DISTRIBUTION OF LIPIDS AND TRACE MINERALS IN DIFFERENT MUSCLE SITES OF FARMED AND WILD TURBOT (*Psetta maxima*)

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SUMMARY

Lipid and trace mineral composition were studied in different sites of the edible flesh of farmed and wild turbot (*Psetta maxima*). Lipid matter (total content, sterols, tocopherols) showed to accumulate in the edge zone, except for phospholipids (PL) that provided a distribution that was found to be independent from the kind of turbot and the zone considered. Fatty acid composition of total lipids showed a non-homogeneous distribution, since the edge zone exhibited a different fatty acid group composition (higher monounsaturated and lower polyunsaturated and ω3/ω6 ratio values) than the other zones considered; less differences were observed by considering the PL fatty acid composition. Most minerals (Ca, Cu, Fe, Mn and Se) studied showed to be homogeneously distributed along the muscle sites of the wild fish, while more differences were obtained when considering the farmed one. For both kinds of turbots, the most important difference was obtained in the case of Zn, since a largely higher content in the edge zone than in the other zones was detected. A close relationship between Zn and total lipid contents (r^2=0.90 and r^2=0.76 for farmed and wild turbots, respectively) was observed.

Keywords: Turbot, muscle sites, farmed, wild, lipids, minerals

Running title: Distribution of lipids and minerals in turbot

INTRODUCTION

Turbot (*Psetta maxima,* also known as *Scophthalmus maximus)* is a flat fish species of high commercial value found in North Europe waters (North Sea, specially) widely appreciated for its firm, white, and flavourful flesh (FAO, 2004a), whose consumption undergoes a marked decrease because of its low availability and high cost. In recent years, the increasing production of this species as an aquaculture product (FAO, 2004b) has made it more available to consumers. Previous work has been carried out on the effects of diet on turbot growth (Danielssen & Hjertnes, 1993; Regost *et al*., 2001), the development of tools for the identification of turbot with respect to other fish species (Prost *et al*., 1998; Etienne *et al*., 2000), comparison between wild and farmed composition (Sheehan *et al*., 1994; Sérot *et al*., 1998) and quality loss during processing (Chevalier *et al*., 2001; Ruff *et al*., 2002; Aubourg *et al*., 2005).

Seafood products are known to provide high contents of important constituents for the human diet (Piclet, 1987; Simopoulos, 1997). The lipid fraction is now the subject of a great deal of attention due to its high content on ω3 polyunsaturated fatty acids, which have shown potential benefits to human health, particularly in the prevention of cardiovascular diseases (Illingworth & Ullmann, 1990; Ackman & Ratnayake, 1990). Marine organisms have shown to accumulate minerals from the diet and deposit them in their skeletal tissues and organs (Lal, 1989), so as to be considered a good source of essential minerals (Piclet, 1987; Alasalvar *et al*., 2002).

Fish constituents have been shown to be inhomogeneously distributed along the body of a fish (Pearson *et al*., 1977; Aubourg *et al*., 1989). Previous research has shown wide differences in lipid content and composition according to the body zone considered in fatty (Gallardo *et al*., 1989; Body & Vlieg, 1989) and lean (Ingemansson *et al*., 1991; Aubourg *et al*., 1999) fish. Further, several studies have indicated that the concentration of trace minerals in fish is influenced not only by seasonal factors (food source, environment) but also by biological aspects (anatomical, physiological) (Bodsha & Sainsbyry, 1978; Farmer *et al*., 1979; Lal, 1995; Alasalvar *et al*., 2002).

The present work was focused on the lipid and main trace mineral compositions. Its objective was to identify elements of differentiation that characterise wild and farmed turbot. Such differentiation is considered important if cultured turbot is to be used as a replacement for the wild one in the seafood European marketing system. Different edible sites of the turbot body were considered for the present study, according to a non-homogeneous distribution of constituents in the fish body.

MATERIALS AND METHODS

Fish material and sampling

Wild turbot (*Psetta maxima)* specimens (n=6) were caught (June, 2003) near the Galician Atlantic coast and obtained in a local market 10 h after being caught. From catching till arrival to laboratory the fish were kept in ice.

Two-year old farmed turbot specimens (n=6) were obtained from Stolt Sea Farm, S. A. (Carnota, Spain). The feed employed contained 54 % protein, 20 % fat, 8 % carbohydrate, 9 % ash and 9 % moisture. Fish specimens were sacrificed in a water-ice mixture and then kept in ice for 10 h until they arrived at our laboratory.

For both kinds of fish specimens, the length was in the 40-45 cm range, while the width was in the 30-34 cm range; the weight was 1.6-1.8 kg. The white muscle corresponding to the turbot eyed side was considered for the present study and divided into the three following sites (Figure 1): i) the muscle zone placed in the body margin (edge zone, EZ); ii) the muscle zone placed between the head and the end of viscera (ahead zone, AZ); and iii) the muscle zone placed between the end of viscera and the tail (back zone, BZ). Chemical analyses were carried out separately on each of the selected zones. Each individual fish was studied independently (n=6) in order to carry out the statistical analysis.

Water and lipid contents

Water content was determined by weight difference of the homogenised muscle (1-2 g) before and after 24 h at 105 ºC; the results were calculated as g water kg^{-1} muscle. The lipid fraction was extracted by the Bligh & Dyer (1959) method. Quantification results were calculated as g lipid kg^{-1} wet muscle.

Lipid analyses

The phospholipid (PL) fraction was purified from the total lipid extract according to the Aubourg *et al*. (1996) procedure. Total lipid extract and PL fraction were converted into fatty acid methyl esters (FAME) according to the Lepage & Roy (1986) method. FAME were analysed by GC (Perkin-Elmer 8700 chromatograph) employing a fused silica capillary column SP-2330 (0.25 mm i.d. x 30 m, Supelco, Inc., Bellefonte, Pa, USA) according to Aubourg *et al*. (1996). Content of each fatty acid is expressed as g/ 100g total FAME.

Total PL were quantified by measuring the organic phosphorus on total lipid extracts according to the Raheja *et al*. (1973) method based on a complex formation with ammonium molybdate. Results are expressed as g kg^{-1} wet fish tissue.

Total sterols were determined on total lipid extracts by the method of Huang *et al*. (1961) based on the Liebermann-Buchardt reaction. Results are expressed as g kg^{-1} wet fish tissue.

Tocopherol isomers were analysed according to the Cabrini *et al*. (1992) method. The presence of the different tocopherol isomers was checked. Only the α-tocopherol isomer was detected, and its content was expressed as mg kg^{-1} wet fish muscle.

Trace mineral analysis

Seven essential minerals (Ca, Cu, Fe, Mg, Mn, Se and Zn) were chosen for the present study (Sheehan *et al*., 1994; Alasalvar *et al*., 2002). Edible flesh samples were dried in a stove at 50º C until constant weight and later ground in a mortar. Then, a fraction of 300-400 mg of each sample was weighed in a Teflon vessel of 40 ml and a mixture of 4 ml of 65 % HNO_3 and 1 ml of H_2O_2 was added to carry out the microwave digestion. To be digested, the different muscle mixtures were introduced in a Milestone 1200 Mega microwave grouped in series of seven samples plus one blank and one certified material reference (DORM-2, National Research Council Canada) to verify the correct sample dissolution (Table 1).

Mineral contents of the digested samples were determined by atomic absorption. Ca, Fe, Mg and Zn were analysed by means of flame atomic absorption spectrometry (FAAS) using a Varian 220 FS apparatus. Cu, Mn and Se by means of electrothermal atomic absorption spectrometry (ETAAS) using a Varian 220 apparatus equipped with Zeeman background correction. Analyses were carried out in triplicate. Quantification results are expressed as mg kg^{-1} wet flesh muscle, except for Ca and Mg (g kg^{-1} wet flesh muscle).

Statistical analysis

Data from the different chemical measurements were subjected to the ANOVA one-way method ($p<0.05$) to explore differences among the different edible flesh sites considered and between wild and farmed turbot (Statsoft, 1994); comparison of means was performed using a least-squares difference (LSD) method. Correlation values between lipid and mineral contents were also analysed.

RESULTS AND DISCUSSION

Water and lipid contents

Moisture results are given in Figure 2. Water content did not provide significant ($p>0.05$) differences among the three sites considered under study for the wild turbot. However, in the case of the farmed fish a lower ($p<0.05$) content was obtained for the edge zone when compared to the two others, while no differences ($p>0.05$) were obtained between the ahead and back zones. Comparison between both kinds of turbots led to a lower ($p<0.05$) water content for each zone of farmed fish when compared to its counterpart site in wild fish.

Lipid contents are shown in Figure 3. Comparison among the three sites under study provided a higher ($p<0.05$) lipid content in the edge zone than in the two others, while no differences ($p>0.05$) were observed between the ahead and back zones; these conclusions were found valid for farmed and wild fish. Comparison between both kinds of turbots led to a higher ($p<0.05$) lipid content for the farmed one in the edge zone, when compared to its

counterpart in the wild fish; no differences ($p>0.05$) could be assessed in the two other sites between both turbots.

An inhomogeneous fat distribution has been found for turbot, in the sense that the edge zone would play the role of acting as a fat depot zone (Pearson *et al*., 1977) according to a similar behaviour observed in other kind of fish species (Ke *et al*., 1977; Aubourg *et al*., 1989). In the present experiment, this accumulation was specially great in the case of the farmed fish.

In previous research (Sheehan *et al*., 1994; Sérot *et al*., 1998; Grigorakis *et al*., 2002; Orban *et al*., 2003), where a differential zone study had not been carried out, a higher lipid content in farmed than in wild fish was observed; in such cases, the diet was identified as the main reason for this difference (Bell *et al*., 1985; Robin *et al*., 2003) and the higher lipid content was followed by a lower water content, according to a known inverse ratio between water and lipid constituents (Piclet, 1987).

Fatty acid analysis

Fatty acid composition of total lipids and PL was studied in the different chosen sites of farmed and wild turbot; results are included in Tables 2-3. In all cases, the most abundant fatty acids were 22:6ω3, 16:0 and 20:5ω3. Discussion concerning distribution differences among the different sites and between farmed and wild fish is now focused to fatty acid group contents and fatty acid ratio values.

The fatty acid study on total lipids (Table 2) showed a higher content in monounsaturated fatty acids (MUFA) and a lower content in polyunsaturated fatty acids (PUFA) for the edge zone when compared to the two other zones, being both conclusions valid for the farmed and wild fish. Comparison between both kinds of turbots led to lower

contents in saturated fatty acids (SFA) (except for the edge zone) and MUFA and higher in PUFA for the different sites of wild fish when compared to their counterpart sites in the farmed one; results on MUFA and PUFA agree to previous research carried out on different fish species where no zonal differences had been carried out (Sérot *et al*., 1998; Grigorakis *et al*., 2002; Alasalvar *et al*., 2002).

When considering the fatty acid composition of the total PL (Table 3), a more homogeneous composition among sites was obtained than in the case of total lipids, so that less differences were obtained. Thus, a higher SFA content and a lower PUFA value for the edge zone than in the back zone is to be mentioned for the farmed fish; in the case of the wild fish, only a higher SFA content was detected for the ahead zone when compared to the two other sites. PL is a lipid group known to develop a structural role in living bodies, so that its composition is known to reflect less variations as a result of internal (anatomic, physiological and other aspects) and external (diet, water temperature) factors (Pearson *et al*., 1977; Sérot *et al*., 1998).

A majority of the Western population does not consume adequate levels of ω3 fatty acids through natural dietary sources, such as fish. In this sense, a great interest has been accorded recently to the ω3/ω6 ratio of foods included in human diet. Its value has shown a great effect on the development of certain health problems (Illingworth & Ullmann, 1990; Weber, 1992), being the recommended ratio for the whole diet near to 1:6 (ω3:ω6) (Simopoulos, 1994). In the present work, the site comparison showed a lower ω3/ω6 ratio in the edge zone when compared to the two others for the total lipid in farmed turbot, while no differences were obtained for the wild fish. Comparison between both kinds of turbots led to a higher ratio for the wild fish in each zone when compared to each counterpart zone in farmed turbot, according to previous research where no site distribution had been

considered (Sheehan *et al*., 1994; Sérot *et al*., 1998; Alasalvar *et al*., 2002; Grigorakis *et al*., 2002). The PL study did not provide differences ($p>0.05$) for the ω3/ω6 ratio as a result of the site distribution or kind of turbot. It is concluded that both cultivated and wild turbots provide a profitable ω3/ω6 ratio in order to maintain the recommended value for the whole human diet. However, a better value ($p<0.05$) is obtained for the wild fish.

According to the interest on the ω3 series fatty acids, the 22:6ω3 / 20:5ω3 ratio distribution in the different sites and kinds of turbots was studied. The content ratio showed a lower value in the edge zone than in the two other zones for the farmed and wild total fatty acids, while no site differences were obtained when considering the PL fraction. Comparison between both kinds of turbots led to lower values for farmed fish in total and PL fractions, according to previous research where no site distribution study had been attained (Sheehan *et al*., 1994; Alasalvar *et al*., 2002).

Lipid group analysis

Total PL contents obtained (Figure 4) are included in the range 1.7-2.2 g kg^{-1} wet flesh muscle. Results did not provide differences ($p>0.05$) among zones or by comparing both kinds of turbots. PL are known to be an important constituent of cell membranes and, although its content and composition has shown to be altered by the diet (Igene, 1976), the changes are relatively small as compared to those of depot fats (Pearson *et al*., 1977). This homogeneous distribution of PL content in different sites of fish flesh muscle agrees to previous research carried out on fatty fish (Aubourg *et al*., 1989) and to the above mentioned results on fatty acid composition.

Figure 5 shows the results obtained for total sterols. Values in the range 0.2-1.1 g kg^{-1} wet flesh muscle were obtained, that agree to common values for lean fish species

(Piclet, 1987). The site distribution study showed a higher ($p<0.05$) content in the edge zone when compared to the two other zones for both kinds of turbots. This result agrees to previous research on albacore fish where a higher total sterol content was obtained in the lipid depot site (Aubourg *et al*., 1989). Comparison between both farmed and wild fish did not provide differences ($p>0.05$) for the ahead and back zones, but a higher ($p<0.05$) content in the edge zone of the farmed fish was obtained when compared to its counterpart site in the wild fish, according to the results obtained for the total lipid contents (Figure 3). Orban *et al*. (2003) obtained no differences for cholesterol content when comparing wild and farmed sea bass and gilthead sea bream; however, a site distribution study was not encountered in such experiment. Present results on total sterols show an inhomogeneous distribution of this lipid group, so that its accumulation is carried out in the edge zone, and this behaviour is particularly strong in the farmed fish.

Alpha-tocopherol contents obtained are shown in Figure 6. The values were within range reported for different fish species (Piclet, 1987; Ruff *et al*., 2002; Aubourg *et al*., 2004). Farmed fish showed an inhomogeneous distribution for this endogenous antioxidant. Thus, a higher ($p<0.05$) content in the edge zone was obtained when compared to the two other zones under study; no differences ($p>0.05$) were obtained between the ahead and back zones. Wild fish provided a higher mean value for the edge zone; however, differences were not significant ($p>0.05$) among the three sites under study. When both kinds of turbots are compared, no differences ($p>0.05$) in the edge zone were obtained, while the ahead and back zones in wild turbot showed higher ($p<0.05$) levels than their counterparts in farmed turbot. Alpha-tocopherol is a known lipid-soluble chain- breaking antioxidant, whose main role is protecting the polyunsaturated fatty acids from oxidation (Kamal-Eldin & Appelqvist, 1996; Kulås & Ackman, 2001). Its higher presence in the edge

zone agrees to the fact that this zone provides the highest lipid content. If the alpha-tocopherol/total lipid ratio is considered, a higher ($p<0.05$) value is observed in each zone of the wild turbot when compared to its corresponding site in the farmed one. This difference, in addition to the one found for alpha-tocopherol content in ahead and back sites could be explained as a result of a different diet intake between both kinds of turbots.

Mineral analysis

Trace mineral contents obtained are expressed in Table 4. Levels obtained for Ca and Mg were higher than in the five remaining minerals according to common distribution in marine species flesh (Piclet, 1987). Expected values were obtained for most minerals (Ca, Mg, Mn, Se and Zn) and low for Fe and Cu, when compared to previous research (Piclet, 1987; Engman & Jorhem, 1998). The relative low value of Fe could be explained as a result of discarding the dark muscle in the present study (Alasalvar *et al*., 2002).

The Ca content revealed an homogeneous distribution among the three sites studied. However, comparison between both kinds of turbots showed a higher content in the farmed one for the back zone.

The Cu distribution did not show differences as a result of the flesh site considered. However, comparison between wild and farmed turbot provided a higher value in the three zones for the farmed one when compared to their counterpart in wild fish.

Large variations from fish to fish were obtained when considering the Fe presence. The farmed fish showed a higher content in the edge zone than in the back zone for this mineral; no site differences could be observed for the wild turbot. Comparison between both kinds of fish did not provide differences at any of the sites studied.

The Mg content showed to be higher in the ahead zone than in the edge zone in both turbots. Comparison between both kinds of fish led to higher values for the farmed one in the ahead and back zones.

A higher Mn content was observed in the edge zone than in the ahead zone for the farmed turbot; no site differences were obtained for the wild fish. Comparison between both turbots showed a higher content in the farmed one in the three sites studied when compared to their counterparts in wild fish.

The Se content provided an homogeneous distribution among the different sites. Also, no differences could be outlined by comparing both kinds of turbots.

A higher Zn content was obtained in the edge zone than in the two other zones for both kinds of turbots. Farmed turbot showed a higher Zn content than the wild one when comparing each site to its counterpart in the wild fish.

Mineral elements corresponding to the transition and electronegative elements from the Periodic Table have been reported to be strongly bound to other constituents (Piclet, 1987; Gordon, 1988). Accordingly, the relationship between mineral presence and lipid content was investigated in the present work. For it, correlation values between fat and mineral contents were studied. Farmed fish showed a good correlation value for Zn (r^2 = 0.90), and fair in the cases of Fe, Mg and Mn (r^2 = 0.71, r^2 = –0.76 and r^2 = 0.78, respectively); poor correlation values were obtained in the case of considering the PL content instead of the total lipid content. For wild fish, fair correlation values were obtained for total lipid content and presence of Cu and Zn (r^2=0.77 and r^2=0.76, respectively); again, unfair correlations were obtained in the case of considering the PL content.

Content relationship between water and mineral elements was also checked. Farmed fish showed a good correlation for Zn (r^2 = –0.85), and fair in the cases of Fe, Mg and Mn

(r^2 = –0.67, r^2 = 0.76 and r^2 = –0.69, respectively). These results agree to the above mentioned comparison to lipid content, and can be explained as a result of the already mentioned inverse ratio between water and lipid contents (Piclet, 1987). For wild turbot, poor correlation values were obtained between water and mineral elements.

CONCLUDING REMARKS

A non-homogeneous distribution of lipid composition among the different sites considered was observed for turbot. Thus, the edge zone showed a higher lipid content, a different fatty acid group contents and fatty acid ratios and a higher total sterol and α-tocopherol contents than the two other sites considered. These differences were more marked in the farmed fish than in the wild one, showing an important effect of diet provided on the development of an heterogeneous lipid distribution. The PL fraction provided far less differences as a result of the zone or the kind of turbot considered, according to its structural role in living bodies and being less influenced by the diet.

Most trace minerals studied showed a more homogeneous distribution among sites than in the case of the lipid matter. According to lipid fraction results, less differences were obtained for the wild fish than for the farmed one. The most interesting difference was found for the Zn content that led to a higher value in the edge zone for both kinds of turbots. Further, fair correlation values were obtained between Zn and total lipid contents.

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FIGURE LEGENDS

Figure 1: Position of the three white muscle zones considered in the present study: ahead (AZ), back (BZ) and edge (EZ) in the turbot body.

Figure 2: Water content (g kg^{-1} wet flesh muscle) obtained in the edge (EZ), ahead (AZ) and back (BZ) zones of farmed and wild turbot.

Figure 3: Lipid content (g kg^{-1} wet flesh muscle) obtained in the edge (EZ), ahead (AZ) and back (BZ) zones of farmed and wild turbot.

Figure 4: Total phospholipid content (g kg^{-1} wet flesh muscle) obtained in the edge (EZ), ahead (AZ) and back (BZ) zones of farmed and wild turbot.

Figure 5: Total sterol content (g kg^{-1} wet flesh muscle) obtained in the edge (EZ), ahead (AZ) and back (BZ) zones of farmed and wild turbot.

Figure 6: Alpha-tocopherol content (mg kg^{-1} wet flesh muscle) obtained in the edge (EZ), ahead (AZ) and back (BZ) zones of farmed and wild turbot.

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TABLE 1

Analysis results of certified reference material (CRM)*

Concentration	Ca	Cu	Fe	Mg	Mn	Se	Zn
Found	5.14±0.53	2.00±0.14	148± 3	1.01±0.04	3.53±0.22	1.30±0.13	27.6±0.5
Certified	NC	2.34±0.16	142±10	NC	3.66±0.34	1.40±0.09	25.6±2.3

* CRM consisted on fish muscle material (DORM-2) from the National Research Council of Canada. Results are expressed in μg g^{-1} flesh muscle. "NC" indicates a value not certified.

TABLE 4

Trace mineral contents in different muscle zones* of farmed and wild turbot**

Trace mineral	Farmed Turbot			Wild Turbot		
	EZ	AZ	BZ	EZ	AZ	BZ
Ca	0.93 (0.20)	[y] 1.05 (0.10)	0.99 (0.12)	0.90 (0.26)	[z] 0.75 (0.17)	0.78 (0.10)
Cu	[y] 0.33 (0.05)	[y] 0.24 (0.09)	[y] 0.23 (0.06)	[z] 0.16 (0.06)	[z] 0.09 (0.01)	[z] 0.11 (0.03)
Fe	1.91 b (0.80)	0.80 ab (0.49)	0.73 a (0.25)	1.78 (0.69)	1.32 (0.82)	1.65 (0.68)
Mg	0.22 a (0.02)	[y] 0.27 b (0.01)	[y] 0.26 ab (0.02)	0.19 a (0.02)	[z] 0.23 b (0.02)	[z] 0.22 ab (0.02)
Mn	[y] 0.41 b (0.12)	[y] 0.22 a (0.05)	[y] 0.29 ab (0.09)	[z] 0.14 (0.06)	[z] 0.12 (0.04)	[z] 0.12 (0.03)
Se	0.27 (0.09)	0.35 (0.10)	0.37 (0.09)	0.29 (0.10)	0.32 (0.06)	0.31 (0.03)
Zn	[y] 12.11 b (2.46)	[y] 6.32 a (0.74)	[y] 6.33 a (0.66)	[z] 7.81 b (1.17)	[z] 3.77 a (0.28)	[z] 4.28 a (0.37)

* Muscle zone abbreviations: Edge (EZ), ahead (AZ) and back (BZ) zones.

** Mean values (mg kg^{-1} wet flesh fish, except for Ca and Mg expressed as g kg^{-1} wet flesh fish) of six independent determinations are expressed. Standard deviations are indicated in brackets. For each mineral and for each kind of turbot, mean values followed by different letters (a-b) indicate significant differences ($p<0.05$) among muscle zones. For each mineral, means preceded by different superscripts indicate significant differences ($p<0.05$) between both kinds of turbot in the muscle zone indicated.