EFFECT OF FREEZE-CHILL TECHNOLOGY AND MODIFIED ATMOSPHERE PACKAGING ON THE QUALITY OF WHITING (*Merlangius merlangus*), MACKEREL (*Scomber scombrus*) AND SALMON (*Salmo salar*) PORTIONS

by

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Abstract

Freeze-chilling involves freezing and frozen storage followed by thawing and chilled storage. It offers logistic benefits for fish packers as it enables packaged fillets to be held frozen and then released into the chill chain as required. Trials with whiting, mackerel and salmon fillets/portions indicated no difference in odour scores (raw samples) between freeze-chilled and chilled samples; however, freeze-chilled salmon portions were inferior in terms of odour to chilled. Fresh fillets received the highest acceptability scores (cooked samples) followed by frozen, chilled and freeze-chilled samples. The pattern in the data was the same for each species and there was no statistically significant difference between the freeze-chilled and chilled samples. The total volatile base nitrogen (TVBN), trimethylamine (TMA) and total viable count (TVC) data were the same for the three species in that the chilled and freeze-chilled samples had the highest values and the fresh and frozen the lowest. However, there was no statistically significant difference between the freeze-chilled and chilled samples. Freeze-chilled samples had the highest free fatty acid (FFA) and peroxide values (PV) but the levels were low and did not influence sensory response. Gravity drip was significant in the frozen and freeze-chilled samples but presented no major visual problems and could readily be absorbed by drip pads. The effects of the four treatments on the colour and texture of the raw samples were small in practical terms.

Freeze-chilling was then combined with modified atmosphere packaging (MAP) to try to extend the shelf-life even further. The MAP packs for mackerel and salmon (60% N_2 / 40% CO_2), and for whiting (30% N_2 / 40% CO_2 / 30% O_2) maintained their shape during

freeze-chilling whereas packs with 100% CO_2 were slightly imploded with concave sides. The chosen chilled shelf-life of 5-7 days in the MAP trials was vindicated by the results as the products were near the end of their shelf-life (in acceptability terms) after 5 (whiting and mackerel) and 7 (salmon) days. This compares with shelf lives of 3 and 5 days respectively for freeze-chilled fillets in air. Samples in MAP had lower total viable counts than samples in air for raw fillets/portions of each of the three species. However, MAP did not influence odour or acceptability scores, but had a variable effect (generally small) on fillet colour, springiness, drip loss, total volatile base nitrogen / trimethylamine content, peroxide values and free fatty acid contents. Freeze-chilling appears to be an acceptable method of preserving fresh fish and should have commercial advantages.

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Chapter 1

1. INTRODUCTION

The market for seafood products in Europe has grown significantly in recent years, fuelled by increases in the average unit value of seafood products. Multiples are pushing suppliers to innovate to allow them to grow their share of the lucrative fresh/chilled seafood markets and eliminate the requirement for low-yield fresh seafood counters (Price Waterhouse Coopers, 2001). Two methods which have considerable potential to extend the shelf-life of raw fish fillets are freeze-chilling and modified atmosphere packaging (MAP), and a combination of the two could have synergistic effects on product quality. Freeze-chilling involves freezing and frozen storage followed by thawing and retailing the product at chill storage temperatures (2-4°C). Freeze-chilling has particular application for prepacked fish fillets which are increasingly replacing the traditional iced counter in some supermarkets. Extensive literature has been published on the effects of freezing and chilling on selected quality parameters of fish (Refsgaard *et al.*, 1998, Nilsson *et al.*, 1995, Magnússon and Martinsdóttir, 1995). However, the use of freeze-chill technology has received relatively less attention (Martinsdóttir and Magnússon, 2001, Bøknæs *et al.*, 2002, Emborg *et al.*, 2002, Guldager *et al.*, 1998; Bøknæs *et al.*, 2000; Bøknæs *et al.*, 2001). In the current freeze-chilling trials (sections 1 to 3), three days in chilled storage was chosen for whiting and mackerel and five days for salmon. This was based on pre-tests and on discussions with supermarkets on the likely shelf-life of prepacked chilled fish portions from

fish currently being landed at Irish fishing ports, or obtained from Irish fish farms (in the case of salmon). This three to five day time span in chilled storage agrees with the recommendations of Farber (1995) and Church (1998), but is considerably shorter than the extended chilled storage times for thawed cod in modified atmosphere reported by Bøknæs *et al.* (2000) and Bøknæs *et al.* (2001). These studies showed inhibition or inactivation of the specific spoilage organism *P. phosphoreum* in cod packed in modified atmosphere. Freeze-chilling maintains the product in a high quality state until it is thawed and sold as a chilled product, however, once thawed, natural spoilage bacteria proliferate on the surface of the fish and these eventually cause spoilage due to the production of ammonia based and sulphur compounds. Short-term freeze-chilling therefore has little impact in the chilled phase of the freeze-chill product life-cycle. Modified atmosphere packaging (MAP) is a proven method to inhibit the natural spoilage bacteria on foods. This involves the replacement of the air in packs by a mixture of different gases (usually N_2, O_2 and CO_2 in different proportions) which inhibits the natural microflora and slows down spoilage. Modified atmosphere packaging (MAP) has been used successfully to extend the raw fillet shelf-life of many species (Cann *et al.*, 1983, Scott *et al.*, 1984, Barnett *et al.*, 1987) and shelf-life extensions of 25-100% have been reported (Gopal *et al.*, 1990, Cann, 1984, Church, 1998). Relatively little work has been done on the combination of freeze-chilling with MAP for extending the shelf-life of raw fish portions (Bøknæs *et al.*, 2000; *Bøknæs et al.* 2001; Bøknæs *et al.* 2002). The objective of the current trials was to assess the suitability of freeze-chilling in combination with modified atmosphere packaging for extending the shelf-life of raw whiting, mackerel and salmon

portions. Combining freeze-chilling and MAP will allow fish fillets to reach distant markets in a frozen condition followed by thawing for sale at retail outlets. The inclusion of a modified atmosphere should be beneficial in the chill storage phase (post-thawing) by inhibiting the natural spoilage organisms on the surface of the fillet thus extending the shelf-life.

In terms of fish quality, certain parameters appear to be more important than others and are closely correlated with consumer preference. Laslett and Bremner (1979) (using a laboratory panel) found that the important predictors of acceptance were fish flavour, off flavour and toughness for fish minces, and off flavour and fish flavour for fish fingers. Procedures for assessing fish freshness have been reviewed by Whittle *et al.* (1990) while the upgraded quality index method (Nielsen and Jessen, 1997) is used as a sensory system for determining the freshness of thawed whole cod and is particularly relevant as much raw material (for further processing) is received as frozen fish. The multilingual guide to EC freshness grades for fishery products (Howgate *et al.*, 1992) uses four freshness grades [E, A, B, C (reject)], and 33 fish species are currently provided for in the regulations. There are also European guidelines, which are interpreted and implemented in different ways in member states governing the sale of raw fish fillets and recommendations are in place (e.g. Department of Health and Children, Ireland (1992) suggest upper TVC limits of log 5 cfu/g). Total viable counts for raw fish should not exceed log 6 cfu/g and TVBN content should not exceed 35mg N/100g (95/149/EC).

Chapter 2

2. LITERATURE REVIEW

2.1 Nutritional composition of fish

The main constituents of fish are water, protein and fat, however fish also contains carbohydrates, minerals and vitamins (Love, 1982). Water can account for up to 80% of the weight of a whitefish fillet, whereas the average water content of fatty fish is about 70 per cent (McCance and Widdowson, 1991). The water in fresh fish muscle is tightly bound to the proteins in the structure in such a way that it is not easily expelled even under high pressure. After prolonged chilled or frozen storage, however, cells become ruptured and the proteins are less able to retain water, and some of it, containing dissolved substances, is lost as drip. Frozen fish that are stored at too high a temperature, for example, will produce a large amount of drip and consequently quality will suffer. The method of freezing also has an impact on cell damage, with rapid freezing methods producing smaller ice crystals and less cell damage. In the living fish, the water content usually increases and the protein content decreases as spawning time approaches; thus it is possible, with cod for example, to estimate the condition of the fish by measuring the water content of the muscle.

The amount of protein in fish muscle is usually somewhere between 15 and 20 per cent, but values lower than 15 per cent or as high as 28 per cent are occasionally

recorded in some species (McCance and Widdowson, 1991). All proteins, including those from fish, are chains of chemical units linked together to make one long molecule. These units, of which there are about twenty types, are called amino acids, and certain of them (eight in total) are essential in the human diet for the maintenance of good health. Two essential amino acids called lysine and methionine are generally found in high concentrations in fish proteins, hence the reason fish is considered to be a source of protein of high biological value. In contrast these amino-acids are usually present in low concentrations in cereals like maize, rice, potatoes and / or wheat. Cereals however contain high concentrations of the non-essential amino-acids and because of their abundance in the diet are a good source of total protein precursors. Thus fish and cereal protein can supplement each other in the diet. Fish protein provides a good combination of amino acids which is highly suited to man's nutritional requirements and compares favourably with that provided by meat, milk and eggs.

In seafood, oils are the second largest component after protein. Oils have a variety of important roles; they serve as concentrated stores of energy, as fuel molecules and as components of membranes. Of major importance are triglycerides, polar lipids and cholesterol. Triglycerides serve as an energy store and polar lipids and cholesterol are structural components of cell membranes. Cholesterol is a factor in coronary heart disease and other disorders when in dietary excess. The main components of the triglycerides and phospholipids are saturated and unsaturated fatty acids. Eicosapentaenoic acid [EPA, 20:5(n-3)] and docosahexaenoic acid [DHA, 22:6(n-3)] are long-chain omega-3 polyunsaturated fatty acids (omega-3

PUFA) present selectively in fish oils and of major nutritional benefit in the human diet. The omega-3 PUFAs are largely obtained through the diet since humans generally are unable to synthesize them. Arachidonic acid [AA, 20:4(n-6)] is an omega-6 PUFA which is a precursor of prostaglandins (which modulate hormone activity) and other eicosanoids (C_{20} physiologically active compounds). This is formed in the human body from linoleic acid [18:2 (n-6)] and linolenic acid [18:3 (n-3)], both of which are found in the oils obtained from cereals and fish.

The marine-derived omega-3 PUFA have a wide range of potential health benefits, particularly with respect to the prevention of coronary heart disease and rheumatoid arthritis (Kritchevsky, 2000; Kelly, 2001). They may also play a role against some forms of cancer and other disorders, although further research and trials are required. Omega-3 PUFA may also be beneficial for infant brain and retina function and development (Robinson, 2001). Increasingly fish oils are recommended as a dietary supplement to the health conscious and are sold in pharmacies and health shops. Even in times past (1940s, 50s 60s) cod liver oil and halibut liver oil were used as daily supplements. They are still recommended to keep joints supple and as excellent sources of soluble vitamins.

The amount of carbohydrate in white fish muscle is generally too small to be of any significance in the human diet. Fish muscle normally contains only traces of carbohydrates, in the form of sugars, sugar phosphates and glycogen. Some other tissues such as liver contain larger amounts as glycogen, and most molluscan

shellfish also contain a fair amount of glycogen. In both white and fatty fish species, carbohydrate is not present. Some molluscs, however, contain up to 5 per cent of the carbohydrate glycogen. (Torry Advisory Note. No. 89, McCance and Widdowson, 1991).

Vitamins can be divided into two groups, those that are soluble in fat, such as vitamins A, D, E and K, and those that are soluble in water, such as vitamins B and C. All the vitamins necessary for good health in humans are present to some extent in fish, but the amounts vary widely from species to species, and throughout the year. The vitamin content of individual fish of the same species, and even of different parts of the same fish, can also vary considerably. Often the parts of a fish not normally eaten, such as the liver and the gut, contain much greater quantities of oil-soluble vitamins than the flesh; the livers of cod and halibut for example contain almost all of the vitamins A and D present in those species. Water-soluble vitamins in fish, although present in the skin, the liver and gut, are more uniformly distributed, and the flesh usually contains more than half the total amount present in the fish (Feng, 1989). Fish therefore contain all the components necessary for a healthy diet and the health benefits of omega-3 PUFA are well documented. Recent health scares including foot and mouth and mad cow disease have impacted favourably on seafood consumption, as seafood is seen as a healthy and green alternative to red meat and has not had any outbreak of infectious diseases, transmittable to man.

2.2 Spoilage of fish

Like mammalian meats, fish spoil through the combined effects of chemical reactions, continuing activity of endogenous degradative enzymes and bacterial growth (Davis, 1993). Bacteria also produce significant quantities of proteolytic, lipolytic, oxidative and other enzymes, all of which also contribute significantly to the spoilage of the fish. Different bacteria have widely different enzymatic content and therefore give rise to very different types of spoilage. Fresh fish and other seafood products are highly susceptible to spoilage by post-mortem microbial growth and constituents (e.g. enzymes) resulting from microbial growth. Their low body temperature (after death) provides a natural environment for psychrotrophic spoilage microflora (Brody, 1989).

It is generally recognised that the predominant spoilage organisms belong to the genus *Pseudomonas.* Shewan (1971) reviewed the literature on the microbiology of fish and fish products between 1930 and 1970 and concluded that the initial flora of fresh North Sea fish consisted mostly of *Moraxella*, *Arthrobacter*, *Pseudomonas*, *Flavobacterium/Cytophaga*, *Acinetobacter* and *Micrococcus* but *Pseudomonas* became successively dominant during prolonged storage (Stenström and Molin 1989), usually when fish was stored on ice between days 0 and 15.

Despite diverse initial microbial populations most bacterial spoilage in air results from Gram-negative psychrophilic organisms, with *Pseudomonas* species and *Alteromonas* species dominant (van Spreekens, 1977). Once fish are filleted or

made into products, spoilage accelerates, mainly due to the fact that more of the body surface is exposed to the air (aerobic spoilage) and cross contamination of the internal body surface occurs during fillet or mince preparation.

The effect of microbial activity on fresh seafood is the eventual breakdown of proteins as the tissues of the fish are colonised by successive bacterial strains. The rate of decomposition is influenced by the initial number and types of bacteria and storage conditions, such as temperature, humidity and gaseous atmosphere (Stenström and Molin 1989). Fish initially contain significant levels of trimethylamine oxide, which has an osmoregulatory function in the live animal. Both *Pseudomonas* and *Alteromonas* species reduce trimethylamine oxide (TMAO) to trimethylamine (TMA), a fishy-smelling volatile base (van Spreekens, 1977). Ammonia is another undesirable volatile base that is produced by both of these species as a degradative oxidation product of non-protein-nitrogen (NPN), such as amino acids and creatine (Bannerjee, 1967). Total volatile base nitrogen (TVBN) is a comprehensive nitrogen value that includes all volatile nitrogen molecules including both TMA and ammonia (Villemure *et al.*, 1986). Ammonia (NH_3) is the dominant contributor in meat while TMA is often the major base in fish deterioration.

In fatty fish, rancidity can be a problem, which can dramatically shorten the remaining shelf-life of the product. Lipids in most fatty fish, for example in the Atlantic mackerel (*Scomber scombrus*) and the Atlantic salmon (*Salmo salar*), readily undergo oxidation on exposure to the air even in frozen storage (Ke *et al.*,

1975). In fatty fish, depot fat occurs as extracellular globules in the muscle and mesentery (Flo *et al.*, 1972). A variable but high proportion of the total lipids are neutral lipids (triglycerides) with substantial contents of unsaturated C-20 and C-22 fatty acids (Hardy and Keay, 1972). These acids are among the constituents of fatty fish, in free or combined forms, most susceptible to autoxidation. The oxidation products of these unsaturated fatty compounds and a number of minor lipid components contribute to the natural aromas, flavours and colour substances which make fish and fishery products attractive in minor proportions, but aesthetically objectionable if present in excess (Ackman, 1967).

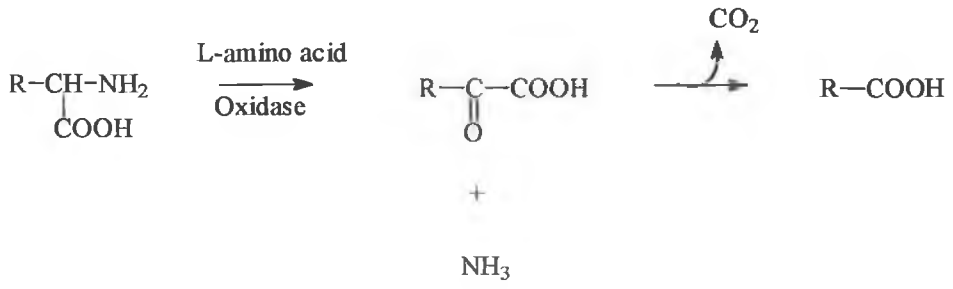
2.3 Microbial spoilage of fish

Microorganisms are found on all the outer surfaces (skin and gills) and in the intestines of live and newly caught fish. The total number of organisms vary enormously, however, Liston (1979) determined the normal range to be in the order of 10^2-10^7 cfu (colony forming units)/cm^2 on the skin surface. The gills and the intestines both contain between 10^3 and 10^9 cfu/g (Shewan, 1962). The first stage of fish spoilage is dominated by endogenous enzymes, whilst the bacterial flora of the gut cavity, gills and skin acclimatise and adapt to the changing environmental conditions (Church, 1998). The sequences of events leading to spoilage have been well documented, and Liston (1979) suggested the following sequence:

1.a. The spoilage bacteria are naturally present on fish.

1.b. Amino acid and other non-protein-nitrogen (NPN) substrate pool are present (ammonia and amines) in the fish.

2. Selective growth of organisms (mostly *Pseudomonas*) which oxidatively deaminate amino acids.



3. Repression of proteinase production is now depressed by selective use of amino acids by the *Pseudomonas* bacteria.

4. Amino acid recruitment to substrate pool by bacterial hydrolysis of protein.

5. Ammonia and volatile fatty acid production sharply increases due to the release of amino acids upon hydrolysis of proteins.

Specific "spoiler" types of bacteria produce S-containing and other odorous compounds. Volatile sulphur-compounds are typical components of spoiling fish and most bacteria identified as specific spoilage bacteria produce one or several volatile sulphides. S. *putrefaciens* and some *Vibrionaceae* produce H_2S (hydrogen sulphide) from the sulphur containing amino-acid L-cysteine (Stenströem and Molin, 1990; Gram *et al.*, 1987).

The composition of the microflora changes quite dramatically during storage. Thus, under aerobic iced storage, the flora is composed almost exclusively of *Pseudomonas* spp. and S. *putrefaciens* after 1-2 weeks. This is believed to be due to their relatively short generation time at chill temperatures (Morita, 1975; Devaraju and Setty, 1985). *Pseudomonas* are psychrotrophic and are ideally suited to refrigeration temperatures. They produce off-odours and off-flavours by producing compounds such as dimethyl disulphide, dimethyl trisulphide, trimethylamine, dimethylamine, butyrate and hexanoate (Miller *et al.*, 1973a,b). However, bacterial action also produces lower chain fatty acids (C_2-C_3) from carbohydrates, aldehydes and ketones from lipids, ammonia, amines, and biogenic polyamines from amino acids and volatile sulphides from sulphur-containing amino acids (Davis, 1993).

Much work has been carried out using these products to assess the quality and acceptability of fish. The main quality parameters used however are trimethylamine (TMA) (Jensen *et al.*, 1980; Woyewoda *et al.*, 1984; Vynke, 1970) and total volatile base nitrogen (i.e. volatile amines plus ammonia) (TVBN) (Vynke, 1970; Villemure *et al.*, 1986). Most marine fish contain trimethylamine oxide (TMAO) which has an osmoregulatory function in the live animal. TMAO is found in concentrations of up to 1% in teleosts (e.g. cod and whiting) and up to 1.5% in elasmobranchs (e.g. sharks and skates) (Church, 1998). After death, spoilage bacteria can use TMAO as a terminal electron acceptor, which allows them to grow when oxygen conditions are depleted (Easter *et al.*, 1983). In this way, TMAO is reduced to TMA and, while TMAO is non-odorous, TMA

(trimethylamine) is a component in the odour of stale fish and is synonomous with what we recognise as a "fishy odour". Quantitative determination of TVBN is perhaps the most widely used indicator of fish and seafood quality (Malle and Tao., 1986; Oehlenschlager., 1997). Total volatile base nitrogen is the ultimate product of proteolytic degradation of muscle protein. It includes ammonia, mono, di and trimethylamines. The former is the ultimate end product of proteolytic degradation and is the main end product in meat spoilage, the latter comes mainly from TMAO and is only produced in fish. Trimethylamine can in fact be the major contributing factor in fish spoilage. The directive 91/493/EC, which establishes the sanitary practices to be adopted during the handling and marketing of seafood, mentions TMA-N and TVBN determinations as additional investigations besides organoleptic tests. They are quantitative measurements and not subject to bias, being objective tests.

2.4 Lipid oxidation and hydrolysis in fish

The polyunsaturated fatty acids found in fatty fish make them particularly susceptible to lipid degradation, which can severely reduce the shelf life of the product. Lipid oxidation in fish is one of the more important factors responsible for quality loss in refrigerated and frozen storage (Flick *et al.*, 1992). The sequence of reactions occuring during the autocatalytic mechanism of fat oxidation can be seen in Figure 1. Lipid oxidation is initiated by abstraction of a hydrogen atom from the central carbon of the pentadiene structure found in most fatty acid acyl chains containing more than one double bond:

$$-CH = CH-CH_2-CH = CH- \longrightarrow CH = CH-CH-CH = CH- + H\cdot$$

Lipid radicals (L·) react quickly with oxygen molecules to produce highly reactive peroxy radicals (LOO·) which again may abstract a hydrogen from another acyl chain resulting in a lipid hydroperoxide (LOOH) and a new radical. This propagation continues until one of the radicals is removed by reaction with another radical or with an *antioxidant* (AH) whose resulting radical (A·) is much less reactive.

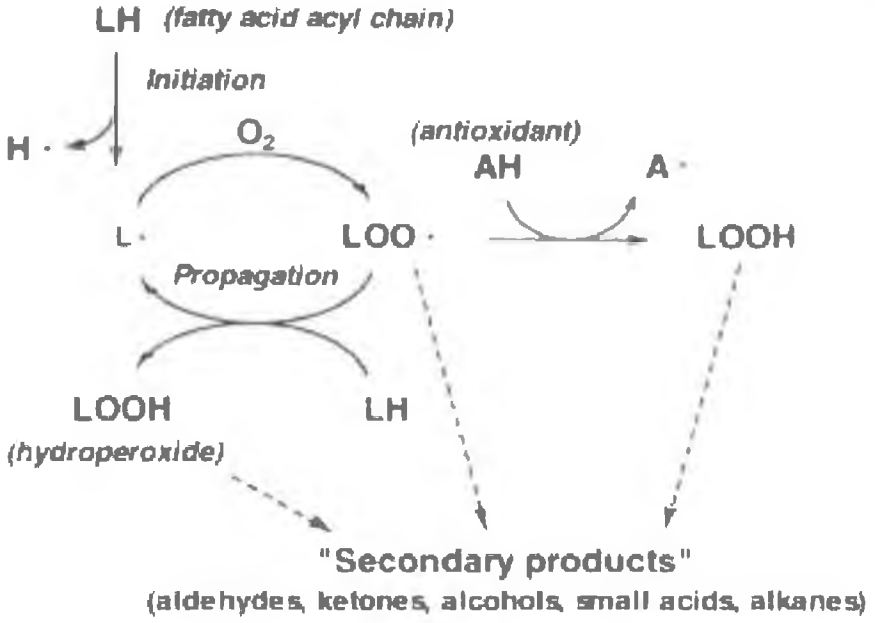


Figure 1. Autocatalytic mechanism for fat oxidation

The hydroperoxides are readily broken down, catalysed by heavy metal ions, to secondary autoxidation products of shorter carbon chain-length. Metal ions are very important in the first step of lipid autoxidation - the initiation process - in catalysing the formation of reactive oxygen species as for example the hydroxyl

radical ($OH^{\bullet}$). This radical immediately reacts with lipids or other molecules at the site where it is generated. The high reactivity may explain why free fatty acids have been found to be more susceptible to oxidation than the corresponding bound ones. The living cells possess several protection mechanisms against lipid oxidation products. An enzyme, glutathione peroxidase, exists which reduces hydroperoxides in the cellular membranes to the hydroxy-compounds. This reaction demands a supply of reduced glutathione and will therefore cease post mortem when the cell is depleted of that substance. The membranes also contain the phenolic compound α-tocopherol (Vitamin E) which is considered the most important natural antioxidant present in fish. Tocopherol can donate a hydrogen atom to the radicals $L^{\bullet}$ or $LOO^{\bullet}$ functioning as the molecule AH in Figure 1. It is generally assumed that the resulting tocopheryl radical reacts with ascorbic acid (Vitamin C) at the lipid/water interface regenerating the tocopherol molecule. Other compounds, for example the carotenoids, may also function as natural antioxidants.

Autoxidation involves the chemical breakdown of fat in the presence of oxygen / air without any additions. Lipid hydrolysis can also occur in fatty fish species impacting adversely on shelf life. During storage, a considerable amount of free fatty acids (FFA) appears, more so in ungutted than in gutted fish, probably because of the involvement of digestive enzymes. Triglyceride in the depot fat is cleaved by triglyceride lipase (TL in Figure 2) originating from the digestive tract or excreted by certain microorganisms. Cellular lipases may also play a minor role.

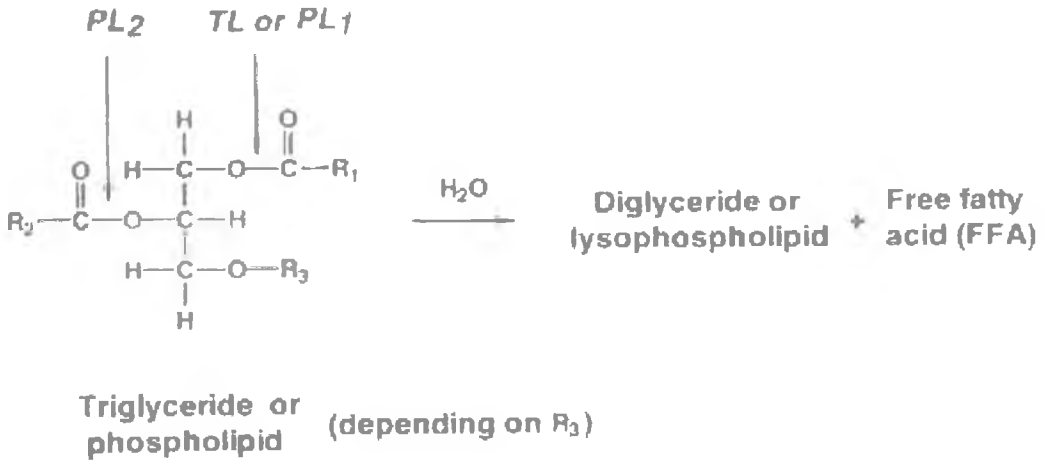


Figure 2. Primary hydrolytic reactions of triglycerides and phospholipids. Enzymes: PL_1 & PL_2 phospholipases;TL, triglyceride lipase

In lean fish, for example Atlantic cod and whiting, production of free fatty acids also occurs, even at low temperatures. The enzymes responsible are believed to be cellular phospholipases - in particular phospholipase A_2 (PL2 in Figure 2) - although a correlation between activity of these enzymes and the rate of appearance of FFA has as yet not been firmly established. The fatty acids bound to phospholipids at glycerol-carbon atom 2 are largely polyunsaturated, and hydrolysis therefore often leads to increased oxidation as the liberated fatty acid is now more prone to oxidation. Furthermore, the fatty acids themselves may cause a "soapy" off-flavour.

2.5 Measurement of spoilage in fish

2.5.1 *Sensory analysis*

Sensory properties are the most important quality index for fish (Stammen *et al.*, 1990). As Regenstein and Regenstein (1981) once put it; " The ultimate measure of the effects of shelf life extension treatments on the final quality of the fish is consumer satisfaction. Is (s)he willing to repurchase the fish product?". When microbiological, chemical and sensory analyses are compared, shelf life and acceptance do not always coincide. In order to assess spoilage, tests must be firstly carried out on a product that is perceived to be "fresh". In the case of fish, freshness can be defined in a number of ways. The Sea Fish Industry Authority (SFIA) (1985) states "it is important that fish are consumed in as fresh a state as possible so that the sweet and desirable flavours are retained". The "flavour" is, perhaps, the most important criterion of freshness and "the acceptability of fish to the consumer decreases markedly when sour flavours develop" (SFIA, 1985).

Flavour is assessed using sensory analysis in which a panel (trained or untrained) of tasters are asked to score samples of fish by various methods. An unstructured hedonic scale can be used in which tasters are asked to mark a 5 or 6cm line from 0 (unacceptable) to 6 (very acceptable). The distance from the start of the line to the mark is proportional to their perception of the degree of freshness of the sample (ISO 4121, 1987).

A trained or untrained taste panel can be used to assess sensory acceptability of seafood products. Trained panels consist of a number of panellists (used to eating seafood) who have been instructed on how to score a particular attribute of the product such as texture, odour etc. Untrained panels consist mainly of consumers who have not been previously instructed on how to score products. Modern consumer evaluations of sensory properties of fish products take into account a wide range of characteristics of the products. Whilst information on consumer acceptance is important, it is also crucial to know what sensory attributes consumers use to describe and discriminate among products. Laslett and Bremner (1979) (using a laboratory panel) found that the important predictors of acceptance for fish minces and fish-fingers were "fish flavour", "off flavour" and "toughness" for minces while "off flavour" and "fish flavour" were most relevant for fish fingers. Connell and Howgate (1971) evaluated the flavour/texture impact on the acceptance of cod and haddock fillets over a wide range of freshness and concluded that flavour was a more important criterion of quality than texture. In a consumer evaluation of sensory properties of fish, Sawyer *et al.* (1988) concluded, "there exists a relatively small set of texture, flavour and appearance attributes that consumers can use to describe and differentiate species". They also concluded, "consumers use a much smaller segment of the perceptual range to describe fish than do trained panellists". It is therefore necessary to try to correlate sensory acceptability with quality measurements such as odour and off flavours, texture analysis, colour etc. to ensure seafood products will be of the highest consumer acceptability, making them a desirable and competitive product.

2.5.2 *Odour*

Volatile amines are the characteristic substances responsible for the fishy odour and flavour encountered in fish having passed the initial phase of freshness. Most consumers consider this aminic smell to be typical for fish in general and the consumer's decision of "like" or "dislike" strongly depends on the concentration of these amines in fishery products (Oehlenschläger, 1997).

The initial burst of volatile amines on opening a pack containing fish of dubious quality can be off-putting and can result in lower demand for that particular seafood product. Amines are present in freshly caught fish at low levels and develop on ageing. The degree to which they develop is dependent on factors such as species, temperature, time and handling and hygiene conditions prior to processing.

During the spoilage of fish, different volatile bases are formed including; ammonia, monomethylamine, dimethylamine, trimethylamine and higher amines (histamine, tryptamine, etc.) (Vyncke, 1970). The most common methods for assessing the freshness of fish, together with the organoleptic tests, are the determination of total volatile basic nitrogen (TVBN) and trimethylamine (TMA) (Civera *et al.*, 1995). Much work has been carried out using TVBN and TMA as indicators of freshness/spoilage (Oehlenschläger, 1997; Vyncke, 1970; Rehbein, 1992; Malle and Poumeyrol, 1989). Directive 91/493/EC, which establishes the sanitary practices to be adopted during the handling and marketing of seafood, mentions TMA and TVBN determinations as additional investigations besides the

organoleptic tests. Directive 95/149/EC specifies the fixed TVBN limits for various categories of fishery products.

The evolution of TVBN and TMA during storage is a well-documented phenomenon (Civera., 1995; Malle and Poumeyrol, 1989). Various methods have been proposed for the determination of TVBN and TMA but perhaps the most rapid technique was proposed by Malle and Tao (1987). The method proposed by the Codex Alimentarius Committee (1968), as reported by Malle and Tao (1987), is frequently used and adopted in this project for TVBN. Protein is removed with trichloroacetic acid, the primary and secondary amines are blocked with formaldehyde and the TMA is distilled over and quantified by titration

A rapid and inexpensive test for odour is a "sniff test" which, as the name suggests, involves smelling the fish/pack to determine the degree of freshness of the product. This technique is very useful as the human nose can be trained to detect fish, which is gone off or of borderline quality. Fishery assessment technicians and purchasers directly employ this method when assessing fish quality and it is a major component of long established quality indices including the Quality Index Method (QIM). QIM is based on the significant sensory parameters for raw fish when using many parameters and a score system from 0 to 4 demerit points (Jonsdottir, 1992).

2.5.3 *Texture Analysis*

Texture, appearance and flavour are the three major components of food acceptability (Bourne, 1978). Texture is as important as aroma, as a criterion of freshness. The degree of softness or hardness is difficult to judge without instrumentation. While it is relatively easy to compare a small number of samples of fish using a combination of sight and touch, comparisons over time of the same samples become difficult without some kind of numerical value assigned to the samples.

Texture of raw salmon fillets is commonly tested in the industry by the "finger method". A finger is pressed on the skin of the fillet and firmness is evaluated the hardness when pressed on the fillet (Sigurgisladottir *et al.*, 1999). Instrumental methods for measuring texture have been investigated since 1861 (Sigurgisladottir *et al.*, 1999). Tests which attempt to imitate with instruments the conditions to which food is subjected in the mouth or on the plate are known as Imitative tests (Szczesniak, 1963). Texture profile analysis (TPA) falls into this category and the major breakthrough in TPA came with the development of the General Foods Texturometer. A small flat-faced cylinder was used to compress a bite-sized piece of food and analyses of the force-time curve led to the extraction of seven textural parameters. These parameters were named (after modification) as follows (Bourne, 1978):

Fracturability: defined as the force at the first significant break in the curve.

Hardness: defined as the peak force during the first compression cycle ("first bite").

Cohesiveness: defined as the ratio of the positive force area during the second compression to that during the first compression.

Adhesiveness: defined as the negative force area for the first bite, representing the work necessary to pull the compression to that during the first compression.

Springiness: defined as the height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite.

Gumminess: defined as the product of hardness multiplied by the cohesiveness.

Chewiness: defined as the product of gumminess multiplied by springiness.

Many adaptations of the TPA instrumentation have been developed in more recent years. Bourne (1968, 1978) adapted the Instron Universal Testing Machine to perform a modified texture profile test by compressing standard-size pieces of food twice in a manner analogous to that of the G.F. Texturometer (Bourne, 1978). Another, and perhaps a more evolved, variation of TPA is the TA-XT2*i* Texture Analyser (Stable Micro Systems, Surrey, UK). This instrument uses the same concepts as the G.F. Texturometer, however it is backed up with powerful computer software that makes it much more user friendly. Much work has been carried out on the texture of fish using this equipment (Schubring, 1999; Sigurgisladottir *et al.*, 1999; Schubring and Munker, 1988).

2.5.4 *Colour*

To a large extent consumers recognise, discriminate and select nutrients with the eye. Through conditioning and association, they expect an item of a certain shape and colour to have a specific odour, taste and texture. In foods, colours are identified with previously experienced quality and serve as instant indicators of good or bad, according to the products and its intended use (Pangborn, 1964).

Colour is very important in food and is used to judge the quality, maturity and age after harvest of many foods (Gormley, 1975). The flesh colour of fish is composed mainly of melanins, pterins and carotenoids (Sinnott, 1988). Melanins may be black, brown, red or yellow in colour and are present in the flesh of fish in the form of melanophores. They are branched cells and the pigment may be aggregated in their centres or dispersed along the branches. When the pigment disperses, the fish appears dark and when the pigment aggregates it appears light. This is used as a defence mechanism in many species and it is important in concealing predators from their prey (Sinnott, 1988). Pterins may be red, yellow or orange in colour and they are granular and are located in the same chromatophores as carotenoids. They are often associated with melanins. Carotenoids are a class of hydrocarbons known as carotenes and their oxygenated derivatives, the xanthophylls. These are yellow, orange or red in colour and are usually found associated with fats (Sinnott, 1988). The discoloration of fish muscle is a combination of variation or change in all three and is also partially caused by Maillard reactions involving ribose with amino-acids which causes browning.

The effects of prolonged chilled storage on fish and seafood products are difficult to assess in the case of whitefish. Changes are less obvious than in more pigmented species such as salmon and mackerel and therefore colour is perhaps the least valuable indicator of spoilage in whitefish species. There is also a risk of discoloration of haem pigments, which leads to browning of flesh in salmonids. In general, whitefish species become more yellow in appearance during chilled storage. This yellowness can be attributed to such factors as microbial growth on the surface of the fillet and discoloration can occur via a bleaching action of cut surfaces probably due to low pH precipitation of sarcoplasmic proteins (Statham and Bremner, 1989).

Very little work has been done on the effects of frozen storage on the colour of whitefish species. If the fish is of high quality prior to freezing and is then rapidly frozen (e.g. blast-frozen) then colour changes should be insignificant. Freezer-burn can occur when raw fish is badly packaged during the freezing process resulting in ice crystals forming on the surface of the fillet/fish which damage the surface. Freezer burn alters the structure of the surface of the fillets and gives a dehydrated looking product of low consumer appeal.

Redness of flesh is one important criterion in salmon (Skrede and Storebakken, 1986). Astaxanthin is the dominant carotenoid pigment in the flesh of wild salmon (Khare *et al.*, 1973), whereas in farmed salmon, synthetic canthaxanthins are added to the diet which provide the colour. Spectral characteristics have frequently been used to measure the xanthin content in salmonids. Most studies

have used the reflectance spectra of raw or processed muscle tissue and further transformance into CIE XYZ tristimulus values and various uniform colour systems (Hunter, 1975). In these systems, salmon colour may be described by the parameters L, a and b, where L represents lightness, a redness and b yellowness.

2.5.5 *Gravity drip-loss*

Gravity drip-loss refers to the natural exudate or drip that comes from fillets and seafood products. Excessive weight loss due to drip has been reported sporadically, but fish stored under traditional methods have drip levels ranging from 3 to 8% (Stammen *et al.*, 1990). Drip and moisture loss is of economic significance, as it not only affects the weight of the final product (and thus the economic gain) but also the perceived freshness of the product. The ability of the fish to retain their intrinsic fluids is of major importance to their commercial value and consumer acceptance (Fennema, 1990). Freezing can cause tissue damage, which results in drip during thawing (Brennan and Gormley, 1999). Moisture loss from the flesh of fish is due primarily to the reduction of the moisture binding capacity of the protein, when the acidity of the muscle is increased by lactic acid formation (Borgström, 1968).

2.5.6 *Centrifugal drip-loss (water holding capacity).*

Centrifugal drip-loss refers to the loss of moisture from a product when a force is applied. In the case of centrifugal drip-loss, samples of fish are centrifuged at 400 x g (Brennan and Gormley, 1999) and any free liquid is forced out of the sample and is retained by glass beads below. As in the case of gravity drip, centrifugal

drip is also expected to be higher in samples which were previously frozen due to cell rupture and tissue damage. Faster freezing rates mean smaller ice crystal formation, so less damage occurs in the cell structure. Therefore when a product is thawed, there is less drip (IIR, 1986). Nilsson and Ekstrand (1994) also found that the fast freezing of rainbow trout resulted in lower drip-loss than slow freezing. The fast frozen trout had a drip of 8.4% while the slow frozen trout had a drip of 9.9%.

2.5.7 *Lipid oxidation*

The deterioration of texture, flavour and odour of stored seafood can be partly but significantly attributed to the oxidation of unsaturated lipids (Flick *et al.*, 1992). Fish lipids are characterised by a high degree of unsaturation in the form of multiple double bonds in the fatty acids and ready susceptibility to attack by molecular oxygen (Olcott, 1962). As discussed earlier, lipid oxidation occurs when unsaturated fatty acids react with molecular oxygen via a free radical chain mechanism to form fatty acyl hydroperoxides commonly known as peroxides. Once these peroxides are formed, they in turn act as free radicals, which in turn can accelerate the rate of lipid oxidation. The hydroperoxides undergo further reactions. Frequently the long chains cleave releasing aldehydes, ketones etc. Being of lower molecular weight, these aldehydes and ketones are volatile and give rise to "off-odours" and "taint". This process is known as "autoxidation" and the process is self-generating and difficult to control. The nature, proportion and degree of unsaturation of fatty acids present in a food will influence the

susceptibility of that product to oxidative rancidity. The presence or absence of natural antioxidants will also influence the susceptibility of the oils to rancidity.

Freezing slows enzyme activity and inhibits the growth of micro-organisms but does not prevent lipid oxidation (Flick *et al.*, 1992). Post-mortem lipolysis can occur in chilled and to a much lesser extent in frozen fish, and the major products are free-fatty acids and glycerol (Hardy and Smith, 1976). Free fatty acids are more susceptible to lipid oxidation than covalently linked acids so the freshness of the fish prior to freezing is of crucial importance as it dictates the potential storage time of the fish in the frozen state. The nature of fishing today means that fatty species such as mackerel and wild salmon can spend several days on ice before reaching the processor. This means that in most cases, lipid oxidation has already occurred and hydroperoxides and peroxides are already present in the flesh before processing. The freezing temperature must therefore be sufficient to slow the oxidation process in order to extend frozen shelf-life and ideally fish should be frozen at sea to reduce temperature abuse. An investigation of oxidative rancidity in mackerel at various temperatures was undertaken by Ke *et al.* (1975) and it was concluded, "some selective and non-selective fat solvent extractable, pro-oxidant compounds are present in the mackerel skin. Their effect is temperature dependent in the frozen condition but their catalytic activity on lipid oxidation can be inhibited by lowering the frozen storage temperature to -40°C". Low temperatures are expensive to maintain over long periods of time. In spite of this, -30°C is commonly used in the processing industry, as it maintains the product/fillets in acceptable condition, while being economically feasible. .

The rancidity of fat is a useful tool for measuring spoilage in fatty species of fish. It is however of little value in lean species as their fat level is so low that large quantities of fat would have to be extracted. The resulting extract would be dilute and would require concentration prior to analysis. Many methods have been proposed for the measurement of rancidity in fish, however the most accurate indicators of rancidity would appear to be peroxide value (PV) and free fatty acids (FFA). Peroxide value measures hydroperoxides, which are primary products of lipid oxidation, that break down to secondary products like aldehydes and ketones, which smell and react with proteins. Lipid is quantitatively extracted from preweighed fillets. The hydroperoxides present in the extracted fat are quantified by an idiometric titration.

Free fatty acids (FFA) are long-chained acids, usually with an even number of carbons (C8-C24). They are released from triglycerides and phospholipids by the action of lipases. They then react more readily, if containing unsaturated bonds with molecular oxygen / air causing the accelerated production of free radicals. The amount of FFA in fat can be determined on the same fat extract used for peroxide value determination. A weighed sample of extracted "oil" is placed into a flask and alcohol is added. This alcohol has been previously neutralised by adding phenopthalein solution and enough NaOH to produce a faint pink colour. This solution is titrated with standardised NaOH with vigorous shaking until a permanent pink colour appears and persists for at least 30 seconds. The amount of NaOH added in the titration is proportional to the FFA (expressed as oleic acid).

2.6 Freeze-chilling of seafood products

The effects of freezing on food are well documented (IIR, 1986) as are the effects of chilling (Gormley, 1990a). Freezing is the crystallisation of liquid water into the solid form of water known as ice. Food is frozen to preserve the initial quality and wholesomeness of the product for consumption at a later date. To achieve the preservation effect a high proportion of the freezable water (> 80%) in the product is converted to ice and is kept in this state during subsequent storage to minimise physical, biochemical and microbial changes which would otherwise lead to deterioration of the product. The effects of freeze-chill technology on the quality of foods, and of fish fillets and portions have received relatively less attention. Freeze-chilling involves freezing and frozen storage followed by thawing and retailing at chill storage temperatures (O'Leary *et al.*, 2000; Redmond *et al.*, 2002). Freeze-chilling offers logistic and other advantages. For example: (i) foods can be prepared in bulk, frozen and stored at deep freeze temperatures until required. Some, or all of the batch can then be thawed as necessary; (ii) freeze-chilling enables chilled foods to reach distant markets in that product can be shipped deep frozen and then thawed when it reaches its destination prior to retail display; (iii) freeze-chilling can reduce the level of product recalls as it enables routine microbiological tests to be completed before the product is released from the factory. In frozen storage, physical and chemical reactions can still occur in foods which lead to a gradual and irreversible reduction in the product quality. There are also concerns, that the freezing step prior to chilling predisposes the product to more rapid spoilage by damaging cell structure. Toughening of the

texture of fish occurs on freezing and cold storage (IIR, 1986). There is also a resultant loss in the ability of the fish to retain tissue fluids on thawing, which leads to drip. The combination of these two factors can lead to fish that is dry, tough, chewy, and rubbery. These effects are principally due to protein denaturation (Jiang and Lee, 1985). Textural changes are mainly due to moisture loss in the tissues of the flesh. The method of freezing can affect the amount of centrifugal and gravity drip in fish portions. Faster freezing rates create smaller ice crystals, which is less damaging and creates less drip than slow freezing. Therefore when the product is thawed there is less drip (IIR, 1986). Nilsson and Ekstrand (1994) found that fast freezing rainbow trout resulted in significantly lower centrifugal drip (8.4%) than slow freezing (9.9%). Redness of flesh is an important criterion in highly pigmented species of flesh. The characteristic red-colour should be maintained when the fish is processed and ready to consume (Skrede and Storebakken, 1986). Walshe and Gormley (1999) found that salmon portions lost colour in frozen storage. In whitefish species of fish, browning of flesh can occur in frozen storage. Discoloration can occur via a bleaching action of cut surfaces (Cann, 1984) probably due to low pH precipitation of sacroplasmic proteins (Stratham and Bremner, 1989). Magnüsson and Martindóttir (1995) have shown that short-term frozen storage (< 5 weeks) has little effect on bacterial counts in cod and ocean perch. This presumably means that the microbial status of the fish prior to freezing will not be affected by short-term frozen storage. In the current trials, samples were frozen for an extremely short time period (3 days) prior to chilling, therefore the frozen step would not be expected to affect the microbial status of the fish portions according to Magnüsson and Martindóttir

(1995). Fish, in particular fatty fish, are very prone to the development of autoxidative rancidity during frozen storage (IIR, 1984). The rate of lipid oxidation can be affected by several factors, including age, maturity, size of fish and the position from where the sample was taken (Flick *et al.*, 1992). Further work needs to be carried out in this area as it is widely accepted that subsequent to an induction period, the peroxide value rises exponentially until it reaches the rancid range (10-20). Thereafter it can disappear completely or rise even further depending on the metabolic pathways used. At this stage however, organoleptically the fish becomes obviously past its "sell by date". According to Stuchell and Krochta (1995) during frozen storage, peroxide values in fish fluctuate over time with no trend. Furthermore, it is known that rancidity continues in frozen storage, albeit at a lower rate. The initial quality of the product prior to freezing is therefore of crucial importance and temperature abuse must be minimised to avoid the production of free fatty acids which will rapidly increase rancidity in frozen storage. Perhaps the definitive test of frozen storage on the quality of fish portions is sensory analysis. Frozen storage is seen to be a necessary step in the production and distribution of fish products due to the extremely short chilled shelf-life of the fresh product. While frozen products may be acceptable, they can still be inferior to a fresh product (Kossovitsas *et al.*, 1973). A compromise situation must therefore be sought, delivering products with a high degree of quality to the consumer. Freeze-chilling would therefore appear to offer the consumer a product of slightly lower yet highly acceptable quality. The quality of the product prior to chilling is of crucial importance as it dictates the remaining shelf-life in the chill phase.

2.7 Modified atmosphere packaging of seafood products

Modified atmosphere packaging (MAP) is the replacement of air in a pack by a different mixture of gases, where the proportion of each component is fixed when the mixture is introduced, but no further control is exercised during storage (Sacks and Gore, 1987). A gas flush or evacuation and backfill is used to replace the air in the package with a specific gas mixture. Carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2) are the most common gases used in MAP.

Nitrogen is used as an inert filler to counteract the pressure when a vacuum is drawn and maintains package integrity to prevent the product from being crushed and/or sticking together (Stammen *et al.*, 1990). Nitrogen has low solubility and appears to exert no direct influence on typical spoilage bacteria, even at concentrations approaching 100% (Coyne, 1932). Oxygen is added to meats to maintain colour and to whitefish to reduce drip, but ideally should be omitted in fatty fish due to the development of oxidative rancidity (Sea Fish Industry Authority, 1985). Carbon dioxide acts as an anti-microbial agent and retards the lag phase of bacterial growth (King and Nagel, 1967).

In 1939, Coyne established that CO_2 could inhibit the normal spoilage flora of fish but found it most effective on *Bacillus*, *Achromobacter*, *Micrococcus* and *Pseudomonas* species. The inhibition level increased with increasing CO_2 levels. CO_2 is both water and lipid soluble and is mainly responsible for the bacteriostatic effect seen on microorganisms in modified atmospheres (Farber, 1991). This

bacteriostatic effect is influenced by the concentration of CO_2, the age and load of the initial bacterial population, storage temperature and type of product to be packaged (Reddy *et al.*, 1992).

The precise mechanism of how CO_2 interacts with bacterial cells is relatively unknown, but the popular theories include:

- Alteration of cell membrane function including effects on nutrient uptake and absorption.
- Direct inhibition of enzymes or decrease in the rate of enzyme reactions.
- Penetration of bacterial membranes, leading to intracellular pH changes.
- Direct changes in the physicochemical properties of proteins (Farber, 1991).

Modified atmosphere packaging has been used successfully to extend the raw fillet shelf-life of many species (Cann *et al.*, 1983; Scott *et al.*, 1984; Barnett *et al.*, 1987) and shelf-life extensions of 25-100% have been reported by many authors (Gopal *et al.*, 1990; Cann, 1984; Church, 1998). Reddy (1992) has tabulated the reported extensions in shelf life obtained by several workers and examples of these are as follows:

Storage temp (°C)	Product	MAP atmosphere	Shelf-life MAP(days)	Shelf-life Air (days)
5	Various whitefish	40% CO_2 30% O_2 30% N_2	9	6
26	Whiting fillets	100% CO_2	2	2
4	Whiting fillets	100% CO_2	16	7.5
0	Mackerel fillets	60% CO_2 40% N_2	6.5	3.5

Many combinations of gas mixtures have been examined for their effects on the shelf-life of fish. In most cases, CO_2 levels above 25% are recommended as suggested by Gill and Tan (1979). There are divergent views with respect to the amount of O_2 that should be included along with N_2 as the diluent gas. Tiffney and Mills (1982) found that O_2 actually increased the shelf-life of white-fish and, they recommended inclusion of 30% O_2, but exclusion for fatty fish and cured products. Lagoin (1985) and Sacks and Gore (1987) made the same suggestions. However, Sacks and Gore (1987) also recommended 40% O_2 with 60% CO_2 as an alternative for white fish. Modified atmosphere packaging therefore has the ability to extend the shelf-life of fishery products, though there are concerns about its effects on the quality of the raw fillets. The effects of MAP on texture are difficult to assess from the literature due to the use of non-complete methodologies by different researchers. Some researchers objectively measure raw texture using a Torymeter or Instron, while others rely on sensory panels for raw or cooked texture analysis (Stammen *et al.*, 1990). The main effects of MAP are on the resultant drip caused by high CO_2. The loss of moisture from the raw product toughens the flesh and alters its overall "mouth-feel". The degree to which MAP alters the texture is dependent upon the initial freshness of the product, the microbial status of the fish prior to packing and the conditions in which it is stored. In general, fillets become tougher in the presence of modified atmospheres and this is as a result of CO_2 diffusing into the tissues of the fillet reacting with water to form carbonic acid, effectively removing some water and producing a weak acid. This can reduce the pH of the flesh thus affecting the water holding capacity and producing excessive amounts of exudate or drip. Lannelongue *et al.*

(1982) reported a drop in surface pH in MAP products due to the disassociation of CO_2 to form carbonic acid ($CO_2 + H_2O \rightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$). Carbonic acid can also have a bleaching effect on fish. Bleaching of pigments and increased opacity of flesh has been reported by Gibson and Davis (Farber, 1995) in high CO_2 environments. Skrede and Storebakken (1986) found that canthaxanthin-pigmented farmed salmon was more yellowish in hue than in astaxanthin-pigmented wild salmon. Canthaxanthin-enriched diets may explain why yellowing was observed in salmon portions stored under modified atmosphere. Sensory acceptability of MAP fish is perhaps the most important factor. The reduction of bound water, which leads to excessive exude or drip, is accompanied by a coarsening of the texture which is described by taste panellists as " slight increases in toughness and dryness" (Tiffney and Mills, 1982), "grainy" (Wang and Brown, 1983) and "powdery" (Haard and Lee, 1982). Tiffney and Mills (1982) reported the flavour of cod under high levels of CO_2 as a "cold store flavour" similar to that which develops in frozen fish. Haard and Lee (1982) referred to a "carbonated" taste of salmon stored using MAP and this may have been due to the higher solubility of CO_2 in the lipids in this high fat species. A final important factor for MAP fish products is odour. Acid-sour odour is the one organoleptic change cited most often in the literature and is attributed to lactic acid bacteria (Stammen *et al.*, 1990). On opening the packs, the characteristic fishy smell can be detected due to the release of compounds such as TVBN and TMA etc. This odour rapidly dissipates on opening the packs. No reports of MAP affecting the odour of the cooked flesh were found. However, these effects would most likely depend on storage conditions, microbial status prior to packing and

condition of the product prior to packing. Many reporters have reported considerable shelf-life extensions using MAP. Claims of shelf-lives of up to 20 days are not very realistic however, in many studies, fish are taken directly from nets by scientists and treated almost aseptically during the trials to produce such long shelf-life. Modern fishing practices have come a long way in recent years and implementation of Hazard Analysis and Critical Control Points (HACCP) on-board trawlers produces fish of relatively high quality. Shelf-life studies must, however, take into account modern on-board practices and fish must be typical of fish sold under normal conditions. It was for these reasons that fish samples used in the current trials were sourced from a fish processor, who obtained samples directly from trawlers.

2.8 Objectives of this project

The objectives of this project were to determine the affects of freeze-chilling (sections 1 to 3) and freeze-chilling in combination with modified atmosphere packaging (sections 4 to 6) on selected quality parameters of whiting, mackerel and salmon portions. The ultimate aim of this project was to extend the shelf-life of individual fish portions using a combination of freeze-chilling and MAP.

Chapter 3

3. MATERIALS AND METHODS

3.1 Introduction

Whiting, mackerel and salmon were used as they are readily available at Howth pier, and it was thought that no problems would be experienced with obtaining very fresh high quality fish on a regular basis. The whiting and mackerel supplied were landed at Donegal and Dunmore East ports (i.e. fish were caught in the Atlantic Ocean) and were shipped by road to Howth for further processing. This journey took approximately four hours and the trucks used for transporting the fish had a controlled temperature of +2 to 4°C. Whiting and mackerel were purchased as skin-on butterfly fillets. Fish spent between one and three days on ice before reaching the port. The salmon used in this experiment came from a fish farm in Donegal and samples were purchased as skinless and boneless fillets.

The project was divided into six trials. Trials 1 to 3 involved whiting, mackerel and salmon with four process treatments i.e. fresh, chill, frozen and freeze-chill. For the fresh process treatment, samples were tested on day of purchase i.e. no previous storage. For chill, samples were placed in a chill-room for three days (whiting and mackerel) or five days (salmon) at 2 - 4°C and then tested. For frozen, samples were blast-frozen at –35°C for 2.5 hours, placed in a freezer at –30°C for 3 days, then placed in a chill-room (2 – 4°C) for fifteen hours before

testing. For freeze-chill, samples were blast-frozen at –35°C for 2.5 hours, placed in a freezer at –30°C for three days (whiting and mackerel) or five days (salmon) followed by three days (whiting and mackerel) or five days (salmon) in a chill-room at 2 to 4°C before testing. Trials four to six involved freeze-chilling with modified atmosphere packaging (MAP) for all three species. Whiting, mackerel and salmon were packed in three gas atmospheres; 1. air, 2. 30%N_2 / 40% CO_2 / 30% O_2 (whiting only) or 40% CO_2 / 60% N_2 (mackerel and salmon) and 3. 100% CO_2 (all three species). The first trial (whiting with four process treatments) began in January 2001 and finished in March 2001; Trial 2. mackerel with the four process treatments (April 2001 - June 2001); Trial 3. Salmon with the four process treatments (July 2001 - September 2001); Trial 4. Freeze-chilled whiting with three gas atmospheres (October 2002 – December 2002); Trial 5. Freeze-chilled mackerel with three gas atmospheres (January 2002 - March 2002); Trial 6. Freeze-chilled salmon with three gas atmospheres (April 2002 – June 2002).

3.2 Sample procurement and preparation

All fish samples were purchased from a fish retailer at Howth (Dublin). This retailer was asked to supply fish of the best possible quality. Approximetely 180 fillets whiting and mackerel and 30 salmon fillets were delivered each time. Upon delivery to The National Food Centre, fish portions were weighed, placed into a 110 x 150 x 55mm plastic tray (Dynopak limited) and the trays were sealed using a MECAPAC 500 modified atmosphere packaging (MAP) machine (Mecaplastic, Bagnolet, France) (Figure 3). The trays were covered in a Dyno AF320 Antifog

film (Dynopak Limited) and four small holes were punched in each pack prior to chilling to maintain atmospheric conditions (in work packages 1 to 3 only). Once the fillets were packed, they were put through the various process treatments as described in section 3.1 above.

Figure 3. Modified atmosphere packaging of whiting fillets in plastic trays using the Mecapac 500 MAP packaging machine

After each process treatment was applied, chemical, physical and sensory tests as detailed in section 3.3 were carried out. For the MAP section, the fillets were packed in each gas atmosphere, freeze-chilled and then the tests were carried out.

3.3 Analysis of samples

3.3.1 Trials 1 to 3 and Trials 4 to 6 experimental design

Trials 1 to 3 consisted of four process treatments (fresh, chill, frozen and freeze-chill) x five replicates x one test date with 19 degrees of freedom. Trials 4 to 6 consisted of three process treatments (air, 60% N_2 / 40% CO_2 (mackerel and salmon) or 30% N_2 / 40% CO_2 / 30% O_2 (whiting only) x four replicates x three test dates (day 0, 3 and 5 for whiting and mackerel fillets or day 0, 5 and 7 for salmon portions) with 35 degrees of freedom.

3.3.2 Odour evaluation

The odour status of the prepacked fish from the chilled, frozen and freeze-chilled treatments was assessed by a sniff test using a panel of three trained assessors. All three assessors were given different but identically treated packs to sniff. The packs (one fillet or portion per pack) were opened and sniffed immediately (time 0) and again after 10 min at room temperature. The data were tested by one way analysis of variance for Trials 1 to 3 over the three process treatments (not measured for the fresh sample), testing at both time 0 and after 10 minutes, with five replicates of each and 29 degrees of freedom. For Trials 4 to 6 the data were tested by two way ANOVA over three process treatments, testing at both time 0 and after 10 minutes, and two test days (3 and 5 for whiting and mackerel or 5 and 7 salmon) with four replicates of each with 47 degrees of freedom. Three packs were assessed per process treatment per replicate. The results of the triplicate

packs were averaged before ANOVA was carried out. The samples were scored on a scale from 1 to 6 (1. fresh seaweed-like smell; 2. odourless; 3. slight fishy odour; 4. significant fishy odour; 5. strong fishy odour; 6. totally-off, i.e. putrid smell).

3.3.3 Acceptability scores

An untrained sensory panel of 25 tasters was used for each of the five replicates. Panellists were asked to score the acceptability of a sample of fish (circa 50g) [steamed for 6 min (whiting), grilled for 6 min (mackerel) or steamed for 30 min (salmon)] on a 6 cm line from 0 (unacceptable) to 6 (very acceptable). The marked point on the line was measured with a ruler and the data were analysed collectively as 4 process treatments x 25 tasters x 5 replicates (Trials 1 to 3) and as 4 process treatments by 25 tasters x 4 replicates (Trials 4 to 6). Only one (single stimulus taste panel; Gormley, 1989) was tasted each time as the samples from the four treatments were due for testing of different days.

3.3.4 Colour

The colour of the fish samples from the four process treatments was measured using a HunterLab model D25A colour difference meter (Hunter Associates Laboratory Inc., Virginia, USA) fitted with a 2.5cm diameter aperture. Colour was expressed in Hunter Lab units and as L/b (white to yellow ratio). The colour difference between the fresh sample and each of the other three treatments was calculated as $\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$. For whiting and mackerel, colour

was measured at three points on each half of the butterfly fillets (i.e. 6 colour measurements averaged per fillet) for five fillets per process treatment per replicate. For salmon, colour was measured at the centre (i.e. the thickest part) of each of 10 portions per process treatment per replicate. The data were examined by one way ANOVA as the average of 5 colour measurements x 4 process treatments x 5 replicates with 19 degrees freedom (Trials 1 to 3) and by two way ANOVA as the average of 5 colour measurements x 3 process treatments x 3 test days x 4 replicates with 35 degrees of freedom (Trials 4 to 6).

3.3.5 Texture measurements

For springiness measurements, raw fillets (whiting and mackerel) or salmon portions were placed on the platform of a TAXT2*i* Texture Analyser (Figure 4) (Stable Micro Systems, Surrey, England) and a P/20P perspex probe (20mm diameter) was selected to simulate finger feel. Constant penetration was applied to a depth of 3mm (Sigurgisladottir *et al.*, 1999) using a probe speed of 1mm/sec at the thickest part of each half of each butterfly fillet and the samples were recompressed after 5 seconds. Springiness was calculated as the re-compression force divided by the compression force x 100. For whiting and mackerel, springiness was measured at the thickest points on each half of the butterfly fillets (i.e. 2 colour measurements averaged per fillet) for five fillets per process treatment per replicate. For salmon, texture was measured at the centre (i.e. the thickest part) of each of 10 portions and the results averaged per process treatment per replicate. The data were examined by one way ANOVA as the average of 10 texture measurements x 4 process treatments x 5 replicates with 24 degrees

freedom (Trials 1 to 3) and by two way ANOVA as the average of 10 texture measurements x 3 process treatments x 3 test days x 4 replicates with 35 degrees of freedom (Trials 4 to 6). Shear values for the raw fillets were assessed using a T-2000 Texture System (Figure 5) (model TP5, Food Technology Corporation, Virginia, USA) fitted with a standard Kramer shear test cell (ram speed 4mm per sec.). Shears were conducted in triplicate (100g samples) per process treatment per replicate.

Figure 4. The TAXT2*i* Texture Analyser used for measuring springiness in fish portions

Figure 5. The T-2000 Texture System with Kramer shear cell attached

3.3.6 Centrifugal drip (%)

The method of Wierbiki and Deatherage (1958) was used for centrifugal drip (CD). Samples (3-5g) of thawed fish were accurately weighed into cup-shaped filter-paper thimbles and were placed over 2 cm of glass beads in centrifuge tubes to allow liquid to drain from the thimbles. The samples were centrifuged at 500 x g for 10 min at 10°C and reweighed. Centrifugal drip was recorded as the weight loss expressed as a percentage of the original weight (w/w). Centrifugal drip was measured in duplicate for each of three fillets per process treatment per replicate and the results were averaged for each process treatment. The data were examined by one way ANOVA as the average of 6 CD values x 4 process treatments x 5

replicates with 19 degrees freedom (Trials 1 to 3) and by two way ANOVA as the average of 6 CD values x 3 process treatments x 3 test days x 4 replicates with 35 degrees of freedom (Trials 4 to 6).

3.3.7 Gravity drip (%)

The amount of gravity drip (GD) in the trays was determined by weighing the fillets pre and post the process treatments. The difference in weight was determined and the percentage gravity drip loss was calculated (w/w). Gravity drip was measured for each of five fillets per process treatments and the results averaged. The data were examined by one way ANOVA as the average of GD values x 4 process treatments x 5 replicates with 19 degrees freedom (Trials 1 to 3) and by two way ANOVA as the average of 5 GD values x 3 process treatments x 3 test days x 4 replicates with 35 degrees of freedom (Trials 4 to 6).

3.3.8 Free fatty acid (FFA)

Free fatty acids and PVs were measured for mackerel and salmon. To extract the fat, equal volumes of petroleum spirit and diethyl ether (500 cm^3 total) were mixed with 300g of fish (taken from 3 fillets) in a blender (Masterchef 500, Moulinex, France) for 5 min. and the solvent plus fat were decanted into a beaker. The 300g of fish were taken from a mixed sample of upper, middle and lower parts of the fillet to obtain a representative sample. After evaporation of the solvents on a water bath, the remaining oil was dried in an oven at 100° C for 1h, allowed to cool, weighed and tested for FFAs and PVs. The AOAC method 940.28 (1990a) was used for FFA in which 7.05g of well mixed oil was weighed

into a 250 cm^3 flask and 50 cm^3 of absolute alcohol. previously neutralised by adding 2 cm^3 of 1% phenolphthalein solution and enough 0.1M NaOH to produce a faint permanent pink colour, was added. This pink colour of the phenolphthalein disappeared due to the acidity in the oil. This was titrated with 0.25 M NaOH, with vigorous shaking until a permanent pink colour appeared and persisted. This gave a value for percentage free fatty acids expressed as oleic acid (i.e. the cm^3 0.25 M NaOH used in titration corresponds to the percentage oleic acid). FFA was calculated in duplicate for each process treatment per replicate. The data were examined by one way ANOVA as the average of 2 FFA values x 4 process treatments x 5 replicates with 19 degrees freedom (Trials 1 to 3) and by two way ANOVA as the average of 2 FFA values x 3 process treatments x 3 test days x 4 replicates with 35 degrees of freedom (Trials 4 to 6).

3.3.9 *Peroxide value (PV)*

For PV the AOAC method 965.33 (1990b) was used in which 5.00 g (+/- 0.05g) of sample (extracted fat) was weighed into a 250 cm^3 erlenmeyer flask. 30 cm^3 of an acetic acid:chloroform mixture (1:3 v/v) was added and the solution was swirled to dissolve the fish oil. 0.5 cm^3 of saturated KI solution was added with occasional shaking over a five minute period then 30 cm^3 of water was added. The iodine released was titrated with 0.01 M Sodium thiosulphate with vigorous shaking until the yellow colour was almost gone. 0.5 cm^3 of 1% starch solution was then added and the solution was shaken vigorously to ensure the release of all the iodine from the chloroform layer, and the titration was continued until the blue colour just disappeared. The peroxide value (milliequivalents of peroxide oxygen

/kg sample) = S x N x 1000/g, where S = cm^3 $Na_2S_2O_3$ and N = normality (= molarity) $Na_2S_2O_3$ solution. PV was calculated in duplicate per process treatment per replicate. The data were examined by one way ANOVA as the average of PV values x 4 process treatments x 5 replicates with 19 degrees freedom (Trials 1 to 3) and by two way ANOVA as the average of 2 PV values x 3 process treatments x 3 test days x 4 replicates with 35 degrees of freedom (Trials 4 to 6).

3.3.10 Total viable count (TVC)

Approximately 2.5 cm^2 of flesh was cut from the tail-end of five fillets of whiting and mackerel, and from three portions of salmon. A representative 10g sample was stomached with 90 cm^3 diluent (1/4 strength Ringers solution, Oxoid, code BR52) and tested by the pour plate method (standard plate count agar, Oxoid, code CM463) to determine Total Viable Counts (TVCs). Incubation was at 30°C for 72h. Microbial loads were expressed as $\log_{10}$ cfu/g. Two fillets per process treatment per replicate were measured. The data were examined by one way ANOVA as the average of 2 TVC values x 4 process treatments x 5 replicates with 19 degrees freedom (Trials 1 to 3) and by two way ANOVA as the average of 2 TVCs values x 3 process treatments x 3 test days x 4 replicates with 35 degrees of freedom (Trials 4 to 6).

3.3.11 Total volatile base nitrogen (TVBN) and trimethylamine (TMA)

Total volatile base nitrogen was measured using the method of Malle and Tao (1986). Samples of fish muscle (100g) were homogenised in aqueous trichloroacetic acid solution (200cm^3, 7.5%) and the homogenate was centrifuged

at 400 x g for 5 min. The supernatant liquid was filtered (Whatman No. 3 filter paper) and the filtrate (25 cm^3) was loaded into a Kjeldahl-type distillation tube followed by 5 cm^3 of 10% (w/v) NaOH. Steam distillation was continued into an aqueous boric acid solution (4%) containing mixed methyl red-bromocresol green indicator until a final volume of 50 cm^3 was obtained in the beaker (40 cm^3 of distillate). The distillate was titrated against aqueous 0.05M sulphuric acid solution and the quantity of TVBN was determined by the formula TVBN = n x 16.8 mg of N/100g, where n was the amount (cm^3) of sulphuric acid required.

The same experimental procedure and reagents were used for the TMA analysis (Malle and Tao, 1986) but formaldehyde (20 cm^3; 38%) was also added to the distillation tube. Increasing volumes of formaldehyde were added to 24 cm^3 of filtrate in order to determine how much formaldehyde was required to block the primary and secondary amines (leaving only tertiary amines to react). Steam distillation was then performed as for the TVBN assay. The amount of volatile amines was measured and the results were expressed in mg nitrogen/100g. When the required amount of formaldehyde was added, only the TMA was distilled. The TMA content was calculated from the volume of 0.05M sulphuric acid used for titration by the formula TMA = n' x 16.8 mg N, where n' was the amount (cm^3) of sulphuric acid required. For the three species, TVBN and TMA were measured in triplicate per process treatment per replicate and the results averaged. Three separate fillets were used each time for whiting and mackerel, and three portions for salmon (taken from approximately the same location on the fillet). The data were examined by one way ANOVA as the average of 3 TVBN/TMA values x 4

process treatments x 5 replicates with 19 degrees freedom (Trials 1 to 3) and by two way ANOVA as the average of 3 TVBN/TMA values x 3 process treatments x 3 test days x 4 replicates with 35 degrees of freedom (Trials 4 to 6).

3.3.12 Moisture content (%)

Samples of fish (3-5g) from each process treatment were dried at 70°C and 74.7 mPa for ~ 15 h in a vacuum oven to constant weight. The moisture content was determined by weight difference and was expressed as a percentage (w/w) of the original thawed mass. Measurements were made in triplicate for each treatment per replicate and the results were averaged. The data were examined by one way ANOVA as the average of 3 moisture values x 4 process treatments x 5 replicates with 19 degrees freedom (Trials 1 to 3) and by two way ANOVA as the average of 3 moisture values x 3 process treatments x 3 test days x 4 replicates with 35 degrees of freedom (Trials 4 to 6).

3.3.13 Temperature logging

Temperature in the chill phase was constantly measured using a Grant 1000 series squirrel (Grant software limited, Cambridgeshire, England). Holes were cut in the base of a plastic tray and the temperature probe was inserted into the flesh of a fillet / darn to monitor temperature over the chilled shelf-life of the fillet. Temperature profiles were used to ensure that fillets were kept within the required temperature range of + 2 to 4 °C over the chill phase.

3.3.14 Statistical analysis of data

All results were analysed by analysis of variance (ANOVA) using SAS (Version 6.12, SAS Institute Inc., Cary, NC, USA).

Chapter 4

4. RESULTS

4.1 Results of Trials 1 to 3; Freeze-chilling of whiting, mackerel and salmon portions

4.1.1 Odour and acceptability scores

The samples which were frozen received better odour scores (lower values) than the chilled or freeze-chilled fish for both whiting ($P< 0.05$) and salmon ($P< 0.001$) (Table 4.1.1). The process treatments had no effect on the odour scores for mackerel. There was no statistical difference between the odour scores for the chilled or freeze-chilled samples of both whiting and mackerel, both of which were in chilled storage at 4°C for three days. However, chilled salmon received a much less favourable ($P<0.001$) odour score than freeze-chilled; the salmon samples were in chill storage at 4°C for 5 days. Time of sniffing (immediately on pack opening vs 10 min after pack opening) had no influence on the odour scores of any of the three species and there were no statistically significant interactions between process treatment and time of sniffing.

Table 4.1.1 Odour (sniff) scores[a,b] for chilled, frozen and freeze-chilled portions of raw whiting, mackerel and farmed salmon

Species	Chilled	Frozen	Freeze-chilled	LSD[c]	*F*-test
Whiting	3.15	2.67	3.13	0.36	P< 0.05
Mackerel	2.86	3.00	2.83	0.28	NS[d]
Salmon	5.61	1.94	3.60	0.62	P< 0.001

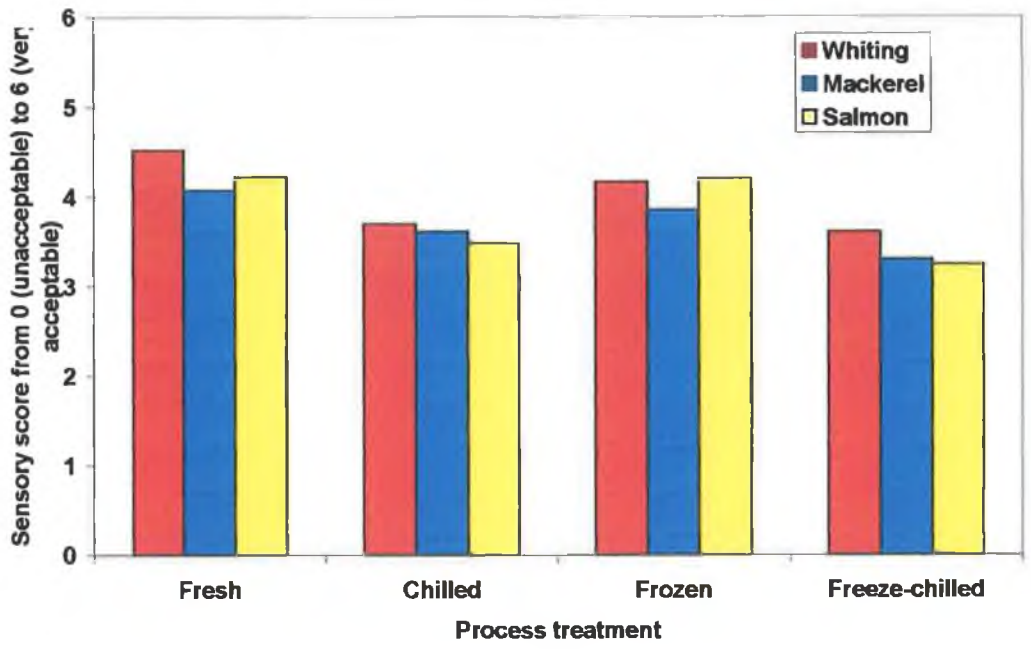
[a] On a 6-point scale from 1 (fresh seaweed-like smell) to 6 (totally off, i.e. putrid smell)
[b] Data analysed over two times of testing (t_o and t_{10})
[c] Least significant difference
[d] Not significant

Fresh and frozen fillets of both mackerel and salmon did not vary significantly in taste panel acceptability scores. The frozen whiting was poorer than its fresh equivalent. In all cases the chilled samples were poorer than their fresh or frozen equivalents. The freeze-chilled was of even lower acceptability (Table 4.1.2; P< 0.001) than for mackerel or salmon. The difference between the chilled and freeze-chilled whiting, however, was again not significant. The pattern is more clearly visualised in Figure 6.

Table 4.1.2 Taste panel acceptability scores[a,b,c] for fresh, chilled, frozen and freeze-chilled portions of whiting (steamed), mackerel (grilled) and salmon (steamed)

Species	Fresh	Chilled	Frozen	Freeze-chilled	LSD[d]	*F*-test
Whiting	4.52	3.70	4.16	3.60	0.32	P< 0.001
Mackerel	4.07	3.61	3.85	3.30	0.35	P< 0.001
Salmon	4.22	3.48	4.20	3.24	0.40	P< 0.001

[a] On a 6 cm line with endpoints of 0 (unacceptable) and 6 (very acceptable)
[b] Single sample hedonic panels; 25 tasters
[c] Collective data for the 5 replicates
[d] Least significant difference



Steamed whiting P< 0.001: LSD 0.32
Grilled mackerel P< 0.001; LSD 0.35
Steamed salmon P< 0.001; LSD 0.40

Figure 6. Sensory acceptability scores for cooked whiting, mackerel and salmon portions subjected to four process treatments

4.1.2 Colour

The process treatments did not influence the whiteness or redness values for whiting (mean L = 51.2; a = +1.33) or mackerel (mean L = 40.6; a = +4.5) fillets

(Table 4.1.3, 4.1.4). However, chilled salmon portions had a higher ($P< 0.05$) lightness value (L value) than the other samples (Table 4.1.5). Salmon from the fresh and frozen treatments was also more red (a value) ($P<0.001$) than the chilled or freeze-chilled samples (Table 4.1.5). Whiting from the freeze-chilled treatment was more yellow (Table 4.1.3; $P< 0.01$) than the other samples but the effect was small in practical terms. In mackerel, the effects were more pronounced with the fresh sample showing the least yellowing (Table 4.1.4; $P< 0.001$). This result was also reflected in the L/b ratios, with the fresh mackerel sample having the highest ($P< 0.001$) ratio. In salmon, the fresh sample was more yellow ($P< 0.001$) and had a lower L/b ratio ($P< 0.001$) than portions from the other three treatments (Table 4.1.5; $P< 0.001$). Total colour difference values (i.e. from the fresh sample) (ΔE) were only different for the mackerel samples where there was a progressive increase in ΔE values from chilled to frozen to freeze-chilled (i.e. 3.44 vs. 4.98 vs. 5.36) respectively (Table 4.1.4).

Table 4.1.3 Effect of process treatments on selected quality parameters[a] of raw whiting fillets

Quality parameter	Fresh	Chill	Freeze	Freeze-chill	LSD[b]	F-test
Kramer Shear (N/100g)	1781	1725	1730	1748	119.6	NS[c]
Springiness (%)	79.6	85.6	84.0	85.4	6.90	*
TVBN (mg N/100g)	13.7	25.0	17.5	25.5	6.36	**
TMA (mg N/100g)	2.40	12.0	6.90	12.1	4.08	***
Centrifugal Drip (%)	7.30	9.20	13.6	13.2	6.20	NS[c]
Gravity drip (%)	-	1.02	9.01	6.03	1.01	***
Moisture (%)	82.1	82.2	82.0	82.5	1.60	NS[c]
Hunter L	50.8	52.3	50.3	51.3	2.80	NS[c]
Hunter a	-2.06	-0.97	-1.12	-1.15	1.00	NS[c]
Hunter b	-0.79	0.23	0.90	2.53	1.39	**
Hunter L/b	Meaningless[d]					
TVC (Log_{10} cfu/g)	4.14	5.54	4.04	5.24	0.50	***
ΔE	-	2.63	3.05	3.89	1.76	NS[c]

[a] Measured over 5 replicates
[b] Least significant difference
[c] No significant difference
[d]. Hunter L/b values were negative which is meaningless

Table 4.1.4 Effect of process treatments on selected quality parameters[a] of raw mackerel fillets

Quality parameter	Fresh	Chill	Freeze	Freeze-chill	LSD[a]	F-test
Shear (N/100g)	1811	1867	1848	1851	59.0	NS[a]
Springiness (%)	92.0	92.0	94.0	88.0	5.40	NS[a]
TVBN (mg N/100g)	15.9	23.1	17.9	21.1	3.70	*
TMA (mg N/100g)	3.90	8.70	3.50	5.30	2.98	*
Centrifugal Drip (%)	5.10	5.70	10.1	9.60	3.18	*
Moisture (%)	70.4	70.7	67.5	69.7	4.60	NS[a]
Hunter L	40.7	40.6	39.9	41.1	3.17	NS[a]
Hunter a	4.40	4.20	4.60	4.70	2.31	NS[a]
Hunter b	4.40	6.90	7.90	7.90	1.43	***
Hunter L/b	9.40	5.90	5.00	5.70	1.45	***
TVC (Log_{10} cfu/g)	4.52	5.34	4.26	5.14	0.56	**
Gravity drip (%)	-	2.00	10.0	4.00	4.00	*
ΔE	-	3.44	4.98	5.36	1.15	*
Peroxide (meq. peroxide/kg sample)	1.66	2.94	0.90	2.28	1.71	NS[a]
FFA (% oleic acid)	0.58	0.51	0.51	2.02	0.39	***

[a] see footnotes Table 4.1.3

Table 4.1.5 Effect of process treatments on selected quality parameters[a] of raw salmon darns

Quality parameter	Fresh	Chill	Freeze	Freeze-chill	LSD[a]	F-test
Shear (N/100g)	1724	1732	1708	1722	24.3	NS[a]
Springiness (%)	96.0	94.0	95.0	94.0	19.0	NS[a]
TVBN (mg N/100g)	17.0	22.7	14.8	20.2	2.3	***
TMA (mg N/100g)	2.06	6.04	1.74	4.06	0.91	***
Centrifugal Drip (%)	2.80	1.02	5.08	1.06	0.79	***
Moisture (%)	68.3	66.8	67.9	66.7	8.20	***
Hunter L	40.3	42.7	40.8	39.8	1.82	*
Hunter a	21.2	17.7	19.0	17.4	1.08	***
Hunter b	14.6	12.2	12.6	12.7	0.74	***
Hunter L/b	2.78	3.52	3.24	3.14	0.24	***
TVC (Log_{10} cfu/g)	5.08	7.36	4.78	7.56	0.20	***
Gravity drip (%)	-	1.70	3.30	3.22	0.35	***
ΔE	-	47.8	46.8	45.9	1.76	NS[a]
Peroxide (meq. peroxide/kg sample)	1.62	2.22	2.18	4.40	0.23	***
FFA (% oleic acid)	0.31	0.74	0.33	1.60	0.26	***

[a] See footnotes Table 4.1.3

4.1.3 Texture

The fresh whiting sample was less springy ($P<0.01$) than samples from the other three treatments (Table 4.1.3). The process treatments had no influence on the springiness values of mackerel (mean 91.5%) (Table 4.1.4) or salmon (Table 4.1.5) (mean 95.0%), or on the shear values of the raw flesh of each species. Mean shear values were 1.75, 1.84 and 1.72 kN/100g for whiting, mackerel and salmon respectively (Table 4.1.3 – 4.1.5).

4.1.4 Drip loss

The pattern in the gravity drip data was the same for each species in that the chilled samples had least, the frozen samples most, while the freeze-chilled packs were intermediate (Table 4.1.3, 4.1.4 and 4.1.5). However, the magnitude of the effects varied from species to species: e.g. the gravity drip in the chilled and freeze-chilled whiting and salmon ($P< 0.001$) samples differed significantly whereas this was not the case with the mackerel samples. Drip loss (gravity drip) appeared to be a very successful method of estimating tissue damage due to freezing in all three fish species studied (Table 4.1.3 – 4.1.5).

Frozen and freeze-chilled mackerel samples had a much higher centrifugal drip ($P< 0.05$) than the fresh or chilled samples (Table 4.1.4). For salmon, the freeze-chilled portions had less ($P< 0.001$) centrifugal drip than the frozen and there was no difference between centrifugal drip of the chilled and freeze-chilled samples (Table 4.1.5). The process treatments had no effect on the centrifugal drip values

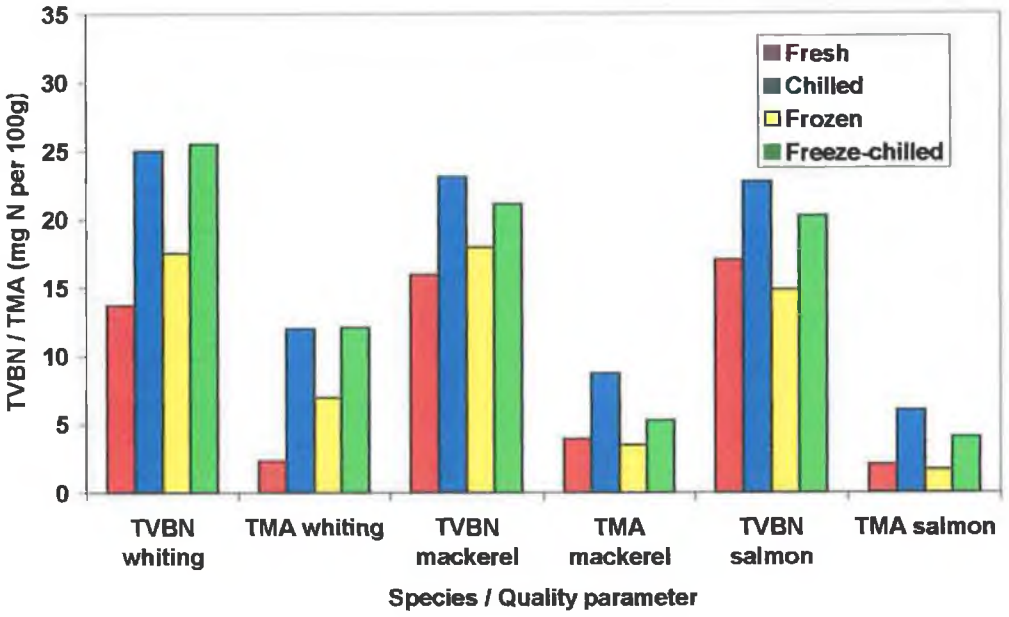
of whiting (mean 7.6%, Table 4.1.3). Centrifugal drip did not appear entirely reliable in estimating tissue damage due to freezing.

4.1.5 Free fatty acids (FFAs) and peroxide values (PVs)

Freeze-chilled mackerel and salmon samples had higher ($P<0.001$) FFA values than samples from the other treatments (Tables 4.1.4 and 4.1.5). Peroxide values were also highest ($P< 0.001$) in the freeze-chilled salmon samples but were not influenced by any of the process treatments in the case of mackerel (mean 1.95 meq. peroxide/kg sample) (Table 4.1.4).

4.1.6 Total volatile base nitrogen (TVBN) and trimethylamine (TMA)

The patterns for the TVBN and TMA data were the same for the three species in that the chilled and freeze-chilled samples had the highest values ($P< 0.05$ to $P< 0.001$) (Tables 4.1.3, 4.1.4, 4.1.5) and the fresh and frozen the lowest values. There was no difference between the TVBN values of the chilled and freeze-chilled samples for any of the species but the chilled mackerel ($P< 0.05$) and salmon ($P< 0.001$) had higher TMA values than the freeze-chilled samples. The TVBN values overall were similar for the three species, whereas TMA values for whiting were generally higher than those for the other species (Figure.7). All values were well below the recommended EU maximum levels (35 mg N per 100g TVBN).



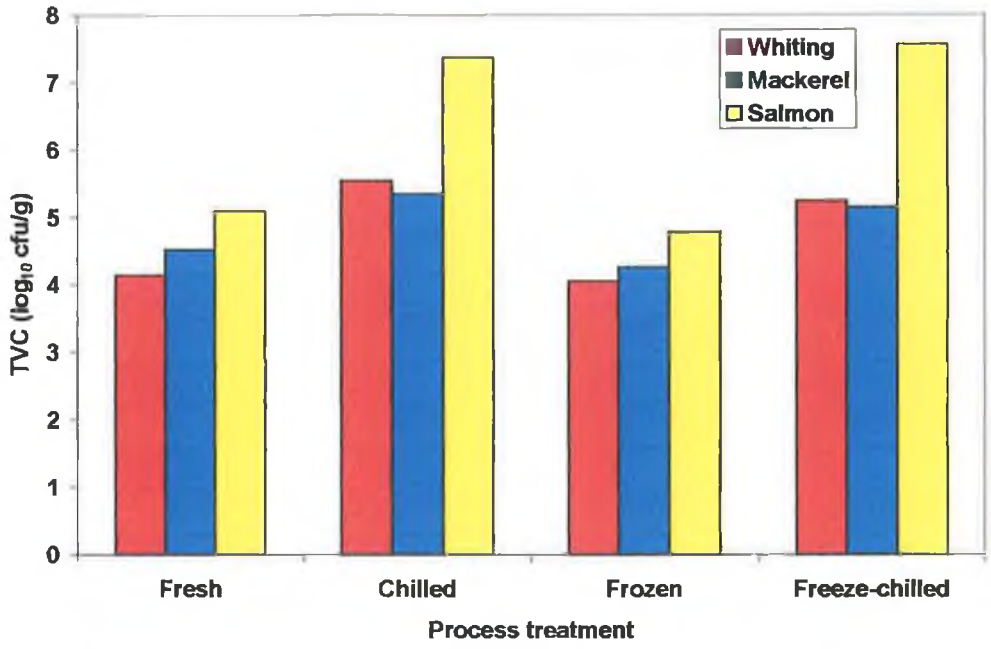
Whiting TVBN P< 0.01; LSD 6.36 mg N /100g; TMA P< 0.001; LSD 4.08 mg N /100g
Mackerel TVBN P< 0.05; LSD 3.70 mg N /100g; TMA P< 0.05; LSD 2.98 mg N /100g
Salmon TVBN P< 0.001; LSD 2.33 mg N /100g; TMA P< 0.001; LSD 0.91 mg N /100g

Figure 7. Total volatile base nitrogen and trimethylamine levels in raw whiting, mackerel and salmon portions subjected to four process treatments.

4.1.7 Total viable count (TVC)

The results of the total viable counts were inversely related to the TVBN results i.e. the chilled and freeze-chilled samples of the three species had higher (P< 0.01 to P< 0.001) counts than the fresh or frozen samples (Tables 4.1.3, 4.1.4, 4.1.5). However, there was no difference between the counts for the chilled and freeze-

chilled samples for each of the species. There was no statistical difference between the microbial loads in the fresh and frozen samples.



Whiting P< 0.001; LSD 0.50 Log_{10} cfu/g
Mackerel P< 0.01; LSD 0.65 Log_{10} cfu/g
Salmon P< 0.001; LSD 0.20 Log_{10} cfu/g

Figure 8. Total viable counts for whiting, mackerel and salmon portions subjected to four process treatments

4.1.8 Moisture

The process treatments had no statistically significant effect on the moisture content of the three species and the mean values were 82.2, 69.6 and 67.4% for whiting, mackerel and salmon portions respectively (Table 4.1.3 – 4.1.5).

4.2 Discussion

Odour testing was utilised in the current study to determine if there was an off-odour at time of pack opening, and if so, whether it remained 10 minutes after pack opening. Such odours are off-putting for the consumer. The results showed no difference between the odour scores at times 0 and 10 minutes. With the exception of the chilled and freeze-chilled salmon samples, all odour scores were below the mid-point (i.e. 3.5) of the odour scale (1 = fresh seaweed-like smell; 6 = putrid) and could be classed as odourless or having a slight fishy odour, i.e. they were quite acceptable. The freeze-chilled samples received more favourable odour scores than the chilled samples but the data were not statistically significant except in the case of salmon. The freeze-chilled salmon sample also received a middle rating (3.60) but the chilled sample score of 5.61 indicated a strong off-odour, and that five days at 4°C was above the upper limit of its shelf-life (Table 4.1.1); these two samples also had the highest TVC values (Table 4.1.5) indicating the correlation between this organoleptic test and acceptability.

There were no statistically significant differences between the chilled and freeze-chilled samples. This suggests that freeze-chilling, in comparison with chilling, was not having a deleterious effect on portion acceptability. The freeze-chilled samples were the oldest and could be expected to have the lowest ratings, whereas the fresh fish should have the highest acceptability rating, as found. However, the acceptability scores for all the samples were above 3.0 on a scale from 0 (unacceptable) to 6 (very acceptable) indicating that all were quite acceptable

(Figure 6). The single stimulus taste panel procedure (Gormley, 1989) used in these tests proved satisfactory and 25 or more tasters were used each time.

The odour and acceptability data related well to the TVBN, TMA and TVC values for the different species from the four process treatments. The slight fishy odours encountered in the whiting, mackerel and salmon were most likely due, in part, to the production of TVBN and particularly TMA. There was no significant difference in the TVBN or TMA values between the chilled and freeze-chilled samples of the three species (Figure 7, Table 4.1.3 – 4.1.5). As expected, the fresh and frozen samples had lower TVBN/TMA values than the chilled or freeze-chilled samples, reflecting the fact that proteolysis and degradation of TMAO occurred at 4°C, catalysed by enzymatic activity from the fish tissue and / or contaminating spoilage organisms. In whiting, the fishy odour can be attributed to the production of TVBN and particularly TMA predominately by spoilage organisms belonging to the genus *Pseudomonas* (Shewan, 1971). The TVBN values were positively correlated with TVC [r = +0.62 (whiting); +0.77 (mackerel); +0.81 (salmon)] and negatively correlated with acceptability scores [r = -0.61 (whiting); -0.63 (mackerel); -0.65 (salmon)]. The TVC values were, in turn negatively correlated with acceptability scores [r = -0.65 (whiting); -0.65 (mackerel); -0.81 (salmon)]. These data show that fillet acceptability decreased as TVBN, TMA and TVC values increased. The role of TVBN/TMA as indicators of fish quality has been discussed by Whittle *et al.* (1990) and by Dalgaard (2000). The TVBN values in the current study were all below the EC guidelines (Council Regulation No. 95/149/EEC of March 1995) for raw fish (35mg N/100g flesh).

The TMA values for mackerel (Table 4.1.4) were much higher than those of 1mg/100g reported by Jhaveri *et al.* (1982) for mackerel stored at 0°C on ice for four days.

The TVC values for the three species from the four process treatments were acceptable for raw fish (Department of Health and Children, Ireland, 1992), with the exception of chilled and freeze-chilled salmon portions, which had values above log 7 cfu/g after five days in chilled storage; the fresh salmon samples had a TVC value of $\log_{10}$ 5.08 cfu/g which is indicative of unacceptable in-factory hygiene practices. Detailed microbiological tests (e.g. specific spoilage organisms) were not conducted in the current study and the TVC data should be interpreted as indicators of hygiene. It was anticipated that freeze-chilling could be conducive to microbial growth as freezing opens up product structure and results in more drip than chilling alone. However, the data showed this was not the case as the freeze-chilled fish samples had insignificantly different TVC values than the chilled with the exception of salmon where the freeze-chilled sample had a slightly higher (non significant) value (Figure 8, Table 4.1.3 – 4.1.5). The TVC values for the chilled and freeze-chilled mackerel samples at 5.34 and 5.14 cfu/g were higher than the value of below log 5 cfu/g reported by Jhaveri *et al.* (1982) for mackerel on ice for four days. Overall, the TVC values for the three species were generally slightly lower than those of Guldager *et al.* (1998) for modified atmosphere packed cod held on ice for 3-4 days. The TVCs in the study of Guldager *et al.* (1998), were measured by the method of Long and Hammer (1941), incubated at 15°C. Therefore, it is not surprising that Guldager *et*

al. (1998) showed higher TVCs compared to the present study as he would have included more psychrotrophs in his counts. However, it must also be noted that comparisons between species and different trials are difficult due to variations in raw material quality and to experimental conditions. The relatively high TVC values found for the raw salmon may be due to the scattering of fish slime during filleting; it is recommended therefore that the body of the salmon be cleaned up using high-pressure water prior to filleting.

Development of off-flavour is one of the major effects of lipid oxidation (Flick *et al.*, 1992). In the current trials, the freeze-chilled samples had the highest FFA and PV values. The levels did not appear to affect sensory responses however, as no taste panellists cited the presence of "rancid off odours" in their results. The FFAs were similar and the PVs lower than those reported by Gormley *et al.* (2002) for frozen salmon and smoked mackerel.

Raw fillet colour in-pack is of major importance as it is highly visible to the consumer both at time of purchase and during cooking in the home. Freeze-chilling promoted some yellowing in whiting fillets, while for mackerel and salmon portions, the chill, freeze and freeze-chill treatments gave an inferior colour compared to the fresh samples. In addition, the biggest colour difference (ΔE) from fresh was in the freeze-chilled sample. However, all of the colour effects were small in practical terms, and should have little effect on consumer acceptability.

Visible drip in the pack can also be off-putting for the consumer. The gravity drip in the current trials was significant for the freeze and freeze-chill treatments (Tables 4.1.3 – 4.1.5) but presented no major visual problems; any potential negative effects could be overcome by the use of drip pads. Gravity drip for the frozen samples was higher than for the freeze-chilled and a similar effect was found for centrifugal drip in mackerel. An explanation may be that the fillets reabsorbed some of the drip generated by the freezing step during the chilled storage period. However, this was not reflected in the moisture contents of the fish samples.

The freezing treatments (i.e. freeze or freeze-chill) did not influence the shear values of the raw fillets of any of the species and the only textural difference detected was the lower springiness value in fresh whiting fillets. The frozen storage time of three days in the current study was short, but some toughening could be anticipated if the time for the freezing component of the freeze-chill treatment was extended to several months (Gormley *et al.*, 1993; Howell, 1995).

The outcome from these trials is that freeze-chilling is a suitable technology for use with prepacked whiting, mackerel and salmon portions. Raw material freshness is of paramount importance and fillet freezing at sea or on-farm would be highly beneficial in delivering an extended shelf-life in the chilled phase of the freeze-chilled process. Combining freeze-chilling with modified atmosphere packaging is also an option. Extended times of frozen storage in the freeze-chill process should be tested to study potential deleterious changes in product quality

including increased drip, toughening, oxidation and changes in colour. In this context Refsgaard *et al.* (1998) found that only minor sensory changes occurred in salmon stored at –30°C for 34 weeks. Gormley *et al.* (2002) produced largely similar findings for salmon and smoked mackerel but showed that fluctuating temperatures below the freezing-point, gave increased rancidity over an 8 month period.

The use of good manufacturing practices (GMP) and hazard analysis of critical control points (HACCP) is imperative in the production, storage, distribution and retailing of freeze-chilled foods. National and EU guidelines should also be adhered to (Gormley and Butler, 2000). Particular attention should also be focused on the thawing step and careful temperature control should be exercised. In the case of freeze-chilled fish fillets, tempering can be achieved by transferring prepacks from the supermarket deep freeze room to the chilled retail display cabinets in the evening time, thus resulting in a tempered (thawed) product the following morning. The normal safety rules for frozen foods prevail in the frozen component of the process, and those for chilled foods in the chill phase. The labelling requirements are those of conventionally chilled foods. However, it is desirable for reasons of consumer information and product liability to label the product as "previously frozen", A use-by-date must also be employed and this label should be attached at the start of the thawing process. This is 3 days (whiting and mackerel) and 5 days (salmon) based on the findings of this study.

4.3 Conclusion

Fresh and frozen fillets of mackerel and salmon and fresh whiting fillets received the highest acceptability scores (cooked samples). There was no significant difference between chilled and freeze-chilled samples of each species. The total volatile base nitrogen (TVBN), trimethylamine (TMA) and total viable count (TVC) data showed the same pattern for the three species in that the chilled and freeze-chilled samples had the highest values and the fresh and frozen the lowest. However, again there was no statistically significant difference between the freeze-chilled and chilled samples. Freeze-chilled samples had the highest free fatty acid (FFA) and peroxide values (PV) but the levels were low and did not influence sensory responses. Gravity drip was significant in the frozen and freeze-chilled samples but presented no major visual problems and could readily be absorbed by drip pads. The effects of the four treatments on the colour and texture of the raw samples were small in practical terms.

4.4 Results of Trials 4 to 6; Freeze-chilling in combination with modified atmosphere packaging for whiting, mackerel and salmon portions.

The MAP packs maintained their integrity during the freeze-chill process using the 60% N_2 / 40% CO_2 (mackerel and salmon) or 30% N_2 / 40% CO_2 / 30% O_2 (whiting only) gas phases and continued to maintain their shape (no pack implosion) throughout storage. All the 100% CO_2 packs however imploded, with the sides of the packs concave in shape. In the preparation of the results below, the term MAP refers to packs with air or the various gas mixtures, while day 0, day 3, day 5 and day 7 refers to the period of time such MAP packs were held at 2 to 4°C.

4.4.1 *Odour and acceptability scores*

Time of sniffing (i.e. on pack opening versus 10 minutes after pack opening) had an effect on odour scores in whiting, with fillets scoring better ($P< 0.05$) 10 minutes after pack opening (Table 4.4.1.1). Variations in the composition of the modified atmosphere packaging (MAP) had no effect on odour scores for the three species whereas storage time (days) did (Table 4.4.1.1). The odour of whiting ($P< 0.001$) and mackerel ($P< 0.05$) deteriorated with time i.e. higher values (Table 4.4.1.1) on day 5 than on day 3. Likewise the odour of salmon ($P<0.01$) was poorer on day 7 than on day 5 (Table 4.4.1.1). Interaction ($P< 0.05$) between MAP treatment and storage time for salmon portions occurred as indicated on Table 4.4.1.1 showing that day 7 salmon portions scored worse at t_0 in air than portions in other gaseous atmospheres.

Table 4.4.1.1. Effect of time of sniff[a] and day of sniff[b] on odour scores[c] of raw whiting, mackerel and salmon portions with modified atmosphere packaging

		Day 3		Day 5		Day 7		
Species	Gas mixture	t_0	t_{10}	t_0	t_{10}	T_0	t_{10}	**Average (gas)**
Whiting	Air	2.65	2.30	3.33	2.75	-	-	**2.76**
	30/40/30[d]	2.65	2.45	3.30	2.98	-	-	**2.84**
	100% CO_2	2.85	2.50	3.20	2.93	-	-	**2.87**
Average (time)		**t_0: 3.0**		**t_{10}: 2.65**		-		
Average (days)		**Day 3: 2.57**		**Day 5: 3.08**		-		
Mackerel	Air	3.12	2.70	2.98	2.65	-	-	**2.86**
	60/40[e]	3.20	2.88	3.85	3.35	-	-	**3.32**
	100% CO_2	3.13	2.75	4.35	3.93	-	-	**3.54**
Average (time)		**t_0: 3.44**		**t_{10}: 3.04**		-		
Average (days)		**Day 3: 2.96**		**Day 5: 3.52**		-		
Salmon	Air	-	-	2.50	2.38	3.83	3.35	**3.01**
	60/40[e]	-	-	2.83	2.45	3.03	2.75	**2.76**
	100% CO_2	-	-	3.08	2.55	3.00	2.78	**2.85**
Average (time)		-		**t_0: 3.04**		**t_{10}: 2.71**		
Average (days)		-		**Day 5: 2.63**		**Day 7: 3.12**		

[a] All samples were sniffed on pack opening (t_0) and 10 minutes later (t_{10})
[b] All samples were sniffed on days 3 and 5 (whiting and mackerel) or day 5 and 7 (salmon).
[c] Samples scored by marking a 6cm line from 0 (Fresh seaweed like smell) to 6 (totally-off, i.e. putrid smell)
[d] 30% N_2 / 40% CO_2 / 30% O_2
[e] 60% N_2 / 40% CO_2

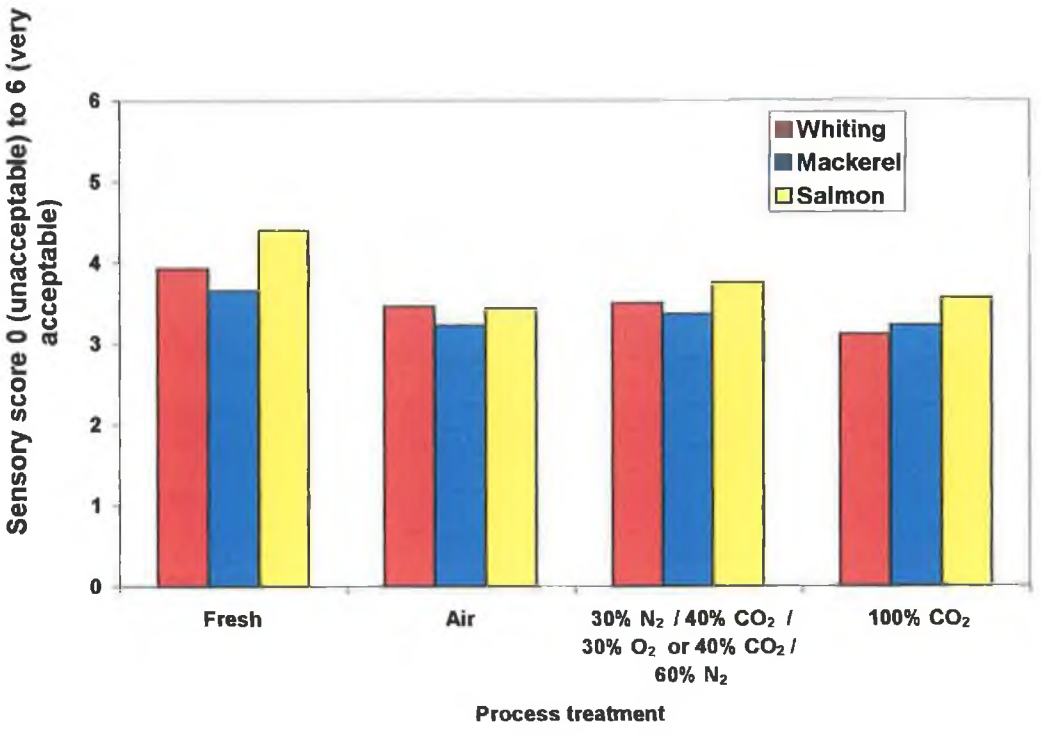
Whiting	Gas mixture	NS;	LSD 0.33
	Day	P< 0.001;	LSD 0.27
	Time	P< 0.05;	LSD 0.27
	Interactions	NS;	

p.t.o

Continued

Mackerel	Gas mixture	NS;	LSD 0.60
	Day	P< 0.05;	LSD 0.49
	Time	NS;	LSD 0.49
	Interactions	NS;	
Salmon	Gas mixture	NS;	LSD 0.42
	Day	P< 0.01;	LSD 0.34
	Time	NS;	LSD 0.34
	Interaction between gas mixture and day of sniff	P< 0.05;	LSD 0.59

Taste panel acceptability on a scale from 0 (unacceptable) to 6 (very acceptable) showed no significant difference between the gaseous atmospheres after 5 (whiting and mackerel) or 7d (salmon). However, all treatments scored lower than the fresh samples (day 0) (Figure 9) for each species (P<0.001; P< 0.05; P< 0.001 respectively) except in the case of mackerel fillets packed in 60% N_2 / 40% CO_2 which were not significantly different to the fresh fillets. (Table 4.4.1.2).



Steamed whiting P< 0.001; LSD 0.34
Grilled mackerel P< 0.05; LSD 0.32
Steamed salmon P< 0.001; LSD 0.61

Figure 9 Sensory acceptability scores for freeze-chilled whiting, mackerel and salmon portions with or without modified atmosphere packaging

Table 4.4.1.2. Taste panel acceptability scores[a] for cooked whiting[b], mackerel[b] and salmon[c] portions following freeze-chilling with or without modified atmosphere packaging

Species	Gaseous atmosphere					LSD	*F*-test
	Fresh[d]	Air	30% O_2 / 40% CO_2 / 30% N_2	60% N_2 / 40% CO_2	100% CO_2		
Whiting	3.93	3.46	3.50	-	3.18	P< 0.001	0.34
Mackerel	3.66	3.23	-	3.37	3.23	P< 0.05	0.32
Salmon	4.40	3.44	-	3.75	3.56	P< 0.001	0.61

[a] Data averaged over four replicates
[b] After chilled storage (4°C) for 5 days
[c] After chilled storage (4°C) for 7 days
[d] Data for the fresh (day 0) sample

4.4.2 *Colour (Hunter Lab values)*

Modified atmosphere packaging had no effect on Hunter L or a values for any of the three species [means (whiting 51.2, -0.73), (mackerel 43.0, 4.40) or (salmon 41.4, 17.9) (Table 4.4.2.1, 4.4.2.2)]. Hunter b values showed air-packed whiting (P< 0.05) and salmon portions (P< 0.01) to be less yellow than those in 100% CO_2 after five days (whiting) and seven days (salmon) (Table 4.4.2.3). There was an interaction (P< 0.05) between MAP and storage time for Hunter b values of whiting fillets (Table 4.4.2.3). Day 3 portions packed in air had lower b values than those packed in 30% N_2 / 40% CO_2 / 30% O_2. Storage time (days) had a deleterious effect on Hunter L (P< 0.05) and a (P< 0.05) values for whiting (Table 4.4.2.1, 4.4.2.2). Whiting (P< 0.01) and mackerel (P< 0.001) portions became more yellow as storage time increased. However, this effect was not observed in salmon portions (mean b value of 14.7) (Table 4.4.2.3).

Table 4.4.2.1 Effect of storage time (days) and gaseous atmosphere on Hunter L values for whiting, mackerel and salmon portions

Species	Day	Gaseous atmosphere				Average
		Air	30% O_2 / 40% CO_2 / 30% N_2	60% N_2 / 40% CO_2	100% CO_2	
Whiting	0	44.9	44.9	-	44.9	**44.9**
	3	51.8	52.7	-	52.5	**52.3**
	5	54.2	56.8	-	58.3	**56.4**
Average		**50.3**	**51.4**	-	**51.9**	
Mackerel	0	42.4	-	42.4	42.4	**42.4**
	3	43.7	-	44.3	43.6	**43.9**
	5	44.1	-	41.1	43.4	**42.9**
Average		**43.4**	-	**42.6**	**43.1**	
Salmon	0	41.1	-	41.1	41.1	**41.1**
	5	41.5	-	41.2	41.3	**41.3**
	7	41.2	-	42.2	42.0	**41.8**
Average		**41.3**	-	**41.5**	**41.5**	

Whiting	Storage time P< 0.05;	LSD 7.2
	Gaseous atmosphere NS;	LSD 1.5
	Interaction NS:	LSD 7.34
	Except when comparing means of the same level of days	LSD 2.6
Mackerel	Storage time NS;	LSD 1.91
	Gaseous atmosphere NS;	LSD 1.47
	Interaction NS;	LSD 2.62
	Except when comparing means of the same level of days	LSD 2.55
Salmon	Storage time NS;	LSD 2.31
	Gaseous atmosphere NS	LSD 0.82
	Interaction NS;	LSD 2.43
	Except when comparing means of the same level of days	LSD 1.41

Table 4.4.2.2 Effect of storage time (days) and gaseous atmosphere on Hunter a values for whiting, mackerel and salmon portions

Species	Day	Gaseous atmosphere				Average
		Air	30% O_2 / 40% CO_2 / 30% N_2	60% N_2 / 40% CO_2	100% CO_2	
Whiting	0	1.69	1.69	-	1.69	**1.69**
	3	-1.70	-2.55	-	-1.25	**-1.83**
	5	1.47	2.28	-	2.45	**-2.07**
Average		**-0.49**	**-1.04**	-	**-0.67**	
Mackerel	0	4.88	-	4.88	4.88	**4.88**
	3	4.88	-	3.98	3.93	**4.26**
	5	3.50	-	4.35	4.38	**4.08**
Average		**4.42**	-	**4.40**	**4.39**	
Salmon	0	18.8	-	18.8	18.8	**18.8**
	5	17.9	-	18.5	19.2	**18.5**
	7	17.2	-	16.6	15.8	**16.5**
Average		**18.0**	-	**18.0**	**17.9**	

Whiting	Storage time P< 0.05;	LSD 2.86
	Gaseous atmosphere NS;	LSD 0.53
	Interaction NS;	LSD 2.89
	Except when comparing means of the same level of days	LSD 0.92
Mackerel	Storage time NS;	LSD 1.02
	Gaseous atmosphere NS;	LSD 1.02
	Interaction NS;	LSD 1.65
	Except when comparing means of the same level of days	LSD 1.76
Salmon	Storage time NS;	LSD 3.74
	Gaseous atmosphere NS;	LSD 1.61
	Interaction NS;	LSD 4.05
	Except when comparing means of the same level of days	LSD 2.79

Table 4.4.2.3 Effect of storage time (days) and gaseous atmosphere on Hunter b values for whiting, mackerel and salmon portions

Species	Day	Gaseous atmosphere				Average
		Air	30% O_2 / 40% CO_2 / 30% N_2	60% N_2 / 40% CO_2	100% CO_2	
Whiting	0	0.39	0.39	-	0.39	**0.39**
	3	2.46	3.64	-	3.67	**3.26**
	5	4.93	4.69	-	5.12	**4.91**
Average		**2.59**	**2.91**	-	**3.07**	
Mackerel	0	4.42	-	4.42	4.42	**4.42**
	3	9.20	-	8.37	8.62	**8.73**
	5	9.77	-	8.12	9.32	**9.07**
Average		**7.80**	-	**6.97**	**7.46**	
Salmon	0	14.6	-	14.6	14.6	**14.6**
	5	13.7	-	14.0	15.9	**14.5**
	7	14.1	-	14.8	16.4	**15.1**
Average		**14.2**	-	**14.5**	**15.6**	

Whiting	Storage time P< 0.01;	LSD 2.19
	Gaseous atmosphere P< 0.05;	LSD 0.36
	Interaction P< 0.05;	LSD 2.20
	Except when comparing means of the same level of days	LSD 0.62
Mackerel	Storage time P< 0.001;	LSD 1.27
	Gaseous atmosphere NS;	LSD 0.71
	Interaction NS;	LSD 1.53
	Except when comparing means of the same level of days	LSD 1.22
Salmon	Storage time NS;	LSD 2.04
	Gaseous atmosphere P< 0.01;	LSD 0.74
	Interaction NS;	LSD 2.14
	Except when comparing means of the same level of days	LSD 1.29

4.4.3 *Texture*

Storage time (days) at 2 to 4°C had no effect on the springiness (%) of whiting (mean 84.7%), mackerel (mean 92.2%) or salmon (mean 94.3%) portions (Table 4.4.3.1). The various MAP treatments also had no effect on the springiness of whiting and salmon portions (Table 4.4.3.1). Mackerel fillets stored in 60% N_2 / 40% CO_2 were less springy ($P< 0.01$) than samples from the other treatments (Table 4.4.3.1). The magnitude of the effect was small in practical terms. There was an interaction ($P< 0.05$) between MAP and storage time for mackerel fillets. On day 3 fillets packed in 60% N_2 / 40% CO_2 were the least springy, however on day 5, fillets packed in air were the least springy (Table 4.4.3.1).

Both day 3 and day 5 whiting fillets had lower shear values ($P< 0.001$) and were significantly less tough than the fresh sample (day 0) (Table 4.4.3.2). Mackerel fillets had higher ($P< 0.05$) shear values on day 3 than on day 0 and day 5. MAP treatment had no effect on the shear values of whiting fillet (mean 1.56 kN) (Table 4.4.3.2). However, mackerel fillets stored in 100% CO_2 had higher ($P< 0.001$) shear values than samples from the other treatments. There was an interaction ($P< 0.05$) between MAP treatment and storage time (days) for mackerel fillets (Table 4.4.3.2) with day 3 fillets having higher shear values than day 0 in each atmosphere and day 5 fillets having lower shear values than day 3. MAP treatment and storage time had no effect on shear values (kN) of salmon portions and the mean value was 1.76 kN. The reasons for the variations in shear values were not investigated and it is likely to be quite complicated at a physiological/biochemical level.

Table 4.4.3.1 Effect of storage time (days) and gaseous atmosphere on Springiness values (%) for whiting, mackerel and salmon portions

Species	Day	Gaseous atmosphere				Average
		Air	30% O_2 / 40% CO_2 / 30% N_2	60% N_2 / 40% CO_2	100% CO_2	
Whiting	0	84.5	84.5	-	84.5	**84.5**
	3	84.3	83.0	-	84.5	**83.9**
	5	85.3	86.8	-	84.8	**85.6**
Average		**84.7**	**84.8**	-	**84.6**	
Mackerel	0	93.3	-	93.3	93.3	**93.3**
	3	92.8	-	90.0	92.3	**91.7**
	5	91.0	-	91.5	93.0	**91.8**
Average		**92.3**	-	**91.3**	**92.8**	
Salmon	0	93.8	-	93.8	93.8	**93.8**
	5	94.3	-	95.0	95.0	**94.8**
	7	94.5	-	94.5	95.0	**94.7**
Average		**94.2**	-	**94.4**	**95.0**	

Whiting	Storage time NS;	LSD 5.55
	Gaseous atmosphere NS;	LSD 1.83
	Interaction NS;	LSD 5.75
	Except when comparing means of the same level of days	LSD 3.16
Mackerel	Storage time (days):NS;	LSD 3.0
	Gaseous treatment P< 0.01;	LSD 0.87
	Interaction P< 0.05;	LSD 2.87
	Except when comparing means with same Level of day	LSD. 1.51
Salmon	Storage time NS;	LSD 1.36
	Gaseous atmosphere NS;	LSD 1.11
	Interaction NS;	LSD 1.92
	Except when comparing means of the same level of days	LSD 1.91

Table 4.4.3.2 Effect of storage time (days) and gaseous atmosphere on shear values (kN) for whiting, mackerel and salmon portions

Species	Day	Gaseous atmosphere				Average
		Air	30% O_2 / 40% CO_2 / 30% N_2	60% N_2 / 40% CO_2	100% CO_2	
Whiting	0	1.61	1.61	-	1.61	**1.61**
	3	1.55	1.55	-	1.52	**1.54**
	5	1.55	1.54	-	1.54	**1.54**
Average		**1.57**	**1.57**	-	**1.55**	
Mackerel	0	1.93	-	1.93	1.93	**1.93**
	3	1.99	-	1.98	2.06	**2.01**
	5	1.94	-	1.90	1.96	**1.93**
Average		**1.95**	-	**1.94**	**1.99**	
Salmon	0	1.78	-	1.78	1.78	**1.78**
	5	1.73	-	1.75	1.75	**1.74**
	7	1.75	-	1.77	1.75	**1.76**
Average		**1.75**	-	**1.78**	**1.76**	

Whiting	Storage time P< 0.001;	LSD 0.024
	Gaseous atmosphere NS;	LSD 0.040
	Interaction NS;	LSD 0.059
	Except when comparing means of the same level of days	LSD 0.069
Mackerel	Storage time P< 0.05;	LSD 0.048
	Gaseous treatment P< 0.001;	LSD 0.022
	Interaction P< 0.05	LSD 0.049
	Except when comparing means with same Level of day	LSD 0.038
Salmon	Storage time NS;	LSD 0.037
	Gaseous atmosphere NS;	LSD 0.042
	Interaction NS;	LSD 0.044
	Except when comparing means of the same level of days	LSD 0.035

4.4.4 *Drip loss*

MAP treatment and chilled storage time (days) had no effect on the total moisture content of whiting, mackerel and salmon portions and mean values were 80.6, 68.3 and 67.1g/100g respectively. MAP treatment did have an effect on gravity drip (GD) values for the three species. Portions packed in 100% CO_2 had higher GD than those packed in 30% O_2 / 40% CO_2 / 30% N_2 (whiting; $P< 0.05$) or in 60% N_2 / 40% CO_2 (mackerel; $P< 0.001$, salmon; $P< 0.001$). In turn, samples packed in air had less GD than in the other two MAP treatments. Storage time at 2 to 4°C had no effect on the GD values for the three species. There was an interaction ($P< 0.05$) between MAP and storage time for mackerel fillets (Table 4.4.4.1). After 3 and 5 days at 4°C fillets packed in 100% CO_2 had the highest GD (%) and there was no difference between GD in air-packed and 60% N_2 / 40% CO_2-packed samples. However air-packed fillets had higher drip ($P< 0.05$) on day 5 than on day 3.

Whiting ($P< 0.001$), mackerel ($P< 0.001$) and salmon ($P< 0.05$) showed an increase in centrifugal drip with time of storage at 2-4°C (Table 4.4.4.2). Mackerel fillets packed in 60% N_2 / 40% CO_2 had less CD ($P< 0.01$) than in other MAP treatments and salmon portions packed in 100% CO_2 had the highest ($P< 0.001$) CD. There was an interaction ($P< 0.05$) between MAP and storage time for mackerel fillets. On day 3, fillets packed in 60% N_2 / 40% CO_2 had the lowest CD and there was no significant difference between CD in air-packed and 100% CO_2-packed fillets.

Table 4.4.4.1 Effect of storage time (days) and gaseous atmosphere on percentage gravity drip for whiting, mackerel and salmon portions

Species	Day	Gaseous atmosphere				Average
		Air	30% O_2 / 40% CO_2 / 30% N_2	60% N_2 / 40% CO_2	100% CO_2	
Whiting	0	-	-	-	-	-
	3	4.47	8.80	-	15.8	**9.69**
	5	4.95	9.98	-	17.0	**10.6**
Average		**4.71**	**9.39**	-	**16.4**	
Mackerel	0	-	-	-	-	-
	3	3.75	-	4.50	6.50	**4.92**
	5	5.50	-	5.50	6.75	**5.92**
Average		**4.63**	-	**5.00**	**6.63**	
Salmon	0	-	-	-	-	-
	5	2.33	-	4.50	5.70	**4.18**
	7	2.58	-	4.85	5.98	**4.47**
Average		**2.45**	-	**4.68**	**5.84**	

Whiting	Storage time NS;	LSD 3.14
	Gaseous atmosphere P< 0.05	LSD 1.47
	Interaction NS;	LSD 3.85
	Except when comparing means of the same level of days	LSD 2.55
Mackerel	Storage time NS;	LSD 1.68
	Gaseous atmosphere P< 0.001;	LSD 0.65
	Interaction P< 0.05	LSD 1.54
	Except when comparing means with same Level of day	LSD. 0.93
Salmon	Storage time NS;	LSD 0.24
	Gaseous atmosphere P< 0.001;	LSD 0.46
	Interaction NS;	LSD 0.56
	Except when comparing means of the same level of days	LSD 0.65

Table 4.4.4.2 Effect of storage time (days) and gaseous atmosphere on percentage centrifugal drip for whiting, mackerel and salmon portions

Species	Day	Gaseous atmosphere				Average
		Air	30% O_2 / 40% CO_2 / 30% N_2	60% N_2 / 40% CO_2	100% CO_2	
Whiting	0	4.37	4.37	-	4.37	**4.37**
	3	12.5	15.5	-	14.2	**14.1**
	5	16.0	21.9	-	18.6	**18.8**
Average		**11.0**	**13.9**	-	**12.4**	
Mackerel	0	5.53	-	5.53	5.53	**5.53**
	3	11.4	-	8.02	10.6	**10.0**
	5	11.2	-	9.82	13.5	**11.5**
Average		**9.36**	-	**7.79**	**9.85**	
Salmon	0	1.00	-	1.00	1.00	**1.00**
	5	2.43	-	3.70	4.85	**3.66**
	7	2.80	-	3.65	4.88	**3.78**
Average		**2.08**	-	**2.78**	**3.58**	

Whiting	Storage time P< 0.001;	LSD 5.11
	Gaseous atmosphere NS;	LSD 2.42
	Interaction NS;	LSD 5.68
	Except when comparing means of the same level of days	LSD 4.19
Mackerel	Storage time P< 0.001;	LSD 2.13
	Gaseous atmosphere P< 0.01;	LSD 1.21
	Interaction P< 0.05;	LSD 2.52
	Except when comparing means with same level of day	LSD 2.10
Salmon	Storage time P< 0.05;	LSD 1.82
	Gaseous atmosphere P< 0.001;	LSD 0.69
	Interaction NS;	LSD 1.93
	Except when comparing means of the same level of days	LSD 1.20

4.4.5 *Free fatty acids (FFAs) and peroxide values (PVs)*

Mackerel fillets packed in 100% CO_2 had the highest percentage of ($P< 0.05$) free fatty acids (Table 4.4.5.1). There was a significant increase in the percentage free fatty acid values with time of staorage at 2-4°C for mackerel in all three atmospheres. This pattern was also obvious for salmon packed in air and 60% N_2 / 40% CO_2 but not in 100% CO_2.

The patterns for the peroxide value were largely similar to those for the FFAs (Table 4.4.5.2). All values for mackerel (1.4 - 2.0) and salmon (0.84 - 0.95) remained relatively constant irrespective of gaseous atmosphere after an interval rise from time 0. There were significant interactions between MAP and time of storage for both FFA ($P< 0.001$) (Table 4.4.5.1) and peroxide values ($P< 0.05$) (Table 4.4.5.2) for salmon portions. Free fatty acid values for salmon portions packed in air and 60% N_2 / 40% CO_2 increased with respect to (storage) time, however there was no significant difference between portions packed in 100% CO_2 on days five and seven. There was no significant difference in PVs on days five and seven. Peroxide values for day 5 portions packed in 60% N_2 / 40% CO_2 were similar to those packed in air and however on day 7 portions packed in 60% N_2 / 40% CO_2 had higher PVs than those packed in air.

Table 4.4.5.1 Effect of storage time (days) and gaseous atmosphere on percentage free fatty acids (expressed as oleic acid) for mackerel and salmon portions

Species	Day	Gaseous atmosphere			Average
		Air	60% N_2 / 40% CO_2	100% CO_2	
Mackerel	0	0.25	0.25	0.25	**0.25**
	3	1.23	1.36	1.66	**1.42**
	5	1.58	1.62	2.15	**1.78**
Average		**1.02**	**1.07**	**1.35**	
Salmon	0	0.31	0.31	0.31	**0.31**
	5	1.00	0.64	0.80	**0.81**
	7	1.04	1.33	0.67	**1.01**
Average		**0.75**	**0.76**	**0.59**	

Mackerel	Storage time P< 0.001;	LSD 0.45
	Gaseous atmosphere P< 0.05;	LSD 0.23
	Interaction P< 0.001;	LSD 0.51
	Except when comparing means with same Level of day	LSD 0.40
Salmon	Storage time P< 0.001;	LSD 0.17
	Gaseous atmosphere P< 0.001;	LSD 0.08
	Interaction P< 0.001;	LSD 0.19
	Except when comparing means of the same level of days	LSD 0.14

Table 4.4.5.2 Effect of storage time (days) and gaseous atmosphere on peroxide value (meq. peroxide per kg^{-1} fat) for mackerel and salmon portions

		Gaseous atmosphere			
Species	Day	Air	60% N_2 / 40% CO_2	100% CO_2	**Average**
Mackerel	0	0.40	0.40	0.40	**0.40**
	3	2.45	1.92	2.44	**2.27**
	5	3.25	1.88	2.65	**2.59**
Average		**2.03**	**1.40**	**1.83**	
Salmon	0	0.31	0.31	0.31	**0.31**
	5	1.45	1.11	0.94	**1.06**
	7	1.31	1.44	1.27	**1.34**
Average		**0.92**	**0.95**	**0.84**	

Mackerel	Storage time P< 0.001;	LSD 0.64
	Gaseous atmosphere NS;	LSD 0.56
	Interaction NS;	LSD 0.95
	Except when comparing means with same Level of day	LSD 0.97
Salmon	Storage time P< 0.01;	LSD 0.46
	Gaseous atmosphere P< 0.01;	LSD 0.06
	Interaction P< 0.05;	LSD 0.48
	Except when comparing means of the same level of days	LSD 0.11

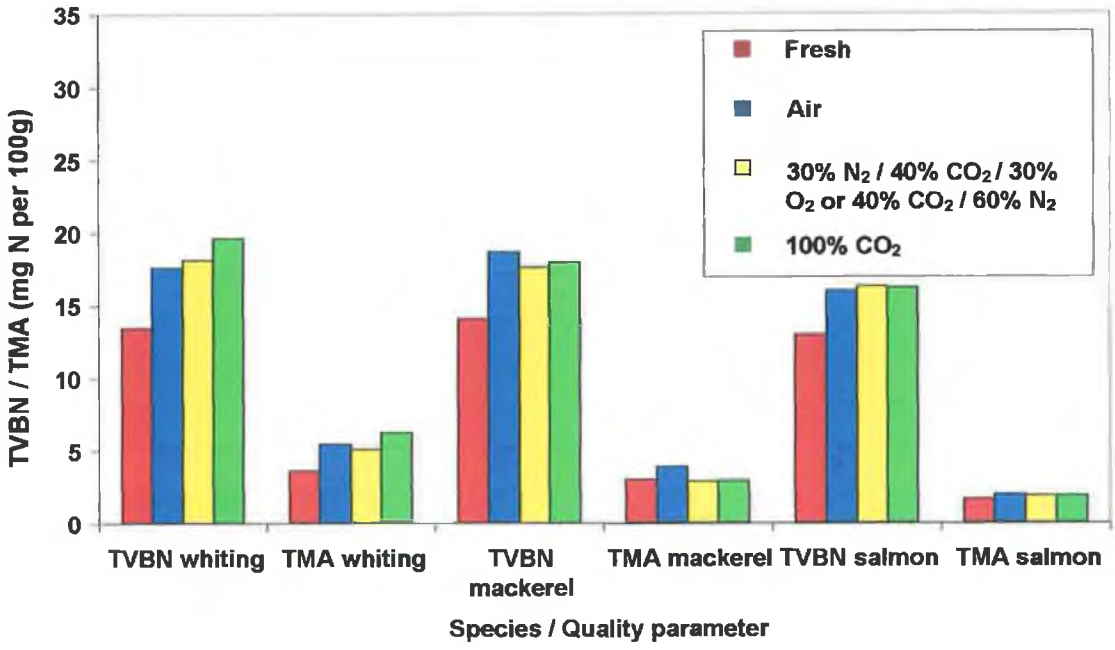
4.4.6 *Total volatile base nitrogen (TVBN) and trimethylamine (TMA)*

Modified atmosphere packaging had no significant effect on TVBN (Table 4.4.6.1) or TMA (Table 4.4.6.2) value in whiting and salmon portions. However, mackerel fillets packed in air had higher TVBN ($P< 0.05$) and TMA ($P< 0.001$) levels than in other treatments (Figure 10). Mackerel chilled for 3 and 5 days and salmon for 5 and 7 days respectively had higher TVBN ($P< 0.001$) levels than day 0 (fresh) samples. There was a progressive increase in TVBN ($P< 0.001$) and TMA ($P< 0.001$) levels with time of chilling in whiting fillets (Table 4.4.6.1). Storage time had no effect on TMA levels in mackerel and salmon portions (Table 4.4.6.2) (mean values 3.21 and 1.90 mg N/100g respectively) however day 0 and day 3 whiting samples had much lower ($P< 0.001$) TMA values than day 5 samples.

Table 4.4.6.1 Effect of storage time (days) and gaseous atmosphere on TVBN levels (mg N per 100g) in whiting, mackerel and salmon portions

Species	Day	Gaseous atmosphere				Average
		Air	30% O_2 / 40% CO_2 / 30% N_2	60% N_2 / 40% CO_2	100% CO_2	
Whiting	0	13.5	13.5	-	13.5	**13.5**
	3	17.0	16.6	-	19.6	**17.7**
	5	22.3	24.4	-	25.9	**24.2**
Average		**17.6**	**18.2**	**-**	**19.6**	
Mackerel	0	14.1	-	14.1	14.1	**14.1**
	3	19.6	-	18.7	19.2	**19.2**
	5	22.3	-	20.0	20.6	**21.0**
Average		**18.7**	**-**	**17.6**	**18.0**	
Salmon	0	12.9	-	12.9	12.9	**12.9**
	5	16.0	-	18.3	18.2	**17.5**
	7	19.0	-	17.6	17.3	**18.0**
Average		**16.0**	**-**	**16.3**	**16.2**	

Whiting	Storage time P< 0.001;	LSD 3.12
	Gaseous atmosphere NS;	LSD 2.24
	Interaction NS;	LSD 4.11
	Except when comparing means of the same level of days	LSD 3.89
Mackerel	Storage time P< 0.001;	LSD 2.47
	Gaseous atmosphere P< 0.05;	LSD 0.87
	Interaction NS;	LSD 2.61
	Except when comparing means with same level of day	LSD 1.51
Salmon	Storage time P< 0.01;	LSD 2.03
	Gaseous atmosphere NS;	LSD 2.23
	Interaction NS;	LSD 3.54
	Except when comparing means of the same level of days	LSD 3.87



Whiting TVBN NS (no sig. difference between gaseous treatments; all significantly higher than fresh)
Whiting TMA NS (no sig. difference between gaseous treatments; all significantly higher than fresh)
Mackerel TVBN $P< 0.05$; LSD 0.87
Mackerel TMA $P< 0.05$; LSD 0.46
Salmon TVBN NS (no sig. difference between gaseous treatments; all significantly higher than fresh)
Salmon TMA NS (no sig. difference between fresh and other gaseous treatments)

Figure 10 Total volatile base nitrogen (TVBN) and trimethylamine (TMA levels in whiting, mackerel and salmon portions with or without modified atmosphere packaging.

Table 4.4.6.2 Effect of storage time (days) and gaseous atmosphere on TMA levels (mg N per 100g) in whiting, mackerel and salmon portions

Species	Day	Gaseous atmosphere				Average
		Air	30% O_2 / 40% CO_2 / 30% N_2	60% N_2 / 40% CO_2	100% CO_2	
Whiting	0	3.60	3.60	-	3.60	**3.60**
	3	4.65	4.00	-	5.28	**4.64**
	5	8.07	7.65	-	9.82	**8.52**
Average		**5.44**	**5.08**	-	**6.23**	
Mackerel	0	3.00	-	3.00	3.00	**3.00**
	3	4.00	-	1.93	1.93	**2.62**
	5	4.63	-	3.60	3.80	**4.01**
Average		**3.88**	-	**2.84**	**2.91**	
Salmon	0	1.58	-	1.58	1.58	**1.58**
	5	1.90	-	2.10	2.30	**2.10**
	7	2.38	-	1.78	1.63	**1.93**
Average		**1.95**	-	**1.88**	**1.86**	

Whiting	Storage time P< 0.001;	LSD 1.70
	Gaseous atmosphere NS;	LSD 1.36
	Interaction NS;	LSD 2.38
	Except when comparing means of the same level of days	LSD 2.35
Mackerel	Storage time NS;	LSD 1.09
	Gaseous atmosphere P< 0.001;	LSD 0.46
	Interaction P< 0.01;	LSD 1.33
	Except when comparing means with same level of day	LSD 0.80
Salmon	Storage time NS;	LSD 1.47
	Gaseous atmosphere NS;	LSD 0.49
	Interaction NS;	LSD 1.52
	Except when comparing means of the same level of days	LSD 0.84

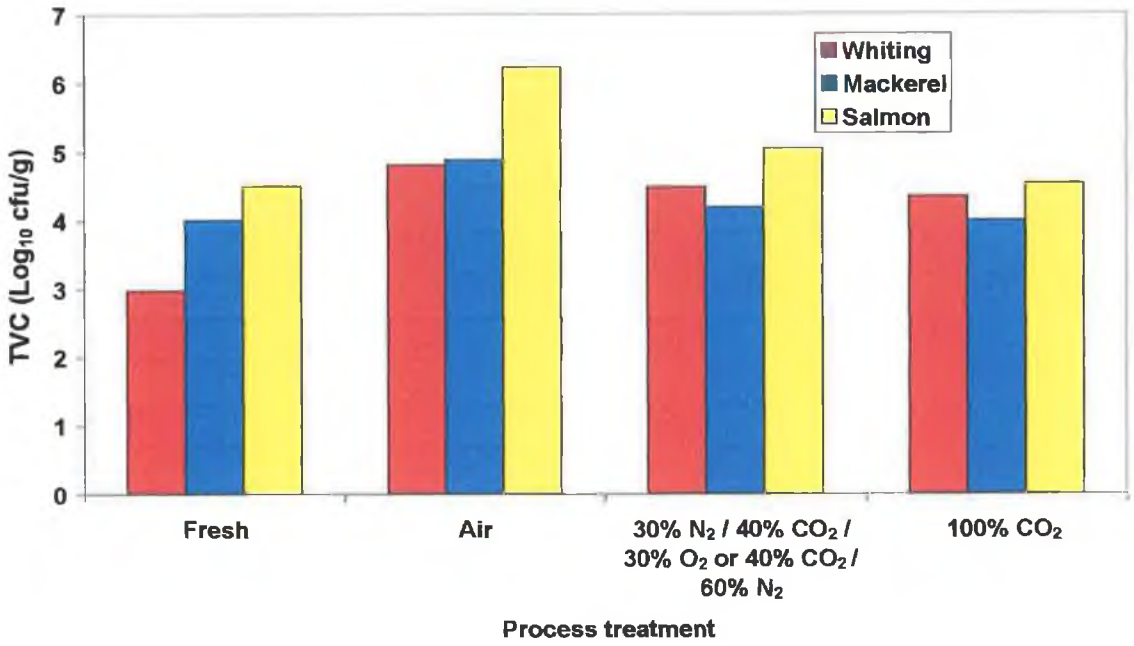
4.4.7 *Total viable count (TVC)*

There was a consistent growth of micro-organisms in both whiting ($P< 0.05$) and salmon ($P< 0.01$) portions (Table 4.4.7.1) over the 5/7 day period. Statistical analysis indicated the rise to be of significance for whiting. The rise was not as large as for mackerel and overall growth did not reach such high levels in this species. Modified atmosphere packs of mackerel ($P< 0.001$) and salmon ($P< 0.001$) portions in 100% CO_2 had the lowest TVCs. There was no difference in TVCs of whiting fillets packed in 30% 0_2 / 40 CO_2 / 30% N_2 and 100% CO_2. However, both these treatments had lower ($P< 0.001$) TVCs than whiting fillets packed in air (Table 4.4.7.1). There were interactions between storage time (days) and MAP treatment for whiting ($P< 0.01$), mackerel ($P< 0.001$) and salmon ($P< 0.001$) portions. In the case of whiting portions, on day 3, 30% N_2 / 40% CO_2 / 30% O_2 and 100% CO_2-packed portions had the lowest TVCs. However on day 5, portions packed in 100% CO_2 had lower TVCs than those packed in 30% N_2 / 40% CO_2 / 30% O_2, and these in turn had lower values than those packed in air. Mackerel and salmon portions had similar TVC patterns in that portions of both species packed in 100% CO_2 had the lowest TVCs on day 3 followed by 60% N_2 / 40% CO_2 (Figure 11). However, on day 5, there was no significant difference between TVCs in the 60% N_2 / 40% CO_2 and 100% CO_2 packs.

Table 4.4.7.1 Effect of storage time (days) and gaseous atmosphere on total viable counts (log_{10} cfu/g) whiting, mackerel and salmon portions

Species	Day	Gaseous atmosphere				Average
		Air	30% O_2 / 40% CO_2 / 30% N_2	60% N_2 / 40% CO_2	100% CO_2	
Whiting	0	2.97	2.97	-	2.97	**2.97**
	3	5.22	4.69	-	4.79	**4.88**
	5	6.24	5.77	-	5.28	**5.76**
Average		**4.81**	**4.48**	-	**4.34**	
Mackerel	0	4.03	-	4.03	4.03	**4.03**
	3	4.83	-	4.13	4.02	**4.32**
	5	5.80	-	4.39	3.93	**4.70**
Average		**4.88**	-	**4.18**	**3.99**	
Salmon	0	4.50	-	4.50	4.50	**4.50**
	5	6.90	-	5.33	4.53	**5.58**
	7	7.28	-	5.30	4.55	**5.71**
Average		**6.23**	-	**5.04**	**4.53**	

Whiting	Storage time P< 0.05;	LSD 1.95
	Gaseous atmosphere P< 0.001;	LSD 0.20
	Interaction P< 0.01	LSD 1.98
	Except when comparing means with same Level of day	LSD 0.35
Mackerel	Storage time P< NS ;	LSD 1.17
	Gaseous atmosphere P< 0.001;	LSD 0.16
	Interaction P< 0.001	LSD 1.19
	Except when comparing means with same Level of day	LSD 0.27
Salmon	Storage time P< 0.01;	LSD 0.62
	Gaseous atmosphere P< 0.001;	LSD 0.32
	Interaction P< 0.001	LSD 0.71
	Except when comparing means with same Level of day	LSD. 0.56



Whiting P< 0.001; LSD 0.20 (all sig. higher than fresh)
Mackerel P< 0.001; LSD 0.15 (all sig. higher than fresh)
Salmon P< 0.001; LSD 0.32 (all sig. higher than fresh)

Figure 11 Total viable counts for freeze-chilled whiting, mackerel and salmon portions with or without modified atmospheres

4.4.8 *Correlations*

Total volatile base nitrogen was negatively correlated with sensory acceptability for the three species (Table 4.4.8.1). TVCs were positively correlated with TVBN and negatively with acceptability score. Most correlation coefficients were relatively low except in the case of whiting, reflecting the complexity of the fish tissues being studied.

Table 4.4.8.1. Correlation's[a] of selected quality parameters for whiting, mackerel and salmon with or without modified atmosphere packaging

	Species		
	Whiting	Mackerel	Salmon
TVBN[b] X ACC[c]	- 0.93	-0.71	-0.91
TVBN[b] X TVC[d]	+0.85	+0.45	+0.48
TVC[b] X ACC[c]	-0.86	-0.44	-0.63

[a] Results averaged over 20 data points
[b] Total volatile base nitrogen (mg N per 100g)
[c] Sensory acceptability (0 (unacceptable) to 6 (very acceptable))
[d] Total viable count (log_{10} cfu/g)

4.5 Discussion

Odour is an attribute immediately sensed by the consumer on opening a pre-packaged fish portion. Fresh fish has a sweet "seaweed-like" odour, which develops into a strong "fishy" odour as compounds such as TVBN and TMA develop from the breakdown of protein and primarily from trimethylamine oxide (TMAO). In pre-packaged fish fillets, the odours concentrate in the headspace of the pack and are evident on pack opening. However, if the fillets are in good condition, these are minimal and will dissipate soon after pack opening as indicated by the results for mackerel (odour score decreased from 3.44 on pack opening to 3.04, 10 minutes later and salmon likewise (3.04 down to 2.71) over 10 minutes. The opposite occurred however for whiting (an increase from 2.57 to 3.01 over 10 minutes). The odour scores in the current study were often below 3 (slight fishy odour) and were never higher than 3.52 (a score of 4 indicates a significant fishy odour, one of 5 a strong fishy odour, and 6 a putrid smell). Five-day-old portions scored worse for odour than three-day samples. However, all samples were below the midpoint of the odour scale (i.e. 3.5), which put them in the "slight fishy odour" category indicating that the MAP samples had an acceptable odour overall after five (whiting and mackerel) and seven (salmon) days. MAP treatment had no effect on the odour scores for the three species however, an interaction was observed for salmon portions between storage time (days) and MAP treatment ($P< 0.05$) which showed that the 60% N_2 /40 % CO_2 and the 100% CO_2-stored samples scored better than samples packed in air. This effect was only observed for salmon portions and might be explained by the

treatment of the samples prior to packing. The farmed salmon used in these trials were tested within 24 hours of catching whereas the whiting and mackerel were on ice on-board trawlers for several days before testing. This is unsatisfactory but is representative of current Irish commercial trawler practice.

The most common methods for assessing the freshness and odour of fish, together with the sensory tests, are the determination of total volatile base nitrogen (TVBN) and trimethylamine (TMA) (Civera *et al.*, 1995). The total volatile bases developed during the storage of unfrozen fish consist primarily of ammonia and trimethylamine (TMA) (Storey *et al.*, 1984). Spoilage of thawed MAP fish portions have received relatively little attention (Bøknæs, 2000, Windsor and Thoma, 1974). However, research on freeze-chilled (thawed) fish portions have shown the previously frozen samples to have a slightly longer shelf-life than the unfrozen samples (Guldager *et al.*, 1998, Shewan, 1961, Simmonds and Lamprecht, 1985). The development of TVBN and TMA during frozen storage depends to a large extent on the freshness of the samples prior to freezing. Bøknæs *et al.* (2000) compared cod fillets stored in air for one or eight days prior to MAP and subsequent frozen storage at –20 and –30°C for 6 weeks and found TMA production was more pronounced in eight day stored samples. They contributed the rise in TMA to the growth of specific spoilage organisms (*P. phosphoreum*), which proliferated during the iced storage prior to freezing. These organisms are rich in enzymes which catalyse the conversion of TMAO to TMA. The TVBN and TMA levels in the current trials were moderately high in whiting and mackerel fillets and much lower in the farmed salmon samples. In the case of

whiting samples, MAP did not affect TVBN and TMA development. However values increased with time of chilling. Mackerel fillets packed in 60% N_2 / 40% CO_2 had the lowest TVBN values while TMA values were lowest in the 100% CO_2 and 60% N_2 / 40% CO_2. The initial high TVBN and TMA levels in whiting and mackerel fillets were most probably due to on-board storage conditions prior to processing resulting in degradation and oxidation of fish tissues due to temperature abuse. The production of TVBN (ammonia) may not require oxygen while TMAO oxidase enzymes responsible for the production of TMA does. The TVBN and TMA levels were lower for the three species in MAP than those recorded for freeze-chilled fish fillets without MAP (sections 1 to 3). This suggests that the combination of MAP and freezing produced fillets of higher quality than freeze-chilling alone. Presumably, the lack of oxygen prevented the catalytic degradation of TMAO. The salmon portions had lower initial TVBN and TMA levels than whiting and mackerel samples and variation in MAP treatment had no effect. Since the salmon had the highest initial microbial load, which increased in both air and N_2 / CO_2 but did not change in 100% CO_2, one can only conclude that the organisms rich in the degradative enzymes which produced the TMA and NH_3 did not grow during the chill phase.

Taste panel acceptability scores on cooked samples showed that there was no significant difference between the three gaseous atmospheres for each species. All gaseous atmospheres scored significantly lower than the fresh samples except in the case of mackerel fillets packed in 60% N_2 / 40% CO_2 which scored as well as the fresh samples. All species/samples scored above the mid-point of the line

which suggested that they were all very acceptable after 5 (whiting and mackerel) or 7 (salmon) days. In the case of the fatty fish species (lipid content ca. 20-30%) such as mackerel and salmon, off-tastes can also be attributed to oxidative rancidity of the fats, the production of free fatty acids, or a combination of both. Rancidity is perceived as an unacceptable taste by the consumer; typically soapy, stale, linseed oil flavours are detected (Church, 1998). In whitefish species (lipid content ca. 1-2%) such as whiting, the fat is mainly membrane-bound and this fish is less prone to oxidation and compounds such as TVBN and TMA contribute more to off-tastes. Rancidity in fatty fish species such as salmon is not well-characterised and has been described as fish oil taste (Sylvia *et al.*, 1995) and as fatty and train oily odours (Milo and Grosch, 1996). Frozen storage does not prevent the development of rancidity in fish fillets (Gormely *et al.*, 2001). Lipid deterioration occurs at a slower rate in frozen storage, but hydroperoxides are still produced during the frozen phase. Zotos *et al.*, (1994) reported extensive lipid oxidation of mackerel fillets (PVs of 108 meq. Kg^{-1}) over periods of 22 to 33 weeks in frozen storage. These high levels do not accumulate at higher temperatures. Presumably the enzymatically catalysed degradative pathways of hydroperoxides are selectively inhibited in frozen storage. Similar results have been noted during the storage of frozen beefburgers (M.M. Uí Mhuircheartaigh 2002, personal communication). Zotos *et al.* (1994) also reported that free fatty acid (%) accumulated to over 60% in mackerel fillets during this extensive frozen storage period. These samples were still acceptable to taste panellists after 33 weeks of frozen storage at –20°C. In the current trials mackerel fillets packed in 100% CO_2 had the highest FFAs, while salmon in this atmosphere had the lowest

values. The peroxide values for both mackerel and salmon increased with storage time. The highest recorded FFAs for mackerel (1.78 % FFA expressed as oleic acid) and salmon (0.81 % FFA expressed as oleic acid) and corresponding PVs (2.59 and 1.34 meq. peroxide/kg sample respectively) were well below the values reported by Zotos *et al.*, (1994) and most probably did not affect the sensory scores of the cooked product. A much more extensive survey would need to have been undertaken to draw any further conclusions.

In the current trials, there was a significant increase in the percentage free fatty acids with time of storage at 2-4°C as enzymatic and oxidative degradation of the fat occurs readily at 4°C. As can be seen from Table 4.4.5.1 and 4.4.5.2, peroxide and free fatty acid levels reached higher values in 100% CO_2 in mackerel fillets than in other gas mixtures. Lipolysis (production of FFAs) does not require oxygen. Most likely the acids produced were not further degraded because aerobic metabolism was inhibited in the CO_2 and 60% N_2 / 40% CO_2 atmospheres therefore allowing free fatty acids to accumulate. Free fatty acids are more readily oxidised by tissue enzymes than those in intact phospholipids or triglycerides. The production of FFAs is enzyme catalysed (lipases) and does not require oxygen, merely time. It is also temperature dependant (rate approximately doubling over time for every 10°C rise in temperature, Q_{10}). The production of peroxides does require oxygen and is enzyme catalysed, which would explain the lower PVs in the modified atmospheres, compared to air-packed samples. In the salmon samples no change in the production of FFAs or peroxides was observed by changing the atmospheric gases.

Overall the peroxide value did not appear to vary significantly with the atmospheric composition. All values for both mackerel (1.4 - 2.0) and salmon (0.84 - 0.95) remained roughly constant (Table 4.4.5.1, 4.4.5.2) irrespective of atmospheric composition. This was surprising considering that oxygen is required for oxidation to occur and therefore samples packed in air would have been expected to have higher values (more oxidation). It is important to remember however, that final values are a compromise between rate of production (oxidation) and rate of further degradation (dependant on other metabolic pathways, some of which are aerobic). All values for both mackerel and salmon remained below the rancid range (10-20).

Colour is an important attribute of both raw and cooked fish. Browning in whitefish species is an indication of spoilage and discoloration can occur via a bleaching action of cut surfaces (Cann, 1984) probably due to low pH precipitation of sacroplasmic proteins (Stratham and Bremner, 1989). Lannelongue *et al.* (1982) reported a drop in surface pH in MAP products due to the dissociation of CO_2 to carbonic acid (proportional to CO_2 concentration). Bleaching of pigments and increased opacity of flesh has been reported by Gibson and Davis (1995) in high CO_2 environments. Whiting and salmon portions stored in 100% CO_2 in the current trials were more yellow than in other treatments, and whiting and mackerel fillets became progressively more yellow over time. Skrede and Storebakken. (1986) found that canthaxanthin-pigmented farmed salmon was

more yellow in hue than astaxanthin-pigmented wild salmon and this may explain why yellowing was observed in the MAP salmon portions.

In the current trials, the springiness and shear values (Kramer shear values) of whiting fillets were not affected by MAP. However three-day and five-day old whiting fillets were less tough than on day 0 (fresh). This could be related in part to the soft muscle structure of this species and in part due to immersion of the fillet in the free moisture in the bottom of the tray i.e. the gravity drip (up to 16% in 100% CO_2 pack) over the three to five day time period. Mackerel portions were toughest in 100% CO_2, while 60% N_2 / 40% CO_2 and 100% CO_2 gave salmon portions with the highest shear values. The toughening of fish during freezing and cold storage is well documented (IIR, 1986) and salmon from frozen storage has been previously reported to be more firm, less juicy and more fibrous (Refsgaard *et al.*, 1998). Frozen storage (Gormely *et al.*, 2002) and the freezing component of the freeze-chill process also leads to moisture loss in the form of increased drips (O'Leary *et al.*, 1999). Some modified atmosphere packagings do likewise according to other workers (Randell *et al.*, 1995; Tiffany and Mills, 1982; Cann, 1984). These findings were confirmed in the current trials where both time at 4°C and the use of MAP increased drip loss in all three species. The problem of in-pack drip can be minimised however by the use of drip pads.

The microbiological status of fish and fish products has been reviewed by Shewan (1971) and low counts are a key indicator of fish freshness and food hygiene practice. In addition *Pseudomonas* tends to become dominant during prolonged

storage (Stenström and Molin 1989). Fish portions in the current trials were packed in MAP and blast-frozen at –35°C and stored at –30°C for three days prior to chilling. It is likely, therefore, that the freezing component of the freeze-chill process had little impact on the microbial status of the fish portions since the frozen storage time was well below the five weeks suggested by other workers (Magnusson and Martinsdottir, 1995; Bøknæs *et al.*, (2000)) as the minimum frozen storage period to reduce bacterial numbers in cod (through cell death). Sections 1 to 3 also showed no significant difference in TVCs between previously frozen and unfrozen air-packed whiting, mackerel and salmon portions. In the current trials, after five days in chill, whiting and mackerel fillets packed in 60% N_2 / 40% CO_2 and 100% CO_2 atmospheres had significantly lower TVCs than those packed in air (freeze-chilled samples) making them a safer and healthier product. The former gave less drip than the latter however so on this basis are aesthetically more pleasing. Pack implosion was also not a problem in the former.

The results from the current trials suggest that whiting, mackerel and salmon portions performed well as freeze-chilled MAP products. Sensory tests indicated that samples were still acceptable after five (whiting and mackerel) or seven days (salmon) which was one to two days longer than in previous trials (sections 1 to 3) when samples were packed in air. European legislative criteria for the sale of raw fish products were maintained for each species packed in modified atmosphere in that the whiting and mackerel samples used in these trials came from commercial fishing trawlers and were kept on ice for approximately three to seven days prior to processing. This situation is typical of Irish commercial practice. Ideally

however, freezing at sea would be a much more favourable option and would result in a longer shelf-life in MAP.

4.6 Conclusions

The MAP packs for mackerel and salmon (60% N_2 / 40% CO_2), and for whiting (30% N_2 / 40% CO_2 / 30% O_2) maintained their shape during freeze-chilling whereas packs with 100% CO_2 were slightly imploded with concave sides. The chosen chilled shelf-life of 5-7d in the MAP trials appeared to be correct as the products were near the end of their shelf-life (in acceptability terms) after 5 (whiting and mackerel) and 7 (salmon) days. This compares with shelf lives of 3 and 5d respectively for similar freeze-chilled fillets in air. Samples in MAP had lower total viable counts than samples in air for raw fillets/portions of each of the three species. However, MAP did not influence odour or acceptability scores, but had a variable effect (generally small) on fillet colour, springiness, drip loss, total volatile base nitrogen / trimethylamine content, peroxide values and free fatty acid contents. From a consumer point of view, the imploded CO_2 packs are unlikely to have appeal. Future studies should perhaps concentrate on the use of the 60% N_2 / 40% CO_2 or 100% CO_2 unless / until better quality packaging has been produced.

Chapter 5

5. FINAL CONCLUSIONS

The objectives of this project were to determine the effects of freeze chilling (Trials 1 to 3) and freeze-chilling in combination with modified atmosphere packaging (Trials 4 to 6) on selected quality parameters of whiting, mackerel and salmon portions. The ultimate aim of this project was to extend the shelf-life of individual fish portions using a combination of freeze-chilling and MAP.

5.1 Freeze-chilling of whiting, mackerel and salmon portions.

Freeze-chilling trials with whiting, mackerel and salmon fillets/portions indicated no difference in odour scores (raw samples) between freeze-chilled and chilled samples; however, freeze-chilled salmon portions were inferior in terms of odour to chilled. Fresh and frozen fillets of mackerel and salmon and fresh whiting fillets received the highest acceptability scores (cooked samples). There was no significant difference between chilled and freeze-chilled samples of each species. The total volatile base nitrogen (TVBN), trimethylamine (TMA) and total viable count (TVC) data showed the same pattern for the three species in that the chilled and freeze-chilled samples had the highest values and the fresh and frozen the lowest. However, there was no statistically significant difference between the freeze-chilled and chilled samples. Freeze-chilled samples had the highest free fatty acid (FFA) and peroxide values (PV) but the levels were low and did not influence sensory response. Gravity drip was significant in the frozen and freeze-chilled samples but presented no major visual problems and could readily be

absorbed by drip pads. The effects of the four treatments on the colour and texture of the raw samples were small in practical terms.

5.2 Freeze-chilling in combination with modified atmosphere packaging.

The MAP packs for mackerel and salmon (60% N_2 / 40% CO_2), and for whiting (30% N_2 / 40% CO_2 / 30% O_2) maintained their shape during freeze-chilling whereas packs with 100% CO_2 were slightly imploded with concave sides. The chilled shelf-life of prefrozen fillets proved to be either five (whiting and mackerel) or seven (salmon) days in these MAP trials. This compares with shelf lives of 3 and 5d respectively for freeze-chilled fillets in air. Samples in MAP had lower total viable counts than samples in air for raw fillets/portions of each of the three species. However, MAP did not influence odour or acceptability scores, but had a variable effect (generally small) on fillet colour, springiness, drip loss, total volatile base nitrogen / trimethylamine content, peroxide values and free fatty acid contents. It may be of value to assess the type of microbial growth in the various phases. Total counts are ideally as low as possible, however the selective growth of a "pathogen" would not be desirable even in very low numbers.

Chapter 6

6. RECOMMENDATIONS TO INDUSTRY

The main findings of this thesis suggest that freeze-chilling in combination with modified atmosphere packaging produces whiting and mackerel fillets with a shelf-life of five days and salmon darns with a shelf-life of seven days at 2 to 4°C. The quality of raw fish portions could however, undoubtedly be increased even further by reducing temperature abuse on board trawler prior to processing. Problems associated with the supply of fresh fish in Ireland are mainly due to an under developed cold chain. The recent imposition of stringent quotas on commercial species has made it necessary for boats to spend longer at sea to make fishing trips viable. Handling and packaging however of raw fish on board the majority of Irish fishing vessels do not effectively safeguard the raw fish against additional time delays between landing and processing. The current practices of storing fish on ice is not an effective method of maximising quality and must be addressed by infrastructural investment in modern vessels and / or new technology. One technology, which has obvious advantages, is the use of liquid ice. This involves pumping an ice-slurry into the hold on top of the fish. The advantages of such a system include; increased surface area contact between fish and ice cooling the fish more rapidly; elimination of "hot spots" which can occur in ice-packed fish; reduction in mechanical damage to fish flesh associated with crushed ice and reduction of temperature abuse and cross contamination due to the

fact that the fish and ice slurry can be pumped out of the hold into crates for delivery to processors.

The use of on board technology could maintain the fish / fillet at temperatures close to 0 °C which could slow down bacterial growth and maintain TVBN and TMA levels at low levels. The rapid temperature lowering might also slow the oxidation process directly reducing the amount of free radicals available to cause fat rancidity. The net result might be fish / fillets of extremely high quality which could then be processed in a HACCP environment producing freeze-chilled MAP products of the highest quality. The main limitation on preserving quality and increasing the shelf-life would be the initial freshness of the fish and the natural biodegradation during / after freezing. The former could be controlled by the treatment at sea, the latter will be slowed down if the initial quality is optimised and should result in an overall increase in shelf-life. This is a separate project in its own right.

Chapter 7

6. BIBLIOGRAPHY

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Chapter 8

8. APPENDICES

8.1 Cooked fish taste panel sheet

Name..... Date.....

Please taste the following fish sample and mark the line at the appropriate place.

Example;

Unacceptable _____ Very acceptable

Sample No.....

Unacceptable _____ Very acceptable

Comments:

.....
.....
.....
.....
.....
.....
.....

Thank you for your time!!

8.2 Publications

8.2.1 National

Fagan, J.D., Gormley, T.R. and Uí Mhuircheartaigh, M.M. (2002). Freeze-chill technology for raw whiting and mackerel fillets. *Farm and Food*, **12**, pp. 14-17

Fagan, J.D., Gormley, T.R. and Uí Mhuircheartaigh, M.M. (2002). Freeze-chill technology and its application to consumer consumption of whiting and mackerel fillets. *The Irish Scientist*, **10**, p. 112

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Fagan, J.D., Gormley, T.R. and Uí Mhuircheartaigh, M.M. (2002). Taste panel acceptability of underutilised fish species. *WEFTA Conference, 12th - 15th May*, Galway, Ireland, Oral presentation

Fagan, J.D., Gormley, T.R. and Uí Mhuircheartaigh, M.M. (2002). Freeze-chill technology for raw whiting and mackerel fillets. *EMERTEC Conference, 11th - 13th March 2002*, Madrid, Spain. Poster presentation

Fagan, J.D., Gormley, T.R. and Uí Mhuircheartaigh, M.M. (2002). Freeze-chill technology for mackerel fillets. *Student seminar*, The National Food Centre. October 2002

Fagan, J.D., Gormley, T.R. and Uí Mhuircheartaigh, M.M. (2002). Freeze-chill technology for whiting (Merlangius merlangis) fillets. *32nd Annual Food Science and Technology Research Conference, Cork, Ireland, September (2002)*

8.2.2 International

Fagan, J.D., Gormley, T.R. and Uí Mhuircheartaigh, M.M. (2003). Freeze-chill technology with modified atmosphere packaging for raw whiting, mackerel and salmon portions. *Lebensmittel-Wissenschaft und-Technologie,* In Press

Fagan, J.D., Gormley, T.R. and Uí Mhuircheartaigh, M.M. (2003). Effect of freeze-chilling in comparison with fresh, chilling and freeze-chilling on some quality parameters of raw whiting, mackerel and salmon portions. *Innovative Food Science and Emerging Technologies*. In Press

8.3 Paper

Submitted to *Lebensmittel-Wissenschaft und-Technologie* November 2002

Effect of freeze-chilling, in comparison with fresh, chilling and freezing, on some quality parameters of raw whiting, mackerel and salmon portions.

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Freeze-chilling involves freezing and frozen storage followed by thawing and chilled storage. It offers logistic benefits for fish packers as it enables packaged fillets to be held frozen and then released into the chill chain as required. Trials with whiting, mackerel and salmon fillets/portions indicated no difference in odour scores (raw samples) between freeze-chilled and chilled samples; however, freeze-chilled salmon portions were inferior in terms of odour to chilled. Fresh fillets received the highest acceptability scores (cooked samples) followed by frozen, chilled and freeze-chilled samples. The pattern in the data was the same for each species and there was no statistically significant difference between the freeze-chilled and chilled samples. The total volatile base nitrogen (TVBN), trimethylamine (TMA) and total viable count (TVC) data were the same for the

three species in that the chilled and freeze-chilled samples had the highest values and the fresh and frozen the lowest. However, there was no statistically significant difference between the freeze-chilled and chilled samples. Freeze-chilled samples had the highest free fatty acid (FFA) and peroxide values (PV) but the levels were low and did not influence sensory response. Gravity drip was significant in the frozen and freeze-chilled samples but presented no major visual problems and could readily be absorbed by drip pads. The effects of the four treatments on the colour and texture of the raw samples were small in practical terms.

Keywords: **freeze-chilling; fish portions; quality; acceptability**

Introduction

The effects of freezing on food are well documented (IIR, 1986) as are the effects of chilling (Howgate, 1987;Gibson, 1994; Huss, 1995). However, the effects of freeze-chill technology on the quality of foods, and of fish fillets and portions have received relatively less attention. Freeze-chilling involves freezing and frozen storage followed by thawing and retailing at chill storage temperatures (O'Leary *et al.*, 2000; Redmond *et al.*, 2002; Fagan *et al.*, 2002). Freeze-chilling offers logistic and other advantages. For example: (i) foods can be prepared in bulk, frozen and stored at deep freeze temperatures until required. Some, or all of the batch can then be thawed as necessary; (ii) freeze-chilling enables chilled foods to reach distant markets in that product can be shipped deep frozen and then thawed when it reaches its destination prior to retail display; (iii) freeze-chilling can reduce the level of product recalls as it enables routine microbiological tests to be completed before the product is released from the factory. The objective of these trials was to assess the suitability of freeze-chilling as a technology for raw whiting and mackerel fillets, and for salmon portions. In the current trials, the frozen storage component of the freeze-chill process was short i.e. 3d, and the samples were then thawed and held at 2-4°C. Freeze-chilling has application at retail level for chilled pre-packed raw fish fillets and portions which are replacing the traditional iced fish counter in some supermarkets (Fagan *et al.*, 2002). Samples were compared as fresh, chilled, frozen and freeze-chilled and were then subjected to a wide range of physical, chemical and sensory tests to determine portion quality.

Materials and Methods

For each species, four process treatments were compared: (i) fresh (tested on day of purchase with no storage); (ii) chilled (4°C for 3d); (iii) frozen (air-blast at -35°C for 3h, stored at –30°C for 3d and thawed overnight at 4°C); and (iv) freeze-chilled (blast frozen at -35°C for 3h, stored at -30°C for 3d followed by 3d of chilled storage at 4°C for whiting and mackerel, and 5d for salmon. Whiting and mackerel were purchased from a fish company in Dublin (Ireland) as skin-on fillets and were transported to the laboratory on ice (internal temperature 0-1°C). The fillets were kept on ice for approximately 3 days before reaching the fish company and had an estimated core temperature of 0-2°C during that period. Whiting were tested between January and March and consisted mainly of 30-36cm specimens. Mackerel were tested between April and June and were mainly of 29-38cm specimens. Farmed salmon were purchased from the same fish company as skinned boneless fillets and were transported to the laboratory on ice. The salmon were bled and gutted the previous day and were transported on ice (estimated core temperature 0-2°C) to the processor within 24 hours. These were tested between July and September and consisted of 2.5-year-old fish (6-7 kg fish). The salmon fillets were cut into portions of ca 250g. Twenty-five fillets were tested within 6h to provide data for the fresh samples. Ten randomly chosen fillets were used for sensory analysis (acceptability) and 15 for physical and chemical tests. The chill, freeze and freeze-chill process treatments also used 25 fillets per replicate. Fillets were weighed, placed individually into plastic (HDPE) trays (Dynopak, Ireland), and the trays sealed with a film (340mm Antifog high barrier film) (Dynopak, Ireland). Six pinholes were punched in the film to

maintain aerobic conditions. The trial was replicated five times over a 10-week period using new batches of fish each time. The experimental design was 4 process treatments x 5 replicates and the results were tested by analysis of variance (ANOVA) with 19 degrees of freedom.

Odour evaluation

The odour status of the prepacked fish from the chilled, frozen and freeze-chilled treatments was assessed by a sniff test using a panel of three trained assessors. The packs (one fillet or portion per pack) were opened and sniffed immediately (time 0) and again after 10 min at room temperature. The samples were scored on a scale from 1 to 6 (1. fresh seaweed-like smell; 2. odourless; 3. slight fishy odour; 4. significant fishy odour; 5. strong fishy odour; 6. totally-off, i.e. putrid smell). The data were tested by ANOVA as 3 process treatments x 2 times of testing (0 and 10 min) x 5 replicates with 29 degrees of freedom. Three packs were assessed per process treatment per replicate.

Acceptability scores

An untrained sensory panel of 25 tasters was used for each of the five replicates. Panellists were asked to score the acceptability of a sample of fish (circa 50g) [steamed for 6 min (whiting), grilled for 6 min (mackerel) or steamed for 30 min (salmon)] on a 6 cm line from 0 (unacceptable) to 6 (very acceptable). The marked point on the line was measured with a ruler and the data were tested by ANOVA as 4 process treatments x 25 tasters for each individual replicate, and also collectively as 4 process treatments x 25 tasters x 5 replicates. Only one

sample (single stimulus taste panel; Gormley, 1989) was tasted each time as the samples from the four treatments came due for testing on different days.

Colour

The colour of the fish samples from the four process treatments was measured using a HunterLab model D25A colour difference meter (Hunter Associates Laboratory Inc., Virginia, USA) fitted with a 2.5cm specimen aperture. Colour was expressed in Hunter Lab units and as L/b (white to yellow ratio). The colour difference between the fresh sample and each of the other three treatments was calculated as $\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$. For whiting and mackerel, colour was measured at three points on each half of the butterfly fillets (i.e. 6 colour measurements per fillet) for five fillets per process treatment per replicate. For salmon, colour was measured at the centre (i.e. the thickest part) of each of 10 portions per process treatment per replicate.

Texture measurements

For springiness measurements, raw fillets were placed on the platform of a TAXT2*i* Texture Analyser (Stable Micro Systems, Surrey, England) and a P/20P perspex probe (20mm diameter) was selected to simulate finger feel. Constant penetration was applied to a depth of 3mm (Sigurgisladottir *et al.*, 1999) at the thickest part of each half of each butterfly fillet and the samples were recompressed after 5 sec. Springiness was calculated as the re-compression force divided by the compression force x 100. This test was conducted on five fillets from each treatment per replicate. Shear values for the raw fillets were assessed

using a T-2000 Texture System (model TP5, Food Technology Corporation, Virginia, USA) fitted with a standard test cell (ram speed 4mm per sec.). Shears were conducted in triplicate (100g samples) per process treatment per replicate.

Centrifugal drip (g/100g)

The method of Wierbiki and Deatherage (1958) was used for centrifugal drip. Samples (3-5g) of thawed fish were accurately weighed into cup-shaped filter-paper thimbles and were placed over 2 cm of glass beads in centrifuge tubes to allow liquid to drain from the thimbles. This was achieved by centrifugation at 500 x g for 10 min at 10°C followed by immediate reweighing. Centrifugal drip was recorded as the weight loss expressed as a percentage of the original weight (w/w).

Gravity drip (g/100g)

The amount of gravity drip in the trays was determined by weighing the fillets pre and post the process treatments. The trays were allowed to drain for 10 min and the difference in weight was determined and the percentage gravity drip loss was calculated (w/w).

Free fatty acids (FFAs) and peroxide values (PVs)

Free fatty acids and PVs were measured for mackerel and salmon. To extract the fat, equal volumes of petroleum spirit and diethyl ether (500 cm^3) were mixed with 300g of fish in a blender (Masterchef 500, Moulinex, France) for 5 min. and the solvent plus fat were decanted into a beaker. After evaporation on a water

bath, the remaining oil was dried in an oven at 100° C for 1h, allowed to cool, and tested for FFAs and PVs. The AOAC method 940.28 (1990a) was used for the former and 965.33 (1990b) for the latter. Free fatty acids were expressed as % oleic acid (i.e. the cm^3 0.25M NaOH used in the titration corresponds to % oleic acid) and PVs as meq. peroxide/kg sample. Free fatty acids and PVs were measured in duplicate per process treatment per replicate.

Total viable count (TVC)

Approximately 2.5 cm of flesh was cut from the tail-end of five fillets of whiting and mackerel, and from three portions of salmon. A representative 10g sample was stomached with 90 cm^3 diluent (1/4 strength Ringers solution, Oxoid, code BR52) and tested by the pour plate method (standard plate count agar, Oxoid, code CM463) to determine TVC. Incubation was at 30°C for 72h. Microbial loads were expressed as $\log_{10}$ cfu/g. This was done for each process treatment per replicate.

Total volatile base nitrogen (TVBN) and trimethylamine (TMA)

Total volatile base nitrogen was measured using the method of Malle and Tao (1986). Samples of fish muscle (100g) were homogenised in aqueous (200cm^3, 7.5ml/100ml) trichloroacetic acid solution and the homogenate was centrifuged at 400 x g for 5 min. The supernatant liquid was filtered (Whatman No. 3 filter paper) and the filtrate (25 cm^3) was loaded into a Kjeldahl-type distillation tube followed by 5 cm^3 of 10g/100ml (w/v) NaOH. Steam distillation was continued into aqueous boric acid solution (4ml/100mla final volume of 50 cm^3 was

obtained in the beaker (40 cm^3 of distillate). Titration used aqueous 0.05M sulphuric acid solution and the quantity of TVBN was determined by the formula TVBN = n x 16.8 mg of N/100g, where n was the amount (cm^3) of sulphuric acid required.

The same experimental procedure and reagents were used for the TMA analysis (Malle and Tao, 1986) but formaldehyde (20 cm^3; 38 ml/100ml) was also added to the distillation tube. Increasing volumes of formaldehyde were added to 24 cm^3 of filtrate in order to determine how much formaldehyde was required to block the primary and second amines (leaving only tertiary amines to react). Steam distillation was then performed as for the TVBN assay. The amount of volatile amines was measured and the results were expressed in mg nitrogen/100g. When the required amount of formaldehyde was added, only the TMA was distilled. The TMA content was calculated from the volume of 0.05M sulphuric acid used for titration by the formula TMA = n' x 16.8 mg N, where n' was the amount (cm^3) of sulphuric acid required. For the three species, TVBN and TMA were measured in triplicate per process treatment per replicate and the results averaged. Three separate fillets were used each time for whiting and mackerel, and three portions for salmon (taken from approximately the same location on the fillet).

Moisture content (g/100g)

Samples of thawed fish (3-5g) were dried at 70°C and 74.7 mPa for ~ 15 h in a vacuum oven to constant weight. The moisture content was determined by weight

difference and was expressed as a percentage (w/w) of the original thawed mass. Measurements were made in triplicate for each treatment per replicate.

Results

Odour and acceptability scores

The samples which were frozen received better odour scores (lower values) than the chilled or freeze-chilled fish for both whiting ($P < 0.05$) and salmon ($P < 0.001$) (**Table 1**). The process treatments had no effect on the odour scores for mackerel. There was no statistical difference between the odour scores for the chilled or freeze-chilled samples of both whiting and mackerel, both of which were in chill storage at 4°C for 3d. However, chilled salmon received a much less favourable ($P<0.001$) odour score than freeze-chilled; the salmon samples were in chill storage at 4°C for 5d. Time of sniffing (immediately on pack opening vs 10 min after pack opening) had no influence on the odour scores of any of the three species.

Fresh fillets received the highest panel acceptability scores followed by frozen, chilled and freeze-chilled samples. (**Table 2**; $P < 0.001$). The pattern in the data was the same for the three species. Inspection of the results indicated no statistically significant difference between the chilled and freeze-chilled samples. Mackerel had lower acceptability scores than the other two species with the exception of the chilled and freeze-chilled samples where the salmon portions

received the lowest acceptability scores (**Table 2**). However, salmon samples were tested after 5d in chill storage compared with 3d for mackerel and whiting.

Colour

The process treatments did not influence the whiteness or redness values for whiting (mean L = 51.2; a = +1.33) or mackerel (mean L = 40.6; a = +4.5) fillets. However, for salmon the chilled portions had a higher ($P< 0.05$) lightness value than the other samples (**Table 5**). Salmon from the fresh and frozen treatments was also redder ($P<0.001$) than the chilled or freeze-chilled samples (**Table 5**). Whiting from the freeze-chilled treatment was yellower (**Table 3**; $P< 0.01$) than the other samples but the effect was small in practical terms. In mackerel, the effects were more pronounced with the fresh sample showing the least yellowing (**Table 4**; $P< 0.001$). Total colour difference values (i.e. from the fresh sample) (ΔE) were only different for the mackerel samples where there was a progressive increase in ΔE values from chilled to frozen to freeze-chilled (i.e. 3.44, vs 4.98 vs 5.36) respectively.

Texture (% recompression / compression)

The fresh whiting sample was less springy ($P<0.01$) than samples from the other three treatments (**Table 3**). The process treatments had no influence on the springiness values of mackerel (mean 91.5%) or salmon (mean 95.0%), or on the shear values of the raw flesh of each species. Mean shear values were 1.75, 1.84 and 1.72 kN/100g for whiting, mackerel and salmon respectively.

Drip loss

The pattern in the gravity drip data was the same for each species in that the chilled samples had least, the frozen samples most, while the freeze-chilled packs were intermediate (**Tables 3-5**). However, the magnitude of the effects varied from species to species; e.g. the chilled and freeze-chilled whiting ($P< 0.001$) samples were different [similarly for salmon ($P< 0.001$) whereas the mackerel samples were not].

Free fatty acids (FFAs) and peroxide values (PVs)

Freeze-chilled mackerel and salmon samples had higher ($P<0.001$) FFA values than samples from the other treatments (**Tables 4 and 5**). Peroxide values were also highest ($P< 0.001$) in the freeze-chilled salmon samples but were not influenced by any of the process treatments in the case of mackerel (mean 1.95 meq. peroxide/kg sample).

Total volatile base nitrogen (TVBN) and trimethylamine (TMA)

The patterns for the TVBN and TMA data were the same for the three species in that the chilled and freeze-chilled samples had the highest values ($P< 0.05$ to $P< 0.001$) (**Tables 3-5**) and the fresh and frozen the lowest. There was no difference between the TVBN values of the chilled and freeze-chilled samples for any of the species but the chilled mackerel ($P< 0.05$) and salmon ($P< 0.001$) had higher TMA values than the freeze-chilled samples.

Total viable count (TVC)

The pattern in the TVC data was similar to that found for TVBN, i.e. the chilled and freeze-chilled samples of the three species had higher ($P< 0.01$ to $P< 0.001$) counts than the fresh or frozen samples (**Tables 3-5**). However, there was no difference between the counts for the chilled and freeze-chilled samples for each of the species. The frozen samples had lower counts than the fresh, but the effect was only statistically significant in the case of salmon.

Moisture

The process treatments had no statistically significant effect on the moisture content of the three species and the mean values were 82.2, 69.6 and 67.4 g/100g for whiting, mackerel and salmon portions respectively.

Discussion

Freeze-chill technology has logistic and other advantages in the preparation, distribution and retailing of chilled foods (O'Leary *et al.*, 2002; Redmond *et al.*, 2002). Freeze-chilling has particular application for prepacked fish fillets which are increasingly replacing the traditional iced counter in some supermarkets (Fagan *et al.*, 2002). The aim of these trials therefore, was to determine whether freezing prior to chilling caused more rapid spoilage of prepacked fish portions during chilled storage at 4°C compared with fish that had not been frozen before chilling. Extensive literature has been published on the effects of freezing and chilling on selected quality parameters of fish (Refsgaard *et al.*, 1998; Nilsson *et al.*, 1995; Magnússon and Martinsdóttir, 1995). However, the use of freeze-chill technology has received relatively less attention (Martinsdóttir and Magnússon, 2001; Bøknæs *et al.*, 2002; Emborg *et al.*, 2002; Guldager *et al.*, 1998; Bøknæs *et al.*, 2000; Bøknæs *et al.*, 2001; Fagan *et al.*, 2002). In the current trials, 3d in chilled storage were chosen for whiting and mackerel and 5d for salmon. This was based on pre-tests and on discussions with supermarkets on the likely shelf-life of prepacked chilled fish portions from fish currently being landed at Irish fishing ports, or obtained from Irish fish farms (in the case of salmon). This 3-5d timespan in chilled storage agrees with the recommendations of Farber (1995) and Church (1998), but is considerably shorter than the extended chilled storage times for thawed cod in modified atmosphere reported by Bøknæs *et al.* (2000) and Bøknæs *et al.* (2001). These studies showed inhibition or inactivation of the specific spoilage organism *P. phosphoreum* in cod packed in modified atmosphere. The mentioned extended storage times for thawed cod products were

related to these findings. The storage temperature of –30°C used in the frozen storage component of the freeze-chill process in the current study is important for maintaining fish quality (Gormely *et al.*, 2002).

In terms of fish quality, certain parameters appear to be more important than others and are closely correlated with consumer preference. Laslett and Bremner (1979) (using a laboratory panel) found that the important predictors of acceptance were fish flavour, off flavour and toughness for fish minces, and off flavour and fish flavour for fish fingers. Procedures for assessing fish freshness have been reviewed by Whittle *et al.* (1990) while the upgraded quality index method (Nielsen and Jessen, 1997) is used as a sensory system for determining the freshness of thawed whole cod and is particularly relevant as much raw material (for further processing) is received as frozen fish. The multilingual guide to EC freshness grades for fishery products (Howgate *et al.*, 1992) uses four freshness grades [E, A, B, C (reject)], and 33 fish species are currently provided for in the regulations. There are also European guidelines, which are interpreted and implemented in different ways in member states (e.g. Department of Health and Children, Ireland suggest upper TVC limits (1992) of log 5 cfu/g) governing the sale of raw fish fillets and guidelines are in place. The TVBN content should not exceed 35mg N/100g flesh (95/149/EC).

Odour testing was utilised in the current study to determine if there was an off-odour at time of pack opening, and if so, did it remain 10 min after pack opening. Such odours are off-putting for the consumer. The results showed no difference

between the odour scores at times 0 and 10 min. With the exception of the chilled and freeze-chilled salmon samples, all odour scores were below the mid-point (i.e. 3.5) of the odour scale (1 = fresh seaweed-like smell; 6 = putrid) and could be classed as odourless or having a slight fishy odour, i.e. they were quite acceptable. The freeze-chilled samples received more favourable odour scores than the chilled ones but the data were not statistically significant except in the case of salmon. The freeze-chilled salmon sample also received a middle rating (3.60) but the chilled sample score of 5.61 indicated a strong off-odour, and that 5d at 4°C was the upper limit of its shelf-life (**Table 1**); these two samples also had the highest TVC values (**Table 5**).

Taste panel acceptability scores indicated that the freeze-chilled samples received the lowest ratings but the results were not statistically different from the chilled samples indicating that freeze-chilling, in comparison with chilling, was not having a deleterious effect on portion acceptability. The freeze-chilled samples were the oldest and could be expected to have the lowest ratings, whereas fresh should have the highest acceptability rating which was the case. However, the acceptability scores for all the samples were > 3.0 on a scale from 0 (unacceptable) to 6 (very acceptable) indicating that all were acceptable. The single stimulus taste panel procedure (Gormley, 1989) used in these tests proved satisfactory and 25 or more tasters were used each time. The acceptability tests used in these trials were both extensive and comprehensive and gave a good indication of the acceptability of the packaged fish.

The odour and acceptability data related well to the TVBN, TMA and TVC values for the different species from the four process treatments. The slight fishy odours encountered in the whiting, mackerel and salmon were most likely due, in part, to the production of TVBN and TMA. The measured TVBN / TMA values after 3-5d at 4°C were relatively high. These were typical of values for commercially landed fish in Ireland. There was no difference in the TVBN values between chilled and freeze-chilled samples of the three species and TMA values followed a similar pattern. As expected, the fresh and frozen samples had lower TVBN/TMA values than the chilled or freeze-chilled samples. In whiting, the fishy odour can be attributed to the production of TVBN and TMA predominately by spoilage organisms belonging to the genus *Pseudomonas* (Shewan, 1971). The TVBN values were positively correlated with TVC [r = +0.62 (whiting); +0.77 (mackerel); +0.81 (salmon)] and negatively correlated with acceptability scores [r = -0.61 (whiting); -0.63 (mackerel); -0.65 (salmon)]. The TVC values were, in turn negatively correlated with acceptability scores [r = -0.65 (whiting); -0.65 (mackerel); -0.81 (salmon)]. These data show that fillet acceptability decreased as TVBN, TMA and TVC values increased. The role of TVBN/TMA as indicators of fish quality has been discussed by Whittle *et al.* (1990) and by Dalgaard (2000). The TVBN values in the current study were all below the EC guidelines (Council Regulation No. 95/149/EEC of March 1995) for raw fish (35mg N/100g flesh). The TMA values for mackerel were much higher than those of 1mg/100g reported by Jhaveri *et al.* (1982) for mackerel stored at 0°C on ice for 4d. In salmon, the lowest TVBN value was in the frozen sample and this could be due to a reduction

in the TMA-producing bacteria caused by blast-freezing followed by storage at –30°C for 3d (Magnüsson and Martinsdóttir, 1995).

The TVC values for the three species from the four process treatments were acceptable for raw fish (Department of Health and Children, Ireland, 1992) with the exception of chilled and freeze-chilled salmon portions, which had values > log 7 cfu/g after 5d in chilled storage; the fresh salmon samples had a TVC value of $\log_{10}$ 5.08 cfu/g which is indicative of in-factory hygiene practices. Detailed microbiological tests (e.g. specific spoilage organisms) were not conducted in the current study and the TVC data should be interpreted as indicators of hygiene. It was anticipated that freeze-chilling could be conducive to microbial growth as freezing opens up product structure and results in more drip than chilling alone. However, the data showed this was not the case as the freeze-chilled fish samples had lower TVC values than the chilled with the exception of salmon where the freeze-chilled sample had a slightly higher (non significant) value. The standard deviations between treatments for TVCs in salmon were similar for the 5 replicates with values of 1.40, 1.49, 1.53, 1.50 and 1.50. The TVC values for the chilled and freeze-chilled mackerel samples at 5.34 and 5.14 cfu/g were higher than the value of < log 5 cfu/g reported by Jhaveri *et al.* (1982) for mackerel on ice for 4d. Overall, the TVC values for the three species were generally slightly lower than those of Guldager *et al.* (1998) for modified atmosphere packed cod held on ice for 3-4d. The TVCs in the study of Guldager *et al.* (1998) were measured by Long and Hammer incubated at 15°C. Therefore, it is not surprising that Guldager *et al.* (1998) showed higher TVCs compared to the present study.

However, cognisance must also be taken of the fact that comparisons between species and different trials are difficult due to variations in raw material quality and to experimental conditions. The high TVC values found for the raw salmon may be due to the scattering of fish slime during filleting; it is recommended therefore that the body of the salmon be cleaned up using high-pressure water prior to filleting.

Development of off-flavour is one of the major effects of lipid oxidation (Flick *et al.*, 1992). In the current trials, the freeze-chilled samples had the highest FFA and PV values but the levels were low and unlikely to effect sensory responses; this was borne out in the odour and taste acceptability panel results as rancid off-notes were never cited by the panellists. The FFAs were similar and the PVs lower than those reported by Gormley *et al.* (2002) for deep-frozen salmon and smoked mackerel.

Raw fillet colour in-pack is of major importance as it is highly visible to the consumer both at time of purchase and during cooking in the home. Freeze-chilling promoted some yellowing in whiting fillets, while in mackerel and salmon portions, the chill, freeze and freeze-chill treatments gave an inferior colour to the fresh. In addition, the biggest colour difference (ΔE) from fresh was in the freeze-chilled sample. However, all the colour effects were small in practical terms.

Visible drip in the pack is also off-putting for the consumer. The gravity drip in the current trials was significant for the freeze and freeze-chill treatments (**Tables 3-5)** but presented no major visual problems; any potential negative effects could be overcome by the use of drip pads. Gravity drip for the frozen samples was higher than for the freeze-chilled and a similar effect was found for centrifugal drip in mackerel. An explanation may be that the fillets reabsorbed some of the drip generated by the freezing step during the chilled storage period. However, this was not reflected in the moisture contents of the fish samples.

The freezing treatments (i.e. freeze or freeze-chill) did not influence the shear values of the raw fillets of any of the species and the only textural difference detected was the lower springiness value in fresh whiting fillets. The frozen storage time of 3d in the current study was short, but some toughening could be anticipated if the time for the freezing component of the freeze-chill treatment was extended to several months (Gormley *et al.*, 1993; Howell, 1995).

The outcome from these trials is that freeze-chilling is a suitable technology for prepacked whiting, mackerel and salmon portions. However, it is important to stress that the samples were held frozen for only 3d prior to thawing and chilling. Raw material freshness is of paramount importance and fillet freezing at sea or on-farm would be highly beneficial in delivering an extended shelf-life in the chilled phase of the freeze-chilled process. Combining freeze-chilling with modified atmosphere packing is also an option. Extended times of frozen storage in the freeze-chill process should be tested to study potential deleterious changes

in product quality including increased drip, toughening, oxidation and changes in colour. In this context Refsgaard *et al.* (1998) found that only minor sensory changes occurred in salmon stored at –30°C for 34 weeks. Gormley *et al.* (2002) produced largely similar findings for salmon and smoked mackerel but showed that fluctuating temperatures below the freezing point gave increased rancidity over an 8 month period.

The use of good manufacturing practices (GMP) and hazard analysis of critical control point (HACCP) is imperative in the production, storage, distribution and retailing of freeze-chilled foods. National and EU guidelines should also be adhered to (Gormley and Butler, 2002). Particular attention should also be focused on the thawing step and careful temperature control should be exercised. In the case of freeze-chilled fish fillets, tempering can be achieved by transferring prepacks from the supermarket deep freeze room to the chilled retail display cabinets in the evening time, thus resulting in a tempered (thawed) product the following morning. The normal safety rules for frozen foods prevail in the frozen component of the process, and those for chilled foods in the chill phase. The labelling requirements are those of conventionally chilled foods. However, it is desirable for reasons of consumer information and product liability to label the product as "previously frozen", A use-by-date must also be employed and this label should be attached at the start of the thawing process. A use-by-date must also be employed and this label should be attached at the start of the thawing process. This is 3d (whiting and mackerel) and 5d (salmon) based on the findings of this study.

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Table 1 Average odour (sniff) scores[a,b] for chilled, frozen and freeze-chilled portions of raw whiting, mackerel and farmed salmon

Species	Chilled	Frozen	Freeze-chilled	LSD[c]	*F*-test
Whiting	3.15	2.67	3.13	0.36	P< 0.05
Mackerel	2.86	3.00	2.83	0.28	NS[d]
Salmon	5.61	1.94	3.60	0.62	P< 0.001

[a] On a 6-point scale from 1 (fresh seaweed-like smell) to 6 (totally off, i.e. putrid smell)
[b] Data analysed over two times of testing (t_o and t_{10})
[c] Least significant difference
[d] Not significant

Table 2 Average taste panel acceptability scores[a,b,c] for fresh, chilled, frozen and freeze-chilled portions of whiting (steamed), mackerel (grilled) and salmon (steamed)

Species	Fresh	Chilled	Frozen	Freeze-chilled	LSD[d]	*F*-test
Whiting	4.52	3.70	4.16	3.60 (steamed)	0.32	P< 0.001
Mackerel	4.07	3.61	3.85	3.30 (grilled)	0.35	P< 0.001
Salmon	4.22	3.48	4.20	3.24 (steamed)	0.40	P< 0.001

[a] On a 6 cm line with endpoints of 0 (unacceptable) and 6 (very acceptable)
[b] Single sample hedonic panels; 25 tasters
[c] Collective data for the 5 replicates
[d] Least significant difference

Table 3 Effect of process treatments on a number of quality parameters for raw whiting fillets

Parameter	Average values				LSD[a]	F-test
	Fresh	Chilled	Frozen	Freeze-chilled		
Yellowness (b)	-0.79	+0.23	+0.90	+2.53	1.39	P< 0.01
Springiness (%)[b]	79.6	85.6	84.0	85.4	6.90	P< 0.05
Gravity drip (g/100g	-	1.00	9.00	6.00	1.13	P< 0.001
TVBN[c] (mg N/100g)	13.7	25.0	17.5	25.5	6.36	P< 0.01
TMA[d] (mg N/100g)	2.40	12.0	6.90	12.1	4.08	P< 0.001
TVC[e] (log_{10} cfu/g)	4.14	5.54	4.04	5.24	0.50	P< 0.001

[a] Least significant difference
[b] Recompression / compression x 100
[c] Total volatile base nitrogen
[d] Trimethylamine
[e] Total viable count

Table 4 Effect of process treatments on a number of quality parameters for raw mackerel fillets

Parameter	Average values				LSD[a]	F-test
	Fresh	Chilled	Frozen	Freeze-chilled		
Yellowness (b)	4.40	6.90	7.90	7.90	1.43	P< 0.001
White/yellow ratio (L/b)	9.40	5.90	5.00	5.70	1.45	P< 0.001
Colour difference (ΔE^b)	-	3.44	4.98	5.36	1.15	P< 0.05
Centrifugal drip (g/100g	5.10	5.70	10.1	9.60	3.18	P< 0.05
Gravity drip (%)	-	2.00	10.00	4.00	4.00	P< 0.05
FFA[c] (g/100g acid)	0.58	0.51	0.51	2.02	0.39	P< 0.001
TVBN[a] (mg N/100g)	15.9	23.1	17.9	21.1	3.70	P< 0.05
TMA[a] (mg N/100g)	3.90	8.70	3.50	5.30	2.98	P< 0.05
TVC[a] ($\log_{10}$ cfu/g)	4.52	5.34	4.26	5.14	0.56	P< 0.01

[a] See footnotes in Table 3

[b] $\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$

[c] Free fatty acids

Table 5 Effect of process treatments on a number of quality parameters for raw prepacked (in air) salmon portions

Parameter	Average values				LSD[a]	F-test
	Fresh	Chilled	Frozen	Freeze-chilled		
Whiteness (L)	40.3	42.7	40.8	39.8	1.82	P< 0.05
Redness (a)	21.2	17.7	19.0	17.4	1.08	P< 0.001
Yellowness (b)	14.6	12.2	12.6	12.7	0.74	P< 0.001
White/yellow (L/b)	2.78	3.52	3.24	3.14	0.24	P< 0.001
Centrifugal Drip (g/100g)	2.80	1.02	5.08	1.06	0.79	P< 0.001
Gravity drip (g/100g)	-	1.70	3.30	3.20	0.35	P< 0.001
Moisture (g/100g)	68.3	66.8	67.9	66.7	0.82	P< 0.001
FFA[b] (g/100gacid)	0.31	0.74	0.33	1.60	0.26	P< 0.001
Peroxide value (meq. perox /kg)	1.62	2.22	2.18	4.40	0.23	P< 0.001
TVBN[a] (mg N/100g)	17.0	22.7	14.8	20.2	2.33	P< 0.001
TMA[a] (mg N/100g)	2.10	6.00	1.70	4.10	0.91	P< 0.001
TVC[a] (log_{10} cfu/g)	5.08	7.36	4.78	7.56	0.20	P< 0.001

[a] See footnotes in Table 3
[b] See footnotes in Table 4

8.4 Paper

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Effect of Modified Atmosphere Packaging with Freeze-chilling on some quality parameters of Raw Whiting, Mackerel and Salmon Portions.

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Abstract

Modified atmosphere packaging (MAP) was combined with freeze-chilling to extend the shelf-life of raw whiting, mackerel and salmon fillets/portions. The MAP packs for mackerel and salmon (60% N_2 / 40% CO_2), and for whiting (30% N_2 / 40% CO_2 / 30% O_2) maintained their shape during freeze-chilling whereas packs with 100% CO_2 were slightly imploded with concave sides. The chosen chilled shelf-life of 5-7d in the MAP trials was vindicated by the results as the products were near the end of their shelf-life (in acceptability terms) after 5 (whiting and mackerel) and 7 (salmon) days. This compares with shelf lives of 3 and 5d respectively for freeze-chilled fillets in air. Samples in MAP had lower total viable counts than samples in air for raw fillets/portions of each of the three species. However, MAP did not influence odour or acceptability scores, but had a variable effect (generally small) on fillet colour, springiness, drip loss, total volatile base nitrogen / trimethylamine content, peroxide values and free fatty acid contents.

Keywords: Modified atmosphere packing, freeze-chilling, fish portions, quality, acceptability

Industrial relevance: Raw fish is highly perishable and has a short shelf-life. The results of these tests showed that raw whiting, mackerel and salmon portions/fillets performed well as freeze-chilled MAP products and that these combined technologies confer logistic benefits in product distribution and retailing.

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1. Introduction

The market for seafood products in Europe has grown strongly in recent years, fuelled by increases in the average unit value of seafood products. Multiples are pushing suppliers to innovate to allow them to grow their share of the lucrative fresh/chilled seafood markets and eliminate the requirement for low-yield fresh seafood counters (Price Waterhouse Coopers, 2001). Two methods which have considerable potential to extend the shelf-life of raw fish fillets are freeze-chilling and modified atmosphere packaging (MAP), and combination of the two could have synergistic effects on product quality. Freeze-chilling of food involves freezing and frozen storage followed by thawing and retailing at chill storage temperatures [(O'Leary, Gormley, Butler, and Shilton. (2000), Redmond, Butler and Gormley (2002), Fagan, Gormley and Uí Mhuircheartaigh (2003))]. Freeze-chilling offers logistic and other advantages as foods can be prepared in bulk, frozen and stored at deep freeze temperatures until required. Freeze-chilling has received some attention as a method of preserving fish fillets [(Guldager, Bøknæs, Østerberg, Nielsen, and Dalgaard (1998); Bøknæs, Østerberg, Nielsen, and Dalgaard (2000); Bøknæs, Østerberg, Sørensen, Nielsen and Dalgaard. (2001); Fagan *et al.*, (2003)]. Farber (1995), Church (1998) and Murray, Gibson and Shewan (1971) have shown an approximate shelf life of 3-5d (at ca 4°C) for prepackaged freeze-chilled fish fillets in chilled storage. However, other authors have reported much longer shelf-life extensions using MAP, for example, Guldager *et al.*, (1998) achieved a shelf-life of >20d on thawed cod fillets packed in MAP and Tiffney and Mills (1982) reported a 15d shelf-life for whiting fillets packed in 100% CO_2 (stored at 4°C). The effects of freeze-chilling on some chemical, physical and sensory properties of raw whiting, mackerel and salmon

portions were assessed in previous trials (Fagan *et al.*, 2003) and the three species performed well as freeze-chilled products with whiting and mackerel fillets having a shelf-life of 3 days and salmon portions 5 days in the chill component of the freeze-chill process. Modified atmosphere packaging (MAP) has been used successfully to extend the raw fillet shelf-life of many species [(Cann, Smith and Houston, (1983), Scott, Fletcher and Summers, (1984), Barnett, Conrad and Nelson, (1987)] and shelf-life extensions of 25-100% have been reported [(Gopal, Nambiar, Bhattarcharyya, Joseph and Prabhu, (1990), Cann, (1984), Church, (1998)]. Relatively little work has been done on the combination of freeze-chilling with MAP for extending the shelf-life of raw fish portions (Bøknæs *et al.*, (2000); Bøknæs, Østerberg, Sørensen, Nielsen and Dalgaard. (2001); Bøknæs, Kristina, Jensen, Charlotte, Andersen and Martens, (2002). The objective of the current trials was to assess the suitability of freeze-chilling in combination with modified atmosphere packaging for extending the shelf-life of raw whiting, mackerel and salmon portions. Combining freeze-chilling and MAP will allow fish fillets to reach distant markets in a frozen condition followed by thawing for sale at retail outlets. The inclusion of a modified atmosphere should be beneficial in the chill storage phase (post-thawing) by inhibiting the natural spoilage organisms on the surface of the fillet thus extending the shelf-life.

2. Materials and Methods

Whiting and mackerel were purchased from a local fish company in Dublin (Ireland) as skin-on butterfly fillets and were transported to the laboratory on ice. Farmed salmon was purchased as skinless and boneless fillets and these were cut

into portions of approximately 250g. Twenty-five fillets/portions of each species were tested within 6 hours to provide data for the fresh samples. Ten were used for sensory analysis and fifteen for physical and chemical tests. One hundred and fifty fillets/portions of each species were individually placed into plastic (HDPE) trays (Dynopak, Ireland) per replicate. Fifty fillets/portions (per species) were packed in air and these conditions were maintained by piercing 6 small holes in the film. Fifty packs were filled with a premixed 60% N_2 / 40% CO_2 (mackerel and salmon) or 30% N_2 / 40% CO_2 / 30% O_2 (whiting) gas and the remaining 50 packs were filled with 100% CO_2. All packs were sealed with a 340mm Antifog High barrier film (Dynopak, Ireland) and were blast-frozen at –35°C for 2.5 hours. Packs were stored at –30°C for 3 days, after which time, they were placed in a chill room at 2 to 4°C for 5 (whiting and mackerel) or 7 (salmon) days. In the case of whiting and mackerel, 25 packs were tested on days 3 and 5, and for salmon, 25 packs were tested on days 5 and 7. For each species, a range of physical, chemical and sensory tests were carried out, as cited by Fagan *et al.*, (2003) and included color (Hunter Lab scale), total volatile base nitrogen (TVBN mg N/100g), trimethylamine (TMA mg N/100g) (Malle and Tao, 1986), moisture content (%) (O'Leary *et al.*, 2000), centrifugal drip (%) (Wierbiki and Deatherage., 1958), gravity drip (%) (Fagan *et al.*, 2003), free fatty acids (FFA) (expressed as % oleic acid) (Official method, AOAC, 1990a), peroxide value (PV) (milliequiv. peroxide/kg sample) (Official method, AOAC, 1990b), total viable count (log_{10} cfu/g) (Fagan *et al.*, 2003), springiness (%) (Sigurgisladottoir, Hafsteinsson, Jonsson, Lie, Nortvedt, Thomassen and Torrissen, 1999), shear (N) (using a standard Kramer shear cell), and odor / sniff tests on pack opening (scale from 1

fresh to 5 putrid) (Fagan *et al.*, 2003). Taste panel acceptability tests (25 tasters) were carried out using a hedonic scale with a 6 cm line (0 unacceptable to 6 very acceptable) as cited by Fagan *et al.*, (2003). On days 3 and 5, panelists were given three samples of fish (circa 50g) i.e. one sample from each gaseous atmosphere and were asked to mark the 6 cm line. The gaseous atmospheres in the packs were measured using a MAP-test 3050-gas analyser (Hitech Instruments, England). Four packs from each treatment per replicate were sampled as they came due for testing and the results were averaged. The experimental design was 3 gaseous atmospheres x 2 test days x 4 reps with 23 degrees freedom and the results were analysed by ANOVA using SAS (Version 6.12, SAS Institute Inc., Cary, NC, USA).

3.0 Results

The MAP packs stood up well to the freeze-chill process and the 60% N_2 / 40% CO_2 (mackerel and salmon) or 30% N_2 / 40% CO_2 / 30% O_2 (whiting) maintained their shape (no pack implosion) throughout the storage. However, all the 100% CO_2 packs imploded slightly, with the sides of the packs being concave. In the presentation of the results, the term MAP refers to packs with air or the various gas mixtures, while day 0 (d0), day 3 (d3), day 5 (d5) and day 7 (d7) refers to MAP packs held at 2 to 4°C, i.e. in the chilled phase of the freeze-chill process.

3.1 Odour and acceptability scores

Time of sniffing (i.e. on pack opening versus 10 min after pack opening) had an effect on odour scores in whiting, with fillets scoring better ($P< 0.05$) 10 min after pack opening (Table 1). Modified atmosphere packaging (MAP) had no effect on odour scores for the three species whereas storage time (days) did. The odour of whiting ($P< 0.001$) and mackerel ($P< 0.05$) deteriorated with time (i.e. higher values; Table 1) on d5 than on d3. Likewise the odour of salmon ($P<0.01$) was poorer on d7 than on d5. There was an interaction ($P< 0.05$) between MAP treatment and storage time for salmon portions which showed that d5 salmon portions packed in 60% N_2 / 40% CO_2 and 100% CO_2 scored lower (better) ($P< 0.05$) than portions packed in air.

Taste panel acceptability on a scale from 0 (unacceptable) to 6 (very acceptable) showed no significant difference between the gaseous atmospheres after 5 (whiting and mackerel) or 7d (salmon). However, all treatments scored lower than

the fresh samples for each species ($P<0.001$; $P< 0.05$; $P< 0.001$ respectively) (Table 2).

3.2 Colour (Hunter Lab values)

Modified atmosphere packaging had no effect on Hunter L or a values for whiting (means 51.2, -0.73), mackerel (43.0, 4.40) or salmon (41.4, 17.9) portions. Hunter b values showed air-packed whiting ($P< 0.05$) and salmon portions ($P< 0.01$) to be less yellow than those in 100% CO_2 (Table 3). There was an interaction ($P< 0.05$) between MAP and storage time for whiting fillets for Hunter b values. On d3 portions packed in air had lower b values than those packed in 30% N_2 / 40% CO_2 / 30% O_2, conversely on d5, portions packed in 30% N_2 / 40% CO_2 / 30% O_2 had lower b values than those packed in air and 100% CO_2. Storage time (days) had an effect on Hunter L ($P< 0.05$) and a ($P< 0.05$) values for whiting (Table 4). Whiting ($P< 0.01$) and mackerel ($P< 0.001$) portions were more yellow on d5 than on d3 than on d0. However, this effect was not observed in salmon portions (mean b value of 14.7).

3.3 Texture

Storage time (days) at 2 to 4°C had no effect on the springiness (%) of whiting (mean 84.7%), mackerel (mean 92.2%) or salmon (mean 94.3%) portions. MAP treatments also had no effect on the springiness of whiting and salmon portions. However, mackerel fillets stored in 60% N_2 / 40% CO_2 were less springy ($P< 0.01$) than samples from the other treatments (Table 3). However, the magnitude of the effect was small in practical terms. There was an interaction ($P< 0.05$)

between MAP and storage time for mackerel fillets. On d3 fillets packed in 60% N_2 / 40% CO_2 were the least springy, however on d5, fillets packed in air were the least springy.

Both d3 and d5 whiting fillets had lower shear values ($P< 0.001$) and were significantly less tough than the fresh sample (day 0) (Table 4). Mackerel fillets had higher ($P< 0.05$) shear values on d3 than on d0 and d5. MAP treatment had no effect on whiting fillet shear values (mean 1.56 kN). However, mackerel fillets stored in 100% CO_2 had higher ($P< 0.001$) shear values than samples from the other treatments. There was an interaction ($P< 0.05$) between MAP treatment and storage time (days) for mackerel fillets with d3 fillets having higher shear values than d0 in each atmosphere and d5 fillets having lower shear values than d3. MAP treatment and storage time had no effect on shear values (kN) of salmon portions and the mean value was 1.76 kN.

3.4 Drip loss

MAP treatment and storage time (days) had no effect on the moisture content of whiting, mackerel and salmon portions and mean values were 80.6, 68.3 and 67.1g/100g respectively. MAP treatment had an effect on gravity drip (GD) values for the three species. Portions packed in 100% CO_2 had higher GD than those in 30% O_2 / 40% CO_2 / 30% N_2 (whiting; $P< 0.05$) and 60% N_2 / 40% CO_2 (mackerel; $P< 0.001$, salmon: $P< 0.001$). In turn, samples packed in air had less GD than in the other two MAP treatments (Table 3). Storage time at 2 to 4°C had no effect on the GD values for the three species. There was an interaction ($P<$

0.05) between MAP and storage time for mackerel fillets. On d3 and d5 fillets packed in 100% CO_2 had the highest GD (%) with no difference between GD in air-packed and 60% N_2 / 40% CO_2-packed samples. However air-packed fillets had higher drip ($P< 0.05$) on d5 than on d3.

Whiting ($P< 0.001$), mackerel ($P< 0.001$) and salmon ($P< 0.05$) showed an increase in centrifugal drip with time of storage at 2-4°C (Table 4). Mackerel fillets packed in 60% N_2 / 40% CO_2 had less CD ($P< 0.01$) than in other MAP treatments and salmon portions packed in 100% CO_2 had the highest ($P< 0.001$) CD (Table 3). There was an interaction ($P< 0.05$) between MAP and storage time for mackerel fillets. On d3, fillets packed in 60% N_2 / 40% CO_2 had the lowest CD and there was no significant difference between CD in air-packed and 100% CO_2-packed fillets. On d5, there was no significant difference between fillets packed in air and 60% N_2 / 40% CO_2 with 100% CO_2-packed fillets having the highest CD.

3.5 Free fatty acids (FFAs) and peroxide values (PVs)

Mackerel fillets packed in 100% CO_2 had the highest percentage of ($P< 0.05$) free fatty acids. In contrast however, salmon fillets packed in this gaseous atmosphere had the lowest ($P< 0.001$) FFA values (Table 3). Storage time had an effect on FFA values for mackerel ($P< 0.001$) and salmon ($P< 0.001$) portions with d3 and d5 samples having higher FFAs than d0 (fresh) (Table 4). The patterns for the peroxide value were largely similar to those for the FFAs (Table 3 - 4). All values for mackerel (1.4 - 2.0) and salmon (0.84 - 0.95) remained roughly constant irrespective of gaseous atmosphere after an interval rise from time 0. There were

significant interactions for both FFA ($P< 0.001$) and peroxide values ($P< 0.05$) for salmon portions. Free fatty acid values for salmon portions packed in air and 60% N_2 / 40% CO_2 increased with respect to (storage) time, however there was no significant difference between portions packed in 100% CO_2 on days five and seven. There was no significant difference in PVs on days five and seven. Peroxide values for day 5 portions packed in 60% N_2 / 40% CO_2 were similar to those packed in air and however on day 7 portions packed in 60% N_2 / 40% CO_2 had higher PVs than those packed in air.

3.6 Total volatile base nitrogen (TVBN) and trimethylamine (TMA)

Modified atmosphere packaging had no effect on TVBN or TMA values in whiting and salmon portions. However, mackerel fillets packed in air had higher TVBN ($P< 0.05$) and TMA ($P< 0.001$) levels than in other treatments (Table 3). Mackerel ($P< 0.001$) chilled for 3 and 5 days and salmon ($P< 0.01$) for 5 and 7 days respectively had higher TVBN levels than day 0 (fresh) samples. There was a progressive increase in TVBN ($P< 0.001$) and TMA ($P< 0.001$) levels with time of chilling in whiting fillets (Table 4.). Storage time had no effect on TMA levels in mackerel and salmon portions (Table 4.) (mean values 3.21 and 1.90 mg N/100g respectively) however day 0 and day 3 whiting samples had much lower ($P< 0.001$) TMA values than day 5 samples Table 4).

3.7 Total viable count (TVC)

Whiting fillets (on day 3 and 5) ($P< 0.05$) and salmon portions (on day 5 and 7) ($P< 0.01$) had higher TVCs day 0. However, there was no difference between

microbial loads on d3 and d5 (whiting) or d5 and d7 (salmon) (Table 4). This effect was not observed for mackerel fillets, which had mean TVCs of $\log_{10}$ 4.35 cfu/g. Modified atmosphere packs of mackerel ($P< 0.001$) and salmon ($P< 0.001$) portions in 100% CO_2 had the lowest TVCs (Table 3). There was no difference in TVCs of whiting fillets packed in 30% O_2 / 40 CO_2 / 30% N_2 and 100% CO_2. However, both these treatments had lower ($P< 0.001$) TVCs than whiting fillets packed in air (Table 3). There were interactions between storage time (days) and MAP treatment for whiting ($P< 0.01$), mackerel ($P< 0.001$) and salmon ($P< 0.001$) portions. In the case of whiting portions, on d3, 30% N_2 / 40% CO_2 / 30% O_2 and 100% CO_2-packed portions had the lowest TVCs. However on d5, portions packed in 100% CO_2 had lower TVCs than those packed in 30% N_2 / 40% CO_2 / 30% O_2, and these in turn had lower values than those packed in air. Mackerel and salmon portions had similar TVC patterns in that portions of both species packed in 100% CO_2 had the lowest TVCs on d3 followed by 60% N_2 / 40% CO_2. However, on d5, there was no significant difference between TVCs in the 60% N_2 / 40% CO_2 and 100% CO_2 packs.

3.8 Correlations

Total volatile base nitrogen was negatively correlated with sensory acceptability for the three species (Table 5). TVCs were positively correlated with TVBN and negatively with acceptability score. Most correlation coefficients were relatively low with the exception of those for whiting.

4. Discussion.

The average unit value of Irish fish exports was 38 per cent below the level across the European Economic Area and was 43 per cent (2001 data) below that in the European Union (Price Waterhouse Coopers, 2001). This has important implications for the Irish fish processing sector. Adding value to a product can be achieved by minimal processing, for example, pre-packaging fish fillets in plastic trays. Raw fish portions have a short shelf-life which presents logistical problems during distribution and retailing. Fagan *et al.*, (2003) have shown that freeze-chilling is an effective method of increasing the shelf-life of raw fish fillets. The product is held / transported in the frozen state and is then tempered back to chill temperatures for retail sale. The decision to use shelf lives of 5d (whiting and mackerel) and 7d (salmon) in the current MAP study was based on a shelf-life of 3 to 4 days for raw prepacked (in air) fillet portions of these species as was found by Fagan *et al.* (2003) and by other researchers conducting shelf-life tests on raw fish (Farber, 1995; Church, 1998), together with a "rule of thumb" often quoted by seafood companies that MAP increases the shelf-life of raw fish portions by up to 50%. The chosen shelf-life of 5-7d in the current MAP trials was vindicated by the results as the products were near the end of their shelf-life (in acceptability terms) after 5 (whiting and mackerel) and 7d (salmon). This is much shorter than the shelf-life of 15d reported by Church (1998) for whiting stored in 100% CO_2 (4°C), and for mackerel (6.5d) in 60% CO_2 / 40% N_2 (0°C) for salmon steaks (13d) packed in 60% CO_2 / 40% N_2 as reported by Cann (1984). This difference in shelf-life may be due to the freshness of the fish at time of packaging and also to

the different experimental conditions prevailing in the various studies. In terms of fish quality, certain parameters appear to be more important than others and are closely correlated with consumer preference. Laslett and Bremner (1979) (using a laboratory panel) found that the important predictors of acceptance were flavour, off flavour and toughness for fish minces, and off flavour and flavour for fish fingers. EU guidelines are in place for TVBN (95/149/EC) which set an upper limit of 35-mgN/100g for several fish species while Irish guidelines (Department of Health, 1992) are in place for TVCs in raw fish and suggest and upper limit of 5.0 $\log_{10}$ cfu/g.

Odour is an attribute immediately sensed by the consumer on opening a pre-packaged fish portion. Fresh fish has a sweet "seaweed-like" odour, which develops into a strong "fishy" odour as TVBN and TMA develop from the breakdown of protein and trimethylamine oxide (TMAO). In pre-packaged fish fillets, the odours concentrate in the headspace of the pack and are evident on pack opening. However, if the fillets are in good condition, these are minimal and will dissipate soon after pack opening as indicated by the results for mackerel (odour score decreased from 3.44 on pack opening to 3.04, 10min later), and salmon (3.04 down to 2.71). However, the opposite occurred for whiting (2.57 increased to 3.01). The odour scores in the current study were often below 3 (slight fishy odour) and were never higher than 3.52 (4 indicates a significant fishy odour, 5 a strong fishy odour, 6 a putrid smell). Five-day-old portions scored worse for odour than d3 samples. However, all samples were below the midpoint of the odour scale (i.e. 3.5), which put them in the "slight fishy odour" category.

This indicated that the MAP samples had an acceptable odour after 5 (whiting and mackerel) and 7 (salmon) days. MAP treatment had no effect on the odour scores for the three species. However, an interaction was observed for salmon portions between storage time (days) and MAP treatment ($P< 0.05$). This showed the 60% N_2 /40 % CO_2 and the 100% CO_2-stored samples scored better than samples packed in air. This effect was only observed for salmon portions and might be explained by the treatment of the samples prior to packing. The farmed salmon used in these trials were tested within 24h of catching whereas the whiting and mackerel were on ice on-board trawlers for several days before testing. This is unsatisfactory but is representative of current Irish commercial trawler practice.

The most common indices for assessing the freshness and odour of fish, together with the sensory tests, are TVBN and TMA (Civera, Turi, Parisi and Fazio, 1995). The volatile bases developed during the storage of unfrozen fish consist primarily of ammonia and TMA (Storey, Davis, Owen and Moore, 1984). Spoilage of thawed MAP fish portions have received relatively little attention (Bøknæs *et al.*, 2000, Windsor and Thoma, 1974). However, research on freeze-chilled (thawed) fish portions have shown that the previously frozen samples have a slightly longer shelf-life than the unfrozen samples (Guldager *et al.*, 1998, Shewan, 1961, Simmonds and Lamprecht, 1985). The development of TVBN and TMA during frozen storage depends to a large extent on the freshness of the samples prior to freezing. Bøknæs *et al.* (2000) compared cod fillets stored in air for 1 or 8d prior to MAP, and subsequent frozen storage at –20 and –30°C for 6 weeks, and found TMA production was more pronounced in the 8d samples. They attributed the rise

in TMA to the growth of specific spoilage organisms (*P. phosphoreum*), which proliferated during the iced storage prior to freezing. The TVBN and TMA levels in the current trials were moderately high in whiting and mackerel fillets and much lower in the farmed salmon samples. In the case of whiting, MAP did not affect TVBN or TMA development. However, values were higher on d3 and d5 than on d0 indicating that enzymatic activity (degradation of protein and TMAO continued at 4°C). Mackerel fillets packed in 60% N_2 / 40% CO_2 had the lowest TVBN values while TMA values were lowest in the 100% CO_2 and 60% N_2 / 40% CO_2. The TVBN and TMA levels for the three species were lower than those recorded for freeze-chilled fish fillets without MAP (Fagan *et al.*, 2003). This suggests that the combination of MAP and freezing maintained fillet quality better than freeze-chilling alone. The salmon portions had the lowest TVBN and TMA levels and this is attributed to the initial freshness of the samples prior to freezing and confirms the conclusions of Bøknæs *et al.* (2000) of the need for high quality raw fish for MAP.

Taste panel acceptability scores on cooked samples showed that there was no difference between the three gaseous atmospheres for each species. All species/samples scored above the mid-point of the line which suggested that they were all acceptable after 5 (whiting and mackerel) or 7 (salmon) days. In the case of fatty fish (lipid content ca 10-30%) such as mackerel and salmon, off-tastes can also be attributed to oxidative rancidity of the fat. Rancidity is perceived as an unacceptable taste by the consumer; typically soapy, stale, linseed oil flavours are detected (Church, 1998). In whitefish species (lipid content ca 1-2%) such as

whiting, the fat is mainly membrane-bound and is less prone to oxidation. In this case TVBN and TMA contribute more to off-tastes. Rancidity in fatty fish such as salmon is not well characterised and has been described as fish oil taste (Sylvia, Morrissey, Graham, and Garcia, 1995) and as fatty and train oily odours (Milo and Grosch, 1996). Frozen storage does not prevent the development of rancidity in fish fillets (Gormely *et al.*, 2002). Lipid deterioration occurs at a slightly slower rate in frozen storage, but hydroperoxides are still produced during the frozen phase. Zotos, Hole and Smith, (1994) reported extensive lipid oxidation of mackerel fillets (PVs of 108 meq. Kg^{-1}) over 22 to 33 weeks of frozen storage and hydrolysis of the lipid (> 60 g/Kg^{-1} FFA) in the mackerel samples. These samples were still acceptable to taste panellists after 33 weeks at –20°C. In the current trials, mackerel fillets packed in 100% CO_2 had the highest FFAs, while salmon in this atmosphere had the lowest values. Storage time (days) influenced PVs for mackerel and salmon with the d5 samples having higher values than the d3 samples. The highest recorded FFAs for mackerel (1.78 %) FFA and salmon (0.81 % FFA expressed as oleic acid) and corresponding PVs (2.59 and 1.34 meq. peroxide/kg sample) were well below the values reported by Zotos *et al.* (1994) and most probably did not affect the sensory scores of the cooked product.

Browning in whitefish species is an indication of spoilage and discoloration can occur via a bleaching action of cut surfaces (Cann, 1984) probably due to low pH precipitation of sacroplasmic proteins (Stratham and Bremner, 1989). Lannelongue, Finne, Hanna, Nickelson and Vanderzandt (1982) reported a drop in surface pH in MAP products due to the dissociation of CO_2 to carbonic acid

(proportional to CO_2 concentration). Bleaching of pigments and increased opacity of flesh has been reported by Gibson and Davis (1995) in high CO_2 environments. Whiting and salmon portions stored in 100% CO_2 in the current trial were yellower than those from the other treatments, and whiting and mackerel fillets became progressively more yellow over time. Skrede and Storebakken (1986) found that canthaxanthin-pigmented farmed salmon was yellower in hue than astaxanthin-pigmented wild salmon and this may explain why yellowing was observed in the MAP salmon portions.

In the current trials, the springiness and Kramer shear values of whiting fillets were not effected by MAP. However, d3 and d5 whiting fillets were less tough than d0 (fresh). This could be related in part to the soft muscle structure of this species, and in part due to immersion of the fillets in the gravity drip (up to 16% in 100% CO_2 pack) over the 3 to 5d time period. Mackerel portions were toughest in the 100% CO_2, while 60% N_2 / 40% CO_2 and 100% CO_2 gave salmon portions with the highest shear values. The toughening of fish during freezing and cold storage is well documented (IIR, 1986) and salmon from frozen storage was more firm, less juicy and more fibrous (Refsgaard, Brockhoff and Jensen, 1998). Frozen storage (Gormely *et al.*, 2002) and the freezing component of the freeze-chill process also leads to moisture loss in the form of increased drips (O'Leary *et al.*, 1999; Fagan *et al.*, 2003) as does MAP treatments (Randell, Ahvenainen and Hattula, 1995; Tiffany and Mills, 1982; Cann, 1984). These findings were confirmed in the current trials where both time at 4°C and the use of MAP

increased drip loss in all three species. However, the problem of in-pack drip can be minimised by the use of drip pads.

The microbiological status of fish and fish products has been reviewed by Shewan (1961) and low counts are a key indicator of fish freshness and good food hygiene practice. In addition *Pseudomonas* tends to become dominant during prolonged storage (Stenström and Molin 1989). Fish portions in the current trial were packed in MAP, blast-frozen at –35°C, and stored at –30°C for 3d prior to chilling. It is likely, therefore, that the freezing component of the freeze-chill process had little impact on the microbial status of the fish portions since the frozen storage time was well below the 5 weeks suggested (Magnüsson and Martinsdóttir, (1995); Bøknæs *et al.*, (2000)) as the minimum frozen storage period to reduce bacterial numbers in cod. Fagan *et al.* (2003) also found no difference in TVCs between previously frozen and unfrozen air-packed whiting, mackerel and salmon portions.

The results from the current trials suggest that whiting, mackerel and salmon portions performed well as freeze-chilled MAP products. Sensory tests indicated that samples were still acceptable after 5 (whiting and mackerel) or 7d (salmon) which was 1 to 2d longer than in previous tests (Fagan *et al.*, 2003) for samples packed in air. European guidelines governing the sale of raw fish products were complied with as indicated by TVBN values below 35 mgN/100g (EC 95/149/EC). The TVC values were less than log 6 (cfu/g) which is the maximum advocated by the Department of Health and Children Guidelines, Ireland (1992).

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Table 1. Odour scores[a] for raw whiting, mackerel and salmon portions subjected to modified atmosphere packaging with or without freeze-chilling

	Species		
	Whiting	**Mackerel**	**Salmon**
Time of sniffing[b] (min)			
0	2.99	3.44	3.04
10	2.65	3.04	2.71
F-test	P< 0.05	NS[e]	NS[e]
LSD[c]	0.28	0.51	0.36
Day of sniffing[d]			
3	2.57	2.96	-
5	3.01	3.52	2.63
7	-	-	3.12
F-test	P< 0.001	P< 0.05	P< 0.01
LSD[e]	0.28	0.51	0.36

[a] Scale from 1 to 6 (1. fresh seaweed-like smell; 2. odourless; 3. slight fishy odour; 4. significant fishy odour; 5. strong fishy odour; 6. putrid smell)
[b] Data averaged over days
[c] Least significant difference
[d] Data averaged over times
[e] No significant difference

Table 2. Taste panel acceptability scores[a,b] for cooked whiting, mackerel and salmon portions subjected to modified atmosphere packaging with or without freeze-chilling

	Gaseous atmosphere						
Species	**Fresh[c]**	**Air**	**30/40/30**	**60/40**	**100% CO_2**	***F*-test**	**LSD[d]**
Whiting	3.93	3.46	3.50	-	3.18	P< 0.001	0.34
Mackerel	3.66	3.23	-	3.37	3.23	P< 0.05	0.32
Salmon	4.40	3.44	-	3.75	3.56	P< 0.001	0.61

[a] Scale from 0 (unacceptable) to 6 (very acceptable)
[b] For 5 replicates
[c] Day 0
[d] Least significant difference

Table 3. Effect of modified atmosphere packaging with or without freeze-chilling on raw fish quality parameters[a]

	Gaseous atmosphere					
	Air	**30/40/30[b]**	**60/40[c]**	**CO_2[d]**	***F*-test**	**LSD[e]**
Whiting						
Hunter b value	2.59	2.91	-	3.07	P< 0.05	0.36
Gravity drip (g/100g)	4.71	9.40	-	16.4	P< 0.05	1.47
TVC[f]	4.81	4.48	-	4.34	P< 0.001	0.20
Mackerel						
Shear (kN)	1.95	-	1.94	1.99	P< 0.001	0.02
Springiness (%)	92.3	-	91.3	92.8	P< 0.01	0.87
TVBN[g]	18.7	-	17.6	18.0	P< 0.05	0.87
TMA[h]	3.88	-	2.84	2.91	P< 0.001	0.46
CD(g/100g)[I]	9.36	-	7.79	9.86	P< 0.01	1.21
Gravity drip(g/100g)	4.63	-	5.00	6.63	P< 0.001	0.65
FFA[k]	1.02	-	1.07	1.35	P< 0.05	0.23
TVC[f]	4.88	-	4.18	3.99	P< 0.001	0.16
Salmon						
Hunter b value	14.2	-	14.5	15.7	P< 0.01	0.74
Hunter L/b	2.96	-	2.90	2.70	P< 0.05	0.18
CD (g/100g)[I]	2.08	-	2.78	3.58	P< 0.001	0.69
Gravity drip (g/100g)	2.45	-	4.68	5.84	P< 0.001	0.46
PV[j]	0.92	-	0.95	0.84	P< 0.01	0.06
FFA[k]	0.75	-	0.76	0.59	P< 0.001	0.08
TVC[f]	6.23	-	5.04	4.53	P< 0.001	0.32

[a] Data for five replicates.
[b] 30% O_2 / 40% CO_2 / 30% N_2 only used for whiting.
[c] 60% N_2 / 40% CO_2 only used for mackerel and salmon.
[d] 100% CO_2.
[e] Least-significant difference.
[f] Total viable count (log_{10} cfu/g)
[g] Total volatile base nitrogen (mg N/100g)
[h] Trimethylamine (mg N/100g flesh).
[i] Centrifugal drip (g/100g)
[j] Peroxide value (milliequiv. Peroxide/kg fat).
[k] Free fatty acids (% FFA expressed as oleic acid).

Table 4. Effect of storage time[a] (days) on the quality parameters of raw fish portions subjected to modified atmosphere packaging with or without freeze-chilling

	Time (days)					
	0	**3**	**5**	**7**	***F*-test**	**LSD**
Whiting						
Hunter L	44.9	52.3	56.4	-	P< 0.05	7.20
Hunter a	1.69	-1.83	-2.07		P< 0.05	2.86
Hunter b	0.39	3.26	4.91	-	P< 0.01	2.19
Shear (kN)	1.61	1.54	1.54	-	P< 0.001	0.02
TVBN	13.5	17.7	24.2	-	P< 0.001	3.12
TMA	3.60	4.64	8.52	-	P< 0.001	1.70
CD (g/100g)	4.37	14.1	18.8	-	P< 0.001	5.11
TVC	2.97	4.88	5.76	-	P< 0.05	1.95
Mackerel				-		
Hunter b	4.42	8.73	9.07	-	P< 0.001	1.27
Shear (kN)	1.93	2.01	1.93	-	P< 0.05	0.05
TVBN	14.1	19.2	21.0	-	P< 0.001	2.47
CD (g/100g)	5.53	10.0	11.5	-	P< 0.001	2.13
PV	0.40	2.27	2.59	-	P< 0.001	0.64
FFA	0.25	1.42	1.78	-	P< 0.001	0.45
Salmon						
TVBN	12.9	-	17.5	18.0	P< 0.01	2.03
CD	1.00	-	3.66	3.78	P< 0.05	1.82
PV	0.31	-	1.06	1.34	P< 0.01	0.46
FFA	0.31	-	0.81	1.01	P< 0.001	0.17
TVC	4.50	-	5.58	5.71	P< 0.01	0.62

[a] See footnotes Table 3.

Table 5. Correlation coefficients[a] for selected quality parameters of whiting, mackerel and salmon portions subjected to modified atmosphere packaging with or without freeze-chilling

	Species		
	Whiting	**Mackerel**	**Salmon**
TVBN[b] X ACC[c]	- 0.93	-0.71	-0.91
TVBN[b] X TVC[d]	+0.85	+0.45	+0.48
TVC[b] X ACC[c]	-0.86	-0.44	-0.63

[a] Based on 20 data points
[b] Total volatile base nitrogen (mg N per 100g)
[c] Sensory acceptability (0 (unacceptable) to 6 (very acceptable))
[d] Total viable count ($\log_{10}$ cfu/g)