symmetry

MDPI

Article

Simultaneous Quantification of Mixed-Acid Triacylglycerol Positional Isomers and Enantiomers in Palm Oil and Lard by Chiral High-Performance Liquid Chromatography Coupled with Mass Spectrometry

Toshiharu Nagai [1,*], Tetsuaki Kinoshita [2], Erika Kasamatsu [1], Kazuaki Yoshinaga [1,3], Hoyo Mizobe [1], Akihiko Yoshida [1], Yutaka Itabashi [4] and Naohiro Gotoh [2]

[1] Research and Development Division, Tsukishima Foods Industry Co. Ltd., 3-17-9 Higashi Kasai, Edogawa-ku, Tokyo 134-8520, Japan; er-kasamatsu@tsukishima.co.jp (E.K.); kyoshinaga@agri.fukushima-u.ac.jp (K.Y.); h-mizobe@tsukishima.co.jp (H.M.); a-yoshida@tsukishima.co.jp (A.Y.)

[2] Department of Food Science and Technology, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan; kino.tetsu.11@gmail.com (T.K.); ngotoh@kaiyodai.ac.jp (N.G.)

[3] Faculty of Food and Agricultural Sciences, Fukushima University, 1 Kanayagawa, Fukushima-shi, Fukushima 960-1296, Japan

[4] Faculty of Fisheries Sciences, Hokkaido University, Hakodate 041-8611, Japan; yutaka@fish.hokudai.ac.jp

* Correspondence: t-nagai@tsukishima.co.jp; Tel.: +81-3-3689-3054

Received: 26 July 2020; Accepted: 18 August 2020; Published: 19 August 2020

Abstract: Palm oil and lard are edible fats which are rich in palmitic (P) and oleic acids (O). In this study, triacylglycerol (TAG) positional isomers (symmetric and asymmetric isomers) and enantiomers (asymmetric isomers) in palm oil and lard were quantified simultaneously by using liquid chromatography/mass spectrometry. The CHIRALPAK IF-3 column used in our previous study recognized the difference of TAG isomers consisting of P and O in palm oil and lard, separated *sn*-OPP/*sn*-PPO/*sn*-POP and *sn*-OPO/*sn*-OOP/*sn*-POO into each isomer peak, and enabled the quantification of these TAG isomers with good recovery (95–120%). Although *sn*-POP and *sn*-OPO were the major TAGs in palm oil and lard, a comparison of the abundance ratios of TAG enantiomers such as *sn*-PPO/*sn*-OPP and *sn*-OOP/*sn*-POO revealed that there were slightly more TAG enantiomers with O at the *sn*-1 position and P at the *sn*-3 position in palm oil and P at the *sn*-1 position and O at the *sn*-3 position in lard. These results were consistent with previous reports for the positional distribution of fatty acids of palm oil and lard. This is the first study that has enabled all TAG isomers consisting of P and O in natural oils and fats to be individually quantified by mass spectrometry.

Keywords: triacylglycerol; isomer; palm oil; lard; chiral HPLC

1. Introduction

Oils and fats originated from plants and animals are widely used in a variety of industries. Triacylglycerol (TAG) is a main component of edible oils and fats, and their physical and nutritional properties depend on TAG compositions. TAG is composed of one glycerol and three fatty acids [1] and not only their composition, but also binding positions on the glycerol backbone, affect their properties. TAG compositions are specific to each oil and fat species. The binding positions of fatty acids on the glycerol backbone of TAG are distinguished as *sn*-1, 2 and 3, as shown in Figure 1 [2]. The possible permutations of three fatty acids with repetition on the glycerol backbone of TAG composed of two

kinds of fatty acids, A and B, are AAA, *sn*-AAB, *sn*-ABA, *sn*-ABB, *sn*-BAA, *sn*-BAB, *sn*-BBA, and BBB. '*sn*-' is a prefix meaning stereospecific numbering. When '*sn*-' is prefixed to abbreviated TAG (ex. AAB), *sn*-AAB means the TAG molecule binding two As and B at the *sn*-1, 2 and 3 positions in this order. Therefore, *sn*-AAB and *sn*-BAA are in an enantiomeric relationship. *sn*-ABA is a positional isomer for *sn*-AAB (or *sn*-BAA). AAB without '*sn*-' means TAG including two As and one B without considering the binding positions.

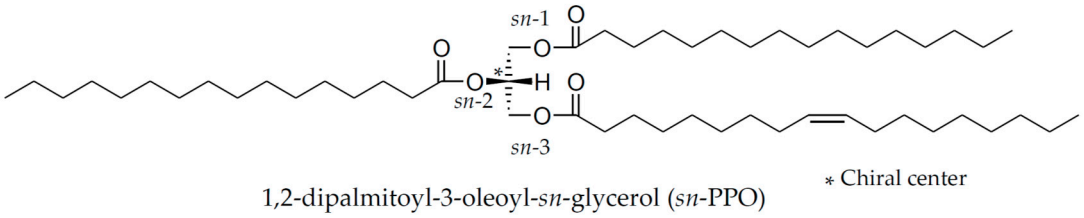

Figure 1. Fischer projection of triacylglycerol (TAG) (ex. 1,2-dipalmitoyl-3-oleoyl-*sn*-glycerol).

Palm oil is the most heavily produced edible plant oil in the world [3], and is used in many applications, including food in the world. The major fatty acids consisting of palm oil is palmitic acid (P) and oleic acid (O), which are representative saturated and unsaturated fatty acids that are widespread in nature. Lard is also composed of P and O and used as edible fat. However, palm oil and lard have been reported to have different binding positions on the glycerol backbone for P and O. The positional distributions of fatty acids on the glycerol backbone of fats and oils analyzed so far indicate that palm oil has more P in the *sn*-1,3 position and more O in the *sn*-2 position, and lard has more O in the *sn*-1,3 positions and more P in the *sn*-2 position [4,5]. In fact, palm oil is known to contain high levels of *sn*-POP [6], and lard is known to contain high levels of *sn*-OPO [7]. Therefore, these two fats and oils were sometimes compared in terms of nutrition [5,8]. In addition, adulteration of palm oil with lard can be a problem [9–11] because both palm oil and lard are used as semi-solid fat in food use. For example, differential scanning calorimetry (DSC) and near-infrared (NIR) spectroscopy were used to detect the adulteration of oils and fats with different physical properties such as lard and palm oil. This difference is due to the unique TAGs of palm oil and lard, which can be measured to distinguish between different types of fats and oils. Silver-ion high performance liquid chromatography (HPLC), which can separate TAG positional isomers, enables us to determine symmetric TAGs such as *sn*-POP and *sn*-OPO in distinction from asymmetric TAGs.

In 2011, we developed a reversed phase (RP) HPLC method using Sunrise C28 column (ChromaNik Technologies Inc., Osaka, Japan) [12] and a chiral HPLC method using CHIRALCEL OD-RH column (Daicel Corporation, Osaka, Japan) [13], which enable to separate TAG positional isomers and enantiomers, respectively. Sunrise C28 column separated TAG positional isomers containing two saturated and one unsaturated fatty acids such as *sn*-POP/*sn*-PPO (or *sn*-OPP). On the other hand, CHIRALCEL OD-RH column recognized the difference between saturated and unsaturated fatty acids at the *sn*-1 and 3 positions of the pair of TAG enantiomers; *sn*-PPO/*sn*-OPP, *sn*-OOP/*sn*-POO, and *sn*-PPL/*sn*-LPP and separated these pairs into each enantiomer peak for the first time. Moreover, the combination of the RP HPLC and chiral HPLC enabled the quantification of naturally occurring TAG positional isomers and enantiomers [14–16]. However, the use of two kinds of separation modes made the analysis procedure complicated and the analysis time very long. For this reason, we have developed a new chiral HPLC method using CHIRALPAK IF-3 column (Daicel Corporation) which can simultaneously separate TAG positional isomers and enantiomers such as *sn*-OPP/*sn*-PPO/*sn*-POP and *sn*-OPO/*sn*-OOP/*sn*-POO [17]. In this study, we applied this chiral HPLC with mass spectrometry (MS) to the simultaneous quantification of naturally occurring TAG isomers, *sn*-OPP/*sn*-PPO/*sn*-POP and *sn*-OPO/*sn*-OOP/*sn*-POO in palm oil and lard. No study has ever separated all these TAG positional and enantiomers in natural oils and fats by chiral HPLC and quantified them individually by MS.

2. Materials and Methods

2.1. Materials and Reagents

1,3-Dipalmitoyl-2-oleoyl-*sn*-glycerol (*sn*-POP), 1,2-dipalmitoyl-3-oleoyl-*rac*-glycerol (*rac*-PPO), 1,3-dioleoyl-2-palmitoyl-*sn*-glycerol (*sn*-OPO), 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol (*rac*-OOP), and 1,2,3-triundecanoylglycerol (C11C11C11) were our in-house product (Tsukishima Foods Industry Co., Ltd., Tokyo, Japan). Palm oil and lard used were commercial products. All of the other reagents used were analytical grade and purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

2.2. Preparation of Standard Solutions

Twenty-five milligrams of *sn*-POP and 50 mg of *rac*-PPO ('*rac*-' means racemic mixture, regarded as 25 mg of *sn*-PPO and 25 mg of *sn*-OPP) were dissolved in acetone and their concentrations were adjusted to 1000 μg/mL using a 25 mL-measuring flask. Then, 2.5 mL of 1000 μg/mL standard solution was diluted with acetonitrile and its concentration was adjusted to 50 μg/mL using a 50 mL-measuring flask. Next, 0.1, 0.2, 1, 2, 3, 4, or 5 mL of 50 μg/mL standard solution and 2 mL of 50 μg/mL internal standard solution (C11C11C11) were diluted with acetonitrile and each of standard's concentration was adjusted to 0.5, 1, 5, 10, 15, 20, or 25 μg/mL with 10 μg/mL of C11C11C11 using a 10 mL-measuring flask. Standard solutions of *sn*-OPO and *rac*-OOP were prepared in the same way.

2.3. Preparation of Sample Solution

Approximately 40 mg of palm oil (or lard) was weighed and dissolved in acetone and filled up using 25 mL-measuring flask (1600 μg/mL). One milliliter of 1600 μg/mL sample solution was diluted with acetonitrile and filled up using 25 mL measuring flask with 5 mL of 50 μg/mL internal standard (C11C11C11) solution to be adjusted the concentration of the sample solution to 64 μg/mL with 10 μg/mL internal standard. In the case of the recovery test, 5 mL of 50 μg/mL standard solution was spiked to the sample solution.

2.4. LC/MS Analysis Using MRM (Multiple Reaction Monitoring) Mode

A setup of LC/MS system used for the quantification is as follows: an HPLC system, Alliance e2695 (Waters Corporation, Milford, MA, USA); a MS with electrospray ionization (ESI) probe, Quattro micro API tandem quadrupole system (Waters Corporation); and a post column pump to promote ionization, LC-10AD (Shimadzu Corporation, Kyoto, Japan). LC/MS conditions were as follows: column, CHIRALPAK IF-3 (2.1 mm i.d. × 250 mm, 3 μm, Daicel Corporation); column temperature, 25 °C; mobile phase, acetonitrile; flow rate, 0.2 mL/min; injection volume, 5 μL; ionization mode, ESI-positive; capillary voltage, 3 kV; source block temperature, 120 °C; desolvation temperature, 450 °C; cone gas flow rate, 50 L/h; desolvation gas flow rate, 800 L/h; cone voltage, 35 V; collision energy, 25 V; data acquisition mode, MRM mode (*m/z* 850.8 > 577.5 for ammonium adduct of PPO, *m/z* 876.8 > 577.5 for ammonium adduct of OOP, and *m/z* 614.7 > 411.5 for ammonium adduct of C11C11C11); and dwell time, 0.1 s. Eluent from HPLC was mixed with 0.l mL/min of 0.1 M ammonium formate methanol solution using a tee-connector in front of ESI probe. The measurements were repeated three times for each standard and sample solution.

2.5. Calculation of the Concentration and Recovery of Each TAG Isomer in Palm Oil and Lard

The values of slope (a), intercept (b), and R^2 of calibration curves were calculated from the concentrations of standard solutions and the peak areas of samples divided by internal standard, C11C11C11 (A/A_{IS}) shown below. The concentrations of each TAG isomer in sample solutions were calculated using Equation (1). Recoveries were calculated by the Equation (2). The concentration of the

standard solution spiked into each sample solution was 10 μg/mL for the recovery test. The contents of each TAG in palm oil or lard were obtained by Equation (3).

$$C\ (\mu g/mL) = (A/A_{IS} - b)/a \quad (1)$$

$$\text{Recovery } (\%) = (C_{\text{measured (sample + spiked standards)}} - C_{\text{measured (sample)}})/C_{\text{calculated (spiked standard)}} \times 100 \quad (2)$$

$$\text{Content } (wt\%) = C/64 \times 100 \quad (3)$$

(A: peak area of each TAG, A_{IS}: peak area of C11C11C11, a: slope, b: intercept, $C_{\text{calculated (spiked standard)}}$ = 10 μg/mL)

3. Results and Discussion

In general, natural fats and oils contain various types of TAGs, and each TAG should exhibit different physical properties. Although fats and oils are aggregates of many TAGs, it is expected that the physical properties of semi-solid fats such as palm oil and lard can be evaluated by examining the isomers of the major TAGs that affect the overall physical properties. In this study, TAG isomers composed of P and O were selected as the analytes for quantitative analysis, because P and O are two fatty acids that are most widely distributed in organisms and are particularly prevalent in palm oil and lard. The TAG isomers of interest here consist of both saturated and unsaturated fatty acids and exhibit a semi-solid state at room temperatures.

For the quantification of these TAG isomers, we selected the MRM mode because of its high selectivity and sensitivity. Chiral HPLC using CHIRALPAK IF-3 column (2.1 mm i.d.) which has the same chiral stationary phase as that we used in our previous study (4.6 mm i.d.) was able to separate *sn*-OPP/*sn*-PPO/*sn*-POP (Figure 2a) and *sn*-OPO/*sn*-OOP/*sn*-POO (Figure 2b) into individual isomers, although the peaks were slightly overlapping. The chromatograms of palm oil and lard spiked with 10 μg/mL of each standard TAG isomer were shown in supplementary data (Figures S2 and S3). The elution orders of the TAG isomers were confirmed from our previous study, but the retention times were slower overall than our previous ones. This would be due to the difference of the condition of the chiral stationary phase.

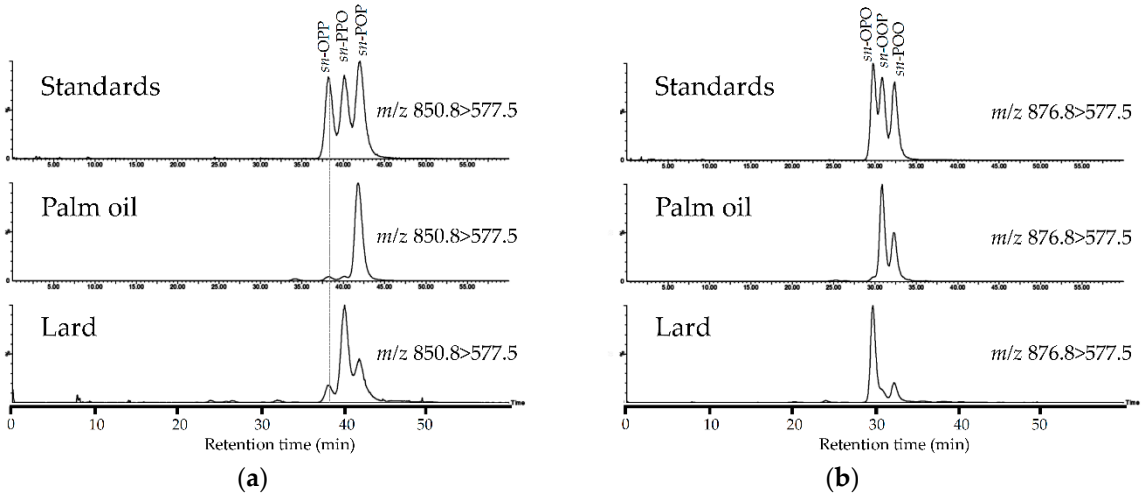

Figure 2. Multiple reaction monitoring (MRM) chromatograms of TAG isomers of (**a**) palm oil and (**b**) lard.

Calibration curves for each TAG in Table 1 showed good linearity ($R^2 > 0.995$) in the range of 0.1–25 μg/mL. The limit of quantification (signal-to-noise ratio >10) of each TAG was approximately 0.05 μg/mL (data not shown). The recovery rates were in the range of 95 to 120% for these TAGs

(Tables 2 and 3). The present analytical conditions were considered to be applicable for the quantitative analysis of TAG isomers consisting of P and O of palm oil and lard. However, the relative standard deviations (RSD) of the recovery rates of *sn*-OPO and *sn*-OOP in palm oil and *sn*-OPO in lard were greater than those of other TAG isomers. It was presumably due to insufficient separation of *sn*-OPO and *sn*-OOP in the MRM chromatograms of palm oil and lard. In this case, the peak areas were determined by vertically dividing partially overlapping peaks, which is not inherently a correct method. Therefore, even if the peaks on the same MRM chromatogram, the slope of the calibration curve for TAG positional isomers is different. If possible, the column should be extended to improve the separation, but in this study, the data were obtained with this resolution.

Table 1. The slopes, intercepts, and square of corelation coefficients (R^2) of calibration curves.

	***sn*-OPP**	***sn*-PPO**	***sn*-POP**	***sn*-OPO**	***sn*-OOP**	***sn*-POO**
Slope (a)	0.0206	0.0227	0.0299	0.0335	0.0339	0.0344
Intercept (b)	−0.0110	−0.0100	−0.0112	−0.0179	−0.0126	−0.0156
R^2	0.9958	0.9953	0.9956	0.9977	0.9978	0.9976

Table 2. The contents and recovery rates of TAG isomers composed of palmitic acid (P) and oleic acid (O) in palm oil.

TAG	***sn*-OPP**	***sn*-PPO**	***sn*-POP**	***sn*-OPO**	***sn*-OOP**	***sn*-POO**
Content (wt%)	2.1 ± 0.04	1.8 ± 0.03	19.0 ± 0.36	1.2 ± 0.01	9.0 ± 0.13	6.5 ± 0.03
Recovey (%)	95	97	113	97	98	106
RSD(%) of recovery	7.1	8.0	2.2	19.8	25.0	4.0

The values of TAG contents (wt%) in palm oil are described as MEAN ± SE (n = 3).

Table 3. The contents and recovery rates of TAG isomers composed of palmitic acid (P) and oleic acid (O) in lard.

TAG	***sn*-OPP**	***sn*-PPO**	***sn*-POP**	***sn*-OPO**	***sn*-OOP**	***sn*-POO**
Content (wt%)	1.2 ± 0.01	2.6 ± 0.01	1.4 ± 0.00	12.8 ± 0.15	2.6 ± 0.01	4.2 ± 0.02
Recovey (%)	95	103	102	120	96	102
RSD(%) of recovery	6.4	4.4	2.9	15.3	3.4	5.6

The values of TAG contents (wt%) in lard are described as MEAN ± SE (n = 3).

Among these TAGs, *sn*-POP was the most common in palm oil at 19.0% (Table 2). The total content of 22.9% for *sn*-POP, *sn*-OPP, and *sn*-PPO was close to the previously reported 24.5% as TAG molecular species, which means the sum of all the isomers [6]. The *sn*-PPO/*sn*-OPP and *sn*-OOP/*sn*-POO ratios in palm oil were about 1 and 3/2, respectively, which was very close to the *sn*-OOP/*sn*-POO ratio measured in our previous study [13]. In the case of lard, 12.8% of OPO was detected in the present analysis (Table 3). The total content of *sn*-OPO, *sn*-OOP, and *sn*-POO was 19.6%, which was in good agreement with the previously reported value 20.8% [7]. A comparison of the abundance ratios of TAG enantiomers (*sn*-PPO/*sn*-OPP and *sn*-OOP/*sn*-POO) between palm oil and lard suggested that P tends to be present in the *sn*-3 position in palm oil, while it is present in the *sn*-1 position in lard, which is in good agreement with previous reports for the positional distribution of fatty acids of palm oil and lard [4]. These differences of the binding positions of P and O on the glycerol backbone between palm oil and lard would be caused by the substrate selectivity of each acyltransferase in glycerol-3-phosphate pathway [18], which would result in the synthesis of nutritionally and physically suitable TAGs for the energy storage tissues of each organism. In future study, the evaluation of various tissues of organisms by this chiral HPLC method will reveal the characteristic features of such TAG synthesis in detail.

4. Conclusions

Chiral HPLC on a CHIRALPAK IF-3 column enabled all TAG isomers consisting of P and O in palm oil and lard to be separated and individually quantified by MS. Although the resolution of *sn*-OPO and *sn*-OOP needed to be improved, it was confirmed that this analytical method has a high potential for application to other natural lipid analyses.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-8994/12/9/1385/s1, Figure S1: MRM chromatograms of blanks; *m/z* 850.8 > 577.5 for PPO and *m/z* 876.8 > 577.5 for OOP, Figure S2: MRM chromatograms of palm oil (64 μg/mL) with standards (10 μg/mL) added; *m/z* 850.8 > 577.5 for PPO and *m/z* 876.8 > 577.5 for OOP, Figure S3: MRM chromatograms of lard (64 μg/mL) with standards (10 μg/mL) added; *m/z* 850.8 > 577.5 for PPO and *m/z* 876.8 > 577.5 for OOP.

Author Contributions: Conceptualization, T.N. and N.G.; methodology, T.K. and T.N.; validation, T.K. and T.N.; investigation, T.N., E.K. and T.K.; resources, K.Y. and H.M.; writing—original draft preparation, T.N. and T.K.; writing—review and editing, T.N.; supervision, A.Y., Y.I. and N.G.; Project administration, N.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Botham, K.M.; Mayes, P.A. Lipids of physiologic significance. In *Harper's Illustrated Biochemistry*, 29th ed.; Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W., Weil, P.A., Eds.; McGraw-Hill Companies: New York, NY, USA, 2012; pp. 140–150.
2. Nawar, W.W. Lipid. In *Food Chemistry*, 3rd ed.; Owen, R.F., Ed.; Marcel Dekker Inc.: New York, NY, USA, 1996; pp. 230–231.
3. OECD-FAO Agricultural Outlook. Available online: http://www.fao.org/3/CA4076EN/CA4076EN_Chapter4_Oilseeds.pdf (accessed on 27 April 2020).
4. The Lipid Web. Available online: https://www.lipidhome.co.uk/lipids/simple/tag1/index.htm (accessed on 27 April 2020).
5. Renaud, S.C.; Ruf, J.C.; Petithory, D. The positional distribution of fatty acids in palm oil and lard influences their biologic effects in rats. *J. Nutr.* **1995**, *125*, 229–237. [PubMed]
6. Lísa, M.; Holčapek, M. Triacylglycerols profiling in plant oils important in food industry, dietetics and cosmetics using high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. *J. Chromatogr. A* **2008**, *1198*, 115–130. [CrossRef] [PubMed]
7. Dugo, P.; Kumm, T.; Fazio, A.; Dugo, G.; Mondello, L. Determination of beef tallow in lard through a multidimensional off-line non-aqueous reversed phase–argentation LC method coupled to mass spectrometry. *J. Sep. Sci.* **2006**, *29*, 567–575. [CrossRef] [PubMed]
8. Teh, S.S.; Ong, A.S.H.; Choo, Y.M.; Mah, S.H. Sn-2 hypothesis: A review of the effects of palm oil on blood lipid levels. *J. Oleo Sci.* **2018**, *67*, 697–706.
9. Marikkar, J.M.N.; Lai, O.M.; Ghazali, H.M.; Che Man, Y.B. Detection of lard and randomized lard as adulterants in refined-bleached-deodorized palm oil by differential scanning calorimetry. *J. Am. Oil Chem. Soc.* **2001**, *78*, 1113–1119. [CrossRef]
10. Marikkar, J.M.N.; Lai, O.M.; Ghazali, H.M.; Man, Y.C. Compositional and thermal analysis of RBD palm oil adulterated with lipase-catalyzed interesterified lard. *Food Chem.* **2002**, *76*, 249–258. [CrossRef]
11. Basri, K.N.; Hussain, M.N.; Bakar, J.; Sharif, Z.; Khir, M.F.A.; Zoolfakar, A.S. Classification and quantification of palm oil adulteration via portable NIR spectroscopy. *Spectrochim. Acta Part A* **2017**, *173*, 335–342. [CrossRef] [PubMed]
12. Nagai, T.; Gotoh, N.; Mizobe, H.; Yoshinaga, K.; Kojima, K.; Matsumoto, Y.; Wada, S. Rapid separation of triacylglycerol positional isomers binding two saturated fatty acids using octacocyl silylation column. *J. Oleo Sci.* **2011**, *60*, 345–350. [CrossRef] [PubMed]
13. Nagai, T.; Mizobe, H.; Otake, I.; Ichioka, K.; Kojima, K.; Matsumoto, Y.; Gotoh, N.; Kuroda, I.; Wada, S. Enantiomeric separation of asymmetric triacylglycerol by recycle high-performance liquid chromatography with chiral column. *J. Chromatogr. A* **2011**, *1218*, 2880–2886. [CrossRef] [PubMed]

14. Nagai, T.; Matsumoto, Y.; Jiang, Y.; Ishikawa, K.; Wakatabe, T.; Mizobe, H.; Yoshinaga, K.; Kojima, K.; Kuroda, I.; Saito, T.; et al. Actual ratios of triacylglycerol positional isomers and enantiomers comprising saturated fatty acids and highly unsaturated fatty acids in fishes and marine mammals. *J. Oleo Sci.* **2013**, *62*, 1009–1015. [CrossRef] [PubMed]
15. Nagai, T.; Watanabe, N.; Yoshinaga, K.; Mizobe, H.; Kojima, K.; Kuroda, I.; Odanaka, Y.; Saito, T.; Beppu, F.; Gotoh, N. Abundances of triacylglycerol positional isomers and enantiomers comprised of a dipalmitoylglycerol backbone and short-or medium-chain fatty acids in bovine milk fat. *J. Oleo Sci.* **2015**, *64*, 943–952. [CrossRef] [PubMed]
16. Nagai, T.; Ishikawa, K.; Yoshinaga, K.; Yoshida, A.; Beppu, F.; Gotoh, N. Homochiral asymmetric triacylglycerol isomers in egg yolk. *J. Oleo Sci.* **2017**, *12*, 1293–1299. [CrossRef] [PubMed]
17. Nagai, T.; Kinoshita, T.; Kasamatsu, E.; Yoshinaga, K.; Mizobe, H.; Yoshida, A.; Itabashi, Y.; Gotoh, N. Simultaneous separation of triacylglycerol enantiomers and positional isomers by chiral high Performance liquid chromatography coupled with mass spectrometry. *J. Oleo Sci.* **2019**, *68*, 1019–1026. [CrossRef] [PubMed]
18. Botham, K.M.; Mayes, P.A. Metabolism of acylglycerols. In *Harper's Illustrated Biochemistry*, 29th ed.; Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W., Weil, P.A., Eds.; McGraw-Hill Companies: New York, NY, USA, 2012; pp. 229–236.