doi: 10.17221/448/2016-CJFS

Visceral Oil from Farmed *Sparus aurata*, *Dicentrarchus labrax* and *Diplodus puntazzo* as a Source of ω-3 PUFA

Vassilia SINANOGLOU, Dimitra HOUHOULA, Vasiliki KYRANA and Vladimiros LOUGOVOIS*

Department of Food Technology, Technological Educational Institution (T.E.I.) of Athens, Athens, Greece

**Corresponding author: dhouhoula@teiath.gr*

Abstract

Sinanoglou V., Houhoula D., Kyrana V., Lougovois V. (2017): Visceral oil from farmed *Sparus aurata*, *Dicentrarchus labrax* and *Diplodus puntazzo* as a source of ω-3 PUFA. Czech J. Food Sci.,35: 414–423.

Crude oils recovered from the viscera of conventionally and organically farmed gilthead sea bream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), and sharpsnout sea bream (*Diplodus puntazzo*) were characterised. Triacylglycerols (TAG) and phospholipids (PL) were the major lipid classes. Visceral oils contained high levels of *n*-3 polyunsaturated fatty acids (PUFA), in particular docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The DHA/EPA ratios (range 1.66–2.46) were higher in organically farmed fish. Total PUFA and *n*-3 fatty acid levels varied according to both species and rearing system, and were higher in the conventionally farmed sparids. The ratios of *n*-3 to *n*-6 PUFA (1.42–2.19) were comparable to the values reported for muscle lipids, while the PUFA/SFA ratios (1.07–1.33) exceeded the recommended value. Visceral oils exhibited good oxidative stability, as judged by monitoring lipid oxidation products during storage at 63°C. These data indicate that the viscera of all three species may represent a good source for the production of omega-3 rich oils.

Keywords: farmed fish; viscera; lipid composition; oxidative stability

The farming of gilthead sea bream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) is well established in many Mediterranean countries. Over the past two decades, this primary sector industry has expanded rapidly and in recent years production increased dramatically, exceeding 300 000 t in 2011 (FAO 2014). Sharpsnout sea bream (*Diplodus puntazzo*), a sparid fish with attributes similar to those of gilthead sea bream, has also attracted attention and is commercially available, although in much lesser quantities. Unlike other farmed fish, e.g., salmon, where the processing discards and by-products are fully utilised, the rest raw materials from *S. aurata*, *D. labrax*, and *D. puntazzo* (mostly offal) are currently discarded, despite the fact that they represent a potential source of valuable components with nutritional, functional and bioactive properties (Rustad *et al*. 2011). As cultured species deposit large amounts of fat in their internal organs, marine lipids rich in long-chain omega-3 polyunsaturated fatty acids (*n*-3 PUFA) can be melted out and used to meet the growing world demand for high-quality fish oils. Lipids from fish and other marine organisms are the major dietary sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are essential for the development and functioning of the nervous and reproductive systems, as well as in photoreception. Cultured fish viscera have been considered a reliable source for the extraction of lipid at an industrial scale throughout the year, exhibiting minimal seasonal variation in composition. A number of studies have reported on the physicochemical characterisation and production of oil from the viscera of cultured freshwater fish, such as carp (Crexi *et al*. 2010), trout (Zhong *et al*. 2007; Fiori *et al*. 2012) and catfish (Sathivel *et al*. 2002; Thammapat *et al*. 2010). However, information on the chemical composition of the visceral oil from the

doi: 10.17221/448/2016-CJFS

major euryhaline fish cultured in the Mediterranean basin, namely *S. aurata*, *D. labrax*, and *D. puntazzo*, is limited. To the best of our knowledge, no attempt has been made so far to add value to the viscera of these commercially important cultured species, which are currently being wasted instead. Accordingly, this study was initiated with a view to characterising the lipid classes and FA profiles of crude oils recovered from the viscera of conventionally and organically farmed *S. aurata*, *D. labrax*, and *D. puntazzo*, and to identifying components that could be converted to value-added products. An additional aim of the study was to examine the oxidative stability of the visceral oils, by monitoring changes in primary and secondary oxidation products and FFA over a 30-day storage period at 63°C (oven test).

MATERIAL AND METHODS

Viscera samples. Whole viscera from freshly harvested *S. aurata*, *D. labrax*, and *D. puntazzo*, reared under organic (farm KO) or conventional (farms NC and SC) production systems, were collected from a fish processing plant in the course of three independent sampling occasions (late May and early June 2014). Two separate 1-kg batches of whole viscera, which included the digestive tract (intestine and stomach), the liver and visceral depot fat, were collected from each group of fish. The specimens from which the viscera were extracted were in the following weight range: *S. aurata* (KO, NC), 386–598 g (mean weight 501 ± 72 g); *D. labrax* (KO, NC), 385–481 g (mean weight 435 ± 48 g); *D. puntazzo* (SC, conventional fish only), 398–498 g (mean weight 445 ± 50 g). The samples were transported to the laboratory and processed immediately.

Chemicals. All used chemicals were purchased from Sigma-Aldrich Chemicals Co. (UK and USA), Mallinckrodt Chemical Works (USA) or Merck (Germany). The lipid standards and fatty acid methyl esters used as GC-FID standard mixtures were described previously (Sinanoglou *et al*. 2013, 2014). Analytical grade solvents were used for sample preparation; the solvents used for GC and Iatroscan TLC-FID analyses were of HPLC grade (Merck). Double-distilled water was used throughout.

Lipid and moisture determination. Lipids were extracted from the visceral samples using the chloroform/methanol procedure described by Zhong *et al*. (2007). Separation of the chloroform layer, containing the lipid, was achieved by centrifuging the homogenates at 3000 *g* for 10 min using a Z306 Hermle centrifuge (Hermle Labortechnik GmbH; Germany). Total lipid content was calculated gravimetrically, after evaporating the solvent at 40°C in an IKA RV06-ML rotary evaporator equipped with a HB4 basic water bath unit (IKA GmbH; Germany). Moisture determinations were performed in 5-g samples of homogenised whole viscera, using a Kern MLB 50-3 moisture analyser (Kern & Sohn GmbH, Germany).

Analysis of neutral and polar lipids. Lipid classes were separated and quantified by thin-layer chromatography-flame ionisation detection (TLC-FID), using an Iatroscan MK-6 TLC/FID-FPD Analyser (Iatroscan Laboratories, Inc., Japan) as described previously (Sinanoglou *et al*. 2013, 2014).

Analysis of fatty acid methyl esters. Fatty acid methyl esters (FAMEs) were prepared as previously described (Sinanoglou & Miniadis-Meimaroglou 1998). FAMEs were identified and quantified using an Agilent 6890 Series GC (Agilent Technologies, USA) with flame ionisation detector, according to the procedure of Sinanoglou *et al*. (2013).

Determination of hydrolytic and oxidative stability. The hydrolytic and oxidative stability of visceral lipid was evaluated by determining free fatty acids (FFA), peroxide values (PV), conjugated dienes (CD), and 2-thiobarbituric acid-reactive substances (TBARS). Oil samples were placed in open 250-ml conical flasks and allowed to oxidise in an oven (63 ± 0.1°C, natural air circulation) over a period of four weeks. Analyses were conducted on days 1, 8, 15, 21, and 30. Free fatty acids (method Ca 5a-40; AOCS 2005) were determined in neutral ethyl alcohol-ethyl ether oil solutions, by titrating with 0.1 M NaOH in the presence of phenolphthalein indicator, and the results were expressed as oleic acid per 100 g lipid. Peroxide value determination (using method Cd 8-53; AOCS 2005) was performed in oil samples diluted with an acetic acid–chloroform mixture; the oil solutions obtained were treated with potassium iodide and titrated with 0.1 M sodium thiosulphate solution. Results were expressed as milliequivalents per kg oil. To determine the CD content, 0.02–0.03 g of oil were dissolved in 25 ml of isooctane and the absorbance of the resulting solution was read in a 10-mm cuvette at 234 nm, using pure isooctane as a blank (Zhong *et al*. 2007). CD values were expressed as the quotient of the absorbance at 234 nm over the concentration of the oil sample (g/100 ml). TBARS

doi: 10.17221/448/2016-CJFS

were measured spectrometrically (method Cd 19-90; AOCS 2005). Concentrations of malondialdehyde (MDA) equivalents were calculated from a standard curve obtained by reacting known amounts of 1,1,3,3-tetraethoxypropane (TEP) with TBA reagent. Results were expressed as μmol of MDA equivalents per gram of oil.

Statistical analysis. Duplicate measurements from three independent trials were combined and analysed using one-way ANOVA post-hoc tests. Means were subjected to pair-wise multiple comparisons using Tukey's significant difference test at $P < 0.05$. Linear regression and Pearson's correlation coefficients were used to analyse the relationship of moisture, sterol and TAG levels with total lipid content. All statistical calculations were performed using the SPSS statistical analysis software for Windows (IBM SPSS Statistics v19.0; USA).

RESULTS AND DISCUSSION

Lipid and moisture content of whole viscera. Whole viscera from all three species examined in this study were found to harbour high levels of lipids (Table 1). Depending on the species and rearing system, extraction yields (% wet basis) varied considerably; conventional *D. labrax* and *S. aurata* displayed the highest ($P < 0.05$) average visceral oil contents (40.20 ± 4.65 and 42.61 ± 5.29%, respectively), while considerably lower yields were obtained from *D. puntazzo* (30.00 ± 3.09%) and organically farmed *S. aurata* (29.92 ± 3.52%), and *D. labrax* (26.53 ± 3.42%). Similar visceral lipid contents have been reported for cultured steelhead trout, *Oncorhynchus mykiss* (40.2%) (Zhong *et al*. 2007) and American catfish, *Ictalurus punctatus* (33.6%) (Sathivel *et al*. 2002). However, with most species, the reported yields of visceral oil are much lower, e.g., 13–17% in carp, *Cyprinus carpio* (Crexi *et al*. 2009), 14.3% in walleye pollock (Oliveira & Bechtel 2005), 9.2% in Indian mackerel, *Rastrelliger kanagurta* (Zuta *et al*. 2003) and 4.9% in sardine, *Sardina pilchardus* (Dumay *et al*. 2006). An exceptionally high oil yield (93%) has been reported for Asian catfish, *Pangasius bocourti* (Thammapat *et al*. 2010). With cultured fish, differences in production systems, diet formulations and feeding regimes are known to affect composition (Majolini *et al*. 2009; Trocino *et al*. 2012) and the amount of fat deposits laid down in the internal organs (Grigorakis 2007). In the present study, perivisceral fat was consistently more abundant in conventional *D. labrax* and *S. aurata* (as judged by visual observation of the extracted viscera), which could account for the higher lipid levels found in those specimens. Visceral moisture content was found to decrease proportionally with increasing lipid level, irrespective of species or rearing system. Overall, a highly significant ($r = 0.929$, $P < 0.001$) inverse relationship existed between the two components, which was expressed by the following equation: lipid content (%) = −1.03 × moisture content (%) + 84.8.

Neutral and polar lipids. Data from the analysis of lipid classes in the viscera of conventionally and organically farmed *S. aurata*, *D. labrax*, and *D. puntazzo* are summarised in Table 1. Neutral lipids consisted primarily of triacylglycerols (TAG), which accounted for 85.3–89.4% of the total visceral lipid, and, to a far lesser extent, sterols; other classes of neutral lipids, e.g., free fatty acids (FFA), 1,2-diacylglycerols (DAG), and monoacylglycerols (MAG) were only detected in conventional *D. labrax* as minor components. Much lower TAG contents than those presented here have been reported for the viscera of steelhead trout (74.1%) (Zhong *et al*. 2007) and walleye pollock (72.1%) (Oliveira & Bechtel 2005). However, in those studies, substantial FFA and/or DAG contents were also present in the viscera and it is likely that the observed differences could have resulted, at least in part, from lipase activity during storage. The action of endogenous enzymes has been reported to increase the FFA content of visceral oil (Crexi *et al*. 2009), and tissues displaying lower lipid contents are characterised by higher percentages of FFA (Oliveira & Bechtel 2005). Phospholipids (PL) represented the second most important lipid class next to TAG, accounting for 9.6–13.3% of the total visceral lipid. Phosphatidylcholine (PC) was the predominant phospholipid component, followed by phosphatidylethanolamine (PE) and minor proportions of phosphatidylinositol (PI), phosphatidylserine (PS), and sphingomyelin (Sphm). Lyso-phosphatidylcholine (l-PC) was also detected in *D. labrax* and organic *S. aurata*. Significant differences ($P < 0.05$) in the major lipid classes were observed between species. However, the rearing system did not affect the relative proportion of neutral and polar lipids. The sparids displayed higher levels of TAG and lower levels of PL than *D. labrax*. Accordingly, the TAG/PL ratio was lowest ($P < 0.05$) in *D. labrax* and highest in *S. aurata*, with *D. puntazzo* ranking in-between (Table 1). Significant differences were observed also

doi: 10.17221/448/2016-CJFS

Table 1. Total lipid and lipid class profile (% of TL) of visceral oils from conventionally and organically farmed *D. labrax*, *S. aurata* and *D. puntazzo*

	D. labrax (KO)	*D. labrax* (NC)	*S. aurata* (KO)	*S. aurata* (NC)	*D. puntazzo* (SC)
Total lipids (% w/w)	26.53 ± 3.42^{a}	40.20 ± 4.65^{b}	29.92 ± 3.52^{a}	42.61 ± 5.29^{b}	30.00 ± 3.09^{a}
Neutral lipids (% of TL)	86.74 ± 0.93^{a}	87.26 ± 1.05^{ab}	90.20 ± 1.24^{b}	90.44 ± 1.01^{b}	89.28 ± 1.23^{b}
TAG	85.61 ± 0.89^{a}	85.34 ± 1.03^{a}	89.09 ± 1.23^{b}	89.37 ± 0.99^{b}	88.17 ± 1.07^{b}
FFA	–	0.53 ± 0.05	–	–	–
Sterols	1.13 ± 0.08^{a}	1.05 ± 0.07^{a}	1.11 ± 0.10^{a}	1.07 ± 0.11^{a}	1.11 ± 0.11^{a}
1,2-DAG	–	0.18 ± 0.02	–	–	–
MAG	–	0.16 ± 0.02	–	–	–
Polar lipids (% of TL)	13.26 ± 0.46^{a}	12.74 ± 0.52^{a}	9.80 ± 0.38^{b}	9.56 ± 0.34^{b}	10.72 ± 0.46^{c}
PE	3.84 ± 0.36^{a}	3.65 ± 0.28^{a}	3.23 ± 0.37^{a}	3.38 ± 0.31^{a}	3.47 ± 0.29^{a}
PS and PI	0.72 ± 0.07^{a}	0.61 ± 0.05^{a}	0.40 ± 0.02^{b}	0.28 ± 0.02^{c}	0.31 ± 0.02^{c}
PC	7.84 ± 0.41^{a}	7.67 ± 0.45^{a}	5.64 ± 0.28^{b}	5.72 ± 0.33^{b}	6.69 ± 0.35^{c}
Sphm	0.62 ± 0.05^{a}	0.56 ± 0.04^{a}	0.34 ± 0.02^{b}	0.18 ± 0.01^{c}	0.25 ± 0.01^{d}
l-PC	0.24 ± 0.03^{a}	0.25 ± 0.02^{a}	0.19 ± 0.02^{b}	–	–
TAG/PL	6.45 ± 0.13^{a}	6.70 ± 0.17^{a}	9.09 ± 0.25^{b}	9.35 ± 0.27^{b}	8.22 ± 0.22^{c}
PC/PE ratio	2.04 ± 0.05^{a}	2.10 ± 0.06^{a}	1.75 ± 0.03^{b}	1.69 ± 0.04^{b}	1.93 ± 0.04^{c}

1,2-DAG – 1,2-diacylglycerides; FFA – free fatty acids; l-PC – lyso-phosphatidylcholine; MAG – monoacylglycerides; PC – phosphatidylcholine; PE – phospatidylethanolamine; PI – phosphatidylinositol; PS – phosphatidylserine; Sphm – sphingomyelin; TAG – triacylglycerols; results represent means ± SD (n = 6 separate samples); means in the same row bearing different letters differ significantly according to ANOVA ($P < 0.05$); KO – farm under organic production; NC, SC – farms under conventional production

in the PC/PE ratio, with *D. labrax* displaying the highest ($P < 0.05$) and *S. aurata* the lowest values. The results indicate that the TAG/PL ratio in the visceral oils of the three species had an inverse relationship with the PC/PE ratio. The relative proportions of the sterols were very similar, irrespective of the species or rearing system. However, when calculated on a wet viscera basis, sterol concentrations varied significantly ($P < 0.05$) among samples (300–456 mg/100 g), being much lower in organically farmed fish. Further, they were found to increase linearly ($r = 0.998$, $P < 0.01$) with increasing total lipid content. Similar observations were made with regard to TAG concentrations, which ranged from 22.7 g/100 g viscera in organic *D. labrax* to 38.1 g/100 g viscera in conventional *S. aurata* (data calculated from Table 1) and correlated significantly ($r = 0.994$, $P < 0.01$) with total visceral lipid content. Thus, increasing TAG and sterol concentrations were associated with lipid deposition. Whole viscera from all three species were found to be good sources of PC for industrial and/or biomedical applications, providing 1.7–3.1 g PC per 100 g of raw material (data calculated from Table 1).

Fatty acid profile. The fatty acid profiles (% of the total fatty acid methyl esters) of visceral oils from conventionally and organically farmed *D. labrax*, *S. aurata* and *D. puntazzo* are shown in Table 2. Monounsaturated fatty acids (MUFA) were predominant in all three species (42.25–48.35%), followed by polyunsaturated fatty acids (PUFA; 26.68–32.97%) and saturated fatty acids (SFA; 24.7625.79%). Oliveira and Bechtel (2005) reported that MUFA accounted for 43.1% of the total fatty acids in walleye pollock viscera, while PUFA and SFA were present at levels of 27.8 and 21.4%, respectively; Zhong *et al*. (2007) reported that trout visceral oil consisted of 24.2% SFA, 39.6% MUFA and 25.2% PUFA, and Crexi *et al*. (2009, 2010) found that crude, bleached and refined carp visceral oils contained 25–27% SFA, 42% MUFA, and 26–28% PUFA, values which are close to the results presented here. The major fatty acids identified in the visceral oil samples were oleic (C18:1 *n*-9), palmitic (C16:0), DHA (C22:6 *n*-3), linoleic (C18:2 *n*-6), palmitoleic (C16:1 *n*-7), myristic (C14:0), EPA (C20:5 *n*-3), and eicosenoic acids (C20:1 *n*-9), which constituted 75–80% of the total FA content. Palmitic acid was dominant among saturated fatty acids, accounting for more than 56% of the SFA in all species studied (14.07–15.59% of the total FA), which

doi: 10.17221/448/2016-CJFS

Table 2. Fatty acid composition (% of the total fatty acid methyl esters) of visceral oils from conventionally and organically farmed *D. labrax*, *S. aurata*, and *D. puntazzo*

Fatty acids	*D. labrax* (KO)	*D. labrax* (NC)	*S. aurata* (KO)	*S. aurata* (NC)	*D. puntazzo* (SC)
C14:0	3.61 ± 0.08^{a}	4.87 ± 0.12^{b}	4.08 ± 0.15^{c}	5.00 ± 0.10^{b}	3.99 ± 0.09^{c}
C14:1	0.34 ± 0.00^{a}	0.47 ± 0.01^{b}	0.44 ± 0.01^{c}	0.38 ± 0.00^{d}	0.40 ± 0.01^{e}
C15:1 *n*-5	–	0.10 ± 0.00	–	–	–
C16:0	14.91 ± 0.24^{ac}	14.07 ± 0.22^{b}	15.26 ± 0.18^{ad}	14.75 ± 0.23^{c}	15.59 ± 0.26^{d}
iso-C16:0	1.29 ± 0.02^{a}	0.55 ± 0.01^{b}	0.56 ± 0.01^{b}	0.37 ± 0.01^{c}	0.30 ± 0.01^{d}
C16:1 *n*-9	1.20 ± 0.05^{a}	1.66 ± 0.02^{b}	0.64 ± 0.02^{c}	0.40 ± 0.01^{d}	0.84 ± 0.02^{e}
C16:1 *n*-7	5.00 ± 0.11^{a}	5.87 ± 0.16^{b}	4.41 ± 0.13^{c}	5.56 ± 0.19^{b}	5.66 ± 0.14^{b}
iso-C17:0	0.49 ± 0.02^{a}	0.38 ± 0.02^{b}	0.71 ± 0.03^{c}	0.39 ± 0.02^{b}	0.25 ± 0.01^{d}
anteiso-C17:0	0.29 ± 0.01^{a}	0.47 ± 0.02^{b}	0.42 ± 0.01^{c}	0.52 ± 0.04^{b}	0.34 ± 0.01^{d}
cyclo-C17:0	0.25 ± 0.01^{a}	0.33 ± 0.01^{b}	0.44 ± 0.01^{c}	0.29 ± 0.01^{d}	0.34 ± 0.01^{b}
C17:0	0.16 ± 0.00^{a}	0.42 ± 0.01^{b}	0.46 ± 0.01^{c}	0.37 ± 0.01^{d}	0.30 ± 0.00^{e}
C17:1 *n*-7	0.27 ± 0.01^{ac}	0.32 ± 0.01^{b}	0.29 ± 0.01^{ad}	0.26 ± 0.01^{c}	0.30 ± 0.01^{bd}
C18:0	2.85 ± 0.16^{ab}	2.62 ± 0.14^{a}	3.18 ± 0.17^{b}	2.65 ± 0.12^{a}	2.95 ± 0.18^{b}
C18:1 *n*-9	30.42 ± 0.61^{a}	30.11 ± 0.58^{a}	25.24 ± 0.47^{b}	24.97 ± 0.35^{b}	27.65 ± 0.41^{c}
C18:1 *n*-7	3.16 ± 0.14^{a}	2.88 ± 0.12^{b}	2.69 ± 0.13^{bc}	2.63 ± 0.09^{c}	2.34 ± 0.11^{d}
CLA *trans*-10,*cis*-12	0.51 ± 0.01^{a}	0.15 ± 0.00^{b}	0.18 ± 0.00^{c}	0.21 ± 0.01^{d}	0.12 ± 0.01^{e}
CLA *cis*-9,*trans*-11	0.59 ± 0.03^{a}	0.15 ± 0.00^{b}	0.15 ± 0.00^{b}	0.13 ± 0.00^{c}	0.20 ± 0.01^{d}
CLA *cis*-11,*trans*-13	0.18 ± 0.01^{a}	0.07 ± 0.00^{b}	0.05 ± 0.00^{c}	0.06 ± 0.00^{d}	–
C18:2 *n*-6	7.63 ± 0.33^{a}	9.95 ± 0.41^{b}	7.88 ± 0.22^{a}	9.77 ± 0.37^{b}	8.03 ± 0.28^{a}
C18:3 *n*-6	0.37 ± 0.01^{a}	0.25 ± 0.01^{b}	0.23 ± 0.01^{bc}	0.22 ± 0.01^{c}	0.30 ± 0.01^{d}
C18:3 *n*-3	1.82 ± 0.11^{a}	2.49 ± 0.15^{b}	1.62 ± 0.14^{a}	2.46 ± 0.16^{b}	2.54 ± 0.16^{b}
C18:4 *n*-3	0.44 ± 0.01^{a}	0.70 ± 0.03^{b}	1.09 ± 0.06^{c}	1.35 ± 0.06^{d}	1.46 ± 0.08^{d}
C19:0	0.24 ± 0.01^{a}	0.30 ± 0.01^{b}	0.28 ± 0.01^{b}	0.20 ± 0.01^{c}	0.21 ± 0.01^{c}
C20:0	0.59 ± 0.02^{a}	0.53 ± 0.01^{b}	–	0.79 ± 0.02^{c}	0.26 ± 0.01^{d}
C20:1 *n*-9	4.48 ± 0.35^{a}	2.67 ± 0.25^{b}	4.57 ± 0.36^{a}	3.13 ± 0.27^{b}	3.24 ± 0.32^{b}
C20:2 *n*-6	0.85 ± 0.02^{a}	0.54 ± 0.01^{b}	0.83 ± 0.01^{a}	0.51 ± 0.01^{c}	0.80 ± 0.01^{d}
C20:3 *n*-6	0.39 ± 0.01^{a}	0.29 ± 0.01^{b}	0.57 ± 0.02^{c}	0.36 ± 0.01^{d}	0.30 ± 0.01^{b}
C20:4 *n*-6	0.23 ± 0.01^{ab}	0.23 ± 0.01^{ab}	0.24 ± 0.01^{a}	0.21 ± 0.01^{b}	0.30 ± 0.00^{c}
C20:3 *n*-3	0.52 ± 0.01^{a}	0.65 ± 0.02^{b}	0.63 ± 0.01^{b}	0.88 ± 0.02^{c}	0.78 ± 0.02^{d}
C20:5 *n*-3 (EPA)	3.45 ± 0.24^{a}	3.58 ± 0.27^{a}	5.06 ± 0.32^{b}	4.90 ± 0.31^{bc}	4.45 ± 0.21^{c}
C22:1 *n*-11	2.48 ± 0.08^{a}	1.49 ± 0.07^{b}	5.03 ± 0.18^{c}	4.30 ± 0.12^{d}	1.50 ± 0.06^{b}
C22:1 *n*-9	0.51 ± 0.03^{a}	0.48 ± 0.02^{a}	0.62 ± 0.02^{b}	0.72 ± 0.03^{c}	0.18 ± 0.00^{d}
C23:0	0.18 ± 0.01^{a}	0.22 ± 0.01^{bd}	0.31 ± 0.01^{c}	0.31 ± 0.01^{c}	0.21 ± 0.01^{d}
C22:4 *n*-6	0.11 ± 0.00^{a}	–	0.11 ± 0.00^{a}	0.12 ± 0.00^{b}	0.14 ± 0.00^{c}
C22:5 *n*-6	0.13 ± 0.00^{a}	0.25 ± 0.00^{b}	0.25 ± 0.01^{b}	0.14 ± 0.00^{c}	0.14 ± 0.00^{c}
C22:5 *n*-3	0.99 ± 0.03^{a}	1.19 ± 0.08^{b}	1.91 ± 0.04^{c}	1.96 ± 0.05^{c}	2.53 ± 0.09^{d}
C24:0	0.11 ± 0.00^{a}	–	0.09 ± 0.00^{b}	–	0.05 ± 0.00^{c}
C22:6 *n*-3 (DHA)	8.47 ± 0.33^{a}	8.26 ± 0.41^{a}	8.73 ± 0.29^{a}	8.12 ± 0.38^{a}	10.90 ± 0.31^{b}
C24:1 *n*-9	0.49 ± 0.01^{a}	0.44 ± 0.01^{b}	0.75 ± 0.02^{c}	0.61 ± 0.01^{d}	0.14 ± 0.01^{e}
SFA	24.97 ± 0.25^{a}	24.76 ± 0.23^{a}	25.79 ± 0.21^{b}	25.64 ± 0.22^{b}	24.78 ± 0.22^{a}
MUFA	48.35 ± 0.58^{a}	46.49 ± 0.47^{b}	44.68 ± 0.45^{c}	42.96 ± 0.34^{d}	42.25 ± 0.29^{e}
PUFA	26.68 ± 0.31^{a}	28.75 ± 0.29^{b}	29.53 ± 0.29^{c}	31.40 ± 0.36^{d}	32.97 ± 0.31^{e}
Σ *n*-3	15.69 ± 0.30^{a}	16.87 ± 0.38^{b}	19.04 ± 0.27^{c}	19.67 ± 0.35^{d}	22.65 ± 0.34^{e}

doi: 10.17221/448/2016-CJFS

Table 2 to be continued

Fatty acids	*D. labrax* (KO)	*D. labrax* (NC)	*S. aurata* (KO)	*S. aurata* (NC)	*D. puntazzo* (SC)
Σ *n*-6	10.99 ± 0.28^{a}	11.88 ± 0.38^{b}	10.49 ± 0.21^{c}	11.73 ± 0.33^{b}	10.32 ± 0.23^{c}
n-3/*n*-6	1.43 ± 0.02^{a}	1.42 ± 0.02^{a}	1.82 ± 0.02^{b}	1.68 ± 0.02^{c}	2.19 ± 0.01^{d}
MUFA/SFA	1.94 ± 0.02^{a}	1.88 ± 0.02^{b}	1.73 ± 0.02^{c}	1.68 ± 0.02^{d}	1.70 ± 0.01^{cd}
PUFA/SFA	1.07 ± 0.01^{a}	1.16 ± 0.02^{b}	1.14 ± 0.02^{b}	1.22 ± 0.02^{c}	1.33 ± 0.02^{d}
DHA/EPA	2.46 ± 0.01^{a}	2.31 ± 0.01^{b}	1.73 ± 0.01^{c}	1.66 ± 0.02^{d}	2.45 ± 0.01^{a}
TFA (g/g fat)	0.79 ± 0.04^{a}	0.80 ± 0.03^{a}	0.84 ± 0.03^{a}	0.85 ± 0.04^{a}	0.84 ± 0.04^{a}

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; TFA – total fatty acids; results represent means ± SD (n = 6 separate samples); means in the same row bearing different letters differ significantly according to ANOVA ($P < 0.05$); KO – farm under organic production; NC, SC – farms under conventional production

is in accord with the findings for carp (Crexi *et al*. 2010), trout (Zhong *et al*. 2007; Fiori *et al*. 2012) and catfish (Sathivel *et al*. 2002; Thammapat *et al*. 2010) visceral oils. Myristic acid (C14:0) and stearic acid (C18:0) were present in much lesser proportions, with the former increasing almost linearly (r = 0.993, $P < 0.01$) with total visceral lipid content. Palmitic and myristic acids are also the major SFA in the muscle lipid of *S. aurata*, *D. labrax*, and *D. puntazzo* (Testi *et al*. 2006; Trocino *et al*. 2012), with their relative proportions being very similar to those reported here for visceral lipid. Odd-chain SFA such as margaric acid (17:0), isomethyl branched fatty acids such as *iso*-C16:0, *iso*-C17:0, and *cyclo*-C17:0 were present as minor components in all samples. Oleic acid was the most abundant monounsaturated fatty acid in all samples analysed, constituting 25–30% of the total FA content. This is in agreement with the findings reported for the visceral oil of steelhead trout (28.6%) (Zhong *et al*. 2007), carp (25.8–26.5%) (Crexi *et al*. 2010), horse mackerel *Magalaspis cordyla* (27.7%), and croaker *Otolithes ruber* (26.0%) (Nazeer *et al*. 2012). The relative proportion of oleic acid was not affected by the rearing system; however, significant ($P < 0.05$) differences were observed between species, with *D. labrax* displaying the highest and *S. aurata* the lowest value. Palmitoleic acid (C16:1 *n*-7) was the second most important MUFA, next to oleic, and its proportion was significantly ($P < 0.05$) higher in conventional *D. labrax* and *S. aurata*. Eicosenoic and cetoleic (C22:1 *n*-11) acids were also present in the visceral oil of all three species examined, in proportions similar to those found in their muscle tissues (Trocino *et al*. 2012). Eicosenoic acid was affected by the rearing system, and was always more abundant in organically farmed fish. Cetoleic acid seemed to be affected both by species and rearing system and its relative proportion was also higher ($P < 0.05$) in the visceral oil of organically farmed fish. Cetoleic acid was accompanied by a minor proportion of its isomer C22:1 *n*-9. The visceral oil from all three species studied was characterised by high proportions of polyunsaturated fatty acids, *n*-3 PUFA in particular, which accounted for 15.69–22.65% of the total FA content. The major *n*-3 fatty acids were DHA and EPA, but α-linolenic (C18:3 *n*-3), docosapentaenoic (DPA, C22:5 *n*-3) and steriadonic (18:4 *n*-3) acids were also present in notable amounts. Total PUFA and *n*-3 fatty acid contents of the visceral oils were affected both by species and rearing system, with conventionally farmed fish displaying significantly ($P < 0.05$) higher proportions than their organic counterparts. The observed differences in PUFA levels between species resulted mainly from the higher percentages of EPA, DPA and steriadonic acid in the visceral oils of the sparid fish. *D. puntazzo* also displayed higher DHA levels than its counterparts, whereas no significant ($P < 0.05$) difference in DHA was observed between *S. aurata* and *D. labrax*. The proportion of α-linolenic acid was not affected by species, but rather by the rearing system, being higher in conventionally farmed fish. The proportion of *n*-6 PUFA was also higher in conventional fish. Linoleic acid (C18:2 *n*-6), the principal *n*-6 PUFA, was found in significantly ($P < 0.05$) higher proportions in conventional *D. labrax* (9.95%) and *S. aurata* (9.77%). Similar values (9.0–9.7%) have been reported for trout and carp visceral oils (Zhong *et al*. 2007; Crexi *et al*. 2010), but higher levels (13.5 to 19.1%) may be found in catfish species (Sathivel *et al*. 2002; Thammapat *et al*. 2010). Increasing levels of *n*-6 PUFA are known to result from the supplementation of fish feed with vegetable oils, which

doi: 10.17221/448/2016-CJFS

increases the proportion of dietary C18:2 *n*-6 (Grigorakis 2007; Turchini *et al*. 2009; Benedito-Palos *et al*. 2011). The low proportions of γ-linolenic (C18:3 *n*-6) and arachidonic (C20:4 *n*-6) acids in the visceral oils analysed were probably due to the minimal amounts of these fatty acids in the fish diet. The main CLA isomers detected were the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers. The DHA/EPA ratios of the oil samples ranged from 1.66 to 2.46 and were affected by the rearing system, being higher ($P < 0.05$) in organically farmed fish. These values compare favourably with those reported for the visceral oils of Asian catfish (0.11) (Thammapat *et al*. 2010), carp (0.31) (Crexi *et al*. 2010), rainbow trout (0.94) (Fiori *et al*. 2012), and Indian mackerel (1.08) (Sahena *et al*. 2010). Increasing DHA/EPA ratios are thought to be beneficial, since DHA is more efficient than EPA in reducing the risk of coronary heart disease and the progression of coronary atherosclerosis (Holub 2009). The ratio of *n*-3 to *n*-6 PUFA, often used to compare the relative nutritional value of fish oils, ranged from 1.42 to 2.19, being much higher than the 1 : 4 to 1 : 1 ratio that has been recommended for a balanced human diet (Simopoulos 2002). Comparable *n*-3/*n*-6 ratios have been reported for the muscle lipid of *S. aurata* (1.19–1.60) (Anedda *et al*. 2013) and organically reared *D. labrax* (1.44–1.75) (Trocino *et al*. 2012), as well as for the ordinary muscle (1.69–2.13) and perivisceral fat (1.29–1.80) of cultured *D. puntazzo* (Rondan *et al*. 2004). According to Trocino *et al*. (2012), the *n*-3/*n*-6 ratio in the muscle lipid of organically farmed *D. labrax* was three times higher than in conventional fish. In the present study, the visceral oil of organic *S. aurata* displayed a higher *n*-3/*n*-6 ratio than its conventional counterpart, but no significant difference was observed in the case of *D. labrax*. The PUFA/SFA ratios of the visceral oils from all three species (1.07–1.33) exceeded the value of 0.45 and was highest in *D. puntazzo*, while the ratios of MUFA to SFA ranged from 1.68 to 1.94 and was highest in organic *D. labrax*. The results indicate that the viscera of farmed *S. aurata*, *D. puntazzo* and *D. labrax* could be used in the production of high-quality fish oil for the food and pharmaceutical industries. The contents of SFA, MUFA, PUFA, *n*-3, and *n*-6 fatty acids, expressed as g/100 g of viscera, were calculated from the data shown in Table 2. The largest amounts of *n*-3 fatty acids, especially EPA and DHA, were obtained from conventional *S. aurata* (4.71 g/100 g wet weight) followed by *D. puntazzo* (3.87 g/100 g wet weight) and conventional *D. labrax* (3.81 g/100 g wet weight). From a nutritional standpoint, the high DHA + EPA content of the visceral oils is beneficial and desirable for the manufacture of *n*-3-supplemented foods and fish oil *n*-3 concentrates.

Products of lipid oxidation and hydrolysis. Data regarding the oxidative and hydrolytic stability of the visceral oils, as assessed by determining primary (CD, PV) and secondary (TBARS) oxidation products and FFA content over the 30-day storage period at 63°C, are presented in Figures 1–4. The trend in lipid oxidation observed in the present study was similar to that reported for steelhead trout (Zhong *et al*. 2007) and mackerel (Zuta *et al*. 2007). Levels of CD increased almost linearly during storage in all three species, with the sparid fish (conventional *S. aurata* and *D. puntazzo*, in particular) displaying the highest values at the end of the trial. Peroxide values showed a similarly increasing trend up to 20 days of storage, but remained largely unchanged thereafter. The initial PVs and CD levels were similar to those reported for the visceral oil of carp and steelhead trout (Zhong *et al*. 2007; Crexi *et al*. 2009). Muscle lipids are more susceptible to oxidation and usually exhibit higher initial levels of peroxides than the visceral oils; thus, PVs in the range of 3.09–10.28 meq O_2/kg have been reported for the unrefined oils of such species as tuna (*Thunnus* sp.), hoki (*Macruronus novaezelandiae*), menhaden (*Brevoortia patronus*), horse mackerel (*Trachurus trachurus*), shad (*Alosa fallax*), golden mullet (*Mugil auratus*), and garfish (*Belone belone*), while considerably higher values (up to 15–20 meq/kg) may be found in the refined oil (Boran *et al*. 2006; Crexi *et al*. 2010; Yin & Sathivel 2010). In the present study, PVs were higher in the visceral oils of conventional fish at every time interval, and correlated significantly ($P < 0.05$) with CD levels. The increase in absorbance at 234 nm provides an indication of auto-oxidation, and is closely associated with the uptake of oxygen and the formation of hydroperoxides in the early stage of oxidation (Zuta *et al*. 2007). However, peroxides are unstable, particularly during prolonged storage at elevated temperatures, which imposes limitations on their usefulness as a measure of oxidative deterioration in lipids. The concentration of TBARS increased slowly over the 30-day storage period and correlated significantly ($P < 0.05$) both with CD levels and PVs. Similarly to the conjugated dienes, TBARS levels were higher in the visceral oil of the sparids. The presence of

doi: 10.17221/448/2016-CJFS

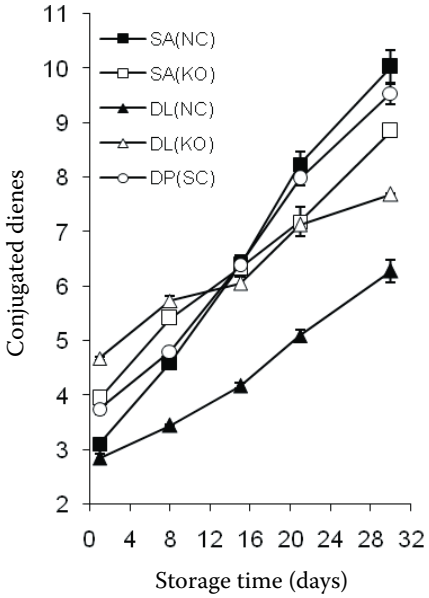

Figure 1. Conjugated diene levels in the visceral lipid of conventional *S. aurata* SA(NC) and *D. labrax* DL(NC), organic *S. aurata* SA(KO) and *D. labrax* DL(KO), and conventional *D. puntazzo* DP(SC), during storage at 63°C for 30 days; means of three samples analysed in duplicate; where error bars are not visible, error is within the size of the caps

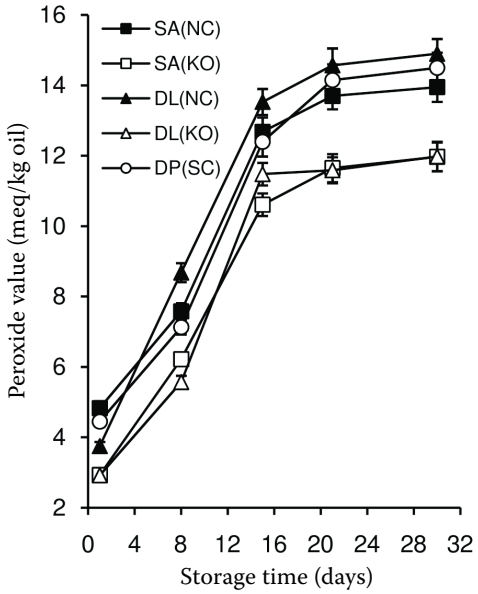

Figure 2. Peroxide values in the visceral lipid of conventional *S. aurata* SA(NC) and *D. labrax* DL(NC), organic *S. aurata* SA(KO) and *D. labrax* DL(KO), and conventional *D. puntazzo* DP(SC), during storage at 63°C for 30 days; means of three samples analysed in duplicate; where error bars are not visible, error is within the size of the caps

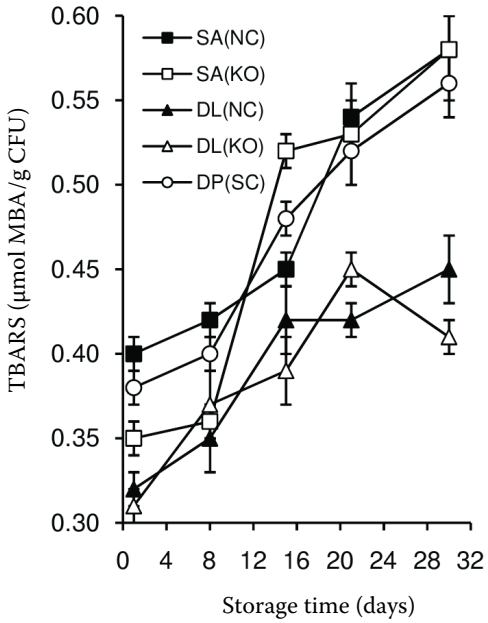

Figure 3. TBARS levels in the visceral lipid of conventional *S. aurata* SA(NC) and *D. labrax* DL(NC), organic *S. aurata* SA(KO) and *D. labrax* DL(KO), and conventional *D. puntazzo* DP(SC), during storage at 63°C for 30 days; means of three samples analysed in duplicate; where error bars are not visible, error is within the size of the caps

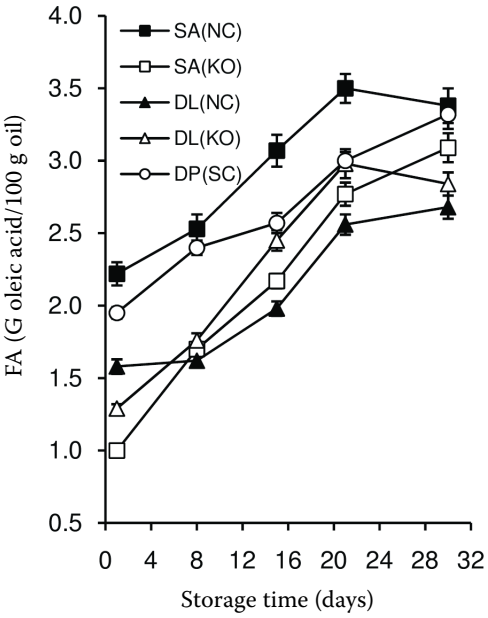

Figure 4. FFA content in the visceral lipid of conventional *S. aurata* SA(NC) and *D. labrax* DL(NC), organic *S. aurata* SA(KO) and *D. labrax* DL(KO), and conventional *D. puntazzo* DP(SC), during storage at 63°C for 30 days; means of three samples analysed in duplicate; where error bars are not visible, error is within the size of the caps

doi: 10.17221/448/2016-CJFS

appreciable PE levels in the visceral oil of all three species examined might have contributed to the oxidative stability of the samples, via the formation of antioxidative compounds such as pyrroles, resulting from non-enzymatic browning reactions between the PE primary amine group and products of PL oxidation (Lu *et al*. 2012). Lipid oxidation and non-enzymatic browning reactions constitute major deteriorative mechanisms in marine phospholipids, and follow parallel reaction pathways. The presence of PE in marine oils has been reported to cause the formation of pyrroles, generate Strecker-derived volatiles and decrease the yellowing index and lipid oxidation levels (Lu *et al*. 2013). Carotenoids were not detected in the analysed samples (data not shown), but other antioxidants that might be present in the visceral oil, e.g., α-tocopherol originating from the feed, could have an impact on lipid stability. The initial FFA levels were in the range of 1.00–2.22%. The visceral oil of conventionally farmed sparid fish displayed the highest initial FFA contents, whereas lowest levels were obtained with the oil of organic *S. aurata*. Much of the initial FFA content results from the hydrolysis of triacylglycerols by endogenous and/or bacterial lipases present in the digestive tract. Thus, low FFA levels would be anticipated in the crude oil if early gutting and immediate processing of the viscera would take place. During storage, the FFA content increased gradually in all examined samples, reaching levels of 2.68–3.38% by the end of the trial. Higher FFA contents (4.26–4.53%) have been reported for the crude visceral oils of carp (Crexi *et al*. 2009) and catfish (Sathivel *et al*. 2003). The formation of FFA has been associated with off-flavours, increased foaming of the oil during mixing and heating and lower smoke points; an acceptable level of FFA in refined fish oil is between 1.8–3.5% (Sathivel *et al*. 2003).

CONCLUSIONS

The composition of visceral oils from conventionally and organically farmed *S. aurata*, *D. labrax*, and *D. puntazzo* was determined. Neutral lipids, mainly triacylglycerols, represented the major lipid class, followed by phospholipids. The proportions of lipid classes were influenced more by species than by rearing system. Visceral oils from all three species studied were found to be an excellent source of PC (lecithin) and PE, providing 1.7–3.1 and 1.00–1.50 g of PC and PE per 100 g of raw material, respectively. MUFA were predominant in all three species (42.25–48.35%), followed by PUFA (26.68–32.97%), and SFA (24.76–25.79%). Oleic acid was the major MUFA, whereas palmitic acid was dominant among SFA. Visceral oils were characterised by high proportions of polyunsaturated fatty acids, which were affected both by species and rearing system, with conventional fish displaying significantly ($P < 0.05$) higher proportions than their organic counterparts. The major *n*-3 fatty acids were DHA and EPA, but α-linolenic, docosapentaenoic and steriadonic acids were also present in notable amounts. Organic fish displayed higher DHA/EPA ratios. During storage at 63°C, visceral oils exhibited good oxidative stability, as assessed by conjugated dienes, peroxide values, thiobarbituric acid-reactive substances (TBARS) and free fatty acid determination. According to the results of this study, the whole viscera of farmed *S. aurata*, *D. labrax* and *D. puntazzo* are an important source of functional nutrients that could be converted to value-added products.

References

Anedda R., Piga C., Santercole V., Spada S., Bonaglini E., Cappuccinelli R., Mulas G., Roggio T., Uzzau S. (2013): Multidisciplinary analytical investigation of phospholipids and triglycerides in offshore farmed gilthead sea bream (*Sparus aurata*) fed commercial diets. Food Chemistry, 138: 1135–1144.

Benedito-Palos L., Bermejo-Nogales A., Karampatos A.I., Ballester-Lozano G.F., Navarro J.C., Diez A., Bautista J.M., Gordon Bell J., Tocher D.R., Obach A., Kaushik S., Pérez-Sánchez J. (2011): Modelling the predictable effects of dietary lipid sources on the fillet fatty acid composition of one-year-old gilthead sea bream (*Sparus aurata* L.). Food Chemistry, 124: 538–544.

Boran G., Karaçam H., Boran M. (2006): Changes in the quality of fish oils due to storage temperature and time. Food Chemistry, 98: 693–698.

Crexi V.T., Souza Soares L.A., Pinto L.A.A. (2009): Carp (*Cyprinus carpio*) oils obtained by fishmeal and ensilage processes: characteristics and lipid profiles. International Journal of Food Science and Technology, 44: 1642–1648.

Crexi V.T., Monte M.L., Souza Soares L.A., Pinto L.A.A. (2010): Production and refinement of oil from carp (*Cyprinus carpio*) viscera. Food Chemistry, 119: 945–950.

Dumay J., Donnay-Moreno C., Barnathan G., Jaouen P., Bergé J.P. (2006): Improvement of lipid and phospholipid recoveries from sardine (*Sardina pilchardus*) viscera using industrial proteases. Process Biochemistry, 41: 2327–2332.

doi: 10.17221/448/2016-CJFS

Fiori L., Solana M., Tosi P., Manfrini M., Strim C., Guella G. (2012): Lipid profiles of oil from trout (*Oncorhynchus mykiss*) heads, spines and viscera: Trout by-products as a possible source of omega-3 lipids? Food Chemistry, 134: 1088–1095.

Grigorakis K. (2007): Compositional and organoleptic quality of farmed and wild gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) and factors affecting it: a review. Aquaculture, 272: 55–75.

Holub B.J. (2009): Docosahexaenoic acid (DHA) and cardiovascular disease risk factors. Prostaglandins, Leukotrienes and Essential Fatty Acids, 81: 199–204.

Lu F.S.H., Nielsen N.S., Baron C.P., Jacobsen C. (2012): Oxidative degradation and non-enzymatic browning between oxidized lipids and primary amine groups in different marine PL emulsions. Food Chemistry, 135: 2887–2896.

Lu F.S.H., Nielsen N.S., Baron C.P., Diehl B.W.K., Jacobsen C. (2013): Impact of primary amine group from aminophospholipids and amino acids on marine phospholipids stability: Non-enzymatic browning and lipid oxidation. Food Chemistry, 141: 879–888.

Majolini M., Trocino A., Xiccato G., Santulli A. (2009): Near infrared reflectance spectroscopy (NIRS) characterization of European sea bass (*Dicentrarchus labrax*) from different rearing systems. Italian Journal of Animal Science, 8 (Suppl. 2): 860–862.

Nazeer R.A., Satya N., Kumar S. (2012). Fatty acid composition of horse mackerel (*Magalaspis cordyla*) and croaker (*Otolithes ruber*). Asian Pacific Journal of Tropical Disease, 2: S933–S936.

Oliveira A.C.M., Bechtel P.J. (2005): Lipid composition of Alaska pink salmon (*Oncorhynchus gorbuscha*) and Alaska walleye pollock (*Theragra chalcogramma*) byproducts. Journal of Aquatic Food Product Technology, 14: 73–91.

Rondán M., Hernández M.D., Egea M.A., García B., Rueda F.M., Martínez F.J. (2004): Effect of feeding rate on fatty acid composition of sharpsnout sea bream (*Diplodus puntazzo*). Aquaculture Nutrition, 10: 301–307.

Rustad T., Storrø I., Slizyte R. (2011): Possibilities for the utilisation of marine by-products. International Journal of Food Science and Technology, 46: 2001–2014.

Sahena F., Zaidul I.S.M., Jinap S., Yazid A.M., Khatib A., Norulaini N.A.N. (2010): Fatty acid compositions of fish oil extracted from different parts of Indian mackerel (*Rastrelliger kanagurta*) using various techniques of supercritical CO_2 extraction. Food Chemistry, 120: 879–885.

Sathivel S., Prinyawiwatkul W., Grimm C.C., King J.M., Lloyd S. (2002): FA composition of crude oil recovered from catfish viscera. Journal of the American Oil Chemists' Society, 79: 989–992.

Sathivel S., Prinyawiwatkul W., King J.M., Grimm C.C., Lloyd S. (2003): Oil production from catfish viscera. Journal of the American Oil Chemists' Society, 80: 377–382.

Simopoulos A.P. (2002): The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacotherapy, 56: 365–379.

Sinanoglou V.J., Miniadis-Meimaroglou S. (1998): Fatty acid of neutral and polar lipids of (edible) Mediterranean cephalopods. Food Research International, 31: 467–473.

Sinanoglou V.J., Strati I.F., Bratakos S.M., Proestos C., Zoumpoulakis P., Miniadis-Meimaroglou S. (2013): On the combined application of Iatroscan-TLC-FID and GC-FID to identify total, neutral and polar lipids and their fatty acids extracted from foods. ISRN Chromatography, Article ID 859024. doi: 10.1155/2013/859024

Sinanoglou V.J., Proestos C., Lantzouraki D.Z., Calokerinos A.C., Miniadis-Meimaroglou S. (2014): Lipid evaluation of farmed and wild meagre (*Argyrosomus regius*). European Journal of Lipid Science and Technology, 116: 134–143.

Testi S., Bonaldo A., Gatta P.P., Badiani A. (2006): Nutritional traits of dorsal and ventral fillets from three farmed fish species. Food Chemistry, 98: 104–111.

Thammapat P., Raviyan P., Siriamornpun S. (2010): Proximate and fatty acids composition of the muscles and viscera of Asian catfish (*Pangasius bocourti*). Food Chemistry, 122: 223–227.

Trocino A., Xiccato G., Majolini D., Tazzoli M., Bertotto D., Pascoli F., Palazzi R. (2012): Assessing the quality of organic and conventionally-farmed European sea bass (*Dicentrarchus labrax*). Food Chemistry, 131: 427–433.

Turchini G.M., Torstensen B.E., Ng W.K. (2009): Fish oil replacement in finfish nutrition. Reviews in Aquaculture, 1: 10–57.

Yin H., Sathivel S. (2010): Physical properties and oxidation rates of unrefined menhaden oil (*Brevoortia patronus*). Journal of Food Science, 75: E163–E168.

Zhong Y., Madhujith T., Mahfouz N., Shahidi F. (2007): Compositional characteristics of muscle and visceral oil from steelhead trout and their oxidative stability. Food Chemistry, 104: 602–608.

Zuta P.C., Simpson B.K., Chan H.M., Philips L. (2003): Concentrating PUFA from mackerel processing waste. Journal of American Oil Chemists' Society, 80: 933–936.

Zuta P.C., Simpson B.K., Zhao X., Lecler L. (2007): The effect of α-tocopherol on the oxidation of mackerel oil. Food Chemistry, 100: 800–807.

Received: 2016–11–28
Accepted after corrections: 2017–09–13
Published online: 2017–10–10