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# Occurrence of Conjugated Cyclopropanoid Acid in Purified Fish Oil

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**Abstract** The presence of conjugated trienoic acids were found in the purified fish oil from the Japanese sardine by an analysis of high performance liquid chromatography equipped with a photodiode-array detector. Gas chromatography–mass spectrometry indicated that main conjugated trienoic acids were those of 16:3, 20:5, and 22:6. Conjugated trienoic acid of 16:3 was identified as a cyclopropanoid acid with conjugated triene by NMR analysis. All conjugated trienoic acids were formed by heat treatment during the deodorization of fish oil, but not found after de-gumming, alkaline refining, and bleaching. Conjugated trienoic acids of 16:3, 20:5, and 22:6 originated from 16:4n-1, 20:5n-3 (EPA), and 22:6n-3 (DHA) in the fish oil during the heat treatment for deodorization.

**Keywords** HPLC · Conjugated triene · Fish oil · 16:4n-1 · EPA · DHA

## Introduction

Increased interest in conjugated linoleic acid (CLA) is due to the beneficial health effects [1]. Dairy products are the most important source of CLA. Conjugated linoleic acid in ruminant meat and milk originates from the incomplete bio-hydrogenation of linoleic acid in the rumen. Fatty acids having conjugated diene, triene, and tetraene also occur naturally in various seed oils and in aquatic plants, especially those of marine origin. The aquatic plants lipids have been found to contain conjugated fatty acids, with carbon chain lengths varying from 16 to 22 carbon atoms [2–7]. However, conjugated fatty acids in the seed oils are usually 18 carbon compounds with conjugated triene [8–10]. The only well known conjugated diene and tetraene of plant origin are 10*trans*(t),12t-18:2 [11] and α-parinaric acid (9*cis*(c),11t,13t,15c-18:4) [12, 13]. Various enzymes are responsible for the endogenous formation of conjugated trienes/tetraenes in terrestrial and aquatic plants. The enzymes responsible for the formation of conjugated fatty acids include conjugases, oxidases and isomerases [14].

Apart from biological pathways, conjugated fatty acids are formed during purification of edible oils. Crude oil is refined by a series of processes to remove impurities that affect taste, smell, appearance and stability of the oil. The refining processes involve de-gumming, alkali refining, bleaching, and deodorization. Yurawecz et al. [15] found traces (up to 0.2%) of conjugated linolenic acid (CLN) in their study of 27 vegetable oils. The isomers were identified as α-eleostearic acid (9c,11t,13t-18:3), β-eleostearic acid (9t,11t,13t-18:3), and 8t,10t,12t-18:3. The possible mechanism for formation of these CLN isomers involves linoleate oxidation, reduction of hydroperoxide to the hydroxide, and dehydration [16, 17]. We also found that 8,10,12 and 9,11,13-CLN were formed during bleaching of soybean oil [18].

Production of edible fish oil involves oil extraction from the fish body and a refining process, which is basically the same as that for vegetable oils. Therefore, conjugated fatty

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acids may be formed during the refining processes of fish oil. The objective was to analyze conjugated fatty acids in fish oils by high performance liquid chromatography (HPLC) with a photodiode-array detector. Furthermore, the conjugated trienoic fatty acid with cyclopropane ring was characterized.

## Materials and Methods

### Preparation of Methyl Esters from Fish Oil

Purified fish oil and fish oil at different processing stages were kindly donated by the Nihonsuisan Co. (Tokyo, Japan). Methyl esters from fish oils were prepared by transesterification using 0.5 M sodium methoxide in methanol. After putting the fish oil (10 g) into a 300-mL flask, 100 mL methanol containing 0.5 M sodium methoxide was added and the transesterification was done by heating the mixture at 60 °C for 1 h. The methyl esters were then extracted with *n*-hexane and the hexane solution was washed with water. The solution was dried over sodium sulfate and concentrated in a rotary evaporator under vacuum below 30 °C. The recovered methyl esters (ca. 8 g) were purified on a silicic acid column (22 × 2.1 cm i.d.) (Silicagel 60, Merck, Darmstadt, Germany) by eluting with *n*-hexane (100 mL) and a mixture of diethyl ether/*n*-hexane solution 5:95 v/v (600 mL) and 10:90 v/v (200 mL). The fraction eluted with diethyl ether/*n*-hexane 5:95 v/v was used in the characterization of the fish oil methyl esters. The purified ester gave only a single spot on TLC with normal-phase silica plates developed with diethyl ether/*n*-hexane/acetic acid (30:70:1 v/v/v). The detection of the spot on TLC was done by spraying with 50% aqueous $H_2SO_4$ and heating on a hot plate to clear the organic material. Methyl oleate (Merck, Darmstadt, Germany) was used as a standard.

### Gas Chromatographic (GC) Analysis

Methyl esters thus prepared were separated on a GC to determine the fatty acid composition. Shimadzu GC-14B (Shimadzu Seisakusho, Kyoto, Japan) equipped with a flame-ionization detector and a capillary column (Omegawax 320, 30 m × 0.32 mm i.d., Supelco, Bellefonte, PA, USA) was employed for the GC analysis. The column temperature was set at 200 °C while the injector and detector were held at 250 and 260 °C, respectively. Helium at 50 kPa was used as the carrier gas.

### Analytical HPLC

Analytical HPLC was carried out with a reversed-phase column (Develosil C30 UG-5, 250 × 4.6 mm i.d., 5.0-μm particle size, Nomura Chem. Co., Seto, Aichi, Japan) protected with a 10 × 4.0 mm i.d. guard column containing the same stationary phase. A mixture of methanol and water (85:15 v/v) at a flow rate of 1.0 mL/min was used as a mobile phase. The HPLC analysis was carried out on a Hitachi L-7000 system equipped with pump (L-7100) and an auto-sampler (L-7200). The instrument also housed a photodiode-array spectrophotometric detector (Hitachi L-7455) and an online analysis software (Hitachi HPLC system-5-manager; Model D-7000).

### Preparative HPLC

Analysis of fish oil methyl esters by HPLC with a photodiode-array detector showing the presence of fatty acids having conjugated trienes were subjected to preparative HPLC. A preparative HPLC was used for the separation of these conjugated trienoic acids using the HPLC system above. The HPLC conditions were the same as those mentioned in the analytical HPLC except that the column size was 250 × 10 mm i.d. and the solvent flow was 3.0 mL/min.

### Identification of Conjugated Trienoic Fatty Acids

The structural analysis of conjugated trienoic acid methyl ester separated by HPLC was done by nuclear magnetic resonance (NMR) and by gas chromatography–mass spectrometry (GC–MS) after the conversion of the methyl esters to dimethyloxazoline (DMOX) derivatives [19]. The NMR experiments were conducted on an ECX-400-1 instrument (Japan Electric, Japan). Gas chromatography–mass spectrometry was performed using a Hewlett-Packard HPG1800A instrument (Hewlett-Packard Co., Palo Alto, CA, USA) under the following conditions: Omegawax-250 column (Supelco, Inc., Bellefonte, PA, USA; 30 m × 0.25 mm i.d.); helium carrier gas (40 mL/min); injector, detector and column temperatures of 230, 240, and 198 °C, respectively.

### Isomerization of EPA and DHA

Methyl esters of EPA and DHA (99+% purity) were purchased from Nu-Chek-Prep (Elysian, MN, USA). Potassium *tert*-butoxide (Nacalai Tesque, Kyoto, Japan) was used as a catalyst for the isomerization of double bonds in EPA and DHA ester so as to produce conjugation. The reaction was carried out by stirring the ester (0.1 g) with potassium *tert*-butoxide (0.05 g) in dimethylformamide (50 mL) at 30 °C for 1 h. After incubation, the reaction mixture was neutralized with 2N HCl. The reaction products were extracted with *n*-hexane and fractionated by silicic acid column chromatography, eluting with *n*-hexane

and diethyl ether/*n*-hexane (5:95 v/v). Isomerized ester was eluted with diethyl ether/*n*-hexane (5:95 v/v). The composition of conjugated dienes and trienes in isomerized EPA and DHA was evaluated by the AOCS official method Cd 7–58 [20]. The isomerized esters were analyzed by an analytical HPLC. The HPLC was done as described in above section (analytical HPLC).

### Fractionation of 16:4n-1

Ethyl esters rich in 16:4n-1 (16:4n-1 > 30%) were kindly donated by Nihonsuisan Co. This ester mixture was obtained in the production of EPA ethyl ester for pharmaceutical purpose. The purified EPA ethyl ester was prepared by distillation and urea fractionation of mixed ethyl esters of Japanese sardine oil. 16:4n-1 rich fraction was obtained in this procedure as a by-product. Ethyl esters containing 16:4n-1 (>30%) were saponified with 0.2 N KOH at room temperature under nitrogen overnight. After acidifying the aqueous solution with 1 N HCl, free fatty acids were extracted with diethyl ether. The free fatty acids were methylated with 7% boron trifluoride in methanol. The methyl ester mixture containing 16:4n-1 was subjected to preparative HPLC. The HPLC condition was the same as described in above section (preparative HPLC). Purity of the 16:4n-1 obtained was more than 93% by GC.

### Heat Treatment of Methyl Esters

A small portion (10 mg) of each methyl ester of 16:4n-1, EPA, and DHA was put into a tube. After purging the sample with nitrogen, the tube was sealed and placed in a block heater at 200 °C for 2 h. After cooling, the sample was diluted with *n*-hexane and then subjected to analytical HPLC. The HPLC was done as described in the section above (analytical HPLC).

## Results and Discussion

Crude fish oil was obtained by cooking, pressing, and centrifuging from the whole body of Japanese sardines. After de-gumming, the oil was winterized to increase the polyunsaturated fatty acid contents. The winterized oil was purified by alkali refining, bleaching, and deodorization. Gas chromatography analysis showed that the major fatty acids of the purified fish oil were 20:5n-3 (EPA; 28.3%), 22:6n-3 (DHA; 13.5%), 16:1n-7 (8.5%), 18:1n-9 (7.6%), 16:0 (7.1%), 14:0 (4.7%), 16:4n-1 (3.9%), 18:4n-3 (3.5%), 22:5n-3 (2.8%), and 16:3n-4 (2.7%). The higher percentage of EPA over that of DHA and the presence of significant amounts of 16:4n-1 and 16:3n-4 reflected the characteristics of sardine oil [21–23], although winterization increased polyunsaturated fatty acid content and decreased saturated fatty acid contents.

The presence of highly unsaturated fatty acids having 16–18 carbons and 3–6 double bonds, namely 16:4n-1, 16:3n-4, 18:4n-3, 20:5n-3, 22:6n-3, and 22:5n-3, were detected on reverse-phase HPLC by UV detection at 210 nm (Fig. 1). Some other peaks were observed through UV detection at 233 or 274 nm. Figure 2 shows the HPLC chromatogram detected at 274 nm and the spectrum obtained by scanning over 200–400 nm range. The spectrum with absorption maxima at 262.5, 272 or 273.3, and 282.7 or 284.0 indicates the presence of conjugated trienoic structures in these peaks. Main peaks of conjugated trienoic fatty acids were fractionated by reversed-phase HPLC into fractions $F_1$–$F_3$ (Fig. 1). After separation, each peak was analyzed by GC and HPLC. $F_1$ showed a single peak on chromatograms of HPLC and GC, while $F_2$ and $F_3$ gave several peaks on both chromatograms.

The DMOX derivatives of $F_1$ also showed a single peak on the GC–MS chromatogram. Molecular ion at *m*/*z* 301 and fragment ion at *m*/*z* 286 (M-$CH_3$) indicated that $F_1$ would be a conjugated trienoic isomer of a 16:4 or a 16:3 with a ring. Although several peaks appeared on the GC chromatograms of $F_2$ and $F_3$ DMOX derivatives, these peaks showed the same parent ion at *m*/*z* 355 ($F_2$) and *m*/*z* 381 ($F_3$). Both numbers were consistent with molecular weights of EPA and DHA, respectively, suggesting that $F_2$ and $F_3$consists of conjugated trienoic isomers of EPA and DHA, respectively. To confirm that $F_2$ and $F_3$ were conjugated trienoic isomers of EPA and DHA, methyl esters of EPA and DHA were isomerized by an alkaline catalyst. The compositions of conjugated dienes, trienes, and tetraenes were 54.8, 18.6, and 8.5% for isomerized EPA, and 64.3, 14.2, and 5.5% for isomerized DHA, respectively. When both isomerized mixtures were analyzed by reversed phase HPLC with UV detection at 274 nm, several peaks

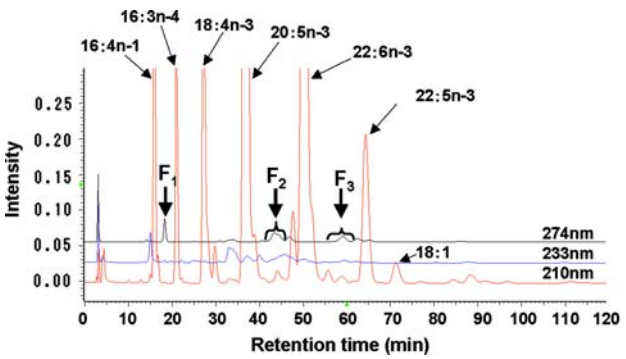


**Fig. 1** Analysis of purified fish oil methyl esters by HPLC with a photodiode-array detector. Separation was completed on a Develosil C30 column and methanol/water (85:15 v/v) at a flow rate of 1.0 mL/min

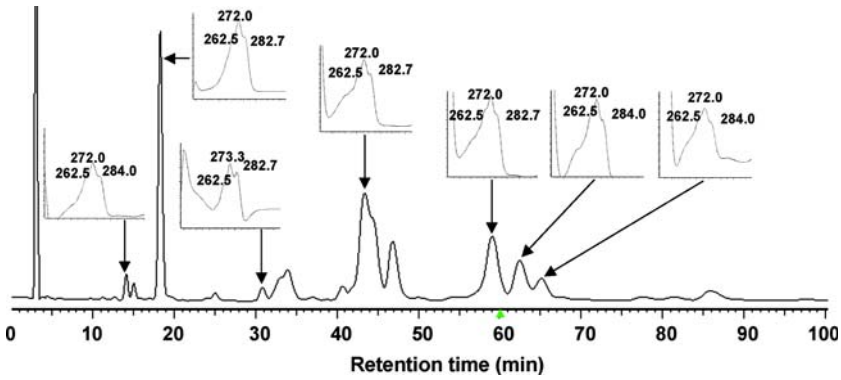


**Fig. 2** HPLC chromatogram of purified fish oil methyl esters using 274 nm detection. The inset shows the UV spectrophotometric scans of each peak

corresponding to conjugated trienoic isomers of EPA and DHA were detected at the same retention times of $F_2$ and $F_3$, respectively.

The DMOX derivatives are convenient to identify the double bond positions of a conjugated fatty acid isomer by GC–MS. Double bond positions of CLA and CLN can be identified by the characteristic loss of 12 daltons [18, 24]. However, it was difficult to assign a 12-amu gap in the MS spectrum of DMOX derivatives of $F_1$, $F_2$ and $F_3$. This might be due to the high unsaturation of these conjugated fatty acids. Retention time of fatty acids on reversed-phase HPLC are affected by chain length, number of double bonds, position and geometry of double bonds. Generally, a fatty acid with *cis* double bonds elutes faster than one with *trans* double bonds. $F_2$ and $F_3$ were eluted later than their non-conjugated forms, EPA and DHA, respectively (Fig. 1). Therefore, these conjugated trienoic fatty acids contain *trans* double bonds in their molecule.

Table 1 shows the NMR analysis of $F_1$. All protons were clearly resolved and this was confirmed by $^1$H–$^1$H 2D shift correlation spectroscopy (COSY) (Fig. 3). Resolution of the higher field signal (–0.240) of H-14, than that of TMS, indicated the presence of cyclopropane structure [25]. $^{13}$C NMR and DEPT spectra indicated that $F_1$ was a methyl ester of a 16 carbon fatty acid with one ester carbonyl, two methyl (-$CH_3$) carbons, six methylene (-$CH_2$) carbons, and eight methine (-CH) carbons. Configuration within the conjugated trienes were suggested by the coupling between allylic methylene proton signals and the olefinic signals and the symmetry of the COSY pattern. These results yielded a structure of $F_1$ as methyl (6*Z*,8*E*,10*Z*)-12-(2-methylcyclopropan-1-yl)-dodecatrienoate (Fig. 4). Judging from the

**Table 1** H1 NMR Data for *F*1

| Carbon No. | $\delta$ | m | J (Hz) |
|---|---|---|---|
| 1 | – | – | – |
| 2 | 2.322 | t | 7.2, 2.0 |
| 3 | 1.658 | q | 8.2, 2.0 |
| 4 | 1.427 | q | 7.7, 2.0 |
| 5 | 2.221 | q | 8.6, 2.0 |
| 6 | 5.421 | dt | 10.87, 7.7 |
| 7 | 6.067 | m | 10.42 |
| 8 | 6.464 | m | – |
| 9 | 6.464 | m | – |
| 10 | 6.067 | m | 10.42 |
| 11 | 5.564 | dt | 10.42, 7.7 |
| 12 | 2.199 | d | 2.0 |
| 13 | 0.737 | m | – |
| 14 | 0.637 | m | – |
| | –0.240 | m | – |
| 15 | 0.799 | m | – |
| 16 | 1.057 | dt | 5.89, 3.0 |
| 1′ | 3.668 | s | 3.0 |

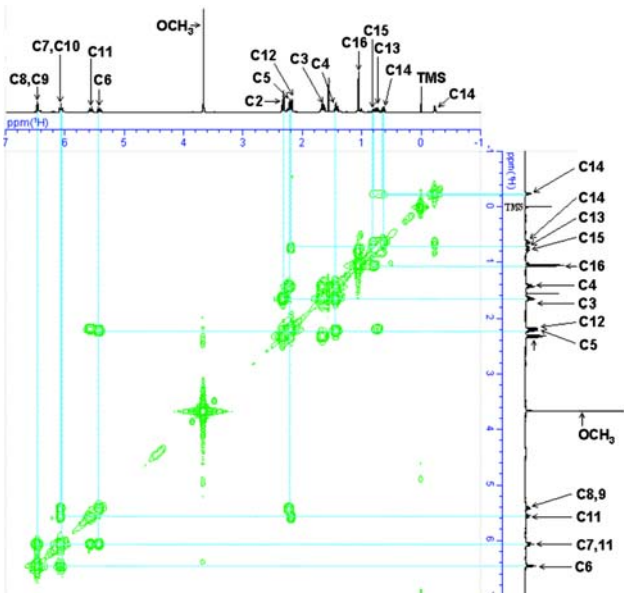


**Fig. 3** 1H–1H 2D-shift correlation spectroscopy of $F_1$ (methyl (6*Z*,8*E*,10*Z*)-12-(2-methylcyclopropane-1-yl)-dodecatrienoate)

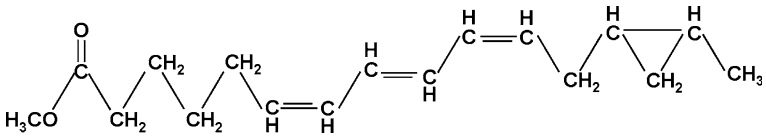

**Fig. 4** Structure of methyl (6*Z*,8*E*,10*Z*)-12-(2-methylcyclopropane-1-yl)-dodecatrienoate

fatty acid composition of the fish oil, this conjugated cyclopropanoid acid originates from 16:3n-4 or 16:4n-1. The presence of the cyclopropane structure suggested that 16:4n-1 might be the origin of the conjugated cyclopropanoid acid.

The HPLC chromatogram of processed fish oil at different stages is shown in Fig. 5. The formation of conjugated trienoic acids was observed after deodorization, but not after de-gumming, alkali refining, and bleaching. Some other peaks were observed through UV detection at 233 nm in fish oils at all stages of refining. After deodorization, two new peaks (retention times: 15 and 32–35 min) appeared. The spectrum obtained by scanning over the 200–400 nm range in the photodiode-array suggested that all these peaks contained a conjugated diene structure. Sources of these conjugated dienoic acids would be some of PUFA in fish oils. However, the structural analysis of the conjugated dienoic acids was not completed in this study.

The formation of conjugated trienoic acids during deodorization suggested the occurrence of the double bond migrations in PUFA of fish oil by heating. To confirm this, purified methyl ester of 16:4n-1, EPA, or DHA was heated at 200 °C for 2h, and then, the formation of conjugated fatty acids analyzed by analytical HPLC (Fig. 6). Conjugated dienoic and trienoic acids were detected in every chromatogram after heating. These peaks were identical to those that appeared after deodorization of fish oil (Fig. 5), confirming that conjugated trienoic acids in the fish oil originated from 16:4n-1, EPA, and DHA.

In vegetable oils, linolenic acids with a conjugated triene (CLN) are produced from trace amounts of linoleate hydroperoxides remaining in the oils during bleaching [16–18]. This reaction involves reduction of hydroperoxides to the hydroxide and dehydration of the hydroxide by heating and acid treatment during bleaching. Furthermore, we have reported that high temperature in deodorization of soybean oil induced migration of double bonds of linoleate and linolenate to produce their conjugated dienoic isomers [18].

However, the formation of conjugated triene from linolenic acid was not observed during the deodorization of soybean oil. In contrast, conjugated trienoic acids were detected after deodorization of fish oil (Fig. 5), which originated from 16:4n-1, EPA, and DHA. In the formation of conjugated trienes from EPA and DHA, migration of double bonds should occur at least twice. Structural study of each isomer of the conjugated trienes of EPA and DHA is required to clarify the mechanism for the formation of these conjugated trienoic acids. The 16:4n-1 has four double bonds of which one is located at the methyl terminal. This characteristic structure might produce only one

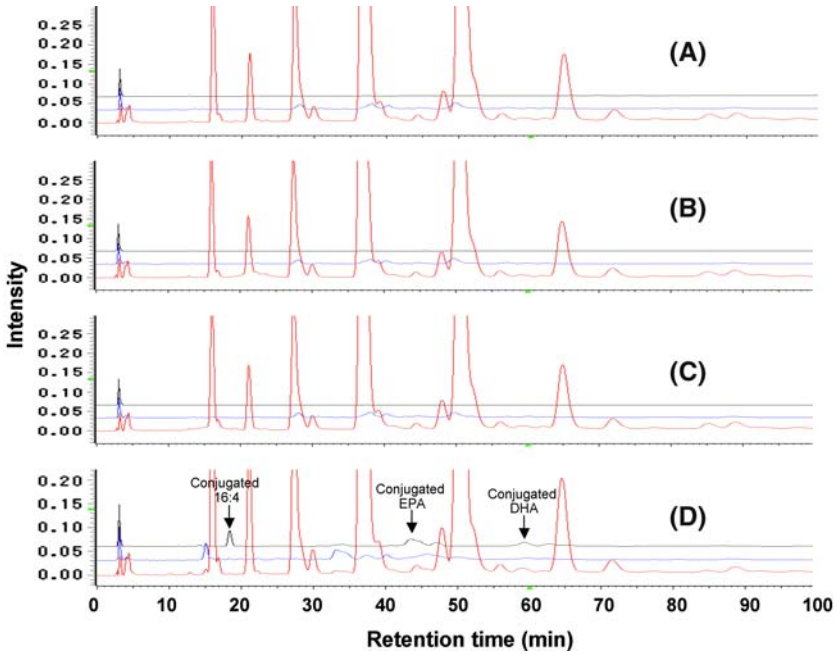


**Fig. 5** **a** HPLC chromatogram of methyl esters from fish oil after de-gumming. **b** Alkali refining. **c** Bleaching. **d** Deodorization

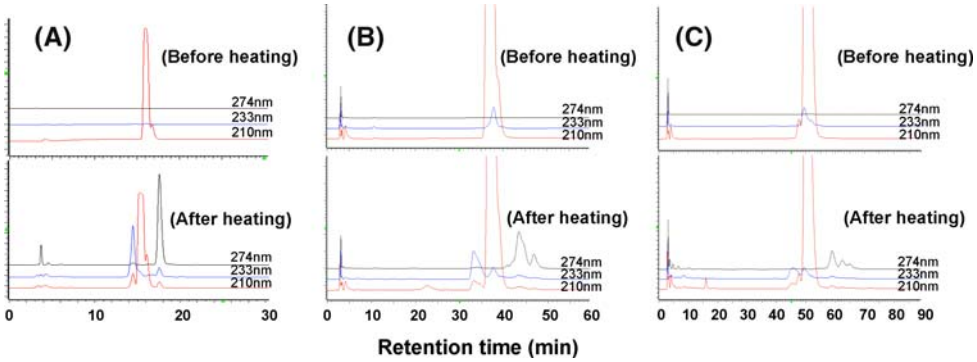


**Fig. 6** **a** HPLC chromatogram of methyl esters of 16:4n-1. **b** EPA. **c** DHA before and after heating at 200 °C for 2 h

specific conjugated isomer, conjugated cyclopropanoid acid, during heat treatment for deodorization.

## References


1. Pariza MW, Park Y, Cook ME (2001) The biologically active isomers of conjugated linoleic acid. Prog Lipid Res 40:283–298
2. Lopez A, Gerwick WH (1987) Two new icosapentaenoic acids from the temperate red seaweed *Ptilota filicina*. J Agardh Lipids 22:190–194
3. Wise ML, Hamberg M, Gerwick WH (1994) Biosynthesis of conjugated triene containing fatty acids by novel isomerase from the red marine algae *Ptilota filicina*. Biochemistry 33:15223–15232
4. Bhaskar N, Kinami T, Miyashita M, Park S, Endo Y, Fujimoto K (2004) Occurrence of conjugated polyenoic fatty acids in seaweeds from the Indian Ocean. Z Natuforsch 59c:310–314
5. Burgess JR, De la Rosa RI, Jacobs RS, Butler A (1991) A new eicosapentaenoic acid formed from arachidonic acid in the coralline red algae *Bassiella orbigniana*. Lipids 26:162–165
6. Hamberg M (1992) Metabolism of 6,9,12-octadecatrienoic acid in the red alga *Lithothamnion corrallioides*: mechanism of formation of a conjugated tetraene fatty acid. Biochem Biophys Res Commun 188:1220–1227
7. Mikhailova MV, Bemis DL, Wise ML, Gerwick WH, Norris JN, Jacobs RS (1995) Structure and biosynthesis of novel conjugated polyene fatty acids from the marine green alga *Anadyomene stellata*. Lipids 30:583–589
8. Smith Jr CR, Occurrence of unusual fatty acids in plants. Prog Chem Fats Other Lipids 11:137–177
9. Takagi T, Itabashi Y (1981) Occurrence of mixtures of geometrical isomers of conjugated octadecatrienoic acids in some seed oils: analysis by open-tubular gas liquid chromatography and high performance liquid chromatography. Lipids 16:546–551
10. Suzuki R, Arato S, Noguchi R, Miyashita K, Tachikawa O (2001) Occurrence of conjugated linolenic acid in flesh and seed of bitter gourd. J Oleo Sci 50:753–758
11. Hopkins CY, Chisholm MJ (1964) Isolation of natural isomer of linoleic acid from seed oil. J Am Oil Chem Soc 41:42–44
12. Bagby MO, Smith Jr CR, Wolff IA (1966) Stereochemistry of α-parinaric acid from *Impatiens edgeworthi* seed oil. Lipids 1:263–267
13. Spitzer V, Marx F, Pfeilsticker K (1994) Electron impact mass spectra of the oxazoline derivatives of some conjugated diene and triene $C_{18}$ fatty acids. J Am Oil Chem Soc 71:873–876
14. Narayan B, Hosokawa M, Miyashita K (2006) Occurrence of conjugated fatty acids in aquatic and terrestrial plants and their physiological effects. In: Shahidi F (ed) Nutraceuticals and specialty lipids and their co-products. CRC Press, Boca Raton, pp 201–218
15. Yurawecz MP, Molina AA, Mossoba M, Ku Y (1993) Estimation of conjugated octadecatrienes in edible fats and oils. J Am Oil Chem Soc 70:1093–1099
16. Parr LJ, Swoboda PAT (1976) The assay of conjugable oxidation products applied to lipid deterioration in stored foods. J Food Technol 11:1–12
17. Fishwick MJ, Swoboda PAT (1977) Measurement of oxidation of polyunsaturated fatty acids by spectrophotometric assay of conjugated derivatives. J Sci Food Agric 28:387–393
18. Kinami T, Horii N, Narayan B, Arato S, Hosokawa M, Miyashita K, Negishi H, Ikuina J, Noda R, Shirasawa S (2007) Occurrence of conjugated linolenic acids in purified soybean oil. J Am Oil Chem Soc 84:23–29
19. Sehat N, Yurawecz MP, Roach JAG, Mossoba MM, Kramer JKG, Ku Y (1998) Silver-ion high-performance liquid chromatographic separation and identification of conjugated linoleic acid isomers. Lipids 33:217–221
20. AOCS official method Cd 7–58 (1998) Official methods and recommended practices of the American Oil Chemists' Society. In: Firestone D (ed), 5th edn. AOCS Press, Champaign
21. Bandarra NM, Batista I, Nunes ML, Empis JM, Christie WW (1997) Seasonal changes in lipid composition of sardine (*Sardine pilchardus*). J Food Sci 62:40–42
22. Gámez-Meza N, Higuera-Ciapara I, Calderon de la Barca AM, Vázquez-Moreno L, Noriega-Rodríquez J, Angulo-Guerrero O (1999) Seasonal variation in the fatty acid composition and quality of sardine oil from *Sardinops sagax caeruleus* of the gulf of California. Lipids 34:639–642
23. Ando Y, Ota T, Takagi T (1989) Japanese sardine oils as a source of 16:3n-4 and 16:4n-1 fatty acids. J Am Oil Chem Soc 66:1323–1325
24. Spitzer V (1999) Gas chromatography/(electron impact) mass spectrometry analysis of conjugated linoleic acid (CLA) using derivatization techniques. In: Yurawecz MP, Mossoba MM, Kramer JKG, Pariza MW, Nelson GJ (eds) Advances in conjugated linoleic acid research. vol 1, AOCS, Champaign, pp 110–125
25. Hartmann S, Minnikin DE, Römming HJ, Baird MS, Ratledge C, Wheeler PR (1994) Synthesis of methyl 3-(2-octadecylcyclopropen-1-yl)propanoate and methyl 3-(2-octadecylcyclopropen-1-yl)pentanoate and cyclopropane fatty acids as possible inhibitors of mycolic acid biosynthesis. Chem Phys Lipids 71:99–108