CHAPTER I

INTRODUCTION

The United States (U.S.) is one of the largest seafood markets in the world (ARS, 2002). Historically, a variety of seafood has been accessible to the public through fishing and depletion of wild populations in marine and freshwater bodies. The imminent need to keep wild fish and shellfish populations from extinction is creating a shift from wild harvest to aquaculture (Harvey, 2003).

Fish have always been considered to be an excellent source of protein, minerals and a low-fat product, especially in the U.S. where low-fat fish are preferred by consumers (Kinsella, 1988). Fish, among other nutritive properties are the best source of n-3 (omega-3) fatty acids; consumption of fish may be related to lower risk of cardiovascular diseases (American Heart Association, 2002) and promote other health benefits. Because of this, the American Heart Association has recommended the consumption of fatty fish (mackerel, salmon, tuna) at least twice a week. In the U.S., cardiovascular diseases are the leading cause of death (American Heart Association, 2003).

The aquaculture industry in the U.S. peaked in production during the 90's and since yields have remained steady. It has been predicted that aquaculture will be supplying increased products in the future due to the decline in wild captures and growing demand. The U.S. has established programs to increase knowledge in areas that could efficiently increase aquaculture production without sacrificing product quality (ARS, 2002). An interesting and promising area in aquaculture is biotechnology, which has already shown great research advances. Fast-growing fish (transgenic fish) are among the many biotechnology applications that are being researched in variety of species (Atlantic, Pacific and coho salmon, tilapia, carp, channel catfish). Transgenic fish have not been approved for human consumption in the U.S., however advances in technology and continuing research could assure safety for human consumption (risk assessment) in future years.

Besides biotechnology, the aquaculture industry has many traditional technologies that can be used efficiently to enhance fish and shellfish quality and nutritive properties. Among the most important and researched factors are: farming systems, control of growth rate, water chemistry and water temperature, feeding behavior and nutrition. Nutrition is the most important tool that may be utilized to modify properties such as fatty acid profile, fat content, flavor, color and texture to deliver a more nutritious and appealing product to consumers (Haard, 1992a; Rasmussen, 2001).

Farmed fish have been slowly accepted by consumers, since they have been thought to have lower quality and poor flavor compared to their wild counterparts (Haard, 1992a; Rasmussen, 2001). It is important to farmers to know the differences and similarities between farmed and wild fish of different species; this could lead them to understand the chemical, physical, nutritional and sensorial profile of the wild animal and try to reproduce it in their farmed products. Differences and similarities between wild fish and their farmed counterparts have been found in a variety of species (Jahncke et al., 1988; Nettleton and Exler, 1992; George and Bophal, 1995; Chen et al., 1995; Rahman et al., 1995; Rueda et al., 1997; Cox and Karahadian, 1998; Alasalvar et al., 2002; Grigorakis et al., 2002; Grigorakis et al., 2003; Orban et al., 2003 Jankowska et al., 2003). Wild fish tend to be leaner and firmer when compared to farmed fish. The fatty acid profile differs between wild and farmed fish according to their diet. This may have direct impact on flavor and aroma compounds.

In this research three different species were used to compare their farmed and wild counterparts: yellow perch (*Perca flavescens*), southern flounder (*Paralichthys lethostigma*) and coho salmon (*Oncorhynchus kisutch*).

Yellow perch

Yellow perch is a freshwater fish that belongs to the Percidae family. They can be found in Canada (Nova Scotia and Quebec) and in the North-Eastern U.S. (Page and Burrr, 1991). Yellow perch is a high priority species in the U.S., most specifically in the Great Lakes area, where demand is high. The decline of yellow perch populations around the 1970 and 1980’s stimulated the aquaculture industry to increase yellow perch production to satisfy regional demands. Commercial aquaculture of yellow perch is based on ponds and intensive-tank rearing systems (Burden, 2004).

Southern flounder

Southern flounder is a member of the Family Bothidae (lefteye flounders) and an important recreational and commercial fish in the Southeastern United States. This species is distributed from North Carolina to northern-most eastern and western seaboards of Florida through to Texas and Mexico (Reagan et al., 1985; Daniels, 2000). This marine species is of interest to the aquaculture industry because of its commercial value, wide salinity and temperature tolerance and decreased number of wild landings. Southern flounder feed on crabs, shrimp and mainly fish (Robins and Ray, 1986). Aquaculture production of southern flounder is still in its infancy; however a variety of research concerning spawning, pond rearing, weaning and nutrition has been attained (Jenkins and Smith, 1999).

Coho salmon

Coho salmon (silver salmon) belongs to the Salmonidae family and is an important commercial and recreational fish. This species is distributed in the North Pacific from Anadyr River in Russia to Hokkaido, Japan and from Point Hope in Alaska to Chamalu Bay in Baja California, Mexico. Coho salmon can be found in the ocean or in lakes and it returns to the streams to spawn. They live in freshwater for the first 1-2 years and then migrate to the sea (Page and Burr, 1991). Coho salmon are cultured extensively and recently most of the research on coho salmon has focused on biotechnology, to produce transgenic coho (Devlin et al., 1994, 1995a, 1995b, 1999, 2004).

The objectives of this research are:

1. Determine differences and similarities in quality chemical, physical and sensorial properties between wild and farmed fish of the same species
2. Determine the impact nutrition has on growing rates and quality of fish fillets
3. Analyze quality properties of genetically modified coho salmon (F4 generation of salmon with an inserted growth hormone gene) when compared to their wild and farmed counterparts

CHAPTER II

LITERATURE REVIEW

Approximately 1,000 fish and shellfish species are consumed in the world (Fraser and Sumar, 1998), varying in nutritional and sensorial characteristics. Fish can be classified depending on the environment where they live: freshwater or marine; pelagic (next to the ocean surface) or demersal (bottomfish) (Flick et al., 1990; Kaylor and Learson, 1990). Fish have also been classified according to their lipid content (Ackman, 1990):

- Lean fish (cod, yellow perch): < 2% fat
- Low fat fish (flounder, halibut): 2-4 % fat
- Medium fat fish (salmon): 4-8 % fat
- High fat fish (mackarel): > 8% fat

Fish are a good source of protein, amino acids, minerals and n-3 fatty acids. More specifically, fatty fish (higher amounts of n-3 fatty acids delivered) are valuable food products recommended to help lower triglycerides and prevent heart attacks and stroke in humans (American Heart Association, 2002).

Historically marine fatty fish have been acknowledged as a good source of n-3 fatty acids (tuna, mackerel and even salmon). However, with the help of aquaculture, other fish (freshwater, lean) could be an important source of n-3 fatty acids by increasing the lipid concentration of the muscle and n-3 content in the edible muscle, through diet and feeding regime. In this section, the impact of aquaculture on fish quality and fish quality parameters will be discussed.

2.1 AQUACULTURE STATUS IN THE U.S.

The U.S. is a major market for aquacultured seafood being the second largest seafood importer in the world (Harvey, 2004). U.S. seafood consumption has been constant for the past years at 16 pounds per person in a year.

Aquaculture is defined as “the cultivation of aquatic animals and/or plants in a controlled environment for all or part of their life cycle” (Selock, 2001). Aquaculture production in the U.S. increased approximately 400% between 1980-1998 (Harvey, 2004) and in 2001, aquacultured products in the U.S. accounted for 800 million pounds of the national seafood supply (Selock, 2001).

The major fish species being farmed in the U.S. are: channel catfish (half of total sales; major states producers: Mississippi, Arkansas, Alabama and Louisiana); rainbow trout, different species of salmon (mainly Atlantic salmon), hybrid striped bass, tilapia, yellow perch, walleye, sturgeon, and bait minnows (Selock, 2001; Harvey, 2004).

In 2001, approximately $935 million were produced from aquaculture products, however a steady behavior in national aquaculture production has been observed recently, while an increase in seafood imports has taken place. In 2002, 45% of the seafood consumed in the U.S. was acquired from foreign suppliers. One billion pounds of those imported products were farmed, with a value of $2.7 billion (Harvey, 2003). The most important imported seafood products in the U.S. are: shrimp (frozen and fresh: 946 million pounds (2002)), Atlantic salmon (413 million pounds) and tilapia (148 million pounds) (Harvey, 2003).

In the U.S., aquaculture research attention has focused on growth rates, feed efficiencies, disease and mortality rates, rather than product development or quality (Harvey, 2004). The importance of the creation of new seafood products with wider shelf-life and attractive quality and nutritive properties is a main component of the USDA action plan from the National Program of Aquaculture (ARS, 2002).

The increasing human population and limited natural resources are encouraging different sectors in agriculture to develop methods to improve and accelerate animal production without compromising quality and, most importantly, safety. Global aquaculture is believed to be an agricultural sector that will play a major role in the years to come to deliver the food products needed to satisfy the demands of the increasing population. Therefore, in the U.S. research to improve and increase domestic aquaculture production is an important goal of the National Program of Aquaculture. Some of the program components are dedicated to specific research areas such as: the utilization of recirculating aquaculture systems (RAS) to improve production and establish more environmentally friendly processes; development of feeds that are not only low in cost but can improve production, reduction of diseases and improvement to quality of the final product. Another important area of focus on is biotechnology, to create and improve genetic germplasm from important aquaculture species. The identification and preservation of genetic maps of these species is one of the objectives, as well as, using genetic improvements and nutrition to produce high quality and safe aquaculture products.

2.1.1. Biotechnology in Aquaculture

The importance of fish as a nutritive and health-promoting food products, the increasing production of aquaculture products to satisfy global food demand and the concern of food safety are leading research to focus on the faster production of high-quality products (Bartley and Hallerman, 1995; McLean and Devlin, 2000; Hew and Fletcher, 2001; Maclean, 2003). Aquatic biotechnology has been playing an important role in aquaculture research to improve growth rates, increase cold tolerance, and improve disease resistance. The main fish species that have been used in biotechnology research are: Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*), common carp (*Cyprinus carpio*), tilapia (*Oreochromis niloticus*), and channel catfish (*Ictalurus punctartus*) (Maclean, 2003).

Increase in growth rate of fish has been accomplished using exogenous growth hormone and insertion of growth hormone genes (McLean and Devlin, 2000). Exogenous growth hormones (recombinant bovine placental lactogen, recombinant bovine growth hormone) increase appetite, accelerate growth, increase feed conversion efficiencies, and impact proximate composition of fish (Devlin et al., 1994; McLean et al., 1997; Rasmussen et al., 2001).

Growth enhancement of fish by transgenesis has been researched extensively, using either mammalian or piscine promoters (McLean and Devlin, 2000). The insertion of growth hormone genes using an antifreeze (ocean pout) or metallothionein (salmon) promoter in salmon, has also shown higher and faster growth rates (0- 10 fold) (Devlin et al., 1994, 1995a, 1995b, 2001, 2004; Du et al., 1992); higher feed conversion efficiencies, precocious smoltification, morphological abnormalities, and inferior swimming ability (McLean and Devlin, 2000; Devlin et al., 1995b, Farrell et al., 1997).

The effects of insertion of growth hormone genes on quality parameters of the muscle has not been researched extensively (Chatakondi et al., 1995; Guillén et al., 1999; Cook et al., 2000), however it is an important research area that needs to be addressed in order to evaluate the safe consumption of these fish.

The potential use of transgenic fish for commercial purposes raises several environmental and safety concerns such as: escape of transgenic fish to the environment, adverse nutritional effects, and adverse effects on fish (Maclean, 2003). To monitor all aspects of transgenesis, regulations are being developed. In the U.S., the FDA will regulate any product derived from transgenic animals for use in drugs, animal feed and human food. The Center for Veterinary Medicine (CVM) from the FDA will specifically regulate fish with the growth hormone gene (Lewis, 2001).

In this research, the impact of nutritional and environmental conditions (wild, farmed or genetically modified) on final product quality was evaluated.

2.2 FISH QUALITY

Fish quality is a very difficult concept to explain due to the variety of factors that must be considered. Population, fish species, spawning period, season, nutrition, post-harvest handling, and storage are some of the key factors that will impact the quality of a fish product (Kinsella, 1988; Nielsen et al., 2002). Quality of fish involves nutritional, microbiological, biochemical and physicochemical properties, however, consumers will decide to buy a fresh seafood product based solely on its “freshness”. To determine freshness of a fish product, consumers use their senses for evaluation and will make a decision based on appearance (color, surface appearance) aroma, flavor and texture. Sensory analysis is considered to be the most important tool to determine freshness of a fish product by inspection services and fish industry in the European Union (Parisi et al., 2002). Sensory properties of fish will be reviewed further in this chapter.

The most important indicators of flesh quality are: safety, fat content and distribution, color and texture (Gill, 1990), however, nutritional factors such as n-3 (omega-3) fatty acids and mineral content (essential and heavy metals), also will play important roles in quality attributes.

With the introduction of farmed fish into the market, a variety of differences in their composition and quality have been observed when compared with their wild counterparts. The most common difference between wild and farmed fish is the fillet lipid content (Haard, 1992a, Rasmussen, 2001).

2.3 PROXIMATE COMPOSITION OF FISH

Proximate composition of fish involves the determination of moisture, lipid, protein and ash content. Carbohydrate is determined by difference. The proximate composition of fish is affected by a diversity of factors such as: size, sexual maturation, temperature, salinity, exercise, ration, time and feeding frequency, starvation, type and amount of dietary ingredients (Shearer, 1994).

Protein and ash contents do not vary as often as lipid, since it is not impacted by diet, but mainly is determined by the species type, genetic characteristics and size (Haard, 1992a, Shearer, 1994, Morris, 2001).

Lipid content of fish flesh, on the other hand, is directly related to the nutrition of the fish. When comparing wild and farmed fish, higher lipid contents are found in farmed fish mostly because of the accessible and well formulated diets (Higgs et al., 1989; Nettleton and Exler, 1992; George and Bophal, 1995; Rueda et al., 1997; Cox and Karahadian, 1998; Alasalvar et al., 2002; Grigorakis et al., 2002; Ruff et al., 2002; Grigorakis et al., 2003; Jankowska et al., 2003; Orban et al., 2003). The lipid content of wild fish, however, cannot be manipulated by the fisherman and will be mainly influenced by the prey type and availability, among other factors (Haard, 1992a). The importance of nutrition in farmed fish is enormous and enables farmers with a powerful tool to design products that can not only impact human health positively, but also generate products preferred by consumers.

2.4 IMPACT OF NUTRITION IN FARMED FISH QUALITY

Nutrition in farmed fish and its impact on flesh quality has been researched extensively (Haard, 1992a; Shearer 1994, 2001; Morris, 2001; Rasmussen, 2001; Lie, 2001). Each ingredient has a unique effect on a variety of quality properties. Type, amount of dietary ingredients in the feed, feeding regimes, and ration can all impact flesh quality positively or negatively.

2.4.1. Dietary protein

Dietary protein is the most expensive ingredient in aquafeeds. Fish meal has been the major protein source in these diets due to its optimum amino acid and fatty acid profile, good palatability to fish and other desirable nutritional properties (Bassompierre et al. 1997; Watanabe

2002). A decline in the global production of fish meal, high prices and the need to reduce nitrogen and phosphorus loads are motivating researchers to identify alternative protein sources (vegetable or animal origin) that can be efficiently utilized to optimize growth, reduce feed cost, and lower ammonia and phosphorus outputs. The most common alternative protein sources being investigated are: full-fat and defatted soybean meal (at present the most cost-effective alternative), canola, sunflower, cottonseed, lupin, corn gluten meal, squid, crab, shrimp and meat meal (Kaushik, 1995, 2004; Kikuchi, 1999; Hunter et al., 2000; Cho and Bureau, 2001; Yang et al., 2002, 2003; Cheng et al., 2003; Jahan et al., 2003; Chou et al., 2004; Francesco et al., 2004). There are some considerations that must be taken into account when varying the type of dietary protein, such as: appropriate amino acid profile (vegetable sources are often deficient in lysine and methionine); digestibility; protein value (biological value); availability and steady supply; price; palatability; effects on overall performance characteristics of the animal (growth rate; feed efficiencies) and impact on flesh quality (Watanabe, 2002; Chou et al., 2004). It has been observed that variations in protein source has influenced organoleptic properties of fish fillets by changing the color or altering the flavor (Adelizi et al., 1998; Francesco, 2004; Chou et al., 2004; Oliveira et al., 2004).

Research on varying protein concentrations (same protein source) in diets are focused on optimizing weight gain and determining protein requirements. There is contradictory evidence on the effect of varying dietary protein concentrations on fillet lipid content. Some authors have reported increases in lipid concentration in whole body and muscle when fed different protein concentrations (isocaloric diets) (Huang, 1991; Al Hafedh, 1999; Kaushik, 2004); while others have attributed no impact of protein concentration in fillet proximate compositions implying that dietary carbohydrate (added to keep diets isocaloric) was influencing the lipid content (Shearer, 1994; Robinson and Li, 1999; Alvarez-González, 2001; Yang, 2002, 2003).

2.4.2. Dietary carbohydrate

Dietary carbohydrate can be utilized as an energy source by some fish. Omnivores are known to utilize carbohydrates as an energy source and create a protein-sparing effect, unlike carnivores (Morris, 2001; Hemre et al., 2002). The addition of carbohydrates to feeds to deliver energy to fish and create a protein-sparing effect is being investigated. Most of the research shows that high energy diets and increasing carbohydrate concentrations (dextrin, starch, wheat flour) increases liver lipid and size and lipid content of the fillet (Cho and Kaushik, 1990; Rasmussen et al., 2000; Rasmussen, 2001; Kikuchi and Takeda, 2001; Lee et al., 2002, Luzzana et al., 2002; Yang et al., 2002, 2003). According to Hemre (2001), approximately 15% of the carbohydrate ingested will be stored as liver glycogen (1% of total glycogen) and around 8-15% will be stored in the muscle as glycogen (8% or total muscle glycogen). Carbohydrates are related to lipid deposition in that they play an important role in lipogenesis, increasing lipid biosynthesis from dietary lipid (Hemre, 2001). The ability to take advantage of carbohydrates to spare protein is a multifactor process, which includes: species, strain of fish, temperature, light regime and type and amount of dietary carbohydrate. Protein sparing effects occur when complex sugars and gelatinized forms of carbohydrates are used rather than simple sugars (Hemre et al., 2001).

2.4.3. Dietary lipid

Dietary lipid provides essential fatty acids, phospholipids and energy to fish diets to promote growth, health, metabolic pathways and are the precursors of eicosanoids (physiological functions) (Sargent et al., 1999).

Dietary lipid, just as dietary carbohydrate, can act as an energy source to spare protein utilization for energy and also increase palatability to the diet. The development of high-energy diets (high lipid content) to reduce nitrogen and phosphorus loads from high-protein diets and improve feed conversion ratios, among other factors, is rapidly gaining popularity (Rasmussen et al., 2000; Huang et al., 2001; Chaiyapechara et al., 2003). High dietary lipid in feeds increases the amount of fillet lipid (Shearer, 1994; Bjerkeng et al., 1997; Huang et al., 2001; Rasmussen, 2001; Luzzana et al., 2002; Chaiyapechara et al., 2003; Gaylord et al., 2003; Liu et al., 2004;), which could be considered a quality defect, because of possible higher oxidation rates, lower fillet yields and softer texture.

The most common oil used in fish diets is fish oil due to palatability, but most importantly for its fatty acid profile. The high amounts of n-3 fatty acids present in fish oil impact the fatty acid profile of aquafeeds and subsequently the fish muscle, increasing the nutritional value of the final product (Haard, 1992a; Rasmussen, 2001; Steffens, 1997; Sérot, 1998; Oliveira et al., 2004). Additions of oils from plant sources have been researched as well to determine the effect on fish quality. The fatty acid profile from oils of plant origin differs from fish oil and subsequently will change the fatty acid profile of the fish (reduction in amounts of n-3 fatty acids). The addition of fish oils to commercial diets and increases in fat content of farmed fish can beneficially increase the amount of n-3 fatty acids delivered per fish serving size.

2.5 FATTY ACID PROFILE

Lipids are one of the major organic components of fish, and currently attract the attention of consumers due to the importance of their fatty acid profile.

Lipids are glycerol esters of fatty acids (99%) (Nawar, 1996). Fatty acids are important in human and animal nutrition because of their impact upon a variety of physiological processes and participation in the production of important metabolites that are of biological importance. (Nawar, 1996; Tocher, 2003). Fatty acids can be divided in saturated (single bonds in carbon chain) and unsaturated fatty acids (double bonds in carbon chain). Unsaturated fatty acids are classified and named depending on the chain length, number and position of the double bonds present. If more than one double bond exists in the chain, fatty acids are called polyunsaturated fatty acids (PUFAs) (Nawar, 1996). Fish oils have high concentration of PUFAs, most specifically n-3 PUFAs.

Fatty acids of the n-3 family have been positively related to human health, since benefits of n-3 fatty acids were first reported in 1985(Ackman, 1990). After considerable research, the American Heart Association (AHA, 2002) has recommended the consumption of fatty fish because of their high content of n-3 fatty acids, at least twice a week. For those individuals with coronary heart disease, the AHA recommends the consumption of 1g eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) per day, preferably from fatty fish. Fatty acids from the n-3 family have been related to decreases of: risk of arrythmias, thrombosis, triglyceride levels, growth rate of atherosclerotic plaque and also with the slight lowering of blood pressure (American Heart Association, 2002). The optimal amount of n-3 to deliver the desired positive health effects depends also on the concentration of n-6 fatty acids. Arachidonic acid (AA; 20:4n-6) is the precursor of eicosanoids that can influence the behavior of immune cells and inflammatory reactions and their overproduction might be detrimental to human health (Kinsella, 1988). DHA and EPA regulate the conversion of linoleic acid (18:2n-6) to AA and produce eicosanoids themselves, thereby displacing eicosanoids derived from AA. The ratio of n-3 to n-6(n-3/n-6) fatty acids is often reported and this ratio should be relatively high in order to be considered advantageous for human health (Kinsella, 1988; Stephens, 1997).

The amount and kind of fatty acids in wild fish species varies according to their environment and nutrition. Marine fish lipids generally have lower levels of linoleic acid and linolenic acid (18:3n-3), however, they contain higher levels of n-3 fatty acids. Marine fish consume phytoplankton (algae) high in EPA and DHA or fish, mollusks and crustacean that have fed on algae (Steffens, 1997). Freshwater fish, however, have higher levels of n-6 fatty acids (18:2n-6) and AA. The different profile is due to dietary sources, since freshwater fish feed on fresh water algae, crustaceans, and aquatic larvae of insects that are rich in 18:2n-6, 18:3n-3 and EPA (20:5n-3). Freshwater fish generally have an increased ability to elongate and desaturate essential fatty acids 18:2n-6 and 18:3n-3 to AA and EPA and DHA, respectively. This process either does not occur or is severely depressed in marine fish due to the abundance of 20:5n-3 and 22:6n-3 in dietary sources as well as the lack of efficiency of enzyme systems needed for this bioconversion (Stephens, 1997; Tocher, 2003). Linoleic acid competes for elongases and desaturases during the production of eicosanoids (prostaglandins, thormboxanes and leucotrienes) when 18:3n-3 requires them to produce EPA and DHA.

Farmed fish, if fed the right type of protein and lipid source, could have a robust supply of n-3 fatty acids and, even better, increase the content of n-3 fatty acids in the fillet that could positively impact human health, without altering the chemical, physical and sensorial properties of the farmed products.

One of the major disadvantages of having high amounts of PUFAs in fish is the impact these compounds have on oxidation reactions and subsequent production of off-aromas and off-flavors, thereby decreasing the shelf-life of fresh and frozen fish fillets (Flick et al., 1992).

2.6 LIPID OXIDATION IN FISH

Lipid oxidation involves the reaction of oxygen with free radicals from unsaturated fatty acids, which leads to the production of off-flavors and off-aromas. The most common method to determine lipid oxidation in fish is the measurement of thiobarbituric reactive substances (TBARS), in which carbonyl products react with thiobarbituric acid to develop a color which is measured by absorbance on a spectrophotometer (Freeman and Hearnsberger, 1993). There are different types of lipid oxidation: photoxidation, thermal oxidation and enzymatic oxidation, but autoxidation of unsaturated fatty acids is the most common (Labuza, 1975). Lipid oxidation is influenced by different factors: fatty acid profile, oxygen, light, metals, temperature, and water activity, among others.

The main steps of autoxidation are: initiation, propagation and termination. Hydroperoxides are the first oxidation products (no flavor) which will subsequently form compounds such as aldehydes, alkanols, alkanes, alkenes that produce an off-flavor (Nawar, 1996; deMan, 1999a). Volatile aldehydes are responsible for the rancid flavor in catfish fillets, most specifically pentanal, hexanal, cis-hexenal and nonenal (Freeman and Hearnsberg, 1993) Off-flavors due to oxidation will be discussed in the flavor section. Rancidity is a major problem in frozen fish, while in fresh fish the main quality problem is microbiological spoilage (Ackman and Ratnayake, 1992).

The high content of PUFAs in fish increases the rate of oxidation, since the increased double bonds accelerates oxidation (Flick et al., 1992; Nawar, 1996).

Lipid oxidation in fish is a major quality problem because of the decrease in shelf-life through off-flavor production and color and texture modification (Lie, 2001). The aquaculture industry and researchers are investigating options to reduce lipid oxidation by including antioxidants in the diets (vitamin E (α-tocopherol) or injecting mixtures of antioxidants (ascorbic acid, citric acid) in a tripolyphosphate sodium solution to fish fillets (Freeman and Shanon, 1994; Stéphan et al., 1995). Other antioxidants used in seafood to prevent lipid oxidation are: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), ethylene diamine tetraacetic acid (EDTA; chelating agent) (Flick et al., 1992). Fish naturally possess antioxidants that can help retard lipid oxidation and these include α-tocopherol, ubiquinol and carotenoid pigments (Undeland, 2001).

2.7. SENSORY PROPERTIES OF FISH

Sensory evaluation is "The scientific method that measures analyzes and interprets responses from products through sight, smell, touch, taste and hearing" (Lawless and Heymann, 1999a) Sensorial attributes of food products are perceived by consumers in a specific order (Meilgaard et al., 1999):

1. Appearance: color, size, shape, clarity, surface
2. Odor (Aroma): "Aroma is the odor of a food product". Volatile compounds of products are affected by temperature and biochemistry of the compounds
3. Consistency and texture: viscosity, consistency and texture. Texture (hardness/ firmness, cohesiveness, dryness, moistness): sensory perception of mechanical or physical properties of the food product by the sense of touch (hand, tongue, lips, jaw)
4. Flavor: sensorial perceptions of chemical compounds of the food product by tasting in the mouth. Flavor is a complex concept that integrates the aroma (volatile substances), tastes (salty, sweet, sour and bitter) in the mouth and chemical factors (astringency, heat, metallic flavor, umami taste).

Sensory analysis, as mentioned before, is of vital importance to the fish industry, in order to assess freshness of products. Fish consumers expect a product that is safe and has good appearance, odor, taste and texture (Warm et al., 2000; Parisi et al., 2002) and their decision to purchase a fish product is based first on appearance, followed by flavor and then texture (Rasekh and Kramer, 1970). Some properties in fish that are relevant to fresh and frozen fish quality that are tracked analytically and could be related to sensorial perceptions are: texture (mechanical determination of hardness/firmness, rigidity), off-flavor production (measurement of trimethylamine (TMA) and total volatile bases (TVB)), color (Minolta cholorimeter) and microbiological characteristics (specific spoilage organisms, total plate count) (Parisi et al., 2002).

2.7.1 Flavor and aroma

Flavor is a broad concept that considers all the compounds in food that have a smell and taste, the interaction between these compounds and the effect on human senses (DeMan, 1999b). Aromas and flavors of fish can be influenced by a variety of factors and therefore can be grouped as follows (Lindsay, 1990):

1. **Fresh fish:** Fresh fish aroma and flavor are characterized for being mild, melon, green, sweet and depending on the species-specific aroma and flavor characteristics would be encountered. The flavor of fish is influenced by free amino acids, minerals and fatty acids (Haard, 1992a). The fatty acid profile is most likely is responsible for fish aroma volatile compounds. The action of enzymes (lipoxygenases) on PUFAs, produces carbonyls,

aldehydes and ketones which are responsible for some of the fresh like odors of fish (Grigorakis et al., 2003; Morita et al., 2003). Some of the fresh fish volatile compounds obtained from enzymatic action on PUFAs are: hexanal (green); t2- hexenal (green); 1-octen-3-ol (raw mushrooms); 1, c5-Octadien-3-ol (earthy, mushrooms, green); t2, c6-Nonadienal (cucumber-like); t2, c6-Nonadienol (cucumber, melon-like) (Lindsay, 1980). When comparing odors in wild and farmed cooked turbot after 1 week of frozen storage (Prost et al., 1998), differences were found in aroma compounds between treatments, mainly due to variations in the fatty acid profiles. The main aroma compounds identified, included: TMA (fishy); 2,3 butanedione (buttery); 4-heptenal (boiled potato); 4-methyl-2-pentanol (mushroom) and 2,6- nonadienal (cucumber). Grigorakis et al. (2003) compared wild and farmed gilthead sea bream after 1 day of storage and reported production of volatile alcohols (1-butanol, 1-penten-3-ol) that contributed to smooth, melon and cucumber-like aroma of fresh seafood. Aldehydes (some found: hexanal, 2-methyl-1-butanal, nonanal, 2,4-heptadienal) and ketones (some found: 2-octanone, 2-decanone, 2-propanone) were also detected. These carbonyls are known to deliver the fresh-like aroma. In this study, wild and farmed gilthead sea bream also varied in fatty acid composition, which explained differences in some aldehydes and alcohols and a significant difference in flavor. A higher concentration in AA in wild fish, increased concentrations of hexanal, 1-octen-3-ol and (E)-2 nonenal (Grigorakis et al., 2003). Differences in volatile compounds are to be expected between farmed and wild fish, as well as between freshwater and saltwater species due to dietary differences and environmental conditions (Lindsay, 1990).

2. **Oxidized flavors:** Oxidation of lipids (PUFAs) in fish produces volatile compounds that are commonly referred to as fishy, cod liver-like, burnt, and whale oil-like (Gill, 1990; Lindsay, 1990). Autooxidation of n-3 fatty acids produces t,c,c-2,4,7-decatrienal (18:3, 20:5 and 22:6 n-3) and t,t,c-2,4,7 decatrienal, which are partly responsible for oxidation off-flavors. Fatty acids of the n-6 family are responsible for the production of hexanals (cut grass, green), (E2)-octenal (lemon), (E)2-nonenal and (E,E)-2,4-decadienal, while oxidation of n-3 fatty acids produce c-4 heptenal (cold-storage flavor, cold boiled potato, cardboardy), pentenylfuran (orange) and (E,Z)-2,6-nonadienal ((Lindsay, 1990; Prost et al., 1998). There are a variety of compounds responsible for off-aromas in fish and one

that is very common is TMA (fishy odor; indicator of fish spoilage), which is produced by the action of microorganisms on trimethylamine oxide (TMAO) (Lindsay, 1990; Grigorakis et al., 2003). This compound serves an osmoregulatory function in saltwater fish (freshwater fish have minimum quantities of TMAO). In frozen fish, TMAO can be degraded to dimethylamine (DMA) by enzymatic action and cause not only off-flavors but toughening of the meat by reaction of formaldehyde (by-product of DMA enzymatic reaction) with muscle proteins.

3. **Processing flavor:** Volatile compounds produced by the action of cooking and processing seafood products are generally related to the flavors obtained from canned seafood products (canned tuna-like).
4. **Environmentally derived:** These are volatile compounds produced from the action of the environment. The most common example of environmentally influenced off-flavor is "muddy, musty or blue-green". These off-flavors are caused by methylisoborneol (MIB; 1-R-exo-1,2,7,7-tetramethylbicyclo-[2-2-1]-heptan-2-01) or geosmin (trans-1-10-dimethyl-trans-9-decalol), produced by cyanobacteria, actinomycetes, and algae (Linday, 1990; Grimm et al., 2004). In the catfish industry muddy flavor accounts for 80% of the rejections due to off-flavor in the meat. Farmers must contend with the production of off-flavors most commonly identified as: muddy, musty, sewage, rotten plants, stale, metallic, lagoon, which most of the time are produced by algae; other common farmed fish off-flavors are "hatchery" (soybean oil) and "fishy" (fish (menhaden oil [TMA]) (Haard, 1992a).

The importance of sensory techniques in the fish industry has led to the development of a sensory wheel, similar to the wine sensorial wheel, for specific species (Warm, 2000) in which several odor, flavor, textural, and appearance (color) attributes are presented to make sensorial evaluation easier.

2.7.2. Color

Color is an appearance property that is perceived by the action of light on the object (Lawless and Heymann, 1999b) and is an important factor impacting consumer's acceptability (Haard, 1992a).

Skin and flesh color will vary depending on pigments and chromatophore cells (cells that hold the pigments). Fish color is influenced by biological pigments in the skin, flesh or obtained by diet (heme proteins, carotenoids); postharvest pigments, which are caused by post-harvest handling and processing; and/or by addition of colorants (injection of food dyes, dipping in carotenoids solutions) (Haard, 1992b).

Chromatophores can be found in the skin, subdermis and dermis of fish. They can be classified depending on the pigment that they hold: melanophores (brown melanin), erytrophores (red carotenoids), and xantophores (yellow carotenoids).

Probably the most well known and studied pigment in the seafood industry is the group of carotenoids (yellow, orange, red), that are responsible for the color of valuable species: shrimp, salmon, rockfish, red snapper, trout, crab, lobster and crayfish (Haard, 1992b). The two main classes of carotenoids are carotenes and xanthophylls (oxygenated carotenes).

De novo synthesis of carotenoids can only be accomplished by green plants, bacteria and fungi. Fish obtain carotenoids from dietary sources, from which phytoplankton is the main source in aquatic environments. Once fish have ingested the carotenoids different biochemical processes can occur to obtain the final color on skin and flesh color, and this is dependent upon fish species and type of carotenoids.

Astaxanthin

Astaxanthin (oxygenated carotenoid) and canthaxanthin are responsible for the distinctive color of some important fish species. Canthaxanthin, an intermediate in the metabolism of β-carotene to astaxanthin, is found in smaller concentrations in wild salmon (Buttle et al., 2001; Nickel and Springate, 2001). However, this pigment is also used in farmed salmon and shrimp feeds to improve pigmentation. In studies with Atlantic salmon, canthaxanthin showed better deposition than astaxanthin (Buttle et al., 2001). In wild fish, however the typical red-orange color is mainly due to astaxanthin rather than canthaxanthin (Buttle et al., 2001).

Sources of astaxanthin are crustacean wastes (crawfish, crab, shrimp, krill) red yeast (*Phaffia rhodozyma*), algae and alfalfa (Haard 1992b; Nickel and Springate, 2001). Astaxanthin is an expensive pigment, therefore cheaper options are being researched to achieve pigmentation in farmed salmon and shrimp feeds. In farmed shrimp feeds, synthetic forms of astaxanthin and canthaxanthin, crustacean meal, paprika, marigold, capsicum, red yeast, microalgae

(*Haematococcus pluvialis, H. lacustris*) and spirulina, are added to achieve the desired pigmentation (Liñán-Cabello et al., 2002).

Carotenoid deposition can be summarized in the following steps: absorption of the pigment in the digestive tract, transportation of pigment in blood, retention in muscle and metabolism of carotenoids (Johnston et al., 2000; Buttle et al., 2001; Nickel et al., 2001). The deposition of the carotenoid in the muscle depends on muscle properties as well. Carotenoids will bind non-specifically to hydrophobic sites on the actomyosin by weak hydrophobic bonds. Deposition of the pigments is affected by numerous factors such as: genetics, age, growth rate and muscle texture. The amount and size of muscle fibers also are important in pigment deposition, since they could affect the number of binding sites. Fiber density also plays an important role in the color of fish, since the firmer the muscle is (higher fiber density), the better color is appreciated by the lower scattering light effect (Jonhston et al., 2000).

2.7.3. Texture

Texture is difficult to conceptualize, however some authors refer to it as the mechanical or physical properties of the food (Coppes et al., 2002). Texture of fish muscle is an important attribute for consumers and fish processors. Fish muscle is composed of segments (myotomes) separated by connective tissue (myocommata or myosepta) and can be found along the fish (long axis). Muscle fibers are inserted in the myosepta and are surrounded by collagen (Johnston, 1999, 2000, 2001). In fish, red and white fibers play an important role in mechanical properties of the muscle. Red fibers (high myoglobin content) are small (cross-sectional area) and are used for long periods of swimming (migration) and use aerobic metabolic pathways. Red fibers are present in the red or dark muscle (superficial) and contain specific myofibrillar proteins. Dark muscle is more active and higher in fat content when compared to white muscle (Fauconneau et al., 1995). White fibers are most present in the edible muscle or white muscle (deep muscle) and are used by the fish for fast swimming and use anaerobic metabolic pathways. White fibers are composed of myofibrils that contain protein filaments of actin and myosin (Jonhston, 2001).

Fish muscle properties are dependent on type of muscles and protein, depending on genetics, growth rates and lipid content. However, the changes in texture of the fish muscle (firmness/hardness) due to post-harvest handling are very important for fishermen and farmers. Texture is affected by the rate and duration of rigor mortis, pH, proteolysis and storage (Haard, 1992a). Farmed fish generally have a softer texture than wild fish and this could be attributed to factors like lipid content and amount of exercise (Haard, 1992a).

After death, muscle goes through a variety of chemical processes that could impact the texture of the fillet. The amount of glycogen and stress can impact duration of rigor and pH of the meat which will yield either a soft flesh or a very firm meat. Rigor mortis is the binding process of myosin to F-actin, which causes a contraction of the muscle, leading to rigidity. The contraction will last until all energy reserves (glycogen) are consumed with a subsequent production of lactate and decrease in pH. Rigor can start 6 to 24 h post-mortem and it can last until all energy has been consumed (5-7 days) (Fauconneau et al., 1995). Fish stress at death can impact flesh quality, by depleting glycogen before rigor, leading to an earlier rigor and a reduction in the concentration of lactate and a higher final pH in the muscle, leading to soft flesh.

Texture can be measured as force (Puncture, shear, compression), time (variation of food deformation by application of force) and distance (measure of strain). The most common instruments used to measure texture are: Instron Universal Testing Machine, Texture Analyzer and the Reograph Gel (Coppes et al., 2002).

2.8 OTHER NUTRITIONAL PROPERTIES OF FISH

2.8.1 Minerals

Mineral content in fish is roughly estimated by ash determination, where minerals are in the form of oxides, sulfates, phosphates, nitrates and chlorides. Minerals are deposited in fish flesh from the aquatic environment and nutritional sources (Haard 1992a; Miller, 1996; deMan, 1999c). Minerals can be classified depending on their essentiality to humans:

Essential macro minerals (required amount > 100 mg/day): calcium; potassium; sodium; magnesium; chloride and phosphorus. Other major salts present in food are: sulfate and bicarbonates (Mayes, 1993; deMan, 1999c).

Trace elements (micro minerals) (required amount < 100 mg/day): chromium; cobalt; cupper; iodine; iron; manganese; molybdenum; selenium; silicon; zinc, fluoride and nickel.

Non-nutritive and toxic: mercury, lead, arsenic and cadmium

Some of the most talked about minerals in human nutrition are sodium (excessive consumption can cause hypertension), calcium (important mineral in bones and teeth, its deficiency is related to osteoporosis), and iron (important component of hemoglobin, deficiency can cause anemia).

Mercury levels in fish have gained recent media attention after the FDA published a consumer advisory (2001) where recommendations were made to pregnant women and young children to restrict consumption of shark, swordfish, king mackerel and tilefish because of a probable risk of high methylmercury concentrations.

Fish are good sources of iron, zinc, phosphates and cobalt (Miller, 1996). However the environment where they live will influence in the final mineral content. For instance, fish from acid waters have higher contents of manganese, zinc and heavy metals like mercury. Minerals not only play an important role in the nutritive value of fish, but also could impact sensorial properties such as flavor (Haard, 1992a).

2.8.2 Amino Acids

Amino acids are joined together by peptide bonds to conform proteins (deMan,1999d). There are 9 essential amino acids that need to be included in the adult human diet, since they can not synthesize amino acids to support growth or health (Rodwell, 1993). The essential amino acids (for adults) are: histidine, isoleucine, leucine, lysine, methionine (and/or cysteine), phenylalanine (and/or tyrosine), threonine, tryptophan and valine.

The quality of a protein is determined by its amino acid profile (higher recommended levels) and digestibility (better or similar to egg or milk). Usually animal proteins are better quality proteins than plant proteins. Fish is considered a high quality protein (100% chemical score, according to FAO/WHO) (Damodaran, 1996).

In farmed fish nutrition, the substitution of protein sources can become a problem if the amino acid profile of the protein is unknown or the right amount of amino acids is not supplemented in the diet. Essential amino acids for fish are: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

The concentration of amino acids in fish flesh is not determined by dietary effects but it is affected by growth and genetic information. In farmed and wild fish, the amount and quality of protein is very similar (Haard, 1992a, Shearer, 1994). However, there have been differences reported in free amino acid content between farmed and wild fish and this could impact fish flavor (Haard, 1992a).

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