

Full Length Research Paper

Antioxidant property of wild and farmed sea bream (*Sparus aurata*) cooked in different ways

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The antioxidant activity of water-soluble extracts of raw and cooked wild and farmed sea bream was evaluated. The cooked sea bream were steamed, boiled, grilled, oven cooked and fried with olive, corn, soybean and sunflower oils. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and hydroxyl radical-scavenging activity (RSA) as well as the reducing power activity of different extracts increased linearly with increasing concentration. DPPH scavenging activity was more effective with farmed fish extracts. Extracts of oven cooked wild and grilled farmed fish exhibited the best DPPH scavenging activity. Otherwise, measurement of hydroxyl radicals scavenging activity showed that extracts of oven cooked wild and farmed fish were the most effective. Extracts of raw and cooked wild and farmed sea bream had similar reducing power activity. Consequently, the observed variability in our results confirmed that the *in vitro* antioxidant assays for the evaluation of radical scavenging activity gave different values that reflected the different chemical properties of the different extracts.

Key words: Wild and farmed sea bream, cooking, radical-scavenging activity (RSA) 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl radicals, reducing power.

INTRODUCTION

Regular consumption of seafood is efficient for the prevention of several degenerative diseases, including cardiovascular and inflammatory disorders, cancer and stroke (Kris-Etherton et al., 2003; Din et al., 2004). Recently, more attention has been focused on the evaluation of the activities of naturally occurring antioxidants in different food products. Biological antioxidants are molecules which when present at low concentrations as compared to those of an oxidizable

substrate, significantly delays or prevent oxidation of that substrate (Halliwell, 1990). The antioxidant activity of a food could be a useful index for predicting oxidative stability and potential health benefits (Ninfali et al., 2002; Del Carlo., 2004; Serafini and Del Rio, 2004). In food products, oxidative reactions are affected by several factors, among which are lipid composition and processing, and could be delayed by endogenous or exogenous antioxidants (Del Carlo., 2004; Serafini and Del Rio, 2004).

To date, most of the natural antioxidants that have been studied are non-protein compounds in plants but protein hydrolysate or peptides from fish and shellfish, as well as their by-products, have also been shown to

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exhibit antioxidant activity (Mendis et al., 2005; He et al., 2006; Dai-Hung et al., 2010). There is a remarkable variation in the chemical composition among fish species, which depends on several factors such as geographic location, season, age and particularly diet (Luzia et al., 2003; Mnari et al., 2007). Several studies have examined the scavenging radical activity of pork, poultry and salmon cooked with different oils (Maikhunthod and Intarapichet, 2005; Zhang, 2005; Sacchetti et al., 2008). The gilthead sea bream is very popular in Tunisia and the production of cultivated fish has become intensive in order to meet consumer demand. There is some available information about the antioxidant properties of wild and farmed fish flesh as well as the effects of the cooking process.

The objective of this study was to evaluate the antioxidant properties of water-soluble extracts of wild and farmed sea bream and to examine the variation of these properties according to different cooking methods.

MATERIALS AND METHODS

Cultured gilthead sea bream (*Sparus aurata*) were collected in the autumn of 2008 from the station of Tunisian aquaculture located in the region of Hergla (Center East). The finfish was characterized by a temperature of 22°C; a salinity of 40‰; a pH of 8.2 with an average weight and total length of 85 ± 2.3 g and 17.5 ± 0.1 cm, respectively. It was raised in the usual farming conditions, with the same feed and feeding techniques.

Wild gilthead sea bream (average weight and total length of 71 ± 0.9 g and 17.5 ± 0.2 cm, respectively) were caught in the same season from the coastal waters of Monastir (Central East) characterized by a temperature of 25°C; a salinity of 38‰ and a pH of 7.8. Specimens were transported in ice to the laboratory where they were weighed and immediately processed. Four fish from each kind were used in each analysis: control, steaming, grilling, oven cooking and frying with four oil varieties: olive, soybean, sunflower and corn oils.

Butylated hydroxy toluene (BHT), potassium ferricyanide, 1,1-diphenyl-2-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), trichloroacetic acid (TCA), 2-deoxy-D-ribose, ethylenediamine tetracetic acid (EDTA), ferrous chloride, ascorbic acid and ferric chloride were purchased from Sigma Aldrich (France), Scharlau (Barcelona, Spain) Normapur or Prolabo VWR (France). All other used reagents (analytical and HPLC grade) were obtained from Sigma Aldrich (France).

Cooking

The common ways of cooking were used. Grilled fish were prepared in a "Black and Decker" grill with the thermostat set at 200°C. After the set temperature was attained, the fish were grilled for 10 min (5 min for each side). For steaming, the water was poured into the bottom of the pot and heated to the boiling point. Then, the fish were put on the middle layer of the pot and steamed for 10 min. Oven cooked fish were prepared in the oven (180 to 200°C for 10 min). For the boiling process, the fish were dipped into boiled water for 10 min. For frying experiments, a "Tefal" frying pan ($\varnothing = 20$ cm) was used on the same electrical heating unit. After each frying process, the pan was cleaned with a paper towel and the fat that remained in the pan was collected. The samples were inserted into the frying pan after the oil has reached the desired

temperature (180°C) controlled by a digital thermometer. Samples were fried in the four oil varieties used for 10 min and turned each 5 min. The standardization of the cooking steps has been achieved in pre-experiments. For different cooking process, each fish was cooked separately.

Preparation of soluble fraction from raw and cooked fish

Samples of each wild and farmed sea bream, raw and cooked with different ways, were taken and lyophilized. The distilled water was used to extract the soluble substances from raw and cooked fish. One gram of each lyophilized sample (raw, grilled, steamed, oven cooked and fried with the four oil varieties) was mixed with 100 ml of deionized water. The mixture was stirred at room temperature for 30 min and then centrifuged at 5,000 g for 15 min at room temperature using a "Universal 32 Hettich" (France) to remove undissolved debris. The supernatant was used for further analysis.

Chemical analysis

DPPH radical scavenging activity

DPPH radical scavenging activity was determined by DPPH assay as described by Wu et al. (2003) with slight modifications. The sample (1.5 ml) was added to 1.5 ml of 0.15 mM DPPH in 95% ethanol. The mixture was vigorously mixed and allowed to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was measured at 517 nm using a UV visible spectrophotometer JASCO V-530 (France). The blank was prepared in the same manner, except that distilled water was used instead of the sample. The percentage of remaining DPPH ($DPPH_R$) was calculated as follows:

$$DPPH_R (\%) = (DPPH_S / DPPH_0) \times 100$$

Where, $DPPH_S$ is the DPPH concentration of the sample at 30 min and $DPPH_0$ is the DPPH concentration of the blank (initial concentration).

Hydroxyl radical scavenging activity

The deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium (Halliwell et al., 1987). The reaction mixture containing $FeCl_3$ (100 μ M), EDTA (104 μ M), H_2O_2 (1 mM) and 2-deoxy-D-ribose (2.8 mM) was mixed with or without various concentrations of different extracts (2 to 10 mg/ml). The reaction mixture was diluted to 1 ml final reaction volume with 20 mM potassium phosphate buffer at pH 7.4 and incubated for 1 h at 37°C. Then, the mixture was heated to 95°C in a water bath for 15 min followed by the addition of 1 ml each of TCA (10%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.025% BHA). Finally, the reaction mixture was cooled in ice and centrifuged at 5319 g for 15 min. The absorbance of the supernatant was measured at 532 nm. All readings were corrected for any interference from the brown colour of the extract or antioxidant by including appropriate controls. The negative control was made without any antioxidant or extract. Decreased absorbance of the mixture indicated scavenging ability and the scavenging percent was calculated as follows:

$$\text{Scavenging percent } (\%) = [(A_0 - (A_s - A_c)) / A_0] \times 100\%$$

Where, A_s is the presence of deoxyribose and test compounds; A_0 , is the presence of deoxyribose but without test compounds and A_c , is the presence of test compounds but without deoxyribose. EC50 value (mg extract/ml) is the effective concentration at which DPPH

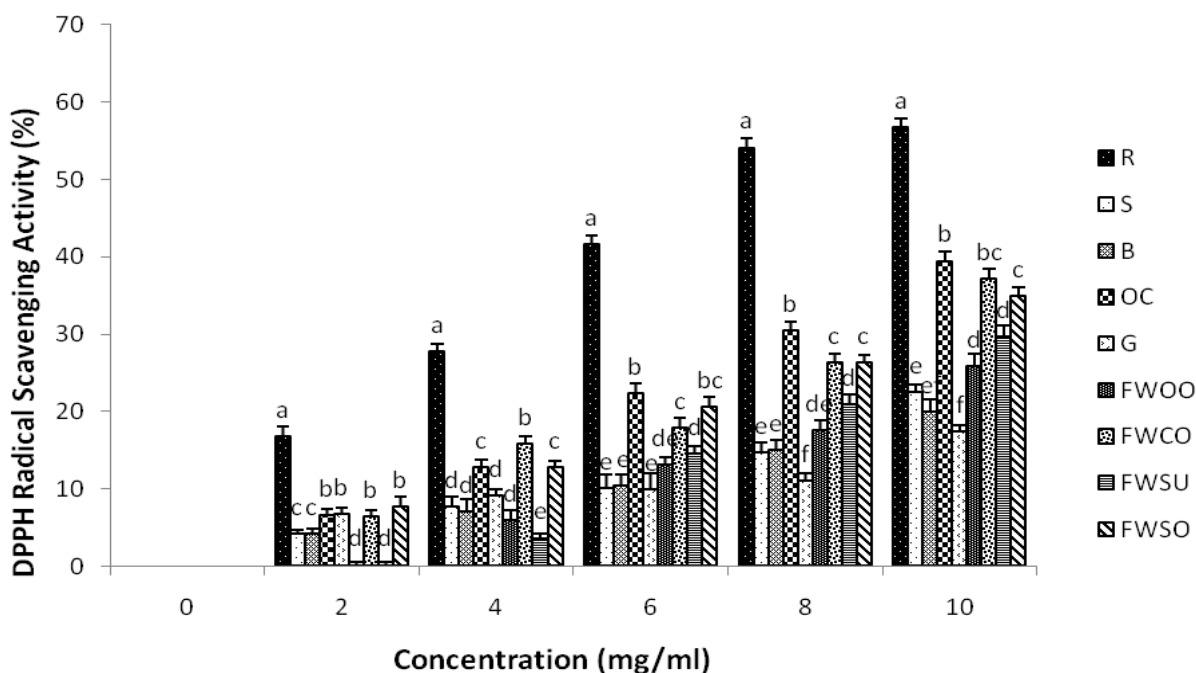


Figure 1. Antioxidant activity of water soluble fractions of wild sea bream cooked in different ways at different concentrations as determined by DPPH radical scavenging activity. Each value is expressed as mean \pm standard deviation ($n = 4$). Different small letters within histogram are significantly different ($p < 0.05$) with respect to the concentration of the extract. R: raw; S: steamed; B: boiled; OC: oven cooked; G: grilled; FWOO: fried with olive oil; FWCO: fried with corn oil; FWSU: fried with sunflower oil; FWSO: fried with soybean oil.

or hydroxyl radicals were scavenged by 50% and were obtained by interpolation from linear regression analysis.

Test for ferric ion reducing capacity (Fe_3^+ to Fe_2^+)

The ferric ion reducing capacity was estimated according to the procedure of Wang et al. (2003) with minor modifications. One hundred microlitres of potassium ferricyanide solution ($\text{K}_3\text{FeC}_6\text{N}_6$) (4 mM) were mixed with 200 μl of 20 mM phosphate buffer, pH 6.5 with or without sample extracts at various concentrations ranging from 2 to 10 mg/ml. The resulting solution was incubated at 50°C for 20 min. Two hundred microlitres of 10% trichloroacetic acid were added to the reaction mixture and centrifuged at 5319 g. The resulting supernatant was mixed with 100 μl of ferric chloride solution FeCl_3 (2 mM) and the final volume was made up to 1 ml with water. The solution was incubated at ambient temperature for 10 min and then the absorbance was recorded at 700 nm. Absorbance increases with an increase in ferric ion reducing capacity.

Statistical analysis

The descriptive statistics (mean standard values from 4 samples \pm standard deviation) were conducted using the SPSS program, release 12.0 for Windows (SPSS, Chicago, IL, USA). The experimental data were subjected to an analysis of variance for a completely random design. To evaluate the changes in antioxidant activity after processing wild and farmed fish, a two way ANOVA was carried out as well as a post hoc analysis using Tukey's test. Differences were considered to be significant when $p < 0.05$.

RESULTS AND DISCUSSION

Scavenging activity on 1,1-diphenyl-2-picrylhydrazyl radicals

The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability (Siddhuraju and Becker, 2007). The results show radical scavenging activity (RSA) in water-soluble extracts of raw and cooked sea bream (Figures 1 and 2). It might be that the water-soluble extracts contained substances that acted as electron donors and could react with free radicals to convert them to more stable products. The cooked wild and farmed sea bream showed marked differences ($p < 0.05$) in the RSA as compared to the raw fish. Generally, DPPH radical scavenging activity was significantly higher ($p < 0.05$) in all extracts of the farmed sea bream as compared to the wild fish. At 10 mg/ml of extracts, after grilling, the RSA loss from farmed fish was 8.92%, significantly different from the loss of 79.53% in wild fish. After steaming, the RSA losses were 29.86% in farmed fish and 72.76% in wild fish. After boiling, the loss of RSA was 59% from farmed fish and 72.23% from wild fish whereas after grilling and steaming, the RSA loss was significantly greater ($p < 0.05$) from wild fish as compared to farmed fish. It appears that the oxidative compounds present in the wild sea bream are affected differently by different cooking methods. The antioxidants

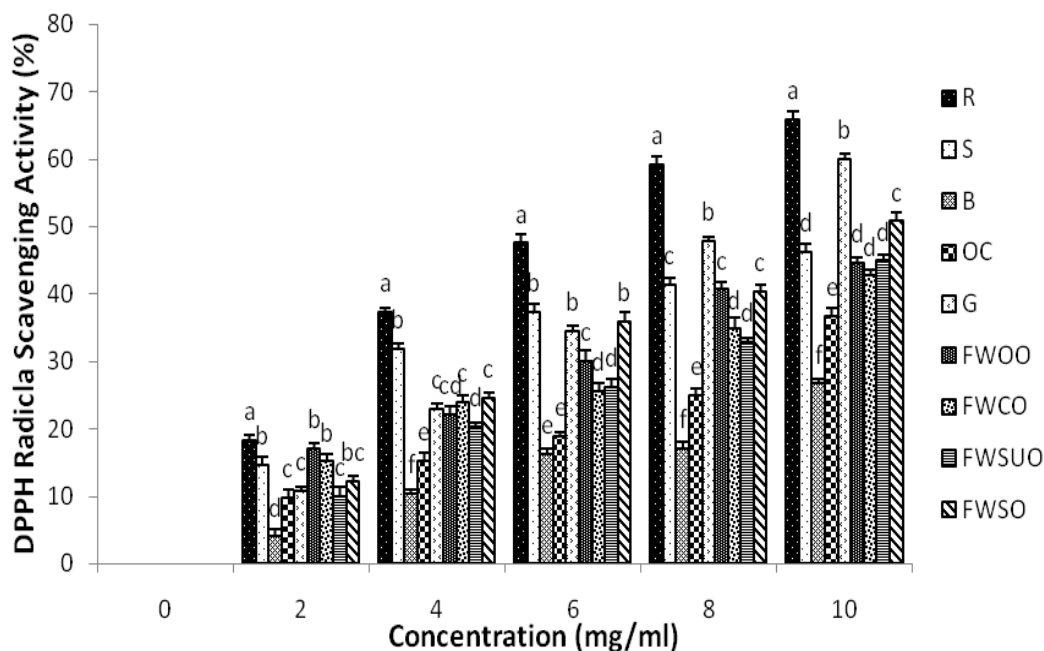


Figure 2. Antioxidant activity of water soluble fractions of farmed sea bream cooked in different ways at different concentrations as determined by DPPH radical scavenging activity. Each value is expressed as mean \pm standard deviation ($n = 4$). Different small letters within histogram are significantly different ($p < 0.05$) with respect to the concentration of the extract. R: raw; S: steamed; B: boiled; OC: oven cooked; G: grilled; FWOO: fried with olive oil; FWCO: fried with corn oil; FWSUO: fried with sunflower oil; FWSO: fried with soybean oil.

might be water-soluble peptides; indeed, peptides have been reported to have antioxidant activity (Wu et al., 2003; Je et al., 2005; Rajapakse et al., 2005).

The frying process decreased the RSA property in both farmed and wild fish extracts with all the types of oil tested; however, the loss of RSA was significantly greater ($p < 0.05$) from wild sea bream versus that from farmed fish. For farmed fish, the frying process had more impact on the loss of RSA property than that found after grilling and steaming. The observed differences were statistically significant ($p < 0.05$). Obviously, the fried extracts of farmed fish contained antioxidant components that could react rapidly with DPPH radicals and reduce most DPPH radical molecules. Moreover, antioxidants are reactive substances that could easily react with radicals but also react with some oxygen species. Heating could enhance the reaction rate between antioxidant and oxidant and could also determine the antioxidant degradation and consumption through different pathways (Sacchetti et al., 2009). Thus, differences in the heating temperature might also explain the difference in antioxidant activity observed between samples cooked by different methods.

The measured RSA in both fishes was, in part, due to qualitatively and/or quantitatively different hydrophilic compounds. The wild and the farmed fish have different compositions, including different fatty acid, mineral and

amino acid contents. This difference was particularly due to their different diet and environmental conditions (Grigorakis et al., 2002; Luzia et al., 2009). Also, the farmed fish were given a diet supplemented with vitamins and other compounds in order to increase their immunity. With oven cooking, water-soluble extracts of both farmed and wild fish lost approximately half of their RSA property: 44.14 and 43.66%, respectively. This method of cooking had a similar impact on measured DPPH-activity for farmed and wild fish. Finally, grilled farmed fish and oven-cooked wild fish showed the best DPPH activity.

Hydroxyl radical scavenging activity

Oxygen radicals induced some oxidative damage to biomolecules, example carbohydrates, proteins, lipids and nucleic acids, and this damage is associated with aging, cancer and several diseases (Suematsu et al., 1977; Je et al., 2004; Calabrese et al., 2005). Among the oxygen radicals, the hydroxyl radical is the most reactive and severely damaged adjacent biomolecules. Therefore, it is important and urgent to search for hydroxyl radical scavengers in food materials for prevention of several diseases. Water-soluble extracts of all cooked wild and farmed sea bream showed effective scavenging ability on

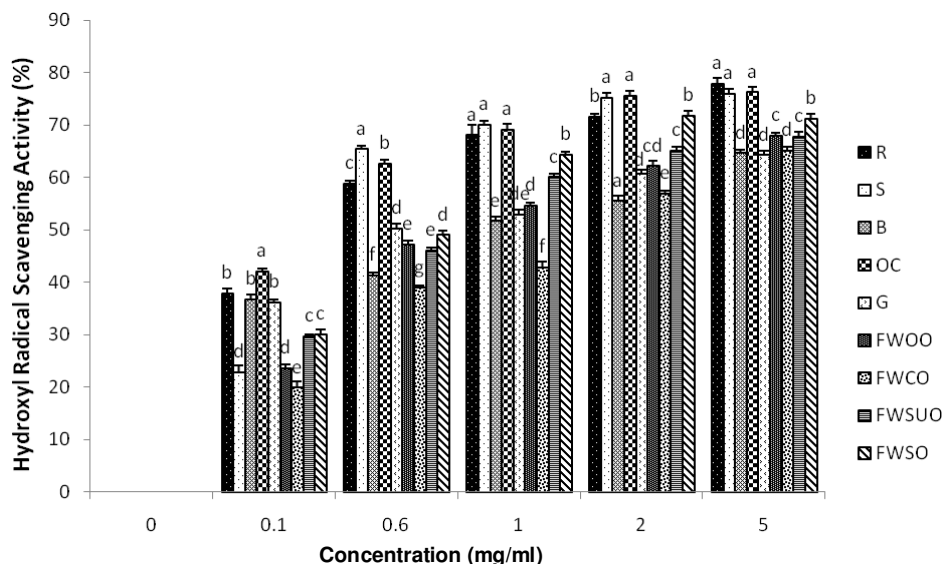


Figure 3. Antioxidant activity of water soluble fractions of wild sea bream cooked in different ways at different concentrations as determined by hydroxyl radical scavenging activity. Each value is expressed as mean \pm standard deviation ($n = 4$). Different small letters within histogram are significantly different ($p < 0.05$) with respect to the concentration of the extract. R: raw; S: steamed; B: boiled; OC: oven cooked; G: grilled; FWOO: fried with olive oil; FWCO: fried with corn oil; FWSUO: fried with sunflower oil; FWSO: fried with soybean oil.

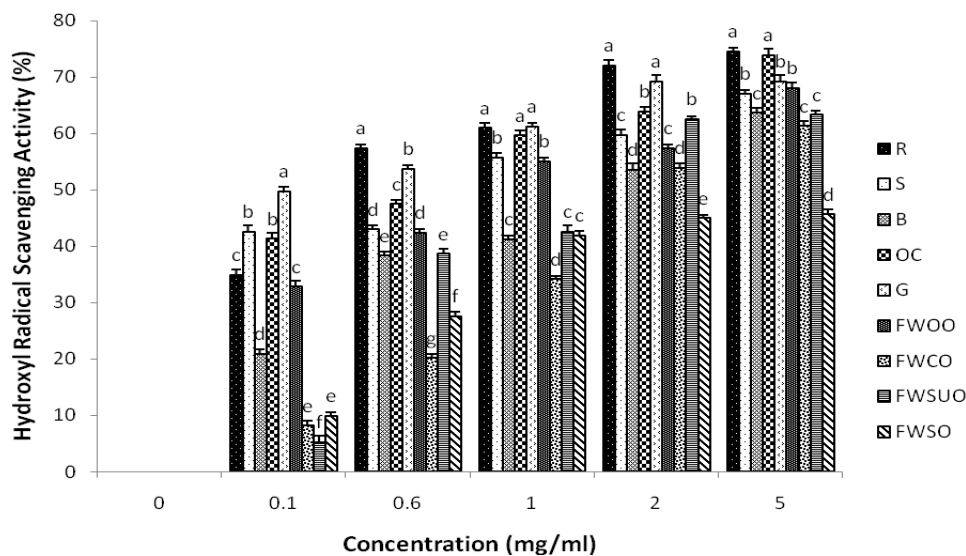


Figure 4. Antioxidant activity of water soluble fractions of farmed sea bream cooked in different ways at different concentrations as determined by hydroxyl radical scavenging activity. Each value is expressed as mean \pm standard deviation ($n = 4$). Different small letters within histogram are significantly different ($p < 0.05$) with respect to the concentration of the extract. R: raw; S: steamed; B: boiled; OC: oven cooked; G: grilled; FWOO: fried with olive oil; FWCO: fried with corn oil; FWSUO: fried with sunflower oil; FWSO: fried with soybean oil.

hydroxyl radicals in a concentration-dependent manner (Figures 3 and 4).

In wild fish, at 1 mg/ml of extracts, the RSA loss after

cooking as compared to that in raw fish was: fried in corn oil 43%, boiled 24%, grilled 22.2%, fried in olive oil 19.6%, fried in sunflower oil 11.7% and fried in soybean

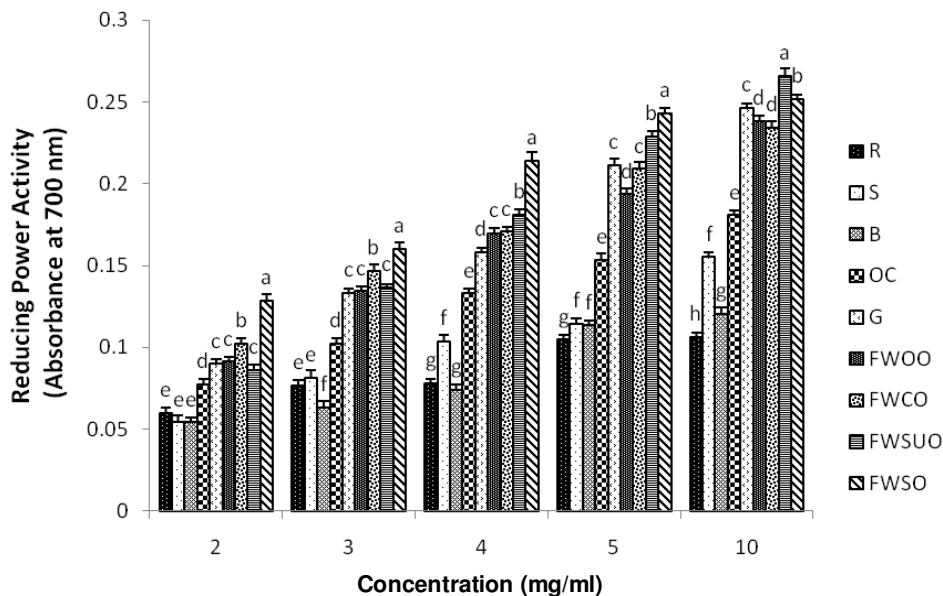


Figure 5. Reducing power activity of water soluble fractions of wild sea bream cooked in different ways at different concentrations. Each value is expressed as mean \pm standard deviation ($n = 4$). Different small letters within histogram are significantly different ($p < 0.05$) with respect to the concentration of the extract. R: raw; S: steamed; B: boiled; OC: oven cooked; G: grilled; FWOO: fried with olive oil; FWCO: fried with corn oil; FWSUO: fried with sunflower oil; FWSO: fried with soybean oil.

oil 5.3%. Meanwhile, the measured RSA of oven-cooked and steamed wild fish was stable. In farmed fish, at 1 mg/ml of extracts, the loss of RSA as compared to that in raw fish was: fried in corn oil 44%, boiled 32.3%, fried in soybean oil 31.3%, fried in sunflower oil 30.1%, steamed 9.75% and fried in olive oil 9.7%. Boiling and frying process caused significant ($p < 0.05$) loss of antioxidants from the samples to cooking water and oil in all the tested wild and farmed samples (Figures 3 and 4). This is an indication that the activity could have leached into the boiling water and frying oil.

There was no loss of RSA from water-soluble extracts of grilled or oven-cooked farmed fish. The water-soluble extracts of oven-cooked wild and farmed fish showed a higher hydroxyl radical scavenging activity. It was due to the inhibition of desoxyribose degradation and protection of carbohydrate from oxidation provoked by $\cdot\text{OH}$. This protection might be due to the active hydrogen donor ability of hydroxyl substitution.

Test for ferric ion reducing capacity

The antioxidant activity of natural components might have a reciprocal correlation with their reducing power (Duh et al., 1999; Yildirim et al., 2001). Figures 5 and 6 show the reducing power of the water-soluble extracts of raw and cooked wild and farmed sea bream. The reducing power of all samples increased with concentration and showed similar reducing power activity. These results indicate

that reductone-related products appeared to be present in the water-soluble extracts. In both farmed and wild fish at 10 mg/ml of extracts, the reducing power in the water-soluble extracts of fried and grilled fish was significantly different ($p < 0.05$) from that obtained from oven-cooked, steamed and boiled fish. It is suggested that water-soluble extracts of fried and grilled fish have a high tendency to donate electrons to reactive free radicals, converting them into more stable products and terminating the free radical chain reaction. It seems that the dual heating process and the high temperature affected proteins and peptides containing free hydroxyl groups that could have contributed to the reducing activity (Wong and Kitts, 2003). The RSA values from the reducing power test were in contrast with those obtained by DPPH and the hydroxyl tests. Thus, media with different polarity might contribute to the extraction of different antioxidative compounds with a varying mode of functions. Also, it is very important to quantify the amounts of compounds present in extracts before any firm conclusions can be reached.

EC50 in antioxidant properties

The antioxidant properties assayed on DPPH and hydroxyl radicals are summarized in Table 1. The results have been normalized and are expressed as EC50 values (mg various extracts per ml) for comparison. The effectiveness of antioxidant properties was inversely

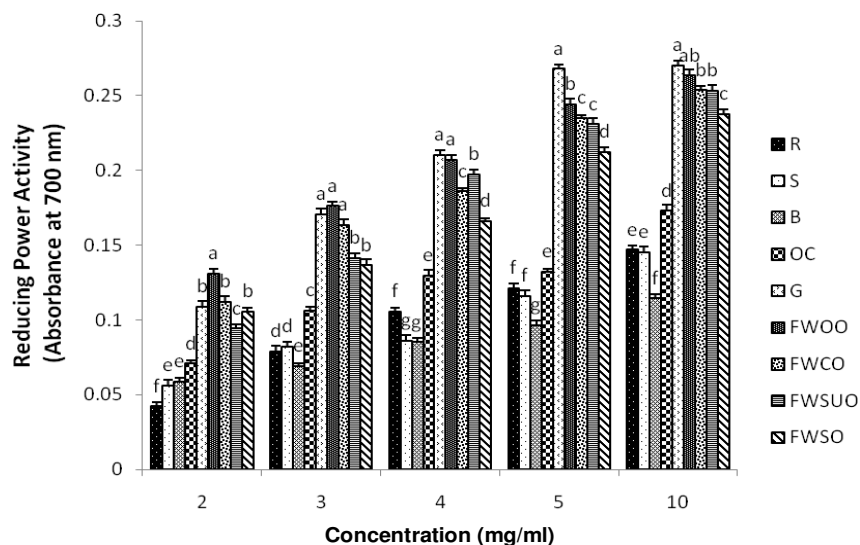


Figure 6. Reducing power activity of water soluble fractions of farmed sea bream cooked in different ways at different concentrations. Each value is expressed as mean \pm standard deviation (n = 4). Different small letters within histogram are significantly different ($p < 0.05$) with respect to the concentration of the extract. R: raw; S: steamed; B: boiled; OC: oven cooked; G: grilled; FWOO: fried with olive oil; FWCO: fried with corn oil; FWSUO: fried with sunflower oil; FWSO: fried with soybean oil.

TABLE 1. EC₅₀ values of soluble water extracts from cooked wild and farmed sea bream

		R	B	S	OC	G	OO	CO	SUO	SO
Scavenging activity on DPPH radicals	<u>Wild fish</u>	7.28 $\pm 0.34^A$	30.25 $\pm 1.34^A$	24.1 $\pm 1.28^B$	12.9 $\pm 0.25^B$	33.9 $\pm 1.48^A$	20.8 $\pm 1.35^B$	14.24 $\pm 0.48^A$	14.6 $\pm 0.52^A$	17.02 $\pm 0.36^A$
	<u>Farmed fish</u>	6.01 $\pm 0.1^B$	19.9 $\pm 0.1^B$	9.9 $\pm 0.08^B$	14.73 $\pm 0.23^B$	8.4 $\pm 0.15^B$	10.63 $\pm 0.14^B$	11.7 $\pm 0.08^B$	11.5 $\pm 0.13^B$	9.6 $\pm 0.1^B$
Scavenging activity on hydroxyl radicals	<u>Wild fish</u>	2.67 $\pm 0.1^{bc}$ B	3.36 $\pm 0.11^A$	2.61 $\pm 0.03^A$	2.59 $\pm 0.01^A$	3.2 $\pm 0.03^B$	3.16 $\pm 0.03^A$	3.28 $\pm 0.08^A$	2.89 $\pm 0.02^B$	2.8 $\pm 0.02^B$
	<u>Farmed fish</u>	3.49 $\pm 0.06^A$	3.65 $\pm 0.03^A$	2.66 $\pm 0.02^A$	2.3 $\pm 0.06^B$	2.83 $\pm 0.23^A$	2.6 $\pm 0.02^B$	3.03 $\pm 0.01^B$	3.54 $\pm 0.07^A$	3.65 $\pm 0.04^A$

EC₅₀: the effective concentration at which the antioxidant activity using DPPH or hydroxyl radicals were scavenged by 50%. EC₅₀ value was obtained by interpolation from linear regression analysis. Each value is expressed as mean \pm standard deviation (n=4). Means with different small letters within a row are significantly different ($p < 0.05$). Means with different capital letters within a column at a specific antioxidant attribute are significantly different ($p < 0.05$).

R: raw; S: steamed; B: Boiled; OC: oven cooked; G: grilled; OO: fried with olive oil; CO: fried with corn oil; SUO: fried with sunflower oil; SO: fried with soybean oil.

correlated with the EC₅₀ value. Water-soluble extracts of raw and cooked wild and farmed sea bream were more effective in scavenging ability on hydroxyl radicals than those on DPPH radicals, as shown by their lower EC₅₀ values. Among the water-soluble extracts of the wild fish, the oven-cooked extract had the best scavenging activity

on DPPH and hydroxyl radicals. The grilled extract was most effective in scavenging ability among the farmed fish extracts on DPPH radicals; whereas the oven-cooked extracts were more effective in scavenging ability on hydroxyl radicals. Water-soluble extracts of boiled wild and farmed sea bream had a lower level of scavenging

ability on DPPH and hydroxyl radicals.

Conclusion

Three methods: reducing power, DPPH radicals and hydroxyl radical-scavenging activities were used in this study for measuring the antioxidant property of water-soluble extracts of wild and farmed sea bream (*S. aurata*) cooked in different ways. It appears that different methods involved reaction with different antioxidant compounds in the water-soluble extracts of cooked wild and farmed sea bream. The measured DPPH radicals' activity was more important in farmed fish than in wild fish. Water-soluble extracts of oven-cooked wild and grilled farmed fish showed the best scavenging activity. RSA on hydroxyl radicals of oven-cooked exhibited the best activity. The data of reducing power activity were similar for water soluble extracts of wild and farmed sea bream. Finally, *in vitro* tests are not sufficient to evaluate the radical scavenging activity and cannot to be extrapolated to the *in vivo* effect (Houghton et al., 2007). Thus, it will be interesting to identify the bioactive compound(s) responsible for the observed antioxidant activity and some tests need to be performed in biological models, as animal systems.

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