CHARACTERIZATION OF SEA BREAM (*Sparus aurata*) FROM DIFFERENT FARMS: STUDY OF THE PROTEINS

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RESUME

Le présent travail s'est intéressé à la qualité des protéines dans les produits aquacoles en étudiant les variations du profil électrophorétique réalisé par SDS-PAGE, des protéines sarcoplasmiques (SPP) et myofibrillaires (MFP) chez les daurades royales (*Sparus aurata*) issues de différentes stations A, B et C d'élevage aquacole de la région Nord de la Tunisie. L'analyse de la fraction sarcoplasmiques n'a montré aucune différence significative. Alors que pour la fraction myofibrillaire, l'analyse en composante principale (ACP) des lectures densitométriques a permis de révéler une différence significative ($P<0.05$) entre la station C et les deux stations A et B.

ABSTRACT

The present work analyses the quality of proteins in aquaculture products, by studying changes in the electrophoretic profile performed by SDS-PAGE, for the sarcoplasmic (SPP) and myofibrillar (MFP) proteins fractions in sea bream (*Sparus aurata*) issued from different aquaculture farms (A, B and C) of the Northern region of Tunisia. Analysis of the sarcoplasmic fractions didn't show any significant differences. While for myofibrillar fractions, the principal component analysis (PCA) of densitometric readings showed significant differences ($P <0.05$) between station C and the two others stations, A and B.

INTRODUCTION

The fish muscle texture may be used to assess the freshness (Cheret et al., 2006) and more generally the quality of fish flesh. It is mostly associated with changes in the chemical composition of fish muscle and the muscle protein breakdown (Aussanasuwannakul et al., 2010). In the present work we focused on the qualitative aspects related to the quality of protein in aquaculture products by focusing on the sea bream (*Sparus aurata*) which is widely used in Tunisia. In this sense, the aim of present work is to determine the protein quality of sea breams sampled from three different farms in the Northern region of Tunisia.

MATERIALS AND METHODS

Nine specimens of fresh gilthead sea bream (*Sparus aurata*) were sampled in 3 different aquaculture farms (stations A, B and C) in the Northern region of Tunisia. Fish were killed by immersion in ice water (hypothermia) and transported within 2 hours to the laboratory in a cooler with crushed ice. Upon arrival, the length and weight of the samples were taken, and the internal organs (viscera, gonad and liver) were weighed. The breams were then deheaded and filleted. The masses of the head, the skeleton and the fillets were measured. The sea bream fillets were then chopped and stored in aliquots of 5 g at - 80 ° C.

Fractionation of proteins

A portion of 0.5 g from the chopped fish fillets were suspended with 5 ml of chilled phosphate buffer (15.6mM Na_2HPO_4, 3.5mM KH_2PO_4, pH=7.5) and then centrifuged at 5,000 g for 15 min at 4 °C. The pellet was re-suspended in 5 ml of the same buffer and again centrifuged as mentioned above. Supernatants of both consecutive centrifugations were mixed together to constitute the sarcoplasmic proteins fraction (SPP). The remaining pellets were again extracted twice with 5 ml of chilled phosphate buffer containing potassium chloride (15.6mM Na_2HPO_4, 3.5 mM KH_2PO_4, 0.45 M KCl, pH=7.5) and centrifuged as previously indicated. Supernatants were collected to constitute the myofibrillar proteins fraction (MFP). Samples were kept on crushed ice during the process in order to prevent heating. The SPP and the MFP fractions were split respectively into 3 microtubes, 2 ml each one, and were stored at −80 °C until analysis.

Determination of soluble protein concentrations

The concentrations of the soluble proteins in the SPP and the MFP fractions were determined by using a micro method derived from that of Bradford (1976), using bovine serum albumin (BSA) as standard. In each well of a 96 wells microplate, 250 µl of filtered Bradford reagent (50 mg of Coomassie blue G250, 25 ml of ethanol 95% and 50 ml 85% H3PO4, supplemented with bi-distilled water until 500 ml) reacted with 10 µl of protein extract diluted with the appropriate buffer. After stirring and incubation for 10 min at room temperature, the absorbance was measured at 595 nm using spectrophotometer (µQuant,BioTek, Vermont, USA).

Determination of total protein concentrations

The total protein concentrations were determined according using the method of Lowry modified by Hartee (1972). The lecture was carried out with the spectrophotometer (µQuant,BioTek, Vermont, USA) at 650 nm absorbance, using an Elisa plates.

Electrophoresis SDS–PAGE

The SDS–PAGE (Sodium dodecyl sulphate–polyacrylamide gel) electrophoresis of the SPP and the MFP fractions were applied under denaturing conditions, using the method described by Laemmli (1970). The electrophoretic separation was performed on vertical polyacrylamide gels with a Mini PROTEAN® Tetra System (Bio-Rad, Hercules, CA), using 5% poly-acrylamide for the stacking gels and 15% poly-acrylamide for the separating gels. For pre-treatment, it was used a volume of 20 µL of the SPP or the MFP fractions mixed with 5 µL of sample buffer (60 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS, 0.1% bromophenol blue, 14.4 mM β-mercaptoethanol) which was boiled for 5 min at 100°C. To each gel, it was added proteins molecular weight markers 225, 150, 100, 75, 50, 35, 25 and 10 kDa (Promega, Madisson , USA), and the well were charged with adequate volumes of pre-treated samples. Electrophoresis was performed at a constant current of 30mA per gel. Gels were then stained using Coomassie method Brilliant Blue (R-250), then photographed with a photo-documentation device Digi-Doc-IT (UPV, Upland, CA) and analyzed by Gel-Pro Analyzer software, V4.0 (Media Cybernitics, Bethesda, MD).

Statistics

All of the data obtained were analyzed by analysis of variance (ANOVA), the test of Duncan and the test of Turkey ($P < 0.05$ and $P < 0.01$) with the statistical package SPSS 17.0 (SPSS Chicago, IL, USA).

RESULTS AND DISCUSSION

Total protein, sarcoplasmic (SPP) and myofibrillar (MFP) fractions

-*Different superscripts indicate significant differences ($p<0.05$) between samples.*

The total protein, the SPP and the MFP proteins contents of the gilthead *Sparus aurata* fillets are shown in table I. The total protein and the MFP values differed between fish ($p < 0.05$), the values being the highest for the fish from the station of aquaculture C. The SSP contents didn't differ between the stations.

Table I : Concentrations of total protein, sarcoplasmic (SSP) and the myofibrillar (MFP) protein fractions, in gilthead *Sparus aurata* fillets issued from 3 different aquaculture farms from Northern region in Tunisia.

	A	B	C
Protein (g/100g)	16,51±0,19[a]	16,72±0,33[a]	17,15±0,4[b]
SSP (g/100g)	4,78±0,33[a]	4,76±0,17[a]	5,03±0,44[a]
MFP (g/100g)	10,81±0,14[a]	11,02±0,61[a]	11,36±0,3[b]

-*Values mean ±SEM; n = 3.*

SDS-PAGE separation and densitometry quantification of SSP and MFP protein fraction

The electrophoretic profiles of the SPP fraction (figure 1) allowed to identify 14 major bands in all the profiles made the different samples. These bands corresponded to molecular weights of 100, 62, 50, 41, 36, 33, 26, 24, 23, 22, 18, 15, 10; and 9 KDa. No significant difference were observed between the aquaculture farms. According to Tayohara (1990), the sarcoplasmic proteins bands corresponding to the molecular weights 94, 50, 43, 40 and 35 kDa, represent respectively the enolase phosphorylase, the creatine kinase (CK), the aldolase and the glyceraldehyde-phosphate dehydrogenase GAPDH. While the proteins bands corresponding to approximately 13 and 12 kDa have been assumed to be paravalbumines (Ladrat et al., 2003). The SPP corresponding to molecular weights of 26, 40 and 94 kDa, have been reported as sarcoplasmic proteins responsible for the gelling in fish (Tadpitchayangkoon et al, 2009).

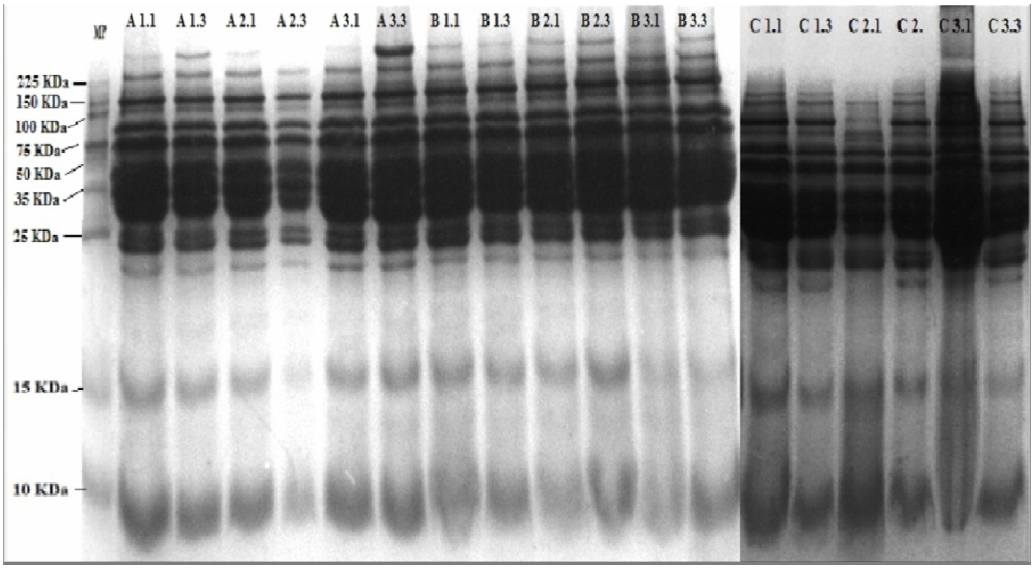

Figure 1 : SDS-PAGE patterns of sarcoplasmic (SSP) proteins fractions extracted from *Sparus aurata*, issued from 3 different aquaculture farms from Northern region in Tunisia.

PM: molecular weight marker of proteins

A, B and C: for the station and number for the sample

Concerning the electrophoretic profiles of the MFP fractions (figure 2) they enabled to identify the following groups of bands: a band with a molecular weight of 200 kDa corresponding to the heavy chain of myosin (MHC), a band at 110 kDa corresponding to the α -actinin (α -ACN), a group of bands between 51 and 60 kDa corresponding to vimentin and paratropomyosine, a band at 45 kDa corresponding to actin (AC) for, a group of bands included between 32-39 kDa corresponding to tropomyosin (TMP) and finally another group of strips of low molecular weight at 22,23 and 16 kDa.

The proteins electrophoretic profiles did not show major differences in bands distributions between sea bream *Sparus aurata* from the different farms. In order to go deeper in the examination of the proteins profiles of the samples, an analysis of the optical density of the gels was performed. An averages of 6 readings were made for each sample, knowing that according to Martinez et al. (2007) small differences in band intensity can be assigned to individual polymorphisms or difference of the relative amount of protein loaded on the gel electrophoresis.

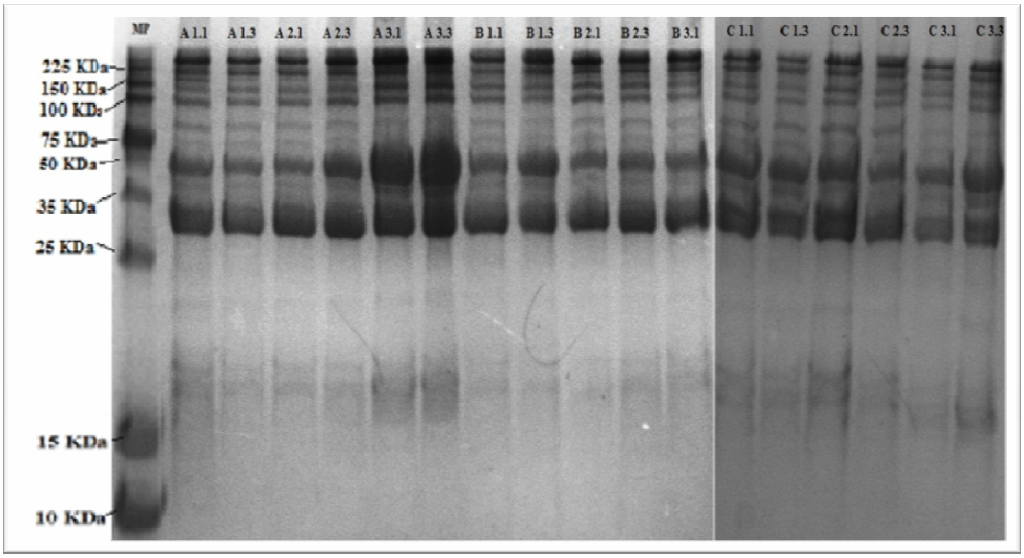

Figure 2 : SDS-PAGE patterns of Myofibrillar (SSP) protein fraction extracted from fillets of *Sparus aurata*, issued from 3 different aquaculture farms from Northern region in Tunisia.

PM: molecular weight marker of proteins

A, B and C: for the station and number for the sample

The major changes of proteins that have been reported during storage of fish flesh are the weakening of the Z line, the degradation of titin, nebulin, dystrophin and desmin and the release of α-actinin and the disruption of collagen junctions between myotomes (Delbarre-Ladrat et al., 2006). All these changes were not visible in this study as the samples were treated upon arrival at the laboratory, and stored at -80 ° C. In addition, later, proteins fractions were collected under ice cold conditions and were stored at -20°C. According to Bonnal et al. (2001), 60% of the binding between the extracellular actin cytoskeleton and the matrix is cleaved during the first 24 hours and disappear after 2-3 days of storage at 4 ° C. The main groups of enzymes involved in the degradation of muscle proteins of fish during *post-mortem* storage are: the calpain, the cathepsins and the metalloproteinases matrix (Delbarre-Ladrat et al., 2006; Chéret et al., 2007). Calpains and cathepsins degrade the myosin heavy chain, α-actinin, desmin, actin and tropomyosin (Delbarre-Ladrat et al., 2006).

The results of densitometry study were analyzed using the program Gel-Pro Analyser (V 4.5). For the SSP fraction, no significant differences were noted between fish lots and the results of densitometric readings of proteins bands were similar to those reported for the species *Sparus aurata* (Addis et al. 2010; Attouchi and Sadok, 2011). These studies have also shown that changes in the electrophoretic profiles of the SSP fractions are characteristic of species and that no differences could be identified between the reared and the wild fish. Contrastingly, for the MFP fractions, densitometric readings, permitted to reveal significant differences ($P < 0.05$) between the fish from the station C and those from the two other stations A and B. In order to visualize this differences all the data were treated with a PCA(figure 3). The observation of the position pattern (Plot in Rotated Space) supports the densitometric differences detected in the bands of MFP fraction for the samples of sea bream (*Sparus aurata*) from station C comparatively to those from the stations A and B

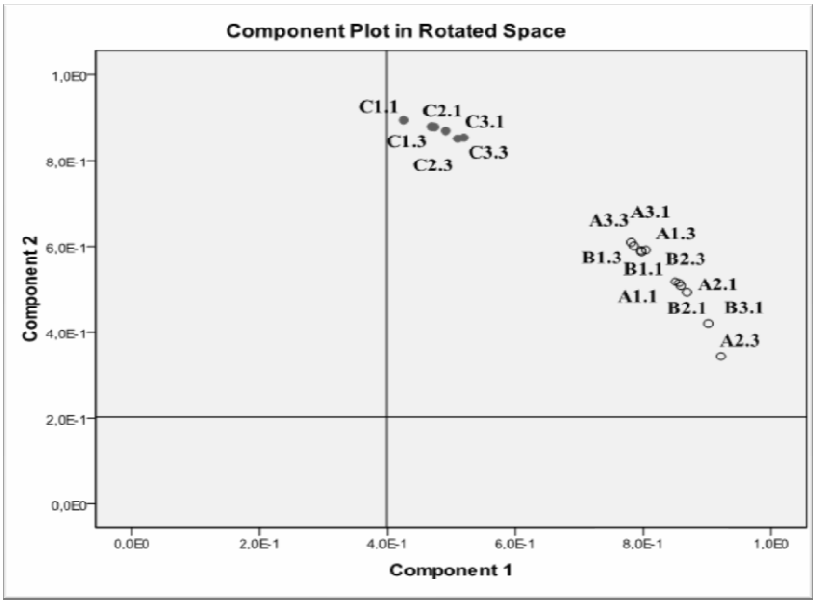

Figure 3 : position diagram (Plot in Rotated Space) obtained by principal component analysis (PCA) for bands of MFP (myofibrillar proteins) fractions, detected by the Gel- Pro Analyzer program.

A, B, C: studied specimens

CONCLUSION

The electrophoretic profiles performed by SDS-PAGE analysis of the SSP fractions didn't show any differences between fish lots, and the results of densitometric readings of bands were similar to those reported for the species *Sparus aurata*Contrastingly, for the MFP fractions, densitometric readings, permitted to reveal significant differences between the fish from the station C and those from the two other stations A and B. .

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