Original Research Paper

Seasonal variations in proximate and fatty acid composition of golden grey mullet *Liza aurata* (R, 1810) from the Tunisian coast

Ilhem Ketata Khitouni¹, Nourhène Boudhrioua Mihoubi², Abderrahmen Bouain¹ and Faouzi Ben Rebah³*

¹UR Biodiversité et Ecosystèmes Aquatiques, FSS, Sfax, Tunisia.
²UR Ecophysiology et Procédés Agroalimentaires, ISB-Sidi Thabet, Tunisia.
³Laboratoire de Biochimie et de Génie Enzymatique des Lipases, ENIS, Sfax-Tunisia.

* Corresponding Author
Email: benrebahf@yahoo.fr
Tel.: +21674675055

The aim of this work was to determine the seasonal variations of chemical composition of *Liza aurata* of the coastal catch from the Gabes Gulf, Tunisia. Maximal water were reached in July for females (77.89 ± 0.27 mg / 100 g of fresh fish) and in May for males (77.68 mg / 100 g of fresh fish). Minimal water values were reached during September for both females and males (63.77 ± 0.45 and 64.86 ± 0.16 mg /100 g of fresh fish, respectively). Negative correlations between fat and water contents were observed. The higher fat contents were recorded during September for males and females (10.13 ± 0.07 % and 15.07 ± 0.12 mg / 100 g of fresh fish, respectively). Higher protein contents were observed over the year (except May, July and August for males and after the spawning period for females, from July to September). Palmitic acid was the most abundant fatty acid ranging from 20.03% to 31%, apart from the female in summer where oleic acid and palmitic acid were estimated at 28.98 and 23.35%, respectively. Oleic acid was the main unsaturated fatty acid, ranging from 13.36% to 28.98%. *Liza aurata* muscle contained appreciable levels of Omega-3 polyunsaturated fatty acids (14.47 % - 23.23 %) suggesting the use of this fish as a source of healthy diet for humans.

Key words: water, fat, protein, mineral element, fatty acids, fish muscle, *Liza aurata*.

INTRODUCTION

The chemical composition of fish muscle varies greatly depending on species, sexual cycle, age, feed, stage of maturity, environment, season, organs and muscle location (FAO, 2002; Noël et al., 2011 and Roy et Lall, 2006). Actually, seafood products are well known to provide significant amounts of different beneficial nutrients such as proteins, essential minerals and lipid with highly unsaturated fatty acids (Simopoulos, 1997). However, various fish species do not provide the same nutrient profile depending on the seasons. Generally, fish is the excellent source of protein, because of the amino acid composition and degree of digestibility (Louka et al., 2004). Moreover, fish fat is subject of a great deal of attention due to its high content of ω-3 polyunsaturated fatty acids (ω-3 PUFA) having a positive effect in the prevention of certain human diseases like hypertension, inflammation, psoriasis, aggression, depression and cancer (González et al., 2006; Haliloglu et al., 2004). Interestingly, great seasonal variations of fatty acid composition for several marine organisms were reported in response to various factors. For example, the saturated fatty acids increased during the months of high fat content; period of the enhanced feeding activity (Gockse et al., 2004; Zlatanos and Laskaridis, 2007).

To ensure the nutritional value as well as eating quality of fish, the seasonal biochemical evaluation is necessary. In Tunisia, Gabes Gulf coast is considered a main seafood resource (Ben Rebah et al., 2009; DGPA, 2004), very little information is available on the biochemical constituents in
relation to reproductive cycle of commercial Tunisian fishes. *Liza aurata* constitutes the large portion of catches and the highly consumed fish species in Tunisia (Fischer, 1987). In order to use this specie as good as possible, the need for further studies aiming to elucidate many factors such as the amount and the variation of its composition over the year are of significance. Hence, the objective of this study was to examine the seasonal changes over the year in the chemical composition of *Liza aurata* caught in the Gabes gulf area.

**MATERIALS AND METHODS**

**Raw material and samples preparation**

This study was carried out from December to November 2011. Fish samples of *Liza aurata* species were obtained monthly from Gabes gulf area, Tunisia. Fishes were rapidly transported on ice to the laboratory for preparation to chemical analyses. The total length (20-30 cm) and the total weight (80-200 g) of fish were measured in order to select homogenous samples (superior to sexual maturity size). Weight of samples was determined by using a precision scale 10⁻⁴ g (Sauter). The total muscles, latero-dorsal and latero-ventral were dissected and used in the present work. Further, females and males were analyzed separately because the species shows sexual dimorphism. Water content was measured for every ten samples (both males and females) by drying in an oven at 105°C to a constant weight for 48 h. Dry samples were crushed by a Moulinex® blender. The fish dry powder of fish muscle was divided in 3 parts to determine the protein, fat and ash contents and repeated three times. All chemical analyses were expressed in g/100 g of fresh fish. According to the Association of official analytical chemists (AOAC, 2000) methods, fat content was quantified by Soxhlet extraction, proteins by Kjeldahl procedure, and ash by incineration in a muffle furnace at 550°C. Analyses were performed over the year.

In order to determine fatty acid composition, the method of Bligh and Dyer (1959) was used. A homogenized fresh sample (25g) was extracted using chloroform/methanol/water mixture (5V/10V/5V). Fats extracts were converted into fatty acid methyl esters (FAME) using acetylchloride and then analyzed by gas-liquid chromatography (Perkin-Elmer 8700 chromatograph, Madrid, Spain) according to Aubourg et al., (1991). A fused silica capillary column SP-2330 (0.25 mm i.d.× 50 m, Supelco, Inc., Bellefonte, PA, USA) was employed and the temperature program was as follows: increased from 145 to 190 °C at 1.0 °C/min and from 190 °C to 210 °C at 5.0 °C/min; held for 13.5 min at 210 °C. The carrier gas was nitrogen at 10 psig and detection was performed with a flame ionization detector at 250 °C. A programmed temperature vaporizer injector was employed in the split mode (150:1) and was heated from 45 to 275 °C at 15 °C/min. Peaks corresponding to FAME were identified by comparing their retention times with those of standard mixtures (Qualmix Fish, Larodan, Malmo, Sweden; FAME Mix, Supelco, Inc.). Peak areas were automatically integrated; 19:0 fatty acid was used as internal standard for quantitative purposes. Content of each fatty acid was expressed as percentage weight of total fatty acids (% wt).

The mineral element contents (Ca, Na, Mg, Fe, Zn and K) were determined by an inductively coupled plasma optical emission spectrophotometer (ICP-OES) (Perkin-Elmer, Model 4300 DV, Norwalk, CT) according to the AOAC (2000) method.

**Estimation of the energetic value (calorific value) of fish muscle**

The energetic value of each sample was determined by multiplying the percentages of protein (PC) and fat (FC) contents with their respective standard factors of 4 and 9 kcal/100 g of fish sample (Jabeen and Chaudhry, 2011) using the following equation:

\[
\text{Caloric value} = (4PC + 9FC) \text{kcal/100 g weight.}
\]

**Statistical analysis**

Statistical analysis were performed by using SPSS software® version 11.0 (Statistical Package for Social Sciences). Values are expressed as mean ± standard deviation. Variance analysis was performed for water, protein, fat and ash contents measured in the muscle according to the factors sex, size of the fish species to determine the pertinent factors affecting the chemical contents (p < 0.05). Correlation matrixes were established between the measured variables (water, protein, fat and ash). Honestly, significant difference (HSD) with ANOVA one factor was performed for establishing the index of significance for histograms plate. Every factor presenting a p-value (p) inferior to 0.05 was considered significant.

**RESULTS**

**Global chemical composition**

Variations in chemical composition of *Liza aurata* for both fish sexes are presented in Figure 1. Water content ranged from 64.86 in September to 77.89 % in July for females (Figure 1A), whereas the lowest levels for both sexes were recorded in September. The highest values of the fat content (10.13 % for males and 15.07 % for females) were observed for the same period of time, (Figure 1B). Water content correlated negatively with fat content (R=−0.91; p< 10⁻³). In addition, the lowest values of the fat content were found in the period from February to July. The average annual fat content of the *Liza aurata* muscle is 4.58% and 5.09% for males and females, respectively. The protein
Figure 1: Seasonal variations of Water content MC (A), fat content FC (B) protein content PC (C), and ash content AC (D) of *Liza aurata*; mean values ± standard deviations; Values followed by the same small letters (a, b, c, d, e, f, and g) do not share significant differences at p < 0.05.

Table 1. Variance analysis of water, protein, fat and ash contents of *Liza aurata* according to sexes and season

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Content</th>
<th>F (Fisher number)</th>
<th>p (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Water</td>
<td>69.558</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>1.47</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>45.61</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Ash</td>
<td>154.80</td>
<td>0.001</td>
</tr>
<tr>
<td>Season</td>
<td>Water</td>
<td>7.25</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>1.43</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>6.11</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Ash</td>
<td>5.48</td>
<td>0.04</td>
</tr>
<tr>
<td>Sex × Season</td>
<td>Water</td>
<td>0.51</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>2.87</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>0.48</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>Ash</td>
<td>0.06</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Season: Winter (Dec-Jan-Feb), Spring (Mar, Apr, May), Summer (June, July, Aug) and Autumn (Sept, Oct, Nov).

content was high for the fish muscle over the year (19.93% for males and 20.20% for females). For the ash content (Figure 1D), *Liza aurata* muscle showed a fluctuation especially in October (2.65% and 2.95% for males and females, respectively). The average annual ash content of the *Liza aurata* muscle is about 1.56% for males and 1.59% for females (Figure 1C). Protein content correlated positively with ash content (R=0.51; p< 10^{-3}). The variance analysis of water, protein, fat and ash contents according to fish sex and season factors, and the fish sex season interaction is presented in Table 1. Season had a significant effect on ash content. However, the fish sex had a significant effect on water and ash contents. Moreover protein content varied significantly as a function of fish sex season interaction. Similarly, the fish energetic values (Figure 2) varied over the year.

### Fatty acids composition

The fatty acid profile of muscle of male and female *Liza*
Figure 2: Seasonal variations of energetic values of *Liza aurata*; Values followed by the same small letters (a, b, c, d, e, f and g) do not share significant differences at p < 0.05.

*a. aurata* exhibited a dominance of the saturated fatty acids (SFAs) < polyunsaturated fatty acids (PUFAs) < monounsaturated fatty acids (MUFAs) (Table 2). The lowest SFAs values presented in spring with 35.62% for males and 40.08% for females and the highest values observed in summer with 55.58% for males and 45.96% for females. For the total MUFAs, values ranged between 16.74% in summer and 30.83% in autumn (for males) and between 26.48% in winter and 34.37% in summer (for females). However, the total PUFAs varied between 23.53% in winter and 34.59% in spring (for males) and between 19.67% in summer and 33.09% in spring (for females). ω-3 fatty acids are the most important fat fraction of the PUFAs in fish muscle, which their content varied between 16.72% in spring and 21.84% in summer (for males) and between 14.47% in summer and 23.23% in winter (for female). The PUFAs contained essentially the eicosapentaenoic (C20:5) and the docosahexaenoic acids (C22:6) with maximum values 3.92% for males in spring and 7.09% for females in winter; and 18.82% for males in summer and 17.36% for females in autumn. PUFAs are also formed by other minor fatty acids such as: linoleic acid (C18:2), linolenic acid (C18:3), arachidonic acid (C20:4) and docosapentaenoic acid (C22:5). In all the year, the fish muscle presented the higher value of ω-3 fatty acids for males and females. A high $\Sigma \omega 3/\Sigma \omega 6$ ratio of fish muscle was observed essentially in winter (8.44% for males and 5.35% for females) showing the predominance of ω-3 fatty acids. The sum of eicosapentaenoic (EPA, C20:5 ω-3) and docosahexanoic (DHA, C22:6 ω-3) acids varied between 16.37% in spring and 21.34% in summer (for males) and between 13.97% in summer and 22.90% in winter (for females). The main SFA was palmitic acid (C16:0) with values varying from 20.03% to 30.98% of the total SFAs (Table 2). The MUFA fraction was predominated by oleic acid (C18:1) for males, ranging from 35.15% in spring to 24.11% in winter and for females from 13.36% in spring to 28.98 in summer.

**Mineral composition**

Mineral composition of Fe, Zn, K, Mg, Na and Ca in muscle of *Liza aurata* are shown in Table 3. The highest mineral content was observed in spring with 74.30 mg/100 g for males and in summer with 51.51 mg/100 g for females. Interestingly, the relative abundance of minerals was Ca > K > Na > Mg > Fe > Zn, except in spring K was higher then Ca. The highest Ca content was found in summer for males (28.02 mg/100 g) and in autumn for females (25.59 mg/100 g). However, K content varied between 9.67 mg/100 g in winter and 36.25 mg/100 g in spring for males and between 14.49 mg/100 g in spring and 18.47 mg/100 g in winter for females. Fe contents varied between 0.03 mg/100 g (in spring for females) and 0.20 mg/100 g (in winter for males). Mg had the highest values in spring (5.24 mg/100 g for males). In spring, the Na element showed the highest values for males (10.94 mg/100 g) and in winter for females (8.61 mg/100 g). Generally, season, sex and season/sex interaction illustrated significant effect on Fe,
Zn, Na, Ca, K and Mg variations (p < 10^-4).

DISCUSSIONS

From this study, dedicated to specimens of *Liza aurata* caught from Gabes gulf area (Tunisia), it can be concluded that the proximate composition present seasonal variations in the water, fat, protein, and ash contents in response to various factors. Commonly, the chemical composition variations have been described and reported to be related to many factors (season, temperature, location, breeding cycle, diet, age, size, sex, etc.) (Rajasilta, 1992; Bandarra et al., 1997). Water was the major constituent in all parts of muscle and the highest value was found in May for males and July for females (>77%). As reported by diverse studies (Garcia-Arias et al., 1994), water content varied widely with seasons, and in inverse proportion to fat content (minimal water content and maximal fat content found in September). Protein content remained constant at a high level over the year as reported by many studies (Ben Rebah et al., 2009; Njinkoue et al., 2002; Tzikas et al., 2007). Heterogeneous distribution of protein content in female specie was also observed before spawning (January-August). The period of the gonadic maturation extending between September and December corresponded to fish muscle having a higher nutritional value (20.69% < protein < 21.54%; 4.57% < fat < 10.13% and 1.31% < ash < 2.65% for males; and 18.75% < protein < 22.75%; 4.28% < fat < 15.07% and 0.98% < ash < 2.95% for females).

Before the gonadic maturation (from January to August) the fish samples showed a medium nutritional value (17.74% < protein < 21.94%; 1.18% < fat < 8.14% and 1.22% < ash < 1.65% for males; and 17.41% < protein < 21.91%; 1.22% < fat < 10.77% and 1.28% < ash < 1.68% for females). Therefore, *Liza aurata* could be considered as a good fish quality with higher nutritional values over the year (121.03 Kcal/100 g of fresh fish for males and 126.70 Kcal/100 g of fresh fish for females annually). This result is in agreement with these reported for *Mugil cephalus* specie belonging to the same family (Udo and Arazu, 2012). Nevertheless, during the period of the gonadic maturation (between September and December), the average nutritional values were 153.97 Kcal/100g and 156.04.
Kcal/100 g for males and females, respectively. Before the gonadic maturation, both males and females have medium nutritional values. The high fat and protein content in the gonadic maturation can be explained by the fact that fish use their protein and fat contents during the spawning period (Sharer, 1994).

Golden grey mullet is considered as a fatty fish (> 5% fat) as reported by Boyer et al. (1995) and fat content is stored in the muscle (Bougis, 1952). In this perspective, it was reported that muscles of highly active species are richer in lipids as compared with species characterized by low functional activity (Yuan et al., 2005). Furthermore, ecological and physiological peculiarities of fishes significantly affect the lipid fatty acids composition. The high amounts of saturated and monounsaturated fatty acids in Liza aurata muscles are almost in agreement with data obtained from other studies (Dey et al., 1993). It seems that the fish diet contain higher SFA and MUFA, but deficient in PUFA (Mnari et al., 2010). Palmitic acid (C16:0) was the main fatty acid contributing of the total SFA content of lipid for all Liza aurata samples in spite of the one exception of females in summer (Oleic acid is 28.98 ± 0.1 while palmitic acid is 23.35 ± 0.11). This result is in good agreement with those reported in previous research (Erdem et al., 2009; Mnari et al., 2010). It is also important to mention that the ω3/ω6 ratio is important in the Tunisian Liza aurata. This ratio is very important in human dietary, because is implicated in reducing of cancer risk and in the prevention of heart disease, shock syndrome and cardiomyopathy (Kinsella et al., 1990; Bell et al., 1991). Therefore, this ratio is a good index to compare fish oil relative nutritional values (Piggott and Tucker, 1990). Interestingly, the total ω-3 fatty acid content (14.47-23.23%) was at acceptable levels compared to the marine fish oil from the Parangipettai, Southeast Coast of India, Mugil cephalus (21.83%) (Kumaran et al., 2012). Liza aurata seems to be a good source of ω-3 fatty acids recognized for their health benefits (Leaf and Weber, 1988). Among the ω-6 fatty acids, Liza aurata accumulated arachidonic acid (C20:4) in spring (13.55% for males and 12.31% for females) which is a precursor for prostaglandin and thromboxane biosynthesis. It influences blood clot formation and its attachment to the endothelial tissue during wound healing (Pompeia et al., 2002).

Concerning the mineral content of Liza Aurata, among the essential elements analyzed, Ca, K, Na and Mg were found to be the most abundant, whereas Zn and Fe were presented at very low levels. The most important elements are essential to cellular metabolism and commonly found at high concentrations in biological tissues (Wagner and Boman, 2003). Element with lower levels (Zn and Fe) are widely known to be present in enzyme active centers that are responsible for the development of important functions in all animals. Thus, marine-derived foods can serve as a good source of essential elements (Johnson and Fischer, 1994; Lal, 1995). In fact, marine organisms absorb minerals from their diet and the surrounding water and deposit them in their skeletal tissues and organs. As reported for protein and lipid, mineral content varied with seasons. Generally, the concentration of minerals in fish is influenced by a number of factors such (species, size, dark/white muscle, age, sex, sexual maturity, area of catch, food source, water chemistry, salinity, temperature and contaminants, etc.) (Ketata Khitouni et al., 2011; Noël et al., 2011; Yildiz, 2008). Furthermore, it has been reported that intrinsic factors such as growth (size, weight), age, sex, sexual maturity, fish mobility physiology and stress influence the accumulation of trace metals in marine organisms (Phillips and Rainbow, 1993).

A season is an important factor that could affect considerably the chemical composition of fish and consequently the quality of fish species. Liza aurata species from the Gabes Gulf (Tunisia) was found to be a good source of protein and fat. It contained essential fatty acids, particularly ω-3 with beneficial effects on health. The high energetic and nutritional values over the year and spatially in the gonadic maturation period, indicates the important nutritional quality of this fish species. The significant seasonal differences observed in the fish contents (water, fat, protein, mineral elements, etc.) are mainly related to the fish diet but also to various environmental factors.

### Table 3. Seasonal variation of mineral element contents in the muscle of Liza aurata

<table>
<thead>
<tr>
<th>Season</th>
<th>Sex</th>
<th>Fe</th>
<th>Zn</th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>Male</td>
<td>0.20 ± 0.01 d</td>
<td>0.04 ± 0.00 a</td>
<td>18.88 ± 0.03 b</td>
<td>9.67 ± 0.2 a</td>
<td>1.6 ± 0.01 a</td>
<td>8.02 ± 0.8 b</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.20 ± 0.0007 d</td>
<td>0.05 ± 0.001 c</td>
<td>18.2 ± 0.01 b</td>
<td>18.7 ± 0.01 d</td>
<td>2.1 ± 0.05 b</td>
<td>8.61 ± 0.04 d</td>
</tr>
<tr>
<td>Spring</td>
<td>Male</td>
<td>0.09 ± 0.002 b</td>
<td>0.07 ± 0.0007 c</td>
<td>21.69 ± 0.006 c</td>
<td>36.25 ± 0.005 d</td>
<td>5.24 ± 0.003 d</td>
<td>10.94 ± 0.06 d</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.03 ± 0.0009 a</td>
<td>0.004 ± 0.0006 a</td>
<td>9.21 ± 0.003 a</td>
<td>14.49 ± 0.008 a</td>
<td>1.80 ± 0.1 a</td>
<td>3.89 ± 0.1 a</td>
</tr>
<tr>
<td>Summer</td>
<td>Male</td>
<td>0.06 ± 0.002 a</td>
<td>0.09 ± 0.0007 d</td>
<td>28.02 ± 0.06 d</td>
<td>14.81 ± 0.01 b</td>
<td>4.72 ± 0.01 c</td>
<td>7.41 ± 0.1 a</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.05 ± 0.001 b</td>
<td>0.09 ± 0.001 d</td>
<td>22.93 ± 0.005 c</td>
<td>17.31 ± 0.002 c</td>
<td>3.58 ± 0.01 d</td>
<td>7.53 ± 0.007 b</td>
</tr>
<tr>
<td>Autumn</td>
<td>Male</td>
<td>0.19 ± 0.001 c</td>
<td>0.06 ± 0.001 b</td>
<td>17.42 ± 0.009 a</td>
<td>18.90 ± 0.001 c</td>
<td>2.46 ± 0.004 b</td>
<td>8.52 ± 0.007 c</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.17 ± 0.002 c</td>
<td>0.03 ± 0.001 b</td>
<td>25.59 ± 0.006 d</td>
<td>15.31 ± 0.001 b</td>
<td>2.27 ± 0.001 c</td>
<td>7.63 ± 0.008 c</td>
</tr>
</tbody>
</table>

Mean values ± SD; Values in the same row with different letters are significantly different (p<0.05).
REFERENCES


quality of wild and farmed sea bass, *Dicentrarchus labrax* L. Aquacul., 249: 175-188.


