NUTRITIONAL EVALUATION OF FILLETS, PULP AND CROQUETTES OF WILD CAUGHT LAKE TRASIMENO GOLDFISH (*CARASSIUS AURATUS* L.)

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ABSTRACT

Processing is required to render the products of some fish species edible, thus enhancing their economic value. The aim of the present study was to evaluate the nutritional value of goldfish (*Carassius auratus* L.) products from Lake Trasimeno (Umbria, Italy). The same number of fillets (n=20), raw and pre-cooked pulp samples, as well as of croquettes were used to evaluate the proximate composition, total lipid fatty acid profile and the protein and lipid oxidative stability. The water holding capacity (WHC) and the percentage of cooking loss (CL) were also assessed. Despite the low amount of lipids (1.85%), fresh goldfish fillets were characterized by a high PUFA level (54.30%), with DHA being the most abundant long-chain fatty acid (15.60%) followed by EPA (9.45%). Fillet processing reduced both WHC and CL of the final products, while the production of TBARS and carbonylated proteins increased, especially in croquettes (+ 59 and + 77%, respectively). Thrombogenic and atherogenic indexes also increased due to the PUFA reduction, reaching statistical significance only for the croquettes (0.53 *vs* 0.21 and 0.46 *vs* 0.34, respectively). These results suggest that goldfish fillets are characterized by a high nutritional value and a good oxidative stability. However, more efforts are needed to preserve these characteristics throughout processing.

INTRODUCTION

Trasimeno is one of the largest and shallowest lakes of the Italian peninsula with an average surface area of about 122 km² and a maximum depth of less than 6 m. It is located about twenty kilometres west of Perugia, in central Italy. It is a closed lake with no natural outlets. Its catchment basin is located entirely on lithotypes with a low permeability and almost corresponds to a hydro-geological basin (DRAGONI *et al.*, 2003). The water, seeping through the pleistocenic and recent sediments in the basin, probably reaches the lake via the aquifer surrounding it. Because of these characteristics, the water level of the lake undergoes considerable variations in accordance with the rate of local rainfall and, thus, it strictly depends on meteorological and climatic conditions (BURZIGOTTI *et al.*, 2003). Despite the high variability in the water level, Lake Trasimeno is very rich in fish such as chub (*Leuciscus cephalus* L.), rudd (*Scardinius erythrophthalmus* L.), eel (*Anguilla anguilla* L.), pike (*Exos lucius*), carp (*Ciprinus carpio*), tench (*Tenca tenca*), goldfish (*Carassius auratus* L.) and herbivore carp (*Ctenopharyngodon idellus Val.*) (NATALI, 1989).

Goldfish is one of the most common autocthonous fish species in Lake Trasimeno (MEARELLI *et al.,* 1990; GHETTI *et al.*, 2007; LORENZONI *et al.*, 2007). The high invasive capacity of this fish is due to its notable tolerance to extreme environmental conditions (low temperatures, high rate of pollution, murky water with a low percentage of oxygen), high sexual fertility and a wide feeding regimen (LUSHCHAK *et al.*, 2001, 2005, 2007; FORD *and* BEITINGER, 2005). It usually lives in shallow ponds, lakes rich in vegetation and slow moving rivers. During the dry season or winter it burrows into the mud. Goldfish feed on plants, insect larvae and plankton. Because of these characteristics, the goldfish population, as well as other similar autocthonous fish species, such as carp and tench, has greatly increased over the last years to the detriment of the autochthonous fish species, causing an alteration in the equilibrium of the Lake Trasimeno fish community (MEARELLI *et al.*, 1990; LORENZONI *et al.*, 2007). For this reason, it is of great importance to find solutions to reduce the number of goldfish and simultaneously benefit the local fishermen's yearly income.

One of the major disadvantages of goldfish is the presence of a high number of fine inter-muscular bones which limits its direct consumption. Moreover, consumers' perception of goldfish, which is generally considered as a pet animal, should also be taken into account. Raw goldfish fillets could be a solution to the first problem, as they can be easily processed into more edible products and these products could be a further stimulus for goldfish fishing and marketing. The second limitation could be more difficult to overcome.

The goals of this study were to determine the nutritional value of the fillets of the Lake Trasimeno goldfish, taking into account the proximate and fatty acid composition and to investigate potential modifications of the chemical composition, oxidative stability and physical properties of the goldfish products (pulp and croquettes). Differences between pulp samples before and after pre-cooking were also evaluated.

MATERIAL AND METHODS

The study was carried out in collaboration with the "Albatrasimeno" Fisherman Cooperative of S. Feliciano (Perugia, Italy). Forty goldfish were caught by net in April 2008 and taken to the laboratory of the cooperative for processing as described below.

Fillets, pulp samples and croquette preparation

Goldfish, of an average live weight of 550 g, were eviscerated after washing with running water and the heads and tails were removed, dorsal and ventral fillets were dissected and twenty of them (mean weight of 100 ± 13 g) were taken for analysis. The others were double minced (∅ 4.5 and 2.5 mm) and the pulp samples obtained were used for nutritional evaluation before (mean weight of 100 ± 3 g, each) and after cooking (weight of 100 ± 2 g, each). In detail, forty pulp samples were pre-cooked at 72° C for 6 min and twenty were used for the croquette preparation (25 ± 1 g, each). The following ingredients were used to prepare the goldfish croquettes: pre-cooked goldfish pulp (65%), red potatoes (16%), grated bread (6%), parmesan cheese (6%), egg (5%), parsley (1%) and salt (1%).

All fillet samples, raw and pre-cooked pulp and croquettes (n=20) were taken to the laboratory of the Department of Applied Biology for nutritional evaluation.

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 (1995. N. 950.46B, 920.153, and 928.08, respectively). Total protein was calculated by Kjeldahl nitrogen using 6.25 as the conversion factor. Total lipids were extracted in duplicate from 5 g of each homogenised sample and calculated gravimetrically (FOLCH *et al.*, 1957).

Fatty acids were determined by gas chromatography after lipid extraction according to the method of FOLCH *et al.* (1957). One mL of lipid

extract was evaporated under a stream of nitrogen and the residue was derivatized by adding 3 mL of sulfuric acid (3% in methanol). After incubating at 80°C for 1 h, methyl esters were extracted with petroleum ether and 1 μL was injected into the gas chromatograph (Fisions Mega 2 Carlo Erba Gas Chromatograph, model HRGC Milano, Italy), equipped with a flame ionization detector. Separation of fatty acid methyl esters (FAME) was carried out on an Agilent (J&W) capillary column (30 m x 0.25 mm I.D, CPS Analitica, Milan, Italy) coated with a DB-Wax stationary phase (film thickness of 0.25 μm). The operating conditions were those described by DAL BOSCO *et al.* (2007). Individual fatty acid methyl esters (FAME) were identified by reference to the retention time of FAME authentic standards. The relative proportion of each fatty acid in the samples was expressed as a percentage.

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):

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

The concentration of each fatty acid (mg/100 g of fish) was calculated from the lipid content of the fish using 0.91 as the conversion factor according to JOHANSSON *et al.* (2000).

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

ples (cooled for 30 min at 15°C) with respect to the weight of the raw samples.

Assessment of oxidative stability

The extent of lipid oxidation was quantified by spectrophotometry (Hitachi U-2000) as thio-barbituric acid reactive substances (TBARS) according to the method of KE *et al.* (1977) and using a molar extinction coefficient of 156 x 10^3 M/cm at 532 nm. Results are expressed as malondialdehyde (MDA) equivalents (mg MDA/kg).

The amount of the products of oxidative damage to proteins (protein carbonyl groups, PCGs) were measured spectrophotometrically by reaction with 2,4-dinitrophenylhydrazine (DNPH) according to the method proposed by LUSHCHAK *et al.* (2005). In detail, samples were homogenized (1:10 w/v) using a Potter-Elvehjem glass homogenizer in 50 mM potassium phosphate buffer, pH 7.0, containing 0.5 mM EDTA. A few crystals of phenylmethylsulfonyl fluoride were added prior to homogenization to inhibit proteases. A 250 mL aliquot of this homogenate was then mixed with 0.5 mL of 10% (final concentration) trichloroacetic acid and centrifuged for 5 min at 13,000 g. The resulting pellets were mixed with 1 mL of 10 mM DNPH in 2 M HCl and incubated for 1h at room temperature. Control samples were mixed with 1 mL of 2 M HCl. After washing three-times with 1ml of ethanol-butylacetate (1:1 v/v), pellets were dissolved in 1.5 mL urea (6M) and PCGs were measured by means of a spectrophotometer (Hitachi U-2000) set at 370 nm, using a molar extinction coefficient of 22 x 10^3 M/cm. The values are expressed as nanomoles

- atherogenic index = (C12:0 + 4xC14:0 + C16:0)/[(ΣMUFA + Σ (n-6) + Σ (n-3)];
- thrombogenic index = (C14:0 + C16:0 + C18:0)/[(0.5 x ΣMUFA + 0.5 x (n-6) + 3 x (n-3) + (n-3)/(n-6)].

Evaluation of pH, water holding capacity and cooking loss

The pH was measured with a Knick digital pH-meter (Broadly Corp., Santa Anna, CA, USA) after homogenizing the samples with iodoacetate (KORKEALA *et al.,* 1986).

The Water Holding Capacity (WHC) was evaluated (NAKAMURA and KATOH, 1985) by centrifuging one gram of sample, placed on a tissue paper inside a tube, for 4 min at 1,500 x g. The water remaining after centrifugation was quantified by drying the samples at 70°C overnight. WHC was calculated as follows: (weight after centrifugation - weight after drying)/initial weight x 100.

To evaluate cooking loss, samples of about 20 g were placed in open aluminium pans and cooked in an electric oven (pre-heated at 200°C) for 15 min to an internal temperature of 80°C (CYRIL *et al.*, 1996). Cooking loss was estimated as the percentage of weight of the cooked sam-

of PCGs per milligram protein in the urea solution. Protein concentration was measured by the BRADFORD (1976) method with Coomassie Brilliant Blue G-250 and using bovine serum albumin as a standard.

The sum of thiol groups (SH) was spectrophotometrically measured at 412 nm (molar extinction coefficient of 13,6 x 10^3 M/cm) in supernatants after reaction with 5,5'-dithio-bis (2-nitrobenzoic acid) as described by LUSHCHAK and BAGNYUKOVA (2006). Sample homogenates were prepared as described for the PCG assay and supernatants were obtained after centrifugation at 4°C for 15 min at 15,000 *g*. The thiol levels are expressed as micromoles of SH-groups per gram.

Reagents

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

Statistical evaluation

The data were analysed with a one way linear model (Statacorp®, 2005) evaluating the effect of technological treatment. Differences among goldfish products were evaluated by t-test, reporting the mean and standard error of the mean (SEM), at P<0.05.

RESULTS AND DISCUSSION

Proximate composition and physical traits of goldfish fillets, pulp and croquettes

The proximate composition of natural goldfish (fillets) and processed products (pulp and croquettes) is reported in Table 1. The amount of protein in the raw fillets (17.15%) was similar to the levels observed by TAKEUCHI et al. (1979) and by HOSSIAN and JAUNCEV (1989) in carp (Ciprinus carp; 16-19%), whereas it was much higher with respect to the protein content of sutchi catfish (Pangasius hypophthalmus; 12.6%, ORBAN, 2007). The latter fish species is commonly used for croquettes or other processed products because of its very low cost.

The lipid level of the goldfish fillets (1.85%) was much lower than that of brown (2.8%) and of rainbow trout (4.20%), which are both freshwater fish (DAL BOSCO et al., 2007). It was also lower than the crucian carp (Carassius carassius L.), which represents about 4.10% of the total weight, including the skin (KOLAKOWSKA et al., 2000). On the other hand, the lipid level of goldfish fillets was very similar to the lipid content of other lean freshwater fish species, such as burbot (Lota lota L.), zander (Stizostedion lucioperca L.) and roach (Rutilus rutilus L.) and marine garfish (Belone belone L.), having a lipid content less than 2% (KOLAKOWSKA et al., 2000).

Grinding did not cause any significant modification in the proximate composition of goldfish fillet, while cooking of raw pulp caused a significant reduction in the moisture content and a consequent increase in the amounts of protein and lipids. The addition of ingredients to the pre-cooked pulp for croquette preparation negatively affected (P<0.05) all of the aforementioned parameters.

The pH value of fillets was also affected by processing. The pH was significantly increased after cooking, whereas it was reduced after the addition of various ingredients to the cooked pulp (Table 1). Heat is known to cause variations in the electric charge of acid groups, separation of peptide chains and production of new compounds, reducing the concentration of hydrogen-ions (GRAU, 1978). SHERMAN (1961) has also attributed the increased pH of cooked pork meat to the fission of protein chains at labile linkages involving imidazole, -SH and -OH groups, followed by hydrogen bonding between carboxyl and amino groups. On the contrary, addition of various ingredients to the cooked pulp probably increased the number of acid groups, decreasing the pH of the croquettes.

Regarding the physical traits of goldfish fillet and processed products, it was found that grinding reduced (P<0.05) the WHC of fillets/pulp most probably by breaking down the muscle cell membranes (Table 1). This parameter was further reduced by thermal treatment, increasing the amount of water lost by cooking. It is well known that protein denaturation and coagulation, induced by heat, may reduce the space within the myofibril protein resulting in water loss (HAMM, 1969) and reduction in WHC (HONIKEL, 1998). The lower cooking loss of the cooked pulp, despite the lower WHC, could be justified by the reduced moisture level.

Nutritional characteristics

The fatty acid profile of the total lipids of fillets, pulp and croquettes is reported in Table 2. The percentage of total SFA in the goldfish fillet (29.00%) is similar to that of crucian carp (29.55%) (KOLAKOWSKA et al., 2000), but much lower than that of sutchi catfish (41.1%). On the contrary, the total PUFA (54.30%) of goldfish was 1.6 and 4.3 times higher than that

Table 1 - Proximate composition and physical traits of goldfish fillets, pulp and croquettes.

		Fillet	Pulp		Croquette	SEM
			Raw	Cooked		
Moisture	%	79.86[c]	78.57[c]	75.93[b]	72.05[a]	2.11
Protein		17.15[a]	18.20[b]	20.11[c]	24.10[d]	0.31
Lipids		1.85[a]	1.95[a]	2.15[ab]	2.54[b]	0.42
Ash		1.14	1.28	1.82	1.31	0.36
pH		6.46[b]	6.40[b]	6.68[c]	6.16[a]	0.12
WHC*	"	54.41[c]	50.51[b]	47.10[a]	48.69[a]	2.07
Cooking loss	"	30.49[b]	31.44[b]	22.55[a]	23.58[a]	1.86

N=20 per group; a..d: P<0.05.
Legend: *WHC stands for water holding capacity.

Table 2 - Fatty acid profile (%) of goldfish fillets, pulp and croquettes.

	Fillet	Pulp		Croquette	SEM
		Raw	Cooked		
C14:0	1.08[a]	1.78[a]	1.73[a]	2.89[b]	0.92
C16:0	19.19[a]	18.67[a]	18.64[a]	24.01[b]	2.14
C18:0	6.30[a]	5.65[a]	7.71[b]	8.94[c]	1.01
Others	2.43[a]	2.62[a]	2.00[a]	3.16[b]	0.87
Total SFA*	29.00[a]	28.72[a]	30.08[a]	39.00[b]	2.65
C14:1n-6	0.03[a]	0.11[ab]	0.06[a]	0.21[b]	0.12
C16:1n-7	3.55[a]	5.97[b]	5.09[b]	5.08[b]	0.45
C18:1n-9	9.28[a]	10.94[a]	11.44[a]	19.12[b]	1.89
Others	3.82[b]	4.64[b]	5.08[c]	2.78[a]	0.85
Total MUFA**	16.68[a]	21.66[b]	21.67[b]	27.19[c]	1.80
C18:2n-6	5.57[a]	6.86[a]	7.05[a]	12.64	2.03
C20:3n-6	0.73[c]	0.60[b]	0.66[bc]	0.07[a]	0.10
C20:4n-6	9.05[c]	7.41[b]	9.07[c]	4.56[a]	0.57
C18:3n-3	5.84[c]	8.57[d]	4.18[b]	3.09[a]	0.41
C20:3n-3	0.58[c]	0.66[d]	0.44[b]	0.22[a]	0.02
C20:5n-3	9.45[b]	7.83[c]	7.20[b]	3.40[a]	0.32
C21:5n-3	0.68[b]	0.40[a]	0.63[b]	0.14[a]	0.31
C22:5n-3	1.33[b]	2.49[c]	3.08[d]	1.46[a]	0.29
C22:6n-3	15.60[d]	10.17[b]	11.63[c]	5.94[a]	0.79
Others	5.47[d]	4.61[c]	4.29[b]	2.29[a]	0.27
Total PUFA***	54.30[c]	49.60[b]	48.23[b]	33.81[a]	3.12
Total PUFA n-3	36.22[b]	32.43[b]	29.31[b]	15.40[a]	9.08
Total PUFA n-6	18.09[b]	17.18[a]	18.93[c]	18.42[b]	0.74

N=20 per group; a..d: P<0.05.
Legend: *SFA stands for saturated fatty acids; **MUFA stands for monounsaturated fatty acids; ***PUFA stands for polyunsaturated fatty acids.

found in crucian carp (32.98%, KOLAKOWSKA *et al.*, 2000) and sutchi catfish (12.5%, ORBAN, 2007), respectively. Goldfish was poorer in total MUFA than crucian carp (16.68 *vs* 32.98%, KOLAKOWSKA *et al.*, 2000).

The major fatty acids of goldfish fillet were: palmitate (16:0) for SFA, oleate (C18:1n-9) for MUFA, arachidoneate (C20:4n-6), eicosapentoneate (C20:5n-3, EPA) and docosahexaeonate (C22:6n-3, DHA) for PUFA. A similar fatty acid profile was also observed in two other fish species from lakes in central Italy, such as the Common whitefish (*Coregonus lavaretus*) and the European perch (*Perca fluviatilis*) (Lazio Region, PRAL 1999-2001). In particular, it was found that the fat of European perch, fished in Lake Trasimeno, contains about 33.32% PUFA, of which 9.78 and 19.94% are represented by EPA and DHA, respectively (POLI *et al.*, 1994). Compared with other fish species, the level of these n-3 PUFA in goldfish fillet was much higher than in crucian carp (4.98 *vs* 9.45% and 9.93 *vs* 15.60% for EPA and DHA, respectively KOLAKOWSKA *et al.*, 2000). Moreover, the percentage of EPA in goldfish fillet was similar to that reported for gilthead sea bream (*Dicentrarchus labrax*, 10.91%) and European sea bass (*Aurata sparus*, 10.94%), while the percentage of DHA was even higher (15.60 *vs* 10.69% and 13.99%, respectively; TURCHETTO *et al.*, 1994). In sutchi catfish, both of these long-chain PUFAs are lower, while the amount of linoleic acid is very high, representing about 44% of the total PUFA (ORBAN, 2007). Differences in the fatty acid profile among fish species are due to the dietary habits, phytoplacton and zooplacton availability and other environmental factors, such as temperature and oxygen availability, as well as endogenous factors, like age and behaviour/metabolism/physiology.

The fatty acid profile of the fillet was affected in various ways by the three processing steps. Grinding of fillet increased the percentage of MUFA, by increasing the amount of palmitoleic acid (C16:1n-7), while reduced the percentage of PUFA, especially arachidonic acid (C20:4n-6), EPA and DHA. Consequently, the amounts of total n-3 and n-6 PUFA in the raw pulp decreased, reaching a statistical significance only for the n-6 series. The reduction of PUFA in raw pulp could be ascribed to exposure of a higher surface area of meat to oxygen after mincing, which was also confirmed by the higher TBARS level as discussed below (Table 3). PUFA are known to be more susceptible to oxidation than the other fatty acid groups because of the higher number in double bonds.

Apart from the reduction in EPA and DHA levels, cooking did not produce any further modification in the main fatty acid of the pulp.

On the contrary, addition of ingredients to the cooked pulp for croquette preparation resulted in a considerable increase in SFA and MUFA, especially oleic acid (C18:1n-9), and a concomitant decrease in PUFA (P<0.05), especially the n-3 group. Among the ingredients used, Parmesan cheese is very rich in oleic acid and might have contributed to the final concentration of this fatty acid in the croquettes. Eggs are also rich in various fatty acid groups, chiefly C16:0, C18:1n-9 and C18:2n-6.

The quantitative levels of the main fatty acid groups are reported in Table 3. It is worth noting that 100 g of goldfish fillet, containing only 1.85 g of total lipids, may provide a considerable amount of n-3 fatty acids, approximately 610 mg, of which 159 mg are represented by EPA and 263 mg by DHA. These findings are in agreement with those reported by HEPBURN *et al.* (1986), WESTRE *et al.* (1993) and HYVONEN *and* KOIVISTOINEN (1994). HEPBURN *et al.* (1986) found that crucian carp fillet provides the same level of PUFA as goldfish, but it contains a higher amount of lipid (5%).

The n-6/n-3 ratio of fresh goldfish fillet, an acknowledged nutritional index for human health, was lower than that reported for the rainbow trout living in the Valnerina Lake, Central Italy (0.50 *vs* 0.62, DAL BOSCO *et al.*, 2007), but higher than in crucian carp (0.22, KOLAKOWSKA *et al.*, 2000). When fillets were processed into croquettes, the value was doubled (P<0.05). Also the other nutritional indexes (atherogenic and trombogenic) changed during processing, reaching their highest value for goldfish croquettes (P<0.05). These changes are in accord with the changes in the fatty acid composition (SFA increased to the detriment of PUFA) which oc-curred after each treatment (mincing, cooking, addition of ingredients).

Finally, the thrombogenic index of fresh goldfish fillets was identical to that of Valnerina rainbow trout, whereas the atherogenic index was much lower (0.34 *vs* 0.45, DAL BOSCO *et al.*, 2007), most probably due to the lower level of SFA.

Oxidative stability

The Peroxidability Index of goldfish fillet, measuring the relationship between the fatty acid composition of a tissue and its susceptibility to oxidation, was quite high (273.12), in agreement with its high content in long-chain polyunsaturated fatty acids (Table 3). These results are similar to the findings of TESTI *et al.* (2006), but higher with respect to the value reported for the Valnerina rainbow trout (273.12 *vs* 230.03, DAL BOSCO *et al.*, 2007). This index tended to drop after grinding and cooking, reaching statistical significance only after the addition of ingredients for croquette production. This trend was similar to that observed for PUFA during transformation.

The extent of lipid and protein oxidation of goldfish fillet, pulp and croquette were evaluated by measuring the end products of lipid peroxidation (TBARS) and protein carbonylation (PCGs). Accordingly, it was found that both mechanical and thermal treatments seriously compromised the oxidative stability of processed products, as verified by the increase in production either of TBARS or PCGs (Table 3). Food products rich in PUFA (the primary targets for free radical attack), as the Lake Trasimeno goldfish, are more prone to undergo oxidation if no precau-

Table 3 - Quantitative fatty acid contents, oxidative status and nutritional indexes of goldfish fillets, pulp and croquettes.

		Fillet	Pulp		Croquette	SEM
			Raw	Cooked		
SFA*	mg/100 g	488.21[a]	509.64[a]	588.51[a]	901.45[b]	54.62
MUFA**	"	280.81[a]	384.36[b]	423.97[b]	628.47[c]	65.47
Σ n-3	"	609.68[b]	575.38[b]	573.35[b]	355.84[a]	47.81
Σ n-6	"	304.46[a]	304.77[a]	370.27[ab]	425.64[b]	56.69
EPA	mg/100 g	159.09[c]	138.94[b]	140.87[b]	78.59[a]	12.49
DHA	"	262.63[b]	180.47[ab]	227.54[b]	137.30[a]	53.24
PUFA***	"	914.14[b]	880.15[b]	943.62[b]	781.48[a]	87.98
n-6/n-3		0.50[a]	0.53[a]	0.63[a]	1.19[b]	0.35
Thiols	μmol/g	0.27[a]	0.22[a]	0.10[b]	0.08[b]	0.11
PCGs[§]	nmol/mg protein	1.43[a]	3.13[b]	4.22[b]	6.23[c]	1.21
TBARS[¥]	mg MDA/kg	0.54[a]	0.79[b]	0.83[b]	1.33[c]	0.42
Peroxidability index		273.12[b]	216.98[b]	226.78[b]	80.27[a]	39.87
Atherogenic index		0.34[b]	0.37[b]	0.23[a]	0.46[c]	0.10
Trombogenic index		0.21[a]	0.38[b]	0.27[a]	0.53[c]	0.13

N=20 per group; a..d: P<0.05.
Legend: *SFA stands for saturated fatty acids; **MUFA stands for monounsaturated fatty acids; ***PUFA stands for polyunsaturated fatty acids; [§]PCGs stands for protein carbonylated groups; [¥]TBARS stands for thiobarbituric acid reactive species.

tion is taken during processing. In fact, the level of TBARS significantly increased after grinding, as a consequence of a greater oxygenation of the minced fish fillet. On the other hand, heat shock has been demonstrated to increase lipid oxidation, and the rate of TBARS production depends on the temperature of cooking and duration (DAL BOSCO *et al.*, 2001). The conditions used in the present study for the cooking of raw pulp of goldfish only slightly elevated the TBARS level. In contrast, addition of ingredients like salt and parmesan cheese during croquette preparation resulted in a considerable increase in both lipid and protein oxidation products ($P<0.05$), also because of higher amounts of protein (24.10 *vs* 17.15%) and lipids (2.54 *vs* 1.85%). However, TBARS production never exceeded the threshold level (2.5 mg MDA/kg, CASTELLINI *et al.*, 2006), confirming no sign of sensorial deterioration in the processed products. Finally, it was found that PCG levels were significantly correlated with TBARS concentration ($r=0.89$, $P<0.05$, data not shown), throughout goldfish processing, suggesting a possible relationship between these two oxidative stress markers. Hence, it is likely that production of toxic aldehydes, such as MDA, during lipid oxidation, might have triggered protein carbonylation.

In addition to carbonylation, involving protein-NH_2 groups, processing of goldfish fillet modified the protein-SH groups as well (Table 3). Thiols are known to play a key role in oxidative stress by donating a hydrogen atom to the reactive free radicals and may protect, along with other antioxidants, biomacromolecules against oxidative damage (DRÖGE, 2002). The chief low-molecular weight thiol is glutathione, which plays a key role also in the maintenance of high levels of other protein-related thiols by reducing the formation of disulfides in collaboration with thioredoxin and glutaredoxin (SCHAFER and BUETTNER, 2001). As for the other two oxidative stress indexes, the level of total thiols underwent a noticeable reduction during cooking, underlying the necessity of additional antioxidants, such as vitamin E or C, to preserve the oxidative stability of goldfish proteins in the processed products. Finally, transformation of NH groups into carbonyls and oxidation of SH, might also have contributed to the changes in pH, WHC and CL values, during processing.

CONCLUSIONS

The data of the present study, taken together, suggest that goldfish from Lake Trasimeno are characterized by a high nutritional quality (high levels of EPA and DHA) and good oxidative stability, both comparable to that of other valuable fish species. Nevertheless, the presence of a large number of bones limits direct consumption of goldfish. For this reason, it is necessary to process them into more easily edible products using various technological processes which may alter the original nutritional quality. Among these, grinding and pre-cooking seem to slightly reduce the nutritional value, while addition of salt, parmesan cheese and eggs to the pre-cooked pulp results a significant reduction of PUFA, mainly of the n-3 series and a reduction of the oxidative stability of the final product with a consequent increase in both atherogenic and thrombogenic indexes. Even so, the goldfish croquettes can be considered as a good source of n-3 PUFA, providing an amount of approximately 78.59 mg of EPA and 137.30 mg of DHA per 100 g of consumed product; this latter value is higher than the daily recommended value for adult men (120 mg day^{-1} of DHA) as specified by NRF (2001). Further studies are needed to optimize the processing in order to preserve the nutritional characteristics of goldfish fillets.

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