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Lipidomic quantitation of oxidized fatty acids in plant and algae oils

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Abstract

Linoleic acid (LA) and alpha-linolenic acid (ALA) in plant or algae oils are precursors to oxidized fatty acid metabolites known as oxylipins. Liquid chromatography tandem mass spectrometry was used to quantify oxylipins in soybean, corn, olive, canola and four high-oleic acid algae oils at room temperature or after heating for 10 minutes at 100°C. Flaxseed oil oxylipin concentrations were determined in a follow-up experiment that compared it to soybean, canola, corn and olive oil. Published economic disappearance data for soybean, canola, corn and olive oil were used to estimate daily oxylipin intake. The LA and ALA fatty acid composition of the oils was generally related to their respective oxylipin metabolites, except for olive and flaxseed oil which had higher LA-derived mono-hydroxy and ketone oxylipins than other oils, despite their low LA content. Algae oils had the least amount of oxylipins. The change in oxylipin concentrations was not significantly different amongst the oils after short-term heating. Estimated oxylipin intake from non-heated soybean, canola, corn and olive oil was 1.1 mg per person per day. These findings suggest that oils represent a dietary source of LA- and ALA- derived oxylipins and that the response of oils to short-term heating does not differ amongst the various oils.

Graphical Abstract

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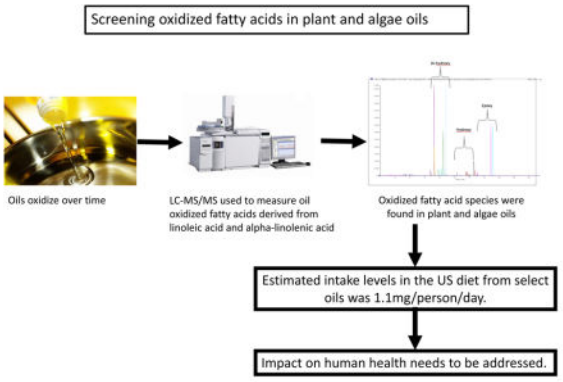
Conflicts of interest

The authors have no conflicts of interests to declare.



Keywords

Linoleic acid; alpha-linolenic acid; oxidized fatty acids; oxylipins; lipidomics; plant oils; soybean; corn; canola; flaxseed; olive; algae oils; mass spectrometry

Introduction

Plant oils are a major dietary source of the essential polyunsaturated fatty acids, linoleic (LA, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3). LA and ALA are required for infant development and supporting optimal nutrition. The estimated requirements are 0.5–1% of calories for ALA and 2% for LA [1, 2]. Currently, however, LA accounts for approximately 7% of caloric intake in the US due to the widespread consumption of high-LA oils such as soybean and corn [3]. Estimated US intake of ALA is 0.72% energy [3], provided mainly through soybean oil and to a lesser extent, canola oil [3].

LA and ALA are precursors to bioactive oxidized fatty acid metabolites known as oxylipins. LA-derived oxylipins are involved in inflammatory cascades, pain perception and skin barrier integrity [4–7]. Little is known about the role of ALA-derived oxylipins in mammals, although plants produce them enzymatically to regulate root development and defense against pathogens [8, 9].

Oxylipins can be synthesized *in vivo* from their precursor fatty acids via oxygenase enzymes [10–12], or obtained through food or dietary oils [13–16]. Ingested hydroxyl, epoxy and dihydroxy oxylipins are bioavailable [17–21]. LA-derived hydroperoxides have been shown to degrade in the stomach into aldehydes, which accumulate in liver [22, 23].

The oxidation of fatty acids in oils is non-enzymatic [24], although enzymatic oxidation via lipoxygenase enzymes occurs during the extraction of oils from seed sources [25]. Studies reported the formation of LA-derived hydroxy, hydroperoxy, ketone dienes or epoxy monoenes in oils heated at 40, 100 or 180°C for 10–264 hours or up to 156 days [13–15]. Oils with higher LA content, such as safflower oil, produced more LA-derived oxylipins as compared to low-LA oils [13–15]. In these studies, oxylipins were detected after prolonged heating of the oils but not at room temperature, likely because the analytical methods involving high performance liquid chromatography with an ultraviolet detection, gas

chromatography coupled to a flame ionization detection (GC-FID) or nuclear magnetic resonance, lacked sensitivity [13–15]. Fankhauser-Noti et al., however, detected the presence of LA-derived mono-epoxy and di-epoxy fatty acids with GC-FID in fresh (non-heated) and 4-year old olive and sunflower oil [26]. We are not aware of studies that reported on ALA-derived oxylipins in oils.

The advent of lipidomic analysis with ultra-high pressure liquid chromatography tandem mass spectrometry (UPLC-MS/MS) has enabled the separation, detection and quantitation of many oxylipin species at picomolar concentrations [27]. To gain detailed analytical insight into the type and quantity of oxylipins in oils, the aim of the present study was to measure LA and ALA derived oxylipins in plant and algae oils containing varying amounts of LA and ALA at room temperature or after heating for 10 minutes with UPLC-MS/MS [27]. Algae oils were tested because they are an emerging and potentially sustainable source of dietary fat [28]. Thus, like plants, algae synthesize unsaturated fatty acids and contain oxygenase enzymes that may contribute to lipid oxidation during the extraction [29]. Oxylipins were measured in oils at room temperature or after heating for 10 minutes at 100°C to simulate low-heat cooking or simmering conditions. This allowed us to test whether highly unsaturated oils were more vulnerable to oxidation compared to less saturated oils. Daily intake of LA and ALA derived oxylipins was also estimated based on published consumption data of commonly consumed plant oils in the US[3].

We hypothesized that concentrations of LA and ALA derived oxylipins at room temperature will be proportional to their precursor fatty acid concentrations in oils, and that high LA or ALA oils will produce more oxylipins following short-term heating for 10 minutes compared to low LA or ALA oils. Understanding the dietary contribution of oils to potentially bioactive oxylipins is likely to have significant implications for human health and disease.

Materials and Methods

Chemicals and Reagents

Methanol, ethyl acetate, chloroform, toluene, and hexane were obtained from Fisher Scientific (Hampton, NH). Methanol was LC-MS grade, whereas all other solvents were HPLC grade. Acetic acid, butylated hydroxytoluene, sodium carbonate, glycerol, triphenylphosphine, ethylenediaminetetraacetic acid (EDTA), and hydrochloric acid were purchased from Sigma-Aldrich (St. Lous, MO). Oxylipin standards were purchased from Cayman Chemicals (Cayman Chemical, Ann Arbor, MI) or synthesized by Dr. Bruce Hammock’s lab at UC Davis. The synthetic standards included the following ALA-derived oxylipins: 15(16)-EpODE, 12(13)-EpODE, 9(10)-EpODE, 15,16-DiHODE, 12,13-DiHODE and 9,10-DiHODE. Fatty acid standards were purchased from NuCheck Prep (Elysian, MN). Oils were purchased from local stores.

General Study Design

Two experiments were carried out to test the aforementioned hypotheses. In the first experiment, oxylipins and fatty acids were measured in soybean, corn, canola and olive oil

as well as 4 algae oils provided by TerraVia Holdings, Inc. that were high in oleic acid, low in LA and ALA, and either lacked or contained 1000 ppm of mixed tocopherols (FORTIUM® MT70 IP Liquid from Kemin IA, USA) as an antioxidant. The algae oils tested were a High Stability Algae Oil (HSAO), HSAO without added antioxidants (w/AO), Ultra Omega-9 Algae oil ("Thrive"), and "Thrive" w/AO. Oxylipins were measured at room temperature or after heating for 10 minutes at 100°C. Heat was applied at 100°C (212°F), because it is below the smoke point range of the oils used in the present study (140–244°C; 280–471°F) [30, 31].

In the second experiment, we confirmed oxylipin concentrations in off-the-shelf oils tested in Experiment 1 (corn, canola, soybean and olive oil), and compared them to flaxseed (linseed) oil, which may be prone to oxidation due to its high ALA (54% of total fatty acids) content [32]. Estimated oxylipin intake levels in the US diet were then calculated from published consumption data for soybean, corn, canola and olive oil [3] and measured oxylipins in these oils. The fatty acid composition of the oils in both experiments was confirmed with GC-FID.

The same soybean, canola, and corn brands were used in both experiments. They were purchased from different markets, however. Experiment 1 oils were purchased from local supermarkets in San Francisco. Experiment 2 oils were purchased from local supermarkets in Davis. Spectrum and STAR extra virgin olive oil brands were used in Experiments 1 and 2, respectively.

Experiment 1—Five mL of each of the soybean, corn, canola, olive and 4 algae oils were added to 8 mL glass vials (17 mm diameter, 64 mm height) and heated, uncapped at 100°C on a heating block for 10 minutes. Temperature was measured each minute. Samples were staggered 1 minute apart to allow time for temperature recording (Supplementary Figure 2). Two 10 μL samples were taken from each oil at baseline (room temperature) and after 10 minutes of heating for fatty acid and oxylipin analysis (methodological details below). There were three replicates per oil in total, and each was performed on a separate day (i.e. all 8 oils heated and sampled on day 1 and then on days 2 and 3). Oils were stored in a 4°C fridge and thawed prior to aliquoting samples each day.

Experiment 2—Soybean, corn, canola, olive and flaxseed oils were purchased from various supermarket outlets in Davis (CA, USA). Supplementary Table 1 shows the oil brand, store they were purchased from, date they were purchased and expiry date. For each oil, 5 bottles from the same brand but with different lot numbers were purchased from the various stores, except for flaxseed oil, because we were not able to find the same brand in different stores. Therefore, 3 different brands of flaxseed oil were purchased, with one brand being purchased in duplicate from the same store to increase the sample size to 4 (Supplementary Table 1). The reason we aimed to keep the oil company source consistent was to confirm the validity of our methods on the same oil brand. Oxylipins were extracted and measured within a week after purchasing the oils. Fatty acid composition of the oils was confirmed a few weeks later.

Oxylipin Analysis with UPLC-MS/MS

A total of 8 LA and 8 ALA oxylipins were measured by targeted UPLC-MS/MS (Supplementary Table 2). Ten μL of oil sample were mixed with 200 μL of methanol containing 0.1% acetic acid and 0.1% butylated hydroxytoluene (BHT) after adding 10 μL surrogate standard solution (purity ≥ 95%) containing 5 pmol (per sample) of d11-11(12)-EpETrE, d11-14,15-DiHETrE, d4-6-keto-PGF1α, d4-9- HODE, d4-LTB4, d4-PGE2, d4-TXB2, d6-20-HETE and d8-5-HETE in methanol[27] and 10μL of antioxidant solution (0.2 mg/mL BHT, ethylenediaminetetraacetic acid (EDTA), and triphenylphosphine (TPP) in water/methanol (1:1 v/v)). While the test oils did not contain arachidonic and docosahexaenoic acid, d4-6-keto-PGF1α, d4-LTB4, d4-PGE2 and d4-TXB2, which are standards for arachidonate or docosahexaenoate derived oxylipins, constituted part of our routinely used surrogate standard mix [10, 11]. They were included in the assay but were not used in any of the LA or ALA oxylipin calculations.

Oils were hydrolyzed by adding 200 μL of 0.25 M sodium carbonate solution (1.13 g in 21.3 mL water and 21.3 mL methanol) to 10 μL oil aliquot containing antioxidant and surrogate standard [10, 33, 34]. The samples were vortexed and heated for 30 minutes at 60°C under constant shaking. The samples were allowed to cool to room temperature and 25 μL acetic acid and 1575 μL of Millipore water were added to each sample. Litmus paper was used to confirm that the pH was below 7 after adding the acetic acid.

Oxylipins were extracted using solid phase extraction (SPE) Waters Oasis HLB columns (60 mg, 3cc cartridges; Waters, Milford, MA). The SPE columns were washed with 4 mL ethyl acetate and twice with 4 mL of methanol and pre-conditioned twice with 4 mL of SPE wash buffer containing 95/5/0.1 v/v/v Millipore water/methanol/acetic acid. The hydrolyzed oil samples were loaded onto the column and washed with 4 mL SPE buffer twice. The SPE filter was dried under high vacuum for 20 minutes. Oxylipins were then eluted from the filter with 0.5 mL methanol and 1.5 mL ethyl acetate into collection tubes containing 6 μL of 30% glycerol (in methanol). Samples were dried with vacuum centrifugation and reconstituted in 50 μL methanol containing 200 nM 1-cyclohexyl ureido, 3-dodecanoic acid (CUDA) as a surrogate recovery standard. The reconstituted samples were filtered using Ultrafree-MC VV Centrifugal Filter (0.1 μm;EMD Millipore, Bedford, MA, USA) tubes and transferred to LC-MS/MS vials.

A surrogate standard mix containing 10, 50 or 200 nM of d11-11(12)-EpETrE, d11-14,15-DiHETrE, d4-6-keto-PGF1α, d4-9-HODE, d4-LTB4, d4-PGE2, d4-TXB2, d6-20-HETE and d8-5-HETE was spiked with 200 nM CUDA and used to calculate accuracy and sample percent recovery. The accuracy was determined by dividing the observed concentrations of the surrogate standards using CUDA, by the expected concentrations and multiplying by 100 (n=3). The observed concentration of the surrogate standards was calculated as follows:

$$\text{Observed surrogate concentration} = (\text{Surrogate standard area}/\text{CUDA area}) \times (1/\text{slope}) * 200\,\text{nM CUDA}.$$

The percent recovery in our samples was calculated as follows:

$$\text{Percent recovery} = (\text{Surrogate area in oil sample}/\text{CUDA area in oil sample}) \times (1/\text{slope of surrogate standard curve}) \times 200\ \text{nM CUDA}/100\ \text{nM surrogate concentration} \times 100.$$

The slope of surrogate standard curve was derived from a plot of the standard surrogate concentration / CUDA concentration versus surrogate area / CUDA area,

Oxylipins were analyzed on an Agilent 1200 SL LC series UPLC system (Agilent Corporation, Palo Alto, CA, USA) connected to a 4000 QTRAP tandem mass spectrometer (Applied Biosystems Instrument Corporation, Foster City, CA, USA) equipped with an electrospray source (Turbo V). The system was operated in negative electrospray ionization mode and used optimized multiple reaction monitoring (MRM) conditions [27]. Oxylipins were separated on an Agilent Eclipse Plus C-18 reverse-phase column (2.1 × 150 mm, 1.8 μm particle size). The auto-sampler temperature was kept constant at 4°C and the column at 50°C. Mobile phase A contained Millipore water containing 0.1% glacial acetic acid, and mobile phase B contained acetonitrile/methanol (80/15 v/v) with 0.1% glacial acetic acid. The flow rate was 250 μL/min. Solvent B was held at 35% for 0.25 min, and then increased to 45% between 0.25 and 1 min, 55% from 1 to 3 min, 66% from 3 to 8.5 min, 72% from 8.5 to 12.5 min, 82% from 12.5 to 15 min and 95% from 15 to 16.5 min. It was maintained at 95% to 18 min, lowered to 35% from 18 to 18.1 min and held at 35% between 18.1 and 21 min. The retention time, MRM conditions, collision energy, limits of quantitation and surrogate standard used for each oxylipin are presented in Supplementary Table 3. The limits of detection were set at 3 times the signal to noise ratio, whereas the limits of quantitation were set at 10 times the signal to noise ratio.

Oil hydrolysis under air or nitrogen

To ensure that heat applied during the hydrolysis process described above does not cause oxylipin artefacts, we performed the hydrolysis reaction described above with soybean oil samples (10 uL per sample) capped under air (n=3) or flushed with nitrogen prior to capping (n=3) at 60°C for 30 minutes. The hydrolyzed oxylipins were subjected to SPE and measured on an Agilent 1290 Infinity ultrahigh-pressure liquid chromatography system interfaced to a 6460 triple-quadrupole mass spectrometer with electrospray ionization LC-MS/MS. The same oxylipin method was adapted from the 4000 QTRAP tandem mass spectrometer system (Applied Biosystems) to the Agilent 6460 tandem mass spectrometer. The data shown in Supplementary Table 4 confirm no differences between oxylipins hydrolyzed under air or nitrogen.

Fatty Acid Analysis with gas chromatography

Ten μL of oil sample was nonadecanoic acid (19:0) ethyl ester in chloroform/methanol (2:1 v/v; Experiment 1) or free heptadecanoic acid in methanol (Experiment 2) as internal standards. TFatty acids were analyzed according to the method of Ichihara et al. [35]. Four hundred μL of toluene, 3 mL methanol and 600 μL of 8% hydrochloric acid in methanol solution were added to each sample before placing vials on a dry heating block at 90°C for 60 minutes. The samples were allowed to cool at room temperature for approximately 10 minutes. One mL of hexane and 1 mL of water were added to each sample. The samples

were vortexed and the hexane and water layer were allowed to separate by leaving the sample undisturbed for 15 minutes. Six hundred μL of the upper hexane layer were transferred to a micro-centrifuge tube containing sodium sulfate as a drying agent. The hexane layer containing fatty acid methyl esters (FAMEs) was transferred into new micro-centrifuge tubes and stored at −80°C until analysis.

FAMEs were analyzed on a Varian 3800 gas-chromatography system equipped with a DB-23 fused silica capillary column (30 m × 0.25 mm inner diameter, 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA, USA). The injector and detector temperature were set at 250°C and 300°C, respectively. The initial oven temperature was held at 50°C for 2 minutes, and was increased by 10°C/min to 180°C, held at 180°C for 5 min, increased to 240°C at 5°C/min and held at 240°C for 5 min. The total run time was 37 min. The carrier gas was helium, which was maintained at a flow rate of 1.3 mL/min. A custom mix of 31 fatty acid methyl ester standards (NuChek Prep, Elysian, MN, USA) was used to identify the individual fatty acids. Retention times of the main fatty acids in oils are presented in Supplementary Table 5. Fatty acid concentrations were determined by comparison of the GC peak areas to the internal standard area. Data were expressed as percent of total identified fatty acid peaks or absolute concentrations.

Estimation of dietary LA and ALA-derived oxylipin intake levels

US oil intake data reported by Blasbalg et al. (2011)[3] was used to derive the mean amount of oxylipins consumed in the US diet. The amount of soybean, corn, canola and olive oil consumed in grams per person per day based on the Blasbalg et al study is 31.8, 2.2, 2.2 and 1.9, respectively (Supplementary Table 6). This amount was multiplied by the measured mean of oxylipin concentrations in each oil, to estimate LA and ALA oxylipin intake levels, after correcting for oil density.

Statistical Analysis

Statistical analysis was performed on GraphPad Prism v. 6.05 (La Jolla, CA, USA). For Experiment 1, the effect of heating on the rise in temperature of the 8 oils over time was determined using a two-way repeated measures analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The differences in fatty acid and oxylipin composition and in change of oxylipin concentration after heating between the different oils were assessed using a Kruskall-Wallis one-way ANOVA. No post-hoc tests were performed in Experiment 1 because the sample size was too small (n = 3 per oil) to provide accurate comparisons without risking a type I or II statistical error. Data from Experiment 1 were presented as median and range of the lowest and highest points.

Data for Experiment 2 were analyzed by Kruskall-Wallis test, followed by Dunn's multiple comparison test. The sample size (n= 4–5 per group) was sufficient to allow meaningful comparisons. Fatty acid and oxylipin data for Experiment 2 were presented as median and interquartile ranges (25^{th} and 75^{th} percentiles). Spearman's correlation analysis was used to correlate LA and ALA concentrations (μM) to LA- and ALA-derived oxylipins (nM).

Oxylipin intake data was expressed as mean without standard deviation, because it is a calculated value so true variability cannot be established. Hence, no statistical comparisons were done for calculated oxylipin intake data.

Statistical significance was set at $p<0.05$.

Results

Experiment 1

Standard recovery—Mean accuracy for d11-11(12)-EpETrE, d11-14,15-DiHETrE, d4-9-HODE and d8-5-HETE, the surrogates used to quantify LA- and ALA-derived oxylipins was 82%, 81%, 92% and 82%, respectively. The correlation coefficient reflecting linearity of the standard curve was above 0.99 for all measured oxylipins. Mean percent recovery of d11-11(12)-EpETrE, d11-14,15-DiHETrE, d4-9-HODE and d8-5-HETE, the surrogates used to quantify LA- and ALA-derived oxylipins was 39%, 70%, 55% and 48%, respectively. The low percent recovery of the surrogate standards in the oils is likely due to their partial degradation during base hydrolysis and matrix effects caused by ion suppression [36, 37]. However, the use of the surrogate standards added prior to base hydrolysis and SPE corrects for these losses [36]. Although a reduced percent recovery may decrease sensitivity, only peaks with a signal to noise ratio above 10 were included in the analysis to minimize the risk of quantitating noise. A representative LCMS/MS chromatogram of peaks detected in soybean oil before and after 10 minutes of heating is shown in Supplementary Figure 1.

Temperature—Two-way repeated measures ANOVA showed a significant effect of time on oil temperature ($F(10,160)=3204$; $P<0.0001$) during the 10-minute heating period. Oil temperature increased significantly within the first minute compared to baseline and reached a steady-state level of 100°C within 7 minutes (Supplementary Figure 2).

Baseline oil fatty acid composition—Oil fatty acid percent composition is shown in Figure 1. Concentration data are presented in Supplementary Figure 3. Differences in fatty acid percent composition amongst the oils were reflected in concentration data. Therefore, only statistical analysis of the percent composition data will be discussed in this section.

Kruskall-Wallis test showed significant differences amongst the groups for myristic acid (14:0; $p=0.014$), palmitic acid (16:0; $p=0.003$), stearic acid (18:0; $p=0.007$), oleic acid (18:1n-9; $p=0.003$), vaccenic acid (18:1n-7; $p=0.013$), LA ($p=0.002$) and ALA ($p=0.01$). Myristate, stearate and vaccinate were generally low in composition (<5%) or not detected in some oils. Palmitate was highest in olive (12%), corn (11.5%) and soybean oils (11%), followed by canola oil (4%), HSAO (neat and w/AO; ~3.5%) and “Thrive” (neat and w/AO; ~1.5%). Oleate was highest in the 4 algae oils (~91%), followed by olive (74%), canola (63.5%), soybean (21%) and corn (29%) oil.

LA composition was highest in corn and soybean oil (55%) followed by canola (19%), olive (7%), “Thrive” (neat and w/AOs; ~3.9%) and HSAO (neat and w/AO; ~1.5%). ALA was highest in canola oil (7.2%), followed by soybean oil (6.5%), corn oil (0.8%) and olive oil (0.4%) It was negligible or not detected in the 4 algae oils.

Baseline oxylipin concentrations—The LA and ALA oxylipin data are presented in Figures 2-A and 2-B, respectively. Overall, high LA or ALA oils had high concentrations of their respective LA or ALA metabolites, except for olive oil, which had comparable levels of LA and ALA monohydroxylated products compared to corn oil despite being low in LA (7%) and ALA (0.4%). The presence or absence of α-tocopherol in the algae oils did not appear to affect oxylipin concentrations.

Results from one-way ANOVA indicated that LA-derived oxylipin median values differed significantly among the groups for 9-HODE ($p = 0.006$), 13-HODE ($p = 0.006$), 9-oxo-ODE ($p = 0.003$), 13-oxo-ODE ($p = 0.007$), 9,10-DiHOME ($p = 0.006$) and 12,13-DiHOME ($p = 0.006$) (Figure 2-A). Monohydroxylated metabolites (9-HODE and 13-HODE) were 19 to 24 times higher in olive and corn oil than soybean and canola oil, which were both 3 to 4 times higher than the 4 algae oils. LA dihydroxylated metabolites (9,10-DiHOME and 12,13-DiHOME) were at least 11 times higher in corn and soybean oil as compared to other oils. No significant differences were observed for epoxy-metabolites of LA (9(10)-EpOME and 12(13)-EpOME).

Kruskall-Wallis test also showed statistically significant differences amongst the groups in ALA-derived monohydroxy (9-HOTrE, $p = 0.019$; 13-HOTrE, $p = 0.005$), epoxy (9(10)-EpODE, $p = 0.04$; 15(16)-EpODE, $p = 0.035$) and dihydroxy metabolites (9,10-DiHODE, $p = 0.008$; 12,13-DiHODE, $p = 0.004$; 15,16-DiHODE, $p = 0.004$) (Figure 2-B). Monohydroxylated ALA metabolites (9- and 13-HOTrE) were highest in olive oil, followed sequentially by corn, soybean and canola oil, compared to algae oils. Epoxidized ALA metabolites (9(10)-EpODE, 15(16)-EpODE), and the dihydroxylated ALA metabolite 15,16-DiHODE, were 5- to 22- fold higher in canola and soybean oil compared to the other oils. Dihydroxylated ALA-derived 9,10-DiHODE was 4-fold higher in soybean oil compared to corn oil, which was 4- to 19- fold higher compared to other oils. 12,13-DiHODE was detected in corn, canola and soybean oils, but was negligible or undetected in the remaining oils.

Effect of oil type on the change in oxylipin and fatty acid concentrations after short-term heating—Heat was applied at 100°C for 10 minutes to test whether oxylipins increased more in oils with higher levels of LA and ALA compared to less unsaturated oils such as algae. Kruskall-Wallis test found no significant differences amongst the oils in the change (from baseline) in LA or ALA oxidized metabolites (Supplementary Figure 4-A and 4-B). There were no significant differences amongst the oils in the change in fatty acid concentrations or percent composition from baseline (data not shown).

Experiment 2

Fatty acid composition of various off-the-shelf oils—The fatty acid percent composition data for olive, corn, canola, soybean and flaxseed oils obtained from 4 to 5 stores (one oil per store) are presented in Table 1. Fatty acid analysis confirmed the composition of the oils[13, 38]. Flaxseed oil contained the highest amount of ALA (56%), followed by canola (9%), soybean (7%), corn (1%) and olive (0.8%) oil. Corn and soybean

oil contained the highest amount of LA (55–57%) followed by canola (20%), flaxseed (15%) and olive (9%) oil. Significant differences among the various oils are shown in Table 1.

Oxylipin concentrations of various off-the-shelf oils—Oxylipin concentrations of five off-the-shelf oils are shown in Table 2. Kruskall-Wallis test followed by Dunn's multiple comparison test revealed that LA- and ALA- oxidized metabolite concentrations differed significantly amongst the different oils.

With regard to LA metabolites, concentrations of LA-derived monohydroxy (9- and 13-HODE) and ketone metabolites (9- and 13-oxo-ODE) were highest in flaxseed and olive oil relative to corn, soybean and canola oil. 9- and 13-HODE and 13-oxo-ODE were significantly higher by 15–316 fold in flaxseed oil as compared to canola ($p<0.001$) and soybean oil ($p<0.05$). These metabolites were also significantly higher by 37–97 fold in olive oil as compared to canola oil ($p<0.05$). 9-oxo-ODE was significantly higher in olive oil as compared to both canola ($p<0.001$) and soybean oils ($p<0.05$) by 15 and 12 fold, respectively.

Flaxseed and corn oil had the highest concentration of LA-derived epoxides (9(10)- and 12(13)- EpOME). Both of these oils had an 11-fold significantly higher concentration of 12(13)-EpOME relative to olive oil ($p<0.01$). 9(10)-EpOME concentration was significantly higher than canola ($p<0.01$) and olive oil ($p<0.001$) by 7 and 69 fold, respectively.

Dihydroxy products of LA-derived epoxides (9,10-DiHOME and 12,13-DiHOME) were significantly higher in soybean ($p<0.001$) and corn oil ($p<0.01$) by 159–330 and 200–317 fold, respectively, as compared to olive oil.

With regard to ALA metabolites, flaxseed oil had the highest concentration of monohydroxylated ALA-derived metabolites, followed by olive oil (Table 2). 9- and 13-HOTrE were significantly higher in flaxseed oil than soybean, corn and canola oil by 121–1208 fold. 9-HOTrE was significantly higher in olive oil than canola oil by 510-fold, whereas 13- HOTrE was significantly higher than corn oil by 22-fold.

Epoxy-ALA metabolites (15(16)-, 12(13)- and 9(10)-EpODE) were highest in flaxseed oil, followed by canola oil. All three epoxidized ALA metabolites were 116–1109 and 6–10 times higher in flaxseed and canola oil ($p<0.05$), respectively, than olive oil, which had the lowest concentration of epoxidized ALA metabolites amongst the five oils. Soybean, corn and canola oil had similar concentrations of epoxidized ALA metabolites; however, 12(13)-EpODE was 98% lower in corn oil relative to flaxseed oil ($p<0.05$) and 9(10)-EpODE was 83% lower in soybean oil than flaxseed oil ($p<0.05$).

Dihydroxy ALA metabolites (15,16-, 12,13-, and 9,10-diHODEs) were highest in soybean oil and lowest in olive oil. The differences were statistically significant between soybean and olive oil for all three diHODEs, which were lower in olive oil by 98–99.9% ($p<0.001$). Soybean oil also had a higher concentration of 12,13-DiHODE (+85%; $p<0.05$) and 9,10-DiHODE (+98%; $p<0.05$) compared to canola oil.

Reproducibility between experiments—Supplementary Table 7 summarizes mean oil oxylipin concentrations from Experiments 1 and 2, and the experiment comparing air to nitrogen capping during hydrolysis ("Experiment 3"). As shown, oxylipin concentrations were similar between Experiments. Epoxides in canola oil, however, differed by 4–7 folds between Experiments 1 and 2, suggesting they are unstable in this particular oil. The oils used came from different outlets (San Francisco or Davis) and likely had different expiration dates. Fankhauser-Noti et al. reported similar fluctuations in epoxides between fresh and old olive and sunflower oil[26].

Estimated daily oxidized fatty acid intake—Mean daily intake of oxidized LA and ALA metabolites from soybean, corn, canola and olive oil was estimated, based on published mean consumption (kg/p/year) data on these oils [3]. Consumption data for flaxseed oil and the four algae oils were not available.

As shown in Figures 3-A and 3-B, of the four oils, soybean oil contributed the most of LA and ALA oxylipins in the diet, because it is the most consumed plant oil in the US (Supplementary Table 6) [3]. The majority of oxylipins in the diet were in the form of epoxy and dihydroxy metabolites of LA and ALA.

Estimated daily intake of total oxylipins from olive, corn, canola and soybean oils averaged 1.1 mg/day (Figure 3-C). Of the measured oils, soybean oil contributed most oxylipins in the diet, followed by corn, olive and canola oil (Figure 3-C).

Correlations—Oil LA and ALA concentrations positively correlated with concentrations of their respective metabolites as shown in Table 4. LA correlated positively with LA-derived 9(10)- and 12(13)-EpOME and 9,10- and 12,13-DiHOME ($P<0.05$). It also correlated with ALA-derived 9-HOTrE and DiHODEs ($P<0.05$).

ALA did not correlate with LA-derived oxylipins, but positively correlated with ALA-derived hydroxy, epoxy and dihydroxy metabolites ($P<0.05$).

Discussion

The present study reported the presence of LA- and ALA- derived oxylipin species in plant and algae oils. Concentrations of LA- and ALA- derived oxylipins were generally proportional to the concentrations of their precursor fatty acids except for flaxseed and olive oil, which had higher oxylipin concentrations than other oils. Estimated oxylipin intake levels based on available consumption data on soybean, corn, canola and olive oils averaged 1.1 mg per person per day. Oxylipin concentrations were comparable between experiments and to one study which reported epoxy-LA metabolites in olive oil[26], thus confirming the validity and reproducibility of our measurements.

The oxylipins detected in non-heated oils were likely formed by non-enzymatic or enzymatic pathways during the seed extraction process. Non-enzymatic auto-oxidation is known to be influenced by storage or processing conditions [39]. Enzymatic oxidation is mediated in part by lipoxygenase, which is activated when barrier integrity of the seed, fruit or algae is compromised by homogenization during the oil extraction process [25, 29, 40]. Other

plant or algae enzymes involved in oxylipin formation include soluble epoxide hydrolase, cytochrome P450 or pathogen-inducible oxygenases [41–44], although their activation during the oil extraction process is not known.

There were no significant differences amongst the oils in the change in oxylipin concentrations following 10 minutes of heat relative to baseline (Experiment 1, Supplementary Figure 4). Previous studies reported the formation of LA-derived oxylipins after heating high LA oils at 40, 100 or 180°C for 10–264 hours [13–15]. We predicted, however, that using UPLC-MS/MS, we might detect changes in oxylipins in high LA or ALA oils within 10 minutes of heating at 100°C. The lack of difference between the oils could be due to the short heating duration as previous work has demonstrated that prolonged heating is required to oxidize oils [13, 15]. It is also possible that non-enzymatic oxylipin products of heat-induced oxidation (HODEs, oxoODEs) increased in the high LA or ALA oils during heating, but were rapidly degraded into secondary volatile compounds within the 10 minute heating period [45, 46].

The LA and ALA content of the oils from Experiment 2 were related to the concentration of their respective oxylipin metabolites (Table 4). LA also correlated highly with ALA-derived oxylipins. This association was likely driven by olive and flaxseed oil which unexpectedly contained a high amount of LA-derived oxylipins potentially caused by processing, storage or handling conditions that need to be further investigated. Overall, however, the findings suggest that precursor fatty acid pool in oils is an important determinant of oxylipin concentrations, consistent with the observation that the low LA and ALA algae oils had the least concentration of LA- and ALA- derived oxylipins compared to other plant oils, irrespective of vitamin E content (Experiment 1, Figure 2). The presence of vitamin E may not be critical for low polyunsaturated fatty acid oils when heated for a short period of time (10 minutes) at 100°C.

In both experiments, olive oil had higher concentrations of LA-derived ketones (9- and 13-oxo-ODE), and monohydroxylated LA (9- and 13-HODE) and ALA (9- and 13-HOTrE) metabolites, than soybean, canola and corn oil, despite being low in LA (9%) and ALA (0.8%). This could be due to enhanced lipoxygenase activity upon homogenizing the olive fruit to extract the oil compared to seeds (soybean, canola or corn)[40]. However, Jarén-Galán et al. reported higher lipoxygenase activity from soybean compared to olives [47]. It is possible that other oxygenase enzymes that differ in activity between soybeans and olives account for the unexpected high oxylipin concentrations in olive oil. Other factors such as processing or storage conditions may also explain the high mono-hydroxylated oxylipin metabolites detected in olive oil relative to other higher-LA or ALA oils.

LA-derived monohydroxy and ketone metabolites, and ALA-derived monohydroxy and epoxy metabolites were highest in flaxseed oil as compared to other oils (Experiment 2). The high concentration of ALA-derived oxylipins is expected because flaxseed oil contained the highest concentration of ALA (54%) compared to the other oils tested in this study (2–6%). Flaxseed oil has less LA relative to soybean oil (14% versus 50%), yet LA-metabolites were 3-fold higher in flaxseed oil compared to soybean oil. It is not known whether these metabolites were formed during processing, storage or flaxseed crushing. It is possible that

the high concentrations of ALA epoxides may have catalyzed the oxidation of LA via electrophilic attack of the allylic carbon next to the double bonds, although this remains to be determined.

The consumption of LA has increased from 2% to 7% of energy over the past century, due to increased consumption of high LA plant oils, such as soybean oil [3]. Soybean oil is the most commonly consumed plant oil in the US and the fourth major contributor of total calories [3]. It is likely that the consumption of oxidized LA metabolites concomitantly increased over the past few decades with increased soybean oil intake. Approximating the levels of dietary oxylipins will allow future studies evaluating the bioavailability and effect of oxylipins on health and disease to utilize relevant doses.

Oxylipins are bioavailable, and circulating LA-derived oxylipins in particular have been associated with atherosclerosis, pain syndromes and hypertension, consistent with their role in mediating pro-inflammatory signaling in tissues or vasculature [4, 48, 49]. A recent meta-analysis reported that fried food consumption was associated with hypertension and weight gain [50]. In vivo, the concentration of circulating oxylipins depends on the availability of their precursor fatty acid [10, 11, 51]. However, studies have demonstrated that dietary oxylipins are absorbed [17–20] and incorporated into blood chylomicrons [52]. The relative plasma contribution of dietary oxylipins compared to endogenously produced oxylipins is not known and merits future evaluation.

Estimated US daily intake of LA and ALA oxylipins was 0.64 and 0.44 mg, respectively, and amounted to a total of 1.1 mg per person per day. These values are likely underestimated, however, because 1) oxylipins in this study were quantified in off-the-shelf oils maintained at room temperature, 2) oxylipin content of commonly consumed foods such as peanut butter and French Fries was not measured [53, 54], and 3) a targeted UPLC-MS/MS approach was used to quantify oxylipins, which means that other oxylipin species such as hydroperoxides of LA or ALA or oleic acid-derived compounds were not accounted for in our estimates. Accounting for the amount and type of oxylipins produced during food processing, cooking or prolonged storage, in relation to water, metal and antioxidant content will provide a better estimate of daily oxylipin consumption levels [39, 55, 56]. The use of non-targeted mass-spectrometry methods may identify other oxylipin species in commonly consumed oils that can be quantified with targeted UPLC-MS/MS as performed in the present study [57].

Limitations of this study include the low sample size and lack of information on the processing methods used to produce the oils and storage conditions and duration since date of production. The risk of statistical errors associated with the low sample size or number of replicates in Experiment 1 was mitigated by reproducing the measurements of some oils (corn, soybean, olive and canola) in Experiment 2. Information on processing and storage would require coordination with each of the oil manufacturers in future studies.

In summary, this study quantified LA and ALA oxylipins in various oils and found them to be related to LA and ALA fatty acid composition with a notable exception being olive and flaxseed oils. The amount of oxylipins consumed through commonly consumed plant oils in

the US was estimated to be 1.1 mg per person per day, but this value is underestimated because it does not account for oxylipins in commonly consumed oils not measured in this study, or processing, frying or storage effects. Knowing the amount and type of oxylipins chronically consumed through dietary oils is important for understanding their health implications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ALA	alpha-linolenic acid
AO	antioxidants
CUDA	1-cyclohexyl-dodecanoic acid urea
DiHODE	dihydroxyoctadecadienoic acid
DiHOME	dihydroxyoctadecamonoenoic acid
EDTA	triphenylphosphine, ethylenediaminetetraacetic acid
EpODE	epoxyoctadecadienoic acid
EpOME	epoxyoctadecamonoenoic acid
FID	flame ionization detector
GC	gas-chromatography
GC-FID	gas chromatography coupled to a flame ionization detection
HODE	hydroxyoctadecadienoic acid
HOTrE	hydroxyoctadecatrienoic acid
HSAO	High Stability Algae Oil
LA	Linoleic acid
LC-MS/MS	liquid chromatography tandem mass spectroscopy
MRM	multiple reaction monitoring
NMR	nuclear magnetic resonance
oxo-ODE	oxo-octadecadienoic acid

PUFAs	polyunsaturated fatty acids
SPE	solid-phase extraction
UV	ultraviolet
w/AO	without added antioxidants

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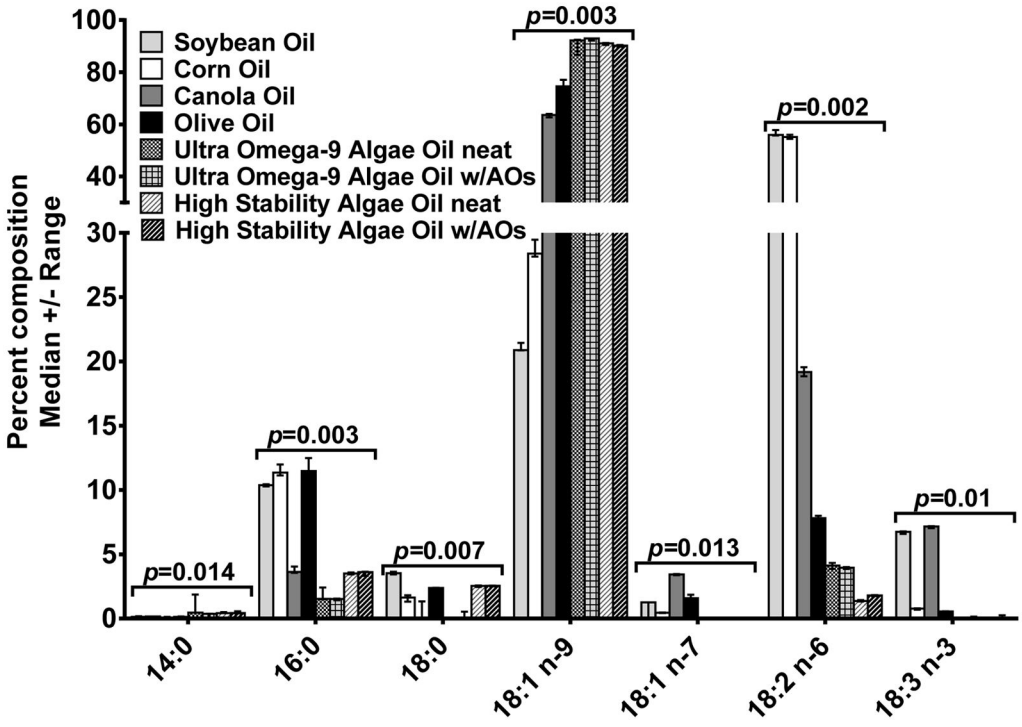

Figure 1. Oil fatty acid percent composition

Fatty acid composition of the different oils at room temperature. Data are presented as median ± range. Significant differences between oils were assessed using a nonparametric Kruskall-Wallis test. Significant P values (<0.05) are reported on the figure. Only the main fatty acids present in the oils are represented in the figure, namely, myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1 n-9), vaccenic acid (18:1 n-7), linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3).

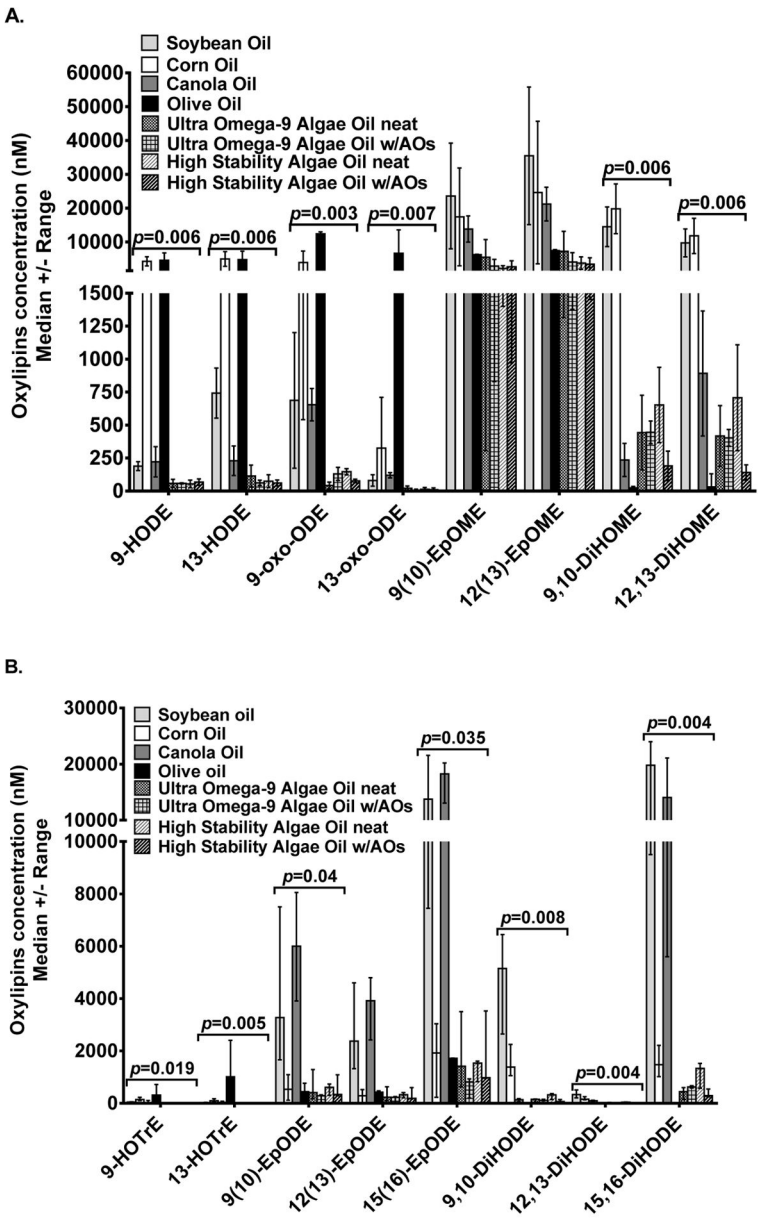

Figure 2. Oil LA (A) or ALA (B) derived oxylipin concentrations at room temperature

Oxidized linoleic acid (A.) and α-linolenic acid (B.) metabolite concentrations (nM) in the different oils at room temperature. Data are presented as median ± range. Significant differences between oils were assessed using a nonparametric Kruskall-Wallis test. Significant P values (<0.05) are reported on the figure. HODE, hydroxyoctadecadienoic acid; oxo-ODE, oxo-octadecadienoic acid; EpOME, epoxyoctadecamonoenoic acid; DiHOME, dihydroxyoctadecamonoenoic acid; HOTrE, hydroxyoctadecatrienoic acid; EpODE, epoxyoctadecadienoic acid; DiHODE, dihydroxyoctadecadienoic acid.

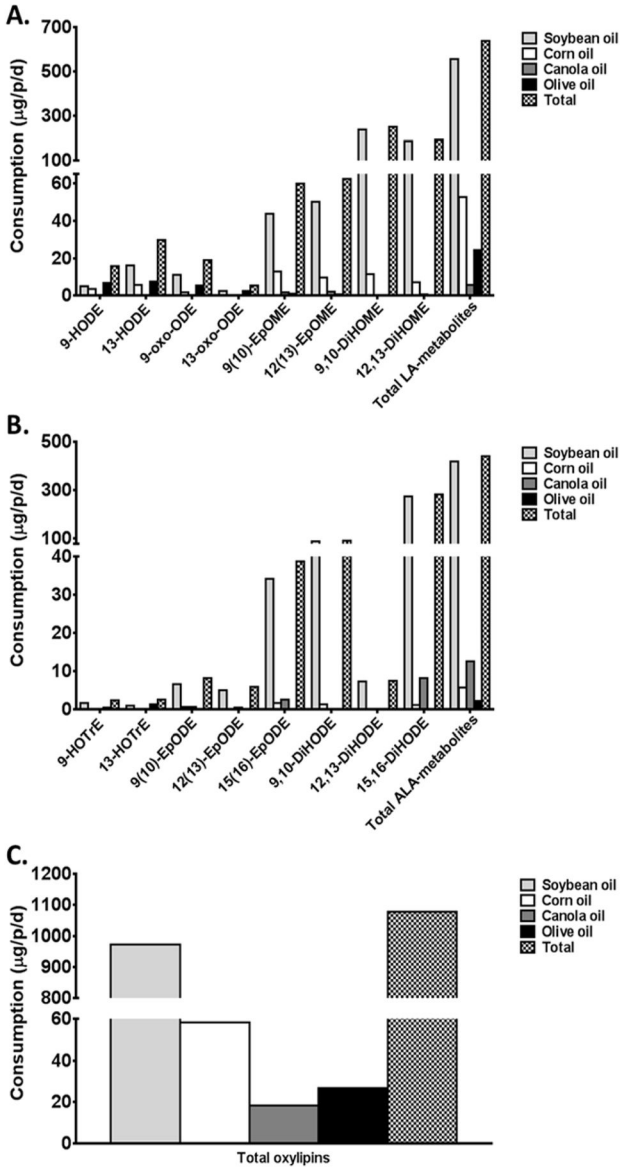

Figure 3. Estimated US consumption of LA (A) and ALA (B) derived oxylipins

Mean intake of the linoleic acid (LA)-derived (A.), α-linolenic acid (ALA)-derived (B.) and total oxylipins (C.) through the consumption of the four commonly consumed oils in US. Values are expressed as µg of oxylipins per person per day (µg/p/d). HODE, hydroxyoctadecadienoic acid; oxo-ODE, oxo-octadecadienoic acid; EpOME, epoxyoctadecamonoenoic acid; DiHOME, dihydroxyoctadecamonoenoic acid; LA, linoleic acid; HOTrE, hydroxyoctadecatrienoic acid; EpODE, epoxyoctadecadienoic acid; DiHODE, dihydroxyoctadecadienoic acid; ALA, alpha-linolenic acid.

Table 1

Fatty acid composition of the different oils (soybean, corn, canola, olive and flaxseed)

	Soybean Oil	Corn Oil	Canola Oil	Olive Oil	Flaxseed Oil
16:0	10.2 (10.0 – 10.5)^{ab}	11.9 (11.8 – 11.9)^{ac}	4.0 (4.0 – 4.2)^{b}	13.7 (12.5 – 14.3)^{a}	5.3 (5.2 – 5.5)^{bc}
18:0	3.8 (3.7 – 4.0)^{a}	1.5 (1.5 – 1.6)^{b}	1.6 (0.0 – 1.7)^{ab}	2.6 (2.6 – 3.1)^{ab}	3.7 (3.4 – 4.1)^{ab}
18:1 n-9	22.5 (22.4 – 22.8)^{ac}	28.5 (28.1 – 28.7)^{bc}	61.5 (60.9 – 63.1)^{ab}	71.3 (67.4 – 73.2)^{b}	20.2 (18.9 – 21.4)^{c}
18:1 n-7	1.2 (1.1 – 1.2)^{ab}	0.0 (0.0 – 0.0)^{a}	1.6 (1.5 – 2.2)^{b}	1.6 (1.3 – 1.8)^{b}	0.0 (0.0 – 0.0)^{a}
18:2 n-6	55.0 (54.7 – 55.0)^{ac}	56.9 (56.8 – 57.3)^{a}	19.7 (19.6 – 19.8)^{ab}	8.6 (7.2 – 11.2)^{b}	15.0 (14.5 – 15.5)^{bc}
18:3 n-3	7.1 (7.0 – 7.1)^{ab}	1.0 (1.0 – 1.1)^{ac}	8.9 (8.7 – 8.9)^{bc}	0.8 (0.8 – 0.9)^{a}	55.7 (54.2 – 57.0)^{b}
18:2 n-6/18:3 n-3	7.8 (7.6 – 7.9)^{abc}	54.3 (52.3 – 61.6)^{a}	2.2 (2.2 – 2.3)^{bc}	12.0 (8.6 – 12.5)^{ac}	0.3 (0.3 – 0.3)^{b}

Data (% of total detected fatty acids) are expressed as median and interquartile range (25th and 75th percentiles). Data were analyzed by Kruskall-Wallis test followed by Dunn's multiple comparison post-hoc test. For each row, different alphabetical superscripts mean that the oils differed significantly ($P<0.05$) from each other. Only main fatty acids present in the oils are presented, namely, palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1 n-9), vaccenic acid (18:1 n-7), linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3).

Table 2

Concentrations of linoleic acid (LA) and α-linolenic acid (ALA)-derived metabolites in nM in the different oils (olive, corn, canola, soybean and flaxseed).

	Soybean Oil	Corn Oil	Canola Oil	Olive Oil	Flaxseed Oil
13-HODE	1570 (1025–2095)^{ab}	8400 (6085–10205)^{bc}	147 (101–232)^{b}	14300 (6235–17600)^{ac}	24100 (16900–45925)^{c}
9-HODE	333 (260–795.5)^{ab}	5450 (4270–6065)^{bc}	215 (165.5–303)^{b}	14000 (4915–16000)^{ac}	68050 (40900–78100)^{c}
13-oxo-ODE	277 (147.5–352.5)^{ab}	446 (330.5–545)^{bc}	120 (64.4–181)^{b}	4420 (2980–5240)^{ac}	25800 (20275–50000)^{c}
9-oxo-ODE	892 (722–1590)^{a}	2320 (1470–3485)^{ab}	679 (444.5–963)^{a}	10300 (4850–12250)^{b}	2880 (1640–3805)^{ab}
12(13)-EpOME	5100 (3510–6140)^{ab}	13000 (11050–16600)^{a}	2880 (2445–3210)^{ab}	1210 (609–1445)^{b}	12970 (7905–18575)^{a}
9(10)-EpOME	4430 (3135–5345)^{ab}	17300 (16250–21200)^{a}	2550 (2025–2975)^{b}	250 (939–2685)^{b}	9200 (6368–11950)^{ab}
12,13-DiHOME	18800 (10870–22700)^{a}	9030 (5230–13565)^{a}	982 (906–1065)^{ab}	56.9 (40.85–78.1)^{b}	352.5 (159–868.5)^{ab}
9,10-DiHOME	23900 (14200–28800)^{a}	15400 (9130–21050)^{a}	357 (314.5–414)^{ab}	75.5 (59.8–99.5)^{b}	444 (158–934)^{ab}
Total LA-metabolites	**56590 (37475–63568)**^{ab}	**72802 (60932–84871)**^{a}	**7922 (6850–8963)**^{b}	**46244 (20954–55257)**^{ab}	**143157 (111198–193904)**^{a}
9-HOTrE	99 (59–140)^{ab}	231 (208–283)^{bc}	42 (26–63)^{b}	2090 (1915–2955)^{ac}	50750 (28275–77800)^{c}
13-HOTrE	160 (113–243)^{ab}	42 (28–53)^{a}	95 (77–114)^{ac}	908 (604–1260)^{bc}	19300 (11890–34975)^{b}
15(16)-EpODE	3590 (2525–4095)^{ab}	2430 (2155–3055)^{ab}	3840 (3210–4075)^{a}	224 (194–235)^{b}	248500 (141500–384750)^{a}
12(13)-EpODE	574 (353–628)^{ab}	213 (206–260)^{ac}	851 (710.5–1024)^{bc}	85 (64–116)^{a}	15050 (9165–21325)^{b}
9(10)-EpODE	742 (457–791)^{ab}	1010 (809–1370)^{ac}	1030 (899–1375)^{bc}	178 (122–231)^{a}	20650 (13950–27500)^{c}
15,16-DiHODE	28000 (16250–32900)^{a}	1530 (935–2630)^{b}	11200 (9710–12600)^{ab}	15 (11–27)^{b}	7990 (2600–14700)^{ab}
12,13-DiHODE	720 (415–914)^{a}	187 (126–277)^{ab}	103 (85–114)^{bc}	14 (10–17)^{b}	381 (225–519)^{ac}
9,10-DiHODE	9160 (5000–11150)^{a}	1720 (939–2775)^{ab}	146 (132–173)^{bc}	7 (6–13)^{c}	306 (180–507)^{ac}
Total ALA-metabolites	**43127 (26067–49923)**^{ab}	**7073 (6362–9890)**^{ac}	**17192 (16166–18277)**^{bc}	**3570 (2942–4810)**^{c}	**355205(279990–497593)**^{b}

Data are expressed as median and interquartile range (25th and 75th percentiles). HODE, hydroxyoctadecadienoic acid; oxo-ODE, oxo-octadecadienoic acid; EpOME, epoxyoctadecamonoenoic acid; DiHOME, dihydroxyoctadecamonoenoic acid; OxLAM, oxidized linoleic acid metabolites; HOTrE, hydroxyoctadecatrienoic acid; EpODE, epoxyoctadecadienoic acid; DiHODE, dihydroxyoctadecadienoic acid; ALA, α-linolenic acid. Data were analyzed by Kruskall-Wallis test followed by Dunn's multiple comparison tests. For each row, different alphabetical superscripts mean that the oils differed significantly ($P<0.05$) from each other.

Table 4

Spearman's correlation between fatty acid and oxylipin concentrations from Experiment 2

	Correlation Between Fatty Acid and Oxylipin Concentration			
	LA concentration		ALA concentration	
	r-value	p-value	r-value	p-value
LA-oxylipins				
9-HODE	−0.3191	0.1285	−0.00956	0.9646
13-HODE	−0.2643	0.2119	−0.01565	0.9421
9-oxo-ODE	−0.3148	0.1341	−0.3696	0.0755
13-oxo-ODE	−0.3765	0.0698	0.04174	0.8465
9(10)-EpOME	0.4991	**0.0130**	0.06174	0.7744
12(13)-EpOME	0.4652	**0.0220**	0.2565	0.2263
9,10-DiHOME	0.8652	**<0.0001**	0.1104	0.6075
12,13-DiHOME	0.86	**<0.0001**	0.06174	0.7744
ALA-oxylipins				
9-HOTrE	−0.4904	**0.0150**	0.4304	**0.0358**
13-HOTrE	−0.3852	0.0630	−0.0087	0.9678
9(10)-EpODE	0.01566	0.9421	0.5214	**0.0090**
12(13)-EpODE	0.04001	0.8527	0.855	**<0.0001**
15(16)-EpODE	0.1339	0.5327	0.7896	**<0.0001**
9,10-DiHODE	0.8339	**<0.0001**	0.2009	0.3466
12,13-DiHODE	0.6551	**0.0005**	0.492	**0.0146**
15,16-DiHODE	0.5466	**0.0057**	0.6458	**0.0007**

Table depicts correlations between total LA and ALA concentration and total LA- and ALA-oxylipin concentrations in all oils. Data was analyzed using Spearman Correlation Analysis. Significant p-values were determined to be <0.05. HODE, hydroxyoctadecadienoic acid; oxo-ODE, oxo-octadecadienoic acid; EpOME, epoxyoctadecamonoenoic acid; DiHOME, dihydroxyoctadecamonoenoic acid; OxLAM, oxidized linoleic acid metabolites; HOTrE, hydroxyoctadecatrienoic acid; EpODE, epoxyoctadecadienoic acid; DiHODE, dihydroxyoctadecadienoic acid; ALA, α-linolenic acid.