CHAPTER VI

VARIATIONS IN CHEMICAL, PHYSICAL AND SENSORIAL PROPERTIES OF WILD AND CULTIVATED SOUTHERN FLOUNDER (*Paralichthys lethostigma*)[1]

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ABSTRACT

A comparison of the chemical, physical and sensorial properties of aquacultured and wild southern flounder was undertaken. Wild fish were derived from a commercial harvest in North Carolina, whereas cultured animals were fed a diet containing their calculated dietary protein requirement (50% crude protein). Proximate composition, color, texture and fillet fatty acid content were analyzed together with mineral presence and sensory evaluation. Wild southern flounder fillets exhibited higher protein content, lower fat content and higher texture values ($p \leq 0.05$) than their cultured counterparts. A higher content of unsaturated and n-6 fatty acids was found in wild southern flounder when compared to farmed southern flounder. Differences ($p \leq 0.05$) were detected with respect to fillet color, with overall, farmed fish being lighter. Wild flounder fillets expressed higher levels ($p \leq 0.05$) of sodium, potassium, sulfur and iron.
KEYWORDS. Fatty acids, minerals, color, quality, composition, sensory analysis

INTRODUCTION

Southern flounder (*Paralichthys lethostigma*), a member of the Family Bothidae (lefteye flounders), is an important sport and commercial fish in the Southeastern United States. This species is distributed from North Carolina to northern-most eastern and western seaboards of Florida through to Texas and Mexico (Reagan and Wingo, 1985; Daniels, 2000). Most wild-caught southern flounder are of 30-40 cm, with 35 cm having been established as the minimum legal harvest length (Stokes, 2003). The diet of wild southern flounder consists of shrimp and small fish (mullet, anchovies, menhaden), while farmed flat fish are provided with feeds that contain protein concentrations of 50-55% crude protein (CP). Marine flatfish generally require dietary protein levels ranging between 40-60% (Guillaume et al., 1991). Published CP requirements for cultured Bothidae, including southern, summer (*P. dentatus*) and olive (*P. olivaceus*) flounder fall in the range of 45-55% (Lee et al., 2002; Daniels and Gallagher, 2000; Kim et al., 2002; González et al., 2003a).

Unlike olive flounder, research upon southern and summer flounders has been restricted. However, interest in these species as candidates for intensive cultivation, especially in recirculating aquaculture systems, has increased (Schwarz et al., 2002), resulting in an enhanced knowledge upon their basic biology. These include studies on induced ovulation, spermiation and spawning, larval rearing and weaning, and salinity tolerance and thermal preferences (Jenkins and Smith, 1999; Watanabe and Carroll, 2001; Benetti et al., 2001a,b).

At present, aquaculture production of both southern and summer flounders is limited, with most of the harvest being destined for domestic sushi restaurants and for export to Asia (Bengtson, 1999). Expansion of domestic and international markets for farmed flounder will rely heavily upon whether farmed flounder are perceived as being of equal or superior quality to their wild counterparts. Imperative in this respect therefore, will be to determine whether formulated diets impact final product quality. Accordingly, the objective of this study was to compare physical, chemical and sensorial properties of farmed and wild southern flounder.

MATERIALS AND METHODS

Animals

Fresh southern flounder (*Paralichthys lethostigma*) were obtained from the North Carolina shore and immediately shipped on ice to the Department of Food Science and Technology at Virginia Polytechnic Institute and State University (Virginia Tech). Upon arrival, fillets were skinned and frozen (-20° C). Cultured southern flounder, reared in a recirculating system using a 50% CP diet (comprising a mixture of low temperature menhaden fishmeal {Special Select®, Omega Protein, Hammond, LA, USA}, casein {50:50 w/w} and 14% lipid {370 kcal available energy/100 g diet}; González et al., 2003a) were skinned, filleted and frozen pre-rigor (-20° C).

Compositional analyses

Wild and cultured fillets (n = 3 per treatment) were freeze-dried and subsequently analyzed in triplicate for lipid (Soxhlet method; AOAC, 1990), Kjeldahl protein (AOAC, 1990) and moisture (AOAC, 1990).

Color

The color of raw fillets (n = 6 wild/cultured) was measured at three locations (head, center and tail). Minced fish fillets (n=3) were also analyzed. Fillets were minced with a knife and homogenized; color was measured on minced and whole fillet samples by colorimetry (CR-2000; Konica-Minolta Photoimaging Inc., Tokyo, Japan) to determine L***** (white), a* (green to red), and b* (blue to yellow) values.

Minerals

Fillets were thawed (n = 3 per treatment) and digested by wet ashing and mineral contents (Al, Fe, Cu, Mn, Zn, Cr, Ni, As, Se, Cd, Pb, Hg, Ba, Co, S, Na, Mg, P, K and Ca) determined using the flame emission method (AOAC, 1990; Spectro Flame Modula Tabletop ICP with autosampler; Fitchburg, MA, USA). Approximately 4 g of each sample were dried for 2.5 h at 110º C, digested in concentrated nitric acid and further diluted with hot water to 100 ml. Samples were stored at 3º C prior to testing. Minerals were reported as μg/g of fish muscle.

Firmness

Shear force (model 1101, Canton, MA, USA) was measured using the Instron Universal Testing Machine with a 10-blade Lee-Kramer cell with data being reported as total energy/g fish muscle (J/g), using a 500 kg load transducer. Crosshead speed was set as 100 mm/min and a 20% load range was used. Fish fillets from each treatment (n = 9) were thawed and placed on ice prior to testing. Fish fillets were cut into rectangles to fit the Lee-Kramer cell and the weight of each sample was recorded. Fish samples showed similar thickness. The total energy used to penetrate the sample was divided by the sample weight and firmness was reported as total energy per gram of sample.

Sensory Analysis

A triangle test (Meilgaard et al., 1999) was used to determine an overall difference in flavor between wild and farmed fish. Fillets were minced separately and baked in aluminum foil at 177° C for 7 min. Approximately 28 g of minced fish was placed in 28 g cups with lids and served to panelists (n = 30) at room temperature. Test sensitivity parameters were set as: $\beta = 0.10$; $\alpha = 0.05$ and P_d = 40 % (proportion of distinguishers) (Meilgaard et al., 1999). Red illumination was employed to avoid color bias and panelists were seated in individual booths. Panelists included students, staff and faculty of Virginia Tech. Each panelist received one triangle test (3 samples) with each sample, identified by a randomly chosen three digit code. A balanced design was used to randomly present the samples to the panelists.

Fatty Acid Profile

Fillet lipids (n=3 wild/farmed) were extracted by the Folch procedure (Folch et al., 1957). Fatty acids were transesterified to methyl esters with 0.5N NaOH in methanol and 14% Boron trifluoride in methanol (Park and Goins, 1994). In addition 120 µg undecenoic acid (Nu-Check Prep) was added prior to methylation as an internal standard. All samples were analyzed on a 6890N gas chromatograph with a 7683 autoinjector, split/splitless capillary injector and flame ionization detector (Agilent Technologies, Palo Alto, CA, USA). The carrier gas was ultrapure hydrogen, with a gas velocity of 29 cm/sec and flow at 1.4 ml/min. The injection volume was 0.5 µl**,** and a split ratio of 65:1 was used. A Chrompack CP-Sil 88 100 m x 0.25 mm id capillary

column was used to separate fatty acid methyl esters (Chrompack, Middleburg, The Netherlands). The temperature program for separation began at 70º C, was held for 1 min, increased to 100 at 5º C/min, held for 3 min, increased to 175º C at 10º C/min, held for 45 min, increased to 220º C at 5º C/min and held for 15 min. Total analysis time was 86.5 minutes. Temperatures for injector and detector were 250 and 300º C respectively. Data were integrated and quantified using a Chem DataStation (Agilent Technologies, Palo Alto, CA, USA). Fatty acid data was reported on total percent terms.

Statistical Analysis

All data were subjected to One-way Analysis of Variance (ANOVA) using Jump (JMP®) (SAS Institute, Cary, NC). Sensory analysis was analyzed according to number of correct responses based on table T8 in Meilgaard et al (1999).

RESULTS AND DISCUSSION

Muscle lipid and protein content differed ($p \leq 0.05$) between farmed and wild southern flounder (Table 1). These results concur with previous studies that compared wild and farmed marine round (Rueda et al., 1997; Grigorakis et al., 2002; Grigorakis et al., 2003; Orban et al., 2003) and flatfish (Sérot et al., 1998; Ruff et al., 2002). Elevated fillet lipid content of cultured fish has generally been attributed to the amount and type of diet, strain, rearing temperature, exercise, and other environmental conditions (Haard, 1992; Gines et al., 2004). Elevated lipid content in farmed fish has been associated with a decline in eating quality (Chaiyapechara et al., 2003). However, elite dietary formulation and feeding strategies could become valuable tools to permit production of value-added products that contain enhanced levels of n-3 fatty acids without impacting product quality (Rasmussen et al., 2000). The benefits of manipulating n-3 fatty acid content of fish fillets relate to their health, and especially cardiovascular, benefits (Gohlke, 2004).

The color of wild and farmed fillets differed for the a* value (red to green; Table 2; $p \leq 0.05$), with cultured flounder exhibiting higher green (low red values) values. This influenced the lightness of the fillet, which was visibly noticeable. Differences in color between wild and aquacultured fish likely occur due to dietary effects (Lindsay, 1980; González et al., 2003b) although seasonal changes, muscle type and muscle water content are also known to influence color (Haard 1992, Rahman et al., 1995). Fillet color is an important factor since it can influence consumer choice and acceptability of certain fish products (Hatae et al., 1989). Fillet color control therefore, becomes a challenge for aquaculture when attempting to deliver products that are comparable to their wild counterparts.

Among the minerals measured in the present study, only 4 varied ($P < 0.05$) between treatments (Table 3). Iron was higher ($p \leq 0.05$) in wild animals. Similar observations have also been made for sea bass (*Dicentrarchus labrax*: Alasalvar et al., 2002). Differences in iron content likely relate to the presence of a higher proportion of dark muscle in wild fish, which acquires its dark or red color due to the presence of myoglobin (heme pigment). The higher iron content may also have had some influence upon the wild flounder a* values.

Heavy metals measured in this study were present in low concentrations and no significant differences were found between treatments ($p > 0.05$; Table 3). Mercury was below the limits of detection of the method employed (0.024 ppm in solution). The relative concentrations of

microminerals in fish may be influenced by a variety of factors: including species, season, size, type of muscle, sex, age, diet and environment (Haard, 1992; Alasavar et al., 2002). Certain of the macrominerals also differed between wild and cultured fish, with wild southern flounder fillets exhibiting higher concentrations ($p \leq 0.05$) of sodium, potassium and sulfur. Similar differences for sodium and sulfur have also been observed between cultured and wild yellow perch (González et al., 2003b). The mineral content of fish muscle may influence flavor (Haard, 1992), and differences in sodium, potassium, sulfur and iron content recorded herein could have had a bearing on perceived flavor distinction between wild and cultured southern flounder.

Overall differences in flavor were found between wild and farmed southern flounder ($p \leq 0.05$). Some of the descriptors that panelists used to comment on wild fish samples were: "bland", "moist", "mild", and "sweet". In contrast, descriptors employed for farmed southern flounder included: "fishy", "fish-like taste", "fishy smell", "earthy", and "muddy". Other comments related to fillet firmness although the "firm" was used for both treatments equally. Nevertheless, texture analysis determined higher firmness values for wild (0.33 ± 0.11 J/g) when compared to farmed fish (0.24 ± 0.08 J/g) ($p \leq 0.05$). Similar findings have been made during comparisons of wild and cultured yellow perch and gilthead sea bream (González et al., 2003; Grigorakis et al., 2003). The softer texture of farmed fish, which represents a common characteristic, has been ascribed to fillet fat content, water holding capacity, and amount, or lack thereof, of exercise (Haard, 1992; Rasmussen, 2001).

Several fatty acids differed ($p \leq 0.05$) between wild and farmed southern flounder (Table 4). The fatty acids with highest concentrations present in wild fillets were: docosahexaenoic acid (DHA, 22:6n-3), palmitic acid (16:0), arachidonic acid (20:4n-6), oleic acid (18:1n-9), stearic acid (18:0), docosapentaenoic acid (22:5n-3), and lignoceric acid (24:0). While the major fatty acids present in farmed southern flounder were: docosahexaenoic acid (DHA, 22:6n-3), palmitic acid (16:0), lignoceric acid (24:0), palmitoleic acid (16:1n-7), oleic acid (18:1n-9), myristic acid (14:0), docosapentaenoic acid (22:5n-3) and stearic acid (18:0). Overall, farmed fish expressed higher concentrations of saturated and lower concentrations of unsaturated fatty acids when compared to wild southern flounder ($p \leq 0.05$). The primary fatty acid in both treatments was DHA, which was expected since marine fish usually have high concentrations of long chain n-3 fatty acids (Steffens 1997). However, elevated ($p \leq 0.05$) arachidonic acid content was also recorded in wild fish, which likely resulted due to differences in diet. Adult flounder feed on

shrimp and fish while traveling between the estuaries and the Gulf of Mexico. According to Burke (1995), southern flounder prefer low estuarine salinities during feeding. The variation of habitats, salinities and available feed can influence the fatty acid profile. For instance, marine shrimp have a high content of arachidonic, oleic, EPA, palmitic, stearic and palmitoleic acid (Chanmugam et al. 1986) and could therefore influence arachidonic content. Verification of this supposition however would require stomach content analysis. Elevated arachidonic acid levels have also been recorded for wild turbot (Sérot et al. 1998). While few differences were found in n-3 fatty acids between treatments (Table 4), the n-3/n-6 ratio was significantly higher ($p \leq 0.05$) in farmed fish, thereby concurring with the findings of Chen et al. (1995) on their studies with sturgeon (*Acipenser oxyrinhus desotoi*).

Although there were differences in some important fatty acids between both treatments, the variation in lipid content affected the amount of fatty acids delivered in 100 g of fish fillet (dry weight basis). Because of the higher lipid content in farmed southern flounder a higher concentration of n-3 fatty acids was delivered (0.88g/100g muscle), while wild southern flounder only delivered 0.20g/100g. The same amount of arachidonic acid was delivered by both treatments (0.05g/100g), while different amounts of n-6 fatty acids were provided by fish fillets due to differences in lipid content (farmed: 0.17g/100g; wild: 0.09g/100g). According to the American Heart Association, amounts ranging from 0.5 – 1.8 g/day of EPA and DHA should be consumed from fatty fish to reduce death from heart disease (American Heart Association, 2002).

CONCLUSIONS

Farmed and wild southern flounder both represent good sources of essential minerals and n-3 fatty acids. Noteworthy however, was that a diet of 50% CP (fishmeal: casein) and 14% lipid (menhaden fish oil) impacted the fatty acid profile of farmed southern flounder, beneficially increasing the content of n-3 fatty acids and lipid content in the muscle.

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TABLES

Table 1. A comparison of the proximate composition of farmed and wild southern flounder.

Proximate composition	Farmed	Wild
	% dry weight basis	
Moisture	79.83 ± 1.62[a]	81.01 ± 1.29[a]
Crude protein	93.95 ± 0.42[a]	97.05 ± 0.02[b]
Lipid	3.04 ± 0.14[a]	1.61 ± 0.30[b]
Fiber (acid)	3.89 ± 0.69[a]	2.99 ± 0.57[a]

[ab]Data represent means ± standard deviation (n = 3). Different superscripts in the same row are significantly different ($p \leq 0.05$).

Table 2. Comparison of color of farmed and wild southern flounder fillets using a Minolta CR-200.

Color	Farmed	Wild
L*	60.79 ± 2.79[a]	57.94 ±4.48[a]
a*	-2.41 ± 0.29[a]	-0.50 ± 0.94[b]
b*	-1.19 ± 1.60[a]	-1.71 ± 1.47[a]

[ab]Data represent means ± standard deviation (n = 3) with different superscripts in the same row being significantly different ($p \leq 0.05$).

* L = lightness extends from 0 (black) to 100 (white); a* = redness- greenness: extends from – a* (green) to + a* (red); b* = yellow-blue: extends from – b* (blue) to + b* (yellow)

Table 3. A comparison of fillet mineral content between farmed and wild southern flounder.

Minerals	Farmed	Wild
Micro minerals	µg/g fish muscle	
Aluminum (Al)	1.36 ± 0.58	1.87 ± 0.04
Iron (Fe)	4.03 ± 0.80[a]	11.45 ± 1.35[b]
Cupper (Cu)	0.25 ± 0.10	0.18 ± 0.04
Manganese (Mn)	0.30 ± 0.05	0.27 ± 0.02
Zinc (Zn)	7.70 ± 1.35	5.91 ± 1.38
Chromium (Cr)	1.01 ± 0.96	1.77 ± 0.72
Nickel (Ni)	0.61 ± 0.30	0.84 ± 0.46
Arsenic (As)	0.21 ± 0.08	< 0.16
Selenium (Se)	0.51 ± 0.08	0.69 ± 0.15
Cadmium (Cd)	0.02 ± 0.01	0.02 ± 0.01
Mercury (Hg)	< 0.05	< 0.05
Lead (Pb)	1.12 ± 0.09	1.03 ± 0.14
Cobalt (Co)	0.11 ± 0.02	0.07 ± 0.03
Barium (Ba)	< 0.01	< 0.01
Macro minerals		
Sodium (Na)	147.11 ± 34.76[a]	414.38 ± 41.16[b]
Magnesium (Mg)	248.40 ± 22.48	254.12 ± 17.04
Phosphorus (P)	1132.86 ± 326.90	1635.79 ± 30.71
Sulfur (S)	2055.97 ± 68.65[a]	2507.81 ± 144.82[b]
Potassium (K)	1939.79 ± 739.18[a]	3268.18 ± 59.48[b]
Calcium (Ca)	119.84 ± 4.11	110.32 ± 9.94

[ab]Data represent means ±SD (n = 3). Different superscripts in the same row are significantly different ($p \leq 0.05$).

Table 4. Concentrations of selected fatty acids in fillets of farmed and wild southern flounder.

Fatty acids	Farmed	Wild
	% of total fatty acids*	
14:0	5.33 ± 1.30 [a]	1.12 ± 0.09[b]
16:0	20.5 ± 0.68	21.6 ± 0.56
18:0	4.85 ± 1.25[a]	7.01 ± 0.39[b]
24:0	10.1 ± 0.32[a]	4.74 ± 0.20[b]
16:1n-7	7.40 ± 1.94[a]	2.72 ± 0.19[b]
18:1n-7	2.10 ± 0.62	1.69 ± 0.27
18:1n-9	6.83 ± 4.86	7.56 ± 0.39
20:1n-9	1.07 ± 0.12[a]	0.48 ± 0.11[b]
18:2n-6	1.70 ± 0.19[a]	2.94 ± 0.26[b]
18:3n-6	0.20 ± 0.17	0.11± 0.12
20:4n-6	1.63 ± 0.36[a]	8.92 ± 0.79[b]
22:4n-6	0.22 ± 0.03[a]	1.99 ± 0.10[b]
18:3n-3	0.71± 0.22 [a]	0.44 ± 0.07[a]
20:5n-3	0.07 ± 0.02[a]	0.20 ± 0.07[b]
22:5n-3	5.05 ± 0.27	5.56 ± 0.66
22:6n-3	24.2 ± 3.61	27.7 ± 0.51
Saturated fatty acids	41.8 ± 0.66[a]	35.9 ± 0.86[b]
Unsaturated fatty acids	58.2 ± 0.67[a]	64.1 ± 0.85[b]
n-3 fatty acids	30.0 ± 3.18	33.9 ± 0.88
n-6 fatty acids	4.74 ± 0.31[a]	15.2 ± 0.44[b]
n-3/n-6 ratio	6.36 ± 0.96[a]	2.23 ± 0.12[b]

[ab]Data represent means ± SD. Different superscripts in a row indicate significant differences ($p \leq 0.05$).

*One or more of the fatty acid concentrations reported in this table might be erroneous (provided by service laboratory). Please refer to published manuscript for correct information.