Discrimination of origin of farmed trout by means of biometrical parameters, fillet composition and flavor volatile compounds

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ABSTRACT

To date it is well known that the quality of farmed trout is affected by diet composition, by feeding regime, by husbandry practices and by rearing conditions and environment. The trout processing industry and the large-scale retail trade, in consideration of the wide variability of trout quality and characteristics, have imposed, or will soon impose, quality criteria for the end product. Moreover, recent food scares and the malpractices of some food producers have increased public requests for traceability. The aim of the present study was to evaluate the main chemical quality and the biometrical characteristics of rainbow trout produced in three different farms in Italy (two intensive farms, located one on mountain and one on plain, and an extensive farm in which fish fed only on naturally available nutrients) and to establish whether farmed trout origins could be differentiated by these parameters. Trout farmed in the intensive mountain farm (IMF) showed the highest crude lipid content in the fillets and the fatty acids of their fillets were characterized by the highest percentage of MUFA. Trout farmed in the intensive plain farm (IPF) were characterized by low dressing percentage, and the lipid of their fillets was rich in n-6 fatty acids. Trout stocked for the last year of their life in the extensive farm (EF) were leaner both in the carcass and in the fillets. The analysis of flavor volatile compounds showed some differences in the bouquet design, particularly differences in the amounts of n-3 and n-6 derivates volatile aldehydes and alcohols. All data significantly different ($P<0.05$) were subjected to Linear Discriminant Analysis (LDA) and 8 variables were chosen to create two discriminant equations generating a strong prediction model for classification of farmed trout respective to their origins.

Key words: Rainbow trout, *Oncorhynchus mykiss*, Fillet quality, Flavor volatile compounds, Linear Discriminant Analysis (LDA)

RIASSUNTO

DIFFERENZIAZIONE DELL'ORIGINE DI TROTE D'ALLEVAMENTO TRAMITE PARAMETRI BIOMETRICI, COMPOSIZIONE CHIMICA E COMPOSTI AROMATICI VOLATILI DEL FILETTO.

Oggigiorno è ben noto che la qualità delle trote di allevamento può essere influenzata dalla composizione delle diete, dal regime alimentare, dalle pratiche di allevamento e dalle condizioni e caratteristiche ambientali dell'allevamento. L'industria di trasformazione e commercializzazione, in considerazione della discreta variabilità delle principali caratteristiche qualitative del prodotto trota, hanno imposto, o ben presto imporranno, dei criteri e limiti qualitativi sul prodotto finale. Inoltre, le recenti paure alimentari e le cattive pratiche da parte di alcuni produttori, hanno incrementato la richiesta di tracciabilità da parte dei consumatori. Lo scopo del presente studio è stato quello di valutare le principali caratteristiche chimiche/qualitative del filetto e le caratteristiche biometriche di trote iridee allevate in tre differenti aziende italiane (due allevamenti intensivi, uno di montagna e l'altro di pianura, e un allevamento estensivo in cui i pesci si nutro-

no esclusivamente dei nutrienti naturalmente disponibili nel bacino) e di stabilire se l'origine di produzione delle stesse fosse differenziabile tramite l'analisi di questi parametri. Le trote allevate nell'allevamento intensivo di montagna (IMF) sono risultate essere quelle con il più alto contenuto lipidico (5.32 ±0.67%) nel filetto e il profilo acidico dei lipidi del filetto era caratterizzato dal maggiore contenuto percentuale in acidi grassi monoinsaturi (32.22±1.21%). Le trote ottenute dall'allevamento intensivo di pianura (IPF) sono state caratterizzate da una bassa resa all'eviscerazione (84.21 ±0.94%) ed i lipidi del loro filetto erano ricchi in acidi grassi del gruppo n-6 (16.38 ±2.79%). Le trote che avevano trascorso almeno l'ultimo anno della loro vita nell'allevamento estensivo (EF) sono risultate essere le più magre sia dall'analisi biometrica delle carcasse (coefficiente di grassezza 2.67 ±0.49%) sia come minor contenuto lipidico nel filetto (3.23 ±0.72%). Inoltre l'analisi delle sostanze volatili responsabili della formazione dell'aroma delle carni ha mostrato l'esistenza di alcune differenze tra le tre tipologie di campioni, particolarmente differenze nelle quantità di aldeidi e alcoli derivati dagli acidi grassi della serie n-3 e n-6. Tutti i dati statisticamente significativi (P<0.05) sono stati sottoposti all'Analisi Discriminante Lineare (LDA) e 8 variabili sono state scelte per creare due equazioni in grado di generare un modello di previsione affidabile per la classificazione dell'origine delle trote di allevamento.

Parole chiave: *Trota iridea,* Oncorhynchus mykiss*, Qualità del filetto, Composti volatili, Analisi Discriminante Lineare (LDA)*

Introduction

Global production of rainbow trout (Oncorhynchus mykiss) was estimated to be 448,142 t in 2000. Chile, Norway, Italy and France accounted for 17.8%, 10.9%, 9.9%, and 9.2%, respectively (FAO, 2001). Over the past two decades, trout diets have changed in several ways. Dietary protein and fat level have increased and trout diets are now produced mainly by cooking extrusion (Hardy, 2002). High-energy diets, which may offer environmental benefits (Midlen and Redding, 1998) and protein sparing (Hardy, 1995), are therefore widely used in salmonids farming (Torstensen *et al.*, 2000; Rasmussen *et al.*, 2000a), and, at the same time, the partial replacement of fish meal and fish oil with alternative sources is generally used for cost savings (Guillou *et al.*, 1995; Turchini *et al.*, 2000; Hardy, 2002). Their use, particularly for rainbow trout (Johansson, 2001), has been associated with negative aspects related to a lower quality of the end product arising from increased lipid deposition (Rasmussen *et al.*, 2000b; Shearer, 2001) and from the change in the fatty acids composition (Steffens, 1997; Turchini *et al.*, 2003a).

Farming systems for rainbow trout are similar throughout the world. Usually fish are raised in flowing water in earthen or concrete raceways, with stocking densities depending upon water flow and water quality (Hardy, 2002). Italian intensive trout farms are distinguishable in two main different categories: the mountain farms in the Alps and in the Apennines, and the plains farms, mainly in the Padana valley in the north of Italy. Usually mountain farms are small (2 hectares) with a yearly water temperature range from 6 °C to 18 °C and with a stocking density of 15-25 kg/m^3; plain farms are larger (4-10 hectares) with a smaller yearly water temperature range from 12 °C to 18 °C and with a stocking density of 20-40 kg/m^3 (Anonymous, 1992). In both categories fish are generally fed with commercially available high-energy extruded pellets.

To date it is well known that end product quality of the trout farms is mainly affected by the diet composition and the feeding regime (Shearer, 2001), but also husbandry practices and environmental characteristics may compromise that quality (Pottinger, 2001). The European trout processing industry and the large-scale retail trade, in consideration of the wide variability of trout qualities, have imposed, or will soon impose, quality criteria for the end product (Rønsholdt and McLean, 1999; Greenhalgh, 2001). For these reasons many trout farmers have recently begun using a finishing period prior to slaughter to reduce fat content and also ameliorate the fatty acids profile (Einen *et al.*, 1998; Rasmussen *et al.*, 2000b; Regost *et al.*, 2001). Moreover, recent food scares (i.e. TSE) and the malpractices of some food producers have increased public requests for safe-

ty and the importance of the traceability issue, in fishery and aquaculture product, is dramatically increasing nowadays and there is a need for research which can deliver a product-specific and general analytical fish products traceability system (Moretti *et al.*, 2003).

The aims of the present study were to evaluate the main chemical qualities, such as proximate composition, fatty acids composition and flavor volatile compounds composition of the fillets, and the biometrical characteristics of Italian rainbow trout from three different farms (an intensive mountain farm, IMF; an intensive plain farm, IPF; and an extensive farm, EF), and to establish whether farmed trout origins could be differentiated by their basic biometrical and chemical parameters using supervised linear discriminant analysis (LDA) chemometrics. The extensive farm, in which fish feed only on naturally available nutrients for at least the last year of their life, represents a new particular farming system which produces trout sold as high quality / high value trout for restoration and is very much appreciated by consumers (Puzzi, 2002). Moreover, the extensive farm could be considered as a finishing strategy for trout previously reared in an intensive farm.

Material and methods

The extensive farm (EF)

The extensive farm (EF) (Azienda Agricola Torrettone, Truccazzano, MI, Italy) is essentially an artificial lake located in the North of Italy and stands inside the area of the "North Adda River Regional Park". The lake is a disused stone quarry filled by the ground water table and its perimeter is 1540 m and its surface is 4.6 ha. The maximum depth is 13 m, the average depth is 6.6 m and the total water volume is 306,000 m^3. The "Torrettone" lake was stocked in the sixties with different vertebrate and invertebrate preys and was for recreational fishing in the past used. To date the lake has rich and diversified fish communities and some species are perfectly acclimatized and naturally spawning, maintaining equilibrated populations. These include chub *(Leuciscus cephalus)*, bleak *(Alburnus alburnus alborella)*, "savetta" *(Chondrostoma soetta)* and Eurasian Perch *(Perca fluviatilis)*. Other fish species present, and regularly introduced in the lake, are common carp *(Cyprinus carpio)* and Siberian sturgeon *(Acipenser baeri)*. Rainbow trout *(Oncorhynchus mykiss)*, purchased from the Intensive Mountain Farm (IMF), are introduced in the lake annually at an average weight of 170-180 grams, and then trout are left to grow for one or two years until they reach an average weight of 500-600 grams. At this size trout are fished out and sold to high quality food establishments. From 1999 to 2002, the water temperature varied from a minimum of 8 °C due to the thermal water column during winter, to a maximum of 13 °C and 17 °C on the bottom and on the surface, respectively. Dissolved oxygen varied from 8 to 12 ppm on the surface to 2 to 6 ppm on the bottom of the lake, and in accordance with the Vollenweider-OECD classification system, the waters of "Torrettone" lake are classified as mesotrophic (OECD, 1982). No organic matter of any kind of organic was introduced to the lake for over four years and fish fed only on naturally available nutrients.

The intensive mountain farm (IMF)

The intensive mountain farm (Azienda Agricola Fonti del Dal, Trent, Italy) is a typical family-owned mountain trout farm located on the Alps in the northeastern Italy at 550 m above sea level. Fish are raised in flowing bore water in concrete and earthen raceways. The raceways are 50 m long,5 m wide and 0.6 m deep and the water flow rate assures complete water replacement every 3 hours. The stocking density is 25 kg/m^3 and dissolved oxygen is to saturation in the ingoing water to 70% in the effluent. The water temperature varies from a minimum of 5 °C during winter to a maximum of 11 °C during summer. Fish are manually fed twice a day, seven days a week, at 1% ratio. The feed used in the IMF was a commercial extruded pellet (Biomar, Ecolife 21, Denmark); the labeled composition, in decreasing order, was as follows: fish products, fish oil, cereals, products and waste products of oleaginous seeds, products and waste products of leguminous seeds, vitamins and minerals. The feed labeled proximate composition was: PG, 47.0 %; LG, 26.0 %; NFE, 9.8 %; fiber, 0.6 %; Ash, 9.7 %; Energy 23.0 KJ/g.

The intensive plain farm (IPF)

The intensive plain farm (SalmoPan s.r.l., Cremona, Italy) is a typical trout farm located in the Padana Valley in the North of Italy at 85 m above sea level. Fish are raised in flowing bore water in concrete raceways. The concrete raceways are 100 m long, 5 m wide and 0.5 m deep and the water flow rate assures complete water replacement every 3 hours. The stocking density is 40 kg/m^3 and liquid oxygen is added in the ingoing water to guarantee an optimal oxygen concentration always superior to 7 ppm, even in the effluent. The water temperature varies from a minimum of 12 °C during winter to a maximum of 16 °C during summer. Fish are fed twice a day, seven days a week, at 1% ratio. Feed is distributed in the raceways using automated feeders that throw 15 g of feed every 30 seconds. The feed used in the IPF was a commercial extruded pellet (Hendrix, Selected BE, Norway). The labeled composition, in decreasing order, was as follows: fish products, products and waste products of oleaginous seeds, oils and fats, cereals, products and waste products of cereals seeds, vitamins and minerals. The feed labeled proximate composition was: PG, 40.0 %; LG, 21.0 %; fiber, 2.0 %; Ash, 8.4 %; Energy 21.5 KJ/g.

Animals and samplings

The bigger trout produced and marketed by the three farms were of a fairly different mean size, varying from the 500 to 800 g of total weight and from 350 to 390 mm of total length. Even if the average size of the samples were not very homogeneous, we opted to choose trout at the size at which they are usually sold by the three farms. In this way it was possible to estimate the characteristics of fish that the consumers could really find on the market. Nine fish from each farm were culled for analysis. Fish were sampled in different times of the year, from January to September, but each sampling was contemporaneous in each farm. Fish were killed by immersion in ice slurry, then refrigerated and immediately transported to the University facilities for analysis. In the same day of the sampling fish were analyzed for biometrical parameters as below; then each trout fillet was vacuum-sealed packed, deep frozen and stored at -80 °C until analysis.

Biometric parameters calculations

Each fish was wiped with a dry cloth and weighed to the nearest 0.001g (TW, total weight) and total length (TL) was measured. The total offal was accurately removed and weighed. Afterwards the liver and the abdominal fat were both carefully isolated and individually weighed (AFW, abdominal fat weight; LW, Liver weight). The gutted carcass was then weighed (CW, gutted carcass weight), then the fish was skinned and all the edible muscle was attentively recovered and weighed (FW, weight of both fillets). The calculations for biometrical parameters were: condition factor, $K=(10^5 \times TW)/TL^3$, (g/mm); Hepato Somatic Index, $HSI=(LW/TW)\times 100$; Dressing percentage, $DP=(CW/TW)\times 100$; Fillet Yield, $FY=(FW/TW)\times 100$, Coefficient of Fatness, $C.Fat.=(AFW/TW)\times 100$.

Chemical and fatty acid analysis

The fillets of nine trout for each sampling were analyzed for moisture, protein, lipid and ash using standard methods (AOAC, 1996); briefly, moisture was determined by heating at 80 °C to constant weight, protein by estimating the Kjeldahl nitrogen (× 6.25) in an automated distillation unit (Büchi 339, Switzerland), lipid by chloroform/methanol extraction (Bligh and Dyer, 1959), ash by incinerating in a muffle furnace at 550 °C for 18 h. After the extraction of total lipids, the preparation of fatty acid methyl esters, for fatty acid analysis, was performed according to Christie (1982). Fatty acid analysis was carried out on an Agilent gas-chromatograph (Model 6890) fitted with an automatic sampler (Model 7683) and FID detector. The conditions used were the following: HP-Innowax fused silica capillary column (30 m x 0.25 mm I.D., 0.30 μm film thickness; Agilent Technologies), temperature programmed from 150°C to 180 °C at 3°C/min, then from 180°C to 250°C at 2.5 °C/min, held for 10 min. Carrier gas was helium at 1.0 ml/min, inlet pressure 16.9 psi. Fatty acids were identified relative to known external standards. All analyses were done in duplicate. The index of atherogenicity was calculated with the following equation, as proposed by Ulbricht and Southgate (1991) taking into account the different effect of different fatty acids on human health:

Index of atherogenicity (IA)

IA = [(12:0) +(4 × 14:0)+ (16:0)] × [(PUFA n-6 and n-3) + MUFA)]$^{-1}$

Volatile compounds analysis

The volatile compounds analysis was performed as previously described (Mentasti *et al.*, 1997) on nine right fillets for each trout sampling. Ten grams of accurately 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 2 hours. A 10 μl solution of undecane (2 mg ml^{-1}) was added as internal standard. All reagents and solvents were from Merk (Darmstadt, Germany). Compounds were analyzed in an Agilent 6890 Series GC system coupled to a 5973N mass selective detector. The separation was performed on a DB-5MS capillary column (30 m x 0.25 mm I.D.., 0.25μm film thickness) (Supelco, Bellefonte, PA, USA). The carrier gas was helium with a linear flow rate of 1 ml min^{-1}. The oven temperature program was: 35 °C held for 1 minute, from 35 °C to 60 °C at 120 °C min^{-1}, from 60 °C to 100 °C at 1.5 °C min^{-1}, then from 100 °C to 280 °C at 5 °C min-1. Samples of 1 μl were injected in pulsed splitless mode (purge flow 20 ml min^{-1} at 0.9 min). Mass spectra were obtained under EI condition at 70 eV in the 35-300 amu range. Ion source was held at 230 °C and quadrupole at 150 °C. Identification of compounds was based on mass spectra from library database (NIST 98, WILEY 275) and comparing GC retention times and retention index with those of known standards. The retention index (RI) proposed by Kovàtz was calculated for comparison of retention data from literature (Castello, 1999). The data were recorded and analyzed with the HP Chemstation Software.

Statistical analysis

Data are reported as mean values ± standard error of mean (s.e.); N=9. Homogeneity of variance was confirmed and comparison between means was by one-way ANOVA. Student-Newman-Keuls was used as post hoc test for comparison of the means among different sampling. Significance was accepted at probabilities of 0.05 or less. All variables found to be significant at the 95 % confidence level in the ANOVA were used for origin recognition of farmed trout by Linear Discriminant Analysis (LDA). The supervised LDA was applied to classify the samples in clusters according their variety and to identify variables important to the distinguishing of trout origins. Linear discriminant analysis is a statistical method used to distinguish in groups a collection of objects, having a set of cases whose group membership is known a priori (Giansante *et al.*, 2003). All variables found to be significant (P<0.05) in the ANOVA were used for LDA computation (Aursand *et al.*, 2000) and selected variables were firstly transformed into natural logarithms, in such a way to have a normal data distribution. In the LDA the algorithm chosen to select the variables was stepwise selection, which combines forward selection and backward elimination using the minimization of Wilks' lambda. Wilks' lambda is a measure of a variable's potential and smaller values indicate the variable is better at discriminating between groups. The F significance level was chosen as the variable entry (less than 0.05) and removal (greater than 0.10) criterion. The number of functions obtained by LDA is equal to the number –1. So the two discriminant equations (LDA1 and LDA2), which are a linear combination of the independent variables selected by the stepwise method, are expressed as:

$$D_j = B_{0j} + B_{1j}X_1 + \ldots + B_{nj}X_n$$

Where Dj is the discriminant score (j=1,...,m-1, where m is the number of groups), B_{0j} is a constant term and B_{ij} and X_i (i=1,...,n) are respectively the coefficients estimated from the data and the values of each independent variable chosen by stepwise LDA.

LDA were performed four times on four different datasets obtained i) by simple biometric parameters and fillet proximate composition (dataset A), ii) by only the flavor volatile compounds (dataset B), iii) by only the fatty acid composition (dataset C), iv) all the available data (dataset D), to evaluate if one of this group of variables could be used to effectively discriminate

between groups. Finally a cross-validated test was used to validate the accuracy of the LDA classifications. All the statistical analysis were performed by SPSS 11.5 (SPSS Inc. Chicago, Illinois).

Results and discussion

Biometric parameters

Total weight and total length values of the sampled trout were significantly different (Table 1). IMF trout showed the highest total weight, but IMF trout length and EF trout length were statistically not different. IPF trout were the shortest but their total weight was not different from the EF trout weight. Therefore, it seems possible to assert that the sampled trout, even if some differences in total weight and total length were noted, could be comparable and registered differences are just ascribable to differences in the general fish shape. This is, of course, reflected in the wide variability in the condition factor (k) values, varying from 1.30±0.04 to 1.04±0.06 for IMF and EF trout, respectively. The condition factor previously reported for adult rainbow trout by Chaiyapechara *et al.* (2003) fed, to apparent satiation, 15% or 30% lipid diets were higher (from 1.40 to 1.46) with respect to the data recorded for the sampled trout. The condition factor reflects the nutritional state of an individual fish (Busacker *et al.* 1990) and, also in the consideration that no differences were noted for the hepatosomatic index (HSI), it seems that all the studied farms did not exceed in feeding. In any case, observing the abdominal fat weight, the dressing percentage (DP) and the coefficient of fatness (C.Fat) of trout from intensive farms (IMF and IPF), and considering that both farms fed their fish at the 1% ratio seven days a week but that feed used in IMF was higher in total lipid and energy, it also seems possible to assert that IMF trout could have been overfed. The highest carcass weight was recorded for IMF trout (675.37 ±57.87 g), while the highest total offal weights were for IMF and IPF trout (102.98 ±8.72 and 109.23 ±34.22 g, respectively).

Table 1. The biometric parameters of trout reared in the three different farms: the intensive mountain farm (IMF); the intensive plain farm (IPF) and the extensive farm (EF).

		Culture Systems		
		IMF	IPF	EF
Total weight	g	791.17^{b} ±66.08	498.00^{a} ±61.42	580.78^{a} ±41.47
Total Length	mm	391.33^{b} ±11.37	347.44^{a} ±13.04	381.00^{b} ±6.46
Offal weight	g	102.98^{b} ±8.72	109.23^{b} ±34.22	51.27^{a} ±6.48
Carcass weight	"	675.37^{b} ±57.87	415.59^{a} ±47.36	519.40^{a} ±35.70
Liver weight	"	10.43^{b} ±1.04	6.72^{a} ±0.85	6.81^{a} ±0.58
Abdominal fat weight	"	48.82^{b} ±4.71	28.28^{a} ±4.54	16.61^{a} ±3.53
Fillets weight	"	395.22^{b} ±38.51	234.56^{a} ±32.19	283.40^{a} ±28.82
K[1]		1.30^{b} ±0.04	1.14^{a} ±0.03	1.04^{a} ±0.06
HSI[2]		1.32 ±0.07	1.37 ±0.08	1.17 ±0.06
DP[3]		85.20^{a} ±0.56	84.21^{a} ±0.94	89.60^{b} ±0.49
FY[4]		49.53 ±1.43	46.64 ±1.03	48.02 ±1.99
C.Fat [5]		6.26^{b} ±0.45	5.46^{b} ±0.41	2.67^{a} ±0.49

Means within rows without superscript or with the same superscript are not significantly (P> 0.05) different from each other.

[1] *K: Condition factor*

[2] *HSI: Hepato somatic index*

[3] *DP: Dressing percentage*

[4] *FY: Fillet Yield*

[5] *C.Fat: Coefficient of fatness*

Dressing percentage and the coefficient of fatness were significantly higher and lower (89.60±0.49% and 2.67 ±0.49%, respectively) for EF trout with respect to IMF and IPF trout. These could be considered as indicators of a superior quality of EF trout (Sinnott, 2001), but it also seems interesting to point out that the higher (not significant) fillet yield (FY) was noted for IMF trout.

Proximate composition

All the proximate composition values recorded for the fillets of different trout samples were significantly different (Table 2), but in any case within the normal ranges of variability reported for the edible portion of rainbow trout (Souci *et al.*, 1994). It is worth noting that sampled trout had different carcass weights and some parameters could have been affected by that. Moisture percentage was highest for EF trout (75.34 ±0.83%), protein percentage was highest for IMF and IPF trout (20.67 ±0.27% and 20.06±0.23 %, respectively) and ash content was highest (1.60 ±0.04) for IMF trout. Fillets lipid content was lowest for EF trout (3.23 ±0.72%), intermediate for IPF trout (4.21 ±0.51%) and highest for IMF trout (5.32 ±0.67%). The protein content is considered to be pre-determined by the genetic characteristics of the species (Shearer, 1994) and unaffected by the diet (Morris, 2001) and therefore the differences noted in crude protein content of the fillets are likely due to the combined effect of changes in the fat and moisture percentage of the fillets (Shearer, 2001). Generally the moisture content declines in response to increased fat in the fillets (Jobling *et al.*, 1998); so it seems possible to argue that the real differences between the three fillets were mainly due to the variability in the fat content. IMF trout fillets were fatter while EF trout fillets were leaner, and then preferable from the general consumer point of view even if generally the fat contained in fish fillet has high nutritional qualities. IMF trout were also biggest and usually lipid content of the fillet increase with size, but IPF and EF trout did not show significant differences in size and therefore the extensive farming seems extremely effective in total fat containment, probably due to the high energy requirements for active swimming and for catching prey.

Fatty acid composition

The fatty acid composition (% on total fatty acids) of fillets of trout reared in the three different culture systems is reported in Table 3. No significant differences between samplings were noted for C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C20:4n-3, C22:6n-3 (docosahexaenoic acid, DHA), total saturated fatty acids (ΣSFA), the sum of all the n-9 fatty acids (Σn-9), the sum of all the n-3 fatty acids (Σn-3) and also the n-3/n-6 ratio. All the other fatty acids and fatty acid factors of the trout fillets were different between samples and in particular this could be ascribable, as well known from literature, to the different diets (Steffens, 1997). Total amount of SFA was unaffected by sample origin and this could indicate that the level of SFA in the muscle has a limited potential range, as previously shown for fish fed a diet containing different lipid sources (Turchini *et al.*, 2003a; b).

The fatty acids found in highest percentage in

Table 2. The proximate composition of fillets of trout reared in the three different farms: the intensive mountain farm (IMF); the intensive plain farm (IPF) and the extensive farm (EF).

		Culture Systems		
		IMF	IPF	EF
Moisture	%	71.57^{a} ±0.67	73.44^{ab} ±0.57	75.34^{b} ±0.83
C. Protein	"	20.67^{b} ±0.27	20.06^{ab} ±0.23	19.66^{a} ±0.22
C. Lipid	"	5.32^{c} ±0.67	4.21^{b} ±0.51	3.23^{a} ±0.72
Ash	"	1.60^{c} ±0.04	1.44^{b} ±0.04	1.28^{a} ±0.02

Means within rows without superscript or with the same superscript are not significantly (P> 0.05) different from each other.

Table 3. The fatty acid composition (% on total fatty acids) of fillets of trout reared in the three different farms.

	Culture Systems		
	IMF	IPF	EF
C12:0	0.05^{b} ±0.01	0.04^{ab} ±0.01	0.03^{a} ±0.01
C14:0	3.97^{b} ±0.21	2.84^{b} ±0.16	2.58^{a} ±0.21
C15:0	0.37^{a} ±0.01	0.33^{a} ±0.02	0.20^{b} ±0.05
C16:0	17.36^{b} ±0.20	16.80^{ab} ±0.35	16.25^{a} ±0.30
C17:0	0.20^{ab} ±0.02	0.26^{b} ±0.03	0.14^{a} ±0.04
C18:0	3.71^{a} ±0.10	4.39^{ab} ±0.06	4.99^{b} ±0.43
Σ SFA	25.67 ±0.30	24.66 ±0.51	24.18 ±0.44
C16:1n-7	5.87^{b} ±0.29	3.89^{a} ±0.32	3.69^{a} ±0.33
C18:1n-7	2.78^{b} ±0.13	2.42^{ab} ±0.15	2.09^{a} ±0.11
C18:1n-9	14.89^{ab} ±0.46	16.82^{b} ±0.62	14.32^{a} ±0.89
C20:1n-9	4.42^{b} ±0.23	2.67^{a} ±0.23	2.33^{a} ±0.33
C22:1n-9	0.49^{b} ±0.04	0.28^{a} ±0.02	0.22^{a} ±0.06
C22:1n-11	3.77^{b} ±0.18	1.86^{a} ±0.25	2.12^{a} ±0.19
Σ n-7	*8.65^{b} ±0.40*	*6.32^{a} ±0.47*	*5.77^{a} ±0.44*
Σ n-9	*19.80 ±0.65*	*19.77 ±0.71*	*16.88 ±1.10*
Σ MUFA	32.22^{b} ±1.21	27.94^{a} ±1.14	24.77^{a} ±1.69
C18:2n-6	7.68^{a} ±0.53	14.51^{b} ±2.69	14.57^{b} ±1.25
C18:3n-6	0.12 ±0.02	0.18 ±0.03	0.11 ±0.04
C20:2n-6	0.49 ±0.02	0.65 ±0.08	0.47 ±0.12
C20:3n-6	0.18 ±0.02	0.34 ±0.05	0.24 ±0.06
C20:4n-6	0.60 ±0.02	0.71 ±0.05	0.82 ±0.10
C18:3n-3	1.41^{a} ±0.07	2.22^{b} ±0.25	1.90^{ab} ±0.16
C18:4n-3	1.58^{b} ±0.06	1.03^{a} ±0.04	1.04^{a} ±0.11
C20:4n-3	0.13 ±0.02	0.12 ±0.03	0.09 ±0.03
C20:5n-3	7.28^{c} ±0.14	5.10^{a} ±0.34	6.14^{b} ±0.40
C22:5n-3	2.29^{b} ±0.03	1.87^{a} ±0.18	2.18^{ab} ±0.09
C22:6n-3	20.34 ±1.21	20.68 ±1.42	23.48 ±2.43
Σ n-6	9.08^{a} ±0.54	16.38^{b} ±2.79	16.21^{b} ±1.36
Σ n-3	33.04 ±1.20	31.02 ±1.65	34.84 ±2.52
Σ PUFA	42.11^{a} ±1.29	47.40^{b} ±1.47	51.05^{b} ±1.30
n3/n6	3.76 ±0.30	2.56 ±0.50	2.40 ±0.38
IA[1]	0.45^{b} ±0.01	0.37^{a} ±0.01	0.35^{a} ±0.01

Means within rows without superscript or with the same superscript are not significantly (P> 0.05) different from each other.
[1]*IA : index of atherogenicity*

trout fillets, irrespective of the different origins, were palmitic acid (C16:0), oleic acid (C18:1n-9; OLA), linoleic acid (C18:2n-6; LA), eicosapentaenoic acid (C20:5n-3; EPA) and docosahexaenoic acid (C22:6n-3), as previously reported for rainbow trout (Souci *et al.*, 1994) and also for other carnivorous freshwater fish (Turchini *et al.*, 2003a; b.). C16:0 and C18:1n-9 were highest in IMF and IPF trout fillets, 17.36 ±0.20% and 16.82 ±0.62%, respectively. The lowest values of total monounsaturated fatty acids (ΣMUFA; 24.77 ±1.69% and 27.94 ±1.14%) were recorded in the

fillets of EF and IPF trout, respectively. High levels of OLA, LA and ΣMUFA in the fish fillet are usually due to the supply of feed containing vegetable oil (Hertrampf and Piedad-Pascual, 2000; Caballero *et al.*, 2002; Turchini *et al.*, 2003a). The amount of OLA and ΣMUFA were higher for trout cultured in the two intensive farms (IMF and IPF) and then fed commercial extruded pellet containing vegetable oil, with respect to trout reared in the extensive farm (EF), where fish fed only on available vertebrate and invertebrate preys. The same differences were noted between cultured and wild fish (Sheikh-Eldin *et al.*, 1996; Ahlgren *et al.*, 1999). Surprisingly, the fillets of the IMF trout showed a very low content of LA, while showing the highest value of eicosapentaenoic acid (EPA; C20:5n-3), 7.68 ±0.53% and 7.28 ±0.14%, respectively. The fillets of the EF trout showed the highest amount of total polyunsaturated fatty acids (ΣPUFA; 51.05 ±1.30%).

No significant differences were noted for the percentage of Σn-3 between trout fillets, but in consideration of the different total lipid amount of the fillets, the IMF trout seems preferable from a nutritional point of view for humans because their flesh contained the highest amount of Σn-3 (Connor, 2000). Moreover, the significant lower percentage of Σn-6 of IMF trout fillets, and the consequent highest (not significant) n3/n6 ratio, makes the IMF trout fillets preferable for this peculiarity of fish meat (Pike, 1999) that could help in increasing the total n3/n6 ratio of the human food. On the other hand, the relatively higher amount of ΣSFA and the lower amount of ΣPUFA registered for the IMF trout fillets have contributed in obtaining the statistically highest value (worst for human health) of the index of atherogenicity (IA) for this samples (Ulbricht and Southgate, 1991).

Volatile compounds isolated and identified

The results of volatile compounds analysis (Table 4) showed that the major classes of compounds isolated and identified in the fillets of trout reared in the three different culture systems were aldehydes, alcohols, ketones and hydrocarbons. The volatile compounds class found in highest percentage in trout fillets, irrespective of the different origins, was the sum of all aldehydes; varying from 66.8 ±1.6% to 72.5±2.6% of total volatile compounds for IMF and EF trout, respectively. The sum of total alcohols isolated in fillets of EF trout was significantly lower while the sum of total ketones was significantly higher for fillets of IPF trout. A total of 38 compounds were isolated and identified in the trout fillet. The most frequently cited drawback inherent in SDE is the possible presence of artifacts in its extract, mainly due to oxidation and thermal reactions but, in a recent and exhaustive review, Chaintreau (2001) underlined that SDE still remains an irreplaceable and trustworthy isolation method for recovering volatile from a matrix. Moreover, trout are usually consumed after cooking and the SDE extraction technique could simulate a boiling process.

The higher (not significant) value of the sum of total aldehydes was recorded in EF trout fillets and interestingly the same fillets showed the high-

Table 4. The volatile compounds classes isolated and identified in rainbow trout fillets (% on total volatile compounds) of trout reared in the three different farms.

	Culture Systems		
Volatile Compounds Classes	IMF	IPF	EF
∑ aldehydes	66.8 ±1.6	70.5 ±0.9	72.5 ±2.6
∑ alcohols	3.9^{b} ±0.3	3.6^{b} ±0.1	2.8^{a} ±0.1
∑ ketones	8.4^{a} ±0.4	9.2^{b} ±0.4	7.2^{a} ±0.5
∑ hydrocarbons	16.4 ±2.4	12.7 ±1.4	13.7 ±3.3
∑ other compounds	4.6 ±0.5	4.0 ±0.2	3.8 ±0.1

Means within rows without superscript or with the same superscript are not significantly (P> 0.05) different from each other.

er percentage value of n-3 fatty acids. A direct correlation between n-3 fatty acids and total volatile aldehydes was previously shown in fillets of brown trout *(Salmo trutta)* fed a diet containing different lipid sources by Turchini *et al.* (2003c). A total of 18 volatile aldehydes were isolated and identified in all the samples (Table 5) and the odor thresholds of aldehydes are generally low (Spurvey *et al.*, 1998), therefore they have a great potential effect on total fish flavor. The typical fresh fish flavor is mainly due to volatile aldehydes which are commonly derived from the PUFA degradations via specific lipoxygenase activity or thermal degradation (Durnford and Shahidi, 1998).

As regards the aldehydes generally attributed to n-3 PUFA oxidation, it was possible to isolate and identify in all the samples a major compound such as 2,4-heptadienal and three minor compounds such as 2-hexenal, 2,4 octadienal and 2,6-nonadienal. 2,4-Heptadienal was the aldehyde found in greatest percentage in all the samples and it was significantly higher (19.9 ±0.8%) for IMF trout fillets. 2,4-Heptadienal is well reported to be generated during the DHA and other n-3 fatty acids autoxidation process (Belitz and Grosch, 1987; Durnford and Shahidi, 1998) and it was reported to have an oxidized, cod liver oil, but also green, cucumber-like odors (Ólafsdòttir *et al.*, 1997). In addition, 2,6-Nonadienal, associated with a typical green and cucumber odor, was previously reported as an important compound responsible for the aroma of food because of its very low threshold (0.01-1 ppb) (Leffingwell and Leffingwell, 1991; Piveteau *et al.*, 2000) and 2,4-Octadienal was described as responsible for a potent aroma in fresh fish such as anchovy (*Engraulis encrasicholis* L.) and turbot (*Scophtalamus maximus*) with deep-fried fat and pine/resin attributes (Prost *et al.*,

Table 5. Volatile aldehydes isolated and identified in rainbow trout fillets (% on total volatile compounds) of trout reared in the three different farms.

		Culture Systems			
RI[1]	Volatile aldehydes	IMF	IPF	EF	Odor description[2]
769	2-Pentenal	4.8^{b} ±0.2	3.7^{a} ±0.2	3.7^{a} ±0.2	green, grass, tomato
805	Hexanal	6.2^{a} ±0.4	10.9^{b} ±1.1	11.5^{b} ±2.0	herbaceous, cut grass, oxidized
854	2-Hexenal	5.1^{b} ±0.4	4.8^{ab} ±0.2	4.1^{a} ±0.2	moss, mushroom
898	4-Heptenal	4.2^{b} ±0.7	3.3^{ab} ±0.3	2.2^{a} ±0.2	powerful green, biscuit
901	1-Heptenal	1.7 ±0.3	1.9 ±0.3	2.6 ±0.2	oily, fatty
957	2-Heptenal	0.9^{a} ±0.1	1.2^{b} ±0.1	1.4^{b} ±0.1	cooked fish, sulphuric, soapy
962	Benzaldehyde	1.6^{b} ±0.1	1.4^{ab} ±0.1	1.2^{a} ±0.1	candy, sweet, almond
1005	Octanal	1.4 ±0.1	1.5 ±0.1	1.5 ±0.1	soapy, oily, fatty
1013	2,4-Heptadienal	19.9^{b} ±0.8	15.9^{a} ±0.7	14.8^{a} ±1.1	green, cucumber, oxidized
1059	2-Octenal	1.8^{a} ±0.1	2.5^{b} ±0.2	2.3^{b} ±0.1	oily, green leaf
1107	Nonanal	3.2^{a} ±0.2	3.5^{a} ±0.3	5.4^{b} ±0.5	floral, waxy, soapy, tallowy
1116	2,4-Octadienal	2.3^{b} ±0.1	1.8^{a} ±0.1	1.7^{a} ±0.1	pine, resin, cucumber
1193	2,6-Nonadienal	2.7 ±0.1	2.7 ±0.2	2.4 ±0.1	green, cucumber
1206	4-Ethil-benzaldehyde	1.4 ±0.1	1.2 ±0.1	1.4 ±0.1	sweet, minty, anisy
1358	2-Decenal	0.5 ±0.1	0.6 ±0.1	0.6 ±0.1	orange, fatty fried, tallowy
1408	2,4-Decadienal	7.6^{a} ±0.3	11.9^{b} ±0.9	12.7^{b} ±1.3	green, fat fried, cod oil
1438	2-Undecenal	0.8 ±0.1	1.0 ±0.2	1.3 ±0.2	tallowy, sweet
1791	Tetradecanal	0.7 ±0.2	0.7 ±0.2	1.6 ±0.4	-

Means within rows without superscript or with the same superscript are not significantly (P> 0.05) different from each other.

[1] RI: Kovàts retention indeces (Castello, 1999) for MDN-5 capillary column

[2] Odor description from literature: Belitz and Grosch, 1987; Serrini et al, 1994; Kaway, 1996; Durnford and Shahidi, 1998; Prost et al., 1998; Le Guen et al., 2000; Chung et al., 2001; Sérot et al., 2001; 2002.

1998). All of these molecules could play a key role in the various fish aroma development (Milo and Grosch, 1996).

Three volatile aldehydes, reported as oxidation products of n-6 PUFA, were identified in all the samples: hexanal, 2-octenal and 2,4 decadienal; and these molecules are often found in the aroma of many aquatic foods such as fish and crustaceans (Milo and Grosch, 1996; Prost *et al.*, 1998). A key role in the seafood and fish aroma characterization is attributed to these molecules because of their low threshold of perception, that is reported between 0.05 and 10 ppb in water (Josephson, 1991; Leffingwell and Leffingwell, 1991). 2,4-Decadienal is generally considered to contribute to the aromas of a variety of freshly prepared foods: as the main impact odorant of boiled cod (Prost *et al.*, 1998), it was, on the other hand, found in significantly higher percentage in the fillets of IPF and EF trout, 11.9 ±0.9% and 12.7 ±1.3%, respectively. 2,4-Decadienal is reported as one of the principal compounds formed by autoxidation of LA (Belitz and Grosch, 1987) and in general n-6 fatty acids (Serrini *et al.*, 1994). 2-Octenal was found in the volatile compounds of diverse aquatic food (Milo and Grosch, 1996; Prost *et al.*, 1998), and it is responsible for an aroma described in various terms such as oily, green leaf, lemon, cucumber, seaweed and also nut-like odor (Josephson, 1991; Milo and Grosch, 1996; Piveteau *et al.*, 2000).

These observations and the measured percentage of the flavor volatile compounds derived by n-3 or n-6 fatty acids, are in accordance with the fatty acids composition of the samples: IMF trout fillets were rich in n-3 fatty acids, while IPF and EF trout fillets were richest in LA and n-6 fatty acids.

Benzaldehyde, hexanal, 2-hexenal, 2,4 octadienal, 2,6-nonadienal and 2-decenal were previously reported as high in many different fish samples (Sérot et al, 2002; Turchini *et al.*, 2003c) and were also isolated and identified in this study. Another volatile compound arising from the degradation of other fatty acids is octanal. Octanal could be related to n-9 MUFA oxidation and generally, it is associated with a soapy, oily, fatty and also citrus odor and it has been isolated and identified in the aroma of different fish and crustaceans (Milo and Grosch, 1996).

In addition to aldehydes, 3 alcohols, 6 ketones, 7 hydrocarbons and 4 other compounds were isolated and identified in trout fillets (Table 6). The sum of total alcohols (Table 4) was significantly lower (2.8 ±0.1%) for EF samples. Alcohols may have been produced by lipid oxidation, (chemical or enzymatic) or thermal degradation of unsaturated fat (Kaway, 1996; Hsieh and Kinsella, 1989; Chung *et al.*, 2001) but generally they do not contribute to the overall flavor because of their high threshold values, unless they are unsaturated (Le Guen *et al.*, 2000). Unsaturated alcohols from C6 to C9 are responsible for the fresh fish, planty, cucumber and mushroom-like odors (Kaway, 1996; Ólafsdòttir *et al.*, 1997; Chaintreau, 2001) This is the case of 1,5-Octadien-3-ol which was reported to contribute actively with a fresh mushroom and moss odor and has a low odor threshold value (10 ppb) (Kaway, 1996) and 1,5-Octadien-3-ol was proposed as an intermediate in the EPA degradation via the specific 12-lipoxygenase activity (Durnford and Shahidi, 1998). Differently, 1-octen-3-ol was reported as n-6 PUFA oxidation product and was associated with a mushroom-like odor (Josephson, 1991).

EF trout fillets were therefore characterized by a bouquet design generally different from the trout reared in both the intensive farm, in consideration of high contents of aldehydes and low contents of alcohols probably due to the different fatty acids composition and total lipid content of the fillets.

The total sum of ketones was highest in IPF trout (9.2 ±0.4%). Ketones may be produced by thermal oxidation/degradation of PUFA or aminoacid degradation (Kaway, 1996; Spurvey *et al.*, 1998). 3,5-Octadiene-2-one was isolated only in IPF and EF samples and it was one of the most abundant ketones previously found in all fish by-product by Karahadian and Lindsaly, (1989). It contributes a fatty-fruity odor, and is reported, together with the 2,4-hepadienal, as the main product of the degradation of EPA via lipid autoxidation (Kawai, 1996). 2,3-Octanedione, which represented roughly 1% of all the total volatile compounds for all the analyzed samples, has been previously reported as a characteristic ketone of the volatile compounds of brown trout (Turchini *et al.*, 2003c) and its amount could be influenced by the diet. Three cyclic ketones were also isolated and

identified in rainbow trout fillets and similar compounds were previously isolated and identified in steamed clams, crayfish by-products and roasted shrimp (Spurvey *et al.*, 1998).

Seven hydrocarbons were detected in the trout samples but hydrocarbons generally do not provide much odor to food (Chung *et al.*, 2002). The two more important, in terms of percentage, were 3,5-octadiene, significantly highest for IMF sample, and 2,4,10,14-tetramethylpentadecane, commonly named pristane. Pristane was reported to contribute a green, sweet aroma to crayfish and originates from lipid autoxidation processes through alkyl radicals or from decomposition of carotenoids (Spurvey *et al.*, 1998) or may also be derived from diet (Bon *et al.*, 1988). Pristane is a common hydrocarbon, originating from fossil and biogenic sources, present in contaminated aquatic environments and in fish (Pineiro *et al.*, 1996).

Two furan compounds were detected in all the

Table 6. Volatile alcohols, ketones, hydrocarbons and other compounds isolated and identified in rainbow trout fillets (% on total volatile compounds) of trout reared in the three different farms.

		Culture Systems			
RI[1]	Volatile Compounds	IMF	IPF	EF	Odor description[2]
	Alcohols				
974	1,5-Octadien-3-ol	2.5^{b} ±0.3	2.0^{b} ±0.1	1.4^{a} ±0.1	moss, mushroom
981	1-Octen-3-ol	0.8 ±0.1	1.1 ±0.1	1.0 ±0.1	mushroom, herbaceous, spicy
1463	2-Octen-1-ol, 3,7-dimethyl	0.6 ±0.1	0.5 ±*4	0.4 ±0.1	-
	Ketones				
966	5-Ethylcyclopent-2-en-1-one	0.7^{b} ±0.1	0.7^{b} ± *	0.5^{a} ±0.1	-
987	2,3-Octanedione	1.3 ±0.3	1.2 ±0.1	1.1 ± *	metallic
1070	2-Cyclopenten-1-one, 3-(1-methylethyl)	3.2 ±0.4	3.1 ±0.1	2.5 ±0.2	-
1083	Cyclohexen-1,4-dione, 2,6,6-trimethyl	2.9^{b} ±0.3	2.0^{a} ±0.2	1.5a ±0.2	-
1092	3,5-Octadien-2-one	n.d.3	1.8 ±0.5	1.1 ±0.3	fatty, metallic, fruity
1424	3-Undecen-2-one	0.4 ±0.1	0.5 ± *	0.4 ±0.1	-
	Hydrocarbons				
812	3,5-Octadiene	4.9^{b} ±0.4	3.3^{ab} ±0.9	1.9a ±0.7	-
1364	1,4-Octadiene	1.0 ±0.2	1.0 ±0.1	0.7 ±0.1	-
1447	1,3,6-Octatriene	1.2^{b} ±0.1	0.8^{a} ± *	0.5^{a} ±0.1	cheese, plastic
1500	Pentadecane	2.4 ±0.4	2.5 ±0.3	3.6 ±1.6	alkane
1626	Cyclopentene, 1-(2-propenyl)	0.6 ±0.1	0.7 ±0.1	0.8 ±0.1	-
1700	Heptadecane	1.9 ±0.7	2.2 ±0.5	1.3 ±0.3	alkane
1703	Pentadecane, 2,6,10,14-Tetramethyl	4.2 ±1.2	2.1 ±0.6	4.9 ±1.5	green, sweet, crayfish
	Other compounds				
916	Phenol, 3-ethyl-	n.d.	0.2 ±0.1	0.2 ±0.1	phenolic, sheepy, medicinal
991	2-Pentyl-furan	0.9 ±0.1	1.0 ±0.1	1.1 ±0.1	liquorice, orange-like
1000	2-(1-pentenyl) furan	3.3^{b} ±0.4	2.7^{ab} ±0.1	2.5^{a} ±0.1	grass, butter
1483	Butylated Hydroxytoluene	0.4 ±0.1	nd	nd	-

Means within rows without superscript or with the same superscript are not significantly (P> 0.05) different from each other.

[1] RI: Kovàts retention indices (Castello, 1999) for MDN-5 capillary column

[2] Odor description from literature: Belitz and Grosch, 1987; Serrini et al, 1994; Kaway, 1996; Durnford and Shahidi, 1998; Prost et al., 1998; Le Guen et al., 2000; Chung et al., 2001; Sérot et al., 2001; 2002.

[3] nd : Not detected

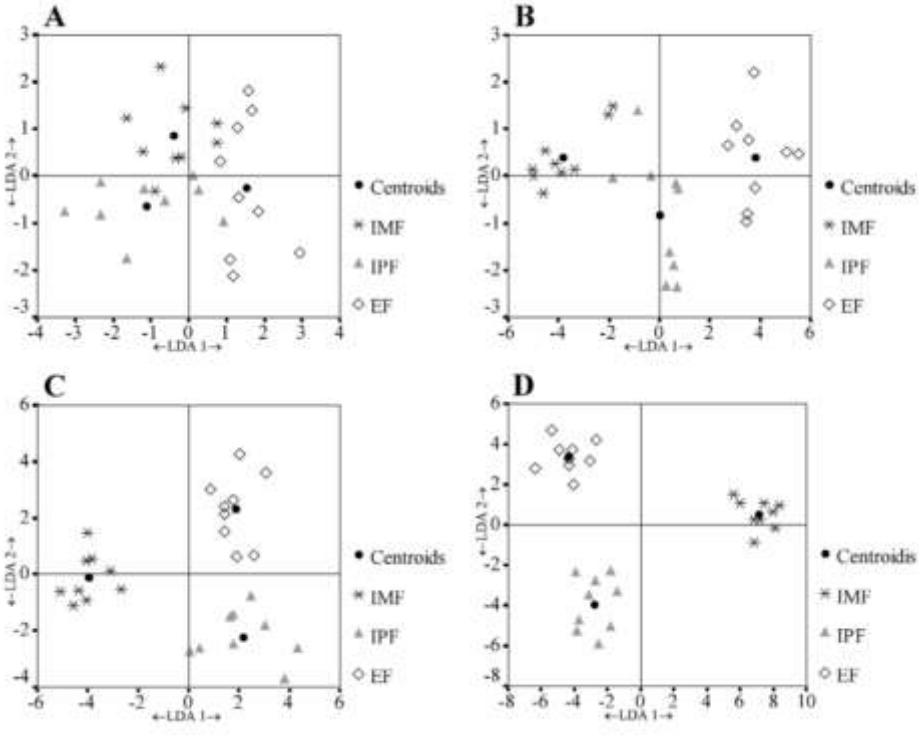
*[4] *: SEM <0.05*

trout samples: 2-pentyl furan varying from 0.9 ±0.1% to 1.1±0.1% and 2-(1-pentenyl)-furan, which was significantly higher for IMF samples (3.3 ±0.4%) and lower for EF samples (2.5 ±0.1%). Furans may originate in the Maillard reaction (Chung *et al.*, 2002) but 2-pentyl furan is also reported to derive from the oxidation of n-6 PUFA (Frankel, 1982) and it was for the first time reported as a seafood volatile in turbot (Prost *et al.*, 1998); the positive or negative furans contribution to food aroma is a debated question (Spurvey *et al.*, 1998). The 3-ethyl phenol was isolated only in IPF and EF trout, while buthylated hydroxytoluene (BHT) only in IMF trout. BHT is a synthetic antioxidant commonly used in aquafeed and it is highly lipophilic (Hertrampf and Piedad-Pascual, 2000). Surprisingly in IPF trout fillets BHT was not detected even if these fish were fed a commercial diet containing this antioxidant. Probably the higher content of total lipid of the IMF, with respect to IPF, trout fillet has to be taken into consideration in looking for an explanation of its presence in the volatile compounds fraction of the trout fillets.

Linear Discriminant Analysis

The LDA distribution of samples on a plane given by the two linear discriminant functions (first LDA factor, LDA1 and second LDA factor, LDA2) are reported in Figure 1. From the canonical representation it is immediately evident how the use of only biometrical parameters and proximate composition (A) is not useful to discriminate, while the use of flavor volatile compounds (B) and fatty acids (C) can discriminate the three group mainly on just one axis : LDA1 and LDA2 for B

Figure 1. Canonical representation of the three groups of farmed trout by the LDA distribution of samples on plane given by the two linear discriminant functions (LDA1 and LDA2) using different datasets: biometrical parameters and proximate composition (A); flavor volatile compounds (B); fatty acids (C); all datasets (D).



and C, respectively. A clear discrimination between groups through both axes LDA1 and LDA2 was evident using all the data available (D).

The variables chosen, by the stepwise selection, were, in decreasing order of their contribution to the first LDA factor (LDA1), two for dataset A: ln(DP %) and ln(K); four for dataset B: ln(2,4-Octadienal %), ln(Nonanal %), ln(2,4-Decadienal %) and ln (total ketones %); six for dataset C: ln(C22:1n-11 %), ln(C20:5n-3 %), ln(C18:2n-6), ln(C18:3n-3), ln(C18:1n-7) and ln(C16:0); nine for dataset D: ln(DP %), ln(C22:1n-11 %), ln(C20:5n-3 %), ln(4-Heptenal %), ln(C18:2n-6), ln(Moisture %), ln(2,4-Heptadienal %), ln(2,4-Decadienal %) and ln(Nonanal %).

The first and second factor (LDA1 and LDA2) coefficients of linear discriminant analysis performed using the four different datasets are reported in Table 7. The explained percentages of variance were, for the first factor, 75.6%, 96.7%, 69,8% and 74,0% for A, B, C and D datasets, respectively. The Wilks' lambda values calculated on LDA factor 1 were 0.284, 0.061, 0.021 and 0.003 for A, B, C and D datasets, respectively. Dataset A seems therefore not useful to discriminate within trout origins, while dataset D seems extremely effective because a Wilks' lambda of 0.003 indicates the greatest discriminatory ability of the function. Using dataset D the first factor explained 74.0% of the total variance and the fol-

Table 7. First and second factor (LDA1 and LDA2) coefficients, the explained percentage of variance, the Wilks' lambda and the percentage of correctly classified samples of linear discriminant analysis performed using different datasets: biometrical parameters and proximate composition (A); flavor volatile compounds (B); fatty acids (C); all datasets (D).

		A		B		C		D	
	Chosen variables	LDA 1	LDA 2	LDA 1	LDA 2	LDA 1	LDA 2	LDA 1	LDA 2
	Constant	-200.274	-97.933	-11.323	2.926	-48.312	19.318	46.919	-311.227
Biometrics and proximate	ln (K)	1.470	9.782						
	ln (DP)	44.883	21.665					23.772	53.239
	ln (Moisture)							-38.437	9.798
Flavor volatile compounds	ln (Nonanal)			5.724	0.378			-3.482	2.038
	ln (2,4-Octadienal			-5.970	4.357				
	ln (2,4-Decadienal)			5.712	0.752			-5.287	2.422
	ln (Total Ketones)			-2.758	2.926				
	ln (4-Heptenal)							2.509	0.443
	ln (2,4-Heptadienal)							5.522	-1.486
Fatty acids	ln (C16:0)					6.543	-22.163		
	ln (C18:1n-7)					14.494	6.084		
	ln (C22:1n-11)					-8.751	-1.921	3.254	4.873
	ln (C18:2n-6)					14.153	10.875	-0.366	3.060
	ln (C18:3n-3)					-14.093	-11.808		
	ln (C20:5n-3)					-0.663	11.190	5.095	8.671
Explained % of variance		75.6	24.4	96.7	3.3	69.8	30.2	74.0	26.0
Wilks' lambda		0.284	0.686	0.061	0.725	0.021	0.206	0.003	0.089
Samples correctly classified		77.8%		88.9%		96.3%		100%	

lowing equation was obtained:

D_1 = 46.919 +23.772[ln(DP %)] +3.254[ln(C22:1n-11 %)] +5.095[ln(C20:5n-3 %)] +2.509[ln(4-Heptenal %)] -0.366[ln(C18:2n-6)] -38.437[ln(Moisture %)] + 5.522[ln(2,4-Heptadienal %)] -5.287[ln(2,4-Decadienal %)] -3.482[ln(Nonanal %)]

The validations of the models were performed by classifying each case while leaving it out from the model calculations and the percentage of cross-validated grouped cases correctly classified were 77.8%, 88.9%, 96.3% and 100% for dataset A, B, C and D, respectively. Consequently it seems possible to assert that simple observations of biometrical parameters and fillet proximate composition values are not useful for discriminating between farmed trout of different origins and this probably because of the high influence of size, genetic strain and feed management on these parameters (Shearer, 1994; De Silva and Anderson, 1995). The observations of fatty acid composition could be used for discriminating farmed trout, but the high influence of dietary fatty acids on these parameters (Steffens, 1997; Turchini *et al.*, 2003a, b) could affect the interpretation of the model and the discrimination obtained is probably more related to the feed suppliers than to the farms. But the LDA model constructed using combined dataset from biometrical parameters, fillet proximate and fatty acids compositions and the flavor volatile compounds, contemplating the effects of different factors such as size, genetic strain, environment, feed management and feed composition, could be a useful tool in discriminating between farmed trout origins.

Conclusions

The three typologies of trout produced in the three different culture systems analyzed in the present study showed important differences. Trout farmed in the intensive mountain farm (IMF) showed the highest coefficient of fatness and the highest crude lipid content in the fillets. The fatty acids of their fillets were characterized by the highest percentage of palmitic acid and MUFA. Trout farmed in the intensive plain farm (IPF) were characterized by the worst (lowest) dressing percentage and the lipid of their fillets was richest in n-6 fatty acids. Trout stocked for the last year of their life in the farm (EF) were leaner both in the carcass and in the fillets. They showed the best carcass dressing percentage and the lipid fraction of the fillets was characterized by high level of PUFA and by the lowest value for the index of atherogenicity. Moreover, the analysis of flavor volatile compounds showed some differences in the bouquet design of the three samples, particularly as regards to the aroma compounds derived from n-3 or n-6 fatty acids, and this could influence the consumer choice. The model constructed by guided LDA method, following the selection of variables by ANOVA and using the most discriminating variable between all the measured variables, could provide an effectively useful tool in answering the question of the origin of Italian farmed trout.

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