CHAPTER VII

CHEMICAL, PHYSICAL AND SENSORIAL DIFFERENCES IN FARMED SOUTHERN FLOUNDER (*Paralichthys lethostigma)* FED COMMERCIAL OR CRAB MEAL-SUPPLEMENTED DIETS[1]

[1]To be submitted as a research note to the Journal of Aquatic Food Product Technology

ABSTRACT

A 12- week feeding trial was conducted to determine the effects of commercial or crab meal–supplemented diets on sensory properties of southern flounder (*Paralichthys lethostigma*). Proximate, color, fatty acid composition, texture and sensory analyses were performed on the fillets.

A significant difference in flavor was found in fillets fed a commercial or a crab-meal supplemented diet. No significant difference was found between the two treatments when comparing fat, protein, fiber content and texture. However, fatty acid composition and color were significantly affected by dietary treatment.

The data suggests that crab-meal supplemented finishing diets could be utilized to enhance final product quality

KEYWORDS. Southern flounder, crab, sensory, color, fatty acids.

INTRODUCTION

Final product quality of fish is a complex term that relates nutritional, microbiological, biochemical and physicochemical properties; however, consumers will establish their quality criteria when buying fish based on appearance (color, surface appearance) aroma, flavor and texture. Sensory properties (flavor, aroma, texture and color) of fish are affected by factors such as: environment (water temperature, pH), age, feeding type and techniques, stress, and slaughtering techniques; but the most influential factor on flavor and color is the type of dietary ingredients in the feed (Haard, 1992; Lie, 2001; Morris, 2001; Rasmussen, 2001; Sinnot 2001).

Flavor of fish is influenced by free amino acids, minerals, fatty acids (enzymatic or oxidation products) and peptides (Haard, 1992). The variation of protein or oil sources has been found to influence fat content, fatty acids color and flavor of a variety of fish species (Liu et al., 2004; Adelizi et al., 1998; Rasmussen et al., 2000; Oliveira et al., 2004; Francesco et al., 2004). In fish feeds, fishmeal is the most common protein source; however a decline in the global production of fish meal, high prices and the need to reduce nitrogen and phosphorus loads are motivating researchers to identify optional protein sources.

In order to improve final product quality in fish, procedures like starvation and feeding fish in the last weeks prior to slaughter "finishing diets" are some of the most used (Rasmussen et al., 2000). In this project, crab meal was added in fish meal-based diets to corroborate reports that have confirmed the variation in flavor and color of wild and farmed fish fed crustaceans and /or crustacean waste (Haard, 1992; Lyons et al., 2001). Crab meal, which is high in unsaturated fatty acids, carotenoid pigments, flavor components and minerals, is already being used as a protein source and flavor enhancer in cattle, swine and extruded snacks (Murphy et al., 2003).

In this project, crab meal (5%) was incorporated to a fish meal-based diet and fed to southern flounder (*Paralichthys lethostigma*) to analyze the impact of crab meal on fillet flavor.

MATERIALS AND METHODS

Animals

Southern flounder (n= 40), fed a 50% protein diet for twelve weeks were randomly selected and placed into a two-compartment cage and suspended in a 8,706 L recirculation aquaculture system (RAS). Fish were filleted, skinned and frozen pre-rigor at -15º C.

Diets and feeding

Fish in the first compartment were fed a commercial diet while a crab meal supplemented diet (5%) was fed to fish in the second compartment (n = 20/treatment; Table 1). Both groups were fed twice daily to apparent satiation for 12 weeks.

Compositional Analyses

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

Color

Color was measured in raw fish fillets from each treatment (n = 6 per treatment). Three different positions of the fillet (head, center and tail) were measured, using a Minolta (CR-2000, Japan) colorimeter to determine L***** (white), a* (green to red), and b* (blue to yellow) values.

Firmness

Shear force was measured using a 10-blade Lee-Kramer cell in an Instron Universal Testing machine (model 1101, Canton, MA). The data were reported as total energy (J), 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 = 12) were thawed and placed on ice prior to testing. Fish fillets were cut in rectangles to fit the Lee-Kramer cell and the weight of each sample was recorded. Thickness of each sample was similar. The total energy used to penetrate the sample was divided by the sample weight and firmness was reported as total energy (J) per gram of sample.

Sensory Analysis

A triangle test (Meilgaard et al., 1999) was used to determine an overall difference in flavor between fillets of southern flounder fed the commercial and crab meal-supplemented diet. Fish fillets of each treatment were minced with a knife separately and baked in aluminum foil at 177° C for 10 min. Approximately 28 g of minced fish were placed in 28 g cups with lids and served to panelists at room temperature (n = 30). Test sensitivity parameters were established as: β = 0.10; α = 0.05 and P_d = 40 % (proportion of distinguishers). Each panelist received one triangle test (3 samples), identified by a three digit code. A balanced design was used to randomly present the samples to the panelists. Red light and individual booths were used to avoid bias. Panelists were chosen from students, faculty and staff from the Food Science and Technology Department.

A one-sided paired comparison test also was conducted to determine which treatment had the strongest "fish" flavor. The number of panelists used in this study was 42. Test sensitivity parameters were set up to be α = 0.05; β = 0.40 and P_d = 30 % (proportion of distinguishers).

Fatty Acid Profile

Lipids from three different frozen fillets from each treatment were extracted by the Folch procedure (Folch et al., 1957). Fatty acids were transesterified to methyl esters with 0.5 N 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 100º C 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 min. Temperatures for injector and detector were 250º C and 300º C, respectively. Data were integrated and quantified using a Chem DataStation (Agilent Technologies, Palo Alto, CA, USA). Fatty acids were reported as total percent of fatty acids.

Statistical Analysis

All data were subjected to One-Way Analysis of Variance (ANOVA) using Jump (JMP®, SAS Institute, Cary, NC). Differences in means were analyzed using Tukey's HSD ($p \leq 0.05$). Sensory analysis was analyzed according to number of correct responses based on table T8 and T10 in Meilgaard et al. (1999).

RESULTS AND DISCUSSION

There were no differences in the lipid and protein contents ($p > 0.05$) of southern flounder fillets fed the commercial and crab meal-supplemented diets (Table 2), even though the proximate composition of the diets was different (Table 1). In the present study proximate composition was assessed on the edible muscle only, discarding the finray muscle. Gaylord et al. (2003) reported that lipid deposition in summer flounder (*Paralichthys dentatus*) occurred in the finray muscle, rather than the white edible muscle. Fish store excess lipid in different sections of their body and as summer flounder, southern flounder must have stored the excess lipid from the commercial diet in the finray muscle, which was not analyzed in the present study.

Other studies, have reported higher feed consumption in yellow perch when fed diets that included squid and krill meal as flavor additives (Gould et al., 2003). Research, partially replacing fish meal with crab meal (5-14%) in Atlantic salmon diets increased feed efficiency and protein efficiency, as well (Lyons et al., 2001).

Another property that was not affected by diet was texture (flounder fed commercial diet: 0.04 J/g; flounder fed crab meal-supplemented diet: 0.05 J/g) ($p > 0.05$). No texture variations were expected since fillets from fish fed the two diets possessed similar lipid and protein concentrations.

The color of fillets fed the crab meal-supplemented diet was changed by the carotenoid content in the crab meal. In different species addition of a variety of pigments in diets is used to impact the color of fish fillets, most specifically in the salmon and shrimp industry (Simpson, 1982). Lyons et al. (2001) also observed color differences in salmon fillets when crab meal was partially replacing fish meal in diets. In the present study, yellow (b*) was increased in the crab meal-supplemented diet and in the fish fillets fed this diet (Table 3). Appearance and acceptability of fish fillets are influenced strongly by color of fillets, since 40% of consumer's decision to buy seafood is based on color (Rasekh and Kramer, 1970). Variations in type of dietary protein and type of oil have influence fillet color in other studies (Liu et al., 2004; Oliveira et al., 2004; Francesco et al., 2004; Adelizi et al., 1998; Kaushik et al., 1995)

The formulation of the diets affected the fatty acid profile of fish fillets (Table 4). Recently, variations in oil and protein sources in fish (Rasmussen, 2001) and other animals diets (Wood et al., 2003) to increase n-3 (omega-3) fatty acids in the meat has been a topic of interest in research, due to the important human health benefits that n-3 fatty acids deliver (American Heart

Association, 2002; Kinsella, 1988; Minnis et al., 1998). The most important fatty acids that varied between treatments were 18:1n-9, 20:1n-9, 20:2n-6, 20:4n-6 and 22:4n-6 ($p \leq 0.05$); Table 4), from which only 20:4n-6 and 22:4n-6 were higher in southern flounder fed the crab meal-supplemented diet. Overall the n-3/n-6 ratio was significantly higher ($p \leq 0.05$) in flounder fed the crab meal-supplemented diet, increasing the nutritional value of the fish fillet. Ingredients of the commercial diet are unknown, but usually vegetable protein sources are utilized and this could explain the lower n-3/n-6 ratio and higher content of linoleic and oleic acid. Further research needs to be done to determine the effects of a higher crab meal inclusion level on fatty acid profile.

Flavor also was affected by differences in dietary ingredients. An overall difference in flavor was determined by panelists who tasted the fish fillets fed the two diets. Some of the comments that panelists described included that the fish fed the crab meal-supplemented diet tasted "milder" and not as "fishy" or "stronger" as the flounder fed the commercial diet. Due to the differences in color a red light was used to avoid bias. The second sensory test, showed no significant differences ($p > 0.05$), when panelists were asked which sample had a stronger "fish flavor". These results show that the difference in flavor previously found, was not related to "fish flavor" but other flavor attributes were not examined in this study.

CONCLUSIONS

Crab meal supplementation influenced sensory properties of southern flounder fish fillets and the n-3/n-6 ratio. The carotenoid content of the crab meal influenced fillet color, enhancing the yellow hues and decreasing whiteness.

This data demonstrates that finishing diets can effectively impact final product quality and with further research, nutritional value of fish fillets could be improved by utilization of different protein sources.

Further research is needed to identify volatile compounds that could be responsible for aroma and flavor enhancement.

ACKNOWLEDGEMENTS

This work is the result of research supported in part by the NOAA Office of Sea Grant, U.S. Department of Commerce, under Grant No. NA56RG0141 to the Virginia Graduate Marine Science Consortium and the Virginia Sea Grant College Program. This work also was sponsored in part by a grant of the U.S. Department of Agriculture, Cooperative State Research Education and Extension Service

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TABLES

Table 1. Composition of crab meal-supplemented diet and proximate analysis of crab meal-supplemented and commercial diet

Ingredient	%
Protein	45
Fish meal	40
Crab meal	5
Dextrin	4
Lipid	10
Mineral	4
Vitamin	3
CMC	1

Proximate composition (dry weight basis)		
	Commercial diet*	Crab meal-supplemented diet
Protein	52.48 ± 0.12[b]	43.23 ± 0.76[a]
Lipid	18.63 ± 0.29[b]	10.49 ± 0.17[a]
Fiber	10.11 ± 0.24	13.04 ± 0.23

[a]Means ± standard deviation (n = 3) with different letters in the same row are significant different at $p \leq 0.05$

*Commercial diet was formulated as 45:16 (protein:lipid %) as fed.

Table 2. Proximate composition of flounder fillets fed a commercial or a crab meal-supplemented diet

Proximate composition	Flounder fed commercial diet	Flounder fed a crab meal-supplemented diet
	% dry weight basis	
Moisture	77.18 ± 0.42[a]	78.45 ± 0.67[b]
Crude protein	89.31 ± 1.59	89.14 ± 2.20
Lipid	5.17 ± 2.38	4.50 ± 2.17
Fiber (acid)	3.73 ± 2.00	2.46 ± 1.32

[ab]Means ± standard deviation (n = 3) with different letters in the same row are significant different at $p \leq 0.05$

Table 3. Color of commercial, crab meal-supplemented diets and flounder fed a commercial or the crab meal-supplemented diet

Color	Commercial diet	Crab meal-supplemented diet	Flounder fed commercial diet	Flounder fed crab meal-supplemented diet
L*	21.01 ± 4.45[a]	34.99 ± 3.62[b]	46.14 ± 2.58 [a]	41.71 ± 5.25[b]
a*	3.08 ± 0.85[a]	2.92 ± 0.98[a]	0.88 ± 1.14[a]	1.28 ± 0.57[a]
b*	5.52 ± 1.86[a]	17.92 ± 4.11[b]	1.93 ± 1.92[a]	3.51 ± 0.71[b]

[ab]Means ± standard deviation (n = 3) with different letters in the same row are significant different at $p \leq 0.05$. Diets compared separately and fish fillets compared separately.

* 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 4. Fatty acid composition of southern flounder fed a commercial or a crab meal-supplemented diet

Fatty acid	Fillets of southern flounder fed	
	Commercial diet	Crab meal-supplemented diet
	g/100g fatty acids (dry weight basis)*	
14:0	2.77 ± 0.69	1.98 ± 0.64
16:0	22.9 ± 1.88	24.2 ± 2.00
18:0	6.76 ± 0.66	7.18 ± 0.33
24:0	7.58 ± 0.60	7.20 ± 0.40
16:1n-7	4.07 ± 0.99	3.07 ± 0.82
18:1n-7	2.24 ± 0.26	2.22 ± 0.16
18:1n-9	9.70 ± 1.42[a]	6.68 ± 0.76[b]
20:1n-9	0.85 ± 0.07 [a]	0.63 ± 0.04[b]
18:2n-6	3.90 ± 1.87	1.39 ± 0.73
18:3n-6	0.13 ± 0.11	0.46 ± 0.37
18:3n-3	0.55 ± 0.11[a]	0.32 ± 0.10[b]
20:2n-6	0.72 ± 0.03[a]	0.43 ± 0.03[b]
20:4n-6	2.57 ± 0.03[a]	3.31 ± 0.08[b]
20:5n-3	0.08 ± 0.03	0.09 ± 0.04
22:5n-3	3.62 ± 0.33	3.45 ± 0.28
22:6n-3	26.8 ± 4.76	32.9 ± 5.71
Saturated fatty acids	41.1 ± 2.88	41.5 ± 3.31
Unsaturated fatty acids	58.6 ± 2.83	58.0 ± 3.54
n-3 fatty acids	31.1 ± 4.35	36.8 ± 5.40
n-6 fatty acids	8.86 ± 1.45	7.15 ± 0.26
n-3/n-6 ratio	3.53 ± 0.40[a]	5.16 ± 0.90[b]

[ab]Means ± standard deviation (n = 3) with different letters in the same row are significantly different at $p \leq 0.05$

*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.