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Season and cooking may alter fatty acids profile of polar lipids from Blue-Back Fish

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**Abstract**

Polar lipids (PoL) represent a new promising dietary approach in the prevention and treatment of many human diseases, due to their potential nutritional value and unique biophysical properties. This study investigates the effects of catching season and oven baking on the fatty acid profiles (FAP) of PoL in four species of blue-back fish widely present in the North Adriatic Sea: anchovy (*Engraulis encrasicholus*), sardine (*Sardina pilchardus*), sprat (*Sprattus sprattus*) and horse mackerel (*Trachurus trachurus*).

PoL levels (427–652 mg/100 g flesh) varied among the four species, with no significant seasonal variations within species. FAP of raw fillets were particularly high in PUFA, especially DHA and EPA; total PUFA was constant in all species throughout the year, while n-3 PUFA rose in spring (except in sprat), especially due to the contribution of DHA. The FAP response for PoL to oven baking was species-specific and, among n-3 PUFA, DHA exhibited the greatest heat resistance; the influence of oven baking on FAP was found to be correlated with the catching season, especially for anchovy and sardine, while sprat PoL were not affected by cooking processes.

The four species analyzed in this study presented very low n-6/n-3 fatty acid ratios and highly favorable nutritional indices, emphasizing their PoL qualities and promoting their role in increasing human n-3 PUFA intake. The four species can be considered as superior sources of n-3 PUFA and can be employed as supplements in functional food manufacturing and in pharmaceutical and cosmetic industries.

**Keywords**

Blue-back fish · Polar lipids · Neutral lipids · Fatty acid composition · Season influence · Cooking effects · Baking · Nutritional quality

**Abbreviations**

| | |
|---|---|
| AI | Atherogenicity index |
| ALA | α-Linolenic acid |
| ARA | Arachidonic Acid |
| DHA | Docosahexaenoic acid |
| EPA | Eicosapentaenoic acid |
| FAME | Fatty acid methyl esters |
| FAP | Fatty acid profile |
| HH | Hypocholesterolemic to hypercholesterolemic fatty acid ratio |
| LNA | Linoleic acid |
| MUFA | Monounsaturated fatty acid(s) |
| n-3 PUFA | Long-chain n-3 polyunsaturated fatty acid(s) |
| n-6 PUFA | Long-chain n-6 polyunsaturated fatty acid(s) |
| NL | Neutral lipids |
| PL | Phospholipids |
| PoL | Polar lipids |
| PUFA | Polyunsaturated fatty acid(s) |
| SFA | Saturated fatty acid(s) |
| TI | Thrombogenicity index |
| TL | Total lipids |

## Introduction

Fish is not only a valuable source of high quality animal protein, but it is also well known that fish lipids are rich in long-chain n-3 polyunsaturated fatty acids (n-3 PUFA), especially 20:5n-3 (EPA) and 22:6n-3 (DHA); these fatty acids are unanimously considered as molecules with high nutritional value for the human diet and represent a focus of nutritionists, for the prevention and treatment of obesity (Fernandes et al., 2014; Parmentier, Al Sayed Mahmoud, Linder, & Fanni, 2007; Schneedorferová, Tomčala, & Valterová, 2015). Several studies have demonstrated that n-3 PUFA may be considered as nutraceuticals, i.e. medicinal foods that play a role in maintaining well-being, enhancing health and modulating immunity and thereby able to prevent as well as to treat specific diseases such as cardiovascular diseases, rheumatoid arthritis, diabetes, ulcerative colitis, allergies, eczema, thickening of the skin, weight gain, premature labor, depression and cancer (Fernandes et al., 2014; Hossain, Hosokawa, & Takahashi, 2009; Loftsson, Ilievska, Asgrimsdottir, Ormarsson, & Stefansson, 2016; Murakawa et al., 2007; Taylor, Pletschen, Arends, Unger, & Massing, 2010).

The fatty acid profile (FAP) of marine fish can vary among species and among individuals, depending on their diet, size, age, gender, environmental conditions, season and method of capture (Fernandes et al., 2014).

Several studies have observed that the total lipid (TL) content of fish and the FAP may vary throughout the year, due to exogenous and endogenous factors, and may also be affected by processing or cooking methods (Kaçar & Başhan, 2015); in fact, cooking may significantly alter the content, composition, biological activity and nutritional value of fish lipids, due to water loss, oxidation, loss of fatty acids by leaching, and fatty acid exchange between fish and other oils (Little, Armstrong, & Bergan, 2000). The FAP of TL is influenced by the lipid classes by which they are constituted: neutral lipids (NL) and polar lipids (PoL), which are different in composition and role. NL mainly consist of triacylglycerols, storage lipids used for energy purposes, for the maturation of gametes during the breeding season and as a temporary PUFA reservoir which can be forwarded to structural lipids or directed to specific metabolic pathways (Varljen, Baticic, Sincic-Modric, Obersnel, & Kapovic, 2004). The PoL include mainly phospholipids (PL) (De Leonardis & Macciola, 2004; Fanni, Linder, & Parmentier, 2004), important structural components of cell membranes and eicosanoid precursors. The

FAP of NL seems to depend mainly on the quality and availability of dietary intake and on the reproductive status of the subject, while the PoL fraction is less representative of dietary composition and can vary mainly due to environmental conditions such as temperature and salinity (Cordier, Brichon, Weber, & Zwingelstein, 2002).

The n-3 PUFA of PoL are important precursors of beneficial prostaglandins and platelet-activating factors and have a number of interesting biological activities, ranging from involvement in inflammatory and allergic reactions and antihypertensive responses to physiological processes (reproduction, fetal development, childbirth) (Blank, Cress, Smith, & Snyder, 1992). Conversely, oxylipins formed from n-3 and n-6 PUFA have essential roles in normal physiology and function, but can also have detrimental effects (Gabbs, Leng, Devassy, Monirujjaman, & Aukema, 2015). In mammalian systems, longer-chain n-3 and n-6 PUFA are synthesized from two essential precursors: α-linolenic acid (ALA; 18:3 n-3), leading to n-3 PUFA, and linoleic acid (LNA; 18:2 n-6), resulting in n-6 PUFA. The precursors compete for the enzymatic system, thus a high intake of ALA supports the elongation of n-3 PUFA and reduces the elongation of n-6 PUFA (Brenner, 1977). The n-6/n-3 PUFA ratio is the main important index of nutritional value of fish lipids; the intake of an appropriate ratio of essential fatty acids appears to be critical for the prevention and reduction of obesity, and nutritionists recommend human intakes of PUFA with ratios below 4 (Santos-Silva, Bessa, & Santos-Silva, 2002). Currently, the n-6 PUFA content in the human diet is from 3.75 to 10 times higher than recommended value, which is strongly correlated to an increased incidence of chronic non-transmissible diseases. Western diet, composed of high contents of red meat, refined flours and industrial products (with high levels of n-6 precursors and low levels of n-3 ones), enhances the production of long-chain n-6 PUFA and suppresses the production of n-3 PUFA (Adkins & Kelley, 2010; Fernandes et al., 2014). Moreover, the amount of DHA available for the global body metabolism remains insufficient for a large proportion of consumers (Parmentier et al., 2007), so that a higher dietary intake of n-3 and a reduction of n-6 consumption is strongly recommended for the correction of an unbalanced diet (Fernandes et al., 2014).

Currently, there is increasing attention on marine PoL, especially on PL, because of their potential nutritional value and their unique biophysical properties, so that their administration seems to be a promising new dietary approach in the prevention and treatment of many human diseases. Compared to

NL, PL of marine origin show greater nutritional value, higher bioavailability and higher intestinal absorption of n-3 PUFA, properties due to the esterification in the sn-2 position of ARA, EPA and DHA (Parmentier et al., 2007); PL also present better resistance to oxidation compared to triacylglycerols from the same source (Adkins & Kelley, 2010). Fish species with a high PL content are considered valuable food products due to their high levels of PUFA. Previous studies suggested that fish meals rich in PL could be used as a valuable functional ingredient of food or nutraceuticals (Henna Lu, Nielsen, Timm-Heinrich, & Jacobsen, 2011). The demand for PUFA in health-related products is increasing; modern biotechnology and engineering make it possible to design and purify marine PL for a wide range of applications (e.g. in nutrition as dietary supplements, and in drug delivery in the form of liposomes) (Loftsson et al., 2016; Mika, Swiezewska, & Stepnowski, 2016). Despite the nutritional, pharmaceutical and cosmetic value of marine PL having been widely discussed (Burri, Hoem, Banni, & Berge, 2012), there are very limited studies on the FAP of PoL in blue-back fish, including species widely caught and commonly consumed; these small pelagic fish may provide a valuable source of n-3 PUFA due to the abundance of the stocks. The aim of this study was the market promotion of these species, currently with a low commercial value, and the divulgation of their nutritional properties. Moreover, since these species analyzed are generally consumed cooked in the area subjected to study, the effects of cooking process have been assessed. An increment in the consumption of local fish in place of imported or over-exploited fish species would represent an example of sustainable utilization of the natural marine resources, and would also have a positive environmental impact (Guo, Vikbjerg, & Xu, 2005). Therefore, the objectives of this study are: (1) characterizing the PoL content in four species of blue-back fish from the Adriatic Sea; (2) highlighting any changes in PoL content and FAP due to the seasonal period and/or cooking process; (3) providing information about the best season for catching and consuming the fish species considered.

## Materials and Methods

### Sample Collection and Preparation

Four fish species were included in this study: anchovy (*Engraulis encrasicholus*), sardine (*Sardina pilchardus*), sprat (*Sprattus sprattus*) and horse mackerel (*Trachurus trachurus*). Fish caught in the

145 North Adriatic Sea were obtained from wholesale fish markets in Cesenatico and Rimini, in three
146 different seasons, fall (four batches for anchovy, sardine and horse mackerel) or winter (three batches
147 for sprat), and in the following spring, as described in the study of Pirini, Testi, Ventrella, Pagliarani
148 and Badiani (2010). Within each batch, one fillet out of each of 30 specimens was retained to constitute
149 the pooled raw reference.

150 The contralateral fillets were oven-baked as described by Pirini, Testi, Ventrella, Pagliarani and
151 Badiani (2010). Briefly, the oven temperature was set at 190°C; cooking time ranged from a minimum
152 of 5 min for sprat to a maximum of 9 min for horse mackerel to reach a core temperature of 70 °C, as
153 checked by an iron–constantan thermocouple connected to a digital potentiometer (Type J/K
154 Thermometer mod. 421502, Extech Instruments Corp., Waltham, MA, USA).

155 Raw and cooked fillets were either skinned (sardine, sprat and horse mackerel) or left unskinned
156 (anchovy), as customarily prepared in Italy, then cut into small pieces, homogenized using a stainless-
157 steel meat mincer, and stored at −20 °C until lipid analysis.

158

159 **Lipid Analysis**

160 Lipid analyses were carried out in duplicate on 5 g of homogenized fillet, both raw and cooked. TL
161 were extracted according to the method of Folch, Lees and Sloane Stanley (1957) and weighed after
162 complete evaporation of the solvent. TL were separated into NL and PoL fractions by SPE (solid phase
163 extraction) using Phenomenex Strata SI-1 Silica normal phase columns (55 μm, 70 A) (Torrance, CA,
164 USA) with a capacity of 12 mL, containing 2 g silica, according to the method described by Bayır et al.
165 (2010), with some modifications as shown below. The sample of TL diluted in 3 mL of chloroform was
166 applied to the column equilibrated by four aliquots of 3 mL of chloroform. The NL fraction was eluted
167 by adding 3 mL of chloroform eight times with a flow of two drops per second; PoL were eluted by four
168 aliquots of 3 mL of methanol and four aliquots of 3 mL of a chloroform/methanol mixture (3:7 v:v),
169 with a flow of two drops per second. PoL content was evaluated gravimetrically after completely solvent
170 evaporation. Colorimetric determination of phosphorus content confirmed gravimetrical results, thus
171 confirming that the PoL fraction coincided with PL (Bayır et al, 2010). Fractions obtained were weighed,
172 diluted in a specific volume of chloroform and methanol in the ratio 1:1, and stored at −20 °C prior to

use for further analysis. The methyl esters of the PoL fatty acids were prepared by transmethylation with acid catalyst (Christie, 1989) and analyzed on a Varian 3380 gas chromatograph equipped with a J&W Scientific DB-23 fused silica capillary column (30 m × 0.25 mm), under the conditions reported by Pirini et al. (2010).

Fatty acid identification was accomplished by comparing the retention time of unknown FAME with those of known FAME standard mixtures (Sigma-Aldrich Corp., St. Louis, MO, USA; PUFA No.1, Marine Source, and PUFA No.3, Menhaden Oil, SUPELCO, Inc., Bellefonte, PA, USA).

**Lipid Quality Indices**

A qualitative assessment of the nutritional profile of the PoL fraction was done through computation of the ratio between the percentages of n-6 and n-3 PUFA, often highlighted as a key element for a healthy diet (Simopoulos, 2006). Moreover, the nutritional quality of the PoL fraction was assessed by three indices: atherogenicity index (AI), thrombogenicity index (TI) and hypocholesterolemic to hypercholesterolemic fatty acid ratio (HH), as described by Ulbricht and Southgate (1991) and Santos-Silva, Bessa, & Santos-Silva (2002). AI indicates the relationship between the sum of the main saturated fatty acids (14:0 and 16:0) and that of the main classes of unsaturated fatty acids (MUFA and n-3 and n-6 PUFA); AI was calculated by applying the following equation:

$$\mathrm{AI} = \frac{[(4 \times 14{:}0) + 16{:}0]}{\mathrm{MUFA} + \mathrm{n}-6 + \mathrm{n}-3}$$

IT is expressed as the relationship between pro-thrombogenic (saturated) and anti-thrombogenic fatty acids (MUFA, n-6 and PUFA) and was calculated by applying the following equation:

$$\mathrm{TI} = \frac{(14{:}0 + 16{:}0 + 18{:}0)}{(0.5 \times \mathrm{MUFA}) + (0.5 \times \mathrm{n}-6) + (3\,\mathrm{n}-3) + \frac{\mathrm{n}-3}{\mathrm{n}-6}}$$

The HH ratio is related to cholesterol metabolism and was calculated according to the following equation:

$$\mathrm{HH} = \frac{[(18{:}1\mathrm{n}-9 + 18{:}2\mathrm{n}-6 + 18{:}3\mathrm{n}-3 + 20{:}5\mathrm{n}-3 + 22{:}5\mathrm{n}-3 + 22{:}6\mathrm{n}-3)]}{(14{:}0 + 16{:}0)}$$

**Analytical Quality Assurance**

The analytical quality was controlled for three times by analyzing the standard reference material "Meat Homogenate" (SRM 1546; NIST, Gaithersburg, MD) for lipid content as well as FAP, following the same analytical procedure used in this work. The means determined were always within the certified (or reference) intervals.

### Statistical Analysis

For each species, data (lipid content and FAP, expressed as mole%) were arcsin-transformed and analyzed using a two way "between group/within subjects" analysis of variance (ANOVA). "Season of catch" (fall/winter vs spring) was the between-group factor, whereas "State" (raw vs cooked) was the within-subjects (repeated measures) factor. Means were separated at, or below, the 5% probability level using Tukey's HSD *post hoc* test. All statistical computations were performed using the Statistica® software package (Release 7, 2005; StatSoft Inc., Tulsa, OK, USA).

## Results

### Polar Lipids Content

The percentages of PoL in raw and cooked fillets of the blue-back fish examined are shown in Table 1. Levels of PoL, calculated as g/100 g of TL (or %PoL) in fresh fillets, ranged from 4.83% (sprat in spring) to 22.8% (sardine in spring). In sardine, these values varied significantly among fishing seasons: they were higher in spring, when the lower amount of TL characterizes this species in this period. The same parameters expressed in absolute terms (mg of PoL/100 g of raw fillet) showed levels between 427 and 652 mg/100 g tissue, with no statistically significant seasonal variation within species, suggesting variability in TL profile during the year.

The cooking process significantly influenced total PoL content (expressed in absolute terms) in all species, due to the loss of water. The %PoL of anchovy increased significantly in both seasons, while in sardine and in horse mackerel this effect was only appreciable in subjects collected in fall.

### Fatty Acid Profile of Polar Lipids in Raw Samples

226 The FAP of PoL fractions of the four species analyzed in fall/winter and in spring is shown in Tables
227 2 and 3. The n-3 PUFA were the major fraction, with percentages ranging between 42.40% and 57.06%,
228 followed in decreasing order by SFA (26.70-30.85%), MUFA (8.58-13.89%) and n-6 PUFA (2.85-
229 8.28%). The SFA were high in palmitic acid (16:0), with a peak of 35,40% in the spring in sardine;
230 among the MUFA, oleic acid (18:1 n-9) was predominant, with values ranging between 2.90% and
231 6.75%; ARA (1.07-3.70%) was the most represented n-6 PUFA, while DHA (30.50-45.23%) and EPA
232 (4.67-13.22%) were the major ones among n-3 PUFA. The species that contains the highest PUFA
233 content is horse mackerel although sprat is richer in n-3 PUFA, followed by horse mackerel and anchovy
234 fished in spring.
235
236 **Seasonal Dynamics of Fatty Acid Profile of Polar Lipids**
237 The SFA, MUFA and PUFA composition of PoL (Tables 2 and 3) generally showed a remarkable
238 pattern of seasonality, reflecting fatty acid fluctuations. Almost all SFA tended to decline from fall to
239 spring, i.e. the 14:0 in sardine (from 3.98% to 0.68%, about 1/6 of the fall value). An exception is the
240 increase of 16:0 content in sardine (from 18.0% in fall to 31.2% in spring) and, to a lesser extent, in
241 anchovy (from 20.39% in fall to 35.40% in spring). However, these changes had no effect on the overall
242 value of SFA, except in sardine (from 30.85% in fall to 41.73% in spring). Significant variations in the
243 percentages of MUFA were found in anchovy, sardine and horse mackerel caught in fall, mainly due to
244 the increase in both 16:1n-7 and 18:1n-9. In these very same species, seasonal changes in fatty acid
245 composition of MUFA were also recorded for NL. Furthermore, fall-caught horse mackerel showed an
246 increase in SFA (especially 16:0), as it can be seen in Table 5 and Table 6.
247 The percentage of total PUFA, instead, did not change seasonally: in anchovy, sardine and horse
248 mackerel caught in fall, the reduction in DHA content was compensated for by increases in the
249 percentage of EPA or of n-6 PUFA. In fact, anchovy and sardine were richer in n-6 PUFA in fall, while
250 in sprat and in horse mackerel seasonal rates remain unchanged. The levels of n-3 PUFA were
251 significantly higher in spring, except for sprat. In anchovy, this trend reflected that of the major n-3
252 PUFA (EPA and DHA), while in sardine and horse mackerel it was connected to a marked increase in
253 DHA, which compensated for the significant decline of EPA in spring. A similar pattern was observed

for NL PUFA, where higher levels of n-6 are detected in anchovy caught in fall and increased n-3 in spring horse mackerel (see Table 5 and Table 6).

**Effect of Cooking on Fatty Acid Profile of Polar Lipids**

Upon cooking, significant changes in percentages of fatty acids, connected to season and species, were frequently observed (see Tables 2 and 3). As was observed for raw samples, the n-3 PUFA fraction was the highest, with percentages ranging from 39.38% to 57.38%, followed by SFA (26.73-38.10%), MUFA (5.93-11.75%) and n-6 PUFA (2.69-7.99%). In particular, cooking seemed to cause a significant reduction in SFA level in spring sardine, mainly due to the remarkable decrease of 16:0; on the contrary, in fall in anchovy, and in spring in horse mackerel, the same fatty acid tended to achieve notably higher values. In sprat and horse mackerel, the quantities of SFA were less influenced by cooking and season, while in anchovy and in sardine the interaction (Sc*St) between season and status was significant. The cooking process induced an important decrease in MUFA content in sardines, particularly those collected in fall; on the contrary, an increase could be observed in anchovy. Cooking caused a significant drop in total PUFA only in the fall anchovy. For n-6 PUFA, the cooking effect seemed to consist of a small decrease in the total concentration in horse mackerel fished in spring and in anchovy and sardine fished in fall, in which the interactions Sc*St are significant for many fatty acids. The effects of the cooking process on n-3 PUFA mainly involved fall anchovy and sardine; in the former species, cooking caused significant loss of DHA and thus a significant decrease in n-3 PUFA; in the latter, all n-3 PUFA, excluding DHA, incurred significant losses, while in spring, EPA and DHA appeared to increase.

NL showed a different behavior towards cooking processes compared to PoL; in each species analyzed, SFA, MUFA and n-6 PUFA from NL are quite stable, or they can be even found concentrated in cooked fillet, due to the water loss. On the contrary, the trend of n-3 PUFA is similar to that of PoL, since drops are recorded in fall anchovy and spring sardines, mainly due to loss in DHA (see Table 5 and Table 6).

**Nutritional Indices**

The n-6/n-3 PUFA ratios (see Tables 2 and 3) were steadily below the value of 1 (0.05 to 0.16), given the clear predominance of n-3 PUFA. In particular, a seasonal reduction in n-6/n-3 ratio was found in raw anchovy (0.11 in fall to 0.06 in spring), while a less important decrease was shown for horse mackerel (0.16 to 0.12). Cooking finally produced slight but significant increases in n-6/n-3 ratio only in fall anchovy.

Table 4 shows the nutritional profile of PoL of the four species in the two seasonal periods in the raw and cooked states. Within each species, the comparison between seasons did not reveal significant differences in AI and in TI. The values of AI in subjects in the raw state varied from a minimum of 0.21 in horse mackerel to a maximum of 0.56 in sardine, both species caught in spring; in sprat (in both seasons) and in anchovy caught in spring, the TI were in the range 0.11–0.25, respectively. After baking, the values of AI were included in the range 0.23 to 0.59, respectively in spring sprat and in fall sardine. Within each species, comparison between seasons showed a significant decrease of AI in spring anchovy and sardine only after cooking. Finally, the highest HH value observed was 4.49 in spring horse mackerel; the lowest was 1.64 in spring sardine. In raw anchovy and sardine, HH values tended to be slightly higher in fall. In all other cases, the differences between seasons and between raw and cooked fish seemed to be minor.

## Discussion

### Seasonal Effect on Polar Lipids Content and Fatty Acid Profile

The proportions of PoL (g/100 g of TL) did not differ from those reported in the literature (De Leonardis & Macciola, 2004; Fanni, Linder, & Parmentier, 2004; Shahidi & Miraliakbari, 2006): the highest values of PoL were detected during the “lean period”, when the amount of NL in the flesh decreases in favor of gonadal maturation. Even the amount of PoL agrees by and large with the data from other authors (Kolakowska, Olley & Dunstan, 2006), ranging between 300 and 500 mg/100 g of flesh (see Table 1). The lack of significant seasonal variation in PoL content confirms that the variations in TL detected in the course of the year should be mostly attributable to cyclical variations of NL, as reported by other authors (Fanni, Linder & Parmentier, 2004). As far as we know, this is one of the very

few studies reporting seasonal changes of PoL fatty acids of blue-back fish from the Adriatic Sea; therefore the comparison with literature data is fragmented and incomplete.

Overall, the results of this survey indicate that the FAP of raw PoL exhibits a preponderance of PUFA, especially DHA and EPA. Total PUFA remains substantially constant in all species during the year, while the n-3 PUFA content (except in sprat) tends to rise in spring, especially due to the contribution of DHA. Furthermore, n-6 PUFA show significant seasonal variations only in anchovy and sardine, with lower values in spring compared to fall. These findings are consistent with the results obtained in the study by Passi et al. (2002), where the FAP of PoL were analyzed in sardine and horse mackerel from the central Tyrrhenian Sea, without specifying the season of capture. Other investigations on PoL from *Anguilla anguilla* reported low levels of MUFA and very high levels of PUFA, mostly n-3 (Ciappellano, Erba, Colombo, Testolin & Bolis, 1999). Similarly, in a study of seasonal fluctuations in FAP in three anadromous subspecies (*Salmo trutta caspius*, *S. t. labrax* and *S. t. macrostigma*), Bayır et al. (2010) reported PoL fractions with very high percentages of PUFA and SFA (due to the high levels of 16:0). Seasonal changes in the various groups of fatty acids varied depending on the species considered, in agreement with the lack of homogeneity occurring in changes of PoL in blue-back fish. Given the large number of variables involved, it is difficult to distinguish the origin of PoL fluctuations, especially since in aquatic animals this fraction was found to be susceptible to the dietary quality, although to a lesser extent than NL (De Souza et al., 2008; Standal, Axelson & Aursand, 2010).

On this basis, the seasonal changes we observed in PoL of blue-back fish can be attributed to the different seasonal availability of fatty acids in the aquatic food chain rather than homeoviscous adaptation. On the other hand, high levels of n-3 PUFA, typical in winter or early spring due to the adaptation of aquatic animals at low temperatures, were detected only in horse mackerel.

**Effect of Cooking on Fatty Acid Profile**

Important changes in the FAP of fish muscle, caused by culinary treatments, consist of considerable loss of DHA and EPA and depend on the cooking method (Larsen, Quek & Eyres, 2010; Türkkan, Cakli & Kilinc, 2008). Baking is considered by many authors the best heat treatment for preservation of all lipid features of fish meat, including the PUFA content and n-3/n-6 ratio (García-Arias et al., 2003); it

has been found to be the best preparation technique with regard to retention of EPA and DHA of TL, maintaining over 80% for both fatty acids (Al-Shagir et al., 2004; Mierke-Klemeyer et al., 2008). The great increase of PoL content in cooked fillets (see Tables 2 and 3) when compared to the raw counterpart is rationally connected to the loss of moisture and storage lipids, subjected to melting or leaching from the flesh with the so-called "cook-out" (Little, Armstrong, & Bergan, 2000).

The heat resistance of many fatty acids found in this study, including n-3 PUFA (especially in spring sardine, sprat and horse mackerel), can be related to some extent of "internal protection" of n-3 fatty acids that prevent the degradation of PUFA during heat treatments. This protective effect may be due to the presence of NL and PoL incorporated in lipoprotein structures, such as biological membranes (Küllenberg, Taylor, Schneider, Massing and Ulrich, 2012), and to the presence of high levels of natural antioxidants (vitamin E, astaxanthin) in the flesh (Gladyshev et al., 2006; Larsen, Quek & Eyres, 2010). From the results of this study (Tables 2 and 3), the response to baking is species-specific, as also reported by other authors (García-Arias et al., 2003; Schneedorferová, Tomčala & Valterová, 2015); it seems to be correlated to the catch season in anchovy and sardine: in baked anchovy caught in fall, significant increments in the percentages of SFA, MUFA and n-6 PUFA were detected, together with a decrease in the global percentage of PUFA (due to significant reductions in n-3 PUFA), and a consequent increase in n-6/n-3 ratio. In baked sardine, significant increments were detectable in the MUFA and n-6 PUFA percentages of subjects caught in fall. These findings agree with those reported in the study of Martelli et al. (2013), in which the FAP was influenced by both catch season and cooking treatments.

**Nutritional Indices**

The n-6/n-3 ratio has been suggested to be the best index for comparing the relative nutritional value of fish oils (Simopoulos, 1991). The typical Western diet is characterized by a high intake of n-6 PUFA and a low intake of n-3 PUFA. According to the recommendations of nutritional advisers, the attempt is to increase the levels of n-3 PUFA in the diet, such that the n-6/n-3 ratio does not exceed 4.0 (Santos-Silva, Bessa, & Santos-Silva, 2002). Analysis of PoL in the fish species included in this study reported an n-6/n-3 ratio between 0.05 and 0.17 (see Tables 2 and 3), which are values 2-15 folder lower than those reported by Simonetti et al. (2008) in a study of PoL in four freshwater species; these findings

suggest excellent nutritional quality in terms of FAP of PoL in the fish sources investigated, also confirmed by the AI and indices always lower than 1 (between 0.10 and 0.59, among raw and cooked samples). These indices (see Table 4) indicate the potential for stimulating platelet aggregation and are primarily related to a decrease in cardiovascular disease risk; thus, the smaller the values, the greater the protective potential for coronary artery disease (Turan, Sönmez & Kaya, 2007). From the findings of this study, sardine was the species with the highest index values. AI and TI indices were not influenced by seasonality or by cooking, with the exception of cooked sardine and anchovy which showed a decrease of both AI and TI indices in spring compared to the raw state.

The HH index refers to the ratio between the sum of hypercholesterolemic fatty acids and the sum of hypercholesterolemic fatty acids, and indicates the specific effects of fatty acids on the cholesterol metabolism; from a nutritional point of view, high HH values are considered beneficial to human health (Testi, Bonaldo, Gatta & Badiani, 2006). HH index values from this study (means ranging from 1.64 to 4.49, see Table 4) were higher than those obtained in similar investigations on black needle, white needle, mackerel and sardine from a Brazilian market (Fernandes et al., 2014). HH indices tended to be higher in horse mackerel and sprat and showed significant seasonal variability in raw and cooked sardine; furthermore, in every species analyzed, they exhibited a decrease after the cooking process, with the exception of sprat, for which baking increased the index.

Due to the very low n-6/n-3 fatty acid ratios and the highly favorable nutritional indices found in the blue-back fish analyzed in this study, the PoL qualities of the four species examined may play an important role in the human diet, helping to increase significantly the n-3 fatty acid intake and improving consumer health (Hossain, Hosokawa & Takahashi, 2009; Murakawa et al., 2007; Ulbricht & Southgate, 1991; Taylor et al., 2010). Furthermore, the existence of such high levels of EPA and DHA could make blue-back fish PoL biologically valuable in the food industry for enrichment and production of functional foods with higher nutritional value (Burri, Hoem, Banni, & Berge, 2012). This study contributes to finding new commercialization strategies for these species, possibly as value-added products. This study promotes the importance of marine PoL for the human diet, as a valuable source of PUFA, and highlights the nutritional quality of four popular blue-back species from the Adriatic Sea (*E. encrasicholus*, *S. pilchardus*, *S. sprattus* and *T. trachurus*). This manuscript presents, for the first time,

data on the influences of catch season and cooking treatments on the FAP of PoL, and therefore on the nutritional quality of the species analyzed. Sprat was the only species not affected by seasonal variations or by cooking processes. For the other species, especially sardine and anchovy, it is difficult to delineate the best season for capture and consumption because, although the data indicate a higher level of PUFA (in particular n-3) in the specimens caught in spring, the nutritional indices of cooked samples were generally higher in those collected in fall.

The increased accessibility of marine lipids during recent years has opened up new possibilities for the use of PoL not only as a superior nutritional source of n-3 PUFA, but also for use in the pharmaceutical, cosmetic and functional food industries (Burri, Hoem, Banni, & Berge, 2012). Since the only available source of n-3 PUFA comes at present from marine lipids, there is a need to scale-up productions of these compounds and to identify new affordable resources, natural or synthetic (Løvaas et al., 2006); future investigations should explore the PoL content in other marine species, krill, fish roe, fish by-products (viscera and skin) and even in non-animal marine sources such as microalgae, in order to meet the growing demands of the market. In addition, in order to complete the assessment of the role of PoL in food manufacturing and in pharmaceutical and cosmetic applications, other important aspects which need to be considered and deepened are the bioavailability of PoL from different sources, and the identification of reliable physiological markers to confirm and determine their bioactivity.

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**Conflict of interest**

The authors declare that there is no conflict of interest regarding the publication of this article.

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562 **Table 1** Polar lipid (PoL) content in blue-back fish fillets from the four species, in the raw and cooked
563 state, and from different seasons

| Fish species | *Season* | PoL (g/100g TL) | | PoL (mg/100g flesh) | |
|---|---|---|---|---|---|
| | | *Raw* | *Cooked* | *Raw* | *Cooked* |
| **Anchovy** | *Fall* | y11.1 ± 2.25 | x18.9 ± 1.61 | y486 ± 24 | x1028 ± 174 |
| | *Spring* | y7.71 ± 1.31 | x15.1 ± 2.12 | y442 ± 56 | x977 ± 81 |
| **Sardine** | *Fall* | y6.70 ± 1.38b | x11.9 ± 1.46b | y486 ± 58 | x827 ± 46 |
| | *Spring* | 22.8 ± 2.21a | 24.9 ± 3.65a | y652 ± 68 | x927 ± 71 |
| **Sprat** | *Winter* | 14.1 ± 4.22 | 25.9 ± 14.4 | y482 ± 130 | x967 ± 154a |
| | *Spring* | 4.83 ± 0.43 | 6.08 ± 0.73 | 539 ± 31 | 662 ± 30b |
| **Horse mackerel** | *Fall* | y20.5 ± 5.41 | x29.6 ± 3.58 | y427 ± 34 | x679 ± 122 |
| | *Spring* | 20.4 ± 4.56 | 25.0 ± 6.93 | y595 ± 37 | x845 ± 86 |

564

565 *Values are means of four determinations ± Std Dev; values in the same row, with trait preceded by*
566 *different letters (x, y), differ significantly ($p < 0.05$) for state (raw or cooked); values within a column,*
567 *followed by different letters (a, b), differ significantly ($p < 0.05$) for catch season.*

568

569 **Table 2** Content of selected fatty acids (mole%) in polar lipids from skin-on fillets of anchovy and
570 skinned sardine in the raw (R) and cooked (C) state (St)

| Fatty acids [A, B, C] | St | **Anchovy** | | Statistical analysis | | | | **Sardine** | | Statistical analysis | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Fall* | *Spring* | RSD | Sc | St | Sc*St | *Fall* | *Spring* | RSD | Sc | St | Sc*St |
| **14:0** | R | 3.00a | 1.29b | 0.940 | *** | * | n.s. | x3.98a | 0.68b | 0.267 | *** | ** | * |
| | C | 3.30a | 1.41b | | | | | y2.47a | 0.65b | | | | |
| **16:0** | R | x19.54b | 25.83a | 0.762 | *** | * | *** | y20.39b | x35.40a | 2.078 | ** | n.s. | *** |
| | C | x23.23 | 24.51 | | | | | x28.68 | y27.95 | | | | |
| **18:0** | R | y5.46a | 4.36b | 0.384 | *** | n.s. | ** | 5.83 | x5.26 | 0.451 | ** | * | ** |
| | C | x6.60a | 3.79b | | | | | 6.21a | y3.76b | | | | |
| **Σ SFA[D]** | R | y29.62 | 32.65 | 1.253 | n.s. | * | ** | 30.85b | x41.73a | 2.773 | n.s. | n.s. | ** |
| | C | x35.10a | 30.84b | | | | | 38.10 | y32.72 | | | | |
| **16:1n-7** | R | 2.71a | 1.21b | 0.092 | *** | * | n.s. | x3.26a | 0.64b | 0.181 | *** | ** | ** |
| | C | 2.98a | 1.28b | | | | | y1.99a | 0.62b | | | | |
| **18:1n-9** | R | y3.71a | x2.90b | 0.069 | *** | n.s. | *** | x6.19a | 3.19b | 0.524 | *** | ** | n.s. |
| | C | x4.16a | y2.51b | | | | | y4.16a | 2.73b | | | | |
| **18:1n-7** | R | x2.94 | x3.23 | 0.082 | n.s. | n.s. | *** | x2.66 | x2.85 | 0.234 | n.s. | ** | n.s. |
| | C | x3.26 | y2.90 | | | | | y2.04 | y2.17 | | | | |
| **Σ MUFA[E]** | R | y9.84a | x7.70b | 0.167 | *** | n.s. | *** | x13.89a | 6.90b | 0.901 | *** | ** | * |
| | C | x11.16a | y6.93b | | | | | y8.94 | 5.93b | | | | |
| **18:2n-6** | R | 1.15a | 0.54b | 0.049 | *** | n.s. | n.s. | x1.29a | 0.96b | 0.083 | * | *** | ** |
| | C | 1.10a | 0.53b | | | | | y0.79 | 0.88 | | | | |
| **20:4n-6** | R | 2.20a | 1.29b | 0.032 | *** | n.s. | ** | 1.59a | 1.07b | 0.200 | *** | n.s. | n.s. |
| | C | 2.12a | 1.35b | | | | | 1.64a | 1.13b | | | | |
| **22:5n-6** | R | x1.52a | 0.96b | 0.033 | *** | *** | ** | 1.11 | 1.07 | 0.112 | n.s. | n.s | n.s. |
| | C | y1.28a | 0.93b | | | | | 1.12 | 1.31 | | | | |
| **Σ n-6 PUFA[F]** | R | x5.76a | 3.33b | 0.053 | *** | *** | ** | x5.01a | 3.78b | 0.216 | *** | ** | ** |
| | C | y5.34a | 3.29b | | | | | y4.08 | 3.91 | | | | |
| **18:3n-3** | R | 0.66a | 0.26b | 0.023 | *** | n.s. | * | x0.80a | 0.43b | 0.046 | ** | *** | *** |
| | C | 0.61a | 0.29b | | | | | y0.34 | 0.42 | | | | |
| **18:4n-3** | R | x0.69a | 0.26b | 0.025 | *** | ** | *** | x1.02a | 0.22b | 0084 | *** | *** | *** |
| | C | y0.53a | 0.30b | | | | | y0.24 | 0.23 | | | | |
| **20:5n-3** | R | 8.52b | y10.15a | 0.276 | *** | ** | *** | x8.26a | y4.67b | 0.525 | *** | n.s. | *** |
| | C | 7.79b | x12.45a | | | | | y5.78 | x5.88 | | | | |
| **22:5n-3** | R | x0.98a | 0.86b | 0.028 | * | *** | ** | x1.24a | 0.67b | 0.051 | *** | *** | *** |
| | C | y0.83 | 0.83 | | | | | y0.62 | 0.74 | | | | |
| **22:6n-3** | R | x38.39 | 42.41 | 0.911 | *** | *** | *** | 30.50 | 37.71 | 4.221 | ** | * | n.s. |
| | C | y32.74 | 42.71a | | | | | 32.08b | 47.23a | | | | |
| **Σ n-3PUFA[G]** | R | x49.49 | 54.00 | 1.140 | *** | * | *** | 42.40 | 44.07 | 4.681 | * | n.s. | * |
| | C | y42.71 | 56.70a | | | | | 39.38b | 54.82a | | | | |
| **Σ PUFA[H]** | R | x55.76 | 57.56 | 1.221 | ** | ** | *** | 47.96 | 48.18 | 4.778 | * | n.s. | * |
| | C | y48.44b | 60.18a | | | | | 43.74b | 58.99a | | | | |
| **Unknown** | R | y8.53a | 4.47b | 0.207 | *** | * | * | 11.88 | 5.18 | 1.699 | *** | n.s. | n.s. |
| | C | x9.48a | 4.20b | | | | | 11.85a | 4.30b | | | | |
| **n-6/n-3** | R | x0.12a | 0.06b | 0.002 | *** | n.s. | ** | 0.12 | 0.09 | 0.007 | ** | * | n.s. |
| | C | y0.13a | 0.06b | | | | | 0.10 | 0.07 | | | | |

571

572 *Values are means of four determinations (each one in duplicate on pooled samples) per catch season*
573 *and state; means within a column and trait preceded by different letters (x, y) differ significantly ($p \leq$*
574 *0.05); means in the same row followed by different letters (a, b) differ significantly ($p \leq 0.05$); RSD =*
575 *residual standard deviation; Sc = catch season; A: includes 15:0 and 17:0; B: includes 20:1n-11, 20:1n-*
576 *9, 20:1n-7, 22:1n-11 and 22:1n-9; C: includes 18:3, 20:2, 20:3 and 22:4n-6; D: includes 16:4n-3 and*
577 *20:4n-3; E: includes 16:2n-4; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; n.s. = not significant.*

578

579 **Table 3** Content of selected fatty acids (mole%) in polar lipids from skinned fillets of sprat and horse

580 mackerel in the raw (R) and cooked (C) state (St)

| Fatty acids [A,B,C] | St | **Sprat** | | Statistical analysis | | | | **Horse mackerel** | | Statistical analysis | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Winter | Spring | RSD | Sc | St | Sc*St | Autum | Spring | RSD | Sc | St | Sc*St |
| **14:0** | R | 1.02 | y1.31 | 0.074 | ** | ** | ** | 0.42 | 0.38 | 0.037 | n.s. | n.s. | n.s. |
| | C | 0.97b | x1.84a | | | | | 0.33 | 0.38 | | | | |
| **16:0** | R | 22.53 | 22.29 | 1.129 | n.s. | n.s. | n.s. | 17.07 | y16.74 | 1.121 | n.s. | * | * |
| | C | 22.86 | 21.70 | | | | | 17.78 | x20.79 | | | | |
| **18:0** | R | 3.49 | x3.47 | 0.169 | n.s. | *** | ** | 8.51 | 8.67 | 0.558 | n.s. | n.s. | n.s. |
| | C | 3.36 | y2.28 | | | | | 8.30 | 9.00 | | | | |
| **Σ SFA[D]** | R | 27.96 | 27.95 | 1.182 | n.s. | n.s. | n.s. | 27.07 | 26.70 | 1.737 | n.s. | * | n.s. |
| | C | 28.06 | 26.73 | | | | | 27.33 | 31.19 | | | | |
| **16:1n-7** | R | 1.44 | y1.78 | 0.072 | ** | * | *** | 1.21a | 0.77b | 0.077 | *** | n.s. | n.s. |
| | C | 1.27b | x2.21a | | | | | 1.04a | 0.75b | | | | |
| **18:1n-9** | R | 4.83 | y6.25 | 0.170 | * | * | ** | x6.75 | 5.74 | 0.279 | n.s. | * | * |
| | C | 4.70 | x7.05 | | | | | y5.80 | 5.59 | | | | |
| **18:1n-7** | R | 1.96 | x2.18 | 0.094 | n.s. | ** | *** | 2.35a | 1.57b | 0.176 | *** | n.s. | n.s. |
| | C | 2.04 | y1.43 | | | | | 2.32a | 1.56b | | | | |
| **20: n-9** | R | 0.20 | y0.30 | 0.024 | n.s. | * | n.s. | | | | | | |
| | C | 0.22 | x0.38 | | | | | | | | | | |
| **22:1n-11** | R | 0.20 | y0.33 | 0.057 | n.s. | ** | ** | | | | | | |
| | C | 0.20 | x0.58 | | | | | | | | | | |
| **Σ MUFA[E]** | R | 8.69 | 10.93 | 0.303 | * | n.s. | * | 11.00a | 8.58b | 0.521 | ** | n.s. | n.s. |
| | C | 8.54 | 11.75 | | | | | 9.81 | 8.44 | | | | |
| **18:2n-6** | R | 0.76 | x0.72 | 0.018 | n.s. | ** | ** | 1.02 | 0.81 | 0.073 | n.s. | n.s. | n.s. |
| | C | 0.78 | y0.61 | | | | | 0.92 | 0.80 | | | | |
| **20:4n-6** | R | 1.15 | 1.11 | 0.072 | * | n.s. | n.s. | 3.70 | 2.87 | 0.098 | * | n.s. | n.s. |
| | C | 1.25a | 1.08b | | | | | 3.68 | 2.72 | | | | |
| **22:5n-6** | R | 0.76 | 0.65 | 0.046 | n.s. | n.s. | n.s. | 1.97 | 2.17 | 0.128 | n.s. | * | n.s. |
| | C | 0.72 | 0.68 | | | | | 1.87 | 1.97 | | | | |
| **Σ n-6 PUFA[F]** | R | 3.01 | 2.85 | 0.065 | n.s. | n.s. | * | 8.28 | x7.16 | 0.152 | * | ** | n.s. |
| | C | 3.12 | 2.69 | | | | | 7.99 | y6.76 | | | | |
| **18:3n-3** | R | 0.49 | x0.48 | 0.010 | n.s. | * | * | 0.22 | 0.20 | 0.027 | n.s. | n.s. | n.s. |
| | C | 0.49 | y0.45 | | | | | 0.22 | 0.23 | | | | |
| **18:4n-3** | R | 0.50 | y0.47 | 0.026 | n.s. | ** | *** | 0.12 | 0.12 | 0.009 | n.s. | n.s. | n.s. |
| | C | 0.48 | x0.67 | | | | | 0.12 | 0.12 | | | | |
| **20:5n-3** | R | 10.62 | 13.22 | 0.675 | n.s. | * | n.s. | 8.38 | 7.43 | 0.302 | * | n.s. | n.s. |
| | C | 11.70 | 14.69 | | | | | 8.93 | 7.39 | | | | |
| **22:5n-3** | R | 0.70 | 0.70 | 0.032 | n.s. | n.s. | n.s. | 3.00 | 2.54 | 0.114 | ** | * | * |
| | C | 0.67 | 0.72 | | | | | 2.97a | 2.20b | | | | |
| **22:6n-3** | R | 44.46 | 40.21 | 1.661 | * | n.s. | n.s. | 38.02b | 45.23a | 2.198 | ** | n.s. | n.s. |
| | C | 43.70 | 39.64 | | | | | 38.13 | 41.58 | | | | |
| **Σ n-3 PUFA[G]** | R | 57.06 | 55.50 | 1.273 | n.s. | n.s. | n.s. | 49.62b | 55.38a | 2.490 | ** | n.s. | n.s. |
| | C | 57.38 | 56.50 | | | | | 50.24 | 51.51 | | | | |
| **Σ PUFA[H]** | R | 60.48 | 58.72 | 1.273 | n.s. | n.s. | n.s. | 58.23 | 62.83 | 2.585 | n.s. | n.s. | n.s. |
| | C | 60.81 | 59.52 | | | | | 58.55 | 58.42 | | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Unknown** | R | 2.87 | x2.40 | 0.162 | n.s. | ** | n.s. | 3.70a | 1.89b | 0.599 | *** | n.s. | n.s. |
| | C | 2.59 | y2.00 | | | | | 4.30a | 1.95b | | | | |
| **n-6/n-3** | R | 0.05 | 0.05 | 0.001 | n.s | n.s | n.s. | 0.17a | 0.13b | 0.004 | ** | n.s. | n.s. |
| | C | 0.05 | 0.05 | | | | | 0.16 | 0.13 | | | | |

*Values are means of four determinations (each one in duplicate on pooled samples) per catch season and state; means within a column and trait preceded by different letters (x, y) differ significantly ($p \leq 0.05$); means in the same row followed by different letters (a, b) differ significantly ($p \leq 0.05$); RSD = residual standard deviation; Sc = catch season; A: includes 15:0 and 17:0; B: includes 20:1n-11, 20:1n-7 and 22:1n-9; C: includes 18:3, 20:2, 20:3 and 22:4n-6; D: includes 16:4n-3 and 20:4n-3; E: includes 16:2n-4; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; n.s. = not significant.*

589 **Table 4** Seasonal variation of nutritional quality indices AI (atherogenicity index), TI (thrombogenicity
590 index) and HH (hypocholesterolemic/hypercholesterolemic fatty acid ratio) in polar lipids in the raw
591 (R) and cooked (C) state (St)

| Fish species | St | AI | | TI | | HH | |
|---|---|---|---|---|---|---|---|
| | | *Fall/Winter* | *Spring* | *Fall/Winter* | *Spring* | *Fall/Winter* | *Spring* |
| **Anchovy** | R | y0.38±0.02 | 0.38±0.03 | 0.14±0.02 | y0.14±0.02 | x3.20±0.05 | 2.62±0.04 |
| | C | x0.49±0.02a | 0.36±0.04b | 0.19±0.03 | x0.25±0.04 | y2.22±0.02 | 2.39±0.02 |
| **Sardine** | R | y0.47±0.02 | x0.56±0.04 | 0.17±0.02 | x0.22±0.03 | x2.46±0.04a | y1.64±0.04b |
| | C | x0.59±0.04a | y0.38±0.02b | 0.17±0.02 | y0.14±0.02 | y1.75±0.03a | x2.53±0.02b |
| **Sprat** | R | 0.31±0.02 | 0.31±0.02 | 0.11±0.02 | 0.11±0.02 | 3.25±0.06 | 2.97±0.05 |
| | C | 0.31±0.03 | 0.33±0.02 | 0.13±0.02 | 0.11±0.02 | 3.26±0.06 | 3.31±0.05 |
| **Horse mackerel** | R | 0.22±0.03 | 0.21±0.02 | 0.13±0.02 | 0.12±0.02 | 4.04±0.03 | x4.49±0.05 |
| | C | 0.23±0.02 | 0.24±0.02 | 0.10±0.02 | 0.15±0.03 | 3.88±0.02 | y3.38±0.06 |

592

593 *Values are means of four determinations ± Std Dev. Means within a column and trait preceded by*
594 *different letters (x, y) differ significantly ($p \leqslant 0.05$); means in the same row followed by different letters*
595 *(a, b) differ significantly ($p \leqslant 0.05$).*

596

**Table 5** Content of selected fatty acids (mole%) in neutral lipids from skin-on fillets of ANCHOVY and skinned SARDINE at raw (R) and cooked (C) state.

| Fatty acids [A,B,C] | St | **Anchovy** | | Statistical analysis | | | | **Sardine** | | Statistical analysis | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Fall* | *Spring* | RSD | Sc | St | Sc*St | *Fall* | *Spring* | RSD | Sc | St | Sc*St |
| **14:0** | R | y6.90b | 8.54a | 0.257 | *** | n.s. | ** | 6.93a | 4.75b | 0.267 | *** | ** | * |
| | C | x7.88 | 8.06 | | | | | 6.95 | 7.06 | | | | |
| **16:0** | R | 19.39 | 19.68 | 1.316 | n.s. | n.s. | n.s. | 21.69 | y20.89 | 2.078 | ** | n.s. | *** |
| | C | 21.62 | 20.24 | | | | | 22.96 | x24.06 | | | | |
| **18:0** | R | 4.97 | 3.91 | 0.334 | ** | * | n.s. | y5.13a | 4.58b | 0.451 | ** | * | ** |
| | C | 5.48 | 4.37 | | | | | x6.09a | 4.61b | | | | |
| **Σ SFA[D]** | R | 33.66 | 34.07 | 1.729 | n.s. | * | n.s. | 35.86 | y32.24 | 2.773 | n.s. | n.s. | ** |
| | C | 37.71 | 34.56 | | | | | 38.31 | x38.05 | | | | |
| **16:1n-7** | R | y6.08b | 9.37a | 0.302 | *** | n.s. | ** | 5.50a | y3.08b | 0.181 | *** | ** | ** |
| | C | x6.89b | 9.03a | | | | | 5.43 | x4.06 | | | | |
| **18:1n-9** | R | y4.73 | 5.32 | 0.183 | n.s. | ** | n.s. | 8.24 | 7.38 | 0.524 | *** | ** | n.s. |
| | C | x5.20 | 5.63 | | | | | 8.71 | 7.84 | | | | |
| **18:1n-7** | R | 2.79b | 3.35a | 0.116 | *** | * | n.s. | y2.76 | 2.07 | 0.234 | n.s. | ** | n.s. |
| | C | 3.01b | 3.38a | | | | | x3.27a | 2.08b | | | | |
| **Σ MUFA[E]** | R | y14.79b | 19.52a | 0.471 | *** | * | ** | y18.38a | y14.49b | 0.901 | *** | ** | * |
| | C | x16.26b | 19.16a | | | | | x19.33a | x16.42b | | | | |
| **18:2n-6** | R | 1.54a | 1.18b | 0.052 | *** | * | n.s. | 1.61 | 1.64 | 0.083 | * | *** | ** |
| | C | 1.61a | 1.23b | | | | | 1.66 | 1.72 | | | | |
| **20:4n-6** | R | 1.90a | 0.90b | 0.085 | *** | n.s. | n.s. | 1.25 | 0.92 | 0.200 | *** | n.s. | n.s. |
| | C | 1.74a | 0.92b | | | | | 1.21 | 0.76 | | | | |
| **22:5n-6** | R | x1.09a | 0.43b | 0.054 | *** | * | ** | 0.72 | x0.83 | 0.112 | n.s. | n.s. | n.s. |
| | C | y0.90a | 0.45b | | | | | 0.72 | y0.64 | | | | |
| **Σ n-6 PUFA[F]** | R | 5.56a | 3.13b | 0.215 | *** | n.s. | n.s. | 5.44 | 5.00 | 0.216 | *** | ** | ** |
| | C | 5.33a | 3.26b | | | | | 5.51a | 4.71b | | | | |
| **18:3n-3** | R | 1.28 | 1.11 | 0.038 | ** | n.s. | n.s. | 1.21 | y1.26 | 0.046 | ** | *** | *** |
| | C | 1.29a | 1.08b | | | | | 1.12 | x1.47 | | | | |
| **18:4n-3** | R | 1.76b | 2.66a | 0.392 | *** | n.s. | n.s. | 1.68 | 1.78 | 0.084 | *** | *** | *** |
| | C | 1.70b | 2.53a | | | | | 1.46 | 1.86 | | | | |
| **20:5n-3** | R | 12.12b | 15.88a | 0.606 | *** | * | n.s. | 10.35 | 8.19 | 0.525 | *** | n.s. | *** |
| | C | 10.64b | 15.90a | | | | | 9.76a | 7.14b | | | | |
| **22:5n-3** | R | x1.27a | 0.80b | 0.071 | *** | n.s. | ** | 1.47 | x1.11 | 0.051 | *** | *** | *** |
| | C | y1.06a | 0.86b | | | | | 1.44a | y0.91b | | | | |
| **22:6n-3** | R | x20.46a | 13.04b | 0.995 | *** | ** | ** | 17.99b | x26.60a | 4.221 | ** | * | n.s. |
| | C | y16.12a | 13.32b | | | | | 17.52 | y19.51 | | | | |
| **Σ n-3PUFA[G]** | R | x37.34 | 33.89 | 1.549 | n.s. | ** | ** | 33.42 | x39.59 | 4.681 | * | n.s. | * |
| | C | y31.19 | 34.17 | | | | | 31.95 | y31.43 | | | | |
| **Σ PUFA[H]** | R | x43.65a | 37.73b | 1.575 | * | ** | ** | 39.65 | x45.44 | 4.778 | * | n.s. | * |
| | C | y37.42 | 38.11 | | | | | 38.35 | y36.92 | | | | |
| **Unknown** | R | 7.74 | 8.36 | 0.007 | *** | * | n.s. | x6.67 | 8.56 | 0.007 | ** | * | n.s. |
| | C | 8.41 | 8.00 | | | | | y4.71b | 9.20a | | | | |
| **n-6/n-3** | R | y0.15a | 0.09b | 0.821 | n.s. | n.s. | n.s. | 0.16 | y0.13 | 1.699 | *** | n.s. | n.s. |
| | C | x0.17a | 0.10b | | | | | 0.17 | x0.15 | | | | |

599

600 [A] Values are means of 4 determinations (each one in duplicate on pooled samples) per season
601 of catch and state; [B] Means within a column and trait preceded by different letters (x,y) differ
602 significantly ($P \leq 0.05$); means on the same row followed by different letters (a,b) differ
603 significantly ($P \leq 0.05$); [C] RSD = Residual Standard Deviation; Sc = Season of catch; St = State;
604 [D] Includes: 15:0 and 17:0; [E] Includes: 20:1n-11; 20:1n-9; 20:1n-7; 22:1n-11 and 22:1n-9; [F]
605 Includes: 18:3, 20:2, 20:3 and 22:4n-6; [G] Includes: 16:4 and 20:4n-3; [H] Includes: 16:2n-4;
606 *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; n.s. = not significant.
607

608 **Table 6** Content of selected fatty acids (mole%) in neutral lipid from skinned fillets of SPRAT

609 and HORSE MACKEREL at raw (R) and cooked (C) state.

| Fatty acids [A,B,C] | St | **Sprat** | | Statistical analysis | | | | **Horse mackerel** | | Statistical analysis | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Winter | Spring | RSD | Sc | St | Sc*St | Autum | Spring | RSD | Sc | St | Sc*St |
| **14:0** | R | 5.97 | 5.17 | 0.220 | n.s. | n.s. | n.s. | 4.16 | 3.31 | 0.157 | * | n.s. | n.s. |
| | C | 6.41 | 5.16 | | | | | 4.15 | 3.62 | | | | |
| **16:0** | R | 25.23 | 22.08 | 0.747 | n.s. | n.s. | n.s. | 28.61a | 20.34b | 1.043 | *** | n.s. | * |
| | C | 24.92 | 23.12 | | | | | 26.64a | 21.53b | | | | |
| **18:0** | R | 2.97 | y2.62 | 0.058 | n.s. | ** | n.s. | 8.06 | 7.31 | 0.458 | n.s. | n.s. | n.s. |
| | C | 3.05 | x2.81 | | | | | 8.02 | 8.14 | | | | |
| **Σ SFA[D]** | R | 35.67 | 30.73 | 0.640 | n.s. | n.s. | n.s. | 42.76a | 32.58b | 1.418 | *** | n.s. | * |
| | C | 35.79 | 32.07 | | | | | 40.73a | 34.96b | | | | |
| **16:1n-7** | R | 7.18 | 6.88 | 0.293 | n.s. | n.s. | n.s. | 8.40a | 4.93b | 0.493 | *** | n.s. | n.s. |
| | C | 7.81 | 6.74 | | | | | 8.38a | 5.07b | | | | |
| **18:1n-9** | R | 16.17 | 16.91 | 0.962 | n.s. | n.s. | n.s. | y16.61 | 15.92 | 0.414 | n.s. | * | * |
| | C | 17.59 | 17.78 | | | | | x18.03 | 15.81 | | | | |
| **18:1n-7** | R | 2.02a | y1.29b | 0.078 | ** | ** | ** | y4.31 | 3.44 | 0.095 | * | * | * |
| | C | 2.07 | x1.73 | | | | | x4.68 | 3.41 | | | | |
| **20:1 n-9** | R | 1.30 | 1.77 | 0.187 | n.s. | n.s. | n.s. | 0.39 | 0.74 | 0.051 | * | n.s. | n.s. |
| | C | 1.52 | 1.55 | | | | | 0.36 | 0.70 | | | | |
| **22:1n-11** | R | 2.34 | 3.84 | 0.042 | n.s. | n.s. | n.s. | - | 0.57 | - | - | - | - |
| | C | 2.58 | 3.49 | | | | | - | 0.57 | | | | |
| **Σ MUFA[E]** | R | 29.59 | 31.31 | 1.887 | n.s. | n.s. | n.s. | 30.49 | 26.26 | 0.819 | * | n.s. | n.s. |
| | C | 32.15 | 31.90 | | | | | 32.14 | 26.22 | | | | |
| **18:2n-6** | R | 1.00 | 0.90 | 0.028 | n.s. | n.s. | n.s. | 1.08 | 1.28 | 0.267 | n.s. | n.s. | n.s. |
| | C | 1.03 | 0.89 | | | | | 1.38 | 1.27 | | | | |
| **20:4n-6** | R | 0.44 | 0.36 | 0.061 | n.s. | n.s. | n.s. | 1.32 | 1.42 | 0.094 | n.s. | n.s. | n.s. |
| | C | 0.35 | 0.36 | | | | | 1.34 | 1.54 | | | | |
| **22:5n-6** | R | 0.21 | 0.17 | 0.069 | n.s. | n.s. | n.s. | 0.33b | 0.68a | 0.049 | *** | n.s. | n.s. |
| | C | 0.18 | 0.24 | | | | | 0.31b | 0.59° | | | | |
| **Σ n-6 PUFA[F]** | R | 3.11a | 2.58b | 0.095 | ** | n.s. | n.s. | 3.48 | 4.35 | 0.291 | * | n.s. | n.s. |
| | C | 3.06 | 2.64 | | | | | 3.93 | 4.35 | | | | |
| **18:3n-3** | R | 1.09 | x0.98 | 0.005 | * | *** | ** | 0.49 | 0.67 | 0.035 | n.s. | n.s. | n.s. |
| | C | 1.07 | y0.94 | | | | | 0.53 | 0.66 | | | | |
| **18:4n-3** | R | 2.34 | 2.06 | 0.045 | n.s. | * | n.s. | 0.67 | 0.91 | 0.040 | n.s. | n.s. | * |
| | C | 2.26 | 1.94 | | | | | 0.73 | 0.82 | | | | |
| **20:5n-3** | R | 7.66 | 9.62 | 0.479 | n.s. | * | n.s. | 6.26 | 7.66 | 0.403 | n.s. | n.s. | * |
| | C | 6.64 | 9.23 | | | | | 6.88 | 6.81 | | | | |
| **22:5n-3** | R | 0.41 | 0.65 | 0.030 | * | n.s. | n.s. | 1.24b | 2.28a | 0.178 | ** | n.s. | n.s. |
| | C | 0.39 | 0.67 | | | | | 1.34 | 2.04 | | | | |
| **22:6n-3** | R | 12.96 | 15.86 | 1.017 | n.s. | n.s. | n.s. | 7.11b | 17.99a | 1.513 | *** | n.s. | n.s. |
| | C | 11.63 | 15.47 | | | | | 7.09b | 15.77a | | | | |
| **Σ n-3 PUFA[G]** | R | 24.81 | 29.62 | 1.463 | n.s. | n.s. | n.s. | 15.96b | 29.97a | 2.106 | *** | n.s. | n.s. |
| | C | 22.32 | 28.71 | | | | | 16.83b | 26.46a | | | | |
| **Σ PUFA[H]** | R | 28.74 | 32.87 | 1.497 | n.s. | n.s. | n.s. | 20.09b | 34.69a | 2.198 | *** | n.s. | n.s. |
| | C | 26.16 | 32.04 | | | | | 21.28b | 31.18a | | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Unknown** | R | 6.69 | 5.58 | 0.666 | ** | n.s. | n.s. | 6.54 | 6.32 | 0.688 | n.s. | n.s. | * |
| | C | 6.42a | 4.68b | | | | | 5.65 | 7.52 | | | | |
| **n-6/n-3** | R | 0.13 | 0.09 | 0.015 | n.s. | n.s. | n.s. | 0.22a | 0.15b | 0.021 | ** | n.s. | n.s. |
| | C | 0.14 | 0.09 | | | | | 0.23a | 0.16b | | | | |

[A] Values are means of 4 determinations (each one in duplicate on pooled samples) per season of catch and state; [B] Means within a column and trait preceded by different letters (x,y) differ significantly ($P \leq 0.05$); means on the same row followed by different letters (a,b) differ significantly ( $P \leq 0.05$); [C] RSD = Residual Standard Deviation; Sc = Season of catch; St = State; [D] Includes: 15:0 and 17:0; [E] Includes: 20:1n-11; 20:1 n-7 and 22:1n-9; [F] Includes: 18:3, 20:2, 20:3 and 22:4n-6; [G] Includes: 16:4 and 20:4n-3; [H] Includes: 16: 2n-4; *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; n.s. = not significant.