FATTY ACID NUTRITIONAL PROFILES IN GULF OF MEXICO FISHES

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INTRODUCTION

The fatty acids, linoleic, linolenic and arachidonic, cannot be synthesized by the body therefore are considered to be essential components in our diet. They are needed for maintaining the function and integrity of membrane structure, for fat metabolism, for growth and for synthesis of certain prostaglandins. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both omega-3 fatty acids, are currently considered essential fatty acids for all marine organisms. Fish obtain omega-3 fatty acids from the phytoplankton or algae in their diet. There is evidence that omega-3 polyunsaturated fatty acids (n-3 PUFA) found in fish oils provide many potential health benefits when added to the human diet. Research reports have indicated that cold water fish species are rich sources of omega-3 fatty acids. The implications have been to the general public that warm water fishes may not contain omega-3 fatty acids or that they do not contain enough to be utilized as a source of omega-3 fatty acids. Current research reported in this manuscript is funded by the National Marine Fisheries Service and has as study objectives: to determine the fatty acid content, particularly the omega-3 acids, as well as cholesterol and the proximates of over 40 Gulf fishes, most of which have no state-of-the-art fatty acid identification; to provide this information in the format that can be utilized by the consumer; to promote consumption of a broad spectrum of seafood especially highlighting the lesser known species; and to format the data in such a way that it can be used for nutritional labeling. The species that were chosen for the first year of this study are shown in Table 1.

Diets extremely rich in land plant foods and oils and animals and poultry fed on grain products provide almost exclusively omega-6 fatty acids. Omega refers to any of 12 families of naturally occurring acids having more than one double bond where unsaturation is entirely methylene-interrupted and of cis (Z) configuration. Each family is designated by the number of carbon atoms from the last double bond to the terminal methyl group. The fatty acids most often found in foods belong either to the omega-6 or to the omega-3 families, the omega-3 fatty acids found almost exclusively in seafood. The letter n has now replaced the term omega and will be used in this manuscript. Most land plants and vegetable oils contain large quantities of linoleic (C18:2) acid which can be quickly metabolized through elongation and desaturation to form arachidonic acid, an n-6 fatty acid which is further metabolized to form various potent hormone-like derivatives called prostaglandins and leukotrienes sometimes referred to as eicosanoids. Some of these hormones are necessary for certain body functions while others play a specific role which leads to a variety of diseases including thrombosis, arthritis, asthma and other immune-related diseases. Two events led to the significant interest and intense medical research in n-3 fatty acids: the epidemiological investigations of Bang and Dyerberg (1972) and later the discovery by Needleman (1979) that prostaglandins derived from n-3 fatty acids had different biological properties than those derived from arachidonic acid, an n-6 fatty acid. Research data supports the evidence that n-3 fatty acids both suppress eicosanoid production and antagonize the biosynthesis of arachidonic acid from linoleic acid. Therefore, by eating
Table 1. Gulf of Mexico Fishes Examined for Nutritional Content in 1988.

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cynoscion arenarius*</td>
<td>sand seatrout</td>
</tr>
<tr>
<td>Pogonias cromis</td>
<td>black drum</td>
</tr>
<tr>
<td>Anchoa mitchilli</td>
<td>bay anchovy</td>
</tr>
<tr>
<td>Anchoa hepsetus</td>
<td>striped anchovy</td>
</tr>
<tr>
<td>Carcharhinus limbatus</td>
<td>blacktip shark</td>
</tr>
<tr>
<td>Arius felis</td>
<td>hardhead catfish</td>
</tr>
<tr>
<td>Dasyatis sabina</td>
<td>Atlantic stingray</td>
</tr>
<tr>
<td>Lagodon rhomboides</td>
<td>pinfish</td>
</tr>
<tr>
<td>Orthopristis chrysoptera</td>
<td>pigfish</td>
</tr>
<tr>
<td>Mugil cephalus</td>
<td>striped mullet</td>
</tr>
<tr>
<td>Leiostomus xanthurus</td>
<td>spot</td>
</tr>
<tr>
<td>Rachycentron canadum</td>
<td>cobia</td>
</tr>
<tr>
<td>Euthynnus alletteratus</td>
<td>little tunny</td>
</tr>
<tr>
<td>Caranx cryosom</td>
<td>blue runner</td>
</tr>
<tr>
<td>Bagre marinus</td>
<td>gafftopsail catfish</td>
</tr>
<tr>
<td>Thunnus atlanticus</td>
<td>blackfin tuna</td>
</tr>
<tr>
<td>Morone saxatilis x</td>
<td>striped bass, hybrid</td>
</tr>
<tr>
<td>Morone chrysops</td>
<td></td>
</tr>
<tr>
<td>Callinectes sapidus</td>
<td>blue crab</td>
</tr>
</tbody>
</table>


more seafood rich in n-3 acids, the body would synthesize fewer eicosanoids, and the risk for getting these diseases would be lessened (Lands, 1986).

METHODS

Sample design. There are a number of factors that can lead to variations in the fatty acid content of fish. Seasonal changes, state of reproductive maturity, feeding habits, spawning versus non-spawning and geographic differences are but a few of the conditions that may affect content and distribution of these fatty acids (Exler and Weihrauch, 1976; Exler et al., 1975; Jangaard et al., 1967; Joseph, 1985; Stansby, 1973). A collection scheme was devised that would permit a measure of the variability associated with most of these factors. Each species was collected during four seasons in sufficient quantities to yield information on seasonal variability of fatty acid concentration and distribution for each of the species.

One species was selected in 1988, spot (Leiostomus xanthurus), for an in-depth examination of other factors leading to fatty acid variability. Collections of this fish were made from several locations to examine geographic variability; both juvenile and adult specimens were collected to measure the effect of maturity on fatty acid profiles.

Though previous investigators have recognized the importance of many of the factors leading to variability in fatty acid content of fish, few have expressed awareness of a very important source of variability, individual variability. Stansby (1981) has stressed the need to look at individual specimen
variability in the design of a proper sampling program. Without knowledge of specimen to specimen variability, it is impossible to assess with what reliability reported values of fatty acids in fish estimate the true mean content for any particular species. Furthermore, this measure of individual variability is essential in establishing upper and lower limits of the mean which are necessary for establishing nutritional guidelines for fish.

The task of establishing a sample size that would lead to a statistically valid sampling program was discussed with Al Rainosek, biostatistician of the National Marine Fisheries Service, Pascagoula Laboratory. Most means of setting proper sample size (i.e. No. of individual specimens) are based upon some knowledge of expected individual variability. With no previous information available on the individual variability of fatty acids in Gulf fishes, it was felt necessary that some background information needed to be generated. Striped mullet (Mugil cephalus) and spot (Leiostomus xanthurus) were chosen as representative of Gulf fishes included in this study. Ten individual fish of both species were collected and analyzed separately. After careful statistical evaluation of this data, it was decided that collections each season would consist of at least nine separate fish of each species taken from one location at one time. These fish were grouped into three subgroups of three fish each and a composite of fish tissue prepared within each subgroup. If following analyses of the three subgroups, lower limits of means proved to be unacceptable, additional composites were prepared and analyzed as needed until an acceptable level of statistical reliability was achieved.

Sample preparation. Immediately after delivery of fresh fish to the laboratory, the fish were measured, weighed, sexed and state of health recorded. The fish were filleted and given a unique identification number, placed in plastic containers, flushed with nitrogen to remove oxygen and then frozen at -40°C. Before compositing any of the fish, an examination was made of the entire collection of each species made during the previous season. As many unique characteristics (e.g. size, sex, location) of each species as possible were used in sorting the individuals into the three subgroups. Identical quantities of tissue from each of the fish in the subgroups was used in the composites so that no bias was placed upon size of fish. Tissues were taken from the entire longitudinal cross section of fillet so that the sample was representative of all of the edible tissue of each fish. All tissue from individual fish not used for compositing was archived.

Analytical procedures. Samples for compositing were thawed and thoroughly homogenized before 0.5 g aliquots were placed into 30 ml centrifuge tubes for saponification using 1 ml 50% KOH and 4 ml 95% ethanol. Duplicate analyses were made on all composite samples. After saponification and separation of non-saponifiable material, pH was adjusted to 1 and extraction was made of the saponifiable fraction containing the fatty acid salts. Fatty acids were converted to methyl esters using 7% BF₃–MeOH by the method of Metcalfe and Schmitz (1966). A nitrogen atmosphere was maintained throughout all procedures to minimize oxidation. Gas-liquid chromatography (GLC) of methyl esters was carried out using a Perkin-Elmer Model Sigma 2000 gas chromatograph with an all-glass split injection system onto a 30 m x 0.25 mm i.d. fused silica capillary column coated with 0.25 micron film thickness of Dura Bond WAX (J & W Scientific). Helium was used as the carrier gas. The data was quantified by internal standardization using a Perkin-Elmer Sigma 10 data system. Tentative identification of peaks was by comparison of retention time with those of standards.
RESULTS AND DISCUSSION

During the first half of 1988, most analytical attention was directed towards the individual variability study used in setting sample size and towards performance evaluation studies of analytical techniques. Only data from two seasonal collections of fishes from the Gulf will be discussed in this report. Sufficient information is available from these analyses to make a number of valuable observations.

Consumers have been erroneously led to believe that cold water fishes are the only sources of n-3 acids. The overall results indicate that the levels of n-3 fatty acids in Gulf fishes listed in Table 1 fall within 0.25 to 1.0 g per 100 g portion of fish fillet. This compares favorably with the 0.1 to 3.0 g per 100 g portion concentrations reported for cold water fishes (Nettleton, 1985; USDA, 1987). Warm water species examined in 1988 do contain somewhat less absolute quantities of n-3 acids than those cold water fishes such as mackerel and salmon continually mentioned in nutritional news releases. However, most of the Gulf species examined thus far have a fairly low fat content as compared to some of the fattier cold water fishes. Because the American diet is high in fat (Kinsella, 1988), larger portions of leaner fish could be consumed to obtain the same amount of n-3 fatty acids as the cold water fishes but with perhaps fewer fat calories.

In order to assess the fatty acids in seafood products, we looked at several quality indicators. The total polyunsaturated fatty acids (PUFA) are compared to total saturated fatty acids since an increase in saturated fat intake as a percent of total fat intake is associated with an increase in total plasma cholesterol, and appears to raise equally low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol (Grundy, 1986). Biochemical and physiological findings show that when n-3 fatty acids are a significant fraction of total fat intake, plasma triglycerides are lowered with little effect on LDL and HDL (Parks and Bullock, 1987), therefore we include data on both the total n-3 fatty acid concentration and the total concentration of EPA and DHA, the two primary n-3 fatty acids in seafood, per 100 g fish serving. Lands (1986) has stressed the importance of balancing our dietary n-3 and n-6 PUFAs. We have provided the ratio of the concentrations of the two families of acids in Gulf fishes for the consumer who is interested in increasing intake of these n-3 dietary acids.

Fatty acid quality parameters computed for four representative species of Gulf fishes are shown in Table 2. The greatest distinction among these fishes is seen in the quantity of EPA and DHA expressed as percent of total fatty acids. Thirty-nine percent of the fatty acids of bay anchovy (Anchoa mitchilli) are made up of EPA and DHA, the highest value among these four fishes. These two acids comprise but 0.22% of the tissue weight, therefore, this fish contains both high levels of n-3 fatty acids as well as a low quantity of total fatty acids. The n-3/n-6 ratio for this fish is also the highest of the four fishes. Based strictly upon percentage of EPA and DHA in total fatty acid and n-3/n-6 ratios, bay anchovy would be the clear choice, however, the other three fishes are distinctive in other fatty acid quality parameters. Cobia (Rachycentron canadum), a popular game fish, has a high concentration of PUFA. Striped mullet also has high PUFA concentrations as well as fatty acids enriched in n-3 acids. Black drum (Pogonias cromis) has moderately high values in all of the quality parameters.

In Table 3a-c are the fatty acid quality parameters computed for subgroups of cobia, black drum and hardhead catfish (Arius felis). The unique features
Table 2. Fatty Acids in Gulf Finfish.

<table>
<thead>
<tr>
<th></th>
<th>Striped Mullet</th>
<th>Bay Anchovy</th>
<th>Black Drum</th>
<th>Cobia</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUFA, wt%</td>
<td>0.46</td>
<td>0.26</td>
<td>0.39</td>
<td>0.51</td>
</tr>
<tr>
<td>(EPA + DHA), wt%</td>
<td>0.31</td>
<td>0.22</td>
<td>0.22</td>
<td>0.26</td>
</tr>
<tr>
<td>(EPA + DHA)/total fatty acids, %</td>
<td>20</td>
<td>39</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>n-3/n-6</td>
<td>5.8</td>
<td>9.7</td>
<td>3.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Unsat/sat</td>
<td>2.4</td>
<td>2.3</td>
<td>2.2</td>
<td>1.7</td>
</tr>
</tbody>
</table>

1Fatty acid quality parameters tabulated as follows: PUFA, wt% -- total polyunsaturated fatty acids expressed as wt% of wet tissue weight; (EPA+DHA), wt% -- combined wt. of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as % of wet tissue wt; (EPA+DHA)/total fatty acids, % -- EPA+DHA as % of total fatty acid fraction; ratio of total n-3 acids to total n-6 acids; unsat/sat -- ratio of total unsaturated acids to total saturated acids.

Table 3a. Fatty Acids, Cobia1

<table>
<thead>
<tr>
<th></th>
<th>10-21 lbs</th>
<th>30-31 lbs</th>
<th>34-50 lbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUFA, wt%</td>
<td>0.28</td>
<td>0.35</td>
<td>0.91</td>
</tr>
<tr>
<td>(EPA + DHA), wt%</td>
<td>0.15</td>
<td>0.20</td>
<td>0.42</td>
</tr>
<tr>
<td>(EPA + DHA)/total fatty acids, %</td>
<td>13</td>
<td>11</td>
<td>9.3</td>
</tr>
<tr>
<td>n-3/n-6</td>
<td>2.2</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Unsat/sat</td>
<td>1.9</td>
<td>1.6</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Table 3b. Fatty Acids, Black Drum1

<table>
<thead>
<tr>
<th></th>
<th>9-10 lbs</th>
<th>11-12 lbs</th>
<th>13-15 lbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUFA, wt%</td>
<td>0.43</td>
<td>0.43</td>
<td>0.30</td>
</tr>
<tr>
<td>(EPA + DHA), wt%</td>
<td>0.23</td>
<td>0.28</td>
<td>0.16</td>
</tr>
<tr>
<td>(EPA + DHA)/total fatty acids, %</td>
<td>10</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>n-3/n-6</td>
<td>3.1</td>
<td>4.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Unsat/sat</td>
<td>2.1</td>
<td>2.2</td>
<td>2.4</td>
</tr>
</tbody>
</table>

1See Table 2 for parameter explanation.
Table 3c. Fatty Acids, Hardhead Catfish

<table>
<thead>
<tr>
<th></th>
<th>212-432 g (M &amp; F)</th>
<th>465-549 g (F)</th>
<th>612-675 g (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUFA, wt%</td>
<td>0.21</td>
<td>0.29</td>
<td>0.19</td>
</tr>
<tr>
<td>(EPA + DHA), wt%</td>
<td>0.12</td>
<td>0.16</td>
<td>0.12</td>
</tr>
<tr>
<td>(EPA + DHA)/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total fatty acids, %</td>
<td>17</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>n-3/n-6</td>
<td>2.6</td>
<td>2.3</td>
<td>3.4</td>
</tr>
<tr>
<td>Unsat/sat</td>
<td>2.4</td>
<td>2.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

1See Table 2 for parameter explanation.

of these subgroups were weights. In addition the hardhead catfish was subdivided by sex. For both the hardhead catfish and blackdrum, no clear trends with weights were observed for the fatty acid quality parameters. For cobia there was a direct relationship of fatty acids and size of fish. In larger fish, PUFAs comprise a higher concentration in the fish tissue. EPA and DHA concentrations increase in larger fish expressed as percent of fish tissue but shrink as a percent of the total fatty acids.

Fatty acid quality parameters in juvenile and adult spot are shown in Table 4. The trend seen with size in the cobia was also observed for the spot with a dramatic increase in PUFAs going from juvenile to adult stages as well as large increases in EPA and DHA expressed as percent of tissue. Note that EPA and DHA comprise 36% of the total fatty acids in the juvenile stage with a decline to 8.6% in the adult.

Table 4. Spot Fatty Acids

<table>
<thead>
<tr>
<th></th>
<th>Juvenile</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUFA, wt%</td>
<td>0.15</td>
<td>0.78</td>
</tr>
<tr>
<td>(EPA + DHA), wt%</td>
<td>0.10</td>
<td>0.46</td>
</tr>
<tr>
<td>(EPA + DHA)/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total fatty acids, %</td>
<td>36</td>
<td>8.6</td>
</tr>
<tr>
<td>n-3/n-6</td>
<td>3.4</td>
<td>4.4</td>
</tr>
<tr>
<td>Unsat/sat</td>
<td>2.9</td>
<td>3.6</td>
</tr>
</tbody>
</table>

1See Table 2 for parameter explanation.

A listing of the individual fatty acid composition of black drum are shown in Table 5 which contains data from two seasonal collections of this fish. Fatty acid data in this study are computed both as absolute weight concentrations in tissue, shown here, and as percent of the total fatty acids. The latter form of data presentation is more often seen in the literature but is also of less use in evaluating nutritional information. Also included in Table 5 are proximate data, detailing other nutritional characteristics of black drum. The
### Table 5. Fatty Acids in Black Drum (Pogonias cromis)

<table>
<thead>
<tr>
<th></th>
<th>Winter (January 16, 1988)</th>
<th></th>
<th></th>
<th></th>
<th>Spring (May 10, 1988)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>Mean</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>Mean</td>
</tr>
<tr>
<td><strong>Saturated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>449.52</td>
<td>342.50</td>
<td>222.05</td>
<td>336.02</td>
<td>312.01</td>
<td>233.98</td>
<td>392.46</td>
<td>339.48</td>
</tr>
<tr>
<td>16:0</td>
<td>5167.99</td>
<td>3972.34</td>
<td>2331.76</td>
<td>3780.69</td>
<td>2822.19</td>
<td>3597.15</td>
<td>4000.44</td>
<td>3606.59</td>
</tr>
<tr>
<td>18:0</td>
<td>1573.98</td>
<td>1123.93</td>
<td>639.62</td>
<td>1179.24</td>
<td>960.49</td>
<td>1169.70</td>
<td>1256.51</td>
<td>1135.24</td>
</tr>
<tr>
<td>20:0</td>
<td>99.44</td>
<td>90.72</td>
<td>38.84</td>
<td>76.32</td>
<td>46.82</td>
<td>46.00</td>
<td>57.17</td>
<td>49.92</td>
</tr>
<tr>
<td>22:0</td>
<td>64.43</td>
<td>49.59</td>
<td>21.70</td>
<td>45.24</td>
<td>28.92</td>
<td>18.35</td>
<td>26.09</td>
<td>24.66</td>
</tr>
<tr>
<td>23:0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td><strong>Monounsaturated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:1</td>
<td>5265.09</td>
<td>3934.78</td>
<td>2263.61</td>
<td>3829.82</td>
<td>3400.46</td>
<td>4073.06</td>
<td>3679.77</td>
<td>3718.45</td>
</tr>
<tr>
<td>18:1</td>
<td>5175.42</td>
<td>3468.38</td>
<td>2388.52</td>
<td>3677.69</td>
<td>2450.25</td>
<td>5713.29</td>
<td>3591.41</td>
<td>3255.99</td>
</tr>
<tr>
<td>20:1</td>
<td>802.09</td>
<td>457.35</td>
<td>390.60</td>
<td>550.08</td>
<td>446.00</td>
<td>453.67</td>
<td>531.56</td>
<td>471.21</td>
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<tr>
<td>21:1</td>
<td>121.10</td>
<td>72.32</td>
<td>81.82</td>
<td>91.75</td>
<td>0.00</td>
<td>0.00</td>
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<td><strong>Polyunsaturated (PUFA)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:2n-6</td>
<td>259.31</td>
<td>154.91</td>
<td>99.55</td>
<td>167.69</td>
<td>105.94</td>
<td>116.64</td>
<td>121.70</td>
<td>117.43</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>137.23</td>
<td>68.66</td>
<td>30.50</td>
<td>78.75</td>
<td>46.28</td>
<td>61.00</td>
<td>65.97</td>
<td>57.76</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>120.52</td>
<td>120.49</td>
<td>74.62</td>
<td>105.21</td>
<td>62.62</td>
<td>100.49</td>
<td>109.40</td>
<td>97.50</td>
</tr>
<tr>
<td>20:5n-3 (EPA)</td>
<td>115.29</td>
<td>77.12</td>
<td>66.57</td>
<td>86.33</td>
<td>64.19</td>
<td>67.90</td>
<td>84.27</td>
<td>72.12</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>642.79</td>
<td>524.52</td>
<td>496.83</td>
<td>534.71</td>
<td>449.91</td>
<td>461.87</td>
<td>524.51</td>
<td>476.92</td>
</tr>
<tr>
<td>20:4n-3</td>
<td>1041.91</td>
<td>854.64</td>
<td>625.47</td>
<td>640.64</td>
<td>707.72</td>
<td>563.82</td>
<td>764.72</td>
<td>605.42</td>
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<td>22:2n-6</td>
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<td>19.32</td>
<td>0.00</td>
<td>13.20</td>
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<td>31.94</td>
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### Proximates

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**Notes:** For each season, three subgroups of fish were analyzed, A, B, and C. The data for each subgroup represents means for duplicate analyses. Each fatty acid is denoted by carbon chain length: No. of double bonds. For explanation of entries under profile variables, see note in Table 2.
seasonal means for all the fatty acids give the impression that there is a slight decline in the concentration of most of the fatty acids going from the winter to spring season though the decline is generally less than 10%. This decline in fatty acid concentration is accompanied by somewhat lower protein and cholesterol also. One should note, however, that the relative concentrations change little between these two seasons. The profile variables which reflect relative contributions of various groupings of fatty acids such as n-3/n-6, unsat/sat, and (EPA+DHA)/n-3,2 show no significant differences between the seasons. The data within the three subgroups comprising each seasonal analysis show variations among subgroups that are much larger than the variations between seasonal means. Therefore most likely the variation between the two seasonal collections is more a reflection of individual to individual variation than true seasonal variation.

More comprehensive information on seasonal, biological (age, size, sex) and geographic variations on the 20 species of fishes chosen for study in 1988 will be presented in future reports.

ACKNOWLEDGMENTS

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REFERENCES


Characterization of Volatile Odor Components in Crude Fish Oils from Gulf and Atlantic Menhaden (*Brevoortia* spp.)

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INTRODUCTION

The world's total fish catch in 1986 was 85 million metric tons, 30% of which was processed into fish oil and fish meal. In the U. S., fish landing in 1986 totaled 2.7 million metric tons and a larger percentage, 44%, was reduced to fish oil and fish meal. Ninety-nine percent of this oil was manufactured from menhaden, and most was produced in the Gulf states. In the same year, 90% of the U. S. fish oil was exported to Europe and refined and partially hydrogenated to make margarine and as ingredients for use in the bakery industry (Bimbo, 1987). Recently, there has been a large increase in the public's interest in fish oils and omega-3 fatty acids due to potential benefits in the reduction of cardiovascular risks. Fish oils are rich in polyunsaturated fatty acids including omega-3 acids such as eicosapentaenoic acid and docosahexaenoic acid. Since refined American fish oil is not yet available, the capsules containing refined and deodorized fish oil have been imported from Japan and Europe. It is estimated that as much as 300 million dollars will be spent on these imported capsules in 1988. In 1986, the National Fish Meal and Oil Association filed a petition to FDA to affirm GRAS status of menhaden oil as a direct human food ingredient. If menhaden oil is approved as GRAS, a tremendous economic potential exists for Louisiana, which is the largest fish oil producing state in the U. S.
However, there is a major problem with fish oils, namely, formation of fish oil odors due to degradation of lipids, proteins, and amino acids by microbial spoilage, autoxidation and other reactions. Nutritional and health benefits can not be realized if fish oils are unacceptable due to their objectionable odors.

The overall objectives of this project were (1) to identify the odorous components in the U.S. fish oils, (2) to determine the efficiency and to monitor the reduction of odorous components in refining and processing leading to food grade oil, and (3) to study the flavor stability of food grade oil during storage. In this paper, we report results obtained from both the Gulf and Atlantic menhaden oils to achieve the first objective.

MATERIALS AND METHODS

Zapata Haynie Corp. provided crude oils from Gulf menhaden (Brevoortia tyrannus) and Atlantic menhaden (Brevoortia patronus). Volatile compounds were collected from the samples by a Tekmar dynamic headspace sampling system (Cincinnati, OH) using helium as a purging gas and Tenax TA (Chrompack, Inc., Raritan, NJ) as a trapping sorbent. Figure 1 shows a schematic diagram of the dynamic headspace sampling system. The trapped volatiles were then flash-desorbed into a cryogenically focussed high resolution capillary column (Supelcowax 10, 60 m x 0.25 mm i.d. x 0.25 μm film thickness) for gas chromatography. The separated volatile compounds were identified by electron ionization mass spectrometry and computer mass spectral matching using a Hewlett Packard Mass Selective Detector. Authentic compounds were used for confirmation of the identification under comparable experimental conditions. The odors of the components were also perceived as they were eluted from the column. Details of this combined analytical procedure have been described elsewhere (Hsieh et al, 1989).

RESULTS AND DISCUSSION

Figure 2 shows the total ion chromatograms, or the "finger print profiles", of the volatiles from the crude oils of Gulf and Atlantic menhaden. Both samples were 1988 oils. The compounds identified and the odor perceived are labelled at the appropriate chromatographic retention times. Many of the compounds noted derive from
lipid oxidation, and contribute negatively to the flavor quality of the fish oils. It also was observed that Gulf crude oil contained more volatiles than did the Atlantic oil.

The analytical procedure used did not involve high temperature heating for a prolonged period or organic solvent extraction with potential impurity contamination. It has decreased possibility of artifact formation in comparison with conventional analytical techniques. This procedure will be used to determine the efficiency of refining and deodorization of the crude menhaden oils leading to food grade oil with acceptable flavor quality as well as the flavor stability of refined and deodorized oil in storage. This procedure may also be used to characterize the critical volatile components that affect flavor quality in other oils.

REFERENCES


ACKNOWLEDGMENTS

Manuscript No. 88-21-2783 of the Louisiana Agricultural Experiment Station. This study was supported, in part, by a grant from the Louisiana Sea Grant College Program, an element of NOAA, Office of Sea Grant, U. S. Department of Commerce. The authors wish to thank S. S. Williams and W. Vejaphan for their assistance in the earlier part of this project, and S. P. Meyers for helpful discussions.
Figure 1. Schematic diagram of Toker dynamic headspace sampler coupled to a gas chromatograph.
Figure 2: Total Ion Chromatograms of Dynamic Headspace Volatiles from Gulf (Top Panel) and Atlantic (Bottom Panel). Crude Marine Oil: compound identification and total ion intensity.

Retention Time (min)

0 10 20 30

Total Ion Intensity

0 10 20 30

xylene

2-penten-1-ol: grassy green, musty

4-pentanol

2,4-dimethyl benzene

3-hexanol: cut grass, green

2-methyl disulfide

(E)-2-pentenal: painty

(E)-2-butenal: painty

3-penten-1-one

2-ethyl turan

2-propanol

2-pentanol

decane

pentanal

pentane

nonane

butanal

2-butanone

cycloctane

Total Ion Intensity
Figure 2. (Continued)

Retention Time (min)

Total Ion Intensity

3-heptenone: sickly sweet, cooling

4-o-xylene

Heptanal: waxy green, grassy

Limonene

Dodecane

Propyl benzene

(E)-2-hexenal: sharp green, oily

2-pentyl furan
terpene

2,1,3,5-trimethyl benzene: pesticide-like

2-ethyl toluene

1,2,4-trimethyl benzene: pesticide-like

Octanal: citrus, fatty, orange

Tridecane

C4-alkyl benzene

Butyl benzene

(E)-2-heptenal: sharp green, grassy

1,2,3-trimethyl benzene: pesticide-like

1,2-diethyl benzene

C4-alkyl benzene

1-hexanol

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Figure 2. (Continued)

- 2-nonanone: musty with citrus topnote
- (E,E)-2,4-hexadienial
- (E)-2-hexen-1-ol
- C₅-alkyl benzene
- (E)-2-octenal: musty, waxy floral
- 1,4-dichlorobenzene
- 2-octanol
- (E,Z)-2,4-heptadienal
- Decanal: sweet, green fruity, fatty
- (E,E)-2,4-heptadienal
- Pentadecane
- Benzaldehyde: cherry, almond, sweet fruity
- 2-nonanol
- 2-nonenal: fatty, waxy, musty
- (E,Z)-2,4-octadienal
- (E,E)-2,4-octadienal
- 3,5-octadiene-2-one
- Hexadecane
Figure 2. (Continued)

Retention Time (min)

Total Ion Intensity

100

110

120

100

110

120

decenal
phenyl acetaldehyde
2,6-dimethyl pentadecane
heptadecane
3-hexadecene
n-decanol
(E,Z)-2,4-decadienal: oxidized oil
(E,E)-2,4-decadienal: oxidized oil
SHRIMP SPECIES IDENTIFICATION USING UREA GEL
ISOELECTRIC FOCUSING SYSTEM


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INTRODUCTION

Isoelectric focusing (IEF) has been extensively used for seafood species identification because it provides reliable and reproducible protein patterns for differentiating closely related species. Using thin layer polyacrylamide gel IEF, various fish species have been readily identified (Hamilton, 1982; Lundstrom, 1981 and 1983).

Identification of cooked or processed seafood has not been extensively studied using this methodology. Most difficulties encountered are due primarily to alterations of protein molecules by heat treatment. The extractability of heat denatured proteins can be increased by the use of sodium dodecyl sulfate (SDS) or urea. Krzynowek and Wiggin (1979 and 1981), using urea to extract crab meat proteins and IEF, identified with 98% accuracy the genus of cooked or frozen crab meat among 79 samples. Recently An et al. (1988a) used 8M urea or 1% SDS to extract boiled shrimp proteins and they properly identified the genus of cooked shrimp using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).

Urea has been incorporated in electrophoretic gels to enhance protein separation and resolution (Boulikas, 1985; Keung et al., 1985; O'Farrell, 1975; Tuszyński et al., 1978). By utilizing a 9.2 M urea IEF gel for the first dimension, O'Farrell (1975) successfully separated an Escherichia coli lysate into over 1,000 components by two–dimensional gel electrophoresis. Therefore the objectives of this study were (1) to investigate if the addition of urea in IEF polyacrylamide gels would enhance the resolution of protein patterns for shrimp species identification, (2) to optimize urea IEF conditions for speciation of raw and cooked shrimp, and (3) to examine the species–specificity of shrimp protein banding patterns by combining the protein extraction systems with urea gel IEF.

MATERIALS AND METHODS

Sample preparation

Three shrimp species, pink (Penaeus duorarum, Key West, FL), white (Penaeus setiferus, Jacksonville, FL), and rock shrimp (Stenodyne brevirostris, Port Canaveral, FL), were obtained and stored at -33°C until needed. Individual shrimp were thawed under tap water, peeled, and deveined. For the cooking treatment, shrimp were placed in boiling water for 5 min.

Water and 1% SDS (w/v) were used to extract proteins from raw or cooked shrimp, respectively (An et al., 1988a). The solvents contained 0.1 mM phenylmethylsulfonfluoride (PMSF), 10 mM EDTA and 0.01% (w/v) sodium
azide to inhibit proteases and microbial growth. Both raw and cooked shrimp samples were chopped and each combined in separate beakers with the individual solvents at a ratio of 1:3 (w/v). Samples were homogenized at room temperature for 1 min using a Polytron (Brinkmann Instrument), and then centrifuged for 20 min at 26,900 x g (20°C). The supernatants were collected and the protein concentration determined (Lowry et al., 1951), and 100 μg of protein was applied on the IEF gel. For mixture samples, shrimp extracts were mixed in a 1:1 protein ratio (w/w) between pink and white, pink and rock, or white and rock and a 1:1:1 ratio between all three species. The mixtures were processed as above and subjected to IEF analysis.

Optimization of IEF conditions

The IEF method of O'Farrell (1975) was modified for a slab gel system. Various conditions were compared to establish the best conditions for species identification using banding patterns of water extracts from raw pink shrimp and protein standards (Broad pH kit, pH 3–10, Pharmacia). The protein standards contained: trypsinogen, pI 9.30; lentil lectin—basic band, pI 8.65; —middle band, pI 8.45; —acidic band, pI 8.15; horse myoglobin—basic band, pI 7.35; —acidic band, pI 6.85; human carbonic anhydrase B, pI 6.55; bovine carbonic anhydrase, pI 5.85; beta—lactoglobulin A, pI 5.20; soybean trypsin inhibitor, pI 4.55; and amyloglucosidase, pI 3.50.

Urea concentration: A non-denaturing (without urea) and a denaturing gel system with 6 or 9.2 M urea were compared on a polyacrylamide gel containing 6.2% (v/v) ampholytes (Pharmalytes pH 3–10, Pharmacia). Proteins were focused at room temperature for 17 hr at 400 V with a circulating tap water coolant system.

Ampholytes concentration: Banding patterns of the water extract of raw pink shrimp and protein standards were compared using a 9.2 M urea polyacrylamide gel containing 5.0 or 6.2% (v/v) ampholytes. The gel was focused for 17 hr.

Focusing time: Two focusing times, 10 and 17 hr, were compared using a 9.2 M urea gel containing 6.2% (v/v) ampholytes.

Electrolytes: Two different electrolyte systems, 20 mM sodium hydroxide–10 mM phosphoric acid (O'Farrell, 1975) and 10 mM histidine–10 mM glutamic acid (Anonymous, 1988) were compared for narrow pH range gel containing 100% ampholytes of pH 4–6.5 (Pharmalytes 4–6.5).

Ampholytes mixtures: Ampholytes were mixed to focus the bands at the anodic side of the pH 3–10 gel. Ampholytes 4–6.5 (Pharmalytes 4–6.5) at 100, 80, and 60% were mixed with 0, 20, and 40% ampholytes of pH 3–10 (Pharmalytes 3–10); the mixture was added into the urea gel at the final concentration of 6.2% (v/v).

Isoelectric focusing

A gel mixture containing 4% (w/v) acrylamide, 2% (w/v) Triton X–100 and 9.2 M urea was heated to 37°C with shaking for five min to dissolve urea. Ampholytes at a final concentration of 6.2% (v/v) was added, the gel mixture was degassed for 5 min, then combined with fresh persulfate solution and TEMED (final
concentration: 0.02% (v/v) and 0.14% (v/v), respectively) and poured into 16 x 20 cm slab gel plates (0.75 mm thick) assembled with a comb. The gel was allowed to polymerize for 1 hr. Lysis buffer containing 9.5 M urea, 2% (w/v) Triton X-100 and 2% (v/v) ampholytes was overlayed on the gel following the removal of the comb. The gel was allowed to sit for another hour. After the lysis buffer was replaced with a fresh one, prefocusing of the gel was done at 200 V for 15 min, 300 V for 30 min and then 400 V for 30 min in a Protean II electrophoresis slab gel unit (BioRad) using a 10 mM phosphoric acid as the anode electrolyte solution and a 20 mM sodium hydroxide as the cathode solution.

Following prefocusing, protein samples were applied on the gel, and an aqueous solution containing 2% (w/v) Triton X-100 and 2% (v/v) ampholytes was overlayed atop the protein samples. The gel plate was reassembled in the electrophoresis unit and fresh cathode solution was added to the chamber. Proteins were focused at room temperature for 17 hrs at 400 V with circulating tap water. After the IEF run, the gel was stained with Coomassie blue R-250 and destained (Anonymous, 1986). Positive image was developed from the stained gel using EDP paper (Electrophoresis Duplicating Paper, Eastman Kodak). Protein profiles of the IEF gel was scanned using the video densitometer (Model 620, BioRad) with the developed pictures on EDP paper. The densitometer was set at the reflectance mode.

**pH Measurement of the gel and determination of the apparent pI values of shrimp protein bands**

Apparent pI values of shrimp proteins were determined indirectly by comparing their R_f values on the gel with those of the protein standards. A pH profile of the whole gel was determined at room temperature (25 ± 2°C) before protein fixation using a micro-surface pH electrode (Ingold Electrodes Inc.). A linear relationship of gel pH and the R_f values of protein standards enabled determination of the apparent pI values for the corresponding standards. From the apparent pI and the R_f values of the protein standards and the R_f values of the shrimp proteins on the gel, the apparent pI values of shrimp proteins were determined.

**RESULTS AND DISCUSSION**

**Optimization of IEF gel conditions**

Urea concentration: The presence of urea in the gel greatly increased protein resolution. Among the three different urea concentrations used (0, 6 and 9.2 M), the 9.2 M gel provided the best protein banding pattern for raw pink shrimp and standards (Fig. 1). Proteins were well focused at the anode side and the bands were highly resolved. Good protein separation also occurred with the 6 M urea gel, but the resolution was not as good as the 9.2 M gel. Four major bands were found with shrimp samples run on gels containing no urea. In addition, the banding patterns and the pI values of the protein standards were different in the gels with or without urea.
**Figure 1 (Left).** Protein banding patterns of the water extract of raw pink shrimp (P) and protein standards (Std) focused on polyacrylamide gel containing 0, 6, or 9.2 M urea (cathode on top). The numerical designations indicate apparent pI values of the protein bands.

**Figure 2 (Right).** IEF patterns of the water extract of raw pink shrimp (P) and protein standards (Std) focused on polyacrylamide gel containing (1) 5% ampholytes for 17 hr, (2) 6.2% ampholytes for 10 hr, and (3) 6.2% ampholytes for 17 hr (cathode on top).
Ampholytes concentration: More stable protein banding patterns were obtained on gels containing 6.2% ampholytes (Fig. 2). Compared to the bands on the 5% ampholytes gel, protein bands on the 6.2% gel migrated less from the cathodic end where the sample was applied. Protein bands were more evenly distributed on the 6.2% ampholytes gel, while they were primarily focused near the anode side in the gel containing 5% ampholytes. In addition diffusion of protein bands occurred on the gel containing 5% ampholytes.

Focusing time: Focusing for 17 hr provided better band resolution than did a 10 hr period (Fig. 2). Proteins focused for only 10 hr were not completely resolved. This observation supported the results of Duncan and Hershey (1984) who showed that most proteins were completely localized by 6,400 volt—hour.

IEF patterns of raw shrimp for pH 3–10

IEF patterns of water extracts of raw pink, white and rock shrimp were compared. Water extracts gave rise to excellent banding patterns for species identification (Fig. 3). The most diverse and species-characteristic bands occurred at the low pl region. Bands with pl values of 5.48, 5.38, and 5.11 were specific for pink; 5.30, 5.22, and 4.97 for white; and 5.43, 5.20, and 5.06 for rock shrimp. Rock shrimp also had an additional specific band with a pl of 6.57.

Although most of the major bands at the anodic side of the gel were resolved and could be used effectively for species comparison, the minor protein bands at the lower pl region of the gel were clustered together due to the use of the pH 3–10 ampholytes. Since these minor protein bands may also provide useful information to further aid species identification, anodic region of the gel was expanded by incorporating into the gel a narrower range (pH 4–6.5) ampholytes.

Optimization of IEF gel conditions for narrow pH range

Electrolytes: The histidine—glutamic acid electrolyte systems recommended by the manufacturer interfered with proteins focusing at the narrower pH range of 4–6.5. There was no stable pH gradient formation in the gel nor was protein separation (data not shown). The interference still occurred even when proteins were focused at 4°C on the gel containing no urea. It was speculated that the low pH of histidine might cause protein precipitation on the gel and thus interfering their entering into the gel. The precipitated proteins might also prevent electrolytes from moving continuously. This problem was alleviated by the use of the sodium hydroxide—phosphoric acid electrolyte system; a stable pH gradient and a reproducible banding patterns were obtained. Therefore the sodium hydroxide—phosphoric acid system was used for future studies.

Ampholytes mixtures: Different ampholytes mixture affected the pH profile of the gel at the cathodic region. Incorporation of 40% pH 3–10 ampholytes with 60% pH 4–6.5 ampholytes caused the increase of about one pH unit at the very end of the cathodic region as compared to the gel that contained only pH 4–6 ampholytes (Fig. 4). Thus the actual pH of the gel containing only pH 4–6.5 ampholytes was determined to range from 4.3 to 7.2; from pH 4.6 to 7.7 for the one containing 80% pH 4–6.5 ampholytes; and from pH 4.3 to 8.3 for containing only 60% pH 4–6.5 ampholytes.

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Figure 3 (Left). IEF patterns of water extracts of raw pink (P), white (W), and rock (R) shrimp, and their mixtures (cathode on top). Protein standards (Std) are also included. The numerical designations indicate apparent pI values of the protein bands.

Figure 4 (Right). pH profiles of IEF gel containing three different ratios of wide and narrow pH range of ampholytes.
The change of the gel pH range also affected the resolution of raw pink shrimp proteins and protein standards. The distance between two specific protein bands was increased on the gel containing higher percentages of the pH 4–6.5 ampholytes. Among the three different mixtures, the 80% mix (of pH 4–6.5 ampholytes) was found to efficiently separate the raw shrimp proteins. Most of the protein bands useful for shrimp species identification, including those bands with low pI values to provide most information for species identification were well resolved and distributed throughout the gel (Fig. 5). The use of only the narrow pH range ampholytes (4–6.5) showed a good protein band separation; but some protein bands at the cathodic end were excluded from the gel due to the narrower gel pH range. Although most of the protein bands found on the gel containing only pH 3–10 ampholytes were present on the gel containing 40% pH 3–10 and 60% pH 4–6.5 ampholytes mixture, the protein bands were not separated as clearly as those on the gel containing 20% pH 3–10 and 80% pH 4–6.5 ampholytes mixture. Therefore the latter ampholytes mixture was used to separate shrimp proteins for further studies.

**IEF patterns of raw shrimp for narrow pH range**

Banding patterns of the water extracts of raw pink, white and rock shrimp and their mixtures were compared for species specificity (Fig. 6). As reported previously using only the pH 3–10 ampholytes (An et al., 1988b), species identification could be achieved by checking the presence of the major species-specific protein bands that were present at the anodic side. These included the protein bands with pI values of 6.97, 6.79, 5.52 and 5.45 for pink shrimp, the 6.87, 5.45 and 5.42 bands for white shrimp, and the 5.54 and 5.31 bands for rock shrimp.

Species identification could also be achieved by comparing among the species patterns of the minor bands that were well separated. The bands with pI values of 7.41, 7.28 and 7.26 for pink shrimp, the 7.41, 7.29, 7.02, 6.77, 6.66 and 5.96 bands for white shrimp, as well as the 7.16, 6.78, 6.75, 6.33, 6.24 and 6.20 bands for rock shrimp were shown to be specific for each species. The presence of these bands together with the major bands could be used effectively in identifying the species as well as in detecting the presence of a specific shrimp species in the mixture. For example, the presence of rock shrimp in the pink + rock, white + rock, or pink + white + rock shrimp mixture could be determined from the presence of the 7.16, 6.75, 6.33, 6.24 and 5.31 protein bands that were specific for rock shrimp.

The difference in protein banding patterns of the three shrimp species was well reflected by the diversity of their densitometric scanning profiles (Fig. 7). Since better protein separation occurred in these gels containing 20% pH 3–10 and 80% pH 4–6.5 ampholytes mixture, species-specific peaks were distributed throughout the profiles. Peaks at the 5–40 and 90–110 nm regions appeared to be more useful for species differentiation.

**IEF patterns of cooked shrimp for narrow pH range**

The water extracts of the cooked shrimp also showed different protein patterns (Fig. 8). Less shrimp proteins were extracted due to heat treatment. Thus many minor protein bands that were used for species identification in fresh sample
Figure 5 (Left). IEF patterns of water extracts of raw pink and protein standards focused on urea polyacrylamide gel containing 100, 800, and 60% of pH 4-6.5 ampholytes with addition of 0, 20, and 40% those of pH 3-10 (cathode on top).

Figure 6 (Right). IEF patterns of water extracts of raw pink (P), white (W), and rock (R) shrimp, and their mixtures (cathode on top). Protein standards (Std) are also included. The numerical values indicate apparent pI values of the protein bands.
Figure 7. Densitometric profiles of water extracts of pink, white, and rock shrimp extracted with water.
Figure 8 (Left). IEF patterns of water extracts of cooked pink (P), white (W), and rock (R) shrimp, and their mixtures (cathode on top). Protein standards (Std) are also included. The numerical values indicate apparent pI values of the protein bands.

Figure 9 (Right). IEF patterns of SDS extracts of cooked pink (P), white (W), and rock (R) shrimp, and their mixtures (cathode on top). Protein standards (Std) are also included. The numerical values indicate apparent
case were not present on the gel. The bands with low pI values, such as the 5.56, 5.51 and 5.48 for pink shrimp, the 5.48, 5.43 5.38, 5.07 and 4.98 for white shrimp, and the 5.76, 5.56, 5.51, 5.49 5.42, 5.20 and 5.14 for rock shrimp, were most useful for species identification. Identification of shrimp species in mixture samples, however, was complicated because of the similar patterns between mixture samples.

SDS enhanced protein extractability and thus caused the increase of protein band numbers on the IEF gel (Fig. 9). The protein bands with pI values of 6.83, 6.79, 6.03 and 5.13 were specific for rock shrimp; these rock shrimp bands could easily be distinguished even in mixture samples. Species could also be identified by checking the presence of the major protein bands with pI values of 5.33 and 5.26 for pink shrimp, and 5.40 and 5.23 for white shrimp. Due to the clustering of these bands, the identification of species specificity in mixture samples was difficult except for rock shrimp.

CONCLUSION

Incorporation of urea (9.2 M) in the gel greatly increased the protein separation and the band resolution. On the optimized condition using gels containing 9.2 M urea and 6.2% ampholytes and focused for 17 hrs, shrimp species were identified by the patterns of major protein bands.

Furthermore, the use of a 20% pH 3–10 and 80% pH 4–6.5 ampholytes mixture in IEF gels produced a narrower pH gel ranging from pH 4.7 to 7.7, and greatly enhanced protein separation as compared to gels containing only the pH 3–10 ampholytes. Not only the major bands but also the species–specific minor bands were well separated, so that species were easily differentiated. Presence of the species–related protein bands of raw samples was helpful to identify species present in a mixture samples. However the differentiation of the specific shrimp species in mixture of cooked samples was not effective due to the loss of some species–related protein bands by the heat treatment.

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IDENTIFICATION OF FISH SPECIES USED IN SURIMI PRODUCTS BY ELECTROPHORETIC TECHNIQUES


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INTRODUCTION

Surimi is a mechanically deboned, minced fish meat which has been washed to remove blood, fat, soluble pigments and other odoriferous substances, and mixed with cryoprotectants such as sugar and/or sorbitol (Lee, 1984 and 1986a). The washing process greatly improves the color and the odor of the minced fish and stabilizes the functional properties of surimi during frozen storage (Rasekh et al., 1980). Due to its light color, bland odor and unique gelling properties, surimi is used as a functional protein ingredient in the manufacture of a variety of fabricated seafood products such as crab legs, scallops, lobster and shrimp analogs (Lanier, 1986; Regenstein and Lanier, 1986).

To formulate the fabricated seafood products, surimi is combined with natural shellfish meat, shellfish flavoring agents, salt, water and starch, and/or egg white to modify the functionality and to enhance the flavor and texture. The finished products must be labeled properly to meet the Food and Drug Administration (FDA) guidelines which reflect the nature of the products. Martin (1986) has also stressed the need to establish proper nomenclature and labeling for newly developed seafood analogs. These must meet FDA requirements which have an impact on the two major ingredients in the fabricated seafood products: the fish species as the main ingredient and the other species, such as snow crab meat for crabmeat analog, as the additional ingredient. Problems exist regarding the labeling of the content of the specific seafood components. Products with claims of 35% crabmeat are widely sold when the use of over 10% crabmeat is known to show detrimental effect to the product (Regenstein and Lanier, 1986). Thus the establishment of useful methods to identify species origin, and, if possible, the content of surimi product is of great need for regulatory purposes and for protection of consumer’s values.

Our previous studies using 1% sodium dodecyl sulfate (SDS) or 8M urea to extract proteins (An et al., 1988a and b) have shown that the species and genus of cooked shrimp could be successfully identified on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and urea gel isoelectric focusing (IEF). This study was carried out to determine if the combined application of extractants (water, 1% SDS or 8M urea) and the SDS–PAGE and IEF techniques could effectively differentiate the protein patterns of Alaska pollock and red hake and to identify these two species used in surimi.
MATERIALS AND METHODS

Alaska pollock (Theragra chalcogramma) and red hake (Urophycis chuss) were used. Red hake fillet, and surimi prepared at the Department of Food Science and Nutrition Department (Lee, 1986b) at the University of Rhode Island were shipped frozen to the Food Science and Human Nutrition Department at the University of Florida. Frozen Alaska pollock fillet was provided by Arctic Alaska Seafoods, Inc. (Seattle, WA), while surimi was manufactured by the Alaska Pacific Seafoods (Kodiak, AK). The samples were stored at -33°C until needed. Heat treatment of the samples was done by cooking in boiling water for 5 min. All experiments were repeated at least twice.

Protein extraction and sample preparation

Raw fish fillet and surimi samples were extracted with water only, while cooked samples were extracted with water, 1% SDS or 8 M urea. All solvents contained 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM EDTA and 0.01% sodium azide (An et al., 1988a). The chopped samples of the fish fillet or surimi were combined with each solvent at a ratio of 1:3, and homogenized at room temperature for 1 min using a Polytron (setting 5.5). The homogenates were centrifuged at 26,900 g for 20 min at 20°C, the supernatants collected, and the protein contents determined (Lowry et al., 1951). For IEF, the protein content in the supernatant was adjusted with water to 5 μg/μL. Sucrose (3–4 granules) was added to each 100 μL extract, and 100 μg protein was applied on a IEF gel. For SDS–PAGE, 35 μg protein was loaded on the gel after the protein content of the supernatant was adjusted to 1.75 μg/μL with Tris–HCl buffer (pH 6.8) and SDS solution, heat–denatured, then finally sucrose and bromophenol blue were added (O’Farrell, 1975). Mixture samples were prepared by mixing the fish and surimi extracts in a 1:1 protein ratio.

Isoelectric focusing

A gel mixture containing 4% acrylamide, 2% Triton X–100 and 9.2 M urea was heated to 37°C. Ampholyte was added at a final concentration of 6.2%. After being combined with fresh persulfate solution and N,N,N′,N′–tetramethyl-ethylene–diamine (TEMED) (final concentration: 0.02 and 0.14%, respectively), the mixture was poured into 16 x 20 cm slab gel plates (0.75 mm thick) assembled with a comb. Following polymerization, lysis buffer containing 9.5 M urea, 2% Triton X–100 and 2% ampholyte was overlayed on the gel following the removal of the comb. The gel was allowed to sit for another hour. Prefocusing of the gel was done, after the lysis buffer was replaced with a fresh buffer, at 200 V for 15 min, 300 V for 30 min, and then 400 V for 30 min in a Protean II electrophoresis slab gel unit (Bio–Rad) using 0.01 M phosphoric acid as the anode solution and 0.02 M sodium hydroxide as the cathode solution.

Both the lysis buffer and cathode solution were removed after prefocusing. Following the application of protein samples, an aqueous solution containing 2% Triton X–100 and 2% ampholyte was overlayed on the top of the protein samples. After the gel plate was reassembled in the electrophoresis unit, fresh cathode
Figure 1 (Left). SDS-PAGE patterns of intact fillet and surimi of Alaska pollock and red hake extracted with water. Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate molecular weights (kD) of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.

Figure 2 (Right). IEF patterns of intact fillet and surimi of Alaska pollock and red hake extracted with water (cathode on top). Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate apparent pI values of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.
patterns can hardly be differentiated as being AS alone or as the combination of AS and RS. However, the use of the IEF patterns, as indicated in the later sections, will help the identification process.

The study with IEF showed that Alaska pollock had specific protein bands with pI values of 6.61 and 5.13 while the red hake had specific bands of 6.85, 6.79, 6.67, 6.35 and 4.63 (Fig. 2). These specific bands were evident in the fish and fish–surimi mixtures. The 5.22 band of Alaska pollock and red hake, the 5.13 band of Alaska pollock, and the 4.63 band of red hake were missing in the surimi samples. Two bands with pI values of 5.71 and 5.62 were found in Alaska pollock surimi. The reason for their presence in surimi and not in the corresponding fish is not known.

The identification of fish species used in surimi was made possible using a urea IEF system. The patterns of AS and RS could be differentiated from each other using the specific bands of 5.71 and 5.62 for AS and 6.85 and 4.63 for RS. These specific bands were also present in the AS+RS mixture; this makes the differentiation of AS, RS, and AS+RS possible.

Water extraction of cooked fish and surimi

Heat treatment of fish and/or surimi greatly reduced the number of water–extractable protein bands present on the SDS–PAGE and IEF gels (Figs. 3 and 4). Minor bands with MW’s of 21.7 and 23.6 kD were specific for the cooked surimi of Alaska pollock and red hake, respectively (Fig. 3). Therefore they could be used for species identification of a surimi in mixture (AS+RS) containing Alaska pollock and red hake. The 19.9 kD band was specific for the cooked Alaska pollock fillet and could be used to indicate the presence of Alaska pollock in a AF+RF fish mixture.

The IEF study also indicated that a protein band with a pI of 5.03 was specific for Alaska pollock whereas the one at 4.56 was specific for red hake (Fig. 4). The 5.09 band was detected only in the water extracts of the two cooked fish samples but not in the surimi, whereas the 4.70 band was detected only in cooked Alaska pollock surimi. The 4.82 minor band of the Alaska pollock fillet was not detected in the corresponding surimi sample. Differentiation of AS, RS and AS+RS mixture could be achieved from the presence of the 4.70 band for AS and the 4.82 band for RS.

SDS and urea extracts of cooked fish and surimi

The use of SDS and urea greatly enhanced protein extraction from cooked samples than that of water (An et al., 1988a and b). Fish and surimi also showed an increased number of protein bands on the electrophoretic gels after treating cooked samples with SDS and urea. In addition, the banding patterns of the SDS and urea extracts were very similar on the SDS–PAGE gels. The SDS–extractable bands with MW’s of 47.1, 45.9, 35.5, 23.8 and 21.2 kD were specific for Alaska pollock fillet and surimi, and the 25.3 and 20.3 kD bands for red hake fillet and surimi (Fig. 5). For urea extracts, the 46.1, 45.1, 23.6, and 20.7 kD bands, and the 58.2, 24.7 and 19.5 bands were specific for Alaska pollock and red hake, respectively (Fig. 6).
solution was added to the chamber. Proteins were focused at room temperature for 17 hrs at 400 V with circulating water. The gel was stained with Coomassie blue R–250 and destained.

The apparent pI values of the fish and surimi proteins were determined indirectly by comparing their R_f values on the gel with those of the protein standards following the previously described procedures (An et al., 1988b). The protein standards (Broad pI kit, pH 3–10, Pharmacia) contained: trypsinogen, pI 9.30; lentil lectin–basic band, pI 8.65, —middle band, pI 8.45, —acidic band, pI 8.15; horse myoglobin–basic band, pI 7.35, —acidic band, pI 6.85; human carbonic anhydrase B, pI 6.55; bovine carbonic anhydrase, pI 5.85; β-lactoglobulin A, pI 5.20; soybean trypsin inhibitor, pI 4.55; and amyloglucosidase, pI 3.50.

Sodium dodecyl sulfate—polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was performed according to the modified procedure of Laemmli (1970) and O’Farrell (1975) using a Protean II (vertical slab) unit. Slab gels consisted of a running gel (10.4%) and a stacking gel (3.1%). The protein samples were run initially at a constant current of 15 mA/slab and then increased to 30 mA/slab when the marker front reached the running gel. Following electrophoresis, the proteins were stained with Coomassie Brilliant Blue R–250 and destained. Electrophoretic patterns were recorded by developing the positive image using a Kodak Electrophoresis Duplicating Paper. Molecular weights of the protein bands were determined according to the method of Weber and Osborn (1966) and Weber et al. (1972) using a low molecular weight protein kit (Pharmacia) containing phosphorylase b (MW 94 kD), bovine serum albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), soybean trypsin inhibitor (21.1 kD) and α-lactalbumin (14.4 kD).

RESULTS AND DISCUSSION

Water extracts of intact fish and surimi

SDS–PAGE and IEF were shown to be effective in demonstrating species differences in protein patterns of the water extracts of raw fish and surimi samples (Figs. 1 and 2). Using SDS–PAGE, Alaska pollock (AF) showed characteristic bands with MW’s of 94.9, 89.5, 88.3 and 79.2 kD while the red hake (RF) showed specific bands with MW’s of 87.0, 68.3, 22.7 and 21.9 kD. All these species specific bands were found in the fish and fish–surimi mixtures (Fig. 1).

Generally, the surimi samples still showed the basic patterns as those of the fish fillet from which they were made. Some of the characteristic protein bands, such as the 94.9, 88.3 and 79.2 kD of Alaska pollock and the 68.3, 22.7 and 21.9 kD of red hake, were missing in the corresponding Alaska pollock and red hake surimi (AS and RS, respectively) samples possibly due to the washing process during surimi preparation. New bands with MW of 51.3, 23.4 and 20.8 kD were found in Alaska pollock surimi. These bands were also present in the AS+RS mixture and they can be used with reservation to differentiate surimi mixtures. Due to the similarities of the AS and RS banding patterns, the surimi mixture (AS+RS)
Figure 3 (Left). SDS-PAGE patterns of cooked fillet and/or surimi of Alaska pollock and red hake extracted with water. Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate molecular weights (kD) of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.

Figure 4 (Right). IEF patterns of cooked fillet and/or surimi of Alaska pollock and red hake extracted with water (cathode on top). Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate apparent pl values of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.
Figure 5 (Left). SDS-PAGE patterns of cooked fillet and/or surimi of Alaska pollock and red hake extracted with SDS. Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate molecular weights (kD) of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.

Figure 6 (Right). SDS-PAGE patterns of cooked fillet and/or surimi of Alaska pollock and red hake extracted with urea. Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate molecular weights (kD) of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.
Figure 7 (Left). IEF patterns of cooked fillet and/or surimi of Alaska pollock and red hake extracted with SDS (cathode on top). Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate apparent pI values of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.

Figure 8 (Right). IEF patterns of cooked fillet and/or surimi of Alaska pollock and red hake extracted with urea (cathode on top). Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate apparent pI values of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.
From the specific banding patterns and the presence of species-specific protein bands displayed on IEF gels, the species origin of the SDS and urea extracts of fish and/or surimi samples could be achieved (Figs. 7 and 8). More protein bands were extracted by urea from cooked samples than by SDS or water (Figs. 4, 7 and 8). For SDS extracts, the bands with pI values of 5.72, 5.12 or 4.80 were found specific for Alaska pollock surimi, fish fillet, or both, respectively; whereas the bands of 6.85, 6.74, 5.22 and 4.64 were specific for red hake fish sample but not the surimi. The 5.49 band of red hake was specific both for the fish and surimi. Differentiation of AS, RS and AS+RS mixture could be achieved from the presence of the 5.49 band of RS and 4.80 band of AS (Fig. 7). For urea extracts, the protein patterns on the IEF gels were similar between the fish and surimi samples even though some specific bands were missing in surimi samples. The 5.65 band was specific for Alaska pollock, whereas the 5.59 and 5.54 bands were specific for red hake (Fig. 8). The 5.10 and 4.94 bands of the Alaska pollock fish, the 5.17 and 4.71 bands of the Alaska pollock fish, and the 5.17 and 4.71 bands of red hake were missing in their respective surimi samples and therefore could be used for identification purposes in this instance.

CONCLUSION

The SDS–PAGE and IEF methods were shown in this study to be effective in distinguishing the species specificity between Alaska pollock and red hake of raw or cooked fish and surimi samples. Although water is a good protein extractant for raw samples, it does not provide the specificity needed for cooked samples. The use of SDS or urea, however, will increase the number of proteins extracted from cooked samples of fish or surimi and thus improve the effectiveness of species differentiation with SDS–PAGE or IEF. This method appears to have potential application for identifying raw and cooked fish species and surimi samples.

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