



## Oxidative stability of cod liver oil in the presence of herring roe phospholipids

Liang, Peng; Akoh, Casimir C.; W. K. Diehl, Bernd; Jacobsen, Charlotte

*Published in:*  
Food Chemistry

*Link to article, DOI:*  
[10.1016/j.foodchem.2019.125868](https://doi.org/10.1016/j.foodchem.2019.125868)

*Publication date:*  
2020

*Document Version*  
Peer reviewed version

[Link back to DTU Orbit](#)

### *Citation (APA):*

Liang, P., Akoh, C. C., W. K. Diehl, B., & Jacobsen, C. (2020). Oxidative stability of cod liver oil in the presence of herring roe phospholipids. *Food Chemistry*, 310, [125868]. <https://doi.org/10.1016/j.foodchem.2019.125868>

---

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

## Journal Pre-proofs

Oxidative stability of cod liver oil in the presence of herring roe phospholipids

Peng Liang, Casimir C. Akoh, Bernd W. K. Diehl, Charlotte Jacobsen

PII: S0308-8146(19)32004-7

DOI: <https://doi.org/10.1016/j.foodchem.2019.125868>

Reference: FOCH 125868

To appear in: *Food Chemistry*

Received Date: 17 June 2019

Revised Date: 13 October 2019

Accepted Date: 5 November 2019



Please cite this article as: Liang, P., Akoh, C.C., W. K. Diehl, B., Jacobsen, C., Oxidative stability of cod liver oil in the presence of herring roe phospholipids, *Food Chemistry* (2019), doi: <https://doi.org/10.1016/j.foodchem.2019.125868>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Published by Elsevier Ltd.

## **Oxidative stability of cod liver oil in the presence of herring roe phospholipids**

Peng Liang <sup>a,d</sup>, Casimir C. Akoh <sup>b</sup>, Bernd W. K. Diehl <sup>c</sup>, Charlotte Jacobsen <sup>d\*</sup>

<sup>a</sup>College of Food Science, Fujian Agriculture and Forestry University, 15 Shangxiadian Road, Fuzhou 350002, Fujian, China

<sup>b</sup>Department of Food Science & Technology, University of Georgia, 30602 Athens, GA, USA

<sup>c</sup>Spectral Service AG, Emil-Hoffmann-Straße 33, D-50996 Köln, Germany

<sup>d</sup>Division of Food Technology, National Food Institute (DTU Food), Technical University of Denmark, Building 204, Kemitovet, DK 2800 Kgs Lyngby, Denmark

\*Correspondence should be addressed to Charlotte Jacobsen (chja@food.dtu.dk), Division of Food Technology, National Food Institute (DTU Food), Technical University of Denmark, DK 2800 Kgs Lyngby, Denmark.

\*Phone: +45 45 25 25 59.

**1 Abstract**

2 The aim of this research was to investigate the effect of herring roe phospholipids  
3 (PLs) on the oxidative stability of cod liver oil during storage. The effect of PLs on  
4 the oxidative stability of cod liver oil was assessed in terms of peroxide value, free  
5 fatty acids, secondary oxidation products and pyrrolisation. The results show that the  
6 PV was lower in cod liver oil containing PLs ( $P < 0.05$ ) than in the control without  
7 PLs. Benzaldehyde, 2,5-dimethylpyrazine, 2-methyl-2-pentenal, 1-penten-3-ol and  
8 3-methylbutanal were the main volatiles. In addition, significant pyrrolisation was  
9 observed after 28 days when PLs were added to cod liver oil. The results suggested  
10 that cod liver oil with dispersed PLs was oxidized during storage followed by  
11 non-enzymatic browning reactions. The findings indicated that the ratio between  
12 pyrroles formed and  $\alpha$ -tocopherol may influence the formation of new peroxides and  
13 secondary oxidation products.

14 **Keywords:** marine phospholipids, oxidation, cod liver oil, non-enzymatic browning  
15 reactions, strecker aldehydes.

## 16 **1. Introduction**

17 Marine phospholipids (PLs) have received much attention by researchers due to  
18 their high content of polyunsaturated fatty acids (PUFAs), especially  
19 docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Belhaj,  
20 Arab-Tehrany, & Linder, 2010). Marine PLs are naturally present in high  
21 concentration in fish heads (Gbogouri, Linder, Fanni, & Parmentier, 2006), fish roe  
22 (Shirai, Higuchi, & Suzuki, 2006) and krill oil (Burri, Hoem, Banni, & Berge, 2012)  
23 with different fatty acid compositions. Many studies have shown that marine PLs  
24 have better oxidative stability than marine triacylglycerol (TAG) available from fish  
25 oil due to (a) their tight intermolecular packing conformation with the PUFA at the  
26 sn-2 position of PLs and (b) a synergistic effect of PLs on the antioxidant activity of  
27  $\alpha$ -tocopherol. In addition, pyrroles formed from non-enzymatic browning reactions  
28 between oxidized PLs/amino acids and fatty acid oxidation products in slightly  
29 oxidized marine PLs have protective effects against oxidation (Lu, Nielsen, Baron,  
30 Diehl, & Jacobsen, 2012).

31 Numerous studies have shown that natural PLs possess antioxidant activity.  
32 Hudson and Mahgoub (1981) reported that PLs from eggs were effective  
33 antioxidants in lard. Saito and Ishihara (1997) reported that phosphatidylethanolamine  
34 (PE) and phosphatidylcholine (PC) were good antioxidants in a sardine oil system.  
35 King et al. (1992) showed that the more egg yolk phosphatidylcholine (PC) added to  
36 salmon oil, the higher the oxidative stability obtained. They suggested that  
37 Maillard-type reaction products may have improved the oxidative stability of  
38 PL-supplemented fish oils.

39 Marine PLs contain higher amount of PUFAs compared to PLs from egg and  
40 soybean and may therefore be more susceptible to lipid oxidation than these PL

41 sources. Despite their polyunsaturated nature, marine PLs may still provide  
42 antioxidative effects when added to other lipids. Belhaj, Arab-Tehrany, & Linder  
43 (2010) evaluated the oxidative stability of salmon oil added with marine PC. Their  
44 results indicated that when marine PC was added as an emulsifier in salmon oil, it  
45 could increase the stability of salmon oil via its antioxidant activity despite an  
46 increase in long-chain PUFAs, especially with DHA due to addition of marine PC.

47 Moreover, the effect of temperature on lipid oxidation and non-enzymatic  
48 browning reactions in krill oil (rich in marine PLs) were investigated upon storage  
49 (Lu, Bruheim, Haugsgjerd, & Jacobsen, 2014) The authors suggested that the  
50 formation of pyrroles might help to protect the krill oil against lipid oxidation. These  
51 antioxidative compounds were formed from non-enzymatic browning reactions  
52 between the primary amine group of PE or amino acids with the lipid oxidation  
53 products in marine PLs (Lu, Nielsen, Baron, Diehl, & Jacobsen, 2013; Lu, Nielsen,  
54 Baron, & Jacobsen, 2012).

55 Herring roe is an underutilized source of marine PLs. Approximately, 600,000 t  
56 herring are caught in Norway per year, but only a small amount of herring roe is  
57 consumed by humans. Thirty percent of the lipids in herring roe are marine PLs of  
58 which most are in the form of PC (75%) (Bjørndal, Strand, Gjerde, Bohov, Svardal,  
59 Diehl, et al., 2014). Besides, the total EPA and DHA constituted more than 35% of  
60 total fatty acids. Oxidative stability of herring roe lipids was found to be higher than  
61 fish oils prepared from sardine and tuna (Moriya, Kuniminato, Hosokawa, Fukunaga,  
62 Nishiyama, & Miyashita, 2007). The authors suggested that the higher oxidative  
63 stabilities of herring roe lipids would be mainly due to the presence of PLs.

64 To the best of our knowledge, the oxidative stability of cod liver oil with purified  
65 marine PLs from herring roe added has not been studied previously. The **main**

66 objective of the present study was to investigate the effect of herring roe PLs on lipid  
67 oxidation of cod liver oil upon storage. The secondary objective is to investigate the  
68 non-enzymatic browning reactions in cod liver oil with herring roe PLs added upon  
69 storage. This is the first time the storage stability of cod liver oil with herring roe PLs  
70 added has been studied. Herring roe PLs were purified by acetone precipitation from  
71 herring roe oil to eliminate the effect of other factors on lipid oxidation, such as  
72 content of TAG, antioxidants or other residues that might be present in marine PLs.

## 73 2. Materials and methods

### 74 2.1 Materials

75 Herring roe oil was kindly provided by Novastell (Etrépagny, France).  
76 Commercial cod liver oil was obtained from Maritex A/S, subsidiary of TINE, BA  
77 (Sortland, Norway) without addition of antioxidant. The peroxide value (PV) of cod  
78 liver oil was < 0.1 mequiv/kg, free fatty acids content (FFA) was 0.10%. Contents of  
79  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherol were  $141 \pm 9$ ,  $0 \pm 0$ ,  $95 \pm 5$ ,  $42 \pm 2$   $\mu\text{g}$  toc/g oil, respectively.  
80 Herring roe oil was stored at -40 °C until use. The fatty acid composition of cod liver  
81 oil was as follows (% w/w): 8.90% palmitic acid (C16:0), 8.20% pamtoleic acid  
82 (C16:1n-7), 1.86% stearic acid (C18:0), 16.0% oleic acid (C18:1n-9), 5.16% vaccenic  
83 acid (C18:1n-7), 1.79% linoleic acid (C18:2n-6), 0.84%  $\alpha$ -linolenic acid (C18:3n-3),  
84 11.59% gadoleic acid (C20:1n-11), 9.27% eicosapentaenoic acid (C20:5n-3), 6.06%  
85 cetoleic acid (C22:1n-11), 11.64% docosahexaenoic acid (C22:6n-3) and 7.78%  
86 others.

### 87 2.2 Methods

#### 88 2.2.1 Purification of phospholipids (PLs) from herring roe oil by acetone 89 precipitation

90 PLs were isolated from herring roe oil by using acetone precipitation as

91 described by Mozuraityte and coworkers (2007) and Liang et al. (2017) with a few  
92 modifications. Briefly, 100 g herring roe oil was dissolved in 200 mL chloroform.  
93 This solution was then poured into 1000 mL of acetone under vigorous stirring at  
94 room temperature without light for 30 min. Afterwards, the mixed solution was kept  
95 at  $-18\text{ }^{\circ}\text{C}$  overnight to allow PLs precipitation. The acetone was decanted and the PLs  
96 precipitates were redissolved in chloroform. The isolation step was repeated twice.  
97 The purified PLs were dried under nitrogen for 1 h to evaporate the acetone and  
98 chloroform.

### 99 *2.2.2 Determination of phospholipids classes by $^{31}\text{P}$ NMR*

100 The classes of PLs were determined through  $^{31}\text{P}$  NMR by Spectral Service  
101 GmbH (Cologne, Germany) using the same method as in our previous study (Lu,  
102 Nielsen, Baron, Diehl, and Jacobsen, 2012). [See also data provided as supplementary](#)  
103 [material.](#) ~~However, the~~ The measurement was performed only once [due to limited](#)  
104 [amount of sample material available.](#)

### 105 *2.2.3 Preparation of cod liver oil with different levels of PLs dispersion*

106 Five different formulations of PLs dispersion in cod liver oil were prepared with  
107 different levels of purified PLs (as shown in Table 1). To avoid the effect of  
108 tocopherol on the oxidative stability of cod liver oil, it was removed from PLs during  
109 acetone purification. Therefore, the oxidation of cod liver oil only depended on the  
110 effect of PLs and the synergism between tocopherol (from cod liver oil) and PLs.

### 111 *2.2.4 Oxidative stability determination*

112 The cod liver oil with different levels of PLs was incubated at  $40\text{ }^{\circ}\text{C}$  in darkness,  
113 exposed to air for up to 28 days and were stirred at regular intervals. For each type of  
114 cod liver oil, a beaker with 25.0 g of oil containing PLs were prepared. The  
115 experiment was performed in triplicate. Samples were taken from the same beaker on



116 day 0, 7, 21, and 28. Samples were flushed with nitrogen and stored at -20°C in  
117 ~~refrigerator~~ until analysis, and the maximum storage time for the samples before  
118 analysis ~~should be~~ was ~~less than 10 days~~.

119 *2.2.5 Determination of peroxide value (PV) and free fatty acids (FFA) of herring roe*  
120 *oil and purified PLs*

121 PV was determined in herring roe oil and purified PLs using the colorimetric  
122 ferric-thiocyanate method at 500 nm as described by Shantha and Decker (1994). FFA  
123 was determined using the AOCS method Ca 5a-40 (1998). The measurement was  
124 performed in duplicate.

125 *2.2.6 Determination of tocopherol*

126 The tocopherol content was determined according to AOCS method Ce 8-89  
127 (1989b). Approximately 0.05 g of herring roe oil and purified PLs were dissolved in  
128 heptane (10 mL) and from this, 1.0 mL samples were analyzed by HPLC-FLD  
129 (Agilent 1100 series, Agilent Technologies, Santa Clara, CA) on a Water Spherisorb  
130 (R) 3 µm silica column (4.6 × 150 mm). The mobile phase consisted of heptane:  
131 isopropanol (100:0.4, v/v). The flow rate was 1 mL/min. Tocopherols were detected  
132 by fluorescence (FLD) detector at 290 nm excitation and emission at 330 nm. The  
133 determination was repeated twice and quantified by authentic standards.

134 *2.2.7 Determination of fatty acid composition of herring roe oil and purified PLs*

135 The fatty acid composition was determined according to AOCS Ce 2-66 (2000)  
136 with a minor modification. In detail, 30-60 mg of herring roe oil and purified PLs  
137 were weighed into vials, respectively. To each sample, 100 µL of internal standard  
138 (2%, w/v, C23:0 in heptane), 200 µL heptane with 0.01% BHT, and 100 µL of  
139 toluene were added and mixed. Afterwards, 1 µL of methanolic BF<sub>3</sub> (20% solution)  
140 was added, and the methylation glass was sealed with a Teflon cap and a screw lid.

141 The mixture was vortexed for 10 s and placed into a microwave oven (Multiwave  
142 3000 SOLV, Anton Paar, Graz, Austria). The conditions of the microwave for  
143 methylation was 5 min at 100 °C with a power of 500 W followed by cooling 10 min.  
144 Next, 1 mL of saturated NaCl solution and 0.7 mL of heptane were added and mixed  
145 for 10 s. Later, approximately 1 mL of upper layer of heptane was transferred to a GC  
146 vial for fatty acid composition analysis. During analysis, the fatty acid methyl esters  
147 were separated by GC-FID (HP 5890 A, Hewlett Packard, Palo Alto, CA) with a  
148 DB127-7012 column (10 m × ID 0.1 mm × 0.1 mm film thickness) using AOCS  
149 Official Method Ce 1b-89 (AOCS, 1989a). Quantification of DHA, EPA and total n-3  
150 polyunsaturated fatty acid (PUFAs) were conducted by using internal standard of  
151 C23:0. The analysis was carried out in duplicate.

#### 152 *2.2.8 Determination of volatiles by Dynamic Headspace GC-MS*

153 The procedure for volatiles determination was performed as described by  
154 Thomsen, et al. (2017) and Rørbæk (1994) with minor modifications. Approximately  
155 1.0 g of sample was weighed and mixed with 30 mg of internal standard solution (30  
156 µg/g of 4-methyl-1-pentanol in rapeseed oil). The volatiles were collected on Tenax®  
157 tubes (Gerstel, GmbH & Co. KG) for 30 min at 45°C with a nitrogen flow of 150  
158 mL/min. The collected volatiles were desorbed by using an automatic thermal  
159 desorber (ATD-400, PerkinElmer, Norwalk, CT, USA) at 220°C combined with  
160 Agilent 5890 IIA model (Palo Alto, CA, USA) GC connected to a MS HP 5972 mass  
161 selective detector. The initial oven temperature was 35°C for 3 min, with increment at  
162 3.0°C/min to 140°C, and increment at 5.0°C/min to 170°C, then increment at 10.0  
163 °C/min to 240°C, where it was held for 8 min. Measurements were made in triplicate  
164 in each sample. The individual compounds were confirmed by mass-spectrometry  
165 (HP 5973 inert mass-selective detector, Agilent Technologies, USA; Electron

166 ionization mode, 70 eV, mass to charge ratio scan between 30 and 250).

### 167 *2.2.9 Determination of pyrroles content*

168 The pyrroles content in cod liver oil containing different levels of PLs were  
169 determined according to Lu et al. (2012). Approximately 0.3 g of sample was weighed  
170 and 6 mL of chloroform–methanol (2:1) was added followed by addition of 2 mL of  
171 distilled water. After centrifugation (2500 rpm), the chloroform phase was analyzed  
172 for pyrroles content in duplicate. Pyrroles content were quantified using an authentic  
173 external standard [1-(4-methoxyphenyl)-1H-pyrrole] (at 570 nm). The pyrroles  
174 concentration are given as millimoles of 1-(4-methoxyphenyl)-1H-pyrrole per gram of  
175 sample.

176 **Statistical analysis.** The data were analyzed by analysis of variance (ANOVA)  
177 with Bonferroni's post-test for multiple comparisons using Minitab 16 statistical  
178 package (Minitab Inc., State College, PA). Significant differences were accepted at p  
179 < 0.05.

## 180 **3. Result and discussion**

### 181 *3.1 Formulation of cod liver oil with different levels of herring roe PLs*

182 To evaluate the effect of herring roe PLs on the oxidation stability of cod liver  
183 oil, different levels of herring roe PLs was added to cod liver oil as shown in Table 1.

### 184 *3.2 Chemical composition of herring roe oil and purified phospholipids (PLs)*

185 In this work, PLs were purified from herring roe oil, which contained high  
186 amount of PLs (approximately 30% of PLs in total herring roe oil) through acetone  
187 precipitation. Under this condition, the TAG and other nonpolar lipids were removed.  
188 The total PLs percentage increased from 33.75% to 72.30% (Table 2). In general, the  
189 purified PLs had higher content of PC, PI, LPC, LPE and APE than unpurified herring  
190 roe oil. The purified PLs also had a higher level of total LysoPLs (5.38%) than

191 unpurified sample (2.08%), indicating some level of hydrolysis of PLs during  
192 purification. This phenomenon was also observed in our previous work (Lu, Nielsen,  
193 Baron, Diehl, & Jacobsen, 2012). Also, the content of FFA increased when compared  
194 with untreated sample confirming that hydrolysis took place during purification.

195 As seen in Table 3, after purification, the PLs contained higher amounts of EPA  
196 and DHA when compared with the unpurified herring roe oil. The total content of  
197 EPA and DHA of purified PLs were 45.22% compared to 39.88% in unpurified  
198 herring roe oil. The DHA and EPA level in purified PLs were 33.24% and 11.98%,  
199 respectively. Mozuraityte et al. (2007) determined the isolated PLs from the total lipid  
200 of cod roe by acetone precipitation method. They found that the isolated PLs  
201 contained  $98 \pm 2\%$  of PLs, traces of cholesterol, and unknown compounds. On  
202 average, the isolated PLs was rich in DHA with  $26 \pm 9\%$  and EPA with  $11 \pm 2\%$  of  
203 total fatty acids. In our study, the purified herring roe PLs contained higher levels of  
204 DHA, but lower levels of PL. Tocher et al. (1984) analyzed the fatty acids  
205 composition in seven different types of fish roes and reported that the DHA and EPA  
206 levels in herring roe were 31.4% and 13.7%, respectively. Furthermore, the DHA  
207 level was higher than EPA level in different kinds of fish roe.

208 In addition, the peroxide value of herring roe oil was also determined before and  
209 after purification, and it was 0.62 mequiv/kg and 0.64 mequiv/kg, respectively. The  
210 result suggests that the purification method did not affect the oxidation of herring roe  
211 oil.

### 212 *3.3 Storage experiment of cod liver oil/herring roe phospholipids dispersion*

#### 213 *3.3.1 PV and FFA.*

214 As seen in Fig. 1 (a), at the beginning of storage, PV increased significantly with  
215 an increase in content of PLs. This phenomenon may be due to the removal of

216 tocopherol in purified PL. However, the PV showed significantly lower ( $P < 0.05$ )  
217 increment during storage after 21 days when FO with added PLs was compared with  
218 FO. This result may be due to the antioxidant activity of PLs, or due to faster  
219 decomposition and reaction to secondary and tertiary oxidation products when FO  
220 was replaced with herring roe PLs.

221 The proportion of FFA in purified PLs was significantly higher than herring roe  
222 oil after purification. Thus, the FFA in FO with PLs added increased with increasing  
223 levels of PL added (Fig. 1(b)). ~~As the storage proceeded, the content of FFA was~~  
224 ~~slightly increased slightly but significantly in FO with low levels of PLs added ( $P >$~~   
225 ~~0.05), but whereas it increased more in significantly in FPL5 ( $P < 0.05$ ).~~ This finding  
226 ~~may due to the high content of FFA in FO with 50% of PLs added could indicate that~~  
227 ~~lipases in herring roe PL were still active during storage, but further investigations are~~  
228 ~~needed to confirm this hypothesis.~~

### 229 3.3.2 Secondary lipid oxidation products: volatiles

230 In order to further study the oxidative stability of FO with PLs, the concentration  
231 of volatiles were measured during storage. Fig. 2 show the changes in benzaldehyde,  
232 2,5-dimethylpyrazine, 2-methyl-2-pentenal, 1-penten-3-ol, and 3-methylbutanal.  
233 Benzaldehyde was detected in FPL1~FPL5 after 7 days incubation at 40°C. In  
234 particular, the level of benzaldehyde in FPL5 was the highest one from 7 days to 28  
235 days followed by FPL3>FPL1>FPL2>FPL4. Benzaldehyde was only observed in FO  
236 after 21 days storage. It was reported that benzaldehyde was strongly correlated with  
237 sensory properties of FO (Guillén, Carton, Salmeron, & Casas, 2009), and Giogios et  
238 al. (2009) assumed that benzene compounds could be decomposition products of  
239 amino acid or sugars due to their low level in FO. As illustrated by benzaldehyde in  
240 Fig. 2, lipid oxidation occurred in FO after 21 days storage, and the degradation of

241 PLs may play a key role in producing more benzaldehyde in FO.

242 Furthermore, Fig.2 (B) showed an appreciable increase in pyrazines as  
243 demonstrated by the presence of 2,5-dimethylpyrazine ~~in this study~~. There was no  
244 2,5-dimethylpyrazine in FO and FPL1. However, the content of 2,5-dimethylpyrazine  
245 ~~was~~ increased with increasing content of herring roe PLs in FO, especially FPL5 was  
246 higher than other treatments. This finding demonstrated that herring roe PLs played  
247 an important role in producing pyrazines. The effect of thermal treatment towards  
248 pyrazines formation was also reported in other studies. For instance, Lu and  
249 co-workers reported that 2,5-dimethylpyrazine and 2-ethylpyridine increased during  
250 storage ( $P < 0.05$ ) in krill oil incubated at 40°C (Lu, Bruheim, Haugsgjerd, &  
251 Jacobsen, 2014). In addition, Baek and Cadwallader reported that  
252 2,5-dimethylpyrazine increased drastically after enzymatic hydrolysis of crayfish  
253 hydrolysate at 65°C (Baek & Cadwallader, 1996). The formation mechanism of all  
254 those pyrazines, pyridines and their alkyl derivatives were discussed in detail in our  
255 previous work on non-enzymatic browning reactions in krill oil upon storage (Lu,  
256 Bruheim, Haugsgjerd, & Jacobsen, 2014). The possible pathways may involve lipid  
257 oxidation followed by reaction of lipid oxidation products and amino acids in marine  
258 PLs. They are important reactants for pyrazines formation. Thus, marine PLs may  
259 produce pyrazine when added to FO due to lipid oxidation.

260 As shown in Fig. 2(C), the content of 2-methyl-2-pentenal was increased with an  
261 increase in the amount of PLs added to FO at day 0, and further increased during  
262 storage. 2-methyl-2-pentenal was suggested to be the major volatile Strecker aldehyde  
263 resulting from a reaction between secondary lipid oxidation products originating from  
264 (*E,E*)-2,-4-heptadienal with lysine (Zamora, Ríos, & Hidalgo, 2010). Hence, the  
265 higher formation of 2-methyl-2-pentenal during storage with increasing content of

266 herring roe PLs suggested that lipid oxidation followed by non-enzymatic browning  
267 reactions took place to a higher degree when higher amounts of herring roe PLs were  
268 present.

269 1-Penten-3-ol is a typical lipid oxidation product (Eymard, Baron, & Jacobsen,  
270 2009) and is formed from the decomposition of hydroperoxides of the omega-3 fatty  
271 acids (Olsen, Vogt, Saarem, Greibrokk, & Nilsson, 2005). Lu et al. (2014) compared  
272 the increment rate of 1-penten-3-ol from Day 0 to 7 (in area per day) during  
273 incubation of krill oil at 40°C and found a 3-fold increment of 1-penten-3-ol  
274 compared to 20°C.

275 In this work, we also determined the compound of 1-penten-3-ol as the typical  
276 lipid oxidation product. As shown in Fig. 2(D), 1-penten-3-ol was observed in all  
277 samples after day 7 in this study. Therefore, lipid oxidation occurred in each sample  
278 from day 7. In particular, FO and FPL5 contained higher levels of 1-penten-3-ol when  
279 compared to other treatments after 28 days. Interestingly, the dramatic increase of  
280 1-penten-3-ol in FO and FPL5 from day 21 was probably related to their high degree  
281 of unsaturation. Further research is however still needed to understand why the  
282 formation of 1-penten-3-ol changed so dramatically between FPL4 and FPL5.

283 As shown in Fig. 2(E), 3-methylbutanal was observed in all samples, and  
284 followed by FPL1>FPL2>FPL3>FPL4>FPL5. In the present study,  
285 3-methylbutanal increased with the increment of PLs in FO over 28 days of  
286 incubation at 40°C. 3-Methylbutanal is a degradation product from amino acids, such  
287 as valine, isoleucine and leucine (Lu, Nielsen, Baron, Diehl, & Jacobsen, 2013). It is  
288 formed from the reaction between these amino acids with tertiary lipid oxidation  
289 products, namely unsaturated epoxy keto fatty esters, epoxyalkenals, and  
290 hydroxyalkenals. The mechanism was discussed in detail by Lu et al.(2012). It is

291 necessary to further understand the relationship between FO and PLs for forming  
292 3-Methylbutanal during storage.

293 Taken together, our finding suggested that cod liver oil with dispersed herring  
294 roe PLs oxidized during storage, and non-enzymatic browning reactions also took  
295 place after oxidation reaction.

### 296 3.3.3 Pyrrolisation

297 In this study, pyrrolisation in FO containing different levels of herring roe PLs  
298 was investigated via measurement of hydrophobic pyrroles (Fig. 3), since the  
299 hydrophobic pyrroles contributed more to browning than hydrophilic ones (Hidalgo,  
300 Nogales, & Zamora, 2005). No pyrroles was found in any sample at the beginning of  
301 storage, and no pyrroles were formed in FO during storage up to 28 days.

302 It was clear that the hydrophobic pyrroles increased with increasing herring roe  
303 PLs addition from FPL1 to FPL5 (Fig. 3) confirming the results obtained for  
304 pyrazines and Strecker aldehydes in Fig. 2. When comparing the concentrations of  
305 pyrroles in FPL4 and FPL5 (0.67 mM vs 2.45 mM in FPL5), it is surprising that the  
306 content of pyrroles in FPL5 was more than twice as high as in FPL4 despite the fact  
307 that the content of marine PL in FPL5 was less than twice as high as in FPL4. Results  
308 from the analysis of pyrazines and 2-methyl-2-pentenal (Fig. 2) also suggested a  
309 much larger difference between FPL4 and FPL5 than what could be expected from  
310 differences in the content of marine PL in these samples. This may be related to the  
311 relatively lower content of tocopherol in FPL5 as [asis](#) also discussed below. Further  
312 studies are needed to explain this accelerating effect of the non-enzymatic browning  
313 reactions of increasing the content of marine PL from 20 to 33 % of total lipids.

314 The presence of hydrophobic pyrroles might offer additional protection to FO  
315 against lipid oxidation. Based on the lower PV, the relative oxidative stability of FO



316 containing PLs may be partly affected by the higher content of pyrroles. However, the  
317 PV of FPL5 was higher than other dispersions containing PLs. It can be speculated  
318 that this was due to the lower content of tocopherol in FPL5 compared with other  
319 treatments (FPL1-FPL4). It can be calculated that the content of  $\alpha$ -tocopherol in FO  
320 was 141  $\mu$ g/g, but 93.06 mg/kg in FPL5. As we mentioned before, the tocopherol in  
321 herring roe oil was removed during precipitation with cold acetone. It has been shown  
322 that the antioxidant activity of pyrroles can be improved by  $\alpha$ -tocopherol (Hidalgo,  
323 León & Zamora, 2007; Lu, Nielsen, Baron, Diehl & Jacobsen, 2012). Hence, the ratio  
324 between pyrroles and  $\alpha$ -tocopherol may have influenced the formation of peroxides  
325 and secondary oxidation products in FPL1-FPL5. This deserves further investigation.

#### 326 **4. Conclusions**

327 The purified herring roe PLs contained high level of DHA, and the main  
328 component was PC, followed by PE. In addition, the present work evaluated the effect  
329 of purified herring roe PLs on the oxidation stability of FO during 28 days of storage  
330 at 40°C. The results demonstrated that after 21 and 28 days PV was lower in FO  
331 containing purified PLs, and the FFA in FO with PLs added increased with FPL1 to  
332 FPL5. Furthermore, the concentration of volatiles, including benzaldehyde,  
333 2,5-dimethylpyrazine, 2-methyl-2-pentenal, 1-penten-3-ol, and 3-methylbutanal were  
334 monitored to evaluate the formation of volatile oxidation and non-enzymatic reaction  
335 products. The results suggested that lipid oxidation was followed by non-enzymatic  
336 browning reactions. Furthermore, pyrroles, which may protect against formation of  
337 new peroxides, were formed. However, the ability of pyrroles to confer this protection  
338 may be influenced by the level of  $\alpha$ -tocopherol and pyrroles formed.

#### 339 **AUTHOR INFORMATION**

#### 340 **Corresponding Author**

341 \*Phone: +45 45 25 25 59. E-mail: chja@food.dtu.dk.

## 342 **Notes**

343 The authors declare no competing financial interest.

## 344 **FUNDING**

345 This work is supported by the National Natural Science Foundation of China (Grant  
346 No. 31801465) and Outstanding Young Scientific Research Talent Program of Fujian  
347 Agriculture and Forestry University (xjq20180).

## 348 **ACKNOWLEDGMENTS**

349 We thank Thierry COSTE (Novastell, France) for free marine phospholipid samples.  
350 We also greatly appreciate Inge Holmberg and Thi Thu Trang Vu's kind help for the  
351 volatiles and lipid chemical composition analysis.

## 352 **References**

- 353 AOCS.Official Method Ca 5a-40. Free fatty acids method. In Official methods and recommended  
354 practices of the American Oil Chemists' Society (4th ed.). AOCS Press: Campaign, IL. USA.  
355 1989a.
- 356 AOCS.Official Method Ce 8-89. Determination of tocopherols and tocotrienols in vegetable oils and  
357 fats by HPLC. AOCS Press, Champaign, IL. USA. 1989b.
- 358 AOCS.Method Ca 5a-40: Free Fatty Acids. In: Firestone, D. (Eds.), Official Methods and  
359 Recommended Practices of the American Oil Chemists' Society, fifth edition, American Oil  
360 Chemists' Society, Champaign, IL, USA. 1998.
- 361 AOCS. (2000). Official Method Ce 2-66 Preparation of Methyl Esters of Fatty Acids. In): American  
362 Oil Chemists' Society Urbana, IL, USA.
- 363 Baek, H., & Cadwallader, K.Volatile compounds in flavor concentrates produced from

- 364 crayfish-processing byproducts with and without protease treatment. *Journal of Agricultural*  
365 *and Food Chemistry*[J], 1996.44(10), 3262-3267.
- 366 Belhaj, N., Arab-Tehrany, E., & Linder, M. Oxidative kinetics of salmon oil in bulk and in  
367 nanoemulsion stabilized by marine lecithin. *Process Biochemistry*[J], 2010.45(2), 187-195.
- 368 Bjørndal, B., Strand, E., Gjerde, J., Bohov, P., Svardal, A., Diehl, B. W., Innis, S. M., Berger, A., &  
369 Berge, R. K. Phospholipids from herring roe improve plasma lipids and glucose tolerance in  
370 healthy, young adults. *Lipids in Health and Disease*[J], 2014.13(1), 82.
- 371 Burri, L., Hoem, N., Banni, S., & Berge, K. Marine omega-3 phospholipids: metabolism and biological  
372 activities. *International Journal of Molecular Sciences*[J], 2012.13(11), 15401-15419.
- 373 Eymard, S., Baron, C. P., & Jacobsen, C. Oxidation of lipid and protein in horse mackerel (*Trachurus*  
374 *trachurus*) mince and washed minces during processing and storage. *Food Chemistry*[J],  
375 2009.114(1), 57-65.
- 376 Gbogouri, G. A., Linder, M., Fanni, J., & Parmentier, M. Analysis of lipids extracted from salmon  
377 (*Salmo salar*) heads by commercial proteolytic enzymes. *European Journal of Lipid Science*  
378 *and Technology*[J], 2006.108(9), 766-775.
- 379 Giogios, I., Grigorakis, K., Nengas, I., Pappasolomontos, S., Papaioannou, N., & Alexis, M. N. Fatty  
380 acid composition and volatile compounds of selected marine oils and meals. *Journal of the*  
381 *Science of Food and Agriculture*[J], 2009.89(1), 88-100.
- 382 Guillén, M. D., Carton, I., Salmeron, J., & Casas, C. Headspace composition of cod liver oil and its  
383 evolution in storage after opening. First evidence of the presence of toxic aldehydes. *Food*  
384 *Chemistry*[J], 2009.114(4), 1291-1300.
- 385 Hidalgo, F. J., León, M. M., & Zamora, R. Effect of tocopherols in the antioxidative activity of oxidized

- 386 lipid- amine reaction products. *Journal of Agricultural and Food Chemistry*[J], 2007.55(11),  
387 4436-4442.
- 388 Hidalgo, F. J., Nogales, F., & Zamora, R.Changes produced in the antioxidative activity of  
389 phospholipids as a consequence of their oxidation. *Journal of Agricultural and Food*  
390 *Chemistry*[J], 2005.53(3), 659-662.
- 391 Hudson, B. J., & Mahgoub, S. E.Synergism between phospholipids and naturallyoccurring antioxidants  
392 in leaf lipids. *Journal of the Science of Food and Agriculture*[J], 1981.32(2), 208-210.
- 393 King, M., Boyd, L., & Sheldon, B.Antioxidant properties of individual phospholipids in a salmon oil  
394 model system. *Journal of the American Oil Chemists Society*[J], 1992.69(6), 545-551.
- 395 Liang, P., Cheng, X., Xu, Y., Cheng, W., & Chen, L.Determination of fatty acid composition and  
396 phospholipid molecular species of large yellow croaker (*Pseudosciaena crocea*) roe from  
397 China. *Journal of Aquatic Food Product Technology*[J], 2017. 26(10),1259-1265.
- 398 Lu, F., Bruheim, I., Haugsgjerd, B., & Jacobsen, C.Effect of temperature towards lipid oxidation and  
399 non-enzymatic browning reactions in krill oil upon storage. *Food Chemistry*[J], 2014.157,  
400 398-407.
- 401 Lu, F., Nielsen, N. S., Baron, C. P., Diehl, B., & Jacobsen, C.Impact of primary amine group from  
402 aminophospholipids and amino acids on marine phospholipids stability: Non-enzymatic  
403 browning and lipid oxidation. *Food Chemistry*[J], 2013.141(2), 879-888.
- 404 Lu, F., Nielsen, N. S., Baron, C. P., & Jacobsen, C.Oxidative degradation and non-enzymatic browning  
405 due to the interaction between oxidised lipids and primary amine groups in different marine  
406 PL emulsions. *Food Chemistry*[J], 2012.135(4), 2887-2896.
- 407 Lu, F. H., Nielsen, N. S., Baron, C. P., Diehl, B. W., & Jacobsen, C.Oxidative stability of dispersions

- 408 prepared from purified marine phospholipid and the role of  $\alpha$ -tocopherol. *Journal of*  
409 *Agricultural and Food Chemistry*[J], 2012.60(50), 12388-12396.
- 410 Lu, F. S. H., Nielsen, N. S., Baron, C. P., Diehl, B. W. K., & Jacobsen, C. Oxidative Stability of  
411 Dispersions Prepared from Purified Marine Phospholipid and the Role of  $\alpha$ -Tocopherol.  
412 *Journal of Agricultural & Food Chemistry*[J], 2012.60(50), 12388-12396.
- 413 Lu, F. S. H., Nielsen, N. S., Baron, C. P., Diehl, B. W. K., & Jacobsen, C. Impact of primary amine  
414 group from aminophospholipids and amino acids on marine phospholipids stability:  
415 Non-enzymatic browning and lipid oxidation. *Food Chemistry*[J], 2013.141(2), 879-888.
- 416 Moriya, H., Kuniminato, T., Hosokawa, M., Fukunaga, K., Nishiyama, T., & Miyashita, K. Oxidative  
417 stability of salmon and herring roe lipids and their dietary effect on plasma cholesterol levels  
418 of rats. *Fisheries Science*[J], 2007.73(3), 668-674.
- 419 Mozuraityte, R., Rustad, T., & Storror, I. The role of iron in peroxidation of polyunsaturated fatty acids  
420 in liposomes. *Journal of Agricultural and Food Chemistry* [J], 2007.56(2), 537-543.
- 421 Olsen, E., Vogt, G., Saarem, K., Greibrokk, T., & Nilsson, A. Autoxidation of cod liver oil with  
422 tocopherol and ascorbyl palmitate. *Journal of the American Oil Chemists' Society*[J],  
423 2005.82(2), 97-103.
- 424 Rørbæk, K. (1994). *Oxidation and flavours in fish oil*. Ph. D. thesis. Technological Laboratory Ministry  
425 of Fisheries and Center for Food Research, Technical University of Denmark, Lyngby.
- 426 Saito, H., & Ishihara, K. Antioxidant activity and active sites of phospholipids as antioxidants. *Journal*  
427 *of the American Oil Chemists' Society*[J], 1997.74(12), 1531-1536.
- 428 Shantha, N. C., & Decker, E. A. Rapid, sensitive, iron-based spectrophotometric methods for  
429 determination of peroxide values of food lipids. *Journal of AOAC International*[J],

- 430 1994.77(2), 421-424.
- 431 Shirai, N., Higuchi, T., & Suzuki, H. Analysis of lipid classes and the fatty acid composition of the  
432 salted fish roe food products, Ikura, Tarako, Tobiko and Kazunoko. *Food Chemistry*[J],  
433 2006.94(1), 61-67.
- 434 Thomsen, B., Horn, A., Hyldig, G., Taylor, R., Blenkiron, P., & Jacobsen, C. Investigation of Lipid  
435 Oxidation in High-and Low-Lipid-Containing Topical Skin Formulations. *Journal of the*  
436 *American Oil Chemists' Society*[J], 2017.94(10), 1287-1300.
- 437 Tocher, D. R., & Sargent, J. R. Analyses of lipids and fatty acids in ripe roes of some northwest  
438 European marine fish. *Lipids*[J], 1984.19(7), 492-499.
- 439 Zamora, R., Ríos, J. J., & Hidalgo, F. J. Formation of volatile pyrrole products from  
440 epoxyalkenal/protein reactions. *Journal of the Science of Food & Agriculture*[J], 2010.66(4),  
441 543-546.

443 **Figure captions**

444 **Fig. 1** Measurement of PV and FFA in FO and FO with added levels of PLs during 28  
445 days of storage at 40 °C. Values are mean  $\pm$  standard deviation (n = 3). (a) PV; (b)  
446 FFA. Note: Letters in lower case indicate comparisons between values on the same  
447 day with different concentrations, and letters in upper case indicate comparisons  
448 between values of the same concentrations on different days. Note: FO, cod liver oil  
449 as control; FPL1, 2.5% of PLs added in FO; FPL2, 10% of PLs added in FO; FPL3,  
450 20% of PLs added in FO; FPL4, 25% of PLs added in FO; FPL5, 50% of PLs added  
451 in FO. Values are the mean  $\pm$  standard deviation (n = 3).

452 **Fig. 2** Measurement of benzaldehyde, 2,5-dimethylpyrazine, 2-methyl-2-pentenal,  
453 1-penten-3-ol, 3-methylbutanal in FO and FO with added levels of PLs during 28 days  
454 of storage at 40 °C. Values are the mean  $\pm$  standard deviation (n = 3).

455 **Fig. 3** Comparison of pyrroles in 50% of PLs in FO (FML5) after 28 days of storage  
456 at 40 °C. Values are the mean  $\pm$  standard deviation (n = 2). Note: Letters in lower case  
457 indicate comparisons between values on the same day with different concentrations of  
458 PLs. Note: FPL1, 2.5% of PLs added in FO; FPL2, 10% of PLs added in FO; FPL3,  
459 20% of PLs added in FO; FPL4, 25% of PLs added in FO; FPL5, 50% of PLs added  
460 in FO.

461 **Tables:**

462 Table 1. Experimental design for the cod liver oil containing herring roe PLs

463 Table 2. Composition of herring roe oil before and after acetone precipitation

464 Table 3. Fatty acid compositions of herring roe oil PLs before and after acetone

465 precipitation

Journal Pre-proofs



Fig. 1.

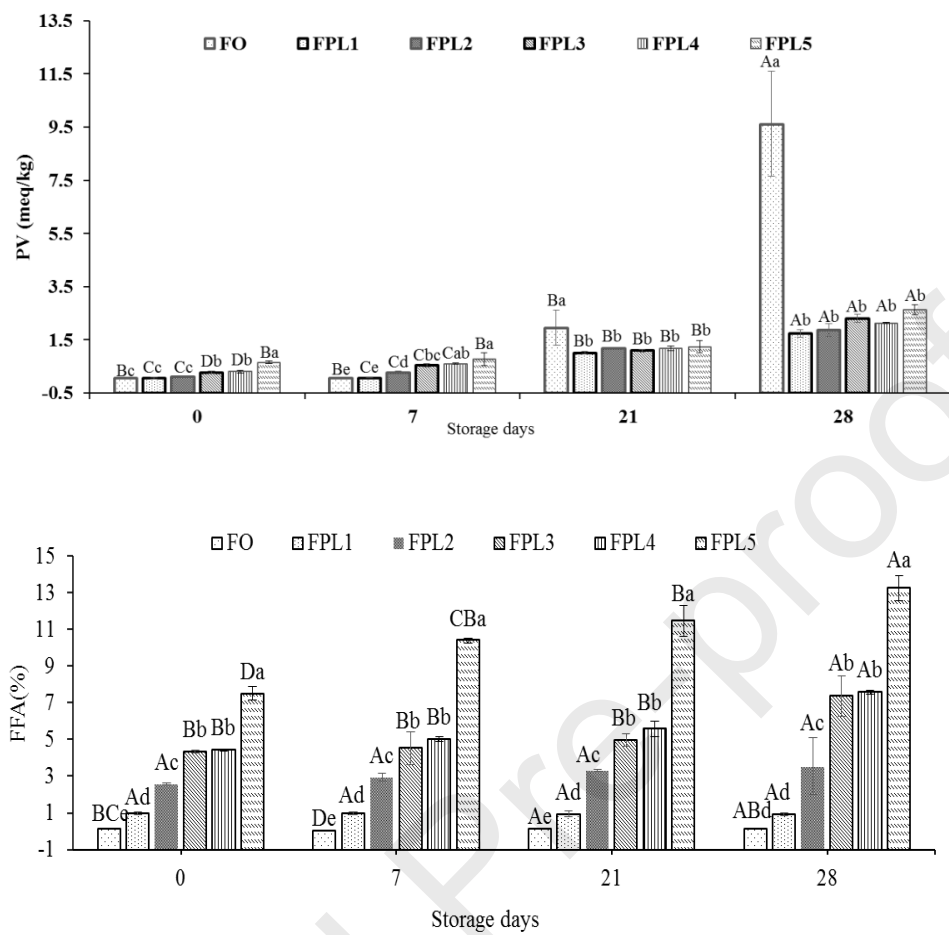


Fig. 2.

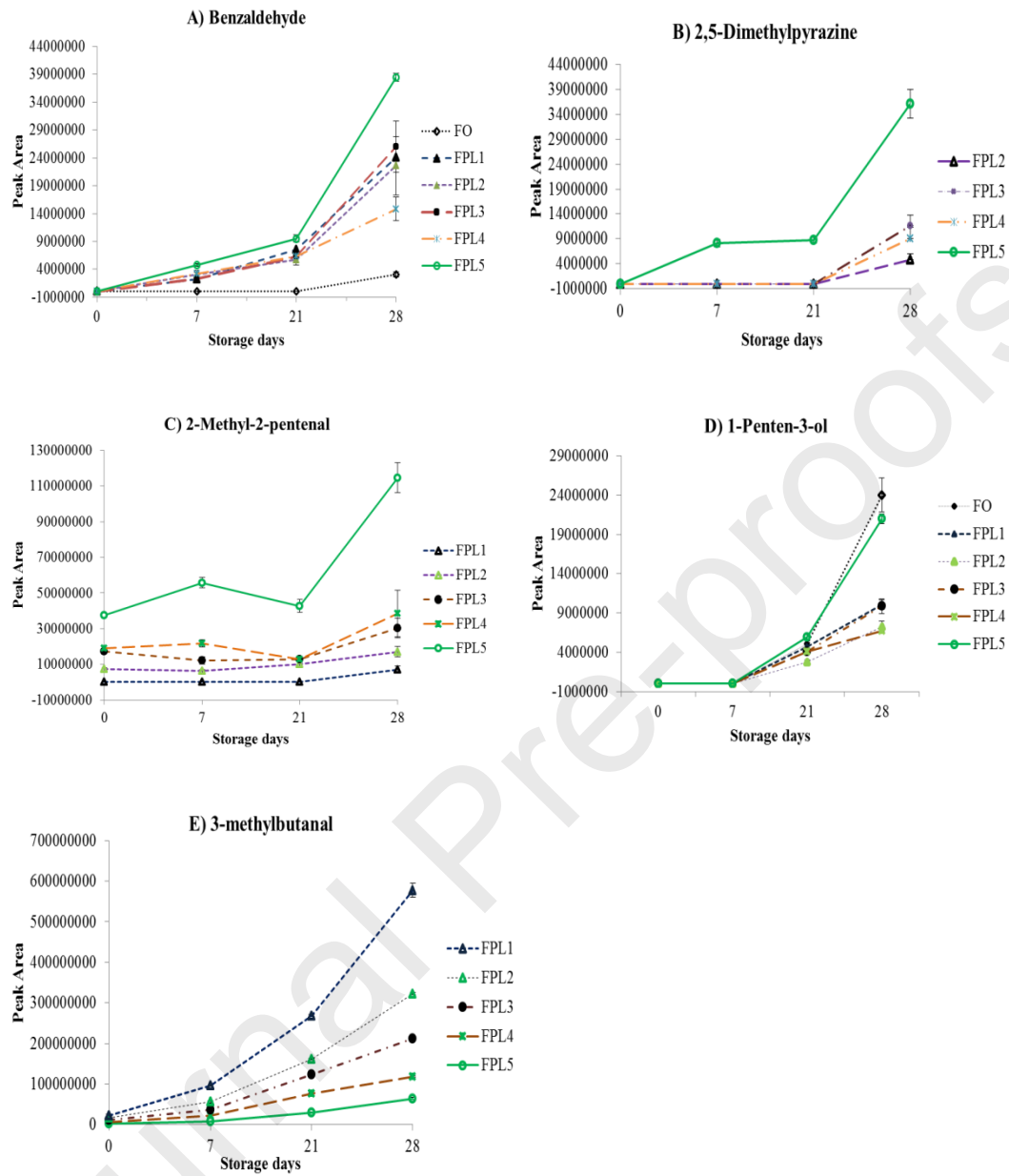


Fig. 3.

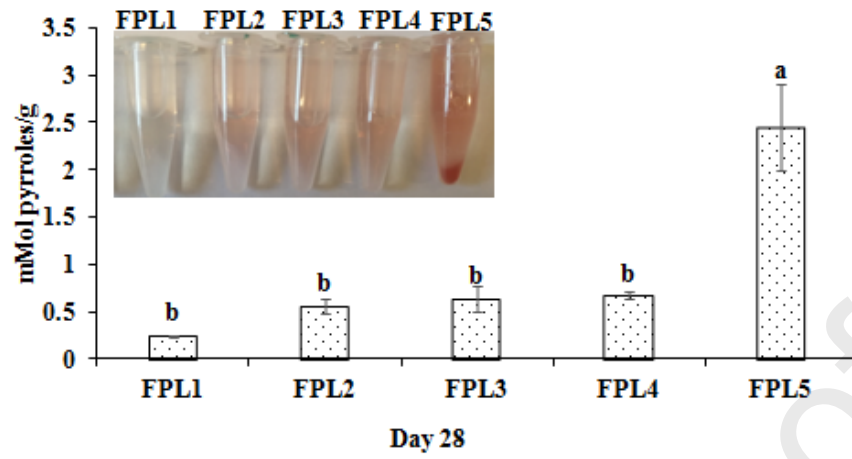


Table 1.

items	Cod liver oil (g)	Added PLs to cod liver oil (g)	PLs relative to cod liver oil (%)
FO	100.0	0.0	0.0
FPL1	97.56	2.44	2.50
FPL2	90.91	9.09	10.00
FPL3	83.33	16.67	20.00
FPL4	80.00	20.00	25.00
FPL5	66.67	33.33	50.00

Note: FO, cod liver oil; FPL1, 2.5% of PLs added in cod liver oil; FPL2, 10% of PLs added in FO; FPL3, 20% of PLs added in FO; FPL4, 25% of PLs added in FO; FPL5, 50% of PLs added in FO.

Table 2.

	Before purification	After purification
	herring roe oil	herring roe PLs
Total phospholipids of total lipid (%)	33.75	72.30
Phosphatidylcholine (PC) of total PLs (%)	26.94	55.40
Lysophosphatidylcholine (1-LPC) of total PLs (%)	0.20	0.41
Lysophosphatidylcholine (2-LPC) of total PLs (%)	1.58	4.06
Phosphatidylinositol (PI) of total PLs (%)	0.87	2.44
Phosphatidylethanolamine (PE) of total PLs (%)	3.33	5.49
Lysophosphatidyl ethanolamine of total PLs (LPE) (%)	0.30	0.81
Acylated phosphatidyl ethanolamine (APE) of total PLs (%)	ND	0.61
Phosphatidate (PA) of total PLs (%)	0.18	0.40
Lysobisphosphatidic acids (LPA) of total PLs (%)	ND	0.10
Other phospholipids of total PLs (%)	0.34	2.63
Peroxide value (mequiv/kg)	0.62±0.01	0.64±0.03
Free fatty acid (%)	22.61±1.09	31.37±6.43
α-Tocopherol (mg/kg)	222±1.05	ND
γ-Tocopherol (mg/kg)	319±1.23	ND
δ-Tocopherol (mg/kg)	112±0.74	ND

PLs=phospholipids

ND=not detected

Table 3.

	Herring roe oil (% of total fatty acids)	Herring roe PLs (% of total fatty acids)
C14:0	2.28±0.10	2.43±0.01
C14:1	0.13±0.00	0.15±0.00
C15:0	0.46±0.03	0.56±0.00
C16:0	16.77±0.90	23.18±0.90
C16:1(n-7)	2.92±0.02	1.56±0.01
C16:2(n-4)	0.09±0.00	0.06±0.00
C16:3(n-4)	0.64±0.00	0.36±0.00
C17:0	0.20±0.00	0.31±0.00
C17:1	-	0.20±0.00
C16:4(n-3)	0.07±0.00	0.38±0.00
C18:0	1.40±0.01	2.86±0.02
C18:1(n-9)	4.24±0.10	5.47±0.08
C18:1(n-7)	2.67±0.09	3.52±0.04
C18:2(n-6)	0.20±0.00	0.81±0.00
C18:2(n-4)	-	0.06±0.00
C18:3(n-6)	0.13±0.00	0.08±0.00
C18:3(n-4)	-	0.12±0.00
C18:3(n-3)	0.37±0.00	0.32±0.00
C18:4(n-3)	0.59±0.00	0.30±0.00
C18:5(n-3)	0.04±0.00	0.05±0.00
C20:0	-	0.06±0.00
C20:1(n-9, n-11)	-	0.70±0.00
C20:1(n-7)	0.93±0.00	0.73±0.00
C20:2(n-6)	0.07±0.00	0.13±0.00
C20:3(n-6)	0.06±0.00	0.05±0.00
C20:4(n-6)	0.59±0.00	0.86±0.00
C20:3(n-3)	0.16±0.00	0.05±0.00
C20:4(n-3)	0.76±0.00	0.42±0.00
<b>C20:5</b>	<b>10.37±0.50</b>	<b>11.98±0.80</b>
<b>(n-3)EPA</b>		
C22:1(n-11)	0.03±0.00	0.03±0.00
C22:1(n-9)	0.20±0.00	0.37±0.00
C21:5(n-3)	7.85±0.70	0.16±0.00
C22:5(n-3)	1.34±0.00	0.99±0.00
<b>C22:6</b>	<b>29.51±1.20</b>	<b>33.24±1.40</b>
<b>(n-3)DHA</b>		
C24:1(n-9)	1.69±0.00	-
C24:0	3.34±0.08	1.02±0.00
Others	9.90±0.85	6.43±0.04
<b>EPA + DHA</b>	<b>39.88±1.70</b>	<b>45.22±2.20</b>
ΣSAFA	24.45±0.31	30.42±0.93
ΣMUFA	12.81±0.11	12.73±0.13
ΣPUFA	52.84±2.40	50.42±2.20

PLs=phospholipids

Values are means ± SD, n=2.

### Highlights:

- The effect of herring roe phospholipids on the oxidative stability of cod liver oil was investigated systematically.

- Benzaldehyde, 2,5-dimethylpyrazine, 2-methyl-2-pentenal, 1-penten-3-ol and 3-methylbutanal were the main volatiles during storage at 40°C.
- Significant pyrrolisation was observed after 28 days when herring roe phospholipids were added to cod liver oil.
- The cod liver oil with dispersed herring roe phospholipids was oxidized during storage followed by non-enzymatic browning reactions

**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships

that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered

as potential competing interests