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Full Length Research Paper

Biochemical composition of three Tunisian silverside (fish) populations caught in open sea, lagoon and island coasts

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Fatty acid and amino acid profiles were determined in three silverside populations caught in Tunisian waters *Atherina boyeri* (open sea), *Atherina lagunae* (lagoon) and *Atherina* sp. (island coasts). Saturated fatty acids reached in total lipids 43.54%, 36.96% in marine and 33.64% in insular silverside and *A. lagunae*, in which eicosapentaenoic acid, docosahexaenoic acid and linoleic acid were the prominent fatty acids. The n-3/n-6 index showed a significant level indicating a tendency to accumulate n-3 fatty acids in *A. boyeri* and *A. lagunae* and n-6 fatty acids in *Atherina* sp. Total amino acid content ranged from 528 to 588 mg/g crude protein, in which, glutamic acid was the most abundant. Methionine had the lowest essential amino acid score in *A. boyeri* and *Atherina* sp. (0.73 and 0.71, respectively) while tryptophan had the lowest in *A. lagunae* (0.07).

Key words: Silverside populations, fatty acids, amino acids, biochemical composition.

INTRODUCTION

Fish is a major source of food for human nutrition providing an important amount of dietary protein and lipid diet in many countries. Fish flesh is easily digestible because it contains long muscle fibres. Furthermore, it has been linked to health benefits, such as the prevention of cardiovascular diseases and some types of cancer, including colon, breast and prostate (Rose and Connoll, 1993; Marchioli, 2001, 2002; Sidhu, 2003). These effects are largely attributable to the polyunsaturated fatty acids (PUFA) found in fish oils especially the n-3 family including the eicosapentaenoic acid (EPA or 20:5 n-3), the docosapentaenoic acid (DPA or 22:5 n-3) and the docosahexaenoic acid (DHA or 22:6 n-3). It is also reported that n-3 PUFA have been recognized as important substances with beneficial properties for the improvement of visual function (Carlson and Werkman, 1996) and also for the prevention of atherosclerosis and thrombosis development (Calder, 2003). Furthermore, arachidonic acid (20:4 n-6) and eicosa-pentaenoic acid can be metabolised to a variety of eicosanoids for hormonal substances synthesis. These beneficial PUFA can be divided into two biochemical families, n-3 and n-6, with different biological effects (James and Cleland, 1996).

Atherina is a genus of small inshore fishes with many populations living in brackish, marine and freshwater. It is distributed in the Eastern Atlantic Ocean and Mediterranean Sea, extending to the south along the African coast into the Indian Ocean (Quignard and Pras, 1986). Works on *Atherina boyeri* (Risso, 1810), in the eastern and western Mediterranean Sea (Kiener and Spillmann, 1969; Marfin, 1982; Trabelsi and Kartas, 1985; Trabelsi

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Abbreviations: PUFA, Polyunsaturated fatty acids; **EPA**, eicosapentaenoic acid; **DPA**, docosapentaenoic acid; **DHA**, docosahexaenoic acid; **FAMEs**, fatty acids methyl esters; **HPLC**, high performance liquid chromatography; **SFA**, saturated fatty acids; **MUFA**, monounsaturated fatty acid; **EAA**, essential amino acid; **P-PER**, predicted protein efficiency ratio.

Table 1. Total length and body weight of three Tunisian silversides populations: *A. boyeri* (Bizerta Sea), *A. lagunae* (Bizerta Lagoon) and *Atherina* sp. (Kerkannah's Islands).

Parameters	*At. boyeri*	*A. lagunae*	*Atherina* sp.	AV
Total length (cm)	8.18 ± 0.18 a	8.02 ± 0.13 a	4.65 ± 0.10 b	***
Body weight (g)	4.82 ± 0.27 a	3.64 ± 0.18 b	0.76 ± 0.06 c	***

Means (n = 88) with the same letter within rows are not significantly different (p > 0.05); AV: analysis of variance; * significant at 0.05; ** significant at 0.01; *** significant at 0.001.

et al., 2002) showed that the species includes two distinct groups of populations, a homogenous group of marine atherinids and a heterogeneous group of atherinids populating lagoons.

Despite the numerous cited studies on molecular and biometric analysis of the different groups of atherinids, no detailed biochemical studies of the lipid and protein contents of these small inshore fish are available. *A. boyeri* and *Atherina lagunae* constitutes a moderate portion of catches and are most often consumed after solar dryness essentially in the south of Tunisia. The objective of the present work is to evaluate the nutritional value (fatty acids and amino acids profiles) of three silversides populations originated from marine, lagoon and island Tunisian coasts.

MATERIALS AND METHODS

Samples collection and preparation

The three Tunisian silverside populations *A. boyeri*, *A. lagunae* and *Atherina* sp. were caught respectively from Bizerta Sea, Bizerta Lagoon and Kerkannah's islands in September 2007. Samples were kept in ice, transported immediately to the laboratory where they were rapidly weighted, measured, frozen in liquid nitrogen and stored at -40°C until analysis. The average weight and length of sampled fish ranged between 0.76 to 4.82 g and from 4.65 to 8.18 cm, respectively (Table 1).

Biochemical analysis

Moisture

Moisture of the fish samples (5 g) was determined according to the AOAC (1990) method by drying in an oven at 105°C (n = 6). Results were expressed as percentage of wet weight.

Ash

Ash content was determined by burning sample (5 g) for 12 h in a furnace at 525°C (n = 6) according to the AOAC (1995) method. Results were expressed as percentage of wet weight.

Protein

Total protein content in the homogenized samples (5 g) was determined using Kjeldahl method (AOAC, 1990). Results were expressed as percentage of wet weight (n = 3).

Lipids extraction

Total lipids were extracted according to the method of Folch et al. (1957), using chloroform/methanol (2/1). Aliquots of the chloroform layer extract were evaporated to dryness under nitrogen and the lipids were quantified gravimetrically.

Fatty acid analysis

Fatty acids methyl esters (FAMEs) were obtained by the method described by Metcalfe et al. (1966). A fraction of the lipid extract was saponified with 0.5N NaOH in methanol followed by methylation in 14% boron trifluoride in methanol (BF_3/MeOH). The methylated sample was then extracted with 8 ml n-hexane. All of these reactions were performed in quadruplet for each sample. The resulting methyl esters were analysed using an Agilent Gaz chromatograph system 6890 N equipped with a flame ionization detector (FID), a splitless injector and a polar INNOWAX fused silica capillary column (30m * 0.25 mm i.d. * 0.25 µm film thickness). The temperature of the injector and the detector were 250 and 275°C respectively. Helium was used as a carrier gas with a flow rate of 1.5 ml/mn. Peaks were identified by comparison of their retention times with PUFA 3 FAMEs standards (SUPELCO).

Amino acids analysis

Total amino acid composition was determined using an Agilent chromatograph L1100 high performance liquid chromatography (HPLC) equipped with a quaternary pump, a 20 µl injection valve and a diode array and fluorescence detectors. Mobile phase A was 10% of acetonitrile/methanol/water (45:45:10; v/v/v) and B was 90% of sodium phosphate buffer Na_2HPO_4 (pH 6.5). The flow rate was constant at 1 ml/min, and the column temperature was set at 25°C. The fluorescence excitation and emission wavelengths were 340 nm of λ_{ex} and 450 nm of λ_{em}, respectively. Samples were hydrolysed in 6 M HCl in evacuated sealed tubes at 110°C for 24 h. After derivatization by O-phtalaldehyde, amino acids were identified by comparison of their retention times with those of standards (Sigma) and quantified with the software EZChrom Elite™ CDS Chromatography, using Sigma amino acids as external standard. The results were expressed in term of mg amino acid per g of protein. The predicted protein efficiency ratio (P-PER) was determined using one of the equations developed by Alsmeyer et al. (1974)

$$\text{P-PER} = -0.468 + 0.454\ (\text{Leu}) - 0.105\ (\text{Tyr})$$

The essential amino acid index is calculated using the ratio of the quantity of each essential amino acid in a protein to the quantity of the same amino acid in a reference protein and this ratio was multiplied by 100. Next, the $\log_{10}$ of each ratio and the 8 values obtained were summed and divided by 8 to obtain a mean value.

Table 2. Proximate composition (% wet weight) of three Tunisian silversides populations: *A. boyeri* (Bizerta Sea), *A. lagunae* (Bizerta Lagoon) and *Atherina* sp. (Kerkannah's Islands).

Parameters	*A. boyeri*	*A. lagunae*	*Atherina* sp.	AV
Moisture (%)	71.99 ± 0.54 a	73.31 ± 0. 1 b	74.48 ± 0.22 b	**
Protein (%)	19.41 ± 1.44 a	19.11 ± 1.63 a	17.86 ± 1.14 a	NS
Lipid (%)	6.20 ± 0.46 a	6.09 ± 0.12 a	5.03 ± 0.03 b	**
Ash (%)	1.98 ± 0.11 a	1.86 ± 0.08 a	2.25 ± 0.13 b	**

Means (n = 6) with the same letter within rows are not significantly different (p > 0.05). AV: analysis of variance; NS: non significant; *significant at 0.05; ** significant at 0.01; *** significant at 0.001.

Finally the antilog of this mean was obtained. By convention, the essential amino acid pattern (gAA/100 g protein) for whole egg was used, which is: Lysine (6.98), methionine + cystine (5.79), threonine (5.12), isoleucine (6.29), leucine (8.82), valine (6.85), phenylalanine + tyrosine (9.89), tryptophan (1.49).

Statistical analysis

Statistical analysis was performed using SPSS software, version 10.0. The comparison of different biochemical parameters were tested using Duncan's test (95% confidence interval) with one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Proximate composition

Table 2 shows the proximate composition of three silversides populations from Bizerta sea *A. boyeri*, Bizerta lagoon *A. lagunae* and Kerkannah's islands *Atherina* sp. during the same period. The lipid and protein contents found in all examined fresh atherines ranged from 5.03 to 6.20% and from 17.86 to 19.41% respectively. The lowest ($p < 0.05$) fat content was found in island silverside *Atherina* sp. The high protein contents and the moderate lipids levels in this small inshore fish are similar to that found in other species such sardine, horse-mackerel and sarda (Bandarra et al., 1997, 2001; Mourente et al., 2001; Zaboukas et al., 2006). Ash content ranged between 1.86 and 2.25%. These levels are higher than those found in other species (Ben-Gigirey et al., 1999; Mazorra-Manzano et al., 2000; Selmi and Sadok, 2008). The high ash content is probably more related to the size of fish and the presence of bones in the samples.

Fatty acids analysis

Fatty acids composition of the three silversides populations is presented in Table 3. Saturated fatty acids (SFA) of total lipid atherina extract constitute the majority of the fatty acids pool, followed by monounsaturated fatty acid (MUFA) and polyunsaturated fatty acids (PUFA). The saturated fraction ranged between 33.64% and 43.54% with palmitic acid C16:0 as the most important fatty acid within this fraction, followed by stearic acid C18:0 and myristic acid C14:0.

The dominance of palmitic acid in fish lipid has been reported by other authors (Bandarra et al., 2001; Osman et al., 2001; Aidos et al., 2002; Passi et al., 2002). This fatty acid percentage seems to be rather constant throughout the year. Ackman (1964, 1966) reported similar data in a study with herring and concluded that this compound did not seem to be influenced by the diet. Total monounsaturated fatty acids (especially C16:1 and C18:1) were more abundant in *A. lagunae* and *Atherina* sp. (36.2 and 32.65%, respectively) and significantly ($p < 0.05$) less important in *A. boyeri* (25.37%).

N-3 polyunsaturated fatty acids content in *A. boyeri* (18.24%) was higher than that found in *Atherina* sp. (11%) and *A. lagunae* (11.04%). Eicosapentaenoic, docosahexaenoic and linoleic acids were the prominent PUFA. The high proportion of n-6 PUFA was found in insular *Atherina* sp. reaching 14.44%. The n-3/n-6 index showed a tendency to accumulate n-3 PUFA in *A. boyeri* and *A. lagunae* and n-6 PUFA in *Atherina* sp.

Amino acids analysis

Table 4 shows the amino acid profile of each silverside population. Both acidic amino acids (glutamic and aspartic acid) had the highest concentrations. Such results are similar to that found by others authors (Adeyeye, 2009; Selmi et al., 2009) in sardine flesh and three Nigerian fish (*Clarias anguillaris*, *Oreochromis niloticus* and *Cynoglossus senegalensis*). Treonine and isoleucine constituted the highest essential amino acid (EAA) concentration in *A. boyeri* (71.53 and 45.6 mg/g protein) and *Atherina* sp. (55.89 and 46.75 mg/g protein), while leucine and isoleucine had the highest concentration of EAA in *A. lagunae* (71.53 and 47.33 mg/g protein). The World Health Organisation recommended valine and isoleucine requirements for school children aged from 10 to 12 years, at the rate of 33 and 30 mg amino acid/kg body weight/day respectively (FAO/WHO/UNU 1985). For example, a 30 kg child will require 990 and 900 mg of valine and isoleucine per day, respectively. The protein values for the three silversides populations (from proximate composition, g/100 g on dry weight basis) were 69.3 (*A. boyeri*), 71.6 (*A. lagunae*) and 69.98

Table 3. Fatty acids composition (% total fatty acids) of three Tunisian silversides populations: *At. boyeri* (Bizerta Sea), *A. lagunae* (Bizerta Lagoon) and *Atherina* sp. (Kerkannah's Islands).

FA	*A. boyeri*	*A. lagunae*	*Atherina* sp.	AV
C14:0	6.41 ± 0.24 a	5.96 ± 0.05 a	4.38 ± 0.02 b	***
C16:0	30.02 ± 0.42 a	25.54 ± 0.01 b	23.03 ± 0.10 c	***
C18:0	5.53 ± 0.04 a	3.85 ± 0.04 b	4.95 ± 0.01 c	***
C16:1 n-7	7.33 ± 0.28 a	17.45 ± 0.09 b	10.62 ± 0.03 c	***
C18:1 n-9	13.82 ± 0.15 a	12.34 ± 0.59 b	18.44 ± 0.27 c	***
C18:1 n-7	1.51 ± 0.02 a	4.17 ± 0.44 b	2.49 ± 0.03 c	***
C20:1 n-9	2.71 ± 0.12 a	2.24 ± 0.01 a	1.11 ± 0.01 b	***
C16:2 n-4	1.02 ± 0.01 a	0.74 ± 0.02 b	1.13 ± 0.08 a	**
C16:3 n-4	1.00 ± 0.02 a	0.79 ± 0.03 b	0.81 ± 0.05 b	*
C18:2 n-6	4.21 ± 0.04 a	4.83 ± 0.08 b	11.54 ± 0.02 c	***
C18:4 n-3	1.80 ± 0.11 a	1.58 ± 0.07 ab	1.32 ± 0.01 b	*
C20:4 n-6	1.22 ± 0.02 a	1.52 ± 0.01 b	2.41 ± 0.01 c	***
C20:5 n-3	6.14 ± 0.12 a	3.62 ± 0.02 b	3.21 ± 0.01 b	***
C22:5 n-3	0.92 ± 0.03 a	0.94 ± 0.01 a	0.86 ± 0.03 a	NS
C22:6 n-3	8.46 ± 0.37 a	4.39 ± 0.14 b	5.16 ± 0.08 c	***
SFA	43.54 ± 0.68 a	36.96 ± 0.09 b	33.64 ± 0.11 c	***
MUFA	25.37 ± 0.41 a	36.20 ± 0.07 b	32.65 ± 0.28 c	***
PUFA	26.57 ± 0.68 a	19.37 ± 0.01 b	27.63 ± 0.10 a	***
n-3	18.24 a	11.04 b	11.00 b	***
n-6	5.97 a	6.78 b	14.44 c	***

Means (n = 4) with the same letter within rows are not significantly different (p > 0.05). AV: analysis of variance; NS: non significant; * significany at 0.05; ** significant at 0.01; *** significant at 0.001.

(*Atherina* sp.). Consumption of 100 g *A. boyeri* would provide about 4280 and 4560 mg of Val and Ile, respectively. If a 30 kg child therefore consumes 100 g of *A. boyeri* per day, his FAO/WHO daily requirements of valine and isoleucine would be met by 432 and 506%, respectively.

The predicted protein efficiency ratio (P-PER) in the three silversides populations (Table 4) were similar to sorghum *ogi* 0.27 (Oyarekua and Eleyinmi, 2004) and lower than that found in three Nigerian fish, *C. anguillaris* 2.22, *O. niloticus* 1.92 and *C. senegalensis* 1.89 (Adeyeye, 2009). A common feature of sorghum and maize is that the proteins of these grains contain a relatively high proportion of leucine, and was suggested that an amino acid imbalance from excess leucine might be a factor in the development of pellagra (FAO 1995). Clinical, biochemical and pathological observations in experiments conducted in humans and laboratory animals showed that high leucine in the diet impairs the metabolism of tryptophan and niacin and is responsible for niacin deficiency (Ghafoorunissa and Narasinga, 1973). It has been showed that biochemical and clinical manifestations of dietary excess of leucine could be counteracted not only by an increasing the intake of niacin or tryptophan but also by supplementation with isoleucine (Belavady and Udayasekhara, 1979). These studies suggested that the leucine/isoleucine balance is more important than dietary excess of leucine alone in regulating the metabolism of tryptophan and niacin and hence the disease process. In the present study, the ratio Leu/Ile was low in value and did not exceed 0.5 in all studied silverside. Table 5 shows that methionine had the lowest essential amino acid score (EAAS) in *A. boyeri* and *Atherina* sp. (0.73 and 0.71 respectively), and tryptophan had the lowest score in Atherina lagunae (0.07). While, threonine and phenylalanine had the highest EAAS in Atherina boyeri, Atherina sp. and *Atherina lagunae*. The lower methionine levels in all silversides populations are probably caused by partial destruction during the amino acid analysis by acidic hydrolysis.

CONCLUSION

Despite the low economic value of atherinids fish, we can assume that *A. boyeri*, *A. lagunae* and *Atherina* sp. are a good source of n-3 and n-6 polyunsaturated fatty acids. The high levels of essential amino acids in *A. boyeri*, *A. lagunae* and *Atherina* sp. will make them a good food source in complementing cereals for weaning foods and their high utilisable energy due to protein will prevent

Table 4. Amino acids profiles (mg/g crude protein) of three Tunisian silversides populations: *At. boyeri* (Bizerta Sea), *At. lagunae* (Bizerta Lagoon) and *Atherina* sp. (Kerkannah's Islands).

Amino acid	*A. boyeri*	*A. lagunae*	*Atherina* sp.
Aspartic acid	42.85	42.99	45.76
Glutamic acid	83.55	84.64	87.39
Serine	21.85	20.18	19.31
Glutamine + Histidine	11.12	13.12	17.99
Alanine + Arginine	91.59	82.40	89.19
Glycine	79.34	63.06	82.4
Treonine	71.53	38.32	55.89
Valine	42.80	43.95	42.33
Methionine	3.13	2.15	2.06
Lysine	15.67	14.44	12.44
Tyrosine	21.75	24.11	23.90
Tryptophan	14.64	11.64	20.45
Phenylalanine	17.95	18.10	18.12
Leucine	20.63	21.80	20.67
Isoleucine	45.60	47.33	46.75
P-PER	0.24	0.27	0.22

P-PER: Predicted protein efficiency ratio.

Table 5. Essential amino acid scores of three Tunisian silversides populations: *A. boyeri* (Bizerta Sea), *A. lagunae* (Bizerta Lagoon) and *Atherina* sp. (Kerkannah's Islands).

Amino acid	*A. boyeri*	*A. lagunae*	*Atherina* sp.
Lysine	1.35	0.81	1.29
Methionine + Cystine	0.73	0.60	0.71
Threonine	2.15	0.44	2.31
Isoleucine	1.86	0.81	1.85
Leucine	1.37	0.97	1.35
Valine	1.80	0.85	1.78
Phenylalanine + Tyrosine	1.60	1.02	1.60
Tryptophan	1.99	0.07	2.24
Mean value	1.61	0.70	1.64

protein-energy malnutrition in their consumers.

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