BIOCHEMICAL AND DESCRIPTIVE PROFILING OF GRAYLING (*Thymallus thymallus*) AS A NOVEL AQUACULTURE PRODUCT

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Biochemical and descriptive profiling of grayling
(*Thymallus thymallus*) as a novel aquaculture product
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ABSTRACT

Grayling (*Thymallus thymallus*) is a white-fleshed salmonid that remains, for the most part, an unknown fish to the majority of the Finnish population due to the sparsity of its habitats, depletion of populations and earlier restrictions on grayling commercial activity that were imposed in an effort to preserve some local grayling populations. It is, however, a favourite among recreational and sports fishermen.

The Fish Innovation Centre (FIC) set up the Grayling Project (September 2006 - December 2007), which was realized together with the Kuopio University's Department of Biosciences, Fish Research Unit and Department of Physiology with the aim of assessing grayling as a novel aquaculture product based on descriptive analysis, fatty acid composition and shelf life.

Grayling is a fish that has a typical salmonid fatty acid profile, which reflects its diet. It has high amounts of ω3 fatty acids and polyunsaturated fatty acids. The latter, makes it suspect to lipid oxidation and rancidity. The shelf-life of hot-smoked grayling is typical for hot-smoked fish products. Data suggested that hot-smoked whitefish (*Coregonus lavaretus*) deteriorated quicker than hot-smoked grayling, but this cannot be confirmed due to differences between samples regarding size and processing. Microbial results were surprising because it was expected that bacterial levels would be high, due to visible mould on the surface of fish at times and also due to results from the sensory evaluation. However, samples were taken from the flesh which had dried out and had few or no microbes. Data suggested that age was a factor in the quality of grayling flesh, but other differences such as differing diet and water quality may also have contributed to observations made.

Grayling, as a novel aquaculture food product is a potential competitor for whitefish given the properties and attributes analysed. However, there are questions that remain open for future study, which include quantification of lipid content and its possible seasonal variation, exploring the differences between the sensory profiles of whitefish and grayling in greater detail and investigating consumer attitudes towards grayling products and preference for processed grayling products compared to other fish products

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TIIVISTELMÄ

Harjus (*Thymallus thymallus*) on valkolihainen lohikala, joka on jäänyt suurimmalle osalle suomalaisista tuntemattomaksi laikuittaisen esiintymisen ja kantojen vähenemisen takia. Lisäksi joidenkin vesistöjen paikallista harjuskantaa on suojeltu rajoittamalla harjuksen myyntiä. Tietyille vapaa-ajankalastajille harjus on tuttu ja arvostettu kala.

Kalatietokeskus/Fish Innovation Centre (FIC) aloitti Harjus-projektin (syyskuu 2006-joulukuu 2007), joka toteutettiin yhdessä Kuopion yliopiston biotieteiden laitoksen, kalantutkimusyksikön ja fysiologian laitoksen kanssa. Projektin tarkoitus oli arvioida harjusta uutena kalaviljelylajina käyttäen aistinvaraista tutkimusta, rasvahappoanalyysia sekä säilyvyyskoetta.

Harjuksella on tyypillinen lohikalan rasvahappoprofiili, joka heijastaa sen ruokavaliota. Harjuksella on korkea pitoisuus ω3 rasvahappoja sekä monityydyttömiä rasvahappoja. Näin harjus on altis rasvan hapettumiselle ja härskiintymiselle. Lämminsavustetun harjuksen säilyvyysaika on samanlainen kuin muillakin lämminsavustetuilla kalavalmisteilla. Aineisto näytti antavan sellaisen kuvan, että lämminsavustettu siika (*Coregonus lavaretus*) pilaantui nopeammin kuin lämminsavustettu harjus, mutta tätä ei voida vahvistaa näytteiden kokoerojen ja prosessoinnin mahdollisten vaihtelujen takia.

Mikrobiologiset tulokset yllättivät. Kalojen pinnoilla oli välillä havaittavissa hometta ja todettavissa aistinvaraista laadun heikentymistä, joten korkeita bakteeripitoisuuksia oli odotettavissa. Näytteet kuitenkin otettiin kalan lihan syötävistä osista, jotka olivat kuivuneet ja joissa oli hyvin vähän mikrobeja. Aineisto osoitti, että kalan ikä on vaikuttava tekijä harjuksen lihan laadussa, mutta myös muita eroja kuten eri ruokavalio ja veden laatu saattoi olla vaikuttamassa havaintoihin.

Harjus voisi olla mahdollinen kilpailija siialle elintarvikemarkkinoilla. Jatkossa olisi määritettävä tarkkaan harjuksen rasvojen määrä ja niiden mahdollinen vaihtelu vuodenajan mukaan. Lisäksi aistinvaraiset erot siian ja harjuksen välillä olisi määritettävää tarkemmin. Tärkeää olisi tietää myös kuluttajien suhtautuminen harjustuotteisiin sekä mahdollinen mieltymys muihin kalatuotteisiin verrattuna harjukseen.

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The Grayling Project did not just touch on a possible new aquaculture product, it also opened up, for me, a whole new outlook on fish and food products. It has been an interesting journey where I have learnt a whole new set of skills starting with how to fillet a fish.

The process began with a meeting in 2005 between Liisa Nurminen, of the Kuopio University, and me, and a follow-up with Heikki Koskinen, who then worked at the Fish Innovation Centre. About a year later, the actual work and research began, and the final analyses completed this year, 2008. The grayling were kept at the Fish Research Unit, which is where I took samples of dorsal muscle, as well as gathered length and weight data. Sensory analysis was done at the Kuopio Univerisity's Department of Clinical Nutrition, at the Department of Biosciences Nutrition and Food Biotechnology laboratory and also at the Kuopio City Environmental Laboratory. Microbial analysis was done at the Nutrition and Food Biotechnology laboratory and fatty acid analysis was done at the Department of Physiology and Department of Clinical Nutrition. The fish feed was provided by the company Biomar and fish processing conducted at Kuopion Kalatuote Oy, Markkasen Kala Oy and Lihaniekka Oy.

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Roseanna Avento

TABLE OF CONTENTS

1 INTRODUCTION

Diversification in the food fish sector has been acknowledged as a way to developing aquaculture and meeting market demands for fish. According to FAO (2007), the global per capita fish consumption has increased over the past four decades, rising from 9.0 kg in 1961 to an estimated 16.5 kg in 2003. In Finland, the average annual consumption of domestic fish per person, in the year 2005, was 5.2 kg filleted weight (Nylander, 2006). Rainbow trout (*Oncorhynchus mykiss)* and carp (*Cyprinus carpio*) dominate European inland aquaculture and it has been recommended that investigations of under-utilized species be conducted (FAO, 2007; FAO, 2001).

Finnish aquaculture production is focused on both rainbow trout and European whitefish (*Coregonus lavaretus*), though rainbow trout dominates the industry (Nylander, 2006). The Finnish Game and Fisheries Research Institute (FGFRI) has helped the Finnish aquaculture industry diversify from trout production to production of whitefish due to demands from the market for new fish species, and whitefish which has been seen as a good alternative to red-fleshed salmon, is greatly valued for its white flesh and easy production methods (FGFRI, 2006a).

Grayling (*Thymallus thymallus*) is a white-fleshed salmonid. Grayling's scientific name is attributed to the slight aroma of thyme (*Thymus vulgaris*) given off by its flesh. Grayling is also commonly referred to as the "Lady of the Stream". The description of grayling given by John Bickerdyke as 'a very beautifully colored, yet somewhat grotesque dace' fits its like a glove, according to Foyle (1976), who says that it is a beautiful fish of delicate and graceful symmetry, with its prominent outsized dorsal fin and its lovely coloring, shot with purple, gold and gray. Grayling inhabits cold, running, well-oxygenated riverine and lake waters and can be found in the northern hemisphere incuding England, Wales, Northern Europe and in the Ural Mountains (Koli, 1998).

Grayling remains, for the most part, an unknown fish to the majority of the Finnish population due to the sparsity of its habitats, depletion of populations and earlier restrictions on grayling commercial activity that were imposed in an effort to preserve some local grayling populations. It is, however, a favorite among recreational and sports fishermen and is a common catch especially in the fresh, cold waters in Lapland.

Recreational catches of grayling in Finland were valued at 3.21€/kg in 2002, 2.43€/kg in 2004 and 2.98€/kg in 2006; while in comparison, recreational catches of whitefish were valued at 3.00€/kg in 2002, 2.82€/kg in 2004 and in 2006 the value rose to 2.98€/kg (FGFRI 2002; FGFRI, 2004; FGFRI, 2006b). Lehtonen (1989) maintains that grayling spoils quickly and cannot therefore be transported over long distances after slaughter, which has also contributed to its absence from the fish stalls outside northern Finland.

Grayling culture is a relatively new activity in Finland, conducted primarily for stocking purposes, to conserve original strains, maintain diversity and introduce the species into waters where recreational fishing and fishing tourism is practiced (Nissinen, 2003a).

In Finland, grayling is more commonly farmed in natural dams than in tanks. The expense of tank farming has curbed its growth even though it is more successful than dam farming when aquaculture results are considered. There are, for example, lower mortality losses among fry. Farming conditions and feeding are also more controlled in indoor tank farming. (Nissinen, 2003b).

Grayling culture is considered similar to that of other salmonids because its water quality requirements and farming methods do not differ (Nissinen, 2003a). In particular, grayling culture is similar to that of arctic char (*Salvelinus alpinus*), and both do well in high density tank farming, due to their calm nature. In addition, during the first stages of ongrowing, grayling and whitefish culture are quite similar (Nissinen, 2003b).

One of the challenges in grayling tank farming relate to fish egg quality (Maukkonen and Pursiainen, 2004) and in the FGFRI Taivalkoski station, quality may vary greatly, with survival rates ranging from 7-8% to 20-30% (Karjalainen, M., personal communication). The lack of suitable feeds also makes grayling culture challenging. Carlstein (1993) has demonstrated that grayling can, during the start-feeding phase, be successfully reared on artificial dry diets, developed for other species with small fry such as whitefish. In addition, artificial dry diets can be used exclusively throughout the first summer. Previously, this was not thought possible, for instance, Ocvirk and Vovk (1986) did not consider it possible to start-feed grayling fry without using live or zooplankton as supplementary feeds (Carlstein, 1993).

Feed suitability is considered an issue of significance because fatty acid patterns have been shown to differ greatly between wild and cultured grayling and this has been attributed to the variations in fat quality discerned in natural food available to wild fish and artificial feeds given to cultured fish (Ahlgren et al., 1999).

Pathological problems also pose challenges in grayling culture. Disease-causing agents in grayling culture include parasites, bacteria (especially Atypical *Aeromonas salmonicida*, ASA) and fungi. (Nissinen, 2003b).

Nissinen and the Fish Innovation Centre (FIC) together with the Kuopio University have, in the year 2003, successfully compiled a “Guide for Grayling Aquaculture” with the aim of developing grayling culture (Nissinen, 2003b). Further investigation into the market entry possibilities for grayling is required for exploration of expanding grayling culture from stocking purposes to food fish production (Nissinen, 2003b). This may in turn merit interests in increasing and developing grayling culture.

Interest in examining the potential of introducing new aquaculture products to the market, has formed the basis of the Fish Innovation Centre’s Grayling Project, and was realized together with the Kuopio University’s Department of Biosciences, Fish Research Unit and Department of Physiology from September 2006 to December 2007. The aim of the project was to profile grayling on the basis of descriptive analysis, fatty acid composition and shelf life in order to gain scientific information that could be used to evaluate its selling points and thus its marketability.

2 CONCEPTUAL FRAMEWORK

2.1 LAUNCHING NEW AQUACULTURE PRODUCTS

FAO (2007) attributes 43% of the total amount of fish in the world available for human consumption in 2004 to aquaculture. Between 15% and 35% of the aquatic products consumed in southern European countries, are aquaculture products (Harache, 2002). In Finland, 39% of the value of primary fish production in 2005 was attributed to aquaculture, compared to 41% from recreational fishing and 21% from commercial fishing (Nylander, 2006).

The share of aquaculture products on the market will continue to grow and diversify despite the conflicts for development. Some species have an exceptional aptitude to farming and may have a possibility to gain ground in the export industry towards European markets, which in turn stimulates new demands from consumers and opens new market segments for species that are, at present, niche products. (Harache, 2002).

Through the years, changes in the market have taken place leading to the need for new product development. These changes include a slow and steady rise in demand, increased imports from third countries due to stagnant European production, rising demand for fresh and value-added products and reduced demand for unsophisticated frozen and canned products (FAO, 2001).

The concept of a new product is complex because it means different things to different people in different markets (Mariojouls and Young, 2002). A product is regarded as new when prior exposion to the product is lacking or when the product has not been available on the market at all (Iandoli and Cozzolino, 2002; Mariojouls and Young, 2002). Products may also be regarded as new in terms of degree of innovation (Mariojouls and Young, 2002; Möller, 2003).

Six categories of new products according to degree of innovation, are given by Möller (2003) thus:

i. Classically innovative products (New products that create an entirely new market)
ii. New product lines (New products that allow a company to enter an established market for the first time)
iii. Product line extensions (New products that supplement a company's line through variations in package size, recipes etc.)
iv. Improved and revised existing products (New products that provide improved performance and greater perceived value)
v. Repositioned products (Existing products that are targeted for new markets or market segments)
vi. Cost-reduced products (New products that provide similar performance at lower cost than the original product)

Launching new aquaculture products is not a simple endeavor. Only 10% of all new products are successful in the market (Brethauer, 2002). Failure in new product launch is attributed to poor product definition and the absence of product definition (Möller, 2003). Young and Smith (2002) propose three other reasons for the low success rate in launching new aquatic products. First, producers of aquatic foods compete not only with other aquatic products, both captured and cultured, but they also compete with products from other food categories. Second, organizations producing competing food products are large and have access to extensive resources that can be used in new product development, while many aquaculture producers are comparatively small-scale and lack similar resources. Third, many aquaculture producers tend to focus on the technical issues of fish husbandry rather than adopting market-oriented perspectives that are significant for the successful launch of new aquaculture products.

Markets today expect that products fit certain criteria. Trends that are of interest when marketing new aquaculture products, vary from a growing affinity for diversity in flavors to demand for simple convenience foods. The main drivers for demand in seafood and aquaculture products are the quest for convenience in preparation, product quality and the healthy image that seafood has gained in terms of nutritive value and special health benefits. (Möller, 2003).

To succeed on the market, aquaculture producers are encouraged to be proactive rather than reactive, and expend time and effort in researching, creating and communicating about new product options and collecting good quality market data (Papageorgiou, 2002; Young and Smith, 2002).

New product development firstly requires that consumers are involved early in the process. In addition, real-life studies of consumer liking for food products should be made and these should involve multi-disciplinary cross-functional teams that enable the process effective. Management should also be involved to provide leadership, adequate resources, empowerment and a clear view on strategy and objectives. (Möller, 2003).

Four themes that must be considered by aquaculture producers in an effort to understand the market needs, manage their markets and finally implement their commercial strategies are outlined by Monreal (2002) as product quality and health, environmental concerns, socio-economic aspects and finally, marketing. Aquaculture products will have to provide strategically sound arguments like freshness and traceability and proudly claim their farmed origin and demonstrate that the responsible practices of their production are environmentally friendly (Harache, 2002).

These factors will, together, provide a holistic view of customer needs during product development and also provide commitment from the producer organizational leaders, which is imperative for new product success on the market.

2.2 VALUE-ADDITION IN AQUACULTURE

Consumer and market trends have initiated a number of structural changes that have occurred in aquaculture, to maintain and improve efficiency and profitability. These changes include the emergence of high-performance large-scale distributors and retailers, the improved performance of logistics and transport companies that permit suppliers to reach important market sites efficiently and effectively, the modification of national and international trading routes, pressure from consumers and distributors on producers to supply quality products and finally a wide range of product availability and increased consumer bargaining power. (Papageorgiou, 2002).

Market demand has shown progressive evolution, requiring diversified products under diversified forms for instance some regional markets for example in Europe, still appreciate unique round gutted fish, but at the same time complete or replace their demand with several references to the same product: steaks, fillets, skinned fillets, pre-packed, brochettes, cold or heat smoked, marinated, ready to cook and so forth. (Harache, 2002).

Whereas diversification refers to the introduction of new species to develop the culture of species that are not present in all countries, differentiation refers to the introduction of products that have added value in terms of new packaging, new processing etc. (Iandoli and Cozzolino, 2002).

Value-addition for fish is mostly a highly subjective concept, but there is a general consensus regarding the attributes involved which include convenience of use, good basic quality, and interesting and pleasing sensory properties that are often associated with coatings and accompaniments (Möller, 2003). Adding value through some sort of "transforming" is a particularly important issue with regard to fish products, considering that the increase in consumption of fresh fish in most European countries is due to steaks and fillets rather than to whole fish (Papageorgiou, 2002; Paquotte, 1998).

Differences between consumer habits in Northern European countries and Southern European countries have been observed in the way they add value that they require for seafood products, in reference to species diversification and product differentiation. In the Southern European countries, there is a significant demand for fresh and frozen products. On the other hand, in Northern European countries, there is a significant demand for processed and frozen products. (Iandoli and Cozzolino, 2002).

Although the aquaculture sector is developing, an in-depth transformation of market conditions is also ongoing in Europe. The most important factors are the rapidly expanding role of supermarkets in the distribution chain, the new development of new processing techniques like pre-packed fresh fish and the growing importance of catering and the evolution of more sophisticated food consumer behavior. The analysis of the general trends of the market for food and seafood products must, therefore, be undertaken with regard to the specific constraints of aquaculture in terms of production costs, dependence on environmental factors and zootechnical constraints. (Paquotte, 2002)

2.3 QUALITY OF FISH PRODUCTS

Quality is a concept commonly employed in aquaculture. Suppliers often purport to deal only in quality products made from fresh fish, without clearly defining what quality in that instance is (Bremner, 2002). It is without a doubt that whenever product development occurs, quality should be a factor of consideration since it plays a significant role in determining the success or failure of a new product launch. Quality and freshness are however, in Bremner's (2002) words, concepts that can only be measured when quality is defined in certain terms. Quality descriptors for the product will ultimately determine and define the quality of the said product.

For the purposes of this study, quality will be considered hand in hand with safety of fish products for the reasons outlined in sections 2.1 and 2.2 where it has been established that consumer trends today demand high quality, safe and convenient value-added products.

In addition, Piiroinen and Järvelä's (2006) study, concerning food choices made by consumers, suggested that food safety and flavor played major roles in consumer food choice. Food is required to be delicious and healthy as well as inexpensive. The quality descriptors for fish that will be covered in this study are descriptive analysis, shelf-life and chemical composition, specifically fatty acid composition.

Descriptive analysis has been used in the food industry for many years to evaluate the quality and consistence of many different products (Johnsen et al., 1987). Descriptive analysis involves sensory evaluation, which is a scientific method used to evaluate a food product on the basis of the attributes of flavor or taste, aroma, texture and appearance, and provides information about how consumers perceive and react to food products in real life (Lawless and Heymann, 1999).

Sensory evaluation can also give indicators about the quality of the product, providing information about the suitability of the product for sale, for human consumption or whether the product meets a particular standard (Lawless and Heymann, 1999). In fact, one of the first steps in quality control in processing fish is sensory evaluation.

In the processing of cat-fish (*Ictalurus punctatus*), for example, flavor evaluation is carried out to check for the absence of off-flavors and the presence of desirable flavor, and only fish which are judged to possess suitable flavor quality are processed (Johnsen and Kelly, 1990). Shelf-life can also be measured through sensory evaluation and is normally paired with microbial analysis (Lawless and Heymann, 1999).

Lipid composition, which can be measured using gas-liquid chromatography, is regarded as significant in assessing fish quality because fish is considered a good source of long-chain polyunsaturated ω3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have been implicated in an array of health benefits such as reduced cardio-vascular disease, lowering blood pressure and reduction of the blood lipid level (Nesheim and Yaktine, 2007; VRN, 2005).

Homeoviscous adaptation responses to cold temperatures and pressure have been implicated as the reason for the high content of polyunsaturated fatty acids (PUFA) in fish since they reduce membrane order and increase fluidity (Hall et. al, 2002; Snyder and Hennessey, 2003). Prior research has indicated that the content of PUFA in both captivated and wild fish vary according to temperature, for instance in similar species found in higher and lower latitudes, higher PUFA content is found among fish from colder geographical regions (Olsen, 1999), whereas Farkas and Csengeri (1976) have demonstrated that in temperate zones fish collected in winter months have higher PUFA content than those collected from the same locations in warmer months. (Snyder and Hennessey, 2003).

Fish are living creatures and their growth and well-being also ultimately affects their quality. The condition, fatness or wellbeing of fish can be compared using the condition index which is based on the hypothesis that the heavier the fish of a given length, the better the condition (Bagenal and Tesch, 1978). The condition index (K) can be calculated from the following equation: K= 100* (weight/length^3). Forslund (1991) states that for most fish an index of 1 shows that the fish exhibit normal growth, though this changes due to seasonal changes in the fish weight. The length-weight relationship in fish is likely to change with metamorphosis, the onset of maturity and does vary between fish from different localities, of different sexes and at different growth stanzas (Le Cren, 1951).

2.3.1 Descriptive analysis of fish

Descriptive analysis, using sensory evaluation, involves using a trained panel to taste, smell and evaluate a food sample, and tests these attributes in terms of intensity. However, consumer perception of fisheries is poorly understood and infrequently studied, and issues related to how consumers perceive edible fish products, what sensory attributes they use to describe and discriminate among different species of fish and what sensory factors contribute most to consumption or non-consumption are often left unaddressed (Sawyer et al., 1988).

Sensory descriptors for fish, especially those that describe flavor, in Finnish literature are limited and those that occur in international literature are limited to only specific species (Luoma and Latva-Kala, 1998). Marked differences between descriptors used for different species have been noted in Luoma and Latva-Kala’s study (1998). Grayling is a species for whom, few or no descriptors have been found in literature, with the exception of aroma, where grayling is said to possess a slight aroma of thyme.

Descriptive terms, which characterize components of products, should be selected so that they bring to mind specific attributes rather than causative agents (Johnsen et. al, 1987). Attributes for fish that have been defined in prior studies (Chambers and Robel, 1993; Johnsen et. al, 1987; Luoma and Latva-Kala, 1998; Prell and Sawyer, 1988; Sawyer et al., 1988) are summarized in Table 1.

Table 1. Summary of descriptors for different attributes of fish

ATTRIBUTE	DESCRIPTORS
Aroma	sweet, briny, sour, fresh, scorched, stale, rancid, moldy, grainy, musty, putrid, cardboard, cucumber (associated with cucumber), odorless
Flavor	sweet, fresh, sour, mouth drying, briny, earthy, metallic, bitter, oily, blue-green (associated with blue-green algae growth in water) nutty-buttery, stale, rancid, moldy, grainy, musty, putrid, cardboard, rubbery, fishy, tasteless, meaty, flat, sharp, off taste, old taste, tasty, mellow, medicinal, lemon, perky taste, not-oily, chicken-like
Appearance	flaky, gelatinous, sticky, firm, fibrous, juicy, rubbery, off white, dark, grey, brown, neutral, off-gray, light color, uniform, translucent, yellowish, mottled look, pale
Texture	flaky, gelatinous, sticky, firm, fibrous, juicy, rubbery, bony, stringy, slimy, chunky, watery, lumpy, greasy, spongy, granular, loose, fragile, heavy, tight, dried out, soggy, juicy, wet, fine, tender

2.3.2 Shelf-life of fish products

Barbosa et al. (2002) have discussed the concept of shelf-life, saying that it is a complex concept, generally meaning the storage life, keeping time or storage time or the length of time a product can be kept under specified storage conditions, until it becomes significantly poorer in quality or unsuitable for sale or consumption,

There have also been discussions on the use of the terms “storage life” or “shelf-life” and their differences. Storage life in Howgate’s (1985) words refers to the storage of material for instance a whole fish, from the point of harvest to the point of final sale or of transformation into another product, for example packaged fillets. Shelf-life on the other hand has been used to refer to packaged or processed products and commercial shelf-life is thereby the period that fish can be offered for sale. (Barbosa et al., 2002)

Shelf-life is thought of to begin when fish die, but the moment a fish dies is controversial. A fish’s heart does not stop beating immediately after death. It is also difficult to estimate the time of death for fish that have been caught in bulk. Also, some fish products are sold alive and therefore the beginning of shelf-life is difficult to define. In addition, the end of shelf-life is also controversial. For instance, in open dating, the “use by dates” are not the same as “sell by dates”. The “use by date” is the date when foods become, from the microbiological point of view, no longer acceptable for human consumption. (Barbosa et al., 2002).

It should be noted that open dating as required by the European Union’s Directive 97/4/EEC, Article 9 of 79/112/EEC does not directly indicate safety from pathogen growth in foods, but requires dating for foodstuffs that are perishable due to microbiological growth and therefore likely to present an immediate danger to human health (Labuza, 2007).

In Finland, the Food Act (23/2006) requires that food is handled safely and that consumers are protected from health hazards. The Fish Hygiene Decree (16/EEO/2000) also requires that the shelf-life of fish products be determined using sensory evaluation and microbiological analysis. In addition, the Decree on Food Hygiene of Food Products made from Animals (37/EEO/2006) recommends that fish are stored at temperatures between 0 - + 3°C.

Further, the Finnish Food Safety Authority (EVIRA, 2000) recommendation E11/212/2000 also recommends that vacuum-packed fish products should be stored in as chilly conditions as possible and at a maximum of + 3°C. The same document also recommends a maximum of 10-14 days selling time for vacuum-packed fish products, but if temperatures of maximum + 3°C can be achieved by sellers, then selling time can be extended to a maximum of 3 weeks. The microbiological limits for vacuum-packed hot-smoked fish and for ready food products given by EVIRA (2002) are shown in Table 2.

Table 2. Microbiological limits for food products given by EVIRA-
The Finnish Food Safety Authority in 2002

Product	Good Quality	Poor Quality
Vacuum-packed hot-smoked fish	Total aerobic plate count: below 10^5 CFU/g	Total aerobic plate counts: 10^7 CFU/g and above
Ready food products	Total aerobic plate count: below $5*10^5$ CFU/g	Total aerobic plate counts: 10^7 CFU/g and above
	Yeast plate count: below 10^4 CFU/g	Yeast plate counts: 10^5 CFU/g and above
		Mould limits to be detected using sensory techniques

Three reasons for food spoilage are given by Singh and Anderson (2004). The first of these is due to physical changes or instability. For fish, this would be for example, damaging the surface of the fish or loss of moisture or water. The second reason is due to reaction or breakdown of its chemical components including proteins, lipids and carbohydrates. The third reason is due to microbial spoilage from potential microorganisms like bacteria, fungi (mould and yeast), viruses and parasites.

Since spoilage is always reflected in the appearance, texture, aroma and taste of the product being tested, shelf-life can be scientifically measured using microbial analysis based on counts of microbes as well as sensory evaluation by a trained panel. (Singh and Cadwallader, 2004).

Factors that affect shelf-life include the quality of the raw fish material for instance the fish species and its condition, the chilling method and time, processing time, processing methods and conditions, packaging of the fish products, temperature of the product and maintenance of the cold-chain. Shelf-life can be shortened or extended when any of these factors is impacted.

The beginning of the end of shelf-life, can actually be said to start when the fish is pulled out of the water and stunned. If the initial quality of the raw fish material is poor, it is hardly likely that the quality of the final food product will have better quality. Also, if the cold-chain is not maintained, the quality of fish will begin to deteriorate very fast.

Different processing methods and preservation techniques can be used to extend the shelf-life of fish, mostly by inhibiting the growth of micro-organisms. Traditional means of extending the shelf-life of fish include chill and frozen storage, reduction in water activity by addition of sodium chloride (brining), removal of oxygen and air by vacuum- and modified atmosphere packing, heat treatment, smoking and addition of a few inorganic and organic preservatives like nitrite, sorbate and benzoate (Nilsson and Gram, 2002). In Finland and the EU, however, the use of nitrite as an additive for preservation has not been extended to fish products (European Parliament and Council Directive 95/2/EC) mainly due to its possible carcinogenic effects (Penttilä, 2001).

2.3.3 Lipid and fatty acid composition

Lipids are a chemically diverse group of compounds that are insoluble in water and are soluble in nonpolar solvents like ester and chloroform and include fats, oils and waxes and are the derivatives of fatty acids (Webster and Lim, 2002). According to Nelson and Cox (2000), fatty acids are carboxylic acids with hydrocarbons chains ranging from 4 to 36 carbons long (C_4 to C_{36}) and these chains may be fully saturated and unbranched, or they may contain one or more double bonds. As has been mentioned earlier in section 2.3, fish is a rich source of fatty acids owing to the physiological necessity to have sufficiently fluid membranes that facilitate adaptive responses against temperature and pressure changes from the ecosystem (Hall et al., 2002) and health authorities frequently urge people to include fish in their diets in order to stay healthy.

The amount of PUFA in the human diet is important, as is the balance between ω3 and ω6 fatty acids. Western diets are usually too high in ω6 in comparison to ω3 content. A beneficial fish diet should not only maintain high amounts of ω3 fatty acids, but should also have a low saturated fatty acid content (SAFA) and a relatively high ω3/ω6 ratio. (Ahlgren et al., 1994).

Measuring and characterizing total lipid content of foodstuffs is considered an important and necessary issue because of health concerns, food labeling requirements and the impacts of lipid oxidation on shelf-life and food safety (Pike, 1998). A good criterion for fat quality of the fish is the ω3/ω6 ratio that also best reflects the quality of what the fish itself ate (Ahlgren et al., 1994).

It is well established that oily fish are particularly susceptible to lipid oxidation and rancidity development because of the high content of polyunsaturated fatty acid in their lipids, particularly the nutritionally important ω3 fatty acids eicosapentaenoic acid (20: 5 n-3) (EPA) and docosahexaenoic acid (22: 6 n-3) (DHA). The rate and extent of rancidity development in fish is mainly determined by the lipid level and their fatty acid composition as well as the levels of endogenous antioxidants and endogenous oxidative catalysts. Other external influencing factors include oxygen concentration, surface area exposed to atmospheric oxygen, storage temperature and processing procedures that lead to tissue damage. (Ashton, 2002; Hamilton, 2003).

The levels of lipids in fish flesh vary depending on species, diet, seasonal fluctuation and tissue, ranging from lean fish (<2% total lipids) such as cod, haddock and pollack, to high lipid species (8 to 20% total lipid) such as herring, mackerel and farmed salmon. Body and Vlieg (1989) suggest that a cut-off point between low fat and high fat is a total lipid level of 5% (Ashton, 2002; Hamilton, 2003).

The lipids of freshwater fish in boreal regions have been shown to contain considerable amounts of ω3 series fatty acids throughout the year and that seasonal variations in fish muscle are small for perch (*Perca fluviatilis*) and vendace (*Coregonus albula*), where DHA slightly decreased during the summer. In rainbow trout (*Oncorhynchus mykiss*), no clear seasonal differences have been observed in the muscle tissue and the fatty acid spectrum follows the pattern of the commercial feed they received. (Ågren et al., 1987).

Clear seasonal variation in fatty acid composition in vendace, which correlated to its diet and development of gonads has also been detected by Muje (1988). Ahlgren et al. (1999) report that the quality of dietary fat greatly influences the fatty acid patterns of grayling muscles. In addition, they determined that grayling continued to produce DHA despite their being fed on pellets rich in DHA, suggesting that grayling does not have the capacity to break down DHA in contrast to rainbow trout (Kiessling and Kiessling, 1993).

The ω6 composition of wild and cultured grayling differs according to results from Ahlgren et al.'s (1999) study. Levels of linoleic acid (18:2 n-6) (LA) in wild grayling corresponded to those in their diet, while in cultured grayling levels of LA were much lower than in their feed. This was interpreted as incapacity of cultured grayling to elongate and desaturate linoleic acid into arachidonic acid (20:4 n-6) (AA). Consequently, cultured grayling also have low AA in contrast to high DHA levels, which is also reflected in fin erosion and other abnormalities.

Lipid composition can be analysed using gas-liquid chromatography. The methodology described by Folch (1956) and Ågren et al. (1992) basically consists of lipid extraction from tissue using an organic solvent, washing of the crude extract and collection of the lipid extract and finally analysis by a gas chromatograph. Though Kozlova (1998) maintains that the liver and muscle in fish serve as fat depots and that the liver is the main lipid storage organ in the body of ray fish species, Kiessling et al. (1991) have reported that muscle and adipose tissue are the main fat depots in salmonids (Görgün and Akpinar, 2007).

3 OBJECTIVES

The main objective of this thesis is to compile a biochemical and descriptive profile of grayling as a novel aquaculture food product, reflecting on the following:

i. The condition of the grayling used for experiments
ii. The effect of age and weight on the quality of grayling flesh
iii. A descriptive analysis of grayling flesh
iv. The shelf-life of hot-smoked grayling and
v. An investigation of seasonal variation in the fatty acid composition in grayling dorsal muscle

This profile can then be used to evaluate the selling points of grayling as a novel product on the market. This profile can also be used to determine at which point in time grayling flesh quality is at its highest and therefore discern the most favorable time for slaughter.

As established in section 2.1, when considering new product launches it is imperative to consider other competing products on the market. In the experiments considered here, whitefish has been chosen as a comparison factor. Whitefish is not only a salmonid, like grayling, but the two fish look very much alike and they are both white-fleshed and whitefish is an already established aquaculture food product.

If grayling were to be on the market, it would most likely face competition from another white-fleshed species rather than from red-fleshed species like salmon and rainbow trout. The profile will therefore characterize grayling flesh in terms of aroma, appearance, texture and flavor and the results will be compared to those received from a similar analysis of whitefish flesh.

The shelf-life analysis will determine if there are differences between the shelf-life of hot-smoked grayling and whitefish. As mentioned in sections 2.3.2, shelf-life depends on factors such as product, processing and packaging. This means that the shelf-life of one product is not automatically the same for another product.

For the purposes of this thesis, a pilot project was conducted, in co-operation with fish processing companies, aiming at determining which and what grayling products would be focused on. A small panel evaluated 7 different grayling products and based on the scores given, a decision was made to use hot-smoked grayling as a case example. Packaging is also considered in this thesis and for this reason the shelf-life analysis encompasses both vacuum-packed products as well as products packed in expanded polystyrene (EPS) fish boxes.

The fatty acid analysis focuses on the composition and variation of the fatty acid composition in grayling flesh over a time frame of one year.

4 MATERIALS AND METHODS

4.1 THE EVALUATION PANEL

An evaluation panel was compiled to conduct sensory analysis. The panel was composed of individuals from the student body and staff of Kuopio University. The panelists took tests in September 2006 to examine their recognition of basic flavors and aromas. In addition, the panelists took part in training instruction on testing for the freshness of fish and other sensory evaluation topics arranged in a Food Microbiology course organized by the Kuopio University's Department of Biosciences.

In total, there were 12 panelists involved in this project. However, only 9 were used per evaluation session. The ideal case would be to have the same panelists at each session, but due to issues such as illness or other pressing engagements, it was necessary to use stand-ins. The procedure that developed during the sessions basically entailed 9 regular panelists, who were then replaced by a stand-in panelist if necessary.

All the panelists were always briefed on the procedures, before the sessions, and given other necessary information as per the session. In addition, they were instructed to refrain from smoking, drinking coffee or using chemicals and eating foods that would interfere with the evaluation process by dulling their senses. There were 2 male and 10 female panelists, in total, aged between 20 and 59.

Sensory evaluation was conducted at the sensory evaluation facilities belonging to the Kuopio City Environmental Laboratory. The facilities allow 8 people to partake in evaluation at one go. The evaluation procedure basically entailed evaluating samples in closed petri dishes by looking to assess the outward appearance, opening one side of the petri dish and taking in a deep breath to perceive the aroma of the sample, assessing texture first with a fork and knife and then by putting the sample in the mouth to assess flavor. Panelists were required to eat a cracker and rinse their mouths with water, before and in between sample assessment.

4.2 GENERAL DESCRIPTION OF GRAYLING

Upon the start of the experiments described in this thesis, grayling at the Kuopio University Fish Research Unit were roughly about 5-6 years in age and weighed 220 g on average. These fish are of the Lake Kitkajärvi strain. Fish were spread out in 12 indoor tanks, covered with nets, with a maximum volume of 320 liters, with average densities of roughly 40 kg/m^3. A health assessment from the Finnish Food Safety Authority (EVIRA) in August 2006 determined that the fish were free from bacterial and parasitical infection (Appendix 1). The fish were fed twice a day by hand, 5 days a week, on starter feed for whitefish (Biomar's Bio-Optimal C74 of particle size 4.5 mm) for the entire length of the experiment. Water temperatures ranged from 8-14° C over the length of the experiment. No extra lighting was provided.

To further describe the condition of grayling at the Fish Research Unit, data on the population weight was gathered in September 2006, April 2007 and September 2007. Four tanks, 9-12, were monitored closely for the duration of the experiment and data gathered to deduce changes in average weight. Data on weight, weight without internal organs and length, from random samples of the grayling population, was gathered in November 2006, April 2007 and November 2007. This data was used to determine the condition factor of the fish according to the formula:

Condition Factor K= 100 * Weight (g) / Length^3 (cm)

4.2.1 Age-weight factor reflection in flesh quality

It was recognized that the grayling at the Fish Research Unit were quite small in relation to their age (5-6 years old and average weight 220g). It was necessary to determine whether the grayling at the Fish Research Unit can be used for food production and whether its quality would be similar to that of younger, similar-sized grayling.

Grayling aged 4 years old and weighting 200-300 g was obtained from the FGFRI Taivalkoski station to be used in a triangle test to evaluate whether the panel could tell the difference between the samples from the Fish Research Unit and the FGFRI Taivalkoski station. This experiment was carried out twice in the autumn of 2006 and in the spring of 2007.

In the triangle test, panelists received coded samples, which consisted of two fish samples T (grayling from Taivalkoski) and F (grayling from the Fish Reseach Unit), measuring 4 cm in width, in six combinations TTF, TFT, FTT, FFT, FTF and TFF. The samples were cooked in water according to the boil-in-the-bag procedure, where each fish sample was placed in a polythene bag, which was slit to avoid cooking the sample in its own juices, and the samples were placed in boiling water and cooked for ~15 minutes, cooled to room temperature and served. Each panelist was asked to determine the odd sample and the process was repeated thrice.

4.2.2 Descriptive analysis of grayling and whitefish

Panelists were engaged in a descriptive analysis of the two samples T and F, mentioned in section 4.2.1., as well as sample W, whitefish. Whitefish samples for this experiment originated from the FGFRI, Tervo station.

Samples measured ~ 4 cm in width and were cooked in water according to the boil-in-the-bag procedure, described in section 4.2.1, and cooled to room temperature. Panelists used adjectives to describe the attributes of the two samples according to aroma, appearance, texture, flavor in the manner described in section 4.1. The panelists were not told what species was in each sample.

4.3 SHELF-LIFE ANALYSIS OF HOT-SMOKED GRAYLING AND WHITEFISH

Shelf-life analysis compared hot-smoked grayling and whitefish according to packing method. Analyses began with a preliminary pilot phase, which determined the planning of the actual experiment and determined the frequency of testing and evaluation. This was also a chance for the panel to practice their sensory evaluation skills and get more acquainted with the two fish.

Fish for the experiment was processed (brined in an 8% salt solution and hot-smoked) by Kuopion Kalatuote Oy, based in Kuopio, and Markkasen Kala Oy based in Tervo. The two companies worked together to ensure that they processed the fish in a similar fashion.

There were three major differences between the pilot phase and the actual experiment. The first was the processing of the fish. In the pilot phase, grayling from the Fish Research Unit was stunned, using a sharp blow to the head, gutted, put on ice and delivered to the two companies, for processing. The two companies obtained (wild) whitefish from an unknown third party in northern Finland. Both companies, thereby provided vacuum packed grayling and whitefish as well as grayling and whitefish packed in EPS fish boxes.

In the actual experiment, grayling from the Fish Research Unit was stunned, using a sharp blow to the head, gutted, put on ice and delivered to Kuopion Kalatuote for processing, while whitefish obtained from the FGFRI station in Tervo, was stunned and transported to Markkasen Kala for gutting and processing.

Kuopion Kalatuote was responsible for hot-smoking and packing the grayling into EPS fish boxes and vacuum-packages, while Markkasen Kala was responsible for hot-smoking and packing the whitefish into EPS fish boxes. Markkasen Kala delivered the hot-smoked whitefish to Kuopion Kalatuote for packing into vacuum-packages.

The second difference involved the size of the fish. In the pilot stage, both species were of roughly the same size. However, in the actual experiment, the grayling were about half the size of the whitefish. The third difference is related to the origin of whitefish. In the pilot stage, the whitefish used was from natural stock, while in the actual stage the whitefish used was cultured stock.

When the fish was received from the companies it was stored in a refrigerator which was, for the entire length of the experiment, maintained at 3°C, according to the recommendations for storage of fish products (Decree on Food Hygiene of Food Products made from Animals 37/EEO/2006; ETL 2003).

4.3.1 Vacuum-packed, hot-smoked grayling and whitefish

The panel was asked to evaluate, according to the procedure described in section 4.1, 18 samples of grayling and 18 samples of whitefish on the basis of appearance, aroma, texture and flavor on a scale of 0-5 (0=not fit for human consumption and 5=excellent). The panelists were asked to note down instances of poor quality, defects or signs of spoilage, on giving a score of 3 and below. The evaluations were conducted on days 0, 14, 20, and 28. Microbial analysis was also conducted on the same days as well as on day 33.

Samples were tested for total counts of aerobic, mesophilic bacteria according to the Standard NMKL Method No. 86 3rd Edition 1999, yeasts and moulds according to the Standard ISO 7954:1987, and lactic acid bacteria according to the Standard NMKL Method No. 140 1991 modified to aerobic conditions. The tested samples were taken from the edible parts of fish flesh.

4.3.2 Hot-smoked grayling and whitefish packed in EPS fish boxes

The panel was asked to evaluate, according to the procedure described in section 4.1, 18 samples of grayling and 18 samples of whitefish on the basis of appearance, aroma, texture and flavor on a scale of 0-5 (0=not fit for human consumption and 5=excellent). The panelists were asked to note down instances of poor quality, defects or signs of spoilage on giving a score of 3 and below. The evaluations were conducted on days 0, 6, 12, 16, 20. Microbial analysis was also conducted on the same days as well as on day 23.

Samples were tested for total counts of aerobic, mesophilic bacteria according to the Standard NMKL Method No. 86 3rd Edition 1999 and yeasts and moulds according to the Standard ISO 7954:1987. The tested samples were taken from the edible parts of fish flesh.

4.4 FATTY ACID ANALYSIS OF GRAYLING DORSAL MUSCLE

Fatty acid analysis was conducted over a time frame of 1 year. Samples were collected from grayling at the Fish Research Unit at three different points in time thus: November 2006, April 2007 and November 2007. At each sampling stage, 10 samples were collected. Sample collection involved stunning the fish with a sharp blow to the head and cutting a piece of dorsal muscle, which was then stored at -70°C pending analysis. Feed samples were also analyzed, in addition to the samples of grayling dorsal muscle.

Sample analysis was conducted in January 2008 according to the methodology described by Folch (1956) and Ågren et al. (1992). 100 mg of tissue was mechanically ground and vigorously shaken with 3 ml of chloroform-methanol (2:1). The homogenate was mixed thoroughly with 750 µl of 0.88% potassium chloride (KCl) and the mixture allowed to separate into two phases by centrifugation at 3000-4000 r.p.m. for 5 min at 25°C. The volume of the lower phase was collected into a glass tube and the liquid sample was evaporated under a stream of nitrogen at 23°C. The lipid extract was dissolved in 1 ml of chloroform and analyzed by gas-liquid chromatography in the following procedure described.

100-200 μl of the lipid extract dissolved in chloroform was evaporated to dryness. 100 μl of toluene and 500 μl of 14% boron trifluoride in methanol was added and the mixture incubated for 1 hour at 100°C. Methyl esters of the fatty acids formed were analyzed in a gas chromatograph (HP 5890 Series II, Hewlett-Packard, Waldbroon, Germany) equipped with a HP-FFAP capillary column (0,2 mm i.d,, 25m, 0.33 μm film thickness).

4.5 STATISTICAL ANALYSIS

Data collected in the previous sections was subsequently computed and analyzed using Microsoft Excel 2002 and SPSS v.14 software. The data on length, weight, gutted weight and condition factor of the grayling as well as the data on fatty acid composition was statistically tested using the One-Way Analysis of Variance (ANOVA) and Tukey's post hoc test. The One-Way ANOVA is a statistical test used to compare the means of more than two populations or treatments assuming that the data (*k*) distribution is normal and have indentical variances, the samples are random and selected independently of one another (Devore and Peck, 1993). Tukey's post hoc test is used to identify where differences occur between the means (Devore and Peck, 1993; Rasi et al., 2007).

To infer differences between the flesh quality of two grayling populations from the Fish Research Unit and the FGFRI, Taivalkoski station mentioned in section 4.2.1 a triangle test was used and the significance of differences was inferred from the probability table for triangle test in Tuorila and Applebye (2005).

Sensory data in the shelf-life analysis of hot-smoked grayling and whitefish was collected as described in sections 4.3.1 and 4.3.2. Means of the scores awarded to the hot-smoked grayling and whitefish were compared using the Mann-Whitney U-test, which is a non-parametric test for comparing 2 independent samples and assumes that the population distributions have the same shape and spread, though one may be shifted to one side of the other (Devore and Peck, 1993; Rasi et al., 2007).

5 RESULTS

5.1 CONDITION OF GRAYLING

Weight data gathered at the Fish Research Unit revealed that average weight of the grayling in September 2007 had risen by 30% on average (tanks 9-12), as compared to the situation in September 2006.

Length, weight and gutted weight data was gathered in September 2006, April 2007 and September 2007 in conjuction with sampling for fatty acids of dorsal muscle. The sample size was 10 fish at each sampling session. During sample collection in the spring, it was noted that all the female fish had barely any flesh at all and were merely skin and bones. Also, large differences were noted between the weight of male and female fish. The trend throughout the year, was that the difference between weight and gutted weight of the female fish was greater than that of the male fish (Table 3).

Table 3. Mean difference between the weight and gutted weight of grayling at the Fish Research Unit (Kuopio University) according to sex

Time	**Sex**	**Weight and Gutted Weight Mean Difference %**
September 2006	F	28.9% ± 7.8
	M	11.2% ± 0.5
April 2007	F	35.8% ± 8.2
	M	12.1% ± 4.5
September 2007	F	30.7% ± 6.1
	M	9.7% ± 1.1

M=Male, F= Female

Based on this information, it was decided to use the gutted weight, instead of the weight with internal organs, to calculate the condition factor (Table 4). The data suggests that there were significant differences ($P<0.05$, One-Way ANOVA, Tukey's test) between the mean length and weight in September 2006 and September 2007 of the sampled fish. However, the modified condition factor (K) of the fish did not significantly change ($P>0.05$, One-Way ANOVA, Tukey's test) in the one year period and ranged from 0.6-0.7.

Table 4. Summary of means (±SD) of length, weight, gutted weight and condition factor of grayling at the Fish Research Unit (Kuopio University). n= 10

Time	Length (cm)	Weight (g)	Gutted Weight (g)	Modified Condition Factor K
September 2006	33.5 ± 3.0^{a}	318.4 ± 52.6^{a}	257.1 ± 66.8^{a}	0.7 ± 0.0^{a}
April 2007	31.2 ± 4.2^{ab}	286.6 ± 96.7^{ab}	215.6 ± 77.1^{ab}	0.6 ± 0.1^{a}
September 2007	28.2 ± 31.4^{b}	212.2 ± 66.3^{b}	164.9 ± 53.8^{b}	0.7 ± 0.1^{a}

Means indicated with the same letter in each column do not differ significantly (P>0.05 (ANOVA, Tukey's test). Condition factor has been calculated using gutted weight.

5.2 AGE-WEIGHT REFLECTION IN GRAYLING FLESH QUALITY

Experiments to infer the flesh quality (aroma, appearance, structure and flavor) of two grayling populations of similar size but different age were conducted in the autumn of 2006 and in the spring of 2007. The experiment conducted in the autumn was intended to be a triangle test, but did not succeed due to a mishap in the set-up of the experiment. The results were inconclusive, but the exercise functioned well as an introduction to the sensory evaluation process for both the panelists and the thesis executor and provided valuable information on how to improve the setup of the experiment in the following spring.

In April 2007, panelists evaluated samples of grayling from the FGFRI Taivalkoski station and Kuopio University Fish Research Unit in a triangle test. The triangle test resulted in 17 correct observations out of 27. According to the probability table for triangle tests in Tuorila and Applebye (2005), the difference between the two samples is significant (P<0.05).

5.3 DESCRIPTION OF GRAYLING AND WHITEFISH

Aroma, appearance, structure and flavor attributes of grayling and whitefish were given by the panel as summarized in Table 5. Both grayling samples were described as having a fishy, fresh, herbal, sweet and woody aroma and appeared white and glossy. They both were described as having a compact, crumbly, soft structure that also broke up easily. The flavor of the grayling samples were described as having a dry, earthy, fishy flavor with herbal, rich and sweet taints.

There were no major differences between the attributes given to the grayling samples, with the exception of flavor where the Taivalkoski grayling samples were described as 'very dry' as opposed to just 'dry'. Other attributes given to describe the flavor of the Taivalkoski grayling that also suggest dryness are cardboard and newspaper.

Whitefish was described as having a fresh, woody, grassy, reedy or shore/lake-water aroma and flavor and had a smooth, white/greyish, soft and mushy appearance and tender, soft structure that broke up easily.

Noted differences between grayling and whitefish are in regard to the herbal aroma and flavor of grayling and grassy/reedy or shore/lake-water aroma and flavor of whitefish. Another difference regarded structure and appearance where whitefish was described as mushy and tender, while grayling from the Fish Research Unit was described as compact and tender and grayling from Taivalkoski was tough.

Table 5. A summary of sensory attributes for grayling and whitefish, frequencies in parantheses

SAMPLE	AROMA	APPEARANCE	STRUCTURE	FLAVOR
Grayling (Fish Research Unit- Kuopio University)	Briny (1) Cardboard (1) Earthy (1) Fishy (3) Fresh (1) Herbal (2) Sweet (1) Woody (1)	Bright (1) Glossy (2) Greyish (1) Oily (1) Watery (1) White (1)	Breaks up easily (3) Compact (3) Crumbly (1) Juicy (2) Soft (1) Tender (4)	Dry (3) Earthy (1) Fishy (2) Fresh (1) Herbal (1) Metallic (1) Muddy (1) Paper (1) Rich (1) Sweet (1)
Grayling (FGFRI) Taivalkoski	Fishy (2) Fresh (1) Fruity (1) Herbal (2) Muddy (1) Sweet (2) Woody (1)	Compact (2) Glossy (1) White (1)	Breaks up easily (4) Compact (1) Crumbly (2) Crunchy (1) Juicy (1) Soft (3) Tough (1)	Cardboard (1) Fishy (2) Metallic (1) Muddy (1) Newspaper (1) Rich (1) Sweet (1) Very Dry (4)
Whitefish	Briny (1) Fishy (2) Fresh (1) Grassy (2) Metallic (1) Muddy (1) Reeds (1) Rich (1) Shore water (1) Sweet (1) Woody (1)	Anaemic (1) Bright (1) Dry (1) Glossy (1) Greyish (1) Mushy (2) Smooth (1) Soft (1) White (1)	Breaks up easily (5) Dry (1) Compact (1) Crumbly (2) Soft (1) Tender (3) Tough (1)	Bitter after-taste (1) Grassy (2) Cardboard (1) Dry (2) Fishy (1) Grainy (1) Lake water (1) Metallic (1) Paper (1) Rich (1)

5.4 SHELF-LIFE ANALYSIS OF HOT SMOKED GRAYLING AND WHITEFISH

5.4.1 Vacuum-packed hot-smoked grayling and whitefish

Microbial growth (Table 6) was monitored from day 0-33 and was relatively small, never exceeding recommended limits, though it should be noted that the samples were taken from the edible parts of fish flesh. Microbial growth varied greatly from one sample to the other and there was no real trend followed, except that there was hardly any yeast or mould growth in the vacuum-packed fish.

Table 6. Range of microbial growth (log CFU/g) in samples of vacuum-packed hot-smoked grayling and whitefish

Day	Total Anaerobic Bacteria		Lactic Acid Bacteria		Yeasts and Moulds	
	Grayling	Whitefish	Grayling	Whitefish	Grayling	Whitefish
0	> 2.00	> 2.00	> 2.00 - 3.13	> 2.00	> 2.00	> 2.00
14	> 2.00	>2.00 - 3.40	> 2.00 - 3.13	> 2.00 -3.51	> 2.00	> 2.00
20	> 2.00	> 2.00	> 2.00	> 2.00	> 2.00	> 2.00
28	>2.00 - 2.36	>2.00 - 3.04	> 2.00	> 2.00 -3.16	> 2.00	> 2.00
33	> 2.00	>2.00 - 3.06	> 2.00	> 2.00	> 2.00	> 2.00

Data on the sensory evaluation of the vacuum-packed, hot-smoked grayling and whitefish suggests that the quality of fish deteriorated progressively from day 0-28 (Figure 1). The quality of whitefish was generally given a lower score than that of grayling from day 0 to day 28. Day 14 appears to be the point where the quality of both fish deteriorated drastically (Figure 1).

Descriptive data (Appendix 2) given by the panelists on day 14 focused on structure where observations of dryness and toughness were given for both fish. On day 20, the panelists gave observations of sharp, old and metallic aromas for both fish. In addition, there were plenty of comments on wet and slimy appearance, dry and tough structure and old and rancid flavor. By day 28 the samples scored quite low on aroma, appearance, structure and flavor. The Mann-Whitney U test implies significant differences between mean evaluation scores of vacuum packed, hot smoked grayling and whitefish ($P<0.05$) at day 0 for aroma; day 14 for aroma, structure and taste; and day 28 for aroma, structure and flavor.

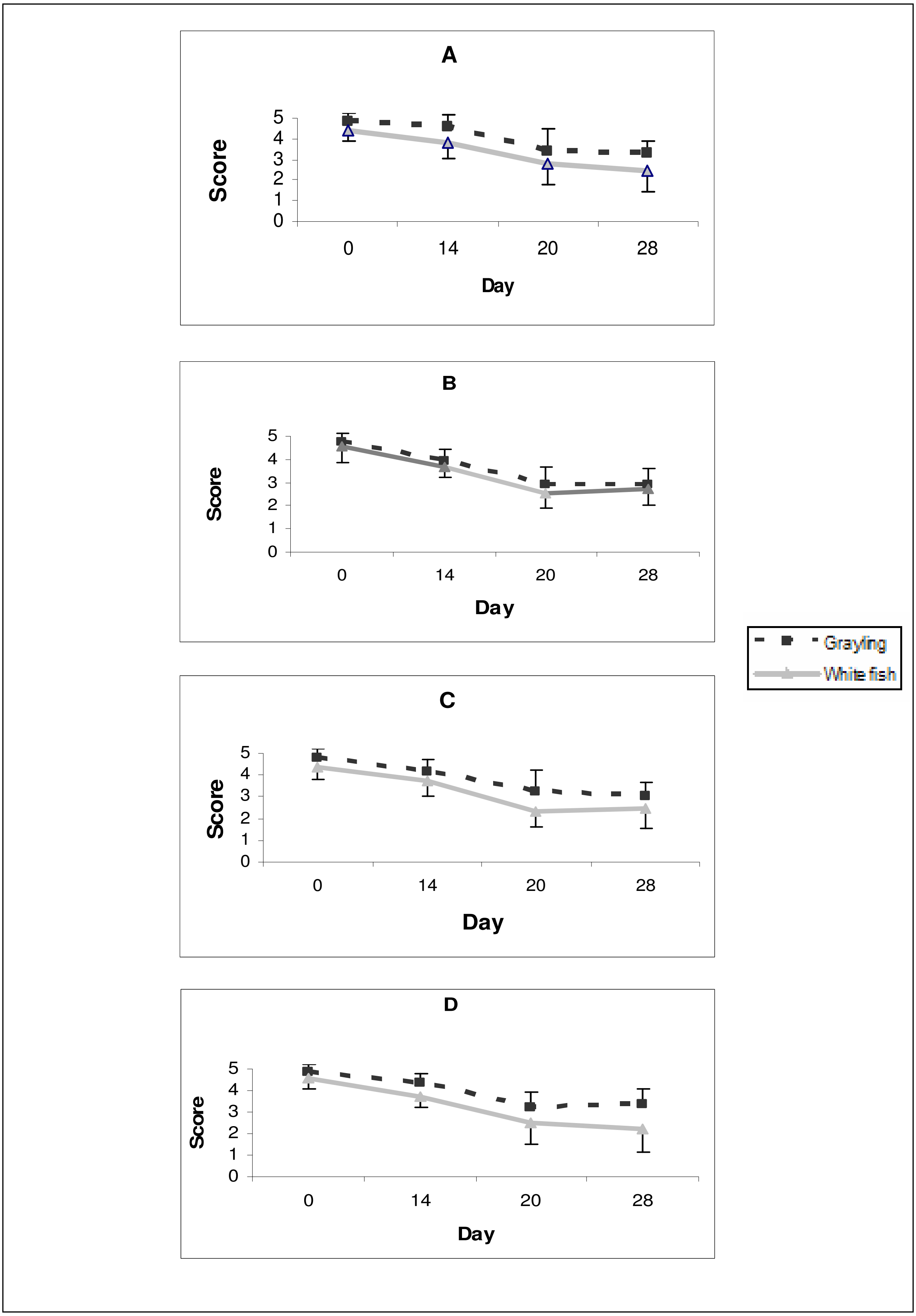

Figure 1. Sensory evaluation of vacuum-packed hot-smoked grayling and whitefish according to A= Aroma, B= Appearance, C= Structure and D= Flavor from day 0-28 [score] 0-5 (5=excellent quality and 0= not fit for human consumption)]

5.4.2 Hot-smoked grayling and whitefish packed in EPS fish boxes

Microbial growth (Table 7) was monitored until day 23 and never exceeded recommended limits, with the exception of one sample on day 20 and one on day 23 which came close to the limit. It should be noted that the tested samples were taken from the edible parts of fish flesh. Visible mould was detected by day 16 on the skin of the fish), but this did not noticeably affect the microbial growth of the flesh samples analyzed. Microbial growth varied greatly from one sample to the other and there was no real trend followed.

Table 7. Range of microbial growth (log CFU/g) in samples of hot-smoked grayling and whitefish packed in EPS fish boxes

Day	Total Anaerobic Bacteria		Yeasts and Moulds	
	Grayling	Whitefish	Grayling	Whitefish
0	> 2.00 - 2.77	> 2.00 - 2.26	> 2.00	> 2.00
6	> 2.00	> 2.00	> 2.00	> 2.00
12	> 2.00	> 2.00 - 3.43	> 2.00	> 2.00 - 3.36
16	> 2.00 - 213	> 2.00	> 2.00	> 2.00
20	> 2.00	< 3	> 2.00	> 2.00 - 2.86
23	> 2.00	> 2.00 - 4.55	> 2.00	> 2.00 - 2.82

Data on the sensory evaluation of the hot-smoked grayling and whitefish packed in EPS fish boxes suggests that the quality of fish deteriorated progressively from day 0-20 (Figure 2). The quality of whitefish was generally given a lower score than that of grayling from day 0 to day 12, when the quality of grayling deteriorated more than whitefish. Day 6 appears to be the point where the quality of both fish deteriorated drastically. The quality of grayling then seems to have deteriorated quite steadily from then on. Whitefish, however, had another point of drastic deterioration at day 16. Day 20 results were slightly odd in regards to the scores awarded grayling, which were higher than those of day 16. This is not logical and does not follow the trend of the data. On day 20, there were 3 scores given that were much higher than the others, thereby raising the average. These were traced back to stand-in panelists who had not attended the previous evaluation session on day 16 and it was assumed that they performed the evaluation with day 12 as the reference. For this reason, day 20 results for grayling are rejected.

Descriptive data (Appendix 3) given by the panelists on day 6 is focused on structure where observations of mushiness and softness were given for whitefish, while grayling was described as having lost elasticity. On day 12, panelists described both fish as appearing wet and slimy. In addition, they described aromas of both fish as pungent and sharp. Whitefish was also described as having a milky aroma. The structure of both fish on day 12 was described as tough and flaky, while grayling was described as having a grassy flavor and whitefish as either sour or tasteless. On day 16, the panelists described both samples as having sharp, stale or pungent aromas, slimy and yellowish or grey coloration, dry and tough structures and tastes were sharp and rancid or old. By day 20, the samples scored low on aroma, appearance, structure and taste and 5 of the grayling samples and half (9) of the whitefish samples were not evaluated for flavor.

The Mann-Whitney U test implies significant differences between the mean evaluation scores for hot smoked grayling and whitefish (P<0.05) at day 0 for flavor; day 6 for structure; day 12 for aroma, structure and flavor; day 16 for appearance and day 20 for aroma, appearance, structure and flavor.

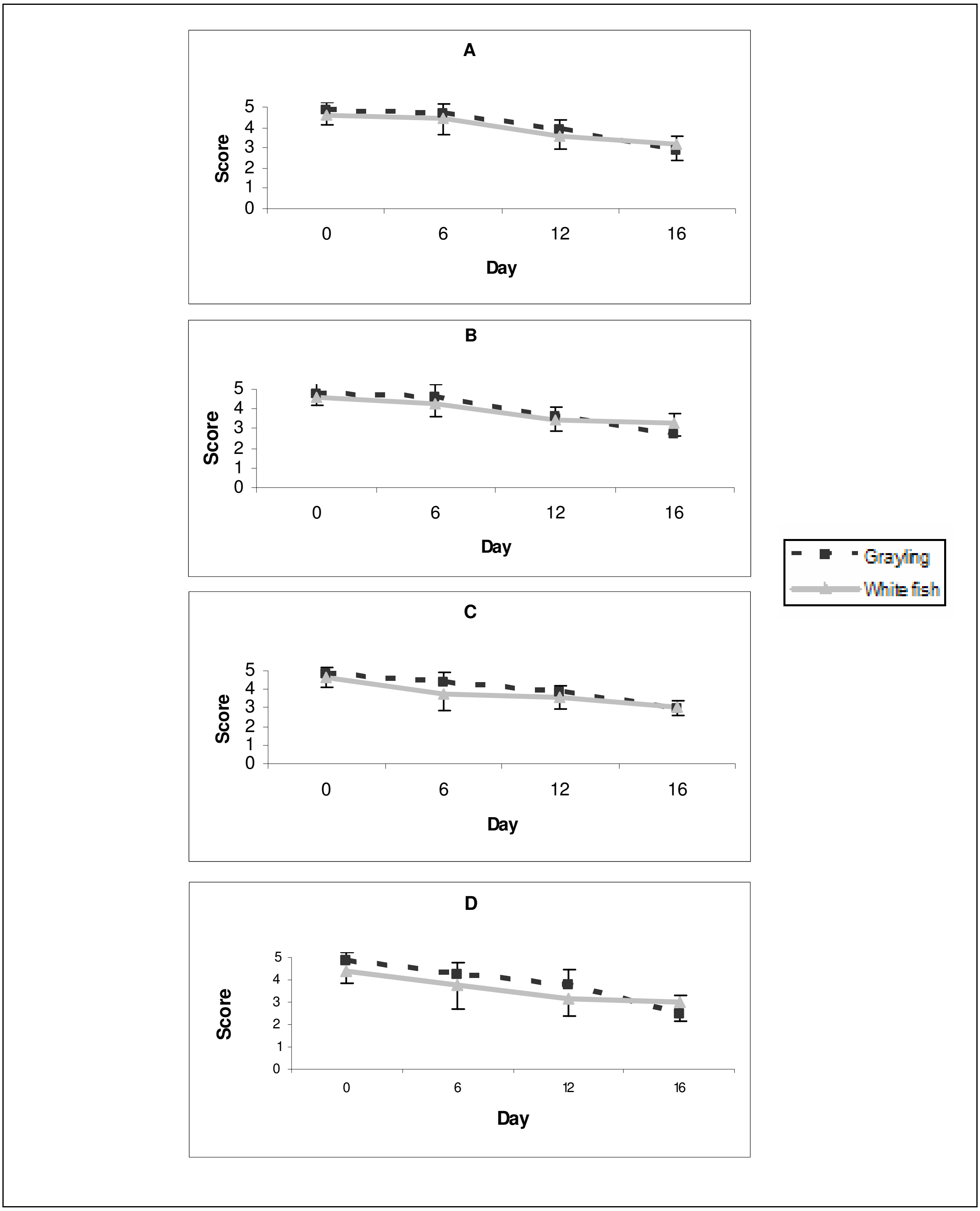

Figure 2. Sensory evaluation of hot-smoked grayling and whitefish packed in EPS boxes according to A= Aroma, B= Appearance, C= Structure and D= Flavor, from day 0-20 [score 0-5] (5=excellent quality and 0= not fit for human consumption)]

5.5 FATTY ACID ANALYSIS OF GRAYLING DORSAL MUSCLE

Palmitic acid (C16:0), oleic acid (C18:1 ω-9) and docosahexaeonic acid (C22:6 ω-3) (DHA) were the major fatty acid components in dorsal muscle tissue of grayling during the whole year period. The data in Table 8 suggests that the predominant fatty acid, in the dorsal muscle tissue of grayling, is DHA. This did not vary significantly over the year (P>0.05, One-Way ANOVA, Tukey's test) .

Amounts of saturated fatty acids (SAFA) varied, over the year, between 18.7%- 26.2% total fatty acids. The proportion of SAFA in the spring was significantly lower in the spring 2007 than in the autumn of 2006 (P<0.05). It was also lower than in the autumn of 2007, but this difference was not statistically significant (P>0.05, One-Way ANOVA, Tukey's test).

The monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were more varied in amounts ranging from 13.3%-32.2% and 48.8%-63.7% total fatty acids respectively. MUFA exhibited a slight increase in level in the spring, whereas PUFA exhibited a small decrease in level in the spring. These changes were not, however, statistically significant (P>0.05, One-Way ANOVA, Tukey's test)

ω3 in the dorsal muscle tissue of grayling ranged from 31.8%-53.3% of the total fatty acids while ω6 ranged from 7.6%-17.4% of the total fatty acids. ω3 and ω6 values did not vary significantly (P>0.05, One-Way ANOVA, Tukey's test) over the year.

The ω3/ω6 ratios varied from 1.8-6.7 over the whole year. There were significant differences (P<0.05 One-Way ANOVA, Tukey's test) between the ω3/ω6 ratio in the first autumn and the periods that followed thereafter when the ratio increased.

The fatty acid profile of the artificial feed used to feed the grayling was also analyzed. The feed had high amounts of eicosenoic acid (C20:1 n-9) and docosenoic acid (C22:1 n-9+11) amounting to 15.6% and 19.6% total fatty acids respectively. These fatty acids were not among the major components of the grayling flesh. The ω3/ω6 ratio of the feed analyzed was 1.70

Table 8. Proportions of fatty acids in samples of artificial feed and tissue samples from the dorsal muscle of grayling collected between November 2006 and October 2007

Sample	Grayling Muscle	Grayling Muscle	Grayling Muscle	Artificial Feed
No. of samples	10	10	10	2
Sampling date	6.11.2006	24.4.2007	29.10.2007	15.1.2008
Fatty Acid (FA)	Mean % of total FA	Mean % of total FA	Mean % of total FA	Mean % of total FA
14:0	2.1 ± 0.5[a]	2.6 ± 0.6[a]	2.5 ± 0.8[a]	6.3 ± 0.0[b]
16:0	18.2 ± 1.9[a]	15.0 ± 2.0[a]	17.0 ± 2.6[ab]	11.5 ± 0.1[b]
16:1(ω-7)	2.4 ± 0.0[a]	3.5 ± 1.0[a]	3.0 ± 1.2[a]	7.8 ± 0.1[b]
18:0	3.7 ± 0.8[a]	2.9 ± 0.3[a]	3.5 ± 0.5[a]	1.6 ± 0.0[b]
18:1(ω-9)	11.4 ± 4.2[a]	16.1 ± 4.0[a]	12.3 ± 4.6[a]	11.5± 0.1[a]
18:1(ω-7)	2.2 ± 0.7[a]	2.1 ± 0.4[a]	2.0 ± 0.3[a]	2.7 ± 0.0[a]
18:2(ω-6)	7.6 ± 2.4[a]	11.8 ± 3.7[a]	10.0 ± 3.7[a]	7.6 ± 0.0[a]
18:3(ω-3)	1.7 ± 0.4[ab]	2.2 ± 0.5[b]	1.6 ± 0.5[ab]	1.2 ± 0.0[a]
18:4(ω-3)	0.9 ± 0.3[a]	1.1 ± 0.4[a]	0.9 ± 0.4[a]	1.9 ± 0.1[b]
20:1(ω-9)	1.5 ± 0.6[a]	2.2 ± 0.6[a]	1.9 ± 0.7[a]	15.6 ± 0.0[b]
20:1(ω-7)	0.2 ± 0.1[a]	0.3 ± 0.1[a]	0.3 ± 0.1[a]	0.9 ± 0.0[b]
20:2(ω-6)	0.3 ± 0.1[ab]	0.3 ± 0.1[ab]	0.4 ± 0.1[b]	0.2 ± 0.0[a]
20:3(ω-6)	0.1 ± 0.0[a]	0.2 ± 0.0[a]	0.2 ± 0.2[a]	0.1 ± 0.0[a]
20:4(ω-6)	1.3 ± 0.2[a]	1.0 ± 0.3[a]	1.2 ± 0.3[a]	0.3 ± 0.0[b]
20:4(ω-3)	0.6 ± 0.1[a]	0.6 ± 0.1[a]	0.6 ± 0.1[a]	0.3 ± 0.0[b]
20:5(ω-3)	8.8 ± 1.4[a]	7.2 ± 1.0[ab]	7.8 ± 1.2[a]	5.7 ± 0.1[b]
22:1(ω-9+11)	1.2 ± 0.4[a]	1.3 ± 0.4[a]	1.3 ± 0.5[a]	19.6 ± 0.2[b]
22:4(ω-6)	0.2 ± 0.1[a]	0.3 ± 0.1[a]	0.3 ± 0.1[a]	0.3 ± 0.0[a]
22:5(ω-6)	0.4 ± 0.1[a]	0.4 ± 0.1[a]	0.4 ± 0.1[a]	0.0 ± 0.0[b]
22:5(ω-3)	1.9 ± 0.3[a]	2.1 ± 0.2[ab]	2.4 ± 0.2[b]	0.6 ± 0.0[c]
22:6(ω-3)	32.9 ± 6.2[a]	26.6 ± 8.3[a]	30.3 ± 8.9[a]	4.5 ± 0.1[b]
24:1(ω-9)	0.4 ± 0.1[a]	0.3 ± 0.1[a]	0.3 ± 0.1[a]	0.2 ± 0.0[b]
∑SAFA	24.1 ± 2.1[a]	20.5 ± 1.8[b]	22.8 ± 2.3[ab]	19.4 ± 0.0[b]
∑MUFA	19.3 ± 6.0[a]	25.8 ± 6.4[a]	21.2 ± 7.3[a]	58.1 ± 0.2[b]
∑ PUFA	56.7 ± 7.0[a]	53.7 ± 4.9[a]	56.0 ± 5.2[a]	22.5 ± 0.3[b]
∑ω3	46.8 ± 6.5[a]	39.8 ± 8.0[a]	43.6 ± 8.7[a]	14.0 ± 0.3[b]
∑ω6	9.8 ± 2.2[ab]	13.9 ± 3.5[a]	12.4 ± 3.5[ab]	8.5 ± 0.0[b]
∑ω3/∑ω6	5.1 ± 1.6[a]	3.2 ± 1.4[b]	4.0 ± 1.9[b]	1.7 ± 0.0[c]

Means indicated with the same letter in each row do not significantly differ (P>0.05, One-Way ANOVA, Tukey’s test). SAFA: Saturated fatty acids, MUFA: Monosaturated fatty acids, PUFA: Polyunsaturated fatty acids

6 DISCUSSION

Competitiveness in aquaculture is a challenge and solutions for profitability depend highly on consumer demand and successful product cycle management. Interesting implications can be received by value-addition. In the Chilean market, for example, when jack mackerel (*Trachurus symmetricus*) landings dropped from 8 million tonnes per season by half, value-addition was used to save the industry and now frozen fillets are sold to Africa and canned products worldwide, resulting in soaring export revenues (Neilson, 2007).

Building value-added aquaculture products is a process that implies producer contemplation and action on decisions regarding species and/or product diversification. The driving forces for value addition are determined in the market by lifestyle and demographic trends and are not presumed to be the same in all important areas (Möller, 2003). It is therefore important to consider how a new aquaculture product is received in the market and how it develops in the long run.

Creating a profile of grayling can be regarded as one step in the product development cycle and launch of a new aquaculture product. Fatty acid analysis can be used to give information about the fat quality of the product, an important issue in present-day society when health implications of products are scrutinized carefully. Shelf-life analysis establishes the selling time for the product and is of great importance as far as consumer protection is concerned. Finally, generating a sensory profile that encompasses aroma, appearance and flavor provides information that can be used in development of value-added products. This can involve, for instance, processing the fish product and creating a description of the product that gives actual and factual information, about the product, that has a base in scientific methodology.

These are issues of importance today, when consumers are aware of, and demand product labeling. Consumers need to have sufficient and reliable details about fish origins and other essential characteristics and these must be easily visible and legible (Asensio and Montero, 2008).

In addition, it has been established that consumers make food product choices on the basis of a mix of intrinsic attributes they expect to receive such as food safety, nutrition, taste, texture, function, convenience, production methods; and on extrinsic quality indicators and cues the product carries such as certification, labeling, price, and brand name, as well as the store in which the product is sold (Caswell, 2006).

Keeping these factors in mind, it is clear that value-addition and product development in aquaculture needs to take more than just the processing chain into consideration. The farm where fish are held and the final consumer needs and demands need to be simultaneously considered. This was one of the drivers for this research.

The quality of the grayling at the Fish Research Unit was assessed before, and during product development. In addition, during the planning stage of the research, scores given to 7 different grayling products were used to determine which grayling product (hot-smoked grayling) would be used as the case example in the final experiments. Finally, the shelf-life of this product was assessed.

Grayling at the Fish Research Unit were generally relatively small in comparison to their age. Intensive feeding on starter feed for whitefish did, however, result in a 30% increase in the average weight the fish. Questions have been presented as to why the grayling at the Fish Research Unit are small in size (about 200-300g), in relation to their age (5-6 years). The fish are kept in cold water conditions all through the year, in the attempt to curb diseases and parasites. This aim has been realized because, as the health assessment conducted by EVIRA (Appendix 1) showed, the grayling at the Fish Research Unit were free from disease and parasites.

Basic fish biology dictates that as water temperatures rise, metabolic rates in fish rise and, as such, fish eat more and growth accelerates to some extent. It is therefore logical, that the grayling at the Fish Research Unit did not, despite their age, gain much in size since the water temperatures were too low to enact any increase in metabolism. It should be noted, however, that the Fish Research Unit functions as a research station and not as a commercial fish farm.

Similar observations are made for the question of condition factor of the grayling at the Fish Research Unit. There were no significant changes depicted as far as the modified condition factor (ranging from 0.6-0.7) of the fish was concerned. Comparative length and weight data of 11 year old grayling received from the FGFRI, Taivalkoski station resulted in very close values as regards condition factor, which ranged from 0.9-1.2 (Karjalainen, M., personal communication). These figures are quite close to those given by Forslund (1991), who states that for most fish, an index of 1 shows that fish exhibit normal growth. The unchanging condition factor of the grayling at the Fish Research Unit is attributed to the stable conditions they are kept in.

Grayling at the Fish Research Unit differed significantly from grayling from the FGFRI, Taivalkoski station with regards to aroma, appearance, structure and flavor. From the characterization data, it appears that the FGFRI samples (younger) may have been drier and tougher than the Fish Research Unit samples.

Difference in age and growth patterns may have accounted for the sensory differences between the two grayling samples. However, the quality of fish also has its roots in its origins, growth and diet. In addition, genetic factors and stress levels of fish which are determined by factors like density and physical activity also affect the final product quality (Børresen, 2007). It is therefore feasible that differing diet between the samples and water quality in their habitats, may also have affected the quality of the flesh. This is supported by Johnsen and Kelly (1990) who have determined that some flavor attributes are related to diet and others for instance, grassy, are related to environmental factors.

Luoma and Latva-Kala (1998) also report that different fish species have different odors, structures and flavors and these can be affected by the habitats. A characterization of whitefish was also conducted and this was compared to grayling, but how much whitefish and grayling differed, was not measured in this study. The herbal aroma and flavor and compact, tender structure of grayling in comparison to the grassy/reedy aroma and flavor and mushy tender structure of whitefish were the notable differences that stood out. It is interesting to note that Luoma and Latva-Kala's (1998) sensory evaluation of whitefish had similar findings as regards the aroma. In their paper, whitefish is described as having a cucumber-like aroma, which was also likened to shore-water and fresh, cut grass.

The fish processing companies involved in the project gave some comments on their experiences during processing the grayling and whitefish. Grayling was more difficult to flake than whitefish. Grayling was not difficult to fillet, especially when compared to perch, owing to its thick skin. Grayling, however, does have small bones and it was considered difficult to make a boneless fillet. The companies generally wondered if it would be possible to get bigger grayling than those delivered. However, as has been established earlier, the grayling at the Fish Research Unit was small in relation to its age.

The sensory analysis of hot-smoked grayling and whitefish did not produce surprising results as regards the shelf-life of fish. Both grayling and whitefish began to exhibit signs of deterioration in quality at about 6 -7 days for fish packed in EPS fish boxes and 14 days for vacuum-packed fish. Fish packed in vacuum-packages has a longer shelf-life due to the fact that atmospheric oxygen and air are eliminated from the package, and thereby limit microbial activity.

The sensory data suggested that whitefish may have begun to deteriorate earlier than grayling. However, this cannot be upheld as a full truth since there were many differences between the samples, beginning with size and processing facility.

The whitefish was twice as big as the grayling. This is a factor that comes into play during processing (brining and hot-smoking). Brining and hot-smoking are both preservative methods and are affected by brining time and hot-smoking time. The immersion time of fish in brine varies with fish size, thickness and fat content (Bannerman, 2001).

In addition, two companies were involved in processing the fish, allowing more room for errors, human or otherwise. The preliminary data, even though it was not presented in this thesis, indicated that there was no clear difference between whitefish and grayling as regards shelf-life in a case where the fish were basically of the same size. The preliminary experiment, however, had whitefish from wild stock and not cultured.

Microbiological data did not give results even close to the recommended limits, with the exception of one or two samples. This was surprising because mould at times was clearly visible on the surface of the fish. Microbial plating was done with samples of the flesh and not the surface (skin) of the fish. Therefore, it can be presumed that the flesh itself had no or few microbes. According to Curiale (1998), sometimes a food may exhibit an exceptionally long shelf-life even though the temperature, pH, water and nutrient levels permit microbial growth and this may be due to the absence of microorganisms in the samples tested, or because the contaminating organisms will not grow in the particular product formulation.

This suggests that brining and hot-smoking of the samples worked very effectively as preservation techniques and reduced the water content very well, and also kept a good protective cover over the flesh. Despite the fact that there were no or few microbial data, sensory data suggests that spoilage occurred early on. Where a similar experiment is set-up in the future, it is recommended that samples be taken from both the flesh and the surface (skin) of the fish.

Fatty acid analysis data suggests that grayling is a fish with a high PUFA content making it suspect to lipid oxidation and rancidity. The fatty acid profile is similar to that of other salmonid fish like vendace, whose fatty acid make-up is also predominantly made up of PUFA (Muje, 1988). Grayling had a high ω3:ω6 ratio ranging from 3-7 which is in line with previous studies (Ahlgren, 1999). For comparison purposes, perch, vendace and rainbow trout, according to Muje's (1988) thesis, had ω3:ω6 ratios of 4.8, 3 and 4.79, respectively

There was little seasonal variation in the fatty acid spectrum, but this is reflective of the grayling's diet which was constant throughout the year. It was, however, noted that the female fish had practically no flesh in the spring. This is attributed to energy allocation for egg production which has been demonstrated in other fish species where some fatty acids are transferred to eggs (Henderson, 1995).

Lipid quantity in grayling was not assessed, thus there is no scientific evidence to support claims of variation of lipid quantity by season or at spawning time. However, earlier research has shown that muscle lipid in vendace, for instance, is consumed in preparation for spawning (Ågren, et al., 1987).

Water temperature did not play a part in the fatty acid profile because the water temperature at the Fish Research Unit hardly varied over the year. This may have interfered with growth for one, and secondly with spawning which may have an effect on the variation of fatty acids, as has been seen with other fish like vendace (Muje, 1988).

One of the objectives of this study was to discern the time at which grayling flesh was of the highest quality regarding fatty acid composition and thereby infer the optimal slaughter time. Since the fatty acid composition did not vary significantly over the year, owing to the stability of conditions and lack of variation in diet, it was not possible to infer the optimal time for slaughter. Ahlgren et al. (1999) have shown in their research that the fatty acid profile of grayling varied with commercial and natural diets. It is thereby reasoned that because during culture, conditions can be monitored and affected easily for example through controlling temperature and feeding times and types of feeds, it is possible to 'assure' consumers that cultured grayling from a given institute will have a given fatty acid composition, which cannot be guaranteed from wild stock of a similar species on natural diets that vary seasonally.

Based solely on its scarcity, grayling has potential to enter the market as a niche product. A niche product infers that a certain type of product is available for a given time at very high market prices. However, there are questions that remain open for future study which include: quantification of lipids, the influence of spawning on the lipid content and fatty acid profile of grayling that needs to be explored further as the results of this study were inconclusive on that account, exploring the differences between the sensory profiles of whitefish and grayling and consumer attitudes towards grayling products and finally preference for processed products made from grayling as compared to other similar fish products.

Competition is high in the aquaculture sector, and white fleshed species that can equal salmon as regards value-adding potential are being continually sought for. Without considering grayling's would-be competitor, whitefish, on the Finnish market, global outlook has already moved on from *Tilapia* and pangasius *(Pangasius hypophthalmus)* to cobia (*Rachycentron canadum*), which is already being enthusiastically referred to as "tropical salmon" (Eurofish, 2007).

Furthermore, there are a few factors, mentioned by FAO (2008), which make the strategy of value-addition quite complex. Value addition frequently involves large investments in buildings and equipment, which decreases flexibility and it channels large amounts of scarce economic resources into fixed capacity, increasing vulnerability in situations with uncertain supplies of raw material, which is the case for grayling currently. In addition, production, sales and distribution of value added products often require large economic resources for marketing and promotion, which smaller companies usually do not possess.

As demand for aquaculture product grows, however, developments need to be made as regards product definition, product diversification and market targets, in a move towards increased efficiency and profitability Further studies on these issues could pave the way for interesting opportunities and prospects in a field facing declining prices despite growing demand, not only for diversity, but for safe, high quality and competitive products. Fuller potential and capacity can still be achieved in the aquaculture product development sector and value-added production is a strategy that needs attention.

7 CONCLUSIONS

The main objective of this thesis was to compile a biochemical and descriptive profile of grayling focusing on the quality of the grayling material used, the effect of age and weight on the quality of grayling flesh, a descriptive analysis of grayling flesh including its fatty acid composition and finally, the shelf-life of hot-smoked grayling. The findings described in section 5, the results, and discussion in section 6, have laid ground for the conclusions made in this section.

The grayling material used for product development was suitable for food production. The condition factor of the fish 0.6-0.7 did not vary over the year implying that conditions were stable. In addition, the fish were free from disease and parasites.

Grayling is a fish with a fishy, fresh, herbal, sweet and woody aroma, that has a compact, crumbly and tender structure that breaks up easily and has a dry, earthy, fishy flavor with rich, sweet, herbal taints. It is a fish rich in polyunsaturated fatty acids (PUFA), mainly docosahexaenoic acid (DHA), and has a high content of ω-3 acids, which may range from 32%-54% of the total fatty acids.

The fatty acid composition of cultured grayling is quite stable. This is attributed to dietary conditions which are stable and unvaried. Spawning and gonad formation may affect the lipid content and fatty acid composition, but concrete scientific evidence of this could not be attained because of the experiment set-up and conditions of the facility where the grayling were kept.

Age may play a factor in the quality of grayling flesh. Younger grayling was distinguishable from older grayling. The data findings suggested that the flesh of younger grayling may be tougher and drier than older grayling. However, age is not the only factor that may affect the quality of grayling flesh. Environmental conditions, water quality, quality and diversity of feeds as well as differing growth patterns, stress and genetic factors may also affect the quality of grayling flesh (Børrensen, 2007).

Grayling products are subject to rancidity and can spoil quickly in the absence of a cold-chain. Hot-smoked grayling products have a shelf-life similar to other fish products, 6-7 days for unpacked products and 14 days for vacuum-packed products when storage is at maximum +3° C.

Grayling may have potential to be introduced to the market as a niche product, but customer attitudes and preferences need to be researched before product development and value-added production are invested in. Whitefish is a potential competitor product already well-established on the market. Differences between different grayling and whitefish value-added products need to be explored in depth as should consumer preferences for these products. These issues together can further clarify the potential of grayling as a novel product on the market. Information retrieved will be useful for market definition, product specification and pricing as well as for rationalizing possible future development in grayling culture.

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Evira

ELINTARVIKETURVALLISUUSVIRASTO

TUTKIMUSTODISTUS

1 (1)

KUOPIO 11.9.2006
DN:o 10.8.2006/2, 2
ba 5369-5428
pa 4309-4368

Kuopion Yliopisto,
Kalantutkimusyksikkö
Ikäheimo Mikko
PL 1627
70211 KUOPIO

Tilaaja:	Kuopion Yliopisto, Kalantutkimusyksikkö, Ikäheimo Mikko, PL 1627, 70211 KUOPIO
Omistaja:	Kuopion Yliopisto, Kalantutkimusyksikkö, Ikäheimo Mikko, PL 1627, 70211 KUOPIO, vesiviljelyrekisterinumero 08-006
Näytteet:	harjus, kokonainen eläin, tuoreena, 60 kpl kala/tarkkailuun kuuluva P1-kalalaitos
Saapumispvm:	10.8.2006
Tutk.aloituspvm:	10.8.2006
Näytteenottopvm:	10.8.2006
Tutkimuksen syy:	kalaterveystarkkailuohjelman mukainen tutkimus
Tutkittu/menetelmä:	Aerobi-infektion osoittaminen bakteriologisesti / Viljely Patologis-anatomisesti

Tutkimuksesta vastaa: ELL Satu Viljamaa-Dirks
Eläinlääkäri Henry Kuronen

Kyselyihin vastaa: ELL Satu Viljamaa-Dirks, 020 77 24962

Tutkimustulos:
Spesifistä bakteeritartuntaa ei todettu.

Näytetiedot:
1 - 60 (ba 5369-5428, pa 4309-4368)
150 g, kala/tarkkailuun kuuluva P1-kalalaitos

Silmämääräinen tutkimus:
Näytekaloissa ei todettu silmämääräisiä muutoksia.

Makroskooppinen diagnoosi:
Ei spesifisiä muutoksia.

Bakteriologia:
Tutkimustulos:

Spesifistä bakteeritartuntaa ei todettu.

ELINTARVIKETURVALLISUUSVIRASTO EVIRA * LIVSMEDELSSÄKERHETSVERKET EVIRA *

*-merkityt menetelmät ovat akkreditoituja. Menetelmäkuvaukset ja mittausepävarmuus, mikäli se ei käy ilmi tutkimustodistuksesta, toimitetaan pyydettäessä.
Tulokset pätevät vain tutkituille näytteille. Asiakirjan julkaisu tai osittainen kopiointi on sallittu ainoastaan Eviran luvalla, kokonaisuudessaan asiakirjan saa kopioida.

Kuopion tutkimusyksikkö
PL 92 (Neulaniementie 4) 70701 Kuopio
Puh: 020 77 24952 Fax: 020 77 24970

Summary of descriptive sensory data of vacuum packed hot smoked grayling (n=18) and whitefish (n=18)

Day		AROMA	APPEARANCE	STRUCTURE	FLAVOR
0	Grayling	-	-	-	-
	Whitefish	-	-	Porridge-like Soft	Coaly
14	Grayling	Mild, Musty	Glossy, Watery, Slimy	Compact, Fat distinguished on the flesh, Tough	-
	Whitefish	Cardboard, Metallic, Mild, Stale, Sweet, Inspecific	Glossy, Watery, Yellowish, Pale, Dry	Dryish, Grainy, Not juicy, Crumble, Mushy, Rubbery, Tough	Porridge-like, Fades, Old, Tasteless
20	Grayling	Salmiakki, Old, Sharp, Spoilt, Old, Strong, Sweet, Solvent-like	Glossy, Slimy, Wet, Yellowish, Dark	Rubbery, Soft, Crumbly, Loose, Tough, Fat between layers	Plastic, Papery, Rancid, Grassy, Tough, Old (*2 samples were not tasted or evaluated*) .
	Whitefish	No aroma, Faded, Rancid, Sharp, Metallic, Stale, Sulphur, Sweet and Sour, Woody	Dry, Yellowish, Colorless, Greyish, Dirty, Anaemic, Glossy, Wet	Dry, Mushy, Tough, Crumbly, Soft, Hard, Fibrous, Rubbery	Mushy, Grassy, Putrid, Sharp, Tasteless *(3 samples were not tasted or evaluated)*
Day 28	Grayling	Bitter, Metallic, Not fresh, Oily, Old, Sharp, Eggy, Sour, Stale	Dry, Greyish, Slimy, Fat on the surface, Wet, Faded color	Dryish, Slimy, Mushy, Soft, Breaks up easily, Crunchy	Old, Rancid, Stuffy, Sharp and sour aftertaste, Oily, Paper, Tasteless *(2 samples were not tasted or evaluated*).
	Whitefish	Bitter, Grassy, Old, Rancid, Sour, Sharp, Stale, Sweet and Sour, No Aroma	Dry, Grey, Yellowish, Woody, Yellowish fat, Greyish-green, Slimy, Dark	Dry, Mushy, Crumbly, Rubbery, Tough, Chewy, Sticky, Breaks up easily	Mild, Old, Rancid, Spoilt, Stale, Sweet. *(4 samples were not tasted or evaluated*)

APPENDIX 3(3)

Summary of descriptive sensory data of hot smoked grayling (n=18) and whitefish (n=18) packed in EPS fish boxes

Day		AROMA	APPEARANCE	STRUCTURE	FLAVOR
0	Grayling	-	-	-	-
	Whitefish		-	Soft but not mushy	Tasteless
6	Grayling	Sweet	Glossy, Wet	Breaks up, Inelastic	-
	Whitefish	Untypical	Watery, Glossy, Pale	Mushy, Soft, Grainy, Crumbly	Chewy, Sour, Bitter, Faded taste, Burbot-like
12	Grayling	Sharp, Stuffy, Pungent	Wet, Slimy, Glossy, Fat distinct on the surface	Slimy, Tough, Flaky	Grassy
	Whitefish	Not fresh, Sharp, Sour, Cardboard, Milky, Metallic, Pungent	Wet, Slimy, Inelastic, Yellow, Fat distinct on the surface	Flaky, Damp, Mushy, Soft, Dry, Chewy	Grassy, Sour, Old, Spoilt, Sharp, Faded taste
16	Grayling	Sour, Like Raw fish, Sharp, Old, Bitter, Spoilt, Egg, Stale, Rancid, Sharp,Yeast	Slimy, Greyish, Wet, Yellowish, Damp, Fat distinct on the surface	Flaky, Chewy, Tough, Dry, Mushy, Slimy, Crumbly, Tough	Sour, Old, Sharp, Rancid, Tasteless, Stale, Spoilt (*1 sample was not tasted or evaluated*).
	Whitefish	Pungent, Sour, Sharp, Stuffy, Sweet, Faded aroma, Mild, Stale	Slimy, Watery, Glossy, Slimy, Yellowish, Damp, Distinct fat on the surface	Dry, Chewy, Flaky, Tough, Hard	Sour, Spoilt, Woody, Cardboard, Faded taste, Rancid
20	Grayling	Old, Rancid, Sharp, Stale, Sulphuric, Metallic	Wet, Slimy, Damp, Grey, Anaemic, Glossy	Distinct fat on the surface, Flaky, Tough, Dry, Not compact	Old, Grainy, Sweet and Sour, Old, Woody, Tasteless *(5 samples were not evaluated)*
	Whitefish	Spoilt, Sharp, Decayed, Fat, Old herring, Old, Stale, Rancid, Sweet	Dry, Green, Grey, Yellow, Slimy, Pasty, Damp	Dry mush, Tough, Flaky, Not compact, Hard	Rancid *(9 samples were not evaluated)*