NUTRITIONAL VALUE OF RAW AND PROCESSED FILLETS OF BOLSENA LAKE WHITEFISH (*COREGONUS LAVARETUS* L.)

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ABSTRACT

The aim of the study was to investigate and compare the nutritional value of raw and processed fillets (marinated and smoked) of whitefish from Bolsena Lake (Italy). The study was carried out in collaboration with the "Lago vivo" Fisherman Cooperative using 40 whitefish caught by net. The chemical composition showed increased nutrient (protein, lipid and carbohydrate) concentration with processing due to dehydration. Regarding the fatty acid profile of fillets, marinating was associated with higher levels of n-6 PUFAs, whereas the smoked and raw fillets showed higher concentrations of α-linolenic, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. Whitefish fillets were characterized by high nutritional value and good oxidative stability, mainly as smoked products.

Keywords: fillet, nutritional quality, processed, whitefish, fatty acids, food value

1. INTRODUCTION

Bolsena Lake is the largest volcanic lake in Europe and the fifth largest in Italy. It is located in the province of Viterbo in Northern Lazio on the border with Umbria and Tuscany. It has a drainage basin of 243 km^2, nearly half of which is occupied by the lake itself. Unlike other Italian lakes, Bolsena Lake is populated by a large number of fish species, including native herbivorous or insectivorous species (i.e. tench, chub, carp, rudd and sand smelt) and some predatory species such as pike, perch and eel. These are supported by other fish species added for specific human interest, such as whitefish and gambusia, or placed unconsciously as in the cases of bluegill, a voracious predator of young fish and eggs.
The European whitefish (*Coregonus lavaretus*), widely distributed in the freshwaters of Northern Europe (GOEBEL *et al.*, 2016) and introduced into the major lakes of Northern Italy, is present in the lakes of Central Italy mainly as result of stock enhancement practices. However, it is an uncommon species in our country and rarely present on consumer tables. In addition, the reduced surface area of the Bolsena Lake basins and the strong national tradition linked mainly to marine species of our country are limiting marketing and thus the spread of freshwater fish species in the domestic markets. As result, there is very little knowledge of whitefish nutritive characteristics among consumers.
The high value of meat and the economic interest in the Bolsena Lake whitefish has placed a spotlight on the return of the product under the collective brand "*Tuscia-Viterbese*", which is also very popular. A license for this kind of product can be issued by fishing companies and/or companies that process and/or market the species covered by this brand; for this reason, it is considered a local product. Furthermore, considering the extreme perishability of foodstuffs, conservation techniques for whitefish products are needed (YEANNES and CASALES, 2007), some of which have already come up from fishermen 2000 years ago.
Nowadays, the various storage systems are also largely used to distinguish products and to attract consumers (SOGN-GRUNDVAG *et al.*, 2014). Accordingly, a potential contribution to the economy of the fishermen's cooperative is certainly represented by the possibility of placing on the market differentiated products, such as smoked or marinated fillets, and to define their nutritional characteristics. Indeed, in accordance with EC Regulation 1169/2011, nutrition labelling on food is mandatory to achieve a high level of health protection for consumers and to guarantee access to accurate information.
In a previous work, ORBAN *et al.* (2006) studied the nutritional quality and safety of whitefish caught in three different Italian lakes, underlining their good protein and mineral contents and low lipid levels in the fillet of such species; beginning with this study, we wanted to deepen the existing knowledge on nutritional aspects of whitefish living in Bolsena Lake, both raw and processed (marinated and smoked), in order to add value to local products on the market.

2. MATERIALS AND METHODS

The study was carried out in collaboration with the "*Lago vivo*" Fisherman's Cooperative of Bolsena (Viterbo, Italy). Forty whitefish were caught by net in April 2016. All fish were immediately dipped in a mixture of water and ice and successively transferred to polystyrene boxes containing ice and transported under refrigerated conditions to the laboratory of the cooperative for processing as described below.

2.1. Fillets and processed preparation

Whitefish with an average live weight of 250 g were eviscerated after washing with running water, the heads and tails were removed, dorsal and ventral fillets were dissected and 20 of them were taken for analysis. The preparation procedure of the threads and the process (smoking and marinating) adopted provided for manual filleting with subsequent removal of the skin and scraps. At this point, the process followed two different paths: firstly, for the smoked product, the obtained fillets (approximately 100 g per fish) were salted and then smoked at 60 ± 5°C over beech wood for 4 hours; the next step was vacuum packaging and storage in a special cold storage (0-4°C) awaiting shipment to the various sales outlets.
Concerning the production of processed fillets, these were marinated for almost 12 hours in vinegar, lemon juice and spices with addition of olive oil, onion, celery, pepper and salt (approximately 30 g/kg of fillet), then the whole fillet was packed in packages of various weights (from 100 to 1000 g) in a modified atmosphere and stored in special refrigerated cells (0-4°C) awaiting shipment to different outlets. All fillet samples, raw and processed (n=20 per type), were stored at -30°C at the laboratories of the Department of Agricultural, Food and Environmental Science and Department of Veterinary Medicine of the University of Perugia until analysis (2 weeks later).

2.2. Proximate analyses and fatty acid composition

All samples were analysed in duplicate to determine the proximate composition. In detail, moisture, ash and total nitrogen were assessed using the AOAC methods (2000 N. 950.46, 923.03 and 991.15, respectively). Total protein nitrogen was calculated by the Kjeldahl method using 6.25 as the conversion factor. Total lipids were extracted in duplicate from 10 g of each homogenized sample and calculated gravimetrically (FRANCESCHINI *et al.*, 2015). The energy value and caloric value of the raw and processed fillets expressed in kJ/g and kcal/g, respectively, was calculated following the criteria of EU Regulation 1169/2011.
Fatty acids (FA) were determined by gas chromatography after lipid extraction according to the method proposed by FRANCESCHINI *et al.* (2015). Approximately 10 g of fish were homogenized with 5 mL of 0.5 M sodium acetate-water solution using an Ultraturrax (T25 basic, IKA, Labortechnik, Germany). Then, another 8 mL of sodium acetate solution, 8 mL of methanol and 4 mL of chloroform were added to 4 g of this mixture and mechanically shaken for 3 minutes. After addition of 4 mL of chloroform, the mixture was shaken again for 2 minutes, and 8 mL of water was added in order to separate the chloroform and methanol phases. After centrifugation, the lower phase was collected in a flask.
Fatty acid methyl esters (FAME) were obtained as described by BRANCIARI *et al*. (2017). Fifty milligrams of the lipid fraction were dissolved in 2 mL of hexane, then 1 mL of hexane containing internal standard (methyl nonadecanoate 0.4 mg/mL; Sigma-Aldrich, Bellefonte, PA, USA) and 2 N KOH in methanol (0.5 mL) were added. The mixture was then shaken vigorously for 3 min, water (3 mL) was added and the upper organic phase was dried over anhydrous sodium sulphate, cooled in an ice bath and immediately injected into a high-resolution gas chromatograph. Separation of FAME was carried out on a capillary column CP-Select CB (100 m x 0.25 mm i.d., 0.39 μm, J&W, Agilent Technologies, Palo Alto, CA, US). We used helium as a carrier gas at a flow rate of 1.6 mL/min. The injector temperature was set at 270°C and the detector at 300°C. The oven temperature program was the following: starting from 60°C (maintained for 1 minute), the temperature was increased to 30°C/min up to 150°C; after 3 min, with an increase of 0.5°C/min, then, after 1 minute, it was increased to 220°C in increments of 1.5°C/min and

then maintained for 15 min. One μL of sample was injected in a split/splitless system (split ratio 1:5). Individual FAME were identified by comparison with a standard mixture (PUFA No. 1, Marine Source, 37 FAMEs by Sigma, methyl *cis*-7,10,13,16,19-docosapentaenoate, *trans*-11-vaccenic methyl ester, *cis*-11-vaccenic methyl ester, Supelco, Bellefonte PA, USA). The percentage of each FA was calculated by using the peak area of the samples corrected with the respective correction factors (AOAC, 2012). To assess the actual nutritional quality of fish fillets, fatty acids were quantified and expressed in mg/100 g tissue using the internal standard method outlined by JOSEPH and ACKMAN (1992). The following equation was applied:

$$\text{Fatty acids (mg/100 g food)} = [(A_x \times W_{is} \times \text{CRF}_x \times \text{CNF}x)/(\text{A}_{is} \times \text{W}s)] \times 1000 \times \text{W}_L,$$

where A_x is the eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) area, A_{is} is the internal standard area, CRF_x is the theoretical correction factor for EPA and DHA, CNF*x* is the conversion factor from FAME to the corresponding fatty acid, W_{is} is the weight of the internal standard added to the lipids, W*s* is the weight of the derivatized lipids and W_L is the percentage of sample lipid.
Based on current knowledge regarding the effect of specific fatty acids on cholesterol metabolism, the ratio between hypocholesterolaemic and hypercholesterolemic fatty acids (HH) was calculated using the following mathematical equation (SANTOS-SILVA *et al.*, 2002):

$$\text{HH} = (\text{C18:1n-9} + \text{C18:2n-6} + \text{C20:4n-6} + \text{C18:3n-3} + \text{C20:5n-3} + \text{C22:5n-3} + \text{C22:6n-3})/(\text{C14:0} + \text{C16:0})$$

The mean value of each fatty acid was used to calculate the sum of the saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids and to calculate the peroxidability index (PI) according to the equation proposed by ARAKAWA and SAGAI (1986):

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

The amount of each fatty acid was also used to calculate the atherogenicity (AI) and thrombogenicity (TI) indexes as proposed by ULBRICHT and SOUTHGATE (1991):

$$\text{AI} = (\text{C12:0} + 4\text{xC14:0} + \text{C16:0})/[(\sum\text{MUFA} + \sum(\text{n-6}) + \sum(\text{n-3})]$$
$$\text{TI} = (\text{C14:0} + \text{C16:0} + \text{C18:0})/[(0.5 \text{ x } \sum\text{MUFA} + 0.5 \text{ x (n-6)} + 3\text{x (n-3)} + (\text{n-3})/(\text{n-6})]$$

The index of nutritional quality (INQ) was calculated on the basis of the EPA + DHA acid level using the formula suggested by GODBE (1994).

2.3. Assessment of oxidative stability

The extent of lipid oxidation was quantified by spectrophotometry (Shimadzu 2025, Kyoto, Japan) as thiobarbituric acid reactive substances (TBARs) according to the method reported by KE *et al*. (1977) and using a molar extinction coefficient of 156 x 10^3 M/cm at λ = 532 nm. Results are expressed as malondialdehyde (MDA) equivalents per kg of tissue (mg MDA/kg).

2.4. Reagents

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

2.5. Statistical evaluation

The data were analysed with a one-way linear model (Statacorp®, 2015) evaluating the effect of technological treatment. Differences among whitefish products were evaluated by multiple samples *t*-test, reporting the mean and standard error of the mean (SEM). Results were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSIONS

3.1. Food labels of raw and processed whitefish fillets

The chemical characteristics of analysed whitefish fillets are reported in Table 1. The processing of whitefish, i.e. smoking and marinating, significantly affected the chemical parameters of fillets.
In particular, smoking reduced the water content of muscle fibres with respect to raw filets (64.82% vs. 76.53%) due to surface drying of the fillet and consequently, the fillets showed nutrient concentration. Even in marinated fillets, an increase in nutrient content due to dehydration was found (65.26%).

Table 1. Raw and processed whitefish fillets food label.

	Fillets			
	Raw	**Smoked**	**Marinated**	**SEM**
Moisture	76.53^{b}	64.82^{a}	65.26^{a}	4.52
Lipids	1.97^{a}	2.86^{a}	9.77^{b}	0.59
of which				
- saturates	30.82^{b}	30.21^{b}	14.50^{a}	1.18
- monounsaturates	33.60	33.54	36.35	2.11
- polyunsaturates	35.57^{a}	36.23^{a}	49.14^{b}	2.56
Carbohydrates	0.97^{a}	1.05^{a}	2.63^{b}	0.29
of which				
- sugar	n.d	n.d.	0.41	0.18
Protein	19.23^{a}	28.53^{b}	20.44^{a}	2.01
Ash	1.30^{a}	2.74^{b}	1.90^{ab}	0.35
Energy*	98^{a} 416^{a}	144^{b} 602^{b}	180^{c} 754^{c}	20.15 41.18

N=20 per group.
$^{a..c}$ Values within a row with different superscripts indicate a significant difference at $p < 0.05$.
Proximate composition was expressed as %; Energy was expressed as kcal/100 g on the first line values and kJ/100 g on the second line values.
* EU regulation 1169/2011.

The percent protein content of smoked fillets was significantly higher than that of raw and marinated ones (28.53% vs. 19.23% and 20.44%, respectively). However, the smoking process can cause a reduction of protein digestibility attributable to the formation of oxidized forms of sulphur amino acids and carbohydrate-protein complexes called Maillard compounds (MENSA-WILMOT *et al.*, 2001), as demonstrated by the low percentage of carbohydrates relative to that in the marinated fillets (1.05% vs. 2.63%). Instead, the higher proportion of carbohydrates in marinated samples was the result of the addition of onion and celery as ingredients, which contain approximately 5.7% and 2.4% carbohydrates, respectively (MARLETTA and CARNOVALE, 2000).
A prominent difference was found in the lipid content, which was higher in the marinated fillet (9.77%), probably because of the addition of olive oil; indeed, the main fatty acids were represented by PUFA and MUFA. The total mineral content was significantly different only between raw and smoked fillets, with lower values in the former (1.30% vs. 2.74%), possibly attributed to the concentration process previously cited.
FUENTES *et al.* (2010) reported a strong correlation of the smoking process with several physico-chemical parameters (moisture, water activity, pH and colour) in commercial smoked products (anchovy and salmon). They suggested that the ability of the smoking process to preserve fish is due to the synergistic action of salt incorporation, smoke compounds and dehydration during the smoking process.
Similarly, marinated fish are products consisting of raw, frozen or salted fish or portions of fish processed by treatment with edible organic acids, usually acetic acid, and salt and added to sauces, creams or oil (MEYER, 1965). They represent semi-preserved fish products, ready-to-eat with no heat treatment (GRAM and HUSS, 1996), and they are considered as a high-value delicacy, as are cold-smoked fish. In the present study, marinated fish were prepared with lemon juice and vinegar, then with natural citric and acetic acids. HAMM (1960) outlined that, in the presence of acid alone, the pH of the muscle was on the acidic side of the isoelectric point, and the electrostatic repulsion allowed for an increase in water holding capacity and a decrease in firmness. However, when salt was added (as in our case), the repulsion decreased and the structure became firmer.
The differing proportions of nutrients in the raw and processed fillets also affected the energy content of the studied products, with higher values found in marinated fillets, followed by smoked and non-processed whitefish (180, 144 and 95 kcal/100 g and 754, 602 and 416 kJ/100 g, respectively).

3.2. Fatty acid profiles of raw and processed whitefish fillets

The fatty acid composition (mg/100 g) of the samples is shown in Table 2. A total of 33 fatty acids were identified in the present study. The fatty acid composition of raw and smoked fillets was nearly identical. The slight differences in concentration were due to the lower moisture content of the smoked fillets, as demonstrated by chemical composition analysis. In raw and smoked fillets, the most represented fatty acid was oleic acid (C18:1n-9cis); this MUFA was present in an amount equal to 327.26 mg/100 g in raw fillets, whereas in smoked ones, a concentration of 483.85 mg/100 g was recorded, followed by palmitic acid (C16:0). Conversely, ORBAN *et al.* (2006) found a higher proportion of the latter fatty acid, followed by oleic (C18:1n-9) and palmitoleic (C16:1) acids in raw whitefish fillets.
In the marinated fillet, the most represented fatty acid was linoleic acid (C18:2n-6, LA; 4005.84 mg/100 g). This higher value, compared with that recorded in raw and smoked fillets, was justified by the marinating preparation method, which included the addition of olive oil with an average linoleic acid value of 12%. The same was also true for oleic acid

(2999.25 mg/100 g), which is present at almost 83% in olive oil, and for palmitic and stearic acids (786.81, 296 and 17 mg/100 g, respectively), which are present from 5% to 20% (BOSKOU, 2015).

Table 2. Fatty acid composition (mg/100 g tissue) of raw and processed whitefish fillets.

	Fillets			
	Raw	**Smoked**	**Marinated**	**SEM**
C12:0	1.18[a]	1.68[a]	8.73[b]	0.98
C13:0	0.57[b]	0.86[b]	0.00[a]	0.12
C14:0	119.08[b]	174.69[c]	63.93[a]	15.21
C14:1	22.62[b]	33.77[c]	12.74[a]	1.25
C15:0	15.19[b]	22.21[c]	9.70[a]	1.05
C15:1	0.80[b]	1.07[b]	0.00[a]	0.08
C16:0	319.22[a]	444.88[a]	786.81[b]	50.16
C16:1	214.52[b]	303.12[c]	147.19[a]	17.51
C17:0	9.08[a]	13.24[b]	8.11[a]	0.89
C17:1	0.54[b]	0.73[b]	0.00[a]	0.10
C18:0	50.42[a]	77.03[a]	296.17[b]	20.31
C18:1n-9t	4.52[b]	7.25[b]	0.00[a]	0.32
C18:1n-9c	327.26[a]	483.85[a]	2999.25[b]	124.21
C18:1n-7c	67.31[a]	93.46[b]	124.66[c]	24.15
C18:2n-6t	0.62[b]	0.97[b]	0.00[a]	0.12
C18:2n-6c	93.52[a]	136.69[a]	4005.84[b]	66.58
C18:3n-6	8.93[a]	13.54[b]	6.00[a]	1.24
C18:3n-3	138.30[b]	198.28[c]	82.65[a]	26.21
C20:0	3.22[a]	4.45[a]	24.20[b]	2.14
C18:4n-3	86.75[b]	128.08[c]	38.69[a]	15.48
C20:1n-9	8.98[a]	12.80[a]	20.39[b]	2.36
C21:0	5.30[a]	7.35[b]	7.32[b]	0.87
C20:3n-6	3.46[a]	5.20[b]	3.13[a]	0.69
C20:4n-6	41.20[b]	59.20[c]	28.71[a]	3.25
C20:3n-3	8.96[b]	12.12[c]	4.58[a]	1.05
C22:0	5.49[a]	8.15[a]	48.87[b]	2.36
C22:1n-9	1.37[b]	1.93[b]	0.00[a]	0.14
C20:5n-3	128.64[b]	186.87[c]	70.58[a]	3.36
C22:2	0.94[a]	1.32[b]	2.73[c]	0.25
C24:0	0.98[a]	1.40[a]	17.46[b]	1.28
C24:1	1.47[a]	2.09[a]	7.44[b]	0.86
C22:5n-3	38.10[b]	59.11[c]	27.32[a]	3.72
C22:6n-3	61.96[b]	105.25[c]	37.68[a]	3.14

N=20 per group.

[a-c] Values within a row with different superscripts indicate a significant difference at $p < 0.05$.

Oleic acid plays an important role in physical well-being and is responsible for the reduction of plasma cholesterol levels and the improvement of the low density/high

density lipoprotein ratio (LDL/HDL, SECCHIARI, 2008), both of which are well-known, important risk factors for cardiovascular disease (ALTHAUS *et al.*, 1988). Furthermore, considering the cultural value of olive oil in our country (DE LEONARDIS, 2014), its use in fishery products could represent an important value added.
Concerning PUFA, the fatty acid with the highest concentration was α-linolenic acid (ALA, C18:3n-3), followed by EPA (C20:5n-3) in all studied samples. Even the ALA content, both in raw and smoked fillets was higher than that in marinated ones (138.30 and 198.28 vs 82.65 mg/100 g, respectively). EPA and DHA are long-chain n-3 fatty acids, synthesized by the human body only in small amount (DE FILIPPIS and SPERLING, 2006); therefore, their dietary consumption is required. In particular, EPA and DHA intake has demonstrated physiological benefits in terms of blood pressure, heart rate, triglycerides and inflammation; moreover, a reduced risk of foetal coronary heart disease (CHD) and sudden cardiac death has been associated with the consumption of ~250 mg/day of EPA plus DHA (GISSI-HF, 2008; MOZAFFARIAN and RIMM, 2006). Accordingly, whitefish fillet is an excellent source of long-chain n-3 PUFA, as 100 g of raw fillet provides approximately 190 mg of EPA + DHA, and smoked filet exceeds the recommended requirement (292.18 mg/100 g).

3.3. Nutritional indexes and oxidative stability of raw and processed whitefish fillets

The total amounts of different fatty acid series, nutritional indexes and oxidative stability of whitefish fillets are reported in Table 3. When the three processing methods were compared, raw and smoked fillets had different amounts of SFA (529.73 vs 755.94 mg/100 g, respectively), due, as previously mentioned, to the concentration of nutrients during the smoking process. The same trend was observed in the MUFA and PUFA levels.

Table 3. Total saturated, monounsaturated and polyunsaturated fatty acids (mg/100 g), nutritional indexes and oxidative status of raw and processed whitefish fillets.

	Fillets			
	Raw	**Smoked**	**Marinated**	**SEM**
SFA	529.73^{a}	755.94^{b}	1271.28^{c}	50.26
MUFA	577.57^{c}	839.37^{b}	3187.01^{c}	62.52
PUFA	611.39^{a}	906.63^{b}	4307.91^{c}	59.85
Σ n-3	462.71^{b}	689.70^{c}	261.50^{a}	21.36
Σ n-6	147.74^{a}	215.61^{b}	4043.68^{c}	78.15
n-6/n-3	0.32^{a}	0.31^{a}	15.46^{b}	2.26
INQ	5.95^{b}	6.24^{b}	1.85^{a}	1.85
HH	1.16^{a}	1.21^{a}	5.00^{b}	5.00
Peroxidability index	112.60^{b}	117.83^{b}	91.14^{a}	21.14
Atherogenic index	0.67^{b}	0.66^{b}	0.14^{a}	0.14
Trombogenic index	0.28	0.27	0.26	0.26
TBARs	0.22^{a}	0.11^{a}	3.60^{b}	0.60

N=20 per group.
$^{a-c}$ Values within a row with different superscripts indicate a significant difference at $p < 0.05$.
SFA, MUFA, PUFA, Σ n-3 and Σ n-6 are expressed as mg/100 g tissue; TBARs are expressed as mg MDA/kg tissue.

Several studies have shown a direct relationship between the consumption of SFA in the diet and the risk of cardiovascular disease (DAYTON *et al.*, 1968). The negative effect of dietary SFA is mainly due to the increase in blood LDL cholesterol (MENSINK *et al.*, 1992). However, the heterogeneity of the saturated fatty acids and their effect as risk factors are worthy of note. For example, stearic acid, little represented in the raw fillet, is not a hypercholesterolemic agent (HUNTER *et al.*, 2010), whereas the myristic acid, which was present in a higher amount than stearic acid, is dangerous to human health because it increases serum cholesterol by four times more than palmitic acid (SECCHIARI, 2008).
Regarding the marinated fillet, the levels of SFA, MUFA and PUFA were significantly higher respect to control or smoked one. The SFA and MUFA amounts were 1271.28 mg/100 g and 3187.01 mg/100 g, respectively, mainly due to the addition of olive oil to the product (Table 3). This ingredient affected also the PUFA levels (4307.91 mg/100 g), which were greatly elevated (BOSKOU, 2015). The prevalence of PUFA over MUFA and SFA may reflect a high inherent capability of freshwater fish to desaturate and elongate enzymatically dietary precursors into long-chain highly unsaturated fatty acids (HENDERSON and TOCHER, 1987).
The smoked fillet had 689.70 mg/100 g of n-3 fatty acids, whereas during the marinating process, the n-3 fatty acid concentration decreased to 261.50 mg/100 g, of which 70.58 mg was EPA, and 37.68 mg was DHA. These results were not due to the oxidation of the product resulting from manipulation (FRANKEL *et al.*, 2014) but rather to the high quantity of PUFA found. In fact, the lipid oxidative status, evaluated with the TBARs test, was worse in marinated than in raw and smoked fillets (3.60 in marinated vs. 0.22 and 0.11 mg MDA/kg in raw and smoked samples, respectively), whereas the IP index, which measures the susceptibility to oxidation on the basis of the fatty acid composition, was lower in marinated than in raw and smoked fillets (91.14 in marinated vs. 112.60 and 117.83, in raw and smoked samples, respectively).
We observed a correlation between the amount of long chain n-3 fatty acids and oxidative status. As already mentioned, the raw and smoked fillets had a higher content of n-3 fatty acids than the marinated fillet. For fish products subjected to processing after slaughter, it is necessary to take precautions to avoid over-oxygenation of the product, which would make it more susceptible to oxidation. The higher TBARs value was related to the worse oxidative status found in the marinated samples, and to the content of unsaturated fatty acids, which are the primary targets of the free radicals (DAL BOSCO *et al.*, 2010).
The trend for n-6 fatty acids was different to that of n-3 fatty acids. Raw and smoked fillets showed a lower content of total n-6 fatty acids (147.74 and 215.61 mg/100 g, respectively), while in the marinated fillet, the concentration is almost 20-times higher, as also shown by the n-6/n-3 ratio (Table 3).
Nowadays, fish products are the main source of n-3 PUFA in the human diet and, in consideration of the high intake of n-6 PUFA in industrialized countries, an increment of their consumption is recommended by dietary guidelines in order to re-establish a healthy balance between n-3 and n-6 PUFA (SIMOPOULOS, 2003). Several studies have demonstrated the role of n-3 fatty acids in the prevention of human diseases. In particular, these compounds also have a beneficial effect on the control and prevention of cardiovascular diseases and play crucial roles in brain development and visual activity (RIZOS *et al.*, 2012).
All the studied indexes reflect the composition of single fatty acids of fillets (Table 3).
The HH index measures the ratio of hypocholesterolemic and hypercolosterolemic fatty acids. The marinated fillet contained a higher concentration of hypocholesterolemic fatty acids (MUFA + PUFA), with a HH value of 5.00, than raw and smoked fillets. This result is noteworthy considering that the main factor in the onset of cardiovascular diseases is the oxidation of low-density lipoproteins (LDL cholesterol; HU and WILLETT, 2002);

therefore, fish products have an important role in the prevention of such pathologies (VALFRÈ *et al.*, 2003).
The INQ index describes the nutritional quality of a food, based on the satisfaction of the daily requirements of EPA + DHA. In the present study, the raw and smoked fillet had higher values than the marinated ones (5.95 and 6.24 vs. 1.85, respectively), in agreement with the EPA + DHA contents previously described.
Finally, the atherogenic (AI) and thrombogenicity indexes (TI), which measure the quality of the lipids in the fillets, are inversely correlated with the ability of fatty acids to reduce the lipid content in the blood and to reduce platelet activity, respectively (ULBRICHT and SOUTHGATE, 1991). The results obtained differed significantly between all three sample treatments, and they were better in the marinated fillet. In raw and smoked fillets, AI values of 0.67 and 0.66, respectively, were recorded, whereas for the marinated fillet, this value was 0.14. However, the TI value remained constant in all the samples (0.28, 0.27 and 0.26 in raw, smoked and marinated, respectively). These similar results could be explained by n-3 and n-6 PUFA values considered in the equations suggested by ULBRICHT and SOUTHGATE (1991). Indeed, they were equally weighted for the AI index, but not for the TI, where n-3 PUFA obtained a higher weight and then, equalizing the ratio between n-6 and n-3, was not significantly different between groups.

4. CONCLUSIONS

The data from the present study suggest that whitefish from Bolsena Lake is characterized by high nutritional quality (mainly due to EPA and DHA content) and a good oxidative stability of raw fillets. However, nutritional characteristics of fillets differed between processing methods: the smoked fillet showed a higher INQ and a lower n-6/n-3 ratio, HH index and TBARs value compared with the marinated one. In contrast, the marinated fillet offered an added value given by olive oil presence (lowest PI and highest PUFA content).
To conclude, the nutritional characterization of local products represents a good strategy to increase their economic value. Further studies focusing on the environment in which the fish lives and the one from which it originates, or on its history (as it is caught and processed on board the grounded boats and transformed yourself), are needed to evaluate such Umbrian fishery products (raw or processed).

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