

## Chemical composition and nutritional value of raw and cooked black scabbardfish (*Aphanopus carbo*)

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**SUMMARY:** The objective of the present study was to follow the seasonal chemical changes and to study the effect of culinary treatments on the nutritional value of black scabbardfish (*Aphanopus carbo*). The proximate chemical composition of black scabbardfish (BSF) landed in Sesimbra (Portugal) was followed for one year. The nutritional quality (proximate chemical composition, amino acid and fatty acid profiles, cholesterol and minerals) of raw, fried and grilled BSF was evaluated in one period of the year. BSF is a semi-fatty species, the protein content was 15-17.5% and the most abundant amino acids were glutamic acid, aspartic acid and lysine. The dominant fatty acids were monounsaturated (66%), followed by saturated (19%) and polyunsaturated (10%) ones. Potassium, phosphorus and sodium were the most abundant minerals. Some dehydration occurred in cooked BSF but fat content was the most affected nutrient, particularly in fried fish. These results may suggest that the absorption of frying oil led to important changes in the fatty acid profile, and particularly in the linoleic acid level. The highest protein losses were recorded in fried BSF. In general, the nutritional quality of grilled fish seemed to be more balanced than that of fried fish.

**Keywords:** black scabbardfish, raw, cooked, fatty acids, amino acids, cholesterol and minerals.

**RESUMEN:** VARIACIÓN ESTACIONAL DE LA COMPOSICIÓN QUÍMICA Y DEL VALOR NUTRITIVO DE SABLE NEGRO (*APHANOPUS CARBO*) CRUDO Y COCIDO. – El objetivo del presente trabajo fue seguir los cambios químicos y estudiar el efecto de los tratamientos culinarios sobre el valor nutritivo de sable negro (*Aphanopus carbo*). La composición química proximal de sable negro (BSF) desembarcado en Sesimbra (Portugal) fue seguida durante un año. La calidad nutricional (composición química proximal, perfiles de aminoácidos y ácidos grasos, colesterol y minerales) del pescado crudo, frito y asado fue evaluada en un período del año. BSF es una especie semi-grasa, el contenido de proteína fue de 15 y 17,5% y los aminoácidos más abundantes fueron glutámico, aspártico y lisina. Los ácidos grasos dominantes fueron los monoinsaturados (66%), seguidos por saturados (19%) y polinsaturados (10%). Potasio, fósforo y sodio son los minerales más abundantes. Se observó alguna deshidratación en el BSF cocinado, pero el nutriente más afectado fue la grasa, sobre todo en el pescado frito. Estos resultados permiten concluir que la absorción de aceite de freír da lugar a importantes cambios en el perfil de ácidos grasos, en particular en el nivel de ácido linoleico. Las máximas pérdidas de proteínas se observaron en el BSF frito. En general, la calidad nutricional del pescado a la parrilla parece estar más equilibrada que la del pescado frito.

**Palabras clave:** sable negro, crudo, cocido, ácidos grasos, aminoácidos, colesterol, minerales.

### INTRODUCTION

In the last few decades fishing of deep-sea species has become common in several European countries, especially in the North Atlantic (Gordon *et al.*, 2003). As a result, a market has been developed and some products are relatively popular, such as the

backs of leafscale gulper shark and Portuguese dogfish (Kjerstad *et al.*, 2003).

Among the deep-sea species, there are very few studies describing the proximate fish muscle composition and the effect of cooking (Brennan and Gormley, 1999; Gormley *et al.*, 1994; Maier *et al.*, 1997; Økland *et al.*, 2005). In addition, concerns are often

raised about the presence of contaminants (Ruiter, 1995; Afonso *et al.*, 2008). Thus, the gathering of available knowledge, the development of studies about the biochemical composition and the dissemination of such information towards the processors and consumers is of utmost importance for the upgrading of these species.

In Portugal, the black scabbardfish (*Aphanopus carbo* Lowe, 1839) (BSF) is the most important commercially exploited fish of Portuguese deep-water fisheries. In the last decade landings have increased to about 6000 tonnes (3000 tonnes each off Madeira and mainland Portugal). This species is available as fresh gutted fish, fillets and portions and frozen fillets, and BSF now plays an important role in the human diet of the Madeira Islands and Sesimbra (Portugal mainland). Moreover, in recent years it has gained an important role in Portuguese gastronomy through the preparation of very special dishes.

As for other fish species, the chemical composition of BSF flesh is influenced by a number of factors, but culinary treatments are also responsible for major changes in fish muscle, especially affecting sensory attributes, chemical characteristics and nutritional composition.

The aim of this study was to characterise the seasonal changes in proximate chemical composition of fillets with and without white skin, to evaluate the nutritional aspects of the flesh (main constituents, amino acid and fatty acid profiles, and cholesterol, mineral and vitamin contents) and to determine the effect of grilling and frying on its biochemical composition.

## MATERIALS AND METHODS

### Sample preparation and cooking

Black scabbardfish (BSF) caught off Sesimbra (Portugal) in 2007-2008 (Fig. 1) was obtained from a local retailer. Samples were transported in ice to the laboratory in Lisbon. For seasonal chemical characterisation, 10 individuals were analysed on a routine monthly basis. On arrival at the laboratory, fresh fish was hand processed, i.e. filleted and skinned (removal of the thin black skin). Two lots of fillets were prepared: one (skin on) with the white skin (the one under the black skin) and one (skin off) without the skin. All fish in each lot were homogenised. Fish for

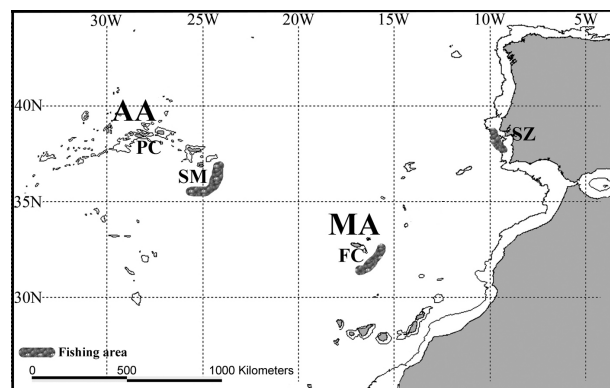


FIG. 1. – Map of the southern northeast Atlantic with the sampling locations of black scabbardfish and the 1000 m isobath. AA, Azores Archipelago; FC, Funchal; MA, Madeira Archipelago; PC, Pico Island; SM, Santa Maria Island; SZ, Sesimbra (mainland Portugal).

nutritional characterisation and culinary treatments was also filleted and skinned by hand (only the black skin was removed). Culinary treatments followed the usual household preparation. For grilling, fish portions (about 7 cm length) were previously spiked with 1.5% salt, which was partially removed after 15 minutes; grilling was done in an electric grill (2000 W) for 10 minutes. For frying, fish portions were prepared and salted as for grilling, then coated with wheat flour and deep-fried in vegetable oil (total saturated fatty acids 10%, total monounsaturated fatty acids 29%, total polyunsaturated fatty acids 61%). The oil was discarded after each batch frying. All assays were conducted in triplicate samples and the results are expressed in wet weight.

### Analytical methods

In this study we analysed the concentration of the different nutritional components in raw, fried and grilled muscle. The results are expressed in g per 100 g wet weight of raw, fried or grilled muscle, respectively. Moisture, ash, protein and lipid contents were determined in each specimen's tissue according to the Association of Official Analytical Chemists procedures (AOAC, 2000). Briefly, the moisture content was obtained by drying the sample overnight at 105°C, ash was quantified after combustion for 16 h at 550°C, crude protein content was determined by the Kjeldahl method (AOAC, 2000) using a conversion factor of 6.25, and total lipid was determined with the Soxhlet extraction method (AOAC, 2000) using ethyl ether. The energy value, expressed as kcal and kJ/100 g edible part, was estimated using FAO (1989) factors: 9.02 and 4.27 kcal/g for fat and protein, respectively.

The amino acid profile was determined by ion exchange chromatography of hydrolysed proteins (6 N hydrochloric acid containing 0.1% phenol) in an MLS-1200 Mega Microwave System (Milestone) at 800 W and 160°C for 10 min. The hydrolysis was performed under inert and anaerobic conditions to prevent oxidative degradation of amino acids. The hydrolysates were filtered and dissolved in pH 2.2 sodium citrate buffer. It is noteworthy that with this hydrolysis procedure tryptophan was not determined. Amino acids were separated by ion exchange liquid chromatography in a Biochrom 20 automatic analyser (Amersham Biosciences) equipped with a column filled with a polysulphonated resin (250 x 4.6 mm), using three sodium citrate buffers, pH 3.20, 4.25 and 6.45 (Amersham Biosciences), and three different temperatures (50°C, 58°C and 95°C). The detection of amino acids was done at 440 nm and 570 nm after reaction with ninhydrin (Amersham Biosciences). Amino acids were identified by comparison of their retention time with those of specific standards (Sigma) and quantified with the software EZChrom™ Chromatography Data System, version 6.7. (Scientific Software Inc.) using norleucine (Sigma) as an internal standard.

The determination of fatty acid profile methyl esters was based on the experimental procedure described by Lepage and Roy (1986), modified by Cohen *et al.* (1988). The fatty acid methyl esters were analysed in a CP 3800 Varian gas chromatograph, equipped with an auto-sampler and fitted with a flame ionisation detector (FID). The separation was carried out with helium as the carrier gas in a DBWax polyethylene glycol column (30 m x 0.25 mm id) programmed to start at 180°C for 5 min, heat at 4°C/min for 10 min and hold up at 220°C for 25 min, with a detector at 250°C. A split injector (100:1) at 250°C was used. Fatty acid methyl esters were identified by comparison of their retention time with those of chromatographic Sigma standards. The values were expressed as mg/100 g edible part, using the conversion factors proposed by Weihrauch *et al.* (1977). The total unsaturation index (UI) was generated by summing the individual fatty acid unsaturation indices, which were calculated by multiplying the number of double bonds of each fatty acid by its percentage and dividing by 100 (Huynh and Kitts, 2009).

Cholesterol was assayed by gas chromatography after saponification in alkaline conditions, based on the method of Naemmi *et al.* (1995) modified by Oehlenschläger (2000). It was analysed in a Cx 3400

VARIAN gas chromatograph and the separation was carried out with helium as the carrier gas in an HP5 column (30 m x 0.5 mm id). The temperatures of the oven, injector and detector were 280°C, 285°C and 300°C, respectively. Cholesterol was identified and quantified by comparison with a pure standard (Sigma, Portugal) from which a calibration curve was prepared.

Calcium and magnesium were measured by flame atomic absorption spectrometry in the ash hydrochloric solution, and using lanthanum as the releasing agent (AOAC, 2000). Chloride was determined by a volumetric method (IPQ, 1988) after precipitation of chloride with a silver nitrate solution in excess and titration of the excess with ammonium thiocyanate in the presence ferric indicator. Copper, iron and zinc were analysed by flame atomic absorption spectrophotometry in the ash hydrochloric solution (AOAC, 2000). Manganese was determined in an ash nitric solution by atomic absorption spectrometry equipped using a graphite furnace (AOAC, 2000). Phosphorus was analysed by spectrophotometry, after reaction of molybdovanadate reagent with the ash hydrochloric solution (AOAC, 2000). Potassium and sodium were quantified by flame photometry in the ash hydrochloric solution, using lithium solution as an ionisation buffer (AOAC, 2000). All results are expressed as 100 g of edible part.

The thrombogenic potential of the fish studied was evaluated according to Ulbricht and Southgate (1991) using the thrombogenic index [TI = (14:0 + 16:0 + 18:0)/(0.5 MUFA + 0.5 n-6 PUFA + 3 n-3 PUFA + n-3 PUFA/n-6 PUFA)]. For TI calculations the fatty acids concentrations were expressed as g/100 g of total fatty acids. The hypercholesterolemic-atherogenic potential of the samples was evaluated using the cholesterol-saturated fat index (CSI) according to Connor *et al.* (1986), where CSI = (1.01 × g of SFA 100 g<sup>-1</sup> of fresh matter) + (0.05 × mg of cholesterol 100 g<sup>-1</sup> of fresh matter).

### Statistical analysis

One-way analysis of variance (ANOVA) was used to compare biochemical compositions. Normality and homogeneity of variances were verified by Kolmogorov–Smirnov and Bartlett tests, respectively. A paired t-test was used to compare the apparent retention factors. All statistical tests were performed using the Statistics Version 6.0 for Windows XP (Microsoft, USA) package.

RESULTS

Raw material

The average values of the four main constituents—moisture, fat, protein and ash—and the energy values in black scabbardfish fillets with (skin-on) and without white skin (skinless) are shown in Table 1 and the seasonal changes of these four compounds over one year are shown in Figures 2 and 3. The moisture content (% wet weight) was lower in skin-on fillets (75.3%) than in skinless fillets (79.0%). The crude protein content was of the order of 16.0% and the ash between 1.1% and 1.2%. As can be seen, the total lipid content was always higher in skin-on fillets (6.7%) than in skinless products (2.5%), due to the removal of a thin layer of subcutaneous fat during the skinning. The energy values were low, 95 and 129 kcal/100 g for skinless and skin-on fillets, respectively.

The amino acid profile (g/100 g protein) of this species, that of a standard (FAO/WHO, 1991) and the recommended daily intakes (Usyodus *et al.*, 2009)

TABLE 1. – Proximate chemical composition (% wet weight; mean ± standard deviation) and energy values (kcal/100 g wet weight; mean ± standard deviation) of skin-on and skinless black scabbardfish fillets.

|                     | Skinless fillets | Skin-on fillets |
|---------------------|------------------|-----------------|
| Moisture (%)        | 79.0 ± 1.8       | 75.3 ± 2.3      |
| Fat (%)             | 2.5 ± 1.2        | 6.7 ± 2.7       |
| Protein (%)         | 16.9 ± 0.7       | 16.1 ± 0.8      |
| Ash (%)             | 1.1 ± 0.1        | 1.2 ± 0.2       |
| Energy (kcal/100 g) | 94.7 ± 14.2      | 129.2 ± 17.8    |

are shown in Table 2. The protein of this species contained high amounts of non-essential amino acids (NEAA) such as glutamic acid, aspartic acid and alanine: 15.29, 10.19 and 6.37 g/100 g of protein, respectively. The main essential amino (EAA) acids were lysine and leucine, accounting for 10.19 and 8.28 g/100 g of protein, respectively. Within semi-essential amino acids (SEAA), arginine was the most abundant (6.37 g/100 g of protein). The main fatty acids (FA) detected in the total lipids of black scabbardfish are shown in Table 4. The saturated fraction (SFA) represented 19.4% of total fatty acids, with

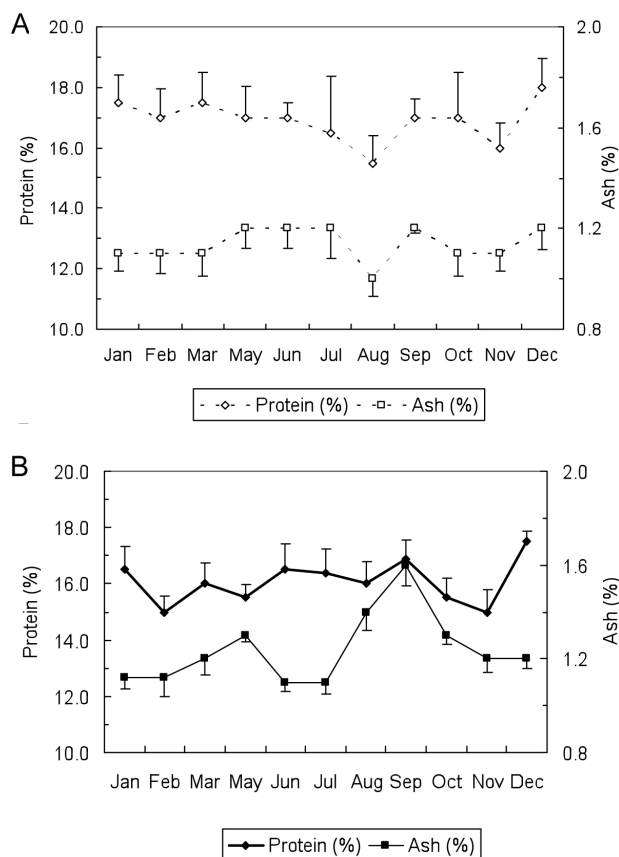


FIG. 2 – Seasonal evolution of protein and ash content in black scabbardfish fillets (A, skin-on; B, skinless).

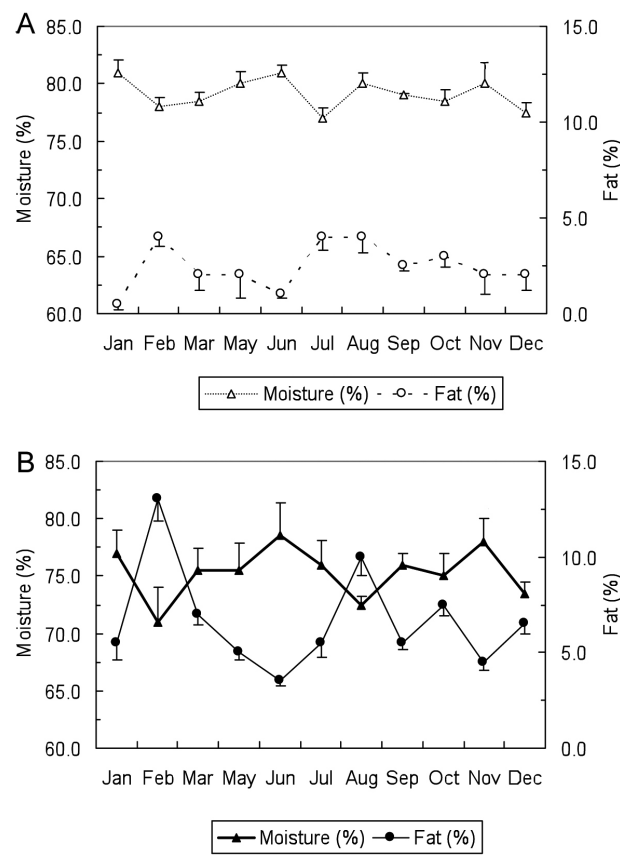


FIG. 3 – Seasonal evolution of moisture and fat content in black scabbardfish fillets (A, skin-on; B, skinless).

TABLE 2. – Amino acid composition (g/100 g protein; mean ± standard deviation) of black scabbardfish edible part, protein standard and human recommended daily intake.

| Amino acid          | Content (g/100 g protein) | Protein standard <sup>1</sup> (g/100 g protein) | Recommended daily intake (mg/kg body weight) <sup>2</sup> |
|---------------------|---------------------------|---|---|
| <b>EAA and SEEA</b> |                           |   |   |
| Threonine           | 4.46±1.26                 | 3.4   | 6.5   |
| Valine              | 5.73±0.98                 | 3.5   | 11.4  |
| Methionine          | 3.18±0.45                 | 2.5 <sup>a</sup>                                | 12.1 <sup>a</sup>   |
| Isoleucine          | 5.10±0.57                 | 2.8   | 15.7  |
| Leucine             | 8.28±1.35                 | 6.6   | 9.5   |
| Phenylalanine       | 3.82±0.50                 | 6.3 <sup>b</sup>                                | 12.1 <sup>b</sup>   |
| Lysine              | 10.19±1.85                | 5.8   | 9.4   |
| Histidine           | 1.91±0.43                 |   |   |
| Arginine            | 6.37±0.00                 |   |   |
| Serine              | 3.82±0.49                 |   |   |
| Σ EAA               | 40.8±6.80                 | 32.0 <sup>c</sup>                               | 79.6  |
| Σ SEEA              | 12.1±2.33                 |   |   |
| <b>NEAA</b>         |                           |   |   |
| Aspartic acid       | 10.19±2.10                |   |   |
| Glutamic acid       | 15.29±1.87                |   |   |
| Glycine             | 5.10±0.45                 |   |   |
| Alanine             | 6.37±0.43                 |   |   |
| Tyrosine            | 3.82±0.29                 |   |   |
| Proline             | 5.10±0.28                 |   |   |
| Σ NEAA              | 45.9±4.92                 |   |   |

<sup>1</sup>FAO/WHO (1991); <sup>2</sup>(Usyduş *et al.*, 2009).  
 EAA-Essential amino acids; SEEA-Semi essential amino acids;  
 NEAA-Non essential amino acids. <sup>a</sup>Methionine+Cysteine;  
<sup>b</sup>Phenylalanine+Tyrosine; <sup>c</sup> including Tryptophan.

palmitic acid (16:0) as the most important FA within this fraction, followed by stearic acid (18:0).

Monounsaturated fatty acids (MFA) were the main group, at over 65%. Oleic acid (18:1n-9) was the dominant one, followed by 22:1 and 20:1. The presence of nervonic acid (24:1n-9) was recorded. Polyunsaturated fatty acids (PUFA) were the smallest group and the n-3 PUFA was the dominant one compared with n-6 PUFA, showing an n-3/n-6 ratio of 8.8. Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) represented around 80% of total PUFA and DHA accounted for over 59%.

The mineral content and range observed for potassium (K), phosphorus (P), sodium (Na), sulphur (S), magnesium (Mg), calcium (Ca), zinc (Zn), copper (Co) and chloride (Cl) are given in Table 3. As can be seen, potassium and phosphorus were the most abundant.

**Cooked products**

Values related to raw BSF and cooked products—chemical composition, energetic value, main fatty acids, cholesterol and minerals—are shown in Table 5.

TABLE 3. – Mineral content (mg/100 g wet weight muscle; mean ± standard deviation), range of black scabbardfish muscle and recommended daily intakes (RDI).

| Elements              | Content (mg/100 g WWM) | Range       | RDI          |
|-----------------------|------------------------|-------------|--------------|
| <b>Macroelements</b>  |                        |             |              |
| Potassium (K)         | 296.6±55.0             | 215.3-432.6 | 4700 mg      |
| Phosphorus (P)        | 175.9±22.0             | 163.2-185.6 | 700 mg       |
| Sodium (Na)           | 156.8±50.2             | 103.8-273.7 | 1500 mg      |
| Sulphur (S)           | 124.2±33.6             | 74.7-171.8  | NS           |
| Chloride (Cl)         | 121.8±27.3             | 86.5-163.7  | 2300 mg      |
| Magnesium (Mg)        | 28.0±1.8               | 24.6-29.9   | (413-316) mg |
| Calcium (Ca)          | 11.9±3.7               | 7.6-17.9    | 1000 mg      |
| <b>Trace elements</b> |                        |             |              |
| Zinc (Zn)             | 0.27±0.02              | 0.25-0.30   | (11-8) mg    |
| Copper (Cu)           | 0.06±0.02              | 0.01-0.17   | 900 µg       |

Values in brackets for Mg and Zn refer to differences in genders: the first and second values correspond to male and female, respectively. NS, has not been set.

TABLE 4. – Main fatty acids (relative area percentage of total fatty acids) in lipids of black scabbardfish muscle, SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), FA (fatty acids).

| Fatty acids (% of total FA) | Raw muscle  |
|-----------------------------|-------------|
| 14:0                        | 2.12±0.078  |
| 15:0                        | 0.38±0.021  |
| 16:0                        | 12.20±0.247 |
| 17:0                        | 0.34±0.006  |
| 18:0                        | 4.24±0.070  |
| 20:0                        | 0.03±0.030  |
| Σ SFA <sup>a</sup>          | 19.36±0.340 |
| 16:1n-7                     | 3.26±0.050  |
| 17:1                        | 0.24±0.205  |
| 18:1n-9                     | 26.06±0.172 |
| 18:1n-7                     | 6.28±0.249  |
| 20:1n-9                     | 14.10±0.187 |
| 22:1n-11                    | 15.48±0.326 |
| 24:1n-9                     | 0.19±0.031  |
| Σ MUFA <sup>a</sup>         | 65.67±0.418 |
| 18:2n-6                     | 1.09±0.256  |
| 18:4n-3                     | 0.11±0.087  |
| 20:2n-6                     | 0.14±0.248  |
| 20:5n-3                     | 1.94±0.066  |
| 22:5n-3                     | 0.84±0.079  |
| 22:6n-3                     | 5.65±0.303  |
| Σ PUFA <sup>a</sup>         | 9.51±1.870  |
| Σ n-3 FA <sup>a</sup>       | 8.54±0.352  |
| Σ n-6 FA <sup>a</sup>       | 0.97±0.619  |
| Ratio n-3/n-6 <sup>a</sup>  | 8.80±1.30   |
| Other FA <sup>b</sup>       | 5.46±1.12   |

STD - Standard deviation (n=5).  
<sup>a</sup>Data relative to the total FA identified.  
<sup>b</sup>Traces and unidentified

Culinary treatments strongly affected the moisture content (P<0.05). The protein levels of raw, grilled and fried products were also significantly different (P<0.05). Fat values were significantly higher in fried products (16.6%) than in raw products (2.8%) and grilled products (3.2%). The fat content in fried

TABLE 5. – Nutritional data of raw, fried and grilled black scabbardfish (mean ± standard deviation).

| Nutritional data    | Raw<br>(/100g wet weight) | Fried<br>(/100g wet weight) | Grilled<br>(/100g wet weight) |
|---------------------|---------------------------|-----------------------------|-------------------------------|
| Moisture (g)        | 79.7±0.02 <sup>a</sup>    | 50.0±0.02 <sup>b</sup>      | 74.4±0.05 <sup>c</sup>        |
| Protein (g)         | 15.7±0.04 <sup>a</sup>    | 24.2±0.20 <sup>b</sup>      | 20.5±0.41 <sup>c</sup>        |
| Total fat (g)       | 2.8±0.01 <sup>a</sup>     | 16.6±0.08 <sup>b</sup>      | 3.2±0.03 <sup>a</sup>         |
| 16:0 (mg)           | 301.2±10.01 <sup>a</sup>  | 1113.5±30.05 <sup>b</sup>   | 357.9±14.10 <sup>c</sup>      |
| Total SFA (mg)      | 478.0±11.07 <sup>a</sup>  | 1846.0±40.10 <sup>b</sup>   | 630.0±50.06 <sup>c</sup>      |
| 18:1 (mg)           | 643.4±47.60 <sup>a</sup>  | 4388.1±89.10 <sup>b</sup>   | 810.5±52.34 <sup>c</sup>      |
| Total MUFA (mg)     | 1621.8±97.13 <sup>a</sup> | 4858.7±102.26 <sup>b</sup>  | 1696.7±83.15 <sup>a</sup>     |
| 18:2 n-6 (mg)       | 20.4±3.76 <sup>a</sup>    | 8116.9±112.78 <sup>b</sup>  | 75.2±5.98 <sup>c</sup>        |
| 20:5 n-3 (mg)       | 47.9±3.83 <sup>a</sup>    | 61.4±8.37 <sup>b</sup>      | 56.8±9.84 <sup>ab</sup>       |
| 22:6 n-3 (mg)       | 139.5±8.91 <sup>a</sup>   | 280.8±12.06 <sup>b</sup>    | 256±11.02 <sup>b</sup>        |
| Total PUFA (mg)     | 234.8±22.34 <sup>a</sup>  | 8610.5±112.28 <sup>b</sup>  | 439.2±37.81 <sup>c</sup>      |
| Total PUFA n-3 (mg) | 210.8±10.23 <sup>a</sup>  | 358±12.29 <sup>b</sup>      | 350.9±9.53 <sup>b</sup>       |
| Total PUFA n-6 (mg) | 24.0±3.45 <sup>a</sup>    | 8252.4±136.71 <sup>b</sup>  | 88.3±11.33 <sup>c</sup>       |
| Cholesterol (mg)    | 24.2±0.53 <sup>a</sup>    | 36±1.03 <sup>b</sup>        | 29±0.70 <sup>c</sup>          |
| Ash (g)             | 1.2±0.01 <sup>a</sup>     | 2.6±0.01 <sup>b</sup>       | 2.3±0.02 <sup>c</sup>         |
| Calcium (mg)        | 14±4.21                   | 22±5.12                     | 18±5.44                       |
| Phosphorus (mg)     | 181±10.72 <sup>a</sup>    | 249±23.64 <sup>b</sup>      | 208±19.51 <sup>b</sup>        |
| Magnesium (mg)      | 29±8.07 <sup>a</sup>      | 40±13.19 <sup>b</sup>       | 33±9.46 <sup>b</sup>          |
| Iron (mg)           | 0.1±0.03                  | 0.2±0.04                    | 0.1±0.03                      |
| Sodium (mg)         | 138±2.54 <sup>a</sup>     | 437±24.50 <sup>b</sup>      | 475±23.76 <sup>b</sup>        |
| Potassium (mg)      | 332±9.92 <sup>a</sup>     | 428±10.37 <sup>b</sup>      | 361±8.55 <sup>c</sup>         |
| Manganese (mg)      | <0.02                     | <0.02                       | <0.02                         |
| Copper (mg)         | <0.03                     | <0.03                       | <0.03                         |
| Zinc (mg)           | 0.5±0.09 <sup>a</sup>     | 0.8±0.16 <sup>b</sup>       | 0.6±0.09 <sup>a</sup>         |
| Chloride (mg)       | 122±3.38 <sup>a</sup>     | 386±11.38 <sup>b</sup>      | 420±13.83 <sup>c</sup>        |

Different superscript letters within rows represent significant differences among raw, fried and grilled fish (ANOVA) ( $P < 0.05$ ).

products reflected the absorption of frying oil. Ash almost doubled in fried and grilled products in comparison with raw BSF ( $P < 0.05$ ).

Relatively to the fatty acids contents, the main changes were due to absorption of vegetable oil and the leaching out of water. The absorption of frying oil was also well evidenced in the considerable increase in MUFA and PUFA as a result of the absorption of oleic and linoleic acids, respectively. The cholesterol contents in raw, grilled and fried products were 24, 29 and 36 mg/100 g, respectively.

The most relevant minerals in cooked products were potassium, phosphorus and sodium (Table 5). The main changes were due to water loss and the addition of salt before cooking.

## DISCUSSION

### Raw material

It is well known that the chemical composition of fish muscle varies according to sex, age, environment and season (Love, 1980). In the present study only the seasonal effect and the presence of white skin was studied. The reason for characterising the fillets with white skin on was that this is the usual form of household use in fish dishes. The results in

Table 1 and Figures 1 and 2 allow us to conclude that black scabbardfish is a semi-fatty species because its fat content is between 5 and 10%. A negative correlation between fat and moisture was observed in the present study (results not shown), as has been reported for a number of fish species (Love, 1980; Suzuki, 1981). These seasonal lipid changes are usual in fish and depend on factors such as time of year (Dawson and Grimm, 1980), environmental conditions (Gill and Weatherly, 1984), stage of maturity of the gonads (Weatherley and Gill, 1987), state of nutrition (Elliot, 1976; Corraze, 2001) and age (Parker and Vanstone, 1966). The stage of maturity is one of the most important factors because during gonadal maturation stored lipids or muscle proteins are mobilised to gonads (Sharer, 1994). A decreasing trend of BSF fat content (Fig. 3A) in the period from July to January was observed; Neves *et al.* (2009) found an increase of the gonadal maturation percentage in the period between July and November, suggesting an inverse relation between lipid content and sexual maturation in this species.

The protein and ash contents were in the range found for most deep water fish species, such as silver scabbardfish (Bandarra *et al.*, 2004) a species belonging to the same family of BSF. The amino acid composition of the edible part of black scabbardfish was similar to that of other deep-water fish

species (Bandarra *et al.*, 2004). The essential amino acids values allow us to conclude that the protein of this species is well balanced, since the amounts were greater than those in the standard protein (FAO/WHO, 1991), as can be seen in Table 2. In addition, limiting amino acids in black scabbardfish, such as lysine and methionine, expressed as g per 100 g of protein, also occurred at levels higher than those in the standard protein (FAO/WHO, 1991). The total of SEAAs in BSF is substantial (12.1 g/100 g protein), namely arginine. These amino acids are not normally required in the human diet but must be exogenously supplied to specific populations under special conditions, such as intensive growth, stress, or in some diseases state. As in most fish species, the most important NEAAs were glutamic and aspartic acid. The amounts of the amino acids cysteine and tryptophan were not determined, but they would sum about 1.28 g per 100 g of black scabbardfish protein. The essential amino acid requirement for an adult man weighing 70 kg is about 5.6 g per day (Usyduş *et al.*, 2009). Therefore, this fish species can be considered a significant source of amino acids in terms of both quality and quantity, because 100 g of the edible part is sufficient to meet this daily requirement.

The influence of diet on the fatty acid profile has been recognised and may thus be related to the stomach contents. However, in the case of black scabbardfish the stomach is frequently empty or everted and it was not possible to analyse any remains in the stomach. Therefore, the discussion takes into account the fatty acid profile of species living in deep-sea waters in the NE Atlantic. In most fish species the omega 3 polyunsaturated fatty acids (n-3 PUFA) are the dominant group, but in BSF the monounsaturated fatty acids were the largest fraction. This exception has also been found for meagre and silver scabbardfish (Nunes *et al.*, 2003) and for sablefish (Oliveira *et al.*, 2008). In BSF oleic acid was the dominant monounsaturated fatty acid, and the percentage of 18:1n-9 and 18:1n-7 isomers was higher than 30% of the total of fatty acids, which is an unusual result in fatty acid profiles of fish species (Bandarra *et al.*, 2004). It is also important to note that relevant percentages of 20:1 and 22:1 were recorded, contrary to the findings of Sirot *et al.* (2008) in more than fifty species. This difference is probably related to the feed available because its exogenous origin is recognised (Ackman, 1982). The high MUFA percentage found in BSF may result from the assimilation of fatty alcohols

from the wax esters of the prey, which are directly converted into fatty acids, as also mentioned by Stowasser *et al.* (2009) for *Coryphaenoids armatus*, a deep-sea fish living at depths of 2500 to 4800 m. The unsaturation index (UI) of total unsaturated fatty acids and the unsaturation index of only EPA + DHA in the lipids of BSF were 1.27 and 0.44, respectively. These values are much lower than those reported by Huynh and Kitts (2009) for species with a similar fat content as a result of the high level of MUFA in BSF.

The presence of nervonic acid (24:1n-9) is not usually detected in most fish species, but in BSF it was about 0.2%. Ota *et al.* (1995) also reported the presence of this fatty acid in flathead flounder lipids and Shanta and Ackman (1991) in nine different marine fish oils. The results obtained by the latter authors support the hypothesis that nervonic acid is biosynthesised by fish. Thus, the presence of this fatty acid in BSF suggests that this species is able to biosynthesise this MUFA. The importance of nervonic acid was highlighted by Seargent *et al.* (1994), who demonstrated some medicinal significance based on its essential role as a nutrient for growth, development and maintenance of the brain. Within the saturated group, palmitic acid was the most abundant, with a level of 12%. This fatty acid is quite constant over the life cycle of marine animals and is considered a key metabolite in the synthesis of other fatty acids (Ackman, 1982).

Although BSF has an unusual fatty acid profile due to its high MUFA percentage and low PUFA content, it has to be stressed that the n-3/n-6 ratio found in BSF falls within the range reported by Huynh and Kitts (2009) for several fish species. Moreover, the percentage of EPA and DHA of the total n-3 PUFA was similar to that reported by the previous authors. This similar percentage of n-3 fatty acids of total PUFA among several species living in quite different habitats shows its importance in the structural lipid fraction.

The average mineral content in the edible part was very close to 1.1 g/100 g though, as can be seen in Table 4, great variations between individuals were found. The order in terms of amounts was: potassium > phosphorus > sodium > sulphur > chloride > magnesium > calcium > zinc > copper. As in most species, potassium was the most abundant mineral. Considering the daily intake recommendations, the edible part of black scabbardfish is a good source of several minerals.

## Cooked products

The proximate chemical composition of fried and grilled BSF showed that both cooking methods resulted in water loss, though the greatest loss was observed in frying. The higher protein level recorded in fried BSF in comparison with the other two methods was also observed by Türkkan *et al.* (2008) in fried, beheaded and eviscerated sea bass (*Dicentrarchus labrax*). The water loss that occurred during frying was responsible for this apparent higher concentration. Furthermore, the high fat content of fried fish reflects the absorption of oil (Table 5). The use of wheat flour to cover fish before frying contributes to an additional uptake of oil. The water loss and the higher contents of the other constituents in the cooked fish are in accordance with the findings of other studies (Gall *et al.*, 1983; García-Arias *et al.*, 2003; Gokoglu *et al.*, 2004; Kalogeropoulos *et al.*, 2004; Rosa *et al.*, 2007). Limited changes in the fat content of BSF occurred during grilling, probably due to the preferential location of lipids in membranes. This is particularly evidenced by the higher level of PUFAs, especially DHA (an important structural fatty acid), which were not lost during grilling.

The absorption of the vegetable oil by fish during frying was responsible for the high level of MUFA and PUFA, because frying oil was very rich in oleic and linoleic acids. The intake of vegetable oil by fish is clearly evidenced by the amounts of these two fatty acids in the fried BSF. The absorption of vegetable oil during frying led to an important decrease in the n-3/n-6 ratio, which is in agreement with previous findings (Echarte *et al.*, 2001). In fact, the n-3/n-6 ratio in grilled fish was 4.0, whereas in fried fish it was only 0.04, indicating that grilled fish provides higher levels of n-3 fatty acids. However, the thrombogenic index calculated for fried and grilled fish showed very similar levels: 0.22 and 0.18, respectively. It is also to be stressed that a dose of 150 g of fried BSF or grilled BSF can provide 513.2 mg or 469.2 mg of EPA plus DHA respectively, which are close to the daily dose (500 mg) recommended by the ISSFAL (2004) to prevent cardiovascular diseases.

The water lost during grilling and frying were also reflected in the relative increase in cholesterol level in the cooked products. This effect was also particularly evident in fried BSF and has been reported for other fish species (Ewaida, 1993; Echarte *et al.*, 2001).

The CSI was calculated for raw, fried and grilled BSF because it has been used to compare different foods in terms of their hypercholesterolaemic-atherogenic potential (Connor *et al.*, 1986). This index was 1.68, 3.66 and 2.09 for raw, fried and grilled fish, respectively. Both culinary treatments, and particularly frying, led to an increase in this index. However, the levels attained in the present study were considerably lower than those reported by Kalogeropoulos *et al.* 2004, who used olive oil as the frying medium. It should be stressed that both raw and cooked BSF had a cholesterol level considerably below the maximum recommended daily intake (RDI), which is 200-300 mg.

The rise in the ash content in cooked products was influenced by the addition of salt and by the water loss during the culinary treatments. Higher levels of calcium, phosphorus, magnesium, potassium and zinc in fried than in grilled fish also reflect the higher water loss during frying. The very high level of sodium and chloride resulted from the addition of sodium chloride to fish during its culinary preparation.

The results obtained by Gokoglu *et al.* (2004) in rainbow trout and by Rosa *et al.* (2007) and Ersoy and Özeren (2009) in African catfish did not show similar trends between the effect of frying and grilling on the mineral fraction, which may be related to the specific characteristics of each fish species and/or to the procedure followed in each culinary treatment.

Finally, taking into consideration the RDI values indicated in Table 3, a dose of 150 g provides a good contribution of minerals to satisfy human requirements.

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