

Nutritional Fatty Acid Quality of Raw and Cooked Farmed and Wild Sea Bream (*Sparus aurata*)

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The effects of steaming, grilling, and frying in corn and sunflower oils, respectively, on the fatty acid compositions of farmed and wild sea bream were evaluated. The lipid content increased with frying in both oil types. The maximum moisture value was found in steamed fish (P < 0.05). Fried sea bream in corn and sunflower oils contained a lower content of n-3 polyunsaturated fatty acids (P < 0.05) (3.87 and 5.32% of total fatty acids (TFA) in farmed fish and 2.96 and 2.14% TFA in wild fish). The n-3/n-6 ratio decreased significantly after cooking, particularly after frying in corn and sunflower oils, respectively: from 2.51 to 0.08 and 0.12 in farmed fish and from 0.94 to 0.06 and 0.04 in wild fish. The trans fatty acid levels remain stable after steaming and grilling, but they were significantly affected by frying. Our results reveal that the cooking process has considerable effect on the fatty acid compositions of farmed and wild sea bream.

KEYWORDS: Farmed and wild sea bream; cooking procedures; EPA; DHA trans fatty acids

INTRODUCTION

Aquatic ecosystems are known to be the main source of n-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Humans obtain the principal part of EPA and DHA by consuming fish and aquatic invertebrates (1). PUFA can reduce blood LDL cholesterol and have antithrombotic, antiinflammatory, antiarrhythmic, and vasodilatory properties (2). Regular consumption of food with an appropriate content of EPA and DHA provides prevention and treatment of depression and cardiovascular and other diseases (3-7). Sea bream (*Sparus aurata*) is very popular among Tunisian consumers and has become extensively cultured species in Tunisia and most of the Mediterranean region. Fish is commonly consumed as fried, grilled, boiled, or steamed. Generally, most information about PUFA content is available for raw fish; thus, the consumer has little knowledge about the nutritive values of cooked fish.

Several studies have mentioned that unsaturated fatty acids are more succeptible to oxidation than their saturated analogues and PUFA content in aquatic species has been shown to decrease during frying (8, 9). However, it was found that in some kinds of fish, the PUFA levels remained unchanged after boiling, roasting, and frying (10, 11).

Furthermore, during frying oil, some of the cis fatty acids are converted to trans isomers (12). These unsaturated fatty acids do not have the same nutritional value as the cis-unsaturated fatty acids, as they have not been shown to be of any benefit in the prevention of disease, although conjugated linoleic acid (CLA) is the exception. Ascherio and Willet (13) showed that trans fatty acids (trans FA) increase plasma concentrations of low-density lipoprotein cholesterol and reduce concentrations of high-density lipoprotein cholesterol.

The effects of frying (8, 9, 11, 14), grilling (15, 16), boiling (11, 14), and oven cooking (9, 17) on the nutritive values of different fish species have been previously studied. However, there is little information regarding the changes in the fatty acid compositions of sea bream, including trans FA. Thus, the aim of this study was to examine how different cooking methods affect the fatty acid profiles and concentrations of farmed and wild sea bream with the focus on EPA, DHA, n-3/n-6 ratio, and trans FA contents.

MATERIALS AND METHODS

Materials. Sunflower and corn oils used for frying were purchased at local stores. These particular oils were selected because they are the most frequently used by Tunisian consumers. All chemicals used (chloroform, methanol, hexane, KOH) were of analytical or chromatographic grade (Sigma, France). The standard fatty acid methyl esters and nonadecanoic acid methyl ester used as the internal standard with purity specific for GC were purchased from Sigma (France).

Fish Samples. Cultured gilthead sea bream (*Sparus aurata*) obtained in the autumn of 2008 from the Station of Tunisian Aquaculture located in Hergla, Tunisia (temperature, 22 °C; salinity, 40%; pH, 8.2), with average weight and total length of 85 ± 2.3 g and 17.5 ± 0.1 cm, respectively, were raised under the usual farming conditions, using the same feed and feeding techniques. The composition of the feed used in this study is given in **Table 1**. Wild gilthead sea bream (average weight and total length of $71 \pm$ 0.9 g and 17.5 ± 0.2 cm) were caught in the same season off the coastal waters of Monastir (Central East of Tunisia) (temperature, 25 °C; salinity, 38%; pH, 7.8). Specimens were transported in ice to the laboratory, where they were weighed and immediately processed. Four fish of each kind were used in each analysis, with eight fish being subjected to each

Table 1. Fatty Acid Content (g/100 g of Fresh Weight) in Farmed Sea Bream after Different Methods of Cooking^a

	raw	steamed	grilled	corn oil	sunflower oil
C12:0	$0.005\pm0.001^{\text{a}}$	$0.007\pm0.002^{\rm a}$	nd	nd	nd
C14:0	0.313 ± 0.016^{a}	$0.393\pm0.065^{\text{a}}$	0.334 ± 0.040^{a}	$0.034\pm0.008^{\rm c}$	$0.060\pm0.005^{\rm b}$
C16:0	0.980 ± 0.053^{a}	1.260 ± 0.264^{a}	1.025 ± 0.064^{a}	$0.763\pm0.128^{\text{b}}$	$0.582\pm0.038^{ ext{b}}$
C17:0	0.091 ± 0.004^{a}	0.107 ± 0.024^{a}	$0.085\pm0.006^{\rm a}$	$0.016 \pm 0.002^{\rm b}$	$0.021 \pm 0.001^{ t b}$
C18:0	0.200 ± 0.005^{a}	0.224 ± 0.027^{a}	0.199 ± 0.001^{a}	0.144 ± 0.023^{b}	0.238 ± 0.021^{a}
C20:0	0.015 ± 0.002^{a}	$0.018\pm0.002^{\text{a}}$	0.010 ± 0.005^{a}	0.027 ± 0.006^{a}	0.017 ± 0.001^{a}
C22:0	0.053 ± 0.011^{a}	$0.040\pm0.013^{\rm a}$	0.030 ± 0.016^{a}	$0.009\pm0.003^{\rm b}$	$0.012\pm0.002^{ ext{b}}$
SFA	1.661 ± 0.410^{a}	2.051 ± 0.380^{a}	1.683 ± 0.210^{a}	$0.993\pm0.060^{\rm b}$	$0.931\pm0.050^{ ext{b}}$
C14:1	0.026 ± 0.001^{a}	$0.031\pm0.008^{\rm a}$	$0.026\pm0.003^{\rm a}$	$0.004\pm0.001^{\rm b}$	$0.005\pm0.001^{ ext{b}}$
C16:1 n-7t	0.019 ± 0.001^{a}	$0.022\pm0.004^{\text{a}}$	$0.010\pm0.005^{\text{a}}$	$0.005\pm0.001^{\rm b}$	$0.005\pm0.001^{ ext{b}}$
C16:1 n-7c	0.415 ± 0.026^{a}	0.492 ± 0.114^{a}	$0.396\pm0.038^{\text{a}}$	$0.050 \pm 0.011^{ m b}$	$0.083\pm0.006^{ ext{b}}$
C17:1	0.083 ± 0.004^{a}	0.094 ± 0.02^{a}	0.068 ± 0.005^{a}	0.011 ± 0.002^{b}	$0.017 \pm 0.001^{ m b}$
C18:1 n-9t	0.010 ± 0.001^{a}	0.008 ± 0.001^{a}	0.007 ± 0.004^{b}	0.004 ± 0.001^{b}	$0.003 \pm 0.001^{ m b}$
C18:1 n-11t	0.017 ± 0.001^{a}	0.033 ± 0.014^{a}	0.044 ± 0.028^{a}	0.023 ± 0.02^{a}	0.040 ± 0.023^{a}
C18:1 n-9c	0.879 ± 0.025^{a}	1.082 ± 0.151^{a}	0.850 ± 0.035^{a}	1.721 ± 0.313 ^b	1.886 ± 0.200^{b}
C18:1 n-7	0.202 ± 0.009^{a}	0.224 ± 0.031^{a}	0.193 ± 0.002^{a}	0.057 ± 0.006^{b}	0.081 ± 0.002^{b}
C20:1t	0.017 ± 0.001^{a}	0.019 ± 0.003^{a}	$0.009 \pm 0.005^{\rm b}$	0.006 ± 0.001^{b}	0.004 ± 0.001^{b}
C20:1 n-9	0.095 ± 0.002^{a}	0.104 ± 0.01^{a}	0.096 ± 0.009^{a}	0.030 ± 0.006^{b}	0.031 ± 0.001^{b}
C22:1 n-9	0.000 ± 0.002^{a}	0.023 ± 0.007^{a}	0.009 ± 0.005^{a}	nd	nd
MUFA	1.781 ± 0.36^{a}	2.132 ± 0.007	1.708 ± 0.38^{a}	1.913 ± 0.22^{a}	2.168 ± 0.36^{a}
C18:2n-6 (t9,t12)	0.015 ± 0.001^{a}	0.012 ± 0.001^{a}	0.010 ± 0.007^{a}	nd	0.003 ± 0.001^{b}
C18:2n-6 (c9,t12)	nd	nd	nd	0.006 ± 0.003^{a}	$0.000 \pm 0.001^{\circ}$ $0.012 \pm 0.002^{\circ}$
C18:2n-6 (t9,c12)	0.005 ± 0.001^{a}	0.012 ± 0.004^{a}	0.031 ± 0.017^{a}	0.000 ± 0.000 0.012 ± 0.004^{a}	0.012 ± 0.002 $0.052 \pm 0.032^{\circ}$
C18:2n-6(c9,c12)	0.388 ± 0.021^{b}	0.012 ± 0.004 0.457 ± 0.066^{b}	0.031 ± 0.042^{b}	2.940 ± 0.525^{a}	$3.067 \pm 0.371^{\circ}$
C18:3 n-6	0.015 ± 0.001^{a}	0.437 ± 0.000 0.015 ± 0.004^{a}	0.007 ± 0.003^{a}	0.022 ± 0.015^{a}	nd
C20:2	0.013 ± 0.001^{a}	0.013 ± 0.004 0.019 ± 0.002^{a}	0.007 ± 0.003 0.010 ± 0.005^{a}	0.022 ± 0.013 0.005 ± 0.001^{b}	0.003 ± 0.001^{b}
C20:2 C20:3 n-6	0.017 ± 0.001^{a}	0.019 ± 0.002 0.013 ± 0.001^{a}	0.010 ± 0.003^{a}	0.005 ± 0.001 nd	0.003 ± 0.001 nd
C20:3 II-6 C20:4	0.012 ± 0.001 0.054 ± 0.003^{a}	0.013 ± 0.001 0.056 ± 0.006^{a}	0.000 ± 0.003 0.050 ± 0.002^{a}	0.007 ± 0.001^{b}	0.011 ± 0.002^{b}
C20:4 C22:2 n-6					
	0.003 ± 0.001^{a}	0.005 ± 0.001^{a}	nd	nd	0.003 ± 0.001^{a}
C22:4 n-6	0.003 ± 0.001^{b}	0.009 ± 0.005^{b}	nd	0.020 ± 0.008^{a}	nd
PUFA n-6	0.520 ± 0.100^{b}	0.610 ± 0.080^{b}	0.492 ± 0.022^{b}	3.017 ± 0.150^{a}	3.168 ± 0.160^{a}
C18:3 n-3t	0.021 ± 0.040^{a}	0.024 ± 0.003^{a}	0.013 ± 0.006^{a}	0.007 ± 0.002^{b}	0.005 ± 0^{b}
C18:3 n-3c	0.073 ± 0.003^{a}	0.070 ± 0.009^{a}	0.052 ± 0.007^{a}	0.053 ± 0.013^{a}	0.015 ± 0.001^{b}
C20:3 n-3	0.008 ± 0.002^{a}	0.009 ± 0.001^{a}	nd	nd	nd
C20:5t	0.087 ± 0.005^{a}	0.080 ± 0.007^{a}	0.055 ± 0.002^{a}	0.018 ± 0.002^{b}	0.052 ± 0.007^{a}
C20:5	0.727 ± 0.049^{a}	0.661 ± 0.061^{a}	0.492 ± 0.020^{b}	0.075 ± 0.015^{d}	$0.135 \pm 0.011^{\circ}$
C22:5 n-3	0.033 ± 0.001^{a}	0.034 ± 0.004^{a}	$0.016 \pm 0.008^{ m b}$	$0.006 \pm 0.002^{ m b}$	0.007 ± 0.002^{b}
C22:6t	0.020 ± 0.003^{a}	0.023 ± 0.005^{a}	0.012 ± 0.006^{a}	0.004 ± 0.001^{b}	0.005 ± 0.001^{b}
C22:6	0.631 ± 0.036^{a}	0.599 ± 0.105^{a}	0.370 ± 0.047^{b}	$0.075 \pm 0.027^{\circ}$	$0.116 \pm 0.008^{\circ}$
PUFA n-3	1.701 ± 0.160^{a}	1.501 ± 0.200^{ab}	1.011 ± 0.270^{b}	$0.238 \pm 0.045^{\circ}$	$0.357 \pm 0.051^{\circ}$
PUFA	2.261 ± 0.240^{b}	2.101 ± 0.150^{b}	$1.501 \pm 0.340^{\circ}$	3.255 ± 0.275^{a}	3.525 ± 0.268^{a}
trans FA	$\textbf{0.215}\pm\textbf{0.009}^{a}$	$0.235\pm0.009^{\text{a}}$	0.191 ± 0.003^{b}	$0.085\pm0.008^{\rm c}$	$0.181\pm0.004^{ m d}$
n-3/n-6	$3.260\pm0.430^{\text{a}}$	$2.450\pm0.310^{\text{a}}$	$2.050\pm0.160^{\text{b}}$	$0.078 \pm 0.080^{\circ}$	$0.112 \pm 0.093^{\circ}$
PUFA/SFA	1.210 ± 0.020^{a}	1.024 ± 0.130^{a}	0.910 ± 0.070^{a}	$3.277 \pm 0.310^{ m b}$	$3.786 \pm 0.250^{ ext{b}}$

^a Mean values from four samples \pm SD. Values in the same row bearing different letters are significantly different ($p \le 0.05$). nd = not detected.

treatment: steaming, grilling, frying in corn oil (FWCO), and frying in sunflower oil (FWSO).

Cooking. Common ways of cooking were used. Grilled fish were prepared in a Black and Decker griller with the thermostat set at 300 °C. After the set temperature was attained, the fish were grilled for 10-15 min. For steaming, the water was poured into the bottom of the pot and heated to the boiling point, and then the fish was put on the middle layer of the pot. The fish was steamed for 15 min. The core temperature of the samples during cooking was 78-82 °C. For the frying experiments, a Tefal frying pan (o.d. 20 cm) was used on the same electrical heating unit. After each frying process, the pan had been cleaned with a paper towel after collecting the fat that remained in the pan. The samples were placed into the frying pan after the oil had reached the desired temperature (180 °C), which was controlled by a digital thermometer. Samples were fried in sunflower or corn oil for 10 min. The cooking steps have been tested and standardized in pre-experiments. Four samples from each species were cooked in each method.

Sample Preparation. Flesh samples for fatty acid analyses were collected before and after cooking. Muscle tissues under the dorsal fin were used as the samples. One gram of the sample was cut and minced. Total lipids were extracted with chloroform/methanol (2:1) according to

the method of Folch et al. (18), as modified by Bligh and Dyer (19). To measure the moisture content, samples were taken and dried to a constant weight at 105 °C. Raw samples were analyzed in the same way.

Analysis of Fatty Acid Composition. The fatty acid compositions of raw and cooked fish were determined by gas chromatography. Fatty acid methyl esters were prepared using 2 M KOH in methanol and n-hexane according to the method described by Ichihara et al. (20) with minor modifications: 10 mg of extracted fat was dissolved in 2 mL of hexane followed by 4 mL of 2 M methanolic KOH. The mixture was then vortexed for 2 min at room temperature and the hexane layer was used for GC analyses. The chromatographic separation was carried out using a Hewlett-Packard (HP 5890) chromatograph, a split/splitless injector, and a flame-ionization detector (FID) linked to an HP Chemstation integrator. A DB23 fused silica capillary column (60 m length \times 0.32 mm i.d. $\times 0.25 \,\mu$ m film thickness; HP-Agilent Technologies, Wilmington, DE) was used with nitrogen as the carrier gas, the flow rate of which was set at 0.44 mL/min; the temperature of the flame ionization detector was maintained at 280 °C and that of the injector at 270 °C. The column temperature program was as follows: from 130 to 170 °C at 6.5 °C/min, from 170 to 215 °C at 2.8 °C/min, 12 min isotherm, 230 °C at 40 °C/min and 20 min isotherm. The standard fatty acid methyl esters (FAMEs) were

	raw	steamed	grilled	corn oil	sunflower oil
C12:0	$0.003\pm0.001^{\text{a}}$	nd	$0.004\pm0.001^{\text{a}}$	nd	nd
C14:0	$0.067\pm0.013^{\rm a}$	0.048 ± 0.013^{a}	$0.012\pm0.010^{\rm b}$	$0.010\pm0.001^{\rm b}$	$0.009 \pm 0.001^{ ext{b}}$
C16:0	$0.564\pm0.154^{\rm b}$	$0.357 \pm 0.061^{ m b}$	0.684 ± 0.179^{a}	$0.375\pm0.038^{\text{b}}$	$0.294 \pm 0.012^{\circ}$
C17:0	0.047 ± 0.016^{a}	0.026 ± 0.005^{a}	$0.075\pm0.016^{\rm a}$	$0.010\pm0.001^{\text{b}}$	$0.007\pm0.002^{\rm k}$
C18:0	0.255 ± 0.105^{a}	$0.129\pm0.019^{\rm b}$	0.464 ± 0.097^{a}	$0.089 \pm 0.005^{\circ}$	0.138 ± 0.004^{t}
C20:0	$0.009\pm0.003^{\rm a}$	0.004 ± 0.001^{a}	0.020 ± 0.005^{a}	0.012 ± 0.001^{a}	$0.009\pm0.003^{\circ}$
C22:0	0.008 ± 0.004^{a}	nd	$0.021\pm0.008^{\rm a}$	nd	nd
SFA	0.904 ± 0.140^{a}	$0.565\pm0.070^{\rm b}$	1.280 ± 0.540^{a}	$0.496\pm0.090^{\rm c}$	$0.457\pm0.090^{\circ}$
C14:1	0.025 ± 0.009^{a}	$0.008\pm0.004^{\rm b}$	$0.033\pm0.009^{\rm a}$	$0.004\pm0.002^{\rm b}$	nd
C16:1 n-7t	$0.011 \pm 0.002^{ m b}$	$0.007\pm0.002^{\rm b}$	$0.027\pm0.009^{\rm a}$	nd	nd
C16:1 n-7c	$0.138\pm0.028^{\rm a}$	0.098 ± 0.025^{a}	$0.264\pm0.078^{\rm a}$	$0.026\pm0.003^{ m b}$	0.020 ± 0.003^{t}
C17:1	0.025 ± 0.006^{a}	0.010 ± 0.004^{a}	$0.038 \pm 0.007^{ ext{a}}$	$0.005 \pm 0.001^{ m b}$	0.003 ± 0.001^{t}
C18:1 n-9 t	0.015 ± 0.007^{a}	$0.004 \pm 0.001^{ m b}$	$0.023\pm0.006^{\rm a}$	nd	nd
C18:1 n-11t	$0.020\pm0.01^{\mathrm{ab}}$	0.015 ± 0.005^{a}	0.029 ± 0.017^{ab}	$0.016\pm0.006^{ ext{b}}$	0.051 ± 0.007^{a}
C18:1 n-9c	$0.652\pm0.27^{\rm b}$	$0.297\pm0.058^{\rm c}$	1.086 ± 0.032^{a}	$0.828\pm0.078^{\rm b}$	1.094 ± 0.029^{a}
C18:1 n-7	0.109 ± 0.019^{b}	$0.085\pm0.028^{\rm b}$	0.242 ± 0.076^{a}	$0.034 \pm 0.004^{\circ}$	$0.038 \pm 0.002^{\circ}$
C20:1t	0.044 ± 0.020^{a}	0.014 ± 0.007^{a}	0.059 ± 0.030^{a}	0.013 ± 0.003^{b}	$0.007 \pm 0.003^{\text{k}}$
C20:1 n-9	0.071 ± 0.042^{a}	0.034 ± 0.005^{a}	0.075 ± 0.016^{a}	0.013 ± 0.002^{b}	0.009 ± 0.003^{t}
C22:1 n-9	0.009 ± 0.002^{a}	0.003 ± 0.001^{b}	0.015 ± 0.003^{a}	nd	nd
MUFA	1.120 ± 0.130^{b}	$0.575 \pm 0.270^{\circ}$	2.521 ± 0.780^{a}	0.945 ± 0.220^{b}	1.225 ± 0.170^{t}
C18:2n-6 (t9.t12)	0.005 ± 0.001^{b}	nd	0.022 ± 0.011^{a}	nd	nd
C18:2n-6 (c9.t12)	0.008 ± 0.005^{a}	nd	0.011 ± 0.006^{a}	0.004 ± 0.001^{a}	$0.006 \pm 0.001^{\circ}$
C18:2n-6 (t9.c12)	0.003 ± 0.001^{b}	nd	0.003 ± 0.001^{b}	0.049 ± 0.019^{a}	$0.056 \pm 0.023^{\circ}$
C18:2n-6(c9.c12)	$0.121 \pm 0.049^{\circ}$	0.026 ± 0.008^{d}	$0.069 \pm 0.010^{\circ}$	1.314 ± 0.184^{b}	$1.683 \pm 0.054^{\circ}$
C18:3 n-6	0.007 ± 0.001^{a}	nd	0.009 ± 0.002^{a}	nd	nd
C20:2	0.018 ± 0.003^{a}	0.007 ± 0.003^{b}	0.027 ± 0.01^{a}	$0.003 \pm 0.001^{\rm b}$	nd
C20:3 n-6	$0.009 \pm 0.001^{\rm b}$	$0.004 \pm 0.002^{\circ}$	0.017 ± 0.001^{a}	nd	nd
C20:4	0.162 ± 0.017^{a}	0.173 ± 0.040^{a}	0.197 ± 0.019^{a}	$0.045 \pm 0.007^{\rm b}$	0.047 ± 0.005^{t}
C22:2 n-6	nd	nd	0.009 ± 0.004^{a}	nd	nd
C22:4 n-6	0.007 ± 0.001^{a}	nd	0.004 ± 0.002^{a}	nd	nd
PUFA n-6	$0.222 \pm 0.080^{\circ}$	0.211 ± 0.070 ^c	0.370 ± 0.780^{b}	1.450 ± 0.110^{a}	1.801 ± 0.320 ^e
C18:3 n-3t	0.006 ± 0.002^{a}	nd	0.01 ± 0.001^{a}	0.006 ± 0.002^{a}	nd
C18:3 n-3c	0.011 ± 0.002^{b}	$0.006 \pm 0.002^{\rm bc}$	0.024 ± 0.007^{a}	0.024 ± 0.001^{a}	$0.004 \pm 0.001^{\circ}$
C20:3 n-3	0.002 ± 0.001^{a}	nd	0.008 ± 0.004^{a}	nd	nd
C20:5t	0.017 ± 0.003^{b}	$0.004 \pm 0.001^{\circ}$	0.028 ± 0.007^{ab}	$0.007 \pm 0.003^{\circ}$	0.031 ± 0.004^{a}
C20:5	0.093 ± 0.017^{ab}	$0.072 \pm 0.001^{\text{b}}$	0.020 ± 0.007 0.090 ± 0.013^{a}	$0.023 \pm 0.004^{\circ}$	$0.024 \pm 0.004^{\circ}$
C22:5 n-3	0.005 ± 0.002^{a}	nd	0.011 ± 0.006^{a}	nd	nd
C22:6t	0.000 ± 0.002^{a}	nd	0.010 ± 0.002^{a}	nd	nd
C22:6	0.004 ± 0.002 0.199 ± 0.068^{a}	0.086 ± 0.012 ^b	$0.010 \pm 0.002^{\circ}$ $0.150 \pm 0.045^{\circ}$	$0.021 \pm 0.007^{\circ}$	$0.013 \pm 0.001^{\circ}$
PUFA n-3	0.139 ± 0.000^{a} 0.340 ± 0.080^{a}	0.000 ± 0.012 0.171 ± 0.090^{a}	0.130 ± 0.043 0.331 ± 0.060^{a}	0.021 ± 0.007 0.084 ± 0.005^{b}	0.073 ± 0.001
PUFA	0.340 ± 0.000 0.570 ± 0.110^{a}	0.171 ± 0.090 0.383 ± 0.120^{a}	0.331 ± 0.000 0.731 ± 0.130^{a}	$1.534 \pm 0.190^{ m b}$	0.074 ± 0.008 1.847 ± 0.150^{t}
Trans FA	0.370 ± 0.110 0.134 ± 0.030^{a}	0.303 ± 0.120 0.100 ± 0.041^{a}	0.731 ± 0.130 0.263 ± 0.110^{a}	0.103 ± 0.070^{a}	$0.138 \pm 0.030^{\circ}$
n-3/n-6	0.134 ± 0.030 1.510 ± 0.300^{a}	0.100 ± 0.041 0.810 ± 0.300^{a}	0.203 ± 0.110 0.940 ± 0.270^{a}	$0.060 \pm 0.010^{ m b}$	0.138 ± 0.030 0.040 ± 0.001^{t}
PUFA/SFA	0.630 ± 0.090^{a}	0.670 ± 0.070^{a}	0.940 ± 0.270 0.570 ± 0.060^{a}	3.110 ± 0.010^{b}	0.040 ± 0.001 4.050 ± 0.110^{t}
	0.030 ± 0.030	0.070 ± 0.070	0.570 ± 0.000	3.110 ± 0.010	4.000 ± 0.110

^a Mean values from four samples \pm SD. Values in the same row bearing different letters are significantly different ($p \le 0.05$). nd = not detected.

run under the same conditions. The FAME peaks p were identified by comparing their retention times with those of the standard mixtures, and the areas under the peaks were automatically integrated using nonadecanoic acid methyl ester (C19:0) as the internal standard.

Statistical Analysis. The descriptive statistics (mean values from four samples \pm standard deviation) and one-way ANOVA were carried out using a statistical analysis system (SPSS Version 12). To evaluate the changes in fatty acid content before and after processing, post hoc analysis was carried out using Tukey's test. Differences were considered to be significant when p < 0.05.

RESULTS AND DISCUSSION

The profile and levels of the fatty acid which were identified in raw and cooked samples for each of the wild and farmed sea bream are mentioned in **Tables 1** and **2**. Saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) were abundant in wild raw fish (P < 0.05) while PUFA were found to have accumulated in farmed sea bream (P < 0.05). Oleic acid (C18:1 n-9) and palmitic acid (C16:0) were the major SFA and MUFA, respectively. Farmed sea bream contained more n-3 PUFA than the wild variety (P < 0.05). In contrast, a concentration of n-6 PUFA was found in wild sea bream (P < 0.05). Similar results were observed for sea bream (21, 22). EPA was the primary n-3 PUFA in farmed sea Bream. In contrast, in wild fish, DHA was found to be the principal n-3 PUFA. The fatty acid profile of farmed sea bream reflected the fatty acid composition of feed used which was rich in n-3 PUFA (32.3%) particularly EPA and DHA (18.6% and 10.4%, respectively). The level of n-6 PUFA was low (10.1%). Linoleic acid (C18:2 n-6) was the major n-6 PUFA in farmed fish (P < 0.05). However, in wild sea bream arachidonic acid (C20:4 n-6) showed the highest level (P < 0.05). In fact, linoleic acid was found in high concentrations in the feed used for farmed fish (21-23). However, in farmed sea bream, the level of C20:4 n-6 was low because the feed used contained minimal amounts of this fatty acid. This finding confirms that the fatty acid profile of fish reflects the fatty acid composition of fish diet. Several studies have reported the strong relationship between the lipid composition of fish and their diet (23, 24). Farmed sea bream exhibited a higher n-3/n-6 ratio than their wild counterparts. Our results revealed also that trans fatty acid (trans FA) contents were

Table 3. Moisture and Fat Contents in Farmed and Wild Sea Bream after Different Methods of Cooking^a

	raw	steamed	grilled	fried with corn oil	fried with sunflower oil
		Farme	d Sea Bream		
moisture content (%)	$73.9\pm0.5^{\text{a}}$	71.6 ± 0.9^{a}	$56.3\pm2.6^{ m b}$	$35.5\pm4^{ m c}$	$35.3\pm3^{\circ}$
fat content (%)	$49.2\pm8.8^{\text{a}}$	$50\pm10^{\text{a}}$	$150\pm30^{\text{b}}$	$180\pm30^{\text{b}}$	$150\pm30^{\text{b}}$
		Wild	Sea Bream		
moisture content (%)	77 ± 0.3^{a}	76.6 ± 0.3^{a}	64.2 ± 2^{b}	$66.5\pm2.2^{\mathrm{b}}$	$62.3\pm2.1^{ ext{b}}$
fat content (%)	$47.7\pm7.4^{\rm a}$	49 ± 10^{a}	50 ± 0^{a}	$120\pm20^{\mathrm{b}}$	$130\pm10^{\text{b}}$

^a Mean values from four samples \pm SD. Values in the same row bearing different letters are significantly different ($p \le 0.05$).

4.8% and 3.7% of the total fatty acids (percent of the TFA) in raw wild and farmed fish, respectively. In wild sea bream, the prominent trans FA was C20:1t, while in farmed fish, C20:5t showed the highest level.

The effects of steaming, grilling, and frying in corn and sunflower oils, respectively, on the moisture and fat content in farmed and wild sea bream are presented in Table 3. After the cooking process, the minimum moisture value was the characteristic of fish fried with sunflower $(35.3 \pm 3 \text{ and } 62.3 \pm 2.1\%)$ and corn oils $(35.5 \pm 4 \text{ and } 66.5 \pm 2.2\%)$, respectively. The maximum moisture was found in steamed fish, in farmed and wild sea bream (71.6 \pm 0.9 and 76.6 \pm 0.3%, respectively). The lipid content increased significantly after grilling only in farmed fish (P < 0.05). Frying sea bream in corn and sunflower oils, respectively, resulted in a large and significant increase in the fat content. These compositional changes were due to the water loss during the cooking process and also to the absorption of lipids from the frying medium. In general, the possible mechanisms for the changes which may take place during culinary preparation include the absorption of the cooking fat, moisture loss, and the leaching of fat-soluble molecules from the fish together with oxidation reactions which generate free radicals in the hot cooking fat (25).

The changes in the fatty acid content after different cooking processes were marginal, in particular when focusing on the most important n-3 PUFA, EPA, and DHA. The concentration of SFA decreased significantly in wild and farmed fish fried with corn and sunflower oils (P < 0.05). Palmitic acid (C16:0) was the major SFA in the raw fish, but during the frying process it decreased, producing the most important reduction in wild and farmed sea bream. This finding was also reported by Candella et al. (9) for fried mackerel and sardine, by Gladyshev et al. (11, 14) for fried humpback salmon and herring, and by Turkkan et al. (17) for fried sea bass.

No significant differences in the total MUFA content have been observed after the different cooking processes in farmed sea bream. In wild fish, the concentration of MUFA decreased after steaming but increased significantly after grilling (P < 0.05). The concentration of oleic acid (C18:1 n-9c) increased after all cooking processes, except in steamed wild sSea bream. Our results are similar to those mentioned by Candella et al. (9) for fried mackerel and sardine, by Al Saghir et al. (25) for steamed and fried sSalmon, by Gladyshev et al. (11, 14) for fried humpback salmon and herring, and by Turkkan et al. (17) for fried sea bass. The significant increase (P < 0.05) observed for fried fish in corn and sunflower oils, respectively, was probably a consequence of absorption from the frying medium which was rich in this fatty acid. Different effects of frying on SFA and MUFA could be explained by the kind of analyzed fish (initial fat content) as well as the cooking oil selected (9).

The concentration of n-6 PUFA remained stable after steaming in wild and farmed sea bream. However, after grilling, n-6 PUFA decreased in farmed fish but increased significantly in wild fish (P < 0.05). In contrast, after the frying process, the concentration of n-6 PUFA increased significantly in fried fish, especially linoleic acid (C18:2) (P < 0.05). The concentration of lonoleic acid (C18:2) increased in both wild and farmed fish fried in corn and sunflower oils, respectively, by 85% and 83% and by 88% and 86%. Our results are similar to those reported by Candella et al. (8) in sole (*Solea solea*), codfish (*Gadus morrhua*), and hake (*Merluccius merluccius*) fried in sunflower oil, by Al-Saghir et al. (25) in salmon fried in corn oil, and by Weber et al. (26) in silver catfish (*Rhamdia quelen*) fried in soybean oil. Agren and Hanninen (27) have also reported that the greatest increase took place in the content of linoleic acid in rainbow trout (*Oncorhynchus mykiss*), vendace (*Coregonus albula*), and pike (*Esox lucius*) fried in sunflower and rapeseed oils.

For the n-3 PUFA group, all cooking processes lead to a decrease of levels in wild and farmed fish. The decrease after frying with corn and sunflower oils, respectively, was assessed to be significant (P < 0.05). The DHA level decreased significantly after steaming in wild sea bream and after grilling in the farmed fish. In fact, in both types of fish, our results showed a significant important effect of frying process on the DHA level in fried fish. The percentages of loss of DHA after steaming, grilling, and frying with corn and sunflower oils, respectively, were 5%, 41%, 88%, and 82% in farmed fish and 57%, 25%, 90%, and 94% in wild fish. The levels of EPA decreased significantly (P < 0.05) in both types of fish fried in corn and sunflower oils, respectively, in steamed wild fish, and in grilled farmed sea bream. For EPA, the percentages of loss in farmed fish were 9% after steaming, 32% after grilling; 90% after frying with corn oil, and 81% after frying with sunflower oil. In wild fish the percentages of EPA losses after steaming, grilling, and frying with corn and sunflower oils, respectively, were 23%, 4%, 75%, and 74%. This reduction is in agreement with the results of Gladyshev et al. (11, 14), who observed the loss of DHA and EPA in humpback salmon (Oncorhynchus gorbuscha) and Norwegian trout (Salmo trtta) fried in sunflower oil. In general, there was a significant effect of frying on the EPA and DHA levels in fried fish. The decrease in the EPA and DHA levels after frying can be explained by the oil absorption during frying. The oils used in the frying process contained no DHA or EPA. Hence, oil absorption would reduce the level of these fatty acids. Additionally, the long-chain polyunsaturated acids in fish and fish oils, such EPA and DHA, are considered to be highly susceptible to oxidation during heating and other culinary treatments (9, 28-30).

The significant increase in n-6 PUFA and loss of n-3 PUFA in fried fish explain the low n-3/n-6 ratio and the higher value of the PUFA/SFA ratios particularly in fried fish compared to raw fish in wild and farmed sea bream (**Figures 1** and **2**). The PUFA/SFA ratio increased significantly after frying with both oils (P < 0.05). This finding can be attributed to the high content of mono- and polyunsaturated fatty acids in the frying oils used. Thus, the fat composition of fried fish tends to be similar to that of culinary fat (31).

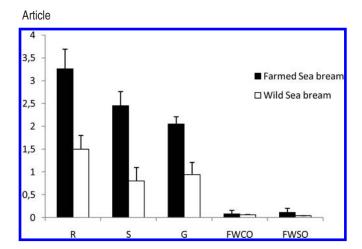


Figure 1. n-3/n-6 ratios in farmed and wild sea bream after different methods of cooking (means of four smaples): R, raw; S, steamed; G, grilled; FWCO, fried with corn oil; FWSO, fried with sunflower oil.

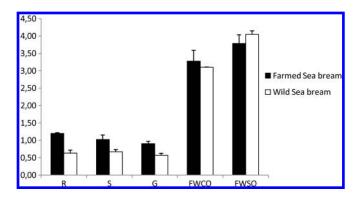


Figure 2. PUFA/SFA ratios in farmed and wild sea bream after different methods of cooking (means of four samples): R, raw; S, steamed; G, grilled; FWCO, fried with corn oil; FWSO, fried with sunflower oil.

The method used for cooking the fish affected the n-3/n-6 ratio, which is of a considerable dietetic importance. In our study, the ratio decreased significantly in wild and farmed fish as a result of frying in corn and sunflower oils, respectively. The decrease in this ratio is an undesired effect, since it affects the health benefits related to the intake of EPA and DHA. A high dietary fat intake, especially of n-6 fatty acids, is related to an increased incidence of breast, prostate, and colon cancers (32, 33). However, diets high in n-3 fatty acids, particularly EPA and DHA, are considered to be important because of their role in the prevention of several degenerative diseases, including cardiovascular and inflammatory dosorders, cancer, and stroke (5, 6).

Our results also revealed that trans FA were affected differently by steaming, grilling, and frying in both types of fish. The consumption of these fatty acids is very worrying, because they can cause a higher risk of coronary disease, sudden death, and possibly diabetes mellitus (34). In wild sea bream, trans FA remain stable after steaming, grilling, and frying with both oil types. Meanwhile, in farmed fish, trans FA decreased after all cooking processes but significant differences (P < 0.05) were observed after grilling and frying, especially in fish fried in corn oil. Candella et al. (9) have also reported that the trans FA content decreased from 7.9 to 0.76% in Sardine (Sardine pilchardus), from 16.7 to 2.4% in mackerel (Scomberomorus *commersoni*), and from 12.41 to 8.86% in salmon (Salmon salar) after frying with sunflower oil. In general, vegetable oils have different susceptibilies toward oxidative degradation, due to differences in their fatty acid unsaturation and the varying type and content of antioxidants (35). Therefore, the use of different fat sources for thermal treatments results in different effects on the oxidative stability of the treated products. trans-Elaidic acid (C18:1 n-9t and C20:1t) decreased significantly (P < 0.05) after frying with both oils in both fish types. trans-Vaccenic acid (C18:1 n-11t) remained stable after all cooking processes in farmed fish. However, in wild fish, the concentration of trans-vaccenic acid increased significantly in fish fried with sunflower oil. The decrease in the individual trans FA observed in wild and farmed sea bream after frying with corn and sunflower oils, respectively, was probably due to the gradient change between the fish being fried and the oils used. In fact, trans FA are formed at a relatively high temperature. Some of the cis fatty acids are converted to trans isomers during the partial hydrogenation of oil to make margarines, shortenings, and frying oils (12). The resulting unsaturated trans FA from vegetable oils are mostly 18-carbon acids of monoene and diene type with trans-elaidic acid (C18:1 n-9t) as one of the major trans FA. However, hydrogenation of fish oils results mainly in monoene type trans FA of 20 and 22 carbons (36). After steaming and grilling, the majority of trans FA remain stable compared to those observed in raw wild and farmed sea bream.

In conclusion, frying wild and farmed sea bream in corn and sunflower oils decreased the SFA, n-3 PUFA, and trans FA contents but increased particularly the level of n-6 PUFA, limiting the benefits of the high n-3 PUFA level in raw fish. Meanwhile, despite containing a significant level of trans FA, steamed and grilled sea bream were the best source of n-3 PUFA, especially EPA and DHA, which probably resulted from their relative stability during steaming and grilling processes.

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LITERATURE CITED

- (1) Arts, M. T.; Ackman, R. G.; Holub, B. J. Essential fatty acids in aquatic ecosystems: a crucial link between diet and human health and evolution. *Can. J. Fish. Aquat. Sci.* **2001**, *58*, 122–137.
- (2) Lombardo, Y.; Chicco, A. G. Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. A review. J. Nutr. Biochem. 2006, 17, 1–13.
- (3) Okita, M.; Sasagawa, T.; Tomioka, K.; Hasuda, K.; Ota, Y.; Suzuki, K.; et al. Habitual food intake and polyunsaturated fatty acid deficiency in liver cirrhosis. *App. Nutr. Inves.* 2002, *18*, 304–308.
- (4) Silvers, K. M.; Scott, K. M. Fish consumption and self-reported physical and mental health status. *Pub. Health. Nutr.* 2002, *5*, 427– 431.
- (5) Kris-Etherton, P.; Harris, W. S.; Appel, L. J. Fish Consumption. Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23*, 20–31.
- (6) Din, J. N.; Newby, D. E.; Flapan, A. D. Science, medicine, and the future-Omega 3 fatty acids and cardiovascular disease-fishing for a natural treatment. *Br. Med. J.* 2004, *28* (7430), 30–35.
- (7) Ruxton, C. H. S.; Calder, P. C.; Reed, S. C.; Simpson, M. J. A. The impact of long-chain n-3 polyunsaturated fatty acids on human health. *Nutr. Res. Rev.* 2005, *18* (1), 113–129.

- (8) Candela, M.; Astiasaran, I.; Bello, J. Effects of frying and warmholding on fatty acids and cholesterol of sole (*Solea solea*). codfish (*Gadus morrhua*) and hake (*Merluccius merluccius*). Food Chem. **1996**, 58 (3), 227–231.
- (9) Candella, M.; Astiasaran, I.; Bello, J. Deep-fat frying modifies highfat fish fraction. J. Agric. Food. Chem. 1998, 46, 2793–2796.
- (10) Montano, N.; Gavino, G.; Gavino, V. C. Polyunsaturated fatty acid contents of some traditional fish and shrimp paste condiments of the Philippines. *Food. Chem.* **2001**, *75*, 155–158.
- (11) Gladyshev, M. I.; Sushchik, N. N.; Gubanenko, G. A.; Demirchieva, S. M.; Kalachova, G. S. Effect of way of cooking on content of essential polyunsaturated fatty acids in muscle tissue of humpback salmon (*Oncorhynchus gorbuscha*). Food Chem. 2006, 96, 446–451.
- (12) Hunter, J. E.; Applewhite, T. H. Reassessment of trans fatty acid availability in the US diet. Am. J. Clin. Nutr. 1991, 54, 363–369.
- (13) Ascherio, A.; Willet, W. C. Health effects of trans fatty acids. Am. J. Clin. Nutr. 1997, 66, 1006–1010.
- (14) Gladyshev, M. I.; Sushchik, N. N.; Gubanenko, G. A.; Demirchieva, S. M.; Kalachova, G. S. Effect of boiling and frying on the content of essential polyunsaturated fatty acids in muscle tissue of four fish species. *Food Chem.* **2007**, *101*, 1694–1700.
- (15) Garcia-Arias, M. T.; Alvarez Pontes, E.; Garcia-Linares, M. C.; Garcia-Fernandez, M. C.; Sanchez-Muniz, F. J. Grilling of Sardines fillets. Effects of frozen and thawed modality on their protein quality. *Lebensm.-Wiss.-Technol.* **2003**, *36*, 763–769.
- (16) Garcia-Arias, M. T.; Alvarez Pontes, E; Garcia-Linares, M. C.; Garcia-Fernandez, M. C.; Sanchez-Muniz, F. J. Cooking- Freezing-Reheating (CFR) of sardine (*Sardina pilchardus*) fillets. Effect of different cooking and reheating procedures on the proximate and fatty acid compositions. *Food Chem.* **2003**, *83* (3), 349–356.
- (17) Turkkan, A. U.; Cakli, S.; Kilinc, B. Effects of cooking methods on the proximate composition and fatty acid composition of Sea bass (*Dicentrarchus labrax*, Linnaeus, 1758). *Food Bioprod. Process.* 2008, 86 (3), 163–166.
- (18) Folch, J.; Lees, M.; Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal's tissues. J. Biol. Chem. 1957, 2, 497–509.
- (19) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917.
- (20) Ichihara, K.; Shibahara, A.; Yamamoto, K.; Nakayama, T. An improved method for rapid analysis of the fatty acids of glycerollipids. *Lipids* **1996**, *31*, 535–539.
- (21) Saglik, S.; Alpasian, M.; Gezgin, T.; Cetinturk, K.; Tekinay, A.; Guven, K. C. Fatty acids composition of wild and cultivated gilthead Sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). *Eur. J. Lipid Sci. Technol.* 2003, 105, 104–107.
- (22) Mnari, A.; Bouhlel, I.; Chraief, I.; Hammami, M.; Romdhane, M. S.; El Cafsi, M.; Chaouch, A. Fatty acids in muscles and liver of Tunisian wild and farmed gilthead Sea bream. *Sparus aurata. Food Chem.* 2007, 100, 1393–1397.

- (23) Grigorakis, K.; Alexis, M. N.; Taylor, A.; Hole, M. Comparison of wild and cultured gilthead sea bream (*Sparus aurata*): composition, appearance and seasonal variations. *Int. J. Food. Sci. Technol.* 2002, *37*, 1–8.
- (24) Pirini, M.; Gatta, P. P.; Testi, S.; Trigari, G.; Monetti, P. G. Effect of refrigerated storage on muscle lipid quality of sea bass (*Dicentrarchus labrax*) fed on diets containing different levels of vitamin E. Food Chem. 2000, 68, 289–293.
- (25) Al-Saghir, S.; Thurner, K.; Wagner, K. H.; Frisch, G.; Luf, W.; Razzazi-Fazeli, E.; Elmadfa, I. Effects of different cooking procedures on lipid quality and cholesterol oxidation of farmed Salmon fish (*Salmon salar*). J. Agric. Food. Chem. 2004, 52, 5290– 5296.
- (26) Weber, J.; Bochi, V. C.; Ribeiro, C. P.; Victorio, A. M.; Emanuelli, T. Effect of different cooking methods on the oxidation. Proximate and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets. *Food Chem.* 2008, *106*, 140–146.
- (27) Agren, J. J.; Hanninen, O. Effects of cooking on the fatty acids of three freshwater fish species. *Food Chem.* **1993**, *46*, 377–382.
- (28) Sant'Ana, L. S.; Mancini-Filho, J. Influence of the addition of antioxidants in vivo on the fatty acid composition of fish fillets. *Food Chem.* **2000**, *68*, 175–178.
- (29) Kulas, E.; Ackman, R. G. Different tocapherols and the relationship between two methods for determination of primary oxidation products in fish oil. J. Agric. Food. Chem. 2001, 49, 1724– 1729.
- (30) Tarley, C. R. T.; Visentainer, J. V.; Matsushita, M.; De Souza, N. E. Proximate composition cholesterol and fatty acids profile of canned sardines (Sardinella brasiliensis) in soybean oil and tomato sauce. *Food Chem.* 2004, 88, 1–6.
- (31) Ohgaki, S.; Kannei, M.; Morita, S. Quantitative and qualitative changes in sardine lipid by cooking. *Annu. Rep. Osaka City Inst. Publ. Health Environ. Sci.* 1994, 56, 24–31.
- (32) Chen, Y. Q.; Edwards, J. J.; Kridel, S. J.; Thornburg, T.; Berquin, I. M. Dietary fat gene interactions in cancer. *Cancer Metastasis Rev.* 2007, 26, 535–551.
- (33) Larsson, S. C.; Kumlin, M.; Ingelman-Sundberg, M.; Wolk, A. Dietary long chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. *Am. J. Clin. Nutr.* 2004, 79, 935– 945.
- (34) Mozaffarian, D. Trans fatty acids Effects on systemic inflammation and endothelial function. *Atheros. Suppl.* 2006, 7, 29–32.
- (35) Kamal-Eldin, A. *Lipid Oxidation Pathway*; AOCS Books and Special Publications Committee: Champaign, IL, 2003.
- (36) Hayakawa, K.; Linko, Y. Y.; Linko, P. The role of trans fatty acids in human nutrition. *Starch/Starke* 2000, 52, 229–235.

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