oxPC(16:0/18:2[3OH])	oxPC(16:0/18:2[O,OOH])	oxPC(16:0_18:2[3O])	
852.5	oxPC(16:0/18:1[3OH])	oxPC(16:0/18:1[OH,OOH])		

Dataset 2 **-** With the help of an R script, an LDA target mass list for lipids with up to four hydroxy modifications was created, which was subsequently used for data analysis. The relative number of lipids identified by LDA shows the same pattern as in the original paper (Table 4): Unoxidized species are most frequent and species get less frequent the more hydroxy modifications they carry. The same pattern holds true for the abundance of lipids *vs.* oxidized lipids. Neither LPPtiger nor LipidMatch reported results coinciding with the manual annotations shown by Riewe *et al*. [16].

Table 4. Number of unique lipid species identified in dataset 2 across 12 lipid classes by LDA vs. reported by Riewe *et al.* [16]; columns show the amount of hydroxy modifications added through lipid peroxidation.

Lipid Class	**LDA**					**Riewe *et al.***				
	0 OH	**1 OH**	**2 OH**	**3 OH**	**4 OH**	**0 OH**	**1 OH**	**2 OH**	**3 OH**	**4 OH**
TG	68	41	14	8	2	71	58	23	12	9
DG	27	9	10	1	1	35	17	18	11	6
MG	0	0	0	0	0	6	1	0	0	0
PC	2	0	0	0	0	3	1	0	2	1
PE	7	0	0	0	0	8	2	0	0	0
PG	0	0	0	0	0	7	0	0	0	0
PI	5	0	0	0	0	5	0	0	0	0
LPC	0	0	0	0	0	12	4	1	0	0
LPE	0	0	0	0	0	7	0	0	0	0
LPG	0	0	0	0	0	2	0	0	0	0
MGDG	6	3	2	0	0	6	4	3	0	0
DGDG	20	3	4	0	0	20	4	5	0	0

3.6. Comparison

Apart from the unsupported $[M\text{-}CH_3]^-$ adduct, LPPtiger reported similar results as LDA for dataset 1, whereas LipidMatch reported significantly less species. As a measure of performance, the F_1 score was calculated for each tool. Here, LDA clearly outperforms the other tools with an F_1 score of 0.86; the scores for LPPtiger and LM are 0.67 and 0.22 respectively. False positives reported by LDA and the missed identification at *m/z* of 836.5 are consequences of unoptimized parameters of LDA's 3D peak detection algorithm. In this study, we utilized existing settings optimized for other instruments to quickly obtain a proof of principle of our MS^n extension for oxidized lipids. *E.g.*, when using the *QTOF* settings, a lipid PC(16:0/18:1[2OH]) is identified at *m/z* of 836.5 but other lipid species are missed. Differences in reported lipids at identical *m/z* values between LDA and LPPtiger are due to ambiguities in notation only, where LPPtiger is sometimes able to rank modifications by their probability (due to so called fingerprint spectra). For dataset 2, neither LPPtiger nor LipidMatch showed satisfying results: LPPtiger only works for phospholipids in negative ion mode and LipidMatch terminated prematurely without returning a result, probably because it does not explicitly support Bruker MS setups.

4. Discussion

LDA mass lists and decision rules were extended, which proved to be a reliable addition for the identification of MGDG, DGDG, SQDG, MGMG and DGMG, as demonstrated with dataset 2. Furthermore, LDA now supports identification of fragments of oxidized lipids in MS^2 spectra. Benchmarking with LPPtiger and LipidMatch proved the validity of the concept and supplied evidence for LDA's superior performance.

4.1. Summary of the Challenges

The two datasets were selected as they originate from well-conceived studies on oxidized lipids that allowed us to generate valid results and comparisons. However, neither LDA's 3D algorithm nor its decision rules were developed specifically for the instruments that recorded the data, whereas LPPtiger's settings were optimized for dataset 1.

In dataset 1, the identifications of LPPtiger and LDA are very similar, though LDA covers more adducts. The false positives can be clearly attributed to the inaccuracy of the data (masses differ up to 0.1Da) and non-optimized settings for handling the data.

Currently, we assume that the small differences in the number of identifications in dataset 2 between the manual analysis by Riewe *et al.* and LDA originate either from stricter rules and mass tolerances used by LDA, or from non-optimized parameters of LDA's 3D algorithm, or from both.

4.2. Nomenclature and Ambiguity

Even after immense efforts to create a more unified nomenclature (for example by the research team behind LipidHome [30], LIPID MAPS [31,32] or the group around Liebisch *et al*. [29]), and owing to the vast number of lipid species, it is still hard to compare lipid species reported by different authors. This is particularly true for more "exotic" lipid species, such as oxidized ones.

For oxidized lipids, biochemists often use elaborate terms describing a molecule at high structural resolution, *e.g.* 1-Palmitoyl-2-(9-keto-12-oxo-10-dodecenoic acid)-PC [33]. It seems that using *ox* as a prefix has prevailed for shorthand notation of lipid classes, *e.g.* oxPC as used in the works of Reis *et al.* [3]. Following our suggestions for shorthand notation in LDA, the aforementioned oxidized lipid would be reported as oxPC(28:1[2O])) at the MS^1 level, oxPC(16:0/12:1[2O]) at the MS^2 level with known *sn*-positions of the FA chains and oxPC(16:0/12:1[9-O,12-O]) when the structural resolution allows assignment of modification positions. Oxo, keto, epoxy and furan modifications will not be discernible, as they have the same masses, and oxo/keto/epoxy/furan and hydroxy modifications differ only in two hydrogen atoms, which could be interpreted as the mass difference of a single double bond. A similar issue emerges when the fatty acyl includes a hydroperoxy modification which has the same mass as a hydroxyl-hydroxy modification, and when the chain includes epidioxide, which has the same mass as an oxo-hydroxy modification. This ambiguity cannot be resolved by typical high-throughput MS techniques, as the complexity increases the more oxidation modifications a lipid carries. Taking an extreme case, oxTG(54:6[4OH]) has the exact same mass as oxTG(54:2[4O]). Due to a lack of adequate nomenclature for such cases, researchers currently do not have any means to report such ambiguities. Nevertheless, information to resolve said uncertainties can be obtained by studies of fragmentation patterns or elution profiles, but such studies are lacking for most of the "exotic" oxidation products.

The possible permutations of oxidized fatty acyls and resulting lipid species is enormous, but with this extension of LDA, an important step towards high-throughput oxidative lipidomics was taken. We anticipate to provide modification-specific fragmentation rule sets in future versions of LDA, offering yet another layer of verification. In the future, lipidomics approaches will replace outdated oxidative stress tests and provide personalized strategies in clinical practice.

Acknowledgements

We are grateful to Zhixu Ni and Maria Fedorova (Universität Leipzig) and David Riewe (Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben) for providing datasets 1 and 2, respectively.

References

[1] Moss GP, Smith PAS, Tavernier D. Glossary of class names of organic compounds and reactivity intermediates based on structure (IUPAC Recommendations 1995). *Pure and Applied Chemistry* 1995;67, 1307-75.

[2] Fahy E, Cotter D, Sud M, Subramaniam S. Lipid classification, structures and tools. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* 2011;1811(11), 637-47.

[3] Reis A, Spickett CM. Chemistry of phospholipid oxidation. *Biochimica et Biophysica Acta - Biomembranes* 2012;1818(10), 2374-87.

[4] Milic I, Griesser E, Vemula V, Ieda N, Nakagawa H, Miyata N, et al. Profiling and relative quantification of multiply nitrated and oxidized fatty acids. *Analytical and Bioanalytical Chemistry* 2015;407(5), 5587-2.

[5] Domingues MRM, Reis A, Domingues P. Mass spectrometry analysis of oxidized phospholipids. *Chemistry and Physics of Lipids* 2008;156(11), 1-12.

[6] Pierre JL, Favier AE, Cadet J, Kalyanaraman B, Fontecave M. *Analysis of Free Radicals in Biological Systems*. Basel, BS, CH: Birkhäuser, 1995.

[7] Shrestha R, Hui SP, Miura Y, Yagi A, Takahashi Y, Takeda S, et al. Identification of molecular species of oxidized triglyceride in plasma and its distribution in lipoproteins. *Clinical Chemistry and Laboratory Medicine* 2015;53(1), 1859-69.

[8] Leitinger N. The Role of Phospholipid Oxidation Products in Inflammatory and Autoimmune Diseases. in *Lipids in Health and Disease*. Edited by Quinn PJ, Wang X. Dordrecht, ZH, NL: Springer Netherlands 2008;325-50

[9] Keller J, Camaré C, Bernis C, Astello-García M, Rosa APB, Rossignol M, et al. Antiatherogenic and antitumoral properties of Opuntia cladodes: inhibition of low density lipoprotein oxidation by vascular cells, and protection against the cytotoxicity of lipid oxidation product 4-hydroxynonenal in a colorectal cancer cellular model. *Journal of Physiology and Biochemistry* 2015;71(4), 577-87.

[10] Megli FM, Russo L. Different oxidized phospholipid molecules unequally affect bilayer packing. *Biochimica et Biophysica Acta - Biomembranes* 2008;1778(1) , 143-52.

[11] Borst JW, Visser NV, Kouptsova O, Visser AJWG. Oxidation of unsaturated phospholipids in membrane bilayer mixtures is accompanied by membrane fluidity changes. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* 2000;1487(8), 61-73.

[12] Gross RW. The evolution of lipidomics through space and time. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* 2017;1862(8), 731-39.

[13] Ferreri C, Chatgilialoglu C. *Membrane Lipidomics for Personalized Health.* Hoboken, NJ, USA: John Wiley & Sons Ltd, 2015.

[14] Hartler J, Triebl A, Ziegl A, Trötzmüller M, Rechberger GN, Zeleznik AO, et al. Deciphering lipid structures based on platform-independent decision rules. *Nature Methods* 2017;14(23), 1171-80.

[15] Ni Z, Angelidou G, Hoffmann R, Fedorova M. LPPtiger software for lipidome-specific prediction and identification of oxidized phospholipids from LC-MS datasets. *Scientific Reports* 2017;7(11), 15138.

[16] Riewe D, Wiebach J, Altmann T. Structure Annotation and Quantification of Wheat Seed Oxidized Lipids by High-Resolution LC-MS/MS. *Plant Physiology* 2017;175(8), 600-18.

[17] Kessner D, Chambers M, Burke R, Agus D, Mallick P. ProteoWizard: open source software for rapid proteomics tools development. *Bioinformatics* 2008;24(7), 2534-36.

[18] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria 2017, [https://www.R-project.org/].

[19] RStudio Team. *RStudio: Integrated Development Environment for R*. RStudio, Inc., Boston, MA 2016, [http://www.rstudio.com/].

[20] Mirai Solutions GmbH. *XLConnect: Excel Connector for R* 2018, [https://CRAN.R-project.org/package=XLConnect]. [R package version 0.2-15].

[21] Firke S. *janitor: Simple Tools for Examining and Cleaning Dirty Data* 2019, [https://CRAN.R-project.org/package=janitor]. [R package version 1.2.0].

[22] Wickham H. The Split-Apply-Combine Strategy for Data Analysis. *Journal of Statistical Software* 2011;40, 1-29.

[23] Borchers HW. pracma: *Practical Numerical Math Functions* 2019, [https://CRAN.R-project.org/package=pracma]. [R package version 2.2.5].

[24] Dowle M, Srinivasan A. *data.table: Extension of 'data.frame'* 2017, [https://CRAN.R-project.org/package=data.table]. [R package version 1.10.4-3].

[25] Koelmel JP, Kroeger NM, Ulmer CZ, Bowden JA, Patterson RE, Cochran JA, et al. LipidMatch: an automated workflow for rule-based lipid identification using untargeted high-resolution tandem mass spectrometry data. *BMC Bioinformatics* 2017;18, 331.

[26] Pluskal T, Castillo S, Villar-Briones A, Orešič M. MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinformatics* 2010;11(7), 395-6.

[27] Laeter JR, Böhlke JK, Bièvre PD, Hidaka H, Peiser HS, Rosman KJR, et al. Atomic weights of the elements. Review 2000 (IUPAC Technical Report). *Pure and Applied Chemistry* 2003;75(1), 683-800.

[28] Sala P, Pötz S, Brunner M, Trötzmüller M, Fauland A, Triebl A, et al. Determination of Oxidized Phosphatidylcholines by Hydrophilic Interaction Liquid Chromatography Coupled to Fourier Transform Mass Spectrometry. *International Journal of Molecular Sciences* 2015;16(4), 8351-63.

[29] Liebisch G, Vizcaíno JA, Köfeler H, Trötzmüller M, Griffiths WJ, Schmitz G, et al. Shorthand notation for lipid structures derived from mass spectrometry. *Journal of Lipid Research* 2013;54(4), 1523-30.

[30] Foster JM, Moreno P, Fabregat A, Hermjakob H, Steinbeck C, Apweiler R, et al. LipidHome: A Database of Theoretical Lipids Optimized for High Throughput Mass Spectrometry Lipidomics. *PLoS ONE* 2013;8(5), e61951.

[31] Fahy E, Subramaniam S, Brown HA, Glass CK, Merrill AH, Murphy RC, et al. A comprehensive classification system for lipids. *Journal of Lipid Research* 2005;46(2), 839-62.

[32] Fahy E, Subramaniam S, Murphy RC, Nishijima M, Raetz CRH, Shimizu T, et al. Update of the LIPID MAPS comprehensive classification system for lipids. *Journal of Lipid Research* 2008;50(12), 9-14.

[33] Reis A, Domingues MRM, Amado FML, Ferrer-Correia AJV, Domingues P. Separation of peroxidation products of diacyl-phosphatidylcholines by reversed-phase liquid chromatography-mass spectrometry. *Biomedical Chromatography* 2005;19(2), 129-37.