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## Nutritional composition of raw and fried big-scale sand smelt (*Atherina boyeri*) from trasimeno lake

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### ABSTRACT

The aim of this research was to investigate the nutritional composition of raw and fried big-scale sand smelt (*Atherina boyeri*) from Trasimeno Lake. Four hundred big-scale sand smelts were caught with nets and analysed immediately. We created a total of 20 batches with 20 whole fish in each batch. Ten batches were analysed as raw samples, while the other 10 batches were analysed after being fried in sunflower oil at a temperature of 190 °C for 3 min (deep fat frying). The pH, proximate composition, fatty acid profile, oxidative stability and nutritional indexes of both groups were assessed. As expected, cooking strongly influenced the characteristics of the meat, mainly in terms of lipids, which were seven times greater in the fried product due to the oil. Frying also affected the fatty acid profile of the meat because oil absorption caused a significant increase in oleic and linoleic acids. Furthermore, we found a slight reduction in long chain n-3 fatty acids (eicosapentaenoic and docosahexaenoic acids). Frying increased oxidative processes and decreased the nutritional value of sand smelt. The obtained results can be considered preliminary because the effects of the fishing season and different physiological phases of sand smelt require further analytical confirmation.

### HIGHLIGHTS

- The aim of the research was to investigate the nutritional composition of raw and fried big-scale sand smelt (*Atherina boyeri*) from Trasimeno Lake.
- The fried big-scale sand smelt had a meat lipids content 7-times greater than the control, due to the oil adsorption.
- Frying increased oxidative processes and decreased the nutritional value of big-scale sand smelt.

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## Introduction

Big-scale sand smelt (*Atherina boyeri*) is a euryhaline species that is present in brackish, fresh and marine waters. It is present in a vast geographical area extending along the coasts of the Mediterranean Sea, the Black Sea, the Sea of Azov and the Caspian Sea. (Bianco et al. 2013; Lorenzoni et al. 2015)

In Italy, big-scale sand smelt is autochthonous, and it is very common along the coasts of the Tyrrhenian and Adriatic Sea, especially in the Venetian lagoon. Moreover, humans have expanded its original range through introductions in numerous lakes (Kottelat and Freyhof 2007). Sand smelt were introduced into Trasimeno Lake in 1920, which probably occurred

inadvertently (Moretti 1959) with the juvenile stages of other species (mullet) of commercial interest (Natali 2002). Trasimeno Lake is one of the largest and shallowest lake of the Italian peninsula with an average surface area of about 122 km<sup>2</sup> and a maximum depth of less than 6 m. Sand smelt were also introduced into other Italian lakes such as Bracciano, Bolsena, Vico, Albano, Nemi, Fondi and Carinola (Bianco et al. 2013). Currently, 19 species of fish are present, 5 of which are native (pike, cavedano chub, tench, rudd and eel), whereas many others are introduced species such as goldfish and topmouth gudgeon, which are fishes of commercial interest and European perch and largemouth bass, which are invasive and dangerous for the lake's ecosystem (Lorenzoni et al. 2015).

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The big-scale sand smelt has a significant economic importance for professional fishing, especially in the winter months. In the Lake Trasimeno, this species represented almost 27% of the total catch, when fish withdrawal reached 240 tons a year. In recent years, the quantity of fish has decreased, probably due to the lowering of the hydrometric levels of the lake (Ludovisi et al. 2013).

As has already occurred for other species in Italian lakes, it is fundamental to define the nutritional characteristics of the fish to valorise them and their relative products (Dal Bosco et al. 2010, 2012; Pompei et al. 2012; Franceschini et al. 2015; Mattioli et al. 2017). Indeed, the disappearance of local productive companies indicates losses, not only at the economic level, but also from historical, social and cultural points of view.

Frying is a common culinary preparation of fish, and it is widely used in Italy both through pan-frying and deep fat frying. This cooking procedure deeply modifies the composition and nutritional properties of fish, reducing the moisture and increasing the fat due to absorption of the frying oil (Weber et al. 2008; Ansorena et al. 2010).

To the best of our knowledge, there is a very little information concerning the quality of big-scale sand smelt meat (Bilgin et al. 2011; Izci et al. 2011a, 2011b). The present investigation could be considered a basis for any future insights on Lake Trasimeno big-scale sand smelt meat and on its main gastronomic utilisation. For these reasons, the aim of this research was to define the nutritional characteristics of raw and fried big-scale sand smelt fish.

## Material and methods

This study was carried out in collaboration with the Fisherman Cooperative of Trasimeno Lake (Perugia, Italy). Four hundred sand smelts (average weight:  $1.50 \pm 0.35$  g; length:  $8 \pm 3$  cm) were caught with nets in November 2017 and immediately transported on ice to the laboratory of the Department of Agricultural, Food and Environmental Science of Perugia. The fish was part of the daily catch of the cooperative (commonly sold for food), and therefore it did not require specific ethic legislation on animal used for experiment purposes.

A total of 20 batches, of 20 whole fish each batch, were constituted. Ten batches were analysed as raw samples, while the other 10 batches were analysed after being fried in sunflower oil (Table 1), at a temperature of 190 °C for 3 min (deep-fat frying).

**TABLE 1.** Sunflower oil nutritional declaration.

Average value	100 mL
Energy	3404 kJ 828 kcal
Fat	92 g
Of which	
Saturated	9 g
Monounsaturated	42 g
Polyunsaturated	41 g
Carbohydrate	0 g
Protein	0 g
Salt	0 g

## pH and proximate composition evaluations

The pH was measured with a Knick digital pH-meter (Broadly Corp., Santa Anna, CA) after homogenisation of the samples with iodoacetate (Korkeala et al. 1986).

All samples were analysed in duplicate to determine the proximate composition. Moisture, ash, and total nitrogen were assessed in detail using the AOAC's methods (1995. N. 950.46B, 920.153, and 928.08, respectively). Total protein was calculated using the Kjeldahl nitrogen method, using 6.25 as the conversion factor. Total lipids were extracted in duplicate from 5 g of each homogenised sample and were calculated gravimetrically (Folch et al. 1957).

## Fatty acid profile and nutritional indexes assessment

The fatty acids content was determined by gas chromatography after lipid extraction according to the method developed by Folch et al. (1957). One millilitre of lipid extract (from 0.2 to 0.3 mg lipids) was evaporated under a stream of nitrogen, and we added 3 mL of sulphuric acid (3% methanol) to derivatise the residue. After incubation at 80 °C for 1 hour, we extracted methyl esters with petroleum ether, then we injected 1 µL into a gas chromatograph (Fisons Mega 2 Carlo Erba Gas Chromatograph, model HRGC Milan, Italy) that was equipped with a flame ionisation detector.

We used an Agilent capillary column (30 m × 0.25 mm I.D, CPS Analitica, Milan, Italy) coated with a DB-Wax stationary phase (film thickness of 0.25 µm) to separate the fatty acid methyl esters (FAME). The operating conditions of the column injection were as follows: the temperatures of the injector and detector were 270 °C and 280 °C, respectively and the detector gas flows were 50 mL/min for H<sub>2</sub> and 100 mL/min for air.

The oven temperature was programmed to give good peak separation; the initial temperature was set at 130 °C and then increased at a rate of 4.0 °C/min until reaching a temperature of 180 °C, which was held for 5 min. The temperature was subsequently increased at a rate of 5.0 °C/min until it reached

230°C, which was held for 5 min. Helium was used as a carrier gas at a constant flow rate of 1.5 mL/min. Individual FAME were identified with reference to the retention time of the FAME mixture (Sigma-Aldrich, Bornem, Belgium) and were calculated with the internal standard method, so tridecanoic acid (C13:0) methyl ester was added before extraction. The concentration of each fatty acid (mg/100 g of fish) was calculated from the lipid content of the fish and a conversion factor of 0.91 according to Johansson et al. (2000). The mean value of each fatty acid was used to calculate the sum of the saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

The peroxidability index (PI) was calculated according to the equation proposed by Arakawa and Sagai (1986):

$$PI = (\% \text{monoenoic} \times 0.025) + (\% \text{dienoic} \times 1) + (\% \text{trienoic} \times 2) + (\% \text{tetraenoic} \times 4) + (\% \text{pentaenoic} \times 6) + (\% \text{hexaenoic} \times 8)$$

The amount of each fatty acid was used to calculate the Atherogenicity (AI) and Thrombogenicity (TI) indexes, as proposed by Ulbricht and Southgate (1991):

$$AI = (C12 : 0 + C14 : 0 \times 4 + C16 : 0) / (MUFA + PUFA(n-6) + PUFA(n-3))$$

$$TI = (C14 : 0 + C16 : 0 + C18 : 0) / (MUFA \times 0.5 + PUFA(n-6) \times 0.5 + PUFA(n-3) \times 3 + PUFA(n-3) / PUFA(n-6))$$

### Assessment of oxidative stability

The tocopherol ( $\alpha$ -tocopherol and its isoform  $\gamma$  and  $\delta$ ) content of raw and fried fish was quantified by a HPLC/FD system according to Hewavitharana et al. (2004). Five millilitres of distilled water and 4 mL of ethanol were added to 2 g of sample and vortexed for 10 sec. After mixing, 4 mL of hexane that contained BHT (200 mg/L) was added and the mixture was carefully shaken and centrifuged at 8000×g for 10 min. An aliquot of supernatant (3 mL) was dried under a stream of nitrogen and dissolved in 200 µL of acetonitrile, then 50 µL were injected into the HPLC system (Jasco, pump model PU-1580, equipped with an autosampler system, model AS 950-10, Tokyo, Japan) on an Ultrasphere ODS column (250 × 4.6 mm internal diameter, 5 µm particles size; CPS analytic, Milan, Italy). Tocopherols and tocotrienols were identified using a FD detector (model Jasco, FP-1520) that was set at excitation and emission wavelengths of

295 nm and 328 nm, respectively and were quantified using external calibration curves prepared with increasing amounts of pure tocopherols in ethanol.

The extent of lipid oxidation was evaluated in fillets as thiobarbituric acid reactive substances (TBARS) by a spectrophotometer (set at 532 nm, Shimadzu Corporation UV-2550, Kyoto, Japan). A calibration curve with tetraethoxypropane was plotted according to the modified method of Ke et al. (1977). The oxidation products were quantified as malondialdehyde (MDA) equivalents (mg MDA/kg muscle).

### Reagents

Unless otherwise noted, all chemicals were analytical and were purchased from Sigma Chemical Co (St Louis, MO).

### Statistical evaluation

The data were analysed with one-way linear model (Statacorp® 2015) that evaluated the effect of cooking and reported the mean and standard error of the mean at  $p < .05$ .

### Results and discussion

The present research represents a contribution to the literature that increases our knowledge of the nutritional characteristics of both raw meat and deep fat fried big-scale sand smelt, which is the most popular gastronomic preparation of this meat.

In Table 2, the pH and chemical characteristics of fresh and fried big-scale sand smelt are reported. As expected, cooking strongly influenced the characteristics of the meat, mainly in terms of lipids, which, by virtue of the frying oil, were seven times greater in the cooked fish. Kalogeropoulos et al. (2004) observed that frying caused significant changes in the meat proximate composition of different fish species. In particular, in agreement with our results, they observed a significant increase in total fat and protein, in pan-fried *Atherina boyeri*, mainly as a result of water loss.

**TABLE 2.** pH and proximate composition (%) of raw and fried whole big-scale sand smelt.

	Raw	Fried	SED
pH	6.42	6.40	0.23
Dry matter	24.63 <sup>a</sup>	43.13 <sup>b</sup>	4.13
Protein	18.40 <sup>a</sup>	24.20 <sup>b</sup>	2.08
Lipids	2.20 <sup>a</sup>	14.20 <sup>b</sup>	1.86
Ash	3.03	2.73	0.40

N = 10 per group.

<sup>a,b</sup> $p < 0.05$ .

SED: standard error of the difference.

**Table 3.** Fatty acid profile (% of total fatty acids) of raw and fried whole big-scale sand smelt.

Fatty acid		Raw	Fried	SED
SFA		34.65 <sup>b</sup>	20.45 <sup>a</sup>	1.12
	C14:0	4.99 <sup>b</sup>	1.27 <sup>a</sup>	0.32
	C16:0	21.84 <sup>b</sup>	13.86 <sup>a</sup>	1.11
	C18:0	5.05	4.11	0.26
	Others	2.77 <sup>b</sup>	1.21 <sup>a</sup>	0.14
MUFA		31.56 <sup>a</sup>	38.31 <sup>b</sup>	1.90
	C16:1n-7	10.72 <sup>b</sup>	2.99 <sup>a</sup>	0.25
	C18:1n-9	14.06 <sup>a</sup>	33.35 <sup>b</sup>	1.26
	C20:1n-9	1.35 <sup>b</sup>	0.52 <sup>a</sup>	0.18
	Others	5.58 <sup>b</sup>	1.47 <sup>a</sup>	0.52
PUFA		33.71 <sup>a</sup>	41.23 <sup>b</sup>	1.58
	C18:2n-6	8.18 <sup>a</sup>	24.87 <sup>b</sup>	1.62
	C20:4n-6	5.14 <sup>b</sup>	2.84 <sup>a</sup>	0.82
	C18:3n-3	5.45 <sup>b</sup>	3.59 <sup>a</sup>	0.55
	C20:5n-3	3.86 <sup>b</sup>	2.17 <sup>a</sup>	0.36
	C22:6n-3	10.36 <sup>b</sup>	7.74 <sup>a</sup>	0.40
	Others	0.72 <sup>b</sup>	0.02 <sup>a</sup>	0.17

N = 10 per group.

<sup>a,b</sup>p < 0.05.

SED: standard error of the difference; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; LA: linoleic acid; ALA: alpha-linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

Gokoglu et al. (2004), studied the effects of different cooking systems (boiled, fried) on rainbow trout fillets and observed significant changes in the level of water, proteins and ashes. No lipid differences were shown when fish were boiled (Puwastien et al. 1999; Gokoglu et al. 2004) whereas frying modified their values due to oil absorption. Other changes were mainly related to the loss of moisture that occurred during frying, which also occurs as a result of oil absorption (Gall et al. 1983; Steiner-Asiedu et al. 1991; Ruiz-Roso et al. 1998). Steiner-Asiedu et al. (1991) reported similar results in fried *Sardinella* sp., *Dentex* sp. and *Tilapia* sp., and similar trends were also shown in other processing methods, such as hot smoking (Steiner-Asiedu et al. 1991; Unlusayin et al. 2001; Mattioli et al. 2017).

The analysis of the fatty acid composition of raw big-scale sand smelt (Table 3) highlighted some important peculiarities of this meat. In fresh fish, the main SFA was palmitic acid (C16:0), while among the MUFA, oleic (C18:1n-9) and palmitoleic (C16:1n7) acids were the most represented. Regarding PUFA, the eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic (DHA, C22:6n-3) acids content were high. This represents a desirable outcome, considering that the main benefits of fish consumption are the EPA and DHA intake. Adequate levels of EPA and DHA reduce cardiovascular risks and Alzheimer disease neuropathology, and are required for normal brain functioning and for optimal cognitive function (Nooyens et al. 2018).

The fatty acid profiles reported here are similar to those published by other authors (Kalogeropoulou et al.

et al. 2004; Bilgin et al. 2011; Bouriga et al. 2011; Izci et al. 2011a, 2011b) with some minor differences. In particular, it should be noted that big-scale sand smelt from Trasimeno Lake showed percentages of SFA similar to those from other places including the Kerkennah Islands of Tunisia in the Mediterranean Sea (Bouriga et al. 2011), Greece on the Aegean Sea (Kalogeropoulou et al. 2004) and Eğirdir Lake in Turkey (Bilgin et al. 2011; Izci et al. 2011a, 2011b). The MUFA of big-scale sand smelt from Trasimeno Lake were higher in C18:1n-9 than the other lake fishes, but lower than other fish in the Tunisian sea (Bouriga et al. 2011). Unexpectedly, Bouriga et al. (2011) and Kalogeropoulou et al. (2004) reported lower values of PUFA (26.49% and 19.2%, respectively) than we found in our samples.

The n-3/n-6 ratio values were highly variable and ranged from 2.47 (Izci et al. 2011a) to 0.53 (Kalogeropoulou et al. 2004), and our value (1.47) falls in the middle of this range. Frying significantly influenced the acidic composition of big-scale sand smelt, mainly due to oil absorption, as was previously observed in research carried out in other species (Dal Bosco et al. 2001). There was a significant increase in oleic and linoleic acids. Furthermore, we observed a slight reduction of EPA and DHA, even if the total PUFA (expressed as a percentage, Table 3) was higher than that observed in the fresh product (mainly affected by the higher percentage of C18:2n-6). It should be noted that the oil used had a fatty acid profile mainly characterised by linoleic acid (48–74%), oleic acid (14–40%), palmitic acid (4–9%) and stearic acid (1–7%) (Table 1). Clearly, the modification of the fatty acid profile and the oxidative process of fried fish depend on the oil used and on the fish species (lean vs. fat fish). Weber et al. (2008) observed that the n-3/n-6 ratio increased after frying in canola oil, which is rich in alpha-linolenic acid (ALA), while the content of PUFA increased when using soybean oil. Candela et al. (1998) observed a reduction of EPA and DHA in mackerel and sardines after frying in sunflower oil. On the contrary, when using canola oil there is an increase in the n-3/n-6 ratio due to ALA. Ohgaki et al. (1994) and Sánchez-Muñiz et al. (1992) confirmed these results by stating that the lipid composition of fried fish tends to be similar to that of the frying oil mainly in lean fish, but no major changes in fatty fish. Extra virgin olive oil led to a higher fat absorption than sunflower oil in both fishes. On the contrary, frying did not significantly affect the lipid profile of farmed salmon regardless of the oil used, but it did affect the lipid profile in fried cod. In fact, the n-6/n-3 ratio increased from 1.01



in raw cod to 6.63 in fried cod. The use of extra virgin olive oil was more efficient in containing oxidative processes during frying in cod, but not in salmon (Ansorena et al. 2010).

Another relevant aspect of fresh big-scale sand smelt is the low level of oxidation (Table 3). The TBARS values can be considered moderate if we consider the high levels of PUFA (430.41 mg/100 g). Al-Kahtani et al. (1996) assessed that meat products can still be considered in a good oxidative state when they have values below 3 mg MDA/kg of product.

However, in comparison to goldfish (*Carassius auratus*) from Trasimeno Lake, the oxidative state of sand smelt (Dal Bosco et al. 2010; Dal Bosco et al. 2012) that were fished during the same time of year was worse. Such different oxidative statuses are mainly related to the feeding behaviours of these fishes; big-scale sand smelt show a more pronounced oxidative muscular metabolism and are exclusively carnivorous fish, and conversely goldfish are omnivores (Dal Bosco et al., 2012; Giannetto et al., 2014). As consequence, the big-scale sand smelt is more exposed to oxidative risk and it does not intake enough antioxidants (algae or marine plants) like the goldfish.

Nevertheless, frying negatively affected the oxidative status of big-scale sand smelt. The TBARS value was almost double (0.39 vs. 0.19 mg MDA/kg) in comparison to raw fish and the antioxidants were lower, showing values for  $\alpha$ -tocopherol and  $\gamma$ -tocopherol of 1/3 and 1/7 the amount in raw fish, respectively. Congruently, Choe and Min (2007) reported that the tocopherols contained in palm oil were totally decomposed during 8 h of frying. The degradation rate of tocopherols is commonly considered an indicator of the oil's stability during frying (Normand et al. 2006).

Izci et al. (2011b) found changes in some quality parameters of fried fish chips produced from sand smelt (*Atherina boyeri*) that were stored at  $-18^{\circ}\text{C}$  for 6 months. However, the authors did not report differences between the pH and oxidative status of raw fish and pre-fried chips. Similarly, Weber et al. (2008) did not detect variations in the TBARS values after frying and assumed that this occurred due to a deactivation of the MDA and the formation of protein-cheated products.

Surprisingly, frying reduced the AI and TI (Table 4), which could be considered an estimation of coronary heart disease (CHD) risk due to lipid intake. It is assumed that these indicators are correlated with the cholesterol content in the blood and with the platelet aggregation, respectively (Ulbricht and Southgate, 1991). It should be noted that these indexes only consider the content of

**TABLE 4.** Fatty acid (mg/100 g of fish) and antioxidant ( $\mu\text{g}/\text{kg}$ ) contents, TBARS (mg MDA/kg) and nutritional indexes of raw and fried big-scale sand smelt.

	Raw	Fried	SED
Fatty acids			
$\Sigma\text{SFA}$	771.45 <sup>a</sup>	3026.64 <sup>b</sup>	28.21
$\Sigma\text{MUFA}$	640.64 <sup>A</sup>	6684.77 <sup>B</sup>	19.78
$\Sigma\text{PUFAn-3}$	250.76 <sup>A</sup>	801.55 <sup>B</sup>	9.70
$\Sigma\text{PUFAn-6}$	179.64 <sup>A</sup>	1379.53 <sup>B</sup>	9.70
$\Sigma\text{PUFA}$	430.41 <sup>A</sup>	2181.08 <sup>B</sup>	10.24
Antioxidants			
$\delta$ -tocopherol	0.019	0.005	0.003
$\gamma$ -tocopherol	0.22 <sup>B</sup>	0.03 <sup>A</sup>	0.01
$\alpha$ -tocopherol	2.50 <sup>B</sup>	0.77 <sup>A</sup>	0.08
Lipid oxidation			
TBARS	0.19 <sup>a</sup>	0.39 <sup>b</sup>	0.15
Nutritional indexes			
Peroxidability index	273.12	226.52	25.81
Atherogenic index	0.64 <sup>B</sup>	0.24 <sup>A</sup>	0.05
Thrombogenic index	0.38 <sup>B</sup>	0.26 <sup>A</sup>	0.03

*N* = 10 per group.

<sup>A,B</sup> *p* < 0.01.

<sup>a,b</sup> *p* < .05.

SED: standard error of the difference;  $\Sigma\text{SFA}$ : sum of total saturated fatty acids;  $\Sigma\text{MUFA}$ : sum of total monounsaturated fatty acids;  $\Sigma\text{PUFAn-3}$ : sum of total polyunsaturated fatty acids from n-3 series;  $\Sigma\text{PUFAn-6}$ : sum of total polyunsaturated fatty acids from n-6 series;  $\Sigma\text{PUFA}$ : sum of total polyunsaturated fatty acids; TBARS: thiobarbituric acid reactive substances.

some PUFAs (Material and methods section) without including their oxidative status. Indeed, although the AI and TI indexes were lower in fried big-scale sand smelt, the TBARS were higher, and frying probably generates some other degradation products (e.g. aldehyde- or epoxy-products). In agreement with this idea, Velasco et al. (2004) reported that during thermoxidation at  $180^{\circ}\text{C}$  of olive and sunflower oils, monoepoxy fatty acid arose from oleic (*cis*-9,10- and *trans*-9,10-epoxystearate) and linoleic (*cis*-9,10-, *trans*-9,10-, *cis*-12,13-, and *trans*-12,13-epoxyoleate) acids. As was also reported by Kalogeropoulos et al. (2004), it is common in fish and molluscs, which are mainly consumed fried, for nutritional quality to worsen in relation to cooking method.

## Conclusions

This research can be considered a first attempt to characterise fresh big-scale sand smelt from Trasimeno Lake and its main gastronomic preparation (fried).

These preliminary results require further analytical confirmation in different seasons and physiological phases of the fish because, in the wild species, they have a great impact on the fish condition index (Giannetto et al., 2012; Lorenzoni et al., 2012) and on the fillet quality. It is also clear that, given the strong effect of the cooking system on all traits analysed, great attention must be paid to the oil used, its quality and the parameters used for cooking such as temperature, time of frying and times of replace.

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## Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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