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7 The chemical composition of different edible locations
8 (central and edge muscles) of flat fish (*Lepidorhombus*
9 *whiffiagonis*)
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SUMMARY

The chemical composition of the different edible locations (central and edge muscles) of a flat fish (megrim, *Lepidorhombus whiffiagonis*) was comparatively analysed at both body sides. Higher moisture and lower lipid contents were obtained in central muscles than in edge ones. Edge sites showed higher triacylglycerols and sterols contents and lower mean phospholipids values than central muscles. A higher α -tocopherol presence was observed in the lipid fraction of central muscles than in the edge ones. For both fish sides, fatty acids (FA) analysis showed lower monounsaturated FA and higher polyunsaturated FA contents and $\omega 3/\omega 6$ ratios in central muscles. Presence of essential elements (Co, Cu, Mn, Se, Zn) did not provide differences among sites considered. Concerning toxic elements, As content showed greater levels in central muscles than in their corresponding edge sites for both fish sides; upper zones showed higher mean scores in As, Cd, Cr, Hg, Ni, Pb and V.

Key words: *Lepidorhombus whiffiagonis*; edible zones; side distribution; proximate composition; fatty acids; lipid classes; microelements

Running title: Two-side composition of megrim muscle

INTRODUCTION

Chemical constituents in edible parts of marine species have proved to be inhomogeneously distributed as a result of physiological and anatomical factors (Haouas *et al.*, 2010; Thammapat *et al.*, 2010). In this sense, the lipid content and composition have been recognised as the most highly affected, showing wide distribution differences among edible tissues in wild fatty (Nakamura *et al.*, 2007) and lean (Ruff *et al.*, 2004; Prego *et al.*, 2012) species as well as in farmed ones (Testi *et al.*, 2006; Álvarez *et al.*, 2009). Concerning microelements presence in seafood, previous studies have shown that concentration in fish muscle sites may be influenced by different biological factors (anatomical, physiological), as well as by external factors such as food source and environment pollution (Alasalvar *et al.*, 2002; Noël *et al.*, 2011).

Marine lipids have shown to provide many beneficial effects on human health, mostly related to their high content on ω 3 fatty acids (Uauy & Valenzuela, 2000; Komprda, 2012). However, such fatty acids have proved to be especially prone to lipid oxidation development (Rustad, 2010), this leading to a wide range of negative effects on nutritional value (Undeland, 2016; Hes, 2017) and healthy concerns (Vieira *et al.*, 2017; Falade *et al.*, 2017). Therefore, previous research confirmed that an inhomogeneous distribution of lipid content and unsaturation degree in seafood may lead to a different damage development in the different parts of the product (Mørkøre *et al.*, 2002; Bienkiewicz *et al.*, 2013).

Flat fish species as flounders, soles, turbot, halibut and others make up important commercial products in many countries. Such species camouflage themselves on the ocean floor, showing a characteristic asymmetry with both eyes lying on the same side of the head in the adult specimens. Among such species, megrim (*Lepidorhombus*

whiffiagonis) is one of the most fished species in the Grand Sole North Atlantic Fishing bank and is exploited by a wide number of European countries. It can grow up to 60 cm in length, prefers a sandy or muddy sea floor, and eats small fish, as well as squid and crustaceans. In agreement with such economical interest, a wide range of studies have been carried out on this species. This accounts for optimization of on-board storage conditions (Pastoriza *et al.*, 2008), gelatine preparation from skin (Gómez-Guillén *et al.*, 2002), employment of new strategies for quality enhancement (Miranda *et al.*, 2016) and assessment of essential microelements content (Afonso *et al.*, 2013; Cardoso *et al.*, 2015). As being a flat species living in the sea bottom, megrim has also attracted a great attention for its content in toxic elements, such levels being taken as indicators of water pollution and contamination (Lavilla *et al.*, 2008; Storelli *et al.*, 2013).

The present work was focussed on the chemical composition of megrim muscle and its relationship with the nutritional value and health concerns in a further processed product. The primary objective was to investigate the constituents composition of the different edible parts of this fish species, with a special stress on the lipid (fatty acids and lipid classes) and the microelement (essential and toxic) compositions. Two different muscle zones (i.e., central and edge muscles) were differentiated and considered separately at each side of the fish body. To our knowledge, a comparative study concerning the chemical composition of both sides of a flat fish has not been carried out before.

MATERIALS AND METHODS

Fish material and sampling

Megrim specimens (n = 36; 235-300 g weight; 28-30 cm length) were captured in the North Atlantic Fishing bank of the Grand Sole, unloaded in Vigo harbour and

transported to our laboratory. From the time of capture until arrival at the laboratory (around 72 hours), the fish were stored in ice.

The fish were distributed into six groups (six individuals per group), which were considered independently in the statistical analysis ($n = 6$). In each individual fish, four different white muscle zones were taken into account. Thus, in the upper side of the fish, two different zones were considered (Figure 1, Panel a): i) the muscle zone placed in the body margin (upper edge muscle, UEM), and ii) the remaining muscle placed in the centre (upper central muscle, UCM). Similarly, two different muscle zones were considered in the lower side of the body fish (Figure 1, Panel b): LEM (lower edge muscle) and LCM (lower central muscle). In all cases, the absence of bones, blood and dark muscle was verified. Inside each of the six groups considered in the study and for each site taken into account, fish muscle of the different specimens was pooled together and submitted to different chemical analyses.

Solvents and chemical reagents used in this study were of reagent grade (Merck, Darmstadt, Germany); in the case of tocopherol analysis, solvents used were liquid chromatographic grade.

Proximate composition analysis

Moisture content was determined as the weight difference in the homogenised muscle (1-2 g) before and after 4 h at 105 °C (AOAC, 1990). Results were calculated as g water kg^{-1} muscle.

The total lipid fraction was extracted using the Bligh & Dyer (1959) method, which employs a single-phase solubilisation of the lipids using a chloroform-methanol (1:1) mixture. Results were calculated as g total lipids kg^{-1} muscle. Throughout the study, total lipid extracts were kept at -40 °C in a nitrogen atmosphere.

Protein content was measured using the Kjeldahl method (AOAC, 1990) with a conversion factor of 6.25. Results were calculated as g protein kg⁻¹ muscle.

Ash content was measured according to the AOAC (1990) method by heating the fish muscle at 550 °C. Results were calculated as g ash kg⁻¹ muscle.

Lipid classes analysis

Phospholipids (PL) were quantified by measuring the organic phosphorus in the total lipid extracts according to the Raheja *et al.* (1973) method, which is based on a complex formation with ammonium molybdate. Results were calculated as g PL kg⁻¹ muscle and as g PL kg⁻¹ total lipids.

To measure the triacylglycerol (TAG) content, the total lipid extracts were first purified on 20 x 20 cm thin-layer chromatography plates coated with a 0.5 mm-layer of silica gel G from Merck (Darmstadt, Germany) using a mixture of hexane-ethyl ether-acetic acid (90/10/1, v/v/v; two developments) as eluent (Álvarez *et al.*, 2009). Once the TAG fraction was purified, the method of Vioque & Holman (1962) was used to measure the ester linkage content according to the conversion of the esters into hydroxamic acids and subsequent complexation with Fe (III). Results were calculated as g TAG kg⁻¹ muscle and as g TAG kg⁻¹ total lipids.

Sterols (ST) were determined on total lipid extracts by the method of Huang *et al.* (1961) based on the Liebermann-Buchardt reaction. Results were calculated as g ST kg⁻¹ muscle and as g ST kg⁻¹ total lipids.

Free fatty acids (FFA) content of the total lipid extracts was determined following the Lowry & Tinsley (1976) method, which is based on the formation of a complex with cupric acetate-pyridine. In this study, benzene was replaced by toluene as

organic solvent. Results were calculated as g oleic acid kg⁻¹ muscle and as g oleic acid kg⁻¹ total lipids.

The content of tocopherol compounds (α -, β -, γ - and δ -) was determined in the different kinds of fish samples according to the method of Cabrini *et al.* (1992) with some modifications. For it, megrim muscle (1 g) was homogenised with distilled water (3 mL) in an Ultra Turrax homogeniser (twice for 90 s). The homogenate was then mixed with 80 mM SDS (4 mL), absolute ethanol (8 mL) and n-hexane (8 mL), being the mixture vortexed for 2 min. The separated aqueous phase was again extracted with n-hexane, being the organic extracts pooled together and evaporated under nitrogen flux. The residue was dissolved in methanol and injected for HPLC analysis in agreement with previous research (Cabrini *et al.*, 1992). The presence of different tocopherol compounds (α , β , γ and δ) was assessed. Only α -tocopherol was detected, being its content calculated with a calibration curve prepared from commercial α -tocopherol. Results were expressed as mg α -tocopherol kg⁻¹ muscle and as g α -tocopherol kg⁻¹ total lipids.

Fatty acid analysis

Lipid extracts were converted into fatty acid methyl esters (FAME) by using acetylchloride and then analysed by gas-liquid chromatography (Perkin-Elmer 8700 chromatograph, Madrid, Spain) according to an established procedure (Álvarez *et al.*, 2009). For this purpose, a fused silica capillary column SP-2330 (0.25 mm i.d. x 30 m, Supelco, Inc., Bellefonte, PA, USA) was employed and the temperature program was as follows: increased from 145 to 190 °C at 1.0 °C min⁻¹ and from 190 °C to 210 °C at 5.0 °C min⁻¹; held for 13.5 min at 210 °C. The carrier gas was nitrogen at 10 psig and detection was performed with a flame ionization detector at 250 °C. A programmed

temperature vaporizer injector was employed in the split mode (150:1) and was heated from 45 to 275 °C at 15 °C min⁻¹.

Peaks corresponding to FAME were identified by comparing their retention times with those of standard mixtures (Qualmix Fish, Larodan, Malmo, Sweden; FAME Mix, Supelco, Inc.). Peak areas were automatically integrated; C19:0 fatty acid was used as internal standard for quantitative purposes. Content of each fatty acid was expressed as mg kg⁻¹ muscle.

Analysis of essential and toxic microelements

Two groups of microelements were analysed. The first group incorporates five essential elements (Co, Cu, Mn, Se and Zn) which are present in all animal tissues (Johnson & Fischer, 1994; Prego *et al.*, 2012). The second group takes into consideration eight elements (Ag, As, Cd, Cr, Hg, Ni, Pb and V) usually referred to as toxic and susceptible to reflect an exogenous harmful influence related to environmental pollution (Engman & Jorhem, 1998; Noël *et al.*, 2011).

Samples were firstly dried in a stove at 50±5 °C until constant weight and later powdered in an agate ball-mill Fritsch Pulverisette type 06.102 (Idar-Oberstein, Germany). A fraction of 538±21 mg of each sample was weighed in a Teflon vessel of 55 mL and a mixture of 6 mL of 69 % HNO₃, 2 mL of 30 % H₂O₂ and 4 mL of Milli-Q water was added to carry out the microwave digestion. Then, fish samples were introduced into a Mars-X-Press CEM (Matthews, NC, USA) microwave oven plus four blanks and four samples of certified material reference for trace elements (DORM-2). Samples were completely digested. The digested solution was transferred from Teflon vessel to a flask of 50 mL volume; handling of samples was carried out inside a clean ISO 5 laminar flow cabinet (Cruma 670 FL, Barcelona, Spain).

In the digested samples, concentrations of Ag, As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, V and Zn were determined in the Analytical Service of the University of Vigo (CACTI, Vigo, Spain) using a quadrupole ICP-MS (Thermo Elemental, X-Series; Bremen, Germany) equipped with a Peltier Impact bead spray chamber and a concentric Meinhard nebuliser. The experimental parameters were: forward power of 1,200 W, peak jumping mode, 50 sweeps per replicate (5 replicates), dwell time of 10 ms, and dead time of 30 ns. Two procedural blanks were prepared using the analytical procedures and reagents, and included in each batch of 20 samples. Quantification was achieved by single mode, by applying corrections provided by the equipment software for polyatomic and isobaric interferences.

Procedural blanks always accounted for less than 1 % of element concentrations in the samples. Accuracy of the analytical procedures was ensured using certified reference material DORM-2, dogfish muscle prepared by the National Research Council of Canada, as the quality control material (Table 1). Quantification results are expressed as mg kg⁻¹ muscle.

Statistical analysis

Data (n = 6) obtained from the different chemical analyses were subjected to the ANOVA method (p<0.05) to investigate differences among the four different zones taken into account (Statsoft Inc., *Statistica*, version 6.0, 2001). Comparison of means was performed using a least-squares difference (LSD) method.

RESULTS AND DISCUSSION

Proximate composition analysis

Moisture content was included in the 750-797 g kg⁻¹ muscle range (Table 2), which can be considered similar to values already obtained for the current species (Pastoriza *et al.*, 2008), for other flat species (Aubourg *et al.*, 2007) and for lean fish species in general (Piclet, 1987). Both central muscles provided higher values than their corresponding edge samples; however, no differences could be observed as a result of the up/down location of the central or edge muscles. An inhomogeneous moisture distribution was also reported previously. Thus, Testi *et al.* (2006) found a higher moisture content in dorsal muscle than in the ventral one when studying European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*), and rainbow trout (*Oncorhynchus mykiss*). In the case of farmed fatty fish species (Nakamura *et al.*, 2007), cephalal ventral muscle showed lower moisture contents than other ordinary muscles such as cephalal dorsal, caudal dorsal and caudal ventral throughout the whole cycle time of bluefin tuna (*Thunnus orientalis*) cultivation. Concerning wild fatty fish species, belly flap muscle of albacore (*Thunnus alalunga*) showed a lower moisture content than back and ventral muscles (Aubourg *et al.*, 1991).

Lipid content in central muscles was included in the 9.0-10.4 g kg⁻¹ muscle range (Table 2), which can be considered similar to the values obtained for this species (Afonso *et al.*, 2013) and other lean fish species (Piclet, 1987). However, both edge zones provided markedly higher values (44-77 g kg⁻¹ muscle range), similar to values found in muscle tissues belonging to fatty fish species (Piclet, 1987). Additionally, a higher lipid content was obtained in the LEM site than in its corresponding muscle from the upper zone. Taking into account the marked effect that lipid oxidation may have on the loss of nutritional value (Undeland, 2016; Hes, 2017) and healthy concerns (Vieira

et al., 2017; Falade *et al.*, 2017), a great attention ought to be accorded to this damage pathway during megrim technological treatment in both edge sites.

An inhomogeneous fat distribution has already been found in other flat fish species (i.e., farmed and wild turbot; Aubourg *et al.*, 2007), so that the edge zone presented a higher lipid content. This zone distribution of lipid content has already been found in fatty fish species such as bluefin tuna (*Thunnus orientalis*; Nakamura *et al.*, 2007) and albacore tuna (*Thunnus alalunga*; Gallardo *et al.*, 1989). Furthermore, leaner fish species have also provided lower lipid levels in dorsal muscle than in the ventral one, such as European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*), and rainbow trout (*Oncorhynchus mykiss*) (Testi *et al.*, 2006). As a general rule, fish species (especially fatty fish) accumulate lipids in ventral regions and in muscle zones closely located to viscera in order to act as fat storage locations (Piclet, 1987).

Protein level in megrim muscle (165-187 g kg⁻¹ muscle range; Table 2) can be considered as expected for this species (Pastoriza *et al.*, 2008), as well as for teleostean fish in general (Piclet, 1987). Scarce differences could be outlined among the different zones considered, being only found a lower protein content in the LEM site when compared with any other location under study. Accordingly, and on the basis of the total protein content, a lower nutritional value would be accorded to this site. Regarding this difference, a higher protein content in dorsal muscle as compared with the ventral one was obtained in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) (Testi *et al.*, 2006). An inhomogeneous distribution of proteins content was also reported for angler (*Lophius piscatorius*; Prego *et al.*, 2012); thus, the tail muscle showed a higher protein content as compared with the head muscle.

Ash content for all zones considered was included in the 11-14 g kg⁻¹ muscle range (Table 2), in agreement with previous research on this species (Pastoriza *et al.*, 2008) and other lean fish species (Piclet, 1987). Comparison of both edge zones showed a higher content in the muscle corresponding to the upper side; additionally, higher mean values were obtained in upper sites when compared with their counterparts from the lower locations. A differential distribution was also observed by Testi *et al.* (2006) by showing a higher ash content in European sea bass (*Dicentrarchus labrax*) dorsal muscle than in the ventral one.

Analysis of lipid classes

PL content was included in a relatively straight range (Table 3). Differences among zones can be considered scarce, being only found a significantly higher content in the LCM site than in any other muscle place under study. An homogeneous distribution of PL content in different muscle zones has been described for wild and farmed species such as turbot (*Psetta maxima*; Aubourg *et al.*, 2007) and blackspot sea bream (*Pagellus bogaraveo*; Álvarez *et al.*, 2009). PL classes are known to be important constituents of cell membranes providing a structural role in living bodies; consequently, their content in muscle has hardly proved to be affected by internal or external factors (Gallardo *et al.*, 1989; Álvarez *et al.*, 2009). However, if the lipid basis is taken into account in the current study, a higher ($p<0.05$) PL proportion was found in the central muscles (290-329 g kg⁻¹ lipids range) than in the edge zones (37-70 g kg⁻¹ lipids range).

TAG showed to be the most abundant lipid class (Table 3). As being a typical depot lipid class, TAG have shown to be the most abundant lipid class in fatty fish species and in fish fatty tissues (Gallardo *et al.*, 1989). Consequently, a very different distribution apart from PL was obtained in the current study for this lipid class. Thus,

the muscle basis analysis showed a lower content in the central muscles than in the edge zones (Table 3). This result is in agreement with a direct relationship between TAG content and lipid content found in many fish species (Piclet, 1987). No differences could be detected as a result of the up/down localization for both central and edge muscles. Meantime, data analysis on the lipid basis led to a homogeneous distribution of TAG content among the four tissues under study (413-586 g kg⁻¹ lipids range), so that no significant differences (p>0.05) could be implied.

As for the PL group, the ST content in muscle was included in a relatively straight range (Table 3). As a general behaviour, the content of this lipid group proved to be markedly lower than for PL and TAG. Indeed, previous cholesterol assessment in total megrim muscle led to a 18-23 mg/100g muscle range (Afonso *et al.*, 2013), markedly higher than the level found in the current research for total sterols. Additionally, ST values obtained in the actual study can be considered low when compared with other wild flat (turbot, *Psetta maxima*; Aubourg *et al.*, 2007), lean (angler, *Lophius piscatorius*; Prego *et al.*, 2012), and marine species in general (Piclet, 1987). In the current research, both central and edge muscles showed higher values in the lower side of the fish; further, higher values were obtained in the edge zones than in their corresponding central muscle sites. If data are analysed on lipid basis, central muscles provided higher (36-38 g kg⁻¹ lipids range) values (p<0.05) than their corresponding edge sites (7-11 g kg⁻¹ lipids range).

The FFA class showed very low levels compared to the previously mentioned lipid classes (Table 3). Higher mean FFA values were obtained for the lower muscles when compared with their counterparts from the upper side; differences were found significant in the case of the edge zones. Additionally, no differences could be detected between central and edge muscles at both sides of the fish. If the lipid basis is taken into

account, the FFA content is found lower than the one previously mentioned for other lipid classes. A heterogeneous FFA distribution was then found since central muscles showed higher ($p < 0.05$) levels (8.6-12.7 g kg⁻¹ lipids range) than edge ones (1.9-2.6 g kg⁻¹ lipids). This result agrees with previous research where an inverse ratio between FFA content and lipid content had been described (Gallardo *et al.*, 1989; Aubourg *et al.*, 1991). Finally, no differences ($p > 0.05$) could be depicted in the current study by comparison of the up/down location when the lipid basis is taken into account.

The content of tocopherol compounds, well-known lipid-soluble antioxidants (Kamal-Eldin & Appelqvist, 1996), was also analysed in the present study. Different tocopherol compounds (α -, β -, γ - and δ -) have been identified in plants and in most seaweeds, although α -tocopherol has been reported to be the only one which accumulates in higher marine animals fed on natural diets (Sigurgisladóttir *et al.*, 2003). Accordingly, α -tocopherol was the only tocopherol detected in the current study, showing higher average values in the edge zones (12.1-14.1 mg kg⁻¹ muscle) than in the central sites (9.8-11.6 mg kg⁻¹ muscle). Furthermore, the up/down comparison did not provide a general trend since a higher mean value was obtained in the lower muscle for the central muscle, while a lower mean value was obtained in the lower muscle for the edge site. A lower α -tocopherol value has been reported by Afonso *et al.* (2008) when analysing total megrim muscle captured in Portuguese waters (6.6 $\pm$ 8.6 mg α -tocopherol kg⁻¹ muscle range). Current contents, however, can be considered similar to those reported for different fish species (Piclet, 1987; Ruff *et al.*, 2002). An inhomogeneous distribution of α -tocopherol content in muscle has been observed in other wild fish, such as turbot (*Psetta maxima*) (Aubourg *et al.*, 2007) and Atlantic halibut (*Hippoglossus hippoglossus*) (Ruff *et al.*, 2004); in such experiments, higher α -tocopherol values were reported in edible zones which had higher total lipid contents. However, no differences

in α -tocopherol content could be observed when considering muscle zones without marked lipid content differences as for angler (*Lophius piscatorius*; Prego *et al.*, 2012) and blackspot sea bream (*Pagellus bogaraveo*, Álvarez *et al.*, 2009).

If the lipid basis is taken into account in the present study, a marked inhomogeneous distribution of this antioxidant molecule is obtained, this leading to higher values ($p < 0.05$) for the central muscle locations than for the edge ones (Figure 2). Additionally, no differences could be implied as a result of comparing the upper and lower locations for both central and edge muscles. Taking into account the higher lipid content found in the edge zones than in the central ones, it can be concluded that protection against lipid oxidation provided by α -tocopherol will be lower in the edge zones during megrim processing.

Assessment of fatty acid composition

In all muscle zones under study, the following decreasing sequence for the three most abundant fatty acids was obtained (Table 4): C22:6 ω 3 > C16:0 > C18:1 ω 9. Other abundant fatty acids were: C14:0, C16:0, C16:1 ω 7, C20:1 ω 9, C22:1 ω 11 and C20:5 ω 3. This fatty acids distribution agrees with the one obtained for wild lean (turbot, *Psetta maxima*, Aubourg *et al.*, 2007; angler, *Lophius piscatorius*, Prego *et al.*, 2012) and fatty (*Euthynnus affinis*, *Sarda orientalis*, *Elagatis bipinnulata*, Saito *et al.*, 1999; bluefin tuna, *Thunnus orientalis*, Nakamura *et al.*, 2007) species as well as with other marine ones in general (Piclet, 1987). However, such distribution is markedly different from the one obtained for farmed blackspot sea bream (*Pagellus bogaraveo*; Álvarez *et al.*, 2009), in agreement with the high effect of diet on fatty acid composition.

A different distribution of fatty acids groups could be observed when comparing central and edge zones (Table 4). Thus, PUFA proved to be the most abundant group in

the central muscles (251-260 mg g⁻¹ lipids), while MUFA provided markedly highest scores (326-374 mg g⁻¹ lipids) in the edge zones. Previous research focussed on megrim (*Lepidorhombus whiffiagonis*) captured in the Portuguese coast showed MUFA to be the most abundant group (247-654 mg/100g total muscle) (Afonso *et al.*, 2013), while SFA and PUFA accounted for lower values (355±106 and 372±118 mg/100g total muscle ranges, respectively). A significant effect of the up/down location could not be concluded ($p>0.05$) both for the central and edge muscles in the current study.

Concerning nutritional and human health aspects, a great attention is being paid on items such as $\omega 3$ fatty acids content and $\omega 3/\omega 6$ ratio (Nakamura *et al.*, 2007). It has been proved that a majority of the Western population does not consume adequate levels of $\omega 3$ fatty acids through natural dietary sources. Thus, a great stress has been given to such values in foods included in human diet, in agreement with the great effect that has been reported for such ratio on the development of certain health problems (Uauy & Valenzuela, 2000; Komprda, 2012). Greater $\omega 3/\omega 6$ ratios and higher $\omega 3$ fatty acids proportions in lipid matter were obtained for the central muscles than for the edge sites (Table 4), while no differences could be depicted as a result of taking into account the side of the fish. In all cases, $\omega 3/\omega 6$ ratios markedly higher than the minimum recommended for the whole diet (near to 1/6; Simopoulos, 1994) were obtained. However, taking into account the lipid content in the different kinds of muscles under study (Table 2), a greater content of $\omega 3$ fatty acids is obtained in the edge muscles (8.42 and 14.77 mg $\omega 3$ fatty acids g⁻¹ muscle for UEM and LEM sites, respectively) than in central muscles (2.14 and 2.41 mg $\omega 3$ fatty acids g⁻¹ muscle for UCM and LCM sites, respectively).

Previous research carried out on total muscle of megrim (*Lepidorhombus whiffiagonis*) captured in the Portuguese coast showed an $\omega 3/\omega 6$ ratio included in the

6.67-7.14 range (Afonso *et al.*, 2013), similar to the edge muscle values obtained in the actual study and accordingly, lower than values obtained for both central muscles. Focussed on a flat species (turbot, *Psetta maxima*), Aubourg *et al.* (2007) showed that the edge zone included higher MUFA and lower PUFA values than the other muscle zones considered in the same study (i.e., ahead and back muscle zones). Contrary to this differential distribution, Nakamura *et al.* (2007) did not find differences in fatty acids groups composition when comparing different zones (dorsal/ventral and cephalal/caudal zones) of bluefin tuna (*Thunnus orientalis*).

Testi *et al.* (2006) studied differences between dorsal and ventral muscles from European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*) and rainbow trout (*Oncorhynchus mykiss*). An inhomogeneous distribution was obtained so that the ventral muscle of sea bass provided higher and lower contents for MUFA and PUFA, respectively; concerning rainbow trout, a higher MUFA content was obtained in the ventral muscle, while sea bream did not provide differences between both muscle zones considered. Additionally, a higher total $\omega 3$ content and $\omega 3/\omega 6$ value was obtained in dorsal muscle, while no differences were obtained for such parameters in sea bream and rainbow trout.

Microelements analysis

Although some mean values differences could be detected, the presence of essential elements did not provide significant ($p>0.05$) differences (Table 5), so that a homogeneous distribution could be concluded. Zn showed the highest values (13.1-15.9 mg kg⁻¹ muscle), while Co presence was found particularly low (0.010-0.012 mg kg⁻¹ muscle). Intermediate values (0.49-2.31 mg kg⁻¹ muscle) were obtained for Se, Mn and Cu.

As living in a mineral-rich medium, marine organisms accumulate microelements from the diet and the aquatic medium and incorporate them in their skeletal tissues and organs, so as to be considered a good source of essential elements (Piclet, 1987). Previous research has indicated that the concentration of microelements in fish muscle may be influenced not only by external factors (food source, environment), but also by anatomical and physiological aspects (Alasalvar *et al.*, 2002; Noël *et al.*, 2011). Among such elements, Cu, Se, Mn and Zn are contained in enzymes which protect cells against oxidant stress and therefore may be considered biological antioxidants (Johnson & Fischer, 1994); thus, Se has recently been reported to be a health-promoting ingredient in foods as being related to the activity of certain Se-containing proteins (Reilly, 1998).

Previous research on total megrim muscle has revealed lower Se content than in the present study. Thus, 0.41 ± 0.09 and 0.36 ± 0.09 mg kg⁻¹ muscle values were reported for megrim specimens obtained from the Portuguese coast by Afonso *et al.* (2008) and Cardoso *et al.* (2015), respectively; furthermore, a 1.10 ± 0.12 mg kg⁻¹ dry muscle range was obtained in specimens obtained in the Vigo (North-West Spain) harbour (Lavilla *et al.*, 2008). Lower values (mg kg⁻¹ muscle) than the current ones were also reported for Zn (3.4 ± 0.3), Cu (0.13 ± 0.03) and Mn (0.07 ± 0.03) in megrim captured in the Portuguese coasts (Afonso *et al.*, 2013).

Concerning the toxic elements (Table 5), the most abundant one was As, showing levels in the 17.5-24.7 mg kg⁻¹ muscle range, while trace levels could be detected for Ag and Cd in most cases. Intermediate levels (0.07-2.13 mg kg⁻¹ muscle range) were detected for the remaining elements (Cr, Hg, Ni, Pb and V). As content provided an inhomogeneous distribution, showing a higher level in central muscles than in their corresponding edge zones; additionally, the upper zones showed higher mean

scores than their corresponding lower zones. Some distribution differences were also depicted for Cr. Thus, higher mean values were obtained in the edge muscles when compared to their corresponding counterpart sites; differences were found significant when comparing the upper muscles. Concerning other toxic elements, central muscles provided higher mean values of Hg than their corresponding edge sites, while the opposite distribution was depicted for V. When the up/down locations are compared, general trends about elements distribution were scarce; thus, the most remarkable result to be pointed out would be the higher mean value of Hg content in lower muscles.

Marine species have been reported to be good bioindicators of trace element contamination in the marine environment because they occupy different trophic levels and can exhibit large bioaccumulation factors (Engman & Jorhem, 1998; Noël *et al.*, 2011). Thus, uneven distribution of toxic elements in the muscle may depend, as for essential microelements, on internal and external factors (Phillips & Rainbow, 1993; Engman & Jorhem, 1998). According to the Commission Regulation (EC, 2006), present results on Pb content would overpass the recommended limits (i.e., 0.30 mg kg⁻¹ wet weight), while Hg content could be considered in the limit of acceptability (i.e., 1.00 mg kg⁻¹ wet weight). Concerning As content, no limit recommendation is available actually for total As in seafood. However, since toxicity has mostly been related to inorganic arsenical compounds, the Joint Expert Committee of the FAO-WHO (1983) recommended a maximum acceptable daily intake of inorganic As for humans of 2 µg kg⁻¹ of body weight. Since present As results correspond to total As, it is not possible to settle the acceptability levels of the current megrim samples. Because of the great importance of the Grand Sole bank for most West-European countries, further analyses ought to be carried out in order to provide a wider knowledge of the levels of such toxic

elements in their different oxidation states and molecular forms in megrim and other fish species captured in this bank.

As being a flat fish living in the sea bottom, a great attention has previously been paid to toxic elements presence in megrim muscle. Different studies account for As assessment, being all values reported lower than the current ones; such reports account for specimens from the Portuguese coast ($11.6 \pm 4.74 \text{ mg kg}^{-1}$ muscle; Anacleto *et al.*, 2009), and from specimens acquired in Galician (North-West Spain) ($42.5 \pm 1.3 \text{ mg kg}^{-1}$ dry muscle; Lavilla *et al.*, 2008) and Basque (North Spain) ($28.45 \pm 17.71 \text{ mg kg}^{-1}$ dry weight; Muñoz *et al.*, 2000) harbours. In most cases, lower values have also been reported for Hg content in megrim muscle; thus, specimens obtained in the Portuguese coast showed 0.33 ± 0.24 (Afonso *et al.*, 2008) and 0.36 ± 0.18 (Cardoso *et al.*, 2015) mg kg^{-1} muscle levels, while megrim from the Adriatic Sea led to slightly lower levels than in the present study ($0.68 \pm 0.21 \text{ mg kg}^{-1}$ muscle; Storelli *et al.*, 2013). Concerning Pb detection, previous research accounts for lower values than in the actual study, too; these reports take into account specimens from the Portuguese ($0.033 \pm 0.008 \text{ mg kg}^{-1}$ muscle; Afonso *et al.*, 2013) and Adriatic ($0.08 \pm 0.04 \text{ mg kg}^{-1}$ muscle; Storelli *et al.*, 2013) coasts.

On the contrary, previous research accounts for higher contents on some other elements when compared to the present ones; this accounts for Ni ($3.24 \pm 0.15 \text{ mg kg}^{-1}$ dry muscle) and V ($1.25 \pm 0.08 \text{ mg kg}^{-1}$ dry muscle) measured in megrim specimens obtained in Vigo harbour (Lavilla *et al.*, 2008). Finally, previous reports on Cd content focussed on fish captured in the Portuguese ($0.002 \pm 0.001 \text{ mg kg}^{-1}$ muscle; Cardoso *et al.*, 2015) and Adriatic ($0.05 \pm 0.02 \text{ mg kg}^{-1}$ muscle) coast revealed similar levels to the current research.

CONCLUSIONS

An inhomogeneous distribution of most chemical constituents in the different megrim muscle sites was obtained. As a result, differences were found more important by central/edge muscles comparison than in the case of taking into account the side of the fish.

Concerning lipid composition and its possible damage during megrim processing, marked differences have been observed between central and edge zones. Thus, both edge muscles (upper and lower) have shown a greater lipid content, but lower α -tocopherol contents. However, edge muscles have proved a higher $\omega 3$ fatty acids content, this leading to higher C20:5 $\omega 3$ (eicosapentaenoic acid, EPA) and C22:6 $\omega 3$ (docosahexaenoic acid, DHA contents (average values of 3.1 and 7.3 g kg⁻¹ fish muscle, respectively) when compared to central muscles (average values of 0.5 and 1.7 g kg⁻¹ fish muscle, respectively).

Related to the analysis of the toxic elements, values exceeding the recommended levels for Pb were obtained, while values corresponding to Hg could be considered in the limit of acceptability. Concerning As content, analysis of inorganic As ought to be carried out to settle the acceptability of fish. In agreement with the great importance of the Grand Sole bank for most West-European countries, further compositional analyses ought to be focussed on such microelements in their different oxidation states and molecular forms in order to guarantee the safety level of megrim captured in this bank.

ACKNOWLEDGEMENTS

RG Barbosa acknowledges Instituto Federal de Santa Catarina (SC, Brazil) for financial support. Authors thank BSc. Susana Calvo (IIM-CSIC) for her help in microwave digestions and Dr. Jorge Millos (University of Vigo) for carrying out the ICP-MS analysis. This work was supported by the Consejo Superior de Investigaciones Científicas (CSIC; Research Project 2017-70E032) and the “Xunta de Galicia” (“Support to Competitive Reference Groups” program; Ref. IN607A-2016/11).

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FIGURE LEGENDS

Figure 1: Location of the different muscle sites considered: Panel a (upper central muscle, UCM; upper edge muscle, UEM) and Panel b (lower central muscle, LCM; lower edge muscle, LEM)

Figure 2: Content* of α -tocopherol in different muscle sites of megrim**

* Mean values (g kg^{-1} lipids) of six replicates ($n=6$). Standard deviations are indicated by bars. Mean values accompanied by different letters express significant differences ($p<0.05$) among muscle sites.

** Muscle sites abbreviations: UCM (upper central muscle), UEM (upper edge muscle), LCM (lower central muscle) and LEM (lower edge muscle).

TABLE 1: Analysis results* of certified reference material (CRM)**

Microelement	Concentration	
	Certified	Measured
Ag	0.041 (0.013)	0.040 (0.004)
As	18.0 (1.1)	17.9 (3.2)
Cd	0.043 (0.008)	0.048 (0.003)
Co	0.182 (0.031)	0.162 (0.040)
Cr	34.7 (5.5)	30.5 (2.3)
Cu	2.34 (0.16)	2.44 (0.26)
Hg	4.46 (0.26)	4.47 (0.75)
Mn	3.66 (0.34)	3.20 (0.61)
Ni	19.4 (3.1)	17.5 (1.0)
Pb	0.065 (0.007)	0.058 (0.010)
Se	1.40 (0.09)	1.50 (0.11)
V	NC***	0.21 (0.05)
Zn	25.6 (2.3)	28.0 (1.1)

* Mean values (mg kg⁻¹ muscle) of four replicates (n=4). Standard deviations are indicated in brackets.

** CRM consisted on fish muscle material (DORM-2) from the National Research Council of Canada (NRCC).

*** NC: not certified.

TABLE 2: Proximate composition* of different muscle sites of megrim**

Constituent	Muscle site			
	UCM	LCM	UEM	LEM
Moisture	792.7 b (7.2)	796.2 b (5.9)	762.5 a (25.2)	750.2 a (14.7)
Lipids	9.0 a (1.2)	10.4 a (1.9)	44.5 b (21.6)	76.2 c (24.1)
Proteins	186.3 b (6.8)	182.0 b (5.3)	181.7 b (6.2)	165.8 a (11.7)
Ash	12.7 ab (0.6)	12.1 a (0.4)	13.4 b (0.7)	11.7 a (1.1)

* Mean values (g kg^{-1} muscle) of six replicates ($n=6$). Standard deviations are indicated in brackets. For each constituent, mean values followed by different letters express significant differences ($p<0.05$) among muscle sites.

** Muscle sites abbreviations: UCM (upper central muscle), LCM (lower central muscle), UEM (upper edge muscle) and LEM (lower edge muscle).

TABLE 3: Content* of lipid classes and groups in different muscle sites of megrim**

Lipid group/class	Muscle site			
	UCM	LCM	UEM	LEM
PL	0.257 a (0.012)	0.320 b (0.019)	0.248 a (0.019)	0.258 a (0.015)
TAG	0.459 a (0.132)	0.446 a (0.151)	2.656 b (0.419)	2.906 b (0.188)
ST	0.033 a (0.002)	0.038 b (0.001)	0.040 b (0.003)	0.056 c (0.005)
FFA	0.008 a (0.003)	0.013 ab (0.004)	0.008 a (0.007)	0.019 b (0.010)

* Mean values (g kg^{-1} muscle) of six replicates ($n=6$). Standard deviations are indicated in brackets. For each lipid group or class, mean values followed by different letters express significant differences ($p<0.05$) among muscle sites.

** Muscle sites abbreviations as expressed in Table 2. Lipid groups and classes: PL (phospholipids), TAG (triacylglycerides), ST (sterols) and FFA (free fatty acids).

TABLE 4: Content* on individual fatty acids, fatty acids groups and $\omega 3/\omega 6$ ratio in different muscle sites of megrim**

Fatty acid	Muscle site			
	UCM	LCM	UEM	LEM
14:0	37.31 (5.71)	35.21 (6.66)	55.76 (10.97)	62.04 (10.86)
15:0	3.73 (0.32)	3.62 (0.25)	4.79 (0.39)	5.48 (0.31)
16:0	130.76 (5.78)	122.43 (3.01)	119.58 (5.40)	128.24 (9.21)
17:0	8.02 (1.02)	7.84 (0.57)	8.93 (1.28)	10.10 (1.01)
18:0	20.46 (2.85)	19.68 (2.00)	18.00 (2.84)	19.91 (2.12)
Total SFA	200.28 a (7.40)	188.79 a (7.05)	207.06 ab (13.32)	225.78 b (17.99)
16:1 ω 7	38.21 (5.55)	36.52 (6.56)	58.76 (10.58)	64.25 (9.91)
18:1 ω 9	83.39 (9.79)	78.11 (6.80)	96.45 (8.66)	108.99 (7.63)
18:1 ω 7	17.83 (2.09)	18.05 (1.03)	22.58 (1.50)	25.73 (1.49)
20:1 ω 9	41.03 (6.28)	40.35 (6.52)	64.17 (10.02)	75.25 (10.52)
22:1 ω 11	46.99 (8.97)	45.93 (9.26)	79.47 (14.46)	93.11 (15.14)
24:1 ω 9	5.19 (0.37)	5.21 (0.32)	5.23 (0.52)	6.11 (0.50)
Total MUFA	232.64 a (16.26)	224.17 a (25.50)	326.66 b (36.38)	373.44 b (33.39)
18:2 ω 6	8.97 (2.02)	7.59 (0.94)	9.54 (1.30)	10.40 (1.50)
20:2 ω 6	2.63 (0.26)	2.56 (0.28)	3.47 (0.29)	3.91 (0.39)
20:4 ω 6	5.74 (1.56)	5.77 (0.86)	4.92 (1.07)	5.83 (0.91)
20:5 ω 3	47.08 (5.88)	45.92 (4.90)	50.44 (8.26)	51.11 (8.97)
22:4 ω 6	4.19 (0.56)	4.05 (0.17)	4.67 (0.52)	5.36 (0.19)
22:5 ω 3	17.63 (1.66)	17.66 (1.27)	18.09 (1.69)	19.89 (1.28)
22:6 ω 3	173.35 (11.59)	167.89 (9.27)	120.65 (10.20)	122.79 (4.88)
Total PUFA	259.58 b (14.17)	251.43 b (7.75)	211.77 a (10.39)	219.30 a (12.14)
Total ω 3	238.06 b (15.24)	231.47 b (7.18)	189.17 a (10.26)	193.89 a (10.85)
Total $\omega 3$ /Total $\omega 6$ ratio	11.23 b (1.69)	11.51 b (0.51)	8.40 a (0.72)	7.61 a (0.29)

* Mean values (mg g⁻¹ lipids) of six replicates (n=6). Standard deviations are indicated in brackets. For each fatty acid group and $\omega 3/\omega 6$ ratio, mean values followed by different letters express significant differences (p<0.05) among muscle sites.

** Muscle sites abbreviations as expressed in Table 2. Fatty acids groups: SFA (saturated fatty acids), MUFA (monounsaturated fatty acids) and PUFA (polyunsaturated fatty acids).

TABLE 5: Microelements contents* in different muscle sites of megrim**

Essential/toxic microelement	Muscle Site			
	UCM	LCM	UEM	LEM
Co	0.012 a (0.006)	0.008 a (0.003)	0.010 a (0.004)	0.010 a (0.008)
Cu	1.86 a (0.41)	2.13 a (1.11)	2.21 a (0.97)	2.31 a (1.18)
Mn	1.05 a (0.93)	0.49 a (0.23)	0.49 a (0.15)	1.00 a (1.20)
Se	2.25 a (0.43)	2.14 a (0.55)	2.01 a (0.79)	1.52 a (0.40)
Zn	13.64 a (0.74)	15.03 a (0.89)	13.15 a (1.77)	15.83 a (2.68)
Ag	< 0.001			
As	24.67 c (2.30)	22.75 bc (2.06)	19.02 ab (2.53)	17.51 a (2.97)
Cd	< 0.005	0.01 a (0.00)	< 0.005	0.01 a (0.00)
Cr	0.86 a (0.09)	1.43 ab (0.62)	2.13 b (1.05)	1.99 ab (0.73)
Hg	1.02 a (0.47)	1.13 a (0.72)	0.70 a (0.32)	0.78 a (0.44)
Ni	0.07 a (0.58)	0.52 a (0.16)	0.17 a (0.03)	0.11 a (0.79)
Pb	1.24 a (1.41)	0.63 a (0.61)	0.44 a (0.345)	1.60 a (3.85)
V	0.11 a (0.04)	0.12 a (0.04)	0.18 a (0.08)	0.18 a (0.06)

* Mean values (mg kg⁻¹ muscle) of six replicates (n=6). Standard deviations are indicated in brackets. For each microelement, mean values followed by different letters express significant differences (p<0.05) among muscle sites.

** Muscle sites abbreviations as expressed in Table 2.

Figure 2

Figure 1

a) Upper side

b) Lower side