Analysis of Fatty Acid Esters of Hydroxyl Fatty Acid in Nut Oils and Other Plant Oils

Hiroko Takumi, Kazuko Kato, Takayo Ohto-N., Hiroki Nakanishi, Hiroshi Kamasaka, and Takashi Kuriki

Abstract: Oils and lipids are common food components and efficient sources of energy. Both the quantity and the quality of oils and lipids are important with regard to health and disease. Fatty acid ester of hydroxy fatty acid (FAHFA) is a novel lipid class that was discovered as an endogenous lipid; FAHFAs have shown anti-diabetic effects in a mammalian system. We analyzed the overall FAHFA composition in nut oils and other common oils: almond (raw, roasted), walnut, peanut, olive, palm, soybean, and rapeseed oils. We developed a method of liquid chromatography coupled with electrospray ionization triple quadrupole mass spectrometry (LC-ESI/MS/MS) for a comprehensive target analysis of FAHFAs. The analysis revealed wide variation in the FAHFA profiles (15 compounds and 62 peaks). For 7–11 compounds of FAHFA, a total level of 8–29 pmol/mg oil was detected in nuts oils; for 11 compounds, 4.9 pmol/mg oil was detected in olive oil, and for 4–9 compounds, < 2 pmol/mg oil was detected in palm, soy, and rapeseed oils. The major FAHFAs were FAHFA 36:3, FAHFA 36:2, and FAHFA 36:4 in nut oil, FAHFA 36:2, FAHFA 34:1, and FAHFA 36:1 in olive oil, and FAHFA 32:1, FAHFA 34:0, FAHFA 36:0, and FAHFA 36:1 in all of the common oils. The composition of FAHFAs in nut oils is mainly unsaturated fatty acids, whereas those in olive oil are unsaturated fatty acids and saturated fatty acids. The composition of FAHFAs in common oils was mainly saturated fats. This is the first report to demonstrate the quality and quantity of the FAHFAs in the nut oils. Nuts have been described to be a great source of many nutrients and to be beneficial for our health. Our present findings comprise additional evidence that the intake of nuts in daily diets may prevent metabolic and inflammatory-based diseases.

Key words: fatty acid ester of hydroxy fatty acid, FAHFA, nut, almond oil, walnut oil, peanut oil, olive oil, palm oil, soybean oil, rapeseed oil

Abbreviations: FAHFA; fatty acid ester of hydroxy fatty acid, FA (18:3) HOA; fatty acid (18:3) ester of hydroxy oleic acid, FA (18:3) HAS; fatty acid (18:3) ester of hydroxy stearic acid, FA (20:0) HLA; fatty acid (20:0) ester of hydroxy linoleic acid, FA (20:1) HOA; fatty acid (20:1) ester of hydroxy oleic acid, LAHFA (20:0); linoleic acid ester ester of hydroxy fatty acid (20:0); LAHFA (22:0); linoleic acid ester of hydroxy fatty acid (22:0), LAHOA; linoleic acid ester of hydroxy oleic acid, LAHSA; linoleic acid ester of hydroxy stearic acid, LAHLA; linoleic acid ester of hydroxy linoleic acid, OAHFA (20:0); oleic acid ester of hydroxy fatty acid (20:0), OAHFA (20:1); oleic acid ester of hydroxy linoleic acid, OAHFA (20:0); oleic acid ester of hydroxy stearic acid (20:0), OAHFA (22:0); oleic acid ester of hydroxy fatty acid (22:0), OAHFA (22:0); oleic acid ester of hydroxy oleic acid, OAHFA (22:0); oleic acid ester of hydroxy linoleic acid, OAHFA (22:0); oleic acid ester of hydroxy stea

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1 Introduction

Type 2 diabetes (T2D) is a global epidemic characterized by hyperglycemia due to impaired islet function and insulin resistance in peripheral tissues. Despite advances in the understanding of the molecular mechanisms contributing to T2D and the development of new treatment modalities, the medical management of T2D remains inadequate. Many of the currently available treatment strategies (including analogs of the incretin, glucagon-like peptide-1 (GLP-1), and dipeptidylpeptidase-4 inhibitors) work primarily by increasing the body’s secretion of insulin. However, effective and safe agents that enhance insulin sensitivity are still needed, toward the goals of improving glucose control and preventing diabetic complications.

Fatty acid ester of hydroxyl fatty acid (FAHFA) is a novel lipid class that was discovered as an endogenous lipid with anti-diabetic effects in a mouse model. FAHFAs have been shown to have a positive effect on glycemia. Their anti-diabetic effects in a mouse model. FAHFAs have been shown to have a positive effect on glycemia. Their anti-diabetic effects in a mouse model. FAHFAs have been shown to have a positive effect on glycemia. They decrease the production of free fatty acids, thus delaying the onset of diabetes. FAHFA levels are significantly decreased in individuals with insulin resistance and diabetes. An administration of exogenous FAHFA normalized glycemia and insulin sensitivity. FAHFAs also exert an anti-inflammatory effect, i.e., a reduction of macrophage activation. There are only a few reports of the profiles of FAHFAs in food.

We have investigated the beneficial effects of the intake of nuts in the diet for preventing T2D, and FAHFAs in nut oils have thus drawn our interest. A frequent consumption of nuts in the diet for preventing T2D is associated with lower total cancer, and all-cause and cause-specific morris reduction of macrophage activation. We purchased the following 21 FAHFA standards from Cayman Chemical Co. (Ann Arbor, MI, USA): 5-PAHSA, 5-POHSA, 5-SAHSA, 5-OAHSA, 9-PAHFA, 9-POHSA, 9-SAHSA, 9-OAHSA, 10-PAHSA, 10-POHSA, 10-SAHSA, 10-OAHSA, 12-PAHSA, 12-POHSA, 12-SAHSA, 12-OAHSA, 13-PAHSA, 13-POHSA, 13-SAHSA and 13-OAHSA, along with the isotopic internal standard (IS) 12-OAHSA-d17. Acetonitrile, methanol, hexane, and acetic acid of ultra-high-performance liquid chromatography (UPLC)/MS quality were obtained from Sigma-Aldrich (St. Louis, MO). Methyl formate was obtained from Kanto Chemical Co. (Tokyo).

2 Experimental Procedures

2.1 Chemicals and materials

Nonpareil raw California almonds were squeezed to obtain almond oil; roasted almond oil was squeezed after raw almonds were roasted at 145°C for 12 min and then at 165°C for 3 min. We purchased virgin walnut oil, peanut oil, virgin olive oil, palm oil, soybean oil, and rapeseed oil at a market. Immediately after their squeezing or purchasing, the oils were stored at −20°C until the sample preparation.

We purchased the following 21 FAHFA standards from Cayman Chemical Co. (Ann Arbor, MI, USA): 5-PAHSA, 5-POHSA, 5-SAHSA, 5-OAHSA, 9-PAHFA, 9-POHSA, 9-SAHSA, 9-OAHSA, 10-PAHSA, 10-POHSA, 10-SAHSA, 10-OAHSA, 12-PAHSA, 12-POHSA, 12-SAHSA, 12-OAHSA, 13-PAHSA, 13-POHSA, 13-SAHSA and 13-OAHSA, along with the isotopic internal standard (IS) 12-OAHSA-d17. Acetonitrile, methanol, hexane, and acetic acid of ultra-high-performance liquid chromatography (UPLC)/MS quality were obtained from Sigma-Aldrich (St. Louis, MO). Methyl formate was obtained from Kanto Chemical Co. (Tokyo).

2.2 Solid-phase extraction of FAHFAs from nut oils and other plant oils

The nut oils and the other plant oils (10 mg each) were added with 20 pmol 12-OAHSA-d17 (as an internal standard) and 1 mL of methanol, followed by sonication in water. The samples were diluted with 5 mM HCl in water. Oasis HLB 60-ng Vac-RC columns (Waters, Milford, MA) were preconditioned with methanol and 5 mM HCl in water via a vacuum manifold (Waters). The diluted extract was applied on the column and washed with 3 mL of 5 mM HCl in water followed by 3 mL of hexane. Finally, the FAHFAs were eluted from the column using 3 mL of methyl formate, and the fraction was dried under a constant stream of N₂ gas, reconstituted in 40 μL of methanol, and transferred to a sample vial. The extraction efficiency was approx. 50% when evaluated by the recovery rate of the internal standard (12-OAHSA-d17) from the oil samples.
2.3 LC-MS/MS analysis

A 10-µL aliquot of the lipid sample was injected, and the lipids were separated on a Cortecs UPLC C18 1.6 µm 150 mm × 2.1 mm i.d. column (Waters) at 30°C using a gradient solvent system. Solvent A was acetonitrile/methanol/water [18:18:4, v/v/v] supplemented with 5 mM ammonium formate and 0.05% ammonium hydroxide. Solvent B was 2-propanol supplemented with 5 mM ammonium formate and 0.05% ammonium hydroxide. The applied elution gradient is shown in Table 1.

The FAHFAs were measured by multiple reaction monitoring (MRM) in negative ion mode with a liquid chromatograph mass spectrometer (LCMS-8040, Shimadzu, Kyoto, Japan). The data analysis was performed using LabSolutions software (Shimadzu). A deuterated internal standard (12-OAHSA-d17) was used for monitoring the recoveries and for the quantitation of all of the FAHFAs. The quantification of the 21 FAHFAs with commercial standards was performed with the calibration curve for each FAHFA. The one-point quantification method used in this study for the

Table 1  Applied elution gradient of the LC-MS/MS analysis.

| Time (min) | Solvent A (%) | Solvent B (%) | Flow rate (mL/min) |
|-----------|---------------|---------------|--------------------|
| 0         | 90            | 10            | 0.07               |
| 5         | 55            | 45            | 0.07               |
| 35        | 45            | 55            | 0.07               |
| 35.1      | 45            | 55            | 0.05               |
| 40        | 10            | 90            | 0.05               |
| 50        | 10            | 90            | 0.05               |
| 50.1      | 90            | 10            | 0.05               |
| 60        | 90            | 10            | 0.05               |

Solvent A: acetonitrile/methanol/water [18:18:4, v/v/v] supplemented with 5 mM ammonium formate and 0.05% ammonium hydroxide. Solvent B: 2-propanol supplemented with 5 mM ammonium formate and 0.05% ammonium hydroxide.
FAHFA molecules that are not commercially available corresponds to the quantification method equivalent to "absolute quantification Level 2" defined by the Lipidomics Standard initiative.23

3 Results

3.1 Optimization of the LC-MS/MS analysis of FAHFAs

For the optimization of the LC-MS/MS analysis, we used the isotopic internal standard (IS) 12-OAHSA-d17 and the above-mentioned 21 FAHFA standards: 5-PAHSA, 5-POHSA, 5-SAHSA, 5-OAHSA, 9-PAHSA, 9-POHSA, 9-SAHSA, 9-OAHSA, 10-PAHSA, 10-POHSA, 10-SAHS, 10-OAHSA, 12-PAHSA, 12-POHSA, 12-SAHS, 12-OAHSA, 13-PAHSA, 13-POHSA, 13-SAHS, and 13-OAHSA. As an example of an application, 10-PAHSA was detected as a deprotonated ion [M-H]⁻ at the mass-to-charge ratio (m/z) of 537. Three major fragments were detected at m/z 255, m/z 281, and m/z 299; m/z 255 corresponds to palmitic acid, m/z 299 corresponds to hydroxystearic acid, and m/z 281 corresponds to dehydro-

Table 2 Chemical structures and optimized parameters of the FAHFA standards.

| FAHFA standards | Structure      | Q1 [M-H] | Q3 [FA-H] | CE | LLOQ (pmol/mL) | R²  |
|-----------------|----------------|----------|-----------|----|----------------|-----|
| 5-PAHSA         |                | 535.5    | 253.1     | 20 | 3              | 0.0064 |
| 5-POHSA         |                | 535.5    | 253.1     | 20 | 3              | 0.0099 |
| 5-SAHSA         |                | 535.5    | 283.1     | 21 | 3              | 0.0002 |
| 5-OAHSA         |                | 535.5    | 281.3     | 20 | 3              | 0.0002 |
| 9-PAHSA         |                | 509.5    | 253.1     | 27 | 3              | 0.0008 |
| 9-POHSA         |                | 535.5    | 253.1     | 28 | 3              | 0.0002 |
| 9-SAHSA         |                | 535.5    | 283.1     | 31 | 3              | 0.0009 |
| 9-OAHSA         |                | 535.5    | 283.1     | 28 | 3              | 0.0004 |
| 10-PAHSA        |                | 535.5    | 253.1     | 28 | 3              | 0.0009 |
| 10-POHSA        |                | 535.5    | 253.1     | 29 | 3              | 0.0004 |
| 10-SAHSA        |                | 535.5    | 283.1     | 29 | 3              | 0.0006 |
| 10-OAHSA        |                | 535.5    | 283.1     | 29 | 3              | 0.0006 |
| 12-PAHSA        |                | 535.5    | 253.1     | 27 | 3              | 0.0063 |
| 12-POHSA        |                | 535.5    | 253.1     | 28 | 3              | 0.0009 |
| 12-SAHSA        |                | 535.5    | 283.1     | 29 | 3              | 1     |
| 12-OAHSA        |                | 535.5    | 283.1     | 27 | 3              | 0.0093 |
| 13-PAHSA        |                | 535.5    | 253.1     | 28 | 3              | 0.0006 |
| 13-POHSA        |                | 535.5    | 253.1     | 28 | 3              | 0.0007 |
| 13-SAHSA        |                | 535.5    | 283.1     | 28 | 3              | 0.0007 |
| 13-OAHSA        |                | 535.5    | 283.1     | 27 | 3              | 0.0083 |
| 12-OAHSA-d17    |                | 580.6    | 298.4     | 28 | 3              | 0.0088 |

LLOQ: lower limit of quantification.
Analysis of FAHFA in Nut and Other Plant Oils

Analysis of gated hydroxystearic acid (Fig. 1A). The most abundant product ion corresponded to the fatty acid fragment with a peak at m/z 255 (Fig. 1B). The fatty acid product ion was also the most abundant obtained in other FAHFA standards.

The chemical structures and optimized parameters of these FAHFA standards were listed in Table 2. A standard mixture of the FAHFA standards was also used for the optimization of the LC conditions (Fig. 1C). Isomers of FAHFA standards were efficiently separated, with the exceptions of the 12- and 13-FAHFA standards. All FAHFA standards showed excellent calibration linearity with a good correlation coefficient (Table 2). The standard curve of 10-PAHSA is shown as an example (Fig. 1D). The quantification of the 21 FAHFA standards with commercial standards was performed with the calibration curve for each FAHFA. The limits of quantification were 3 pmol/mL for all 21 of the FAHFA standards and the IS, with a signal-to-noise ratio >10.

Based on the optimized parameters, we developed a predicted MRM method for FAHFA standards that enable the detection of all combinations of fatty acids that are abundant in plants, i.e., C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C22:0, and C22:1. The parameters of the predicted method for the 21 FAHFA standards detected in any sample are summarized in Table 3. The FAHFA standards that are not commercially available were calculated by using an internal standard in a one-point quantification method that corresponds to the quantification method equivalent to “absolute quantification Level 2” defined by the Lipidomics Standard initiative. However, the analysis results only suggest the existence of an isomer for the predicted molecules, as verification with standard products is required to determine the ester bond position of each FAHFA.

Based on the product ion pattern, the MRM method suggested that some FAHFA molecules overlap. For example, LAHSA and OAHOA as FAHFA 36:2 have the same precursor (m/z 561) and the same product ions (m/z 281 and m/z 279) (Supplemental Table 1). The m/z 281 is [HFA-H$_2$O-H]$^-$ and [FA-H]$^-$ for LAHSA and OAHOA, and the m/z 279 is [FA-H]$^-$ and [HFA-H$_2$O-H]$^-$ for LAHSA and OAHOA, respectively. Since the fatty acid product ion was the most abundant obtained from the standards, we selected the peaks that may overlap as FAHFA molecules having the fatty acid (Supplemental Table 1, Supplemental Fig. 1).

Table 3 Predicted MRM transition parameters for FAHFA standards.

| compound name | family name | Q1 [M-H]$^-$ | Q3 [FA-H]$^-$ | CE | Mode |
|--------------|-------------|--------------|----------------|----|------|
| FAHFA 32:1   | PAHPO       | 507.5        | 255.2          | 27 | Negative |
| FAHFA 34:1   | PAHOA       | 535.5        | 255.2          | 27 | Negative |
| FAHFA 34:2   | PAHLA       | 533.5        | 255.2          | 27 | Negative |
| FAHFA 36:2   | LAHSA       | 561.5        | 279.2          | 28 | Negative |
| FAHFA 36:2   | OAHOA       | 561.5        | 281.2          | 28 | Negative |
| FAHFA 36:2   | SAHLA       | 561.5        | 283.3          | 28 | Negative |
| FAHFA 36:3   | FA18:3HSA   | 559.5        | 277.2          | 27 | Negative |
| FAHFA 36:4   | FA18:3HOA   | 557.5        | 277.2          | 27 | Negative |
| FAHFA 38:0   | PAHFA22:0   | 593.5        | 255.2          | 29 | Negative |
| FAHFA 38:1   | OAHFA20:0   | 591.5        | 281.2          | 29 | Negative |
| FAHFA 38:2   | FA20:1HOA   | 589.5        | 309.2          | 28 | Negative |
| FAHFA 40:1   | FA22:1HSA   | 619.6        | 337.2          | 30 | Negative |
| FAHFA 40:2   | LAHFA22:0   | 617.6        | 279.2          | 30 | Negative |

Based on the product ion pattern, the MRM method suggested that some FAHFA molecules overlap.
Table 4  FAHFAs in the analyzed oils.

| Component Name | Family Name | Peak Name | Structure | Mass Retention (m/z) | Retention Index (RI) | Polar Oil (%) | Neutral Oil (%) | Saponifiable Oil (%) | Total Oil (%) |
|----------------|-------------|-----------|-----------|---------------------|---------------------|---------------|-----------------|---------------------|--------------|
| Table 4: FAHFAs in the analyzed oils. | | | | | | | | | |
Further, LAHOA as FAHFA 36:3 and OAHOA as FAHFA 36:1 may be overestimated by this analytical method as the sum of two product ions which have the same m/z, i.e., [FA-H]⁻ and [HFA-H₂O-H]⁻ (Supplemental Table 2). Since the concentration of OAHOA as FAHFA 36:1 was calculated using the calibration curve, this overestimation could be avoided.

3.2 FAHFAs in the analyzed oils

Table 4 provides the amount of each FAHFA peak, the total amount of each FAHFA family, the number of FAHFAs, and the dominant types of FAHFA. The peak number was assigned to each peak from MRM chromatograms, and the concentrations of 62 FAHFA peaks are listed. We detected 51 FAHFA peaks in the analyzed oil samples, including six FAHFA peaks with commercial standards and 45 newly identified peaks. Eleven FAHFA peaks with commercial standards were not detected. The content for six commercially available FAHFAs was calculated using the calibration curve with the internal standard.

The sum of the concentrations from each FAHFA peak with the same carbon numbers and the same double bond numbers are given in Table 4, as are the total FAHFAs summarized as FAHFA compounds. The results showed that FAHFAs were abundant in the nut oils, and the various types of FAHFAs were detected in all nut oils. Walnut oil contained the highest overall amount of FAHFAs (29.34 pmol/mg oil), followed by peanut oil (11.56 pmol/mg oil), raw almond oil (11.31 pmol/mg oil), and roasted almond oil (8.30 pmol/mg oil). Olive oil (4.90 pmol/mg oil) contained less FAHFAs than the nut oils. The other common oils (palm, soybean, and rapeseed oils) contained lower amounts of FAHFAs, i.e., 1.24, 0.44, and 0.86 pmol/mg oil, respectively.

Peanut oil contained the highest number of detected FAHFA compounds at n = 15, followed by walnut oil (n = 12), olive oil (n = 11), raw almond oil (n = 10), palm oil (n = 9), roasted almond oil (n = 7), soybean oil (n = 7), and rapeseed oil (n = 4). The composition of FAHFAs in the nut oils was mainly unsaturated fatty acids, i.e., FAHFA 36:3, FAHFA 36:2, and FAHFA 36:4. The MRM chromatograms of FAHFA 36:3, FAHFA 36:2, and FAHFA 36:4 are given in Fig. 2.

![Fig. 2](image-url)

**Fig. 2** MRM chromatograms. The numbers were assigned to the peak numbers in Table 4. A: The m/z 559>279 transition was used for LAHOA as FAHFA 36:3. B: The m/z 561>281 transition was used for OAHOA as FAHFA 36:2. C: The m/z 557>279 transition was used for LAHLA as FAHFA 36:4.
2. The FAHFA 36:3 contents differed among all of the nut oils (≥ 3 pmol/mg) and the other common oils (≤ 0.51 pmol/mg). In particular, under the condition of MRM transition m/z at 559 > 279, which detects mainly LAHOA, six peaks were observed in walnut oil and five peaks were observed in raw almond oil, suggesting the possibility of the presence of isomers (Fig. 2A). Similar results were confirmed for FAHFA 36:2. The content of FAHFA 36:2 was higher in the order of walnut, raw almond, peanut, roasted almond, olive, palm, and rapeseed oil. From the chromatograph of MRM transition at m/z 561 > 281 that detects mainly OAUOA, it appears that nut oil may have a large amount of OAUOA and multiple isomers (Fig. 2B). The FAHFA 36:4 results also show that its content in all of the nut oils was higher than those in the other common oils. MRM transition at m/z 557 > 279, which detects mainly LAHLA, revealed 2–3 peaks in the nut oils (Fig. 2C). The total amount of FAHFAs and the number of detected FAHFAs in the raw almond oil were slightly higher than those in the roasted almond oil, and the compositions of FAHFAs differed slightly. The FAHFAs in olive oil consisted of unsaturated fatty acids and saturated fatty acids: FAHFA 36:2, FAHFA 34:1, and FAHFA 36:1. In contrast, the FAHFAs in the common oils (palm, rapeseed, and soybean oils) were FAHFA 32:1, FAHFA 34:0, FAHFA 36:0, and FAHFA 36:1.

We compared the major fatty acids and those of the major FAHFAs in each oil. The major fatty acids in the nut oils are similar to those of the major FAHFAs. In contrast, the major FAHFAs in the common oils did not reflect the major fatty acids (Table 4). Stearic acid and palmitoleic acid were the main components of FAHFAs, although they were not the major fatty acids in the common oils.

4 Discussion

Functional lipids such as carotenoids, omega-3 and omega-6 fatty acids, and medium chain triglycerides in addition to numerous other compounds have been demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond their basic nutritional functions. FAHFAs were discovered as a novel class of endogenous lipids in 2014, and their anti-diabetic and anti-inflammatory effects have been suggested. FAHFAs activate G-protein-coupled receptor (GPR) 120 and GPR40, which
mediate their augmentation of insulin sensitivity and insulin secretion, and they play a beneficial role in glucose-insulin homeostasis. The FAHFA profiles of only a few foods have been reported, for example, oats, clementines, and garlic were identified as having higher FAHFA levels. The FAHFA profiles of rice and Arabidopsis thaliana were also analyzed minutely. Since the FAHFA profiles in nuts had not been determined, we assessed the quantity and quality of the FAHFA profiles in oil, focusing on nut oils. This is the first report to demonstrate the quality and quantity of the FAHFA profiles in oil, focusing on nut oils. We observed that the nut oils were rich in FAHFA profiles. The total amount of FAHFA detected in roasted almond oil was slightly different from that in the roasted almond oil. The heat process used for roasted almond oil might have contributed to the difference between FAHFA profiles in these two types of oil. Unlike the raw almond oil, in the roasted almond oil, most of the FAHFA profiles were maintained after the roasting process, and the amount of FAHFA profiles in the roasted almond oil was higher than that in olive oil. The numbers of FAHFA profiles detected in the nut oils were large, and our results confirmed that the FAHFA profiles consisted of unsaturated fatty acids. Since unsaturated fatty acids are abundant in nut oils, it seems natural that their FAHFA profiles also consisted of unsaturated fatty acids. We observed that the olive oil contained a lesser amount of FAHFA profiles compared to the nut oils, and the main FAHFA profiles in the olive oil consisted of both unsaturated fatty acids and saturated fatty acids. Other common oils (palm, soybean, and rapeseed oils) contained lower amounts of FAHFA profiles, and the FAHFA profiles consisted of saturated fatty acids. Our results clearly establish the characteristics of each type of oil. It is reasonable that the major fatty acids in oil are similar to those of the major FAHFA profiles. However, the major FAHFA profiles in the common oils were not related to the oils’ major fatty acids. LAHLA was reported as a lipid with anti-inflammatory activity, and structural studies identified three LAHLA isomers (15-, 13-, and 9-LAHLA) in oat oil. Our present analyses showed that nut oils were rich in FAHFA 36:4, and four FAHFA 36:4 isomers were identified in the nut oils examined. Notably, FAHFA 36:4 as the MRM transition at m/z 557 > 279, which detects mainly LAHLA, was detected with 2–3 peaks in the nut oils (Fig. 2C). Thus, nut oils are expected to have high...
anti-inflammatory properties.

Nuts are widely used in cooking and are eaten on their own as a snack, and they are used as ingredients in many processed foods. Nuts are a great source of many nutrients, and they are loaded with antioxidants. They exert effects to decrease cholesterol and triglycerides, prevent T2D and metabolic syndrome, reduce inflammation, and reduce the risks of heart attack and stroke\(^{9–13}\). These beneficial effects of nuts have been attributed mainly to their unsaturated oils, phenolic compound, dietary fiber, minerals, and vitamins. Our present findings indicate that the FAHFAs in nuts could also be considered functional components of nuts. The FAHFAs may act with the aforementioned nutrients and phytochemicals synergistically. Further studies are necessary to obtain more details.

A major limitation of our study lies in the accuracy of the annotation and quantification, since not all of the materials were commercially available. Target lipidomics using LC-MS/MS/MS or differential mobility spectrometry may be effective to analyze the hydroxylation position of hydroxy fatty acids or the structure of fatty acids, such as double bond positions and cis-trans isomers\(^3,^{20}\). A determination of the variation of FAHFAs in nut oil by identifying such structural isomers could be important for elucidating the FAHFAs’ biological effects. In some oil samples, the major fatty acids in the oil are not similar to those of the major FAHFAs. Further studies can be expected to clarify the differences between the major fatty acids and the major FAHFAs.

5 Conclusion

We determined the amount of each FAHFA, the total amount of FAHFAs, the number of FAHFAs, and the dominant types of FAHFA in nut oils and other common oils: almond oil (raw, roasted), walnut, peanut, olive, palm, soybean, and rapeseed oils. We developed an LC-ESI/MS/MS method for the comprehensive target analysis of FAHFAs. Our results revealed a broad variation among FAHFA profiles (15 compounds and 62 FAHFA peaks) in the analysis of different oils: for 7–11 compounds of FAHFA, the total levels of 8–29 pmol/mg oil were detected in nuts oils, whereas for 11 compounds, 4.9 pmol/mg oil was detected in olive oil, and for 4–9 compounds, <2 pmol/mg oil was detected in palm, soy, and rapeseed oils. The FAHFAs in nut oils are mainly unsaturated fatty acids. Several beneficial effects of nuts are reported, including unsaturated oils, phenolic compound, dietary fiber, minerals, and vitamins. Our present findings indicate that the FAHFAs in nuts may be considered additional functional components.

Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Information

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