**Development of a solid-phase microextraction method for
determination of volatile oxidation compounds in fish oil emulsions**

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11 **Abstract**

12

13 Headspace solid-phase microextraction (HS-SPME) is proposed for isolating and determining the
14 headspace volatiles formed during oxidation of fish oil in water emulsions. Three different fiber
15 coatings were tested and compared for sensitivity and reproducibility. A
16 carboxen/polydimethylsiloxane (CAR-PDMS) fiber coating was found to be the most suitable for
17 the analysis of emulsion volatiles. The main factors affecting the microextraction process on CAR-
18 PDMS were then evaluated by a factorial design: amount of sample, time and temperature of
19 extraction and stirring. The incubation of 0.5 grams of emulsion at 60 °C during 30 minutes leads to
20 the most effective extraction of volatiles associated to lipid oxidation of fish oil emulsions. The HS-
21 SPME method coupled to GC-MS allowed the qualitative and quantitative analysis of the volatiles
22 derived from oxidation of real fish oil enriched foods as milk and mayonnaise. The method here
23 proposed is very fast and simple and yields high sensitivity, with good repeatability for all target
24 compounds.

25

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27 Keywords: SPME, emulsion, fish oil, volatiles, milk, mayonnaise

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Eliminado: and accurate

29 1. Introduction

30

31 As the intake of n-3 oils in the western world is relatively low, efforts to increase the consumption
32 of marine fatty acids by incorporating fish oil into different food products such as bread [1]
33 yoghurt drinks [2], salad dressed [3] and mayonnaise [4] are common. However, foods containing
34 n-3 fatty acids are highly susceptible to oxidation, leading to undesirable flavors and loss of
35 bioactive n-3 fatty acids [5, 6]. As a result of lipid oxidation, volatile compounds are formed and
36 affect the sensory perception of food products. Among volatiles associated to marine oil oxidation,
37 1-penten-3-one, (pungent green odor), Z-4-heptenal (fishy odor), (E,E)-2,4-heptadienal and (E,Z)-
38 2,6-nonadienal (cucumber odor) have been characterized as very potent odorants, contributing to
39 the unpleasant rancid and fishy off-flavor in bulk fish oil, fish oil enriched mayonnaise, and fish oil
40 enriched fish emulsion [7, 8]. Other formed volatiles are propanal, resulting from n-3 fatty acids
41 oxidation as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and pentane and
42 hexanal, indicators of n-6 fatty acids oxidation [9]. Previous studies on the oxidation in several fish
43 oil and fish oil derived products revealed the formation of 1-penten-3-ol, E-2-hexenal, E-2-octenal
44 or 1,5-octadien-3-ol by means of enzymatic mechanisms whereas (E,E)-2,4-heptadienal, (E,Z)-2,4-
45 heptadienal or 3,5-octadien-2-one are believed to be derived from autooxidation mechanisms [2].

46 Several GC (gas chromatography) procedures have been used to identify and quantify volatiles in
47 seafood. Solvent extraction, simultaneous distillation/extraction, conventional solid phase
48 extraction, supercritical fluid extraction (SFE), and static (SHS) and dynamic (DHS) headspace
49 sampling have been widely used as isolation methods [7, 10, 11]. Techniques based on SFE use a
50 variety of fluids with organic solvents, high pressures and elevated temperatures. In addition, they
51 are highly matrix-dependents and require well qualified operators [12]. SHS sampling is a simple,
52 fast and cheap technique to implement but usually lacks of enough sensitivity because of the low
53 content of the extracted compounds. It has been widely used for analyzing pentane, hexanal and
54 propanal as indicators of PUFA oxidation in biological samples [13, 14]. Preparation techniques
55 involving headspace concentration through the use of porous polymer absorbents (DHS) have been
56 extensively used for the analysis of aroma compounds in fish oils or fish oil enriched emulsions [15,
57 16].

58 SPME was developed by Pawliszyn and co-workers [17] and combines sampling and sample
59 preparation in one step. It can be a fast, sensitive, solventless and economical method for sample
60 preparation before gas chromatography analysis in comparison to well established techniques for
61 analyzing volatiles in foods. The type of fiber, sample volume, temperature, salting-out effect and

62 extraction or desorption time are parameters affecting the preconcentration efficiency of SPME
63 [18]. Techniques based on the analysis of the head space are very useful for determining volatile
64 compounds. Particularly head space-SPME (HS-SPME) has been successfully used to measure the
65 volatile flavor profiles of foodstuffs as oils [19, 20], fish muscle [21, 22], cooked turkey [23], dry-
66 cured loin [24], potato crisp [25] or tomato and strawberry fruits [26]. HS-SPME methodology
67 appears suitable for automation and is more selective than SPME. In addition, it is a faster method
68 than DHS analysis [27] and can provide better precision and linearity response [28]. It has been
69 used to investigate volatiles during photosensitized and free radical oxidation in fish oil emulsions.
70 [29].

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determining volatile compounds
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polymer coatings

71 The aim of this paper is to optimize a method based on HS-SPME coupled to GC–MS for the rapid
72 analysis and characterization of volatiles resulting from lipid oxidation of fish oil enriched food
73 emulsions. Firstly the main factors affecting the extraction were optimized using an experimental
74 design. Then the suitability of the technique to determine volatiles associated to rancidity in real
75 food emulsions was evaluated during oxidation of fish oil enriched milk and fish oil enriched
76 mayonnaise.

78 2. Experimental

80 2.1. Reagents

81
82 L- α -phosphatidylcholine, butanal *E*-2-pentenal, 2-pentylfuran, (*E,E*)-2,4-heptadienal, and *E*-2-
83 hexenal were obtained from Sigma-Aldrich (Steinheim, Germany). Acetaldehyde, pentane,
84 propanal, 2-ethylfuran, 1-penten-3-one, pentanal, 1-penten-3-ol, octane, hexanal, *E*-2-hexenal,
85 heptanal, *E*-2-heptenal, and 3-methyl-3-buten-1-ol (used as internal standard) were purchased from
86 Fluka (New-Ulm, Switzerland). All chemicals and solvents used were either analytical or HPLC
87 grade (Ridel-Haën, Seelze, Germany).

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hexanal, 2-ethylfuran, pentane, *E*-
2-heptenal, 1-penten-3-one, 1-
penten-3-ol, (*E,E*)-2,4-heptadienal

89 2.2. Materials

91 Three SPME fibers were tested: 75µm Carboxen/polydimethylsiloxane coating (CAR-PDMS),
92 65µm Polydimethylsiloxane/divinylbenzene coating (PDMS-DVB) and 65µm
93 Carbowax/divinylbenzene coating fibers. They were obtained from Supelco (Bellefonte, PA).
94 Cod liver oil obtained from Fluka (New-Ulm, Switzerland) was composed by 5.8% of 14:0, 11.9%
95 of 16:0, 6.7% of 16:1 ω7, 1.8% of 18:0, 2.8% of 18:1 ω7, 11.6% of 18:1 ω9, 1.9% of 18:2 ω6, 3.7%
96 of 18:3 ω3, 7.4% of 20:1 ω9, 1.3% of 20:4 ω3, 0.6% of 20:4 ω6, 14.9% of 20:5 ω3, 8.8% of 22:1
97 ω11, 2.8% of 22:5 ω3 and 17.9% of 22:6 ω3. It was used fresh with good initial quality (peroxide
98 value of 0.5 meq/kg).

99 *Fish oil emulsions:* Fish oil in water emulsions containing 10 % fish oil and 1 % L-α-
100 phosphatidylcholine were prepared as described previously [30]. Briefly, lecithin and fish oil were
101 homogenized with distilled water and emulsified by sonicating for a total of 10 min in an ice bath
102 (Selecta, Spain).

103 *Fish oil enriched real products:* Skimmed milk and vegetal oil mayonnaise were purchased in a
104 local market. The content of fat was 2.9 % and 80.0 %, respectively. The fatty acid content of milk
105 was 13.2% of 14:0, 1.8 of 14:1 ω5, 1.5% of 15:0, 38.0% of 16:0, 2.3% of 16:1 ω7, 0.8 of 17:0,
106 13.6% of 18:0, 23.5% of 18:1 ω9, 1.0 of 18:1 ω7, 3.2% of 18:2 ω6, 0.6% of 18:3 ω3, 0.6% of 20:4
107 ω6. Contents on conjugated dienes and trienes were 2.4 and 0.6 mmol hydroperoxide/Kg lipid
108 respectively. It did not show any of the target volatiles considering in this work. The fatty acid
109 content of mayonnaise was 0.0% of 14:0, 0.0 of 14:1 ω5, 0.0% of 15:0, 7.3% of 16:0, 0.3% of 16:1
110 ω7, 0.0 of 17:0, 4.0% of 18:0, 29.2% of 18:1 ω9, 0.8 of 18:1 ω7, 57.2% of 18:2 ω6, 0.3% of 18:3
111 ω3, 0.8% of 20:4 ω6. It showed a concentration of conjugated dienes and trienes of 1.6 and 1.4,
112 mmol hydroperoxide/Kg lipid respectively and none of the volatiles directing in this study.

113 Milk and mayonnaise were fish oil enriched in the laboratory by adding slowly fresh cod liver oil
114 (5% and 16% respectively) on commercial products with continuous homogenization. After that,
115 they showed the following fatty acid content: milk was composed by 1.2% of 14:0, 0.2% of 15:0,
116 10.9% of 16:0, 0.8% of 16:1 ω7, 9.0% of 18:0, 64.6% of 18:1 ω9, 10.0% of 19:0, 8.4% of 18:2 ω6,
117 0.7% of 18:3 ω3, 0.5% of 20:1 ω9, 0.4% of 18:4 ω3, 0.8% of 20:4 ω6, 1.0% of 20:5 ω3, 0.1% of
118 22.5 ω3, 1.4% of 22:6 ω3; and mayonnaise was composed by 1.1% of 14:0, 8.2% of 16:0, 1.7% of
119 16:1 ω7, 0.2% of 17:0, 3.6% of 18:0, 24.2% of 18:1 ω9, 1.6% of 18:1 ω7, 12.7% of 19:0, 49.4% of
120 18:2 ω6, 0.7% of 18:3 ω3, 2.3% of 20:1 ω9, 0.5% of 18:4 ω3, 0.6% of 20:4 ω6, 1.6 of 22.1 ω11,
121 1.8% of 20:5 ω3, 0.3% of 22.5 ω3, 2.5% of 22:6 ω3.

122

123 2.3. Oxidation

124

Eliminado: Previously to storage, in the oxidation experiments, they

125 Fish oil emulsions were oxidized by incubation at 50°C during 24 hours in a 20 mL crimp top glass
126 with a headspace aluminum cap furnished with a PTFE-faced septum. These oxidized emulsions
127 were used for selecting the fibers and optimizing HS-SPME conditions. Oxidation was determined
128 by sensory detection of off-flavors.

129 Fish oil enriched milk and fish oil enriched mayonnaise were oxidised as follows: 100 grams of
130 enriched milk were introduced in 250 mL flask and stored during 4 days at 30 °C and 100 grams of
131 enriched mayonnaise were introduced in 250 mL flask and stored during 17 days at 40 °C.
132 Oxidative stability of these samples was evaluated periodically by sensory analysis and by
133 measuring the formation of conjugated diene and triene hydroperoxides. Triplicate samples were
134 prepared and analyzed.
135 Induction periods of the formation of oxidation by-products were calculated as the time (in days)
136 required for a sudden change in the rate of oxidation by the method of tangents to the two parts of
137 the kinetic curve [31].

139 2.4. HS- SPME conditions

141 a. Selection of fibers: The oxidized fish oil emulsions were employed for testing the sensitivity and
142 reproducibility of three different fiber coatings: 75µm CAR-PDMS, 65µm PDMS-DVB and 65µm
143 CW-DVB. HS-SPME was performed by incubating 1 g of emulsion in a 20 mL vial to 60 °C during
144 15 minutes.

145 b. Sampling conditions for CAR-PDMS fiber: The oxidized fish oil emulsions were employed for
146 determining the main parameters affecting the HS-SPME efficiency: temperature and time of
147 extraction, stirring and sample amount. For such purpose a two-level factorial design (2^4) was
148 selected [32]. This design enables the study of the main effects and two-factor interactions. Two
149 center points per day (factorial design was realized in four days) were added to ensure enough
150 degrees of freedom for block factor evaluation. The lower and upper limits of temperature and time
151 were set at 40, 60 °C and 10, 30 min respectively. The maximum temperature was 60 °C in order to
152 avoid the oxidation of the emulsion. In addition, sampling time was set at 30 min maximum
153 because this time offers the possibility of analysis automatization. Number of experiments was 24
154 and order was fully randomized (Table 1). Data analysis was performed by means of the statistical
155 package Statgraphics Plus for Windows V. 5.1.

156 Fish oil in water emulsions were placed in headspace vials in which total volume was dependent on
157 the experiment performed. Samples were equilibrated for 10 min to room temperature before HS-

Eliminado: Storage experiments for monitoring the lipid oxidation

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Eliminado: fresh fish oil enriched milk and mayonnaise

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Eliminado: 31

158 SPME. The fiber was exposed to the headspace over the sample at different times and temperatures
159 depending on the experiment. 3-Methyl-3-buten-1-ol was used as internal standard.

160 *c. Real fish oil enriched samples:* Fresh fish oil enriched milk and enriched mayonnaise were
161 analyzing by employing the selected time and temperature described above in section 2.2.. These
162 values were employed since they provided the maximum extraction with no further degradation of
163 samples. The amount of sample was optimized after a set of experiments ranging between 0.5-6
164 grams. After that, the analysis of volatiles resulting from the oxidation experiments of fish oil
165 enriched milk and enriched mayonnaise were performed by incubation 0.5 g of samples spiked with
166 50 μ L of 3-methyl-3-buten-1-ol used as internal standard (15 μ g/g in water) in headspace vials.

Eliminado: The HS-SPME method was applied to follow the oxidation of the not oxidized enriched fish oil milk and mayonnaise, indicated in 2.2.

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Eliminado: and volatile compounds were analyzed in the optimized conditions derived from the above experiments

168 2.5. GC-MS analysis conditions

169
170 GC-MS analysis was performed in a Thermo Finnigan ThermoQuest (San Jose, CA) gas
171 chromatograph equipped with a split/splitless injector and coupled with a Trace quadrupole mass
172 detector (Thermo Finnigan ThermoQuest, San Jose, CA). Compounds were separated on a 30 m x
173 0.32 mm x 1 μ m film thickness, fused silica DB-1701 (Folsom CA) capillary column. The GC oven
174 temperature program was: 35 $^{\circ}$ C for 3 minutes, followed by an increase of 3 $^{\circ}$ C/minute to 70 $^{\circ}$ C;
175 then an increase of 10 $^{\circ}$ C/minute to 200 $^{\circ}$ C and finally an increase of 20 $^{\circ}$ C/minute to a final
176 temperature of 260 $^{\circ}$ C hold for 5 minutes. Helium was employed as carrier gas, with a constant
177 flow of 1.5 mL/min. Transfer line temperature was maintained at 265 $^{\circ}$ C. The quadrupole mass
178 spectrometer was operated in the electron impact mode (70 eV), and the source temperature was set
179 at 200 $^{\circ}$ C. Other experimental parameters influencing the acquisition and analysis of mass spectra
180 were the following: full scan mass acquisition range, 10-190 amu; scan rate, 0.220 s/scan; multiplier
181 voltage, 350 V; filament emission current, 150 μ A.

182 The injector temperature was set at 220 $^{\circ}$ C for CW-DVB fiber, at 250 $^{\circ}$ C for PDMS-DVB fiber and
183 at 300 $^{\circ}$ C for CAR-PDMS fiber, according to technical recommendations. The volatiles were
184 desorbed in the GC injection port for 10 minutes. The presence of artifacts due to compounds
185 remained into the fiber after desorption was also checked.

187 2.6. Sensory analysis

188
189 The same raw samples that were used for chemical determinations were equilibrated during 10
190 minutes to room temperature. Samples were evaluated by an expert panel formed by four trained
191 specialists in descriptive analysis of fishy off-flavours of which 50% were women. Tasting was

Eliminado: 4 panellists trained

192 carried out in a room designed for the purpose. Panellists concentrated on detecting rancidity odors
193 using a hedonic scale from 1 to 9. The odors of fishy, rancid, milk and metallic were immediately
194 evaluated upon opening the flask [33].

Eliminado: 32

196 2.7. Measurement of conjugated diene and triene hydroperoxides

198 250 mg of sample (milk or mayonnaise) were homogenized during 1 minute with 2.5 mL of ethyl
199 acetate. Next, 2.5 mL of ethanol were added and shaken during 1 minute. Then, 2.5 mL of hexane
200 were added and the solution was shaken during 1 minute. Finally the sample was shaken during 1
201 minute after the addition of 2.5 mL of deionized water. The organic phase was separated after
202 centrifugation, and then an aliquot was evaporated using a nitrogen gentle stream and resolved with
203 200 µL of hexane and 5 mL of ethanol. Absorbance was measured at 234 nm for conjugated diene
204 and at 268 nm for conjugated triene hydroperoxides (UV-Vis Spectrophotometer Beckman, model
205 DU 640 (Beckman Instruments Inc., Palo Alto, CA, USA)). Data were expressed as mmol
206 hydroperoxides/kg lipid.

Eliminado: µ

208 2.8. Qualitative and quantitative analyses

210 Identification of components was based on the comparison of the retention times of individual
211 standards and computer matching with the reference mass spectra of the Wiley 6, Mainlib and
212 Replib libraries and standards.

213 Quantitative determination of volatiles was performed by the method of internal standards using 3-
214 methyl-3-buten-1-ol. Calibration curves at six levels and two replicates by level, ranging from 0 to
215 1 µg/g, were built adding standard mixtures of all compounds in both fresh fish oil enriched milk
216 and fresh fish oil enriched mayonnaise. Detection limits of the method were calculated to a signal to
217 noise (S/N) ratio of 3. Repeatability of the analysis was evaluated by analyzing 8 replicates of fish
218 oil emulsion, 6 replicates of the enriched milk and 6 replicates of the enriched mayonnaise.
219 Reproducibility of the analysis was determined in fresh and oxidized fish oil in water emulsions
220 stored at -80 °C during 5 days. Recoveries from samples were evaluated in fresh fish oil enriched
221 milk and enriched mayonnaise spiked with standard solutions at concentrations of 500 and 1000
222 ng/g.

Eliminado: Semiquantitative

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determination

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carried out by using an internal
calibration curve

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curve was

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Eliminado: 3

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Eliminado: Statistical Analysis

224 2.9. Statistical analysis

225

226 All experiments were triplicated and analyses were performed in duplicated. The data were
227 compared by one-way analysis of variance (ANOVA) [34], and the means were compared by a least
228 squares difference method. Linear regressions were employed for the calibration graphs by Pearson
229 coefficients. Significance was declared at $p < 0.05$. Statistical analyses were performed with the
230 statistical package Statgraphics Plus for Windows V. 5.1.

231

232 3. Results and discussion

233

234 3.1. HS-SPME-GC-MS conditions

235

236 The initial part of this work was aimed to identify the most suitable chromatographic separation of
237 the analytes associated to rancidity of fish oil emulsions. For such purpose, volatiles of oxidized
238 fish oil emulsions were extracted with CAR-PDMS 75 μm fiber (1 g incubated at 60 $^{\circ}\text{C}$ during 15
239 min) and injected into the gas chromatograph. As a result, 77 compounds were identified and 16
240 were selected for method evaluation (Table 2) since have been usually reported during oxidation of
241 fish muscle, fish oil and fish oil emulsions [5, 35-37].

242 The different fiber coatings were then tested and compared for sensitivity and reproducibility.
243 Figure 1 represents the analysis of the target analytes selected above. CAR-PDMS 75 μm enabled
244 the extraction of a larger range of compounds with higher sensitivity than the other two fiber
245 coatings. This last issue was considered relevant because of the low amounts of volatiles formed
246 during the first steps of lipid oxidation. In addition, reproducibility by using CAR-PDMS 75 μm
247 was higher than the others: CAR-PDMS fiber showed RSD between 5 and 14 %, PDMS-DVB
248 between 7.2 and 17.3 % and CW-DVB between 5.0 and 22.4 % depending of compound. Duflos et
249 al. [22, 38] have also selected CAR-PDMS fiber to identify and quantify the volatiles associated to
250 freshness and spoilage in different fish species. Brunton et al. [23] found that the PDMS/DVB fiber
251 was the most effective for the analysis of pentanal and hexanal in cooked turkey in terms of
252 reproducibility and linearity of response while CAR-PDMS was the most sensitive.

253 The sampling conditions affecting the microextraction process on CAR-PDMS were then evaluated
254 by a factorial design which gives information about the significance of the experimental parameters.
255 Table 1 shows the number and conditions of the experiments executed. The variable response was
256 the peak areas of the different volatiles. Data for 1-penten-3-one are shown since were
257 representative of those achieved for the other target compounds. These data produced the
258 standardized first-order Pareto charts shown in Fig. 2. The Pareto chart shows each of the estimated

Eliminado: More samples were analysed for repeatability...
Statistical analysis: regression curves were built by using the ... method using the Statgraph package ().

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Eliminado: The analysis of the different 24 experiments described in the experimental design

Eliminado: (Table 1)

Eliminado: , produced the results showed in Table 1 and the standardized first-order Pareto charts shown in Fig. 2 for 1-penten-3-one.

effects in decreasing order of magnitude. The length of each bar is proportional to the standardized effect and the vertical line can be used to judge which effects are statistically significant. Any bars which extend beyond the line correspond to effects statistically significant. The amount of sample was the most significant factor for almost all the analytes and its effect was negative (Fig 2). Górecki et al. [39] established that the head space volume in the vial should be minimized to increase the extraction efficiency. A reduction in the headspace available by incrementing the sample amount up to 6 grams could produce competitive conditions among volatile compounds and therefore a decrease of efficiency [40]. Temperature showed a positive and significant effect on the response obtained (Fig. 2). This means that HS-SPME efficiency improved when the temperature increased from 40 to 60 °C. Extraction time was a significant factor for the analyses of volatiles which were better extracted after 30 minutes. The interaction between factors B and C (sample amount and time) was significant too, and the maximum extraction was obtained when the experimental conditions were 1 g of sample and 30 minutes of extraction time. Factor stirring was not found to be significant for any compound. Some interactions between this factor and the others were found significant. However, the higher efficiency was obtained using the selected conditions described for the other factors with no contribution of the stirring. Time of 30 min and temperature of 60°C were those providing the best extraction of volatiles, since these factors show a positive correlation with the HS-SPME response [17]. According to the conclusions above exposed, temperature and time were fixed at 60°C during 30 min, and the sample amount was optimized by studying the responses obtained after the extraction of 0.1, 0.25, 0.5, 0.75 and 1 g. There was an increment of response between 0.1 and 0.5 g and then, for values ranged between 0.5 and 1 g, no significant increment was achieved for almost all the target compounds (Fig. 3). Only for 5 compounds there was a very slight increment of response by increasing the sample amount up to 1 gram. Consequently, and in order to reduce the amount of sample of the method, 0.5 g was finally selected. Temperature and time of extraction were fixed at 60°C during 30 min in fish oil enriched real samples too, because corresponding to the maximum studied values in the design and these factors show a positive correlation with the HS-SPME response [17]. A study of sample amount was performed in fish oil enriched milk and mayonnaise between 0.5 and 6 grams. The highest peak areas for the most target compounds in both matrices were obtained by using 0.5 g of sample (Fig. 4). A desorption of the CAR-PDMS fiber into the chromatograph during 2 minutes was performed. After that, the fibers were reinserted and blank analyses were run. Any compound was observed in the blank chromatogram for any fiber. A desorption time of 10 min was then fixed for

Eliminado: 1-penten-3-ol results are showed

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Eliminado: were highly correlated with the other target compounds.

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Eliminado: , the best results were achieved with the optimized values of the factors described previously and without

Eliminado: and therefore its interactions with other factors were not considered. Interactions...

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Eliminado: We agree with referee's opinion about the correct use of the optimization methods. The factorial design only explains what factors are significant. We have considered that time and temperatures selected are those that provide the best extraction of volatiles. These considerations are now included in the text. ¶

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Eliminado: amount were selected in both matrix because the highest peak area responses were obtained for almost all the target compounds

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293 automatizing the GC analysis and for assuring the total clean of the fiber. The increment between
294 2 and 10 minutes did not influence the resolution of the peaks.
295 Figures 5 and 6 show the full scan chromatograms of an oxidized fish oil enriched emulsion and of
296 an oxidized fish oil enriched milk (Fig. 6A) and mayonnaise (Fig. 6B) obtained in the optimized
297 conditions.
298

299 3.2. Performance of the methodology

300
301 *Emulsions:* As for as repeatability, the relative standard deviations of the replicates remained
302 between 3 and 13 % depending of the volatile analyzed. Internal standard showed a RSD of 3.4 %.
303 The reproducibility was found to range between 6.7 and 18.1 % RSD for fresh and between 6.3 and
304 18.5 % RSD for oxidized oil in water emulsions. Only propanal showed higher RSD values (28.7
305 %) in the oxidized oil in water emulsion.

306 *Real samples:* Due to matrix effect that often affects the SPME technique, the quantitative
307 measurements in enriched milk and mayonnaise were performed by the standard addition method.
308 As regards to linearity, the calibration graphs show a direct proportional relationship between the
309 responses and their increasing concentrations in the samples (Table 3). The correlation coefficients
310 were satisfactory for all the target compounds detected in both enriched milk and enriched
311 mayonnaise. The detection limits calculated for each type of enriched samples (Table 3) were low
312 and sufficient for detecting lipid oxidation in the early steps of the reaction.

313 The detection limits suggest that the efficiency of the process is affected by the type of matrix
314 sample and provide better sensitivity in fish oil enriched milk than in fish oil enriched mayonnaise.
315 The area of the standards added in the same concentrations to fresh enriched milk was higher than
316 those added to fresh enriched mayonnaise. Peak intensities corresponding to volatiles generated in
317 oxidized fish oil enriched milk were also higher than those obtained in oxidized enriched
318 mayonnaise. This is probably due to a more difficult extraction of volatile compounds from this last
319 matrix.

320 Recoveries were evaluated by spiking standards at two concentration levels in both fish oil enriched
321 samples and appeared in the 80–120 % range. Repeatability analysis showed relative standard
322 deviations lower than 10 % (Table 4). The more volatile compounds such as acetaldehyde, pentane
323 and propanal showed lower recoveries and precision than the other volatiles.
324

325 3.3. Analysis of fish oil enriched products

Eliminado: The method exhibited a direct proportional relationship between the extracted amount of volatiles and their initial concentration in the samples (Table 3). The obtained detection limits were enough for detection of lipid oxidation in fish oil enriched emulsion samples (Table 3). Due to matrix effect that often affects the SPME technique, quantitative measurements in real samples normally require applying the standard addition method. Recoveries were evaluated by spiking standards at two concentration levels in both fish oil enriched real samples and appearing in the 80–120 % range with relative standard deviations lower than 10 % (Table 4). Values of recoveries and precision were not satisfactory for the most volatile compounds studied (acetaldehyde, pentane and propanal).

Eliminado: scarcely

Eliminado: obtaining a

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Eliminado: mayonnaise

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Eliminado: in fish oil enriched milk, also when volatile compounds were added in the same concentrations in both matrices. Probably because it was

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326

327 The suitability of the methodology described above for assessing changes in volatile composition
328 of fish oil enriched foodstuffs as milk and mayonnaise was investigated. Firstly, the full scan
329 chromatograms corresponding to oxidized fish oil enriched milk and fish oil enriched mayonnaise
330 were studied (Fig. 6). Hexanal was the main volatile formed during oxidation of fish oil enriched-
331 milk. 1-penten-3-ol, hexanal, *E*-2-hexenal and 2-ethylfuran were the main signals found during
332 oxidation of mayonnaise according to previous reported data [5, 15]. 1-penten-3-ol and *E*-2-hexenal
333 are formed by means of enzymatic mechanism from EPA (20:5 n-3) [41], hexanal, via linoleic acid
334 13-hydroperoxide [42] and 2-ethylfuran via 12-hydroperoxide of linolenate (18:3 n-3), via 14-
335 hydroperoxide EPA (20:5 n-3) or via 16-hydroperoxide DHA (22:6 n-3) [35].
336 During thermal treatment of enriched milk, rancid off-flavors were detected by the second day of
337 oxidation (Table 5). These data were in agreement with the formation of hexanal and *E*-2-hexenal
338 detected by the HS-SPME method here proposed (Fig. 7). Volatile analysis showed that the
339 induction period or the lag phase of lipid oxidation ranged between 1.2 and 1.5 days according to
340 the sensory detection of rancid off-flavors (Fig. 7). The hydroperoxide values detected were also in
341 high correlation with the volatiles. The HS-SPME method showed a high sensitivity for detecting
342 oxidation as can be inferred from the increase of volatiles formed. Hexanal analysis showed an
343 increment of 50 % between the first and second day and *E*-2-hexenal showed an increment of the
344 relation peak area/ IS peak area of 81 %. Similar results were obtained for other volatile compounds
345 that have been previously identified as important markers of fish oil enriched milk products as
346 propanal, 2-ethylfuran, 1-penten-3-one, 1-penten-3-ol, pentanal, (*E,E*)-2,4-heptadienal, *E*-2-
347 pentenal, heptanal or *E*-2-heptenal [42, 43, 44]. The values of conjugated hydroperoxides in the
348 same period were increment by 28 %.
349 The cod liver oil enriched mayonnaise developed significant rancid off-flavors by the seventh day
350 of oxidation (Table 5) and the values of volatiles associated to lipid oxidation were highly
351 correlated with this finding (Fig. 8). Additionally the formation of volatiles was in agreement with
352 the detection of conjugated hydroperoxides. Levels of hexanal and 1-penten-3-ol showed
353 increments of 126 % and 213 % from the original values by the seventh day of oxidation,
354 respectively. Similar results were observed for other compounds related with lipid oxidation as
355 propanal, 2-ethylfuran, 2-pentylfuran, 1-penten-3-one, pentanal, *E*-2-pentenal, *E*-2-hexenal,
356 heptanal or (*E,E*)-2,4-heptadienal. Conjugated hydroperoxides experimented an increment of 9 % in
357 the same period.

Eliminado: 39

Eliminado: 40

Eliminado: Peak intensities corresponding to volatiles generated in oxidized mayonnaise were lower than those obtained in fish oil enriched milk, also when volatile compounds were added in the same concentrations in both matrices. Probably because it was more difficult to extract volatile compounds from this matrix.¶

Eliminado: 3

Eliminado: conjugated hydroperoxides and the formation of

Eliminado: 6

Eliminado: s detected

Eliminado: an induction period of

Eliminado: reaction

Eliminado: was

Eliminado: in agreement

Eliminado: with

Eliminado: and the formation of hydroperoxides

Eliminado: performed

Eliminado: , 1,5-octadien-3-ol or *E*-2-octenal

Eliminado: demonstrated an increment

Eliminado: between the first and second day of

Eliminado: of the initial values.

Eliminado: Hexanal analysis showed an increment of 50 % in the same period and *E*-2-hexenal showed an increment of the relation peak area/ IS peak area of 81 %. Similar results were obtained for other volatile compounds that have been previously identified as important markers of fish oil enriched milk products as propanal, 2-ethylfuran, 1-penten-3-one, 1-penten-3-ol, pentanal, (*E,E*)-2,4-heptadienal, *E*-2-pentenal, heptanal, *E*-2-heptenal, 1,5-octadien-3-ol or *E*-2-octenal [5, 41 42].

Eliminado: 3

Eliminado: conjugated hydroperoxides and

Eliminado:

Eliminado: , *E*-2-octenal

Eliminado: from the original values by the seventh day of oxidation while hexanal lev... [1]

Eliminado: Similar results were observed for other compou... [2]

4. Conclusions

HS-SPME has proved to be a valuable methodology for determination of volatile compounds associated to oxidation of fish oil enriched emulsion products. HS-SPME method enabled the extraction of a large range of compounds. It was shown to be a reliable, rapid, repeatable and sensitive technique. CAR-PDMS 75 μm was employed as extracting fiber and a factorial design demonstrated that the sample amount, temperature, and exposition time were parameters having significant influence on the efficiency of the extraction. This methodology for volatile studies allows the accurate analysis of several volatile compounds present in real fish oil enriched samples which amounts were strongly correlated with the sensory scores and the formation of conjugated hydroperoxides. Therefore, they were key indicators of lipid oxidation.

Acknowledgements

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433

434 Figure captions

435 Fig. 1: Evaluation of extraction efficiencies from different fiber coatings.

Eliminado: 1

436 Fig. 2: Standardized Pareto chart obtained for 1-penten-3-one. Vertical line indicates the statistical
437 significance bound for the different effects.

Eliminado: 2

438 Fig. 3: Evaluation of extraction efficiencies from different sample amounts in oxidised fish oil in
439 water emulsion samples.

Eliminado: 3

440 Fig. 4: Evaluation of extraction efficiencies from different sample amounts in spiked fish oil
441 enriched milk (A) and fish oil enriched mayonnaise (B) samples.

442 Fig. 5: Full scan chromatogram obtained after analysis of an oxidized 10 % fish oil in water
443 emulsion. Peaks are identified as in Table 2.

Eliminado: 4

444 Fig. 6: Full scan chromatograms obtained after analysis of an oxidized fish oil enriched milk (A)
445 and oxidized fish oil enriched mayonnaise (B) samples. Peaks are identified as in Table 2.

Eliminado: 5

Eliminado: an oxidized

446 Fig. 7: Time course of lipid oxidation of fish oil enriched milk measured by conjugated
447 hydroperoxides and headspace volatiles.

Eliminado: 6

448 Fig. 8: Time course of lipid oxidation of fish oil enriched mayonnaise measured by conjugated
449 hydroperoxides and headspace volatiles.

Eliminado: 7

Editor's comments:

We agree with editor's general recommendation about the appropriate validation and statistical treatment of the data. A more complete statistic study of the linearity, sensitivity, limits of detection, accuracy and precision of all compounds studied in this paper, considered the different real samples analyzed, is now included in the manuscript (page 11, line 299-323 and Tables 3 and 4). Additionally, a more detailed explanation of the experiments, samples and statistics employed for the appropriate performance of the method is now described (page 8, line 224-230).

Comments of Reviewers

Reviewer 1

1. Introduction

-Line 56 and 61: We agree with referee's recommendation about the convenience of clarifying the definition of SPME and HS-SPME. This fact has been clarified in the new manuscript (page 4, lines 63-67). HS-SPME was selected as technique in the present study and this term has been used in the whole paper.

2.3 Oxidation

-Line 114: The extent of each individual experiment is now included in this section (page 6, lines 129-131).

2.4 HS-SPME condition.

c: The internal standard: 3-methyl-3-buten-1-ol is the same for both fish oil enriched real samples, milk and mayonnaise. This compound has been used in both, performance of the methodology and the experiments for monitoring oxidation. This has been clarified in the text (page 7, line 165-166) and the standard is included in the Reagents section.

3.1 HS-SPME-GC-MS condition

- Line 203. CAR-PDMS fiber provided higher reproducibility than the others and data showing the reproducibility of the different fibers are now included in the text. (page 9, line 246-248).

-Line 219. A complete study considering of the volatiles compounds was performed for establishing the conditions of the SPME extraction. The final decision was taken considering the overall results of all compounds. This clarification has been included in the text and data of 1-penten-3-one are shown since they were representative of those obtained for the other target analytes (page 9, line 256-258).

-Line 224: The amount of sample has been changed in Table 1.

-Line 213-233: We agree with referee's recommendation about the importance of presenting the source of data containing in Figure 2. Such source is the peak responses obtained for 1-penten-3-

one in the different experiments which are now shown in Table 1. This is now clarified in the text (page 9, line 253-258) and as has been mentioned above, the behavior of 1-penten-3-one was illustrative of the other volatiles.

-Line 236-238: Only five compounds showed a slight increment of response by increasing the sample amount up to 1 gram. Since it is important to reduce the sample amount in the experiments aimed to monitor lipid oxidation, the amount of sample was fixed as 0.5 g. This consideration is now included in the text (page 10, lines 279-283).

-Line 239: After previous tests using desorption times of 2 minutes, the final desorption time was fixed at 10 minutes in order to assure the total clean of the fiber and for improving the automatization of the analysis. We agree with referee comment and we have corrected the paragraph since this parameter has not been optimized (page 10, lines 290-294).

3.2. Analysis of fish oil enriched products

-Line 252: We agree with referee's opinion about this sentence. We have studied oxidized fish oil enriched samples in both cases. This has been clarified in the text adding "fish oil enriched milk and mayonnaise" (page 12, line 329).

-Line 259: We agree with referee about the comparison between peak intensities corresponding to milk and mayonnaise. This sentence has been deleted from the paragraph. The quantitative analysis of each volatile has been performed by using internal calibration as it is now shown in Table 3.

In order to perform an accurate quantitative analysis of each sample, the calibration of each selected volatile is now presented in the manuscript. Very different responses were obtained in the analysis of fresh fish oil enriched milk and mayonnaise after spiking them with the same quantity of volatile compounds. Therefore, the extraction of analytes was more difficult in fish oil enriched mayonnaise than in milk since the areas obtained were lower. In addition, very different LODs were obtained in both matrices. The comparison of slopes and the Table 3 in the old manuscript have been eliminated and the results have been discussed in the section dedicated to the performance of the method.

-Line 264: A more concretized calculation of the induction period is now shown (page 12, line 338-340). Also the definition of the induction period has been included in the experimental section (page 6, line 135-137).

-Line 264: More details about the procedure for the sensory analysis are now given in the text (page 7, line 187-194) and Table 5 shows the results obtained for the detection of rancid odors.

As regards to the discussion of conjugated hydroperoxides formation, we agree with referee's opinion and have now presented and discussed the data on the basis of the volatile method (page 12, lines 336-348).

3.3. Performance of the methodology:

A statistical and complete performance of the method is presented for all studied compounds including linearity, sensitivity, limits of detection, accuracy and precision (page 11, lines 299-323 and Tables 3 and 4).

-Table 1: “time” has been changed by “extraction time” in Table 1.

-Figures 4 and 5 and Table 2: The internal standard has been now indicated in the corresponding figures (actual figures 5 and 6) and Table.

Reviewer 2:

We agree with the general comment regarding the differences among fish oil in water emulsion and the fish oil enriched milk and mayonnaise. We have fixed the SPME conditions of the emulsion and then, since milk and mayonnaise are also real emulsions, we have studied them as examples of the utility of the method. In order to give the information that referee is requesting, we have now performed a complete study of the range of sample amount in each type of real sample (0.5-6 g) considering that an extraction time of 30 min at 60° C procured the best extraction of volatiles associated to oxidation. These values of time and temperature are the maximum values of the experimental design and there is a positive correlation between these parameters and the efficiency of the extraction (J. Pawliszyn, Solid Phase Microextraction: Theory and Practice. John Wiley and Sons Inc, New York, 1997). All these studies and comments are now included and discussed in the text (page 10, line 284-289 and Figures 4A and 4B). The results confirmed that the optimized sample amount for fish oil enriched milk and mayonnaise was 0.5 g.

-Page 4, line 96, page 6, line 178 and lines 250-252. We agree with referee’s opinion about the convenience of showing the raw composition of the milk and mayonnaise. We have now included in the manuscript a detail study of the composition of the initial milk and mayonnaise before the fish oil enrichment (page 5, lines 103-112). This information is included to understand better the blank matrix. The HS-SMPE method here proposed is aimed to identify and quantify the level of oxidation of fish oil enriched emulsion samples on the basis of the volatiles, so the possibilities of interferences must be investigated. The fatty acid composition, the concentration of conjugated hydroperoxides and the volatiles are now presented in the text. Considering the low values of conjugated hydroperoxides and volatiles of the raw materials, no interferences were expected.

-Page 5, line 125: The effect of salt addition has not been evaluated since salt is a strong promoter lipid oxidation, especially in tissues and muscle (E. N. Frankel, Lipid Oxidation, The Oily Press, West Ferry, Dundee, Scotland, 1998).

-Line 167: More details about the procedure for the sensory analysis are now given in the text (page 7, line 187-194). In addition, Table 5 shows the results obtained for the detection of rancid odors. The results obtained by the expert panel were in high correlation with the chemical determination of

conjugated hydroperoxides that is one of the most common methods employed to follow lipid oxidation in oil emulsion foodstuffs (E. N. Frankel, Lipid Oxidation, The Oily Press, West Ferry, Dundee, Scotland, 1998).

-Line 185: We agree with referee's opinion about the correct identification of compounds. In addition to the comparison of the mass spectra with libraries, individual standards of all studied compounds in this paper were injected for correct evaluation of retention time. This is now explained in the material and method section (page 8, 210-212) and in Table 2.

- In order to provide an accurate analytical performance and validation of the method, a whole statistic study of the linearity, sensitivity, limits of detection, accuracy and precision of all compounds studied in this paper is now included together with a more detailed explanation of the experiments and samples employed for the appropriate performance of the method (page 8, line 208-222 and page 110, line 299-323 and Tables 3 and 4). Table 3 includes the LOD values of each volatile compound and their standard errors.

-Page 7 line 224: The words "can provoke" have been changed by "could produce" (page 10, line 265).

-Line 234-238: We agree with referee's opinion about the correct use of the optimization methods. The factorial design only explains what factors are significant. We have considered that time and temperature selected are those providing the best extraction of volatiles and the maximum values allowed with no further oxidation. Lipid oxidation is a complex reaction in which temperature and time can be critical. As regards to the sample amount, an exhaustive study on fish oil emulsions and fish oil enriched- milk and mayonnaise has been done. In this sense, we have not performed a total optimization of the conditions and this consideration has been made in the title and in the whole manuscript. New title: **Development of a solid-phase microextraction method for determination of volatile oxidation compounds in fish oil emulsions**

-Line 230: Not significant interactions with stirring factor were achieved for almost all the target compounds. In those cases in which these interactions were significant, the higher responses were obtained using the selected conditions described for the other factors with no contribution of the stirring. This has been clarified in the manuscript (page 10, line 272-274).

-Line 258: We agree with referee's opinion about the indicated sentence. The differences were achieved after spiking the fresh enriched samples with the same quantity of volatile compounds. The sentence was clarified and transferred to the performance of the method (page 11, line 315-319).

- Page 8 line 289-Table 3: The comparison of the slopes and Table 3 has been eliminated since we have now discussed the performance of the method on enriched milk and enriched mayonnaise in

terms of the standard addition (page 11, line 308-319). As mentioned above, a more exhaustive study of the calibration procedure and quantitative analysis of the method is now shown.

- Figure 4 and 5: Units in Figure 4 (now Figure 5) has been changed.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Table 1

Table 1: Matrix of experiments realized for optimization of SPME factors.

<i>Block</i>	<i>Experiment</i>	<i>Temperature (°C)</i>	<i>Extraction time (min.)</i>	<i>Amount of sample (g)</i>	<i>Stirring</i>	<i>Peak area^a (counts)</i>
1	1	60	10	1	Yes	975368
	2	60	30	6	No	421527
	3	40	30	6	Yes	268617
	4	40	10	1	No	543504
	5	50	20	3	Yes	732941
	6	50	20	3	No	490055
2	7	60	10	6	No	244291
	8	50	20	3	No	567927
	9	50	20	3	Yes	808374
	10	40	30	1	No	1236136
	11	60	30	1	Yes	1883343
	12	40	10	6	Yes	209514
3	13	50	20	3	Yes	463497
	14	50	20	3	No	384015
	15	40	10	6	No	112755
	16	40	30	1	Yes	929211
	17	60	10	6	Yes	366286
	18	60	30	1	No	1165079
4	19	60	10	1	No	527392
	20	40	10	1	Yes	457886
	21	40	30	6	No	244021
	22	50	20	3	Yes	499165
	23	50	20	3	No	552287
	24	60	30	6	Yes	703626

^a Peak area values corresponding to 1-penten-3-one

Table 2: Identification of compounds by means of GC-MS analysis.

Peak	Compound name	Retention time (min)	Identification ^a
1	acetaldehyde	1.65	MS, STD
2	pentane	1.76	MS, STD
3	propanal	2.45	MS, STD
4	butanal	4.46	MS, STD
5	2-ethylfuran	5.59	MS, STD
6	1-penten-3-one	7.96	MS, STD
7	pentanal	8.22	MS, STD
8	1-penten-3-ol	8.59	MS, STD
9	octane	9.18	MS, STD
10	2-pentenal	12.31	MS, STD
11	hexanal	13.15	MS, STD
12	<i>E</i> -2-hexenal	16.96	MS, STD
13	heptanal	17.64	MS, STD
14	2-pentylfuran	18.85	MS, STD
15	<i>E</i> -2-heptenal	19.38	MS, STD
16	(<i>E, E</i>)-2,4-heptadienal	20.94	MS, STD
Internal standard (IS)	3-methyl-3-buten-1-ol	11.46	MS, STD

^a Compounds were identified by comparison with reference substances on the basis of the following criteria: MS obtained from Mainlib, Wiley 6 and Replib libraries (MS) and retention time and spectra of authentic reference compounds (STD).

Table 3: Regression analysis and LODs of the proposed analytical procedure in fish oil enriched samples.

MILK				
Compound	Slope $\pm$ Standard error	Intercept $\pm$ Standard error	Correlation coefficient	LOD (S/N = 3) (ng/g)
acetaldehyde	6.213E-06 $\pm$ 4.815E-08	0.001 $\pm$ 0.000	0.9948	3.62 $\pm$ 0.00
pentane	4.560E-04 $\pm$ 1.352E-05	0.004 $\pm$ 0.007	0.9964	1.69 $\pm$ 0.01
propanal	2.865E-05 $\pm$ 1.190E-06	0.010 $\pm$ 0.001	0.9949	0.18 $\pm$ 0.02
butanal	1.413E-04 $\pm$ 3.078E-06	-0.001 $\pm$ 0.002	0.9954	0.16 $\pm$ 0.04
2-ethylfuran	7.935E-04 $\pm$ 3.270E-05	-0.027 $\pm$ 0.014	0.9944	0.21 $\pm$ 0.03
1-penten-3-one	3.995E-04 $\pm$ 1.311E-05	-0.007 $\pm$ 0.008	0.9930	0.22 $\pm$ 0.01
pentanal	5.327E-04 $\pm$ 1.460E-05	-0.014 $\pm$ 0.008	0.9936	0.38 $\pm$ 0.09
1-penten-3-ol	1.765E-04 $\pm$ 4.737E-06	0.004 $\pm$ 0.003	0.9929	0.12 $\pm$ 0.00
octane	1.870E-04 $\pm$ 9.194E-06	0.000 $\pm$ 0.005	0.9938	0.72 $\pm$ 0.17
<i>E</i> -2-pentenal	2.392E-04 $\pm$ 6.492E-06	-0.001 $\pm$ 0.004	0.9911	0.25 $\pm$ 0.02
hexanal	8.304E-04 $\pm$ 3.770E-05	-0.024 $\pm$ 0.018	0.9953	0.52 $\pm$ 0.02
<i>E</i> -2-hexenal	1.250E-04 $\pm$ 4.203E-06	-0.001 $\pm$ 0.002	0.9902	0.29 $\pm$ 0.02
heptanal	3.828E-04 $\pm$ 1.263E-05	-0.013 $\pm$ 0.007	0.9853	0.66 $\pm$ 0.03
2-pentylfuran	3.509E-04 $\pm$ 1.263E-05	-0.017 $\pm$ 0.008	0.9927	0.17 $\pm$ 0.04
<i>E</i> -2-heptenal	2.002E-04 $\pm$ 8.749E-06	-0.006 $\pm$ 0.005	0.9904	1.20 $\pm$ 0.32
(<i>E,E</i>)-2,4-heptadienal	2.054E-04 $\pm$ 2.038E-05	-0.014 $\pm$ 0.011	0.9941	0.27 $\pm$ 0.04

MAYONNAISE				
Compound	Slope $\pm$ Standard error	Intercept $\pm$ Standard error	Correlation coefficient	LOD (S/N = 3) (ng/g)
acetaldehyde	2.483E-05 $\pm$ 9.051E-08	0.003 $\pm$ 0.000	0.9945	6.58 $\pm$ 0.53
pentane	1.416E-04 $\pm$ 2.448E-05	0.028 $\pm$ 0.012	0.9959	0.34 $\pm$ 0.01
propanal	4.478E-04 $\pm$ 7.453E-08	0.002 $\pm$ 0.000	0.9977	0.64 $\pm$ 0.07
butanal	4.809E-05 $\pm$ 5.592E-06	0.014 $\pm$ 0.003	0.9964	3.71 $\pm$ 0.43
2-ethylfuran	4.215E-04 $\pm$ 1.765E-05	0.021 $\pm$ 0.010	0.9991	0.44 $\pm$ 0.00
1-penten-3-one	1.037E-04 $\pm$ 3.926E-05	0.023 $\pm$ 0.000	0.9977	0.48 $\pm$ 0.01
pentanal	8.462E-05 $\pm$ 2.532E-06	0.007 $\pm$ 0.001	0.9970	0.58 $\pm$ 0.06
1-penten-3-ol	2.704E-04 $\pm$ 2.266E-05	0.016 $\pm$ 0.014	0.9997	0.24 $\pm$ 0.01
octane	5.040E-04 $\pm$ 1.397E-04	0.039 $\pm$ 0.042	0.9988	1.10 $\pm$ 0.14
<i>E</i> -2-pentenal	1.733E-04 $\pm$ 1.185E-05	0.008 $\pm$ 0.009	0.9985	0.93 $\pm$ 0.07
hexanal	3.495E-04 $\pm$ 2.382E-05	0.084 $\pm$ 0.013	0.9965	0.72 $\pm$ 0.08
<i>E</i> -2-hexenal	8.060E-05 $\pm$ 1.165E-06	0.001 $\pm$ 0.001	0.9948	1.13 $\pm$ 0.04
heptanal	1.737E-04 $\pm$ 7.895E-06	-0.006 $\pm$ 0.005	0.9997	2.91 $\pm$ 0.14
2-pentylfuran	3.688E-04 $\pm$ 7.487E-05	-0.015 $\pm$ 0.059	0.9987	0.30 $\pm$ 0.00
<i>E</i> -2-heptenal	7.323E-05 $\pm$ 4.804E-06	0.000 $\pm$ 0.003	0.9966	3.24 $\pm$ 0.20
(<i>E,E</i>)-2,4-heptadienal	4.970E-05 $\pm$ 6.621E-06	-0.001 $\pm$ 0.005	0.9967	1.84 $\pm$ 0.11

Table 4: Repetibility and recovery study.

Compound	MILK			MAYONNAISE		
	Recovery 1000 ng/g (%)	Recovery 500 ng/g (%)	RSD n=6 (%)	Recovery 1000 ng/g (%)	Recovery 500 ng/g (%)	RSD n=6 (%)
acetaldehyde	80.7	88.3	6.1	54.1	100.4	10.7
pentane	95.1	76.1	15.7	99.8	102.1	8.8
propanal	95.9	113.0	9.2	84.7	87.4	14.7
butanal	103.5	98.9	7.9	97.1	100.5	5.9
2-ethylfuran	115.0	90.2	8.7	90.4	103.8	7.7
1-penten-3-one	109.4	95.3	7.7	86.8	93.6	8.5
pentanal	105.4	88.9	7.8	86.2	82.6	7.8
1-penten-3-ol	105.0	93.9	7.1	101.5	109.5	7.4
octane	117.5	94.7	7.6	84.8	87.9	5.0
<i>E</i> -2-pentenal	106.8	89.5	7.8	109.4	105.1	6.3
hexanal	104.1	84.0	7.5	96.2	91.0	7.2
<i>E</i> -2-hexenal	112.0	90.9	10.2	104.2	100.3	8.6
heptanal	105.4	82.7	9.4	108.3	115.7	6.4
2-pentylfuran	115.1	80.8	8.3	86.5	92.6	8.7
<i>E</i> -2-heptenal	119.6	84.5	9.5	99.2	106.5	6.4
(<i>E,E</i>)-2,4-heptadienal	118.8	85.8	9.7	114.0	116.6	8.6

Table 5

Table 5: Development of rancid odors during storage of fish oil enriched real samples.

	Fish oil enriched milk					Fish oil enriched mayonnaise							
Day	0	1	2	3	4	0	1	3	5	7	9	13	17
Sensory analysis	Not rancid odor	Not rancid odor	Incipient rancid odor	Rancid odor	Rancid odor	Not rancid odor	Not rancid odor	Not rancid odor	Not rancid odor	Incipient rancid odor	Incipient rancid odor	Rancid odor	Rancid odor