J Am Oil Chem Soc (2010) 87:1235–1245
DOI 10.1007/s11746-010-1622-x

ORIGINAL PAPER

Choline and Ethanolamine Decompose Lipid Hydroperoxides into Hydroxyl Lipids

Xiangqing Pan · Anthony J. Irwin · Michael Leonard · David Welsby

Received: 21 October 2009 / Revised: 30 May 2010 / Accepted: 9 June 2010 / Published online: 3 July 2010

Abstract Analysis of lipid hydroperoxides and hydroxyl lipids in food and biological systems has historically been difficult due to the instability and diversity of these compounds as well as the complexity of the associated purification and derivatization processes. A rapid, high throughput and robust method based on mass spectrometry using multiple reaction monitoring mode of LC–ESI–MS/MS has been developed for the simultaneous quantitative analysis of fatty acid hydroperoxides and hydroxyl fatty acids without purification or derivatization. The method has been used to demonstrate that functional groups of phospholipids, choline and ethanolamine, are capable of decomposing lipid hydroperoxides into their corresponding hydroxyl lipids. In model systems where choline and ethanolamine were added, the amounts of lipid hydroperoxide isomers decreased while the amounts of hydroxyl lipids increased. In similar model systems containing normal food antioxidants such as tocopherols and butylated hydroxytoluene, and in the control without antioxidants, the amounts of lipid hydroperoxide and hydroxyl lipids did not change significantly under current experimental conditions. Data also demonstrated that phospholipids have a much lower hydroperoxide decomposition ability than choline and ethanolamine, and that the antioxidant activity of phospholipids is probably due to the ability of choline, ethanolamine and other amines from phospholipid degradation to convert primary oxidation products to the corresponding hydroxyl lipids.

X. Pan (✉) · A. J. Irwin · M. Leonard · D. Welsby
Solae, LLC, 4300 Duncan Avenue, St. Louis, MO 63110, USA
e-mail: xpan@solae.com

Keywords Lipid oxidation · Lipid hydroperoxide · Hydroxyl lipids · Tandem mass spectrometry · LC–ESI–MS/MS · Phospholipids · Choline · Ethanolamine

Abbreviations

MRM	Multiple reaction monitoring mode
HPLC	High performance liquid chromatography
ESI–MS/MS	Electro-spray ionization tandem mass spectrometry
LA	Linoleic acid
ALA	Linolenic acid
BHT	Butylated hydroxytoluene
13-HOT	13-Hydroxy-9,11,15-octadecatrienoic acid
9-HOT	9-Hydroxy-10,12,15-octadecatrienoic acid
13-HpOT	13-Hydroperoxy-9,11,15-octadecatrienoic acid
9-HpOT	9-Hydroperoxy-10,12,15-octadecatrienoic acid
13-HpOD	13-Hydroperoxy-9,11-octadecadienoic acid
9-HpOD	9-Hydroperoxy-10,12-octadecadienoic acid
13-HOD	13-Hydroxy-9,11-octadecadienoic acid
9-HOD	9-Hydroxy-10, 12-octadecadienoic acid
9-HODE-d_4	9-Hydroxy-10, 12-octadecadienoic 9, 10, 12, 13-d_4 acid
13-HODE-d_4	13-Hydroxy-9, 11-octadecadienoic-9, 10, 12, 13-d_4 acid
AMVN	2,2′-Azobis(2,4-dimethylvaleronitrile)
DP	Declustering potential
CE	Collision energy
CXP	Collision cell exit potential
EP	Entrance energy potential
FA-OOH	Fatty acid hydroperoxides

FA-OH Hydroxyl fatty acids
DPPP Diphenyl-1-pyrenylphosphine

Introduction

Lipid oxidation in food is a major cause of quality deterioration in flavor, texture, consistency, and appearance [1–3]. Lipid oxidation is also involved in physiological process in the eukaryotic cell, including aging and disease [4–10]. Much work has been devoted to the development of methods for the measurements of lipid hydroperoxides and other oxidation products since lipid oxidation is one of the important problems in food and biological systems. Lipid hydroperoxides are the primary oxidation products of unsaturated lipids, and the instability and diversity of hydroperoxide species, together with other lipid oxidation products in complex food and biological systems, present a formidable challenge to accurate and specific analysis.

One strategy, involving high-performance liquid chromatography (HPLC) separation of lipid hydroperoxide isomers coupled with UV detection of conjugated dienes at 234 nm and with post column chemiluminescence detection based on the oxidation of luminol during a reaction between hydroperoxides and cytochrome *C*, has been proved to be a useful method [11, 12]. This method has been applied for the analysis of hydroperoxide isomers in food and biological systems [13–21] by some of our coauthors using diphenyl-1-pyrenylphosphine (DPPP) as a fluorescent reagent with excitation and emission wavelengths of 352 and 380 nm, respectively.

Our previous work [22–26] successfully used HPLC coupled to an electro-spray ionization tandem mass spectrometry (ESI–MS/MS) system for the characterization of plant metabolites and plant hormones. This method has the advantage over other methods that it provides high sensitivity and specificity and provides the capacity to simultaneously analyze a variety of different compounds during the same machine run as well as eliminating the need for derivatization and purification before analysis. In this study, a method for simultaneous analysis of hydroperoxy and hydroxy substituted unsaturated lipids in complex food and biological systems was developed using the multiple reaction monitoring mode (MRM) of MS/MS.

Many studies [27–32] demonstrated that phospholipids have antioxidant activities and inhibit lipid oxidation using the peroxide value as an index to follow the oxidative process. Phospholipids have also been reported to have strong synergistic antioxidant effects when combined with other antioxidants, such as α-tocopherol and butylated hydroxytoluene (BHT) [33–37], and it has been proposed that the antioxidant activities of phospholipids are mainly due to their functional groups such as choline and ethanolamine which have been hypothesized as being responsible for the decomposition of lipid hydroperoxides into hydroxyl lipids [32, 38–40]. However, little solid data and direct evidence is available to support this hypothesis, and no studies clearly demonstrate that functional groups of phospholipids, including choline and ethanolamine, can actually decompose a variety of lipid hydroperoxide isomers into their corresponding hydroxyl lipids.

The aim of this work is twofold: (1) to develop a method for simultaneous measurement of lipid hydroperoxides and hydroxyl lipids from oxidation of linoleic acid (LA) and linolenic acid (ALA) in complicated food or biological systems; (2) to use the developed analytical methods to demonstrate that functional groups of phospholipids including choline and ethanolamine have the ability to convert isomeric lipid hydroperoxides into their corresponding hydroxyl lipids and to demonstrate that the antioxidant activities of phospholipids are mainly due to their functional groups.

Materials and Methods

Lipid Standards and Chemicals

13-Hydroxy-9,11,15-octadecatrienoic acid (13-HOT), 9-hydroxy-10,12,15-octadecatrienoic acid (9-HOT), 13-hydroperoxy-9,11,15-octadecatrienoic acid (13-HpOT), 9-hydroperoxy-10,12,15-octadecatrienoic acid (9-HpOT), 13-hydroperoxy-9,11-octadecadienoic acid (13-HpOD), 9-hydroperoxy-10,12-octadecadienoic acid (9-HpOD), 13-hydroxy-9,11-octadecadienoic acid (13-HOD), 9-hydroxy-10,12-octadecadienoic acid (9-HOD), 9-hydroxy-10,12-octadecadienoic 9,10,12,13-d_4 acid (9-HODE-d_4), 13-hydroxy-9,11-octadecadienoic-9,10,12,13-d_4 acid (13-HODE-d_4), ALA and LA were purchased from Cayman Chemical® (MI, USA), Nu-Check Prep, Inc. (MN, USA), or Sigma-Aldrich® (St. Louis, MO, USA), respectively. 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN), methylene blue, BHT, choline hydroxide and α-tocopherol were purchased from Sigma-Aldrich®. Soy L-α-phosphatidylcholine (95%) (soy PC) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Soy lecithin including Solec™ FP 40 (>97%) and Solec P (>98%) were obtained from Solae, LLC (St Louis, MO, USA). Except where noted, all chemicals were of HPLC grade or the highest grade available from Cayman Chemical®, Nu-Check Prep, Inc., or Sigma-Aldrich®.

Optimization of Mass Spectrometers for MRM Mode Quantitative Analysis

To optimize the mass spectrometers for quantitative analysis, authentic compounds (200 ng/ml) dissolved in 80% methanol with 0.1% formic acid were directly infused into the TurboIonSpray® source of a 4000 QTRAP® LC–MS/MS System (Applied Biosystems®, Foster City, CA, USA) using a KDS100 syringe pump (KD Scientific Inc., Holliston, MA, USA) at 1.2 ml/h with a needle temperature of 150 °C, needle voltage −3,500 V, curtain gas 10 psi, nebulizing gas (GS1) 20 psi, focusing gas (GS2) 0 psi, and with the interface heater engaged. Declustering potential (DP) and collision energy (CE) were optimized on a compound-dependent basis for the production of characteristic precursor–product ion transitions in negative ionization modes. The automatic quantitative optimization feature of Analyst 1.5 (Applied Biosystems®) was also employed to generate precursor–product ion transitions for each authentic compound with corresponding DP and CE information [23, 24, 26].

HPLC/ESI–MS/MS Conditions for MRM Quantitative Analysis

The HPLC system consisted of two Shimadzu® LC-20AB (Shimadzu Corp., Kyoto, Japan) pumps connected to an HTC PLA auto-injector (CTC Analytics, Zwingen Switzerland) fitted with a 50-µl sample loop, a 100-µl syringe and a fast wash station with two wash solvents of methanol and water [24, 26]. Flow from the sample injector led to a reverse phase column (C18 Gemini® 5 µm, 150 × 2.00 mm, Phenomenex®, Torana, CA, USA) fitted with a guard cartridge held at 40 °C in a Shimadzu® CO-20A oven (Shimadzu Corp.). The sample was eluted at 0.8 ml/min with a binary gradient system consisting of solvent A (distilled water with 0.1% formic acid) and solvent B (acetonitrile:methanol = 65:35 with 0.1% formic acid) solvent. The initial eluate from the column was directed via a Valco valve to waste for 30 s after which the flow was switched to the TurboIonSpray source of the 4000 QTRAP® LC–MS/MS system (Applied Biosystems®). Separations of lipid hydroperoxides and hydroxy lipids were performed using a gradient of increasing content of solvent B. The initial gradient of solvent B was kept at 50% for 2 min, and it was then increased to 100% within 18 min and kept at 100% for 8 min. The probe was vertically positioned 5 mm from the orifice and charged −3 kV. The temperature was held at 500 °C, and GS1 was set at 35 psi, GS2 at 30 psi, curtain gas at 20 psi, nitrogen collision gas set to high, and the interface heater was engaged. The values of DP, entrance potential and CE optimized by direct infusion for each compound were used for MRM transition, respectively.

Calibration of MS/MS Detection

Serial dilutions were constructed in triplicates for each standard starting from 10 to 0.01 nmol/ml. To verify that peak area increased proportionally as a function of increasing compound concentration (mol/l), solutions with various amounts of compounds (0.01–10 nmol/ml) were analyzed. The dose response curves were linear in the concentration ranges selected for various compounds (R^2 values of 0.976–0.999). The peak area of the diagnostic product ion under optimized conditions was used for quantification. The isotopically-labeled compounds were selected as internal standards. The amounts (mol) of compounds were determined by comparison of the response to the internal standards (mol) corrected by factorials. The inclusion of internal standards reduces quantification problems that might be caused by potential variability in ion yield due to ion suppression. The amounts of analytes were normalized to the mass of initial amounts of samples.

Antioxidant Activity of Choline, Ethanolamine and Phospholipids

To test the ability of choline to behave as a lipid hydroperoxide decomposer, each lipid hydroperoxide (100 µg), including 9-HpOT, 13-HpOT, 9-HpOD and 13-HpOD, was added to a 100-ml Pyrex Glass Media Bottle with 750 µg each of LA and ALA and certain amounts of antioxidants (ca. 1.5 µmol) including either of choline (100 µg), ethanolamine (100 µg), α-tocopherol (600 µg), or BHT (300 µg). The sample bottle was then capped and thoroughly mixed with 50 ml methanol. The sample without addition of antioxidant was used as control. The samples were kept in an oven at 37 °C. At 0, 1, 3, 6, 9 and 12 h, 1 ml of sample was taken by pipette to a vial and 50 µl was injected into LC–MS/MS for analysis. 9-HOD-d_4 (50 µg) and 13-HOD-d_4 (50 µg) were added as internal standards.

To test the hydroperoxide decomposition ability of phospholipids, several commercially-available phospholipids, including soy lecithin, were added directly to the system with LA and lipid hydroperoxides. 9-HpOD and 13-HpOD (100 µg each) was added to a tube (Kimble glass tube, 10 ml) with 1 g LA and certain amounts of antioxidant (ca. 12.5 µmol) including Solec™ P (10 mg), Solec FP 40 (10 mg), Soy PC (10 mg), BHT (2.5 mg) or Toc (5 mg). The sample tubes were then capped and thoroughly mixed by vortex. The samples without addition of any antioxidants were used as control. The samples were kept in an oven at 37 °C. At different intervals 10 µl of oil was taken to be dissolved in 1 ml of methanol. The methanol solution (50 µl) was injected to LC–MS/MS for analysis.

9-HOD-d_4 (50 μg) and 13-HOD-d_4 (50 μg) were added as internal standards.

Data Collection and Analysis

Data were collected using Analyst 1.5 software (Applied Biosystems®). Peaks for individual isomers were assigned based on the elution time and precursor–product ion transitions. Peaks were integrated using the IntelliQuan algorithm with a minimum of three rounds of smoothing and a bunching factor of three. The smoothing and bunching factors were adjusted on a peak-by-peak basis to ensure adequate peak identification. Peak area was used to determine the relative ratio of each peak compared to the added internal standard, multiplied by the total amounts of internal standard added (in mole) and divided by the amounts of solvent (in milliliter) to give an analyte concentration of nmol compound/ml of samples after correction with factorials.

Results

Optimization of Mass Spectrometers for Quantitative Analysis of Hydroperoxides and Hydroxyl Lipids by MRM Mode

The multiple reaction monitoring (MRM) mode of the 4000 Q TRAP® mass spectrometer (Applied Biosystems®) was used to monitor the fatty acid hydroperoxides (FA-OOH) and hydroxyl fatty acids (FA-OH) isomers and provide measurements of their abundance after HPLC separation. In this mode, the machine monitors the specific precursor–product ion pairs with conditions optimized for each transition. By rapidly switching between scan states, the machine is capable of the simultaneous monitoring of numerous transitions. This required construction of a detailed MRM table with parameters for each compound being analyzed. The automatic quantitative optimization feature of Analyst 1.5 was used to generate precursor–product ion transitions for each FA-OOH or FA-OH isomer by infusion of each authentic compound into the mass spectrometry. After selection of scan mode and input of molecular weight information, the Analyst software 1.5 optimized the mass spectrometer parameters. For each compound, several pairs of precursor–product ion transitions were generated, and appropriate precursor–product ion transitions representing a major fragmentation path and unique for each compound were selected.

For the specific precursor–product ion transition, MS/MS conditions were optimized to produce the maximal signal, and a detailed MRM table with focus on specific transitions only including DP, CE, collision cell exit potential and entrance energy was constructed (Table 1).

Once the mass spectrometer parameters were optimized, a 'T'-connection was used to combine the flow from the HPLC system with the flow from the infusion pump in order to optimize the source parameters. Finally, the authentic or purified compound was injected, along with the appropriate standards into the HPLC column, and suitable gradient programs provided sufficient separation of peaks for individual isomeric species to allow rigorous identification.

Separation and Identification of Lipid Hydroperoxides and Hydroxyl Lipids by LC–ESI–MS/MS

To separate individual hydroperoxy and hydroxy lipids from each other, authentic standards including HOTs, HpOTs, HODs, and HpODs were subjected to chromatography on a C18 reverse phase column, followed by 4000 QTRAP LC–ESI–MS/MS as described above. The compounds were monitored by a series of MRM scans with unique precursor–product ion transition and other parameters listed in Table 1. All of the oxidation product isomers of LA and ALA were separated from each other by combination of chromatography and mass spectrometer MRM mode, using specific precursor–product ion transitions which provide the specificity necessary for the quantification of these isomers. The conditions used in this study provide a very good separation of hydroperoxy and hydroxyl lipids (Table 1, Fig. 1).

Calibration of Mass Spectrometer Response

Although mass spectrometers have a large theoretical dynamic range, in practice their response may only be linear across a smaller portion of that range. To determine the linear range of the detector response, standard curves consisting of standards were generated. For the majority of hydroperoxy and hydroxyl lipids, the results showed a distinctly sigmoid curve indicating that quantification was only possible within a defined range. Within that range, however, the response of each compound was linear and proportional to the standard. It was found that the linear range for individual hydroperoxy and hydroxyl lipids was broadly within signal intensities of 10^4–10^7 allowing for a 1,000-fold change in hydroperoxy and hydroxyl lipids levels to be measured. Outside of this range, the non-linear response for the standard tended to overestimate or underestimate the amount of hydroperoxy and hydroxyl lipids (Fig. 2).

The ideal internal standard of each hydroperoxy and hydroxyl lipids to be measured is a compound with identical chemistry that varies by enough mass units to be

Table 1 Optimized MS/MS conditions for the analysis of lipid oxidation products

Analytes	Scan mode	Transition	CE	DP	CXP	EP	Dwell time (ms)	RT (min)
9-HOT	–	293.1 → 170.7	−24	−75	−10	−10	50	15.63
13-HOT	–	293.1 → 195.3	−24	−90	−10	−10	50	16.06
9-HpOT	–	309.2 → 184.8	−22	−65	−8	−10	50	15.54
13-HpOT	–	309.2 → 110.8	−22	−65	−8	−10	50	15.93
9-HpOD	–	311.1 → 184.8	−24	−70	−7	−10	50	21.5
13-HpOD	–	311.1 → 112.8	−24	−70	−7	−10	50	21.32
9-HOD	–	295.2 → 170.7	−26	−85	−10	−10	50	21.69
13-HOD	–	295.2 → 194.8	−26	−65	−10	−10	50	21.52
9-HOD-d_4	–	299.2 → 171.8	−26	−85	−10	−10	50	21.67
13-HOD-d_4	–	299.2 → 197.8	−26	−65	−10	−10	50	21.51

Parameters for precursor and product ions were determined based on the structures and observed fragmentation pattern

13-HOT 13-hydroxy-9,11,15-octadecatrienoic acid; *9-HOT* 9-hydroxy-10,12,15-octadecatrienoic acid; *13-HpOT* 13-hydroperoxy-9,11,15-octadecatrienoic acid; *9-HpOT* 9-hydroperoxy-10,12,15-octadecatrienoic acid; *13-HpOD* 13-hydroperoxy-9,11-octadecadienoic acid; *9-HpOD* 9-hydroperoxy-10,12-octadecadienoic acid; *13-HOD* 13-hydroxy-9,11-octadecadienoic acid; *9-HOD* 9-hydroxy-10,12-octadecadienoic acid; *CE* collision energy; *DP* de-clustering potential; *CXP* collision cell exit potential; *EP* entrance energy

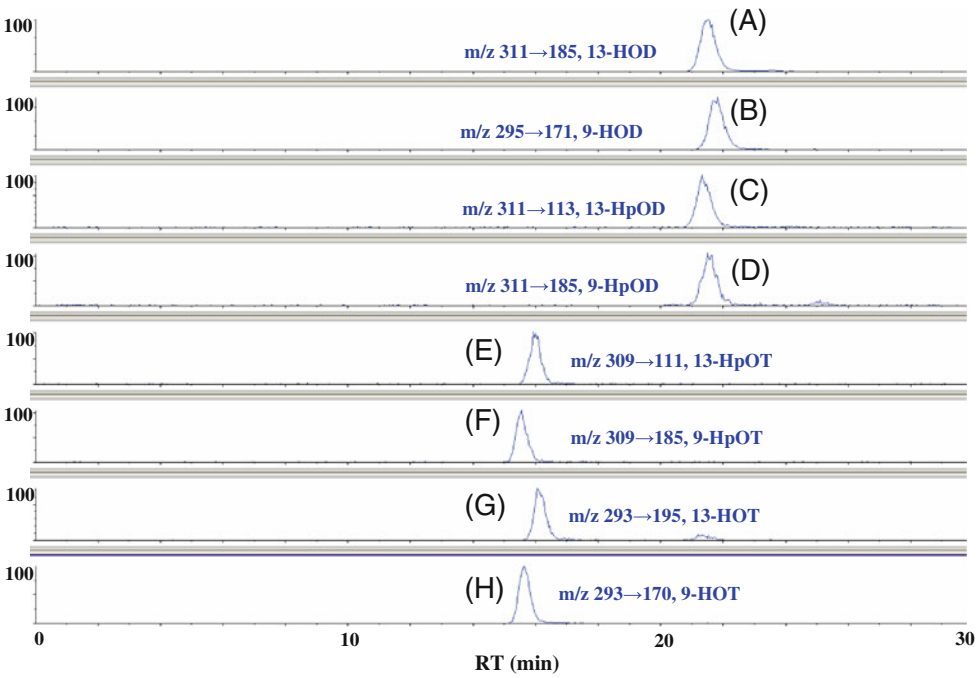

Fig. 1 Typical chromatographic separation of isomeric lipid hydroperoxides and hydroxyl lipids of LA and ALA by LC–MS/MS

distinguishable from the target compounds to be measured. In this study, labeled corresponding hydroxy lipids including 9-HOD-d_4 and 13-HOD-d_4 were used (Table 1). As a result of the different chemistry between the internal standards and the target hydroperoxy and hydroxyl lipids, the target compounds being quantified do not always give the same signal intensity on a mole-for-mole basis as the standard. In order to account for the difference in signal intensity, a factorial (Table 2) was calculated for each hydroperoxy and hydroxyl lipids compared to their corresponding internal standards that would place the regression line for each data set within the 99% confidence limits for the standards.

Effects of Choline and Ethanolamine on Hydroperoxide Decomposition

For control samples and the samples with addition of BHT and α-tocopherol, the levels of hydroperoxide isomers (9-HpOD, 9-HpOT, 13-HpOD, and 13-HpOT) and corresponding hydroxyl fatty acids (9-HOD, 9-HOT, 13-HOD, and 13-HOT) did not change significantly during storage at

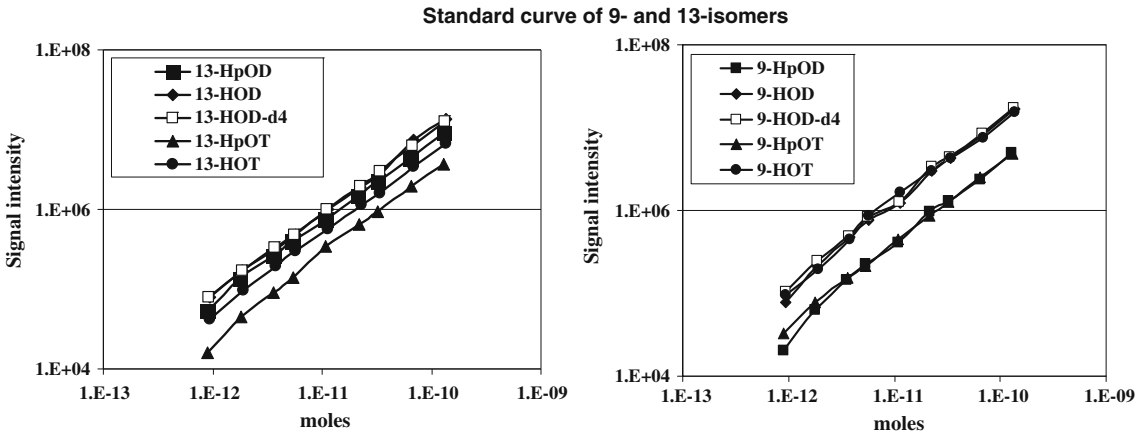

Fig. 2 Standard curves for -OOH and -OH and internal standards. Except where noted, all curves show only the linear portion for each standard and unknown. All scales are logarithmic

Table 2 Signal intensity/mole factorials for each isomer of oxidation products of LA and ALA

Isomers	Factorials	Isomers	Factorials
13-HpOT	0.26	9-HpOT	0.28
13-HOT	0.46	9-HpOD	0.29
13-HpOD	0.62	9-HOT	0.88
13-HOD	1.1	9-HOD	0.99
13-HOD-d_4	1	9-HOD-d_4	1

Factorials were calculated to convert moles of standard into moles of analyte based on the calibration curves for each standard and class of analytes

37 °C (Table 3a–h). In those samples containing choline, the concentrations of LA and ALA hydroperoxides decreased with increase of storage time at 37 °C, while the concentrations of the corresponding hydroxyl LAs and ALAs increased (Table 3a–h) demonstrating that choline decomposed the hydroperoxides into their corresponding hydroxyl lipids. Another functional group of phospholipids, ethanolamine, was also tested and it too demonstrated the ability to decompose the hydroperoxides into their corresponding hydroxyl lipids. However, ethanolamine was not as effective as choline in decomposing the lipid hydroperoxides (Table 3a–h).

Choline was found to have a similar effect on the lipid hydroperoxides generated by AMVN and methylene blue initiated autoxidation and photosensitized oxidation of LA and ALA [18, 20]. The amounts of lipid hydroperoxides (9-HpOD, 13-HpOD, 9-HpOT and 13-HpOT) in samples with the addition of choline decreased with storage time, while their corresponding hydroxyl fatty acids (9-HOD, 13-HOD, 9-HOT and 13-HOT) increased; for the samples with addition of BHT and α-tocopherol, the amounts of hydroperoxide isomers and their corresponding hydroxyl fatty acids did not change significantly and were very similar to the control.

Instead of choline and ethanolamine, several types of commercially-available soy lecithin, including Solec P, Solec FP40 and soy phosphatidylcholine (soy PC) were used to test their ability to decompose hydroperoxides. Soy lecithin and soy PC demonstrated a certain level of ability to decompose lipid hydroperoxides into their corresponding hydroxyl lipids since data showed the slow decomposition of hydroperoxides by lecithin and PC corresponding to a slight increase in hydroxyl lipids (Table 4a–d). However, the ability of lecithin to decompose lipid hydroperoxide does not appear to be as strong as that of choline and ethanolamine (Table 3a–h), and this is probably due to the facts that these two sets of experiments are under different conditions (compound concentration, storage time and reaction system), which may mitigate the validity of direct comparison of the ability of choline and phospholipids to decompose lipid hydroperoxides into hydroxyl lipids.

Discussion

LC–MS/MS is vastly superior to many other analytical methods not only due to the amounts of structural information acquired but also due to the short sample preparation as well as the high throughput analysis. Previous methods using DPPP derivatization for analysis of lipid hydroperoxides [11–21, 42] present practical difficulties in their separation and measurement from the oxidation of multiple essential fatty acids typically found in complicated food or biological systems, and the qualitative confirmation of these measurements requires extensive and time-consuming sample preparation for analysis using methods such as GC–MS. Moreover, previous methods are not suitable for the analysis of hydroxyl lipids since these compounds do not react with DPPP in the post column. The current method is able to report all molecular species of

Table 3 Change of the amounts of lipid hydroperoxides and hydroxyl lipids of LA and ALA with different antioxidants at 37 °C

nmol/ml	0 h	3 h	6 h	9 h	12 h
(a) Change of 9-HpOD					
Control	6.41 ± 0.12	6.40 ± 0.12	7.15 ± 0.15	6.81 ± 0.50	6.93 ± 0.21
Choline	6.41 ± 0.12	3.71 ± 0.23	2.60 ± 0.13	1.61 ± 0.31	1.58 ± 0.19
Ethanolamine	6.41 ± 0.12	5.93 ± 0.05	5.61 ± 0.30	5.21 ± 0.14	4.99 ± 0.25
α-Tocopherol	6.41 ± 0.12	6.02 ± 0.61	6.81 ± 0.21	6.78 ± 0.11	6.68 ± 0.31
BHT	6.41 ± 0.12	6.45 ± 0.12	6.51 ± 0.14	6.68 ± 0.21	6.89 ± 0.10
(b) Change of 9-HOD					
Control	0.04 ± 0.01	0.05 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Choline	0.04 ± 0.01	3.88 ± 0.12	5.42 ± 0.05	6.48 ± 0.16	6.76 ± 0.03
Ethanolamine	0.04 ± 0.01	0.28 ± 0.11	0.47 ± 0.01	0.94 ± 0.02	1.41 ± 0.02
α-Tocopherol	0.04 ± 0.01	0.05 ± 0.09	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
BHT	0.04 ± 0.01	0.05 ± 0.10	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
(c) Change of 13-HpOD					
Control	6.41 ± 0.25	6.66 ± 0.01	7.16 ± 0.19	6.96 ± 0.13	7.21 ± 0.07
Choline	6.41 ± 0.25	3.42 ± 0.02	2.49 ± 0.10	1.53 ± 0.20	1.36 ± 0.11
Ethanolamine	6.41 ± 0.25	5.91 ± 0.21	5.43 ± 0.11	4.99 ± 0.10	4.73 ± 0.09
α-Tocopherol	6.41 ± 0.25	6.36 ± 0.13	7.18 ± 0.09	7.16 ± 0.15	7.06 ± 0.03
BHT	6.41 ± 0.25	6.71 ± 0.19	7.01 ± 0.11	7.06 ± 0.06	7.21 ± 0.08
(d) Change of 13-HOD					
Control	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Choline	0.07 ± 0.01	3.82 ± 0.21	5.33 ± 0.20	6.22 ± 0.17	6.49 ± 0.21
Ethanolamine	0.07 ± 0.01	0.39 ± 0.03	0.55 ± 0.09	0.82 ± 0.02	1.27 ± 0.02
α-Tocopherol	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
BHT	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
(e) Change of 9-HpOT					
Control	6.45 ± 0.23	6.42 ± 0.11	6.99 ± 0.30	6.70 ± 0.23	6.88 ± 0.71
Choline	6.45 ± 0.23	3.77 ± 0.08	2.68 ± 0.25	1.53 ± 0.06	1.55 ± 0.07
Ethanolamine	6.45 ± 0.23	5.67 ± 0.21	5.46 ± 0.35	5.00 ± 0.24	4.80 ± 0.13
α-Tocopherol	6.45 ± 0.23	6.11 ± 0.32	6.94 ± 0.58	6.78 ± 0.60	6.68 ± 0.10
BHT	6.45 ± 0.23	6.54 ± 0.06	6.62 ± 0.91	6.61 ± 0.58	6.80 ± 0.13
(f) Change of 9-HOT					
Control	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Choline	0.02 ± 0.01	3.46 ± 0.06	5.11 ± 0.19	6.08 ± 0.48	6.37 ± 0.56
Ethanolamine	0.02 ± 0.01	0.23 ± 0.01	0.59 ± 0.05	0.93 ± 0.02	1.25 ± 0.32
α-Tocopherol	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.07	0.02 ± 0.01	0.02 ± 0.01
BHT	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
(g) Change of 13-HpOT					
Control	6.45 ± 0.56	6.81 ± 0.38	7.45 ± 0.51	7.15 ± 0.80	7.19 ± 0.21
Choline	6.45 ± 0.56	2.65 ± 0.21	1.61 ± 0.20	0.74 ± 0.21	0.75 ± 0.05
Ethanolamine	6.45 ± 0.56	6.06 ± 0.55	5.73 ± 0.16	5.45 ± 0.30	5.11 ± 0.61
α-Tocopherol	6.45 ± 0.56	6.54 ± 0.43	7.44 ± 0.69	7.40 ± 0.12	7.16 ± 0.31
BHT	6.45 ± 0.56	7.10 ± 0.37	7.17 ± 0.81	7.07 ± 0.38	7.34 ± 0.18
(h) Change of 13-HOT					
Control	0.10 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Choline	0.10 ± 0.01	3.77 ± 0.05	5.08 ± 0.21	5.99 ± 0.05	6.21 ± 0.03
Ethanolamine	0.10 ± 0.01	0.24 ± 0.02	0.55 ± 0.05	0.95 ± 0.03	1.59 ± 0.06
α-Tocopherol	0.10 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
BHT	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01

Table 4 Change of the amounts of lipid hydroperoxides and hydroxyl lipids of LA and ALA with lecithin and antioxidants at 37 °C

nmol/ml	0 h	24 h	48 h	72 h
(a) Change of 13-HpOD				
Solec FP 40	3.20 ± 0.05	1.11 ± 0.07	1.01 ± 0.07	1.59 ± 0.07
Solec P	3.20 ± 0.05	1.12 ± 0.06	1.18 ± 0.09	1.87 ± 0.08
Soy PC	3.20 ± 0.05	1.75 ± 0.03	2.12 ± 0.19	2.29 ± 0.21
α-Tocopherol	3.20 ± 0.05	2.99 ± 0.11	3.21 ± 0.21	4.64 ± 0.08
BHT	3.20 ± 0.05	2.58 ± 0.05	0.77 ± 0.05	1.07 ± 0.05
Control	3.20 ± 0.05	6.49 ± 0.17	10.35 ± 0.17	13.37 ± 0.18
(b) Change of 13-HOD				
Solec FP 40	1.09 ± 0.12	2.21 ± 0.15	2.85 ± 0.15	3.12 ± 0.08
Solec P	1.09 ± 0.12	2.18 ± 0.12	2.77 ± 0.14	4.00 ± 0.08
Soy PC	1.09 ± 0.12	2.12 ± 0.03	2.61 ± 0.13	3.16 ± 0.09
α-Tocopherol	1.09 ± 0.12	1.52 ± 0.22	2.09 ± 0.77	2.81 ± 0.04
BHT	1.09 ± 0.12	1.51 ± 0.08	2.26 ± 0.11	2.50 ± 0.04
Control	1.09 ± 0.12	1.46 ± 0.11	4.24 ± 0.12	60.2 ± 0.18
(c) Change of 9-HpOD				
Solec FP40	3.21 ± 0.10	0.21 ± 0.09	0.93 ± 0.05	1.44 ± 0.01
Solec P	3.21 ± 0.10	0.99 ± 0.03	1.11 ± 0.08	1.64 ± 0.03
Soy lecithin	3.21 ± 0.10	1.06 ± 0.14	1.69 ± 0.11	1.69 ± 0.04
α-tocopherol	3.21 ± 0.10	2.69 ± 0.06	2.67 ± 0.37	3.61 ± 0.14
BHT	3.21 ± 0.10	2.69 ± 0.05	0.78 ± 0.21	1.07 ± 0.16
Control	3.21 ± 0.10	6.06 ± 0.11	9.46 ± 0.02	13.59 ± 0.03
(d) Change of 9-HOD				
Solec FP 40	1.61 ± 0.04	4.49 ± 0.04	5.23 ± 0.16	6.08 ± 0.06
Solec P	1.61 ± 0.04	4.15 ± 0.03	6.54 ± 0.15	8.11 ± 0.14
Soy PC	1.61 ± 0.04	4.21 ± 0.03	5.93 ± 0.12	7.52 ± 0.12
α-Tocopherol	1.61 ± 0.04	2.21 ± 0.06	3.68 ± 0.71	4.3 ± 0.08
BHT	1.61 ± 0.04	2.10 ± 0.07	3.36 ± 0.04	4.41 ± 0.11
Control	1.61 ± 0.04	2.31 ± 0.02	7.42 ± 0.05	9.89 ± 0.12

monohydroperoxy and monohydroxy isomers of LA and ALA in approximately 30 min by LC–MS/MS. This makes high throughput analysis of lipid hydroperoxides and hydroxyl lipids a realistic proposition, thereby enabling the deciphering of oxidation mechanisms.

The LC–ESI–MS/MS method developed in this study can simultaneously measure monohydroperoxides and monohydroxy isomers of LA and ALA without purification or derivatization. The combination of retention time and precursor–product ion transitions provides specific, sensitive, and accurate determination. The use of reversed-phase HPLC conditions to resolve individual species prevents interference between peaks which provide another level of identification in addition to structural information. The calibration lines for the lipid hydroperoxides and hydroxyl lipids generated from oxidation of LA and ALA were found to be linear over three orders of magnitude or more. These results enable the measurement of large changes in their respective concentrations in a sample. With R^2 values from 0.976 to 0.999, the method is very accurate when measuring levels of lipid hydroperoxides and hydroxyl lipids that fall within the linear ranges described above.

To ensure the specific, sensitive and accurate determination of monohydroperoxides and monohydroxy isomers of LA and ALA using LC–MS/MS, proper selection of the precursor–product ion transition is critical. While the precursor–product ion transition showing the maximal signal intensity is preferable for quantitating the analytes, the transition of fatty acid hydroperoxides (FA-OOH) and hydroxyl fatty acid (FA-OH) isomers giving the maximal signal intensity cannot distinguish one isomer from another (Table 5). Such precursor–product ion transitions should not be selected because these transitions are generated without structural information, since the same group of hydroperoxy or hydroxyl isomers have exactly the same precursor ions. For example, the two hydroxyl isomers of LA, 9-HOD and 13-HOD, have identical molecular weight (M_W 296) and formula ($C_{18}H_{32}O_3$) except for the positional difference of the -OH functional group. Both isomers have major fragments of *m/z* 295 and 277 corresponding to $[M–H]^-$ and $[M–H–H_2O]^-$ by loss of H and H_2O (Table 5). Although these major fragments give intensive signals, they do not contain any unique structural information which could be used to distinguish one isomer from another and, therefore, these fragments should not be chosen for the precursor–product ion transitions [4, 6].

Since the structural difference between the 9-HOD and 13-HOD isomers is the unique position of the hydroxyl group, the product ions obtained from cleavage of the characteristic location of the hydroxyl functional group could potentially be different for every isomer and therefore provided unique spectral information for each isomer. The precursor–product ion transitions (Table 1) were selected by consideration of the isomeric structural information, and the selected product ions from the mass spectrum of each compound were identified by the characteristic location of the hydroxyl functional group. 9-HOD and 13-HOD were cleaved adjacent to the hydroxyl group to generate the characteristic fragments specific to each hydroxyl isomer. Fragmentation of the C-12, C-13 bond of 13-HOD with proton transfer produced the fragment ion at *m/z* 195, and fragmentation of C-9, C-10 bond of 9-HOD with proton transfer produced the fragment ion at *m/z* 171 (Table 5). A portion at the side of hydroxyl group was migrated to the other side of the cleavage. The fragments at *m/z* 171 and 195 with unique structural information were used to distinguish 9-HOD from 13-HOD because these fragments are derived by the cleavage of the carbon bond of isomers adjacent to the corresponding uniquely positioned hydroxyl functional group at 9 and 13, respectively [49–52].

Similarly, loss of water and cleavage of the C10,C11 double bond allylic to the hydroperoxide moiety of

Table 5 Fragmentation of lipid hydroperoxides and hydroxyl lipids of LA and ALA

Compound	$[A–H]^-$	$[A–H–H_2O]^-$	Fragment	Fragment	Fragment
9-HpOD	311 (33)	293 (100)	184.8 (22)		
13-HpOD	311 (25)	293 (100)	112.7 (20)		
9-HOD	295.2 (100)	277 (22)	170.8 (19)		
13-HOD	295 (100)	277 (16)	195 (17)		
9-HpOT	309 (10)	290.9 (100)	272.8 (11)	185 (23)	
13-HpOT	309 (40)	291 (100)	194.8 (15)	110.6 (16)	
9-HOT	293.1 (5)	274.8 (100)	230.9 (11)	170.7 (45)	120.8 (25)
13-HOT	293.1 (4)	274.7 (51)	223.4 (50)	195.3 (100)	

m/z (relative abundance). The ion abundance reported is normalized to the most abundant fragment ion

9-HpOT after a two proton shift produced *m/z* 185, and loss of water (Table 5) and cleavage of the C11,C12 double bond allylic to the hydroperoxide moiety of 13-HpOT after a one proton shift produced *m/z* 111 (Table 5). Therefore, the product ions selected for 9-HpOT and 13-HpOT with -OOH functional group at the position of 9- or 13- are position specific and characteristic fragments of *m/z* 185 and *m/z* 111, that are derived by cleavage of the carbon bond of isomers adjacent to the corresponding unique position of the hydroxyl functional group at 9 and 13, respectively. Similarly to HOD and HpOT, HOT and HpOD isomers from oxidation of LA and ALA have different precursor–product ion transitions corresponding to unique positions of the functional group [4, 6, 41, 49–52].

Hydroperoxides are the primary oxidation products of unsaturated lipids which are very unstable and easy to decompose into secondary oxidation products. Lipid hydroperoxides can be converted to their corresponding hydroxyl lipids by reducing agents or reductases [1–4]. In biological systems, formation of hydroxyl lipids from oxidation of unsaturated lipids can be catalyzed by lipoxygenase, P-450 monooxygenase, or cyclooxygenase [43–45]. In food systems, hydroperoxides can be reduced to hydroxyl lipids by metals, amino acids and other reducing agents [1–3, 46]. Some work demonstrated the antioxidant effects of amines [32, 47, 48] and hypothesized that the antioxidant effects of amines are due to their ability to decompose lipid hydroperoxides [40] which has been illustrated by the measurement of peroxide values. However, the present work is the first to clearly demonstrate the ability of choline, ethanolamine and phospholipids to convert lipid hydroperoxides into their corresponding hydroxyl lipids using solid data and detailed chemical structural information of these compounds.

By using developed LC–MS/MS methods, this study clearly demonstrated that choline and phospholipids have the ability to convert lipid hydroperoxides to their corresponding hydroxyl lipids. In the first set of experiments, choline and ethanolamine demonstrated their ability to decompose the added hydroperoxides of LA and ALA into their corresponding hydroxyl LA and ALA. In the control samples, the hydroperoxide and hydroxyl content remained constant which demonstrated that oxidation of the added LA and ALA in these experiments was probably not occurring and hydroxyl LA and ALA detected in this assay arose from the decomposition of the added hydroperoxides of LA and ALA.

However, in the second set of experiments, both hydroperoxide and hydroxyl content in control samples increased and demonstrated that the oxidation of LA and ALA in these experiments was initiated, presumably because of the increased incubation time (72 h). While the hydroxyl contents increased in the samples with added lecithin and PC especially at the early stage of storage (before 24 h), hydroperoxides increased in the control samples but not in those samples with added lecithin and PC. This probably demonstrated that lecithin and PC decomposed the added hydroperoxides into their corresponding hydroxyl compounds. The tested lecithins and PC did not have as dramatic an effect in reducing hydroperoxide contents as seen in the first assays with choline and ethanolamine. This effect is probably due to the fact that the antioxidant activity of phospholipids arises primarily from the choline, ethanolamine and other amines which are present as degradation products in the phospholipids [32]. While data demonstrated the lecithin and PC were capable of decomposing hydroperoxides into hydroxyl lipids, with the increase of storage time (more than 48 h) oxidation of LA and ALA were obviously initiated, which further complicated the comparison of the controls to the test samples.

Due to the different physiochemical properties of choline and phospholipids, the first set of experiments (Table 3a–h) was conducted in methanol solutions to test the ability of choline to behave as a lipid hydroperoxide decomposer since choline and ethanolamine can easily dissolve in methanol; while the second set of experiments (Table 4a–d) was designed to test the lipid hydroperoxide decomposition ability of phospholipids in LA and no methanol was used by consideration of the facts that methanol should not be used in food system as well as phospholipids can dissolve in LA under current experimental conditions. The different

experimental conditions (compound concentration, storage time and reaction system) may be one of the important factors responsible for the different effects of BHT between the first set (Table 3a–h) and the second set of experiments (Table 4a–d), and the different experimental conditions may also mitigate the validity of direct comparison of the abilities of choline and phospholipids to decompose lipid hydroperoxides into hydroxyl lipids. However, this work provide solid data and direct evidence and clearly demonstrates that functional groups of phospholipids, including choline and ethanolamine, can actually decompose a variety of lipid hydroperoxide isomers into their corresponding hydroxyl lipids under current experimental conditions.

In food systems, primary lipid oxidation products are highly reactive and interact readily with proteins, vitamins, cholesterol and many other components, to form new compounds that affect food texture, functionality, nutrition, color and safety. Lipid hydroperoxides can also decompose into small molecular compounds which affect food aroma and flavor. Since the antioxidant activity of choline, amines, and phospholipids is due to their ability to decompose lipid hydroperoxides into corresponding hydroxyl lipids, these compounds actually inhibit lipid oxidation by means of a diversion of the normal lipid oxidation pathway. Although the newly formed hydroxyl lipids still have double bonds which can be further oxidized, they are more stable than corresponding lipid hydroperoxides. Consequently, the system with addition of choline, ethanolamine or phospholipids will have less chance to accumulate lipid hydroperoxides and will show low peroxide values. This work provides further evidence for hydroxyl formation as a possible mechanism for antioxidant activity and for the synergistic effects of phospholipids observed in earlier studies when peroxide values were used as an index.

Acknowledgments We thank Andreas G. Altemueller, Charles Schasteen, Phil Kerr, and Holly M. Amjad for critical reading of the manuscript.

References

1. Erickson CE (1987) Oxidation of lipids in food systems. In: Chan HWS (ed) Autoxidation of unsaturated lipids. Academic Press, Tokyo, pp 207–232
2. Frankel EN (1998) Chapter 10. Foods. In: Frankel EN (ed) Lipid oxidation. The Oily Press, Dundee, pp 187–225
3. Frankel EN (2005) Chapter 11. Foods. In: Frankel EN (ed) Lipid Oxidation. The Oily Press, Dundee, pp 299–354
4. VanRollins M, Murphy RC (1984) Autooxidation of docosahexaenoic acid: analysis of ten isomers of hydroxyldocosahexaenoate. J Lipid Res 25:507–517
5. Deterding LJ, Curtis JF, Tomer KB (1992) Tandem mass spectrometric identification of eicosanoids: leukotrienes and hydroxyeicosatetraenoic acids. Biol Mass Spetrom 21:597–609
6. Wheelan P, Zirroll JA, Murph RC (1993) Low-energy fast atom bombardment tandem mass spectrometry of monohydroxy substituted unsaturated fatty acids. Biol Mass Spectrom 22:465–473
7. MacMillan DK, Murphy RC (1995) Analysis of lipid hydroperoxides and long-chain conjugated keto acids by negative ion electrospray mass spectrometry. J Am Soc Mass Spectrom 6:1190–1201
8. Newby SS, Mallet AI (1997) Rapid simultaneous analysis of prostaglandin E2, 12-hydroxyeicosatetraenoic acid and arachidonic acid using high-performance liquid chromatography/electrospray ionization mass spectrometry. Rapid Commun Mass Spectrom 11:1723–1727
9. Halliwell B, Gutteridge JMC (1999) Free radicals, other reactive species and diseases. In: Halliwell B, Gutteridge JMC (eds) Free Radicals in Biology and Medicine, 3rd edn. Oxford University Press, Oxford, pp 617–783
10. Kempen EC, Yang P, Felix E, Madden T, Newman RA (2001) Simultaneous quantification of arachidonic acid metabolites in cultured tumor cells using high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. Anal Biochem 297:183–190
11. Akasaka K, Ijichi S, Watanabe K, Ohrui H, Meguro H (1992) High-performance liquid chromatography and post-column derivatization with diphenyl-1-pyrenylphosphine for fluorimetric determination of triacylglycerol hydroperoxides. J Chromatogr 592:197–202
12. Akasaka K, Ohrui H, Meguro H (1994) Measurements of cholesterol hydroperoxides of high and combined low and very low lipoprotein in human plasma. Biosci Biotechnol Biochem 58:396–399
13. Ohshima T, Hopia A, German JB, Frankel EN (1996) Determinations of hydroperoxides and structures by high-performance liquid chromatography with post-column detection with diphenyl-1-pyrenylphosphine. Lipids 31:1091–1096
14. Ohshima T, Ushio H, Koizumi C (1997) Analysis of polyunsaturated fatty acid isomeric hydroperoxides by high-performance liquid chromatography with post-column fluorescence detection. In: Shahidi F, Cadwallader KR (eds) Flavor and lipid chemistry of seafood. ACS Symposium Series 674, American Chemical Society, Washington DC, pp 198–217
15. Pan X, Ushio H, Ohshima T (2004) Photo-oxidation of lipids impregnated on the surface of dried seaweed (*Porphyra yezoensis* Ueda): characterization of volatiles. J Am Oil Chem Soc 81(8):773–781
16. Pan X, Ushio H, Ohshima T (2004) Photo-oxidation of lipids impregnated on the surface of dried seaweed (*Porphyra yezoensis* Ueda) hydroperoxide distribution. J Am Oil Chem Soc 81(8):765–771
17. Pan X, Ushio H, Ohshima T (2005) Comparison of volatiles from free radical oxidation and singlet-oxygen oxidation of cod liver oil emulsion. Fish Sci 71(3):639–647
18. Pan X, Ushio H, Ohshima T (2005) Effects of molecular configurations of food colorants on their efficacies as photosensitizers in lipid oxidation. Food Chem 92(1):37–44
19. Pan X, Ushio H, Ohshima T (2005) Effects of food colorants on photooxidation of lipids added to surimi. Fish Sci 71(2):397–404
20. Pan X, Ushio H, Ohshima T (2005) Oxidation of all-*cis*-7,10,13,16,19-docosapentaenoic acid ethyl ester: hydroperoxide distribution and volatile characterization. Eur J Lipid Sci Technol 107(4):228–238
21. Sohn JH, Taki Y, Ushio H, Ohshima T (2005) Quantitative determination of total lipid hydroperoxides by a flow injection analysis system. Lipids 40:203–209
22. Hong Y, Pan X, Welti R, Wang X (2008) Phospholipase Dα3 regulates Arabidopsis response to salinity and water deficits. Plant Cell 20:803–816

23. Yang W, Devaiah SP, Pan X, Isaac G, Welt R, Wang X (2007) A LRR-containing acyl hydrolase mediates jasmonic acid production and arabidopsis resistance to *Botrytis cinerea*. J Biol Chem 282:18116–18128
24. Pan X, Welti R, Wang X (2008) Simultaneous quantification of major phytohormones and related compounds in crude plant extracts by HPLC–ESI–MS/MS. Phytochemistry 69:1773–1781
25. Pan X, Wang X (2009) Profiling of plant hormones by mass spectrometry. J Chromatogr B 877:2806–2813
26. Pan X, Welti R, Wang X (2010) Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography–mass spectrometry. Nat Protoc 5:986–992
27. Olcott HS, Van Veen J (1963) Role of individual phospholipids as antioxidants. J Food Sci 28:313–315
28. Kashima M, Cha GS, Isoda Y, Hirano J, Miyazawa T (1991) The antioxidant effects of phospholipids on perilla oil. J Am Oil Chem Soc 68:119–122
29. Kaitaranta JK (1992) Control of lipid oxidation in fish oil with various antioxidative compounds. J Am Oil Chem Soc 69:810–813
30. King MF, Boyd LC, Sheldon BW (1992) Antioxidant properties of individual phospholipids in a salmon oil model system. J Am Oil Chem Soc 69:545–551
31. Segawa T, Hara S, Totani Y (1994) Antioxidative behavior of phospholipids for polyunsaturated fatty acids of fish oil. II, Yukagaku (Jpn.) 43:515–519
32. Saito H, Ishihara K (1997) Antioxidant activity and active sites of phospholipids as antioxidants. J Am Oil Chem Soc 74:1531–1536
33. Olcott HS, Kuta EJ (1959) Basic substances as synergists for fat antioxidants. Nature 183:1812
34. Hudson BJF, Mahgoub SEO (1981) Synergistic between phospholipids and naturally occurring antioxidants in leaf lipids. J Sci Food Agric 32:208–210
35. Von Schler A, Löschner D (1985) Naturliche Antioxidantien V, Antioxidantien und Synergisten aus Antarktischem Krill. Fette Seifen Anstrichm 87:454–457
36. Schuler P (1990) Natural antioxidants exploited commercially. In: Hudson BJF (eds) Food Antioxidants, Elsevier Applied Science, London, pp 1–18
37. Ohshima T, Fujita Y, Koizumi C (1993) Oxidative stability of sardine and mackerel lipids with reference to synergistic between phospholipids and alpha-tocopherol. J Am Oil Chem Soc 68:119–122
38. O'Brien PJ (1969) Intramolecular mechanisms for the decomposition of a lipid peroxide. I. Decomposition of lipid peroxide by metal ions, heme compounds, and nucleophiles. Can J Biochem 47:485–492
39. Lee JH, Fujimoto K, Kaneda T (1981) Antioxygenic peroxide decomposition properties of antarctic krill lipids. Bull Jpn Soc Sci Fish 47:881–888
40. Miyazawa T, Yamaguchi M, Lee JH, Fujimoto K, Kaneda T (1984) Decomposition of lipid hydroperoxide by choline and ethanolamine. Agric Biol Chem 48:1375–1377
41. Lyberg AM, Adlercreutz P (2006) Monitoring monohydroperoxides in docosahexaenoic acid using high-performance liquid chromatography. Lipids 41:67–76
42. Akasaka K, Suzuki T, Ohrui H, Meguro H (1987) Study on aromatic phosphine for novel fluorometry of hydroperoxides (II)—the determination of lipid hydroperoxides with diphenyl-1-pyrenylphosphine. Anal Lett 20:797–907
43. Horrocks LA, Vanrollins M, Yates AJ (1981) Lipid changes in the aging brain. In: Davison AN, Thompson RHS (eds), The molecular basis of neuropathology, Edward Arnold, London, pp 601–630
44. Capdevila J, Marnett LJ, Chacos N, Prough RA, Estabrook RW (1982) Cytochrome P-450-dependent oxygenation of arachidonic acid to hydroxyl eicosatetraenoic acids. Proc Natl Acad Sci USA 79:767–770
45. Feussner I, Wasternack C (2002) The lipoxygenase pathway. Annu Rev Plant Biol 53:275–297
46. Gardner HW (1987) Reactions of hydroperoxides-products of high molecular weight. In: Chan HWS (ed) Autoxidation of unsaturated lipids. Academic Press, London, pp 51–94
47. Harris LA, Olcott HS (1966) Reaction of aliphatic tertiary amines with hydroperoxides. Am Oil Chem Soc 43:ll–ll14
48. Veen J, Weil JT, Kennedy TE, Olcott HS (1970) Aliphatic hydroxylamines as lipid antioxidants. Lipids 5:509–512
49. Kerwin JL, Torvik JJ (1996) Identification of monohydroxy fatty acids by electrospray mass spectrometry and tandem spectrometry. Anal Biochem 237:56–64
50. Kim HY, Salem NJR (1989) Preparation and the structural determination of hydroperoxy derivatives of docosahexaenoic acid and other polyunsaturated by thermospray LC/MS. Prostaglandins 37:105–119
51. Kim HY, Sawazaki S (1993) Structural analysis of hydroxyl fatty acids by thermospray liquid chromatography/tandem mass spectrometry. Biol Mass Spectrom 22:302–310
52. Seal JR, Porter NA (2004) Liquid chromatography coordination ion-spray spectrometry (LC–CIS–MS) of docosahexaenoate ester hydroperoxide. Anal Bioanal Chem 378:1007–1013