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Effects of dietary lipid source on fillet chemical composition, flavour volatile compounds and sensory characteristics in the freshwater fish tench (*Tinca tinca* L.)

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Abstract

In this study the effects of soybean and linseed oils on chemical and sensory characteristics of fillets were evaluated in the freshwater fish tench (*Tinca tinca* L.). Five experimental diets, differing only in the relative amount of soybean and linseed oil, were formulated and the experiment was conducted on 360 sub-adult tench for 12 weeks. The fatty acid composition of muscle reflected that of the diets and significant correlations were observed. Diets containing higher amounts of $n-6$ fatty acids were responsible for an increased level of $n-6$ fatty acids in the fish flesh. Consequently, an increase in the relative amount of $n-6$-derived volatile aldehydes was also observed. These latter compounds are generally reported to contribute negatively to the general aroma of fish muscle and, consistently, the results of the sensory analysis showed a high value for the "off-flavour" attribute for fish fed the diet containing only $n-6$-rich soybean oil.

Keywords: Linseed oil; Soybean oil; Aquaculture; Fatty acids; Flavour volatile compounds; Sensory analyses

1. Introduction

The incessant increase in the global demand and price of fish meal and fish oil (Tacon, 2004), and the growing controversies concerning the unsustainable use of these commodities in aquafeeds (Naylor et al., 2000) have been reported. Thus, fish nutritionists and farmers are currently looking for alternative ingredients and/or an increase in the culture of low trophic level fish, such as cyprinids, which utilize fish meal and fish oil more efficiently (Lovell, 1998).

It has long been known that, providing their essential fatty acids (EFA) requirements are met, many freshwater fish can be successfully reared on diets containing vegetable or terrestrial animal oils (Sargent, Tocher, & Bell, 2002). As such, practical diets for cyprinids, which contain fish meal and consequently a source of EFA, are usually formulated with just the inclusion of vegetable oil as energy source (Satoh, 1991).

The market value of cultured fish largely depends on their quality and feed composition is one of the factors that affect quality (Morris, 2001). The fatty acid composition of fish tissue usually reflects that of the dietary lipids (Bell, 1998) and this means that fatty acid profiles of tissues can be modified by altering the types of fats and oils used in feed (Francis, Turchini, Jones, & De Silva, 2006; Sargent et al., 2002; Turchini, Gunasekera, & De Silva, 2003). Therefore, there is current interest in the influences of dietary oils on the fatty acid compositions and the possible

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consequent modifications of chemical, organoleptic and sensory attributes of fish fillets (Guillou, Soucy, Khalil, & Abambounou, 1995; Sérot, Regost, Prost, Robin, & Arzel, 2001; Turchini, Mentasti et al., 2003), as well as modifications of the health-related characteristics of fish as food for human use (Seierstad et al., 2005). The available reports for comparing dietary lipid sources and final eating quality in freshwater farmed fish are relative to the substitution of fish oil with vegetable oils and mainly in salmonids. Only a little knowledge is currently available relating to the effects of the use of different vegetable oils with regard to cyprinids and other low trophic level fish.

The aim of the present study was to evaluate the effects of the use of soybean and linseed oils as dietary lipid sources on the fillet chemical composition, fillet volatile compounds and sensory characteristics in farmed tench (*Tinca tinca* L.). Tench is a benthophagous omnivorous cyprinid species of aquacultural interest in European pond fish culture and a possible candidate to complement rainbow trout (*Oncorhynchus mykiss* W.) in a commercial setting (De Pedro et al., 2001; Quirós & Alvariño, 2000; Quirós et al., 2003). The characterization of the final eating quality will enhance the potential marketability.

2. Materials and methods

2.1. Husbandry

The feeding trial was carried out on sub-adult, two summers-old tench in the experimental facilities located in the "SalmoPan s.r.l." hatchery (Cremona, Italy). Fish were reared in 400 l square fibreglass tanks supplied with bore water and the flow rate was set to 7 l min^{-1} for each tank throughout the experiment. Natural lighting, enhanced in the early morning and in the afternoon by artificial lamps, was set at 14:10 light:dark cycle. Temperature and dissolved oxygen were measured daily (16.3 ± 0.3 °C and 7.8 ± 1.4 mg l^{-1}, respectively).

2.2. Experimental diets

The proximate compositions of the main dietary ingredients were determined using standard methods (AOAC, 1990) as described below and, based on the results, five isocalorific (20 kJ g^{-1}), isonitrogenous (350 g kg^{-1} protein) and isolipidic (140 g kg^{-1} lipid) experimental diets were formulated. The protein, lipid and carbohydrate contents of the test diets were based on the previous work on tench (De Pedro et al., 2001). The experimental diets differed only in the relative amount of soybean oil (SO; iodine value 132; free acid 0.06%, specific gravity 0.919) and linseed oil (LO; iodine value 184; free acid 0.06%, specific gravity 0.931), and were designated 100SO (100% SO), 25LO (75% SO and 25% LO), 50LO (50% SO and 50% LO), 75LO (25% SO and 75% LO), 100LO (100% LO) (Table 1). The vegetable oils, added at 10% of the diet weight, represented 71.4% of the total fat of the diets; the remaining 28.6% of total fat was mainly fish oil derived from the lipid contained in the fish meal. The ingredients and proximate compositions of the diets are given in Table 1 and the fatty acid composition of the experimental diets, and that of SO and LO are given in Table 2.

2.3. Experimental design

The experiment was conducted on 360 fish (24 per tank) randomly distributed amongst 15 tanks and assigned to one of the five experimental treatments (three replicates for each treatment). Fish were fed twice daily to apparent satiation, with feed consumption recorded weekly. At the end of the experiment, after 84 days, all fish were individually weighed and 18 fish per tank were culled for the chemical analyses, while another six fish from each tank were culled for the sensory analysis. Seven fish, in unrelated experimental tanks, showed signs of initial gonad development and were consequently discarded from the analyses.

2.4. Chemical analysis

Experimental diets, fish fillets, livers and carcasses were analysed for moisture, protein, lipid and ash using standard methods (AOAC, 1990 codes 930.15; 942.05; 955.04); briefly, moisture was measured by drying tissues at 60 °C to constant weight, protein was measured by estimating the Kjeldahl nitrogen (×6.25) in an automated distillation unit (Büchi 339, Flawil, Switzerland), lipid was measured by chloroform/methanol extraction (Bligh & Dyer, 1959), and ash was measured by incinerating in a muffle furnace at 550 °C for 18 h. All analyses were conducted in triplicate.

2.5. Lipid class separation and fatty acid analysis

Fatty acid analysis was performed on three sub-samples of soybean and linseed oils, three sub-samples of each experimental diet (Table 2) and three pooled muscle samples (three left fillets each) for each replicate. The extraction of total lipids was performed according to Bligh and Dyer (1959). After lipid extraction, an aliquot of the lipids was used for lipid class separation, using aminopropyl columns, as previously described by Kaluzny, Duncan, Merritt, and Epps (1985). Briefly, 5 mg of extracted lipids were evaporated under nitrogen and taken up in a minimal volume of chloroform (500 µl). Aminopropyl 3 ml size Bond Elut LRC columns (NH_2) (Varian, Harbor City, CA, USA) were placed in a vacuum manifold Visiprep (Supelco Inc., Bellefonte, PA, USA) and washed twice under vacuum (~10 kPa) with 2×2 ml portions of hexane. Lipid aliquots in chloroform were then applied to the column under vacuum. Triacylglycerols (TGs) were then eluted with 4 ml of chloroform:2-propanol (2:1, v/v); free fatty acids (FFAs) were next eluted with 4 ml of 2% acetic acid in diethyl ether and finally phospholipids (PLs) were eluted with 8 ml of methanol. Each lipid fraction was col-

Table 1
Diet formulations and proximate composition of the five experimental diets for sub-adult tench

	Diet[a]				
	100SO	25LO	50LO	75LO	100LO
Ingredient g kg^{-1}					
LT Fish meal[b]	308	308	308	308	308
Soybean meal[c]	230	230	230	230	230
Wheat starch[d]	290	290	290	290	290
Vitamin and mineral mix[e]	30	30	30	30	30
Choline[f]	2	2	2	2	2
CMC[g]	20	20	20	20	20
Betaine[g]	10	10	10	10	10
Celite[g]	10	10	10	10	10
Soybean oil[h]	100	75	50	25	–
Linseed oil[h]	–	25	50	75	100
Proximate composition (analysed)					
Moisture g kg^{-1}	43	45	44	31	30
Protein g kg^{-1}	351	355	359	363	362
Lipid g kg^{-1}	141	141	141	139	140
N.F.E. g kg^{-1}[i]	391	387	383	393	391
Ash g kg^{-1}	74	72	73	75	77
Energy kJ g^{-1}[j]	20.6	20.6	20.6	20.8	20.8

[a] Diet abbreviations: 100SO: 100% soybean oil; 25LO: 75% soybean oil and 25% linseed oil; 50LO: 50% soybean oil and 50% linseed oil; 75LO: 25% soybean oil and 75% linseed oil; 100LO: 100% linseed oil.
[b] Low temperature fish meal: Thyborøn Andels Fiskeindustri Amba, Denmark: Moisture 100 g kg^{-1}; Protein 720 g kg^{-1}; Lipid 100 g kg^{-1}; Ash 80 g kg^{-1}.
[c] Soybean meal: Consorzio Agrario Milano e Lodi, Italy: Moisture 120 g kg^{-1}; Protein 437 g kg^{-1}; Fibre 75 g kg^{-1}, Lipid 10 g kg^{-1}; Ash 6 g kg^{-1}.
[d] Gelatinized wheat starch: Idrostarc W, Lameri S.p.A., Cremona, Italy.
[e] Vitamin and mineral mix, Famavit S.p.A., Brescia, Italy; containing, per kg of mix: vitamin C, 8500 mg; vitamin A, 140000IU; vitamin D, 55000IU; vitamin E, 5500IU; vitamin K, 400 mg; thiamin, 400 mg; riboflavin, 800 mg; pyridoxin, 350 mg; niacin, 1650 mg; folate, 200 mg; vitamin B12, 200 mg; biotin, 35 mg; inositol, 13,500 mg; Ca-pantotenate, 1650 mg; Cu, 100 mg; I, 80 mg; Fe, 3500 mg; Mn, 700 mg; Zn, 1000 mg and Se, 3 mg.
[f] Choline chloride: BDH Biochemical, Poole, United Kingdom.
[g] CMC: Carboxymethylcellulose; Betaine: Betaine hydrochloride; Acros Organics, Geel, Belgium.
[h] Soybean oil and linseed oil: G. Ballestrini S.r.l., Oli e Grassi Speciali, Milano, Italy.
[i] N.F.E.: Nitrogen-free extract, calculated by difference.
[j] Calculated on the basis of 19, 36, and 15 kJ g^{-1} of protein, fat and carbohydrate, respectively.

lected in separate collection tubes and particular attention was paid to prevent the columns from becoming completely dry between operations for changing collection tubes. The three major lipid classes obtained (FFAs, TGs and PLs) were then analysed for their fatty acid compositions. Lipid classes were quantified as reported by Christie, Noble, and Moore (1970).

The preparation of fatty acid methyl esters was performed via acid-catalysed transesterification with methanolic hydrogen chloride, according to Christie (2003). Prior to the preparation of methyl esters, 0.5 mg of C23:0, as internal standard, and 0.4 mg of C13:0 ethyl ester, to monitor the precision of transesterification, were added (both standards obtained from Sigma Chemicals, St. Louis, MO, USA). Fatty acid separation and identification was carried out on an Agilent gas-chromatograph (Model 6890; Agilent Technologies, Palo Alto, CA, USA) fitted with an automatic sampler (Model 7683) and FID detector. The conditions used were as follows: HP-Innowax fused silica capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness; Agilent Technologies), split injection ratio 50:1, temperature programmed from 150 °C to 180 °C at 3 °C min^{-1}, then from 180 °C to 250 °C at 2.5 °C min^{-1} and held for 10 min; injector and detector at 280 °C. Carrier gas was helium at 1.0 ml min^{-1}, inlet pressure 16.9 psi. Fatty acids were identified relative to known external standards and the resulting peak areas, corrected by the theoretical relative FID response factors (Ackman, 2002), were quantified relative to internal standard. All analyses were conducted in duplicate.

2.6. Volatile compounds analysis

The volatile compound analysis was performed as previously described (Turchini, Mentasti et al., 2004; Turchini, Giani, Caprino, Moretti, & Valfrè, 2004) on six fillets from each experimental tank. Analyses were carried out in triplicate. Twenty grams of finely minced fish flesh were placed in a 250 ml flask with 100 ml of purified water (Millipore, Bedford, MA, USA) and subjected to simultaneous distillation-extraction (micro SDE apparatus, Chrompack, Middelburg, NL) for 30 min. A 10 ml solution of undecane (2 mg ml^{-1}) was added as internal standard. All reagents and solvents were from Merck (Darmstadt, Germany). Compounds were analysed in an Agilent 6890 Series GC system coupled to a 5973 N mass selective detector. The separation was performed on a DB-5MS capillary column (30 m × 0.25 mm, 0.25 mm film thickness) (Agilent Tech-

Table 2
The fatty acid composition of the experimental diets (mg g^{-1} of diet; w/w% in brackets) and the fatty acid composition of soybean oil (SO) and linseed oil (LO) (mg g^{-1} of lipid; w/w% in brackets)

	Oil[a]		Diet[b]				
	SO	*LO*	100SO	25LO	50LO	75LO	100LO
14:0	*0.6* (*0.1%*)	*0.4* (*0.1%*)	0.9 (0.9%)	1.0 (0.9%)	1.0 (0.9%)	0.5 (0.5%)	0.9 (0.9%)
16:0	*83.9* (*11.2%*)	*42.4* (*5.4%*)	13.3 (12.9%)	12.8 (11.9%)	11.6 (10.8%)	9.3 (9.9%)	9.0 (8.8%)
16:1	*0.6* (*0.1%*)	*0.5* (*0.1%*)	1.0 (1.0%)	1.0 (1.0%)	1.0 (1.0%)	0.9 (1.0%)	0.9 (0.9%)
18:0	*32.4* (*4.3%*)	*35.1* (*4.5%*)	4.1 (4.0%)	4.3 (4.0%)	4.3 (4.1%)	3.9 (4.2%)	4.3 (4.2%)
18:1*n* − 9	*165* (*22.0%*)	*156* (*20.0%*)	20.9 (20.2%)	21.3 (19.8%)	20.8 (19.4%)	18.1 (19.2%)	19.3 (18.9%)
18:1*n* − 7	*10.5* (*1.4%*)	*5.4* (*0.7%*)	1.6 (1.6%)	1.5 (1.4%)	1.4 (1.3%)	1.1 (1.2%)	1.1 (1.0%)
18:2*n* − 6	*401* (*53.3%*)	*120* (*15.4%*)	46.1 (44.6%)	40.4 (37.6%)	32.4 (30.3%)	22.0 (23.3%)	16.5 (16.1%)
18:3*n* − 6	*1.3* (*0.2%*)	*0.7* (*0.1%*)	0.1 (0.1%)	0.1 (0.1%)	0.1 (0.1%)	0.1 (0.1%)	0.1 (0.1%)
18:3*n* − 3	*48.8* (*6.5%*)	*413* (*52.8%*)	5.9 (5.7%)	15.4 (14.4%)	24.8 (23.2%)	30.0 (31.8%)	41.5 (40.6%)
18:4*n* − 3	–	–	0.5 (0.5%)	0.6 (0.5%)	0.6 (0.5%)	0.5 (0.5%)	0.5 (0.5%)
20:0	*2.8* (*0.4%*)	*1.2* (*0.2%*)	0.3 (0.3%)	0.3 (0.3%)	0.3 (0.3%)	0.2 (0.2%)	0.2 (0.2%)
20:1*n* − 9	*1.2* (*0.2%*)	*1.3* (*0.2%*)	0.5 (0.5%)	0.5 (0.5%)	0.5 (0.5%)	0.5 (0.5%)	0.5 (0.5%)
20:4*n* − 6	–	–	0.2 (0.2%)	0.2 (0.2%)	0.2 (0.2%)	0.2 (0.2%)	0.2 (0.2%)
20:5*n* − 3	–	–	2.0 (1.9%)	2.0 (1.9%)	2.1 (1.9%)	1.8 (1.9%)	2.0 (1.9%)
22:0	*3.5* (*0.5%*)	*1.4* (*0.2%*)	0.4 (0.4%)	0.4 (0.4%)	0.3 (0.3%)	0.2 (0.2%)	0.2 (0.2%)
22:1[c]	-	*1.1* (*0.1%*)	0.5 (0.5%)	0.5 (0.5%)	0.5 (0.4%)	0.5 (0.5%)	0.5 (0.5%)
22:5*n* − 3	–	–	0.1 (0.1%)	0.1 (0.1%)	0.2 (0.1%)	0.1 (0.1%)	0.1 (0.1%)
22:6*n* − 3	–	–	4.2 (4.0%)	4.4 (4.1%)	4.3 (4.0%)	3.8 (4.1%)	4.0 (3.9%)
24:1*n* − 9	–	*1.5* (*0.2%*)	-	-	0.1 (0.1%)	0.1 (0.1%)	0.2 (0.1%)
SFA	*123* (*16.4%*)	*80.5* (*10.3%*)	19.5 (18.8%)	19.1 (17.7%)	17.8 (16.6%)	14.4 (15.3%)	14.9 (14.6%)
MUFA	*177* (*23.6%*)	*166* (*21.2%*)	24.6 (23.8%)	24.9 (23.2%)	24.4 (22.8%)	21.2 (22.5%)	22.5 (22.1%)
PUFA	*451* (*60.0%*)	*536* (*68.5%*)	59.3 (57.3%)	63.4 (59.0%)	64.7 (60.5%)	58.6 (62.2%)	65.0 (63.6%)
n − 3	*48.8* (*6.5%*)	*415* (*53.0%*)	12.8 (12.3%)	22.6 (21.0%)	31.9 (29.8%)	36.3 (38.5%)	48.1 (47.1%)
n − 6	*402* (*53.5%*)	*121* (*15.5%*)	46.5 (45.0%)	40.8 (38.0%)	32.8 (30.7%)	22.3 (23.7%)	16.8 (16.5%)
n − 3/*n* − 6	*0.1*	*3.4*	0.3	0.6	1.0	1.6	2.9

Fatty acids accounting for <1 mg g^{-1} in all samples are not reported.
[a] See Table 1 for rate of inclusion of the two oils in the experimental diets.
[b] See Table 1 for diet abbreviations.
[c] 22:1 represent the sum of 22:1*n* − 13, 22:1*n* − 11 and 22:1*n* − 9.

nologies, Palo Alto, CA, USA). The carrier gas used was helium with a linear flow rate of 1 ml min^{-1}. The oven temperature programme was: 35 °C held for 1 min, from 35 °C to 60 °C at 120 °C min^{-1}, then from 60 °C to 280 °C at 3 °C min^{-1}. Samples of 1 ml were injected in a splitless mode (purge flow 19 ml min^{-1} at 1 min). Mass spectra were obtained under EI condition at 70 eV in the 35–300 amu range. The ion source was held at 230 °C and the quadrupole system at 150 °C. Identification of compounds was based on mass spectra from library databases (NIST 98, WILEY 275) and comparing GC retention times and retention indices with those of known standards. The Kovàts retention index (RI) was calculated for comparison of retention data from the literature (Castello, 1999). The data were recorded and analysed with HP Chemstation Software. The data are expressed as undecane equivalents and are reported as mg 100 g^{-1} of edible portion.

2.7. Free malondialdehyde quantification

The susceptibility of the muscle to induced lipid oxidation was assayed by the analysis of free malondialdehyde (MDA). At the end of the feeding trial, six fish from each tank were culled for the estimation of MDA. Analyses were performed on two tench fillets from each of the experimental tanks at day 0, day 3 and day 6 (samples stored at 4 °C until analysed). The determination of MDA in tissue samples was performed according to the method of Gerard-Monnier et al. (1998). The principle of this assay is the measurement of the absorbance at 586 nm, which is obtained with MDA upon reaction with 1-methyl-2-phenylindole. Briefly, 3 g of tissue were homogenized in 10 ml of 20 mM Tris buffer (pH 7.4) containing 5 mM butylated hydroxytoluene to prevent sample oxidation. The homogenate was centrifuged for 10 min at 2000*g* at 4 °C and 0.2 ml of the aqueous supernatant was added to 650 µl of a solution of 1-methyl-2-phenylindole in a mixture of acetonitrile/methanol (3:1, v/v). The final concentration of the reagent was 10 mM. The reaction was then started by adding 150 µl of 37% hydrochloric acid. The absorbance was measured at 586 nm, using a Jasco (Hachioji, Tokyo, Japan) spectrophotometer (model V-530) upon incubation of the reaction mixture at 45 °C for 1 h. The results were expressed as nmol g^{-1} tissue.

2.8. Sensory analysis

Six fish from each tank were culled for sensory analysis in a dedicated laboratory (ISO 8589, 1988). The analysis was performed on fresh samples 2 days after culling by conventional profiling (ISO 13299, 1988) with an 11-member professional panel (ISO 8586, 1983). During training,

Table 3
Sensory descriptors for cooked fillet of tench in order as they were evaluated

Descriptors	Definitions	Range (0 → 9)
Total odour	The total strength of olfactory sensation	Light → strong
Off-flavour	Every perceptible off-flavour not related to the characteristic tench aroma (including earthy, muddy, fatty and fishy)	Light → strong
Salty taste	The taste on the tongue associated with sodium ions	Light → strong
Total flavour	The total oral impression as perceived by taste, smell, feeling in the mouth	Light → strong
Fatty flavour	Impression of fatness perceived as aromatic notes and mouth coating film	Light → strong
Juiciness	Water release during chewing	Dry → juicy
Firmness	Degree of firmness/connection of the fibres during chewing	Scaly → firm
Bitter taste	The taste on the tongue associated with caffeine and similar bitter substances	Light → strong

the assessors developed a vocabulary by describing differences between extreme samples and agreed on a list of eight attributes for profiling (Table 3). Sensory evaluations were recorded via a computer system on 15 cm lines anchored by the numbers 0–9. The anterior and posterior parts of the fillet were removed, while the central part was cut into a single assessor's helping, wrapped in aluminium foil, cooked at 180 °C for 10 min in an air-heated oven and immediately served to the assessors in closed containers following a balanced order.

2.9. Statistical analysis

Data are reported for each dietary treatment as mean values ± standard error of mean (SEM; $N = 3$ tanks/treatment; 24 fish/tank). Homogeneity of variance was confirmed and comparison between means was by one-way ANOVA. Significance was accepted at probabilities of 0.05 or less. The Student–Newman–Keuls was used as a *post hoc* test for comparison of the means among different treatments. The statistical analyses were performed by SPSS 11.5 (SPSS Inc., Chicago, Illinois, USA). Where appropriate, data were subjected to a Pearson correlation, and to linear regression with the software GraphPad Prism 3.0 (GraphPad Software Inc., San Diego, California, USA).

3. Results

3.1. Growth

Throughout the 12 weeks experiment only two fish died, in unrelated tanks, during the first week after initial weighing. Final mean weights of sub-adult tench maintained on the four diets containing different amounts of LO (25LO, 50LO, 75LO and 100LO) were significantly ($P < 0.05$) higher (154.5 ± 0.71 g, 155.9 ± 2.06 g, 156.9 ± 2.06 g and 158.1 ± 1.83 g, respectively) than that of fish on the 100SO (149.0 ± 2.25 g). No significant differences were noted for feed intake, while the feed conversion ratio was highest ($P < 0.05$) for fish on 100SO compared to the other dietary treatments. No significant differences were noted in the fat deposition rate or in the main biometrical parameters, such as hepatosomatic index, condition factors and dress-out percentage.

3.2. Proximate compositions

Data on tissue proximate composition are given in Table 4. No significant effects ($P > 0.05$) of the dietary treatments were observed on carcass proximate composition, while significant differences ($P < 0.05$) were observed for fillet protein percentage and for liver ash content of fish fed different diets.

3.3. Fatty acid compositions

The fatty acid compositions of the two tested oils (Table 2) showed that SO was characterized by a high level of linoleic acid (18:2$n-6$, LA), accounting for 53.1% of the total fatty acids while, in LO, the most abundant fatty acid was the α-linolenic acid (18:3$n-3$, LnA) (52.8%) as is well known from the literature (Hertrampf & Piedad-Pascual, 2000). Therefore, the amounts of LA and LnA in the five experimental diets (Table 2) were directly related to the gradient of substitution of SO and LO, respectively. Values of arachidonic acid (20:4$n-6$, ArA), eicosapentaenoic acid (20:5$n-3$; EPA) and docosahexaenoic acid (22:6$n-3$; DHA) were constant in all the diets, as they were derived mainly by fish meal and not present in the two lipid sources tested. The fatty acids found in sub-adult tench, irrespective of the dietary treatment (Table 5), in order of amount, were oleic acid (18:1$n-9$), DHA, palmitic acid (16:0), LA and palmitoleic acid (16:1$n-7$). The major fatty acid categories of fish muscle were not significantly different among treatments. In all instances the fatty acids in muscle were dominated by polyunsaturated fatty acids (PUFA). In general, there was a decrease in the proportion of monounsaturated fatty acids (MUFA) and $n-6$, and an increase of saturated fatty acids (SFA) and $n-3$ with increasing amounts of LO in the diet. Also, the $n-3/n-6$ ratio increased significantly with increasing amounts of LO in the diet, from 2.13 ± 0.11 (100SO) to 3.08 ± 0.07 (100LO). Significant ($P < 0.05$) linear correlations and regressions were observed among the dietary LA and LnA and their respective amounts in the fillets ($R^2 = 0.86$, $P = 0.028$ and $R^2 = 0.91$, $P = 0.012$). No significant correlations were observed among dietary LA and total ArA of the fillets or between dietary LnA and total EPA and total DHA of the fillets.

Table 4
Carcass, fillet and liver proximate composition (as-is basis; g kg^{-1}) of tench fed the different dietary treatments for 12 weeks (mean ± SEM; $N = 3$tanks/treatment)

	Diet				
	100SO	25LO	50LO	75LO	100LO
Carcass					
Moisture	736.6 ± 9.0	731.5 ± 10.5	742.2 ± 3.5	731.2 ± 4.2	738.8 ± 8.9
Protein	173 ± 7.4	166 ± 1.1	169.1 ± 0.5	172 ± 4.3	173 ± 2.1
Lipid	55.0 ± 4.3	64.2 ± 11.8	52.0 ± 5.3	63.4 ± 3.0	53.5 ± 3.0
Ash	35.8 ± 1.7	38.7 ± 1.9	36.7 ± 3.2	33.9 ± 3.2	35.1 ± 4.2
Fillet					
Moisture	771.7 ± 8.6	790 ± 1.0	790.1 ± 3.5	795.1 ± 8.6	779.9 ± 4.6
Protein	190.0 ± 5.1^{b}	173.3 ± 6.1^{ab}	173.7 ± 5.3^{ab}	166 ± 2.1^{a}	187 ± 4.2^{b}
Lipid	26.6 ± 3.9	25.3 ± 5.7	25.2 ± 4.4	27.5 ± 6.4	22.9 ± 4.1
Ash	11.7 ± 0.5	11.3 ± 0.3	10.9 ± 0.2	11.0 ± 0.2	10.2 ± 0.3
Liver					
Moisture	719.4 ± 17.6	749 ± 9.9	709.7 ± 3.7	729.6 ± 6.6	721.9 ± 14
Protein	143 ± 9.2	125 ± 6.8	146.1 ± 3.7	133 ± 4.7	142 ± 8.6
Lipid	52.5 ± 9.6	34.7 ± 7.5	44.7 ± 7.5	65.7 ± 8.4	58.0 ± 9.0
Ash	9.4 ± 0.7^{a}	9.4 ± 0.4^{a}	08.4 ± 0.6^{a}	13.6 ± 1.7^{b}	14.8 ± 0.3^{b}

Means within rows without superscript or with the same superscript are not significantly ($P > 0.05$) different from each other by one-way ANOVA and S–N–K comparison test.

Table 5
The fillet total fatty acid compositions (mg g^{-1} of lipid) of tench fed the different dietary treatments for 12 weeks (mean ± SEM; $N = 3$tanks/treatment)

	Diet				
	100SO	25LO	50LO	75LO	100LO
14:0	26.6 ± 1.28	24.7 ± 0.76	24.0 ± 0.49	23.4 ± 0.42	25.5 ± 0.70
14:1	1.04 ± 0.10^{b}	0.82 ± 0.03^{A}	0.78 ± 0.03 ^{A}	0.69 ± 0.02 ^{A}	0.79 ± 0.02 ^{A}
15:0	3.29 ± 0.19	3.20 ± 0.08	3.11 ± 0.05	3.07 ± 0.04	3.34 ± 0.07
16:0	104 ± 3.67	111 ± 0.80	111 ± 1.47	110 ± 1.76	111 ± 1.42
16:1$n-7$	68.5 ± 3.91^{b}	58.2 ± 1.52 ^{A}	56.8 ± 1.75 ^{A}	55.2 ± 0.88 ^{A}	59.4 ± 1.41 ^{A}
17:0	1.16 ± 0.06 ^{A}	1.29 ± 0.02 ^{A}	1.27 ± 0.02 ^{A}	1.29 ± 0.03 ^{A}	1.50 ± 0.09^{b}
17:1	3.12 ± 0.10^{b}	2.80 ± 0.08 ^{A}	2.76 ± 0.05 ^{A}	2.69 ± 0.06 ^{A}	2.88 ± 0.08 ^{A}
18:0	14.8 ± 0.93 ^{A}	19.2 ± 0.84^{b}	19.4 ± 0.15^{b}	19.6 ± 0.58^{b}	21.4 ± 0.54^{b}
18:1$n-9$	145 ± 4.70	148 ± 3.28	146 ± 2.56	147 ± 2.18	146 ± 2.56
18:1$n-7$	23.3 ± 0.74	22.8 ± 0.28	22.1 ± 0.41	22.1 ± 0.27	22.0 ± 0.32
18:2$n-6$	81.1 ± 4.42^{b}	78.4 ± 4.20^{b}	77.7 ± 2.02^{b}	70.8 ± 0.97^{b}	60.8 ± 1.15 ^{A}
18:3$n-6$	1.47 ± 0.27	1.41 ± 0.13	1.26 ± 0.06	1.20 ± 0.03	0.91 ± 0.02
18:3$n-3$	11.2 ± 0.60 ^{A}	19.0 ± 1.35^{b}	23.3 ± 1.14^{b}	33.7 ± 1.20^{c}	34.2 ± 2.59^{c}
18:4$n-3$	5.46 ± 0.23^{ab}	5.19 ± 0.25 ^{A}	5.33 ± 0.09^{ab}	5.48 ± 0.17^{ab}	6.02 ± 0.17^{b}
20:0	1.04 ± 0.21	1.18 ± 0.24	1.45 ± 0.04	1.41 ± 0.02	1.39 ± 0.02
20:1$n-9$	21.1 ± 0.85	20.2 ± 0.55	20.4 ± 0.30	19.0 ± 0.73	20.1 ± 0.37
20:2$n-6$	4.20 ± 0.23^{b}	4.76 ± 0.07^{c}	4.43 ± 0.10^{bc}	4.20 ± 0.08^{b}	3.69 ± 0.05 ^{A}
20:3$n-6$	3.56 ± 0.40^{b}	4.05 ± 0.06^{b}	3.60 ± 0.06^{b}	3.37 ± 0.11^{b}	2.28 ± 0.09 ^{A}
20:4$n-6$	6.65 ± 0.26	7.25 ± 0.44	7.21 ± 0.10	7.24 ± 0.30	6.52 ± 0.09
20:4$n-3$	9.99 ± 0.41	9.79 ± 0.16	10.1 ± 0.13	10.1 ± 0.14	10.7 ± 0.20
20:5$n-3$	41.8 ± 1.45	41.2 ± 0.67	41.2 ± 0.65	43.5 ± 1.85	45.3 ± 0.75
22:1 ^{A}	8.29 ± 0.31	7.67 ± 0.27	8.09 ± 0.28	7.41 ± 0.66	8.07 ± 0.54
22:4$n-6$	1.89 ± 0.07	2.24 ± 0.14	2.24 ± 0.03	1.93 ± 0.08	2.09 ± 0.07
22:5$n-3$	15.04 ± 0.57^{b}	14.0 ± 0.34^{ab}	14.2 ± 0.23^{ab}	13.4 ± 0.29 ^{A}	14.2 ± 0.19^{ab}
22:6$n-3$	125 ± 2.97	125 ± 6.41	124 ± 0.95	125 ± 4.19	125 ± 2.05
SFA	154 ± 5.33	162 ± 1.21	161 ± 2.07	161 ± 2.47	165 ± 2.15
MUFA	271 ± 9.34	261 ± 5.44	257 ± 4.19	254 ± 4.55	260 ± 4.75
PUFA	307 ± 8.70	312 ± 3.54	315 ± 3.79	32.0 ± 4.33	311 ± 3.24
$n-3$	208 ± 5.02 ^{A}	214 ± 5.32 ^{A}	218 ± 2.04 ^{A}	231 ± 3.87^{b}	235 ± 3.32^{b}
$n-6$	98.8 ± 5.18^{b}	98.1 ± 3.71^{b}	96.4 ± 2.14^{b}	88.7 ± 0.73^{b}	76.3 ± 1.17 ^{A}
$n-3/n-6$	2.13 ± 0.11 ^{A}	2.20 ± 0.13 ^{A}	2.27 ± 0.04 ^{A}	2.60 ± 0.04^{b}	3.08 ± 0.07^{c}

Fatty acids accounting for <1 mg g^{-1} in all samples are not reported.
Means within rows without superscript or with the same superscript are not significantly ($P > 0.05$) different from each other by one-way ANOVA and S–N–K comparison test.
[A] 22:1 represent the sum of 22:1$n-13$, 22:1$n-11$ and 22:1$n-9$.

The three lipid classes separated (free fatty acids, FFAs; triacylglycerols, TGs; phospholipids, PLs) accounted for 8.6%, 76.9% and 14.5% of the total fatty acid, respectively (Table 6). The amounts of LA, LnA and total $n-6$ PUFA in all three lipid classes separated were significantly ($P < 0.05$) different among fish fed with the different experimental diets. In the TGs, SFA and $n-3$ PUFA were also significantly affected by the dietary treatment and, in the PLs fraction, the EPA content of fish fed the 100LO diet was significantly ($P < 0.05$) higher.

3.4. Flavour volatile compounds

The results of volatile compound analysis showed that 39 compounds were isolated and identified in the fillet of tench fed with the experimental diets (Table 7). The two major categories of flavour volatile compounds were aldehydes (21 compounds) and alcohols (six compounds). No significant differences were noted among dietary treatments for each percent value of all the isolated and identified compounds in the tench fillets. Two factors were calculated, summing the relative percentages of each volatile aldehyde formed by autoxidation of $n-3$ PUFA (Σ $n-3$-derived aldehydes: sum of 2-pentenal, 2-hexenal, 2tr,4c-heptadienal, 2tr,4tr-heptadienal and 2,6-nonadienal) and $n-6$ PUFA (Σ $n-6$-derived aldehydes: sum of hexanal, 2-octenal, 2-decenal, 2tr,4c-decadienal and 2tr,4tr-decadienal) as reported by Belitz and Grosch (1999). The $\sum n-6$-derived aldehydes were significantly ($P < 0.05$) higher for fillets of fish fed 100SO and lower for fillets of fish on 100LO ($20.6 \pm 2.06\%$ and $12.0 \pm 0.47\%$, respectively). The $\sum n-3$-derived aldehydes and the $\sum n-6$-derived aldehydes were positively correlated ($r = 0.74$ and $r = 0.86$, respectively) with the content of $n-3$ PUFA

Table 6
The fillet free fatty acid (FFAs), triacylglycerol (TGs) and phospholipid (PLs) fatty acid compositions (mg g lipid^{-1}) of muscle of tench fed the different dietary treatments for 12 weeks (means ± SEM; $N = 3$tanks/treatment)

	Diet				
	100SO	25LO	50LO	75LO	100LO
FFAs					
SFA	29.0 ± 3.75	28.2 ± 5.39	25.0 ± 2.09	23.2 ± 1.82	18.0 ± 1.12
MUFA	17.4 ± 2.34	15.8 ± 3.15	16.5 ± 1.52	14.1 ± 1.13	10.4 ± 1.32
PUFA	27.2 ± 3.47	25.5 ± 4.37	26.0 ± 2.39	23.0 ± 2.08	17.2 ± 0.35
18:2$n-6$	7.55 ± 1.45^{b}	6.77 ± 1.19^{b}	6.62 ± 0.74^{b}	5.22 ± 0.51^{b}	3.19 ± 0.25 ^{a}
18:3$n-3$	1.00 ± 0.09 ^{a}	1.51 ± 0.24^{ab}	2.38 ± 0.27^{b}	3.16 ± 0.29^{c}	2.26 ± 0.21^{b}
20:4$n-6$	0.89 ± 0.13	0.78 ± 0.14	0.79 ± 0.08	0.77 ± 0.11	0.44 ± 0.10
20:5$n-3$	3.63 ± 0.65	3.67 ± 0.92	3.61 ± 0.38	3.28 ± 0.27	2.83 ± 0.17
22:6$n-3$	9.57 ± 1.22	9.20 ± 1.83	9.63 ± 0.72	8.08 ± 0.79	6.80 ± 0.69
$n-3$	18.0 ± 1.73	17.31 ± 3.13	17.9 ± 1.42	16.28 ± 1.41	13.4 ± 0.47
$n-6$	9.21 ± 1.94^{b}	8.18 ± 1.42^{b}	8.08 ± 0.98^{b}	6.73 ± 0.67^{b}	3.83 ± 0.31 ^{a}
$n-3/n-6$	2.24 ± 0.52	2.22 ± 0.29	2.24 ± 0.09	2.43 ± 0.04	3.89 ± 0.65
TGs					
SFA	101 ± 3.72 ^{a}	114 ± 6.06^{ab}	105. ± 6.49^{ab}	117 ± 2.53^{ab}	122.2 ± 0.48^{b}
MUFA	220 ± 10.87	234 ± 7.57	224 ± 8.49	225.2 ± 9.06	228.1 ± 0.24
PUFA	210 ± 4.15	233 ± 11.28	227 ± 3.68	224 ± 10.53	232 ± 1.72
18:2$n-6$	65.6 ± 3.81^{ab}	70.4 ± 6.36^{b}	65.1 ± 0.74^{ab}	60.7 ± 1.98^{ab}	53.7 ± 0.41 ^{a}
18:3$n-3$	9.46 ± 0.47 ^{a}	16.9 ± 1.99^{b}	19.6 ± 1.04^{b}	28.6 ± 0.53^{c}	32.3 ± 2.61^{c}
20:4$n-6$	3.08 ± 0.23	3.15 ± 0.20	2.95 ± 0.17	3.21 ± 0.06	3.11 ± 0.06
20:5$n-3$	31.3 ± 1.75	34.3 ± 1.58	33.9 ± 1.59	31.7 ± 1.37	37.0 ± 0.52
22:6$n-3$	68.1 ± 2.02	74.3 ± 2.96	72.9 ± 2.26	67.4 ± 6.58	74.3 ± 2.55
$n-3$	135 ± 4.29 ^{a}	150 ± 5.65^{ab}	152 ± 3.27^{ab}	152 ± 9.01^{ab}	170 ± 1.84^{b}
$n-6$	75.4 ± 4.24^{ab}	82.1 ± 6.76^{b}	75.5 ± 0.65^{ab}	71.4 ± 2.21^{ab}	62.8 ± 0.66 ^{a}
$n-3/n-6$	1.78 ± 0.14 ^{a}	1.85 ± 0.11 ^{a}	2.01 ± 0.04 ^{a}	2.13 ± 0.10 ^{a}	2.71 ± 0.05^{b}
PLs					
SFA	29.8 ± 2.77	21.2 ± 4.22	26.0 ± 4.81	26.7 ± 3.45	26.7 ± 1.07
MUFA	18.8 ± 1.25	12.6 ± 2.71	16.8 ± 2.96	16.5 ± 2.03	17.2 ± 0.30
PUFA	77.1 ± 1.09	49.8 ± 9.98	65.3 ± 11.85	64.3 ± 9.48	62.4 ± 3.02
18:2$n-6$	7.45 ± 0.92^{b}	5.07 ± 1.16^{ab}	5.91 ± 1.08^{b}	5.17 ± 0.72^{b}	3.85 ± 0.20 ^{a}
18:3$n-3$	0.27 ± 0.13 ^{a}	0.68 ± 0.18^{ab}	1.41 ± 0.27^{bc}	2.10 ± 0.34^{cd}	2.42 ± 0.17^{d}
20:4$n-6$	3.23 ± 0.10	1.88 ± 0.38	2.51 ± 0.46	2.55 ± 0.43	2.28 ± 0.16
20:5$n-3$	5.73 ± 0.18^{ab}	3.79 ± 0.75 ^{a}	5.46 ± 0.93^{ab}	5.54 ± 0.83^{ab}	6.11 ± 0.31^{b}
22:6$n-3$	54.1 ± 1.21	34.6 ± 6.78	45.5 ± 8.37	44.7 ± 6.49	44.2 ± 2.01
$n-3$	64.6 ± 0.23	41.1 ± 8.17	55.3 ± 9.99	55.3 ± 8.15	55.6 ± 2.64
$n-6$	12.5 ± 1.32^{c}	8.19 ± 1.81^{b}	9.92 ± 1.90^{b}	9.01 ± 1.35^{b}	6.73 ± 0.56 ^{a}
$n-3/n-6$	5.15 ± 0.60 ^{a}	5.19 ± 0.23 ^{a}	5.70 ± 0.31 ^{a}	6.12 ± 0.19 ^{a}	8.36 ± 0.51^{b}

Only major fatty acids and fatty acid classes are reported.
Means within rows without superscript or with the same superscript are not significantly ($P > 0.05$) different from each other by one-way ANOVA and S–N–K comparison test.

Table 7
The fillet flavour volatile compounds (w/w%) of tench fed the different dietary treatments for 12 weeks (means ± SEM; $N = 3$tanks/treatment)

RI[a]		Diet				
		100SO	25LO	50LO	75LO	100LO
143	3-Hydroxy-2-butanone	3.56 ± 1.76	2.94 ± 0.67	3.41 ± 1.12	4.25 ± 0.53	7.83 ± 0.93
167	2-Ethoxy-2methyl-butane	1.93 ± 0.77	2.01 ± 0.32	1.94 ± 0.15	1.90 ± 0.30	1.87 ± 0.22
226	2,3,4-Trimethyl-pentane	1.14 ± 0.18	2.10 ± 0.36	2.22 ± 0.20	2.07 ± 0.80	2.06 ± 0.46
247	2-Pentenal	4.10 ± 0.47	4.65 ± 0.54	4.34 ± 0.18	4.10 ± 0.26	4.85 ± 0.35
271	2-Penten-1-ol	2.17 ± 0.67	3.54 ± 0.71	2.99 ± 0.57	2.23 ± 0.58	2.59 ± 0.55
353	Hexanal	11.7 ± 1.53	7.57 ± 0.37	9.64 ± 2.23	8.49 ± 0.85	6.09 ± 1.19
491	2-Hexenal	1.70 ± 0.03	2.03 ± 0.22	2.15 ± 0.45	2.14 ± 0.35	1.85 ± 0.18
514	Ethylbenzene	2.42 ± 0.40	2.76 ± 0.15	2.83 ± 0.07	2.74 ± 0.44	3.21 ± 0.71
523	3-Methyl-2-hexanol	5.84 ± 1.17	5.91 ± 0.93	5.29 ± 0.13	5.52 ± 0.68	5.07 ± 0.52
602	1,3-Dimethyl-benzene	0.86 ± 0.16	1.15 ± 0.20	1.62 ± 0.08	1.13 ± 0.18	1.62 ± 0.63
612	4-Heptenal	0.94 ± 0.14	0.85 ± 0.05	0.99 ± 0.01	0.90 ± 0.02	0.88 ± 0.01
621	2-Butoxy-ethanol	6.19 ± 1.58	3.24 ± 0.47	5.62 ± 2.35	4.46 ± 0.87	3.92 ± 0.52
635	3-Methylthio-propanol	1.03 ± 0.34	1.31 ± 0.24	1.22 ± 0.16	1.21 ± 0.11	1.20 ± 0.23
765	2-Heptenal	1.41 ± 0.13	2.08 ± 0.66	1.33 ± 0.04	1.11 ± 0.38	0.44 ± 0.36
786	Benzaldheyde	4.97 ± 0.70	9.44 ± 2.11	6.99 ± 0.09	6.47 ± 0.49	5.71 ± 0.75
805	3,5,5-Trimethyl-2-hexene	5.02 ± 2.42	3.98 ± 0.31	3.67 ± 1.48	5.55 ± 2.57	4.02 ± 1.57
821	1-Octen-3-ol	2.24 ± 1.01	3.07 ± 0.23	3.31 ± 0.46	2.56 ± 0.26	2.46 ± 0.39
849	2-Penthyl-furan	1.26 ± 0.20	1.18 ± 0.08	1.10 ± 0.11	1.07 ± 0.02	0.96 ± 0.08
865	2tr,4c-Heptadienal	1.44 ± 0.25	2.40 ± 0.55	2.00 ± 0.45	2.16 ± 0.21	2.21 ± 0.47
881	Octanal	2.24 ± 0.47	1.46 ± 0.06	1.64 ± 0.13	1.71 ± 0.19	1.35 ± 0.27
902	2tr,4tr-Heptadienal	3.04 ± 0.54	4.67 ± 0.46	4.28 ± 0.38	4.81 ± 0.12	5.66 ± 1.21
982	Benzeneacetaldehyde	1.13 ± 0.52	0.81 ± 0.08	0.65 ± 0.09	0.80 ± 0.05	0.85 ± 0.08
1010	2-Octenal	2.70 ± 0.60	2.47 ± 0.32	2.12 ± 0.09	2.38 ± 0.17	1.97 ± 0.17
1023	2-Methyl-decane	1.36 ± 0.03	1.02 ± 0.19	1.37 ± 0.25	1.30 ± 0.14	1.60 ± 0.20
1032	1,3-Cyclooctadiene	5.75 ± 1.11	3.51 ± 0.41	4.80 ± 0.30	5.06 ± 0.58	4.97 ± 1.24
1109	Nonanal	6.18 ± 0.83	3.23 ± 0.52	3.59 ± 0.32	4.97 ± 0.87	3.84 ± 0.94
1121	2,4-Octadienal	0.95 ± 0.07	1.43 ± 0.29	0.85 ± 0.12	0.98 ± 0.01	1.27 ± 0.31
1201	2,6-Nonadienal	1.03 ± 0.15	1.25 ± 0.30	0.92 ± 0.18	0.94 ± 0.05	1.17 ± 0.02
1215	2-Nonenal	1.55 ± 0.43	1.25 ± 0.12	1.08 ± 0.17	1.27 ± 0.06	0.90 ± 0.12
1257	1-(2-Butixyethoxy)-ethanol	3.69 ± 1.78	3.29 ± 1.64	3.11 ± 0.46	2.07 ± 0.43	2.76 ± 0.41
1367	2-Decenal	1.79 ± 0.80	0.96 ± 0.19	0.95 ± 0.21	1.37 ± 0.16	0.89 ± 0.19
1374	1,4-Octadiene	0.74 ± 0.08	0.65 ± 0.13	0.55 ± 0.06	0.77 ± 0.01	0.76 ± 0.13
1406	2tr,4c-Decadienal	1.07 ± 0.29	0.91 ± 0.18	0.82 ± 0.25	1.33 ± 0.10	1.05 ± 0.11
1432	2tr,4tr-Decadienal	2.28 ± 0.49	2.69 ± 0.42	2.66 ± 0.55	2.73 ± 0.58	2.36 ± 0.53
1476	2-Undecenal	1.14 ± 0.31	1.24 ± 0.12	0.95 ± 0.22	1.50 ± 0.04	1.35 ± 0.35
1700	Heptadecane	0.66 ± 0.23	0.84 ± 0.04	0.81 ± 0.15	2.81 ± 2.05	1.72 ± 0.78
1702	Pristane	2.09 ± 0.86	2.05 ± 0.40	1.81 ± 0.65	2.80 ± 0.88	4.02 ± 1.10
1755	Tetradecanal	3.83 ± 0.56	3.16 ± 0.89	2.25 ± 0.96	3.84 ± 0.35	4.37 ± 1.40
1828	9-Octadecanal	2.19 ± 0.33	2.82 ± 1.46	3.37 ± 0.65	4.49 ± 0.26	3.25 ± 0.42
	$\sum n-3$ Derived aldehydes[b]	11.0 ± 1.40	15.0 ± 1.43	13.7 ± 0.27	13.8 ± 0.45	15.4 ± 2.00
	$\sum n-6$ Derived aldehydes[c]	20.6 ± 2.06[b]	16.4 ± 0.43[ab]	17.5 ± 2.17[ab]	16.1 ± 1.15[ab]	12.0 ± 0.47[a]

Means within rows without superscript or with the same superscript are not significantly ($P > 0.05$) different from each other by one-way ANOVA and S–N–K comparison test.

[a] RI: Kovàts retention indices (Castello, 1999) for DB-5MS capillary column.

[b] $\sum n-3$-derived aldehydes: sum of 2-pentenal, 2-hexenal, 2tr,4c-heptadienal, 2tr,4tr-heptadienal and 2,6-nonadienal.

[c] $\sum n-6$-derived aldehydes: sum of hexanal, 2-octenal, 2-decenal, 2tr,4c-decadienal and 2tr,4tr-decadienal.

(mg g^{-1} of diet) and $n-6$ PUFA (mg g^{-1} of diet) of the experimental diets, respectively. Moreover, linear regression accurately described the relationship between $\sum n-6$ derivate aldehydes (Y) of the fillet and $n-6$ PUFA (mg g^{-1} of diet) of the diet (X), and the statistical relationship was described by the following equation: $Y = 0.2129\ X + 9.7374$; $R^2 = 0.73$, $P < 0.05$, with a slope significantly different from zero.

3.5. Free malondialdehyde

At day 0, the MDA values (nmol g^{-1} of fillet) were significantly ($P < 0.05$) higher in the fillet of fish fed 100SO and 50LO (Table 8). After 3 days of storage at 4 °C, the highest oxidation rate was recorded for the fillets of fish fed 100SO. After 6 days, the MDA value was lowest for the fillets of fish fed 75LO. Independent of dietary treatments, the recorded MDA values were always significantly higher ($P < 0.05$) in the fillets stored for 6 days at 4 °C with respect to the fillets stored for only 0 and 3 days.

3.6. Sensory analysis

The results of the sensory analysis performed on fresh samples, stored refrigerated for 2 days after culling, are shown in Table 9. Among the values recorded for the eight

Table 8
Free malondialdehyde values (nmol g^{-1} of fillet) for tench fillets stored at 4 °C for different lengths of time (means ± SEM; $N = 3$ tanks/treatment; two fillets from different fish in each tank for each length of storing time)

	Diet				
	100SO	25LO	50LO	75LO	100LO
Day 0	$6.6 \pm 0.4^{b,A}$	$4.5 \pm 0.3^{a,A}$	$5.8 \pm 0.3^{b,A}$	$4.3 \pm 0.3^{a,A}$	$4.0 \pm 0.2^{a,A}$
Day 3	$7.7 \pm 0.4^{b,A}$	$4.5 \pm 0.3^{a,A}$	$5.3 \pm 0.2^{a,A}$	$5.1 \pm 0.2^{a,A}$	$4.6 \pm 0.2^{a,A}$
Day 6	$9.5 \pm 0.6^{b,B}$	$8.4 \pm 0.4^{a,B}$	$12.4 \pm 2.3^{b,B}$	$8.0 \pm 0.5^{a,B}$	$12.6 \pm 1.3^{b,B}$

Values in the same row that are not followed by the same superscript letter (a,b) are significantly different ($P < 0.05$) by one-way ANOVA and S–N–K comparison test.
Values in the same column that are not followed by the same superscript letter (A,B) are significantly different ($P < 0.05$) by one-way ANOVA and S–N–K comparison test.

Table 9
Sensory characteristics of fillets of tench fed the experimental diets (means ± SEM)

	Diet				
	100SO	25LO	50LO	75LO	100LO
Total odour	6.3 ± 0.3	6.2 ± 0.1	6.2 ± 0.2	6.2 ± 0.1	6.0 ± 0.1
Off-flavour	3.9 ± 0.6^{b}	2.5 ± 0.5^{a}	1.8 ± 0.4^{a}	1.6 ± 0.1^{a}	1.9 ± 0.6^{a}
Salty taste	3.4 ± 0.3	3.1 ± 0.1	3.2 ± 0.2	3.0 ± 0.2	3.2 ± 0.1
Total flavour	5.7 ± 0.1	5.1 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.4 ± 0.1
Fatty flavour	2.4 ± 0.1	2.3 ± 0.1	2.6 ± 0.4	2.7 ± 0.1	2.1 ± 0.1
Juiciness	4.5 ± 0.1	4.1 ± 0.5	4.0 ± 0.2	4.3 ± 0.1	4.1 ± 0.2
Firmness	4.4 ± 0.3	4.6 ± 0.2	4.5 ± 0.2	4.5 ± 0.2	4.8 ± 0.1
Bitter taste	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1

Scores are on a scale of 0–9 (increasing intensity).
Means within rows without superscript or with the same superscript are not significantly ($P > 0.05$) different from each other by one-way ANOVA and S–N–K comparison test.

attributes agreed by the assessors, only the "off-flavour" showed a significant ($P < 0.05$) difference. The "off-flavour" (defined as every perceptible off-flavour not related to the characteristic tench aroma, including earthy, muddy, fatty, fishy; see Table 3) of fillets of tench fed the 100SO was highest (3.9 ± 0.6; in a range form 0 to 9 where 0 is light and 9 is strong) with respect to all the other fillets of tench fed the other experimental diets. High positive correlations were observed between the values recorded by the panellists for the attribute "off-flavour" and the measured $\sum n-6$ derivate aldehydes and the $n-6$ PUFA content of the diet: $r = 0.70$ and $r = 0.81$, respectively.

4. Discussion

In the present study, tench responded well to all the experimental diets, and this, together with their high survival, strongly suggests that both the tested lipid sources did not have any adverse effect on the fish, as previously reported for other species (Hertrampf & Piedad-Pascual, 2000; Regost, Arzel, Robin, Rosenlund, & Kaushik, 2003). Admittedly the overall growth performance of tench in this experiment was relatively small but it seems at this point essential to point out that tench are well known to be a slow-growing species and, in this study, sub-adult specimens close to market size were chosen. Furthermore, the overall growth and feed utilization parameters of sub-adult tench were comparable to the values previously reported for this species (Buchtová, Svobodová, Flajšhans, & Vorlová, 2003; De Pedro et al., 2001; Rennert, Kohlmann, & Hack, 2003).

The $n-3/n-6$ ratio of the five dietary treatments was considerably variable and in any case higher in muscle than in the diets. This could indicate that a threshold level in the muscle was obtained, probably adjusted to a narrowly defined physiological level (Bell et al., 1997; Greene & Selivonchick, 1990; Turchini, Mentasti et al., 2003; Yu, Sinnhuber, & Putnam, 1977). Irrespective of dietary treatment, this minimum level seems to be 2.2. The total fatty acid composition of tench fillet was partially modified by the dietary lipid sources, as is well known for other fish species fed diverse lipid sources (Greene & Selivonchick, 1990; Guillou et al., 1995; Sargent et al., 2002; Turchini, Gunasekara et al., 2003; Turchini, Mentasti et al., 2003; Yu et al., 1977). Significant correlations, and almost perfect linear regressions, were observed between dietary and fillet LA and LnA content, between dietary LA and fillet $18{:}3n-6$ content, and between dietary LA and LnA and their contents in the TGs and PLs fractions.

Comparing the fatty acid composition of FFAs, TGs and PLs, it is important to note that the ArA/EPA/DHA ratios were: 1/5/12 for FFAs, 1/11/23 for TGs and 1/2/18 for PLs, and it is evident that, irrespective of dietary treatment, (i) FFAs accounted for the 8.6% of the total fatty acids and were characterized by SFA, of which some short-chain fatty acids were isolated and

identified; (ii) PLs accounted for 14.5% of the total fatty acids and were characterized by PUFA, particularly by DHA and the $n-3/n-6$ ratio varied from 5.17 to 8.38; (iii) TGs reflected the total fatty acid composition of the fillets, accounting for up to 76.9% of the total fatty acids and the $n-3/n-6$ ratio varied between 1.78 and 2.71. In spite of the dietary treatment, tench fillets were characterized by a high content of $n-3$ PUFA, particularly DHA, and a low SFA/PUFA ratio. For these characteristics, tench could be considered as a high quality food for humans.

The flavour associated with freshwater fish is usually mild, delicate and pleasant and most fish have a common sweet and plant-like aroma (Turchini, Giani et al., 2004). This fresh fish flavour is due to volatile aldehydes and alcohols which are mainly derived from the oxidative deterioration of $n-3$ and $n-6$ PUFA (Durnford & Shahidi, 1998; Kawai, 1996; Prost, Sérot, & Demaimay, 1998; Turchini, Mentasti et al., 2004). The odour thresholds of aldehydes are generally lower than those of other volatile compounds (Spurvey, Pan, & Shahidi, 1998), thus they have a great potential effect on total flavour. The volatile aldehydes formed by autoxidation of $n-3$ PUFA were higher in the flesh of fish fed diets containing higher amounts of $n-3$ PUFA (i.e. 75LO and 100LO) while the volatile aldehydes formed by autoxidation of $n-6$ PUFA were higher in the flesh of fish fed with diets containing higher amounts of $n-6$ PUFA (i.e. 100SO and 25LO). The odour description of volatile aldehydes formed by autoxidation of $n-3$ PUFA, such as 2-pentenal, 2-hexenal and 2,6-nonadienal, are generally pleasant and associated with green, cucumber, apple, mushroom and grass and 2,4-heptadienal is associated with green, cucumber but also oily and fatty notes. On the other hand, the odours of volatile aldehydes derived from $n-6$ PUFA, such as hexanal, 2-octenal, 2-decenal and 2,4-decadienal, are described as tallowy, fatty, herbaceous, nutty, oily and associated with fat-fried, cod oil and oxidized oil (Belitz & Grosch, 1999; Durnford & Shahidi, 1998; Kawai, 1996; Le Guen, Prost, & Demaimay, 2000; Prost et al., 1998; Sérot et al., 2001; Spurvey et al., 1998). It is, therefore, evident that diets containing higher amounts of $n-6$ are firstly responsible for increased levels of $n-6$ PUFA in the muscle of fish and consequently are responsible for the increase of the relative amounts of $n-6$ derivate volatile aldehydes that are generally reported to contribute negatively to the global aroma of fish muscle. O'Keefe, Proudfoot, and Ackman (1995), analysing the flesh quality of chicken fed a diet rich in $n-3$ PUFA, reported the presence of an objectionable hexanal flavour in the $n-3$ PUFA-enriched chicken meat. The authors suggest that the long-chain $n-3$ PUFAs were the initial oxidation sites, but the free radicals then passed on to activate oxidation of 18:2$n-6$ in chicken flesh to produce hexanal. In the present study the reverse situation is apparent: long-chain $n-3$ PUFA is usually abundant in fish flesh and the increased level of 18:2$n-6$, in the fillet of fish fed vegetable oil, may result in the same unpleasant effect.

The results from the chemical characterization of the flavour volatile compounds were in accordance and confirmed by the results of the sensory analysis where the value for the attribute "off-flavour" (described as earthy, muddy, fatty and fishy) was highest for fish fed the diet containing only SO. High positive correlations were observed among the values recorded for this latter parameter and the measured $\sum n-6$ derivate aldehydes, the $n-6$ PUFA content of the fillet and also the $n-6$ PUFA of the diet. Consistent with these general results, Grigorakis, Taylor, and Alexis (2003) showed that the differences in the fatty acid composition of wild and farmed sea bream (*Sparus aurata*) were responsible of different volatile compound profiles and, ultimately, different sensory characteristics of the fish fillet.

In developed countries, aquaculture products are generally available on the market after 1 or 2 days post-harvesting. A few days of domestic refrigeration prior to consumption is also a common practice and therefore, during this time, there is the possibility of a limited but unavoidable activation of lipid oxidation. Lipid oxidation in tench fillets was tentatively estimated by quantification of the free MDA instead of the classical TBARS test (thiobarbituric acid-reactive substances) because of the reported limitations of the TBARS test (Mensink, Temm, & Plat, 1998; Spanier, Vinyard, Bett, & St. Angelo, 1998). Surprisingly, even though it is well known that the rates of autoxidation of LA and LnA, based on their rates of oxygen uptake, are in the order of 1:2 (Sargent et al., 2002) and that MDA is preferentially formed by autoxidation of fatty acids with three or more double bonds (Belitz & Grosch, 1999), the highest amounts of MDA were recorded, at zero and 3 days of storage time, in the fillets of fish fed the diet highest in LA (100SO). After six days at 4 °C, significantly higher amounts of MDA were recorded for the fillets of tench fed the diets 100SO, 50LO and 100LO. Admittedly the two tested oils and the experimental diets were not analysed for their degree of oxidation or for their contents of antioxidant nutrients, such as tocopherols, phenolics, flavonoids, carotenoids and other synergistic antioxidants (Reische, Lillard, & Eitenmiller, 1998; Sargent et al., 2002), which could represent useful information. MDA is just one of the decomposition products of lipid peroxides and the quantification of free MDA seemed to be, in this study, not indicative of the general oxidative state of fish flesh and this suggests that other techniques should be used for assessing lipid oxidation in the muscle of fish fed diets containing different lipid sources (Shahidi, 1998).

5. Conclusions

The present study showed that dietary lipid sources may affect some aspects of final quality of the tench. Particularly, diets rich in $n-6$ PUFA are responsible, not only for increased content of $n-6$ PUFA in the fillets, but also for modification of the flavour volatile compositions and the sensory characteristics of farmed tench. However, independently of the dietary treatment and in consideration of

the high content of $n-3$ PUFA in the fillet, tench could be considered as a high quality food for humans. The effect of dietary lipid sources, particularly linseed oil, on lipid oxidation during storage in the fillets of farmed fish needs further investigation.

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