PAPER

NUTRITIONAL QUALITY OF DIFFERENT FISH SPECIES FARMED IN THE ADRIATIC SEA

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ABSTRACT

We investigated the nutritional quality of commercially important farmed fish species: sea bream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*), dentex (*Dentex dentex*), and turbot (*Scophthalmus maximus*). The omega-3 fatty acid content of dentex was twice as high as that of any other fish species, and its eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid contents were 2-4 times higher. The recommended n-3/n-6 ratio was present in all the fish species, but the recommended polyunsaturated/saturated fatty acid ratio was not present in the turbot. All the fish species, except turbot, met the recommended atherogenic index, thrombogenic index, and ratio of hypocholesterolaemic to hypercholesterolaemic fatty acids, whereas the highest flesh lipid quality value was observed in the dentex.

Keywords: nutritional quality, farmed fish, Adriatic Sea, basic chemical composition, mineral, fatty acid

1. INTRODUCTION

Since 1970, fish farming (aquaculture) has been the world's fastest growing sector in the production of foods of animal origin. Because wild fisheries are stagnating and the human population is growing, aquaculture is expected to fill the gap in supplying fish intended for human consumption, because the demand has continued to increase (FAO, 2016). Consumer habits are also changing continuously, and issues such as convenience, health, ethics, variety, value for money, sustainability, and safety are becoming more important. Health and well-being increasingly influence our decisions about what we consume. Fish have a particularly prominent role in this context because mounting evidence confirms the health benefits of consuming fish. A moderate-to-high fish intake is associated with a reduced prevalence of the chronic diseases that go hand in hand with obesity, such as cardiovascular diseases, diabetes, and some cancers (OEHLENSCHLÄGER, 2012).

Fish is also considered an integral component of a well-balanced diet, providing a healthy source of energy, high-quality proteins, vitamins (D, A, E and B12), essential minerals and particularly n-3 long-chain polyunsaturated fatty acids (LC PUFA), mainly eicosapentaenoic acid (20:5 n-3 EPA) and docosahexaenoic acid, (22:6 n-3 DHA), whose pleiotropic effects on health promotion and disease prevention are well recognized (GIL and GIL, 2015). In fresh fish, the muscle composition is considered to be the most important aspect of quality, whereas its sensory properties and nutritional value, together with freshness, are also important quality parameters in terms of consumer acceptability (GRIGORAKIS, 2007). Fish proteins are easily digested (because the proportion of collagen is low). They are also a good source of essential amino acids. The nutritional value of fish meat is also linked to its lipid composition, as well as its constituent proteins (OEHLENSCHLÄGER, 2012).

Lipid contents are highly variable both between and within fish species. Many factors contribute to this variability, including feed, farm location, fish size and stage of maturity, biological variations, tissue sampled, and starvation (RUEDA *et al.*, 2001). Fish lipids contain a high proportion of unsaturated fatty acids (60%-84%), especially long-chain n-3 fatty acids. The fatty acids (FAs) in fish, especially EPA and DHA, have important effects on the health of the human body and differ from those of meat. The well-known hypotriglyceridaemic effect of n-3 LC PUFA may be beneficial in terms of reducing the percentage of pro-atherogenic small low-density lipoprotein (LDL) particles, and perhaps by ameliorating the inflammatory processes associated with the metabolic syndrome seen in patients with diabetes mellitus or cardiovascular disease (LOPEZ-HUERTAS, 2012). In recent decades, fish nutrition research has devoted much effort to the development of sustainable fish feeds with an optimal FA composition so that the fish provide adequate levels of n-3 FAs for human nutrition (IZQUIERDO *et al.*, 2005; KRIS-ETHERTON *et al.*, 2003; PRATOOMYOT *et al.*, 2010). The minerals in raw farmed fish meat, which occur at overall levels of 0.6-1.5 g/100 g, are also very important in the human diet (ERKAN and ÖZDEN, 2007). According to the literature, the origins of fish and their feeding patterns have no effect on their mineral composition, other than their calcium content (FUENTES *et al.*, 2010). The nutritional value of fish is affected by a low-energy value and high levels of fat-soluble vitamins, especially A and D (MAHAN and ESCOTT-STUMP, 2004).

Because the world's fish stocks are limited, it is now suggested that farmed fish provide an alternative for consumers. Farmed sea-foods have an advantage over captured fishery products because they are produced and harvested under controlled conditions, which allow consumption-related risks to be minimized. In recent years, the Mediterranean aquaculture industry has become interested in farming new species to overcome the problems arising from the overproduction of two main species, the gilthead sea bream (*Sparus aurata*) and the European sea bass (*Dicentrarchus labrax*) and to diversify their products. The common dentex (*Dentex dentex*) is a fast-growing sparid, and a candidate species for fish farming in the Mediterranean (RIGOS *et al.*, 2012). Alternatively, the commercial farming of turbot (*Scophthalmus maximus),* a high-value flat fish, along the Adriatic coast is still in its infancy, and the turbot is mainly farmed along the Atlantic coasts of France and Spain (SÉROT *et al.*, 1998; MANTHEY-KARL *et al.*, 2016). Although the production of this species has achieved high quality and efficiency, very little is known about its nutritional quality relative to our knowledge of other fish species.

Given that the demand for fish is increasing globally and fish consumption is generally recommended by dieticians, it is surprising that there are no data on the FA profiles and FA contents of the different fish species farmed in the Adriatic Sea, especially the turbot and dentex. Therefore, in this study, we investigated the quality parameters of four farmed white fish species sampled from different farms along the Adriatic Coast: the European sea bass (*D. labrax*), gilthead sea bream (*Sp. aurata*), turbot *(S. maximus*), and common dentex (*D. dentex*). The basic chemical parameters, minerals, FA compositions, and health-related lipid indices: atherogenic index [AI], thrombogenic index [TI], the ratio of hypocholesterolaemic to hypercholesterolaemic FAs [HH], and flesh lipid quality [FLQ]) were determined and compared across the four species analyzed.

2. MATERIALS AND METHODS

2.1. Fish farming and sampling conditions

Sea bass and sea bream are the main marine fish species commercially farmed in Croatian sea farms, whereas the cultivation of dentex, and especially turbot, is far less common in these regions. Fish are cultivated in a similar manner at each farming location, consistent with the rules of Mediterranean fish farming, which are as follows: cultivation density of 5-12 kg per cubic meter (for turbot > 20 kg per cubic meter) in 22-28 months; the use of cages; and feeding regimens adjusted to bodyweight, sea temperature, photoperiod, oxygen saturation, etc. The main difference between the northern part of the Adriatic and its middle part is the greater temperature differential between the winter and summer months in the north, whereas in the middle part, the temperature never falls below 12 °C in winter or exceeds 25 °C in summer. The turbot-farming technology has been sufficiently mastered in some geographic areas, but is still in the experimental stage in the Adriatic Sea. The biggest challenge is the extremely high temperatures in the Adriatic Sea, and the most effective modes of manipulation are yet to be determined. Fry are released from different commercial hatcheries in France, Italy, and Greece to on-grow, are cultivated in floating net cages of different sizes, and are fed a commercially available fish feed with different nutritional contents, as shown in Table 1.

Table 1. Declared compositions of feeds used in the production of cultivated fish species.

Parameter (%)	Commercial name of the food/fish species		
	Efico Sigma 870/Turbot/Dentex	Efico YM 854/Sea Bream	Efico YM 868/Sea Bass
Crude proteins	54.0	41.0	40.0
Crude lipids	20.0	18.0	23.0
Crude cellulose	0.4	4.0	3.0
Ash	10.2	6.5	6.6

In this study, samples of sea bass, sea bream, and dentex were collected from different farms situated in the northern (Istria) and middle parts of the Adriatic Sea, whereas turbot was only sampled from the northern part of the Adriatic Sea (Istria). The fish were sampled in March-May 2016 (three groups of samples were collected per species). In total, 72 pieces of fish (18 pieces of each species) were transported to our laboratory in Styrofoam boxes on ice. Before the edible part of the fish flesh was eviscerated and filleted for analysis, the bodyweight and length were measured to the closest g (280-300 g for sea bass, sea bream, and dentex) and cm (28.5-30.5 g for sea bass, sea bream, and dentex), respectively. For the turbot, these parameters were 260-320 g and 24.5-31.0 cm, respectively. Sample preparation involved cleaning the body surfaces, descaling and beheading each specimen, and removing the vertebrae and viscera (not the skin). The fillets and skin of each of the 72 fish samples were homogenized with a laboratory homogenizer (Grindomix GM 200, Retsch, Haam, Germany). The samples were analyzed immediately, first for their basic chemical parameters and then for their FA profiles.

2.2. Determination of basic chemical composition and mineral content

The water content was determined with a gravimetric analysis (ISO 1442:1997) using an Epsa 2000 thermostat (Ba-Ri, Velika Gorica, Croatia). The total protein content was determined by the Kjeldahl method given by International Organization for Standardization (ISO) (ISO 937:1978) using a Digestion Unit 8-Basic (Foss, Höganäs, Sweden) and a Kjeltec 8400 automated distillation and titration device (Foss). The total fat content was determined with the Soxhlet method (ISO 1443:1973), in which the samples are digested with acid hydrolysis and the fats are then extracted with petroleum ether using a Soxtherm 2000 automated device (Gerhardt, Munich, Germany). The ash content was determined according to ISO 936:1998 using a Nobertherm LV9/11/P320 furnace (Lilienthal, Germany). The phosphorus content was determined according to ISO 13730:1996 with a DR/4000U spectrophotometer (Hach, Düsseldorf, Germany). We used the procedures described by PETROVIĆ *et al.* (2015) to determine the calcium and sodium contents. For the basic chemical composition and mineral content analyses, two samples of each of the 72 fish samples (18 samples for each species) were tested in parallel, and the mean values were analysed statistically for percentage weight (%), with an accuracy of 0.01%.

2.3. Fatty acid analysis

The preparation of samples for the analysis of FA methyl esters has been described previously by PLEADIN *et al.* (2015). The FA methyl esters were analysed with gas chromatography (GC) according to ISO 12966-4:2015 and EN ISO 12966-4:2015. A 7890BA gas chromatographer, equipped with a flame ionization detector (FID) and a 60 m DB-23 capillary column with an internal capillary diameter of 0.25 mm and a stationary phase thickness of 0.25 μm (Agilent Technologies, Santa Clara, CA< USA), was used. The components were detected with a FID at a temperature of 280 °C, a hydrogen flow of 40 mL/min, and an airflow of 450 mL/min. Nitrogen was used as the make-up gas at a flow rate of 25 mL/min. The initial column temperature was 130 °C; after 1 min, it was increased by 6.5 °C/min until it reached 170 °C. The temperature was further increased by 2.75 °C/min until it reached 215 °C, where it was maintained for 12 min. The temperature was then increased further by 40 °C/min until the final column temperature reached 230 °C, which was maintained for 3 min. Each sample (1 μL) was injected into a split-splitless injector at a temperature of 270 °C with a split ratio of 1:50. The carrier gas was helium (99.9999%), flowing at the constant rate of 43 cm/s. The FA methyl esters were identified by comparing their retention times with those of FA methyl esters in the standard mixture, as described previously by PLEADIN *et al.* (2015). The FA composition was determined for each of the 72 fish samples (18 pieces of each species). The mean values per species are expressed as the percentage (%) of a particular FA in the total FAs, with an accuracy of 0.01%. The FA methyl ester values were converted into FA values per 100 g of edible part (EP), according to the FAO/INFOODS Guidelines for Converting Units, Denominators and Expressions (2012).

2.4. Determination of lipid quality

The lipid quality indices AI, TI, HH, and FLQ were calculated from the FA compositions. AI indicates the relationship between the total major saturated fats, which are considered pro-atherogenic agents (because they facilitate the adhesion of lipids to the cells of the immune and circulatory systems), and the total major unsaturated fats, which are considered anti-atherogenic agents (because they inhibit the formation of plaques and reduce the levels of esterified FAs, cholesterol, and phospholipids, and therefore prevent micro- and macro-coronary diseases) (ULBRITCTH and SOUTHGATE, 1991). This parameter was calculated as:

$$\text{AI} = ([12{:}0 + (4 \times 14{:}0) + 16{:}0])/(\text{total monounsaturated fatty acids [MUFA]} + \\ + \text{PUFA n-6} + \text{PUFA n-3})$$

TI indicates the tendency for blood to clot in blood vessels. It is defined as the relationship between pro-thrombogenic (saturated) and anti-thrombogenic FAs (MUFA, PUFA n-6, and PUFA n-3) (ULBRITCTH and SOUTHGATE, 1991). The index is calculated as:

$$\text{TI} = (14{:}0 + 16{:}0 + 18{:}0)/([0.5 \times \text{total MUFA} + 0.5 \times \text{PUFA n-6} + 3 \times \text{PUFA n-3}] + \\ + [\text{PUFA n-3}/\text{PUFA n-6}])$$

The ratio between the hypocholesterolaemic and hypercholesterolaemic FAs (HH) takes into account the known effects of certain FAs on cholesterol metabolism (SANTOS-SILVA *et al.*, 2002). It is calculated as:

$$\text{HH} = (\text{C18:1n-9} + \text{C18:2n-6} + \text{C20:4n-6} + \text{C18:3n-3} + \text{C20:5n-3} + \text{C22:5n-3} + \\ + \text{C22:6n-3})/(\text{C14:0} + \text{C16:0})$$

FLQ indicates the proportion of the main PUFAs n-3 (EPA and DHA) in the muscles relative to the total lipid content. The higher this index, the higher the quality of the dietary lipid source (ABRAMI *et al.*, 1992; SENSO *et al.*, 2007). The index is calculated as:

$$\text{FLQ} = (\text{EPA} + \text{DHA in g FAs}/100\text{ g EP})/(\text{total lipids in g}/100\text{ g EP}) \times 100$$

2.5. Statistical analysis

The statistical analysis was performed with SPSS Statistics Software 22.0 (IBM SPSS Statistics 2013, NY, USA). The results were tested for the normality of their distribution ($p > 0.05$) using the Shapiro-Wilks test. To determine the statistical significance of the differences in the chemical and FA compositions between the fish species analysed, one-way ANOVA and the robust Brown-Forsythe test were used. To establish the homogeneity of variance, the Scheffe *post hoc* test or Tamhane's T2 *post hoc* were used. Decisions on statistical relevance were made at the significance level of $p < 0.05$.

3. RESULTS AND DISCUSSIONS

The results presented in this study provide nutritional profiles of four fish species (sea bream, sea bass, turbot, and dentex), with particular emphasis on their FA components and health-related lipid indices. All the fish species are farmed in the Adriatic Sea. The basic chemical and mineral compositions of these fish species are shown in Table 2.

Table 2. Basic chemical and mineral compositions of four species of farmed fish.

Parameter	Sea bass	Dentex	Turbot	Sea bream
Water (%)	70.81 ± 3.28^{c}	69.12 ± 0.39^{c}	$77.88 \pm 1.31^{a,b,d}$	70.16 ± 2.50^{c}
Ash (%)	1.21 ± 0.02^{b}	$1.31 \pm 0.03^{a,c}$	$1.16 \pm 0.05^{b,d}$	1.24 ± 0.07^{c}
Fat (%)	9.11 ± 3.06^{c}	10.46 ± 0.40^{c}	$4.00 \pm 1.20^{a,b,d}$	10.48 ± 3.08^{c}
Protein (%)	19.22 ± 1.46^{c}	18.92 ± 0.43^{c}	$17.59 \pm 1.15^{a,b,d}$	19.09 ± 0.33^{c}
Na (mg/kg)	706 ± 277^{c}	$465 \pm 43.9^{c,d}$	$1212 \pm 292^{a,b}$	968 ± 184^{b}
Ca (mg/kg)	729 ± 189^{b}	$1300 \pm 128^{a,c,d}$	656 ± 151^{b}	540 ± 152^{b}
P (mg/kg)	$2214 \pm 122^{b,c}$	$2740 \pm 195^{a,c,d}$	$1811 \pm 127^{a,b,d}$	$2180 \pm 164^{b,c}$

Results are expressed as mean values (18 samples per species, each of which was analysed in duplicate) ± standard deviations. Significant differences ($p < 0.05$): ^{a}*vs.* sea bass; ^{b}*vs.* dentex; ^{c}*vs.* turbot; ^{d}*vs.* sea bream.

Analysis of the basic chemical and mineral parameters showed that the turbot had a statistically significantly ($p < 0.05$) higher water content and lower fat and protein contents than the other fish analysed. In the remaining three fish species, the basic quality parameters were similar to those presented in earlier studies (ÖZDEN and ERKAN, 2008; ERKAN and ÖZDEN, 2007; KYRANA and LOUGOVOIS, 2002). The descriptive data for the basic chemical fish composition did not clearly correlate with the composition of the feed with which the fish were fed (Table 1), because it is not the only parameter that affects the nutritional composition of a fish species. Nutritional composition may be affected by a number of factors, including age, sex, and environmental factors, such as temperature, salinity, etc. (GRIGORAKIS, 2007). Therefore, although the dentex and turbot were fed the same type of feed, their basic chemical and mineral contents differed significantly.

In a study by ÖZDEN and ERKAN (2008) that compared the properties of sea bream, sea brass, and dentex, the water, ash, protein, and fat contents were in the ranges 69.68%-76.42%, 1.49%-1.95%, 18.21%-21.70%, and 2.29%-8.10%, respectively. In a study of the composition of turbot, these parameters were in the ranges 78.7%-80.2%, 1.0%-1.3%, 18.9%-20.3%, and 1.0%-2.0%, respectively (MANTHEY-KARL *et al.*, 2016). In general, research has shown that the basic chemical compositions of fish vary with the season of cultivation. Because fish are ectothermic poikilotherms, the fat content in a certain period of the year can be attributed to the ongoing physiological process of conserving the fat stock, which occurs in autumn after intense feeding, or the process of spending the fat stock, which occurs during winter. In early summer, when environmental conditions change, primarily as an increase in temperature, the fish metabolism accelerates and the energy taken from food is used for fish growth. PETROVIĆ *et al.* (2015) showed similar proportions of fat and moisture in the sea bass and sea bream, with fat contents of 3.2%-12.3% in the sea bass and 4.2%-15.0% in the sea bream, which depended on and varied strongly across the farming seasons, as explained previously. The fat content is inversely

related to the water content, and these parameters together account for approximately 80% of the total fish meat composition.
The dentex had a significantly higher ($p < 0.05$) proportion of calcium and phosphorus than the other fish species analysed, whereas the turbot had the lowest proportion of phosphorus (significantly lower than in the other fish species, $p < 0.05$). The dentex had a significantly lower sodium content than the turbot and sea bream, and the sodium content of the turbot was significantly higher than that of the common dentex or sea bass. As in earlier studies, the calcium and phosphorus contents were higher in the sea bass than in the sea bream (ERKAN and ÖZDEN, 2007; PETROVIĆ *et al.*, 2015). However, the calcium content determined in this study was significantly higher than the results previously published for the sea bream (ORBAN *et al.*, 2003; PETROVIĆ *et al.*, 2015) and turbot (MANTHEY-KARL *et al.*, 2016), whereas our results for the sea bass were similar to previous results (PETROVIĆ *et al.*, 2015).
The FA compositions, expressed as mean values and standard deviations relative to the total FAs for each species, are shown in Table 3.
The results show that the most strongly represented FA in all four species analysed was oleic acid (C18:1n-9, OA), followed by linoleic acid (C18:2n-6, LA) and palmitic acid (C16:0, PA). The results of this study are consistent with earlier research that demonstrated an increase in C18 FAs, such as OA, LA, and ALA, in farmed fish, in response to the use of vegetable oils in their feed (STROBEL *et al.*, 2012). Among the unsaturated FAs, OA and LA contribute most significantly to the enrichment of aromatic components (ELMORE *et al.*, 1999) and are considered to be of high nutritional value because they protect against cardiovascular disease (HORNSTRA, 1999).
Statistically significant differences in the FA profiles were observed among the fish species analysed ($p < 0.05$). No FA analysis of the commercial feed used was available. The proportion of saturated FAs (SFAs; C14:0, C15:0, and C16:0) was the highest in the turbot. The sea bass and sea bream had significantly higher proportions of MUFA than the dentex or turbot, which is attributable to their oleic acid contents. A significantly lower proportion of PUFA (C18:2n-6c, C18:3n-3) was found in the turbot. In the study by ÖZDEN and ERKAN (2008), the FAs detected in the sea bass, sea bream, and dentex were 28.01%-32.41% SFA, 25.88%-28.62% MUFA, and 24.75%-27.42% PUFA. Compared with these values, our study found significantly higher proportions of MUFA in all the fish species investigated, whereas our SFA and PUFA values were similar to previously reported values, except in the turbot. The samples of turbot analysed in this study contained more SFA and MUFA, and at the same time, less n-3 and less PUFA than previously reported levels, resulting in a less favorable n-3/n-6 ratio than obtained by other researchers (SEROT *et al.*, 1998; MANTHEY-KARL *et al.*, 2016). The n-3/n-6 ratios obtained for turbot in these studies were 3-7-fold higher than that obtained in the present study.
The protective role of fish consumption against coronary heart disease has been widely demonstrated and is mainly attributed to the effects of n-3 FAs and their cardioprotective action (KRIS-ETHERTON *et al.*, 2003; PSOTA *et al.*, 2006). Earlier studies suggested that the n-3/n-6 ratio is a reliable index for interspecies comparisons of relative nutritional values (PIGGOT and TUCKER, 1990). According to SARGENT (1997), the optimum n-3/n-6 PUFA ratio should be 1:5 (0.2). The amounts of the FAs (ALA, EPA, DHA, AA, and LA,) present in the four fish species in our study, as determined by converting the proportion of an individual FA in the total FAs to the amount of the individual FA per 100 g of fish EP, are shown in Table 4.

Table 3. Fatty acid compositions (% of total FAs) of four species of farmed fish.

Fatty acids	Relative FA amount (% of FA) mean±standard deviation			
	Sea bass	Dentex	Turbot	Sea bream
C12:0	ND	0.06±0.00	0.17±0.09	0.06±0.05
C14:0	2.65±0.52^{b,c}	4.13±0.18^{a,c,d}	9.66±0.81^{a,b,d}	2.69±0.78^{b,c}
C15:0	0.33±0.05^{b}	0.45±0.02^{c}	0.75±0.11^{a,b,d}	0.28±0.11^{c}
C16:0(PA)	16.17±1.43^{c}	17.95±0.39^{c,d}	25.36±2.02^{a,b,d}	14.18±1.74^{b,c}
C17:0	0.39±0.08	0.51±0.01	0.49±0.25	0.26±0.11
C18:0	3.97±0.41^{b}	5.50±0.12^{a,d}	4.72±1.37	3.30±0.59^{b}
C20:0	0.33±0.05	0.30±0.15	0.36±0.04	0.35±0.07
C22:0	0.08±0.08	0.10±0.01	ND	0.13±0.11
C14:1	ND	0.08±0.00	0.08±0.09	ND
C16:1n-7t	0.45±0.03	0.44±0.01	0.51±0.07	0.45±0.03
C16:1n-7c	3.46±0.58^{b,c}	5.82±0.11^{a,c}	11.37±1.53^{a,b,d}	4.11±1.25^{c}
C17:1	0.24±0.06	0.36±0.01	0.17±0.19	0.20±0.07
C18:1n-9t	0.34±0.25	0.07±0.07	ND	0.27±0.11
C18:1n-9c (OA)	38.85±3.98^{b,c}	28.10±1.16^{a,c,d}	20.63±2.18^{a,b,d}	42.07±6.23^{b,c}
C18:1n-7	3.16±0.22^{c}	3.41±0.06^{c}	4.66±0.15^{a,b,d}	3.07±0.18^{c}
C20:1n-9	3.10±0.45^{c}	2.53±0.05^{c}	3.87±0.37^{a,b,d}	2.41±0.71^{c}
C22:1n-11	1.42±0.77^{c}	1.15±0.04^{c}	3.35±0.47^{a,b,d}	1.01±0.42^{c}
C22:1n-9	0.49±0.09	0.49±0.02	0.89±0.45	0.62±0.18
C24:1n-9	0.31±0.06	0.40±0.23	0.89±0.86	0.46±0.16
C18:2n-6t	0.08±0.06	0.13±0.01	0.09±0.10	0.05±0.05
C18:2n-6c (LA)	14.60±2.00^{b,c}	10.98±0.02^{a,c,d}	6.08±0.84^{a,b,d}	15.71±0.62^{b,c}
C18:3n-6	0.11±0.06	0.13±0.00	ND	0.12±0.13
C20:2n-6	0.68±0.23^{b}	0.40±0.02^{a}	0.37±0.23	0.68±0.21
C20:3n-6	ND	0.13±0.01	ND	0.11±0.10
C20:4n-6 (AA)	0.26±0.05^{b}	0.57±0.05^{a,c,d}	0.27±0.15^{b}	0.20±0.10^{b}
C18:3n-3 (ALA)	2.92±0.46^{b,c}	1.78±0.04^{a,c}	0.52±0.31^{a,b,d}	3.06±0.99^{c}
C18:4n-3	0.39±0.13^{b}	0.82±0.06^{a}	0.50±0.30	0.33±0.11
C20:3n-3	0.07±0.07^{d}	0.14±0.01	ND	0.22±0.05^{a}
C20:4n-3	0.23±0.07^{b}	0.46±0.02^{a}	0.23±0.19	0.32±0.15
C20:5n-3 (EPA)	1.89±0.53^{b}	4.23±0.42^{a,c,d}	2.01±0.87^{b}	1.12±0.58^{b}
C22:6n-3 (DHA)	3.01±0.89^{b}	8.32±1.12^{a,c,d}	2.06±1.47^{b}	2.19±1.15^{b}
SFA	23.93±2.13^{b,c}	29.06±0.60^{a,c}	41.45±3.04^{a,b,d}	21.24±3.25^{c}
MUFA	51.82±2.45^{b,c}	42.85±1.38^{a,d}	46.43±2.64^{a,d}	54.66±3.74^{b,c}
PUFA	24.25±2.30^{c}	28.09±1.73^{c}	12.12±3.72^{a,b,d}	24.10±1.38^{c}
Total n-6	15.74±2.06^{b,c}	12.34±0.07^{a,c,d}	6.81±1.12^{a,b,d}	16.87±0.37^{b,c}
Total n-3	8.51±1.29^{b}	15.75±1.66^{a,b,c}	5.31±2.61^{b}	7.23±1.48^{b}

Results are expressed as mean values (18 samples per species) ± standard deviations; ND, not detected; LOD, the limit of detection of 0.05%; PA, palmitic acid; OA, oleic acid; LA, linoleic acid; ALA, α-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Significant differences ($p < 0.05$): ^{a}*vs.* sea bass; ^{b}*vs.* dentex¸ ^{c}*vs.* turbot; ^{d}*vs.* sea bream.

Table 4. Absolute quantification of fatty acids (mg FA/100 g edible muscle part) ALA, EPA, DHA, AA, and LA.

Fatty acids (mg/100 g)	Sea bass	Dentex	Turbot	Sea bream
ALA	245.01±38.71^{b,c}	172.28±4.34^{a,c}	18.59±11.01^{a,b,d}	295.29±95.63^{c}
EPA	158.79±44.66^{b,c}	410.40±40.68^{a,c,d}	72.74±31.67^{a,b}	108.27±56.02^{b}
DHA	254.06±75.55^{b,c}	810.32±109.05^{a,c,d}	74.99±53.53^{a,b}	219.90±112.20^{b}
EPA + DHA	418.25±113.42^{b,c}	1220.72±149.60^{a,c,d}	147.73±69.83^{a,b}	321.16±162.62^{b}
AA	22.20±4.29^{b}	55.54±4.48^{a,c,d}	9.69±5.37^{b}	19.57±9.43^{b}
LA	1223.79±167.09^{c,d}	1062.69±2.47^{c,d}	219.33±30.40^{a,b,d}	1517.76±57.98^{a,b,c}

Results are expressed as mean values (18 samples per species) ± standard deviations; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; LA, linoleic acid.

The contents of LA and ALA should depend on the fish's dietary intake because fish lack the *delta* 12 and *delta* 15 desaturase enzymes responsible for the conversion of OA to LA and ALA (RUIZ-LOPEZ *et al.*, 2012). The higher LA and ALA contents presumably indicate the presence of vegetable oils in the feed, such as sunflower, soybean, rapeseed, and linseed oil. LA and ALA were significantly ($p < 0.05$) lower in the turbot, whereas LA was significantly higher ($p < 0.05$) in the sea bream than in the other species (Table 4).

There are various recommendations for both the consumption of fish and the intake of n-3 LCPUFA (primarily EPA and DHA). To meet the recommended EPA and DHA dietary requirements for human nutrition, the American Heart Association (2015) recommends the consumption of two servings of fish (particularly oily fish) twice a week. In terms of the fat content, the German Nutrition Society recommends a smaller serving of oily fish (i.e., 70 g) and a larger serving of lean fish (80-150 g) once a week (BERGLAITER, 2012).

The majority of organizations recommend two servings of fish (approximately 140 g per meal) per week. This would provide approximately 500 mg of EPA and DHA combine a day, which is the intake most countries and organizations recommend (WHO, 2003; KRIS-ETHERTON and INNIS, 2007) for optimal overall health and a reduced cardiovascular risk. However, the lowest daily value set for adults by the European Food Safety Authority (EFSA) is 250 mg of EPA plus DHA (EFSA, 2009). The absolute EPA and DHA contents were significantly higher ($p < 0.05$) in the dentex than in other species analysed, with values of 1220 mg/100 g EP (Table 4). Therefore, to fulfil the lowest-level recommendation set by the EFSA, the amounts of sea bass, dentex, turbot, and sea bream that must be consumed on a daily basis are 61 g, 21 g, 167 g, and 78 g, respectively.

The dietetic value of fish meat is also determined by its lipid quality indices, which reflect the relative proportions of constituent saturated and unsaturated FAs. These indices indicate the global dietetic quality of the lipids and their potential impact on the development of coronary heart disease (ULBRITCTH and SOUTHGATE, 1991). The nutritional quality indices determined for different fish species in this study are shown in Table 5.

The sea bass, sea bream, and turbot had significantly higher proportions of n-6 FAs than the dentex, whereas the dentex had a significantly higher proportion of n-3 FAs (C18:4n-3, C20:4n-3, EPA, DHA) (Table 2). Consequently, the n-3/n-6 ratio was significantly higher for the dentex (Table 5). RIGOS *et al.* (2012) also confirmed the high proportion of n-3 FAs in farmed dentex. They also found a significantly higher n-3/n-6 ratio in farmed dentex than in wild dentex. The n-3 FAs are lower in modern aquaculture products than in wild, naturally caught fish, and the high levels of terrestrial-plant-originating C18:2 n-6 present in feedstuff affect the n-3/n-6 ratio in the EP of farmed fish (GRIGORAKIS, 2007).

Table 5. Nutritional quality indices determined for four species of farmed fish.

Parameter	Sea bass	Dentex	Turbot	Sea bream
n-3/n-6	0.55±0.11[b]	1.28±0.13[a,b,c]	0.74±0.30[b]	0.43±0.09[b]
PUFA/SFA	1.02±0.17[c]	0.97±0.07[c]	0.30±0.10[a,b,d]	1.15±0.19[c]
AI	0.35±0.05[c]	0.49±0.02[c]	1.10±0.13[a,b,d]	0.32±0.07[c]
TI	0.38±0.04[c]	0.36±0.03[c]	0.97±0.33[a,b,d]	0.35±0.06[c]
HH	3.34±0.55[b,c]	2.46±0.08[a,c,d]	0.91±0.13[a,b,d]	3.94±1.03[b,c]
FLQ	4.53±1.25[b]	11.67±1.43[a,c,d]	3.69±1.75[b]	3.06±1.55[b]

Results are expressed as mean values (18 samples per species) ± standard deviations; n-3, omega-3 fatty acids; n-6, omega-6 fatty acids; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AI, atherogenic index; TI thrombogenic index; HH hypocholesterolaemic/hypercholesterolaemic ratio; FLQ, flesh lipid quality. Significant differences ($p < 0.05$): [a]*vs.* sea bass; [b]*vs.* dentex; [c]*vs.* turbot; [d]*vs.* sea bream.

PUFA/SFA values above 0.4-0.5 and n-3/n-6 < 0.25 are required if a diet is to combat various 'lifestyle diseases', such as coronary heart disease and cancer (SIMOPOULOS, 2002). The higher the n-3/n-6 ratio, the more the body is able to use n-3 fats (WOOD *et al.*, 2008). In this study, the recommended n-3/n-6 ratio was met by all the fish species, but the recommended PUFA/SFA ratio was not met by the turbot, which had the lowest PUFA/SFA ratio (0.30 ± 0.10). The n-3/n-6 ratio is suggested to be a good parameter for comparing the relative nutritional values of different species (PIGGOT and TUCKER, 1990). However, this index is of limited value if the FA composition is unknown. Generally, C20 and C22 FAs are more valuable from a nutritional standpoint than C18 FAs (ARTS *et al.*, 2001). Because they are quantitatively predominant, the two FAs EPA and DHA are largely responsible for differences in the n-3/n-6 ratio, a reliable indicator of relative nutritive value of lipids, as was also confirmed in our study (Table 5).

However, some researchers consider that an index such as PUFA/SFA may be inadequate for evaluating the nutritional value of fats, because some SFAs do not increase plasma cholesterol and because the ratio ignores the effects of MUFA (ORELLANA *et al.*, 2009). A recent study suggested that C12:0 and C14:0 more effectively increase total cholesterol than C16:0, whereas C18:0 has no effect on the concentration of total serum cholesterol, and no apparent effect on either LDLs or high-density lipoproteins (MENSINK and KATAN, 1992; DALEY *et al.*, 2010). Therefore, the C12:0, C14:0, and C16:0 FAs present in human diets are associated with increased plasma cholesterol. This association is strongest for C14:0, which has a potentially 4-6 times higher capacity to increase cholesterol concentrations than C16:0 (ULBRITCTH and SOUTHGATE, 1991; MENSINK and KATAN, 1992; BRESSAN *et al.*, 2011).

Based on the discussion presented above, another indicator of nutritional quality, HH, was determined to gain insight into the effects of FAs on blood cholesterol levels. A higher value for the HH index is preferable (SANTOS-SILVA *et al.*, 2002; TESTI *et al.*, 2006). In this study, the HH values ranged from 0.91 ± 0.13 in the turbot to 3.94 ± 1.03 in the sea bream. The HH ratios were significantly higher for the sea bass and sea bream than for the turbot because these two species contain significantly lower proportions of SFA.

Two other indices, AI and TI, were investigated because their effects on the incidence of pathogenic phenomena, such as atheroma and/or thrombus formation, differ from those of single FAs. As expected, AI and TI were always highest for the most atherogenic and thrombogenic dietary components. It is assumed that lipids with AI < 1 and TI < 1 are beneficial to human health. TONIAL *et al.* (2014) suggested that MUFA and PUFA have more profound health benefits because they prevent coronary disease. The AI and TI

values determined in this study were lower than 1, except those for turbot, which had significantly higher ($p < 0.05$) AI and TI values than the other fish species because it has a higher proportion of SFA. In the study conducted by VALFRĖ and co-authors (2003), an AI of 0.45 and a TI of 0.25 were determined for the sea bass, whereas DE FRANCESCO *et al.* (2007), in a study of the sea bream, found AIs of 1.545-1.565 and TIs of 0.196-0.430.
Our results are consistent with previously published data. FLQ was significantly higher in the dentex ($p < 0.05$) than in the other three species. Because this index is related to the proportions of DHA and EPA in the total fish lipid content, it is expected to be highest in the dentex, because this species is the richest source of these two n-3 FAs. However, the lipid quality indices AI and FLQ for the dentex were lower in this study than in another study (SUAREZ and CERVERA, 2010), whereas the TI values were quite similar. However, FLQ for the other species in this study were lower than those reported previously for the gilthead sea bass (SENSO *et al.*, 2007).

4. CONCLUSIONS

This study demonstrates the high variability in the basic chemical and mineral contents of the species studied. The proportion of omega-3 FAs was twice as high in the dentex than in the sea bream, sea bass, or turbot, and the proportions of EPA and DHA are 2-4 times higher in the dentex. The recommended n-3/n-6 ratio was present in all the fish species, but the recommended PUFA/SFA ratio was not present in the turbot, which had the lowest PUFA/SFA ratio. The HH ratio was significantly higher for the sea bass and sea bream than for the turbot. Because the fish species analysed vary in their nutritional quality, the consumption of diverse fish species is advisable. Based on established lipid quality indicators, the dentex, a fish species underexploited in aquaculture, is a highly recommended and important source of the FAs required for human health.

ABBREVIATIONS

AA, arachidonic acid (C20:4 n-6); AI, atherogenic index; DHA, docosahexaenoic acid (C22:6 n-3); EP, edible part; EPA, eicosapentaenoic acid (C20:5 n-3); FAs, fatty acids; FLQ, flesh lipid quality; HDL, high-density lipoprotein; HH, ratio of hypocholesterolaemic to hypercholesterolaemic fatty acids; LA, linoleic acid, C18:2 n-6; LC PUFA, long-chain polyunsaturated fatty acids; LDL, low-density lipoproteins; MUFA, monounsaturated fatty acids; OA, oleic acid, C18:1 n-9; PA, palmitic acid, C16:0; SFA, saturated fatty acids; TI, thrombogenic index.

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