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Characteristics of raw and cooked fillets in species of actual and potential interest for Italian aquaculture: rainbow trout (*Oncorhynchus mykiss*) and meagre (*Argyrosomus regius*)

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Dottorando
Dr.ssa Roberta Martelli

Tutore
Prof.ssa Giuliana Parisi

Anni 2010/2012

Supervisors

Main supervisor:
Associate Professor Giuliana Parisi
Animal Science Section
Department of Agriculture Biotechnology
University of Florence

Project supervisors:
Full Professor Oreste Franci
Animal Science Section
Department of Agriculture Biotechnology
University of Florence

PhD Antonio Bonelli
Animal Science Section
Department of Agriculture Biotechnology
University of Florence

PhD Filippo Faccenda
Centro Trasferimento Tecnologico
FEM-IASMA
Trento

The thesis is based on a literature review and 3 scientific papers submitted but not yet accepted for publication

Eu amo tudo o que foi
Tudo o que já não é
A dor que já não me dói
A antiga e errônea fé
O ontem que a dor deixou
O que deixou alegria
Só porque foi, e voou
E hoje é já outro dia.

(Fernando Pessoa)

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ABSTRACT

Fish is a good source of fat, proteins, vitamins and minerals. It is the major contributor of long-chain omega-3 polyunsaturated fatty acids which are known to have a benefic effect on reducing the incidence of cardiovascular diseases.
The world production of farmed fish has raised in the last decades as a consequence of the overexploitation of fishery resources and the wild stocks depletion. Aquaculture is making an important contribution to the demand of seafood and the sector continues to diversify, to use new species and to modify its system and practices towards safe and quality products.

In Italy a considerable part of fish is sold fresh and intended for domestic consumption after cooking. Heat treatments such as smoking and parboiling are among the most spread processes applied by fish industry.
The effect of cooking on seafood nutritional and organoleptic quality has been described by several researches focusing on both the changes of textural and colorimetric attributes and the retention of nutrients. Heat treatment generally induces structural changes of the muscle involving protein denaturation and gelatinization. Lipid and protein can be also oxidized by heat resulting in losses of nutritional value and formation of volatile molecules responsible for the aromatic profile.

Depending on many intrinsic and extrinsic factors, including species, environmental and rearing conditions, storage time after death and type of heat treatment applied, farmed fish qualitative attributes can be subject to changes.

The overall aim of this study was to assess the effects induced by cooking on quality of fillets from two different farmed species [meagre (*Argyrosomus regius*) and rainbow trout (*Onchorynchus mykiss*)] with reference to changes in physical parameters and nutritional profile. Cooking by boiling was tested and the variation of quality of both raw and cooked fillet depending on species, genetic strain (in rainbow trout), season, rearing system (land-based tank or off-shore cages, in meagre), farming conditions (in rainbow trou) and short-time chilling storage (in meagre) was studied.

Specie-specific differences in fillet physico-chemical traits influenced raw flesh quality and the response to cooking.

In meagre, which was an emerging species in Italian aquaculture, sampling times differences in macronutrients were nullified by cooking. Boiling method induced loss of some macronutrients, mainly lipids and some fatty acids, but

changes detected did not compromise the valuable nutritional traits of this particularly lean species.
Morpho-biometric traits of fish and colour, texture, macronutrients, fatty acids and minerals content of raw fillet were affected by rearing system, as a consequence of the diversified environmental parameters and conditions.
Short time chilling did not affect nutritional profile and minimal changes, mostly involving colour and texture, were detected.

In rainbow trout, which is the major farmed fish species in Italy, cooking by boiling modified physico-chemical profile in a different extent depending on the strain. Differences in some textural properties were nullified by cooking and specific farming conditions resulted to affect fillet quality in a different extent according to the strain.

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PART I

1. INTRODUCTION

1.1. Effects of the most common cooking methods on nutritional content of fish

Fish is a common food in most world countries and gastronomic traditions rarely don't include seafood recipes.

In 2008, the total world fishery production was 142,287,000 tonnes, of which 80.9% (i.e. 115,089,000 tonnes) intended for human consumption (FAO, 2010b).

Though processed and freezing seafood consumption is greatly widespread, a considerable part of fish for human consumption (47.8% according to FAO 2010 database) is marketed as fresh, whole or filleted.

Especially the higher quantity of commodities is represented by fresh and frozen fish and very large differences are found between developed (4% as fresh and 54% as frozen fish) and developing countries (58.6% as fresh and 19.8% as frozen fish) (FAO, 2010b).

Fresh and frozen fish normally undergo different preserving treatments or different cooking processes, while the consumption of raw fish, extremely rare in the Western society, is a traditional custom in the Arctic circle and in most Asiatic countries.

In the last decades, because of its several valuable nutritional components, fish gained popularity in the western countries where cardiovascular diseases (CVD) are one of the main causes of death, provoking only in Europe over 4.30 million of deaths each year (European Cardiovascular Disease Statistics, 2008).

In particular, fish is a good source of easy digestible proteins, vitamins and minerals comparable to those found in meat and poultry, with high quality fat content (Pigott & Tucker,1990).

Concerning fat, fish and mainly oily fish are the major contributors of long-chain omega-3 polyunsaturated fatty acids (ω-3 LC-PUFAs) to the human diet (Mozaffarian & Rimm, 2006a) (Figure 1).

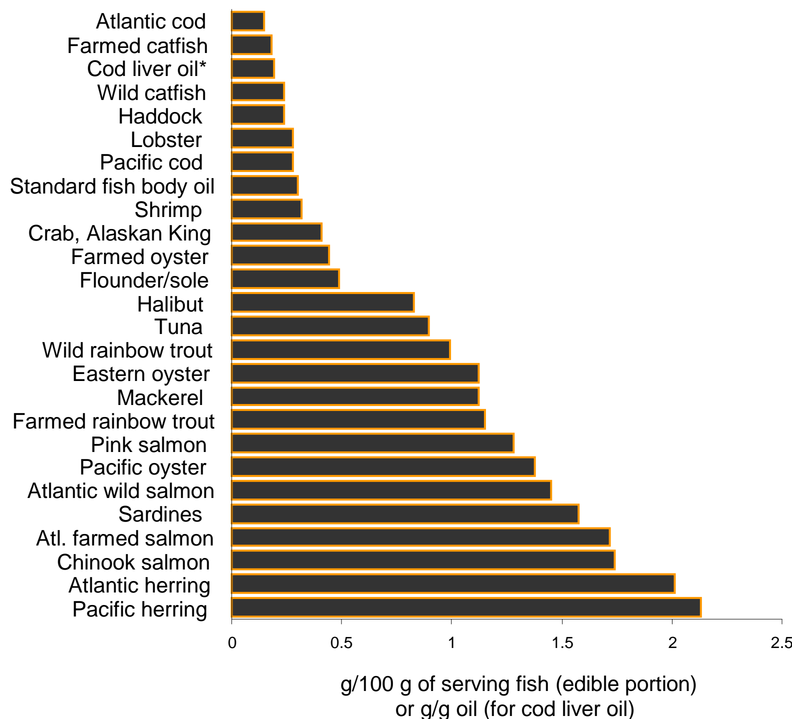
The presence of ω-3 fatty acids with antithrombogenic and antiatherogenic activity gives fish the characteristics of a functional food with a fundamental role in the diet.

In fact epidemiological and clinical trials have shown that ω-3 LC-PUFAs, particularly eicosapentaenoic acid (C20:5 ω-3, EPA), and docosahexaenoic acid (C22:6 ω-3, DHA), have beneficial effects on human health reducing the incidence of cardiovascular diseases and mortality (Burr et al., 1989; Marchioli et al., 2002; Buttriss & Nugent, 2005; Kris-Etherton et al., 2002; Lauritzen et al., 2001; Serhan, 2007).

Although CVD benefit from the consumption of plant- and marine- derived ω-3 fatty acids is now recognized, ideal intakes presently are still unclear (Kris-Etherton et al., 2002).

A reduced risk of fatal CVD and sudden cardiac death has been proved at consumption of 250 mg/day of EPA (C20:5ω-3) plus DHA (C22:6ω-3) (Burr et al., 1989; Mozaffarian & Rimm, 2006a; Yokoyama et al., 2007; GISSI-HF Investigators, 2008, as cited by Smit et al., 2009).

Figure 1 – Amount (expressed as grams) of ω-3 LC-PUFAs eicosapentaenoic acid (C20:5ω-3, EPA) and docosahexaenoic acid (C22:6ω-3, DHA) in fish and fish oils (Kris-Etherton et al., 2002; modified).



*Data from the USDA Nutrient Data Laboratory. The intakes of fish given above are very rough estimates because oil content vary markedly (>300%) with species, season, diet, and packaging and cooking methods. *This intake of cod liver oil would provide approximately the Recommended Dietary Allowance of vitamins A and D.*

U.S. Food and Drug Administration (FDA) recommends not exceeding more than a total of 3 g/day of EPA (C20:5ω-3) and DHA (C22:6ω-3), with no more than 2 g/day from a dietary supplement (FDA, 2000) while the American Heart Association (AHA) recommends eating fish (particularly fatty fish) at least 2 times a week (He, 2009) to guarantee an adequate intake of LC-PUFAs.

EFSA (EFSA, 2010a) recently recommended an Adequate Intake (AI) of 250 mg/day of EPA (C20:5ω-3) plus DHA (C22:6ω-3) for healthy adults with an additional 100-200 mg/day of preformed DHA (C22:6ω-3) for pregnant and lactating women as it plays a fundamental role in development of the brain and retina in foetus and during the first 2 years of life.

Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) suggest the same dose for adults bringing the minimum intake of EPA (C20:5ω-3) plus DHA (C22:6ω-3) to 300 mg/day of which at least 200 mg should be DHA (C22:6ω-3), for pregnant and lactating women (FAO/WHO, 2008).

Similarly the British Food Standards Agency (FSA) recommends for pregnant women a consumption of 2 portions of fish per week of which at least one should be oily fish (FSA, 2004).

But fish can also contribute significantly to the dietary exposure to some contaminants, such as methylmercury, persistent organochlorine compounds, polybrominated flame retardants and organotin compounds (EFSA, 2005). Levels of bioaccumulative contaminants are higher in fish that are higher in the food chain, to such an extent that methylmercury and dioxin-like compounds can exceed the provisional tolerable weekly intake (PTWI) for high consumption of certain species (EFSA, 2005).

In addition to chemical hazards from anthropogenic pollution, especially wild fish can carry biotoxins and parasites, responsible for severe gastrointestinal and neurological damages.

Moreover fish is a very perishable food which can be easily contaminated by a wide range of microorganisms, naturally present in water environments, associated with pollution of water or introduced during post-mortem handling and processing (Gram & Huss, 1996; Huis in't Veld, 1996).

Serious health damages arise from several types of water-borne pathogens and viruses which often are detected in fish and shellfish from polluted water.

Improper storage conditions, bad hygienic practises during manipulation and processing and cross contamination are the most common causes of spread of infection (Huis in't Veld, 1996).

Though they aren't harmful for health, specific spoilage organism (SSO) can rapidly contaminate fillets during storage and contribute to autolytic deterioration, inducing rancidity, colour changes and shortening shelf life.

The complexity of these qualitative factors can be further influenced by cooking.

Since cooking is a quotidian activity, it could not seem apparently a very complicated phenomenon and process. Nevertheless, besides making food safer and preserving it a longer time, cooking is able to trigger several chemical reactions in food and consequently improve or make worse, enhance or suppress the original attributes of raw material, strongly influencing food palatability and

nutritional content (Finley et al., 2006 and Meade et al., 2005 as cited by Larsen et al., 2007) (Table 1).
The temperatures reached during cooking process radically change the characteristics of food which can result very different from the original raw matrix. Qualitative characteristics influencing the consumers' overall judgment of quality, i.e. juiciness, flavour and colour can be significantly influenced by cooking conditions, as the final internal temperature to which the meat is cooked (Wood et al., 1995).

Table 1 - The aims of cooking food

Improvement of food safety	Reduction of microbiological and chemical related risk: ↓ pathogens ↓ heat-labile bacterial toxins ↓ heat-labile micotoxins ↓ heat-labile viral toxins ↓ heat-labile parasites
Extension of shelf life	Reduction of microbiological spoilage and autolysis: ↓ spoilage organisms ↓ endogenous autolytic enzymes
Increase of nutrients availability and digestibility	cell structure degradation biopolymers degradation proteins denaturation and gelatinization removal or inactivation of heat-labile anti-nutritional factors
Improvement of hedonistic attributes and palatability	changes in colour, taste, odour, texture and flavour

Colour and appearance of seafood are very important in terms of consumer perception of seafood quality, and are dominant factors in consumer purchasing decision (Yagiz et al., 2009).
Many changes are desired and expressly wanted by the cooking, especially the increase of meat tenderness due to the changes produced in structure of connective tissue, myofibrillar proteins and soluble proteins significantly

influencing the texture of cooked meat (Rowe, 1974; Bouton & Harris, 1972; Murphy & Marks, 2000).
Textural changes usually are due to the shrinking of myofibrils and pericellular connective tissue and to the loss of the structural integrity of the cell (mitochondria, sarcoplasmic reticulum and nuclei are seriously damaged or disappear) (Lin et al., 2009). Moreover in the interstitial tissues there are small amounts of amorphous materials that coagulate and aggregate by collagen fibres and sarcoplasmic proteins while the percentage of myofiber–myocommata detachments is more than in the raw samples (Lin et al., 2009).
Particularly the effects produced by temperature in texture are useful and providential in the case of meat of terrestrial species, even if the increase of meat tenderness is not only dependent on the level of applied temperature but also on the initial characteristics of raw meat and the duration of the heat application, resulting in conflicting effects (Wood et al., 1995; Lawrie, 1998; Voutila et al., 2007).
On the contrary, scarcity of texture (i.e. beef) and meat toughness are not normally a problem in the case of fish meat.
Cooking is applied on fresh or preserved fish by many different methods, in relation to the kind of fish, the traditional habits and the specific goals of cooking, i.e. a heat treatment can be aimed at preparing a food for direct consumption or preserve it (smoking, canning, etc.).
Raw fish intended for domestic consumption or catering can be cooked in several different methods: boiled, fried, pan-fried, grilled, roasted or baked and microwaved (Table 2). The use of new cooking methods as microwaving, has greatly increased in the last decades (Arias et al., 2003).
Conservation of nutrients is a major consumer concern related to food preparation, and cooking methods can have a detrimental effect on the nutrient composition (Tokur, 2007) and sensory quality of fish (Freeman, 1999).
As claimed by the best chefs, recently supported by pioneering molecular theories, the cooking process really determines which and how many food components will reach the mouth.
Depending on the cooking method, temperature and heat-treatment length, the nutrients of the fillet are partly lost in the cooking medium or concentrate as a consequence of water evaporation, proteins denaturation and gelatinization, while heat trigger oxidative reactions damaging the fragile lipid fraction.
Therefore, it can be important to investigate the differences in nutrients retention among several cooking methods (Ersoy & Özeren, 2009).
Researches on structural and compositional changes that fillet meets during cooking are quite recent, and they sometimes show dissenting opinions about the same phenomenon.
Undoubtedly such discrepancies depend on the compositional differences of the raw fillets owing to species, type of feed, habitat, season of capture, post-mortem preservation method/temperature/duration and cooking method.
Cooking method seems greatly to influence final composition.

Table 2 – Definitions of the main cooking methods provided by on-line encyclopaedias, cooking and producers' associations websites.

Boiling	Cooking in water at 100°C (b.p.) or water-based liquid as stock or milk Simmering: gentle boiling just below boiling point (95°C to 99°C) (www.beeflambnz.co.nz) Poaching: very gentle boiling using a minimum amount of reduced liquid or stock that is kept at just below simmering point (90°C to 94°C) (www.beeflambnz.co.nz)
Pan-frying	Form of frying characterized by the use of the minimal cooking oil or fat, typically using just enough oil to lubricate the pan (www.sensagent.com)
Frying	Cooking in oil or fat Shallow frying: form of frying where the depth of the cooking fat is sufficient to partially submerge the food being cooked (www.sensagent.com) Deep frying: form of frying where food is submerged in oil (www.sensagent.com)
Baking	Cooking by dry heat in a oven (www.sensagent.com)
Roasting	Cooking by dry heat in a oven or on a rotating spit over a fire, gas flame or electric grill bars with small amount of fat or oil (www.beeflambnz.co.nz)
Grilling and barbecuing	Cooking with radiant heat directed from below or above the food put on a grill (www.beeflambnz.co.nz)
Steaming	Cooking by moist heat with food not coming into contact with the cooking liquid but instead with the surrounding steam, sometimes under pressure (www.beeflambnz.co.nz)
Microwaving	Cooking by dielectric heating (www.en.wikipedia.org).

Just skin removal can reduce lipids and change fatty acids profile of cooked fish, as it was found by de Castro et al. (2007) in carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*) and tambacu (*Colossoma macropomum*) fillets. Lipid percentage reduced in all three species, mainly in fresh carp changing from 16.71 to 3.45% and modifying its classification from fat to lean fish, even if it is not specified by which cooking method, baking or steaming, the change is determined.

Even if the variation in percentage of fatty acids was limited, after skin removal fresh cooked carp and Nile tilapia showed a significant decrease ($p<0.05$) in linoleic acid (C18:2ω-6) from 16.6 to 15.4% and 4.7 to 3.5% of the total lipid.

Tambacu was the only species showing a significant increase ($p<0.05$) in EPA (C20:5ω-3) and DHA (C22:6ω-3) after cooking without skin, raising from 0.3 to 1.2% and 1.5 to 8.5% of the total lipid.

In addition, morphological variability of fillet of different species and cuts due to muscle cells difference in diameter and length and bio-molecules specific thermal properties influences flow rate and direction of heat.

Despite cooking conditions from literature references are extremely various, they are likewise useful to describe the main effects of each cooking method on fish fillet chemical composition.

1.1.1. Boiling

Boiling is characterized by hot water upward flows generating inside the body of water and lapping against the fillet surface. Heat from hot water gradually penetrates inside the flesh bringing fish to the water temperature.

Water losses are strongly prevented by the water medium, so that boiled fish generally shows higher water amount than differently cooked fish. This is supported by several researches (Larsen et al., 2010; Weber et al., 2008; Gokoglu et al., 2004).

Both boiled New Zealand King salmon (*Oncorhynchus tshawytscha*) (about 3 minutes) (Larsen et al., 2010) and silver catfish (*Rhamdia quelen*) (98 °C/12 minutes) (Weber et al., 2008) moisture was not significantly different to raw fish, resulting in 63.36±0.17 *vs* 63.86±2.04% (raw) and 74.9±0.17 *vs* 79.6±0.82% (raw), respectively.

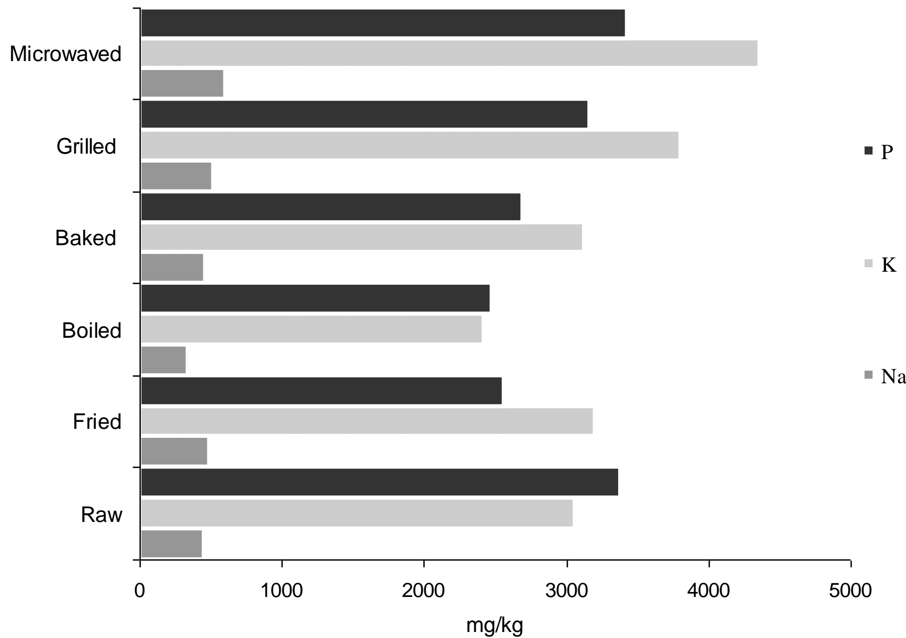
A small concentration of dry matter and protein is usually observed in boiling (Gokoglu et al., 2004; Weber et al., 2008) resulting in a very low retention of hydrosoluble nutrients as vitamins, amino acids and minerals.

Larsen et al. (2007) suggests boiling should be anticipated since the amino acids and creatine can leach out in the boiling water. In particular they found that taurine, glycine, creatine and alanine significantly decreased after boiling in cod (*Gadus morhua* L.), even if the concentrations were not significantly different from fried and baked cod, except for alanine (boiled: 119±42 mg/100 g of dry tissue *vs* baked: 138±40 mg/100 g of dry tissue).

Gokoglu et al. (2004) registered a significant decrease ($p<0.05$) in most minerals (Na, K, P, Zn, Mn) on boiled rainbow trout (*Oncorhynchus mykiss*) (5

minutes), when compared to baked, fried and grilled. In particular, Na and K content decreased from 455±24.5 (raw) to 335.54±7.81 and 3060±56.8 (raw) to 2417±74.2 mg/kg, respectively (Figure 2).

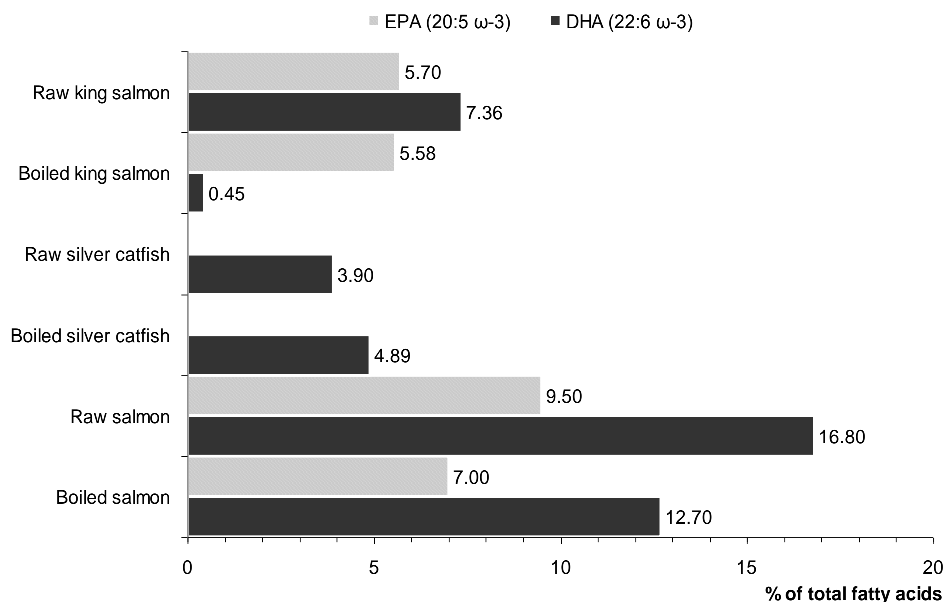
Figure 2 – Phosphorous (P), potassium (K) and sodium (Na) content in boiled rainbow trout (Gokoglu et al., 2004).



The iron content was significantly different ($p<0.05$) between boiled and raw anchovies (*Engraulis encrasicholus*), but the mean total losses resulted lower (11.2% for non heme iron and 30.4% for heme iron) when compared to grilled (52.6% non heme iron and 70.4% for heme iron), microwaved (38.5% for non heme iron and 54.5% for heme iron), and baked (34.4% for non heme iron and 53.7% for heme iron) samples (Turhan et al., 2004).
Anyway some hydrosoluble nutrients do not leach. In salmon (*Salmo salar*), a fish considered as one of the richest in L-carnitine, L-carnitine content is not modified by different cooking methods except with the smoking process in which two thirds of the L-carnitine is lost (Rigault et al., 2008). Similarly the rainbow trout analyzed by Gokoglu et al. (2004) did not significantly change in Ca, Cu and Fe content.
Concerning liposoluble nutrients retention, reference study results are contrasting. King salmon boiled for about 3 minutes registered the most significant decrease ($p<0.05$) in DHA (C22:6ω-3) and DPA content (g fatty acids/100 g fresh) from 0.52±0.06 (raw) to 0.41±0.03 and 1.36±0.09 (raw) to

1.15±0.07% respectively, compared with steamed, microwaved, oven baked, pan and deep fried salmon (Larsen et al., 2010) (Figure 3).

Figure 3 – Variation in LC-PUFA ω-3 content after boiling (Weber et al., 2008; Yagiz et al., 2009; Larsen et al., 2010).



The same was found by Yagiz et al. (2009) who registered a significant ($p<0.05$) decrease at day 0 in saturated and polyunsaturated (ω-3 and ω-6) fatty acids in boiled salmon (72 °C core temperature), where DHA (C22:6ω-3) and EPA (C20:5ω-3) content (% of total fatty acids) ranged from 16.8±0.8 (raw) to 12.7±0.1 and 9.5±0.3 (raw) to 7±0.5 %, respectively (Figure 3).

On the contrary, silver catfish fat content and fatty acids profile were only marginally affected by 12 minutes boiling (core temperature 89±9 °C), similarly to baked and grilled catfish (Weber et al., 2008) (Figure 3). Boiled rainbow trout fat content showed the same behavior without significant changes according to Gokoglu et al. (2004).

Boiling is among the cooking methods that most widely promote lipid oxidation, because of the high temperatures applied. Weber et al. (2008) detected the highest amount of primary and secondary oxidation products in boiled silver catfish even if malonaldehyde did not reach threshold levels for preventing human consumption (3 mg MDA/kg).

According to Yagiz et al. (2009), boiled salmon has significantly ($p < 0.05$) higher levels of TBARS compared to pressure treated (150 and 300 MPa) and untreated (0.1 MPa) samples at the second day of storage.

The oxidative impact of boiling can be noted by the abundance of aldheydes and chetons, mainly arising from unsaturated fatty acids degradation and reflecting fatty acids profile of the muscle.
According to Prost et al. (1998), the greatest amount (expressed as percentage of internal standard, IS%) of (E)-2-octenal (13.68 *vs* 9.74 IS%; $p<0.05$), (E)-2-nonenal (2.77 *vs* 1.90 IS%), and (E,Z)-2,6-nonadienal (13.73 *vs* 6.09 IS%; $p<0.05$) in wild turbot (*Scophtalmus maximus* L.), can be explained by the higher content of the fatty acids precursors C20:4ω-6 and C22:5ω-3 in wild than in farmed fish.
It is nevertheless true that some volatiles compounds from lipid oxidation, like (Z)-4-heptenal and hexanal (Prost et al., 1998), above odour threshold, could be responsible for off-flavours and consequently deteriorate organoleptic profile of cooked fillet.
In particular, after boiling fat fish like salmon (> 3% of total lipid) are able to release such undesirable aldehydes at concentrations far higher than lean fish like cod (Belitz et al., 2009).
Organoleptic profile of boiled fish is usually poorer than differently cooked fish. Several volatiles molecules belonging to the main odorants categories (furanones, pyrazines, aldehydes, chetons and other intermediate products from Maillard reactions such as 5-methylfurfural), can be detected among the substances leaching in the boiling water.
Depending on the fish species, aromas released in the boiling water can change in kind and amount so that Morita et al. (2003) were able to differentiate broths from seawater and freshwater species and from migratory coastal and deep-sea species. Broth from fresh water species like loach (*Misgurnus anguillicaudatus*), pond smelt (*Hypomesus nipponensis*) and carp contains aldehydes like (E)-2-dodecenal, (E,Z)-2,4-decadienal and (E,E)-2,4-decadienal responsible for a strong “green” odour. Broth from seawater species, like slime flounder (*Microstmus achne*), Pacific cod (*Gadus macrocephalus*) and blue-fin tuna (*Thunnus thynnus*) is enriched with chetons (1-hydroxy-2-propanone, 1-hydroxy-2-butanone and 2-pentadecanone) responsible for sweet, roasted and cooked fish aromas.
The “grilled fish” aroma from phenols [phenol and 2-(1-methylpropyl-phenol)], alcohols [alkylalcohol, 2-(2-butoxyethoxy)ethanol] and chetons [2-undecanone and 2-(1-methylpropyl)cyclopentanone] is peculiar to broth from migratory coastal fish like sardine (*Sardinops melanosticta*), banded-blue sprat (*Spratelloides gracilis*) and mackerel (*Scomber japonicus*).
Colour and texture are also affected by boiling. Boiled salmon showed higher L* (lightness) and b* (yellowness index) values but lower a* (redness index) values for dark muscle compared to untreated (0.1 MPa) and high pressure treated (150 MPa) samples after 2, 4 and 6 days of storage in Yagiz et al. (2009) study.

Among texture parameters, hardness decreased from 19.4±5.9 to 13.0±4.3 N, when rectangular shaped pieces of the same fillets were compressed twice to 70% of their original height at 100 mm/min speed and 100 N compression load using a cylindrical-shaped probe.

Lin et al. (2009) described the textural changes in crisp grass carp (*Ctenopharyngodon idellus* C. et V) and grass carp (*Ctenopharyngodon idellus*) after boiling, revealing that after heating for 10 min, changes in the morphological features observed in both samples are strictly dependent on muscle anatomy. Boiled crisp grass carp showed hardness, fracturability, springiness and chewiness greater by 15.11, 10.42, 3.30 and 17.57%, in contrast with grass carp which reduced by 72.48% in hardness, 20.11% in springiness, 78.89% in chewiness, 40.67% in resilience, 12.42% in cohesiveness and 1.35% in fracturability.

Such differences can be explained by the shorter muscle fibres diameter and the denser fibres density of the crisp grass carp, positively correlated to hardness. In addition crisp grass carp had denser collagen layers than grass carp, with a grater mean collagen diameter, leading to high hardness in raw fish muscle.

1.1.2. Steaming

In steam cooking, fish comes into contact with steam produced by boiling water. The difference between steam and fillet temperature enables steam to condense on fish surface and transfer heat on the flesh. Steam condensation prevents dehydration of the surface and development of the crust, keeping fillet tender and uniform.

Moisture content is similar to raw and boiled samples resulting in water losses generally fewer than cooking methods in dry air or oil (Larsen et al., 2010; Erkan et al., 2010). Choubert & Baccaunaud (2010) registered significant differences in moisture between moist heat cooking by steam and dry heat cooking in oven, when compared with raw fillet from asthaxantin-fed rainbow trout (71.25±0.97 *vs* 69.26±1.25 *vs* 73.14±1.01%, in cooked and in raw, respectively).

Similar behaviour was found by Al-Saghir et al. (2004) on steam-cooked salmon showing water losses more reduced than after pan-frying with olive oil, when compared with raw fillet (62.3±0.31 *vs* 57.7±1.07 *vs* 64.4±0.35%, in raw, steam-cooked and pan-fried, respectively).

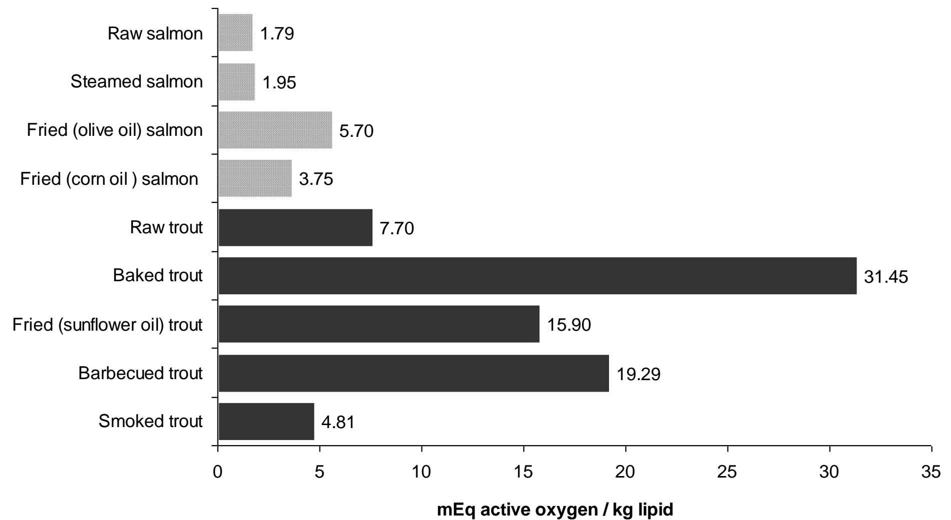
According to Rodriguez et al. (2008) storage at chilling temperature (-1.5 °C) seems not affect moisture retention. Water losses do not show significant differences as a result of the preliminary icing time (0 *vs* 5 *vs* 9 days of chilled storage) in steamed Coho salmon (*Oncorhynchus kisutch*). On the contrary, hardness increases and cohesiveness decreases during chilling storage, affecting textural properties after steaming.

Nutrient losses are greatly more limited in steaming than in the other cooking methods, so that it can be considered among the best cooking method for preserving nutrients.

Erkan et al. (2010) found a significant ($p<0.05$) higher concentration of amino acids after steaming in horse mackerel (*Trachurus trachurus*), inferior only to grilled samples. About vitamins, the same Authors did not register a decrease for vitamin B3 and B6, while vitamins A, E, B1 and B2 significantly reduced so that steaming appears the most damaging method for vitamin B2 preservation (0.144 in raw *vs* 0.078±0.01 mg/100g in cooked fish).
However protein and lipid content generally do not significantly lose nor concentrate because of the low water losses (Al-Saghir et al., 2004; Rodriguez et al., 2008; Erkan et al., 2010; Larsen et al., 2010), and fatty acids profile remains almost unaltered for most of the species of fish. According to Al-Saghir et al. (2004), steaming has not significant influence on fat content and fatty acids profile of salmon fillets. Skinned carp did not significantly change fatty acids profile after cooking by steaming at the 30th day of frozen storage, maintaining high monounsaturated fatty acids levels (56.7%), mainly oleic acid (43.6±1.3%), followed by saturated (26.5%) and polyunsaturated (14.2%) fatty acids (de Castro et al., 2007).
Also skinned Nile tilapia fatty acids profile was similar to that of the raw fish in the same storage and cooking conditions previously described for the carp, confirming a prevalence of saturated and monounsaturated fatty acids. On the contrary skinned tambacu presented a significant ($p<0.05$) decrease of monounsaturated fatty acids, particularly oleic (25.4±3 *vs* 17±1%, in raw and steamed, respectively) and a significant increase of saturated fatty acids, mainly myristic (C14:0) (3.9±0.8 *vs* 5.3±0.3% in raw and steamed, respectively) and palmitic (C16:0) (25.5±2.2 *vs* 27.3±0.5%, in raw and steamed, respectively). However the Authors maintain that saturated fatty acids increase cannot be attributed to the cooking method, since the initial content of myristic acid (C14:0) was higher in the samples submitted to cooking than in the raw ones (de Castro et al., 2007).
Steam-cooking oxidative impact is generally limited on most nutrients. Significant low amount of peroxides (mEq active oxygen/kg lipids) accumulates in steam cooked salmon (1.95±0.11) if compared with olive and corn oil fried samples (5.70±0.70 and 3.75±0.35, respectively) (Al-Saghir et al., 2004) (Figure 4).
Nevertheless some compounds can be markedly oxidized more than in cooking methods usually reckoned more aggressive (i.e. frying or grilling). Al-Saghir et al. (2004) observed the highest Cholesterol Oxidative Products (COPs) formation after steaming (9.88±0.97 μg/g extracted fat) than after frying procedures with olive oil (3.98±0.86 μg/g extracted fat) and corn oil (4.38±2.24 μg/g extracted fat) even if the difference in cooking time of 6 min for frying versus 12 min for steaming and the dilution effect in frying oil could have influenced such results.
Also amines oxidation occurs, as trimethylamine-nitrogen content significantly raised from 0.05±0.01 (raw) to 0.29±0.08 mg/100 of muscle on steamed Coho

salmon because of TMAO thermal breakdown during the steam-cooking process (Rodriguez et al., 2008).
No crust formation and the relatively mild temperatures in comparison with dry air and oil cooking, do not represent a limiting factor for the development of a rich organoleptic profile. Steamed gilthead sea bream (*Sparus aurata*) fillets release several volatiles compounds, among which alcohols contributing smooth flavours (Grigorakis et al., 2003).

Figure 4 – Peroxides accumulation on rainbow trout and salmon cooked by different methods (Al-Saghir et al., 2004; Tokur, 2007).



Differences emerge between wild and farmed gilthead sea breams. Wild gilthead sea bream has a richer organoleptical profile, characterized by higher percentages of alcohols, terpenes, benzenes and chetons, among which 2-heptanone and 2-octanone, having a distinct green and fruity aroma, and E-2-nonenale derived from arachidonic acid (C20:4ω-6), absent in farmed fish (Grigorakis et al., 2003).
The majority of panellists deemed the steam-cooked bighead carp (*Hypophthalmichthys nobilis*) just right (69%) to somewhat too weak (16%) in odour in consumer perception testing led by Freeman (1999). Anyway average hedonic score resulted higher for steamed carp than for the oven-baked one, so that 64% of the panellists liked very much or moderately appearance and flavour of steamed carp.
However steamed fish seems to develop a poor or rich organoleptic profile in relation to the fillet original composition and the extent of cooking loss. The weak odour of steamed horse mackerel in comparison with grilled and fried

could be explained by the concentration at lower levels of alanine, aspartic acid, glutamic acid, glycine, and proline which are responsible for flavour and taste in seafood and seafood products (Ruiz-Capillas & Moral, 2004).

1.1.3 Baking

Baking, or roasting for meat, occurs by means of forced or free convection air heating up from the elements in the oven walls. Some heat is also released by radiant energy from the walls and by conduction from the hot pot surface. Heat penetrates inside the fillet from the surface, while water moves towards the surface and it is gradually removed by evaporation. The air layer surrounding the fish increases the resistance to heat transfer inside and reduces water migration outside the fillet (Fellows, 2000).

Forced convection baking is undoubtedly a more energy efficient option than free convection as forced hot air removes the steady air layer, quickening the heat exchange.

When water loss rate on the fillet surface is greater than the water migration rate towards the surface, the evaporation front moves inside the fillet, the surface dries, overheats and equalizes oven temperature developing the crust. The crust makes baked food more crunchy and firmer (Fellows, 2000). If heating is fast, the crust can preserve nutrients, preventing water and lipid dripping and minimizing proteins degradation. On the contrary slower heating allows the leak of big amounts of water before crust development, making food more dehydrated.

However water losses are not generally too much after baking. Tokur (2007) obtained the highest moisture content (66.11±0.97%) by oven-baking rainbow trout fillets wrapped with an aluminum foil in comparison with frying (63.55±0.50%), barbecuing (63.14±0.31%) and smoking (61.14±0.55%) fillets. Weber et al. (2008) found the moisture content of baked silver catfish (70.2±0.46%) is similar to boiled and microwaved samples but significantly higher than fried (45.4±1.44-47.0±1.50%) and grilled (64.9±1.01%). Similarly Türkkan et al. (2008) in baked sea bass (*Dicentrarchus labrax*) registered a moisture value (66.47±3.08%) intermediate between fried (62.90±4.47%) and microwave-cooked fish (69.29±0.38%).

With regard to nutrients, baked fish generally shows the highest retention (Unusan, 2007; Weber et al., 2008; Wu & Mao, 2008; Chukwu, 2009; Larsen et al., 2010). Neverthless these remarks are not completely supported by other studies (Larsen et al., 2007; Mattila et al., 1999; Türkkan et al., 2008). Wu & Mao (2008) found that protein and ash contents (g/100 g dry weight) increase significantly ($P<0.05$) in baked grass carp on the contrary to fat, that probably exudes with water. From raw to baked samples they also found significant changes for amino acids like aspartic acid, threonine, glutamic acid, valine, isoleucine, phenylalanine, lysine and histidine, even if drying process had no a negative influence on the amino acid score of the fish.

Chukwu (2009) revealed a significant increase ($P<0.05$) in both protein and fat levels (g/100 g of wet weight) of baked tilapia, even if smoking kiln-dried samples retained higher lipids indicating that the fat loss was more intense in the electric oven-dried fish than in smoking kiln-dried samples. Such results were confirmed by Unusan (2007) who detected a significant increase (g/100 g) on protein and fat in baked rainbow trout, with less fat concentration in conventional oven than microwaved cooked fish. Contrary to what was found by Wu & Mao (2008) in grass carp, rainbow trout does not seem to undergo amino acids losses.

On the contrary, Larsen et al. (2007) registered significant losses (TR%) of taurine, glycine, alanine and creatine in baked cod, though glycine, alanine and creatine showed the highest true retention after baking rather than after other cooking methods (frying, boiling, brining+frying, brining+boiling and brining+baking).

Vitamin D content, as fat-soluble vitamin, seems to be correlated to fat content of each fish species (Mattila et al., 1999). In the case of one lot of Baltic herring (*Clupea harengus membras*), in which the fat content was high (15.4%), the loss of cholecalciferol was exceptionally high (23%), probably because it was lost in dripping fat during baking (Mattila et al., 1999). On the contrary, species like perch (*Perca fluviatilis*), vendance (*Coregonus albula*) and rainbow trout, having a lower fat content, showed higher retentions of vitamin D (Mattila et al., 1999).

Baking seems marginally to affect fatty acids profile on species like silver catfish (Weber et al., 2008), King salmon (Larsen et al., 2010) and Nile tilapia (de Castro et al., 2007) for which only saturated fatty acids significantly concentrated (Figure 5).

Also in rainbow trout baking did not affect the percentage of major fatty acids (Jittinandana et al., 2006).

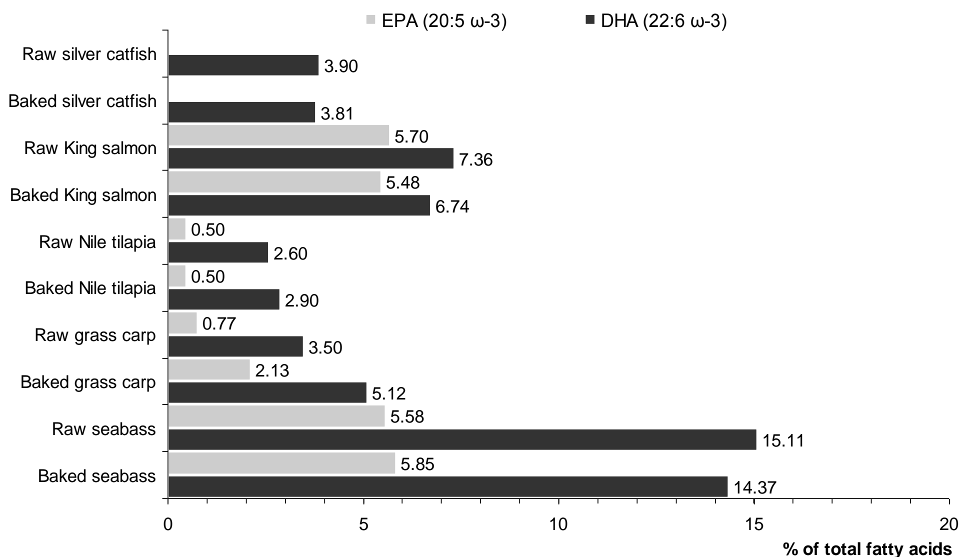
Different species gave different results after baking. de Castro et al. (2007) detected a significant increase ($P<0.05$) in saturated fatty acids of roasted skinned carp and tambacu, while monounsaturated decreased. In particular the former species showed 25.3 (raw) *vs* 26.7% (on total fatty acids) of saturated and 59.4 (raw) *vs* 57.7% of monounsaturated fatty acids. Even if the polyunsaturated fatty acids did not change, DHA (C22:6ω-3) greatly reduced the same.

The greatest increases in long chain ω-3 fatty acids concentrations took place in baked and microwave-cooked vendace, where about 70% increases were found, when calculated on a dry weight basis, according to Ågren & Hänninen (1993).

On the contrary, in baked grass carp both saturated and monounsaturated fatty acids significantly decreased ($p<0.05$), ranging from 32 (raw) to 30.6% and 28 (raw) to 21.5% (on total fatty acids), respectively. Polyunsaturated significantly concentrated ($p<0.05$) after drying processes (from 33.3 to 41.1%), especially linoleic acid (C18:2ω-6), DHA (C22:6ω-3) and EPA (C20:5ω-3) (Wu & Mao, 2008) (Figure 5).

Türkkan et al. (2008) found that in sea bass saturated and monounsaturated fatty acids content decreased after baking, while ω-3 and ω-6 polyunsaturated fatty acids showed an opposite behaviour. The former significantly reduced ($p<0.05$) from 21.22±0.20 (raw) to 20.44±0.14% and the latter increased from 10.50±0.02 (raw) to 13.58±0.06% (Figure 5).
Air drying and high temperatures, until 250°C in the domestic oven, expose fillet to a strong oxidative damage. Such detrimental effects can be seen by the accumulation of oxidation products, mainly from secondary oxidation pathways.

Figure 5 - Variation in LC-PUFA ω-3 content after baking (de Castro et al., 2007; Weber et al., 2008; Wu & Mao, 2008; Türkkan et al., 2008; Larsen et al., 2010).



The highest peroxides content (meq active oxygen per kg lipid) was determined with baking (31.45±1.17) when compared with frying (15.9±1.17), barbecuing (19.29±0.74) and smoking (4.81±1.00) in rainbow trout fillets (Tokur, 2007).
Regarding the secondary oxidation products, baked rainbow trout fillets resulted better (5.78±0.94) than barbecued fillets (8.40±0.51) for TBA value (mg malonaldehyde/kg fish muscle) but significantly worse than fried (4.98±0.74) and smoked (1.82±0.16) ones (Tokur, 2007).
In the case of silver catfish, there was no difference in the peroxide value of boiled, baked or grilled fillets when compared to the raw ones, while a significant increase ($p<0.05$) in TBA values (mg malonaldehyde per kg fish muscle) was observed only after baking (Weber et al., 2008), reflecting the extent of secondary oxidation.

Grass carp fillets significantly decreased in peroxides content both after hot air and microwave baking, but anisidine values significantly increased only in hot air baking, probably because the faster decomposition of hydroperoxides that takes place in this cooking method (Wu & Mao, 2008).
Anyway the lipid stability of baked fish can be affected by the presence of antioxidative agents in dietary treatment. Jittinandana et al. (2006) compared the results between rainbow trout fed with low and high dietary vitamin E supplementation showing a significant difference in TBA after baking (0.67 vs 1.20 mg MDA/kg).
Several molecules responsible for flavour and colour develop during oven-baking. Baltic herring fillets 1-2 days old have a rich organoleptic profile after baking (Aro et al., 2002).
Anyway the most significant effect on flavour was from compounds found in low concentrations like the eight-carbon alcohols, 1,5-octadien-3-ol and 2,5-octadien-1-ol, enzymatically derived from eicosapentaenoic acid (C20:5ω-3) via lipoxygenase action, and responsible for a green and plantlike aroma (Aro et al., 2002), mushroom and geranium-like (Methven et al., 2007).
On the contrary heptadecane (13.81±3.09 *vs* 21.43±7.32 on area %, in raw and cooked, respectively) and 1-heptadecene (22.48±20.39 *vs* 29.51±15.54 on area %, in raw and cooked, respectively) did not have a significant effect on flavour, even if they were the most abundant compounds. Other abundant compounds were 1-pentadecene, nonanal, decanal, and short chain acids (acetic, butanoic, and 3-methylbutanoic).
In particular short chain acids, deriving form aldehydes oxidation and microbial fermentation and partially causing unpleasant odour, increased in herring when baked after storage (48 hours) (Aro et al., 2002).

1.1.4 Frying

In frying the cooking mean is oil. Frying oil reaches temperatures of 200-220 °C, that are clearly higher than boiling water temperature. In such conditions water oil transfers heat to water content of fish, inducing it to evaporate on the surface of the fillet. Oil is an excellent heat transfer medium (Stier, 2000) and the fried food peculiarities, such as crunchiness, acceptable level of oil absorbed by the food and desired delicious odour can be strongly affected by the rapid cooling of the oil. As rapid cooling takes place when fillets are thin and oil viscosity is low, such conditions should be avoided to preserve food crunchiness.
Oil enhances water evaporation on the fillet surface, mainly on the side in contact with the pan, so that water content after frying is lower than in the other cooking methods.
Weber et al. (2008) found that moisture content greatly reduced ($p<0.05$) after frying with soybean (45.4±1.44%), canola (47.0±1.50%) and hydrogenated vegetable oil (46.9±2.30%), if compared with raw (79.6±0.82%), boiled

(74.9±0.17%), oven-baked (70.2±0.46%), microwaved (71.4±0.29%) and grilled (64.9±1.01%) rainbow trout fillets.

This results were confirmed by Türkkan et al. (2008) who detected the most significant change in moisture after frying with sunflower oil (62.90±4.47%) than after baking (66.47±3.08%) and microwaving (69.29±0.38%) in sea bass fillets.

Similarly salmon lost more water after frying with olive (57.7±1.07%), corn (58.0± 0.43%) and partially hydrogenated plant oil (60.2±0.14%) than after pan cooking without oil (59.0±0.21%) and steaming (62.3±0.31%), according to Al-Saghir et al. (2004).

The lowest moisture in salmon after frying was confirmed by Larsen et al. (2010) and the same behaviour was obtained in wall-eye pollock (*Theragra chalcogramma*) (Danowska-Oziewicz et al., 2007) and breaded black pomfret (*Parastromateus niager*) (Moradi et al., 2009) as well.

The oil absorption during the cooking process considerably modifies the initial fish composition. The extent of the changes depends on several aspects as fish lipid content, presence or less of the skin during frying, oil composition, amount and temperature and the frying length. Fish lipid content seems the limiting factor to oil absorption (Sioen et al., 2006). Fatty fish, like salmon, absorb less oil than lean fish, and their lipid content does not significantly seem to change. This has been verified by Larsen et al. (2010) who did not find any significant difference between raw (21.61±3.84%), pan fried (23.14±2.67%) and deep fried (26.30±1.67%) lipid content in King salmon. Similarly Al-Saghir et al. (2004) did not reveal any change on salmon total lipid before (15.6±0.66%) and after frying with olive (18.0±1.85%), corn (18.6±0.13%) and partially hydrogenated plant oil (16.2±0.47%), respectively.

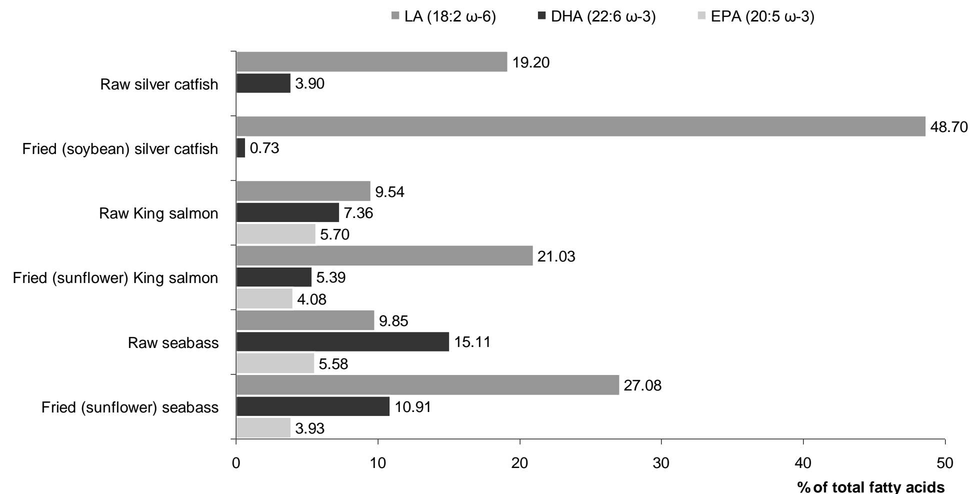
On the contrary, the results obtained by some studies demonstrate that lipid content significantly increases ($P<0.05$) on rainbow trout, which ranged from 3.88±0.08 (raw) to 6.16±0.63% (cooked) (Tokur, 2007) and 3.44±0.013 (raw) to 12.70±0.08% (cooked) on wet-weight basis (Gokoglu et al., 2004).

Studies on catfish revealed that total lipid raises from 2.51±0.45 (raw) to 14.0±0.45, 13.0±0.42 and 14.1±1.13% on wet-weight basis, after frying in soybean, canola and hydrogenated vegetable oil, respectively (Weber et al., 2008) and from 5.7±0.8 to 11.9±1.8% after deep-frying (Wu & Lillard, 1997), supporting the absorption of frying oil by samples. The same behaviour has been found on African catfish (*Clarias gariepinus*) by Ersoy & Özeren (2009).

Changes in fatty acids of fried fish occurred due to fatty acids uptake from the frying oil (Larsen et al., 2010) so that fish fatty acids profile tends to be like the oil one. Especially frying oil largely determines the fatty acid composition of lean fish, stressing the importance of frying oil selection (Ågren & Hänninen, 1993), but changes also occur from the loss of fish lipids into the frying oil (Larsen et al., 2010).

The higher percentage of linoleic acid (C18:2ω-6) from soybean oil (52.0±0.02% of total fatty acids) is responsible for the significant increase of polyunsaturated ω-6 fatty acids in silver catfish fillets, from 29.0±1.10 to 55.1±0.30% of total fatty acids (Weber et al., 2008) (Figure 6). In the same way the higher percentage of oleic acid (C18:1) from canola oil (72.5±0.76% of total fatty acids) brings from 34.2±0.98 to 74.8±0.64% the monounsaturated fatty acids percentage on total fatty acid content of fillets (Weber et al., 2008).

Figure 6 - Variation in LC-PUFA ω-3 and linoleic acid (C18:2 ω-6) content after frying (Weber et al., 2008; Türkkan et al., 2008; Larsen et al., 2010).



Both sea bass (Türkkan et al., 2008) and King salmon (Larsen et al., 2010) fatty acids profile increased in linoleic acid (18:2ω-6) content after frying with sunflower oil, respectively of 9.85±0.07 *vs* 27.08±0.04% and 9.54±0 *vs* 21.03±0.03% of total fatty acids (Figure 6).

The addition of hydrogenated vegetable oils, considered among the main risk factors of coronary disease, sudden death and possibly diabetes mellitus (Mozaffarian et al., 2006b), can be easily detected by the presence of trans fatty acids on fried fillet composition (Weber et al., 2008).

Generally DHA (C22:6ω-3) and DPA (docosapentaenoic acid, C22:5ω-6) levels are significantly reduced after frying (Weber et al., 2008; Larsen et al., 2010) because of the oxidation and the absorption of the oil not containing these fatty acids.

Some liposoluble compounds elute in the frying oil. According to Wu & Lillard (1997), cholesterol decreases from 262.3±15.8 to 162.6±14.0% on a dry weight basis. Instead Ersoy & Özeren (2009) found that liposoluble vitamins A and E (expressed as g/100g) significantly concentrate ($p<0.05$) after frying on African catfish. With regard to the hydrosoluble compounds, the same Authors

registered a significant decrease ($p<0.05$) of B1, B2, niacin and B6 vitamins which are more heat-labile than liposoluble vitamins.
Mineral content (Na, K, Ca, Mg, Fe, Zn, Mn and Cu, expressed as mg/kg) always was significantly higher than in raw fillet. Concerning the oxidative effects of frying, the studies show contrasting results. High temperatures accelerate oxidation but oil dissolves oxidation products, reducing their concentration on fillet and making the oxidative damage difficult to assess (Weber et al., 2008).
The low levels of conjugated dienes (CD), peroxides and malonaldehyde, if apparently could lead to think of a smaller oxidation, actually seem to depend on the more rapid evolution of the oxidative mechanism taking place in this kind of cooking method. The CD and peroxides values decreased for all fried (215-220 °C) samples of silver catfish, when compared with grilled, boiled, oven and microwave baked ones, probably because of their decomposition into secondary oxidation products (Weber et al., 2008). In contrast, no significant differences were observed in the TBARS value between raw and fried samples and Weber at al. (2008) suggested that the MDA eventually formed could have been lost either by dissolution in the frying oil or due to formation of adducts with proteins.
Temperature seems noticeably to affect oxidation, much more than in other cooking methods. Salmon fried with olive, corn or vegetable hydrogenated oil at 180 °C does not bring to a wide oxidative damage, and the significant differences in peroxides content seem to depend on the first oxidation state and degree of unsaturation of the frying oils (Al-Saghir et al., 2004). In particular, olive oil has a high initial peroxides value (11.8±0.03 meq/kg), which provides the explanation for the increased peroxides value of salmon after pan-frying with olive oil (5.70±0.70 meq/kg) (Al-Saghir et al., 2004). In this sense, by reducing time and temperature of frying, it is possible to minimize the oxidation damage and prevent oils nutritional losses with formation of toxic compounds like acrolein.

1.1.5 Microwaving

Microwave oven for cooking has increased greatly during recent decades (Arias et al., 2003). Microwaving exploits the dipolar nature of water. High frequency microwaves emitted by the oven have the same frequency of the water molecules, so that they are absorbed by water. Each water molecule releases the absorbed energy, as heat, to the surrounding water molecules, allowing food to be cooked. Higher is the water content of the food and more efficient is the heating process. Microwave drying offers many advantages in processing, including less startup time, faster heating, energy efficiency, space savings, precise process control, selective heating and final products with improved nutritive quality (Sumnu, 2001).

Heating by electromagnetic radiation prevents large water losses to the extent that microwaving is one of the cooking methods (i.e. with baking) able to retain more water. Most of the studies show the same behavior. Microwave heating resulted in a not significant loss of water on channel catfish (*Ictalurus punctatus*), ranging from 78.8±1.4 to 76.3±1.1% (Wu & Mao, 1997).
According to Weber et al. (2008), the decrease in moisture of microwaved silver catfish, even if statistically significant ($p<0.05$), is lower than after grilling and frying with soybean oil, resulting in 71.4±0.29 *vs* 79.6±0.82 (in cooked and in raw, respectively).
Türkkan et al. (2008) found the maximum moisture for microwave-cooked sea bass, 69.29±0.38 *vs* 71.62±0.23% (raw), if compared with baked and fried ones.
In contrast with most of the studies, moisture significantly decreased in microwaved grass carp, from 77.6±1.06 (raw) to 20.4±1.35% (Wu & Mao, 2008) and rainbow trout (Unusan, 2007) resulting in an increase in protein, fat and ash contents.
Similarly to steaming, liposoluble substances and protein losses are extremely low in microwaving. Wu & Lillard (1997) found that both microwave heating and oven baking result in a minor loss of fat through cook drip in catfish. On the same species Weber et al. (2008) did not register a significant change in fat as in baked, grilled and boiled fish. Microwave-dried fillets retained a higher fat content than hot air-dried samples (6.99±0.17 *vs* 6.43±0.14%; $P<0.05$) in grass carp (Wu & Mao, 2008).
A significant net increase in fat and protein level was detected after microwaving in rainbow trout. Changes in amino acids pattern improved the cholesterolemic ratio arginine/lysine bringing it from 0.54 (raw) to 0.77 and suggesting the most cardiosalutable rainbow trout fillets are the microwave-cooked ones (Unusan, 2007).
Besides when expressed on a dry weight basis, the cholesterol concentrations in microwaved and oven baked fillets were about twice than in deep-fat fried fillets in channel catfish (Wu & Lillard, 1997).
Most minerals (Na, K, Ca, Mg, Zn and Mn) and vitamin E significantly concentrated ($p<0.05$) after microwaving in African catfish analysed by Ersoy & Özeren (2009), that nevertheless demonstrated that vitamin B1, niacin and B6 reduce after microwaving as after frying but, in spite of frying, the microwaving reduces also vitamin A content.
Wu & Mao (2008) suppose that the high temperatures accompanying drying processes could speed up the breakdown of peroxides into their carbonyl components, and thus the peroxide value, and more generally primary oxidation products may remain low. For this reason, conjugated dienes and peroxides do not differ from both raw silver catfish (Weber et al., 2008) and grass carp fillets (Wu & Mao, 2008), while a significant increase in the TBARS value (mg MDA/kg meat) in catfish and anisidine value in carp were observed in the samples baked in microwave and conventional ovens.

The fatty acids profile significantly changed ($p<0.05$) in grass carp (Wu & Mao, 2008) showing a decrease in saturated and monounsaturated fatty acids, mainly affected by the loss of lauric acid (C12:0) and oleic acid (C18:1), respectively. On the other hand polyunsaturated fatty acids concentrated from 16.5±0.18 to 22.6±0.40% of total fatty acids, mainly linolenic acid (C18:3ω-3). Only saturated fatty acids decreased, especially palmitic acid (C16:0), while polyunsaturated fatty acids were not influenced by microwaving in rainbow trout (Unusan, 2007).
With regard to the effect of cooking on nutritional content of fish, it is clearly evident that is difficult finding common elements in rendering the effects of each cooking method. The results from research studies greatly differ from each other preventing an univocal opinion. In particular such discrepancies are useful to avoid rough interpretation of the nutritional impact of each cooking method, in relation to the heating mean used.
The results described above show as in cooking fish it is not enough preferring mild methods like steaming or microwaving to prevent nutritional losses or reduce free radicals development. Temperature applied and cooking length, in addition to the intrinsic qualitative traits of raw fish, should be the most important aspects to consider in cooking fish.

1.2 Effects of the most common cooking methods on texture and color of fish

It is known that cooking consistently modifies physical properties of food. In particular heating is able to change textural properties of fillets and colour, on which overall sensorial perception is based.

1.2.1 Effect of cooking on texture

The properties and concentrations of the structural elements in the fish tissue and their arrangements in the muscle influence the textural properties and mouth feel (Larsen et al., 2011). Myofibrillar and connective proteins are the two types of proteins mainly affecting texture parameters. Anyway because of the low content of connective tissue in fish coupled with the low thermal stability of collagen, the textural properties of fish are dependent predominantly on the state of myofibrillar proteins (Larsen et al., 2011).
Texture has been tested in several fish species applying many different cooking methods. Skinless vacuum packed fillets of Atlantic salmon were placed into a water bath containing boiling water (Yagiz et al., 2009). When the centre temperature of fillets reached 72 °C, they were removed to be analysed for textural parameters. Cooking reduced hardness[1] (13.0 *vs* 19.4N), cohesiveness[2]

[1] Hardness: the force that is required to bite through the sample with incisors (Aussanasuwannakul et al., 2010).

(0.13 *vs* 0.14), gumminess[3] (1.6 *vs* 2.7 N) and chewiness[4] (6.4 *vs* 9.3N*mm) values compared to control samples.
According to Larsen et al. (2011), the reason why the cooked King salmon samples had firmer textures than raw New Zealand King salmon was in part due to myofibrillar toughening during heating. Steaming (0.93 N) gave the softest texture among all the cooking methods although the diference was not statistically significant ($p>0.05$). The highest hardness of pan and deep fried samples (1.93 and 2.52 N) was attributed to the formation of a 'crust' after dehydration in the outer layers. Springness[5] increased in pan fried and deep fried fillets (0.36 and 0.52), while chewiness was the lowest in steamed and poached samples (0.27 and 0.27). The instrumental measurements were linked with sensory analysis finding that the cooking methods that were rated as the most intense and the least intense, deep frying (7.53) and poaching (2.93), respectively, were both the least liked (4.96 and 4.56 on a 10 cm line scale).
In particular poached samples were not liked since they were rated the least intense in flavour, texture, aroma and colour. Surface dehydration characterizing fried food induces the formation of the crust, thicker, more coloured and crunchier than those developing after roasting. The molecules responsible for such attributes (i.e. furfurals, furanones and pirroles) are mainly final and intermediate products of the Maillard reactions which start at frying temperatures (Methven et al., 2007).
Moradi et al. (2009) evaluated the effects induced by pre-frying in sunflower oil (SO) and palm olein (PO) followed by microwaving, baking and frying on breaded black pomfret. Most of the texture profile analysis (TPA) parameters of pre-fried were significantly ($p<0.05$) changed after all types of cooking.
To the higher heating rate applied by frying was due the most pronounced crispness of the fried fillets, that is usually related to hardness. For this the biggest value of the hardness was observed in the final fried (SO: 2715.700 and PO: 2491.800 g/sec force) followed by baked (SO: 1627.200 and PO: 1685 g/sec force) and microwaved (SO: 1440.700 and PO: 1398.100 g/sec force) samples. Similarly chewiness increased, while adhesiveness[6] decreased at a higher level after frying than baking and microwaving. Cohesiveness increased in all cooked samples, less after frying, while fracturability[7] was the texture

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[2] Cohesiveness: the ability to recover from deformation and offer resistance to subsequent deformation (Moradi et al., 2009) or the ratio of the positive force area during the second compression to that during the first compression (Hyldig & Nielsen, 2001).
[3] Gumminess: the quantity to simulate the energy required to disintegrate a semi-solid sample to a steady state of swallowing.
[4] Chewiness: the lenght of time to masticate the sample to a consistency which is appropriate for swallowing at a constant rate of force application (Larsen et al., 2011).
[5] Elasticity or springiness: the degree to which the sample returns to original shape when partially compressed with the molar teeth (Aussanasuwannakul et al., 2010).
[6] Adhesiveness: the negative force area for the first bite, representing the work necessary to pull the compression plunger away from the sample (Hyldig & Nielsen, 2001)
[7] Fracturability: the force at which the material fractures (first significant break in the curve) (Hyldig & Nielsen, 2001).

characteristics found only in the final fried samples. Anyway the type of pan-frying oil employed (sunflower or palm olein) did not affect the change.
Many authors measured texture by cooking fish after storage and demonstrated that storage conditions can modify texture parameters. Rodriguez et al. (2008) used a shear test to evaluate texture in Coho salmon, that was steam cooked after 0, 5 and 9 days of chilling. No results have been reported for raw fillets.
However after cooking firmness increased from 10 to 40 N with longer chilling period, probably because of the decreased water holding capacity that is known to be inversely related to firmness. On the contrary cohesiveness showed a decrease and the more breakable structure was attributed to the denaturation of connective tissue induced by both storage time and cooking and favouring the separation of cells into flakes.
By comparing raw and smoke-baked fillets of rainbow trout, Aussanasuwannakul et al. (2010) found that smoke-baking greatly increased both firmness and energy of shear. Moreover cooked fillets which were refrigerated for 3 and 7 days followed by frozen storage at -25 °C for 30 days were stronger (329.12 and 279.54 g/g sample) than fillets only refrigerated at 2 °C for 3 and 7 days (230.82 and 246.11 g/g sample). The hardening registered in long term frozen fillets after cooking was attributed to the increased moisture loss occurring in larger extent as time of storage increases and temperature decrease. Energy of shear increased after cooking indipendently to storage time and temperature and suggested that fillets were more extensible than their raw state. Nevertheless sensorial analysis was able to detect a significant difference among cooked fillets differently stored since 30 days frozen fillets had higher elasticity than refrigerated. Also pre mortem management of farmed fish seemed to influence textural properties. A significant increase of instrumental hardness, when measured at the 12th day of chilled storage, was found from 0 (7.6 N) to 86 (10.2 N) days of starvation on raw salmon (Einen & Thomassen, 1998). However sensorial analysis which was carried on cooked samples (warmed in bag at 70 °C for 45 min) showed significant opposite results at 13-17th storage day, since the trained assessors assigned 4.6 score at 0 starvation days and 4.2 at 86th using a scale from 1 to 9 (1 = low intensity and 9 = high intensity).
According to Ginés et al. (2004), texture measurements were quite different for raw and steamed Arctic charr (*Salvelinus alpinus*) reared at two temperature levels. After cooking hardness, cohesiveness, gumminess, chewiness and adhesiveness decreased and cooking flattened the differences between fish reared at 10 and 15 °C and fracturability point was not detected any more. Even if lipid content was similar, raw fish reared at 15 °C showed higher hardness (4716.895 *vs* 3120.285 g), gumminess (37306.68 *vs* 26659.54 g), chewiness (6921.515 *vs* 4926.415 g) and fracturability (3202.525 *vs* 2087.255 g) than fish

reared at 10 °C, by compressing samples twice[8]. Moreover elasticity or springness of cooked fillets correlated ($p<0.05$) with four attributes of raw fillets [hardness ($r=0.546$), gumminess ($r=0.507$), chewiness ($r=0.500$), and fracture ability ($r=0.584$)] indicating that harder and more fracturable raw flesh showed springy texture after cooking. The results proved that the effects generated by cooking on texture are extremely various.
Frying differred from the other cooking methods developing a distinctive textural profile influenced by the formation of the crust. Except for frying, hardness resulted highly dependent on fish species since number and size of myotomes and myosepta mainly differ according to the species (and its environment and age-dependent response). Water loss showed to influence hardness more than total lipid (Ginés et al., 2004; Rodriguez et al., 2008; Aussanasuwannakul et al., 2010). However some of the cited authors (Einen et al., 1998; Yagiz et al., 2009; Larsen et al., 2011) did not analyze total lipid content on cooked samples so that such parameter cannot be used to explain the variation of hardness.
Except for breaded fried samples, all cooking methods seemed to reduce the time and energy needed for mastication as chewiness and gumminess generally decrease.

1.2.2 Effect of cooking on colour

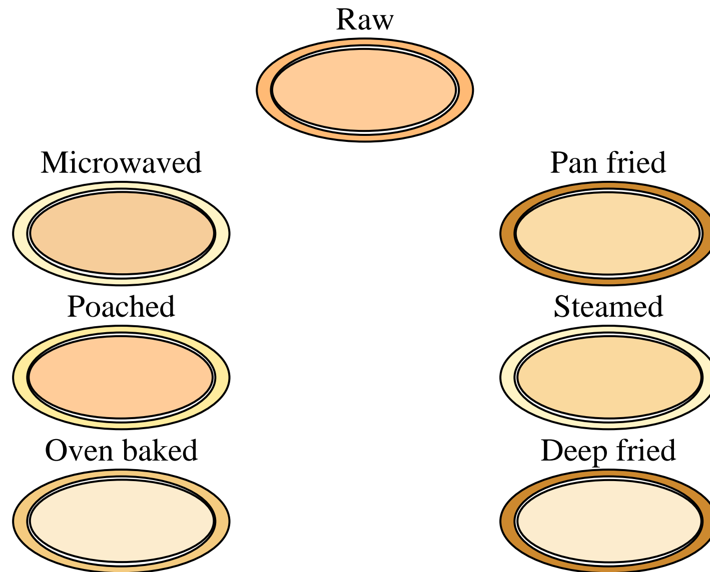
Colour and appearance of seafood are very important in terms of consumer perception of seafood quality, and are dominant factors in consumer purchasing decision (Yagiz et al., 2009). Chemical reactions and structural changes taking place during cooking can consistently affect colorimetric attributes of flesh. By inducing cellular damage, denaturation of proteins, lipid and metals oxidation and nutrients leaching, especially water, fat and liposoluble and metallic pigments, cooking can modify light absorption, refracting properties, lightness (L*), redness (a*), yellowness (b*), tint (Hue°ab) and saturation (Chroma) of the flesh.
In salmonids, cooking can affect carotenoids retention and consequently modify colour of the fillet. Moist and dry heat cooking were tested for asthaxantin and canthaxantin retention by Choubert & Baccaunaud (2010) on rainbow trout. Both asthaxantin and canthaxantin fed fish showed a decrease in L*, a*, b*, croma and Hue after cooking. Fish fed asthaxantin significantly differed from fish fed canthaxantin only for a* and Hue in both moist heat cooking (a*: 0.78 *vs* 2.44; Hue: -9.70 *vs* -12.17°) and dry heat cooking (a*: -2.48 *vs* 0.21; Hue: 12.90 *vs* 16.94°). The decrease in L* values and in b* values during the cooking process indicated that the raw fillet decreased its brightness and lost its yellow component. However cooking procedure (moist *vs* dry) did not influence the amount of carotenoids retained.

[8] The values reported are mean between the two strains (Myvatn and Grenlaekur) of Arctic charr analyzed.

A paper on dark muscle of Atlantic salmon (Yagiz et al., 2009), examined colour changes after 2, 4 and 6 days of storage at 4 °C on vacuum packed boiled fillets. L* value for dark muscle increased slightly after cooking (46.0 *vs* 76.1); however, storage time did not show any major difference in L* values. Also boiling showed higher b* values (16.9 *vs* 21.2) but lower a* values (29.0 *vs* 10.1) for dark muscle compared to control samples.
During frying the development of colour is the result of the chemical browning reactions of reducing sugars and protein sources (known as Maillard reactions) and, to a lesser extent, of the absorption of frying oil and density and thickness of the batter coating. Cooked fish are generally low in typical Maillard reaction flavor volatiles, such as pyrazines, in comparison to levels in most meats, probably due to lower levels of free sugars (Methven et al., 2007). Anyway high molecular weight polymers, called melanoydines and forming through the condensation of cyclic products from Maillard reaction (Ames, 2003) confer to the crust a typical gold-yellow colour.
According to Moradi et al. (2009), pan fried on breaded black pomfret underwent to consistent colour changes after cooking. The b* values increased and L* values decreased after all cooking methods (microwaving, baking and frying). In particular, baking samples had higher L*, a* and b* values compared to the other cooking methods, while the type of oil influenced b* values that was higher by frying with sunflower oil (38.37) than palm oil (36.35).
Larsen et al. (2011) applied several cooking methods (poaching, pan frying, microwaving, oven baking, steaming and deep-frying) on the outer and the inner part of King salmon fillets (Figure 7). For all methods the cooked fillets were lighter, more red and more yellow than raw as reflected by increases in L*, a* and b* values. Heating, inducing protein aggregation, is considered responsible for the reduction of the optical path length in the flesh, so that the light that enters the surface has a lower chance of being selectively absorbed and lightness (and opacity) increases. Non enzymatic browning induced an overall increase of redness (a*). Deep frying and pan frying with sunflower oil darkened the colour of the outer part of the fillet as they showed the lowest L* (56.27 and 57.46) and the highest a* (24.75 and 22.68) values.
More yellow colour was detected on oven baked samples having the highest b* (30.19) followed by pan fried (29.12) and microwaved (27.04). The highest L* value (73.91 and 72.61) and the lowest a* (19.65 and 18.78) and b* (25.82 and 25.05) values were attributed to poaching and steaming. For the inner fillets, the deep fried and oven baked King salmon had the highest L* values (75.05 and 75.38), followed by pan fried (74.19). The poached New Zealand King salmon had the highest a* (22.66) and b* (25.25) values and conversely the microwaved samples had the lowest, but differences between raw and cooked samples were not significant.
Correlation between instrumental colour measurements and the sensory evaluation was not possible as only colour intensity was tested by Larsen et al.

(2011). However the trend showed that the cooking methods which made to become lighter the fillets (poaching and steaming) were the least liked. Consumers seemed to prefer high values of redness like after deep frying, pan fried, baking and microwaving, since fish received 6.26, 6.13, 6.92 and 5.33 of degree of liking on a 10 cm line scale, respectively, when cooked by these last methods.

Figure 7 – Fillet colour in the outer and inner part of King salmon according to Larsen et al. (2011)



Similar L* values were found by Andrés-Bello et al. (2009) on sea bream fillets after steaming at 70, 80 and 90 °C in vacuum conditions (cook-vide) and at 100 °C in atmospheric pressure during 0, 3, 6, 9, 12, 15 and 20 min. Cooking increased L* from 50 to 70-80, independently to the time and temperature of treatment. On the contrary a* increased as time of treatment increased and b* values increased with treatment temperature. No significant differences in color attributes were observed between cook-vide and atmospheric pressure cooking, even if consistency (stiffness) of the flesh was clearly better in 70 °C cooking.
Days of starvation did not significantly affect colour intensity on cooked salmon as the assessors assigned scores in the range 4.2-5.0, according to the 1-9 scale previously reported for texture (Einen & Thomassen, 1998).
To sum up, change in colour of cooked fillets follow opposite trend between pigmented and non-pigmented species. Salmonids tend to lose pigments during cooking and consequently undergo decreases in a* and b* values, showing contrasting results of L* that is also strongly affected by the level of protein denaturation as much as by lipid and water retention. In non pigmented species,

cooking generally induces an increase of L*, a* and b* mainly attributable to the variation in light absorption caused by the denaturated proteins.
Similarly to texture, frying gives different results for colorimetric attributes. In particular the formation of the crust generally darkens the colour of fried fish by lowering L*.

1.2.3 Effects of the most common cooking methods on contaminants of fish

Several kind of contaminants are taken by consuming seafood and European Commission introduced law limits for those contaminants which are a health concern and significantly contribute to total dietary exposure in seafood [Reg. (EC) 1881/2006 and its subsequent amendments] (Table 3) and for biotoxins [Reg. (EC) 853/2004 and its subsequent amendments].
Species, season, diet, location, lifestage and age have a major impact on both the nutrient and contaminant levels of fish (EFSA, 2005). Most of the contaminants are liposoluble molecules that are stored in the lipid deposit of fish. For this reason the variation of storage lipids according to season, lifestage and fish size (Ferreira et al., 2010), greatly affects the presence of toxicants, reaching maximum levels prior to maturation (EFSA, 2005). Moreover, differences in anatomical distribution of fat is a further factor influencing the level of contaminants in edible tissues (EFSA, 2005). For instance, cod tends to accumulate fat in liver, while salmon store most fat in the abdominal peritoneal lipoid tissue, the intermyotomal fascia and particularly in the dermis of the skin.
Also diet represents the main source of exposure of a wide range of contaminants, especially in wild fish that are higher in the food chain (EFSA, 2005). However contaminants were often found in farmed fish as well (Hites et al., 2004; Ferreira et al., 2010), and dioxins (PCDD/Fs) and dioxin-like PCBs (DL-PCBs) are among the most harmful substances present in fish oil and meal, at higher levels when produced by fish caught in the North Atlantic than in the South Pacific (Hites et al., 2004; EFSA, 2005).
Since chemical contaminants principally derive from fish meal and fish oil in farmed fish, the replacement by vegetable protein and oil in fish feed may be a possible means of reducing some contaminants level (EFSA, 2005). Nevertheless the extent of replacement is still a controversial matter in aquaculture, since replacement of marine-derived ingredients by vegetable sources should not affect physiological need of the species nor lead to any adverse effects on digestibility, growth, nutritional quality or organoleptic properties of flesh (EFSA, 2005). Moreover practicality of different procedures for reducing contaminant levels in feed, e.g. by activated carbon treatment, combined with stripping at low pressure and low temperature (De Kock et al., 2004) or short path distillation (Breivik & Thorstad, 2004), needs to be demonstrated as well as the efficiency for the removal of different

contaminants/congeners and maintenance of the nutritional quality of the oil (EFSA, 2005).
Among the farmed fish species, salmon often showed high concentration of several toxicants so that consumption of farmed Atlantic salmon may introduce risks detracting from the beneficial effects of fish consumption. Hites et al. (2004) detected a higher amount of total PCBs, dioxins, toxaphene, and dieldrin in the farmed salmon than in the wild salmon. In particular salmon produced in Europe had significantly higher contaminant levels than those produced in both North and South America, owing to the greater incidence of contaminants in the diet of European salmons.
Also in the muscle of European seabass PCBs and DDT levels were higher in cultured than in wild specimen. On the contrary metals, like Cu, Pb, As and Cd, and PAHs were found to be more abundant in wild seabass (Ferreira et al., 2010).
According to the feeding regime (i.e. carnivorous, herbivorous, omnivorous) each species needs varying ingredients in the compound feed so that DL-PCBs transferred into fish may be up 98% in the carnivorous diet (EFSA, 2005). Moreover biotransformation and/or preferential accumulation of certain congeners led to differences in the congener patterns between feeds and fish (Isosaari et al., 2005). Among polybrominated diphenyl ethers (PBDEs), BDE 209 is accumulated less than 5.2% in rainbow trout, while BDE 47 exceeds 90% in pike (*Esox Lucius*) (EFSA, 2005). Among PCDD/Fs, tetra- and pentachlorinated congeners were found to be preferentially accumulated in salmon, while hepta- and octachlorinated dibenzo-p-dioxins were excreted into the feces.
Contaminants can be exogenous or endogenous. A consistent group of exogenous substances is constituted by toxicants deriving from anthropic activity. Fish can be contaminated by chemical substances due to environmental pollution (metals, organochlorine contaminants) or by biochemical toxic substances deriving from harmful microorganisms, such as algae (i.e. dinoflagellates). Endogenous contaminants can develop during processes, especially during heating treatments.
Even if intakes of contaminants have been calculated from data in the raw product, the effect of cooking on contaminants has been tested by several researches. Some studies investigated the ability of cooking to retain environmental contaminants, while others analysed the endogenous formation of toxic substances.

Table 3 – Annex to the Regulation (EC) N. 1881/2006 setting maximum levels for certain contaminants in foodstuffs (part concerning seafood)

	Foodstuffs	**Contaminant**	**Maximum levels**	**Unit**
3.1.5	Muscle meat of fish	Lead	0.30	mg/kg wet weight
3.1.6	Crustaceans: muscle meat from appendages and abdomen. In case of crabs and crab-like crustaceans (*Brachyura* and *Anomura*) muscle meat from appendages.		0.50	mg/kg wet weight
3.1.7	Bivalve molluscs		1.5	mg/kg wet weight
3.1.8	Cephalopods (without viscera)		1.0	mg/kg wet weight
3.2.5	Muscle meat of fish excluding species listed in points 3.2.6, 3.2.7 and 3.2.8	Cadmium	0.050	mg/kg wet weight
3.2.6	Muscle meat of the following fish: bonito (*Sarda sarda*) common two-banded seabream (*Diplodus vulgaris*) eel (*Anguilla anguilla*) grey mullet (*Mugil labrosus labrosus*) horse mackerel or scad (*Trachurus species*) louvar or luvar (*Luvarus imperialis*) mackerel (*Scomber species*) sardine (*Sardina pilchardus*) sardinops (*Sardinops species*) tuna (*Thunnus species, Euthynnus species, Katsuwonus pelamis*) wedge sole (*Dicologoglossa cuneata*)		0.10	mg/kg wet weight
3.2.7	Muscle meat of bullet tuna (*Auxis species*)		0.20	mg/kg wet weight
3.2.8	Muscle meat of the following fish: anchovy (*Engraulis species*) swordfish (*Xiphias gladius*)		0.30	mg/kg wet weight
3.2.9	Crustaceans: muscle meat from appendages and abdomen. In case of crabs and crab-like crustaceans (*Brachyura* and *Anomura*) muscle meat from appendages.		0.50	mg/kg wet weight
3.2.10	Bivalve molluscs		1.0	mg/kg wet weight

3.2.11	Cephalopods (without viscera)			mg/kg wet weight
3.3.1	Fishery products and muscle meat of fish, excluding species listed in 3.3.2. The maximum level for crustaceans applies to muscle meat from appendages and abdomen. In case of crabs and crab-like crustaceans (*Brachyura* and *Anomura*) it applies to muscle meat from appendages.	Mercury	0.50	mg/kg wet weight
3.3.2	Muscle meat of the following fish: anglerfish (*Lophius species*) Atlantic catfish (*Anarhichas lupus*) bonito (*Sarda sarda*) eel (*Anguilla species*) emperor, orange roughy, rosy soldierfish (*Hoplostethus species*) grenadier (*Coryphaenoides rupestris*) halibut (*Hippoglossus hippoglossus*) kingklip (*Genypterus capensis*) marlin (*Makaira species*) megrim (*Lepidorhombus species*) mullet (*Mullus species*) pink cusk eel (*Genypterus blacodes*) pike (*Esox lucius*) plain bonito (*Orcynopsis unicolor*) poor cod (*Tricopterus minutes*) Portuguese dogfish (*Centroscymnus coelolepis*) rays (*Raja species*) redfish (*Sebastes marinus, S. mentella, S. viviparus*) sail fish (*Istiophorus platypterus*) scabbard fish (*Lepidopus caudatus, Aphanopus carbo*) seabream, pandora (*Pagellus species*) shark (all species) snake mackerel or butterfish (*Lepidocybium flavobrunneum, Ruvettus pretiosus, Gempylus serpens*) sturgeon (*Acipenser species*) swordfish (*Xiphias gladius*) tuna (*Thunnus species, Euthynnus species, Katsuwonus pelamis*)		1.0	mg/kg wet weight
5.3	Muscle meat of fish and fishery products and products thereof, excluding eel. The maximum level for crustaceans applies to muscle meat from appendages and abdomen. In case of crabs and crab-like crustaceans (*Brachyura* and *Anomura*) it applies to muscle meat from appendages	Sum of dioxins (WHO-PCDD/F-TEQ)	4.0	pg/g wet weight
		Sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ)	8.0	pg/g wet weight

5.4	Muscle meat of eel (*Anguilla anguilla*) and products thereof	Sum of dioxins (WHO-PCDD/F-TEQ)	4.0	pg/g wet weight
		Sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ)	12.0	pg/g wet weight
5.10	Marine oils (fish body oil, fish liver oil and oils of other marine organisms intended for human consumption)	Sum of dioxins (WHO-PCDD/F-TEQ)	2.0	pg/g fat
		Sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ)	10.0	pg/g fat
5.11	Fish liver and derived products thereof with the exception of marine oils referred to in point 5.10	Sum of dioxins (WHO-PCDD/F-TEQ)	-	pg/g wet weight
		Sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ)	25	pg/g wet weight
6.1.4	Muscle meat of fish, other than smoked fish	Benzo(a)pyrene	2.0	µg/kg wet weight
6.1.5	Crustaceans, cephalopods, other than smoked. The maximum level for crustaceans applies to muscle meat from appendages and abdomen. In case of crabs and crab-like crustaceans (*Brachyura* and *Anomura*) it applies to muscle meat from appendages		5.0	µg/kg wet weight
6.1.6	Bivalve molluscs		10.0	µg/kg wet weight

1.2.4 Effect of cooking on exogenous contaminants from environmental pollution

Contaminants from environmental pollution, such as methylmercury, persistent organochlorine compounds, brominated flame retardants and organotin compounds, are among the most important toxicants found in fish.

Mercury (Hg) is a persistent toxic pollutant that bioaccumulates and biomagnifies through food chains (Torres-Escribano et al., 2011). Methylmercury is the organic form, more toxic than other forms of mercury after ingestion and accumulating appreciably in fish (Kamps et al., 1972; Joiris et al., 1999; Storelli et al., 2002; Costa, 2007).

Methylmercury is known to be readily absorbed and easily crosses the placentar barrier and the blood-brain barrier (Costa, 2007). It interacts with nervous system and high exposure during pregnancy has resulted in cerebral palsy, blindness or severe mental retardation of the neonate (EFSA, 2005; Costa, 2007). Recent studies have also hypothesized that methylmercury represents a risk factor for cardiovascular disease and atherosclerosis by promoting the formation of free radicals and lipid peroxidation, by binding thiols and inactivating the antioxidant properties of glutathione (Salonen et al., 2000; Guallar et al., 2002; Virtanen et al., 2007 as cited by Costa, 2007).

As methylmercury moves from one trophic level to the next higher trophic level, large old predatory fish as sharks, swordfish, tuna and pike may contain the highest methylmercury level, exceeding 1.0 mg/kg (Costa, 2007). For this reason various federal and state agencies (European Food Safety Authority, US Food and Drug Administration and US Environmental Protection Agency) recommend population groups at risk (pregnant women, young children and breastfeedingwomen) to avoid or reduce consumption of predatory fish (Torres-Escribano et al., 2011).

With regard to methylmercury, cooking does not significantly reduce its concentration (Costa, 2007; Santerre, 2010). On the contrary most of the studies revealed a concentration after cooking. Moretti et al. (1990) analyzed total mercury and methylmercury content in tuna (*Thunnus* sp.), porbeagle (*Lamna nasus*), common smooth-hound (*Mustelus mustelus*), spiny dogfish (*Squalus fernandinus*) and small-spotted catshark (*Scyliorhynus canicula*) revealing that inorganic and organic mercury concentrate after cooking, owing to the drop of fillet weight by releasing of water. In particular, methylmercury level was abundantly above the law level (Reg. CE 1881/2006) in roasted tuna, ranging from 1.12 to 3.14 mg/kg (1.7 times more than raw fish). Despite the levels were below the law limit (Reg. CE 1881/2006), total mercury and methylmercury increased ~1.5 times in both porbeagle and spiny dogfish as well.

Burger et al. (2003) examined the effect of deep-frying on Hg levels in large-mouth bass (*Micropterus salmoides*) caught in the Savannah River (U.S.A.). Mercury levels increased by 45% in breaded and 75% in unbreaded deep-fried

fish, so that U.S. EPA reference dose (RfD) for methylmercury (0.1 μg/kg/d) was greatly exceeded by consuming unbreaded fish in all of the subgroups of fishermen, except for white women.
Also Perelló et al. (2008) detected an increase in Hg levels after frying: from 0.034 to 0.051 μg/g for sardine and from 0.355 to 0.421 μg/g for tuna, respectively. On the contrary frying decreased the level of Hg from 0.142 to 0.121 μg/g in hake, while grilling increased it (0.200 μg/g).
Nevertheless accumulation of Hg and derivatives in the muscle is not indicative of the human intestinal absorption. Some researchers investigated the bioaccessibility of Hg after grilling in certain predatory species commercialized in the Spanish market (Torres-Escribano et al., 2011). Despite Hg concentrated in cooked fish, it was found that bioaccessibility, which is defined as the contaminant fraction that becomes available for intestinal absorption, reduced from an average value of 42 to 26%, leading to a decrease in risk evaluation for cooked bonito (*Sarda* sp.) and tuna (*Thunnus* sp.) (below the Tolerable Daily Intake, TDI, established by WHO/FAO, 2010).
According to the authors, the change in bioaccessibility after cooking was due to the alterations in the structural conformation of the fish muscle proteins produced by temperature, which could cause the loss of the native protein structure. These changes might impede the access of the enzymes to the structures to which Hg is bound in the muscle, low-molecular weight thiols, i.e. sulfhydryl groups containing molecules such as cysteine (Torres-Escribano et al., 2011).
Fish can accumulate toxic metals (i.e. ***Cd***, ***Pb***) and metalloids (i.e. ***As***) to different degree depending on the element, the species and the fish size (He et al., 2010). Metals can enter the human body mainly through inhalation and ingestion and change their chemical form. Nevertheless they cannot be degraded or destroyed (Perelló et al., 2008).
In addition to produce adverse effects on human health (cancer, damage to the nervous system, circulatory diseases), toxic metals can trigger oxidative stress in fish, affecting their flesh quality (Ferreira et al., 2010).
The bioavailability and the toxicity of metals largely depend on the chemical form in which they are found. For example, As(III) and As(V), which together constitute inorganic arsenic, are the most toxic species. The toxicity of the organoarsenical species is lower, and arsenobetaine (AB), a trimethylated species, is recognized to be the least toxic (Devesa et al., 2001).
In general fish species tend to increase metal concentrations after cooking. This is generally due to the concentration of dry matter but it also can be attributable to the absorption of metals from contaminated cooking water similarly to what was reported for other foodstuffs, i.e. rice (Rahman et al., 2006 and Torres-Escribano et al., 2008 as cited by Domingo, 2011).
Perelló et al. (2008) fried and grilled three different fish species to determine the ability to retain some toxic metals. The differences between raw and cooked

were not statistically significant, anyhow sardine presented higher As concentrations when grilled than when fried (3.281 and 2.906 µg/g, respectively), with both values being higher than those observed in raw sardine (2.086 µg/g). In hake (*Merluccius* sp.), the highest As concentrations were noted when this species was cooked and the highest level was found after grilling (1.379 µg/g). With regard to Pb, the concentrations in sardine and tuna were lower in grilled samples in contrast to grilled hake, where the Pb level increased. In turn, when compared to raw samples frying sardine, hake, and tuna meant an increase in Pb concentrations (from 0.043, 0.014 and 0.054 to 0.060, 0.015, and 0.059 µg/g, respectively).

Differences on As content between raw and cooked by grilling, roasting, baking, steaming, stewing, boiling and microwaving, based on the types of seafood in six fish species, bivalves, squid and crustaceans, were found by Devesa et al. (2001). They found a significant As concentration attributable to the decrease in weight that takes place during cooking only in bivalves and squid, with mean increases after cooking of 0.062 and 0.004 µg/g on wet weight, respectively.

Also organic arsenic species, which are less acutely toxic than inorganic arsenic, were detected after cooking (Devesa et al., 2005). Arsenobetaine increased in most of the seafoods analyzed by Devesa et al. (2005), except for hake and crustaceans. Tetramethylarsonium ion (TMA$^+$) was lost only in crustaceans while monomethylarsonic acid (MMA), which was only detected in raw and cooked samples of Atlantic horse mackerel, sardines and bivalves showed an increase of contents after all types of cooking applied (grilling, roasting, baking, steaming, stewing, boiling and microwaving).

However, the bioaccessibility of metals seemed primarily element-dependent, according to He et al. (2010). They found that Fe was the least bioaccessible element in Japanese seabass (*Lateolabrax japonicus*) and red seabream (*Pagrosomus major*), while Cu and Cd were the highest, following the pattern Fe<Se<Zn<As≈Cu≈Cd.

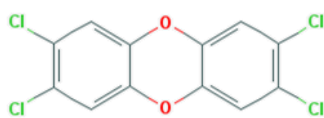
The toxic metals, As and Cd, showed similar percentages of bioaccessibility (70-90%), major than the values obtained by Torres-Escribano et al. (2011) on mercury and its derivatives (6-49%). Similarly to what was noticed on methylmercury (Torres-Escribano et al., 2011), cooking decreased bioaccessibility of all the elements detected by He et al. (2010). If compared to boiling, frying and grilling, steaming resulted to be very favourable to toxic metal absorption, showing As and Cd the highest percentage of bioaccessibility (75.2-88.0%).

Organochlorine contaminants in the environment are polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzo-p-furans (PCDFs), polychlorinated biphenyls (PCBs), camphechlor, dichlorodiphenyltrichloroethane (DDT) and its metabolites (DDD, DDE), chlordane, dieldrin, aldrin, endrin, heptachlor,

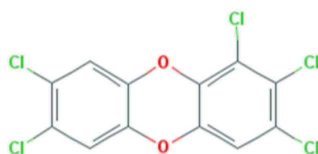
hexachlorobenzene. PCDDs and PCDFs which are often referred to as ***'dioxins' (PCDD/Fs)*** are formed as byproducts or impurities in several industrial and combustion processes (i.e. incineration). They accumulate at high trophic level. 17 PCDD/Fs with chlorine substitution in positions 2, 3, 7 and 8 (Figure 8) are the most toxic (Table 4), among the 210 congeners.
Dioxins can cause reproductive and developmental problems, damage the immune system, they can interfere with hormones and also cause cancer. Dioxins can bring such diseases by triggering a wide variety of toxical and biochemical reactions via aryl hydrocarbon receptor (AhR)-mediated signaling pathways (Figure 9). The AhR is a ligand-activated nuclear transcription protein that can bind to the dioxin. This complex can modulate the gene expression representing the initial steps in a series of biochemical, cellular, and tissue changes that result in the toxicity observed (Mandal, 2005).

Figure 8 – Molecular structures of 4 among the most toxic PCDDs (PubChem).

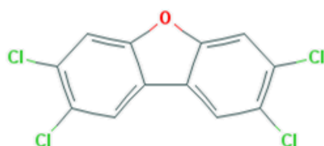
2,3,7,8 – Tetrachlorodibenzo-p-dioxin (TCDD)



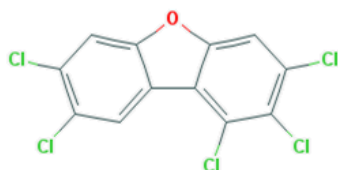
1,2,3,7,8-Pentachlorodibenzo-p-dioxin



2,3,7,8-Tetrachlorodibenzofuran

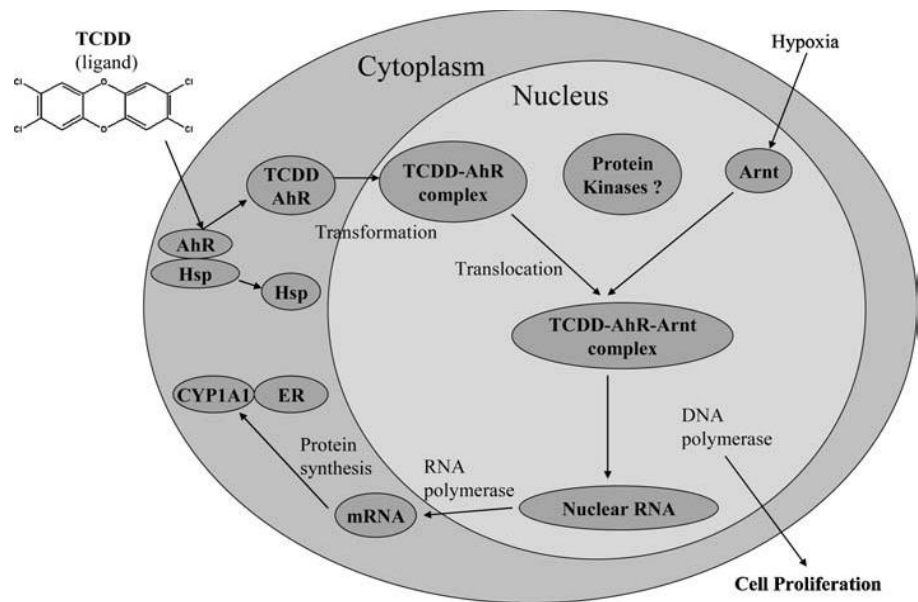


1,2,3,7,8 – Pentachlorodibenzofuran



Polychlorinated biphenyls (PCBs) consist of the biphenyl structure with two linked benzene rings, in which some or all of the hydrogen atoms have been substituted by chlorine atoms. 12 congeners, having chlorine substituents in para- and meta-positions and at the most, in one ortho-position (EFSA, 2005), on 209 possible, are toxic (Table 2). The structures of 4 of them are reported on figure 10. They are also known as dioxin-like compounds because they exhibit toxicological effects similar to dioxins, mediated by activation of the aryl hydrocarbon receptor (Costa, 2007).

Figure 9 - Simple mechanistic model for TCDD toxicity using the AhR pathway. ER endoplasmic reticulum, CYP1A1 cytochrome P450 1A1, AhR aryl hydrocarbon receptor, Hsp heat shock protein, Arnt Ah receptor nuclear translocator, mRNA messenger ribonucleic acid (Mandal, 2005).



Like methylmercury, PCBs pass from the mother to the developing fetus/infant through the placenta/milk, but they take more time (~6 years) to clear from the body (Santerre, 2010). According to Safe (1990) infants exposed to high levels of PCBs show altered postnatal development, lower birth weight, smaller head circumferences and poorer short-term memory.

The presence of dioxins and dioxin-like PCBs is expressed as toxic equivalents (TEQ) after multiplication of congener-specific concentration levels with toxicity equivalency factors (TEF). TEFs were calculated based on their relative

toxicity compared to 2,3,7,8-TCDD (Costa, 2007). The current European legislation is based on TEFs and the last re-evaluation was undertaken in 2005 (EFSA, 2010) (Table 4).

Figure 10 - Molecular structures of 4 among the most toxic PCBs (PubChem).

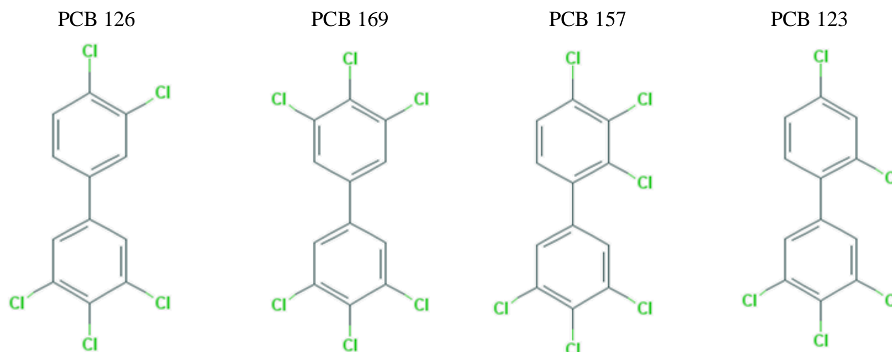


Table 4 - Dioxins (sum of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)) and sum of dioxins and dioxin-like PCBs (sum of PCDDs, PCDFs and polychlorinated biphenyls (PCBs)), expressed as WHO toxic equivalent using the WHO-TEFs [Reg. (EC) 1881/2006]. It is also shown the change in WHO toxicity equivalency factors (TEFWHO98 and TEFWHO05) between assessments in 1998 and in 2005 with changes in bold as reported by the scientific report of EFSA (2010b).

Congenere	Valore TEF	Congenere	Valore TEF
Dibenzo-p-diossine (PCDD)		**PCB diossina-simili: Non-orto PCB + mono-orto PCB**	
2,3,7,8-TCDD	1	*Non-orto PCB*	
1,2,3,7,8-PeCDD	1	PCB 77	0,0001
1,2,3,4,7,8-HxCDD	0,1	PCB 81	0,0001
1,2,3,6,7,8-HxCDD	0,1	PCB 126	0,1
1,2,3,7,8,9-HxCDD	0,1	PCB 169	0,01
1,2,3,4,6,7,8-HpCDD	0,01		
OCDD	0,0001		
Dibenzofurani (PCDF)		*Mono-orto PCB*	
2,3,7,8-TCDF	0,1	PCB 105	0,0001
1,2,3,7,8-PeCDF	0,05	PCB 114	0,0005
2,3,4,7,8-PeCDF	0,5	PCB 118	0,0001
1,2,3,4,7,8-HxCDF	0,1	PCB 123	0,0001
1,2,3,6,7,8-HxCDF	0,1	PCB 156	0,0005
1,2,3,7,8,9-HxCDF	0,1	PCB 157	0,0005
2,3,4,6,7,8-HxCDF	0,1	PCB 167	0,00001
1,2,3,4,6,7,8-HpCDF	0,01	PCB 189	0,0001
1,2,3,4,7,8,9-HpCDF	0,01		
OCDF	0,0001		

Abbreviazioni utilizzate: «T» = tetra; «Pe» = penta; «Hx» =esa ; «Hp» = epta; «O» = octa; «CDD» = clorodibenzo-p-diossina; «CDF» = clorodibenzofurano; «CB» = clorobifenile.

The group of the neurotoxic brominated flame retardants (BFRs) is composed of brominated bisphenols, diphenyl ethers (PCDEs), cyclododecanes, phenols and phthalic acid derivatives, tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCD) and three commercial mixtures of polybrominated diphenyl ethers (PBDEs), referred to as decabromodiphenyl ether (DBDE), octabromodiphenyl ether (OBDE) and pentabromodiphenyl ether (pentaBDE).
The effects of cooking on organochlorine contaminants levels in fish species have been studied by several researches. Changes in organochlorine contaminants levels can be attributable to quite complex factors. The high temperatures of cooking can induce the evaporation of both water and organochlorine contaminants (Witczak, 2009). Even if it is considered unlikely because of the short time of contact between oil and fish, oil can act as an extraction solvent during frying (Bayen et al., 2005; Witczak, 2009). Also they can solubilise in lipid and lipophilic compounds which are often released during cooking.
Generally organochlorine contaminants cooking losses mainly vary according to the fish species (Witczak, 2009; Perelló et al., 2010), the cooking method (Zabik et al., 1996; Moya et al., 1998) and appear to be a function of the initial lipid concentration in the fish (Bayen et al., 2005; Hori et al., 2005).
Perelló et al. (2010) examined the concentrations of the 17 most toxic congeners of PCDD/PCDFs in samples of raw and cooked foodstuffs and the highest level was found in fish, especially in sardine. In this case the type of seafood influenced the ability to retain dioxins, indeed cooking reduced the level in sardine and increased them in hake and tuna. In particular, the sum of PCDDs and PCDFs, expressed as ng WHO-TEQ/kg of fresh weight, decreased from 0.410 to 0.248 and 0.295 in fried and grilled sardine, respectively.
Hot smoking led to a considerable increase of the dioxin content in the edible parts of mackerel (<0.4 WHO-PCDD/F-TEQ ng/kg w.w.) and Greenland halibut (*Reinhardtius hippoglossoides*) (<1 ng/kg w.w.) (Karl & Ruoff, 2008), without exceeding the maximum law level [Reg. (EC) 1881/2006]. According to the authors, the increase was related to the loss of water during processing whereas the fat remained in the muscle meat and the lipophilic dioxins kept in the lipid phase of the muscle meat, respectively.
When fat is released during cooking, PCDD/PCDFs consequently are removed along with fat. This was found by Schecter et al. (1998) in broiled catfish, where PCDD/PCDFs and PCBs TEQ decreased by approximately 50% for each portion. Similarly Hori et al. (2005) showed a decrease of concentration of dioxins both after grilling and boiling in mackerel (*Scomber scombrus*), observing a slight relationship between the estimated fat release and the degree of TEQ reduction.

Concerning PCBs, that are known to show good resistance to thermal decomposition (Witczak, 2009), results are contrasting as some studies did not observed change in concentration (Moya et al., 1998; Salama et al., 1998) while others showed both decreases and increases in PCB levels (Moya et al., 1998; Salama et al., 1998; Witczak, 2009; Perelló et al., 2010).
Perelló et al. (2010) found the highest amount of PCBs in fish than in meat, potatoes, rice and olive oil and fish species affected PCBs content. PCBs decreased after cooking (frying and grilling) in sardine and hake, while slightly increased in tuna. Moreover the higher lipid content in sardine determined the higher loss of PCB after grilling, from 5.2452 to 0.0086 ng WHO-TEQ/kg of fresh weight.
As reported by Sherer and Price (1993), cited by Domingo (2011), processes such as baking, broiling, microwave cooking, poaching and roasting removed approximately 20 to 30% of the PCBs in fish, while frying appeared to remove more than 50%. Witczak (2009) observed that decrease of PCB levels in the fried fish meat was influenced by fat content increase in the final products which occurred due to absorption of oil used for frying. However PCBs reduction after frying depended on the fish species and the initial concentration of PCBs, as well. This last aspect was evidenced by Witczak (2009) by detecting the lowest percentage losses (32.05%) in flounder which was the species at the highest level of total PCBs in raw fillet (6.584±3.099 μg/kg wet weight). On the contrary the highest percentage losses were in cod (81.11%), having a low PCBs level in raw muscle (0.950±0.590 μg/kg wet weight) (Witczak, 2009).
Because the deep frying process creates unique cooking conditions that accelerate drying of the fillets and PCBs evaporation, total PCBs significantly reduced in deep-fried flounder on the contrary to grilled and pan-fried where the reduction was not significant (Moya et al., 1998). However the same effect was not noticed in bluefish (*Pomatomus saltatrix*) that did not decrease in PCBs content after either pan frying and baking resulting in 0.12 and 0.14 mg/kg, respectively.
Also smoking resulted to bring down PCB level to a large extent, probably because of the long, slow processing followed by holding at a higher end temperature (Zabik et al., 1996). The greatest loss of PCBs (65%) was during smoking treatment followed by microwaving (60%) and charbroiling (to broil over charcoal) (37-47%) in bluefish (Salama et al., 1998). The same trend was described by Zabik et al. (1996) who detected the greatest reduction of PCBs (40%) after smoking in lake trout (*Salvelinus namaycush namuycush*) and siscowets (*Salvelinus namaycush siscowet*), when compared to baking and charbroiling method (10-20% of loss).
Also the removal of the skin from the cooked fish resulted in a further loss of PCBs. This was demonstrated in salmon steak starting from 26% and losing 9% (Bayen et al., 2005). The same reduction of total PCBs was evident in the

Chinook salmon cooked without the skin (42%), slightly higher than skin-on cooked fillet (38%) according to Zabik et al. (1995). Moreover the ability to be retained was shown to depend on molecular structure of the congeners: Bayen et al. (2005) described a greater cooking loss of PCBs congeners having higher levels of chlorination.

With regard to brominated flame retardants (BRFs), there are only few contrasting reports on studies in which the influence of cooking on polybrominated diphenyl ethers (PBDEs) concentrations in food was assessed (Domingo, 2011). Bayen et al. (2005) registered a loss of PBDEs in salmon after cooking, even if the cooking method affected the release: skin on fillets showed 42, 25, 32 and 44% of loss after panfrying, microwave cooking, boiling and baking, respectively. Similarly to PCBs, difference in molecular structure affected retention and losses were greater for congeners with high levels of bromination. Perelló et al. (2010) reported an unexpected increase on diphenyl ethers (PCDEs) levels after frying and grilling in sardine (except for frying), hake and tuna. PCDEs reached the concentration of 306.1 ng/kg of wet weight in grilled sardine.

Fish can store organochlorine pesticides in fatty tissue. Those representing the greatest health concern belong to the categories extensively used in the past decades and banned by most of the world countries at present, like DDT and its derivatives. Their ability to persist in the environment and concentrates up the food chain makes them potentially responsible for the onset of many chronicle diseases, like breast cancer (Snedeker, 2001).

The ability to retain pesticides after cooking was assessed by several studies. Zabik et al. (1996) investigated the levels of organochlorine pesticides in skin-off lean lake trout harvested from Lakes Huron, Michigan and Ontario and siscowets from Lake Superior, where fish had previously shown detectable levels of p,p'-DDE, biphenyl, mercury, total PCBs, trans-nonachlor, pentachloroanisole, cis- and trans- chlordane, dieldrin, α-BHC and 1,2,4 trichlorobenzene. Smoking resulted in significantly greater reductions, by 40 to over 65%, while baking and charbroiling reduced the residues to the same extent. In particular the highest residue losses were found for the chlordane complex ranging from 35 to 38%, while modest residue losses of 12-20% were detected for DDT complex, HCB and dieldrin.

The same authors investigated the effect of removal of skin on DDT, DDE and DDD in Chinook salmon and carp demonstrating that the total amount of tDDT was greatly less in skin off fish after baking and charbroiling (Zabik et al., 1995). In particular, the average level of the p,p'-DDT decreased from 0.062 to 0.032 mg/kg of wet weight in skin-on and skin-off cooked salmon. Similar trend was revealed in carp, where only the derivatives of p,p'-DDT were found: the average level of p,p'-DDE ranged from 0.015 to 0.007 mg/kg in skin-on and skin-off deep fried fillets.

1.2.5 Effect of cooking on exogenous contaminants from biological origin

Biotoxins deriving from bacteria and harmful algae (i.e. dinoflagellates) blooming are a health concern, especially in tropical and subtropical regions. Cases are increasingly being reported in temperate regions with the expansion of tourism and trade. Intoxications are relatively uncommon but likely underreported.

Toxins are produced by bacteria or algae which are important food source for molluscs, crustaceans and small herbivorous fish. These may be consumed by larger fish or accumulated by bivalves, that are, in turn, eaten by humans (Sobel & Painter, 2005).

During the algal bloom, that naturally takes place and it is favoured by environmental changes and anthropic pollution, fishing is usually prohibited because of the accumulation of biotoxins in fish. Prevention of intoxication is quite impossible, since toxins are not detectable by sight or smell, and the seafood in which they are found appears normal. For this reason biotoxins diagnosis is generally based on clinical presentation and the history of seafood consumption in the preceding hours (Sobel & Painter, 2005; Abraham et al., 2012).

Molluscs are the category of seafood mostly contaminated by biotoxins because they can easily accumulate great amounts of toxins inside the soft part by filtering marine water. The most common biotoxins present in molluscs are saxitoxin, okadaic acid and domoic acid.

Saxitoxin (STX) is responsible for the onset of a severe disease known as paralytic shellfish poisoning (PSP). The syndrome is neurological and takes place within 30 min after exposure. Tingling, numbness, headache, nausea, vomiting and diarrhoea are the main symptoms. Death may results from respiratory muscle paralysis (letal dose >120-180 μg of the STX). STX intoxication can also be caused by consumption of puffer fish (*Tetradontidae* family), which is the classic food vehicle of tetrodotoxin poisoning (Sobel & Painter, 2005; Abraham et al., 2012).

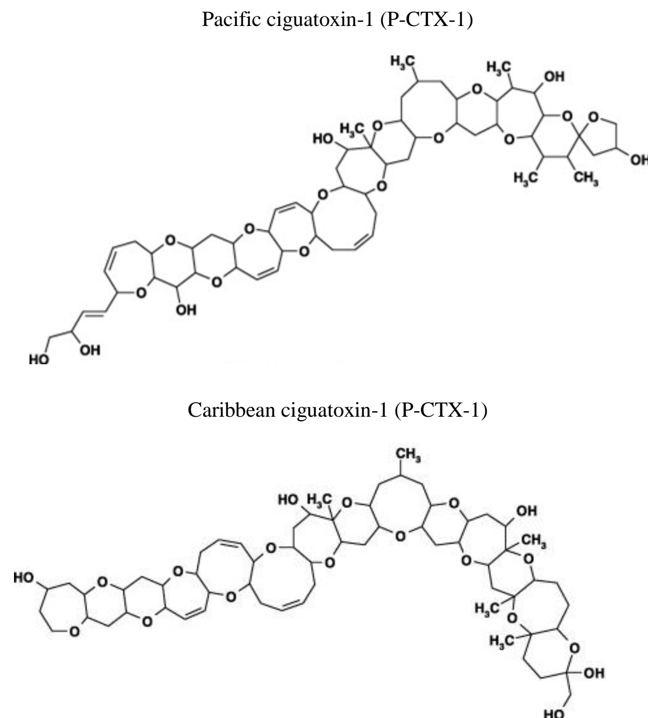
Okadaic acid is the cause of diarrheic shellfish poisoning (DSP), self-resolving illness with exclusively gastrointestinal symptoms. Symptoms usually occur within 30 min after exposure and include diarrhoea and abdominal cramps that typically resolve within 3-4 days after onset.

To ***domoic acid (DA)*** is attributed the amnesic shellfish poisoning (ASP), a rare illness with symptoms ranging from gastrointestinal disturbance to severe and unusual neurological manifestations.

Brevetoxins provoke neurotoxic shellfish poisoning (NSP), characterized by gastro-intestinal and neurological symptoms as perioral parasthesias, diarrhoea, gait deficits. They typically resolve within 48 h.

Biotoxins previously listed can also be found in marine fish, but usually ciguatoxins and azaspiracid are the most spread toxins in marine fish.
Ciguatoxins or ***ciguatera (CTXs)*** (Figure 11) are found in various species of fish of the Pacific and Indian Oceans and Caribbean Sea (intoxication annually estimated: 20000-50000). Symptoms of ciguatera fish poisoning (CFP) are within 12 h and acute phase lasts for 24 hours. It provokes gastrointestinal diseases (e.g. nausea, vomiting and diarrhoea) in the acute phase followed by neurological symptoms (e.g. temperature-related dysesthesias, paresthesias, numbness and tingling of the perioral region and extremities). CFP diagnosis is supported by testing of meal remnants, where available, for CTX contamination (Abraham et al., 2012).

Figure 11 – Structures of Pacific and Caribbean ciguatoxins (Abraham et al., 2012).



Confirmation of CFP is challenging as CTXs are active at very low levels in fish. CFP is confirmed only when CTXs are detected in meal remnants or unused portions of the fish consumed. If a meal remnant is unavailable, traceback analysis of fish from the implicated lot is often unproductive, as fish of the same species harvested from the same region can vary widely in toxin content (Abraham et al., 2012).

Marine biotoxins are heat stable and largely unaffected by cooking (Sobel & Painter, 2005). However difference in storage tissue and molecular structure can influence the tendency to be degraded. Understanding biotoxins distribution and composition within tissue compartments of raw and cooked shellfish is essential, not only as a means of providing essential information on safety and risk assessment of shellfish consumption, but also as a major determinant for health authorities to establish precautionary guidelines and give suitable advice on proper preventive measures to the general public (Wong et al., 2009).

Wong et al. (2009) determined the effect of steam-cooking on saxitoxin content in different tissue compartments of scallop (*Patinopecten yessoensis*) samples. The total PSP toxicity of the raw scallops ranged between 73.1 and 204 STXeq. Steaming favoured the release of STX, which this species generally accumulates in viscera and adductor muscle (more than 70%), and toxins were passed into the soup after the treatment, where they showed a concentration of 32.9-243 µg STXeq (more than 50%). Viscera and gonads did not change in STX content so that selective consumption of soup, viscera and gonads may pose a significant risk of PSP syndrome. However mouse bioassay test showed that all tissue compartments and soup, in both raw and steamed scallops, contained total STX at mice lethal doses. Regarding the toxins profile, there was no significant difference ($p>0.05$) in the levels of each STX congener between raw and steamed samples except the congener GTX3, which decreased significantly ($p<0.05$) in toxin quantity after steam treatment. The authors attributed the decrease to the thermodynamically less stable nature of this congener.

In order to investigate the toxicity decrease of some biotoxins in bivalves (scallops, mussels, clams and cockles), Reboreda et al. (2010) tested the industrial thermal processing, approved by EU on bivalves, which shortly consisted in (1) pre-cooking of the whole bivalve in fresh water for a time >3min at a temperature of 95±5 °C and (2) cooking of the flesh in fresh water for a time >9 min at a temperature of 98±3 °C, cooling, conditioning in containers closed hermetically and (3) sterilization in autoclave at 116 °C for 54 min. STX decreased from 405 µg eq STX/kg to 350 µg eq STX/kg, that was their limit of detection (LOD). Thermal processing on STX contaminated clams and cockles also reduced the STX levels below the detection limit.

Applying the same thermal process previously described, domoic acid was successfully decreased by the treatment (112.50 *vs* 35.33 µg/g) even if the best method removing all the toxins present was hepatopancreas ablation (112.50 *vs* 1.98 µg/g). According to McCarron & Hess (2006), cooking method can influence domoic acid concentration in molluscs. Steaming did not appear to have a decreasing effect on the DA content in fresh mussels (*Mytilus edulis*). An approximate 20% increase in DA concentration was observed as a result of the steaming and autoclaving processes after removal of intervalvular water. On the contrary, boiling (90 °C x 15 min) induced a decrease in DA of 3%.

According to Reboreda et al. (2010), the most resistent toxins were okadaic acid and its derivatives. Thermal processing on mussel whole body increased the total toxin concentration of okadaic acid (94.67 *vs* 162.67 μg/g) and its derivative dinophysistoxins (DTX3) (64.33 *vs* 170.33 μg/g) without changing the OA/total toxin ratio. Nor sterilization of mussels at 121 °C was effective. The author attributed to the lipophilic nature of the toxin, as okadaic acid is mainly stored inside liposomes, the inability to reduce the toxin through the thawing after freezing or hepatopancreas ablation.

Since molecular changes may occur amongst CTX congeners as a result of both metabolic transformation and cooking, the researches on CTX were addressed to identify CTX congeners in raw and cooked muscle and their contribution to total toxicity (MUg), that is known to derive from the toxins concentration (MUg/g flesh where 1 MUg/g is 1 g of mouse killed/g of flesh).

Pottier et al. (2002) analyzed cooked and uncooked horse-eye jacks (*Caranx latus*) and found that total toxin content evaluated by the mouse bioassay was equivalent for cooked or uncooked fish flesh, confirming that fish toxicity is not affected by heating to 120 °C. They detected four classes of Caribbean CTXs: C-CTX-1 and isomers, C-CTX-1143 and isomers, C-CTX-1157 and isomers, and C-CTX-1127. Caribbean ciguatoxin 1 (C-CTX-1) was the major toxin (75–90%) present in the low-toxic flesh (<0.5 MUg/g), while toxic fish (>0.5 MUg/g) contained 5–50% of C-CTX-1, with levels varying independently of mouse toxicity. However the relative content of the four groups of CTXs slightly differed between the cooked and uncooked parts of the same fish. C-CTX-1 decreased after cooking, in conjuction with an equivalent increase of the isomer C-CTX-1141a, suggesting that C-CTX-1141a may arise from C-CTX-1, without changes in toxic level of fish.

Abraham et al. (2012) compared the cooked with the uncooked portion of the same fish (barracuda, *Sphyraena* sp.) implicated in CFP, with respect cytotoxicity and toxins profile (by using LC–MS/MS). They found that composite toxicity of the cooked meal remnant was similar to that of the raw fish and they confirmed C-CTX-1 as the principal CTX congener contributing ~60% of the composite toxicity in barracuda, similar to what was found by Pottier et al. (2002) in horse-eye jacks.

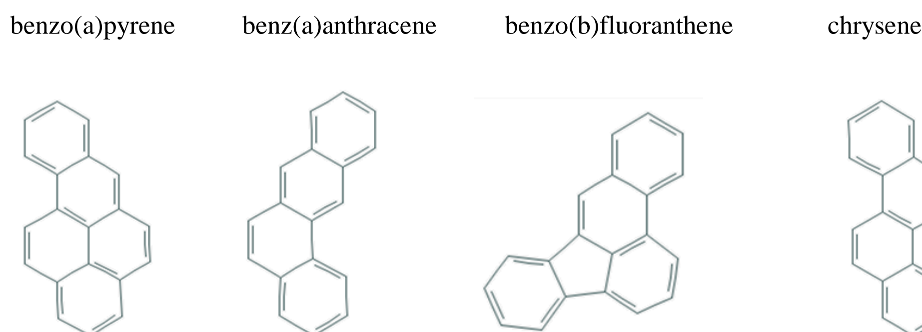
1.2.6 Effect of cooking on endogenous contaminants

Even if environmental pollution may cause contamination with polyciclic aromatic hydrocarbons (PAHs) (Figure 12), they can develop in foods during smoking processes and heating and drying processes that allow combustion products to come into direct contact with food [Reg. (EC) 1881/2006].

The carcinogenic potential of different PAHs is associated with the propensity of phase I enzymes to generate reactive epoxides that may be detoxified through phase II enzymes or bind to other cellular components, such as DNA (Collier et al., 1992 as cited by Visciano et al., 2009).

PAHs carcinogenicity depends on their structure: PAHs containing four fused rings are weakly carcinogenic compounds while PAHs containing five or more rings are regarded as potentially genotoxic and carcinogenic to humans (Alomirah et al., 2011). Moreover the light PAHs, of molecular mass below 216 Da, are regarded as not carcinogenic. Very mutagenic and carcinogenic is benzo(a)pyrene (252 kDa) (Stołyhwo & Sikorski, 2005).

Figure 12 - Molecular structures of the regulated PAHs (PubChem).



Originally European Commission fixed law limits only for benzo(a)pyrene [Reg. (EC) 1881/2006] before that EFSA adopted an opinion (EFSA, 2008) concluding that benzo(a)pyrene is not a suitable marker for the occurrence and effect of carcinogenic PAHs. For this reason, the law limit of the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene was provided for smoked seafood in the Regulation (EC) 835/2011, that has been applied from 1st September 2012.
Most of the studies have been addressed to detect PAHs in smoking process. Although the exact mechanism of formation of PAHs in grilled/smoked foods is not precisely known, it is generally considered that at least three possible mechanisms exist. PAHs can derive from the pyrolysis of organic matter such as fat (mainly), protein and carbohydrates at temperatures above 200 °C. The second mechanism is the formation of volatile PAHs from lipid drip loss, and their adhesion to the surface of the food. The third mechanism is the incomplete combustion of charcoal which can generate PAHs that are brought onto the surface of the food. It has been suggested that low molecular weight PAHs (containing 2–3 aromatic rings) arise from smoke generated during meat grilling as these PAHs are more volatile than high molecular weight PAHs (containing more than 3 aromatic rings) (Alomirah et al., 2011).
Wood smoke contains a large number of PAHs, at least 61, and the most important factor affecting their formation is the temperature of smoke

generation (Stołyhwo & Sikorski, 2005). By lowering the temperature from 425 °C to 300-400 °C and using filters, the content of PAHs in the smoke can be decreased about 10-fold (Stołyhwo & Sikorski, 2005).
Several researches detected PAHs and derivatives, such as nitro-PAH and oxygenated PAH on smoked fish. By holding the internal temperature of the fish at 82 °C x 30 min in the final step of smoking process, pyrene was found in the highest level in lake trout (36.36 ng/g wet tissue) and siscowets (42.62 ng/g wet tissue), followed by fluoranthene (26.84 and 38.68 ng/g wet tissue), phenanthrene (8.35 and 7.61 ng/g wet tissue) and benzo(a)anthracene (9.66 and 15.63 ng/g wet tissue). The level of polynuclear aromatic hydrocarbons formed was higher for fish with higher fat contents (siscowets) (Zabik et al. 1996).
Alomirah et al. (2011) monitored PAHs in the most popular grilled and smoked dishes consumed by Kuwait population, revealing a significant contribution of grilled and smoked food items to the overall dietary exposure to PAHs. Smoked fish samples (species not reported) (0.50 μg kg^{-1}) did not exceed the maximum level of 2 μg/kg for benzo(a)pyrene [Reg. (EC) 1881/2006], while the mean value for the eight genotoxic PAHs, corresponding to the sum of benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, and benzo(ghi)perylene, was above 10 μg/kg, law limit of the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene that is applied from 2012 [Reg. (EC) 835/2011].
Marked differences in PAHs profile species-dependent were described by Visciano et al. (2009). Benzo(a)pyrene was found in all three species examined: Atlantic salmon, swordfish (*Xiphias gladius*) and tuna. Carcinogenic benzo(b)fluoranthene was detected only in Atlantic salmon, whereas benzo(k)fluoranthene was detected in all species at level below 5 ng/g of wet weight. Among species, Atlantic salmon showed the highest levels for all the detected compounds. According to the authors, the prevalence of low molecular weight compounds (acenaphtene, phenanthrene, anthracene, fluoranthene) was due to the environmental pollution representing an important source of three or four rings-PAHs.
Few authors investigated the formation of PAHs during other cooking procedures. One of the most consistent studies was led by Perelló et al. (2009) who detected PAHs after frying, grilling, roasting and boiling in different foodstuffs and found that PAHs, mainly those considered strongly carcinogenic, accumulate in all cooked fish, especially after frying. Fried sardine, hake and tuna reported 35.42, 13.30 and 29.51 μg/kg of fresh weight of total PAHs, respectively. Also roasting, that was tested in hake, resulted in a consistent increase of total PAHs (19.26 μg/kg of fresh weight). PAHs profile in fish was characterized by low level of benzo(a)pyrene which was found three times more in olive oil. Naphthalene, pyrene, phenantrene and fluoranthrene were the most part of PAHs detected in fish. However, the results obtained showed that

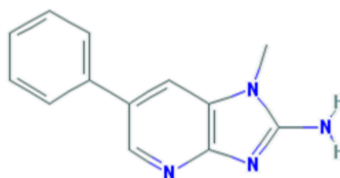
smoking and the other cooking methods can be suitable way of reducing lipophilic contaminants (PCDD/PCDFs, PCBs) but PAHs can be formed the same. For this reason cooking parameters (time/temperature) should be strictly put under control to prevent the formation of PAHs.
Heating processes do not only cause the formation of PAHs, since by cooking several carcinogenic and mutagenic compounds can accumulate.
Heterocyclic aromatic amines (HCAs) are formed naturally during cooking of proteinaceous foods such as fish. Epidemiologic studies have shown that most HCAs are highly mutagenic (Felton et al., 1984, as cited by Oz et al., 2010) and almost all of them are also carcinogenic (Sugimura, 1995, as cited by Oz et al., 2010).
Fish species, cooking procedures, chemical composition of meat, pH are among the main factors affecting the formation of HCAs (Oz et al., 2010; Iwasaki et al., 2010). HCAs formation was investigated by Oz et al. (2010) who compared different cooking methods (microwaving, baking, hot plate, pan frying and barbecuing) and cooking level of doneness (rare, medium, well and very well) in rainbow trout. HCAs were not detected in microwaved and hot plate cooked fillets at every level. Only 2.09 ng/g total HCAs were found in well-done baked fish (15 min). In pan-fried fish, HCAs were formed at well done (6 min) and very well done (8 min) cooking levels.
Barbecuing was the most favourable cooking method for HCAs accumulation at all doneness levels, resulting in 3.19, 3.41, 2.53 and 3.52 ng/g in rare (1.5 min), medium (3 min), well done (4.5 min) and very well done (6 min), respectively. In addition, important part of total HCAs amount belonged to 2-amino-3,4,8-trimethyl-imidazo(4,5-f)quinoxaline(4,8-DiMeIQx) (Figure 13) compounds in both fried and barbecued samples, ranging from 1.76 to 5.48 ng/g.
The same authors (Oz et al., 2007) have already found that the most concentrated compounds was 4,8-DiMeIQx in rainbow and brown trout (*Salmo trutta* L.) analyzed after deep-fat frying, pan-frying, grilling and barbecuing.
Also Iwasaki et al. (2010) detected 4,8-DiMeIQx next to 2-amino-1-methyl-6-phenylimidazo(4-5-b)pyridine (PhIP) and 2-amino-3,8-dimethylimidazo(4,5-f)quinoxaline (MeIQx) (Figure 13) in the majority of very well-done cooked sardine and salmon (max internal temperature: 90 °C). In particular, 0.35 and 0.26 ng/g of 4,8-DiMeIQx were detected in pan fried sardine and salmon, whereas 0.42 ng/g in salmon when cooked according to the traditional Brazilian barbecuing, churrasco. Nevertheless differences in HCAs formation in salmon were found to depend on the cooking method: PhIP values were significantly higher for churrasco (skin-on: 22.5 ng/g; skin-off: 28.8 ng/g) than pan-frying (7.37 ng/g), even if skin on salmon contained significantly lower PhIP levels than skin off samples. On the contrary, Knize et al. (1995) were unable to detect 4,8-DiMeIQx by analyzing fastfood fish (species not reported) and meat so that they were courageously able to assert that fast-food restaurants were contributing very little to the exposure to these potent carcinogens. Similar

results were found by Zimmerli et al. (2001) in steamed salmon. Both of the authors did not detect either MeIQx and PhIP, as well.

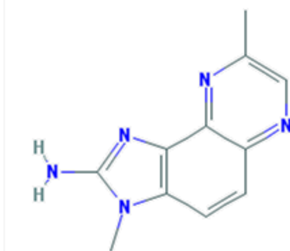
Acrylamide (AA) (Figure 14) is a chemical intermediate used for polyacrilamide, the most common polymers utilized for industrial purposes (Parzefall, 2008). Besides the industrial and laboratory uses humans are exposed to varying amounts of AA via the diet. Analytical studies revealed that processing of food rich in starch and protein is the main source of AA. Specifically, glucose and some amino acids (asparagine>glutamine>methionine>cysteine, in falling efficiency), provided the essential ingredients for AA formation when heated above 120 °C (Parzefall, 2008). AA is known to be mainly neurotoxic in humans (Parzefall, 2008), but it could be associated with a considerable cancer risk (Tareke et al., 2002).

Figure 13 - Heterocyclic aromatic amines (PubChem).

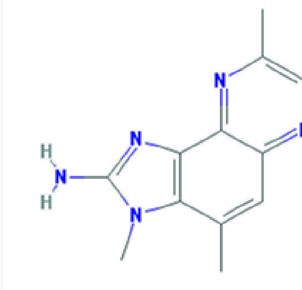
2-amino-1-methyl-6-phenylimidazo(4-5-b)pyridine (PhIP)



2-amino-3,8-dimethylimidazo(4,5-f)quinoxaline (MeIQx)

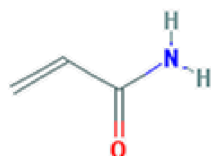


2-amino-3,4,8-trimethyl-imidazo(4,5-f)quinoxaline (4,8-DiMeIQx)



Among the few studies dealing with AA concentration in fish, it is worthwhile mentioning Tareke et al. (2002). In subsequent experiments different types of foodstuffs heated via cooking methods were studied with respect to the formation of AA in the heated foodstuffs. Fish did not develop consistent amount of AA: fried cod and boiled cod showed AA levels of 11 µg/kg and below the detection level of 5 µg/kg, respectively. No detectable levels were found after microwaving. Such results were in contrast with what was found in carbohydrate rich foods, like French-fried potatoes and potato crisps which exhibited relatively high levels of AA [median values of 424 µg/kg (n = 5) and 1739 µg/kg (n = 6), respectively].

Figure 14 – Acrylamide (PubChem).



In conclusion, cooking does not reduce the health risk caused by toxic metals in seafood. However, the risk can be modulated by the decrease of bioaccessibility induced by cooking.

Regarding the health risk attributable to lipophilic contaminants, results are contrasting. Even if loss is congener dependent, increasing lipid percentage in raw flesh (preferably skin-off) and increasing lipid losses during cooking correspond to decreasing amount of organochlorine contaminants and BFRs in cooked fillets. The decrease commonly detected after frying was partly fictitious since lipophilic contaminants just diluted in the frying oil.

Differences in thermostability mainly influence the intake of active biotoxins after cooking, so that cooking conditions generally affect the health risk. CTXs is an exception as it showed to be resistant to all type of treatments. Health risk also resulted to be influenced by difference in tissue distribution of biotoxins.

Hydrophilic biotoxins (i.e. STXs) were easier removed with water loss, on the contrary to lipophilic (i.e. okadaic acid) that are stored in the muscle.

Regarding PAHs and HCAs, increasing lipid content of fish and increasing temperature of cooking correspond to increasing PAHs (mainly low molecular weight congeners) and HCAs formation and accumulation.

This allows us to conclude that the absence of exogenous contaminants in the raw fish is the best way to minimize the health risk. More can be done to prevent endogenous contaminants formation: control time/temperature and avoid overcooking and regulate smoke exposure, humidity, drying, and temperature in smoking practices.

2. AIM OF THE STUDY

The overall aim of this study was to assess the effects induced by cooking on quality of fillets from two different farmed species with reference to changes in physical parameters and nutritional profile.
The study aims to inform both industry and consumers of the differences in the aptitude for heat-treatment of two species characterized by a diverse market background. Moreover the study provides useful indication to fish farmers of which intrinsic and extrinsic factors can influence the quality of their product when intended for both domestic cooking or industrial processing.

The specific goals were to study:

- The effect of cooking on quality of meagre (*Argyrosomus regius*) depending on season of catch (**Paper I**)

- Raw fillet quality depending on meagre (*Argyrosomus regius*) rearing system and short-time chilling storage (**Paper II**)

- The effect of cooking on quality of trout (O*ncorhynchus mykiss*) fillet depending on genetic strain and farm (**Paper III**)

3. MATERIALS AND METHODS

Experimental design

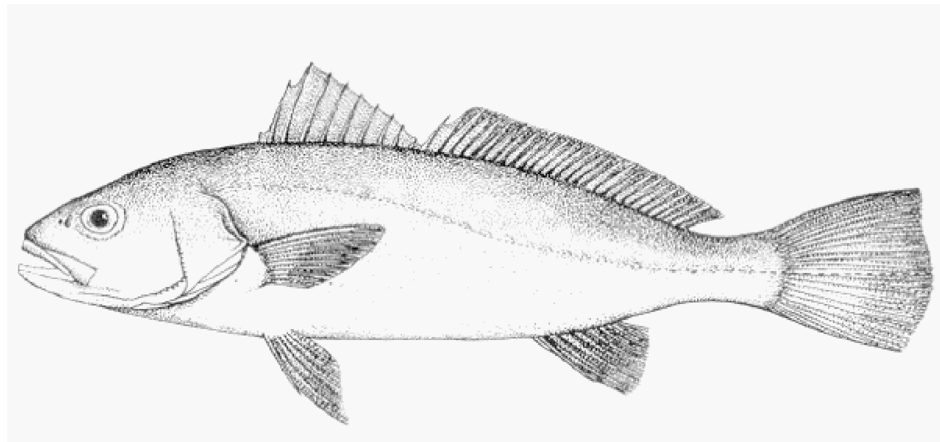
3.1 First research

Influence of the culture technique on physical & chemical characteristics of meagre (*Argyrosomus regius*).

Argyrosomus regius

Meagre (*Argyrosomus regius*; Asso 1801) (Figure 15) is a sea and brackish water fish belonging to the *Sciaenidae* family and it is one of the largest bony fish of the Mediterranean Sea fauna. *Argyrosomus regius* is spread throughout the Mediterranean Sea and along the Atlantic coasts of Europe and West Africa, southern Norway and Sweden, rarely found in the Black Sea and around Italy and Greece (Quéro and Vayne, 1989). The species is found near the shore and shallow water, near the bottom or near the surface, with depth intervals of 15 to 200 meters, in estuaries and coastal lagoons (Chao, 1986). Both adults and juveniles are migratory, moving along the coast or offshore to the coast as a function of changes in water temperature (Quéro and Vayne, 1993). The optimal temperature range for growth is between 17 and 21 °C and meagre reproduction in wild took place in May-July.

Figure 15 – Meagre (*Argyrosomus regius*).



Meagre is one of the newest species in the Mediterranean aquaculture. The first production on a commercial scale was recorded in France in 1997. Meagre

farming has grown also in the Tyrrhenian side of Italian coasts, where the main production centers are La Spezia and Orbetello. Southern France and Italy are the most important markets for this species at 1-3 kg. Italy produced 45 tonnes of cultured meagre in 2010 (FAO, 2010a).
The species is little known until now and still little requested by Italian consumers (Monfort, 2011). Nevertheless meagre has several advantages from both the biological and qualitative point of view. It reaches relatively large commercial sizes quite rapidly, showing promise for the processing industry; it has a high dressing percentage, low adiposity, healthy muscular lipid content, and long shelf life. It is a particularly lean fish, even when grown intensively and receiving the high fat diets that produce high quality marketable products (www.fao.org). Thanks to these promising attributes, meagre has the potential to become a mass market species.

Fish were provided by the fish farm *Il Vigneto* s.r.l. (Ansedonia, Grosseto, Italy) (Figure 16), which is part of the local fish farm cooperative (COOPAM) and commercializes sea bass, sea bream and meagre distributing it throughout the whole national territory.

Figure 16 – Fish farm *Il Vigneto* s.r.l. (aerial view).

Meagre were reared in two different rearing systems: land based tanks located in the farm and submersible sea water cages located two miles far from the coast of Porto Ercole (Grosseto, Italy). Rearing conditions are illustrated in Table 5.

Table 5 – Rearing conditions.

Rearing system	**Geometry of the facilities**	**Fish density (fish/m^3)**	**Water temperature (°C)**	**Sat. O_2 (ppm)**	**Salinity (ppt)**	**Feeding rate (Times per day)**
Land based tank	Shape: circular Volume: 500 m^3	60	19-22*	4-6	16	3-4
Sea water cage	Shape: circular Volume: 2000 m^3	10	13-24	saturated	37	3-4

*geothermal sea water

The experiment involved the Department of Agriculture Biotechnology - Animal Science Section of the University of Florence and the Department of Animal Science of the University of Padua.

Table 6 – Experimental design.

Nr sampling	**Sampling date (yr:2010)**	**Rearing system**	**Nr of fish**	**Day of storage**		
				1	**2**	**3**
1	25th January	Tank	18	6	6	6
2	2nd March	Tank	18	6	6	6
3	4th May	Tank	18	6	6	6
3	8th June	Cage	18	6	6	6

4	27th July	Tank	18	6	6	6
		Cage	18	6	6	6

Fish used in the experiment were from the same tank and cage. As shown in Table 6 fish from the tank were sampled 4 times (January, March, May and July) and those from the cage 2 times (June and July). A total number of 18 fish were collected in each sampling, corresponding totally to a number of 108 fish collected: 72 fish from tank and 36 fish from cage.
The day after the catch, fish underwent to morphometric measurements and physical analyses in the Laboratory of Department of Agricultural Biotechnology – Section of Animal Sciences in Florence. In order to investigate the effect of short-term storage, 6 fish/day were analyzed in the 1st, 2nd and 3rd day after the catch, respectively (Table 6). Fish analyzed the 2nd and 3rd day were kept overnight in melting ice at 1 °C in a cold room.

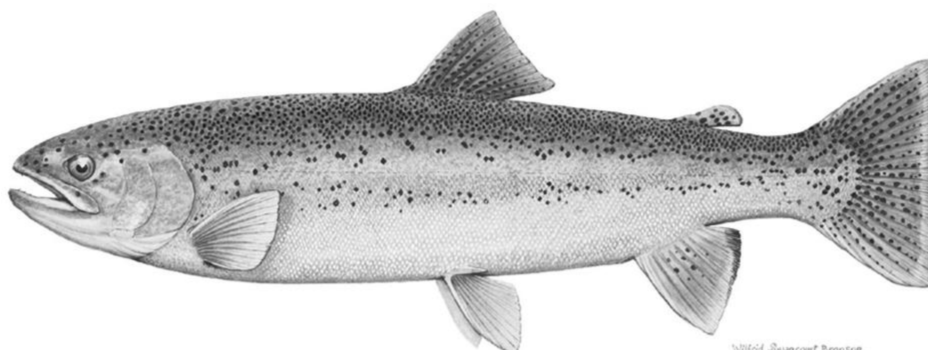
3.2 Second research

Quality characterization of rainbow trout (O*ncorhynchus mykiss*) fillets from different strains and farms: effect of cooking on physico-chemical traits

O*ncorhynchus mykiss*

Rainbow trout (O*ncorhynchus mykiss*) (Figure 17) is a freshwater fish belonging to the *Salmonidae* family. It is native to the Pacific drainages of North America, ranging from Alaska to Mexico. Since 1874 it has been introduced to waters on all continents for recreational angling and aquaculture purposes. Production greatly expanding in the 1950s and several local domesticated strains have developed (www.fao.org). Out of these, numerous improved commercial strains have been bred. The widely cultured commercial strains have been improved from those original rainbow trout populations that possessed advantageous qualities, such as hardness, fast growth, resistance to diseases and reliable reproduction under farm conditions (Woynarovich et al., 2011).

Figure 17 - Rainbow trout (O*ncorhynchus mykiss*)



The optimum water temperature for rainbow trout culture is below 21 °C. Most fish only spawn once, in spring (January-May), although selective breeding and photoperiod adjustment has developed hatchery strains that can mature earlier and spawn all year round.
The rainbow trout farming is greatly spread in Italy and represents the first cultured species with 33,172 tonnes produced in 2010 (FAO, 2010a).

This research was carried out as topic of the project *FILIDEA*, that was a part of the wider research project called *MIGLIORFILETTO - Ricerca integrata per il miglioramento della produzione ittica commerciale trentina in termini di resa sul prodotto lavorato per la specie Oncorhynchus mykiss (trota iridea)*, funded by Provincia di Trento. In the project FILIDEA, the fish analyzed for qualitative parameters were rainbow trouts that belonged to 5 strains selected among those which showed the best growth performances in the project MIGLIORFILETTO.
The farms where the fish were reared were located in unharmed zones and were chosen because representative of the production area in the Provincia Autonoma of Trento and characterized by climatic conditions typical of mountain trout production.
Fish farms were members of ASTRO (Associazione Troticoltori Trentini, Lavis, Trento, Italy) which owns a filleting plant located in Lavis (Trento). In the plant, fish are filleted and/or intended for further different processing like breading, meatball production, smoking,… Processed products have several sales channels: retail market, catering and national department stores.

The fish farms involved in the project (Figure 19) were the following:

- Istituto Agrario di San Michele all’Adige (IASMA) (San Michele all’Adige, Trento, Italy) (Figure 18a)

- Soc. Agr. Troticoltura Foglio (Storo, Trento, Italy) (Figure 18b)
- Troticoltura di L. Tamanini (Vigolo Vattaro, Trento, Italy) (Figure 18c).

Rainbow trout were reared in land-based tank and rearing methods and conditions follow the disciplinary code of ASTRO. Rearing conditions in the 3 fish farms are summarized in Table 7. Temperature fluctuations and dissolved oxygen levels showed the highest variability in Tamanini. This can be firstly explained by the fact that the farm was at higher altitude (800 m on sea level) in comparison with IASMA and Foglio (200 m on sea level) and water supply was also from stream water, generally affected by marked seasonal fluctuations of temperature. IASMA showed the most uniform and standardized parameters.
In order to compare local and foreign stocks, 3 national stocks (2 from Trentino and 1 from Tuscany), 1 from United Kingdom and 1 from U.S.A were tested. The 5 strains analyzed were the following:

- La Frola (origin: Lucca, Italy)
- Troutlodge (origin:U.S.A.)
- Isle of Man (origin: UK)
- Burrini (origin: Trento, Italy)
- Valsugana (origin: Trento, Italy).

Figure 18a – IASMA.

Figure 18b – Troticoltura Foglio.

Figure 18c - Troticoltura di L. Tamanini.

Figure 19 – Iasma (2a), Troticoltura Foglio (2b), Troticoltura di L. Tamanini (2c)

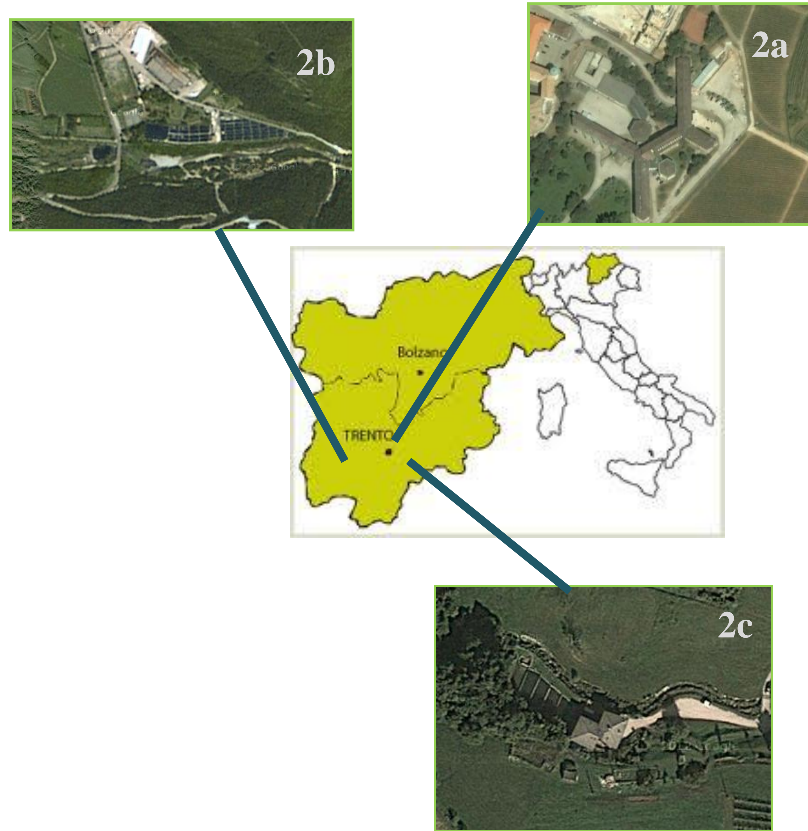


Table 7 – Rearing conditions in the three farms

	Geometry of the facilities	Fish density (fish/m^3)	Water temperature (°C)	Sat. O_2 (ppm)	Feeding rate (Times per day)
Iasma	Shape: rectangular Volume: 12 m^2	50	12.41*	8.24	2-3
Foglio	Shape: rectangular Volume: 7.8 m^2	50	7.80*	7.80	2-3

Tamanini	Shape: rectangular Volume: 43 m^2	50	9.13***	9.24	2-3

*Well water
**Well and spring water
***Spring and stream water.

They refer to batches of rainbow trout belonging to populations which can be geographically and genetically distinguished from each other. The foreign strains were from eggs releasing only female fish.

Fish were caught at commercial size (600-700g). Since fish reached the commercial weight in different period and age, depending on strains and/or farm, samplings took place in different moment of the year. In Table 4 is showed the experimental design. In each sampling 10 fish per stock were caught. Totally 30 fish from each stock were analyzed and 50 fish from each fish farm. Fish were filleted, trimmed and weighted in the processing plant of Lavis; then the right fillets were vacuum packed and labeled, and immediately sent to Florence inside refrigerated box. In the Laboratory of Florence the fillets were newly weighted (about 48 h after slaughtering) and underwent to physical analyses.

Table 8 – Experimental design.

Nr sampling	Farm	Stock	Sex	Sampling date	Nr of fish	Days between the first and the last sampling within each farm
1	IASMA	La Frola	M+F	22.07.2010	10	43
2*		Valsugana	M+F	23.07.2010	10	
2*		Burrini	M+F	23.07.2010	10	
3		Troutlodge	F	04.09.2010	10	
4		Man	F	10.09.2010	10	
5	FOGLIO	La Frola	M+F	23.09.2010	10	50
6		Troutlodge	F	30.09.2010	10	

7*		Valsugana	M+F	14.10.2010	10	
7*		Burrini	M+F	14.10.2010	10	
8		Man	F	11.11.2010	10	
9*	TAMANINI	La Frola	M+F	23.12.2010	10	43
9*		Burrini	M+F	23.12.2010	10	
10		Man	F	21.01.2011	10	
11		Troutlodge	F	27.01.2011	10	
12		Valsugana	M+F	03.02.2011	10	

*Fish from batch of different stocks were caught in the same date of sampling.

3.3 Feed and water sampling

In the first research meagre were fed the same feed (Protec 3P, Skretting Italia S.p.a., Mozzecane, Verona, Italy); it was sampled three times corresponding to the second, third and fourth fish sampling and analyzed for proximate composition (dry matter, crude protein, crude lipid and ash content) and iron and selenium levels in Padua, whereas total lipid content and fatty acid profile analysis was performed in Florence. Rearing water was sampled twice and analyzed for iron and selenium content (Table 9).

Table 9 – Analyses performed on fish and laboratories involved.

Research	Biological matrix	Laboratory	Proximate analysis	Total lipids	Fatty acids profile	Fe, Se	NIRS	Hydroxyproline
Meagre	Fish	Florence		X	X			
		Padua	X			X	X	
	Feed	Florence		X	X			
		Padua	X			X		
	Water	Florence						
		Padua				X		

Rainbow trout	Fish	Florence	X	X	X			X
		Padua					X	
	Feed	Florence	X	X	X			
		Padua						
	Water	Florence						
		Padua				X		

Feed composition and FA profile are shown in Table 10 and 11, respectively.

In the second research the commercial feed Vita (Veronesi S.p.A., Quinto di Valpantena, Italy) was utilised. In the initial phase of the rearing, the fish were fed a commercial feed (moisture 9%; crude protein 62-45%; crude fat 10-21%; ash 11-8%) that differed from the feed utilised during the fattening and the finishing phases. In these last phases feed formulas were those envisaged in the disciplinary code of ASTRO, as follows:

VITA 3: for fish with weight < 100 g
ASTRO 4: for fish with weight > 100 and < 200 g
ASTRO 6: for fish with weight > 200 and < 350 g
ASTRO SALMO 50: for fish with weight > 350 g.

The composition of fattening and finishing phase feeds is shown in Table 12. The FA profile is shown in Table 13.

Table 10 – Feed composition used in meagre research.

	March	**May**	**July**
Moisture (%)	7.95	7.35	6.80
Crude protein (%)	45.30	44.80	44.30
Crude fat (%)	12.95	19.85	18.70
Crude fiber (%)	4.90	3.10	1.75
Ash (%)	6.80	5.45	6.10
NDF (%)	4.55	4.40	7.45
ADF (%)	4.15	2.70	1.40
ADL (%)	0.75	0.45	0.25

Table 11 – FA profile of feed used in meagre research.

Fatty acids (% of total FA)	**January**	**May**	**July**
14:0	5.17	5.11	4.22
16:0	13.92	15.35	14.66
18:0	2.49	3.24	2.74
SFA	***23.04***	***25.23***	***23.17***
16:1n-7	5.39	5.65	3.63
18:1n-9	17.49	12.74	17.45
18:1n:7	2.53	2.61	2.04
20:1n-9	4.47	0.61	2.58
22:1n-11	6.03	0.42	4.39
MUFA	***38.19***	***23.17***	***32.08***
18:2n-6 (LA)	9.52	18.60	19.58
PUFAn-6	***10.99***	***20.12***	***20.78***
18:3n-3 (ALA)	2.60	2.31	3.09
18:4n-3	2.66	1.63	1.95
20:5n-3 (EPA)	7.46	13.45	6.62
22:5n-3	1.36	1.39	0.67
22:5n-6	0.20	0.22	0.21
22:6n-3 (DHA)	10.39	7.46	9.62
PUFAn-3	***25.99***	***27.34***	***22.78***
PUFA	***38.78***	***51.60***	***44.75***
n-3/n-6	2.37	1.36	1.10

The fatty acids C12:0, C15:0, C14:1, C16:2*n*-4, C16:3*n*-4, C17:0, C17:1, C16:4*n*-1, C18:2*n*-4, C18:3*n*-6, C18:3*n*-4, C20:0, C20:1*n*-7, C20:2*n*-6, C20:3*n*-6, C20:3*n*-3, C20:4*n*-3, C21:5*n*-3, C22:1*n*-9, C22:4*n*-6, 6 C22:5*n*-6, detected in percentages lower than 1, are considered in the composite fractions, but not reported in the table for brevity.

Rearing water was sampled one time for selenium and iron analysis, performed in Padua (Table 9).

Table 12 – Feed composition used in rainbow trout research.

	Vita 3	**Astro 4**	**Astro 6**	**Salmo 50**
Moisture (%)	5.35	4.10	5.35	6.95
Crude protein (%)	55.30	45.15	41.10	40.25
Crude fat (%)	18.05	19.25	20.40	20.75
Crude fiber (%)	1.05	3.10	2.95	3.60
Ash (%)	8.50	6.60	6.20	5.85

NDF (%)	2.25	4.30	1.85	3.65
ADF (%)	0.54	2.60	2.40	3.15
ADL (%)	0.12	0.35	0.45	0.75

Table 13 – FA profile of feed used in rainbow trout research.

Fatty acids (% of total FA)	**Vita3**	**Astro4**	**Astro66**	**Salmo50**
C14:0	3.06	3.59	4.92	7.91
C16:0	12.66	13.49	14.64	18.04
C18:0	3.46	3.80	3.49	3.61
SFA	***20.80***	***22.14***	***24.29***	***31.26***
C16:1n-7	3.30	3.80	5.22	8.24
C18:1n-9	16.21	14.24	11.69	9.61
C18:1n-7	1.87	1.94	2.13	3.00
MUFA	***22.64***	***20.95***	***20.35***	***22.47***
C18:2n-6 (LA)	28.54	27.80	16.49	5.86
PUFAn-6	***29.67***	***28.73***	***17.92***	***7.49***
C18:3n-3 (ALA)	3.88	4.52	2.73	1.04
C18:4n-3	0.86	1.29	1.88	1.92
C20:5n-3 (EPA)	10.16	9.96	14.82	17.55
C22:5n-3	1.19	1.05	1.37	1.60
C22:6n-3 (DHA)	7.65	7.90	11.96	10.09
PUFAn-3	***24.47***	***25.43***	***33.71***	***33.54***
PUFA	***56.59***	***56.55***	***54.97***	***45.91***
n-3/n-6	0.82	0.89	1.88	4.48

The fatty acids C12:0, C15:0, C14:1, C16:2*n*-4, C16:3*n*-4, C17:0, C17:1, C16:4*n*-1, C18:2*n*-4, C18:3*n*-6, C18:3*n*-4, C20:0, C20:1*n*-7, C20:2*n*-6, C20:3*n*-6, C20:3*n*-3, C20:4*n*-3, C21:5*n*-3, C22:1*n*-9, C22:4*n*-6, 6 C22:5*n*-6, detected in percentages lower than 1, are considered in the composite fractions, but not reported in the table for brevity.

3.4 Morphometric analyses

The following measurements were taken on both fish species:

- Body weight (BW)
- Total length (TL)
- Body length (BL)
- Head weight
- Fillets weight (left and right).

The following measurements were taken on meagre:

- Liver, visceral fat, viscera weight.

Anatomic indexes

The following anatomic indexes were calculated:

- Condition factor:
  BW (g) / TL^3 (cm)
- Viscerosomatic index (VSI):
  Viscera × 100/ BW
- Visceral fat index (VFI):
  Visceral fat × 100/ BW
- Fat percentage:
  Fat × 100/ BW
- Head percentage:
  Head × 100/ BW
- Frame percentage:
  Frame × 100 / BW
- Percentage of head and frame:
  (Head + Frame) × 100 / BW
- Gonads percentage:
  Gonads × 100 / BW
- Liver percentage
  Liver x 100 / BW.

3.5 Cooking procedure

Each right fillet was wrapped in an aluminum foil and cooked by boiling in a steamer for 10 min. At the end of cooking treatment, liquid leaching out was removed. Fillets were cooled at room temperature and then weighed, to calculate the cooking yield, expressed by the formula:

Cooking Yield = 100 × right fillet weight (cooked) / right fillet weight (raw).

Marketable indexes

Utilising some of the measurements previously detailed, the following marketable indexes were calculated.

- Dressed yield:
  100 × [BW – (viscera + visceral fat + gonads + liver)] / BW
- Fillet yield:
  100 × (left fillet weight + right fillet weight) / BW

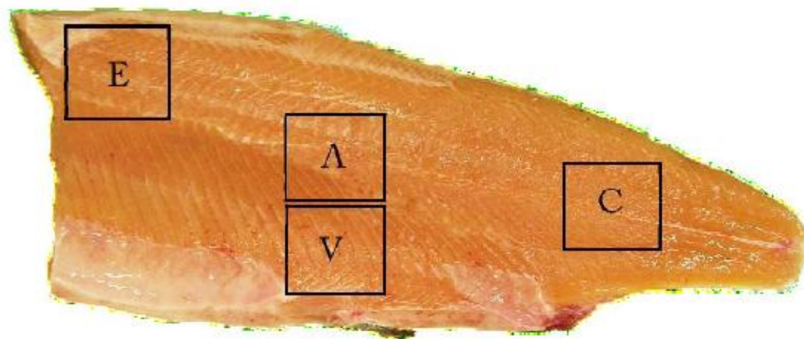
- Cooking losses:
  100 × [right fillet weight (raw) – right fillet weight (cooked) / right fillet weight (raw).

3.6 Physical analyses

3.6.1 Texture analysis

Instrumental texture measurements were collected by using a texturometer Zwick Roell® mod. KAF-TC 0901278, equipped with a 200 N load cell.
Both shear and compression tests were performed on left raw fillets and right cooked fillets. Fillets were cut into four parts (epaxial, central, caudal and ventral, as shown in Figure 20). Shear test was done on central part by using a straight blade (Figure 21a) while compression tests were performed on epaxial, caudal and ventral parts by using a cylindrical probe (diameter: 1 cm; length: 12 cm; Figure 21b). Probe penetrated at a constant speed of 30 mm/min until a 50% of total deformation.

Figure 20 – Fillet part for compression (epaxial, E; ventral, V; Caudal, C) and shear test (central, A).



One cycle compression test was executed on meagre fillets, while cyclic compression test (two cycles) was performed on rainbow trout fillets.
Data were collected and analyzed by the Test-Xpert 2 by Zwick Roell® software version 3.0.

Figure 21a – The shear test with the straight blade.

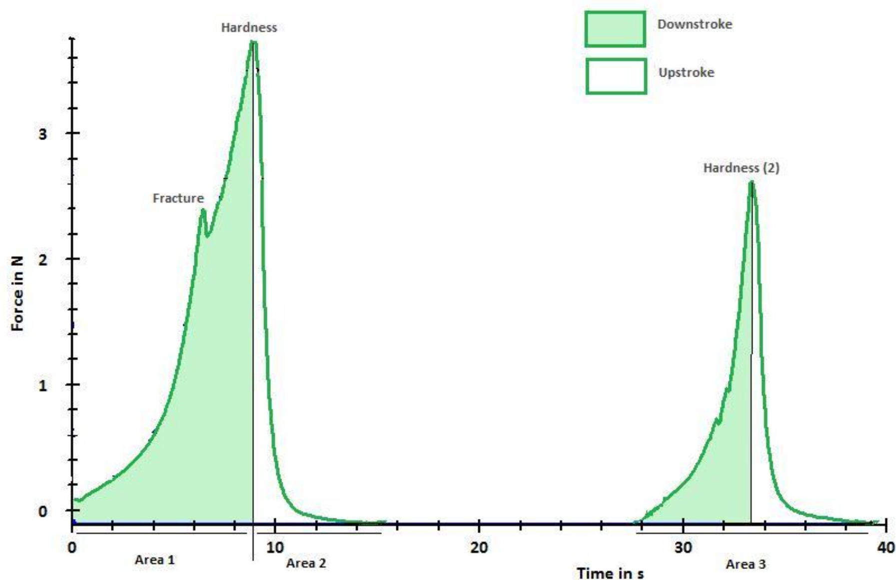
Figure 21b – The compression test with the cylindrical probe.

The following textural attributes were registered and calculated according to the type of the test (Table 14). In Figure 22 is shown an example of force *vs* time graph as registered in real time by software Test-Xpert 2.

Table 14 – Textural parameters and definitions.

	Measurement	Definition	Unit of measurement	Reference
Shear test	Maximum shear force	The highest peak of the curve, which is the maximum resistance of the sample to shearing	N	Veland and Torrissen (1999)
1 cycle compression test	Hardness	The resistance at maximum compression during the first compression. The hardness of the sample at first bite (Figure 22).*	N	Veland and Torrissen (1999)
Cyclic compression test	Hardness	(see above)*		
	Hardness (2)	The resistance at maximum compression during the second compression. The hardness of the sample at the second bite (Figure 22).	N	Veland and Torrissen (1999)
	Fracturability	The force at which the fillet fractures during the first downstroke. Fracturability gives a measure of the breaking strength of the muscle when subjected to tensile, shear and compression tests. The force required to bite through the surface of the fillet (Figure 22).	N	Veland and Torrissen (1999)
	Cohesiveness	The area of the second compression cycle (area 3) relative to the area of the first compression cycle [area(1+2)]. Cohesiveness gives a relative and dimensionless measure of how much of the muscle's strength is retained after the deformation of the first compression. If cohesiveness=1, the muscle has maintained its strength and regained its structure completely during the pause between the compressions, and offers the same resistance to the second compression as to the first. If cohesiveness is <1, the deformation of the first compression has been partly irrecoverable (Figure 22).	Adimensional	Veland and Torrissen (1999)
	Resilience	The area of the first upstroke (area 2) relative to the area of the first downstroke (area 1). Resilience gives a measure of the elasticity of the muscle, and considers not only the distance, but also the force and speed with which the fillets bounces back after the initial deformation. If resilience=1, all the work performed by the probe during the downstroke is returned by the fillet during the upstroke. If resilience is <1, the fillet has not recovered completely to its original thickness, or has recovered with less force or speed than it was compressed with (Figure 22).	Adimensional	Veland and Torrissen (1999)
	Springiness	The time of the second compression cycle relative to the time of the first compression cycle (Figure 5). Thickness that the food matrix recovers during the time that elapses between the two compression cycles.	Adimensional	Ginés et al. (2004); Ayala et al. (2010)
	Gumminess	Hardness multiplied by cohesiveness.	N	Ayala et al. (2010)
	Chewiness	Hardness multiplied by cohesiveness multiplied by springiness.	N	Ayala et al. (2010)
	Adhesiveness	Negative force area under the baseline between the compression cycles.	Joule	Ayala et al. (2010)

Figure 22 – Force (N) *vs* time (s) graph obtained by texturometer analysis in a 2 cycle compression test.

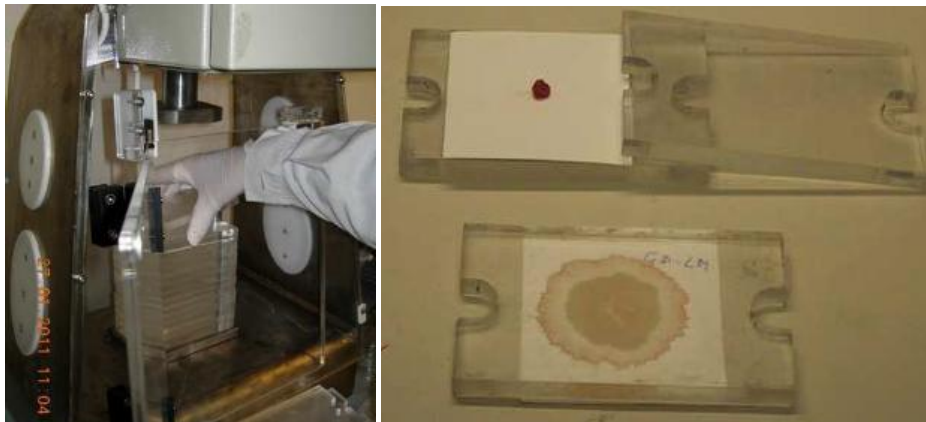


Area 1. The area of the curve during the first downstroke, which is proportional to the work performed by the probe on the fillet during the first compression. The work performed during the first bite.
Area 2. The area under the curve during the first upstroke, which is proportional to the work performed by the fillet on the probe during the first decompression. The work performed during relaxation after the first bite.
Area 1+2. The area under the curve during the first compression cycle. The total work performed during the first compression cycle. Total work performed during the first bite.
Area 3. The area under the curve during the second compression cycle. The total work performed during the second compression cycle. Total work performed during the second bite.

3.6.2 Free water

Free water is constituted by the cellular water physically free, which means that it can move without becoming attracted by proteins (Honikel and Hamm, 1994). Free water was determined in both raw and cooked fillet and performed by filter paper press method of Grau and Hamm (1957). A piece of 300 mg of muscle tissue from epaxial region was placed on a filter paper (8x8 cm) (Whatman No. 1) and pressed by using an hydraulic press (50 bar x 5 min) between two plexiglass plates to a thin film (Figure 23). The water, which is squeezed out, is absorbed by the filter paper. The area of the ring of fluid, which is obtained by subtracting the area of the meat film from the total area, is proportional to the amount of water (Honikel and Hamm, 1994). The area was scanned and measured by using Adobe Photoshop CS4 extended ver. 11 and expressed in cm^2.

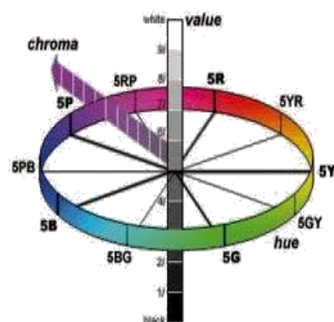
Figure 23 – Hydraulic press (left) and plexiglass plates before and after compression (right).



3.6.3 Colorimetric attributes

Colorimetric attributes were measured according the CIELab colour space (Figure 24). The Commission lnternationale de I'Eclairage - abbreviated as CIE - is an organization devoted to international cooperation and exchange of information among its member countries on all matters relating to the science and art of lighting. In 1976 the CIE proposed a colour space (CIELab) whose main goal was to provide a perceptually equal space. This means that the Euclidian distance between two colours in the CIELab colour space is strongly correlated with the human visual perception (Tkalčič and Tasič, 2003). In the CIELab colour space are measured the values of 5 coordinates: lightness (L*), red index (a*), yellow index (b*), Hue and Chroma.
Measurements were taken in triple in 3 parts of the fillet (epaxial, caudal, ventral) by the Spectro-color® colorimeter and by the software Spectral qc 3.6.

Figure 24 – CIELab colorimetric space.



The following attributes were measured:
Lightness (L*): is a measure of the intensity of light and ranges between 0 and 100
a*: is a measure of the intensity of red colour and ranges between -60 (= green) and +60 (= red)
b*: is a measure of the intensity of yellow colour and ranges between -60 (= blue) and +60 (= yellow)
Hue: is the attribute which tells us whether the colour is red, green, yellow, blue, purple (Tkalčič and Tasič, 2003). It ranges between 0 and 360°. It is calculated according the following formula:

$$Hue = arctg\ (b^*/a^*)$$

Chroma: indicates the saturation of colour which is the level of non-whiteness. An extremely saturated colour has only one spectral component while an unsaturated colour has lots of white added (Tkalčič and Tasič, 2003). It ranges between 0 and 1. It is calculated according to the following formula:

$$\sqrt{}a^2 + b^2$$

Sample treatment
After physical analyses, the fillets were skinned and the muscle was minced. Then the individual ground fillets were frozen at -80/-90 °C and freeze-dried at -40 °C. Freeze-dried samples were removed from the lyophilizer and quickly weighed. They were exposed to air for 24 hours and weighed again. Then they were crushed to a powder by a Moulinex grinder, the powder was collected in plastic bags that were stored in the dark at -22 °C until the moment of the analyses.
In both of experiments a portion of the freeze-dried samples was sent to the Laboratory of the Department of Animal Science of Padua, where the second part of the experiment occurred.
The chemical analyses, in reference to the Laboratory where they were performed, are listed in Table 5.

3.7 Chemical analyses

3.7.1 Moisture (950.46)
Moisture was calculated after freeze-drying according the following formula:

$$Moisture\ \% = 100 - (NWF \times 100/\ NW)$$

where:
NWF = Net weight of freeze dried sample
NW = Net weight of fresh sample.

3.7.2 Proximate composition

Proximate composition (crude protein, crude fat, ashes) was determined according to AOAC (1995).

3.7.3 Crude protein (976.05)

Crude protein as Kjeldahl nitrogen was performed by Kjeldahl method which allows to determine protein, amino, ammonia (NH_3) and urea nitrogen. Procedure consists in a catalytically supported mineralization of muscle and following conversion of organically bonded nitrogen into ammonium sulfate. Alkalizing the solution liberates ammonia which is quantitatively steam-distilled and determined by titration.

An amount of 250-300 mg of freeze dried sample was digested in pyrex tubes by adding tablets of copper sulfate (Cu_2SO_4) and potassium sulfate (K_2SO_4) as catalysts, then 15 ml of sulfuric acid (H_2SO_4) concentrated at 99% were added. Tubes were transferred to a heating plate at 400-430 °C for 1 hour.

In this phase, by reacting with sulfuric acid, amino groups ($-NH_2$) formed ammonium sulfate $(NH_4)_2SO_4$, which is acid-stable. After digestion the sample was allowed to cool room temperature and the glass tube was transferred to a distillation unit. Prior to the distillation the acidic sample was neutralized by adding sodium hydroxide solution (NaOH) at 40%.

Alkaline ambient favored the conversion of ammonium ions (NH_4^+) into ammonia (NH_3) which is transferred into the receiver vessel by means of steam distillation. The equation is the following:

$$NH_4^+ + OH^- \leftrightarrows NH_3\ (gas) + H_2O$$

In the vessel ammonia reacted with 60 mL of boric acid $B(OH)_3$ at 4% in water with methyl red as pH indicator. The reaction products are solvated ammonium ions, according to the equation:

$$B(OH)_3 + NH_3 + H_2O \leftrightarrows NH_4^+ + B(OH)_4^-$$

The concentration of captured ammonium ions in the boric acid was determined by means of an acid base titration using standard solution of sulfuric acid at 0.2%. The chemical reaction is described by the following equation showing the reaction of the tetrahydroxyborate anion $B(OH)_4^-$ with sulfuric acid:

$$B(OH)_4^- + H_2SO_4 \leftrightarrows SO_4^- + B(OH)_3 + H_2O$$

To obtain the percentage of crude protein, the volume of sulfuric acid at 0.2% necessary for the indicator change was multiplied by a correction factor (0.28) corresponding to the nitrogen equivalent of the sulfuric acid. This value was multiplied by 6.25 to have the crude protein content of the analysed sample.

3.7.4 Ash (920.153)

Ash content was determined by weighting 3 g of samples in porcelain capsules, previously weighed when empty, spread to the uniformity and lid in a muffle at 550 °C for 6 hours. After drying, the capsules containing the sample were cooled in the desiccator and re-weighted.
Ash were calculated according the following equation:

$$\text{Ash (\%)} = 100 \times (W1 - W2)/W1$$

where:
W1 = weight (g) of sample before drying
W2 = weight (g) of sample after drying.

3.7.5 Crude fat (991.36)

Crude fat content was determined according to the Soxhlet method and expressed as ether extract (EE). Soxhlet method is a high temperature extraction method which use a reflux apparatus (Soxhlet extractor) with petroleum ether to wash the sample and extract the fat of the sample. An amount of 3 g of freeze-dried sample was put into cellulose thimble (26 x 60 mm). Then cotton wools was placed in top of the thimble. Thimble was transferred to extraction unit and the extraction cup, containing a few glass beads, was weighed and placed on the hot plate. Petroleum ether (40 mL) was poured into the extraction cup, thimble was completely immersed in ether and heated to boiling (40-60 °C). After 1 hour, thimble was removed from the extraction cup and rinsed several times with ether, under reflux, for 1.5 hours. Rinsing extends the contact time between the solvent and the sample and allows it to dissolve all of the fat contained in the sample.
Then the petroleum ether was recovered while the extracted fat remained in the extraction cup. This last was dried in a oven at 103 °C for 30 min, then cooled and weighed.
Ether extract was calculate as follows:

$$EE\% = 100 \times (E2 - E1) / W$$

where:
E1 = weight (g) of the extraction cup prior to extraction
E2 = weight (g) of the extraction cup after drying
W = sample weight (g).

3.7.6 Total lipids

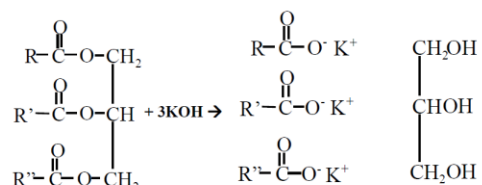
For the quantification of the total lipid content of the samples, modified Folch et al. (1957) method was performed. It is a low temperature extraction method which is known as the most effective for determining fat regardless of fat content of meat products (Peréz-Palacios et al., 2008). Folch et al. (1957) method involves the use of a mixture of selected solvents at different polarity. Maximum yield in extraction of lipid classes of muscle, as trigliceride and membrane lipids, is obtained by using chloroform and methanol in the proportions 2:1 (v/v).
Considering the average moisture content of fillets, 2 g of fresh sample was reconstituted from freeze dried sample by adding 1.5 mL of distilled water to an average amount of 0.5 g of freeze dried sample. Then the reconstituted sample was homogenized with 17-fold volume of solvent mixture (34 mL) in the Omni-Mixer grinder for 5 min at 5000 rpm. The extract was filtered with extra fast Whatman filter paper in a Pyrex test tube and then mixer blade, filter paper and funnel were washed more times (10 mL in total) to ensure quantitative recovery of the lipid extract.
To keep constant the optimal ratio chloroform:methanol:water of 8:4:3, as suggested by the original method, a volume of 9.8 mL (11.3-1.5 mL) of aqueous KCl solution at 0.88% (w/w) was added to the extract (30.3:15.1:11.3). The washing procedure with saline solution is employed to favor the removal from tissue lipids of some non-lipid substances and the formation of a biphasic system with an upper phase (chloroform:methanol:water 3:48:47) containing all the non lipid substances and a lower phase (chloroform:methanol:water 86:14:1) composed by all tissue lipid. The mixtures were kept overnight at refrigerated temperature in order to allow the separation of the two phases. The lower phase was drawn up by glass pipettes in Pyrex tube. Solvents were removed by evaporation (Büchi RE111 Rotavapor) with vacuum pump, until only the fat remained on the bottom of the tube. The fat was dried with pressurized nitrogen and dissolved in 5 ml of chloroform.
An amount of 1 mL of chloroform solution was transferred on an aluminum tray previously placed in an oven to remove humidity and then weighted at room temperature. The tray was placed in a vacuum drying oven at 45 °C to allow chloroform to completely evaporate and then weighted again. The tare weight subtracted from the gross weight gives the amount of lipid in 1 mL.
A further amount of 1 mL of chloroform solution was set for fatty acid profile analysis by gas-chromatography, following the method of Morrison and Smith (1964).

3.7.7 Fatty acids profile

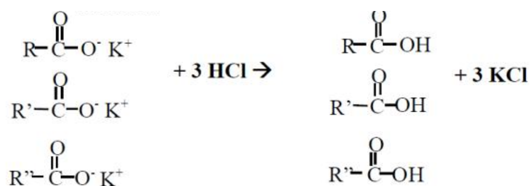
In order to give a final lipid concentration of 3 mg/mL injected into the column, the volume of lipid in chloroform to be taken was calculated according to the following equation:

1 mL : Lipid in 1 mL (g) = x mL : 0.003 g

Each sample volume was then added with a volume of the internal standard tricosanoic acid (C23:0) at the same concentration (0.08 mg/mL) of that found in the external standard used for making the calibration curve. After evaporating chloroform, the residue composed of lipid and C23:0 were re-dissolved in 0.5 mL of cyclohexane.
Saponification was executed by adding to the tubes a solution of 5 mL of 0.5 M KOH in methanol. The tubes were placed in a water bath at 90 °C for 40 minutes and stirred every ten minutes and finally cooled. A mixture of potassium carboxylates and glycerol was formed:

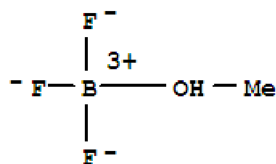


A volume of 1 mL of distilled water and then 2.5 mL of 2 M HCl were added to the saponified fatty acids. Treatments with HCl produces a mixture of fatty acids which can be easily extracted by solvents:



A volume of 2.5 mL of petroleum ether (40-60 °C) was added and the tubes were shaken to favor the solvent extraction of fatty acids. The ether phase was transferred twice and the two steps separated by addition of 2.5 mL of petroleum ether. Petroleum ether with dissolved fatty acids was evaporated.
Esterification was performed according to the modified Morrison & Smith (1964) method. Volumes of 0.5 mL of cyclohexane and 2 mL of BF_3-methanol at 14% were added. When coordinated with methanol (Figure 25), the acidic catalyst boron fluoride becomes a useful reagent for the preparation of fatty acids methyl esters.

Figure 25 – Boron fluoride-methanol complex.



Samples were placed in a water bath at 90 °C for 3 minutes, cooled in water bath and added of 2.5 mL of distilled water and 2.5 mL of petroleum ether. The methylated fatty acids were extracted twice by the addition of 2.5 mL petroleum ether, the solvent has been distilled and methylated fatty acids were dissolved in 1 mL of hexane.

Chromatographic run parameters
Each sample was transferred into the sealed vial and 1 μL was injected into the gas-chromatograph Varian GC 430-GC. The ghromatograph was equipped with a capillary column Supelco Omegawax 320 (length: 30 m; inner diameter: 0.32 mm and thickness of the bonded phase: 0.25 μm), a Ionization Flame Detector (FID) and helium (He) was used as gas carrier.
The chromatographic run was the following:

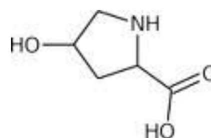
Oven temperature (°C)	Time (min)	Rate (°C/min)
100	2	
160	4	12
220	14	3
220	25	
Total:	*45*	

The injector and the detector temperatures were set at 220 and 300 °C, respectively. The carrier gas was kept at a constant flow of 1.5 mL/min. The split ratio was 1:20. Flow of the gases next to the detector: aux He 25 ml/min, air 300 ml/min, H_2 30 ml/min.

3.7.8 Hydroxyproline content
Collagen is high in proline, glycine and 4-hydroxyproline contents. Hydroxyproline (Figure 26), an amino acid exclusively contained in the

collagen molecules, is used as indicator of collagen content in meat and meat products.

Figure 26 – Molecular structure of 4-hydroxyproline.

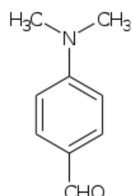


The method applied in these experimental trials was obtained by modifying three different methods (Galasinski, 1978; Bonnet & Kopp, 1984; Bergman & Loxley, 1963). The method utilsed consists of a hydrolysis phase and a colorimetric reaction.
The amount of sample, the volume of acid as well as the dilution volume were half than what is indicated in the original method proposed by Bonnet & Kopp (1984).
An amount of 1.5 g of fresh sample was hydrolyzed in 7.5 mL of perchloric acid (HClO4) at 70% which is the acid suggested by Galasinski (1978) for hydrolysis. Test tubes were kept at 100 °C for 4 hours until solutions were black and viscous.
According to Bonnet & Kopp (1984) method, tubes were cooled and then the hydrolyzed sample was poured into a 50 mL flask and brought to volume. $HCLO_4$ was consequently diluted to 1.8 M. Solution was filtered by filter paper (413-VWR no. 516-0816 porosity 5-13 µm or Durieux 008) into short tubes.
For colorimetric reaction, a calibration curve was prepared by using aliquots of standard solution corresponding to hydroxyproline concentrations between 2 and 20 µg/mL (a quantity characteristic of meat).
In the standard and samples reactions colour development was executed in parallel and each sample was performed twice. Amounts of 0.1 mL of each standard aliquot and 0.1 mL of filtered sample were transferred in Eppendorf tubes (2 mL). NaOH 1.8 M was added to the sample to neutralize $HClO_4$ 1.8 M.
In both standard and samples, hydroxyproline was firstly oxidized by using an oxidant solution composed by 1 volume of Chloramine-T at 7% (w/w) and 4 volumes of buffer solution (pH 6). Buffer solution was necessary to optimize oxidation yield which is known to take place at neutral pH. Chloramine-T reacts with hydroxyproline to give pyrrole-2-carboxylic acid and pyrrole.
In the oxidation phase the following reagents, made according to Bergman & Loxley (1963) method, were added to both standard and samples and left reacting for 25 minutes at room temperature:

- 0.2 mL buffer solution pH 6
- 0.2 mL oxidant solution.

Chloramine-T pyrrole is the starting base for the colour reaction with Ehrlich's reagent. Ehrlich's reagent was prepared according to Bonnet & Kopp (1984) method, by dissolving 20 g p-dimethylaminobenzaldehyde (PDBA, Figure 27) in 30 mL of $HClO_4$ and by mixing 3 volumes of this solution with 13 volumes of isopropanol. The colour of Ehrlich's reagent appeared yellow.

Figure 27 – Molecular structure of PBDA.



When hydroxyproline was oxidized, colour development was induced by adding 1 mL of Ehrlich's reagent on both standard and samples. Then Eppendorf tubes were heated for 25 minutes at 60 °C in a water bath (Bergman & Loxley, 1963). To higher concentration of hydroxyproline corresponds a more intense development of purple-red colour. The absorbance against blank was measured within 4 hour in 0.5 cm cuvette at 558 nm.
Total collagen content was calculated assuming that 17.8% of collagen is constituted of hydroxyproline residues, as was found by Montero et al. (1990) on trout (*Salmo irideus* Gibb) muscle.
The equation utilised was the following:

$$\text{Collagen } \% = \text{Hyp} \times 17.8 / 1000$$

where:
Hyp = mg of Hydroxyproline / 100 g of fresh sample.

3.8 Statistical analysis

The data were analysed statistically with the different procedures (GLM, FACTOR and DISCRIM) from the Statistical Analysis System package (SAS, 2007). Independent variables and number of levels were selected according to the subject of each paper.

4. REFERENCES

A.O.A.C., 1995. Official Methods of Analysis 13th ed., Washington DC, Association of Official Analytical Chemists, 1018 pp.

Abraham, A., Jester, E.L.E., Granade, H.R., Plakas, S.M., Dickey, R.W. 2012. Caribbean ciguatoxin profile in raw and cooked fish implicated in ciguatera. Food Chemistry. 131(1), 192-198.

Ågren, J.J., Hänninen, O. 1993. Effects of cooking on the fatty acids of three freshwater fish species. Food Chemistry. 46, 377-382.

Alomirah, H., Al-Zenki, S., Al-Hooti, S., Zaghloul, S., Sawaya, W., Ahmed, N., Kannan, K. 2011. Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs) from grilled and smoked foods. Food Control. 22(12), 2028-2035.

Al-Saghir, S., Thurner, K., Wagner, K., Frisch, G., Luf, W., Razzazi-Fazeli, E., Elmadfa, I. 2004. Effects of different cooking procedures on lipid quality and cholesterol oxidation of farmed salmon fish (*Salmo salar*). Journal of Agricultural and Food Chemistry. 52, 5290-5296.

Ames, J.M. 2003. BROWNING Nonenzymatic. Encyclopaedia of ecology. Encyclopedia of Food Sciences and Nutrition. 2nd edition. Elsevier Science Ltd. On-line version.

Andrés-Bello, A., Garcìa-Segovia, P., Martinez Monzò, J. 2009. Effects of vacuum cooking (cook-vide) on the physical-chemical properties of sea bream fillets (*Sparus aurata*). Journal of Aquatic Food Product Technology. 18(1-2), 79-89.

Arias, M.T.G., Pontes, E.A., Fernandez, M.C.G., Muniz, F.J.S. 2003. Freezing/defrosting/frying of sardine fillets. Influence of slow and quick defrosting on protein quality. Journal of the Science of Food and Agriculture. 83, 602-608.

Aro, T., Brede, C., Manninen, P., Kallio, H. 2002. Determination of semivolatile compounds in Baltic herring (*Clupea harengus membras*) by supercritical fluid extraction supercritical fluid chromatography-gas chromatography-mass spectrometry. Journal of Agricultural and Food Chemistry. 50, 1970-1975.

Aussanasuwannakul, A., Kenney, P.B., Brannan, R.G., Slider, S.D., Salem, M., Yao, J. 2010. Relating instrumental texture, determined by variable-blade and allo-Kramer shear attachments, to sensory analysis of rainbow trout, *Oncorhynchus mykiss*, fillets. Journal of Food Science. 75(7), s365-s374.

Ayala M.D., Abdel, I., Santaella, M., Martìnez, C., Periago, M.J., Gil, F., Blanco, A. and Lopez Albors, O. 2010. Muscle tissue structural changes and texture development in sea bream, *Sparus aurata* L., during post-mortem storage. *LWT-Food Sci. Technol.* 43, 465-475.

Bayen, S., Barlow, P., Lee, H.K., Obbard, J.F. 2005. Effect of cooking on the loss of persistent organic pollutants from salmon. Journal of Toxicology and Environmental Health, Part A. 68, 253-265.

Belitz, H.D., Grosch, W., Schieberle, P. 2009. Food chemistry. 4th edition. Springer.

Bergman, I., Loxley, R. 1963. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. Analytical Chemistry. 35(12)

Bonnet, M., Kopp, J. 1984. Dosage du collagene dans les tissus conjoctifs, la viande et les produits carnes. Cah. Techn. INRA 5, 19-30.

Bouton, P.E., Harris, P.V. 1972. The effect of cooking temperature and time on some mechanical properties of meat. Journal of Food Science. 37(1), 140-144.

Breivik, H., Thorstad, O. 2004. Removal of organic environmental pollutants from fish oil by short path distillation. The effect of a working fluid. EuroFed Lipid conference, 5-8, September. Edinburgh, U.K.

Burger, J., Dixon, C., Boring, S. 2003. Effect of deep-frying fish on risk from mercury. Journal of Toxicology and Environmental Health. Part A. 66, 817-828.

Burr, M.L., Fehily, A.M., Gilbert, J.F., Rogers, S., Holliday, R.M., Sweetnam, P.M., Elwood, P.C., Deadman, N.M. 1989. Effects of changes in fat, fish and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet. 2, 757-761.

Buttriss, J., Nugent, A. 2005. LIPGENE: an integrated approach to tackling the metabolic syndrome. Proceedings of the Nutrition Society. 64, 345-347.

Chao, L.N. 1986. Sciaenidae. In Fishes of the North-eastern Atlantic and the Mediterranean, Vol. II (Whitehead, P.J.P., Bauchot, M.-L., Hureau, J.-C., Nielson, J. & Tortonese, E., eds), pp. 865–874. Paris: UNESCO.

Choubert, G., Baccaunaud, M. 2010. Effect of moist or dry heat cooking procedures on carotenoid retention and colour of fillets of rainbow trout (*Oncorhynchus mykiss*) fed astaxanthin or canthaxanthin. Food Chemistry. 119, 265-269.

Chukwu, O. 2009. Influences of drying methods on nutritional properties of tilapia fish (*Oreochromis niloticus*). World Journal of Agricultural Sciences. 5(2), 256-258.

Collier, T.K., Singh, S.V., Awasthi, Y.C., Varanasi, U. 1992. Hepatic xenobiotic metabolizing enzymes in two species of benthic fish showing different prevalences of contaminant associated liver neoplasms. Toxicology and Applied Pharmacology. 113, 319–324.

Costa, L. 2007. Contaminants in fish: risk-benefit considerations. Arh Hig Rada Toksikol. 58, 367-374.

Danowska-Oziewicz, M., Karpinska-Tymoszczyk, M., Borowski, J. 2007. The effect of cooking in a steam-convection oven on the quality of selected dishes. Journal of Foodservice. 18, 187-197.

de Castro, F.A.F., Pinheiro Sant'Ana, H.M., Milagres Campos, F., Brunoro Costa, N.M., Coelho Silva, M.T., Salaro, A.L., Do Carmo Castro Franceschini, S. 2007. Fatty acid composition of three freshwater fishes under different storage and cooking processes. Food Chemistry. 103, 1080-1090.

De Kock, J., De Greyt, W., Ayala, J.V., Vanheerswynghels, P., Kellens, M. 2004. Removal of dioxins and PCBs from marine oils: Current status and future developments. Proc. 11th ISNFF, Phuket, Thailand: 53.

Devesa, V., Macho, M.L., Jalon, M., Urieta, I., Muñoz, O., Suñer, M.A., Lopez, F., Velez, D., Montoro, R. 2001. Arsenic in cooked seafood products: study on the effect of cooking on total and inorganic arsenic contents. Journal of Agriculture and Food Chemistry. 49, 4132-4140.

Devesa, V., Súñer, M.A., Algora, S.,Vélez, D., Montoro, R., Jalón, M., Urieta, I., Macho, M.L. 2005. Organoarsenical species contents in cooked seafood. Journal of Agriculture and Food Chemistry. 53, 8813-8819.

Domingo, J. 2011. Influence of cooking processes on the concentrations of toxic metals and various organic environmental pollutants in food: a review of the published literature. Critical Reviews in Food Science and Nutrition. 51, 29-37.

EFSA. 2005. Opinion of the Scientific Panel on Contaminants in the food chain on a request from the European Parliament related to the safety assessment of wild and farmed fish. Question N EFSA-Q-2004-22. EFSA Journal. 236, 1-118.

EFSA. 2008. Polycyclic Aromatic Hydrocarbons in Food. Scientific Opinion of the Panel on Contaminants in the Food Chain. EFSA Journal. 724, 1-114.

EFSA. 2010a. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA Journal. 8(3), 1461-1466.

EFSA. 2010b. Results of the monitoring of dioxin levels in food and feed. Scientific Report of EFSA. EFSA Journal. 8(3), 1385-1421.

Einen, O., Thomassen, M.S. 1998. Starvation prior to slaughter in Atlantic salmon (*Salmo salar*). II. White muscle composition and evaluation of freshness, texture and colour characteristics in raw and cooked fillets. Aquaculture. 169, 37-53.

Erkan, N., Selçuk, A., Özden, Ö. 2010. Amino acid and vitamin composition of raw and cooked horse mackerel. Food Analytical Methods. 3, 269-275.

Ersoy, B., Özeren, A. 2009. The effect of cooking methods on mineral and vitamin contents of African catfish. Food Chemistry. 115, 419-422.

FAO. 2010a. Yearbook 2010. Fishery and Aquaculture Statistics. Rome. FAO 2010.

FAO. 2010b. Yearbook 2008. Fishery and Aquaculture Statistics. Rome. FAO 2010.

FDA. 2000. Consumption of omega-3 fatty acids may reduce the risk of coronary heart disease. Pearson v. Shalala, 164 F.3d 650 (D.C. Cir. 1999).

Fellows, P.J. 2000. Food processing technology: principles and practice. Woodhead Publishing.

Felton. J.S., Knize, M.G., Wood, C., Wuebbles, B.J., Healey, S.K. 1984. Isolation and characterization of new mutagens from fried ground beef. Carcinogenesis. 5, 95-102.

Ferreira, M., Caetano, M., Antunes, P., Costa, J., Gil, O., Bandarra, N., Ferreira, P., Vale, C., Reis-Henriques, M. 2010. Assessment of contaminants and biomarkers of exposure in wild and farmed seabass. Ecotoxicology and Environmental Safety. 73, 579-588.

Finley, J.W., Deming, D.M., Smith, R.E. 2006. Food processing: nutrition, safety and quality. In: Shils, M.E., Shike, M., Ross, A.C., Caballero, B., & Cousins, R.J. (Eds.), Modern Nutrition in Health and Disease (pp. 1777-1788). Lippincott Williams & Wilkins, Philadelphia.

Folch, J., Lees M., Sloane-Stanley G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-509.

Freeman, D.W. 1999. Comparison of moist and dry cooking on sensory quality, consumer acceptance and marketability of canned bighead carp. Journal of Aquatic Food Product Technology. 8(1), 33–44.

Galasinski, W., Gadek, A., Ratkiewicz, A., Rzeczycki, W. 1978. A convenient odification of the method for hydroxyproline determination in proteins Analytical Biochemistry. 85(2), 550-555.

Ginés, R., Valdimarsdottir, T., Sveinsdottir, K., Thorarensen, H. 2004. Effects of rearing temperature and strain on sensory characteristics, texture, colour and fat of Arctic charr (*Salvelinus alpinus*). Food Quality and Preference. 15, 177-185.

GISSI-HF Investigators; Tavazzi, L., Maggioni, A.P., Marchioli, R., Barlera, S., Franzosi, M.G., Latini, R., Lucci, D., Nicolosi, G.L., Porcu, M., Tognoni, G. 2008. Effect of n–3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, doubleblind, placebo-controlled trial. Lancet. 372, 1223–1230.

Gokoglu, N., Yerlikaya, P., Cengiz, E. 2004. Effects of cooking methods on the proximate composition and mineral contents of rainbow trout (*Oncorhynchus mykiss*). Food Chemistry. 84, 19-22.

Gram, L., Huss, H.H. 1996. Microbiological spoilage of fish and fish products. International Journal of Food Microbiology. 33(1), 121-137.

Grau, R., Hamm, R. 1957. Über des wasserbindungsver-mögen des Saügetier musckels. Lebeus Unters. Forsch., 105, 446–460.

Grigorakis, K., Taylor, K.D.A., Alexis, M.N. 2003. Organoleptic and volatile aroma compounds comparison of wild and cultured gilthead sea bream (*Sparus aurata*): sensory differences and possible chemical basis. Aquaculture. 225, 109-119.

Guallar, E., Sanz-Gallardo, I., Van't Veer, P., Bode, P., Aro, A., Gomez-Aracena, J., Kark, J.D., Riemersma, R.A., Martin-Moreno, J.M., Kok, F.J. 2002. Mercury, fish oil, and the risk of myocardial infarction. The New England Journal of Medicine. 347, 1747-1754.

He, K. 2009. Fish, long-chain omega-3 polyunsaturated fatty acids and prevention of cardiovascular disease - eat fish or take fish oil supplement? Progress in Cardiovascular Diseases. 52, 95-114.

He, M., Ke, C., Wang, W. 2010. Effects of cooking and subcellular distribution on the bioaccessibility of trace elements in two marine fish species. Journal of Agricultural and Food Chemistry. 58, 3517-3523.

Hites, R.A., Foran, J.E., Carpenter, D.O., Hamilton, M.C., Knuth, B.A., Schwager, S.J. 2004. Global assessment of organic contaminants in farmed salmon. Science. 303, 226-229.

Honikel, K.O., Hamm, R. 1994. Advances in Meat Research. Vol. 9, Blackie Academic, London. p. 35.

Hori, T., Nakagawa, R., Tobiishi, K., Iida, T., Tsustsumi, T., Sasaki, K., Toyoda, M. 2005. Effects of cooking on concentrations of polychlorinated dibenzo-p-dioxins and related compounds in fish and meat. Journal of Agricultural and Food Chemistry. 53, 8820-8828.

Huis in't Veld, J.H.J. 1996. Microbial and biochemical spoilage of food: an overview. International Journal of Food Microbiology. 33(1), 1-18.

Hyldig, G., Nielsen, D. 2001. A review of sensory and instrumental methods used to evaluate the texture of fish muscle. Journal of Texture Studies. 32, 219-242.

Isosaari, P., Lundebye, A.-K., Ritchie, G., Lie, Ø., Kiviranta, H., Vartiainen, T. 2005. Dietary accumulation efficiencies and biotransformation of poly-brominated diphenyl ethers in farmed Atlantic salmon (*Salmo salar*). Food Additives and Contaminants. 22(9), 829-837.

Iwasaki, M., Kataoka, H., Ishihara, J., Takachi, R., Hamada, G.S., Sharma, S., Le Marchand, L., Tsugane, S. 2010. Heterocyclic amines content of meat and fish cooked by Brazilian methods. Journal of Food Composition and Analysis. 23, 61-69.

Jittinandana, S., Brett Kenney, P., Slider, S.D., Hankins, J.A. 2006. Effect of high dietary vitamin E on lipid stability of oven-cooked and hot-smoked trout fillets. Journal of Food Science. 71(3), 130-136.

Joiris, C.R., Holsbeek, L., Moatemri, N.B. 1999. Total and methylmercury in sardines *Sardinella aurita* and *Sardina pilchardus* from Tunisia. Marine Pollution Bulletin. 38, 188-192.

Kamps, L.R., Carr, R., Miller, H. 1972. Total mercury-monomethylmercury content of several species of fish. Bulletin of Environmental Contaminants and Toxicology. Vol. B, 5, 273-279.

Karl, H., Ruoff, U. 2008. Changes in concentrations of dioxins and dioxin-like PCBs during processing of fish. European Food Research and Technology. 226, 1175-1181.

Knize, M.G., Sinha, R., Rothman, N., Brown, E.D., Salmon, C.P., Levander, O.A., Cunningham, P.L., Felton, J.S. 1995. Heterocyclic amine content in fast-food meat products. Food and Chemical Toxicology. 33(7), 545-551.

Kris-Etherton, P.M., Harris, W.S., Appel, L.J. 2002. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation. Journal of the American Heart Association. 106, 2747-2757.

Larsen, D., Quek, S., Eyres, L. 2011. Evaluating instrumental colour and texture of thermally treated New Zealand King salmon (*Oncorhynchus tshawytscha*) and their relation to sensory properties. LWT-Food Science and Technology. 44, 1814-1820.

Larsen, D., Quek, S.Y., Eyres, L. 2010. Effect of cooking method on the fatty acid profile of New Zealand King Salmon (*Oncorhynchus tshawytscha*). Food Chemistry. 119, 785-790.

Larsen, R., Stormo, S.K., Dragnes, B.T., Elvevoll, E.O. 2007. Losses of taurine, creatine, glycine and alanine from cod (*Gadus morhua* L.) fillet during processing. Journal of Food Composition and Analysis. 20, 396–402.

Lauritzen, L., Hansen, H.S., Jørgensen, M.H, Michaelsen, K.F. 2001. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Progress in Lipid Research. 40, 1-94.

Lawrie, R.A. 1998. Lawrie's meat science. Sixth edition. Woodhead Publishing Limited. Cambridge.

Lin, W., Zeng, Q., Zhu, Z. 2009. Different changes in mastication between crisp grass carp (*Ctenopharyngodon idellus* C. et V.) and grass carp (*Ctenopharyngodon idellus*) after heating: the relationship between texture and ultrastructure in muscle tissue. Food Research International. 42, 271-278.

Mandal, P.K. 2005. Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. Journal of Comparative Physiology B. 175, 221-230.

Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C., Valagussa, F. 2002. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della

Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation. Journal of the American Heart Association. 105, 1897-1903.

Mattila, P., Ronkainen, R., Lehikoinen, K., Piironen, V. 1999. Effect of household cooking on the Vitamin D content in fish, eggs, and wild mushrooms. Journal of Food Composition and Analysis. 12, 153-160.

McCarron, P., Hess, P. 2006. Tissue distribution and effects of heat treatments on the content of domoic acid in blue mussels, *Mytilus edulis*. Toxicon. 47(4), 473-479.

Meade, S.J., Reid, E.A., Gerrard, J.A. 2005. The impact of processing on the nutritional quality of food proteins. Journal of AOAC International. 88, 904-922.

Methven, L., Tsoukka, M., Oruna-Concha, M.J., Parker, J.K., Mottram, D.S. 2007. Influence of sulphur amino acids on the volatile and non-volatile components of cooked salmon (*Salmo salar*). Journal of Agricultural and Food Chemistry. 55, 1427-1436.

Monfort, M.C. 2011. Present market situation and prospects of meagre (Argyrosomus regius), as an emerging species in Mediterranean aquaculture. Studies and reviews. General fisheries commission for the Mediterranean. FAO. N° 89.

Montero, P., Borderias, J., Turnay, J. and Leyzarbe, M.A. 1990. Characterization of hake (*Merluccius merluccius* L.) and trout (*Salmo irideus* Gibb) collagen. *Journal of Agriculture and Food Chemistry*. 38, 604-609.

Moradi, Y., Bakar, J., Syed Muhammad, S.H., Che Man, Y. 2009. Effects of different final cooking methods on physico-chemical properties of breaded fish fillets. American Journal of Food Technology. 4(4), 136-145.

Moretti, G., Marini, V., Bortoli, A. 1990. Mercury and selenium in raw and cooked fish. Rivista della Società Italiana di Scienza dell'Alimentazione. 19(1), 35-38.

Morita, K., Kubota, K., Aishima, T. 2003. Comparison of aroma characteristics of 16 fish species by sensory evaluation and gas chromatography analysis. Journal of the Science of Food and Agriculture. 83, 289-297.

Morrison WR, Smith LM. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with born fluoride-methanol. J Lipid Res 5:600-608.

Moya, J., Garrahan, K.G., Poston, T.M., Durell, G.S. 1998. Effects of cooking on levels of PCBs in the fillets of winter flounder. Bulletin of Environmental Contamination and Toxicology. 60, 845-851.

Mozaffarian, D., Rimm, E.B. 2006a. Fish intake, contaminants, and human health. Journal of the American Medical Association. 296(15), 1885-1899.

Mozaffarian, D., Katan, M.B., Ascherio, A., Stampfer, M.J., Willett, W.C. 2006b. Trans fatty acids and cardiovascular disease. The New England Journal of Medicine. 354, 1601–1613.

Murphy, R.Y., Marks, B.P. 2000. Effect of meat temperature on proteins, texture and cook loss for ground chicken breast patties. Poultry Science, 79(1), 99–104.

Oz, F., Kaban, G., Kaya, M. 2007. Effects of cooking methods on the formation of heterocyclic aromatic amines of two different species of trout. Food Chemistry. 104, 67-72.

Oz, F., Kaban, G., Kaya, M. 2010. Effects of cooking techniques and levels on the formation of heterocyclic aromatic amines in chicken and fish. Journal of Animal and Veterinary Advances. 9(8), 1259-1264.

Parzefall, W. 2008. Minireview on the toxicity of dietary acrylamide. Food and Chemical Toxicology. 46, 1360-1364.

Perelló, G., Martí-Cid, R., Castell, V., Llobet, J.M., Domingo, J.L. 2010. Influence of various cooking processes on the concentrations of PCDD/PCDFs, PCBs and PCDEs in foods. Food Control. 21, 178-185.

Perelló, G., Martì-Cid, R., Llobet, J.M., Domingo, J.L. 2008. Effects of various cooking processes on the concentrations of arsenic, cadmium, mercury, and lead in foods. Journal of Agriculture and Food Chemistry. 56, 11262-11269.

Perelló, G., Martí-Cida, R., Castell, V., Llobet, J.M., Domingo, J.L. 2009. Concentrations of polybrominated diphenyl ethers, hexachlorobenzene and polycyclic aromatic hydrocarbons in various foodstuffs before and after cooking. Food and Chemical Toxicology. 47(4), 709-715.

Peréz-Palacios, T., Ruiz, J., Martín, D., Muriel, E., Antequera, T. 2008. Comparison of different methods for total lipid quantification in meat and meat products. Food Chemistry. 110(4), 1025-1029.

Pigott, G.M., Tucker, B.W. 1990. Seafood effects of technology on nutrition. New York: Marcel Dekker, Inc.

Pottier, I., Vernoux, J., Jones, A., Lewis, R.J. 2002. Characterisation of multiple Caribbean ciguatoxins and congeners in individual specimens of horse-eye jack (*Caranx latus*) by high-performance liquid chromatography/mass spectrometry. Toxicon. 40(7), 929-939.

Prost, C., Serot, T., Demaimay, M. 1998. Identification of the most potent odorants in wild and farmed cooked turbot (*Scophtalmus maximus* L.). Journal of Agricultural and Food Chemistry. 46, 3214-3219.

Quéro, J.C, Vayne, J.J. 1989. Le maigre, Argyrosomus regius (Asso, 1801) (Poissons, Scianidae) en Méditerranée occidentale. Bull. Soc. Zool. France, 114(4), 81-89.

Quéro, J.C, Vayne, J.J. 1993. Nouvel indice sur les peregrinations du maigre. Ann. Soc. Sci. Nat. Charente-Marit. 8(2), 127-128.

Rahman, M.A., Hasegawa, H., Rahman, M.A., Rahman, M.M., Miah, M.A. 2006. Influence of cooking method on arsenic retention in cooked rice related to dietary exposure. Sci. Total Environ. 370, 51-60.

Reboreda, A., Lago, J., Chapela, M., Vieites, J.M., Botana, L.M., Alfonso, A., Cabado, A.G. 2010. Decrease of marine toxin content in bivalves by industrial processes. Toxicon. 55(2), 235-243.

Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.

Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs.

Rigault, C., Mazué, F., Bernard, A., Demarquoy, J., Le Borgne, F. 2008. Changes in Lcarnitine content of fish and meat during domestic cooking. Meat Science. 78, 331-335.

Rodrıguez, A., Carriles, N., Cruz, J.M., Aubourg, S.P. 2008. Changes in the flesh of cooked farmed salmon (*Oncorhynchus kisutch*) with previous storage in slurry ice (-1.5 °C). Food Science and Technology. 41, 1726-1732.

Rowe, R.W.D. 1974. Collagen fibre arrangement in intramuscular connective tissue. Changes associated with muscle shortening and their possible relevance to raw meat toughness measurements. Journal of Food Technology. 9(4), 501-508.

Ruiz-Capillas, C., Moral, A. 2004. Free amino acids in muscle of Norway lobster (*Nephrops novergicus* (L.)) in controlled and modified atmospheres during chilled storage. Food Chemistry. 86, 85-91.

Safe, S.H. 1990. Polychorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Critical Reviews of Toxicology. 21, 51-88.

Salama, A.A., Mohamed, M.A.M., Duval, B., Potter, T.L., Levin, R.E. 1998. Polychlorinated biphenyl concentration in raw and cooked North Atlantic Bluefish (*Pomatomus saltatrix*) fillets. Journal of Agriculture and Food Chemistry. 46, 1359-1362.

Salonen, J.T., Seppanen, K., Lakka, T.A., Salonen, R., Kaplan, G.A. 2000. Mercury accumulation and accelerated progression of carotid atherosclerosis: a population based prospective 4-year follow-up study in men of eastern Finland. Atherosclerosis. 148, 265-273.

Santerre, C.R. 2010. The risks and benefits of farmed fish. Journal of the World Aquaculture Society. 41(2), 250-257.

SAS®. 2007. SAS/STAT software, release 9.1. Cary, NC: SAS Institute, Inc.

Schecter, A., Dellarco, M., Päpke, O., Olson, J. 1998. A comparison of dioxins, dibenzofurans and coplanar PCBs in uncooked and broiled ground beef, catfish and bacon. Chemosphere. 31(9), 1723-1730.

Serhan, C.N. 2007. Resolution phases of inflammation: Novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Annual Review of Immunology. 25, 101-137.

Sherer, R.A., Price, P.S. 1993. The effect of cooking processes on PCB levels in edible fish tissue. Quality Assurance. 2, 396-407.

Sioen, I., Haak, L., Raes, K., Hermans, C., De Henauw, S., De Smet, S., Van Camp, J. 2006. Effects of pan-frying in margarine and olive oil on the fatty acid composition of cod and salmon. Food Chemistry. 98, 609-617.

Smit, L.A., Mozaffarian, D., Willett, W. 2009. Review on fat and fatty acids requirements and criteria for developing dietary guidelines. Annals of Nutrition & Metabolism. 55, 44-55.

Snedeker, S. 2001. Pesticides and breast cancer risk: a review of DDT, DDE, and Dieldrin. Environmental Health Perspectives. 109(1), 35-47.

Sobel, J., Painter, J. 2005. Illnesses caused by marine toxins. Clinical Infectious Diseases. 41(9), 1290-1296.

Stier, R.F. 2000. Chemistry of frying and optimization of deep-fat fried food flavour – an introductory review. European Journal of Lipid Science and Technology. 102, 507-514.

Stołyhwo, A., Sikorski, Z.E. 2005. Polycyclic aromatic hydrocarbons in smoked fish – a critical review. Food Chemistry. 91, 303-311.

Storelli, M.M., Giacominelli-Stuffler, R., Marcotrigiano, G. 2002. Mercury accumulation and speciation in muscle tissue of different species of sharks from Mediterranean Sea. Italy Bulletin Environmental Contaminants Toxicology. 68, 201–210.

Sugimura, T.S. 1995. History, present and future of heterocyclic amines, cooked food mutagens. In: Heterocyclic Amines in Cooked Foods: Possible Human Carcinogens, Adamson, R.H. (Ed.). Princeton Scientific Publishing Co., Princeton, New Jersey, pp. 214-231.

Sumnu, G. 2001. A review on microwave baking of foods. International Journal of Food Science and Technology. 36, 117–127.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., Törnqvist, M. 2002. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. Journal of Agriculture and Food Chemistry. 50, 4998-5006.

Tokur, B. 2007. The effect of different cooking methods on proximate composition and lipid quality of rainbow trout (*Oncorhynchus mykiss*). International Journal of Food Science and Technology. 42, 874-879.

Torres-Escribano, S., Ruiz, A., Barrios, L., Véleza, D., Montoroa, R. 2011. Influence of mercury bioaccessibility on exposure assessment associated with consumption of cooked predatory fish in Spain. Journal of the Science of Food and Agriculture. 91, 981-986.

Turhan, S., Sule Ustun, N., Bogachan Altunkaynak, T. 2004. Effect of cooking methods on total and heme iron contents of anchovy (*Engraulis encrasicholus*). Food Chemistry. 88, 169-172.

Türkkan, A.U., Cakli, S., Kilinc, B. 2008. Effects of cooking methods on the proximate composition and fatty acid composition of seabass (*Dicentrarchus labrax*, Linnaeus, 1758). Food and Bioproducts Processing. 86, 163-166.

Unusan, N. 2007. Change in proximate, amino acid and fatty acid contents in muscle tissue of rainbow trout (*Oncorhynchus mykiss*) after cooking. International Journal of Food Science and Technology. 42(9), 1087-1093.

Veland, J.O,Torrissen, O.J.1999. The texture of Atlantic salmon (*Salmo salar*) muscle as measured instrumentally using TPA and Warner-Brazler shear test. Journal of the Science of Food and Agriculture. 79, 1737-1746.

Virtanen, J.K., Rissanen, T.H., Voutilainen, S., Tuomainen, T.P. 2007. Mercury as a risk factor for cardiovascular diseases. The Journal of Nutritional Biochemistry. 18, 75-85.

Visciano, P., Perugini, M., Manera, M., Amorena, M. 2009. Selected polycyclic aromatic hydrocarbons in smoked tuna, swordfish and Atlantic salmon fillets. International Journal of Food Science and Technology. 44, 2028-2032.

Voutila, L., Mullen, A.M., Ruusunen, M., Troy, D., Puolanne, E. 2007. Thermal stability of connective tissue from porcine muscles. Meat Science. 76(3), 474-480.

Weber, J., Bochi, V.C., Ribeiro, C.P., de M. Victorio, A., Emanuelli, T. 2008. Effect of different cooking methods on the oxidation, proximate and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets. Food Chemistry. 106, 140-146.

WHO (World Health Organization), Joint FAO/WHO Expert Committee on Food Additives (JEFCA), Seventy-second Meeting, Rome, 16–25 February 2010. Summary and conclusions. Available: http://www.who.int/foodsafety/chem/summary72 rev. pdf [Last access: October 2010].

Witczak, A. 2009. Effect of frying on polychlorinated biphenyls content in muscle meat of selected fish species. Polish Journal of Food and Nutrition Sciences. 59(2), 157-161.

Wong, C., Hung, P., Lee, K.L.H., Mok, T., Kam, K. 2009. Effect of steam cooking on distribution of paralytic shellfish toxins in different tissue compartments of scallops *Patinopecten yessoensis*. Food Chemistry. 114(1), 72-80.

Wood, J.D., Nute, G.R., Fursey, G.A.J., Cuthbertson, A. 1995. The effect of cooking conditions on the eating quality of pork. Meat Science. 40, 127-135.

Woynarovich, A., Hoitsy, G., Moth-Poulsen, T. 2011. Small-scale rainbow trout farming. FAO Fisheries and Aquaculture Technical Paper. Rome. 561.

Wu, T., Mao, L. 2008. Influences of hot air drying and microwave drying on nutritional and odorous properties of grass carp (*Ctenopharyngodon idellus*) fillets. Food Chemistry. 110, 647-653.

Wu, W., Lillard, D.A. 1997. Cholesterol and proximate composition of channel catfish (*Ictalurus punctatus*) changes following cooking by microwave heating, deep-fat frying, and oven baking. Journal of Food Quality. 21, 41-45.

Yagiz, Y., Kristinsson, H.G., Balaban, M.O., Welt, A.B., Ralat, M., Marshall, M.R. 2009. Effect of high pressure processing and cooking treatment on the quality of Atlantic Salmon. Food Chemistry. 116, 828-835.

Yokoyama, M., Origasa, H., Matsuzaki, M., Matsuzawa, Y., Saito, Y., Ishikawa, Y., Oikawa, S., Sasaki, J., Hishida, H., Itakura, H., Kita, T., Kitabatake, A., Nakaya, N., Sakata, T., Shimada, K., Shirato, K. 2007. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet. 369, 1090–1098.

Zabik, M.E., Booren, A., Zabik, M.J., Welch, R., Humphreye, H. 1996. Pesticide residues, PCBs and PAHs in baked, charbroiled, salt boiled and smoked Great Lakes lake trout. Food Chemistry. 55(3), 231-239.

Zabik, M.E., Zabik, M.J., Booren, A.M., Nettles, M., Song, J., Welch, R., Humphrey, H. 1995. Pesticides and total polychlorinated biphenyls in Chinook salmon and carp harvested from the Great Lakes: effects of skin-on and skin-off processing and selected cooking method. Journal of Agriculture and Food Chemistry. 43, 993-1001.

Zimmerli, B., Rhyn, P., Zoller, O., Schlatter, J. 2001. Occurrence of heterocyclic aromatic amines in the Swiss diet: analytical method, exposure estimation an risk assessment. Food Additives Contaminants. 18, 533-551.

Internet sources

Cooking methods in www.beeflambnz.co.nz (Last access: 10.04.2011)

European Cardiovascular Disease Statistics – 2008 in www.heartstats.org (Last access: 5.04.2011).

Definitions in:

www.sensagent.com (Last access: 10.04.2011)

www.en.wikipedia.org (Last access: 12.04.2011)

Cultured Aquatic Species Information Programme. Meagre (*Argyrosomus regius* (Asso, 1801)) and Rainbow trout (O*ncorhynchus mykiss*) in www.fao.org (Last access: 12.11.12)

FSA. 2004. Advice on fish consumption: benefits & risks. London TSO in www.food.gov.uk (Last access: 13.04.2011)

FAO/WHO. 2008. Interim summary of conclusions and dietary recommendations on total fat and fatty acids. Joint FAO/WHO expert consultation on fats and fatty acids in human nutrition. November 10-14, WHO HQ, Geneva. In www.WHO.int (Last access: 12.04.2011).

PubChem http://pubchem.ncbi.nlm.nih.gov/ (Last access: 21.11.2011).

Tkalčič, M., Tasič, J.F. 2003. Colour spaces: perceptual, historical and applicational background. In www.cse.unsw.edu.au (Last access: 17.12.2012)

PART II

PAPER I

MACRONUTRIENT AND FATTY ACID PROFILES OF MEAGRE (*ARGYROSOMUS REGIUS*) INFLUENCED BY SAMPLING TIME AND COOKING

Roberta Martelli[a], Antonella Dalle Zotte[b], Antonio Bonelli[a], Paola Lupi[a], Oreste Franci[a], Giuliana Parisi[a*]

[a]*Dipartimento di Biotecnologie Agrarie - Sezione di Scienze Animali, Università di Firenze, Via delle Cascine, 5 - 50144 Firenze, Italy*
[b]*Dipartimento di Medicina Animale, Produzioni e Salute, Università di Padova, Agripolis - 35020 Legnaro (Padova), Italy*

[*]Corresponding author: phone: +39 055 3288265; fax: +39 055 321216.
E-mail address: giuliana.parisi@unifi.it

Keywords
Meagre, Chemical composition, Lipid, True retention

Abstract
The effect of sampling time and of cooking on water, protein, lipid, ash, and fatty acid content of farmed meagre was assessed. Significant differences among sampling times were detected in nutrient content of raw fillets. Cooking by boiling induced loss of some macronutrients, mainly lipids and some fatty acids. The retention of total lipid, C18:2*n*-6 and C18:3*n*-3 decreased significantly with sampling time, while EPA and DHA were retained in similar quantity. DHA retention was higher in comparison to the other FAs considered, in all sampling times. Changes detected did not compromise the valuable nutritional traits of meagre. Despite the losses induced by cooking and the low fat content typical of this species, 100 g portion of fillet guaranteed an intake of EPA and DHA that more than doubles the suggested recommended daily intake for EPA plus DHA (250 mg day^{-1}), at any time during the period examined.
The ability to preserve nutrients is an essential requirement for quality maintenance of meagre and suggests the potential use for fillet heat processing.

1.Introduction

Meagre (*Argyrosomus regius*, Asso 1801) is an emerging farm-raised species in the European seafood market that is not well known to consumers (Monfort, 2010). Fast growth (Quémener, 2002), good feed conversion, high adaptation capacities and resilience against stress factors are among the most important aspects in making meagre one of the best potential candidates for large-scale fish farming in Europe (El Ahdal, 2009; Monfort, 2010). The farming of this

species is also important in diversifying commercial aquaculture in Mediterranean and Eastern Atlantic areas.

In addition to favourable farming aptitudes, the increasing interest in meagre is attributed to its encouraging market and quality traits, such as attractive shape, good processing yield, high nutritional value, and excellent taste (Monfort, 2010). In particular, even in large fish meagre has a low lipid content, which is considered one of the most valuable attributes in extending seafood shelf life (Poli et al., 2003). Moreover, the lipids are composed of a high-quality fatty acid (FA) profile, rich in long-chain omega-3 polyunsaturated FAs (*n*-3 LC-PUFAs), mainly EPA and DHA (Grigorakis et al., 2011; Hernandez et al., 2009; Piccolo et al., 2008; Poli et al., 2003), whose beneficial effects on human health provided by their antithrombogenic and antiatherogenic activity have been greatly recognised (Burr et al., 1989; Buttriss and Nugent, 2005; Kris-Etherton et al., 2002; Lauritzen et al., 2001; Marchioli et al., 2002; Serhan, 2007). Farmed meagre is mainly sold fresh (Monfort, 2010) and prepared for domestic and catering purposes. Previous research has shown that cooking affects fish nutritional value and induces nutrient concentration or losses related to the cooking method employed. In several cases, cooking methods either induced lipid loss and oxidation (Al-Saghir et al., 2004; Rodriguez at al., 2008; Weber et al., 2008) or did not induce any change (Al-Saghir et al., 2004; Erkan et al., 2010; Larsen et al., 2010; Rodriguez et al., 2008).

Regarding FAs, Larsen et al. (2010) and Türkkan et al. (2008) found that DHA and EPA are greatly reduced after cooking, while in other studies, the FA profile was not significantly altered (Al-Saghir et al., 2004; de Castro et al., 2007; Erkan et al., 2010; Rodriguez et al., 2008; Weber et al., 2008).

In light of the present market situation, most research is conducted to amplify the potential market base for meagre by investigating fillet nutritional peculiarities and aptitude for processing. Very little research has been done on nutritional value, however, or addressed the macronutrient content and the FA profile of farmed meagre (Hernandez et al., 2009; Orban et al., 2008; Poli et al., 2003), and no data are available on cooked fillets. Because farmed meagre is characterised by very lean flesh, the ability to retain lipids and *n*-3 FAs after cooking could enhance the quality of this species and provide further information on its nutritional profile.

The aim of this study was to describe the effects of cooking (by boiling) on the proximate composition, lipid content and the FA profile of farmed meagre fillets raised in a tank and sampled at different times during the winter-spring period.

2. Materials and methods

Fish were collected from the "Il Vigneto" farm, located near Ansedonia (Grosseto, Italy - 42°25'45.35"N and 11°16'58.85"E). Meagre were raised in an outdoor land-based circular tank (500 m^3), at a constant density of 60 fish/m^3;

the water temperature during farming ranged from 19 to 22 °C (geothermal water), and salinity was approximately 16 ppt. Fish were reared in natural photoperiod condition.
Fish were fed 3-4 times per day with a commercial extruded feed whose proximate composition and FA profile are shown in Table 1.

Table 1 - Proximate composition and FA profile of the diet.

	Diet
Moisture, %	7.90
Crude protein, %	45.30
Crude fat (EE), %	12.90
Ash, %	6.80
CP/EE ratio	3.50
Fatty acids, % of total FAs	
C14:0	5.17
C16:0	13.92
C18:0	2.49
ΣSFA	***23.00***
C16:1*n*-7	5.39
C18:1*n*-9	17.49
C18:1*n*-7	2.53
C20:1*n*-9	4.47
C22:1*n*-11	6.03
ΣMUFA	***38.20***
C18:2*n*-6 (LA)	9.52
ΣPUFAn-6	***11.00***
C18:3*n*-3 (ALA)	2.60
C18:4*n*-3	2.66
C20:5*n*-3 (EPA)	7.46
C22:5*n*-3	1.36
C22:6*n*-3 (DHA)	10.39
ΣPUFAn-3	***26.00***
n-6/*n*-3 PUFA	0.42

Eighteen fish were sampled 3 times (26th January, 3rd March and 5th May; a total of 54 fish collected; average weight of 994.07 ± 262.41 g). The samplings took place in winter-spring period, when metabolical and physiological changes are more evident, also due to the beginning of the reproduction phase. Fish arrived at the Department of Agricultural Biotechnology in Florence (Italy) the day after the catch. Whole fish weight (W) and total length (TL) were recorded at each sampling. After dissection and filleting, the head, axial skeletal bones, viscera, liver, gonads (when evident and separable), perivisceral fat (the fat stored inside the peritoneal cavity) and right and left fillets were weighed. The condition factor (CF) was determined using the following formula CF = 100 x W (g) / TL^3 (cm).
The visceral fat index (VFI), viscerosomatic index (VSI), hepatosomatic index (HSI), and gonadosomatic index (GSI) were calculated as perivisceral fat, viscera, liver, and gonad percentages of total body weight respectively. Dressing yield and fillet yield were calculated as 100 x [W (g) – visceral weight (g) / W (g)] and 100 x [fillets weight (g) / W (g)], respectively.
Left fillets were analysed as raw, while right fillets were wrapped with aluminium foil, placed on a tray in a fish-steamer, boiled (at 98-100 °C) for 10 min and then cooled at room temperature and weighed. The cooking yield was calculated as 100 x [cooked fillet weight (g) / raw fillet weight (g)].
The determinations of the proximate composition, total lipids and FA profiles were performed on both raw and cooked fillets without skin, homogenised, and freeze-dried prior to analyses.

2.1 Proximate analysis and total lipids

Moisture, crude protein (Nx6.25) and ash content were determined according to AOAC (2000) 950.46, 976.05, and 920.153 methods, respectively.
The total lipid extraction was performed according to a modified Folch et al. (1956) method. Freeze-dried samples, reconstituted fresh by adding distilled water, were homogenised with a 2:1 chloroform-methanol (v/v) solution and filtered. The filter was washed several times, and distilled water with 0.88% KCl was added to the filtrate so that the [Choloroform:Methanol]:water ratio was 4:1. Tubes were stirred, and a biphasic system was obtained by standing overnight. The lower phase containing lipids dissolved in chloroform were siphoned and recovered. The total lipid content was determined gravimetrically, after removal of the solvent (chloroform) by evaporation under vacuum and lipid resuspension in a known volume of chloroform (5 mL). Lipid content was weighed in a crucible (gross weight minus tare) after complete chloroform evaporation.

2.2 Determination of FA composition

FAME (Fatty Acid Methyl Esters) analysis was performed according to the modified method of Morrison and Smith (1964). Lipids were saponified with 0.5 M KOH in methanol, and FAs were hydrolysed by adding 2 N HCl. Methyl esters were prepared by transmethylation using boron fluoride-methanol at 14% concentration. Methylated FAs were dissolved in petroleum ether, dried, and finally resuspended in 1 mL of hexane.

The FA composition was determined by liquid gas chromatography (LGC). A GC Varian 430 gas chromatograph (Varian Inc., Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a Supelco Omegawax™ 320 capillary column (30 m x 0.32 mm i.d., 0.25 μm film and polyethylene glycol bonded phase; Supelco, Bellefonte, PA, USA) was utilised. The oven temperature was held at 100 °C for 2 minutes, increased to 160 °C over 4 minutes at the rate of 12 °C/min, increased to 220 °C over 14 minutes at the rate of 3 °C/min, and kept at 220 °C for 25 minutes. The injector and the detector temperatures were set at 220 °C and 300 °C, respectively. One microlitre of sample in hexane was injected into the column with the carrier gas (helium) kept at a constant flow of 1.5 mL/min. The split ratio was 1:20.

Chromatograms were recorded with a computing integrator software (Galaxie Chromatography Data System 1.9.302.952; Varian Inc., Palo Alto, CA, USA). FAs were identified by comparing the retention time of FAME with the standard Supelco 37 component FAME mix (Supelco, Bellefonte, PA, USA). FAs were quantified through calibration curves, using tricosanoic acid (C23:0) (Supelco, Bellefonte, PA, USA) as an internal standard.

All analytical methodologies were submitted to validation procedures.

2.3 Computation of true retention

True retention measures the proportion of a nutrient remaining in the cooked food in relation to the amount of that nutrient originally present in a given weight of the food before cooking (Murphy et al., 1975). True retention (TR%) of nutrients was calculated according to Murphy et al. (1975), as follows:

TR% = 100 x (nutrient content per g of cooked food × g of food after cooking)/(nutrient content per g of raw food × g of food before cooking).

2.4 Computation of fat quality indexes

The following fat quality indexes have been calculated on raw and cooked fillets to assess the variations induced by the cooking process:

- ratio *n*-6/*n*-3;
- LA/ALA, as linoleic acid (LA; C18:2*n*-6) / alpha-linolenic acid (ALA; C18:3*n*-3) ratio;
- atherogenic index (AI), according to the formula [C12:0 + (4 × C14:0) + C16:0] / (ΣPUFA *n*-3 + ΣPUFA *n*-6 + ΣMUFA) (Ulbricht and Southgate, 1991);

- thrombogenic index (TI) according to the formula [C14:0 + C16:0 + C18:0] / [0.5 × ΣMUFA) + (0.5 × ΣPUFA *n*-6) + (3 × ΣPUFA *n*-3) + (ΣPUFA *n*-3/ΣPUFA *n*-6)]; (Ulbricht and Southgate, 1991).
- Hypocholesterolaemic/hypercholesterolaemic FA ratio (HH), calculated as (C18:1*n*-9 + C18:2*n*-6 + C20:4*n*-6 + C18:3*n*-3 + C20:5*n*-3 + C22:5*n*-3 + C22:6*n*-3) / (C14:0 + C16:0) (Santos-Silva et al., 2002).

2.5 Statistical analysis

Data were analysed using an ANOVA (Analysis of Variance) with the GLM (General Linear Model) procedure of SAS® (2007), using sampling month as the fixed factor. For the fat quality indexes, the condition effect (raw or cooked) was added to the model. The differences between least squares means were statistically tested using the Student's t-test. Values with $p<0.05$ were considered significant.

3. Results and discussion

3.1 Morpho- and somatometric traits

The morpho- and somatometric characteristics of fish are presented in Table 2. Differences were detected among sampling times: fish sampled in January and March had lower weight, length, VFI, VSI and head incidence (%) than fish sampled in May, and conversely, they showed higher dressing and fillet yields. Moreover, an increasing trend from January to May was evident for GSI whereas HSI was higher in January than in March and May.

Table 2 - Morphometric and somatometric measurements of meagre at different sampling times.

	Sampling time			rsd*
	January	March	May	
Weight, g	821.7a	879.6a	1280.8b	147.4
Total length, cm	41.4a	43.0a	48.8b	2.65
Condition Factor (CF)	1.14	1.09	1.09	0.11
Visceral fat index (VFI), % [1]	0.37a	0.46a	1.39b	0.55
Viscerosomatic index (VSI), % [1]	3.69a	3.48a	4.78b	0.87
Head, % [1]	27.83a	28.72a	30.28b	1.86
Frame, % [1]	15.12	16.25	16.04	2.08

Gonadosomatic index (GSI), %[1]	0.07a	0.11ab	0.15b	0.08
Hepatosomatic index (HSI), %[1]	1.45b	1.19a	1.20a	0.28
Dressing yield, %[1]	96.30b	96.51b	95.21a	0.87
Fillet yield, %[1]	50.63b	50.64b	48.04a	2.28

* Residual standard deviation
a, b: means with different letters are different (P<0.05).
[1] In percentage of weight.

Because the rearing water was obtained from geothermal resources and its temperature not subjected to seasonal fluctuation and for such reason constant most of the year, variability in somatometric indexes of fish can mainly be attributed to fish size, the gonad maturation process, and the seasonal photoperiod. Fat deposition is positively related to fish size and age (Fontagné-Dicharry and Médale, 2010; Lanari et al., 1999), and is also affected by season, which justifies the highest VFI in May, when fish were larger. Moreover, the increasing trend of GSI can be explained by considering that summer is the natural reproduction period of wild meagre (Quéro and Vayne, 1987). Finally, the HSI was significantly higher in January and subsequently decreased, probably because energy resources were allocated to gonad production, in agreement with findings in other species of fish (Herland et al., 2010; Piñon et al., 2009).
In this study, VSI results were lower than those observed in farmed meagre of similar weight (Poli et al., 2003) and in other commercially valuable Mediterranean species such as farmed sea bass (*Dicentrarchus labrax*) (Poli et al., 2001) and farmed and wild sea bream (*Sparus aurata*) of standard commercial size (300-400 g) (Yildiz et al., 2008). Conversely, the VFI observed was similar to as found by Piccolo et al. (2008) and Poli et al. (2003) in the same species, whereas it was much lower when compared to sea bass (Grigorakis et al., 2004) and sea bream (Grigorakis et al., 2002). As a consequence of negligible offal, this farmed meagre showed particularly high dressing and fillet yields during all sampling periods, and when compared to meagre of similar body weight analysed in previous research (Grigorakis et al., 2011 and Poli et al., 2003), this fish showed higher fillet yields. Unfortunately, in our study, larger fish showed higher head incidence, which represents a limiting factor among the merchantable traits of meagre (Monfort, 2010; Poli et al., 2003).

3.2 Composition of raw fillets

The proximate composition of meagre raw fillets differentiated by sampling time is reported in Table 3. Similar to somatometric traits, chemical composition varied during the period examined and showed similarities in fish sampled in March and May. Lipid content increased with fish size, in agreement

with the finding of Shearer (1994). Meagre were however characterised by a low muscular lipid content for all sampling times, i.e. 2.49, 2.97 and 3.43 % on wet basis in January, March and May respectively. The limited muscle lipid deposition ability observed in meagre is supported by previous findings on juveniles (Chatzifotis et al., 2010) and adults (Grigorakis et al., 2011; Hernandez et al., 2009; Piccolo et al., 2008; Poli et al., 2003), in this way differentiating meagre from other commonly consumed Mediterranean farmed species (i.e., sea bass and sea bream) whose lipid content is generally greater (Grigorakis et al., 2002; Yildiz et al., 2008). In particular, farmed meagre of 800-1.200 g contained a lipid amount comparable to that found in wild sea bream of 250-450 g caught in January and May, when nutrient availability in the marine environment is lower than in summer (Grigorakis et al., 2002).

Table 3 – Moisture (g 100 g^{-1}), protein, ash, total lipids (g 100 g^{-1}, dry basis), and FA profile of meagre raw fillets by sampling time.

	Sampling time			rsd*
	January	March	May	
Moisture	75.47b	75.26b	74.44a	0.83
Protein	85.39b	83.29a	83.04a	2.21
Ash	5.88b	5.40a	5.24a	0.41
Total lipids	10.16a	12.01b	13.39b	1.98
Fatty acids, % of total FAs				
C14:0	3.88	3.91	3.96	0.23
C16:0	18.42b	18.21b	17.69a	0.46
C18:0	5.16b	4.92ab	4.84a	0.35
ΣSFA	***28.86b***	***28.40ab***	***27.96a***	***0.71***
C16:1*n*-7	5.45	5.44	5.67	0.35
C18:1*n*-9	13.00a	13.96b	14.59c	0.48
C18:1*n*-7	2.65	2.64	2.60	0.07
C20:1*n*-9	1.69a	1.85b	2.12c	0.19
C22:1*n*-11	1.28a	1.50a	1.94b	0.31
ΣMUFA	***25.74a***	***27.26b***	***28.89c***	***1.13***
C18:2*n*-6 (LA)	8.95a	9.49b	9.65b	0.25
C20:4*n*-6	1.09b	1.05ab	1.01a	0.09
C22:5*n*-6	0.36b	0.34ab	0.33a	0.03
ΣPUFAn-6	***11.14***	***12.05***	***11.85***	***1.26***
C18:3*n*-3 (ALA)	0.95a	1.12b	1.23c	0.12
C18:4*n*-3	1.20	1.18	1.20	0.12

C20:5*n*-3 (EPA)	10.54c	9.71b	9.34	0.41
C22:5*n*-3	2.41b	2.39b	2.27a	0.11
C22:6*n*-3 (DHA)	15.46b	14.73ab	13.54a	1.41
ΣPUFAn-3	***31.67c***	***30.29b***	***28.80a***	***1.19***

The fatty acids C12:0, C15:0, C14:1, C16:2*n*-4, C16:3*n*-4, C17:0, C17:1, C16:4*n*-1, C18:2*n*-4, C18:3*n*-6, C18:3*n*-4, C20:0, C20:1*n*-7, C20:2*n*-6, C20:3*n*-6, C20:3*n*-3, C20:4*n*-3, C21:5*n*-3, C22:1*n*-9, C22:4*n*-6, C22:5*n*-6, detected in percentages lower than 0.50, are considered in the composite fractions but not reported in the table for reasons of brevity.
* Residual standard deviation
a, b, c: means with different letters are different (P<0.05).

3.3 FA profile of raw fillets

The dependence of body FA composition on dietary FAs has been reported in previous studies on meagre (Grigorakis et al., 2002; Piccolo et al., 2008; Poli et al., 2003) and on several other farmed species, including sea bream (Cardinal et al., 2011; Grigorakis et al., 2002; Senso et al., 2007; Yildiz et al., 2008) and sea bass (Yildiz et al., 2008). The feed used in this trial (Table 1), which was partially of vegetal origin, did not have a substantial impact on the nutritional value of the fillet however because the farmed meagre expressed a high quality FA profile in all analysed samples.
Total saturated FAs (ΣSFAs) were found at similar proportions in all sampling times, even if a moderately decreasing trend from January to May was evident for C16:0, C18:0 and ΣSFAs (Table 3). Similar to previous research on the same species (Grigorakis et al., 2011; Piccolo et al., 2008; Poli et al., 2003), palmitic acid (C16:0) was the most abundant SFA in fillets. However, myristic acid (C14:0) and stearic acid (C18:0) were also found in sizeable amounts.
ΣMUFAs were found at an increasing percentage from January to May following the trend of intramuscular fat content (Table 3), which is known to be mainly composed of neutral lipids (Nanton et al., 2007). Regarding neutral lipids of meagre, as previously reported by Grigorakis et al. (2011), C16:1*n*-7, C18:1*n*-9, C18:1*n*-7, C20:1*n*-9 and C22:1*n*-11 were the major MUFAs detected. In particular, oleic acid (C18:1*n*-9), derived from both aquatic and vegetable components of the diet, was the most abundant.
In previous research on the same species (Grigorakis et al., 2011; Hernandez et al., 2009; Piccolo et al., 2008; Poli et al., 2003), ΣPUFAs were observed to be present in the greatest amounts during all sampling times, and those belonging to *n*-3 series were three times more present than those of the *n*-6 series. LA represented the most abundant *n*-6 FA in fillets, while marine-derived EPA and DHA were the main FAs of the *n*-3 series. The latter FAs were derived exclusively from the dietary fish oil, as marine carnivorous fish are known to show scarce endogenous biosynthesis of LC-PUFAs (Fontagné-Dicharry and Médale, 2010; Tocher, 2003).
Fish sampled in January registered the highest percentage of ΣPUFA*n*-3, while those sampled in May had the lowest. The conversion of EPA to DHA is limited

in marine carnivorous species and was not likely responsible for the large quantity of DHA found in all sampling times. The high incidence of DHA in all sampling times can be explained as a result of the fact that the fish were lean and poor in deposited fat, and consequently contained more polar lipids, in which DHA is mainly located (Grigorakis et al., 2011). A similar inverse relationship between fillet leanness and DHA has been previously observed in farmed meagre (Poli et al., 2003), on wild sea bream (Grigorakis et al., 2002) and farmed sea bream (Senso et al., 2007).

3.4 Composition of cooked fillets

Cooking yield (%) did not differ among sampling times (89.3 *vs.* 89.4 *vs.* 90.6 in January, March and May, respectively).

Table 4 – Moisture (g 100 g^{-1}), protein, ash, total lipids (g 100 g^{-1}, dry basis), and FA profile of meagre cooked fillets by sampling time.

	Sampling time			rsd*
	January	March	May	
Moisture	72.50	72.33	71.79	0.93
Protein	86.39	85.11	85.73	1.61
Ash	5.08b	4.76a	4.81a	0.19
Total lipids	9.87	11.03	11.15	1.57
Fatty acids, % of total FAs				
C14:0	3.81	3.75	3.78	0.26
C16:0	18.28b	18.02b	17.79a	0.46
C18:0	5.25	5.14	5.16	0.37
ΣSFA	***28.69***	***28.27***	***28.18***	***0.72***
C16:1*n*-7	5.33	5.23	5.42	0.39
C18:1*n*-9	12.89a	13.80b	14.21c	0.54
C18:1*n*-7	2.67	2.64	2.62	0.06
C20:1*n*-9	1.61a	1.78b	2.02c	0.18
C22:1*n*-11	1.16a	1.39a	1.85b	0.03
ΣMUFA	***25.43a***	***26.76b***	***27.91c***	***1.18***
C18:2*n*-6 (LA)	9.07a	9.50b	9.52b	0.31
C20:4*n*-6	1.13	1.10	1.10	0.09
C22:5*n*-6	0.37	0.36	0.35	0.03
ΣPUFAn-6	***11.28a***	***11.69b***	***11.81b***	***0.97***
C18:3*n*-3 (ALA)	0.93a	1.08b	1.15b	0.12
C18:4*n*-3	1.16	1.12	1.10	0.11

C20:5*n*-3 (EPA)	9.92	9.69	9.27	1.42
C22:5*n*-3	2.41b	2.39b	2.29a	0.11
C22:6*n*-3 (DHA)	15.87	15.47	14.77	1.49
ΣPUFAn-3	***32.00c***	***30.89b***	***29.76a***	***1.23***

The fatty acids C12:0, C15:0, C14:1, C16:2*n*-4, C16:3*n*-4, C17:0, C17:1, C16:4*n*-1, C18:2*n*-4, C18:3*n*-6, C18:3*n*-4, C20:0, C20:1*n*-7, C20:2*n*-6, C20:3*n*-6, C20:3*n*-3, C20:4*n*-3, C21:5*n*-3, C22:1*n*-9, C22:4*n*-6, C22:5*n*-6, detected in percentages lower than 0.50, are considered in the composite fractions but not reported in the table for reasons of brevity.
* Residual standard deviation
a, b, c: means with different letters are different (P<0.05).

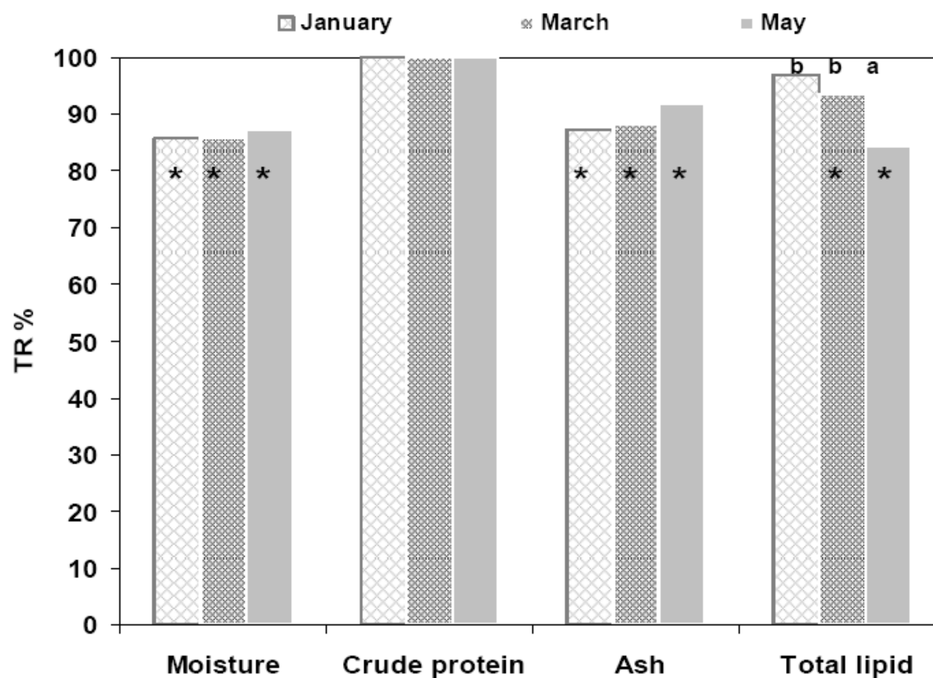
As presented in Table 4, the sampling time differences in moisture, protein, and lipid content found in the raw fillets were nullified by cooking. Moisture loss and protein concentration after cooking were in agreement with the results (expressed on wet basis) obtained for boiled king salmon (*Oncorhynchus tshawytscha*) (Larsen et al., 2010) and for boiled silver catfish (*Rhamdia quelen*) (Weber et al., 2008).
As shown in Table 4, the FA profile of cooked fillets generally followed the same trends as those of raw fillets, with some exceptions. Cooking nullified sampling time differences in C18:0, ΣSFAs, C20:4*n*-6, C22:5*n*-6, EPA and DHA, while generating significant differences in ΣPUFA*n*-6.

3.5 True Retention

TR% is a good estimate of the variation of each nutrient after cooking. As shown in Figure 1, protein TR% was 100% in each sampling time, demonstrating that protein was completely retained. Moisture, ash and lipid were not totally retained, but differences among sampling times were found only for total lipids.
Major lipid losses are more ascribable to intramuscular depot lipids, which are mainly triglycerides and subjected to melting or leaching, rather than to structural lipids (Pirini et al., 2010). For this reason, significantly higher lipid losses ($p<0.05$) were found in meagre sampled in May, which contained more lipids. A similar inverse relationship between lipid retention and intramuscular lipid content was demonstrated by Pirini et al. (2010) in oven-baked wild anchovy (*Engraulis encrasicholus*), sardine (*Sardina pilchardus*), horse mackerel (*Sprattus sprattus*) and sprat (*Trachurus trachurus*).
FA TR% has been calculated on FAs found at concentrations above 4% by total FAME, except in the case of ALA, which was analysed because of its nutritional importance. These FAs were not completely retained in all sampling times (Figure 2), as might have been expected by the total lipid TR%. As shown by the retention in May, the lipid content of fillets and FA retention resulted in an inverse relationship in agreement with the previous finding of Pirini et al. (2010) in the wild species cited above.

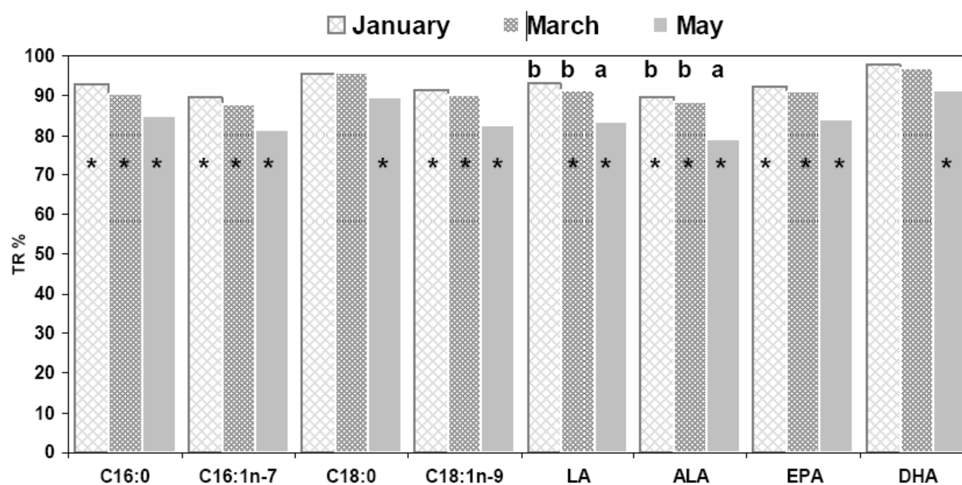
Fish sampled in May had generally lower FA retention when compared to the other samples, but only LA (C18:2*n*-6) and ALA (C18:3*n*-3) retention differed significantly between January and March.
The pronounced decrease registered in May could be the result of different losses of polar and neutral lipids induced by cooking. As previously reported, lipids leaching out during cooking mainly belong to fat deposits that are largely composed of neutral lipids (Nanton et al., 2007). Because meagre neutral lipids are known to be rich in C18:1*n*-9, LA, and ALA (Grigorakis et al., 2011), more C18:1*n*-9, LA, and ALA could have been released after cooking in fish sampled in May with a higher fat content to begin with.

Figure 1 - True Retention (TR%) of macronutrients of meagre fillets after cooking.



a, b : means with different letters are different (P<0.05).
*value different from 100 (P<0.05).

Figure 2 - TR% of main FAs of meagre fillets after cooking.



a, b: means with different letters are different (P<0.05).
*value different from 100 (P<0.05).

The higher TR% expressed by DHA was most likely due to the preponderance of DHA in polar lipids that enhances the retention of this FA. DHA retention represents a positive characteristic of this species, thanks to its well-known cardioprotective effect and especially its efficiency in lowering blood pressure, heart rate, and platelet aggregation, which latter was recently demonstrated to be greater than that of EPA (Cottin et al., 2011).

3.7 Fat Indexes

The most useful indexes of fat quality, as influenced by sampling times and cooking, are reported in Table 5.

Table 5 - Fat quality indexes of meagre fillets at different sampling times and before and after cooking.

	Sampling time			Condition		rsd*
	January	March	May	Raw	Cooked	
n-6/*n*-3 PUFA	0.35a	0.38b	0.40c	0.39	0.38	0.02
LA/ALA	9.60c	8.70b	8.10a	8.62a	8.98b	0.85

AI[1]	0.49c	0.44a	0.48b	0.47a	0.46b	0.02
TI[2]	0.23a	0.23a	0.24b	0.23	0.23	0.01
HH[3]	2.37	2.40	2.40	2.36a	2.41b	0.09

* Residual standard deviation
a, b, c: within criterion, means with different letters are different ($P<0.05$).
[1] atherogenic index
[2] thrombogenic index
[3] hypocholesterolaemic/hypercholesterolaemic fatty acids ratio.

In all samples, the *n*-6/*n*-3 PUFA ratio was less than 1 due to the abundance of EPA and DHA. This ratio was similar to the results of Grigorakis et al. (2011) and Poli et al. (2003) for the same species, and was also in the same range as the values found for cultured sea bream (Grigorakis et al., 2002; Senso et al., 2007; Yildiz et al., 2008) and cultured sea bass (Alasalvar et al., 2002; Yildiz et al., 2008).

Because *n*-6 and *n*-3 PUFAs compete for the same enzymes in the elongation/desaturation endogenous process and have different biological roles (Kris-Etherton et al., 2002; Simopoulos, 2008), the balance between them bears considerable importance in the human diet (FAO, 1994). A diet rich in *n*-6 FAs shifts the physiological state to one that is prothrombotic and proaggregatory, with increased blood viscosity, vasospasm, and vasoconstriction, and decreased bleeding time (Simopoulos, 1999). The recommended balance of *n*-6/*n*-3 PUFA suggested by several authors (Budowski and Crawford, 1985; Nestel, 1987; Russo, 2009; Simopoulos, 1999 and 2008) in preventing cardiovascular diseases, cancer, inflammatory, and autoimmune diseases should therefore be below 5:1.

The LA/ALA ratio was in the range of the recommended balance for human intake, which should be between 5:1 and 10:1 (FAO, 1994) because several studies have demonstrated that a ratio below 10:1 increased EPA content in plasma phospholipids (Liou et al., 2007), decreased total mortality of patients (De Lorgeril et al., 1994), and reduced non-insulin-dependent diabetes mellitus incidence (Raheja et al., 1993). Even though the LA/ALA ratio was within the recommended range in all samples, the results were particularly high because of the abundance of LA and the low proportion of ALA found in the fillets, which reflected the diet's FA profile. The lowest LA/ALA ratio was found in May, when the ALA content was significantly higher than during the other sampling times.

Atherogenic (AI) and thrombogenic index (TI) values were very low during all sampling times. The very low TI of meagre was due to the abundance of EPA and DHA, whose pronounced antithrombogenic activity is ascribed to their

strong effect on inhibiting platelet aggregation via prostanoid PGI_3 production (Ulbricht and Southgate, 1991). The results of this trial were similar to those found by Poli et al. (2003) but lower than those reported by Grigorakis et al. (2011) and Piccolo et al. (2008). Meagre showed a similar TI compared to sea bream (Hurtado-Rodriguez et al., 2010; Senso et al., 2007) but a lower TI than sea bass (Poli et al., 2001). The AI found in our research was higher than that reported by Grigorakis et al. (2011) but lower than that found by Poli et al. (2003) in meagre fillets. Additionally, the AI value was similar to that registered in sea bass (Poli et al., 2001) but higher than that found in sea bream (Hurtado-Rodriguez et al., 2011; Senso et al., 2007).

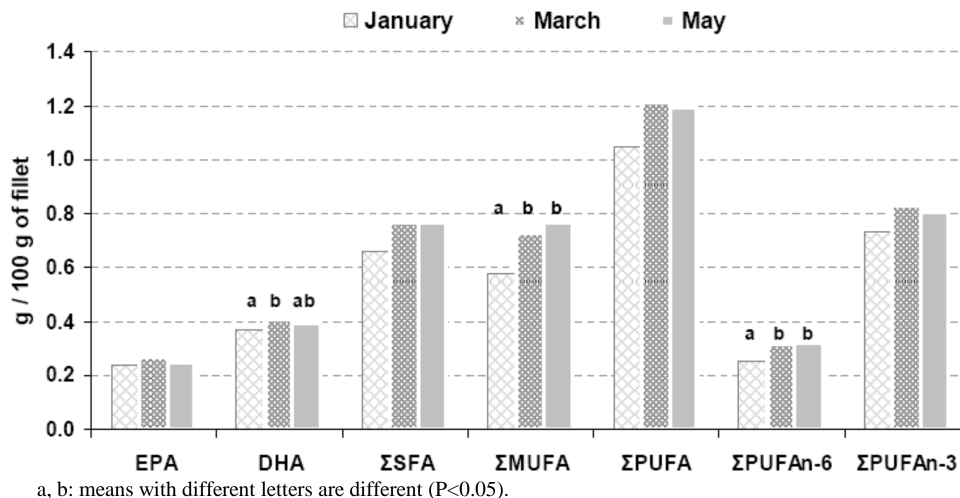
The hypocholesterolaemic/hypercholesterolaemic FA ratio (HH) was always >2, which is higher than reported for seven Brazilian freshwater fish species (Filho et al., 2010), but lower than the value found in white hake (Soriano Sancho et al., 2011). No significant differences were found with respect to sampling time.

Cooking did not affect the *n*-6/*n*-3 PUFA ratio, confirming findings in boiled king salmon (Larsen et al., 2010) and silver catfish (Weber et al., 2008). Although LA and ALA were not totally retained, the higher proportion of LA and the lower proportion of ALA detected after cooking induced a significant increase in the LA/ALA ratio. TI was not significantly modified by cooking, whereas AI significantly decreased. Unlike the results found for cooked white hake (Soriano Sancho et al., 2011), the HH ratio was significantly improved by cooking.

3.8 Intake of FAs from cooked meagre

Cooked farmed meagre was evaluated for FA content based on an average portion size for adults of 100 g. As presented in Figure 3, ΣSFA intake ranged from ~650 mg in January to ~770 mg in May. ΣMUFA intake significantly differed among sampling times, and the highest value (~770 mg) was found in May, despite the low retention. ΣSFA and ΣMUFA are synthesised by the body and are not indispensable nutrients in the diet. Consequently, no intake has been set by nutritionists.

Figure 3 - Dietary FA intake from cooked meagre.



a, b: means with different letters are different ($P<0.05$).

For all sampling periods, ΣPUFA intake exceeded 1 g, with a clear preponderance of ΣPUFA*n*-3. No recommended intakes have been set for ΣPUFA or for the essential FAs LA and ALA because the scientific data available is insufficient to derive an Average Requirement, a Lower Threshold Intake or a Population Reference Intake, in accordance with EFSA (2010). However, the abundance of ΣPUFAs, mainly ΣPUFA*n*-3, represents the main nutritional advantage in meagre consumption, considering that nutritionists generally recommend replacing ΣSFA with ΣPUFA in order to decrease the risk of CHD.

The recommended intake (in grams per day) has only been suggested for EPA plus DHA, with insufficient evidence to set a specific minimum intake of either EPA or DHA alone. According to the EFSA (2010), an intake of 250 mg per day of EPA plus DHA appears to be sufficient for primary prevention of CVD, while the FAO/WHO (2008) sets the intake at 300 mg/day for healthy adults with at least 200 mg of DHA for pregnant and lactating women.

In this study, a 100 g portion of meagre fillet resulted in levels well above the recommended intake of EPA plus DHA. Analogous amounts of EPA were found in January, March and May (239, 264 and 252 mg/100 g, respectively). The intake of DHA was higher than EPA and followed a similar trend, with the lowest amount corresponding to 365 mg in January. Even if the leaner meagre sampled in January provided fillets with the lowest intake of EPA plus DHA per portion, they retained these FAs better than fatter fish sampled in March and May. This suggests that consumption of approximately 2 servings per week of

100 g each of farmed meagre (<2% in lipid content) may fulfil the requirement of EPA and DHA.

4. Conclusions

The results of this study indicate that farmed meagre is a very lean species, that is characterised by a high-quality lipid profile, and rich in ΣPUFA, especially ΣPUFA*n*-3. Although nutritional composition varied according to sampling time, the differences were reduced after cooking.

Cooking by boiling induced the loss of some macronutrients, mainly lipids, but the retention of DHA was higher compared to the other FAs considered.

Despite the losses induced by cooking and the low fat content, the 100 g portion of fillet taken as reference guarantees an intake of EPA and DHA which more than doubled the recommended daily intake for EPA plus DHA (250 mg day^{-1}) suggested by EFSA, at any time during the period taken into examination.

The ability to preserve nutrients is an essential requirement for quality maintenance and represents a positive aspect of meagre quality. Slight nutritional losses demonstrate the aptitude of this species to be sold fresh and suggest that further types of industrial heat processes can be applied to fillets.

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Declaration of Interest

The authors declare to have not any financial and personal relationships with other people or organizations that could have influenced their work.

References

Alasalvar C, Taylor KDA, Zubcov E, Shahidi F, Alexis M. (2002). Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition. Food Chem 79: 145-150.

Al-Saghir S, Thurner K, Wagner K, Frisch G, Luf W, Razzazi-Fazeli E, Elmadfa I. (2004). Effects of different cooking procedures on lipid quality and cholesterol oxidation of farmed salmon fish (*Salmo salar*). J Agr Food Chem 52:5290-5296.

AOAC (2000). Official Methods of Analysis (17th ed.). AOAC International. Washington, DC.

Budowski P, Crawford MA. (1985). Alpha-linolenic acid as a regulator of the metabolism of arachidonic acid: dietary implications of the ratio, n-6:n-3 fatty acids. P Nutr Soc 44:221-229.

Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, Elwood PC, Deadman NM. (1989). Effects of changes in fat, fish and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet 2:757-761.

Buttriss J, Nugent A. (2005). LIPGENE: an integrated approach to tackling the metabolic syndrome. P Nutr Soc 64:345-347.

Cardinal M, Cornet J, Donnay-Moreno C, Gouygou JP, Bergé P, Rocha E, Soares S, Escorcio C, Borges P, Valente LMP. (2011). Seasonal variation of physical, chemical and sensory characteristics of sea bream (*Sparus aurata*) reared under intensive conditions in Southern Europe. Food Control 22:574-585.

Chatzifotis S, Panagiotidou M, Papaioannou N, Pavlidis M, Nengas I, Mylonas CC. (2010). Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*) juveniles. Aquaculture 307:65-70.

Cottin SC, Sanders TA, Hall WL. (2011). The differential effects of EPA and DHA on cardiovascular risk factors. P Nutr Soc 70:215-231.

de Castro FAF, Pinheiro Sant'Ana HM, Milagres Campos F, Brunoro CostaNM, Coelho Silva MT, Salaro AL, Do Carmo Castro Franceschini S. (2007). Fatty acid composition of three freshwater fishes under different storage and cooking processes. Food Chem 103:1080-1090.

De Lorgeril M, Renaud S, Mamelle N, Salen P, Martin J, Monjaud I, Guidollet J, Touboul P, Dalye J. (1994). Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 343:1454-1459.

EFSA (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA Journal 8:1-5.

El Ahdal M. (2009). National Aquaculture Market Report: Morocco. In *National Market Report of Aquaculture, 2003 - 2007*. GFCM-CAQ Working Group on Marketing, p. 58 (unpublished report).

Erkan N, Selçuk A, Özden Ö. (2010). Amino acid and vitamin composition of raw and cooked horse mackerel. Food Anal Method 3:269-275.

FAO (1994). Fats and oils in human nutrition. Joint FAO/WHO expert consultation. 19-26 October 1993 Rome, Italy. Available at: http://www.fao.org. Accessed on 2 September 2011.

FAO/WHO (2008). Interim summary of conclusions and dietary recommendations on total fat and fatty acids. Joint FAO/WHO Expert Consultation on fats and fatty acids in human nutrition. 10-14 November 2008, Geneva, Switzerland. Available at: http://www.who.int. Accessed on 12 April 2011.

Filho MMR, Lima Ramos MI, Aiko Hiane P, Tala de Souza EM. (2010). Nutritional value of seven freshwater fish species from the Brazilian Pantanal. Journal of American Oil Chemists' Society 87:1461-1467.

Folch J, Lees M, Sloane-Stanley GH. (1956). A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509.

Fontagné-Dicharry S, Médale F. (2010). Les lipides des poissons d'aquaculture et leurs facteurs de variation. Oléagineux Corps Gras Lipides 17:209-213.

Grigorakis K, Alexis M, Gialamas I, Nikolopoulou D. (2004). Sensory, microbiological, and chemical spoilage of cultured common sea bass (*Dicentrarchus labrax*) stored in ice: a seasonal differentiation. Eur Food Res Technol 219:584-587.

Grigorakis K, Alexis MN, Anthony Taylor KD, Hole M. (2002). Comparison of wild and cultured gilthead sea bream (*Sparus aurata*); composition, appearance and seasonal variations. Int J Food Sci Tech 37:477-484.

Grigorakis K, Fountoulaki E, Vasilaki A, Mittakos I, Nathanailides C. (2011). Lipid quality and filleting yield of reared meagre (*Argyrosomus regius).* Int J Food Sci Tech 46:711-716.

Herland H, Esaiassen M, Cooper M, Olsen RL. (2010). Quality of farmed Atlantic cod: effects of season and storage. Aquac Res 41:1203-1210.

Hernandez MD, Lopez MB, Alvarez A, Ferrandini E, Garcia Garcia B, Garrido MD. (2009). Sensory, physical, chemical and microbiological changes in aquacultured meagre (*Argyrosomus regius*) fillets during ice storage. Food Chem 114:237-245.

Hurtado-Rodriguez R, Fountoulaki E, Grigorakis K, Alexis M, Flos R. (2010). Season and size effects: changes in the quality of gilthead sea bream (*Sparus aurata* L.). Mediterr Mar Sci 11:117-131.

Kris-Etherton PM, Harris WS, Appel LJ. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 106:2747-2757.

Lanari D, Poli BM, Ballestrazzi R, Lupi P, D'Agaro E, Mecatti M. (1999). The effects of dietary fat and NFE levels on growing European sea bass (*Dicentrarchus labrax* L.). Growth rate, body and fillet composition, carcass traits and nutrient retention efficiency. Aquaculture 179:351-364.

Larsen D, Quek SY, Eyres L. (2010). Effect of cooking method on the fatty acid profile of New Zealand King Salmon (*Oncorhynchus tshawytscha*). Food Chem 119:785-790.

Lauritzen L, Hansen HS, Jørgensen MH, Michaelsen KF. (2001). The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res 40:1-94.

Liou AY, King DJ, Zibrik D, Innis SI. (2007). Decreasing linoleic acid with constant α-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. J Nutr 137:945-952.

Marchioli R, Barzi F, Bomba E, Chieffo C, Di Gregorio D, Di Mascio R, Franzosi MG, Geraci E, Levantesi G, Maggioni AP, Mantini L, Marfisi RM, Mastrogiuseppe G, Mininni N, Nicolosi GL, Santini M, Schweiger C, Tavazzi L, Tognoni G, Tucci C, Valagussa F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.

Monfort MC. (2010). Present market situation and prospects of meagre (*Argyrosomus regius*), as an emerging species in Mediterranean aquaculture. Available at: http://www.fao.org/docrep/013/i1675e/i1675e00.htm. Accessed on 30 November 2011.

Morrison WR, Smith LM. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with born fluoride-methanol. J Lipid Res 5:600-608.

Murphy EW, Criner P, Gray BC. (1975). Comparisons of methods for calculating retentions of nutrients in cooked foods. J Agr Food Chem 23:1153-1157.

Nanton DA, Vegusdal A, Rørå AMB, Ruyter B, Baeverfjord G, Torstensen BE. (2007). Muscle lipid storage pattern, composition, and adipocyte distribution in different parts of Atlantic salmon (*Salmo salar*) fed fish oil and vegetable oil. Aquaculture 265:230-243.

Nestel PJ. (1987). Polyunsaturated fatty acids (n-3, n-6). Am J Clin Nutr 45:1161-1167.

Orban E, Nevigato T, Di Lena G, Masci M, Casini I, Caproni M. (2008). Ricerche di supporto alla innovazione di prodotto e processo della acquacoltura Toscana: opportunità competitive della ombrina (*Argyrosomus regius*) e *Solea solea* in mercati di nicchia. Project funded by ARSIA Toscana 2007-2009. Research Project Report. [in Italian].

Piccolo G, Bovera F, De Riu N, Marono S, Salati F, Cappuccinelli R, Moniello G. (2008). Effect of two different protein/fat ratios of the diet on meagre (*Argyrosomus regius*) traits. Ital J Anim Sci 7:363-371.

Piñon A, Amezcua F, Duncan N. (2009). Reproductive cycle of female yellow snapper *Lutjanus argentiventris* (Pisces, Actinopterygii, *Lutjanidae*) in the SW Gulf of California: gonadic stages, spawning seasonality and length at sexual maturity. J Appl Ichthyol 25:18-25.

Pirini M, Testi S, Ventrella V, Pagliarani A, Badiani A. (2010). Blue-back fish: fatty acid profile in selected seasons and retention upon baking. Food Chem 123:306-314.

Poli BM, Parisi G, Zampacavallo G, Iurzan F, Mecatti M, Lupi P, Bonelli A (2003). Preliminary results on quality and quality changes in reared meagre (*Argyrosomus regius*): body and fillet traits and freshness changes in refrigerated commercial-size fish. Aquacult Int 11:301-311.

Poli BM, Parisi G, Zampacavallo G, Mecatti M, Lupi P, Gualtieri M, Franci O. (2001). Quality outline of European sea bass (*Dicentrarchus labrax*) reared in Italy: shelf life, edible yield, nutritional and dietetic traits. Aquaculture 202:303-315.

Quéméner L. (2002). Meagre (*Argyrosomus regius*): biology, fisheries, market and rearing potential. Plouzané, France. Éditions Ifremer.

Quéro JC, Vayne, JJ. (1987). Le maigre, *Argyrosomus regius* (Asso, 1801) (Poissons, Perciformes, *Sciaenidae*) du golfe de Gascogne et des eaux plus septentrionales. Revue des Travaux de l'Institut des Pêches Maritimes 49:35-66.

Raheja BS, Sadikot SM, Phatak RB, Rao MB. (1993). Significance of the n-6/n-3 ratio for insulin action in diabetes. Ann NY Acad Sci 683:258-271.

Rodriguez A, Carriles N, Cruz JM, Aubourg SP. (2008). Changes in the flesh of cooked farmed salmon (*Oncorhynchus kisutch*) with previous storage in slurry ice (-1.5 °C). LWT-Food Sci Technol 41:1726-1732.

Russo G. (2009). Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. Biochem Pharmacol 77:937-946.

Santos-Silva J, Bessa RJB, Santos-Silva F. (2002). Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. Livest Prod Sci 77:187-194.

SAS® (2007). SAS/STAT software, release 9.1. Cary, NC: SAS institute, Inc.

Senso L, Suarez MD, Ruiz-Cara T, Garcia-Gallego M. (2007). On the possible effects of harvesting season and chilled storage on the fatty acid profile of the fillet of farmed gilthead sea bream (*Sparus aurata*). Food Chem 101:298-307.

Serhan CN. (2007). Resolution phases of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Annu Rev Immunol 25:101-137.

Shearer KD. (1994). Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture 119:63-88.

Simopoulos AP. (1999). Essential fatty acids in health and chronic disease. Am J Clin Nutr 70 (suppl):560S-569S.

Simopoulos AP. (2008). The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 56:365-379.

Soriano Sancho RA, de Lima FA, Guerra Costa G, Barros Mariutti LR, Bragagnolo N. (2011). Effect of annatto seed and coriander leaves as natural antioxidants in fish meatballs during frozen storage. J Food Sci 76:C838-C845.

Tocher D. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fish Sci 11:107-184.

Türkkan AU, Cakli S, Kilinc B. (2008). Effects of cooking methods on the proximate composition and fatty acid composition of seabass (*Dicentrarchus labrax*, Linnaeus, 1758). Food Bioprod Process 86:163-166.

Ulbricht TLV, Southgate DAT. (1991). Coronary heart disease: seven dietary factors. Lancet 338:985-992.

Weber J, Bochi VC, Ribeiro CP, de M Victorio A, Emanuelli T (2008). Effect of different cooking methods on the oxidation, proximate and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets. Food Chem 106:140-146.

Yildiz M, Şener E, Timur M. (2008). Effects of differences in diet and seasonal changes on the fatty acid composition in fillets from farmed and wild sea bream (*Sparus aurata* L.) and sea bass (*Dicentrarchus labrax* L.). Int J Food Sci Tech 43:853-858.

PAPER II

EFFECT OF REARING SYSTEM ON BODY TRAITS AND FILLET QUALITY OF MEAGRE (*ARGYROSOMUS REGIUS*, ASSO 1801) CHILLED FOR A SHORT TIME

running title: rearing system and meagre fillet quality

Roberta Martelli[1], Giuliana Parisi[1*], Paola Lupi[1], Antonio Bonelli[1], Antonella Dalle Zotte[2], Oreste Franci[1]

[1]Dipartimento di Biotecnologie Agrarie - Sezione di Scienze Animali, Università di Firenze - Via delle Cascine 5, 50144 Firenze
[2]Dipartimento di Medicina Animale, Produzioni e Salute, Università di Padova, Agripolis - 35020 Legnaro, Padova, Italy

Corresponding author: phone: +39 055 3288265; fax: +39 055 321216.
E-mail address: giuliana.parisi@unifi.it

Abstract

The purpose of this study was to evaluate qualitative traits of meagre (*Argyrosomus regius*) from two different rearing systems (land-based tank filled with geothermal water *vs* offshore sea cage) and after short-term storage at chilling temperature (1, 2, or 3 days). Fish originated from the same batch were fed the same diet. Morpho-biometric traits, L*, a*, and b* colour parameters, texture, free water, proximate composition, total lipids, fatty acids, iron, and selenium contents were analyzed in the fillets. Most parameters were affected by rearing system. Compared to tank-reared fish, caged fish were shorter, poorer in visceral fat, and had higher incidence in cavity content and liver, lower incidence in gonads and head. Caged fish also had softer fillets in the epaxial site, which showed a higher tendency towards greenish color. Caged fish also showed higher lipid content but lower Fe and Se content. Tank-reared fish fillets were more abundant in PUFAn-3, mainly due to DHA (18.54 *vs* 12.95%; $p<0.001$) and consequently showed the best healthiness indexes. Minimal changes, mostly involving colour and texture, were detected during the first three days of refrigerated storage. During storage, no significant modification of the parameters investigated could be ascribed to the rearing system.

Key words: meagre, *Argyrosomus regius*, rearing system, chilling storage, chemical composition.

1. INTRODUCTION

Farmed fish are known to grow in more stable conditions than wild fish, and different rearing techniques affect fish flesh quality in different ways (Orban *et al.*, 2000). Several studies have recently addressed the effect of rearing systems on quality characteristics, and especially marketable traits, nutrients, texture, and colour (Orban *et al.*, 1997, 2000; Mairesse *et al.*, 2006; Hallier *et al.*, 2007; Jankowska *et al.*, 2007; Roncarati *et al.*, 2010; Valente *et al.*, 2011). Farming time, rearing temperature, stocking density, water current, difference in nutrient availability, and hydrographic and hydrodynamics conditions in off-shore sites proved to be the main factors linked to the rearing system that affected fillet quality.

Lipids, fatty acids, and mineral profile are among the most important nutrients in fish. Seafood is particularly appreciated by consumers as an important source of n-3 polyunsaturated fatty acids (PUFAs) and mineral components, such as selenium and iron, which are essential in preventing disorders, oxidative stress, and cardiovascular disease (Beard *et al.*, 1996; Watanabe *et al.*, 1997; Rayman, 2000; Ruxton *et al.*, 2004). The levels of such nutrients may differ by rearing system because environmental conditions and diet also vary significantly from one system to another (Orban *et al.*, 2000). Similarly, texture and colour, which have gained increasing importance in quality assurance as sensory attributes, can also be affected by rearing system, and in particular, by rearing temperature, which affects the number and size of muscle fibres, lipid deposition, and physical activity, and has been shown to be the factor that influences rheological properties and colorimetric attributes most (Hyldig and Nielsen, 2001; Ginés *et al.*, 2004; Roth *et al.*, 2010).

It might also be presumed that rearing techniques also affect fish quality changes during storage and shelf life due to the above-mentioned documented effects on fillet physical-chemical properties (Orban *et al.*, 1997, 2000; Mairesse *et al.*, 2006; Hallier *et al.*, 2007; Jankowska *et al.*, 2007; Roncarati *et al.*, 2010; Valente *et al.*, 2011). Rearing techniques might, in fact, also affect at the start of the storage the microbiological quality of fish, which is closely linked to the quality of the water from which the fish are harvested. Scientific literature has provided very little information on this topic until now.

It has been recently demonstrated that fish origin (wild or farmed) and rearing techniques both affect consumer perceptions of fillet quality. According to Verbeke *et al.* (2007), a large majority of consumers believes there are no major differences between farmed and wild fish, even if taste perception is mostly in favour of wild fish. With respect to aquacultured products, the type of farming could be relevant in consumer choices. Comparing fish farmed in marine cages to those raised in ponds, for example, mariculture production is perceived more positively than pond production, and this consumer preference is linked to the environmental aspects of fish farming (Stefani *et al.*, 2012).

No studies on how different rearing systems affect the nutrients, colour, and texture of farmed meagre (*Argyrosomus regius*, Asso 1801) fillets have yet been made. Meagre is an emerging species in Mediterranean aquaculture with leanness as its most valuable trait (Poli *et al.*, 2003; Hernandez *et al.*, 2009) that distinguishes it from other marketable farmed fish (i.e. sea bream, sea bass,…) (Lanari *et al.*, 1999; Poli *et al.*, 2001). Less muscle fat than the amounts present in other aquacultured species permits refrigerated storage for longer periods of time. Poli *et al.* (2003) and Hernandez *et al.* (2009) reported a similar shelf life (9 days) for whole fish stored at 1 °C and for fillet wrapped in thin polyethylene film stored at 4 °C. Increasing interest in meagre processing has now been documented (Monfort, 2010), whereas the production of innovative and practical meagre-based seafood products has recently been reported by Ribeiro *et al.* (2012). In Italy, meagre is intensively reared in land-based tanks or in seawater cages. Cage-rearing in particular has provided excellent results at existing commercial hatcheries, which are in the position to reproduce massive quantities of the species (Cardia and Lovatelli, 2007).
Considering the current status of meagre culture in Italy and the potential for its expansion in Mediterranean area, this study aimed at evaluating any possible differences in the qualitative traits of meagre reared by different techniques (land-based tank *vs* sea cage) and identifying which technique provides fillets of the highest quality. Another aim was to evaluate the differences in fillet quality properties induced by short refrigerated storage of whole fish reared with these two systems.

1. MATERIALS AND METHODS

Fish were collected from the farm "Il Vigneto" located near Ansedonia (Grosseto, Italy). Meagre originated from the same batch were raised during the grow-out phase in land-based tanks (Tank) and in seawater cages (Cage). In land-based circular tanks (500 m^3 volume), the density was 60 fish/m^3; water temperature ranged from 19 to 22 °C (geothermal water), and salinity was approximately 16 ppt. In marine circular cages (2000 m^3 volume), the density was 10 fish/m^3, water temperature ranged from 13 to 24 °C, and salinity was approximately 37 ppt. Fish were fed the same commercial extruded feed (crude protein 44%; crude fat 22%; fatty acids (FAs) expressed as a percentage of total FA: SFA 24.2%, MUFA 27.6%, PUFAn-6 20.4%, PUFAn-3 25.0%; Se: 0.85 mg/kg; Fe: 183.9 mg/kg).
After reaching marketable size (average weight of 951.1±259.8 g), 18 fish were sampled from both Tank and Cage in two subsequent times (May and July) with 36 fish collected from each rearing system. After slaughtering, the fish arrived at the Laboratory the day after the catch and were kept on melting ice during the entire experiment in a refrigerated room at 1 °C. Fish were analysed at three

different post-mortem times, i.e. 1st, 2nd and 3rd day (6 fish/day/rearing system/sampling time).

Attention was focused on the period of commercial life considered most important in the fish trade as currently organized in Italy by the large-scale distribution retail market.

Whole body weight (BW) and total length (TL) were recorded at each sampling. Fish were dissected, and the head, axial skeletal bones, total cavity content, liver, gonads (when evident and separable), perivisceral fat (the fat stored inside the abdominal cavity), and the right and left fillets were weighed. The condition factor (CF) was calculated according to the formula

$CF = 100 \times BW (g) / TL^3 (cm)$.

Total cavity content, liver, gonads and perivisceral fat weights were referred as percentage of total BW, obtaining the viscerosomatic index (VSI), hepatosomatic index (HSI), gonadosomatic index (GSI), and fat somatic index (FSI), respectively. Moreover, perivisceral fat was expressed also as a percentage of the total cavity content to obtain the fat visceral index (FVI).

Carcass was calculated as (BW– total cavity content weight), whereas dressing yield (DY) and fillet yield (FY) were determined as the percentages of the carcass and fillets on BW, respectively. Physical characteristics were analysed directly on the left fillets, and chemical composition analyses were carried out on samples taken from the same fillet previously skinned, homogenised, and freeze-dried.

2.1 Free water, texture, and colour

Texture measurements were performed using a Zwick Roell® texturometer (software: Text Expert II) equipped with a 200 N load cell. One cycle compression test was done using a 10 mm diameter cylindrical probe at a constant speed of 30 mm/min to 50% of total deformation. Among textural attributes, hardness was measured on the epaxial, ventral and caudal parts of the fillet. Fillet thickness at the three locations was measured directly by the texturometer at the same time as hardness measurement.

The shear test was performed on the central part of the fillet using a straight blade that moved at a constant speed of 30 mm/min to 50% of the total deformation.

A Spectro-color® colorimeter (using Spectral qc 3.6 software) was utilised for colorimetric measurement carried out according to the CIELab system (CIE, 1976). In this system, lightness (L*) is expressed on a 0–100% scale from black to white; and redness index (a*) ranges from red (+60) to green (-60) while yellowness index (b*) ranges from yellow (+60) to blue (-60). Colour was measured in duplicate on the epaxial, ventral, and caudal fillet positions.

Free water measurement was performed by applying the compression test on filter paper using the Grau and Hamm (1953) method.

2.2 Proximate composition and total lipid content

Moisture, crude protein (Nx6.25), ether extract, and ash content were determined using AOAC (2000) 950.46, 976.05, 991.36, and 920.153 methods respectively.

Total lipid extraction was performed by a modified Folch *et al.* (1956) method. Freeze-dried samples, reconstituted fresh by adding distilled water, were homogenised with a 2:1 chloroform-methanol (v/v) solution and filtered. The filter was washed several times, and distilled water with 0.88% KCl was added to the filtrate until the [Choloroform:Methanol] water ratio was 4:1. The tubes were stirred, and a biphasic system was obtained by standing overnight. The lower phase containing lipids dissolved in chloroform was siphoned and recovered. Total lipid content was determined gravimetrically after removal of the solvent (chloroform) by evaporation under vacuum and lipid resuspension in a known volume of chloroform (5 mL). Lipid content was weighed in a crucible after complete chloroform evaporation. The extracted lipids were used for the FA profile analysis.

2.3 Fatty acid analysis

Fatty Acid Methyl Esters (FAME) analysis was performed using the modified method of Morrison and Smith (1964). Lipids were saponified with 0.5 M KOH in methanol, and FAs were hydrolysed by adding 2 N HCl. Methyl esters were prepared by transmethylation, using boron fluoride- methanol at a 14% concentration. Methylated FA were dissolved in petroleum ether, dried, and finally resuspended in 1 mL of hexane.

FA composition was determined by gas chromatography (GC) using a Varian GC 430 gas chromatograph (Agilent, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a Supelco Omegawax™ 320 capillary column (30 m x 0.32 mm i.d., 0.25 µm film and polyethylene glycol bonded phase; Supelco, Bellefonte, PA, USA) was utilised. The oven temperature was held at 100 °C for 2 minutes, increased to 160 °C over 4 minutes at the rate of 12 °C/min, and then increased to 220 °C over 14 minutes at the rate of 3 °C/min and kept at 220 °C for 25 minutes. The injector and the detector temperatures were set at 220 and 300 °C, respectively. One microlitre of sample in hexane was injected into the column with the carrier gas (helium) kept at a constant flow of 1.5 mL/min. The split ratio was 1:20.

Chromatograms were recorded with the Galaxie Chromatography Data System 1.9.302.952 (Agilent, Palo Alto, CA, USA) computing integrator software. FAs were identified by comparing the FAME retention time with the standard Supelco 37 component FAME mix (Supelco, Bellefonte, PA, USA). FAs were quantified through calibration curves using tricosanoic acid (C23:0) (Supelco, Bellefonte, PA, USA) as an internal standard. FAs were expressed as a percentage of total FAME.

2.4 Computation of fat quality indexes

The following fat quality indexes were calculated:

- n-6/n-3 ratio;
- LA/ALA, as linoleic acid (LA; C18:2n-6)/alpha-linolenic acid (ALA; C18:3n-3) ratio;
- atherogenic index (AI), according to the formula [C12:0 + (4 × C14:0) + C16:0] / (ΣPUFAn-3 + ΣPUFAn-6 + ΣMUFA) (Ulbricht and Southgate, 1991);
- thrombogenic index (TI) according to the formula [C14:0 + C16:0 + C18:0] / [0.5 × ΣMUFA) + (0.5 × ΣPUFAn-6) + (3 × ΣPUFAn-3) + (ΣPUFAn-3/ΣPUFAn-6)] (Ulbricht and Southgate, 1991);
- hypocholesterolaemic/hypercholesterolaemic FA ratio (HH), calculated as (C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3) / (C14:0 + C16:0) (Santos-Silva *et al.*, 2002).

2.5 Selenium and iron content

In order to determine these trace minerals, solutions were prepared for an ICP optical read using the wet microwave digestion method (999.10) (AOAC, 2000). Dry samples were weighed, put into teflon tubes, and then 5 mL of super pure nitric acid and 1-1.5 ml of hydrogen peroxide were added. The teflon tubes were put suitably sealed into the Ethos 900 Microwave Labstation microwave oven (12- position rotor with teflon liner, Milestone Microwave Laboratory Systems) at about 175 °C for 30 min. Samples underwent different time-microwave power combinations: 5 min at 250W, 5 min at 450W, 6 min at 650W, 2 min at 250W, and then 5 min of ventilation in order to cool. At the end of digestion, teflon boxes were left to cool in a water bath and opened under fume, but only after their temperature had reached 40 °C. The walls of the containers were washed with deionized water and the rinse water was poured into 25 cm^3 volumetric flasks. The digested samples were poured in numbered polyethylene bottles and their Fe and Se contents were determined using the MIN 1 method with a (ICP–OES) SPECTRO Ciros Vision EOP spectrometer, a spectrometer with induced coupled plasma source and simultaneous optical detection of emissions in the range of 125 to 770 nm. The instrument had a SPECTRO ADS 500 autosampler and a SPECTRO Smart Analyzer Vision 1.50.534 management software that read Fe and Se levels at absorption lines of 259.940 and 196.090 nm, respectively, with a minimum detection of 0.002 and 0.03 mg/L and a maximum calibrated quantity of 120 and 24 mg/L, respectively.

All analytical methodologies were submitted to validation procedures.

2.6 Statistical analysis

Data were analysed by ANCOVA (Analysis of CoVariance) with the SAS® (SAS, 2007) GLM procedure using rearing system (Tank, Cage), storage time (1, 2 and 3 days), and sampling month (May, July) as the discrete effects, and body weight as the continuous effect. Interaction between rearing system and storage time was tested in a preliminary model and was excluded from the final model because it never attained significance. The differences between least squares means were statistically tested using the Student's t-test.

3. **RESULTS**

Morpho-biometric parameters and indexes

Fish reared in cages showed a similar body weight to those reared in tanks (Table 1). Nevertheless, all subsequent parameters were covaried on BW with the aim of reducing variability and obtaining estimates at the same average BW (951.5 g). After this adjustment, fish reared in cages had significantly ($P<0.001$) lower length and higher CF. Although prerivisceral fat content was negligible in both groups and showed no difference between rearing systems when considered as percentage of BW (FSI), it was higher in tank-reared fish when incidence was referred to cavity content (FVI). VSI and HSI were higher in cage-reared fish, while GSI was significantly higher in fish reared in tanks, consequently DY was also higher in the latter, whereas no differences in FY between rearing systems were detected.

Month of sampling evidenced high variability in morpho-biometric parameters, while the casual sampling of fish in the three days of post-mortem storage did not reveal any substantial differences, in this way indicating the homogeneity of raw material in this experimental thesis. Moreover, as BW increased, length, cavity content, liver percentage, and fillet yield increased proportionately, and only head proportion decreased.

Physical parameters

As shown in Table 2, textural analyses performed on the fillets showed that differences between rearing systems were strictly related to the site of measurement. In the epaxial zone, tank-reared fish showed significantly higher hardness values ($P<0.001$). Also the hardness measured on the caudal and ventral zones and the shear force measured only in the central zone were higher in Tank fish, even though these differences were not significant. In this group of meagre, which showed higher overall hardness values, free water was released in significantly ($P<0.01$) greater amount.

Similarly to texture, differences between rearing systems in colorimetric attributes were also influenced by site of measurement (Table 2). L*, a*, and b* values did not differ significantly in the epaxial zone, whereas L* and a* values in the caudal zone and a* values in the ventral zone were significantly higher in Tank fish fillets.

Table 1. Morphological traits of meagre (means estimated at average body weight of 951.5 g).

	Rearing system		Storage			Significance				RSD
	tank	**cage**	**1 d**	**2 d**	**3 d**	**Rearing**	**Storage**	**Sampling month**	**Weight**	
Body weight, BW (g)	994.61	913.05	958.56	1005.17	897.77	ns	ns	***	/	187.57
Length (cm)	44.85b	43.74a	43.93	44.20	44.75	***	ns	***	*** (+)	1.23
Condition Factor, CF	1.02a	1.11b	1.09	1.07	1.04	***	ns	***	ns	0.09
Body composition (% BW)										
- Cavity content, VSI	3.29a	4.54b	3.67	4.16	3.92	***	ns	*	** (+)	1.02
• **Liver, HSI**	0.90a	1.84b	1.41	1.42	1.32	***	ns	ns	** (+)	0.38
• **Gonads, GSI**	0.26b	0.05a	0.18	0.14	0.15	***	ns	*	ns	0.12
• **Fat, FSI**	0.73	0.54	0.45a	0.87b	0.60ab	ns	*	***	ns	0.56
• **Fat, FVI (% on cavity content)**	17.38b	11.28a	13.57	16.67	12.76	*	ns	***	ns	12.50
- Carcass, DY	96.71b	95.46a	96.33	95.84	96.08	***	ns	*	** (-)	1.02
• **Head**	32.76b	29.67a	31.25	32.77	30.84	**	ns	**	*** (-)	2.04
• **Frame**	15.70	16.29	16.48	16.76	15.85	ns	ns	ns	ns	1.49
• **Fillet, FY**	46.57	47.89	47.21	46.40	48.09	ns	ns	ns	* (+)	2.88

a, b: $p<0.05$ within criterion; *: $p < 0.05$, **: $p< 0.01$,***: $p < 0.001$; ns: not significant.
The symbols (+) and (-) indicate the regression sign on the weight.

Although no differences in hardness among storage days were observed in the epaxial and ventral zones, hardness decreased significantly ($P<0.05$) with storage time in the caudal zone. Shear force and free water were unaffected by days of storage. With regard to colorimetric attributes, L* and a* values were significantly higher at the 2nd day than at the 1st and 3rd days, while b* differed only in the caudal zone between the 2nd and 3rd day.
Body weight affected some of the physical parameters investigated; muscle free water and L* at the epaxial and caudal sites increased with rising BW. The relationship between fillet thickness and BW was obviously positive.

Proximate composition, selenium, and iron contents
The proximate composition of meagre fillets exhibited differences between rearing systems only in ether extract and total lipid content, which were lower in fish reared in tanks where the highest Fe and Se content was present (see Table 3). Other factors, such as day of storage and sampling month, had little or only sporadic influence on fillet chemical composition. The influence of fish weight was more relevant; increased weight negatively affected moisture and ash content while positively affecting fillet lipid content (whether expressed as ether extract and total lipids). A positive relationship between fish weight and Se content was also observed.

Fatty acid profile
The FA profile of fillets from differently-reared fish is reported in Table 4. In both rearing systems, palmitic acid (C16:0) and oleic acid (C18:1n-9) were the predominant saturated and monounsaturated FAs (SFA and MUFA), respectively. Among polyunsaturated FAs (PUFA), C18:2n-6 (LA), C20:5n-3 (EPA), and C22:6n-3 (DHA) were the most abundant. The FA profile on the whole was strongly affected by rearing system, which did not influence only palmitic acid and SFA percentages. On the contrary, as expected, FA variation was never affected by day of storage. The influence of the sampling month, however, was evident. In greater detail, limiting the examination to unsaturated FAs, cage-reared fish showed a significantly higher concentration of C16:1n-7, C18:1n-9, C18:1n-7, LA, EPA, and C22:5n-3 than tank-reared fish, even though the differences in value were generally small. On the contrary, tank-reared fish showed slightly higher amounts of C20:1n-9, C22:1n-11, C20:4n-6, C18:3n-3, and a much higher amount of DHA (about 5.5 percentage points).
As regards healthiness indexes, the higher percentage of PUFAn-3 observed in tank-reared fish was responsible for the superior quality of all such indexes except LA/ALA, which was lower and therefore better in cage-reared fish due to the higher percentage of C18:3n-3. As regards the effect of fish weight, different behavior was observed in each FA and each FA group. All MUFA increased with the increase of BW, similarly to C14:0, ALA and EPA, whereas

C18:0, C20:4n-6 and PUFAn-6, DHA and PUFAn-3 decreased with increasing BW.

Table 2. Physical characteristics of meagre (means estimated at average body weight of 951.5 g).

	Rearing system		Storage			Significance				RSD
	tank	cage	1 d	2 d	3 d	Rearing	Storage	Sampling month	Weight	
Free water (cm^2)	11.90a	10.15b	11.58	10.70	10.80	**	ns	**	* (+)	2.22
Shear force (N)	9.13	8.35	9.64	8.10	8.47	ns	ns	ns	ns	2.19
Epaxial zone										
Thickness (mm)	15.04	15.56	15.46	15.17	15.29	ns	ns	***	*** (+)	1.21
Hardness (N)	9.40b	7.40a	8.29	8.35	8.56	***	ns	*	ns	1.85
L*	31.79	31.55	27.16a	38.64b	29.20a	ns	***	ns	* (+)	4.96
a*	-4.78	-5.43	-5.77a	-3.74b	-5.80a	ns	***	ns	ns	1.49
b*	-0.81	-1.67	-1.37	-0.67	-1.69	ns	ns	ns	ns	2.25
Caudal zone										
Thickness (mm)	7.78	8.65	8.98	7.99	7.67	ns	ns	***	*** (+)	1.91
Hardness (N)	6.53	5.98	7.20b	6.15ab	5.42a	ns	*	***	ns	2.20
L*	36.85b	33.86a	32.03a	41.62b	32.42a	**	***	ns	ns	3.92
a*	-1.95b	-3.80a	-3.76a	-1.07b	-3.79a	**	***	ns	ns	2.42
b*	0.94	0.64	0.73	1.77b	-0.11a	ns	*	*	ns	2.20
Ventral zone										
Thickness (mm)	11.02	11.85	11.02	11.31	11.99	ns	ns	***	*** (+)	1.94
Hardness (N)	10.94	9.92	10.45	8.80	12.03	ns	ns	***	ns	5.17
L*	43.41	43.57	44.19b	46.66b	39.62a	ns	*	***	** (+)	7.68
a*	0.15b	-1.92a	-1.03b	1.49c	-3.11a	*	***	ns	ns	3.51
b*	2.77	1.05	1.81	2.65	1.26	ns	ns	ns	ns	3.73

a, b, c: $p<0.05$ within criterion; *: $p < 0.05$, **: $p< 0.01$,***: $p < 0.001$; ns: not significant.
The symbols (+) and (-) indicate the regression sign on the weight.

Table 3. Chemical composition of meagre, expressed on 100 g of wet weight of fillets (means estimated at average body weight of 951.5 g).

	Rearing system		Storage			Significance				RSD
	tank	cage	1 d	2 d	3 d	Rearing	Storage	Sampling month	Weight	
Moisture (g)	75.99	75.29	75.34	75.68	75.89	ns	ns	ns	*** (-)	1.14
Protein (g)	21.04	20.74	21.00	21.09	20.58	ns	ns	ns	ns	0.78
ether extract (g)	1.51a	2.53b	2.14	1.85	2.07	***	ns	*	*** (+)	0.76
Total lipids (g)	2.12a	3.00b	2.70	2.35	2.64	***	ns	*	*** (+)	0.69
Ash (g)	1.39	1.37	1.40	1.38	1.36	ns	ns	ns	** (-)	0.06
Iron (µg)	265.2b	201.8a	247.8	220.4	232.3	***	ns	ns	ns	43.50
Selenium (µg)	18.3b	15.2a	16.7a	14.2a	19.4b	*	*	***	*** (+)	4.02

a, b: $p<0.05$ within criterion; *: $p < 0.05$, **: $p< 0.01$,***: $p < 0.001$; ns: not significant.
The symbols (+) and (-) indicate the regression sign on the weight.

Table 4. Fatty acid (FA) profile and healthiness indexes of lipids in meagre (means estimated at average body weight of 951.5 g).

	Rearing system		Storage			Significance				RSD
	tank	cage	1 d	2 d	3 d	Rearing	Storage	Sampling month	Weight	
Fatty acids (% of total FA)										
C14:0	3.20a	4.06b	3.63	3.57	3.68	***	ns	**	*** (+)	0.39
C16:0	17.70	17.68	17.69	17.85	17.53	ns	ns	ns	ns	0.57
C18:0	5.95b	5.17a	5.53	5.70	5.44	***	ns	**	*** (-)	0.54
SFA	***28.29***	***28.38***	***28.31***	***28.57***	***28.13***	ns	ns	ns	ns	0.86
C16:1n-7	4.29a	5.85b	5.03	4.99	5.19	***	ns	***	*** (+)	0.59
C18:1n-9	12.77a	13.72b	13.25	13.08	13.41	***	ns	*	*** (+)	0.86
C18:1n-7	2.42a	2.74b	2.57	2.59	2.59	***	ns	***	*** (+)	0.10
C20:1n-9	1.67b	1.39a	1.51	1.48	1.59	***	ns	ns	*** (+)	0.24
C22:1n-11	1.49b	0.99a	1.24	1.17	1.32	***	ns	ns	*** (+)	0.33
MUFA	***24.56a***	***26.47b***	***25.49***	***25.14***	***25.92***	***	ns	*	*** (+)	1.99
C18:2n-6 (LA)	10.23a	11.35b	10.77	10.61	10.97	***	ns	***	ns	0.58
C20:4n-6	1.57b	1.10a	1.33	1.37	1.30	***	ns	***	*** (-)	0.26
PUFAn-6	***13.10a***	***13.58b***	***13.36***	***13.18***	***13.48***	***	ns	***	*** (-)	0.64
C18:3n-3 (ALA)	0.94a	1.18b	1.06	1.01	1.11	***	ns	ns	*** (+)	0.17
C20:5n-3 (EPA)	8.58a	10.12b	9.28	9.35	9.42	***	ns	***	** (+)	0.62
C22:5n-3	2.22a	2.32b	2.26	2.31	2.25	***	ns	***	ns	0.15
C22:6n-3 (DHA)	18.54b	12.95a	15.86	16.17	15.21	***	ns	**	*** (-)	2.19
PUFAn-3	***32.05b***	***28.81a***	***30.46***	***30.80***	***30.04***	***	ns	ns	*** (-)	2.02
PUFA	***47.14b***	***45.08a***	***46.17***	***46.26***	***45.91***	***	ns	*	*** (-)	2.08
Healthiness indexes										
n-6/n-3	0.40a	0.47b	0.44	0.42	0.45	***	ns	**	ns	0.04
LA/ALA	11.98b	9.58a	10.85	11.09	10.40	***	ns	**	*** (-)	1.85
AI	0.44a	0.49b	0.46	0.46	0.46	***	ns	*	*** (+)	0.02
TI	0.09a	0.14b	0.12	0.11	0.12	***	ns	ns	*** (+)	0.02
HH	2.64b	2.42a	2.53	2.52	2.54	***	ns	*	*** (-)	0.15

The fatty acids C12:0, C15:0, C14:1, C16:2n-4, C16:3n-4, C17:0, C17:1, C16:4n-1, C18:2n-4, C18:3n-6, C18:3n-4, C20:0, C20:1n-7, C20:2n-6, C20:3n-6, C20:3n-3, C20:4n-3, C21:5n-3, C22:1n-9, C22:4n-6, C22:5n-6, detected in percentages lower than 0.50, are considered in the composite fractions, but not reported in the table for brevity.
a, b: $p<0.05$ within criterion; *: $p < 0.05$, **: $p< 0.01$, ***: $p < 0.001$; ns: not significant.
The symbols (+) and (-) indicate the regression sign on the weight.

4. **DISCUSSION**

Effect of rearing system

Environmental parameters (e.g. water temperature and salinity) and rearing conditions (e.g. fish density) were different in the two rearing systems. Consequently, most of the different results obtained in Tank or Cage systems can be attributed to the effect of the abovementioned parameters on the metabolism and the physiological condition of the fish initially taken from the same batch.

The differences observed in morpho-biometric parameters could depend on the fact that by producing different swimming activity and feeding behavior, rearing systems influenced fish growth and modified fish shape in different ways. In this trial, the cage-fish reared at lower density and naturally variable water temperature were less slender than tank-reared fish. Flos *et al.* (2002) found that super-intensively raised gilthead sea breams assume a very particular, more compact shape than both fish reared less intensively and wild fish, and that when compared with the latter of similar weight are shorter, wider and higher. Tulli *et al.* (2009) reported that when compared to extensively reared fish, intensively-reared sea bass showed an enlarged ventral zone resulting from reduced swimming activity and the accumulation of perivisceral fat.

Higher percentages of FVI in tank-reared meagre could be ascribed to the higher stocking density that limits swimming activity, whereas the higher feed intake at constant water temperature may have favored visceral fat accumulation. Higher FVI was also reported in sea bass reared in inland basins when compared to those kept in off-shore marine cages (Tulli *et al.*, 2009). Conversely to FVI, the somatic indexes VSI and HSI, were higher in fish raised in cages, where more intense swimming and the higher seawater temperature in summer could have induced increased feed consumption and consequently lipid deposition in the liver and skeletal muscle rather than in the viscera in accordance with the findings of Sheridan (1988). Fish metabolism is largely based on lipids and proteins, storing lipids in the liver, viscera, and muscle, even if the detailed distribution in these body components varies between species (Love, 1970). Moreover, the liver was found to be a depository organ for energy, while muscle seemed to play a lesser role in energy storage in several Sciaenidae species (Craig *et al.*, 2000; Chatzifotis *et al.*, 2006; Shoonbee, 2006). Low HSI values have therefore been observed both after fasting periods (Chatzifotis *et al.*, 2006) and during spawning phases (Herland *et al.*, 2010). In light of these findings, it may be presumed that the physiological state of cage-reared meagre was characterized by increased feed consumption most likely promoted by higher seawater temperature in the final period of the trial. The same group of fish also showed negligible gonadal

development compared to tank-reared fish, and as a result, reserves were accumulated in the liver and muscle.

Differing fish physiological conditions and rearing parameters are also probably responsible for the contrasting results observed in related species in the literature available. Tulli *et al.* (2009) found higher VSI and HSI in sea bass reared in cages than in those raised in inland basins, whereas Roncarati *et al.* (2010) recorded higher VSI and HSI in land-based basins than in offshore and inshore cages.

In this trial, meagre showed particularly high DY and FY in both rearing systems that were higher than those of meagre of similar body weight analyzed in previous research by Poli *et al.* (2003).

Texture measurement results indicated that although rearing systems had no significant influence on hardness at the caudal and ventral sites, tank-raised fish were significantly harder in the epaxial site than cage-raised fish. It is likely that the greater thickness in the epaxial area was responsible for highlighting the difference in hardness due to the rearing system.

Current literature holds that hardness may be influenced by chemical composition, histological muscle characteristics, and animal exercise, which are greatly affected by farming density and temperature. The effect of fillet lipid content on its texture was shown in salmon by Dunajski (1979), Christiansen *et al.* (1995) and Robb *et al.* (2002) and in sea bream by Orban *et al.* (1997), which latter found flesh lipid content and hardness to be inversely related. The higher hardness of tank-reared meagre fillets might therefore be attributed to their overall lower lipid content. As concerns histological muscle characteristics, water temperature is known to influence muscle morphology by affecting the number and size of muscle fibers; more precisely, higher water temperature increases both fibre density and thinning (Ginés *et al.*, 2004; Hallier *et al.*, 2007). Higher fibre density produces an increase in hardness (Hatae *et al.*, 1990). The effect of water temperature could explain the higher hardness values detected in fish reared in tanks, where water temperatures were on average higher and more constant throughout the year than the temperatures in cages, due to the geothermal nature of water. The softer flesh of cage-reared fish may also be attributed to the more intense swimming activity enabled by lower stocking density. Physical exercise, in fact, is known to modify fish muscle structure by stimulating the fibre hypertrophy (Davison, 1997) associated with softer flesh (Hatae *et al.*, 1990; Bugeon *et al.*, 2003).

Another aspect that emerged from this study was the difference in texture in the three measurement sites. Literature reports that fillets have heterogeneous characteristics for textural properties (Botta, 1991; Reid and Durance, 1992) and lipid content (Aursand *et al.*, 1994). The heterogeneity for textural properties could be also explained by the close relationship between fillet thickness and hardness observed also in this trial. In raw salmon fillets Sigurgisladottir *et al.* (1997) found fillet thickness to be significantly and positively correlated with

hardness instrumentally measured by flat cylinder method, a method similar to the one used in this study. The same Authors found a different capacity to identify fish origin through instrumental texture analysis by the different sites where the measurement is made. Although in agreement with the results of this trial, this finding runs contrary to Sigurgisladottir *et al.* (1997), who found the highest discriminating capacity at the most caudal location, whereas in our study the difference between rearing systems was most significant at the epaxial site.
The colour of tank-reared fish fillet did not substantially differ from that of cage-reared fish, apart from the L* in caudal site and the a* in caudal and ventral sites. Since the values of both chromaticity indexes a* and b* were low in all sites, the colour of the fillet was grayish on the whole. The lower a* values seen in cage-reared meagre indicate a higher green colour component tendency, which was most likely due to access to a wider variety of natural food sources and pigments in addition to artificial feed. According to observations on catfish (Hallier *et al.*, 2007) and Arctic charr (Ginés *et al.*, 2004), water temperature differences may also be responsible for colour change.
In both rearing systems, epaxial sites were darker than caudal and ventral sites, whereas ventral sites had a brighter appearance with more yellowish and reddish colour. Since a positive a* value is generally associated with the presence of hemoglobin (Chaijan *et al.*, 2005; Hallier *et al.*, 2007), the higher values of redness index at the ventral site may be attributed to a high level of vascularization in the abdominal cavity wall (Hallier *et al.*, 2007).
The water-holding properties of muscle tissue are very important for commercial value and consumer acceptance. Muscle water-holding capacity is highly influenced by structural changes in muscle proteins, fibril swelling-contraction, and the distribution of fluid between intra- and extra-cellular locations (Jonsson *et al.*, 2001). In this study, tank-reared fish, which showed higher hardness values, released higher amounts of free water, thus confirming the direct relationship between these two parameters found by Jonsson *et al.* (2001) and Rawdkuen *et al.* (2010).
The rearing system significantly affected fillet proximate composition. Similar to as previously reported by Poli *et al.* (2003) and Grigorakis *et al.* (2011) for the same species, the fat content of the fillets that we tested was low. Moreover, cage-reared fish had higher percentages of fat than tank-reared fish, a result that contrasts with what literature commonly reports for other marine species. Sea bass (Roncarati *et al.*, 2010) and sharpsnout sea bream (Orban *et al.*, 2000) reared in cages had leaner fillets than those reared in land-based basins and tanks, respectively, even if comparing different farming systems is always difficult due to the multitude of specific and characteristic factors, however. On the other hand, Davison (1997) reported that in many cases exercise may not necessarily represent increased energy use, and that in many fish, swimming might even be a form of energy saving. An increase of total lipids in red muscle

was detected after exercise training in two cyprinids by Sänger (1992), for example.

An additional assumption may be that the higher lipid content of cage-reared fish is the result of a compensatory growth induced by the consistent increase of sea temperature from the winter to summer period. In the rearing site, sea temperature drops below 20 °C for half the year and is about 14 °C from January to the beginning of March. Since meagre feeding activity is substantially reduced when water temperature falls below 13-15 °C (El-Shebly *et al.*, 2007), caged fish may have resumed feeding in the spring. Ali *et al.* (2003), in a review on compensatory growth in teleosts, provided evidence that periods of food deprivation induce changes in fish storage reserves, particularly lipids, and that the restoration of satiation feeding is followed by significant increases in lipid content in muscles and in the liver and viscera incidences (Miglavs and Jobling, 1989).

Variations in fish mineral composition are known to be closely related to seasonal and biological (species, size, dark/white muscle, age, sex, and sexual maturity) factors, area of catch, food source, environmental pollution (water chemistry, salinity, temperature and contaminant), and processing method (Erkan and Özden, 2007). In this study, the Fe and Se content of the rearing water was always very low (<0.001 and <0.01 mg/L, respectively) and without difference between the two rearing systems. Considering the low content in the water, these trace elements were derived almost entirely from the feed fed to both groups of fish.

Selenium is mostly present in fish in water-extractable form and may be either unbound (i.e. neutral and ionic) or bound to polar materials, such as simple amino acids, peptides, and low molecular weight proteins (Cappon and Smith, 1982). Seafood is known to be a very good source of Se, in which it is present in considerably higher quantity than in other meats (Morris and Levander, 1970). Our study showed meagre Se content to be lower than the values reported by Morris and Levander (1970) in different fish species (40-70 μg/100 g), and lower than those provided by Šatović and Beker (2004) in sea bass (21-33 μg/100 g) and by Erkan and Özden (2007) in sea bass and sea bream (28.2 and 23.6 μg/100 g, respectively).

Seafood, especially marine fish and darker flesh fish, is also a reasonably good source of Fe, even if it does not represent the most important source for humans (Erkan and Özden, 2007; Peterson and Elvehjem, 1928). Tank-reared fish showed a higher Fe level than caged fish, similarly to as observed by Orban *et al.* (2000) in sharpsnout sea bream (*Diplodus puntazzo*) reared in different systems.

The fish flesh FA profile resembles that of the fish feed, and the influence of dietary composition on quality and quantity of FA is reported in the literature (i.e. Tocher, 2003). This study indicates that the rearing system also affects the FA profile however, since differences in fillet lipid content were observed in

fish fed the same diet. The higher lipid content of cage-reared fish can be associated, in fact, with the higher proportion of MUFAs, which are known to abound in meagre neutral lipids (Grigorakis et al., 2011). Conversely, leaner, tank-reared fish displayed higher levels of n-3 PUFAs, foremost of which DHA, which are mainly located in polar lipids, as research by Grigorakis et al. (2011) confirmed for this species as well. The relationship between higher n-3 PUFA content and greater leanness has also been found in other species in similar trials comparing different rearing systems, as in the case of the sea bass studied by Roncarati et al. (2010) and the sharpsnout sea bream studied by Orban et al. (2000).

These FA profiles determined better health lipid indexes in tank-reared fish. Literature offers no data on the values of these indexes in fillets from fish reared under different systems. One comparison might be made with the LA/ALA ratio calculated from the FA profiles reported by Roncarati et al. (2010) and by Orban et al. (2000) for species of fish that could share the same market niche with meagre. Roncarati et al. (2010) found a lower LA/ALA ratio in sea bass farmed in offshore cages than in those reared in land based basins, and this agrees with the findings on meagre in this study. A similar and very low LA/ALA ratio characterized the sharpsnout sea bream analysed by Orban et al. (2000) reared in tanks or cages. The only direct comparison that can be made is with the absolute values of some of these indexes obtained in studies carried out in the same species. In particular, the AI values of tank-reared meagre were abundantly lower than the value (0.69) reported for meagre reared in land-based tanks by Poli et al. (2003). On the contrary, the AI value of cage-reared meagre was higher than the 0.38 value detected by Grigorakis et al. (2011) in the same species reared in sea cages. Also on the contrary, TI values were particularly low compared to those obtained in the same species by the abovementioned studies.

Effect of storage

The deterioration of fresh fish is due to autolytic and bacterial processes (Huss, 1988). During spoilage, fish undergo changes in colour, flavour, and texture (Gram and Huss, 1996) according to an evolution affected by many factors, such as season, feeding, handling, and initial microbiological load.

As expected, the morpho-biometric (Table 1) and chemical characteristics (Table 3) of meagre analysed at different times of storage were the same, demonstrating that the sampling was carried out correctly. Storage had only a limited effect on fillet texture and colour. Only at the caudal site was observed a decrease in hardness, where such softening may be due to the notoriously high collagen content in the tail (Yoshinaka *et al.*, 1988; Johnston, 2001). This may explain the greater detachment of the muscle fibres from the myocommata responsible for tenderization.

The increase of L*, a*, and b* values from the 1st to the 2nd day followed by decrease at the 3rd day of storage may be due to the evolution of *rigor mortis*. The change into a more translucent flesh from the 1st and the 2nd day may be attributed to muscle contraction and the altered muscle light scattering properties known to be responsible for changes in L* during *rigor* development (Erikson and Misimi, 2008). A similar variation in L*, a*, and b* values when *rigor* starts was observed by Erikson and Misimi (2008) in ice-stored salmon.
Minimal changes in the same species during the first three days of refrigerated storage were detected by Poli *et al.* (2003) after measuring non relevant variations in the dielectric properties of muscle and in the *rigor* index in the first four days after death. Also Hernandez *et al.* (2009) observed minimal changes, without detecting variations in colour properties or texture variables in the first 4 days of storage of meagre fillets. The greatest changes actually occurred during the residual period of storage. Applying the EU Sensory Scheme (Rule 2406/EEC), Poli *et al.* (2003) classified the sample of meagre analysed and stored as whole fish at 1 °C under ice cover in Extra class until the 3rd day of storage, and assigned 9 days of shelf life. Equal shelf life was assigned by Hernandez *et al.* (2009) to meagre fillets stored a 4 °C.
The attention in this trial was focused on the parameters that most affect the quality perceived by consumers and the storage duration corresponding to that for the mass distribution and marketing of fish from aquaculture. The overall results on maintaining product quality levels are reassuring and were undoubtedly also partially due to both the storage of meagre in whole fish form that delays changes in intrinsic properties during shelf life and the short refrigerated storage time examined.
Rearing technique did not induce any different behavior during the three days of refrigerated storage. Although the differences in hardness, lipid quantity, and fillet quality attributed to rearing technique described above could probably also induce a different evolution of quality parameters during shelf life, this was not yet evident in the short period of refrigerated storage adopted. It may also be hypothesized that variations in texture were masked by *rigor* resolution condition, and that a different susceptibility to oxidation and rancidity may be manifested only at a more advanced stage of storage.

5. CONCLUSION

In conclusion, the fish from the two rearing systems showed specific characteristics even though the differences detected were not relevant. Compared to fish reared in tanks filled with geothermal water, the fat in fish reared in mariculture cages was distributed more in the muscles than in the perivisceral areas. The higher lipid content of fillets taken from cage-reared fish probably was responsible of higher water holding capacity, lower hardness, a FA profile that was poorer in PUFAn-3, and mainly in DHA, and slightly less favorable healthiness indexes. Short time chilling did not cause significant

changes in flesh quality, while the modifications in colour and texture detected can be attributed to the normal course of *rigor mortis* in the first three days after death when the whole fish is normally sold at full price. Fillets from the two rearing systems presented the same behavior during storage.

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REFERENCES

Ali, M., Nicieza, A., Wootton, R.J., 2003. Compensatory growth in fishes: a response to growth depression. Fish Fish. 4:147-190.

AOAC, 2000. Official Methods of Analysis (17th ed.). AOAC International. Washington, DC.

Aursand, M., Bleivik, B., Rainuzzo, J.R., Jorgensen, L., Mohr, V., 1994. Lipid distribution and composition of commercially farmed Atlantic salmon (*Salmo salar*). J. Sci. Food Agric. 64:239-248.

Beard, J.L., Dawson Harry, B.S., Piñero, D.J., 1996. Iron metabolism: a comprehensive review. Nutr. Rev. 54(10):295-317.

Botta, J.R., 1991. Instrument for nondestructive texture measurement of raw Atlantic cod (*Gadus morhua*) fillets. J. Food Sci. 56:962-968.

Bugeon, J., Lefevre, F., Fauconneau, B., 2003. Fillet texture and muscle structure in brown trout (*Salmo trutta*) subjected to long-term exercise. Aquac. Res. 34(14):1287-1295.

Cappon, C.J., Smith, J.C., 1982. Chemical form and distribution of mercury and selenium in edible seafood. J. Anal. Toxicol. 6:10-21.

Cardia, F., Lovatelli, A., 2007. A review of cage aquaculture: Mediterranean Sea. Cage Aquaculture. Regional reviews and global reviews. FAO Fisheries Technical Paper - 498. FAO, Rome.

Chaijan, M., Benjakul, S., Visessanguan, W., Faustman, C., 2005. Changes of pigments and color in sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) muscle during iced storage. Food Chem. 93(4):607–617.

Chatzifotis, S., Villamor Martin-Prat, A., Limberis, N., Papandoulakis, N., Divanach, P., 2006. First data on growth of cultured brown meagre *Sciaena umbra* using diets with different protein and fat contents. Fish. Sci. 72:83-88.

Christiansen, R., Struksnaes, G., Estermann, R., Torrissen, O., 1995. Assessment of flesh colour in Atlantic salmon *Salmo salar* (L.). Aquacult. Fish. Manage. 26:311-321.

CIE, 1976. Official recommendations on uniform colour space, colour difference equations and metric colour terms. Suppl. n. 2 to CIE Publication n. 15, Colorimetry. Commission International de l´Éclairage, Paris.

Craig, S.R., MacKenzie, D.S., Jones, G., Gatlin, D., 2000. Seasonal changes in the reproductive condition and body composition of free-ranging red drum, *Sciaenops ocellatus*. Aquaculture 190:89-102.

Davison, W., 1997. The effects of exercise training on teleost fish, a review of recent literature. Comp. Biochem. Physiol. A 117(1):67-75.

Dunajski, E., 1979. Texture of fish muscle. J. Texture Stud. 10:301-318.

El-Shebly, A.A., El-Kady, M.A.H., Hussin, A.B., Hossain, Y., 2007. Preliminary observations on the pond culture of meagre, *Argyrosomus regius* (Asso, 1801) (Scieanidae) in Egypt. Journal of Fisheries and Aquatic Science 2(5):345-352.

Erikson, U., Misimi, E., 2008. Atlantic salmon skin and fillet color changes effected by perimortem handling stress, *rigor* mortis and ice storage. Food Chem. 73(2):C50-C59.

Erkan, N., Özden, Ö., 2007. Proximate composition and mineral contents in aqua cultured sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) analyzed by ICP-MS. Food Chem. 102:721-725.

Flos, R., Reig, L., Oca, J., Ginovart, M., 2002. Influence of marketing and different land-based systems on gilthead sea bream (*Sparus aurata*) quality. Aquac. Int. 10:189-206.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1956. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Ginés, R., Valdimarsdottir, T., Sveinsdottir, K., Thorarensen, H., 2004. Effects of rearing temperature and strain on sensory characteristics, texture, colour and fat of Arctic charr (*Salvelinus alpinus*). Food Qual. Prefer. 15(2):177-185.

Gram, L., Huss, H.H., 1996. Microbiological spoilage of fish and fish product. Int. J. Food Microbiol. 33:121–137.

Grau, R., Hamm, R., 1953. Eine einfache methode zur bestimmung der wasserbindung im muskel. Naturwissenschafen 40:29-30.

Grigorakis, K., Fountoulaki, E., Vasilaki, A., Mittakos, I., Nathanailides, C., 2011. Lipid quality and filleting yield of reared meagre (*Argyrosomus regius*). Int. J. Food Sci. Technol. 46:711-716.

Hallier, A., Chevallier, S., Serot, T., Prost, C., 2007. Influence of farming conditions on colour and texture of European catfish (*Silurus glanis*) flesh. J. Sci. Food Agric. 87(5):814–823.

Hatae, K., Yoshimatsu, F., Matsumoto, J.J., 1990. Role of muscle fibers in contributing firmness of cooked fish. J. Food Sci. 55(3):693-696.

Herland, H., Esaiassen, M., Cooper, M., Olsen, R.L., 2010. Quality of farmed Atlantic cod: effects of season and storage. Aquac. Res. 41:1203-1210.

Hernandez, M.D., Lopez, M.B., Alvarez, A., Ferrandini, E., Garcia Garcia, B., Garrido, M.D., 2009. Sensory, physical, chemical and microbiological changes in aquacultured meagre (*Argyrosomus regius*) fillets during ice storage. Food Chem. 114:237-245.

Huss, H.H., 1988. Fresh fish quality and quality changes. FAO Fisheries Series No. 29. Pp. 40. Rome: FAO.

Hyldig, G., Nielsen, D., 2001. A review of sensory and instrumental methods used to evaluate the texture of fish muscle. J. Texture Stud. 32:219-242.

Jankowska, B., Zakęś, Z., Żmijewski, T., Ulikowski, D., Kowalska, A., 2007. Slaughter value and flesh characteristics of European catfish (*Silurus glanis*) fed natural and formulated feed under different rearing conditions. Eur. J. Lipid Sci. Technol. 224:453-459.

Johnston, I.A., 2001. Implications of muscle growth patterns for the colour and texture of fish flesh. Farmed Fish Quality. Fish News Books. Blackwell Science.

Jonsson, A., Sigurgisladottir, S., Hafsteinsson, H., Kristbergsson, K., 2001. Textural properties of raw Atlantic salmon (*Salmo salar*) fillets measured by different methods in comparison to expressible moisture. Aquac. Nutr. 7:81-89.

Lanari, D., Poli, B.M., Ballestrazzi, R., Lupi, P., D'Agaro, E., Mecatti, M., 1999. The effects of dietary fat and NFE levels on growing European sea bass (*Dicentrarchus labrax* L.). Growth rate, body and fillet composition, carcass traits and nutrient retention efficiency. Aquaculture 179:351-364.

Love, R.M., 1970. The Chemical Biology of Fishes. Academic Press, London.

Mairesse, G., Thomas, M., Gardeur, J., Brun-Bellut, J., 2006. Effects of geographic source, rearing system, and season on the nutritional quality of wild and farmed *Perca fluviatilis*. Lipids 41(3):221-229.

Miglavs, I., Jobling, M., 1989. The effect of feeding regime on proximate body composition and patterns of energy deposition in juvenile Arctic charr, *Salvelinus alpinus*. J. Fish Biol. 35:1-11.

Monfort, M.C., 2010. Present market situation and prospects of meagre (*Argyrosomus regius*), as an emerging species in Mediterranean aquaculture. Retrieved November 30, 2011 from the FAO Home Page (Fisheries and Aquaculture Department section) http://www.fao.org/docrep/013/i1675e/i1675e00.htm.

Morris, V.C., Levander, O.A., 1970. Selenium content of foods. J. Nutr. 100:1383-1388.

Morrison, W.R., Smith, L.M., 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with born fluoride-methanol. J. Lipid Res. 5:600-608.

Orban, E., Di Lena, G., Ricelli, A., Paoletti, F., Casini, I., Giambelli, L., Caproni, R., 2000. Quality characteristics of sharpsnout sea bream (*Diplodus puntazzo*) from different intensive rearing systems. Food Chem. 70:27-32.

Orban, E., Sinesio, F., Paoletti, F., 1997. The functional properties of the proteins, texture and the sensory characteristics of frozen sea bream fillets (*Sparus aurata*) from different farming systems. LWT-Food Sci. Technol. 30:214-217.

Peterson, W.H., Elvehjem, C.A., 1928. The iron content of plant and animal foods. Journal of Biological Chemistry 78:215.

Poli, B.M., Parisi, G., Zampacavallo, G., Iurzan, F., Mecatti, M., Lupi, P., Bonelli, A., 2003. Preliminary results on quality and quality changes in reared meagre (*Argyrosomus regius*): body and fillet traits and freshness changes in refrigerated commercial-size fish. Aquac. Int. 11:301-311.

Poli, B.M., Parisi, G., Zampacavallo, G., Mecatti, M., Lupi, P., Gualtieri, M., Franci, O., 2001. Quality outline of European sea bass (*Dicentrarchus labrax*) reared in Italy: shelf life, edible yield, nutritional and dietetic traits. Aquaculture 202:303-315.

Rawdkuen, S., Jongjareonrak, A., Phatcharat, S., Benjakul, S., 2010. Assessment of protein changes in farmed giant catfish (*Pangasianodon gigas*) muscles during refrigerated storage. Int. J. Food Sci. Technol. 45:985-994.

Rayman, M.P., 2000. The importance of selenium to human health. Lancet 356:233-241.

Reid, R.A., Durance, T.D., 1992. Textural changes of canned Chum salmon related to sexual maturity. J. Food Sci. 57:1340-1342.

Ribeiro, B., Cardoso, C., Silva, H.A., Serrano, C., Ramos, C., Santos, P.C., Mendes, R., 2012. Effect of grape dietary fibre on the storage stability of innovative functional seafood products made from farmed meagre (*Argyrosomus regius*). Int. J. Food Sci. Technol. In press. doi:10.1111/j.1365-2621.2012.03151.x.

Robb, D.H.F., Kestin, S.C., Warriss, P.D., Nute, G.R., 2002. Muscle lipid content determines the eating quality of smoked and cooked Atlantic salmon (Salmo salar). Aquaculture 205(3–4):345–358.

Roncarati, A., Sirri, F., Di Domenico, A., Brambilla, G., Lamiceli, A.L., Melotti, P., Meluzzi, A., 2010. Survey of qualitative traits of European sea bass cultivated in different rearing systems. Eur. J. Lipid Sci. Technol. 112:770-779.

Roth, B., Imsland, A., Helge Stien, L., Schelvis-Smit, R., Gunnarsson, S., Foss, A., 2010. The influence of anaerobic muscle activity, maturation and season on the flesh quality of farmed turbot. Aquac. Int. 18:461-474.

Ruxton, C.H.S., Reed, S.C., Simpson, M.J.A., Millington, K.J., 2004. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. J. Hum. Nutr. Diet. 17:449-459.

Sänger, A.M., 1992. Effects of training on axial muscle of two cyprinid species: *Chondrostoma nasus* (L.) and *Leuciscus cephalus*. J. Fish Biol. 40:637–646.

Santos-Silva, J., Bessa, R.J.B., Santos-Silva, F., 2002. Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. Livest. Sci. 77:187-194.

SAS, 2007. SAS/STAT software, release 9.2. Cary, NC: SAS institute, Inc.

Šatović, V., Beker, D., 2004. Selenium content in sea bass of the Adriatic Sea. Eur. Food Res. Technol. 218:111-113.

Sheridan, M.A., 1988. Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. Comp. Biochem. Physiol. B 90(4):679–690.

Shoonbee, W.L., 2006. The qualitative and quantitative description of growth and condition of silver kob, *A. inodorus*. Thesis. University of Stellenbosch (South Africa). Pp. 111.

Sigurgisladottir, S., Torrissen, O., Lie, O., Thomassen, M., Hafsteinsson, H., 1997. Salmon quality: methods to determine the quality parameters. Rev. Fish Sci. 5:1-30.

Stefani, G., Scarpa, R., Cavicchi, A., 2012.. Exploring consumer's preferences for farmed sea bream. Aquac. Int. 20(4):673-691.

Tocher, D., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish Sci. 11:107-184.

Tulli, F., Balenovic, I., Messina, M., Tibaldi, E., 2009. Biometry traits and geometric morphometrics in sea bass (*Dicentrarchus labrax*) from different farming systems. Ital. J. Anim. Sci. 8(2):881-883.

Ulbricht, T.L.V., Southgate, D.A.T., 1991, Coronary heart disease: seven dietary factors. Lancet 338:985-992.

Valente, L.M.P., Cornet, J., Donnay-Moreno, C., Gouygou, J.P., Bergé, J.P., Bacelar, M., Escórcio, C., Rocha, E., Malhão, F., Cardinal, M., 2011. Quality differences of gilthead sea bream from distinct production systems in Southern Europe: intensive, integrated, semi-intensive or extensive systems. Food Control 22(5):708–717.

Verbeke, W., Sioen, I., Brunsø, K., De Henauw, S., Van Camp, J., 2007. Consumer perception versus scientific evidence of farmed and wild fish: exploratory insights from Belgium. Aquac. Int. 15(2):121-136.

Watanabe, T., Kiron, V., Satoh, S., 1997. Trace minerals in fish nutrition. Aquaculture 151:185-207.

Yoshinaka, R., Sato, K., Anbe, H., Sato, M., Shimizu, Y., 1988. Distribution of collagen in body muscle of fishes with different swimming modes. Comp. Biochem. Physiol. B 89(1):147-151.

PAPER III

PHYSICO-CHEMICAL TRAITS OF RAW AND COOKED RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FILLETS FROM DIFFERENT STRAINS AND FARMS

Roberta Martelli, Oreste Franci, Paola Lupi and Giuliana Parisi*
Dipartimento di Biotecnologie Agrarie - Sezione di Scienze Animali, Università degli Studi di Firenze - Via delle Cascine 5, 50144 Firenze

*Corresponding author: phone: +39 055 3288265; fax: +39 055 321216.
E-mail address: giuliana.parisi@unifi.it

ABSTRACT

Marketable indexes, free water, textural properties, color, proximate composition, collagen and fatty acids of five strains of rainbow trout (*Oncorhynchus mykiss*) were measured before and after cooking. The five strains greatly differentiated in physico-chemical parameters in both raw and cooked state. *La Frola* and *Troutlodge* resulted the most valuable strains registering the best yields and the highest n-3 PUFAs content. *La Frola* differed from the other strains showing low valuable qualitative traits in terms of hardness, resilience, gumminess and springiness and brighter and less pigmented flesh with low fat and collagen content. *Troutlodge* registered the most valuable traits in terms of texture and colour, tough higher fat and collagen content in flesh.

Cooking modified physico-chemical profile in a different extent depending on the strain. n-3 PUFAs proportion greatly reduced in all strains. Differences in hardness and chewiness were nullified. *Troutlodge* maintained a positive texture and colour profile after cooking and its quality resulted the best.

KEY WORDS: rainbow trout, strain, cooking, texture, quality

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is a member of the Pacific trouts belonging to the Salmonidae family. It is widely used farming in many countries around the world due to its rapid growth and high nutritional value (Fallah *et al.* 2011). Rainbow trout is the main freshwater fish species farmed in Italy (Ismea, 2010), mainly spread in the north-east regions, where Trentino Alto Adige region historically represents an important area of traditional high-quality production (Faccenda *et al.* 2010).

It has widely reported that genetic differences affect sensory quality of flesh of several salmonids species (Johnston *et al.* 2000; Johnston 2001). For this reason the possible improvement of fillet marketable and physico-chemical quality of rainbow trout by genetic selection has been recently researched (Kause *et al.*

2002, 2003, 2004; Quillet *et al.* 2005; Tobin *et al.* 2006) and several genetic selection programs have been promoted in many countries, except for Italy where a complete program has not been led yet.

It is known that the ability to retain water and textural and colorimetric attributes are between the most important quality characteristics of meat from both terrestrial and aquatic species (Hyldig and Nielsen, 2001; Huff-Lonergan and Lonergan 2005; Steine *et al.* 2005; Bugeon *et al.* 2010). In fish, they are affected by several factors including nutritional state, rearing water temperature, physical activity, muscle structure and composition, post-mortem shrinkage and proteolysis of fibers (Andersen *et al.* 1997; Hyldig and Nielsen 2001; Ginés *et al.* 2004; Huff-Lonergan and Lonergan 2005).

Unacceptable water holding capacity causes the loss of saleable weight and proteins (Huff-Lonergan and Lonergan 2005). Moreover retention of water is important for the texture of fish, since higher water content in the muscle reduces its mechanical strength (Hultmann and Rustad 2008). A too soft or mushy fillets seem to be unpleasant to most consumers (Rasmussen 2001) and softness also represents a problem for the fish industry (Hultmann and Rustad 2008). Moreover lipid and collagen play important role in variation of texture profile influencing firmness, juiciness and palatability (Hyldig and Nielsen 2001; Rasmussen 2001; Fallah et al. 2011).

In addition to the previous parameters, also the uniformity of flesh color is an important quality criterion, mainly in salmonids with pigmented fillets (Bugeon *et al.* 2010). Consumers seem to prefer red-colored products and it has been shown that the redness contributes significantly to the overall enjoyment of cooked salmon (Steine *et al.* 2005).

Concerning nutritional quality, fatty acids profile represents the most valuable trait in freshwater farmed fish as well. In particular farmed rainbow trout is rich in PUFAs, including n-3 PUFAs which are known to be inversely related to cancer, cardiovascular diseases and several chronic diseases like diabetes and asthma (Shahidi and Miraliakbari 2004; Nettleton and Kanz 2005).

Cooking is known to affect physico-chemical parameters of fish, including disintegration of the muscle fiber, loss of water and pigments. Thermal changes of myofibrillar proteins cause increased toughness, whereas heat-induced transformation of collagen to gelatine is responsible for making the flesh more tender since the layered myotomes tend to slide away when applying a compression force (Hyldig and Nielsen 2001; Mørkøre *et al.* 2006a; Aussanasuwannakul *et al.* 2010). For this reason contrasting are the results with salmonids which registered both an hardness decrease (Mørkøre *et al.* 2006a; Aussanasuwannakul *et al.* 2010) and increase (Mørkøre *et al.* 2006a; Ginés *et al.* 2004; Larsen *et al.* 2011) after cooking. Cooking also affects the retention of nutrients, including the healthy long chain fatty acids which can be differently lost (Ågren and Hänninen 1993; Unusan, 2007).

The aim of the present work was to investigate the most important physico-chemical traits of five strains (three Italian strains and two genetically high-selected foreign strains) of rainbow trout farmed in Trentino, which previously had showed the best growth performance. In particular, considering the absence of a complete genetic selection program in Italy, the research intended to point out similarities and differences between the strains to detect which of them showed the best qualitative profile.

MATERIALS AND METHODS

Five rainbow trout strains were obtained from local (*Valsugana-Dellai*, *La Frola*, *Burrini-Batocchi*) and foreign (*Isle of Man*-UK and *Troutlodge*-U.S.A.) suppliers. Eyed-stage eggs were bought and incubated until hatching. Juveniles of the five strains were transferred to three trout farms (F.E.M.-IASMA, Foglio, Tamanini) located in Trentino-Alto Adige region and differing in environmental and managing conditions (Table 1). Regardless of the location, all fish lots were kept at the same density and were fed the same commercial feed: Astro Salmonato 50/50 g (Gruppo Veronesi, Verona-Italy) for the finishing period (moisture 9%, crude protein 42%, crude fat 24%, ash 6%). Feed fatty acid profile is shown in Table 2. The fish were fed six days a week. Nr 10 fish per each strain and farm (50 fish per farm) were slaughtered when they reached marketable size (weight: 700-800 g).

Table 1 – Rearing conditions in each farm.

Farm	**Altitude** (m.a.s.l.)[3]	**Water temperature**[1] **(°C)**	**OD**[2] **(ppm)**
F.E.M.-IASMA	200	11-14	10.18 (Sat. O_2: 97.6%)[2]
Foglio	400	9-11	8.25 (Sat. O_2: 76.83%)[2]
Tamanini	700	3-14	10.35 (Sat. O_2: 96.57%)[2]

[1]Temperature range from a minimum (temperature of water entering the tank) to a maximum (temperature of water coming out of the tank) (data registered in continuum mode)
[2]the values are referred to the water entering the tank (mean of registrations taken in the sampling period)
[3]Meters above sea level.

The morphometric variables measured on the whole fish were: total weight (W), total length (TL), condition factor (CF) calculated as 100 x W (g) / TL^3 (cm). On the basis of W and age, average daily growth (ADG) was calculated as W(g) / age (days). After fish sectioning, head and frame were obtained and weighed to calculate their incidence as percentages of head and frame on W. Dressing yield and fillet yield were calculated as 100 x [W(g) - visceral weight (g) / W(g)] and 100 x [fillets weight (g) / W (g)], respectively. The fillets were sent in refrigerated box to the laboratory of the Department of Agricultural

Biotechnology in Florence and weighed after 24 h from slaughtering. Left fillets were analyzed as raw, while right fillets were wrapped with aluminum foil, placed on a tray in a fish-steamer, boiled (at 98-100 °C) for 10 min and then cooled at room temperature and weighed. The cooking yield was calculated as 100 x [cooked fillet weight (g) / raw fillet weight (g)]. Free water and instrumental colour and texture analyses were carried out on both raw and cooked fillets. The analyses of the proximate composition, total lipids and collagen were performed on both raw and cooked fillets without skin, homogenized and freeze-dried prior to analyses.

Table 2 - Fatty acids composition of the diet

Fatty acids (% of total FA)	**Salmo50**
C14:0	7.91
C16:0	18.04
C18:0	3.61
SFA	***31.26***
C16:1n-7	8.24
C18:1n-9	9.61
C18:1n-7	3.00
MUFA	***22.47***
C18:2n-6 (LA)	5.86
PUFAn-6	***7.49***
C18:3n-3 (ALA)	1.04
C18:4n-3	1.92
C20:5n-3 (EPA)	17.55
C22:5n-3	1.60
C22:6n-3 (DHA)	10.09
PUFAn-3	***33.54***
PUFA	***45.91***
n-3/n-6	4.48

The fatty acids C12:0, C15:0, C14:1, C16:2*n*-4, C16:3*n*-4, C17:0, C17:1, C16:4*n*-1, C18:2*n*-4, C18:3*n*-6, C18:3*n*-4, C20:0, C20:1*n*-7, C20:2*n*-6, C20:3*n*-6, C20:3*n*-3, C20:4*n*-3, C21:5*n*-3, C22:1*n*-9, C22:4*n*-6, 6 C22:5*n*-6, detected in percentages lower than 1, are considered in the composite fractions, but not reported in the table for brevity.

Free water, texture and colour

Free water measurement was performed by applying the compression test on filter paper using the Grau and Hamm (1953) method.

A texture profile analysis (TPA) was carried out using a Zwick Roell® 109 texturometer (software: Text Expert II) equipped with a 200 N load cell. The Warner-Bratzler shear test was performed on the central part of the fillet, using a straight blade that moved down at a constant speed of 30 mm/min to 50% of the total deformation. Maximum shear force, defined as the maximum resistance of the sample to shearing (Veland and Torrisen 1999) was determined.

Two cycle compression test was done using a 10 mm diameter cylindrical probe at a constant speed of 30 mm/min to 50% of total deformation. Textural attributes were measured on the epaxial part of the fillet. Six texture parameters were calculated, as suggested by Veland and Torrisen (1999) and Ayala *et al.* (2010): hardness (peak force of the first compression cycle), cohesiveness (ratio of positive force area during the second compression compared to that obtained during the first compression), resilience (ratio of the area of the upstroke compared to the area of the first downstroke during the first compression cycle), springiness (height that the food recovers during the time that elapses between the two compression cycles), gumminess (hardness multiplied by cohesiveness) and chewiness (hardness multiplied by cohesiveness multiplied by springiness). All measurements were done at room temperature.

A Spectro-color®116 colorimeter (using Spectral qc 3.6 software) was utilised for colorimetric measurement carried out according to the CIELab system (CIE 1976). In this system, lightness (L*) is expressed on a 0–100 scale from black to white; redness index (a*) ranges from red (+60) to green (-60) and yellowness index (b*) ranges from yellow (+60) to blue (-60). Colour was measured in duplicate on the epaxial, ventral, and caudal fillet positions and expressed as average value.

Proximate composition and collagen

Moisture, crude protein (Nx6.25) and ash contents were determined according to AOAC (2000) 950.46, 976.05, and 920.153 methods, respectively. The total lipid extraction was performed according to a modified Folch *et al.* (1956) method. Freeze-dried samples, reconstituted fresh by adding distilled water, were homogenised with a 2:1 chloroform-methanol (v/v) solution and filtered. The filter was washed several times, and distilled water with 0.88% KCl was added to the filtrate so that the [Choloroform:Methanol]:water ratio was 4:1. Tubes were stirred, and a biphasic system was obtained by standing overnight. The lower phase containing lipids dissolved in chloroform were siphoned and recovered. The total lipid content was determined gravimetrically, after removal of the solvent (chloroform) by evaporation under vacuum and lipid resuspension in a known volume of chloroform (5 mL). Lipid content was weighed in a crucible (gross weight minus tare) after complete chloroform evaporation.

The hydroxyproline content necessary to quantify total collagen was obtained by hydrolysing samples with 70% perchloric acid ($HClO_4$) for 4 hours at 100 °C and diluting it into a flask as suggested by Galasinski *et al.* (1978) and Bonnet and Kopp (1984) methods. Quantities for hydrolysis (1.5 g on 7.5 mL) and dilution volume of hydrolyzed sample (50 mL) were one-half of what is suggested by Bonnet and Kopp (1984) method. Diluted samples were then filtered through 413-VWR no. 516-0816 filter papers. For colorimetric reaction, hydroxyproline standard solution, with concentration ranging from 2 to 20 μg/mL, was included. Amounts of 0.1 mL of standard and filtered samples were transferred in Eppendorf tubes (2 mL) and 0.2 mL of acetate/citrate buffer (pH 6) added. Samples were neutralized with 1.8 M NaOH. According to Bergman and Loxley (1963) method, an oxidant solution composed of 1 volume of Chloramine-T at 7% (w/w) and 4 volumes of acetate/citrate buffer (pH 6) was added to the tubes and left reacting for 25 minutes at room temperature. Erlich's reagent solution was prepared according to Bonnet and Kopp (1984) method, by dissolving 20 g p-dimethylaminobenzaldehyde in 30 mL of $HCLO_4$ and by mixing with isopropanol at a ratio 3:13 (v/v). One mL of this solution was added to the tubes. Tubes were heated at 60 °C for 25 minutes in a water bath (Bergman and Loxley 1963). Absorbance was measured using a spectrophotometer (PerkinElmer-Lambda EZ 150) at 558 nm. Total collagen content was calculated assuming 17.8 as conversion factor (Montero *et al.* 1990).

Determination of FA composition

FAME (Fatty acid methyl esters) analysis was performed according to the modified method of Morrison and Smith (1964). Lipids were saponified with 0.5 M KOH in methanol, and FAs were hydrolysed by adding 2 N HCL. Methyl esters were prepared by transmethylation using boron fluoride-methanol at a 14% concentration. Methylated FAs were dissolved in petroleum ether, dried and finally resuspended in 1 mL of hexane.

The FA composition was determined by liquid gas chromatography (LGC). A GC Varian 430 gas chromatograph equipped with a flame ionization detector (FID) and a Supelco Omegawax™ 320 capillary column (30 m x 0.32 mm i.d., 0.25 μm film and polyethylene glycol bonded phase) was utilised. The oven temperature was held at 100 °C for 2 minutes, increased to 160 °C over 4 minutes at the rate of 12 °C/min, and increased to 220 °C over 14 minutes at the rate of 3 °C/min and kept at 220 °C for 25 minutes. The injector and the detector temperatures were set at 220 °C and 300 °C, respectively. One microlitre of sample in hexane was injected into the column with the carrier gas (helium) kept at a constant flow of 1.5 mL/min. The split ratio was 1:20.

FAs were identified by comparing the retention time of FAME with the standard Supelco 37 component FAME mix. FAs were quantified through calibration curves, using C23:0 as an internal standard.

Statistical analysis

Data were analyzed using SAS Proc GLM (SAS, 2007) with the following model:

$$Y_{ijk}= \mu + S_i + F_j + (S*F)_{ij} + b*X_{ijk} + E_{ijk}$$

where

Y= k^{th} observation of the ith strain and the jth farm;
S= strain effect (i= 1…5);
F= farm effect (j= 1, 3);
X= independent variable (body weight);
E= error random effect.

For the texture parameters, the thickness of the fillet was used as further covariate. The differences between least squares means were statistically tested with the Student's t-test. All the statistical analyses on meat traits were performed separately for raw and cooked fillet.

The coefficients of the residual (after the above mentioned model) correlation between the physical and chemical traits were also calculated. Moreover, physical and chemical parameters were subjected to the Principal Component Analysis (Naes et al., 1996), using the SAS Proc FACTOR (SAS, 2007) with Varimax Rotation and the first three factors were subjected at the ANCOVA analysis with the above model. Finally, the discriminant analysis (SAS, 2007) was performed to discriminate fish of different strains or farms on the basis of physical and chemical traits.

RESULTS AND DISCUSSION

The genetic strain influenced the age at which fish reached the marketable size. *Batocchi* resulted the fastest-growing strain while *Man* the slowest-growing, with 60 days of difference (Table 3). Fish belonging to *Troutlodge* showed the highest weight, while the other strains had similar lower weight. *Troutlodge*, *Batocchi* and *La Frola* showed the best ADG, even if *Batocchi* and *La Frola* reached the final weight long before than *Troutlodge*. *La Frola* and *Troutlodge* registered the highest FY, while *Troutlodge* had also the highest CY. On the contrary the lowest FY and CY were found in fish of *Dellai* strain, characterized by a significantly higher head incidence, and of *Man* strain, slightly shorter and stockier in shape as highlighted by CF (Table 3).

The five strains significantly differed in all physical parameters and their response varied depending on the genetic factors and rearing farm. In particular genetic factors are known to affect the structure of the muscle, since many authors found that cell size and fiber diameter vary between population of salmonids (Valente *et al.* 1998; 1999; Johnston *et al.* 2000). As consequence, the genetic diversity may influence the amount of water lost from each cellular compartment (Huff-Lonergan and Lonergan 2005), the ability to endure force

(Hurling *et al.* 1996; Hyldig and Nielsen 2001) and the optical properties (Johnston *et al.* 2000; Johnston 2001) of fillet muscle.
The strain of origin did not significantly influence the free water values registered on raw muscle. Nevertheless *Dellai* tendencially showed the best ability to retain water, conversely *Batocchi* registered a tendency for the highest water losses (Table 4). After cooking the differences became significant ($p<0.01$): similarly to raw fillets, *Dellai* registered the lowest water losses also in the cooked ones, while *Man* strain had the highest (Table 5). In all strains heating induced an increase (from average 10.38 to 14.96 cm^2) of the amount of water released by disgregating the cell structures, as reported by Ofstad *et al.* (1993) and Rørå *et al.* (2003).
As shown in Table 4 shear force and hardness followed the same trend in raw fillet, even if significant differences ($p<0.001$) among the strains were appreciable only for hardness. Fish from *La Frola* strain had the softest flesh, while *Batocchi* the hardest. *La Frola*, showed the lowest gumminess, springiness and chewiness in raw fillet.
By inducing myofibrils disintegration, cooking determined a variation in texture parameters. Cooking nullified the differences in hardness and chewiness among the strains, while for cohesiveness no significant differences were found in both raw and cooked fillets (Table 5). In the current study differences in hardness and chewiness between strains were nullified by the heat-induced structural changes which provoked a reduction of compression force. Differences in resilience, gumminess and springiness between strains were maintained also after the heat treatment (Table 5). A similar behaviour induced by cooking on texture was found by Ginés *et al.* (2004) in strains of arctic charr reared at two distinct water temperatures.
Concerning the constancy in cohesiveness values, it is noteworthy that some authors (Bhattacharya *et al.* 1993; Larsen *et al.* 2011) observed that this parameter, if compared to the others, does not change by applying different cooking temperature and methods.
The five strains significantly differed for color characteristics. In raw fillet, *La Frola* showed a different colorimetric profile compared with that of the other strains (Table 4), since its flesh was brighter and less pigmented. The most intense red-coloured flesh was that from *Dellai* and *Man* strains. After cooking (Table 5) all strains showed a brighter and yellower appearance and differences were highlighted even between fillets with similar colour when raw. However the strains did not differentiate in red component any more. The most yellowish flesh was from *Man*, the least from *Dellai* strain.
It has been demonstrated that colorimetric attributes are strictly influenced by pigments deposition in the flesh of salmonids (Storebakken and Kyoon No 1992). Since pigments derive from the diet and feed composition was the same in all groups of fish of the trial, feed intake of each strain in each farm may have played fundamental role in pigmentation. Pigmentation may also be in part

genetically determined, since each strain differed from the others for growth rate, maturation, age at slaughter, structure and chemical composition of the muscle and consequently in the extent of pigments deposition (Storebakken and Kyoon No 1992; Bjerkeng 2000). Ytrestøyl *et al.* (2006) found that salmon fast growth rate is associated with decreased muscle concentration of astaxanthin due to lower pigment digestibility. In this sense the slowest-growing strains (Table 3), *Dellai* and *Man*, registered the most reddish colour (Table 4). *La Frola*, which is among the fastest growing strains (Table 3), differed from the others showing a weakly coloured meat. Significant differences between strains in colorimetric attributes were appreciated also after cooking (Table 5).
Proximate analysis of raw and cooked fillets is shown in Table 4 and 5, respectively. Fillets from *La Frola* strain were the leanest, while those from *Dellai* and *Troutlodge* had the highest lipid content. No significant differences in collagen content were detected among strains, although *Dellai* tendentially had the lowest amount and *Troutlodge* the highest (Table 4). Variation among the strains in lipid, ash and moisture contents were maintained also after cooking (Table 5).
In *La Frola* strain, the leanness of the flesh may be one of the concomitant factors influencing the low redness and yellow indexes, considering that carotenoids are lipid-soluble compounds. Even if many authors reported a positive relationship between lipid and L* in salmonids (Rørå *et al.* 1998; Mørkøre *et al.* 2001 and 2006b; Bugeon *et al.* 2010), *La Frola* registered significantly higher L* values though having the leanest flesh (Table 4), probably because the physical state and the anatomical structure of the muscle, which are known to modify the light absorption (Larsen *et al.* 2011), may have exerted a stronger influence than fat.

As shown in Table 6, a non-significant negative correlation was found between free water and all TPA parameters. This behaviour is in agreement with the results of Hultmann and Rustad (2008) in salmon and cod, that found that higher water released from the muscle reduces its mechanical strength. Concerning texture, several researches revealed that rainbow trout fillets with high fat content has a softer consistency compared to fillets with lower fat content (Andersen *et al.* 1997; Mørkøre *et al.* 2001, 2006a; Aussanasuwannakul *et al.* 2010). The present study partially confirmed this relationship, since the fattest strains, *Dellai* and *Troutlodge*, had the softest flesh (Table 4). Nevertheless the leanest strain, *La Frola*, showed the same behavior. In this last case the earlier age and the faster growth rate, which are known to affect hardness (Aussanasuwannakul *et al.* 2010; Veland and Torrissen 1999; Folkestad *et al.* 2008) may have nullified the effect produced by the lipid content. Except for shear force and hardness which were positively correlated to lipid, the lipid percentage in muscle did not show a significant correlation with texture parameters in raw fillet (Table 6).

Though no significantly different among strains, cohesiveness resulted abundantly inferior to 1 indicating that the deformation induced by compression was in part irrecoverable (Table 4). Flesh showing less ability to recover the original fillet thickness, registered lower speed and force of recovery ($p<0.05$; $r=0.64$) as expressed by resilience (Table 6). Chewiness showed significant positive linear correlation with gumminess ($p<0.05$; $r=0.29$) and springiness ($p<0.05$; $r=0.60$) (Table 6): the least chewy flesh belonging to *La Frola* strain was also the least gummy and springy (Table 4).
Because of the low content of connective tissue in fish, the textural properties of the muscle tissue are mainly directed by the fibrous proteins, which are present as 70-80% of the total protein content (Larsen *et al.* 2011). In the current study a significant negative correlation was found between total protein and shear force ($p<0.05$; $r=-0.34$), hardness ($p<0.05$; $r=-0.32$), resilience ($p<0.05$; $r=-0.21$) and springiness ($p<0.05$; $r=-0.19$). Since protein fractions were not detected the relationship between myofibrillar protein and texture was not appreciated, as suggested by Larsen *et al.* (2011).
According to Li *et al.* (2005) there is not significant relationship between total hydroxyproline content and hardness of fillet in raw salmon, suggesting a total collagen negligible contribution to the texture if compared to collagen cross-links. Consistently with this, in the present study a non-significant correlation was found between texture parameters and collagen content in raw fillet (Table 6). Accordingly the collagen content measurements of raw fillet may be not useful for the prediction of texture in rainbow trout fillet.
Cooking weakens muscle structure by converting collagen to gelatin (Aussanasuwannakul *et al.* 2010). Even if the contribution of connective tissue to the texture is known to be negligible in cooked fish (Hatae *et al.* 1986), in this study a negative correlation was found between TPA parameters and collagen, which was not significant except for chewiness ($p<0.001$; $r=-0.34$) (Table 6). The influence of collagen on chewiness can be explained by the fact that the cooked flesh of the strains having higher collagen content easily dissolved into flakes becoming softer and providing lower resistance to mastication. An inverse relationship between cooked flesh firmness and collagen content was also observed by Bugeon *et al.* (2010).
With regard to colour, in raw fillet, the intensity of redness and yellowness increased with decreasing L* ($p<0.0001$; $r=-0.67$ and $p<0.0001$; $r=-0.31$, respectively; Table 6), showing a behaviour partly in agreement with Einen and Skrede (1998), who registered an increase of lightness related to decreased perceived colour intensity in salmon, even if the instrumentally achieved intensity in red (a*) remained constant. In the current study, a positive correlation between yellow indexes and both redness ($p<0.05$; $r=0.63$) and lipid content ($p<0.05$; 0.19) was detected (Table 6), similarly to what was found by Rørå *et al.* (1998), Einen and Skrede (1998) and Mørkøre *et al.* (2001, 2006b) in Atlantic salmon.

In this study, redness index was correlated with protein content in both raw and cooked fillets because the reddish pigments, carotenoids and myoglobin, are primarily associated with the muscle protein (Storebakken and Kyoon No 1992; Bjerkeng 2000).
In cooked fillet no correlation was found between L* and a* values (Table 6). The increased levels of of L* and b*, if compared with the raw fillet, was likely attributable to the heat-induced leaching of carotenoid molecules which lead to flesh discoloration (Choubert and Baccanaud, 2010).
Cooking led to a tendencial reduction of a* value according to the results of several authors on heat-treated products (Mørkøre *et al.* 2001; Choubert and Baccanaud 2010). According to the findings of the present study, Larsen *et al.* (2011) found that the fillets were lighter and more yellow in cooked salmon than in raw. Protein aggregation probably induces an increase in opacity and the light that enters the surface has a lower chance of being selectively absorbed (Larsen *et al.* 2011). Conversely Choubert and Baccanaud (2010) found a decrease in L* and b* after dry and moist heat cooking in rainbow trout and associated it with loss of yellow components.

Table 3 - Morphometric characteristics and marketable yields of fish estimated at the average weight of 775.3 g.

	Batocchi	Dellai	La Frola	Man	Troutlodge	Farm	Strain	Farm x Strain	Weight[1]	rsd
Age (days)	607.66a	642.66d	618.33b	657.00e	627.00c	***	***	-	-	12.22
Weight (g)	774.0a	723.4a	783.0a	725.5a	870.9b	*	**	ns	-	162.90
ADG[2] (g/days)	1.28b	1.14a	1.28b	1.10a	1.39b	***	***	*	-	0.26
Trait:										
Length (cm)	37.58b	37.11b	37.16b	36.36a	38.30c	***	***	**	***(+)	0.946
CF[3]	1.44b	1.50b	1.49b	1.58c	1.36°	***	***	**	***(+)	0.111
Head (%)	15.85ab	17.09c	16.77c	16.61bc	15.37a	***	***	***	***(-)	1.557
Frame (%)	15.37b	14.46a	14.48a	14.47a	15.11b	ns	***	***	***(-)	0.929
Raw left fillet (g)	198.4a	195.9a	199.6a	197.6a	206.5b	***	***	***	***(+)	9.212
Raw right fillet (g)	205.5b	196.1a	209.3bc	195.9a	212.1c	**	***	***	***(+)	9.172
Fillet Yield (%)	51.94b	50.28a	52.44bc	50.44a	53.52c	***	***	***	***(+)	2.269
Cooking Yield (%)	83.59a	83.23a	84.16a	84.4a	86.43b	***	**	ns	ns	3.500

[1] in parenthesis, the sign of the linear regression coefficient
[2] Average Daily Growth
[3] Condition factor = 100 x Weight / Length^3
a, b, …: $p<0.05$; *: $p < 0.05$, **: $p< 0.01$, ***: $p < 0.001$; ns: not significant.

Table 4 - Physical and chemical parameters in raw fillet estimated at the average weight of 775.3 g.

	Batocchi	Dellai	La Frola	Man	Troutlodge	Farm	Strain	Farm x Strain	Weight[1]	rsd
Free Water (cm^2)	11.04	9.74	10.52	10.35	10.27	**	ns	***	ns	1.872
Texture parameters										
Shear stress (N)	6.66	6.13	5.93	6.53	5.83	ns	ns	**	ns	1.46
Hardness (N)	5.41c	4.07a	3.75a	4.75b	4.02a	**	***	***	ns	1.223
Cohesiveness	0.37	0.39	0.39	0.36	0.36	ns	ns	*	ns	0.059
Resilience	0.09a	0.12b	0.11b	0.09a	0.09a	*	***	***	ns	0.025
Gumminess (N)	1.98b	2.19b	1.41a	2.20b	2.04b	***	***	***	ns	0.658
Springiness	4.40ab	5.07c	4.10a	4.83bc	4.58ab	ns	*	ns	ns	1.09
Chewiness (N)	9.18c	8.59c	6.09a	8.43bc	6.65ab	*	**	**	ns	3.391
Colour parameters										
L*	33.41ab	34.86b	41.22c	31.68a	36.42b	***	***	***	ns	5.17
a*	9.68ab	10.66b	7.83a	10.27b	9.84ab	***	*	ns	***(+)	4.08
b*	14.27b	12.92b	10.40a	14.32b	14.24b	***	***	***	**(+)	3.20
Chemical parameters (% on fresh weight)										
Total lipid	5.94bc	6.21c	5.04a	5.56b	6.10c	***	***	***	*(+)	0.994
Protein	20.97	20.63	21.05	20.68	20.97	***	ns	**	*(+)	0.818
Ash	1.34a	1.32a	1.36	1.32a	1.40b	*	*	ns	ns	0.108
Moisture	72.61a	72.71a	73.76c	73.22b	72.37a	***	***	***	***(-)	0.997
Collagen		0.65	0.77	0.73	0.82	ns	ns	-	0.143	0.143

[1]in parenthesis, the sign of the linear regression coefficient

a, b….: p<0.05; *: p < 0.05, **: p< 0.01, ***: p < 0.001; ns: not significant.

Table 5 - Physical and chemical parameters in cooked fillet estimated at the average weight of 775.3 g.

	Batocchi	Dellai	La Frola	Man	Troutlodge	Farm	Strain	Farm x Strain	Weight[1]	rsd
Free Water (cm^2)	14.70ab	13.72a	14.57ab	15.99c	15.75bc	***	**	***	ns	2.391
Texture parameters										
Shear stress (N)	6.55b	6.04b	5.14°	6.07b	4.79a	*	***	ns	ns	1.696
Hardness (N)	4.31	4.31	3.89	4.51	3.56	***	ns	**	ns	1.339
Cohesiveness	0.30	0.31	0.31	0.29	0.30	***	ns	**	ns	0.054
Resilience	0.05bc	0.06cd	0.07d	0.04ab	0.04a	***	***	***	ns	0.024
Gumminess (N)	1.08a	1.27ab	1.06a	1.55c	1.32b	***	***	**	ns	0.407
Springiness	5.44c	5.38bc	4.63a	4.69ab	4.48a	*	*	ns	ns	1.379
Chewiness (N)	6.68	6.44	5.62	5.98	4.94	ns	ns	*	*(-)	2.505
Colour parameters										
L*	66.54b	58.13a	69.42c	68.14bc	68.94bc	***	***	***	ns	4.06
a*	8.71	10.65	9.06	9.33	9.05	**	ns	ns	*(+)	2.87
b*	16.90b	13.65a	16.33b	19.00c	16.83b	ns	***	***	ns	2.15
Chemical parameters (% on fresh weight)										
Total lipid	5.55bc	5.92c	4.97a	5.17ab	5.53bc	**	***	**	***(+)	0.793
Protein	24.75a	25.38b	25.32b	25.02ab	24.69a	***	**	***	ns	0.903
Ash	1.45b	1.33a	1.34a	1.34a	1.34a	***	***	***	ns	0.091
Moisture	69.39bc	68.69a	69.78bc	69.84c	69.23ab	***	***	***	***(-)	1.093
Collagen		0.68ab	0.73ab	0.68a	0.80b	ns	*	ns	*	0.094

[1]in parenthesis, the sign of the linear regression coefficient

a, b….: $p<0.05$; *: $p < 0.05$, **: $p< 0.01$, ***: $p < 0.001$; ns: not significant.

The fatty acids profile of muscle total lipids of the five strains in raw and cooked fillets are shown in Tables 7 and 8, respectively. Even though trouts were fed the same diet, each strain had a different fatty acids profile and differences among strains were also maintained after cooking. In all strains the most abundant fatty acids were stearic acid (C16:0), the isomer n-9 of the oleic acid (C18:1n-9), linoleic acid (C18:2n-6), eicosapentaenoic acid (C20:5n-3) and docosaesaenoic acid (C22:6n-3).
In both raw and cooked fillet, *La Frola* strain was found to contain significantly higher value of saturated fatty acids. Conversely it showed the lowest total amount of monounsaturated fatty acids (MUFAs). Concerning PUFAs, *La Frola* and *Troutlodge* had the lowest n-6 polyunsaturated fatty acids content, while *Dellai* the highest. The total amount of n-3 polyunsaturated fatty acids, including EPA and DHA, was higher in *La Frola* and *Troutlodge*.
The fatty acids profile of fillet was presumably influenced by the fatty acids profile of the diet, which was characterized by high proportion of C16:0, C18:1n-9 and C20:5n-3 and C22:6n-3. However significant differences among strains were detected. *La Frola* showed the lowest proportion of MUFAs which are known to decrease during starvation or reduction of feed ration in rainbow trout (Jezierska *et al.* 1982; Kiessling *et al.* 2001). An age-related trend was also detected in MUFAs content, according to what has been reported by Kiessling *et al.* (2001) who found a higher MUFAs proportion at increasing age. PUFAs were abundant in all strains, higher than the proportion detected by Kiessling *et al.* (2001) on fish of similar age.
Secondly, FAs profile may have been partly affected by desaturation and elongation of C18 to C20 and C22 series which generally occur in freshwater fish (Sargent *et al.* 2002). In particular, desaturation of C18:0 to C18:1n-9 may have been taken place, since C18:1n-9 had a higher proportion in the muscle (Table 7) than in the finishing diet (Table 2). The decreased content of C20:5n-3 in the muscle may be attributable to its conversion to C22:5n-3 and C22:6n-3 (Sargent *et al.* 2002). C22:6n-3 clearly was the final product of the elongation/desaturation pathway, considering that the amount in the muscle was twice than in the diet.
Differences among strains were maintained after cooking and the proportion of n-3 PUFAs was greatly reduced (Table 8). This last result is in contrast to the results of Unusan (2007) and Ågren and Hänninen (1993) who found that long chain n-3 FAs were well retained in cooked fillets of rainbow trout.

Table 6 – Residual correlation coefficient (r) between physical and chemical parameters in raw (above the diagonal) and cooked fillet (below the diagonal).

	Free Water	Shear force	Hardness	Cohesiveness	Resilience	Gumminess	Chewiness	Springiness	L*	a*	b*	Lipid	Protein	Ash	Moisture	Collagen
Free Water	--	0.04	-0.09	-0.15	-0.10	-0.02	-0.02	-0.03	-0.06	0.05	0.12	-0.09	0.12	0.03	-0.01	0.15
Shear force	0.03	--	0.39*	-0.02	0.11	0.15	0.28*	-0.01	0.13	-0.19*	-0.04	0.18*	-0.34*	-0.16*	-0.01	-0.12
Hardness	-0.07	0.02	--	-0.38*	0.08	0.04	0.65*	0.01	0.36*	-0.47*	-0.01	0.17*	-0.32*	-0.17*	0.06	-0.10
Cohesiveness	0.04	0.02	0.36*	--	0.65*	0.52*	0.04	-0.03	-0.10	0.08	-0.08	-0.07	-0.03	0.01	0.06	-0.02
Resilience	0.05	0.07	0.23*	0.78*	--	0.41*	0.37*	0.01	0.08	-0.21*	-0.23*	-0.11	-0.22*	-0.08	0.21*	0.05
Gumminess	0.02	-0.00	0.79*	0.62*	0.33*	--	0.29*	0.02	-0.19*	0.10	-0.03	-0.04	0.09	0.05	-0.01	0.13
Chewiness	0.05	0.06	0.74*	0.61*	0.48*	0.73*	--	0.60*	0.21*	-0.29*	-0.04	-0.04	-0.19*	-0.12	0.25*	-0.17
Springiness	0.05	-0.03	0.03	0.08	0.15	-0.00	0.50*	--	0.01	0.03	-0.05	-0.15	0.02	-0.00	0.22*	-0.06
L*	0.12	0.02	-0.02	0.07	0.16	0.03	0.08	0.11	--	-0.68*	-0.31*	0.19*	-0.32*	-0.17*	-0.01	-0.05
a*	0.02	-0.10	-0.01	-0.19*	-0.20*	-0.03	-0.11	-0.08	-0.08	--	0.63*	-0.04	0.39*	0.13	-0.22*	0.01
b*	0.00	-0.02	-0.06	-0.03	-0.06	0.01	-0.11	-0.10	-0.12	0.09	--	0.19*	0.00	-0.10	-0.27*	0.19
Lipid	0.05	0.19*	0.04	0.02	0.11	0.10	-0.04	-0.05	-0.07	-0.06	0.03	--	-0.28*	-0.24*	-0.79*	0.12
Protein	0.01	0.05	0.13	-0.26*	-0.27*	-0.04	-0.04	-0.09	-0.06	0.28*	-0.09	-0.09	--	0.30*	-0.14	-0.31*
Ash	0.08	0.00	0.02	-0.11	-0.09	-0.00	-0.02	-0.12	0.03	0.25*	-0.07	-0.32*	0.47*	--	0.05	-0.07
Moisture	-0.10	-0.13	-0.04	0.11	0.01	0.09	0.07	0.12	-0.01	-0.11	-0.02	-0.74*	-0.45*	0.01	--	0.02
Collagen	-0.15	-0.11	-0.24	-0.21	-0.23	-0.24	-0.34*	-0.08	-0.34*	0.30*	0.18	0.16	-0.04	0.13	-0.09	--

*: $p<0.05$

Table 7 – Fatty acids profile of raw fillets estimated at the average weight of 775.3 g.

	Batocchi	Dellai	La Frola	Man	Troutlodge	Farm	Strain	Farm x Strain	Weight[1]	rsd
Fatty acids (% of total FA)										
C14:0	4.11bc	3.86a	4.18c	4.04b	4.30d	***	***	***	*(+)	0.016
C16:0	13.92c	13.32a	14.06c	13.50ab	13.35a	***	***	***	***(+)	0.517
C18:0	3.30b	3.23b	3.24b	3.26b	3.00a	***	***	***	***(+)	0.159
SFA	***22.41b***	***21.58a***	***22.96c***	***21.74a***	***21.69a***	***	***	***	**(+)	1.011
C16:1n-7	5.77a	5.66a	5.76a	5.92b	6.06b	***	***	***	*(+)	0.273
C18:1n-9	11.25b	11.97c	10.65a	11.71c	11.23b	***	***	**	***(+)	0.624
C18:1n-7	2.57ab	2.54a	2.59b	2.59b	2.66c	***	***	***	**(+)	0.077
MUFA	***20.74b***	***21.27c***	***20.07a***	***21.44c***	***21.19c***	***	***	***	***(+)	0.790
C18:2n-6 (LA)	10.90b	11.94c	10.00a	11.14b	10.21a	***	***	***	ns	1.186
C20:4n-6	1.01b	0.98a	1.01b	1.01b	1.03c	***	*	*	*(-)	0.058
PUFAn-6	***13.08b***	***14.21c***	***12.12a***	***13.39b***	***12.40a***	***	***	***	ns	1.240
C18:3n-3 (ALA)	1.58bc	1.65c	1.45a	1.59bc	1.51ab	***	***	***	ns	0.142
C18:4n-3	1.21b	1.15a	1.21b	1.23b	1.31c	***	***	***	***(-)	0.070
C20:5n-3 (EPA)	12.46b	11.50a	12.78bc	12.48b	13.02c	***	***	***	ns	0.799
C22:5n-3	2.82a	2.96ab	2.92a	2.88a	3.09b	***	*	*	ns	0.313
C22:6n-3 (DHA)	20.79ab	20.98b	21.71c	20.04a	20.28a	***	***	*	***	1.144
PUFAn-3	***40.23ab***	***39.50a***	***41.28c***	***39.76a***	***40.79bc***	***	***	*	**	1.539
PUFA	***56.66***	***56.88***	***56.76***	***56.61***	***56.83***	*	ns	***	***(-)	1.458

The fatty acids C12:0, C15:0, C14:1, C16:2*n*-4, C16:3*n*-4, C17:0, C17:1, C16:4*n*-1, C18:2*n*-4, C18:3*n*-6, C18:3*n*-4, C20:0, C20:1*n*-7, C20:2*n*-6, C20:3*n*-6, C20:3*n*-3, C20:4*n*-3, C21:5*n*-3, C22:1*n*-9, C22:4*n*-6, 6 C22:5*n*-6, detected in percentages lower than 1, are considered in the composite fractions, but not reported in the table for brevity.

[1]in parenthesis, the sign of the linear regression coefficient.

a, b….: $p<0.05$; *: $p < 0.05$, **: $p< 0.01$, ***: $p < 0.001$; ns: not significant.

Table 8 - Fatty acids profile of cooked fillets estimated at the average weight of 775.3 g.

	Batocchi	Dellai	La Frola	Man	Troutlodge	Farm	Strain	Farm x Strain	Weight[1]	rsd
Fatty acids (% of total FA)										
C14:0	4.33bc	4.02a	4.58d	4.17ab	4.46c	***	***	***	*(+)	0.337
C16:0	16.47c	15.74b	17.21d	16.08bc	15.10a	*	***	***	*(+)	1.022
C18:0	3.88bc	3.79b	3.96c	3.88bc	3.41a	**	***	***	***(+)	0.242
SFA	***25.51b***	***24.34ab***	***26.69c***	***24.94b***	***23.82a***	***	***	***	*(+)	1.560
C16:1n-7	6.52b	6.27a	6.80c	6.56b	6.58bc	ns	***	***	*(+)	0.437
C18:1n-9	12.64b	13.21c	12.53ab	12.92bc	12.12a	***	***	**	***(+)	0.884
...C18:1n-7	3.01a	3.01a	3.11b	3.00a	2.95a	ns	*	***	ns	0.161
MUFA	***23.22ab***	***23.71ab***	***23.84b***	***23.89b***	***23.03a***	***	ns	***	**(+)	1.437
...C18:2n-6 (LA)	11.04b	12.12c	9.99a	11.05b	10.29a	***	***	***	ns	1.173
...C20:4n-6	1.00ab	0.98ab	0.97a	1.01b	1.01b	***	ns	**	ns	0.070
PUFAn-6	***13.09bc***	***14.27d***	***12.03a***	***13.24c***	***12.45ab***	***	***	***	ns	1.239
...C18:3n-3 (ALA)	1.45bc	1.51c	1.28a	1.42b	1.41b	***	***	***	ns	0.145
C18:4n-3	1.10b	1.04a	1.05ab	1.09ab	1.22c	***	***	***	ns	0.089
...C20:5n-3 (EPA)	9.43b	8.58a	9.21b	9.31b	11.01c	***	***	***	ns	0.780
C22:5n-3	3.20a	3.39bc	3.22a	3.38ab	3.41bc	***	*	***	ns	0.314
C22:6n-3 (DHA)	18.07ab	18.56b	17.63a	17.88ab	18.32ab	***	ns	*	***	1.517
PUFAn-3	***34.67a***	***34.48a***	***33.90a***	***34.41a***	***36.85b***	***	***	***	**	2.272
PUFA	***51.20b***	***51.91bc***	***49.37a***	***51.04b***	***52.97c***	***	***	***	**(-)	2.757

The fatty acids C12:0, C15:0, C14:1, C16:2*n*-4, C16:3*n*-4, C17:0, C17:1, C16:4*n*-1, C18:2*n*-4, C18:3*n*-6, C18:3*n*-4, C20:0, C20:1*n*-7, C20:2*n*-6, C20:3*n*-6, C20:3*n*-3, C20:4*n*-3, C21:5*n*-3, C22:1*n*-9, C22:4*n*-6, 6 C22:5*n*-6, detected in percentages lower than 1, are considered in the composite fractions, but not reported in the table for brevity.

[1] in parenthesis, the sign of the linear regression coefficient.

a, b….: $p<0.05$; *: $p < 0.05$, **: $p< 0.01$, ***: $p < 0.001$; ns: not significant.

PCA analysis

In order to analyse the joint behavior among the parameters of physico-chemical analyses, the set of data was subjected to the PCA analysis, that was performed using the Varimax method that optimizes and balances the Variance partition among the defined factors (SAS, 2007). Table 9 reveals that the first three Factors explained around 58% in raw fillet and almost the 50% in cooked fillet of total Variance of the examined parameters.

Table 9 – Results of the factor analysis for the physico-chemical parameters in raw and cooked fillets.

	Raw			Cooked		
	Factor1	**Factor2**	**Factor3**	**Factor1**	**Factor2**	**Factor3**
Eigenvalue (Variance explained)	2.96	2.63	2.20	2.69	2.24	1.95
% of total variance	21.14	18.78	15.71	19.21	16.00	13.92
Loadings						
Free Water	0.016	-0.275	-0.186	0.044	0.138	0.175
Shear Force	0.226	**0.585**	-0.110	0.259	-0.133	0.099
Hardness	-0.217	**0.805**	-0.381	**0.697**	0.256	0.05
Cohesiveness	0.180	-0.225	**0.854**	**0.705**	-0.269	-0.180
Resilience	-0.027	0.008	**0.879**	**0.538**	**-0.464**	-0.164
Gumminess	-0.051	0.207	0.045	**0.523**	0.267	**-0.460**
Chewiness	-0.151	**0.806**	0.310	**0.939**	0.139	-0.003
Springness	-0.043	0.335	**0.446**	**0.420**	0.078	0.035
L*	**-0.548**	0.052	0.163	0.040	-0.102	**0.843**
a*	**0.726**	**-0.427**	0.135	-0.090	**-0.425**	0.005
b*	**0.831**	-0.145	-0.094	-0.101	0.142	**0.794**
Lipid	**0.768**	0.222	0.151	-0.011	**-0.702**	-0.210
Protein	0.187	**-0.662**	0.284	0.046	**-0.519**	0.483
Moisture	**-0.812**	0.143	-0.230	-0.044	**0.883**	-0.125

In raw fillet, Factor1 associated the variables colour, moisture and lipid. In particular, lipid, a* and b* were positively associated and showed similar loading values, in the opposite direction of moisture and L*. No significant association was found among the abovementioned parameters and the texture

indicators that influenced the Factor2. In particular shear force, hardness and chewiness were linked and showed high and similar loading values. Such parameters were strictly linked to protein but in the opposite direction. It has to be noted that resilience, cohesiveness and chewiness were not linked to the other textural parameters, being the parameters that more influenced the Factor3.

In the loading plot of raw fillet (Figure 1) are graphically shown the position of the strains and the farms related to the loading values of the physico-chemical parameters. Factor Analysis of raw fillet differentiated with respect to the strain and to the farm. *La Frola* strain was placed on the third quadrant of the figure, next to L* and opposed to lipid, due to the higher lightness and leanness detected in fillets of this strain. *Dellai*, *Batocchi* and *Man* showed the highest similarities in the quality traits, firstly texture, since they were grouped on the positive axis of the Factor2 where textural parameters are loaded positively.

With regard to the farm, Iasma greatly differentiated from Foglio and Tamanini. The characteristics of fish from Iasma, that registered the highest reddish and yellow taints and fat content, explain why Iasma was affected mainly by the Factor1 being located in the same area of lipid, a* and b*.

As shown in Table 9, the variables of cooked fillet had preferentially positive loading values. The Factor1 was influenced by the texture parameters which were all associated, on the contrary to what was found for raw fillets. After cooking, moisture, protein, lipid and a* were associated to the Factor2. If compared to raw state, the chromaticity indexes a* and b* were not linked any more, probably because red component was due to carotenoid content, while the yellow component was indicative of their loss during cooking (Birkeland *et al.* 2006).

In the loading plots of cooked fillet (Figure 2) are graphically shown the position of the strains and the farms related to the loading values of the physico-chemical parameters.

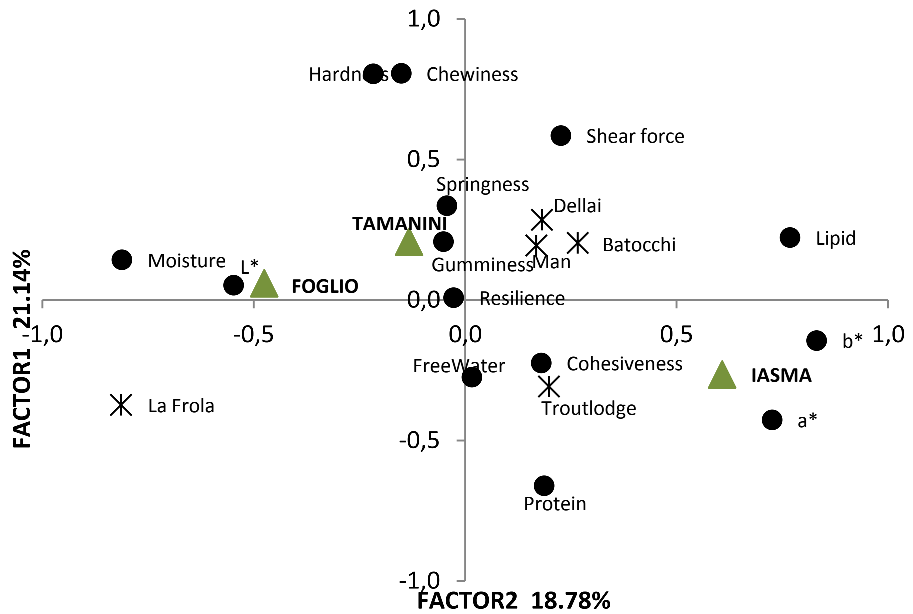
PCA of cooked fillet showed a different response to cooking among strains. *Dellai* strain largely differed from the others. It was located in the bottom of the fourth quadrant, next to lipid, protein and a*. *La Frola* and *Troutlodge* were well defined by the first PC and they were placed opposed to texture parameters. This has meant that the two strains developed a similar softer texture profile after cooking by decreasing in all textural parameters.

Concerning farms, Iasma greatly differentiated from Foglio and Tamanini after cooking as well. Similarly to raw fillet, Iasma was located next to lipid and a* suggesting that fish reared in Iasma had higher lipid content and redness index after cooking.

As shown in Table 10 all the three Factors resulted affected by strain and by farm, both in raw and in cooked fillets. In raw fillet Factor1, that associated colour and chemical parameters, differentiaded *La Frola* from the other strains. *La Frola* and *Troutlodge* showed similar values for Factor2, which is influenced

mainly by the parameters associated with the texture. The similarity between these strains is enhanced after cooking, as shown by the values of all the three Factors.
A significant farm effect was also found. In raw fillets all the farms differed each other for Factor1, whereas Iasma was characterized as different from Tamanini for Factor2, that associated the textural properties. The difference between these farms for texture is fully confirmed in cooked fillets, as the behaviour of Factor1 shown. After cooking, no difference resulted between Foglio and Tamanini for Factor2, whereas the specificity of Iasma for the parameters synthetised in this Factor was confirmed.

Figure 1 – Loading plot of raw fillet.



The discriminant analysis (Table 11) based on the physico-chemical parameters revealed that fish belonging to each strain was quite distinguishable from the others. In raw fillets, the percentage of correct classification for strain of the whole sample was 58%. The strains best classified were *La Frola* and *Troutlodge*, which always showed percentages greater than 66% indicating a good separation of these strains in relation to the physico-chemical parameters.
Fish reared in Tamanini was more clearly distinguishable from the fish reared in the other farms (86% of correct classification), probably because the physico-chemical parameters were affected by the specific rearing conditions of this farm (Table 1). The overall classification accuracy of the discriminant functions

Table 10 – Effect of strain and farm on the three Factors in raw and cooked fillets.

	Strain					Farm			rsd
	Batocchi	Dellai	La Frola	Man	Troutlodge	Iasma	Foglio	Tamanini	
Raw fillet									
Factor1	0.266b	0.182b	-0.814a	0.168b	0.198b	0.608a	-0.475b	-0.132c	0.644
Factor2	0.203b	0.285b	-0.373a	0.193b	-0.308a	-0.266a	0.06	0.207b	0.860
Factor3	0.031b	0.223cb	0.369cb	-0.441a	-0.183ab	0.769a	-0.276b	-0.493	0.662
Cooked fillet									
Factor1	0.238a	0.215a	-0.370b	0.205a	-0.359b	0.258a	0.039a	-0.340b	0.904
Factor2	0.224bc	-0.714a	0.009b	0.394c	0.148bc	-0.797a	0.329b	0.505b	0.646
Factor3	0.119bc	-0.844a	0.244bc	0.448c	0.082b	0.431a	0.112b	-0.513c	0.419

for farm was 83.3%, higher than what was obtained by distinguishing the strains. In particular, Tamanini had the highest percentage of fish correctly classified (86%).

Figure 2 – Loading plot of cooked fillet.

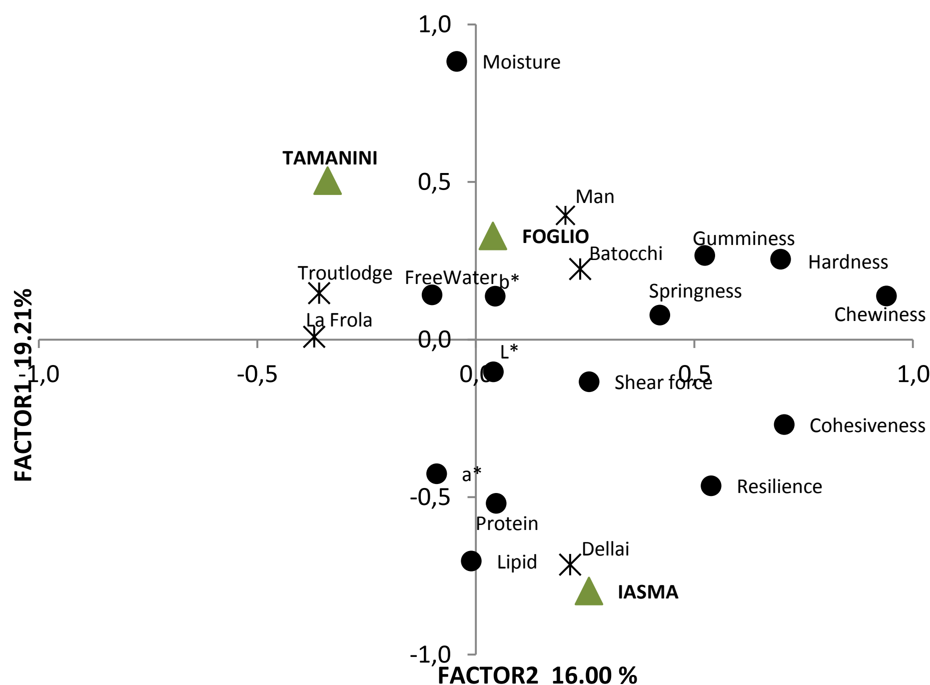


Table 11 - Classification results using linear discriminant function for breed and farm (% correctly classified).

	Raw fillet		Cooked fillet	
	Resubstitution	Crossvalidation	Resubstitution	Crossvalidation
Strain	**58**	**41.3**	**66.7**	**52.1**
- Batocchi	56.7	33.3	76.7	46.7
- Dellai	50	30	73.3	66.7
- La Frola	70	70	64.3	60.7
- Man	46.7	20	60	36.7
- Troutlodge	66.7	53	60	50
Farm	**83.3**	**75.3**	**86.3**	**75.6**
- Iasma	82	76	80	70
- Foglio	82	76	90	84
- Tamanini	86	74	77.1	72.9

The cooking process enhanced the accuracy of classification, since the percentages of correct classification was higher, both for strain (67%) and farm (86%). In contrast to raw fillets, the best scores for strain classification were obtained for *Batocchi* and *Dellai* strains, whereas the best score for farm classification was obtained for fish from Foglio.

CONCLUSION

This study has shown that genetic differences among strains, affecting growth performance and efficiency of feed utilization, had a strong influence on qualitative traits of rainbow trout fillets. *La Frola* and *Troutlodge* resulted the most valuable strains for their market and chemical traits since they registered the best FY, CY and the highest proportion in n-3 PUFAs. Despite this, both raw and cooked fillets from *La Frola* strain differed from Troutlodge, showing a mediocre texture and colorimetric profile, considering that in general consumers prefer firm and elastic fish meat and red-colored tint. Nevertheless the leanness of flesh with high n-3 PUFAs incidence and the low collagen content of the fillets may represent a positive characteristic in *La Frola* strain, since it is known the potential need for lowering the lipid content of farmed fish and for avoiding the unpleasant softening effect induced by the gelatinization of collagen. In this sense, such advantages over *Troutlodge* should be investigated through sensory analysis, to determine if differences in chemical parameters between strains could be spotted by consumers.

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REFERENCES

ÅGREN, J.J. and HÄNNINEN, O., 1993. Effects of cooking on the fatty acids of three freshwater fish species. *Food Chemistry*.46, 377-382.

ANDERSEN, U.B., THOMASSEN, M.S. and RORA, A.M.B. 1997. Texture properties of farmed rainbow trout (*Oncorhynchus mykiss*): effects of diet, muscle fat content and time of storage on ice. *J. Sci. Food Agric*. 74, 347-353.

AOAC, 2000. Official Methods of Analysis (*17th ed.*) AOAC International. Washington, DC.

AUSSANASUWANNAKUL, A., BRETT KENNEY, P., WEBER, G.M., YAO, J., SLIDER, S.D., MANOR, M.L. and SALEM, M. 2010. Effect of sexual maturation on growth, fillet composition, and texture of female rainbow trout (*Oncorhynchus mykiss*) on a high nutritional plane. *Aquaculture*. 317, 79-88.

AYALA, M.D., ABDEL, I., SANTAELLA, M., MARTÌNEZ, C., PERIAGO, M.J., GIL, F., BLANCO, A. and LOPEZ ALBORS, O. 2010. Muscle tissue structural changes and texture development in sea bream, *Sparus aurata* L., during post-mortem storage. *LWT-Food Sci. Technol.* 43, 465-475.

BHATTACHARYA, S., CHOUDHURY, G.S. and STUDEBAKER, S. 1993. Hydrothermal processing of Pacific Chum Salmon: effects on texture and in-vitro digestibility. *Journal of Food Quality*. 16, 243-251.

BERGMAN, M. and LOXLEY, R. 1963. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. *Anal. Chem*. 35(12), 1961-1964.

BIRKELAND, S., HAARSTAD, I. and BJERKENG, B. 2006. Effects of salt-curing procedure and smoking temperature on astaxanthin stability in smoked salmon. *Food Engineering and Physical Properties*. 69 (4), 198-203.

BJERKENG, B. 2000. Carotenoid pigmentation of salmonids fishes – recent progress. *Avances en nutrición acuícola V. Memorias del V Simposium Internacional de Nutrición Acuícola*. 19-22 Noviembre. Mérida, Yucatán.

BONNET, M. and KOPP, J. 1984. Dosage du collagène dans les tissus conjoctifs, la viande et les produits carnes. *Cahiers Techniques.* INRA *5*, 19-30.

BUGEON, J., LEFEVRE, F., CARDINAL, M., UYANIK, A., DAVENEL, A. and HAFFRAY, P. 2010. Flesh quality in large rainbow trout with high or low fillet yield. *J. Muscle Foods* 21(4), 702-721.

CHOUBERT, G. and BACCAUNAUD, M. 2010. Effect of moist or dry heat cooking procedures on carotenoid retention and colour of fillets of rainbow trout (*Oncorhynchus mykiss*) fed astaxanthin or canthaxanthin. *Food Chemistry*. 119, 265-269.

CIE, 1976. Official recommendations on uniform colour space, colour difference equations and metric colour terms. *Suppl. n. 2 to CIE Publication n. 15, Colorimetry*. Commission International de l´Éclairage, Paris.

EINEN, O. and SKREDE, G. 1998. Quality characteristics in raw and smoked fillets of Atlantic salmon, *Salmo salar*, fed high-energy diets. *Aquaculture Nutrition*. 4, 99-108.

FACCENDA, F., GANDOLFI, A., TIBALDI, E. and LUNELLI, F. 2010. Nota breve - Short note. Proposta di Ricerca integrata per il miglioramento della produzione ittica commerciale trentina in termini di resa sul prodotto lavorato per la specie *Oncorhynchus mykiss* (trota iridea). *Studi Trentini di Scienze Naturali*. 87, 193-195. [In Italian].

FALLAH, A.A., SIAVASH SAEI-DEHKORDI, S. and NEMATOLLAHI, A. 2011. Comparative assessment of proximate composition, physicochemical parameters, fatty acids profile and mineral content in farmed and wild rainbow trout (*Oncorhynchus mykiss*). *Int. J. Food Sci. Technol.* 46, 767-773.

FOLCH, J., LEES, M. and SLOANE-STANLEY, G.H. 1956. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. 226, 497-509.

FOLKESTAD, A., RØRVIK, K.A., KOLSTAD, K. and MØRKØRE, T. 2008. Growth rates of individual farmed Atlantic salmon *Salmo salar* L. influence the texture of raw and smoked fillets. Short Communication. *Aquaculture Research*. 39, 329-332.

GALASINSKI, W., GADEK, A., RATKIEWICZ, A. and RZECZYCKI, W. 1978. A convenient modification of the method for hydroxyproline determination in proteins. *Analitical Biochemistry*. 85, 550-555.

GINÉS, R., VALDIMARSDOTTIR, T., SVEINSDOTTIR, K. and THORARENSEN, H. 2004. Effects of rearing temperature and strain on sensory characteristics, texture, colour and fat of Arctic charr (*Salvelinus alpinus*). *Food Qual. Prefer*. 15, 177-185.

GRAU, R. and HAMM, R. 1953. Eine einfache methode zur bestimmung der wasserbindung im muskel. *Naturwissenschafen*. 40, 29-30.

HATAE, K., TOBIMATSU, A., TAKEYAMA, M. and MATSUMOTO, J.J. 1986. Contribution of the connective tissues on the texture differences of various fish species. *Bull. Jap. Soc. Sci. Fish.* 52, 2001-2008.

HUFF-LONERGAN, E. and LONERGAN, S.M. 2005. Mechanisms of water-holding capacity of meat: the role of postmortem biochemical and structural changes. *Meat Science*. 71, 194-204.

HULTMANN, L. and RUSTAD, T. 2008. Textural changes during iced storage of salmon (*Salmo salar*) and cod (*Gadus morhua*). *J. Aquat. Food Prod. Technol*. 11(3-4), 105-123.

HURLING, R., RODELL, J.B. and HUNT, H.D. 1996. Research not: fiber diameter and fish texture. *Journal of Texture Studies*. 6, 679-685.

HYLDIG, G. and NIELSEN, D. 2001. A review of sensory and instrumental methods used to evaluate the texture of fish muscle. Journal of Texture Studies. 32, 219-242.

ISMEA, 2010. Check up ittico 2010.

JEZIERSKA, B., HAZEL, J.R. and GERKING, S.D. 1982. Lipid mobilization during starvation in the rainbow trout, *Salmo gairdneri* Richardson, with attention to fatty acids. *Journal of Fish Biology*. 21(6), 681–692.

JOHNSTON, I.A., ALDERSON, R., SANDHAM, C., DINGWALL, A., MITCHELL, D., SELKIRK, C., NICKELL, D., BAKER, R., ROBERTSON, B., WHYTE, D. and SPRINGATE, J. 2000. Muscle fibre density in relation to the colour and texture of smoked Atlantic salmon (*Salmo salar* L.). *Aquaculture.* 189, 335-349.

JOHNSTON, I.A. 2001. Genetic and environmental determinants of muscle growth patterns. *Fish Physiology*. Vol. 18. Elsevier Inc.

KAUSE, A., RITOLA, O., PAANANEN, T., ESKELINEN, U. and MÄNTYSAARI, E. 2003. Big and beautiful? Quantitative genetic parameters for appearance of large rainbow trout. *Journal of Fish Biology*. 62, 610-622.

KAUSE, A., RITOLA, O., PAANANEN, T., MÄNTYSAARI, E. and ESKELINEN, U. 2002. Coupling body weight and its compositions: a quantitative genetic analysis in rainbow trout. *Aquaculture.* 211, 65-79.

KAUSE, A., RITOLA, O. and PAANANEN, T. 2004. Breeding for improved appearance of large rainbow trout in two production environments. *Aquaculture Research*. 35, 924-930.

KIESSLING, A., PICKOVA, J., JOHANSSON, L., ÅSGÅRD, T., STOREBAKKEN, T. and KIESSLING, K.H. 2001. Changes in fatty acid composition in muscle and adipose tissue of farmed rainbow trout (*Oncorhynchus mykiss*) in relation to ration and age. *Food Chemistry*. 73, 271-284.

LARSEN, D., QUEK, S. and EYRES, L. 2011. Evaluating instrumental colour and texture of thermally treated New Zealand King Salmon (*Oncorhynchus tshawytscha*) and their relation to sensory properties. *LWT - Food Science and Technology*. 44, 1814-1820.

LI, X., BICKERDIKE, R., LINDSAY, E., CAMPBELL, P., NICKELL, D., DINGWALL, A. and JOHNSTON, I.A. 2005. Hydroxylysyl pyridinoline cross-link concentration affects the textural properties of fresh and smoked Atlantic salmon (*Salmo salar* L.) flesh. *Journal of Agriculture and Food Chemistry*. 53, 6844-6850.

MONTERO, P., BORDERIAS, J., TURNAY, J. and LEYZARBE, M.A. 1990. Characterization of hake (*Merluccius merluccius* L.) and trout (*Salmo

irideus Gibb) collagen. *Journal of Agriculture and Food Chemistry*. 38, 604-609.

MØRKØRE, T., VALLET, J.L., CARDINAL, M., GOMEZ-GUILLEN, M.C., MONTERO, P., TORRISSEN, O.J., NORTVEDT, R., SIGURGISLADOTTIR, S. and THOMASSEN, 2001. Fat content and fillet shape of Atlantic Salmon: relevance for processing yield and quality of raw and smoked products. *Journal of Food Science*. 66(9), 1348-1354.

MØRKØRE, T., HANSEN, A.Å., UNANDER, E. and EINEN, O. 2006a. Composition, liquid leakage, and mechanical properties of farmed rainbow trout: variation between fillet sections and the impact of ice and frozen storage. *Journal of Food Science*. 67(5), 1933-1938.

MØRKØRE, T., VALLET, J.L., CARDINAL, M., GOMEZ-GUILLEN, M.C., MONTERO, P., TORRISSEN, O.J., NORTVEDT, R., SIGURGISLADOTTIR, R. and THOMASSEN, M.S. 2006b. Fat content and fillet shape of Atlantic Salmon: relevance for processing yield and quality of raw and smoked products. *Journal of Food Science*. 66(9), 1348-1354.

MORRISON, W.R. and SMITH, L.M. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with born fluoride-methanol. *Journal of Lipid Research.* 5, 600-608.

NAES, T., BAARDSETH, P., HELGESEN, H. and ISAKSSON, T., 1996. Multivariate techniques in the analysis of meat quality. *Meat Science*. 43, S135-S149**.**

NETTLETON, J.A. and KANZ, R. 2005. N-3 long chain polyunsaturated fatty acids in type 2 diabetes: a review. *Journal of American Dietetics Association*. 105, 428-440.

OFSTAD, R., KIDMAN, S., MYKLEBUST, R. and HERMANSSON, A.-M. 1993.Liquid holding capacity and structural changes during heating of fish muscle: cod (*Gadus morhua* L.) and salmon (*Salmo salar*). *Food Structure*. 12, 163-174.

QUILLET, E., LE GUILLOU, S., AUBIN, J. and FAUCONNEAU, B. 2005. Two-way selection for muscle lipid content in pan-size rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 245, 49-61.

RASMUSSEN, R.S. 2001. Quality of farmed salmonids with emphasis on proximate composition, yield and sensory characteristics. *Aquaculture Research. 32*, 767-786.

RØRÅ, A.M.B., KVÅLE, A., MØRKØRE, T. RØRVIK, K., HALLBJOØRN, S. and THOMASSEN, S. 1998. Process yield, colour and sensory quality of smoked Atlantic salmon (*Salmo salar*) in relation to raw material characteristics. *Food Research International*. 31(8), 601-609.

RØRÅ, A.M.B., REGOST, C. and LAMPE, J. 2003. Liquid holding capacity, texture and fatty acids profile of smoked fillets of Atlantic salmon fed

diets containing fish oil or soybean. *Food Research International*. 36, 231-239.

SARGENT, J.R, TOCHER, D.R. and BELL, J.G. 2002. The Lipids. *Fish Nutrition*. Third Edition. Elsevier Science (USA).

SAS 2007. SAS/STAT User's guide. 9.1 version. SAS Institute Inc., Cary, NC, USA.

SHAHIDI, F. and MIRALIAKBARI, H. 2004. Omega-3 fatty acids in health and disease: Part 1 – cardiovasculare disease and cancer. *Journal of Medical Food*. 7, 387-401.

STEINE, G., ALFNES, F. and RØRA, M.B. 2005. The effect of color on consumer WTP for farmed salmon. *Mar. Resour. Econ*. 20, 211-219.

STOREBAKKEN, T. and KYOON NO, H. 1992. Pigmentation of rainbow trout. *Aquaculture*. 100, 209-229.

TOBIN, D., KAUSE, A., MÄNTYSAARI, E.A., MARTIN, S.A.M., HOULIHAN, D.F., DOBLY, A., KIESSLING, A., RUNGRUANGSAK-TORRISSEN, K., RITOLA, O. and RUOHONEN, K. 2006. Fat or lean? The quantitative genetic basis for selection strategies of muscle and body composition traits in breeding schemes of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 261, 510-521.

UNUSAN, N. 2007. Change in proximate, amino acid and fatty acid contents in muscle tissue of rainbow trout (*Oncorhynchus mykiss*) after cooking. *International Journal of Food Science and Technology.* 42, 1087-1093.

VALENTE, L.M.P., GOMES, E.F.S. and FAUCONNEAU, B. 1998. Biochemical growth characterization of fast and slow-growing rainbow trout strains: effect of cell proliferation and size. *Fish Physiol. Biochem*. 18, 213-224.

VALENTE, L.M.P., ROCHA, E., GOMES, E.F.S., SILVA, M.W., OLIVEIRA, M.H., MONTEIRO, R.A.F. and FAUCONNEAU, B. 1999. Growth dynamics of white and red muscles in fast and slow growing strains of rainbow trout. *Journal of Fish Biology*. 55, 675-691.

VELAND, J.O. and TORRISSEN, O.J. 1999. The texture of Atlantic salmon (*Salmo salar*) muscle as measured instrumentally using TPA and Warner-Bratzler shear test. *Journal of the Science of Food and Agriculture*. 79(12), 1737-1746.

YTRESTØYL, T., STRUKSNØS, G., RØRVIK, K.A., KOPPE, W. and BJERKENG, B. 2006. Astaxanthin digestibility as affected by ration levels for Atlantic salmon, *Salmo salar*. *Aquaculture*. 261, 215-224.

5. CONCLUSIONS

The results of the trials carried out on meagre and trout showed that the qualitative traits of each species depended on both intrinsic as genetic strain and extrinsic factors as sampling season, storage and rearing system. Cooking differently affected physical properties and nutrients retention depending on the variation in raw flesh quality of the studied species.

In particular textural properties, colorimetric attributes and water released from the muscle were found to be differently influenced by heating and changes mainly varied depending on the storage time and on the genetic origin.
Concerning the chemical composition, in both species cooking by boiling induced the loss of macronutrients and fatty acids to a different extent depending on the amount of the nutrients found in the flesh.
Concerning lipid, meagre which was a lean species and rainbow trout which showed a moderate-fat content registered different lipid content and fatty acids profile as affected by the diet. Nevertheless in both species, the recommended intake of the healthy long-chain PUFAs by consuming one portion of 100 g of fillet per day was always guaranteed after cooking.

Both species showed high valuable qualitative traits and good attitudes to industrial heat-processing.