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Quality and authenticity assessment of fish and caviar by fatty acid, stable isotope and flavour profiles

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CHAPTER 1

Foreword

1. Fishery and aquaculture production

Fisheries and aquaculture production supplied the world with about 143 million tons of food fish in 2006 (Table 1). Of this total, aquaculture accounted for 51 million of tons.

Around 220 species of aquatic animals and plants are currently cultured worldwide in a vaste range of production system.

Table 1. World fisheries and aquaculture production and utilization

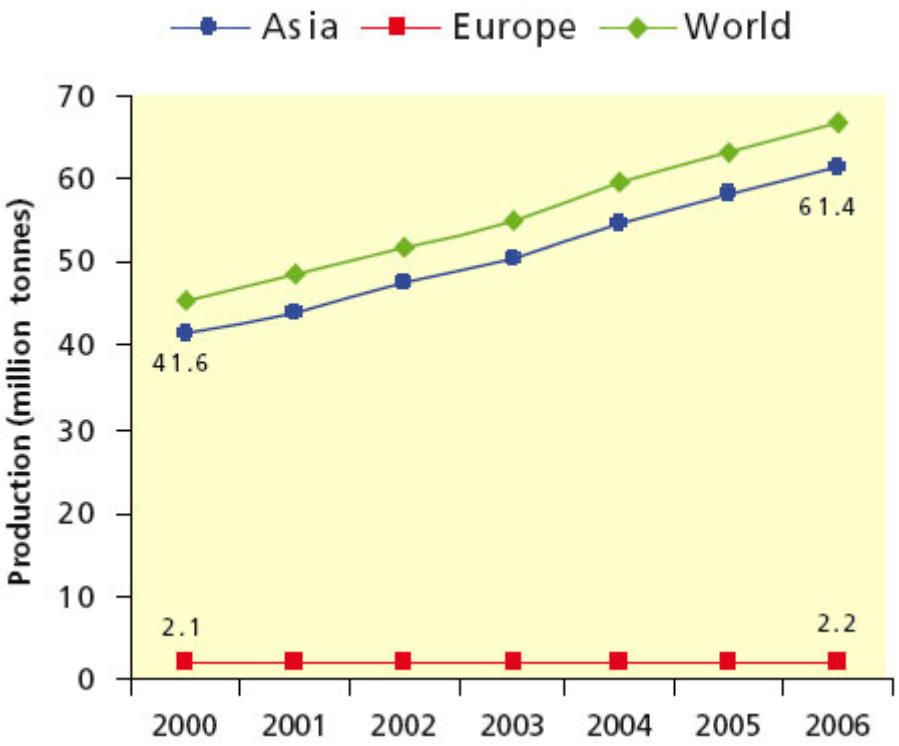
	2002	2003	2004	2005	2006
			Milion tons		
PRODUCTION					
INLAND					
Capture	8.7	9	8.9	9.7	10.1
Aquaculture	24	25.5	27.8	29.6	31.6
Total inland	32.7	34.5	36.7	39.3	41.7
MARINE					
Capture	84.5	81.5	85.7	84.5	81.9
Aquaculture	16.4	17.2	18.1	18.9	20.1
Total inland	100.9	98.7	103.8	103.4	102
TOTAL CAPTURE	93.2	90.5	94.6	94.2	92
TOTAL AQUACULTURE	40.4	42.7	45.9	48.5	51.7
TOATL WORLD FISHERIES	133.6	133.2	140.5	142.7	143.7
UTILIZATION					
Human consuption	100.7	103.4	104.5	107.1	110.4
Non-food uses	32.9	29.8	36	35.6	33.3
Population (billions)	6.3	6.4	6.4	6.5	6.6
Per capita food fish suplly (kg)	16	16.3	16.2	16.4	16.7

Note: excluding aquatic plants

Aquaculture continues to make a significant contribution to total fisheries production over the last few deades. This increasing contribution, however, is largely an Asian phenomenon because Asia accounted for 61.43 million tons or 92 percent of total world aquaculture production in 2006, while Europe contributed 2.20 million tons (Figure 1). In terms of value, the Asian region's share was 80 percent of total value of world aquaculture production. The Asian contribution is significantly influenced and skewed by China.

China infact remains by far the largest producer, with reported fisheries production of 51.5 million tons in 2006 (17.1 and 34.4 million tons from capture fisheries and aquaculture, respectively) (FAO, 2008).

Figure 1. Contributions from Asia and Europe to world aquaculture production



World capture fisheries production has been relatively stable in the past decade with the exception of marked fluctuations driven by catches of anchoveta in the Southeast Pacific, a species extremely susceptible to oceanographic conditions determined by the El Niño Southern Oscillation.

Asian countries accounted for 52 percent of the global capture production. Overall catches in the Western Central Pacific and in the Western Indian Ocean continued to increase, whereas capture production decreased in both the Western and Eastern Central areas of the Atlantic Ocean.

Catches from inland waters have shown a slowly but steadily increasing trend since 1950, owing in part to stock enhancement practices and possibly also to improved reporting. The small increase in production reflects lower production from island sources.

Aquaculture continues to be the fastest growing animal food-producing sector and to outpace population growth, with per capita supply from aquaculture increasing from 0.7 kg in 1970 to 7.8 kg in 2006, an average annual growth rate of 6.9 percent.

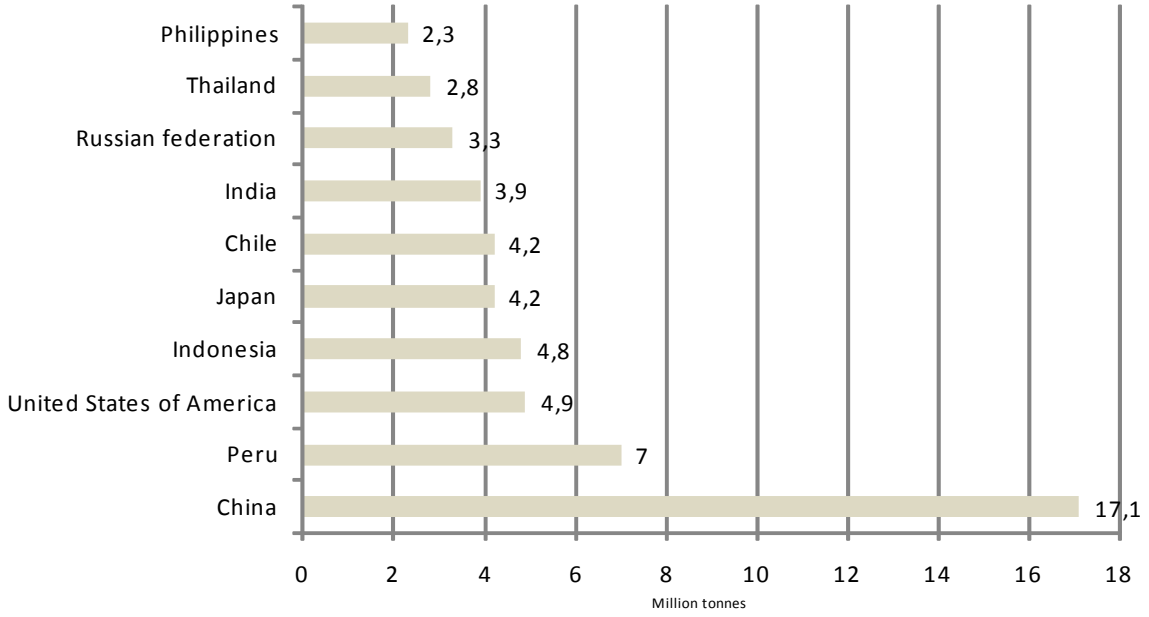
World aquaculture is heavily dominated by the Asia–Pacific region, which accounts for 89 percent of production in terms of quantity and 77 percent in terms of value. This dominance is mainly due to China's enormous production, which accounts for 67 percent of global production in terms of quantity and 49 percent of global value. China produces 77 percent of all carps (cyprinids) and 82 percent of the global supply of oysters (ostreids). The Asia–Pacific region accounts for 98 percent of carp, 95 percent of oyster production, and 88 percent of shrimps and prawns (penaeids). Norway and Chile are the world's two leading producers of cultured salmons (salmonids), accounting for 33 and 31 percent, respectively, of world production. Aquatic plant production by aquaculture in 2006 was 15.1 million tons.

The culture of aquatic plants has increased consistently, with an average annual growth rate of 8 percent since 1970. In 2006, it contributed 93 percent of the world's total supply of aquatic plants, some 72 percent of which was produced by China (FAO, 2008).

1.1. Total capture fisheries production

According to FAO, global capture production in 2006 was about 92 million tons. This represents a decrease of 2.2 million tons in comparison with 2005 (Table 1). This negative trend was mostly caused by fluctuations in anchoveta catches. While without considering anchoveta catches, total inland water chateches increased significantly in 2005 and 2006, total global marine capture production has remained fairly stable since 2002 at between 74.3 and 75.3 million tons.

Figure 2. Marine and inland capture fisheries: top ten producer countries in 2006

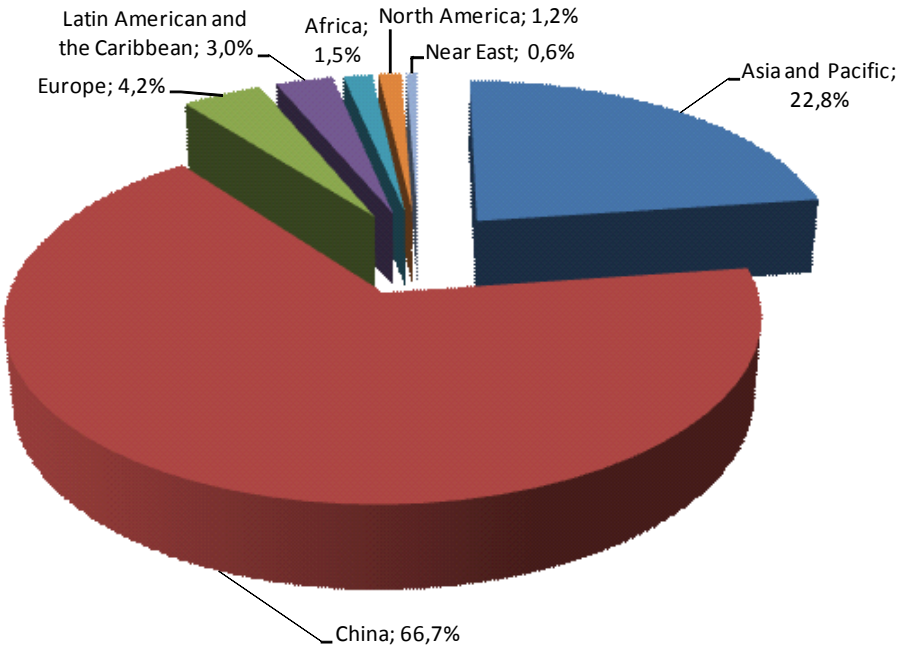


Excluding China, total capture production in 2007 increased by about 3 percent in comparison with 2006. However, China's capture production decreased by more than 2 million tons following the adjustment to the national data collection system.
China has remained by far the global leader with more than 17 million tons (Figure 2) and a very stable capture production, as the variation from one year to the next in its reported total catches was less than 1 percent in the period 1986–2006 (FAO, 2008).

1.2. Aquaculture production

The contribution of aquaculture to global supplies of fish, crustaceans, molluscs and other aquatic animals has continued to grow, increasing from 3.9 percent of total production by weight in 1970 to 36.0 percent in 2006. In the same period, production from aquaculture easily outpaced population growth, with per capita supply from aquaculture increasing from 0.7 kg in 1970 to 7.8 kg in 2006, an average annual growth rate of 7.0 percent. Aquaculture accounted for 47 percent of the world's fish food supply in 2006. In China, 90 percent of fish food production comes from aquaculture (FAO, 2008).
Moreover it is estimated that Asia contributed 88.5 percent of fish in terms of quantity and 71 percent in terms of value to total world fed aquaculture production. In contrast, Europe contributed 4.5 percent of fish in terms of quantity to total world fed aquaculture production.
In 2006, China contributed 67 percent of the world's supply of cultured aquatic animals and 72 percent of its supply of aquatic plants. World aquaculture has grown dramatically in the last 50 years. From a production of less than 1 million tons in the early 1950s, production in 2006 was reported to have risen to 51.7 million tons. This means that aquaculture continues to grow more rapidly than other animal food-producing sectors.
If aquatic plants are included, world aquaculture production in 2006 was 66.7 million tons. In 2006, countries in the Asia and the Pacific regions accounted for 89 percent of production by quantity. Among the world production, China is reported to produce 67 percent of the total quantity and 49 percent of the total value of aquaculture production (Figure 3).

Figure 3. Aquaculture production by region in 2006



Note: excluding aquatic plants

An analysis of production by regions for the period 1970–2006 shows that growth has not been uniform. The Latin America and the Caribbean region show the highest average annual growth (22.0 percent), followed by the Near East region (20.0 percent) and the Africa region (12.7 percent). China's aquaculture production increased at an average annual rate of 11.2 percent in the same period. Recently, China's growth rate has declined to 5.8 percent from 17.3 percent in the 1980s and 14.3 percent in the 1990s. Similarly, production growth in Europe and North America has slowed substantially to about 1 percent per year since 2000. In France and Japan, countries that used to lead aquaculture development, production has fallen in the last decade. It is apparent that, while aquaculture output will continue to grow, the rate of increase may be moderate in the near future.

Table 2 reports the top ten producing countries for cultured aquatic animals in 2006, as well as the top ten countries in terms of annual growth in aquaculture production for the two-years period 2004-06.

Table 2. Top ten aquaculture producers of food fish supply: quantity and growth

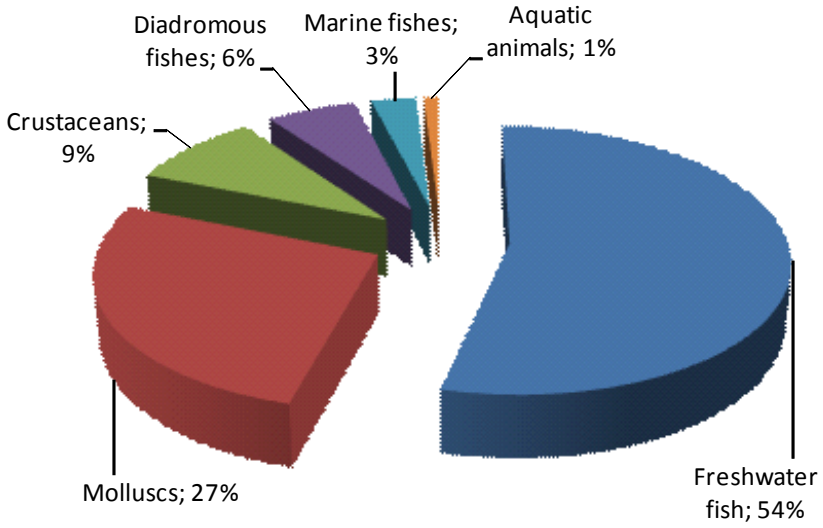
	top ten producers in terms of quantity, 2006			top ten producers in terms of growth, 2006	
	2004	2006		2004	2006
	tons			*tons*	
China	30.614.968	34.429.122	Uganda	5.539	32.392
India	2.794.636	3.123.135	Guatemala	4.908	16.293
Vietnam	1.198.617	1.657.727	Monzabique	446	1.174
Thailand	1.259.983	1.385.801	Malawi	733	1.500
Indonesia	1.045.051	1.292.899	Togo	1.525	3.020
Bangladesh	914.575	892.049	Nigeria	43.950	84.578
Chile	665.421	802.410	Cambodia	20.675	34.200
Japan	776.421	733.891	Pakistan	76.653	121.825
Norway	636.802	708.780	Singapore	5.406	8.573
Philippines	512.220	623.368	Mexico	104.354	158.642

Note: excluding aquatic plants

Most aquaculture production of fish, crustaceans and molluscs continues to come from inland waters (61 percent by quantity). The freshwater environment contributes 58 percent by quantity and the marine environment contributes 34 percent of aquaculture production. While much marine production is high-value finfish, production in this environment also consists of a large amount of relatively low-priced mussels and oysters. Brackish-water production represented only 8 percent of production in 2006 but shows the highest growth in terms of quantity since 2000 (11.6 percent per year). In the same period, the average annual increases in aquatic products from the freshwater and marine water environments have been 6.5 and 5.4 percent in terms of quantity.

In 2006, more than half of global aquaculture production was freshwater finfish. In the same year, molluscs accounted for the second-largest share, 14.1 million tons (27 percent of total production) (Figure 4).

Figure 4. World aquaculture production: major species groups in 2006



The growth in production of the major species groups continues, although the increases seen in the past decade have been smaller than those of the 1980s and 1990s. The period 2000–2006 witnessed strong growth in the production of crustaceans in particular, and in marine fish.

Aquaculture now accounts for 76 percent of global freshwater finfish production and 65 percent of mollusc and diadromous fish production. Its contribution to world supplies of crustaceans has grown rapidly in the last decade, reaching 42 percent of world production in 2006 and, in the same year, it accounted for as much as 70 percent of shrimps and prawns produced worldwide. Most cultured marine species are of relatively high commercial value, sometimes because wild stocks are small or declining. While the overall share of farmed fish in marine finfish production has stayed quite low, for the species that are farmed, aquaculture frequently dominates the market. This is the case for species such as gilthead seabream and seabass. The amounts produced by aquaculture of this species are often substantially higher than in the past higest catch recorded by capture fisheries.

The trend regarding species aquaculture production depends on regions. In the Asia and the Pacific region, aquaculture production from China, South Asia and most of Southeast Asia consists primarily of cyprinids, while production from the rest of East Asia consists of high-value marine fish. In Latin America and the Caribbean, salmonids are now the principal aquaculture species group as a result of the rapid growth in salmon production in Chile. Channel catfish is the top aquaculture species in the United States of America, while Atlantic and Pacific salmon dominate in Canada.

However, in global terms, a few countries still dominate production of major species groups. China produces 77 percent of all carp (cyprinids) and 82 percent of the global supply of oysters (ostreids). The Asia and Pacific region accounts for 98 percent of carp and 95 percent of oyster production and 88 percent Eighty-eight percent of shrimps and prawns also come from this region. Norway and Chile are the world's leading producers of cultured salmons (salmonids), accounting for 33 and 31 percent of world production, respectively. Other European producers supply another 19 percent.
Aquaculture production in Europe contributes 4.5 % of fish in terms of quantity. The products of European aquaculture are diverse and cover many individual species. The most important contributions are the salmonid fish (salmon and trout), demersal marine fish (seabass and seabream), carps (common carp) and mollusks (mussels and oysters) (FAO, 2008).

1.3. Characteristics of aquaculture products

Aquaculture products offer different advantages comparing with fishery products. First of all, aquaculture products can be supplied year round, in contrast most wild-caught seafood is characterized by seasonal fluctuations related to weather and fishing regulations.
Moreover production techniques also allow the aquaculture grower to produce a consistent product. Consistency in supply refers to size, quality and other product characteristics in addition to consistency in the quality supplied. The reliability and the regularity in supply of farmed product should be enable producers to negotiate better price (Asche, 2001). Supply chains of captured fisheries products are more fixed due to the seasonality of supply and cannot respond readily to changes in retail demand.
Another advantage is correlated to the traceability of farmed product than wild-caught product back to its original source. The complexity of market channels for wild-caught product may obscure steps in the supply chain and make tracing products to their source difficult (Asche, 2001).
Fish and other aquaculture production allows to supply fish of a given size and quality grade, in contrast with the uncertainty of what species, size and, to some extent, quality of fish will be caught.
A major disadvantage of aquaculture products when comparing to wild-caught seafood is the price. Costs of production are frequently higher for aquaculture production than for wild-caught seafood. However, as wild fish stocks have declined and boats have had to travel farther on fewer fishing days, costs of capture fisheries have increased. At the same time, research and development have reduced costs of producing a number of aquaculture species. The costs of a number of farmed species are now more competitive than before.

1.4. Market competition between wild-caught and farmed finfish

Fish farming, such as salmon or rainbow trout, has several market advantages over the commercial hunting of wild animals. From price declines in the world market since the advent of farming, the farming process can be more closely than the fishing process to market demands. This include, for example, the ability to control and thus predict supply. Moreover product advantages are features of the aquaculture products produced by the farming process that make it more desirable to the market. For example, a more aesthetically pleasing fish will have a market advantage over a fish that is less pleasing to the eye.
The fisheries production is variable and uncertain. Moreover fishermen are often limited to catch fish in some periods of the year. On the contrary, fish farmers have a greater control over the timing, consistency and quantity of production than do fishermen (Eagle et al., 2003).

1.5. Fish utilization

In 2006, more than 110 million tons (77 percent) of world fish production was used for direct human consumption. Almost all of the remaining 33 million tons was destined for non-food products, in particular the manufacture of fishmeal and fish oil. If China is excluded, the quantities were 72 million tons and 20 million tons, respectively. In China, aquatic products are traditionally most commonly distributed to the domestic market in live and fresh form. However, in recent years, processing has seen significant growth. China processes not only domestic production but also imported fish into an array of fish products, including salted, dried, smoked and various preserved fish products for both domestic and export markets. The Chinese reprocessing industry is labour-intensive and traditionally works on low margins, which have recently tended to narrow further with escalating costs for raw materials (FAO, 2008).
In 2006, 48.5 percent of the fish destined for human consumption was in live and fresh form, which is often the most preferred and highly priced product form. Fiftyfour percent of the world's fish production underwent some form of processing. Seventy-four percent of this processed fish was used for manufacturing products for direct human consumption in frozen, cured and prepared or preserved form, and the rest for non-food uses. Freezing is the main method of processing fish for food use, accounting for 50 percent of total processed fish for human consumption in 2006, followed by prepared and preserved (29 percent) and cured fish (21 percent). Fish is one of the most versatile food commodities and can be utilized in a great variety of ways and product forms. It is generally distributed as either live, fresh, chilled, frozen, heat-treated, fermented, dried, smoked, salted, pickled, boiled, fried, freeze-dried, minced, powdered or canned, or as a combination of two or more of these

forms. However, fish can also be preserved by many other methods. In some parts of Southeast Asia, and particularly in China, the trade is not formally regulated but based on tradition. However, in markets such as the EU, the trade in live fish has to comply with requirements *inter alia* concerning animal welfare during transportation (FAO, 2008).

In many developing countries with tropical ambient temperatures, quality deterioration and significant post-harvest losses occur because of inadequate use of ice, long supply chains, poor access to roads and electricity, and inadequate infrastructure and services in physical markets. Market infrastructure and facilities are often limited and congested, increasing the difficulty of marketing perishable goods. Owing to these deficiencies, together with well-established consumer habits, fish production is utilized in such countries mainly in live/fresh form (representing 60.1 percent of fish destined for human consumption in 2006) or processed by smoking or fermentation. However, in the last few years, there has been a slight increase in the share of frozen products in developing countries (19 percent in 2006, up 7.3 percent since 1996), with a more significant rise in prepared or preserved forms (11.1 percent in 2006, up 41 percent since 1996). In developed countries, the bulk of fish used for human consumption is in frozen and prepared or preserved forms. Freezing is still prominent as the primary form of production, with a proportion that has been constantly increasing, and it accounted for 42 percent of total production in 2006. Processors of traditional products, in particular of canned products, have been losing market shares to suppliers of fresh and frozen products as a result of long-term shifts in consumer preferences. The utilization and processing of fish production have diversified significantly in the last two decades, particularly into high-value fresh and processed products, fuelled by changing consumer tastes and advances in technology, packaging, logistics and transport. These changes include improvements in storage and processing capacity, together with major innovations in refrigeration, ice-making, and food-packaging and fish-processing equipment. Vessels incorporating these improved facilities and able to stay at sea for extended periods have been built. This has permitted the distribution of more fish in live or fresh form. Moreover, improved processing technology enables higher yields and results in a more lucrative product from the available raw material. In developed countries, value-added innovation is mainly focused on increased convenience foods and a wider variety of high value-added products, mainly in fresh, frozen, breaded, smoked or canned form. These necessitate sophisticated production equipment and methods and, hence, access to capital. The resulting fish products are commercialized as ready and/or portion-controlled, uniform-quality meals.

Fish plays an important role not only in terms of its use for direct human consumption but also in the production of animal feeds, particularly fishmeal.

About one-quarter of world fish production is destined for non-food products, with the bulk being converted into fishmeal and fish oil. The remainder, mainly consisting of low value fish, is largely utilized as direct feed in aquaculture and livestock. In 2006, the quantity of fish used as raw material for fishmeal was about 20.2 million tons, down 14 percent on 2005 and still well below the peak levels of more than 30 million tons recorded in 1994. The decrease in fishmeal production in the past decade has been irregular, its considerable fluctuations mainly reflecting annual variations in catches of small pelagics, especially anchoveta.

Another emerging application of fish, crustaceans and other marine organisms is as a source of bioactive molecules for the pharmaceutical industry. Chitin from shrimp and crab shells is already being used in the pharmaceutical industry. Chitin and chitosan have wide-ranging applications in many areas such as water treatment, cosmetics and toiletries, food and beverages, agrochemicals and pharmaceuticals. Japan is the largest market for chitin-derived products. Biomedical products from wastes derived from the fish-processing industry (e.g. skin, bones and fins) are attracting considerable attention from industry. Fish skin as a source of gelatine has attracted interest after bovine spongiform encephalopathy (BSE) and some religious requirements prompted a search for alternatives to mammalian sources of gelatine. It is estimated that about 2 500 tons of fish gelatine was produced in 2006 (FAO, 2008).

2. Feed ingredients in fish farming

Feeds for fish in intensive aquaculture are formulated to be nutrient- and energy-dense, and many are based on ingredients of marine origins. The nutritive requirements of fish offer less flexibility in diet formulation than do those of most land animals. First of all, several species of fish selected for culture are almost pure carnivores, requiring a diet of high protein content. These fish have very poor utilization of carbohydrate as an energy source. For this reason, the ingredients chosen for feed manufacture are generally protein- and energy-rich.
A wide range of raw materials can be used for the manufacture of feeds for aquatic animals (Cho et al., 1982; Tacon and Jackson, 1985; Hardy, 1989; Pigott and Tucker, 1989; Macrae et al., 1993; Cho et al., 1994; Jobling, 1994; Pond et al., 1995; Hardy, 1996; Jobling, 1998).
Fish meal is the major component of aquaculture. In most aquaculture feeds, fish meals are the main source of protein and may constitute up to 60% of the total diet. But faced with the food supply problem for cultured fish, nutritionists have done more work evaluating alternate protein sources in aquaculture diets during the last years. However, their use in aquaculture feed formulation can substitute fish meal only partially.

2.1. Protein supplements

Protein supplements are feed ingredients having a protein content above 20%. There are three general groups: the first group is made up of ingredients having a protein content of 20-30% which contain materials of plant origin that are by-products of the brewing and distilling industries, wheat germ meal and corn gluten feed. The second group is composed of ingredients havening a protein content of 30-50% and includes the oilseed meals, crab meal and dried milk products. The third group contains ingredients of over 50% protein and includes fish meals, blood meal, meat and bone meal, shrimp, poultry by-product meal, soy protein concentrate, wheat gluten and corn gluten meal (Hardy and Barrows, 2002).

2.1.1. Animal by-products

Animal by-products are derived from the meat-packing, poultry processing and rendering industries. The protein content of these products after drying ranges from 50 to over 85%. The essential amino acid composition of animal by-products meal is similar to that of whole-egg protein. These meals are good sources of lysine but poor sources of methionine and cystine, which are usually limited in diet formulation (Hardy and Barrows, 2002)

2.1.1.1. Meat meal and meat and bone meal

These products are dried mammalian tissues, exclusive of hair, hooves, horn and stomach content. The protein content of meal is about 51% while that of meat and bone is closer to 50%. Fat levels in these products average 9.1-9.7%. The principal difference between the two products is the phosphorus level, with meat meal having less than 4.4% phosphorus. The calcium content of meat and bone meal ranges from 8.8 to 12%, while meat meal generally contains about 3% less calcium.

2.1.1.2. Blood meal

Blood meal is a dry product made from clean, fresh animal blood. The most common blood meal is produced by spray-drying after an initial low-temperature vacuum evaporation has reduced the moisture content to about 70%. Other processes of drying blood include flash-drying and conventional drying in a cooker.

Blood meal has a minimum protein content of 85% and a lysine content of 9-11% with a lysine availability of over 80%.

2.1.1.3. Poultry by-product meal

Poultry by-product meal is made from waste generated from poultry processing plants. The material remaining after chickens are dressed, rendered and dried. Regular poultry by-product meal contains about 58% protein and 13% fat.

2.1.1.4. Milk by-products

Several milk by-products are useful in the formulation of fish feeds, including dried whey, dried whey product, casein and dried skim milk. Dried whey product is the residue obtained when a portion of the lactose has been removed, while dried whey contains a minimum of 65% lactose. The protein content of whey products is relatively low (13-17%), yet these products are classified as protein supplements.

Dried skim milk is sometimes used in the starter diets, due primarily to its excellent digestibility and amino acid balance. Its protein content is about 34%.

Casein is the residue obtained by acid coagulation of defatted milk. It contains at least 80% protein (Hardy and Barrows, 2002).

2.1.2. Fish products and by-products

Fish meal is the most important protein ingredients used in diets of fish. It shows an essential amino acid profile that seems to meet the requirements of most teleost species, and nutrient bioavailability is also high. This important feed ingredient contains a high percentage of protein (usually 60-75%), an appreciable amount of mineral ash (10-20%), and a proportion of lipid (5-10%). Most fish

meal is produced from small, pelagic fish species such as sardines, anchovies, capelin, herring and menhaden.
A small percentage of fishmeal is rendered from the by-catch of other fisheries and by-products or trimmings created during processing (e.g. fish filleting) of various seafood products destined for direct human consumption.
Approximately 4 to 5 tons of whole fish are required to produce 1 ton of dry fishmeal. Perù produces almost one-third of the total world fishmeal supply. Olther fishmeal producing countries are Chile, cina, Thailand, U.S.A., Iceland, Norway, Denmark and Japan.
There are several processing methods to produce good quality fishmeal but the basic principles involve separation of solids from the oil.
Processing conditions influence the quality of fish meal, with high processing temperatures (100-150°C) giving meals of lower digestibility than milder conditions (60-70°C) (Asknes and Mundhein, 1997). The differences may arise because of the formation of enzyme-resistant disulphide bridges, or other cross-linkages, between protein chains, the oxidation of amino acids such as methionine and tryptophan or the reaction of lipid oxidation products with amino acids (Macrae et al., 1993).
Thus the best quality meals are produced by processing fresh whole fish under low-temperature conditions, and the quality of the meals produced decreases if stale fish or higher cooking and drying temperatures are used (Pike et al., 1990; Anderson et al., 1993; Jobling, 1994; Hardy, 1996; Asknes and Mundhein, 1997; Jobling, 1998). The meals produced from filleting wastes and other fish processing by-products are considered to be of poor quality due to their relatively high ash, and low protein content.
Addition of fishmeal to fish diets increases feed efficiency and growth through better food palatability and enhances nutrient uptake, digestion and absorption. Fishmeal of high quality provides a balanced amount of all essential fatty acids, phospholipids and fatty acids for optimum development, growth and reproduction especially of larvae and broodstock.
Some promising results have been obtained using acid-preserved fish silage as a protein source, but both beneficial and negative effects have been reported when silages containing free amino acids and partially hydrolysed proteins have been used in fish feeds (Tacon and Jackson, 1985; Hardy, 1996). The silages (which are liquid) can be used as is, and may be used to produce a protein concentrate or even co-dried with other protein ingredients to produce a meal (Hardy, 1996). Fish silages are usually prepared by exploiting lactic acid bacterial fermentation but ensiling process can also involve formic, hydrochloric or sulphuric acid preservation (Arason, 1994; Kristinsson and Rasco, 2000). Use of fish silages prepared using organic or inorganic acids may be associated with a reduction of feed intake in salmonids due to the low pH imparted to the feed by the acids

(Rungruangsak and Utne, 1981). There may be beneficial effects when silage concentrates are used to replace part of the fish meal in feeds for salmonids (Espe et al., 1999). Higher inclusion levels appear however to be less efficacious. There are some additional problems with fish silage, because any deficiencies in ensiling technique may result in the development of bacteria, tryptophan degradation and/or lipid oxidation, all of which would limit the value of using this raw material for the production of fish feeds.
Fish protein concentrates (FCPs) are usually obtained by enzymatic hydrolysis or chemical methods. FCPs can be prepared by relying on autolytic hydrolysis but hydrolysis via the addition of exogenous enzymes is considered to be the best method because it allows good control over the hydrolysis, and thereby the properties of the resulting product (Kristinsson and Rasco, 2000). FCPs are generally of high biological value, and may also contain free amino acids and peptides that act as feeding attractants, stimulants or flavour enhancers and potentiators.

2.1.3. Plant protein sources

Faced with the food supply problem for cultured fish, nutritionists have done more work evaluating alternate protein sources in aquaculture diets during the last years.
The quality of different feedstuffs is greatly dependant on the amino acids profile in their proteins, digestibility of the proteins, freshness of the raw materials and their storage. Plant-based proteins, even when properly processed, are usually not as digestible as fishmeal and their inclusion rate is often limited as it results in depressed growth rates and feed intake. Protein digestibility values for fishmeal are consistently above 95%. In comparison, protein digestibility for many plant-based proteins varies greatly, for example from 77% to 96% depending on the species of plant (Miles and Chapman, 2006).
In addition, they are characterized by a high amounts of carbohydrates, fibre, and other organic molecules such as glucosides, phytates, and cyclopropenes.
The most important protein sources of plant origin are the oilseed meals, prepared from the material remaining after oil has been extracted from soybeans, cottonseed, rape/canola, peanuts, sunflower and safflower seeds (Tacon and Jackson, 1985; Hardy, 1996). It is also usual to include maize (corn) or cereal meals in feed formulations, but these are included as a cheap energy source and for binding properties provided by the starch they contain rather than for their protein content.
Although ingredients of plant origin are being increasingly used in fish feeds, the total replacement of fish meal by plant protein sources has rarely been successful, the high inclusion levels of plant protein sources usually resulting in reduced growth and less efficient feed utilization (De la Higuera et al., 1988;

Robaina et al., 1995; Nengas et al., 1996; Burueu et al., 1998; Burel et al., 1998). The poorer growth commonly observed in fish fed feeds containing high properties of plant proteins may be related to a decrease in feed intake resulting from reduced feed palatability (Reigh and Ellis, 1992; Gomes et al., 1995; Burueu et al., 1998; Refstie et al., 1998; Arndt et al., 1999). However, the possible influences of an essential amino acid imbalance (Médale et al., 1998), low phosphorus availability and the metabolic effects of antinutritional factors (ANFs) can not be ignored (Alarcòn et al., 1999).

Soybean and other legume meals are a good source of lysine and tryphtophan but are limiting in the sulfur-containing of aminoacids as methionine and cystine. Antinutritional factors (ANFs) are compounds that interfere with nutrition digestion, uptake or metabolism and can also be toxic. Soybean meal, for example, contains several ANFs. Some of them, such as the trypsin and chymotrypsin inhibitors, are partially destroyed or inactivated by heating and drying, but others are less affected by the normal processing procedures used in meal production. This incomplete destruction of the AFNs may reduce the potential for using conventional soybean meal in feed formulation and much effort has been expended in devising in processing techniques for improving the nutritional value. Modern processing techniques may employ a range of chemical, enzymatic and physical treatments (Phillips, 1989; Anderson and Wolf, 1995). Once the trypsin inhibitors and allergens have been removed, it seems that the nutritional value of soybean meal is similar to that of high-quality fish meal (Médale et al., 1998).

Moreover, rape/canola and lupin are other plant protein sources. Both plants grow well in cooler climates than soybeans and they are cultivated in Europe and North America for inclusion in animal feeds. Lupin seeds have a high protein content (ca. 35%), although they are deficient in methionine. Unlike soybeans, lupins do not contain high concentrations of many of the ANFs but they contain quinolizidine alkaloids, which make the seeds bitter and potentially toxic even if lupin cultivars with a low alkaloid content have been developed (Burel et al., 1998). Meals prepared from older varieties of rape had limited use in animal feeds due to their content of ANFs. However, now rape cultivars with low levels of ANFs are available. Canola is low in glucosinolates and erucic acid but canola meals have quite high fibre content and also contain stachyose, tannins, phytate and raffinose. Feeds containing low-glucosinolates meals may, nevertheless, have a negative impact on growth such meals have been reported to affect thyroid function in juvenile rainbow trout and the content of other ANFs may have resulted in reduced digestibility and poorer nutrient utilization. Protein concentrates have been prepared from canola to reduce the levels of fibre and other undesirable compounds in the finished products. These concentrates

comprise 60-65% protein and have amino acid profile that resembles that of fish meal.

2.1.4. Other protein sources

Another category of protein supplements is sometimes referred to as unconventional feedstuffs. These products have not reached the level of availability or acceptance that allows them to used routinely in fish feed formulation. They include single-cell proteins derived from yeast or bacteria grown on waste material such as paper mill sludge, sewage, crop, processing wastes. The microorganisms are then harvested, purified, and dried into a product suitable for animal feeding (Hardy and Barrows, 2002). Some concern has been raised about the digestibility of yeast products by salmonids and about the possibility to uptake of heavy metals and other undesirable compounds by single cells organism grown on industrial waste.

Krill, *Euphausia superba* and *E. pacifica*, is a major marine biomass that is increasingly being harvested and processed into supplements for fish feeds. Krill meal is 33-55% protein, 15-20% fat, and 15-28% ash. Besides having a well-balanced amino acid profile, it contains the carotenoid pigment, astaxanthin, which is the natural pigment that colors the flesh of salmonids and the skin of many species of fish. In feeding trials, fish fed diets containing krill meals have grown nearly as well as those fed conventional diets (Gulbrandsen 1979; Hilge 1979; Ibraham et al., 1984).

2.2. Energy source

2.2.1. Carbohydrates (starch)

The upper limit for protein content of basal feeds is 20%, although are in the 10-17%. Grains are generally 68-72% starch and about 10-12% protein. The digestibility of the carbohydrates in grains is highly variable among fish. Carnivorous species, such as salmonids, derive very little energy from unprocessed plant starch. Omnivorous species, such as catfish, and herbivorous species, such as some carp, derive a high amount of energy from grain starch.

Numerous by-products grain processing are used in animal feeds and are also classified as basal feeds. After the nutrient requirements have been met, basal feeds can be used to fill out the energy needs in animal feed formulation. Basal feeds also have excellent binding properties and can help to hold feed pellets together in both dry and semimoist diets. They are relatively indigestible, and this quality is useful in animal production when a reduced rate of growth at a normal feeding level is desired (Hardy and Barrows, 2002).

2.2.2. Fats and oils

The main factor determining which lipid source to use in fish feed formulations are the fatty acid composition of the lipid source and its physical characteristics at ambient temperature.
The principal lipid source used in the formulation of aquaculture feeds is fish oil, which is produced from small marine pelagic fish and represents finite fishery resources. Other than providing a source of energy and essential fatty acids, it is commonly used to coat extruded feed pellets to improve the palatability and appearance of the feed. Aquafeeds currently use about 70% of the global supply of fish oil and it is possible that this percentage will increase reaching about 97% of the world supply in the next years. At present, global fish oil production has reached a plateau and is not expected to increase beyond current levels. For this reason, alternate lipid sources in aquaculture diets are used for the feed aquaculture formulation, with the same consideration reported for the substitution of fishmeal with plant protein sources.
A variety of commercially available fats and oils is suitable as ingredients for fish diets. For example, Salmonid diets normally include fish oils, such as herring, pollock, menhaden, anchovy and capelin. Plant oil, such as soybean, corn and cottonseed, and animal fat such as lard and poultry fat, may also be used as long as the diet contains sufficient levels of essential fatty acids (Hardy and Barrows, 2002).

2.3. Other nutritive ingredients

Protein supplements and basal feeds contain all of the nutrient needed by fish but not at levels sufficient to meet their total nutrients requirements. Additional vitamin and mineral premixes are added to feeds to ensure adequate intake of these nutrients.
Vitamin premixes are concentrates in which stable forms of essential vitamins are mixed with a carrier, usually a basal feed such as a wheat by-product. They are added to practical diets at levels ranging from 0.5 to 4% of the diet, depending on the formulation of the vitamin premix.
Mineral premixes are concentrates of essential elements that are fortified in practical fish diets to make up for low levels in the formulation or to overcome antagonistic interactions among feed ingredients. Additional mineral fortification is required in semipurified, experimental diet since their ingredients are highly refined and do not contain sufficient amounts of minerals to meet the nutritional requirements of fish (Hardy and Barrows, 2002).

3. Chemical composition of farmed fish

The consumer's acceptance of fishery products depends on several attributes of food quality. Some important attributes of food are chemical composition, safety, flavour, color, texture, etc. The fish farmer has some control on physiological factors (growth rate), environmental factors (water T°) and dietary factors such as the presence or absence of specific components. These kinds of controls make possible to manipulate the composition of farmed fish, while it is not possible to control the composition of wild fish. Among factors that affect the fish composition, diet seems to be the most important, regarding the content in protein, lipids, carbohydrate and ash content of the diet and the proportion of nutrient to dietary energy (Haard, 1992).

3.1 The protein content

The protein content of fish is endogenously regulated and pre-determined by genetic characteristics of the species or strain (Shearer, 1994). Furthermore, when expressed as a percentage of the crude protein, the amino acid profile of fish muscle is very similar both amongst fish from the same genus and between genera (Wilson and Cowey, 1985; Cowey, 1993).

It seems that whole body protein content of fish was not easily manipulated amongst fed fish (Shearer, 1994). In fact whole body protein level was unaffected by changes in major nutrient composition in diets fed to Mozambique tilapia (*Oreochromis mossambicus*) (El-Dahhar and Lovell, 1995), hybrid tilapia (*O. niloticus x O. aureus*) (Chou and Shiau, 1996), dentex (*Dentex dentex*) (Tibaldi et al., 1996), and gilthead seabream (*Sparus aurata*) (Company et al., 1999).

The source of the protein appears to have little effects on body protein content. Smith et al. (1988) fed isonitrogenous and isoenergetic diet containing plant or animal protein to rainbow trout and reported no differences in carcass composition, dressing-out percentage or organoleptic properties attributed to diet. Similar results were reported by Kaushik et al. (1995) for rainbow trout fed soy protein. High levels of protein levels of protein may suppress growth.

The amino acid profile of the whole body or the muscle appears to be relatively unaffected by the amino acid content of the diet (Schwarz and Kirchgessner, 1988), although the free amino acid pool can be influenced.

3.2. The ash content

The level of dietary ash appears to have no effects on the ash content of the fish (Shearer, 1994). In fact, the whole body ash level in fish is endogenously regulated and therefore can be little modified by the farmer or the diet. However, whole body ash levels can decline if certain essential elements are deficient in the diet (Schwarz, 1995; Eya and Lovell, 1997). An imbalance of the mineral content of the diet may result in decreased availability of essential minerals from feed. This is exemplified by reduced manganese availability to Atlantic salmon parr fed diets containing relatively modest iron supplements (Andersen et al., 1996). It was generally considered that, provided sufficient minerals in the correct proportions, the dietary ash content does not directly influence the ash content of farmed fish (Shearer, 1994). However, Shearer et al. (1992) indicated that excess ash in diets for Atlantic salmon parr in addition to reducing zinc availability also reduced the availability of energy from the diet with adverse effects on parr growth and feed conversion ratio (FCR). This lower energy availability could therefore result in lower levels of whole body fat.

3.3. The carbohydrate content

It is recognized that fish have no qualitative requirement for dietary carbohydrate (Wilson, 1994) and that, in its absence they can match physiological requirements by gluconeogenesis using amino acid skeletons and selected citric acid cycle intermediates as substrates. However, the carbohydrates still represent the cheapest form of non-protein energy and provide the starch necessary for gelatinization during the feed manufacturing process. The carbohydrate content of fish is very low, generally accounting for less than 1% whole body weight. Typically, the carbohydrates are present as monosaccharides, glycogen or metabolic intermediates.

In general changes in the lipid or protein content of the diet do not directly affect the carbohydrate content of farmed fish. The ability of fish to utilize carbohydrate varies on a species by species basis according to the complexity and quantity of the starch supplied. Cold water and marine fish are believed to have a poor capacity while warm water species have a high capacity for carbohydrate utilization (Wilson, 1994).

3.4. The moisture content

The moisture content of fish is easily modified by changes in the diet. Generally, the moisture content declines in response to increased fat in the whole body, fillets and cutlets (Jobling et al., 1998; Koskela et al., 1998).

3.5. The fat content

Energy intake appears to influence whole lipid stores. Increasing dietary energy by increasing dietary lipid improves growth by sparing protein effect and increases lipid storage (Shearer, 1994).
Rainbow trout fed isonitrogenous diets showed increased lipid deposition with increasing dietary lipid (Tackeuchi et al., 1978; Davies, 1989). In these studies, protein and ash were affected and percentage of moisture was inversely related to the percentage of whole body lipid.
When fish are fed isonitrogenous diets containing equal amounts of energy supplied by lipid or carbohydrate, lipid levels do not differ. Juvenile catfish fed diets containing 6 to 14% lipid or equivalent amounts of energy from corn starch did not differ significantly in percent whole body lipid (Gatlin and Stickney, 1982), similar results were reported by Garling and Wilson (1977) who fed catfish varying levels of lipid (1 to 15%) and carbohydrate (0 to 32%). In this study, however, fish fed more than 28% carbohydrate showed no further increase in lipid deposition, presumably due to the reduced digestibility of this high level of dietary carbohydrate (Spannhof and Plantinkow, 1983).

3.5.1 Fat distribution

The fat content of fish is not uniformly distributed throughout the body but there are regions of the fish which may have very high or low fat content. regarding Atlantic salmon, several studies have focused on the effects of diet on fat localization and on differences in fat content in different regions of the fillet. In general, the muscle tissue at the posterior end of the fish is lean and the fish becomes fatter when moving in the caudal-cranial direction. Very high levels of fat are found in the tissues surrounding the viscera and in the belly flap region (Morris, 2001).

3.5.2 Effects of protein, lipid, carbohydrate and energy on fish fat content

Shearer (1994) emphasized the importance of digestible energy in determining the fat content of fish. Fat can accumulate in fish not only as a consequence of the consumption of lipids but, due to lipogenesis, can be derived by the consumption of protein and carbohydrate. Consequently, it is very difficult to isolate the effects of protein, fat or carbohydrate on fish composition individually and it is common to consider the effects of energy or protein:energy ratio on fish composition.
It is widely accepted that increasing the dietary energy content of fish feeds by increasing the oil content can result in higher levels of fat in the fish. This was clearly demonstrated for rainbow trout, *Onchorhynchus mykiss*, by Jobling et al.

(1998). Two diets were prepared which were either low fat (59% protein, 13% fat, 21.2 Mj kg^{-1} GE). The diets were fed in excess to trout of 90g using automatic feeders for four hours daily. After 11 weeks of feeding, the trout fed the high and low fat diets had grown to 361 and 348 g respectively. Although there were no significant effects of the diets on final weight, the composition of the trout closely reflectes the diets fed. Thus, the trout fed the high fat diet had significantly higher levels of whole body, carcass and visceral fat (and energy) than the trout fed low fat diets. Similarly, the visceral somatic index of the trout fed the high fat diet was higher than that of the fish fed the low fat diet.

Jobling et al. (1998) continued to feed the remaining trout on a mixture of both low and high fat diets simultaneously. During this second period of feeding, both groups of trout increased in weight to approximately 900 g. The whole body and carcass composition of the fish previously fed the high fat diet was little changed while that of the trout formerly fed the low fat diet changed radically. Jobling et al. (1998) showed that, by comparison to a high fat diet, supplying a low fat feed did not restrict trout growth and resulted in a leaner fish. Furthermore, after the fish were offered a choice of feed, it appeared that the already fat fish were capable of maintaining their composition while the lean fish increased in fat content to match that of their counterparts.

Increased body lipid level in response to feeding diets with decreased protein:energy ratio has been documented for numerous species including Atlantic halibut (Aksens et al., 1996), whitefish, *Coregonus lavaretus* (Koskela et al., 1998), gilthead seabream, *Sparus aurata* (Company et al., 1999) and dentex (Tibaldi et al., 1996).

Moreover many protein:energy studies in salmonids involve substitution of protein with oil. Increases in flesh fat content and decreased slaughter yield similar to those recorded for high oil diets have been observed when protein is replaced by carbohydrate in diets for Atlantic salmon. Aksnes (1995) prepared four diets containing a fixed level of lipid (28%) with the following protein/starch ratios: 60/2, 52/10, 45/17 and 38/23. The diets were fed to satiation to Atlantic salmon which grew from 0.6 to 3.5-3.9 kg during a period of 254 days. Increased starch content in the feeds had significant adverse effects on the apparent digestibility coefficient of starch and lipid from the diet such the DP:DE ratios of th e diets were 24.4, 22.9, 20.7 and 19.6g MJ^{-1} for the 60/2, 52/10, 45/17 and 38/23 diets respectively. Since the salmon were fed to satiation, the fish were able to increase feed intake to the effect that there were no significant effects of the protein:energy ratios on fish growth though there were adverse effects on FCR.

3.6. Effects of feeding regime on fish composition

Composition effects attributed to feeding regime appear to be due primarily to differences in feed intake. Kiessling et al. (1991) reported that the levels of protein in red and white muscle increased in rainbow trout with size and age up to 1.4 years, then remained relatively constant regardless of ration level. Fat levels in all tissues examined increased with age and increasing ration. The rate of fat increase in red and white muscle declined in older fish. Much larger changes were seen in visceral fat in relation to ration level. Increasing the feeding rate increased carcass and visceral fat and reduced visceral protein in rainbow trout (Storebakken et al., 1991). Johansen and Jobling (1998) also reported that fish fed to satiation had higher levels on muscle fat than fish fed a lower predetermined ration, but indicates that this could be attributed to differences in fish size. Company et al. (1999) reported that the percentage of body fat was increased by increasing ration in gilthead sea bream (*Sparus aurata*).

Grayton and Beamish (1977) reported increases in weight gain and fat deposition in rainbow trout when they increased the number of daily feedings (one, three or six times to satiation). They attributed this to greater daily feed intake in the fish fed more frequently. When they varied the number of daily feedings and restricted the ration so that all groups had the same daily intake , no differences were reported.

Boujard et al. (1995) examined the effects of feeding time on the composition of rainbow trout, but the effect of meal time is unclear despite the author's claim that both protein and fat were reduced in fish fed at midnight versus those fed at dawn. Composition was reported on a dry weight basis and fish differed in size at the end of the experiment.

Several studies have examined the effects of fasting for a period prior to harvest on body or product composition. Lie and Huse (1992) starved Atlantic salmon for 78 days and observed that weight loss and reduction of fillet fat slowed progressively with time. The largest loss of fat occurred in the viscera and fillet fat decrease was marginal.

Wathne (1995) starved Atlantic salmon of 2.2 kg for five weeks at 8.8°C (308 degree days) with a resultant decrease in ungutted body weight of 4.5%. Dressing out and visceral fat index were significantly improved by starvation and furthermore Wathne (1995) demonstrated that this loss of weight was due to the preferential mobilization of lipids from the adipose tissue though fat levels in the white muscle.

Einen et al. (1998) conducted an extended starvation trial with salmon of 5.0 kg. Initially, all fish were fed twice daily to satiation on a single diet and feeding was suspended 86, 58, 30, 14, 7, 3 and 0 winter days prior to harvest. The salmon

that were fed continuously until harvest increased in weight by 26% while those that were started for 86 days lost 11% of their initial weight. The slaughter yield increased in proportion to the length of starvation for salmon starved for up to 30 days with no further increase in yield for fish starved for between 30 and 86 days. Einen et al. (1998) observed that fillet yield (as %whole body) was not significantly affected for salmon starved up to 30 days though there were significant losses in fillet yield thereafter. Einen et al. (1998) recorded only marginal effects of starvation on the fat content of the large salmon.
By comparison to fish fed continuously until slaughter, 14 and 58 days of starvation were required to significantly lower the fat content of the visceral tissue. Futhhermore, even after 86 days of starvation at winter temperatures, fat in the whole fillet and belly flaps were not significantly reduced and there appeared to be no preferential loss of fat from any of the fillet regions. Einen et al. (1998) concluded that pre-harvest starvation for short periods can be used to increase slaughter yields in large Atlantic salmon though this must be offset against very minor effects on fillet fat levels and decreases in the fillet yield when the starvation period is extendend.

3.7. Fatty acids profile

The fatty acid profile of fish reflects the fatty acid profile of their diet. Kirsch et al. (1998) reported that the fatty acid profile of Atlantic cod (*Gadus morhua*) changed to the fatty acid profile of their new diet after three weeks. Nettleton (1990) also states that the fatty acid profile of cultured fishes can be modified a few week prior to harvest. The fatty acid profile of Atlantic salmon has also been shown to reflect that of their diet, and may modify the organoleptic qualities of the flesh (Bergstrom, 1989; Thomassen and Rosjo, 1989).
Even if fatty acid composition of fillets reflects the fatty acid composition of diets, although the differences in fatty acid composition among groups of fish are less than the differences in fatty acid composition among their respective diets (Hardy et al., 1987). In fact, incorporation of FA into tissues is under various metabolic influences such as preferential incorporation (Linares and Henderson, 1991), β-oxidation (Kiessling and Kiessling, 1993), lipogenic activity, or fatty acid elongation and desaturation processes (Henderson and Sargent, 1985). It is thus difficult to predict fish fatty acid composition from dietary components. The fatty acid composition may also be affected environmental factors (Tocher and Sargent, 1990) or size or age of animals (Kiessling et al., 2001), affecting metabolic activity.
It is also difficult to predict the time required to reach a relatively stable FA composition in fish fed a particular diet. It could be relatively short (Skonberg et al., 1994), or continue to evolve over a longer period (Tidwell and Robinette,

1990). With fast growing young fish, it is possible to obtain a rather complete effect of dietary FA on fish FA composition in a relatively short period of time. In the case of large fish, since the relative weight increment is small, the initial FA composition will continue to have a strong influence on final composition. It is thus essential that following a change in dietary FA source, FA composition must be interpreted taking into account the initial FA content, intake, increase in tissue mass and duration.

The use of vegetable oils in fish diets has an influence on flesh quality (Thomassen and Røsjø, 1989; Sérot et al., 2001), at least in their fatty acid profile. Considering the interest in fish n-3 fatty acids for human health (Nettleton, 1991), fatty acid profile of fish fed plant oils might be considered as having possible negative effects in this regard. After using diets containing vegetable oils, a return to a fish oil diet is one way of reducing this adverse effect (Regost et al., 2003).

4. Essential fatty acids: fish consumption and human health

The consumption of fish and fish-derived products is recommended as a means of preventing cardiovascular and other diseases, and has considerably increased over recent decades. However, as the world's wild fish stocks are limited, consumers are now being proposed farmed fish as an alternative .

The lipid composition of farmed fish is more costant and less affected by seasonal variations than that of of wild fish. As discussed in a previous chapter, vegetable food is increasingly replacing fishmeal in fish feeds, and may induce a relative decrease in omega-3 (n-3) polyunsaturated fatty acids (PUFAs), expressed as a percentage of total fatty acids. However, as farmed fish generally have higher total lipid levels than fish, 100 g of farmed fish fillet can provide a higher amount of n-3 PUFAs than 100g of wild fish (Cahu et al., 2004).

4.1. Omega-6 and Omega-3 Fatty Acids

In addition to protein and trace elements, the high nutritional value of fish is mainly due to their lipid composition: fish in general, but especially cold water sea fish, contain high levels of polyunsaturated fatty acids (PUFAs), mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

There are two classes of essential fatty acids (EFA), omega-6 and omega-3. The distinction between omega-6 and omega-3 fatty acids is based on the location of the first double bond, counting from the methyl end of the fatty acid molecule. In the omega-6 fatty acids, the first double bond is between the 6th and 7th carbon atoms and for the omega-3 fatty acids the first double bond is between the 3rd and 4th carbon atoms. Monounsaturates are represented by oleic acid, an omega-9 fatty acid, which can be synthesized by all mammals including humans. Its double bond is between the 9th and 10th carbon atoms.

Omega-6 and omega-3 fatty acids are essential because humans, like all mammals, can not make them and must obtain them in their diet. Omega-6 fatty acids are represented by linoleic acid (LA; 18:2ω6) and omega-3 fatty acids by α-linolenic acid (ALA; 18:3ω3). LA is plentiful in nature and is found in the seeds of most plants except for coconut, cocoa, and palm. ALA on the other hand is found in the chloroplasts of green leafy vegetables, and in the seeds of flax, rape, chia, perilla and in walnuts. Both EFA are metabolized to longer-chain fatty acids of 20 and 22 carbon atoms. LA is metabolized to arachidonic acid (AA; 20:4ω6), and LNA to EPA (20:5ω3) and DHA (22:6ω3), increasing the chain length and

degree of unsaturation by adding extra double bonds to the carboxyl end of the fatty acid molecule.

Humans and other mammals, except for carnivores such as lions, can convert LA to AA and ALA to EPA and DHA, but it is slow (de Gomez and Brenner, 1975). Premature infants (Carlson et al., 1986), hypertensive individuals (Singer et al., 1984), and some diabetics (Honigmann et al., 1982) are limited in their ability to make EPA and DHA from ALA. These findings are important and need to be considered when making dietary recommendations.

LA, ALA, and their long-chain derivatives are important components of animal and plant cell membranes. In mammals and birds, the omega-3 fatty acids are distributed selectively among lipid classes. ALA is found in triglycerides, in cholesteryl esters, and in very small amounts in phospholipids. EPA is found in cholesteryl esters, triglycerides, and phospholipids. DHA is found mostly in phospholipids. In mammals, including humans, the cerebral cortex, retina, and testis and sperm are particularly rich in DHA.

DHA is one of the most abundant components of the brain's structural lipids. DHA, like EPA, can be derived only from direct ingestion or by synthesis from dietary EPA or ALA.

Mammalian cells can not convert omega-6 to omega-3 fatty acids because they lack the converting enzyme, omega-3 desaturase. When humans ingest fish or fish oil, the EPA and DHA from the diet partially replace the omega-6 fatty acids, especially AA, in the membranes of probably all cells, but especially in the membranes of platelets, erythrocytes, neutrophils, monocytes, and liver cells (Simopoulos, 1991; Simopoulos, 1999). Whereas cellular proteins are genetically determined, the polyunsaturated fatty acid (PUFA) composition of cell membranes is to a great extent dependent on the dietary intake.

Because of the increased amounts of omega-6 fatty acids in the Western diet, the eicosanoid metabolic products from AA, specifically prostaglandins, thromboxanes, leukotrienes, hydroxy fatty acids, and lipoxins, are formed in larger quantities than those formed from omega-3 fatty acids, specifically EPA (Simopoulos, 1991). The eicosanoids from AA are biologically active in very small quantities and, if they are formed in large amounts, they contribute to the formation of thrombus and atheromas; to allergic and inflammatory disorders, particularly in susceptible people, and to proliferation of cells. Thus, a diet rich in omega-6 fatty acids shifts the physiological state to one that is prothrombotic and proaggregatory, with increases in blood viscosity, vasospasm, and vasocontriction and decreases in bleeding time.

There is now a consensus that these omega-3 (n-3) fatty acids protect against cardiovascular diseases, especially the acute complications of coronary heart disease such as the sudden cardiac death syndrome (GISSI-Prevenzione Investigators, 1999; de Lorgeril and Salen, 2002). It is also thought that they can

prevent or delay the clinical manifestations of certain cancers (Senzaki et al., 2001).
The Lyon Heart Study was a dietary intervention study in which a modified diet of Crete (the experimental diet) was compared with the prudent diet or Step I American Heart Association Diet (the control diet) (de Lorgeril et al, 1994; Renaud et al., 1995; de Lorgeril et al., 2000; de Lorgeril et al., 2007). The experimental diet provided a ratio of LA to ALA of 4/1. This ratio was achieved by substituting olive oil and canola (oil) margarine for corn oil. Since olive oil is low in LA whereas corn oil is high, 8% and 61% respectively, the ALA incorporation into cell membranes was increased in the low LA diet.
Cleland *et al.* (1992) have shown that olive oil increases the incorporation of omega-3 fatty acids whereas the LA from corn oil competes. In the Lyon Heart Study, the ratio of 4/1 of LA/ALA led to a 70% decrease in total mortality at the end of two years (de Lorgeril et al., 1994).
The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI, 1999) Prevenzione Trial participants were on a traditional Italian diet plus 850–882 mg of omega-3 fatty acids at a ratio of 2/1 EPA to DHA. The supplemented group had a decrease in sudden cardiac death by 45%. Although there are no dietary data on total intake for omega-6 and omega-3 fatty acids, the difference in sudden death is most likely due to the increase of EPA and DHA and a decrease of AA in cell membrane phospholipids. Prostaglandins derived from AA are proarrhythmic, whereas the corresponding prostaglandins from EPA are not (Li et al., 1997). In the Diet and Reinfarction Trial (DART), Burr et al. (1989) reported a decrease in sudden death in the group that received fish advice or took fish oil supplements relative to the group that did not. Similar results have been obtained by Singh et al. (1997; 2005).

4.2. Lipid content and fatty acids of fish

Unlike mammals, in which most lipids are generally deposited in adipose tissue, fish have lipids in the liver, muscles and perivisceral and subcutaneous tissues. Fish are generally classified into three categories on the basis of their muscle fat content: lean fish, with less then 1% of muscle fat (cod, turbot); an intermediate category with a muscle lipid content of 5-10% (carp, sea bream) and fat fish with more than 10% of muscle fat (salmon, eel) .
In wild fish, the total lipid content and fatty acid composition vary widely during the year. This is true especially for pelagic species, which present the minimum fat value during the spawning period. Moreover the lipid content and fatty acid profile of wild fish are not constant but depend on the species and the catching season (Cahu et al., 2004).

As reported in a previous chapter, farmed fish are fed with commercial diet in which fishmeal is the principal ingredients. The fish feed contains also vegetable meals that partially replaced fishmeal and fish oil, which is now often replaced by vegetables oils. The lipid content of commercial diet can vary from 10% to 30% of dry matter. Fish farmers tend to increase this percentage in order to promote growth and save on proteins. Regarding the fish composition, the high lipid levels in feeds lead to higher lipid concentrations.
Moreover, dietary lipids affect the fatty acid profile of fish (Watanabe, 1982). In particular, dietary intake is mainly responsible for the high EPA and DHA concentrations characterizing fish.
Carnivorous fish such as sea bass and sea bream generally have high EPA and DHA concentrations than herbivorous or omnivorous fish such as carp and catfish, and they have higher omega-3 PUFA levels than freshwater fish (Watanabe, 1982; Bell, 1986). These differences can be attributed to their feeding habits. Freshwater fish are generally more capable of desaturating and elongating oleic, linoleic, and linolenic acids than marine fish and so the fatty acid profile in freshwater species is less affected by the fatty acid profile of their diet. These species can maintain quite high EPA and DHA levels when they are fed a diet containing vegetable oil poor in EPA and DHA.

4.3. Omega-3 intake and beneficial health effects

During last years, some controversy related with the benefits of omega-3 fatty acids has raised. Hooper et al. (2006) concluded that long chain and shorter omega fats did not have a clear effect on total mortality, combined cardiovascular events, or cancer. Moreover, in a prospective study to investigate the relationship between the consumption of fish and intake of long-chain omega-3 fatty acids, and the risk of coronary heart mortality in men and women free of cardiovascular disease (CD), Jarvinen et al. (2006) showed that while for women, their results were in line with the suggested protective effect of fish consumption against CD, a similar association was not found in men. However, Psota et al. (2006) recently reasserted the remarkable cardioprotective effects of omega-3 fatty acids (Din et al., 2004; He et al., 2004, Ismail, 2005; Iso et al., 2006), especially the longer-chain fatty acids from marine sources, suggesting that consumption should be increased in the diet to decrease cardiovascular risk significantly. This is also in agreement with the recent conclusions of Engler and Engler (2006) concerning the beneficial role of omega-3 fatty acids in cardiovascular health and disease. However, these authors suggested caution in the consumption of certain fish species depending on their levels of environmental pollutants (Engler and Engler, 2006). Levenson and Axelrad (2006) concluded that while there is a significant amount of data showing health

benefits of increased fish consumption, there are conflicting reports about the cardiovascular risks of mercury in seafood.

Anyhow, the controversy seems to be served. Dietary recommendations have been made for omega-3 fatty acids, including alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) to achieve nutrient adequacy and to prevent and treat cardiovascular disease. These recommendations are based on a large body of evidence from epidemiologic and controlled clinical studies. The evidence base supports a dietary recommendation of approximately 500 mg/d of EPA and DHA for cardiovascular disease risk reduction. For treatment of existing cardiovascular disease, 1 g/d is recommended. These recommendations have been embraced by many health agencies worldwide. A dietary strategy for achieving the 500 mg/d recommendation is to consume 2 fish meals per week. Foods enriched with EPA and DHA or fish oil supplements are a suitable alternate to achieve recommended intakes and may be necessary to achieve intakes of 1 g/d (Gebauer et al., 2006).

5. Fish aroma and flavour

Flavour perception has been researched widely throughout the years, yet is still not fully understood. It is clear that flavour perception begins with the eyesight, as soon as food is seen. Sending a message to the brain, the eyes tell whether a food looks palatable and should be tasted.
Taste and aroma perception, like many other physical processes, begin at a chemical level, with compounds reacting with certain receptors to elicit a neurological response. This physiological response along with the chemical reactions can be referred to as the sense level of perception. What the brain does with the information received, identifying tastes and odors, perhaps remembering similar odors and even connecting them to memories from childhood, is the cognitive level and can be researched from a psychological point of view.

5.1. Aroma perception

5.1.1. Odorants

Odorants are small molecules, usually less than 1 kDa. They are light enough to be breathed into the nose but heavy enough to be recognized by receptors. Almost all aromas are a result of various such odorants, usually several hundred. Even though a few substances in an aroma may be the key contributors it is still comprised of all the odorants. The human brain is able to recognize a large number of odorants, possibly up to 10.000. Millions of olfactory receptors are at work when a food is smelled and they send messages to the brain, which then processes them and delivers identification of the aroma. It is not yet known whether each odorant has its own receptor, but it is considered likely in light of the fact that these receptors are very many, likely 1.000-10.000. Before reaching the receptor, an odorant must travel through the olfactory mucus, covering the olfactory epithelium. The odorant might take part in many reactions before binding to the receptor cell (Bell, 1996).

5.1.2. Flavour

Flavour is often defined as the sensation arising from the interplay of signals produced as a consequence of sensing smell, taste, and irritating stimuli from a foodstuff (Laing and Jinks, 1996). Flavour is generally thought to consist of the volatile components sensed in the nose, both through the nostrils (orthonasally) and from inside the mouth (retronasally), nonvolatile compounds sensed on the

tongue, and compounds that are perceived in the mouth as texture or mouthfeel. Aroma is considered more important than taste in determining flavour. Flavour analysis has typically focused on measuring volatile compounds, for example by gas chromatography-mass spectroscopy (GCMS) and GC-olfactory methods. But changes in composition occur during eating, through mastication of the food by the teeth and dilution of some component by saliva. Measuring those changes is more difficult. The change a food undergoes is different for each type of food, an aqueous beverage will for example change little as passing through the mouth, while a fat-based food will undergo melting of the fat, temperature changes, and possibly emulsion changes (water-in-oil to oil-in-water), which all cause changes in the release of volatiles (Taylor and Linforth, 1996). Several studies, old and new, also suggest that food flavours play an important role in food digestion. The flavour of food alone, without swallowing, can cause secretion of saliva, gastric acid, enzymes from the exocrine pancreas, and hormones from the endocrine pancreas.
Furthermore, a patient lacking an esophagus, whose food was inserted into the stomach through an artificial opening, needed to also put food in his mouth and taste and chew it in order to gain weight and feel satisfied (Teff, 1996).

5.1.3. Effect of different food constituents on flavour

The main components in foods, carbohydrates, proteins, and lipids all have roles in flavour generation and perception. The taste of amino acids and peptides also been reported to influence flavour and some peptides have their own flavour. A beef peptide, said to be present naturally in beef, is responsible for flavour enhancing properties similar to those of MSG. The predominant influence of proteins on flavour perception is by interactions between flavour components and protein macromolecules. Proteins can bind various aromatic substances or tastants, depending on their polarity and structure, and thus influence flavour release from the foodstuff (Fischer and Widder, 1997).
Lipids have an effect on flavour perception, stability, and generation. A flavour substance may be hydrophilic or lipophilic. When a food contains little or no lipids, a high concentration of lipophilic compounds will be in the air above the food, since it cannot bind to lipids in the food. If lipid content of a food is higher, most of the lipophilic flavourants will be bound to lipids in the food and the concentration in the air above the food is much lower. The headspace concentration of hydrophilic flavorants is not affected as much by the lipid content. Studies have shown that an increase in fat content (for example by adding oil to an aqueous solution of flavorants) results in a decrease of flavour strength and changes the flavour pattern. Flavour release in the mouth from the lipid phase of foods is slower than from the water phase, so the maximum flavour intensity is perceived later than that of hydrophilic flavorants. Lipids in

food also affect the stability of flavours. Reduction in fat content means higher flavour loss during processing and storage because of the higher volatility of flavorants (De Roos, 1997).
Carbohydrates affect food flavours in different ways. They give food sweetness, they undergo reactions that form flavour compounds and they interact with flavour molecules, changing their volatility and consequently the sensory perception of the food. Hundreds of chemicals seem to have the structure required to impart sweetness, but only few of these are used in foods. Fructose and xylitol are the only food carbohydrates that are sweeter than sucrose, commonly used as a reference of sweetness. Maillard browning, the reaction of an amino acid with a reducing sugar, produces flavour in foods both through volatile aromatics formed and bitter-tasting polymers. The flavours produced are typically described as caramelized, bready, nutty, roasted, or meaty. Gums and other thickeners have the effect of reducing sweetness, since the perceived sweetness intensity of sucrose decreases after a certain viscosity is reached. Viscosity affects the diffusion of molecules in a mixture, and altering the viscosity (for example by switching thickeners in a formula) may alter the flavour profile of a food since molecules will reach the retronasal cavity at a different time. Generally carbohydrates decrease slightly the volatility of flavour compounds in water. A few carbohydrates have the opposite effect, especially mono- and disaccharides (Godshall, 1997).

5.2. Fish aromas and flavours

Fish flavours are mainly characterized by the volatile compounds in fish. Ólafsdóttir and Fleurence (1998) present a good review on the main groups of fish odors. These are species related fresh fish odor, microbial spoilage odor, oxidized odor, environmentally derived odor, and processing odor. The last is not of interest when dealing with fresh fish, but is relevant for example in ripening of herring and anchovies. The fresh fish odor is prevalent during the first few days after catching.
After that oxidation products and microbial metabolites dominate the aroma of fish. The compounds associated with fresh fish flavours are mostly 6-, 8-, and 9-carbon aldehydes, ketones and alcohols derived from the unsaturated fatty acids characteristic of fish by lipoxygenase activities. Six carbon compounds (hexanal, *trans*-2-hexenal, *cis*-3-hexenal) provide green plant-like aromas. They are connected to freshwater fish and are usually not found in saltwater species. Eight carbon compounds (1-octen-3-ol, 1-octen-3-one, 1-*cis*-5-octadien-3-ol, 1-*cis*-5-octadien-3-one) seem to occur in most types of fish and seafood and contribute heavy plant-like odors and metallic-like flavours. The nine carbon compounds (3,6-nonadienal, 2,6-nonadienal, 3,6-nonadienal) contribute fresh, green,

cucumber-like odors and flavours and are found in some fish species, particularly freshwater species. These fresh fish compounds are similar to those found in some vegetables, as can be seen from their typical aromas. They are produced by lipoxygenases in plants as well as in fish, though the pathways are somewhat different. The eight carbon alcohols and ketones, which are found in all fish that have been surveyed, are also found in mushrooms. These compounds alone have mushroom or geranium-like aromas, but in freshly harvested fish they contribute heavy plant-like aromas. The nine carbon compounds are found in cucumber and melons and contribute a cucumber and melon-like aroma to the fish in which they are present. Hexanal and 2-hexenal contribute green-plant-like aldehyde aromas. They are found in all freshwater fish and hexanal has been found in 5-6 days old saltwater fish.

It has been found that saltwater fish contain bromophenols, but they are scarce in freshwater species. If low concentrations of bromophenols are mixed into freshwater fish muscle tissue the resulting flavour is marine-like, salty, shrimpy, and iodine-like. Iodine-like off flavours in shrimp have been connected to abnormally high levels of 2,6-dibromophenol (Lindsay, 1990). 2,6-dibromophenol and 2,4,6-tribromophenol provide iodine-, shrimp-, crab-, and sea salt-like flavour to fish and shrimp muscle tissue and oil matrices. Monobromophenols (2-, 3-, 4-) enhance sweet seafood-like flavour in the same matrices. In water solutions all the bromophenols are iodine-like, phenolic or medicinal. Since these chemicals have not been detected in freshwater fish they are believed to be derived from dietary or environmental sources in the ocean (Boyle et al., 1992). Research on wild and cultivated prawns in Australia (Whitfield et al., 1997) further demonstrated that the bromophenols must be derived from the diet of these animals. Total bromophenol content varied in three different wild species from 9,5 to 1114 ng/g, but in the cultivated species it was less than 1 ng/g. Sensory analysis of these prawns showed that the meat of the wild animals had briny, ocean-like, and prawn-like flavours, while the cultivated prawns were bland. For these Australian prawns, the major dietary components of wild prawn are crustaceans, molluscs, protozoans, and marine worms (polychaetes). Small quantities of nematodes and algae and sea grass are also eaten. Of these, only the algae and marine worms are known to synthesize bromophenols. The cultivated prawns feed on fish meal, plant material, prawn meal, and squid meal. The prawn meal is likely themajor contributor of bromophenols in the diet, but it is in much lower concentrations than in the marine worms.

During storage the compounds responsible for the very fresh fish flavours deteriorate through autolytic and microbial reactions. The fresh, planty, and metallic flavours disappear and are replaced with a neutral, flat flavour. When microbes start growing rapidly sulfur compounds, phenols, and certain fatty

acids give spoiled and putrid aromas and flavours. Through microbial breakdown of trimethylamine oxide (TMAO) trimethylamine (TMA) is formed and the resulting odor is fishy in a negative way, reminiscent of old, stale fish or dried fish. Freshwater fish generally do not contain TMAO and TMA is not present in freshly harvested marine fish. When high concentrations of TMA have been formed the fish is very undesirable for consumption. TMAO seems to serve an osmoregulatory function in saltwater fish and is normally not found in freshwater fish above trace amounts. Dimethylamine, which has an ammoniacal aroma, is formed along with formaldehyde from enzymatic activity in fish muscle. It is formed in frozen fish rather than TMA, which is dominantly formed in storage above freezing temperatures. The enzymatic activity is also associated with toughening of fish muscle. It appears that the formaldehyde crosslinks with proteins, thus changing the texture. Dimethyl sulfide and methyl mercaptan have both been reported to contribute off-odors and flavours in fish and are usually formed microbially.

Autoxidation of fish lipids during storage gives off undesirable flavours. *cis*-4-Heptenal is formed through degradation of *trans*-2,*cis*-6-nonadienal by autoxidizing lipid systems. It has been reported to have putty, painty and linseed oil-like aromas at high concentrations, cardboardy at lower concentrations and to possess a cold boiled potato aroma. It does not seem to contribute fishy flavours, but enhances burnt, fishy cod liver oil-like flavours from 2,4,7-decatrienals (Josephson et al., 1983; Josephson et al., 1984; Lindsay, 1990; Kawai, 1996).

The major volatile compounds found in the headspace of some tropical prawns after less than 8 days storage on ice were trimethylamine, 2-methyl-2-propanamine, o-3-methylbutyl hydroxylamine, o-2-methylpropyl hydroxylamine, methyl disulfide, carbon disulfide, methyl (methyl thio) methyl sulfide, and ethyl butyrate. Most of these compounds are formed through bacterial breakdown of amino acids and other bioavailable compounds in the shrimp (Chinivasagam et al., 1998). Prell and Sawyer (1988) documented the flavour profiles of 17 ocean fish species.

They classified the species in a tree diagram according to dominant characteristics and found that haddock, wolffish, tilefish, pollock, cod (market), flounder, and cusk formed one branch, all having relatively low total flavour intensity and fresh fish note and slight sweet, salty, and sour notes. All except flounder also had a slight shellfish flavour. Whiting, white hake, and cod (scrod) formed a second branch, similar to the first but lacking the shellfish flavour and possessing instead an earthy note. Halibut, weakfish, and striped bass had a moderate flavour intensity, and distinctive fish oil, gamey, and sour notes. These comprised the third branch. The fourth branch contained bluefish and mackerel with high flavour intensity, fish oil, and sour notes and low gamey notes.

Monkfish (low flavour intensity, full, shellfish, salty, fresh fish, and sweet), Grouper (low flavour intensity, slight fresh fish, sour and shellfish, sweet and salty, sour), and Swordfish (low flavour intensity, sweet, fresh fish, shellfish, full, nutty-buttery, fish oil) did not seem to fit into any branch and did not make up a branch of their own, suggesting independent flavour groups for these species.
Chambers and Robel (1993) documented flavour profiles of 11 freshwater species. In these species saltiness is much lower than in ocean fish and many have a white meat (as cooked chicken breast) characteristic not noted in ocean fish. Many attributes are similar, for example metallic, bitter, and sour tastes. Fresh fish aftertastes were common in both studies and white meat, metallic, and astringent aftertastes for the freshwater species. In the ocean species a sour aftertaste was often noted.

5.3. Consumer perception of fish flavours

Even though fish and fish products are an important source of nutrients in many countries, and health professionals encourage more fish consumption, research on consumer perception of fish seems to be scant. Many consumers in the Western world feel they should eat more fish and are certain that fish is healthy. Sörensen et al. (1996) tried to connect consumers' attitudes to their likelihood of buying and liking fresh fish in connection with some other products. Most consumers perceived the fresh fish to be healthy and promote well being, but that did not affect their likelihood of buying the fish. The main predictors of buying less fresh fish were the negative consequences associated with fish: that buying it was complicated as it required a trip to the fishmongers and that preparing and eating it was too time consuming and difficult. Many people also associate fish with "fishy" odors and the smell of TMA and do not like the flavour of fish. In parts of the world far away from the ocean fresh saltwater fish is rare and the "fresh" fish is often close to two weeks old when the consumers see it, not helping these assumptions. In an experiment that had the main objective to asses the theory of planned behavior (Bredahl and Grunert, 1997) some conclusions about the behavioral pattern of buying and consumption of fish could be drawn about the Danish consumer. Taste was by far the most influential factor and the consumers' belief of getting a light feeling of satiety was also important. The perceived healthiness was important for deciding to buy frozen fish or shrimp, but not fresh fish. Price and nutritional value had little effect, but the perceived ability to prepare a tasty dish from the raw material was important. The main conclusions of the experiments were that marketing strategies for seafood need to focus less on the health aspects, which consumers seem to be aware of already, and more on making the preparation of seafood meals easy and desirable. Hamilton and Bennet (1983) used small (21-34) groups

of consumers with little training to evaluate nine fish species. Whiting, cod, plaice, haddock, ling, dab, blue whiting, saithe, and lemon sole were evaluated. The consumers could clearly discriminate between all the species and in paired preference tests consumers usually demonstrated a preference for one sample, although it was not significant in most cases. Appearance, texture, flavour and acceptability were rated on a 5 point hedonic scale and regression tests performed to see which factors were most important in determining acceptability. Texture and flavour turned out to be highly correlated and both correlated strongly with acceptability, indicating that flavour alone was enough to model both. Appearance was somewhat correlated to acceptability, but after removing two species appearance had little effect. The strength of preference was not high, indicating that consumers would not react adversely to changing of species, especially in products such as fish fingers.

6. Techiniques for the determination of fish authenticity

Autenthicity testing of food products, such as fish, is very important for labelling and assessment of value. Moreover, it is necessary to avoid unfair competition and assure consumers protection against fraudulent practices, observed sometimes in the food industry.
To ability to identify fish species following the removal of external characteristics by processing, such as canning, smoking or filleting, is of great importance. Once the morphological characters have been removed during processing, the identification becomes difficult and there is the risk for fraudulent substitutions of lower-valued fish species for high-valued fish in seafood products.
To prevent fraudulent fish sunstitutions, food laboratories need to have available techniques to establish the fish species used in the manufacture of fish products (Asensio et al., 2008).

6.1 Nuclear magnetic resonance (NMR) spectroscopy

NMR has shown its applicability as an analytical tool for rapid and non-invasive determination of water and fat (^{1}H-isotope) and sodium (^{23}Na-isotope) contents in various foods. The interactions between the studied molecules and their surrounding environment affect the molecule's behaviour in terms of their mobility and NMR relaxation properties. The method is based on measuring the *relaxation times* (T_1 and T_2) of the nuclei after the system has been subjected to specified pulse sequences. This makes it possible to distinguish and investigate different phases of water in the biological materials. Thus, by using ^{1}H low-field NMR several pools of water have been defined in fresh (Andersen and Rinnan, 2002) and thawed (Jensen et al., 2002) fish muscle, according to their mobility and how tight the water molecules are bound to the muscle structure. One of the latest developments in the use of low-field NMR is a hand-held portable NMR probe (Blümich *et al.*, 1998) able to give fat and water content values by simply placing the probe on top of the sample.

6.2 Isotopic ratio mass spectroscopy (IRMS)

Isotope ratio mass spectrometry (IRMS) has been demonstrated to be a technique capable of revealing the origin of fish (Molkentin et al., 2007). Over the past few years, the determination of stable isotope ratios of light elements,

especially carbon and nitrogen, has been applied to authenticity control and origin assessment of food of animal origin (Rossmann, 2001).
The isotope ratio of animals is primarily determined by diet and, to some extent, reflects their origin (De Niro and Epstein, 1978; De Niro and Epstein, 1981). The carbon isotopic composition of a terrestrial or aquatic organism reflects the isotopic composition of its diet, but the organism is usually enriched in $\delta^{13}C$ by about 1‰, relative to its diet. Thus, carbon isotope ratio remains relatively unaffected by trophic transfer. Similarly, the isotopic composition of nitrogen reflects the isotopic composition of the diet, but with an enrichment much higher than for carbon. Therefore, the consistency of nitrogen enrichment (3–5‰) at each trophic transfer provides a valuable measure of the position of an organism within the food web (De Niro and Epstein, 1976).
Stable isotope analysis has been used as a tool to control the traceability of foodstuff providing good results also for geographical approaches (Kelly et al., 2005). In recent studies, it has been also applied to study the dietary habits of aquatic animals and relative food webs (Domi et al,. 2005; Doucett et al., 1996) and the seasonal variation of the isotopic composition in fish tissues (Perga and Gerdeaux, 2005) to distinguish anadromous and non-anadromous populations of *Salmo trutta* (McCarthy and Waldron, 2000), to establish the rearing location of juvenile salmon (Kennedy et al., 2005), and to investigate the change in isotopic composition during spawning (Doucett et al., 1999). Further and more recently, IRMS has been applied to distinguish wild and farmed gilthead sea bream (Morrison et al., 2007; Moreno Rojas et al., 2007).

6.3 Mid-infrared (MIR) and near-infrared (NIR) spectroscopy

Infrared spectroscopy is a rapid and non-destructive technique for the authentication of food samples. Analysis of a food sample using the MIR spectrum (4000-400 cm^{-1}) reveals information about the molecular bonds present and can therefore give details of the types of molecules present in the food. NIR spectroscopy utilises the spectral range from 14 000 to 4 000 cm^{-1} and provides much more complex structural information related to the vibrational behaviour of combinations of bonds. These techniques are suited for use in an industrial setting due to their ease of use and the relatively low financial cost of obtaining and running the equipment. The IR studies discussed here all employed some form of chemometric analysis, resulting in powerful analytical techniques, which have been successfully employed in classification studies for a wide variety of food products.
Foods that have recently been effectively tested for adulteration using NIR spectroscopy include fruit purées and juices (Rodriguez-Saona et al., 2001; Contal et al., 2002*)*, maple syrup (Paradkar et al., 2002), honey (Downey et al.,

2004), Echinacea root (Laasonen et al., 2002), milk powder (Maraboli et al., 2002) and fish meal (Murray et al., 2001). Differentiation of wines on the basis of grape variety (Cozzolino et al., 2003) yielded correct classification levels of up to 100%. The use of MIR for authentication of red wines on the basis of vintage year enabled correct classification levels of up to 100%, while correct geographical classification of the same wines achieved average levels of 85% (Picque et al., 2005). The adulteration of olive oils with a variety of common adulterants was detected using NIR analysis with very low error limits (Christy et al., 2004). The differentiation of meat from different animal sources (beef, pork, lamb and chicken) using visible and NIR analysis with PCA (Principal Components Analysis) (Cozzolino and Murray, 2004) gave correct classification levels of 80%. Apple juice samples were differentiated on the basis of apple variety using NIR with LDA (Linear Discriminant Analysis) and PLS (Partial Least Squares) (Reid et al., 2005). The results showed correct classification of samples on the basis of apple variety as high as 100%.

With regards to MIR, wine samples have been differentiated on the basis of geographical and varietal origin (Roussel et al., 2003). The use of MIR and chemometrics to detect adulteration of apple juice with beet syrup and cane syrup gave correct classifications of 100 and 96.2% respectively (Sivakesava et al., 2001). Adulteration of honey samples with sugar solutions at levels of 14% w/w has also been detected using MIR and PLS (Kelly et al., 2004). Quantification of saccharides present in honey samples using MIR has been found to compare favourably with high-performance liquid chromatography in terms of accuracy and predictive ability (Tewari and Irudayaraj, 2004). A combination of Fourier-transform mid-infrared (FT-MIR) spectroscopy and a chemometric technique known as potential curves was demonstrated to be capable of classifying apple juice beverages on the basis of the percent of pure apple juice present (Gomez-Carracedo et al., 2004).

6.4 Nuclear magnetic resonance (NMR) spectrometrry

NMR spectroscopy involves the analysis of the energy absorption by atomic nuclei with non-zero spins in the presence of a magnetic field. The energy absorptions of the atomic nuclei are affected by the nuclei of surrounding molecules, which cause small local modifications to the external magnetic field. NMR spectroscopy can therefore provide detailed information about the molecular structure of a food sample, given that the observed interactions of an individual atomic nucleus are dependent on the atoms surrounding it.

High-resolution NMR (HR-NMR; utilises frequencies above 100 MHz) has been applied in many more food authenticity studies than low-resolution NMR (LR-

NMR; uses frequencies of 10-40 MHz). The advantage of HR-NMR over LR-NMR is that it is possible to obtain much more detailed information regarding the molecular structure of a food sample using HR-NMR. The major disadvantage of HR-NMR is that it is one of the most expensive analytical techniques to employ, both in terms of the initial capital outlay and running costs.

Italian olive oil samples were differentiated using ^{1}H-NMR on the basis of their geographical origin in Italy (Mannina et al., 2001/a) and from different areas within Tuscany, itself a relatively small geographical area (Mannina et al., 2001/b).

Chemometrics in conjunction with ^{13}C-NMR was capable of differentiating oils on the basis of botanical origin and, in the case of olive oils, on the basis of processing (Zamora et al., 2001; Zamora et al., 2002; Brescia et al., 2003/a; Mannina et al., 2003). The detection of adulteration of olive oil samples with seed oil was possible using ^{31}P- and ^{1}H-NMR with multivariate discriminant analysis and enabled adulteration levels as low as 5% v/v to be detected (Vigli et al., 2003).

Wines from the Apulia region of Italy were differentiated from wines from Slovenia using ^{1}H-NMR and chemometric analysis (Brescia et al., 2003/b). The addition of pulpwash is a serious quality control issue in the production of orange juice and its detection was possible using a combination of ^{1}H-NMR and PCA (Le Gall et al., 2001). Coffee samples were differentiated according to manufacturer using ^{1}H-NMR and a combination of PCA and LDA (Charlton et al., 2002).

6.5 Chromatographic techniques

Liquid and gas chromatography are capable of separating and enabling identification of almost any type of molecule present in a food sample. Liquid chromatography, in particular high-performance liquid chromatography, can detect compounds such as proteins, amino acids, phenolic compounds and carbohydrates, while gas chromatography is more suited to the analysis of naturally volatile or semi-volatile molecules. The principal disadvantage of the two techniques relates to their use in conjunction with chemometrics. There is often a need to extract the specific analytical data relating to individual compounds and this adds to the time and labour required.

The detection of adulteration of olive oil samples using GC has focused on comparison of their fatty acid composition (Cercaci et al., 2003; Gamazo-Vazquez et al., 2003) and chemometric analysis of their fatty acid contents (Dourtoglou et al., 2003). Moreover, the effects of different types of lipids in the diet on tissue fatty acid composition have been investigated for a number of

cultured and wild species (Bergstrom, 1989; Argyropoulou et al., 1992; Haard, 1992; Shearer, 1994; Rueda et al., 1997; Serot et al., 1998; Grigorakis et al., 2002). In general the farmed fish have a much higher lipid content than their wild counterparts and the fatty acid profiles of farmed fish reflects the fatty acid composition of the diet that the fish has received. As a general rule, cultured fish are characterized by higher levels of monounsaturated (especially 18:1n-9), n-9 and 18:2n-6 fatty acids, whilst wild fish are characterized by higher levels of n-3 fatty acids and higher n-3/n-6 ratios. The presence of increased amounts of 18:1n-9 and 18:2n-6 in cultured fish could be explained by the abundant presence of these fatty acids in plant oil used to partially substitute fish oil in fish feed formulations. These fatty acids are not normal constituents of the marine food chain. Soybean oil, sunflower oil and rapeseed oil are commonly used in aquafeeds and are particularly rich in these fatty acids (Turchini et al., 2000; Rosenlund et al., 2001).

6.6 Dna-based technology

DNA analysis based on the polymerase chain reaction (PCR) has been carried out as an alternative to electrophoretic, chromatographic and immunological techniques. The analysis of nucleic acids (mitochondrial or genomic DNA) presents advantages over protein-based techniques, mainly because DNA assays are not dependent on tissue source, age of individual or sample damage. Although DNA is also degraded, like proteins, under sterilizing conditions, the DNA fragments obtained maintain sufficient differences in their sequence to allow the characterization of even closely related species. Most of the PCR methods for identification of species in fish products are based on the amplification of a conserved region of mitochondrial DNA, followed by sequencing, or restriction fragment length polymorphism analysis (PCR-RFLP) of the amplified fragment (Barlett and Davidson, 1992; Asensio et al., 2000; Russell et al., 2000; Sebastio et al., 2001; Hold et al., 2001; Bellagamba et al., 2001; Rehbein et al., 2002). Alternatively, single strand conformation polymorphism analysis (PCR-SSCP) has been used to generate patterns on gel, detecting unknown sequence variability in the amplified fragment (Rehbein et al., 1997, 1999, 2002).

Polymorphic repetitive DNA sequences, including satellite DNAs and multigene families (rDNAs, SINE, LINE sequences), have also been considered as genetic markers for the identification of eukaryotic species. Recently, species-specific PCR amplification of the nuclear 5S rDNA gene has been studied to identify fish species (Carrera et al., 2000; Asensio et al., 2001; Bellagamba et al., 2003). PCR amplification of anonymous DNA regions by random amplified polymorphic DNA analysis (RAPD) has been shown to be very useful in the study of genetic

polymorphism. Although reproducibility of the PCR reaction represents a very critical point in the RAPD method, the suitability of this method in the identification of fish products has been recently investigated (Martinez et al., 2001; Rego et al., 2002).

6.7 Immunological technology

The majority of reported studies on immunological techniques for food authentication concerns the use of enzyme-linked immunosorbent assay (ELISA). This technique involves the cultivation of antibodies or antisera that are capable of binding to a protein of interest, thereby enabling the detection of that protein, both qualitatively and quantitatively. The major advantage of this approach is that antibodies or antisera can be manufactured to respond specifically to the protein of interest, thereby enabling recognition and quantitation of that protein exclusively. The disadvantages of the ELISA approach include the initial difficulty in producing an antibody specific to a particular protein. However, this is a relatively minor difficulty to overcome when the selectivity of the technique is taken into account.
Recent research using ELISA-based techniques include detecting the presence of meat from different species in food products (Jha et al., 2003) and the presence of vegetable proteins in milk powder (Sanchez et al., 2002). There have also been promising results for the use of ELISA to differentiate milk from different species (Bania et al., 2001; Moatsou and Anifantakis, 2003), as well as to detect the adulteration of sheep and goat milk with cow milk at levels as low as 0.1 % (Hurley et al., 2004). This technique holds much potential for the authentication of food products but, to date, limited advances have been made in extending its authentication capabilities.

7. Traceability issues in fishery and aquaculture products

The availability and the international trade of fish and seafood are strongly influenced by food safety norms. Several European Directives have introduced safety standards into the chain for fishery and aquaculture products with the concept "from farm to fork", usually based on the Codex Alimentarius provisions.

A labelling regulation for fishery and aquaculture products came into effect in the European Union in 2001 (Commission Regulation No. 2065/2001), requiring the identification of the commercial and scientific name, the origin of the fish and its production method (farmed or wild). This regulation aims to provide consumers with a minimum of information on characteristics of such products and it is enforced in Italy by Ministry of Agriculture Decree No. 27.03.02 on the labelling of fish products.

In addition, in regulation No. 178/2002, which lays down procedures in the matter of food safety and establishment of the European Food Safety Authority, the Commission defines traceability as "the ability to trace and follow a food, feed, food-producing animal or substance intended to be or expected to be incorporated into a food or feed, through all stages of production, processing and distribution".

As a consequence of these regulations, various labelling schemes from producers and distributors are now in place for fish products. These aim to promote resource sustainability, distinction of quality and product safety. Typically, such producer's or distributor's labels inform the consumer as to which aquaculture techniques have been used and which type of feed or raw materials have been used in the feed formulation.

The high financial value of fishery products could tempt unscrupulous producers and traders to commit fraud by selling fish products under false authenticity standards. On these bases there is a need for research delivering both a product-specific and general analytical fish products traceability system, verifying existing paper traceability schemes on production methods and the geographical origin of fish. Moreover, there is a need for development of analytical methods for the detection of fish species, according to the EU legislation.

There are many different issues concerning the traceability of fish which it may be desirable to check by performing chemical or physical analysis: the species of origin (fish species), the geographical origin (fish from different regions) and the method of production (wild or farmed, organic or intensive).

7.1. Species of origin

The authentication of fish and seafood products is difficulty for industry, trade and consumers because of the similarity in appearance of many species and the loss of external characteristics during processing. The correct identification of processed seafood has become mandatory not only for correct labelling and assessment of value (i.e. fraudulent addition of species not permitted by law), but also to avoid the poisoning risk related to the presence of neurotoxic species. The identification of species causes serious analytical problems when the usual identifying characteristics are removed during processing, such as cooking, smoking, frying, salting or canning. Many analytical methods have been developed for fish species identification as an alternative to morphological analysis. Traditional and official methods are mostly based on the separation and characterization of specific proteins using electrophoretic techniques, such as isoelectric focusing (IEF) and capillary electrophoresis (CE), high-performance liquid chromatography (HPLC) or immuno-assays systems (Rehbein, 1990; Sotelo et al., 1993; Knuutinen and Harjula, 1998). However, when drastic thermal treatments are used in the production of preserved seafood, proteins lose their biological activity, biochemical structure and chemical properties that allow identification of fish species by the above-mentioned methods. Although electrophoresis in sodium dodecylsulphate polyacrylamide gel (SDS-PAGE) and urea IEF are suitable for the species identification of muscle fish protein in normally-cooked products, they produce no reliable results for sterilized products (Rehbein, 1990; Sotelo et al., 1993). As reported in the previous chapter, DNA analysis based on the polymerase chain reaction (PCR) has been carried out as an alternative to electrophoretic, chromatographic and immunological techniques.

7.2. Geographical origin and method of production

There is much debate and much concern over farmed fish versus wild fish. During the past few years the production of farmed fish has increased compared to wild fish capture. Furthermore, the quality of farmed fish is mainly influenced by the quality of the feed. Consequently there is a growing need to develop appropriate analytical methods allowing discrimination between wild and farmed fish, and determination of their geographical origin.

The detection of origin of fish presents a great technical problem in terms of method of production. Two main approaches could be used to solve this problem. The first approach consists of determining the content of some chemical compounds or the ratio between some chemical constituents (i.e. fatty acids) and assumes that these constituents characterize that particular origin of fish. From this point of view it seems to make sense that any modification of the

diet or environment will modify the value of these ratios and will highlight a difference in its chemical composition. This approach is frequently associated with a large dataset and statistical analysis, in particular chemometric methods. Theoretically, after a multivariate analysis applied to many instrumental measurements, many similarities or differences can be found in the dataset and some particular samples can be distinguished from others for one or more variables.
An alternative approach is to search for a specific marker in the fish, which could be a chemical constituent (i.e. antioxidants, carotenoids) that definitively proves the origin of the fish or fish product.

7.2.1. Fatty acid profile

The effects of different types of lipids in the diet on growth and tissue fatty acid composition have been investigated for a number of cultured and wild species (Bergstrom, 1989; Argyropoulou et al., 1992; Haard, 1992; Shearer, 1994; Rueda et al., 1997; Serot et al., 1998; Grigorakis et al., 2002).
In all cases the farmed fish were found to have a much higher lipid content than their wild counterparts and the fatty acid profiles of farmed fish reflected the fatty acid composition of the diet that the fish has received. As a general rule, cultured fish were characterized by higher levels of monounsaturated (especially 18:1n-9), n-9 and 18:2n-6 fatty acids, whilst wild fish were characterized by higher levels of n-3 fatty acids and higher n-3/n-6 ratios. The presence of increased amounts of 18:1n-9 and 18:2n-6 in cultured fish could be explained by the abundant presence of these fatty acids in plant oil used to partially substitute fish oil in fish feed formulations. These fatty acids are not normal constituents of the marine food chain. Soybean oil, sunflower oil and rapeseed oil are commonly used in aquafeeds and are particularly rich in these fatty acids (Turchini et al., 2000; Rosenlund et al., 2001). Comparison of the fatty acid profiles using multivariate data analysis (PCA) and linear regression showed that the fatty acid profiles in muscle tissue of fish fed different oils were clearly different from their wild counterparts and very similar to those of the diet (Rosenlund et al., 2001; Turchini et al., 2003).

7.2.2. Stable isotope analysis

Isotope ratio mass spectrometry consists of measuring the isotope ratio of an analyte converted into a simple gas, which is isotopically representative of the original sample. The mass spectrometer performs isotope ratio measurements ($^{2}H/^{1}H$, $^{15}N/^{14}N$, $^{13}C/^{12}C$, $^{18}O/^{16}O$) continuously on gases of H_2, N_2, CO_2 and CO, respectively. For example, in the case of carbon, the $^{13}C/^{12}C$ ratio is naturally constant in all biological products but can fluctuate from one C source

to another (atmospheric CO_2, C_4 plants, C_3 plants, animal carbon), so it is possible to detect where these different C sources have been mixed because the $^{13}C/^{12}C$ ratio of the product will have shifted compared to that of the natural product. Examples of many applications of this technique in the investigation of flavours, wine, rice, fruit juice, vegetable oils and honey are reviewed by Meier-Augenstein (1999).

As far as fish are concerned, it is well known that differences exist between the carbon isotope ratios of freshwater and marine fish, because freshwater ecosystems generally are ^{13}C-depleted as compared with marine ecosystems (Doucett et al., 1996; Doucett et al., 1999; Power et al., 2002). Furthermore, terrestrial inputs to freshwater bodies are also more ^{15}N-depleted than marine plankton, leading to differences in nitrogen isotopic composition of these biomes. As the organisms feeding in these biomes inherit a similar isotopic signature in their body tissues, stable isotope analysis can be used to distinguish between different feeding and living environments. Doucett and colleagues (1999) studied isotopic enrichment in migrating salmon and showed that it appeared to result from mobilization, reorganization and catabolism of stored lipid and protein reserves associated with the cessation of feeding upon entering freshwater. They found significant correlations between lipid content and ^{13}C content in red muscle ($r = 0.67$) and protein content and ^{15}N in the liver ($r = 0.32$). They concluded that starvation affects the stable isotope ratios of both carbon and nitrogen in anadromous fish species. Aursand and colleagues (2000) have recently applied isotopic and compositional analysis to the study of different kinds of fish oils and lipids extracted from muscle tissue of wild and farmed salmon. These authors carried out a statistical analysis of fatty acid composition and overall hydrogen and carbon isotope ratios to select the most efficient variables for distinguishing between the different groups of salmon studied. They demonstrated that a classification analysis based on four fatty acids and the overall $(D/H)_{tot}$ isotope ratio of fish oils completely assigned the oils to the correct group. McCarthy and Waldron (2000) used the combined $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratio measurements to identify the eggs as progeny of anadromous or non-anadromous brown trout *(Salmo trutta)*.

7.2.3. Carotenoid profile

Detection of canthaxanthin or of a stereoisomer of astaxanthin deviating from that found in wild salmonids seems to be a reliable method of distinguishing farmed from wild salmonids (Storebakken and No, 1992). This is especially true when a large amount of the (3R,3'S) stereoisomer of astaxanthin is present. Turujman and colleagues (1997) have published an HPLC method to identify the synthetic form of the colour additive astaxanthin in salmon, based on differences in the relative ratios of the configurational isomers of astaxanthin.

The distribution of configurational isomers of astaxanthin in the flesh of wild Atlantic *(Salmo salar)* and wild Pacific *(Oncorhynchus spp.)* are similar, but significantly different from those of cultured salmon. This method can also be used to identify astaxanthin derived from other sources such as *Phaffia* yeast and *Haematococcus pluvialis* algae. Some criticism of these chromatographic methods came from Bjerkeng (1997), who argued that a 1:2:1 distribution of (3S,3'S), (3R,3'S) and (3R,3'R) isomers is not a fundamental property of astaxanthin synthesized for fish feeds. According to this author virtually any ratio of the astaxanthin stereoisomers can be manufactured by modern techniques. Furthermore, the stereoisomer composition of astaxanthin from decapod wastes often resembles that of racemic astaxanthin. It has been argued that no known chromatographic method is able to detect synthesized carotenoid *per se*. This is important in view of the trend to include non-traditional astaxanthin sources in fish feeds.

7.2.4. Antioxidant profile

It is well known that antioxidants have been extensively used to prevent lipid oxidation in raw materials production and fish feed formulations. Of the chemical compounds that have been investigated as antioxidants, three have been found to be effective for feeds and feed ingredients and can be used both efficiently and economically. These are ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline), BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene). EU legislation has established rules for authorizing, marketing and labelling feed antioxidant additives (Council Directive 70/524/EEC and subsequent measures). These antioxidants are lipophylic phenolic compounds, which inhibit the formation of fatty free radicals, which are fundamental to lipid oxidation. Only limited reports are available on the levels of these synthetic antioxidants in cultured fish tissue or fish-based products.

Attempts were made by Hwang and colleagues (1995) to estimate the level of synthetic antioxidants and lipid quality in fish feeds and in cultured fish in Taiwan. Sixty-eight samples of six fish species, thirty-seven feed samples for seven fish species and thirty-three samples of fish meal from six countries were collected from markets and manufacturers and were analysed. BHT was the major antioxidant found in cultured fish, fishery feed and fish meal, ranging from 0-12.3, 0-59.8 and 0-150.0 mg/kg in fish liver (or shrimp hepato-pancreas), feed and fish meal, respectively. According to the authors there was no residue of antioxidant found in fish muscle.

The determination of authorized synthetic antioxidants in fish tissue has yet to be explored as a specific marker method to distinguish cultured fish from wild fish.

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CHAPTER 2

Authentication of Farmed and Wild Turbot (*Psetta maxima*) by Fatty Acid and Isotopic Analyses Combined with Chemometrics

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Authentication of Farmed and Wild Turbot (*Psetta maxima*) by Fatty Acid and Isotopic Analyses Combined with Chemometrics

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1. Abstract

Fatty acid composition and stable isotope ratios of carbon (delta(^{13})C) and nitrogen (delta(^{15})N) were determined in muscle tissue of turbot (*Psetta maxima*). The multivariate analysis of the data was performed to evaluate their utility in discriminating wild and farmed fish. Wild (n=30) and farmed (n=30) turbot of different geographical origins (Denmark, The Netherlands, and Spain) were sampled from March 2006 to February 2007. The application of linear discriminant analysis (LDA) and soft independent modeling of class analogy (SIMCA) to analytical data demonstrated the combination of fatty acids and isotopic measurements to be a promising method to discriminate between wild and farmed fish and between wild fish of different geographical origin. In particular, IRMS (Isotope Ratio Mass Spectrometry) alone did not permit us to separate completely farmed from wild samples, resulting in some overlaps between Danish wild and Spanish farmed turbot. On the other hand, fatty acids alone differentiated between farmed and wild samples by 18:2n-6 but were not able to distinguish between the two groups of wild turbot. When applying LDA isotope ratios, 18:2n-6, 18:3n-3, and 20:4n-6 fatty acids were decisive to distinguish farmed from wild turbot of different geographical origin, while delta(15)N, 18:2n-6, and 20:1n-11 were chosen to classify wild samples from different fishing zones. In both cases, 18:2n-6 and delta(15)N were determinant for classification purposes. We would like to emphasize that IRMS produces rapid results and could be the most promising technique to distinguish wild fish of different origin. Similarly, fatty acid composition could be used to easily distinguish farmed from wild samples.

2. Introduction

Turbot (*Psetta maxima*) is a marine flatfish, naturally distributed in European waters from the Northeast Atlantic to the Arctic Circle including the Baltic, Mediterranean, and Black Seas. Wild and farmed turbot production yields about 6 000 tons annually. In the last years, a considerable proportion of the total production was derived from aquaculture (3 800 and 900 tons from Spain and France, respectively) principally along the Atlantic coast. This species has a high commercial value and is appreciated by consumers for its firm, white, and low-fat flesh.

Turbot lives on sandy, muddy-sandy, and gravel bottoms, most commonly at depths between 1 and 15 m. Adults consume primarily benthic food, such as amphipods, mysid shrimps, and small fish (Aarnio et al., 1996).

The body of turbot is scaleless and studded with numerous isolated tubercles. Tubercles are small, with mineralized conical plates randomly distributed in the eyed side of the body. The blind side of the body is completely white, while the eyed side is from sandy-brown to gray with minute brown, blackish, or greenish specks. From a morphological point of view, only an accurate inspection done by a skillful operator could lead to distinguish between wild and farmed turbot. The blind side of farmed individuals occasionally presents hypermelanosis in the form of dark spots. Environmental, nutritional, and neurological factors seem to be possible causes for the abnormal pigmentation developed in farmed turbot (Venizelos et al., 1999). In the recent years, progress in hatchery-reared flatfish, such as the enhancement of larval nutrition, has minimized these defects in farmed turbot, which are consequently more similar to their wild counterparts.

International fish trade is nowadays strongly influenced by food authenticity and safety norms, and several European Directives have introduced aspects concerning quality and safety standards into the fish chain. Particularly, the labeling regulation for fishery and aquaculture products that came into force in the European Union in 2001 (Commission Regulation (EC) No. 2065/ 2001) requires the statement of the official commercial and scientific name, the geographical origin (FAO fishery zone for wild fish, country of production for farmed fish), and the production method of fish. This regulation was aimed to provide consumers with a minimum of information on the origin of these products.

On the basis of these considerations, a number of recent studies have been published to investigate the potential of different analytical tools in distinguishing wild and farmed fish. Classically, this was carried out using scale pattern analysis (Gudjonsson, 1991) and morphological characteristics or a combination of both methods (Carr et al., 1997). More recently, many analytical procedures have been applied for this purpose, ranging from the determination

of carotenoid stereoisomers (Moretti et al., 2006) to the use of fatty acid profile and compositional analysis (Orban et al., 2003; Alasalvar et al., 2002; Chen et al., 1995; Grigorakis et al., 2002) as well as to the quantification of different levels of organic contaminants (Hites et al., 2004), in conjunction with statistical multivariate analysis (Grahl-Nielsen, 1999).

Wild and farmed fish differ in nutritional (Nettleton and Exler, 1992) sensory, chemical, and physical properties (Grigorakis et al., 2005) and diet is one of the main factors that influence these properties (Rasmussen, 2000). Additional factors such as the nature and availability of the food web, catching area, and production technologies are believed to be important contributors to these variations.

The effects of different lipid sources in the diet on growth and tissue fatty acid composition have been investigated in different cultured and wild species. In all cases, farmed fish were found to have much higher lipid content than their wild counterparts, and the fatty acid profile of farmed fish reflected the fatty acid composition of the diet. As a general rule, cultured fish was characterized by higher levels of n-6 fatty acids (especially 18:2n-6), while wild fish showed higher levels of n-3 fatty acids and a higher n-3:n-6 ratio (Moretti et al., 2003). Reasonably, the high presence of 18:2n-6 in cultured fish might be explained by the abundant presence of this fatty acid in plant oils used to partially substitute fish oil in fish feed formulation.

Isotope ratio mass spectrometry (IRMS) has been demonstrated to be a technique capable of revealing the origin of fish (Molkentin et al., 2007). Over the past few years, the determination of stable isotope ratios of light elements, especially carbon and nitrogen, has been applied to authenticity control and origin assessment of food of animal origin (Rossmann, 2001).

The isotope ratio of animals is primarily determined by diet and, to some extent, reflects their origin (De Niro and Epstein, 1978; De Niro and Epstein, 1981). The carbon isotopic composition of a terrestrial or aquatic organism reflects the isotopic composition of its diet, but the organism is usually enriched in $\delta^{13}C$ by about 1‰, relative to its diet. Thus, carbon isotope ratio remains relatively unaffected by trophic transfer. Similarly, the isotopic composition of nitrogen reflects the isotopic composition of the diet, but with an enrichment much higher than for carbon. Therefore, the consistency of nitrogen enrichment (3–5‰) at each trophic transfer provides a valuable measure of the position of an organism within the food web (De Niro and Epstein, 1976).

Stable isotope analysis has been used as a tool to control the traceability of foodstuff providing good results also for geographical approaches (Kelly et al., 2005). In recent studies, it has been also applied to study the dietary habits of aquatic animals and relative food webs (Domi et al,. 2005; Doucett et al., 2006) and the seasonal variation of the isotopic composition in fish tissues (Perga and

Gerdeaux, 2005) to distinguish anadromous and nonanadromous populations of *Salmo trutta* (McCarthy and Waldron, 2000), to establish the rearing location of juvenile salmon (Kennedy et al., 2005), and to investigate the change in isotopic composition during spawning (Doucett et al., 1999). Further and more recently, IRMS has been applied to distinguish wild and farmed gilthead sea bream (Morrison et al., 2007; Moreno Rojas et al., 2007).
The aim of the present work was to study the potential application of fatty acid analysis and IRMS of carbon and nitrogen in the muscle tissue of wild and farmed turbot to discriminate the production method and the geographical origin of fish. Both linear discriminant analysis (LDA) and soft independent modeling of class analogy (SIMCA) were applied to the data as classification tools. On the basis of analytical results, a reasonable partition of groups was studied and discussed.

3. Materials and methods

3.1. Fish

Wild and farmed turbot from different origins were sampled in March, June, October and February (15 fish per month, for a total of 60 fish). Thirty farmed turbot (FT) were from Spain (average weight 666.5 g) and were collected at the wholesale fish market of Milan (n = 24) and by local retailers (n = 6). Fifteen wild turbot were from Denmark (WT1) (average weight 578.3 g) and were collected from the wholesale fish market of Milan (n = 9) and by local retailers (n = 6). Fifteen wild turbot were from The Netherlands (WT2) (average weight 637.9 g) and were collected from the wholesale fish market of Milan (n = 13) and by local retailers (n = 2). Danish and Dutch wild turbot were caught in the NorthEast Atlantic Ocean, FAO fishery zone No 27. Farmed turbot were from farms located in Galicia waters.
Upon arrival in the laboratory, fish were accurately weighed and measured, and Fulton's K condition index (Gilliers et al., 2001) was calculated using the formula $K = \text{weight}/\text{length}^3$. This morphometric index assumes that heavier fish for the same length are in better nutritional condition. All fish were then filleted by hand,and fillets were skinned. One aliquot of approximately 50 g of each fillet was grounded and freeze-dried to eliminate the water from the tissues. This method has been recognized as the most reliable method for avoiding shifts in δ13C and δ15N isotopic signatures during storage time (Sweeting, 2004). The freeze-dried tissues were used for isotopic analyses. The remaining fillet samples were vacuum packed and stored at –20 °C until analysis.

3.2. Proximate Composition and Fatty Acid Analysis

All assays for proximate composition analysis were performed using standard methods (AOAC, 1996). The moisture content of fillets was determined by drying samples in an oven at 60 °C to constant weight. Total protein was determined by the Kjeldahl method, by which the concentration of nitrogen is measured. A factor of 6.25 was used to convert total nitrogen to crude protein. For the analysis, an automated distillation unit (Büchi 339, Switzerland) was used. The lipid content was determined by extraction with diethyl ether/petroleum benzene (1/1, v/v) in a Soxhlet system extractor (SER 148, Italy). Ash was determined by incineration of sample in a muffle furnace at 550 °C for 18 h. All analyses were done in duplicate.

For fatty acid analysis, the extraction of total lipids was performed according to Bligh and Dyer (1959). The preparation of fatty acid methyl esters (FAMEs) was performed according to Christie (1982). Briefly, the lipid sample (20 mg) was dissolved in 10% methanolic hydrogen chloride (2 mL). A 0.1 mL solution of tricosanoic acid (10 mg/mL) was added as internal standard. The sample was sealed and heated at 50 °C overnight and then 2 mL of a 1 M potassium carbonate solution was added to each sample. The FAMEs were extracted with 2 × 2 mL of hexane and 1 μL was injected into the gas chromatograph, in split mode (split ratio 1:50). Fatty acid analysis was performed on an Agilent gas chromatograph (model 6890), equipped with an automatic sampler (model 7683) and a flame ionization detector (FID). The carrier gas was helium with a flow at 1.0 mL/min and an inlet pressure of 16.9 psi. The column was an HP-Innowax fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) (Agilent Technologies). The oven temperature program was from 100 to 180 at 3 °C/min, then from 180 to 250 at 2.5 °C/min, then held for 10 min. Fatty acids were identified using external standards and quantified using tricosanoic acid as internal standard. Peak areas were corrected according to the theoretical relative FID response correction factors (TRFs) published by Ackman (2002). The results are presented as g/100 g of fatty acids (% by weight). All analyses were done in duplicate.

3.3. Isotope Measurements

$\delta^{13}C$ (signal for reference peaks = 4000 mV) and $\delta^{15}N$ (signal for reference peaks = 4000 mV) values were measured by continuous flow isotope ratio mass spectrometry (CF-EA-IRMS) using an Elemental Analyzer EA 1108 CHN (Carlo Erba, Milan, Italy; oxidation column temperature, 1050 °C; reduction column temperature, 650 °C; and GC-column, 65 °C) coupled to a DeltaPlus mass spectrometer (ThermoFischer, Rodano, Italy). Since fish muscle has a C:N ratio less than 5:1, the CF-EA-IRMS system was operated in the dual isotope

mode, allowing $\delta^{15}N$ and $\delta^{13}C$ to be measured on the same sample. Amounts of 0.60–0.75 mg of sample were weighed into tin capsules for measurements.

3.4. Standards and Equations

The results of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratio analyses are reported in per mil (‰) on the relative δ-scale and referred to the international standards V-PDB (Vienna Pee Dee Belemnite) for carbon isotope ratio and Atmospheric Air, for nitrogen isotope ratio. All results were calculated according to the following equation $\text{Delta}\ (‰) = [(R_{\text{sample}}/R_{\text{reference}}) - 1] \times 1000$ where R is the ratio of the heavy to light stable isotope (i.e., $^{13}C/^{12}C$) in the sample (R_{sample}) and in the standard ($R_{reference}$).

The calibration of the control gases (CO_2, N_2) was performed using the following reference materials:

IAEA−CH7−Polyethylene ($\delta^{13}C$ = –32.15 ‰) and IAEA−CH6−sucrose ($\delta^{13}C$ = –10.4 ‰) for CO2 gas cylinder calibration (used for $\delta^{13}C$ measurements).

IAEA−N1−Ammonium sulfate ($\delta^{15}N$ = 0.4 ‰) and IAEA−N2−ammonium sulfate ($\delta^{15}N$ = 20.3 ‰) for N_2 gas cylinder calibration (used for $\delta^{15}N$ measurements).

The precision (standard deviation) for analysis of laboratory standard (urea) for $\delta^{13}C$ is ± 0.2 ‰ (n = 10) and for $\delta^{15}N$ is ± 0.15 ‰ (n = 10).

The standard deviations of the measurements (n = 10) determined using the respective reference gas were ± 0.05 ‰ for $\delta^{13}C$ and ± 0.08 ‰ for $\delta^{15}N$. Each sample was analyzed in triplicate, and the standard deviations of these analyses were <0.18 ‰ for $\delta^{13}C$ and <0.23 ‰ for $\delta^{15}N$.

International standard, USGS-40 ($\delta^{13}C$ = –26.24 ‰ and $\delta^{15}N$ = –4.52 ‰), was used as the reference material and was analyzed, at intervals in each run, to monitor possible instrumental drift.

One muscle sample of turbot was calibrated with the international reference materials mentioned before and used as a working standard. The working standard was analyzed at regular intervals to control the repeatability of the measurements and to correct possible deviations in the measurements.

3.5. Statistical Analysis

First, basic statistics, both ANOVA and Principal Component Analysis (PCA), were performed. The homogeneity of variance was confirmed, and a comparison between means was performed by one-way ANOVA. Student–Newman–Keuls was used as the post hoc test for comparison of the means among different groups of fish. Significance was accepted at probabilities of 0.05 or less. PCA was performed to study the structure of the data and to detect the most

important variables to be submitted to the following Linear Discriminant Analysis (LDA) and soft independent modeling of class analogy (SIMCA).
The supervised LDA was applied to classify samples in clusters according to their characteristics and to identify variables able to distinguish the origin groups. LDA is a statistical method used to distinguish in groups a collection of objects, having a set of cases whose group membership is known a priori (Aursand et al., 2000). The variables were first transformed into natural logarithms, in such a way to have a normal data distribution. In the LDA, the algorithm chosen to select the variables was stepwise selection, which combines forward selection and backward elimination using the minimization of Wilks' lambda. Wilks' lambda is a measure of a variable's potential, and smaller values indicate which variable is better at discriminating between groups. The F significance level was chosen as the variable entry (less than 0.05) and removal (greater than 0.10) criterion. The number of functions obtained by LDA is equal to the number of groups minus one. So, the discriminant equations, which are a linear combination of the independent variables selected by the stepwise method, are expressed as $Dj = B0j + BijXi + \cdots + BnjXn$ where Dj is the discriminant score (j = 1, … ,m-1, where m is the number of groups), B0j is a constant term, and Bij and Xi (i = 1,…,n) are the coefficients estimated from the data and the values of each independent variable chosen by stepwise LDA, respectively.
A leaving-one-out cross validation procedure was performed to assess the accuracy of the classification rule. In this procedure, the sample data minus one observation were used for calculating discriminant functions, then the omitted observation was classified from them. This procedure was repeated a number of times equal to the number of samples (n = 60). Consequently, each sample was classified from discriminant functions which were estimated without its contribution.
SIMCA classification is based on constructing separately a PC model for each data class. Every considered sample is then assigned to one class according to its distance from the class model. Farmed and wild turbot purchased at the fish market of Milan (n = 46) were used as the training set, while samples purchased by local retailers (n = 14) were used as the testing set, to evaluate the discrimination power of the SIMCA model.
All the statistical analyses were performed by SPSS version 15.0 (SPSS Inc., Chicago, Illinois) and The Unscrambler version 9.7 (Camo, Norway). Data in the tables are reported as mean values ± the standard error of the mean (SEM).

4. Results and Discussion

4.1. Biometric Measurements and Proximate Composition

As presented in Table 1, farmed turbot were lightly heavier than wild turbot. The Fulton's K condition index in wild turbot was lower than in farmed counterparts ($p < 0.05$). These findings are rather frequent when comparing wild and farmed fish.

Table 1. Country of origin and biometric measurements on wild and farmed turbot used in this study

Fish group	N of fish	Country of origin	Eviscerated weight (g±sem)	Minimum weight	Maximum weight	Length (cm)	Fulton's K condition index[A]
WT1[B]	15	Denmark	578.3±38.1	395.0	883.2	32.2±0.6	1.70^{a}±0.16
WT2[C]	15	Netherlands	637.9±17.5	527.3	763.3	32.6±0.4	1.85^{b}±0.19
FT[D]	30	Spain	666.5±15.9	560.9	923.6	32.2±0.4	1.99^{c}±0.21

Data are reported as mean values ± standard error of the meand (SEM). Means within columns with different superscripts are significantly ($P < 0.05$) different by one-way ANOVA and S-N-K comparison test.

[A] $K = Weight/Length^3$

[B] Wild turbot from Denmark. [C] Wild turbot from The Netherlands. [C] Farmed turbot from Spain.

The proximate composition of fillets of wild and farmed turbot is presented in Table 2. The lipid content of farmed turbot was higher when compared to wild turbot. These results are in agreement with those obtained by Sérot (1998). Higher lipid content in cultured species is influenced by different factors, including type of feed, dietary ingredients, and higher energy consumption. Usually commercial feed for turbot contains fish meal and marine oil, wheat and wheat gluten, and vegetable oils (Regost et al., 2003). Its proximate composition is 14–16% of lipid and 53–55% of crude protein. The use of this type of diet produces an increase of lipid level in farmed fish when compared to wild fish. Lipid-rich diets have been demonstrated to increase both visceral and fillet fat content in turbot (Regost et al., 2001).

Table 2. Proximate composition of muscle of wild and farmed turbot

	WT1[A] *(n=15)*	**WT2**[B] *(n=15)*	**FT**[C] *(n=30)*
Moisture	79.25[c] ±78.40	78.40[b] ±0.25	77.42[a] ±0.18
Protein	19.16[a] ±19.35	19.35[a] ±0.23	20.16[b] ±0.17
Lipid	0.60[a] ±1.12	1.12[b] ±0.20	1.36[b] ±0.10
Ash	0.99[a] ±1.14	1.14[b] ±0.04	1.07[ab] ±0.03

Data are reported as mean values ± standard error of the mean (SEM). Means within columns with different superscripts are significantly ($P < 0.05$) different by one-way ANOVA and S-N-K comparison test.
[A] Wild turbot from Denmark. [B] Wild turbot from The Netherlands. [C] Farmed turbot from Spain.

Similarly to lipid, the protein content of farmed turbot was significantly higher when compared to wild turbot. Protein is considered to be a rather stable component of the fish body, depending on fish size and genetic factors. The weight of the individual fish used in our experiment was rather homogeneous (Table 1), thus the differences in protein content were presumably due to the age difference between farmed (younger) and wild (older) turbot or to unknown genetic factors.
The moisture level was higher in wild than in cultured turbot, and indeed it is generally recognized that an inverse relationship exists between water and lipid content in fish (Shearer, 1994).

4.2. Fatty Acid Composition

As a general rule, depending on the species, 18:2n-6 and 18:3n-3 are essential fatty acids for fish and are required for the production of C20 and C22 polyunsatured fatty acids, as precursors of biologically active eicosanoids. In most marine species, including turbot, also 20:5n-3 and 22:6n-3 are required for survival and growth (Owen et al., 1975). Commercial feed used for farmed turbot contain fish meal and marine oil, wheat and wheat gluten, and vegetable oils. Among these ingredients, fish oils are characterized by a high content of EPA and DHA and by the presence of 22:1n-11 (cetoleic acid) and 20:1n-9 (gadoleic acid) fatty acids. These last fatty acids derive from the corresponding fatty alcohols in the wax esters of the zooplankton (Sargent et al., 2004) and are present particularly in the triacylglycerols of pelagic fish caught in the North Sea and used for fish meal production.
Vegetable oils (soybean, corn, linseed, and rapeseed oils) are generally used in fish feed formulations for partial substitution of fish oil, as alternative and economically sustainable feed ingredients (Barlow and Pike, 1998). These oils, in

particular soybean oil, are characterized by a high proportion of n-6 polyunsaturated fatty acids, especially linoleic acid (18:2n-6).
The fatty acid composition of total lipids of muscle of wild and farmed turbot of this study is presented in Table 3.
Theoretically and as shown in data from other species (Alasalvar et al., 2002; Rueda et al., 1997), the fatty acid composition of muscle should reflect and depend on the fatty acid composition of the diet.

Table 3. Fatty acid composition of muscle of wild and farmed turbot (g/100g of fatty acids).

	WT1[A] *n=15*	**WT2[B]** *n=15*	**FT[C]** *n=30*
C14:0	$2.22^{a} \pm 0.16$	$2.51^{a} \pm 0.23$	$3.84^{b} \pm 0.16$
C16:0	$18.88^{b} \pm 0.22$	$19.19^{b} \pm 0.38$	$17.47^{a} \pm 0.31$
C18:0	$6.70^{b} \pm 0.37$	$6.37^{b} \pm 0.52$	$5.25^{a} \pm 0.26$
Σ SFA	27.79 ± 0.44	28.07 ± 0.67	26.57 ± 0.42
C16:1n-7	4.28 ± 0.34	4.84 ± 0.49	4.72 ± 0.21
C18:1n-7	3.91 ± 0.36	3.41 ± 0.16	3.35 ± 0.26
C18:1n-9	11.95 ± 0.34	12.23 ± 0.66	11.67 ± 0.24
C20:1n-9	$1.02^{a} \pm 0.11$	$1.07^{a} \pm 0.11$	$1.59^{b} \pm 0.12$
C20:1n-11	$0.34^{ab} \pm 0.05$	$0.42^{b} \pm 0.07$	$0.25^{a} \pm 0.02$
C22:1n-9	0.21 ± 0.02	0.26 ± 0.04	0.26 ± 0.02
C22:1n-11	$0.28^{a} \pm 0.16$	$0.47^{a} \pm 0.11$	$1.19^{b} \pm 0.14$
Σ MUFA	21.98 ± 1.01	22.70 ± 1.43	23.05 ± 0.71
C18:2n-6	$1.59^{a} \pm 0.12$	$0.97^{a} \pm 0.08$	$8.96^{b} \pm 0.37$
C18:3n-6	0.17 ± 0.02	0.15 ± 0.02	0.20 ± 0.01
C20:2n-6	$0.47^{b} \pm 0.02$	$0.34^{a} \pm 0.03$	$0.64^{c} \pm 0.02$
C20:3n-6	0.15 ± 0.02	0.14 ± 0.01	0.17 ± 0.00
C20:4n-6	$4.34^{c} \pm 0.35$	$2.99^{b} \pm 0.24$	$1.81^{a} \pm 0.08$
Σ N-6	$6.70^{b} \pm 0.32$	$4.59^{a} \pm 0.24$	$11.79^{c} \pm 0.37$
C18:3n-3	$0.77^{b} \pm 0.13$	$0.48^{a} \pm 0.06$	$1.09^{c} \pm 0.05$
C18:4n-3	$0.67^{a} \pm 0.12$	$0.76^{a} \pm 0.14$	$1.31^{b} \pm 0.08$
C20:5n-3	$9.74^{a} \pm 0.32$	$10.12^{a} \pm 0.31$	$11.14^{b} \pm 0.22$
C22:5n-3	$5.85^{b} \pm 0.35$	$6.66^{b} \pm 0.56$	$4.22^{a} \pm 0.08$
C22:6n-3	$26.83^{b} \pm 0.81$	$26.61^{b} \pm 1.20$	$21.28^{a} \pm 0.64$
Σ N-3	$43.85^{b} \pm 0.79$	$44.64^{b} \pm 0.94$	$39.04^{a} \pm 0.54$
Σ PUFA	50.56 ± 0.83	49.23 ± 0.96	50.83 ± 0.46
Σ HUFA	$47.42^{b} \pm 0.84$	$47.15^{b} \pm 1.04$	$39.76^{a} \pm 0.60$
N-3/N-6	$6.80^{b} \pm 0.38$	$10.21^{c} \pm 0.64$	$3.44^{a} \pm 0.13$

Data are reported as mean values ± standard error of the meand (SEM). Means within columns with different superscripts are significantly ($P < 0.05$) different by one-way ANOVA and S-N-K comparison test.
[A] Wild turbot from Denmark. [B] Wild turbot from The Netherlands. [C] Farmed turbot from Spain.

Among saturated fatty acids (SFA), farmed turbot showed a lower percentage of 16:0 and 18:0 and a higher percentage of 14:0 than their wild counterparts. The sum of SFA did not present statistically significant differences between groups. The saturated fatty acids 16:0 and 18:0 can be biosynthesized in fish (Sargent et al., 1989) and can be desaturated by a Δ9 desaturase to 16:1n-7 and 18:1n-9, respectively. These activities are presumably regulated by water temperature and by the presence of essential fatty acids in the diet. In marine wild fish, the final metabolic pathway is not well studied, but it is possible that this enzyme is involved in enhancing monounsaturated fatty acid production in response to a lowered water temperature so as to maintain membrane fluidity, as demonstrated in carp (Tiku et al., 1996).

Monounsaturated fatty acids (MUFA), in particular 20:1n-9 and 20:1n-11, were higher in farmed than in wild animals. These are typical fatty acids of fish oils contained in feed and are transferred to the muscle of farmed fish. These results are consistent with those published by Sérot (1998), who compared the fatty acid composition of muscle from farmed and wild turbot.

All polyunsaturated fatty acids (PUFA) of the n-6 series, except of 20:4n-6, were higher in farmed fish than in other groups. Interestingly, 18:2n-6 was much higher in farmed fish (8.96% vs 0.82%) than in wild fish, while the end product of its desaturation and elongation (20:4n-6) was significantly lower in farmed fish. Also, the intermediates of 20:4n-6 synthesis, 18:3n-6 and 20:3n-6, were at very low levels in farmed groups. Other studies have shown higher percentages of 20:4n-6 in wild fish when compared to its farmed counterpart (Grikorakis et al., 2002; Rueda et al., 1997; Sargent et al., 1989).

This fact could be explained with the reduced capacity of turbot to synthesize highly unsaturated fatty acids (HUFA) from C18 precursors (Owen et al., 1975). Tocher et al. (1989) demonstrated that cultured cells from turbot were unable to elongate C18 into C20. Furthermore, desaturases are known to have a better affinity for n-3 fatty acids than for n-6 fatty acids in turbot (Tocher, 1993). Reasonably the bioconversion of 18:2n-6 into 20:4n-6 is more effective when the diet is deficient in n-3 PUFA (Henderson and Tocher, 1987).

Concentrations of 18:3n-3, 18:4n-3, and 20:5n-3 were higher in farmed fish than in wild fish. In contrast, 22:5n-3 and 22:6n-3 were significantly higher in wild than in farmed turbot. It could be possible that the commercial feed contained a high percentage of EPA, which resulted in a higher amount of these fatty acids in farmed turbot. When comparing the fatty acid composition within wild turbot, the WT1 group showed a higher amount of 20:4n-6 than the WT2 group.

4.3. Isotope Measurements

The results of isotopic analysis of the three groups of wild and farmed turbot are presented in Table 4. Carbon isotopic ratios were statistically different between farmed and wild turbot ($p < 0.01$). When considering separately Danish and Dutch samples, $\delta^{13}C$ of farmed turbot were statistically different from wild Danish but not from wild Dutch fish, whereas nitrogen isotopic ratios had a high variability between groups, showing a significant increment from farmed to wild Danish turbot ($p < 0.01$).

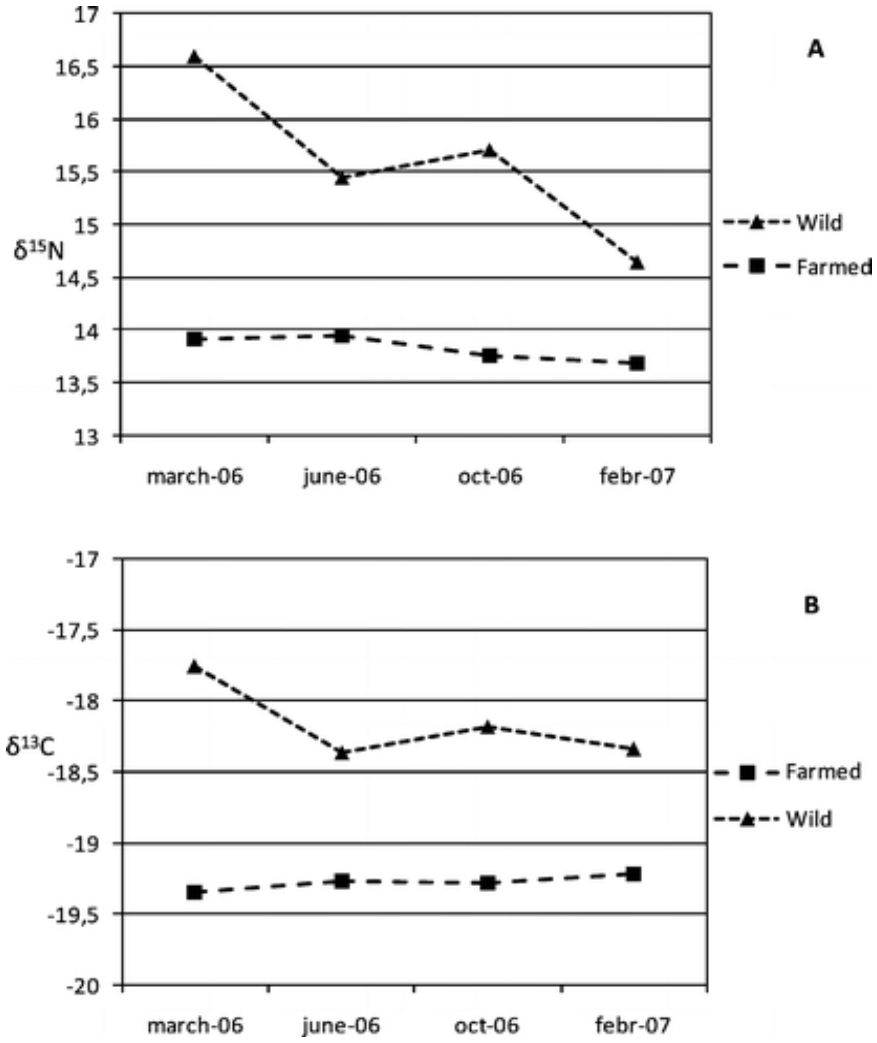
Table 4. Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in wild and farmed turbot

	$\delta^{13}C$				$\delta^{15}N$			
Group	Mean	Min	Max	SEM	Mean	Min	Max	SEM
FT (n=30)	-19.27 a	-20.01	-18.75	0.06	13.82 a	12.18	15.04	0.13
WT1 (n=15)	-18.98 a	-21.58	-15.88	0.41	14.48 b	12.66	17.74	0.34
WT2 (n=15)	-17.33 b	-19.56	-16.50	0.21	16.81 c	14.14	17.87	0.24
Total WT	-18.15	-21.58	-15.88	0.28	15.64	12.66	17.87	0.30

Means within column with different letters are significantly ($P<0.05$) different by one-way ANOVA and S-N-K comparison test.

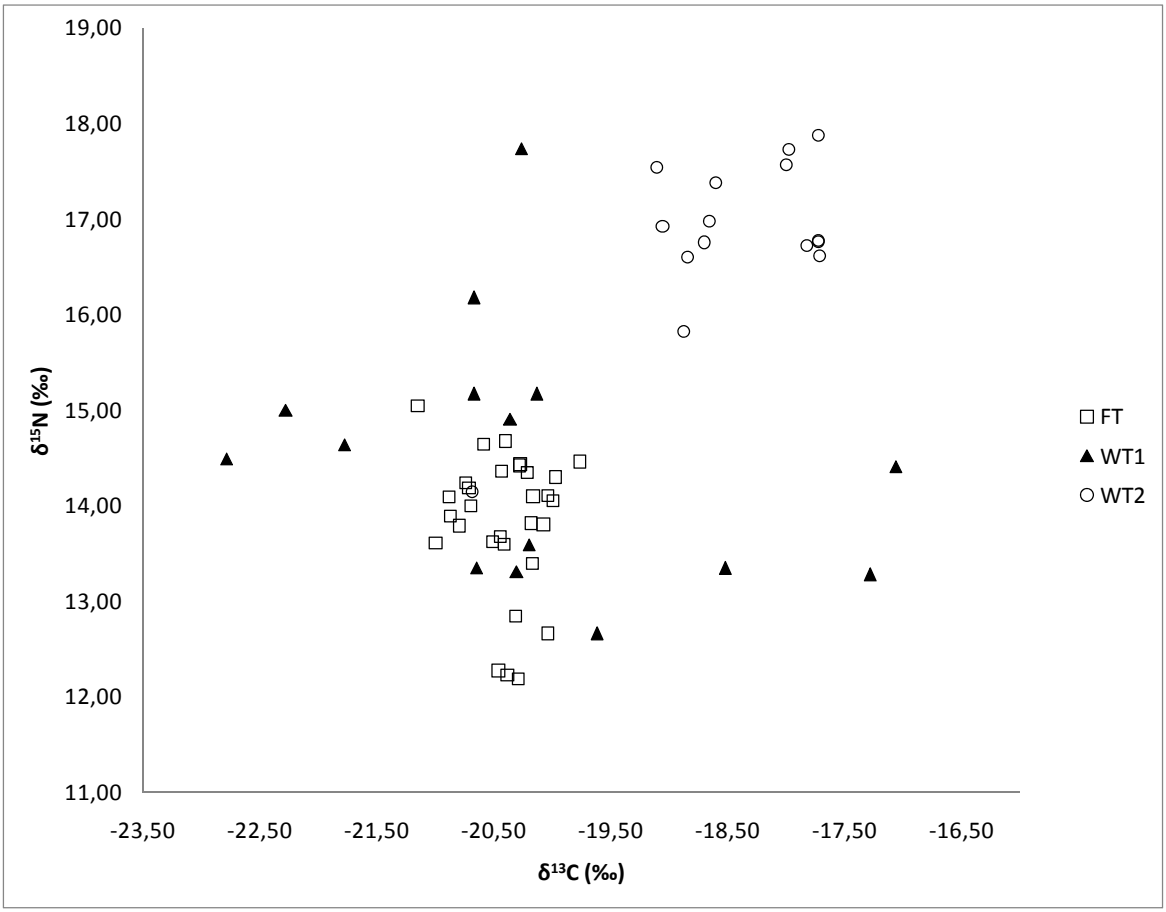
In Figure 1, the isotopic measurements of wild and farmed turbot grouped according to the month of sampling are presented. The isotopic composition of the farmed samples remains relatively constant during the feeding period, due to the application of the same process of production (diet composition and dose). On the other hand, small differences can be observed on the carbon and nitrogen isotopic ratios for wild samples, probably arising from different availability of food during the year and other additional parameters (metabolism). During the periods of scarcity of food, the fish uses the reserves accumulated in its body. Consequently, more positive values in the case of nitrogen and less negative values in the case of carbon are observed. An additional factor that can influence in the same way is the reproductive period of the turbot (spring time) during which the fish does not eat any food for a long time. On the other hand, for the farmed turbot, the type and the quantity of feed received is the same during all the feeding periods, and in addition, the farmed turbot does not reproduce during that period.

Figure 1. Monthly variation of ($\delta^{15}N$) (A) and ($\delta^{13}C$) (B) values in wild and farmed turbot.



As evidenced in Figure 2, isotopes permitted a clear discrimination between Spanish farmed turbot and Dutch wild turbot. There are two homogeneous groups with differences in $\delta^{13}C$ and $\delta^{15}N$ ratios. These differences between farmed and wild fish can be related to the diet of the two groups of fish. Muscle from farmed fish has more negative values of $\delta^{13}C$ than from wild ones, due to a diet less variable and richer in fat. The high-fat diet of farmed fish produces tissues with a higher lipid content, inducing a larger isotopic fractionation of ^{13}C than the one found for wild fish, for which the scarcity of food induces a higher metabolic turnover, resulting in less accumulation of fat in the tissues. For this reason, farmed fish should have lighter $\delta^{13}C$ ratios (values more negatives) than wild ones.

Figure 2. ^{15}N vs ^{13}C values of muscle of wild (WT1 and WT2) and farmed turbot (FT)



The differences in $\delta^{15}N$ values are not as well defined because the values for nitrogen isotope ratios for both groups depend on protein content and mostly on the origin and type of protein of the diets of both types of fish. The natural diet of wild turbot consists mainly of benthic food, such as amphipods, mysid shrimps, and small fish, and its composition could vary depending on the availability of prey and on the geographical origin of fishing areas. Whereas for farmed fish, the diet formulation can change depending on the percentage or the origin of the protein added to the diet (animal or vegetable sources). For this reason, the variation in $\delta^{15}N$ values in our case may be due to the differences between "natural diet" and feed administered.

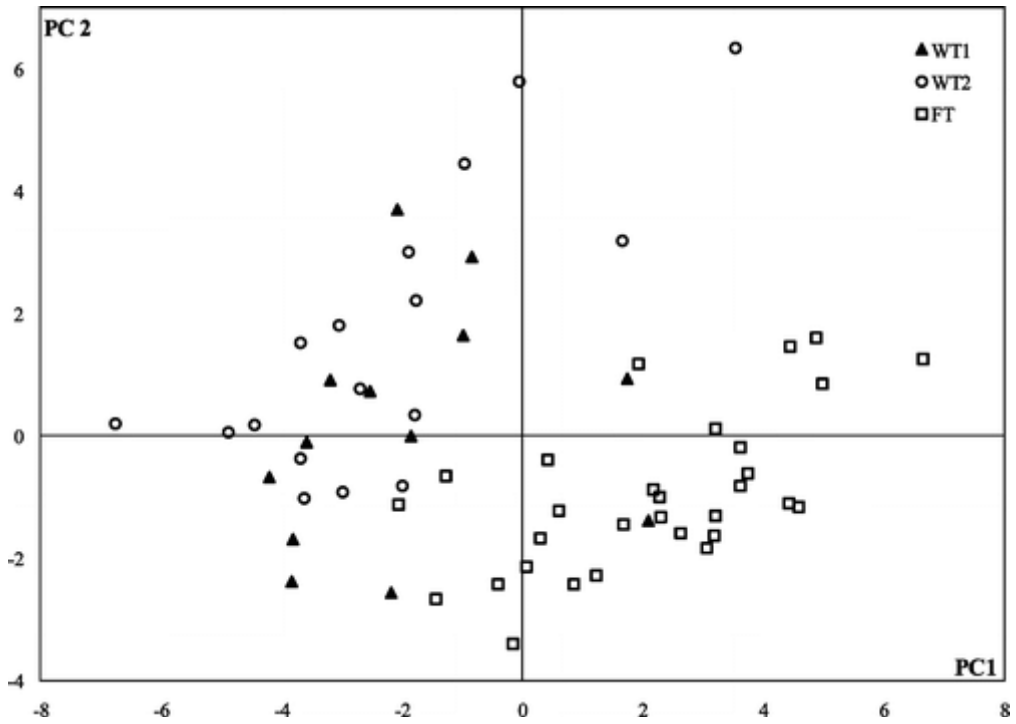
On the other hand, the values obtained for Danish wild turbot are widely spread in Figure 2. The reasons for this behavior are not simple to address. One of the things that would affect the isotopic results obtained in this way could be the different geographical origin of the samples and the different sources and availability of food in places well separated geographically. The fact that the fishery in FAO fishery zone 27 can be done in such different places like Bornholm Island (southeast, between Sweden and Poland), the Faroe Islands

(northwest, between Scotland and Iceland), and Greenland (American continent) would support the previously mentioned hypothesis, and a more extensive sampling in all these specific areas should be done to perform an isotopic map of the samples and to better explain the differences given by our results.

4.4. Principal Component Analysis

PCA was used to provide an overview of the capacity of the variables (chromatographic and isotopic measurements) to discriminate wild from farmed samples and to find the discriminating power of the variables. After applying PCA to our data set, three PCs were extracted. The percentage of variance explained by each PC was 36.9, 18.2, and 9.8%, respectively. According to the loading of the variables in the first PC, the most contributing descriptors were 14:0, 16:0, 18:4n-3, 22:6n-3, and 20:4n-6. Furthermore, the correlation loadings showed strong correlation between 16:0, 18:0, 20:4n-6, 22:6n-3, and between 14:0 and 18:4-n3. When representing the scores of the turbot samples on the two-dimensional space defined by the calculated PCs (Figure 3), farmed samples appeared well distinguished from wild samples. Wild samples coming from Denmark do not seem to separate from those coming from the Netherlands.

Figure 3. Principal component analysis: score plot of the turbot samples in the bidimensional space of the first two PCs



4.5. Linear Discriminant Analysis

First, LDA was used to classify the two groups of turbot (farmed and wild) without considering the different origins of wild samples. After applying LDA, one discriminant function was obtained. Table 5 shows independent variables selected and the calculated discriminate factors. Interestingly, the variables selected by stepwise statistics demonstrated that isotope ratios and n-6 fatty acids gave the most important contribution to discriminate between wild and farmed fish. A complete separation of the two groups was achieved.

Table 5. First factor (LDA1) coefficient, the Wilks' lambda and the percentage of correctly classified samples of linear discriminant analysis applied on fatty acid and isotopic measurements of turbot grouped according to the production method (farmed vs wild turbot)

Variable	Function coefficients
$\delta^{15}N$	0.446
$\delta^{13}C$	-0.482
18:2n-6	-4.287
18:3n-3	1.977
20:4n-6	2.316
Constant	-12.075
Willks' lamba	0.046
Original samples correctly classified	100%
Cross validated samples correctly classify	100%

The recognition ability was 100% for each class. The leaving-one-out cross validation procedure used to evaluate the classification performance confirmed a prediction ability of 100% for the two classes.

When applying LDA to distinguish farmed turbot from wild turbot of different geographical origin (three classes), two discriminant functions were obtained (Table 6). Also, in this case, isotope ratios, 18:2n-6 and 20:4n-6 were selected.

One Danish wild sample was classified as Dutch, and two Dutch wild samples were classified as Danish. All farmed Spanish samples were classified correctly. The recognition ability for all groups was 96.7%, while the leave-one-out cross validation method showed a prediction ability of the functions of 95.0%.

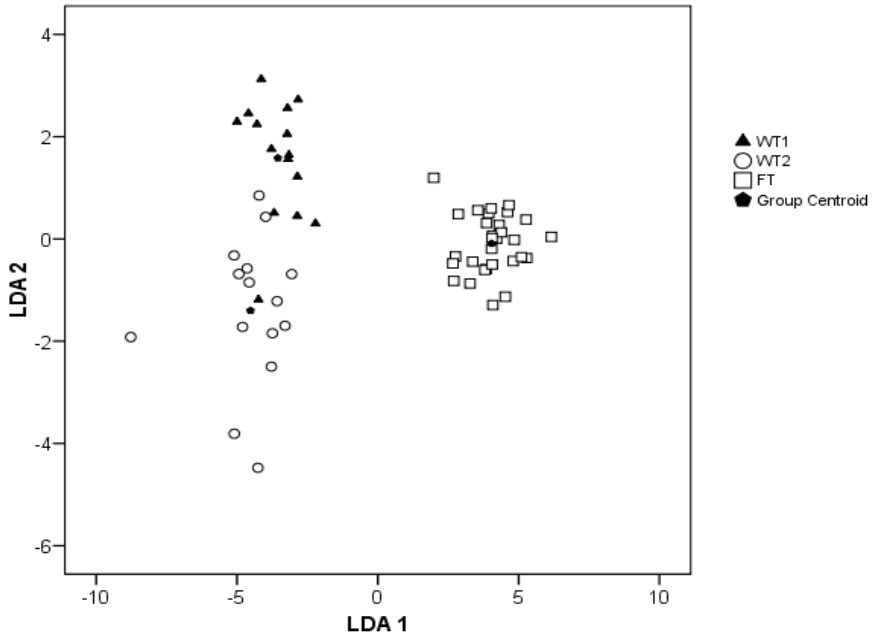
Table 6. First and second factor (LDA1 and LDA2) coefficients, the explained percentage of variance, the Wilks' lambda and the percentage of correctly classified samples of linear discriminant analysis applied on fatty acid and isotopic measurements of wild and farmed turbot, grouped according to the geographical origin.

Variable	Function 1	Function 2
$\delta^{15}N$	-0.700	-0.373
$\delta^{13}C$	-0.346	-0.501
18:1n-9	-3.962	0.059
18:2n-6	4.704	-0.200
18:3n-3	-0.905	1.543
20:1n-11	1.131	0.804
20:4n-6	-1.653	3.119
Constant	23.761	-5.074
Explained of variance (%)	95.3	4.7
Willks' lamba	0.013	0.401
Original samples correctly classified	96.7%	
Cross validated samples correctly classify	95.0%	

Figure 4 shows the plot given by the first and the second discriminant functions accounting for 95.3% and 4.7% of variance of the total between-groups variability, respectively. The discrimination between farmed turbot and wild turbot was clearly displayed along the first linear discriminant function. The second function led, to a minor extent, to the separation of WT1 from WT2 wild samples.

When applying LDA to classify wild samples from different catching zones, the classification results were 93.3% for the two classes, and the leave-one-out cross validation method showed a prediction ability of the functions of 93.3%. Among 30 samples analyzed, only two samples were incorrectly classified.

Figure 4. Canonical discriminant functions of wild turbot (WT1 and WT2) and farmed turbot (FT).



The discriminate function obtained is presented in Table 7. The independent variables selected were $\delta^{15}N$, 18:2n-6, and 20:1n-11. These could be considered the most discriminate variables in distinguishing the two groups of wild turbot.

Table 7. First factor (LDA1) coefficient, the Wilks' lambda and the percentage of correctly classified samples of linear discriminant analysis applied on fatty acid and isotopic measurements of wild turbot grouped according to the country of origin (WT1 vs WT2).

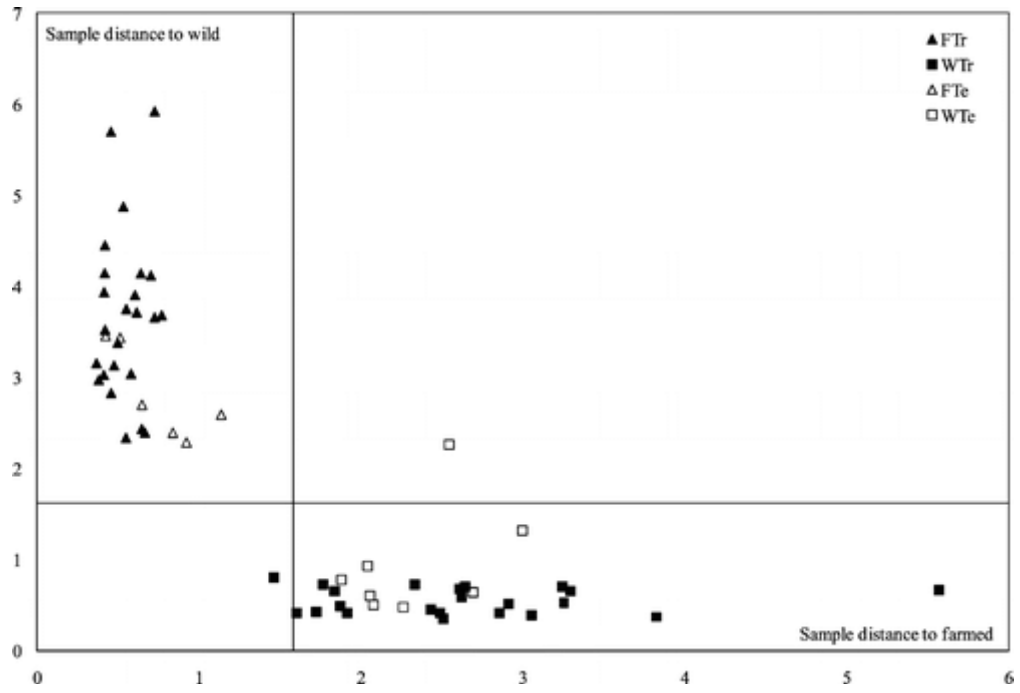
LDA1	**Function coefficients**
$\delta^{15}N$	-0.851
18:2n-6	1.969
20:1n-11	0.910
Constant	14.077
Willks' lamba	0.319
Original samples correctly classified	93.3%
Cross validated samples correctly classify	90.0%

4.6 Soft Independent Modeling of Class Analogy (SIMCA)

To apply SIMCA procedure to the samples, the data set was split into training (46 samples: 22 wild and 24 farmed) and testing (14 samples: 8 wild and 6 farmed) sets. The training set consisted of samples purchased at the wholesale fish market of Milan, while the testing set consisted of samples collected from the local retailers.

The separation of the studied groups can be easily shown by using the Cooman plot, as can be seen in Figure 5.

Figure 5. Cooman's plot of the SIMCA model calculated on wild and farmed samples. Letters F and W indicate farmed and wild samples, respectively. Letters Tr and Te indicate training and testing samples, respectively. Horizontal and vertical lines represent the critical distance (p = 0.05) of the sample from the wild and farmed models, respectively. As evidenced, one wild sample is not correctly classified.



The Cooman plot shows the samples to model distances both for training and for testing samples. If a sample truly belongs to a class, it should fall within the membership limit, that is, to the left of the vertical line and below the horizontal line. The sensitivity and the specificity of the SIMCA model were calculated (Table 8).

Table 8. SIMCA sensitivity and specificity

SIMCA	Sensitivity	Specificity
Trainig set (46)	100	100
Testing set (14)	87.5	100

The sensitivity represents the percentage of samples belonging to a class that are correctly classified by the class model, while specificity is the percentage of samples not belonging to a class that are correctly rejected by the class model. As shown in Figure 5, only one wild sample was incorrectly classified.

5. Conclusions

This study showed that fatty acid composition and isotopic analysis of carbon and nitrogen allowed, if applied together, to discriminate between farmed turbot and wild turbot of different origin. IRMS alone did not permit us to separate completely farmed from wild samples. In fact, there were overlaps between samples, especially between Danish wild and Spanish farmed turbot. Interestingly, Dutch and Danish wild fish showed different behavior in isotopic composition both for carbon and for nitrogen isotopes, reasonably due to the different living environment. Furthermore, seasonal variation of isotopic composition was found according to the month of sampling, reflecting different growth rates of fish and food consumed during these periods.

On the other hand, fatty acids alone differentiated between farmed and wild samples by 18:2n-6 but were not able to distinguish between the two groups of wild turbot. The application of linear discriminant analysis and soft independent modeling of class analogy to various combinations of analytical data demonstrated that the combination of fatty acids and isotopic measurements led to a promising method to discriminate between wild and farmed fish and between wild fish of different geographical origin. When applying LDA to distinguish farmed turbot from wild turbot of different geographical origin, isotope ratios, 18:2n-6, 18:3n-3, and 20:4n-6 fatty acids were decisive, while to classify wild samples from different zone of catching $\delta^{15}N$, 18:2n-6 and 20:1n-11 were chosen. In both case, 18:2n-6 and $\delta^{15}N$ were decisive.

We would like to emphasize that IRMS produces rapid results and could be the most promising technique to distinguish wild fish of different origin. Similarly, fatty acid composition could be simply used to distinguish farmed from wild samples.

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CHAPTER 3

FATTY ACID COMPOSITION AND VOLATILE COMPOUNDS OF CAVIAR FROM FARMED WHITE STURGEON (*ACIPENSER TRANSMONTANUS*)

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Fatty acid composition and volatile compound of caviar from farmed white sturgeon (*Acipenser transmontanus*)

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1. Abstract

The present study was conducted to characterize caviar obtained from farmed white sturgeons (*Acipenser transmontanus*) subjected to different dietary treatments. Twenty caviar samples from fish fed two experimental diets containing different dietary lipid sources have been analysed for chemical composition, fatty acids and flavour volatile compounds. Fatty acid make up of caviar was only minimally influenced by dietary fatty acid composition. Irrespective of dietary treatments, palmitic acid (16:0) and oleic acid (OA, 18:1 n-9) were the most abundant fatty acid followed by docosahexaenoic acid (DHA, 22:6 n-3) and eicopentaenoic (EPA, 20:5 n-3).

Thirty-three volatile compounds were isolated using simultaneous distillation–extraction (SDE) and identified by GC–MS. The largest group of volatiles were represented by aldehydes with 20 compounds, representing the 60% of the total volatiles. n-Alkanals, 2-alkenals and 2,4-alkadienals are largely the main responsible for a wide range of flavours in caviar from farmed white sturgeon.

2. Introduction

The Italian production of farmed caviar from white sturgeon has recently increased, almost exponentially, reaching over 20 metric tons in 2006 and, even though apparently limited, represents the world largest farmed caviar production. The successful caviar production from captive sturgeon has recently attracted increasingly research interest aiming to evaluate the chemical characterization of the final product. Chemical assays together with sensory studies have been carried out with the purpose to determine whether differences might be observed between caviar from farmed and wild sturgeon (Bledsoe et al., 2003; Wirth et al., 2000). For this purpose, different analytical procedures have been applied such as the determination of fatty acid profiles and compositional analysis (Gessner et al., 2002 a; Czesny et al., 2000), the quantification of organic contaminants and metals (Krüger and Pudenz 2002) and sensory evaluation

(Cardinal et al., 2002). In flavour analysis, the isolation of analytes is a crucial step. It usually involves the use of concentration–extraction equipments/methods, such as vacuum distillation, liquid–liquid extraction, simultaneous distillation–extraction (Chaintreau, 2001), static and dinamic head space methods (Prost et al., 2004; Tanchotikul and Hsieh, 1989), solid phase micro-extraction (Guillen et al., 2006; Roberts et al., 2000), supercritical fluid extraction (Aro et al., 2002), pressurized liquid extraction (Carabias-Martinez et al., 2005) and stir bar sorptive extraction (Baltussen et al., 1999; Baltussen et al., 2002). Extraction methods are frequently revised and improved in order to reduce running costs and analytical time, and to increase sensitivity or application versatility. Several comparative studies revealed that the implementation of different sample preparation techniques, and in some cases the simple use of different solvents, may significantly affect the composition and the content of the flavour extract (Wilkes et al, 2000).

Not exhaustive methods based on adsorptive or absorptive extraction, such as SPME or SBSE, are currently increasingly used even though recognised to be strongly influenced by the selectivity of fibre coating and several other factors (Steffen and Pawliszyn, 1996). On the other hand, isolates obtained using exhaustive extraction methods such as solvent extraction and distillation, being responsible of possible artefacts, do not always reflect the exact composition of the odour of the matrix (Steffen and Pawliszyn, 1996).

Thus, the choice of the implemented isolation method should be primarily based on the peculiarities of the samples under investigation and the objectives of the study. For example, in olfactometric applications, it results more advantageous to apply isolation methods which reflect the release of the volatile compounds from the food matrix, rather than determining the overall amount of those volatiles (Prost et al., 2004; Tanchotikul and Hsieh, 1989), and consequently static and dynamic headspace methods should be, in these instances, preferentially used. Conversely, for characterization purposes such as in the present study, an exhaustive method of extraction should be preferred, even in the presence of some limitations.

The simultaneous distillation–extraction (SDE) was created in 1964 when Likens and Nickerson designed an original device for the analysis of hop oil. In 1981, Godefroot et al. brought a decisive improvement to this technique, that became widely used in flavour laboratories. When comparing the recoveries of a flavour model mixture from water using various extraction methods, Reineccius (1993) found that SDE appeared as the method yielding the most representative extract. The presence of artefacts, caused mainly by the oxidation and thermal degradation of some components in the extract, is a well known drawback of SDE and depends on the type of food matrix analysed. Artefacts formation have

been extensively investigated by Chaintreau (2001), and might be minimised using proper operating conditions (Escudero and Etie, 1999).
This study was designed to characterize and typify caviar obtained from farmed white sturgeons (*A. transmontanus*) fed two experimental diets containing different dietary lipid sources. Twenty caviar samples from different fish fed the two diets, for 3 or 6 months before harvesting, have been analyzed for chemical composition, fatty acids and flavour volatile compounds. The effects of the sturgeon diet on the quality of caviar are presented and discussed.

3. Materials and methods

3.1. Animals, husbandry, and experimental diets

The fish used in the present experimentation were white sturgeons (*A. transmontanus*) reared in a large intensive farm (Agroittica Lombarda SpA, Calvisano, Italy). Broodstock fish were reared in ponds with flowing bore water. The ponds were 500 m^2 × 1.6 m depth and the water flow rate assured a complete water replacement every 24 h. The stocking density was 15 kg m^{-2}. Fish were fed at 0.3% (kg feed/100 kg body weight) daily and feed was distributed by automated feeders.
Fish were fed with a commercial sturgeon extruded feed until the beginning of the experiment. Subsequently, 40 white sturgeons were split in two groups, transferred into circular tanks (80 m^2 each) and subjected to two different experimental diets before harvesting and roe collection for caviar production.
The experimental diets consisted of a lean extruded feed (Biomar, Treviso, Italy) (ingredients: fish meal, oily seeds by-products, cereals grains, cereals grains by-products, vitamins and minerals) containing 43% protein 3% lipid, subsequently coated with 10% of two different lipid sources: liver squid oil (SQ diet) and a blend of 50% soybean oil and 50% fish oil (SB diet). Egg collection was carried out after 3 (SB3, SQ3) and 6 months (SB6, SQ6) from the beginning of the experiment.
In Table 1 the proximal and fatty acid composition of the experimental diets are reported.

3.2. Caviar collection

After 3 and 6 months from the beginning of the feeding trial five sturgeons from each experimental group were sacrificed for the collection of the eggs. The roe was removed by opening the body cavity and the eggs were rubbed through a mesh sieve and washed. Finally, the eggs were salted with sodium chloride (NaCl < 3%) and canned in 50 g cans. A total of 20 caviar cans were sampled; 5

cans per treatment. The excellent hygienic conditions implemented to process sturgeon roe into caviar permitted the low salt content and no need for additives. Caviar samples were immediately store at −1 °C in a refrigerated storage room until analyses.

3.3. Proximate composition and fatty acid analysis

Caviar samples were analysed for moisture, protein, lipid, ash using AOAC standard methods (AOAC, 1981). Briefly, moisture by heating at 60 °C to constant weight, protein by estimating the Kjeldahl nitrogen (6.25×) in an automated distillation unit (Büchi 339, Switzerland), lipid by Bligh and Dyer extraction (1959), ash by incinerating in a muffle furnace at 550 °C for 18 h.
After the extraction of total lipids, the preparation of fatty acid methyl esters for fatty acid analysis was performed according to Christie (2003). Briefly, the lipid sample (20 mg) was dissolved 10% methanolic hydrogen chloride (2 mL). A 0.1 mL solution of tricosanoic acid (10 mg mL^{-1}) was added as internal standard. The sample was sealed and heated at 50 °C overnight; then, 2 mL of a 1 M potassium carbonate solution were added to each sample. The FAMEs were extracted with 2 mL × 2 mL of hexane and 1 μL was injected into the gas-chromatograph, in split mode (split ratio 1:50). Fatty acid analysis was carried out on an Agilent gas-chromatograph (Model 6890 Series GC) fitted with an automatic sampler (Model 7683 Series Injector) and FID detector. The conditions used were the following: HP-Innowax fused silica capillary column (30 m × 0.25 mm I.D., 0.30 μm film thickness; Agilent Technologies), temperature programmed from 150 to 180 °C at 3 °C min^{-1}, then from 180 to 250 °C at 2.5 °C min^{-1}, then held for 10 min. Carrier gas was helium at 1.0 mL min^{-1}, inlet pressure 16.9 psi. Fatty acids were identified relative to known external standards. The resulting peak areas were then corrected by theoretical relative FID response factors (Ackman, 2002) and quantified relative to the internal standard. All analyses were done in duplicate. The double bond index (DBI) was obtained calculating the sum of the percentage of each unsaturated fatty acid multiplied by its number of double bonds and divided by the percentage of saturated fatty acids.

3.4. Flavour volatile compounds

The extraction of volatile compounds was performed using a micro simultaneous distillation–extraction apparatus (Chrompack, Middelburg, NL) similar to that described by Godefroot et al. (1981) in the configuration suitable for heavier-than-water solvent, a water bath (Julabo, Seelbach, Germany), and a minichiller (Huber, Offemburg, Germany). Two grams of caviar were placed in a

250 mL round-bottom flask with 100 mL of purified water containing 10 μL *n*-undecane (0.2 mg mL^{-1}) as internal standard, and the flask was attached into the appropriate arm of the SDE apparatus. A 5 mL vial containing 2 mL of methylene chloride in which were dissolved 2,6-di-*tert*-butyl-4-methylphenol (BHT) (50 mg L^{-1}) as antioxidant was linked to the other arm of the SDE apparatus. The separation chamber was filled with 2 mL of methylene chloride and 2 mL of distilled water. The sample was then heated to boiling and simultaneously extracted by methylene chloride heated in a waterbath at 60 °C for 2 h. The condensation was assured by the circulation of polyethyleneglycol at −5 °C. Before GC injection the organic extract was dried over anhydrous sodium sulphate. All reagents and solvents were from Merk (Darmstadt, Germany).

Compounds were analysed in an Agilent 6890 Series GC system coupled to a 5973 N mass selective detector. The separation was performed on a DB-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness) (Supelco, Bellefonte, PA, USA). Carrier gas was helium with a linear flow rate of 1 mL min^{-1}. The oven temperature program was: 32 °C held for 1 min, from 32 to 80 °C at 120 °C min^{-1}, from 80 to 280 °C at 5 °C min^{-1}. Samples of 1 μL were injected in pulsed splitless mode (purge flow 20 mL min^{-1} at 1 min). Mass spectra were obtained under EI condition at 70 eV in the 35–300 amu range. Ion source was held at 230 °C and quadrupole at 150 °C.

Volatile compounds were identified by matching mass spectral data of sample components with those of known compounds from library databases (NIST 98 and Wiley 275). Authentic standards of several detected compounds were analysed in order to confirm the reliability of retention times and tentative identities. The retention index (RI) proposed by Kovàtz was calculated for comparison of retention data from literature (Castello, 1999). The data were recorded and analysed with the HP Chemstation Software. The concentration of compounds was semi-quantitatively determined using *n*-undecane as internal standard and expressed in μg equivalents of undecane for 1 kg of caviar (μg kg^{-1} on a wet basis). All samples were extracted and analysed in duplicate.

3.5. Data handling

Statistical analysis was performed on compositional, fatty acids and volatile compounds by one-way ANOVA. Student–Newman–Keuls was used as post hoc test for comparison of the means among different groups. Significance was accepted at probabilities of 0.05 or less. The statistical analysis were performed by SPSS 15.0 statistical package (SPSS Inc. Chicago, IL).

4. Results and discussion

4.1. Proximate and fatty acid composition of diets

The proximate composition of the experimental diets is shown in Table 1.
Diets were *iso*-lipidic being the total lipid recorded for the two diets 13.2 and 13.6% (g/100 g wet weight) for SQ and SB, respectively. However, they differed in their fatty acid content. The diet enriched with soybean oil (SB) had a higher proportion of n-6 fatty acids and lower proportion of n-3 fatty acids than the diet enriched with squid oil (SQ). The high levels of n-6 were due entirely to an elevated level of linoleic acid (18:2n-6), typical of vegetable oils. Consequently, the high levels of n-6 had a marked effect on the ratio of n-3 to n-6 in the SB diet. On the other hand, docosahexaesanoic acid (DHA, 22:6n-3), one of the most representative polyunsaturated fatty acids (PUFA), was lower in SB diet than the SQ diet, varying from 10.39 to 17.17%, respectively.

Table 1. Proximal composition and total fatty acid composition of the feed fed to sturgeons

Fatty acid (wt %)	SB	SQ
14:00	5.76	5.44
16:00	17.57	18.56
16:1n-7	5.96	6.25
18:00	3.53	3.51
18:1n-9	15.28	13.78
18:1n-7	2.79	3.45
18:2n-6	16.58	7.54
18:3n-6	0.11	0.11
18:3n-3	2.76	1.55
18:4n-3	2.4	2.09
20:1n-9	5.22	5.58
20:4n-6	0.6	1.15
20:5n-3	9.67	12.21
22:5n-3	1.38	1.61
22:6n-3	10.39	17.17
SFA	26.86	27.52
MUFA	29.24	29.06
PUFA	43.9	43.43
Total n-6	17.29	8.8
Total n-3	26.61	34.63
DBI	7.47	8.51
n-3/n-6	1.54	3.94
Moisture (g kg^{-1})	52	55
Protein (g kg^{-1})	426	432
Lipid (g kg^{-1})	136	132
N.F.E. (g kg^{-1})	311	314
Ash (g kg^{-1})	75	67

4.2. Proximate and fatty acid composition of caviar

Biometrical values (fish weight and eggs yield) recorded during the experiment are reported in Table 2, whilst in Table 3 the proximate composition of caviar samples is reported. SQ diet was responsible of significantly increase eggs yield, likely suggesting a more favourable dietary fatty acid composition (higher in n-3 PUFA) compared to SB treatments.

Table 2. Fish weights and eggs yields in farmed white sturgeons (A. transmontanus) fed with different diets (means ± S.E.)

	3 months		6 months	
	SB3 (*n* = 5)	**SQ3 (*n* = 5)**	**SB6 (*n* = 5)**	**SQ6 (*n* = 5)**
Fish weight (kg)	33.5 ± 0.35	33.3 ± 0.37	33.8 ± 0.42	33.3 ± 0.41
Ovary weight (kg)	5.48 ± 0.16	5.58 ± 0.17	5.25 ± 0.21	5.26 ± 0.14
Eggs weight (kg)	4.81 ± 0.12	4.98 ± 0.14	4.53 ± 0.20	4.58 ± 0.14
Ovary yield (%)	16.3 ± 0.47	16.7 ± 0.42	15.5 ± 0.55	15.7 ± 0.36
Eggs yield (%)	14.9 ± 0.38^{ab}	15.4 ± 0.34^{b}	13.9 ± 0.56^{a}	14.2 ± 0.39^{ab}

Value within a row with different letters are significantly different $P < 0.05$.

Since a very low salt content was measured in the final product (from 2.1 to 2.4%, data not shown), the caviar was classified, for the market, as malossol caviar indicating a very high quality product.
Moisture content averaged between 53.8–56.3% and together with lipid (10.2–11.5%) and protein content (23.9–25.4%) were similar to the reported composition of malossol caviar (Gessner et al., 2002 b).

Table 3. Proximate composition (means ± S.E.) in percent wet weight of caviar from different dietary treatment

	SB3	**SQ3**	**SB6**	**SQ6**
Moisture	54.41 ± 0.81	54.96 ± 0.30	56.33 ± 0.90	53.85 ± 0.82
Protein	25.39 ± 0.32	24.87 ± 0.45	23.89 ± 0.47	25.12 ± 0.37
Lipid	11.04 ± 0.56	10.82 ± 0.54	10.19 ± 0.92	11.46 ± 0.65
Ash	3.79 ± 0.10	3.78 ± 0.03	3.46 ± 0.07	3.44 ± 0.15

Value within a row with different letters are significantly different $P < 0.05$.

Proximate composition recorded no significant differences, as expected being the diets *iso*-proteic *iso*-lipidic and *iso*-energetic. Several authors have previously

discussed the role of various ingredients of diet, especially protein and fatty acid, on gonadal development and spawning quality (Bransden, et al., 2007; Morehead et al., 2001; Bell, et al., 1997; Fernandez-Palaci et al., 1996), whilst the role of dietary fatty acids on eggs development and eggs fatty acid make up is less known. It has been reported that in eggs, during gametogenesis, lipoprotein biosynthesis preferentially utilises saturated and monounsaturated fatty acid from adipose and muscle tissues to provide metabolic energy, while n-3 PUFA are incorporated into phospholipids fraction of vitellogen and transferred via the serum into the eggs (Wirth et al., 2002). The fatty acid composition of caviars obtained by different dietary treatments is reported in Table 4.

Table 4. Fatty acid composition (% of total fatty acids) of total lipid in caviar of sturgeon fed different treatment

	SB3	**SQ3**	**SB6**	**SQ6**
C14:0	1.75 ± 0.10	1.61 ± 0.10	1.86 ± 0.07	1.64 ± 0.10
C16:0	20.82 ± 0.16	21.28 ± 0.21	20.62 ± 0.36	21.04 ± 0.27
C16:1n-7	4.35 ± 0.11	4.34 ± 0.17	4.54 ± 0.09	4.57 ± 0.18
C18:0	3.07 ± 0.11	3.39 ± 0.18	3.11 ± 0.15	3.07 ± 0.19
C18:1n-9	29.78 ± 1.29	32.01 ± 1.20	28.54 ± 0.88	30.52 ± 0.62
C18:1n-7	3.59 ± 0.17	3.39 ± 0.12	3.52 ± 0.06	3.50 ± 0.07
C18:2n-6	5.55 ± 0.30^{a}	4.74 ± 0.43^{a}	6.78 ± 0.42^{b}	5.42 ± 0.22^{a}
C18:3n-6	0.33 ± 0.03	0.27 ± 0.07	0.46 ± 0.04	0.37 ± 0.09
C18:3n-3	0.72 ± 0.05^{ab}	0.62 ± 0.05^{a}	0.82 ± 0.05^{b}	0.70 ± 0.02^{ab}
C18:4n-3	0.60 ± 0.03	0.58 ± 0.03	0.64 ± 0.04	0.61 ± 0.03
C20:1n-9	2.82 ± 0.19	2.42 ± 0.12	2.72 ± 0.16	2.32 ± 0.21
C20:2n-6	0.14 ± 0.09	0.05 ± 0.05	0.15 ± 0.09	0.05 ± 0.05
C20:4n-6	1.91 ± 0.15	1.86 ± 0.11	1.98 ± 0.09	1.93 ± 0.05
C20:5n-3	5.89 ± 0.33	5.61 ± 0.24	5.79 ± 0.26	5.66 ± 0.25
C22:1	0.24 ± 0.18	0.05 ± 0.05	0.11 ± 0.11	0.11 ± 0.11
C22:5n-3	1.70 ± 0.10	1.63 ± 0.09	1.76 ± 0.04	1.65 ± 0.03
C22:6n-3	16.62 ± 0.53	16.11 ± 0.55	16.48 ± 0.31	16.72 ± 0.45
SFA	25.76 ± 0.11	26.33 ± 0.23	25.72 ± 0.35	25.86 ± 0.25
MUFA	40.79 ± 0.98	42.21 ± 1.03	39.42 ± 0.73	41.02 ± 0.47
PUFA	33.46 ± 1.07	31.46 ± 0.97	34.86 ± 0.99	33.12 ± 0.58
Total n-6	7.93 ± 0.23^{a}	6.92 ± 0.40^{a}	9.37 ± 0.47^{b}	7.77 ± 0.28^{a}
Total n-3	25.53 ± 0.92	24.54 ± 0.68	25.50 ± 0.54	25.35 ± 0.58
n-3/n-6	3.22 ± 0.10^{b}	3.58 ± 0.17^{b}	2.74 ± 0.10^{a}	3.28 ± 0.17^{b}
EPA/AA	3.11 ± 0.15	3.06 ± 0.25	2.97 ± 0.24	2.94 ± 0.17
DBI	7.92 ± 0.22	7.50 ± 0.13	7.99 ± 0.22	7.86 ± 0.17

Data are expressed as mean ± S.E. Value within a row with different letters are significantly different $P < 0.05$.

The overall fatty acid composition of caviar samples analysed in the present study was similar to the one reported for dorsal muscle of a farmed hybrid sturgeon (Vaccaro et al., 2005). Palmitic acid (16:0) was the most abundant saturated fatty acid in caviar samples, accounting for over 20%. Amongst the unsaturated fatty acids, oleic acid (OA, 18:1 n-9) was the most abundant followed by docosahexaenoic acid (DHA, 22:6 n-3) and eicopentaenoic (EPA, 20:5 n-3). The high levels of 18:1 n-9 and 22:6 n-3 in caviar reflect the importance of these fatty acids as energy reserve and a temporary reservoir of PUFA for future embryonic development, as clearly documented for other species (Almansa et al., 2001; Fernandez-Palacios et al., 1995).

As expected, linoleic acid (LA, 18:2 n-6) levels in caviar samples were significantly higher in soybean-based diet (SB), and total n-6 amounts in caviars mirrored the same result, leading to the lowest ratio between n-3/n-6 series.

However, it is extremely important to underline the fact that while the SB diet had a LA content of over 16%, only less than 7% of LA was deposited in the eggs of fish fed the SB diet for 6 months. Thus, contrary to what commonly happen in other fish tissues such as fillet, liver and adipose tissues in which LA is actively and abundantly deposited, the fatty acid make up of fish eggs are less easily modifiable by dietary LA.

However, significantly higher ($P < 0.05$) amounts of LA and n-6 PUFA were shown in the groups fed the SB diet during the last 6 months before caviar collection (SB6) when compared to those fed diet SQ (groups SQ3 and SQ6) and diet SB given for 3 months only (SB3). Few studies have addressed the issue of eggs quality and nutritional composition of diets; however, the duration of the period during which the broodstock should be fed a diet in order to affect the chemical composition of eggs is not completely clarified. Almansa et al. (2001) showed that 2 months of feeding before the spawning season were not sufficient to alter the chemical composition of egg quality in gilthead seabream (Sparus aurata). Leray et al. (1985) concluded that in rainbow trout (Oncorhynchus mykiss) only a long period of n-3 fatty acid deficiency is able to greatly alter egg lipids.

In the present study the fatty acid composition of the SB6 caviar showed significant differences due to higher content of LA. Nevertheless, these data indicate that 6-month of feeding before caviar collection is necessary to influence the fatty acids profile of eggs, and this influence is rather limited.

On the other hand, oleic acid (OA, 18:1 n-9) was deposited in the eggs at roughly double the concentration compared to the diet. OA is, indeed, well known to be a major fatty acid in crude and polar lipid of marine eggs (Izquierdo, 1996). The highest amount of OA was observed in caviar from sturgeons fed with the SQ diet and this result might agree with the observation previously reported by Fernandez-Palacios et al. (1997), who observed the high

accumulation of OA in eggs from sea bream (*S. aurata*) fed with squid oil and squid meal diet.
The high level of fatty acid from n-3 series recorded in caviar samples, independently form dietary treatments, was expected as n-3 PUFA and especially DHA are well known to be the major component of phospholipids in fish roes (Sargent et al., 2002).
The fatty composition of eggs has been studied in both wild and cultured freshwater populations (Czesny et al., 2002; Dantagnan et al., 2007; Sargent et al., 1997) and it is also known to be affected by dietary and environmental factors. The n-6 series tends to predominate in fresh water derived fish eggs while n-3 characterize fish eggs from salt water environments (De Silva, et al., 1998; Tocher et al., 1995). Recently, Dantagnan et al. (2007) reported how cultured broodstock embryos of "puyen" (*Galaxias maculates*), which were kept in fresh water during the entire cycle, but given diets based on fish meal and oil, showed a n-3/n-6 ratio close to embryos from estuarine fish.
The ratio of n-3 to n-6 PUFA recorded in studied caviar samples ranged from 2.7 to 3.6. These values are within the typical range for freshwater fish eggs (0.5–3.8) (De Silva, et al., 1998).
The n-3/n-6 ratio of caviar from SQ fish was significantly higher when compared to caviar from SB fish. This is in agreement with the relative higher content of n-3 series in SQ diet. However, despite a quite large difference in the dietary n-3 PUFA content (26.6 and 34.6% in SB and SQ diet, respectively), in the caviar the total n-3 PUFA content varied only from 24.5 to 25.5 amongst treatments, and surprisingly EPA and DHA percentages were almost identical in different caviar irrespectively from dietary treatments, clearly showing that the optimal threshold levels were reached. Therefore, it would appear that there was a selective accumulation of DHA and EPA irrespective of the levels in the dietary supply. Similar results were reported by Mazorra et al. (2003) in Atlantic halibut (*Hippoglossus hippoglossus*), where much lower DHA levels in the diets produced concentrations in the eggs similar to the ones from the fish fed with a diet richer in DHA. Other authors (Czesny and K. Dabrowski, 1998) reported a lower concentration of lipids in eggs from domesticated walleye (*Stizostedion vitreum*) than in those from wild stocks but despite this, the concentrations of DHA were the same in eggs from both stocks.

4.3 Flavour volatile compounds in caviar

The volatile substances isolated in caviar by simultaneous distillation–extraction are listed in Table 5.

Table 5. Major volatile compounds identified (μg kg^{-1}) in caviar of sturgeon fed different treatment

Compound	RIa	Means of identification	SB3	SQ3	SB6	SQ6
Hexanal	353	MS, RI, STD	18.8 ± 4.1	29.2 ± 6.4	27.9 ± 6.1	30.5 ± 6.3
3,5-Octadiene (*Z*,*Z*)	372	MS, RI	23.5 ± 5.2	36.6 ± 8.1	34.9 ± 7.6	38.2 ± 7.9
2,4-Octadiene (*Z*,*Z*)	376	MS, RI	10.3 ± 1.9	17.1 ± 4.4	16.0 ± 4.0	20.4 ± 6.4
2-Hexenal	491	MS, RI, STD	7.1 ± 2.0	10.3 ± 1.6	11.1 ± 1.6	12.4 ± 4.9
Ethylbenzene	514	MS, RI	4.0 ± 1.2	4.2 ± 0.3	3.7 ± 0.2	5.2 ± 2.8
4-Heptenal (*Z*)	612	MS, RI, STD	14.9 ± 1.8	12.1 ± 2.6	12.2 ± 1.3	14.9 ± 5.7
3-Methylthio-propanal	635	MS, RI, STD	3.1 ± 0.8	2.3 ± 1.1	3.8 ± 0.4	2.6 ± 0.2
2,4-Hexadienal (*E*,*E*)	639	MS, RI		1.7 ± 0.2	4.4 ± 2.5	1.9 ± 1.1
Heptanal	643	MS, RI, STD	2.4 ± 0.5	5.1 ± 2.4	3.5 ± 1.2	4.0 ± 1.3
2-Heptenal (*Z*)	765	MS, RI	2.2 ± 0.7	2.3 ± 0.4	3.9 ± 1.0	4.4 ± 2.0
Benzaldehyde	786	MS, RI	4.4 ± 1.2	5.5 ± 0.5	6.3 ± 0.3	6.0 ± 2.2
1-Octen-3-ol	821	MS, RI	1.6 ± 0.6	2.1 ± 0.2	3.3 ± 0.6	2.4 ± 0.9
2-Pentyl-furan	849	MS, RI	7.6 ± 1.3	13.7 ± 1.5	13.9 ± 2.4	12.0 ± 2.1
2,4-Heptadienal (*E*,*E*)	865	MS, RI	57.3 ± 12.9	76.3 ± 17.3	90.8 ± 18.7	73.0 ± 6.7
Octanal	881	MS, RI, STD	3.1 ± 0.5	5.4 ± 0.8	4.4 ± 0.5	4.4 ± 0.8
2,4-Heptadienal (*E*,*Z*)	902	MS, RI	91.2 ± 33.4	128.0 ± 27.6	160.7 ± 22.9	127.2 ± 16.2
2-Acetylthiazole	918	MS, RI	2.4 ± 0.7	3.3 ± 0.4	2.5 ± 0.1	3.5 ± 1.5
Benzeneacetaldehyde	982	MS, RI	8.6 ± 3.3	6.6 ± 1.5	9.9 ± 0.2	6.3 ± 1.5
2-Octenal (*E*)	1010	MS, RI	4.2 ± 1.1	5.5 ± 0.7	6.7 ± 0.7	5.4 ± 1.3
3,5-Octadien-2-one	1086	MS, RI, STD	2.2 ± 0.4	2.5 ± 0.3	3.3 ± 0.8	2.7 ± 0.7
Nonanal	1109	MS, RI	21.9 ± 0.5	24.0 ± 5.3	18.6 ± 1.5	18.7 ± 5.3
2,4-Octadienal (*E*,*E*)	1121	MS, RI	6.7 ± 1.9	9.0 ± 2.0	11.8 ± 1.6	10.0 ± 1.4
2,6-Nonadienal (*E*,*Z*)	1201	MS, RI	6.4 ± 1.7	8.8 ± 1.6	11.0 ± 2.4	8.5 ± 1.3
2-Nonenal (*E*)	1215	MS, RI	1.5 ± 0.3	2.2 ± 0.3	2.5 ± 0.7	2.4 ± 0.0
2,4-Decadienal (*E*,*E*)	1406	MS, RI	11.2 ± 1.7	12.5 ± 2.3	18.2 ± 3.6	13.2 ± 1.4
2,4-Decadienal (*E*,*Z*)	1432	MS, RI	50.8 ± 11.2	62.7 ± 11.4	91.9 ± 14.4	69.3 ± 6.7
Pentadecane	1500	MS, RI, STD	2.0 ± 1.0	2.0 ± 1.0	1.1 ± 0.0	1.0 ± 0.5
Heptadecane	1700	MS, RI	3.1 ± 1.2	5.7 ± 2.2	2.8 ± 1.3	4.0 ± 2.0
Pristane	1702	MS, RI, STD	53.2 ± 27.4	45.5 ± 28.8	40.3 ± 19.2	37.3 ± 11.2
Tetradecanoic acid methyl ester	1750	MS, RI	3.2 ± 1.9	45.4 ± 42.0	61.8 ± 60.4	22.3 ± 20.6
Tetradecanal	1755	MS, RI, STD	37.8 ± 11.3	31.4 ± 10.6	29.7 ± 12.7	27.1 ± 10.4
Palmitic acid, methyl ester	1790	MS, RI, STD	165.8 ± 153.6	129.3 ± 123.8	109.7 ± 100.5	46.4 ± 38.1
9-Octadecenal (*Z*)	1828	MS, RI	38.3 ± 11.6	30.4 ± 12.1	22.1 ± 8.8	31.8 ± 14.6
∑ derived oleic acid[c]			25.8 ± 0.7	32.7 ± 8.2	24.2 ± 2.7	24.3 ± 6.5
∑ derived linoleic acid[d]			100.3 ± 19.8	137.0 ± 20.3	170.5 ± 28.4	142.1 ± 11.1
∑ derived linolenic acid[e]			166.5 ± 51.0	228.1 ± 48.7	280.7 ± 47.0	228.2 ± 30.1

Data are expressed as mean ± S.E.M. Value within a row with different letters are significantly different $P < 0.05$. [a] RI: Kovàts retention indices (Bransden et al., 2007) for DB-5MS capillary column;

[b] MS: mass-spectra; RI: retention index; STD: standard compound; [c] Sum of heptanal, octanal, nonanal (Belitz and W. Grosch, 1999).
[d] Sum of hexanal, heptanal, 2-octenal, 2,4-decadienal(*E*,*Z*,/*E*,*E*) (Belitz and W. Grosch, 1999). [e] Sum of 2-hexenal, 2-heptenal 2,4-heptadienal(*E*,*Z*,/*E*,*E*), 2,6 nonadienal and 3,5-octadien-2-one (Belitz and W. Grosch, 1999).

The compounds were identified using both chromatographic (Kovats indices) and spectroscopic (mass spectra, EI, 70 eV) criteria. A representative total ion chromatogram (TIC) of the SDE extract of a caviar sample is illustrated in Fig. 1. The volatile compounds identified resulted not statistically different between groups.

The largest group of volatiles were represented by aldehydes with 20 compounds, representing the 60% of the total volatiles. *n*-Alkanals, 2-alkenals and 2,4-alkadienals have been largely studied in fish products as products of oxidation of lipids and are responsible for a wide range of flavours (Varlet et al., 2007).

Many of the aldehydes can provide several notes to fish flavour (fatty, green, woody, fatty, nutty; floral, citrus, waxy and sweet) depending on the number of carbon atoms and on the degree of unsaturation.

All n-alkanals are generated by oxidation of monounsaturated and n-6 polyunsaturated fatty acids. Hexanal mainly derives from the oxidation of linoleic acid and provides fatty and green notes to fish flavour. This compound is often isolated from food and is used as indicator for the characterization of off-flavours (Durnford and F. Shahid, 1998). Heptanal and octanal may derived either from oleic acid and linoleic acid oxidation, being predominant as oxidation product of oleic acid. Nonanal is also predominant in the volatile fraction formed during the autoxidation of linoleic acid (Durnford and F. Shahid, 1998).

n-Alkanals are considered to play an important role in food aroma, especially for their low odour threshold and for their aromatic notes of grass, fat and tallow. In the analysed caviar their concentration was 55.72 μg IS kg^{-1} and represented 7.54% of the total volatiles isolated.

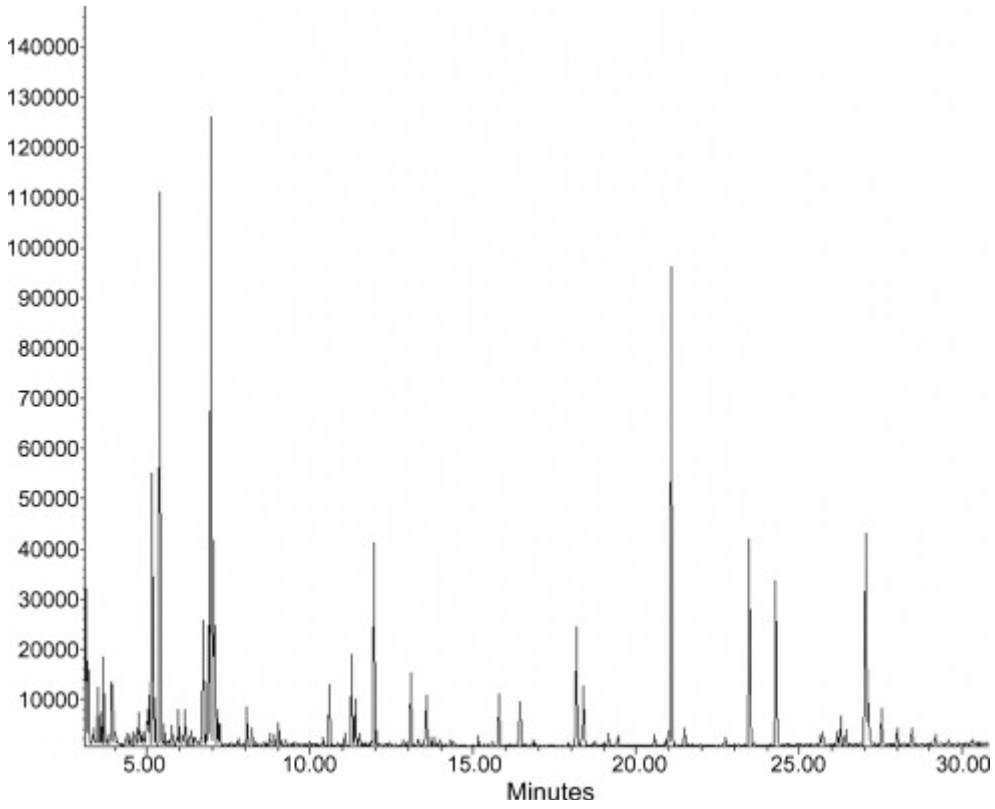
Alkenals are formed by autoxidation of either n-6 PUFA or n-3 PUFA. In particular, 2-hexenal and 2-heptenal are mainly derived from linolenic acid, while 2-octenal and 2-nonenal are mainly derived from linoleic acid. In caviar samples the amount of alkenals found was 34.42 μg IS kg^{-1} with a contribution of 4.66%.

4-Heptenal may derive by (*E*,*Z*)-2,6-nonadienal with a mechanism proposed by Josephson and Lindsay (1987). This compound exhibits at low concentration in water a cardboardy note while at higher concentration its aroma is more putty, paint or oily like.

Both 4-heptenal and 2,6-nonadienal were detected in caviar at concentrations of 13.41 and 8.67 μg IS kg^{-1}, respectively.

Also 2,4-alkadienals were present in the volatile fraction of caviar, mainly 2,4 hexadienal, 2,4-heptadienal, 2,4-octadienal, 2,6-nonadienal and 2,4-decadienal. Among them, the predominant were (*E*,*Z*)-2,4-heptadienal (126.76 μg kg^{-1}) and (*E*,*Z*)-2,4-decadienal (68.67 μg kg^{-1}).
The 2,4-heptadienal is produced from *n*-3 PUFA, such as α-linolenic acid, through 12-hydroperoxide intermediary (Belitz and W. Grosch, 1999). Its odorant threshold is assessed at 778 μg kg^{-1} in water with fried odour for (*E*,*Z*) structure and fatty, nutty odour for (*E*,*E*) structure (Belitz and W. Grosch, 1999). Decadienal isomers come from n-6 PUFA such as linoleic or arachidonic acid and by the intermediary of 9-hydroperoxide deriving from linoleic acid, or 11-hydroperoxide deriving from arachidonic acid. The 2,4-decadienal (*E*,*E*) has a fried, fatty and waxy flavour with an odour threshold at 0.07 μg kg^{-1} in water. Similarly, (*E*,*Z*)-2,4-decadienal is characterized by a fried and fat flavour (Belitz and W. Grosch, 1999).

Fig. 1. Total ion chromatogram of the SDE extract of a caviar sample. For GC–MS conditions see text



A furan compound, 2-pentylfuran, was also detected in caviar. This compound is a well known autoxidation product from linoleic acid (Belitz and W. Grosch, 1999). Furan compounds are not considered to contribute significantly to the basic caviar's aroma, but they contribute in the overall odour of cooked products

(Durnford and F. Shahidi, 1998). Its presence could be caused by the extraction method used, that may lead to the formation of artefacts (Chaintreau, 2001).
As regards other compound isolated from caviar, the 2,4,10,14-tetramethylpentadecane (pristane) is the most abundant. Pristane contribute a green, sweet aroma to crayfish and originates from lipid autoxidation processes through alkyl radicals or from decomposition of carotenoids or may also be derived from the diet. In Table 6 the total amount of volatile compounds found in caviar samples, irrespectively to the experimental groups, their odour threshold in water and the calculated odour activity values (OAVs) for each component are presented. The calculation of OAV was established as the ratio of the concentration of odorant in the matrix and the odour threshold (Rothe and Thomas, 1963) with the aim to overview the effective contribution of volatile compounds identified in the matrix to generate the aroma. Following this thesis and after several olfactometric studies, Grosch et al. (2001) concluded that <5% of volatile identified in food contributed to aroma. The approached calculation of OAVs values allowed to identified 2,4-decadienal (*E*,*Z*), 2,4-decadienal (*E*,*E*) and 2,6-nonadienal (*E*,*Z*), as the aroma compounds with highest OAV values, 981, 867, 197, respectively. It is reasonable to argue that the generation of these above mentioned compounds is caused also by the SDE method applied to caviar samples. Many constituents are sensitive to oxidation, and especially unsaturated fatty acids may produce oxidative products that include 2,4-decadienal, as well as 4 (*Z*)-heptenal, under extraction condition. McGill et al. (1974) showed that the isolation of cod volatiles by SDE led to an odour of cold-stored fish, due to oxidation of lipids into (*Z*)-4-heptenal. As reported above, (*Z*)-4-heptenal is produced by the water-mediated retro-aldol condensation of (*E*,*Z*)-2,6-nonadienal with a mechanism proposed by Josephson and Lindsay (1987). (*E*,*Z*)-2,6-Nonadienal and other compounds such as (*E*)-2-nonenal, belong to the most important odorants of freshwater fish (Durnford and Shahidi, 1998). (*E*,*Z*)-2,6-Nonadienal has been previously reported to contribute to the flavour of ripened anchovy (Triqui and Reineccius, 1995) in ayu fish (Hirano et al., 1992) and in boiled salmon and cod (Milo and Grosch, 1996) through its characteristic cucumber-like aroma and a very low odour threshold. It is worth considering that the contribution of different compounds in aroma determination of a complex matrix resulted from their odorant interaction, that explains how compounds with high OAV might be suppressed in the aroma and on the other hand lower OAV compounds generate the most important contribution (Grosch, 2001).
In this study, simultaneous distillation–extraction has been demonstrated to be a suitable method for the isolation of a wide range of volatiles, mostly aldehydes, which are largely the main responsible for aroma of caviar from farmed white surgeon.

Table 6. Odour impact compounds in caviar (mean of total volatile compound identified in all samples)

Compound	OTa (μg l−1)	Conc.b (μg kg−1)	OAVc	Odour description
Hexanal	4.5–5	26.63 (3.60)	5.92–5.33	Herbaceous, oxidized
3,5-Octadiene (*Z*,*Z*)	–	33.29 (4.50)	–	–
2,4-Octadiene (*Z*,*Z*)	–	15.93 (2.16)	–	–
2-Hexenal	17	10.22 (1.38)	0.6	Moss, mushroom
Ethylbenzene	730	4.27 (0.58)	0.01	
4-Heptenal (*Z*)	0.8–10	13.41 (1.81)	16.76–1.34	Powerful green
3-Methylthio-propanal	0.2	3.03 (0.41)	15.15	Fermented/cooked shrimp
2,4-Hexadienal (*E*,*E*)	10–60	2.68 (0.36)	0.27–0.04	
Heptanal	3	3.99 (0.54)	1.33	Oily, fatty
2-Heptenal (*Z*)	13	3.19 (0.43)	0.25	Cooked fish, sulphury
Benzaldehyde	350–3500	5.54 (0.75)	<0.01	Candy, sweet
1-Octen-3-ol	1	2.36 (0.32)	2.36	Mushroom
2-Pentyl-furan	6	11.80 (1.60)	1.97	Cooked crayfish, raw boiled pasteurized crab meat
2,4-Heptadienal (*E*,*E*)	778	74.35 (10.06)	0.1	Fatty, nutty
Octanal	0.7	4.32 (0.58)	6.17	Cooked potato, fatty, fishy, wax, citrus
2,4-Heptadienal (*E*,*Z*)	778	126.76 (17.15)	0.16	Fry, oily
2-Acetylthiazole	nd	2.93 (0.40)	–	Nutty, toasted cereals
Benzeneacetaldehyde	4	7.85 (1.06)	1.96	Moss, spicy
2-Octenal (*E*)	3	5.44 (0.74)	1.81	Oily
3,5-Octadien-2-one	150	2.68 (0.36)	0.02	–
Nonanal	1	20.78 (2.81)	20.78	Floral, waxy
2,4-Octadienal (*E*,*E*)	nd	9.38 (1.27)	–	Pine, resin, cucumber
2,6-Nonadienal (*E*,*Z*)	0.01	8.67 (1.17)	867	Green, cucumber
2-Nonenal (*E*)	0.08–0.1	2.16 (0.29)	27.00–21.60	Orris-like citrus
2,4-Decadienal (*E*,*E*)	0.07	13.79 (1.87)	197	Green, fat fried, cod oil
2,4 Decadienal (*E*,*Z*)	0.07	68.67 (9.29)	981	Green, fat fried, cod oil
Pentadecane	>1000	1.53 (0.21)	<0.001	–
Heptadecane	>1000	3.73 (0.50)	<0.001	–
2,6,10,14-Tetramethylpentadecane	>1000	44.06 (5.96)	<0.001	Green, sweet, crayfish
Tetradecanoic acid methyl ester	>1000	30.77 (4.16)	<0.001	–
Tetradecanal	>1000	31.48 (4.26)	<0.001	–
Palmitic acid, methyl ester	>1000	112.78 (15.26)	<0.001	–
9-Octadecenal (*Z*)	>1000	30.65 (4.15)	<0.001	–

[a] Odour threshold.
[b] Values within brackets rapresent the percentage of total amount.
[c] Odour activity value.

5. Conclusions

This study contributed to the chemical characterization of caviar from farmed white sturgeon, particularly for fatty acid composition and flavour characteristics. To the authors' knowledge, it is the first time that volatile compounds are isolated from this food matrix and their role in flavour generation discussed. The production of caviar from farmed sturgeon is a feasible way to support the ever-increasing demand for this product and thereby could help in decreasing fishing pressure on the wild stocks.

Consumers are increasingly concerned about quality of farmed product and it is now well documented that farming conditions and dietary treatments can largely modify aquaculture products, thus the significant difference in market price between farmed and wild products. On the contrary, the present study shows that the situation for farmed caviar is quite the opposite. The overall nutritional and organoleptic qualities of caviar obtained from fish fed significantly different diets (marine oil *vs*. vegetable oil) was, indeed, almost unaffected. In a nutshell, it is possible to stress that farmed caviar is able to maintain good eating quality while representing an environmentally friendly, and cost effective, alternatives to wild products. A direct comparison of farmed versus wild products is, at this point, needed to further assess potential differences.

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