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CONSERVATION OF FRESH GOLDEN REDFISH (*SEBASTES MARINUS*) FILLETS: INFLUENCE OF BLEEDING, MODIFIED ATMOSPHERE PACKAGING USING DIFFERENT GAS MIXTURES AND SUPERCHILLING ON QUALITY DETERIORATION

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ABSTRACT

The objective of this study was to evaluate the influence of bleeding, modified atmosphere packaging (MAP) and superchilling on freshness deterioration and sensory shelf life of redfish (*Sebastes marinus*) deskinned fillets. Changes in psychrotrophic microorganisms (TVC), specific spoilage organisms (SSO), their metabolites (TMA, TVB-N), pH, drip loss, headspace gas composition, and sensory characteristics (Torry freshness score and texture) were analysed. The pre-trial conducted involved four gas mixtures as well as vacuum packaging (VP) and air (A) storage at -0.4°C to select the most effective packaging method. The VP fish deteriorated fastest, followed by A treatment. All MAP treatments showed a similar inhibitive effect towards the SSO investigated. The lower (40%) concentration of CO_2 was sufficient to delay SSO growth but the gas to fish ratio of 2.2-2.5 caused some textural problems. The initial quality of the fillets obtained for the pre-trial varied in redness and bruises, affecting the sensory assessment, and resulted in some differences within treatments. For the shelf study, unbled deskinned fillets stored in air were compared to two MAP treatments and to bled air-stored (AB) fillets during storage for 6 days at -1°C and +2°C, thereafter mimicking distribution via sea freight to European markets. Bleeding of the whole fish resulted in fillets with slightly slower TVB-N and TMA formation due to slower growth of *Photobacterium phosphoreum*, an important spoilage bacterium in cold water marine fish. Low pH in MAP fillets resulted in higher drip loss but a lower gas to fish ratio reduced texture problems. *P. phosphoreum* and pseudomonads were the main SSO in MAP and air storage respectively. MAP led to an extension of freshness and shelf life by few days.

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1 INTRODUCTION

Golden redfish (*Sebastes marinus*) is one of the commercially important fish found in Icelandic waters. The main markets are Germany and Belgium but a significant portion is also exported to other Western European countries and Eastern Asia where Japan and China are the largest buyers (Statistics Iceland 2011). Fish products have evolved considerably in recent years (Jónsson and Pálsson 2006). Previously presented as whole fish, an increasing number of processed products are now offered to meet the changes in human life style (Puligundla *et al.* 2012). Nowadays consumers demand for fresh, conveniently packaged, boneless fish products on shelf-service shelves that are also easy to prepare (Masniyom 2011). To ensure a constant supply, chilled fresh fillets are airlifted the same day in boxes while superchilled or frozen fillets and fillet portions are shipped in containers. However, air transportation is very expensive and the high fluctuations in temperature are difficult to control, which can affect the overall quality of fish reaching the consumer (Nga 2010, Margeirsson *et al.* 2012). Over the last years, Icelandic processing companies have increasingly used low cost shipping to international markets such as overseas containers. This method takes longer, but assumes high stability of the goods, and the application of new storage methods to maintain freshness and extend shelf life of the filleted products is required. Therefore, fish processors must develop new methods that suit the current trend and lead to adequate shelf life extension of fish products while taking into consideration their sensory, and microbiological quality as well as safety (Masniyom 2011).

Yellowing and red spots are defects observed in flesh of the filleted redfish products when they arrive to the markets. Flesh colour is an important criterion of choice for consumers as a guarantee of product quality and freshness and would accordingly provide a positive influence at the point of sale (Olsen 2011). The characteristic bluish and translucent colour of unbled redfish products must therefore be maintained throughout their shelf life or else unacceptability leads to rejection and financial losses. Due to high production costs and competitive market process, it is important that loss in quality and value by these defects is avoided.

The production of off-odours and off-flavours observed in fish fillets is mainly due to the spoilage bacteria producing metabolites such as hydrogen sulphide (H_2S), trimethylamine (TMA) and total volatile basic nitrogen (TVB-N), among others (Gram *et al.* 1987). Lipid oxidation also affects the odour and flavour of fatty fish, hence contributing to the spoilage pattern. Shelf life of fish can be considered as the time period from when fish is caught or processed until it is no longer fit for human consumption (Huss 1995). The shelf life of fresh fish is influenced by the raw material quality, cooling methods, processing, packaging and storage conditions they have been submitted to. Studies have revealed that the use of superchilling (-0.5°C to -1°C) delays bacterial growth and prolongs the freshness period and shelf life of fresh fish (Lauzon *et al.* 2010, Liu *et al.* 2010). Further, the use of modified atmosphere (MA) to extend shelf life of fresh fish is well documented (Sivertsvik *et al.* 2002, Beltran Garcia *et al.* 2010) and few studies have addressed MA packaging of redfish fillets (Lauzon *et al.* 2002). Combined application of superchilling and MA storage has been shown to extend the freshness period considerably in cod products (Lauzon *et al.* 2009, Wang *et al.* 2008). However, this has not yet been investigated for redfish fillets. Recent work has demonstrated the positive effect that argon exerts on the pigmentation of trout fillets (Choubert *et al.* 2008) and in meat products (Ruiz-Capillas and Jimenez-Colmenero 2010, Parra *et al.* 2010). Argon use has not yet been investigated for redfish fillets but fish processing companies are interested in knowing more about the use of MAP to preserve the

sensory quality of fish fillets. HB Grandi[1] is also interested in the use of MAP and superchilling to further develop bulk packages to export their products.

The main factor responsible for fillet redness is residual blood due to either poor/no bleeding, and bruises (red spots) result due to damages and harsh or mechanised handling, while yellowing of the flesh is most likely due to oxidation (Thanonkaew *et al.* 2006). Bleeding is a current practice for most white and larger fish species such as cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and saithe (*Pollachius virens*), but not for redfish and small pelagic species. Redfish is a deep-sea species and when captured, they expel their stomach contents due to the pressure difference in the high depths (Begossi *et al.* 2011). In consequence, bleeding and evisceration are not performed on board. These circumstances may contribute to the bluish/reddish colour of newly filleted redfish, as blood may have been blown into the muscle upon pressure change. This residual blood may lead to unfavourable quality deterioration. Research is necessary to assess the impact of bleeding on the quality and shelf life of redfish fillets.

Redfish is a common name for several reef dwelling and deep-water snappers of the genus *Lutjanus* in St Vincent and the Grenadines (SVG). Whole redfish and other fish species are transported from SVG to the USA and other Caribbean islands by air, chilled on ice. These contribute to the export earnings from seafood exportation and are valuable resources. The Fisheries Division (FD) is currently introducing a 'Fish in Ice' project with the assistance of Japanese Volunteers working with a network of local stakeholders. This aims to establish new methods of fresh fish presentation and enhance marketing of high quality fresh fish use. The creation of quality and simple value-added products such as fillets will also enable processors to obtain higher prices compared to whole fish. At present, fish freshness is only assessed organoleptically and no research has been carried out regarding different spoilage patterns, the effects of different packaging methods and storage conditions. Learning new evaluation methods is of great interest, as these can then be used to monitor and study the quality and shelf life of different kinds of seafood products in SVG. Understanding the spoilage process of a fish product will allow the development of tailor-made and efficient packaging and storage methods to maximise value.

In this research, methods to delay quality changes and extend the freshness period of fresh golden redfish fillets in retailed packs were sought in two phases:

- A short trial- to evaluate the effect of different gas mixtures (MA including CO_2, N_2 and/or Ar) on reducing microbial growth and the chemical and physical changes compared to air and vacuum package under subzero temperature storage (-0.4°C). An overall data analysis including the freshness (Torry) score and eating texture quality was conducted to justify the selection of the most appropriate packaging method(s).

- A thorough shelf life study- to evaluate on one hand the effect of bleeding on board, and on the other hand the effect of different atmospheric conditions on quality deterioration of fillets, applying the most promising MAP treatments selected from the preliminary trial compared to air storage at conditions mimicking distribution condition of HB Grandi products during sea freight [superchilled (-1°C) for 6 days followed by cold storage (+2°C)].

[1]HB Grandi is one of the largest fishing companies in Iceland It uses the latest advances in technology for fishing and processing to produce fresh and frozen fillets portions of redfish, cod and saithe.

The specific objectives were to evaluate the influence of bleeding, MAP (different gas mixtures) and superchilling on the development of total psychrotrophic viable microflora (TVC), specific spoilage organisms (SSO), TMA, TVB-N, pH, % drip loss, freshness deterioration and resulting shelf life compared to air storage of golden redfish fillets. The results would be used by the fishing industries in SVG and Iceland to improve the quality of fish products and to design new handling and packaging methods for redfish.

2 LITERATURE REVIEW

2.1 Factors influencing shelf life of fish

The fish muscle is sterile at the time of slaughtering/catch, but becomes quickly contaminated by surface and intestinal bacteria and from equipment and humans during handling and processing (Sivertsvik *et al.* 2002). The shelf life of the fresh raw materials will depend on their intrinsic and extrinsic parameters and will subsequently be influenced by the cooling methods, further processing, packaging and storage conditions they have been submitted to (Huss 1995, Lauzon *et al.* 2010). The shelf life of traditionally iced whole redfish is known to be 16-19 days while that of iced fresh fillets is shorter, about 12-13 days (Martinsdóttir 1995). It is well documented that packed fillets spoil more rapidly and have different spoilage patterns (Lauzon *et al.* 2002, Huss 1995). Fish from tropical countries have a longer shelf life than those from temperate areas because of the greater sensitivity of 'tropical' SSO to cold storage and their slower growth (Huss 1995). This is advantageous since it can permit distribution over considerable distances and possible storage in case of scarcity.

2.2 Handling at sea –Effect of bleeding on fish quality

Bleeding is a current practice for most white and larger fish species such as cod, haddock and saithe, but not for redfish and small pelagic species (Borderías and Sánchez-Alonso 2011). Techniques used for bleeding are cutting the throat, arteries or gill arches and direct gutting. Researchers have shown that the method used has minimal influence on the residual blood in the muscle. However, factors such as the catch method, pre-slaughter crowding or stress, the time from catch to bleeding, bleeding time and if the blood draining takes place in water or air are of greater importance (Olsen 2011). Washing fish after bleeding in cold water prevents clotting and blood spotting (Roth *et al.* 2009) but unbled fish that underwent a lot of mechanical handling may have more apparent bruises. It has been shown that blood, more precisely haemoglobin, can induce oxidation (Jonsdottir *et al.* 2007). Therefore, proper bleeding should be emphasised as one of the prerequisite handling procedures. This not only influences the shelf life of fresh fish products but also greatly affects the appearance and texture of the fish (Olsen 2011). The oxidation of lipids resulted in the formation of yellowness in squid muscle (*Loligo peali*) (Thanonkaew *et al*. 2006). Moreover, blood is enriched with nutrients that can contribute to the rapid proliferation of microorganisms. Indeed, the study of Maqsood and Benjakul (2011) showed that bleeding of Asian seabass (*Lates calcarifer*) delayed lipid oxidation (lower peroxide value and thiobarbituric acid-reactive substances), fishy odour development and microbial growth during storage of sliced products in ice.

2.3 Fish spoilage and specific spoilage organisms

Spoilage can be considered as any change that renders the product unacceptable for human consumption (Dalgaard 2006). Spoilage of fish results from changes caused by oxidation of

lipids, endogenous enzymatic activity, and metabolic activity of microorganisms (Sivertsvik *et al.* 2002). Fish are highly perishable, because of their high water activity (a_w), neutral pH, and presence of autolytic enzymes as well as available nutrients. The rate of deterioration is highly temperature dependent and can be delayed by the use of low storage temperature, e.g. fish stored on ice (Masniyom 2011). Despite a low percentage of carbohydrates, fish is rich in non-protein, low molecular nitrogenous molecules such as free amino acids, trimethylamine oxide (TMAO), urea, nucleotides and creatine which can be rapidly metabolised by microbes, thus making fish a favourable matrix for microbial growth (Adam and Moss 2010). *Rigor mortis* begins after slaughter, followed by autolytic changes where more catabolites become available for bacterial growth. Then processing will open up the fish and cause inescapable microbial contamination of the flesh (Leroi 2010, Huss 1995).

Each fish product has its own SSO and the number of these, as opposed to the total number, is related to the shelf life and can be used as objective indices of spoilage in shelf life determinations (Huss 1995). Spoilage bacteria in temperate water fish are dominated by psychrotrophic, aerobic/ facultative anaerobic, Gram-negative bacteria such as *Photobacterium, Shewanella, Moraxella, Acinetobacter, Aeromonas, Vibrio, Flavobacterium* and *Pseudomonas* (Silvertsvik *et al.* 2002, Leroi 2010). Gram-positive bacteria for example, *Corynebacterium, Bacillus, Lactobacillus, Clostridium,* and *Micrococcus* may also be present in variable proportions. In addition to these Gram-positive microorganisms, *Staphylococcus, Brochothrix,* and *Streptococcus* are dominant in tropical marine fish along with Gram-negative *Vibrio* and Enterobacteriaceae (Masniyom 2011, Al Bulushi *et al.* 2010). Intrinsic and extrinsic parameters of the fish products will allow for the selection of SSO, normally constituting the dominating microflora at spoilage. However, for fish in freshwater the sodium-requiring species of *Vibrio* and *Photobacterium* are very rarely present whereas *Aeromonas*, Enterobacteriaceae and *Flavobacterium-Cytophaga* are relatively more important in those habitats (Dalgaard *et al.* 1996).

Bacteria, like *Photobacterium phosphoreum, Shewanella putrefaciens* (an H_2S-producing bacterium), and pseudomonads can grow in fresh, cold water marine fish stored aerobically (Olafsdottir *et al.* 2006a, Olafsdottir *et al.* 2006b, Reynisson *et al.* 2010). Fish pH is commonly used to follow quality deterioration. The rate of pH decline is highly dependent on primary levels of glycogen and the ATP turnover rate and, due to ATP hydrolysis and lactic acid accumulation, the pH decreases with post mortem time (Gaarder *et al.* 2012, Susanto *et al.* 2011). ATPase has an optimum activity at pH 6 and temperature between -0.8°C and -5°C (the critical zone) in the food item than at >-0.8°C (Gill 2000). TMA production is high with pH above ~6.5 (Dalgaard 2006). A low pH is more likely to prevent oxygenation of haemoglobin and can promote lipid oxidation via acceleration of haemoglobin autoxidation (Maqsood and Benjakul 2010).

Vacuum packaging (VP) and MA packaging (MAP) may favour the growth of anaero-aerotolerant lactic acid bacteria (LAB) and *Brochothrix thermosphacta* (Leroi 2010). These storage conditions still allow the growth of *P. phosphoreum* and *S. putrefaciens* while the growth rate of pseudomonads is much reduced under low oxygen tension. However, MAP involving a CO_2 level up to 40% considerably reduced the growth of *S. putrefaciens* by 50% while *P. phosphoreum* showed high resistance thereby requiring 95% CO_2 to be hindered? (Dalgaard 1995, Debevere and Boskou 1996). Few studies have reported on the proliferation of these bacteria in redfish fillets (Lauzon *et al.* 2011, Semwanga 2010). *P. phosphoreum* and *S. putrefaciens* are able to use TMAO as an electron acceptor for anaerobic respiration. They produce H_2S and other sulphurous compounds from organic and inorganic sources and reduce

TMAO to trimethylamine, which are responsible for the off-odours and off-flavours in spoiled marine fish (Matamoros *et al.* 2006).

2.4 Modified atmosphere packaging (MAP)

MAP is defined as the enclosure of food products in gas-barrier material, in which the gaseous environment has been changed. Studies have shown that MAP has increased the shelf life of fish compared to vacuum packaging (Dalgaard *et al.* 1993) and over-wrap packaging (Randell *et al.* 1997). The effectiveness of this technology depends on a series of factors such as the quality of the raw material used, mixture of gas employed, preservation temperature and type of packaging (Provincial *et al.* 2010). MA generally involves the use of CO_2, N_2 and O_2 in various concentrations. CO_2 dissolves better at lower temperature (as other gases), hence increasing antimicrobial activity. It can also affect texture, drip and possibly colour. A pH decrease in muscle occurs due to CO_2 dissolution in water phase, causing protein denaturation, texture defects, and drip loss (Masniyom 2011, Soccol and Oetterer 2003). The extension of shelf life and freshness should not be at the cost of such attributes, therefore sensory analysis must be conducted to determine the negative effects of any gas mixtures, if any. Recent findings have also indicated that the use of noble gas argon (Ar) in MA could be beneficial as it can remove O_2 better than N_2, hence likely to retard oxidation problems during storage. Choubert *et al*. (2008) applied Ar/CO_2 (60/40) in rainbow trout fillets and observed a positive impact on fillet pigmentation, pH, microbial development and drip loss, hence resulting in an extended storage life. Argon is much heavier, which prevents other gases from entering the package even if the package is left open. Argon is also more soluble in water than nitrogen and oxygen, which could have relevant consequences on its effect on shelf life.

A summary of redfish research is provided in Table 1, showing that the freshness period for redfish may last for 9-11 days in air-stored fish from catch and about 12 days under vacuum. However, extended freshness can only be achieved in MAP using specific gas mixtures. The freshness period has been defined as the time from catch/processing/ packaging until the product loses its freshness characteristics (Torry score of 7 or QDA score $\leq$ 25 for sweet flavour) (Lauzon *et al.* 2010).

2.5 Safety of packaged fish products

Spore forming, anaerobic, psychrotolerant, non-proteolytic *Clostridium botulinum* Type E is pathogenic and able to grow and produce toxin at ~3°C (FDA 2012). It is a natural contaminant and is not affected by CO_2 and could potentially multiply rapidly in MAP products if strict temperature control was not maintained which can result in food poisoning. Moreover, the potential for growth is increased in view of holding temperature of retails cabinets and consumer refrigeration units is commonly >10°C (Doyle 2002) and the products can become spoiled before toxin can be detected (Arritt *et al.* 2007). In general, oxygen delay toxin formation by *C. botulinum*, but aerobic storage or modified atmospheres with oxygen cannot totally prevent toxin formation as an anaerobic micro-environment is formed in the products (Smith *et al.* 2000). However, in the case of fatty fish storage, absence of oxygen is a pre-requisite for better quality maintenance but a threat to safety if temperature cannot be controlled.

Table 1. Summary of earlier redfish fillet research in Iceland (Lauzon *et al.* 2010)

Year	**Known history**	**Raw mat. Age (d)**	Packaging type/storage condition	Packaging conditions (for MAP: CO2/O2/N2)	Storage temp. (°C)	Shelf life (d)	Fresh-ness period (d)	Reference
1990	**deskinned**	**1**	Vacuum bag	air, iced	0	10	8	Blomsterberg and Stefánsson 1994 (Rf 67-94)
				80/20/0	0	**15**	**6**	
				80/0/20	0	**14**	**13**	
				100/0/0	0	**17**	**14**	
				vacuum	0	13.5	11	
1995	**long-line**	**1**	Retail skin pack	air-packed	0	11	9.5	Stefánsson and Halldórsson 1995 (Rf 95-95)
					5	8	6.5	
2000		**2**	10 d whole in air (0.9°C), then filleted, packed	air (open)	0-2	5	1	Lauzon *et al.* 2002
				60/0/40		9	3	
			10 d whole in 60/0/40(0.9°C), then filleted, packed	air (open)		5.5	2	
				60/0/40		11	2	
		2	10 d whole in air (1.8°C), then filleted, packed	air (open)		3	1	Lauzon *et al.* 2002
				60/0/40		8	2	
			10 d whole in 60/0/40(1.8°C), then filleted, packed	air (open)		4	2	
				60/0/40		14	3	

2.6 Superchilling and the extension of shelf life

Studies have revealed that the use of superchilling (-4°C to 0°C) delays bacterial growth and prolongs the shelf life of fresh fish and fish products (Huss 1995). The extension of shelf life of fresh fish fillets is of importance to allow the transport of products to distant markets at a lower cost. The sensory shelf life of superchilled cod fillets has been shown to extend for at least three days compared to the traditional process resulting in a shelf life of 15 days (Olafsdottir *et al.* 2006b). Similarly, the freshness period of superchilled products was extended by 2 days. Such a gain in freshness and shelf life extension is of high economic value and may permit distant transportation of fresh fillets by ship, which is less costly compared to airfreight (Nga 2010). Interestingly, ATP degradation to inosine monophosphate (IMP) occurs at a faster rate in superchilled conditions and can be maintained longer than at chilled (ice) temperatures. IMP is the compound, which contributes to sweet, freshness-like flavour in fish (Gaarder *et al.* 2012).

2.7 MAP combined with superchilling

The combination of modified atmosphere and superchilled conditions can extend both the freshness period and the shelf life of fish fillets (Liu *et al.* 2010). Lauzon and Martinsdóttir (2005) and Martinsdóttir *et al.* (2005) reported that the spoilage microflora of cod fillets was better controlled and the characteristic "fresh fish taste" was maintained longer by superchilling the fillets during processing (a new technique called combined blast and contact freezing, CBC) in combination with MAP and superchilled storage. Low temperature storage not only decreases the growth rate of microbes but also increases the inhibitory effects of MAP by increasing the solubility of CO_2 in the liquid phase surrounding a food product (Liu *et al.* 2010, Lauzon and Martinsdóttir 2005).

2.8 Evaluation of freshness and spoilage in fish

Methods to evaluate fish quality may be divided into sensory, physical, chemical and microbiological methods. Sensory methods rely on trained assessors to objectively assess attributes based on appearance, odour, flavour and texture which are necessary in quality control for evaluation of freshness and for determination of remaining shelf life of seafood (Stone and Sidel 2004). Quantitative Descriptive Analysis (QDA) is used for cooked fillets description to establish a detailed description and quantify product sensory aspects, all very useful in product development. The Torry freshness scheme for measuring sensory parameters of cooked fish products starts at a score of 10 (very fresh) and goes down to 3 (very spoiled). A score of 7 indicates that the freshness characteristics of the fish product are no longer detected, marking the end of the freshness period. The end of shelf life is reached at a score of 5.5, where the fish is considered unfit for consumption. Chemical methods may best be used in resolving issues regarding products of marginal quality and eliminate the need to base decisions regarding product quality on personal opinions (Olafsdottir *et al.* 2006b). The European Union Commission (Regulation (EC) No.1022/2008) specifies the critical limit for unprocessed redfish as 25 mg TVB-N/100 g. TVB-N content accounts for the formation of TMA, dimethylamine, ammonia and other basic nitrogenous compounds associated with seafood spoilage (Huss 1995). TMA is a microbial metabolite and can only be used as an index of spoilage not as freshness, since its rapid formation is generally observed at incipient spoilage. Lower levels of TMA are generally detected under aerobic conditions than in MAP (Sivertsvik *et al.* 2002, Debevere and Boskou 1996), as TMAO transformation to TMA occurs faster under low oxygen tension (Huss 1972).

Physically, knowledge about the pH of fish meat may give valuable information about its condition and degree of spoilage. Microbiological examination of fish products aims to evaluate the possible presence of microbes of public health significance and to give an impression of the hygienic quality of the fish (Huss 1995). Such microbiological data will in general not give any information about eating quality and freshness (Gram *et al.* 1987). However, the number of specific spoilage bacteria or SSO is a better indication of quality deterioration and can be related to the remaining shelf life (Olafsdottir *et al.* 2006b, Huss 1995).

2.9 Microbiological evaluation of fish quality and safety - Common methods used

Isolation and enumeration of SSO proliferating in cold water fish products require techniques that ensure their survival and growth. Many are psychrotolerant, heat labile and require sodium chloride (NaCl) for growth. Consequently, pour plating with agar at ~ 45 °C, or the use of standard plate count agar without NaCl, or the incubation at 30-37°C must be avoided.

The use of chilled diluent followed by spread plating on pre-chilled plates of Long & Hammer's agar, modified to contain 1% NaCl, and incubated aerobically during 5–7 days at 15°C have been appropriate for enumeration of the dominating microflora. No selective agar medium is available for the enumeration of *P. phosphoreum*, except for the conductance method developed by Dalgaard *et al.* (1996). A quantitative Polymerase Chain Reaction (qPCR) method to estimate the levels of *P. phosphoreum* in fish was recently developed at Matís (Reynisson *et al.* 2008). A similar qPCR method has been reported for pseudomonads (Reynisson *et al.* 2008). However, the medium Cephaloridine-Fucidin-Cetrimide (CFC) is selective for pseudomonads but several members of the family Enterobacteriaceae developing in MAP foods may grow on it. To differentiate between the two groups, arginine and the pH indicator phenol red are added to this medium. The pseudomonads produce ammonia from arginine, which causes an alkaline drift in pH and around the colonies to produce a pink colouration whereas Enterobacteriaceae generally do not use this substrate (Stanbridge and Board 1994). A specific count of H_2S-producing bacteria and TVC can be obtained directly on iron agar (IA) medium containing thiosulphate and cysteine. Bacteria capable of forming H_2S from either source of sulphur appear as black colonies and TVC would appear as white colonies (Gram *et al.* 1987). In iced storage fish, most of the H_2S-producing strains were identified as *S. baltica* (Vogel *et al.* 2005). Other SSO have also been reported in some MAP fish products and can be detected on selective media. Streptomycin thallous acetate actidione (STAA) agar is used for the quantitative enumeration of *Brochothrix thermosphacta* on the basis of productivity, selectivity and characteristic appearance of colonies. The combination of antibiotics aims to exclude all other organisms but occasionally, yeast and pseudomonad colonies are found (Gardener 1985). The nitrite actidione polymyxin (NAP) agar is effective in recovering LAB from pure cultures and from foods because most strains are resistant to the inhibitory factors present (Davidson and Cronin 1973, Corry *et al.* 2003). Tryptose Sulphite Cycloserine (TSC) is used for enumeration of *Clostridium perfringens* because research has shown that it provides optimum growth and is the most favourable medium for growth from foods (Byrne, *et al.* 2008, De Jong *et al.* 2003). This pathogen can be monitored for safety purposes in MAP foods.

3 MATERIALS AND METHODS

3.1 Experimental design and sampling procedure

Two experimental phases were conducted to evaluate effectiveness of different methods (bleeding, modified gas packaging and superchilling) in delaying quality changes and extending the freshness period of fresh golden redfish fillets in retailed packs.

3.1.1 Pre-trial

A short trial was carried out on redfish caught on December 6th, 2012 by the vessel Ásbjörn RE where bleeding was not performed. Fillets were processed one day post catch by HB Grandi fish processing company in Reykjavik, Iceland, and 7 styrofoam boxes with an average weight of 5 kg of deskinned fillets were obtained. Different atmospheric conditions were selected based on fish publications to determine the best suitable packaging conditions to achieve the desired effects, i.e. extend freshness period and shelf life of redfish fillets with optimal sensory characteristics (Table 2). Styrofoam trays (Linstar E39-34, 225 x 175 x 30.5 mm; Samhentir-kassagerð ehf., Iceland) were used to package 6 fillets (mean weight: 107.3 ± 6.9 g) per tray, inserted in a vacuum bag (#4031010144, Coex PA/PE, 250 x 500 x 0.120 mm; Plastprent, Iceland), and stored in a cooling chamber at about -0.5°C.

After cooling of the trays for 2 h, the groups to be gas packaged were flushed with a gas mixture according to Table 2 and sealed using a packaging machine (Heavy Duty 2000, HenkoVac, HFE vacuum systems b.v.,The Netherlands). A gas mixer (MAP Mix 9000, PBI Dansensor, Denmark) adjusted at a flow of 125 L min^{-1} was used to achieve the desired gas mixtures prepared from food grade CO_2, N_2, Ar and 75% Ar/25% N_2 (Isaga ehf, Iceland). Testing of the gas mixture was performed, and considered as the value of the headspace analysis on day 0, before a treatment was finally flushed and sealed. At last, the group to be vacuum packaged was sealed. Sampling was done on days 0 (raw material only), 6 and 11 in duplicates using two trays per group, selecting two fillets per tray as one sample replicate for the microbiological and chemical analyses. A third tray was used for another project, for which drip and gas measurements were also done and included in this study. The rest was used for the freshness evaluation of cooked fillets (days 0, 6, 11 and 13).

3.1.2 Shelf life study

The shelf life study was conducted using redfish caught from the same haul by Ásbjörn RE on January 12th, 2013, in the Fjöllin area. One group (A) was not bled and the other was bled (AB) by a cut across the ‘‘neck”, and bled in seawater for 10 min. Fillets were processed by HB Grandi on January 15th, 2013 and 7 (6 from unbled fish and 1 from bled fish) styrofoam boxes (7 kg each) were obtained. Six deskinned fillets (mean fillet weight: 100.9 ± 8.1 g) were placed on styrofoam trays, inserted in a plastic bag and stored in a cooling chamber at -1°C for 2 h until final packaging. Table 3 describes the four treatments investigated.

Table 2. Atmospheric conditions tested.

Treatment	Gas mixture including		
	CO_2 (%)	Ar (%)	N_2 (%)
M1	40	60	
M2	40		60
M3	60	40	
M4	60	30	10
V	Vacuum packed		
A	Air storage with bag over tray (control, not sealed to mimic aerobic storage in styrofoam boxes)		

Group A fillets received three treatments, air-stored and gas packaged using two different gas mixtures (M1 and M2). The air-stored fillets were covered with a polyethylene (PE) bag while MAP fillets were enclosed in a vacuum bag (#4031010142, Coex PA/PE, 250 x 400 x 0.120 mm; Plastprent). Packaging was conducted as described in the pre-trial. Sampling was done in duplicates on the newly processed fillets (day 0 for A and AB raw material sampled from all boxes) and on days 6, 11, 13, and 16 using four trays per group. One fillet was sampled per tray, pooling two fillets into one sample replicate for the microbiological and chemical analyses. The rest was used for the sensory evaluation of cooked fillets and for analyses performed for two other projects.

Table 3. Redfish packaged in different atmospheric conditions and stored up to 16 days (6 days at -1°C; then at 2°C)

Treatment	Packaging condition
Air (A)	PE bag, control
MAP1 (M1)	Vacuum bag + 40% CO_2/60% Ar
MAP2 (M2)	Vacuum bag + 40% CO_2/60% N_2
Air-Bled (AB)	PE bag

3.2 Microbiological analysis

Fillets were aseptically minced, assessing two pooled fillets for each sample. Two replicate samples were evaluated for each group. Minced flesh (20 g) was mixed with 180 g of chilled Maximum Recovery Diluent (MRD, Oxoid, UK) in a stomacher for 1 minute. Successive 10-fold dilutions were done as required with chilled diluent (9 ml). Total viable psychrotrophic counts (TVC) were performed on modified Long and Hammer´s agar (LH) according to van Spreekens (1974) with 1% NaCl and on modified iron agar (IA) as described by Gram *et al.* (1987) with the exception that 1% NaCl was used instead of 0.5% with no overlay. Counts of H_2S-producing bacteria were evaluated on IA. Plates were spread-plated and incubated aerobically at 17°C for 5 days. Counts of all colonies (both white and black) on IA gave the number of total count and counts of black colonies gave the number of H_2S-producing bacteria. Counts are reported as colony-forming units (CFU)/g. The detection limit was 20 CFU/g.

Cephaloridine Fucidin Cetrimide (CFC) agar was modified according to Stanbridge and Board (1994) and used for enumeration of presumptive pseudomonads. Pseudomonas Agar Base (Oxoid) with CFC Selective Agar Supplement (Oxoid) was used. Plates were incubated at 22°C for 3 days. Pseudomonads form pink colonies on this medium.

Estimation of *Photobacterium phosphoreum* (Pp) counts was achieved by a quantitative Polymerase Chain Reaction (qPCR) method developed at Matís (Reynisson, unpublished). Briefly, 1 ml of the 10-fold diluted fish sample in MRD buffer was frozen at -20°C for later DNA extraction. For the DNA extraction, the diluted samples were centrifuged at 12000 x g for 5 min to form a pellet. The supernatant was discarded and DNA was recovered from the pellet using the promeganesil KF, Genomic system (MD1460) DNA isolation kit (Promega Corporation, Madison, USA) in combination with King Fisher magnetic beads automatic DNA isolation instrument (Thermo Lab systems, Waltham, USA) according to the manufacturers' recommendations. All PCR reactions were done using the MX 3005p instrument. The PCR was done using Brilliant QPCR master mix (Agilent, La Jolla, CA, USA). Primers were synthesised and purified with HPLC (MWG, Ebersberg, Germany). The DNA standard used for quantification of *P. phosphoreum* was previously calibrated against the PPDM-Malthus conductance method (Dalgaard *et al*. 1996, Lauzon 2003) using fish samples from storage trials.

In the shelf life study, additional analyses were performed to determine additional members of the microbiota developing from day 10. Nitrite-Actidione-Polymyxin (NAP) agar was used for counts of LAB. The medium was prepared according to Davidson and Cronin (1973). Plates were incubated at 22°C for 5 days after which counted colonies were assessed with hydrogen peroxide. Catalase-negative colonies were regarded as LAB. Counts of *Brochothrix thermosphacta* was assessed on STA selective agar (CM0881 with SR0162, Oxoid) following incubation at 22°C for 4-5 days and colony confirmation done by oxidase test using BBL™

Dry Slide™ Oxidase (#231746, BD) and if negative, followed by catalase test using hydrogen peroxide assessing 5 to 10 colonies per sample. Oxidase-negative and catalase-positive colonies are regarded as *B. thermosphacta*. Presence of *Clostridium perfringens* was evaluated in anaerobic (MAP) treatments at late storage time as a safety indicator. Perfringens agar base (OXOID CM0587) (PAB) with Perfringens selective supplement, Tryptose Sulphite Cycloserine (TSC) (OXOID SR0088), was used. *Clostridium perfringens* colonies may be seen as black colonies on plates incubated at 37°C for 24 h in an anaerobic jar to which one pack of GB Anaerobic System (OXOID AnaeroGem^{TM} AN0035A) was inserted to remove oxygen.

3.3 Chemical analysis

The method of Malle and Tao (1987) was used for total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) measurements in the previously prepared mince. TVB-N was measured by steam distillation (Struer TVN distillatory, STRUERS, Copenhagen) and titration, after extracting the fish muscle with 7.5% aqueous trichloroacetic acid (TCA) solution. The distilled TVB-N was collected in boric acid solution and titrated with sulphuric acid solution. TMA was measured in TCA extract by adding 20 ml of 35% formaldehyde, an alkaline binding mono- and diamine, TMA being the only volatile and measurable amine. All chemical analyses were done in duplicate.

3.4 Physical analysis

The drip loss was measured by gravimetric method using three (pre-trial) or four (shelf life trial) packs. Each empty tray was weighed before packaging with fish, allowing for the evaluation of initial fish weight at sampling. The drip was then calculated as the ratio of the water lost during storage to the original weight of the fish and expressed as a mean percentage (± standard deviation).

The pH was measured in about 5 g of minced fillets mixed with 5 ml of deionised water using the Radiometer PHM 80. Measurements were done within 30-60 min from sampling for MAP fish. The pH meter was calibrated using the buffer solutions of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25 °C).

3.5 Headspace gas analysis and estimation of gas to fish ratio

Headspace gas composition was evaluated in packs using a PBI Dansensor gas analyser (CheckMate 9900, Dansensor A/S, Denmark). A septum was put on the film to enable measurements, gas collected twice via the sampling needle and the latter measurement recorded for each pack. Three (pre-trial) or four (shelf life trial) packs were evaluated for each group at each sampling day.

The gas to fish ratio was calculated for each pack based on the size of the bag, taking into account the width of the sealing seam created at packaging. Volume of fish, assuming a fish density of 1.05 kg m^{-3} (Lowndes 1955), was compared to the headspace gas volume available in the bag containing the fish bulk, resulting in a ratio > 2 in the pre-trial and between 1 and 2 in the shelf life trial due to the smaller size of the bag used. A mean ratio (± standard deviation) was calculated for each MAP treatment at each sampling day, based on three (pre-trial) or four (shelf life trial) packs.

3.6 Sensory analysis

In the shelf life trial, evaluation using the Torry freshness scheme (Shewan *et al*. 1953) and texture using Quantitative Descriptive Analysis (QDA) by Stone and Sidel (2004) was performed to assess cooked samples of the four sample groups of fresh redfish fillets. The scheme used is detailed in Appendices I and II. Nine to ten panellists, all trained according to international standards (ISO 1993, 8586) including detection and recognition of tastes and odours, trained in the use of scales and in the development and use of descriptors, participated in the sensory evaluation. A training session took place prior to the storage trial. Sampling was done on day 0, assessing the raw material before packaging (bled and unbled fillets) and on packed fillets on days 6-10-13 and 16.

Samples weighing ca 40 g were taken from the loin part of the fillets and placed in aluminium boxes coded with three-digit random numbers. The samples were cooked for 5 min in a pre-warmed oven (ConvothermElektrogeräte GmbH, Eglfing, Germany) at 95-100°C with air circulation and steam, and then served to the panel. Each panellist evaluated duplicates of each treatment in a random order. A computerised system (FIZZ, Version 2.0, 1994-2000, Biosystèmes) was used for data recording.

In the pre-trial, assessment of freshness, quality and texture of cooked fillets was performed by few panellists during special sessions (days 0-6-11-13) designed to determine the main differences among the treatments.

3.7 Temperature profile

Product and ambient temperature was monitored throughout both storage trials. A temperature data logger (iButton DS1922L, Maxim Integrated Products Inc, USA) was placed underneath a fillet in one (pre-trial) or three (shelf life trial) trays for each treatment. The ambient temperature was similarly recorded with four loggers positioned on four shelves (2 upper and 2 lower positions) of the cooling chamber. The logger accuracy was ±0.5°C and its resolution 0.0625°C. Temperature was recorded at 10-minute intervals. Mean temperature and standard deviation was calculated for fish products and the ambient environment.

3.8 Data Analysis

Graphical presentation and calculation of means and standard deviations were done using Microsoft Excel 2010. Analysis of variance (one-way ANOVA) was carried out on sensory, microbiological and physico-chemical data in the statistical program NCSS 2000 (NCSS, Utah, USA). Comparisons of treatments were done using the Duncan‘s Multiple-Comparison Test (sensory data), Tukey-Kramer Multiple-Comparison Test (microbiological and physico-chemical data) and Kruskal-Wallis Multiple-Comparison Z-Value Test (for non-parametric independent group comparisons) with a threshold level for significance of 5%. Further, multivariate comparison of all data was conducted in the statistical program Unscrambler (Version X 10.2 Client, CAMO Software AS, Oslo, Norway) with Principal Components Analysis (PCA). Full cross validation was used and the data standardised with 1/SD.

4 RESULTS

4.1 Pre-trial: Selection of gas mixtures

4.1.1 Temperature monitoring

The mean temperature changes taking place in the packages and ambient air are shown in Figure 1. The mean initial temperature of the fish product at packaging was 2.7±0.5°C. The temperature of packaged products was high at the beginning but a subzero temperature (-0.1°C to 0°C) was reached after 8 hours. The incurred fluctuations in ambient temperature throughout the storage period, due to inherent cooling cycles of the cooling chamber, were reflected in the product. These oscillations were highest in M1-3-4 packs (SD=0.4°C) and lowest in air and M2 packs (SD=0.3°C) (Table 4). The mean temperature of the storage chamber and products over the 13-day period was -0.4±0.6°C and -0.3±0.4°C, respectively.

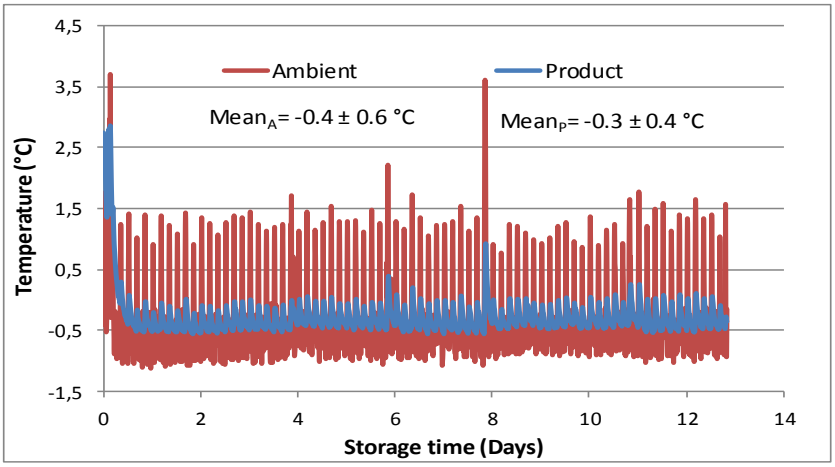

Figure 1. Mean temperature of redfish fillets in different packages (products = Air, Vacuum, M1, M2, M3, M4; n=6 loggers) and ambient air (n=4 loggers) over the 13-day storage period.

Table 4. Variation in temperature for redfish fillets packaged differently and stored at -0.4°C during a 13-day period; M1=40% CO2/60% Ar; M2=40% CO2/60% N_2; M3=60% CO_2/40% Ar; M4=60% CO_2/30% Ar/10% N_2.

	Air	Vacuum	M1	M2	M3	M4
Mean temperature (°C)	-0.24	-0.02	-0.27	-0.36	-0.32	-0.44
SD (°C)	0.34	0.36	0.42	0.34	0.41	0.41

4.1.2 Torry freshness score of cooked products

The Torry freshness scheme for cooked fish products starts at a score of 10 (very fresh) and goes down to 3 (very spoiled). A score of 7 indicates that the freshness characteristics of the fish product are no longer detected, marking the end of the freshness period. The end of shelf life is reached at a score of 5.5, where the fish is considered unfit for consumption. In Figure 2, the Torry freshness score of cooked redfish samples showed that freshness of the fillets was still detected on day 6 with highest score of 9 close to its maximum of 10 for air and M1 groups while similar (lower) scores were given to the other treatments.

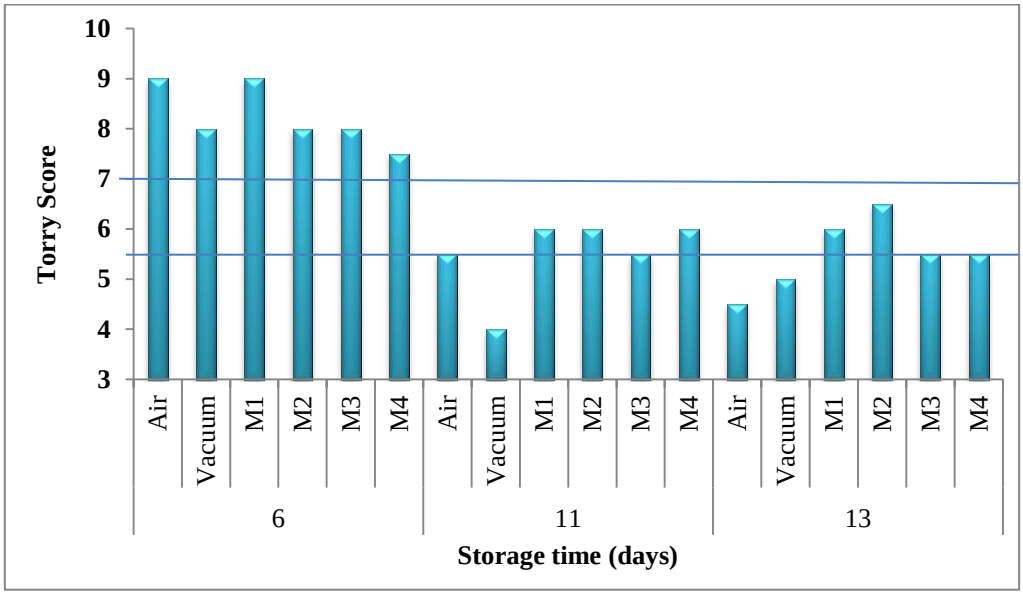

Figure 2. Mean Torry freshness score for cooked redfish fillets packaged differently and stored at -0.4°C. Torry score of 7 is limit for freshness and score of 5.5 is unfit for consumption (shelf life cut off point); M1=40% CO_2/60% Ar; M2=40% CO_2/60% N_2; M3=60% CO_2/40% Ar; M4=60% CO_2/30% Ar/10% N_2.

On day 11, sensory rejection was reached for air and M3 treatments while vacuum score was lower than 5.5. On day 13, M4 was also rejected; however M1 and M2 with a lower concentration of CO_2 still had a score of 6 or slightly above, respectively. Therefore, their shelf life is expected to have ended during the following days. The shelf life of fillets in MAP packs with 40% CO_2 was at least 3 days longer than that for air and vacuum-packed products under the conditions investigated. It should be noted that the quality of the fillets obtained at the beginning of the trial varied in redness and bruises, therefore as the experiment progressed this affected the assessment done by the panellists, resulting in some differences within a treatment.

4.1.3 Drip loss and pH measurements

Drip loss (water loss) was least in the air-stored fillets throughout the study (Figure 3). On day 6, vacuum packaging resulted in significantly higher drip loss (4.2±0.7%) compared to air storage ($p<0.05$), but there was no significant difference between the air and MAP groups. With increased storage time, the exudate also increased with most lost occurring in the MA packages (7.3-7.4%) containing highest % CO_2 in comparison to 5.0 and 6.1% drip loss for the 40% CO_2 packs. At day 11, all treatments had significantly higher drip loss than air-stored fish.

Initial pH of the raw material was almost 6.8 (Figure 3). Following storage, the changes in pH varied among the treatments, being significantly lower (~0.3 units) in all MAP products than in air- and vacuum-packed fish on day 6 ($p<0.05$). On day 11, only M1 and M3 were still significantly lower (~0.2 units) than air- and vacuum-packed fish.

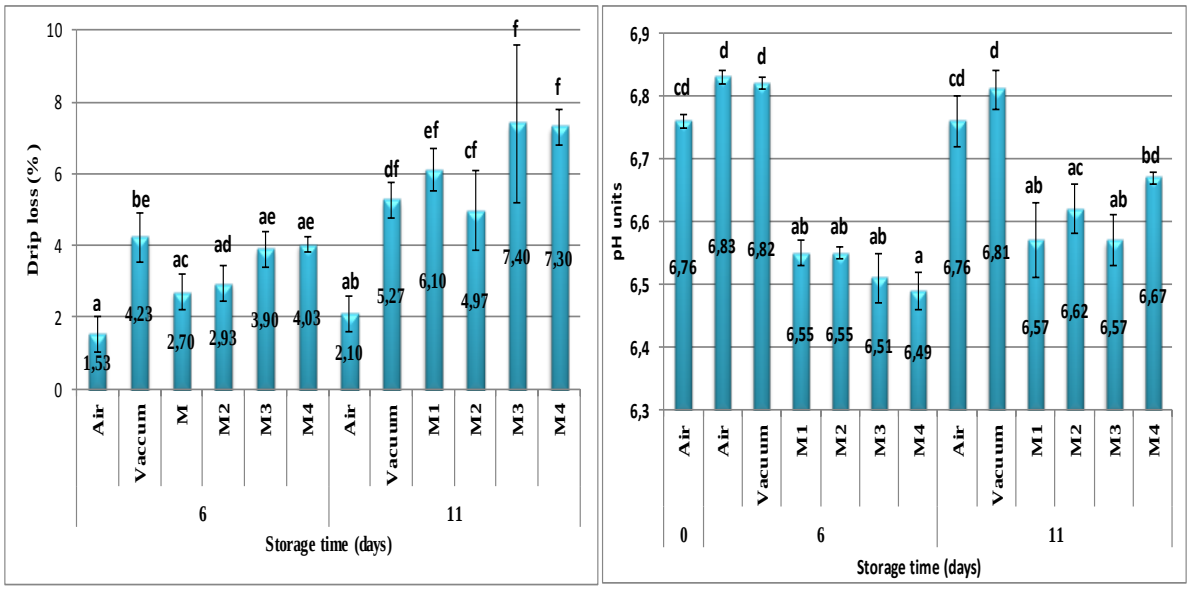

Figure 3. Mean drip loss (%) and pH measurements of raw redfish fillets packaged differently and stored at -0.4°C for 11 days. Error bars show standard deviation (n=3). Symbols (a-f) indicate a significant difference between the group means ($p<0.05$); M1=40% CO_2/60% Ar; M2=40% CO_2/60% N_2; M3=60% CO_2/40% Ar; M4=60% CO_2/30% Ar/10% N_2.

4.1.4 Texture of cooked fillets

The sensory panel also commented on the textural changes observed in redfish fillets during storage. Overall, the texture became more tough and dry as time increased (Table 5). The fillets in low CO_2 (M1 and M2) were less tough and dry than the other MAP treatments on days 6 and 11, while on day 13, M2 fillets were the softest.

Table 5. Texture of cooked redfish fillets assessed during storage under different atmospheric conditions; M1=40% CO_2/60% Ar; M2=40% CO_2/60% N_2; M3=60% CO_2/40% Ar; M4=60% CO_2/30% Ar/10% N_2.

Packaging type		Time (day)			
		0	6	11	13
	Air	Moderately firm, moist, flaky	Tough, dry	Tough, dry	Tough, dry
	Vacuum		Tough, dry	Tough, dry	Tough, dry
	M1		Not firm/tough or dry	More tough and dry than V and A	Very tough and dry
	M2		Less tough and dry compared to M3 and M4	Tough and dry than M1	Less Tough and dry than M1
	M3		Tough, dry, more precipitation	Tough and dry	Tough and dry
	M4		Tough, dry, more precipation	More normal texture, less tough, and dry	Very dry and strange/ abnormal texture

4.1.5 Microbial Analyses

The microbial quality of the raw material at the onset of the trial was fairly good with a mean total viable psychrotrophic count (TVC) of the flesh amounting to log 5.1 CFU/g (1.2 x 10^5 CFU/g) while H_2S-producing bacteria and pseudomonads counts were log 2.3 CFU/g and log 4.0 CFU/g, respectively (Figure 4). *Photobacterium phosphoreum* (Pp) was not detected (< log 1.3 CFU/g) by the PCR method used. Therefore, pseudomonads constituted most of the SSO population early post-processing. Statistical analysis showed there was no difference between TVC obtained on LH and IA media over the storage period ($p>0.05$); they increased significantly in air and vacuumed packages but remained mostly unchanged in the MAP groups. On day 6, all presumed SSO enumerated were detected, except for *P. phosphoreum* in the fillets of two MAP groups (M2 and M3). On day 11, levels of pseudomonads and *P. phosphoreum* were highest in vacuumed and air packages, i.e. at least 100-fold higher than in MAP products. Growth of H_2S-producing bacteria was not as rapid, but these SSO were detected at significantly higher levels in air- and vacuum-packed fish compared to MA packs on day 11. It was noted that the pseudomonad load remained unchanged (insignificant increase) in MAP fish during the course of storage while *P. phosphoreum* levels increased. This indicates that all MAP treatments were effective in controlling the growth of pseudomonads while *P. phosphoreum* was able to grow rapidly between days 6 and 11 (with a 1000-fold increase). The H_2S-producers developed best in air and vacuum and least in 60% CO_2, which are M3 and M4 (Figure 4). Nevertheless, all MAP treatments showed a similar inhibitive effect towards the SSO investigated. Therefore, none of the gas mixtures was significantly better with respect to microbial control. This indicates that a lower (40%) concentration of CO_2 was sufficient to delay SSO growth.

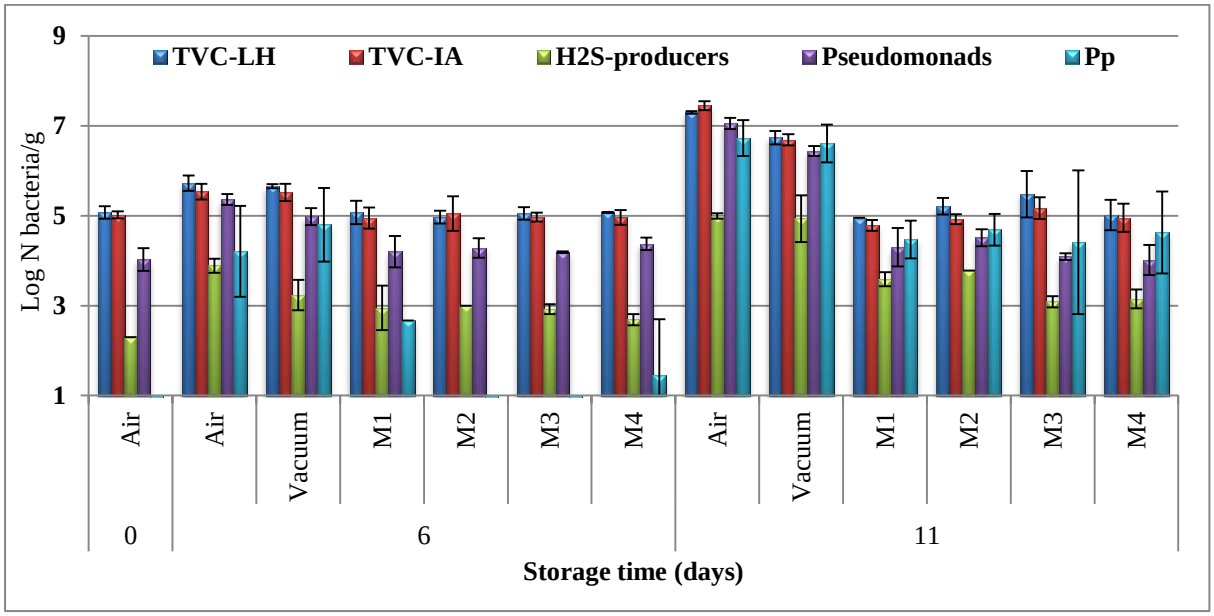

Figure 4. Microbial load in redfish fillets stored under different atmospheric conditions for 11 days at -0.4°C. Error bars indicate standard deviation; M1=40% CO_2/60% Ar; M2=40% CO_2/60% N_2; M3=60% CO_2/40% Ar; M4=60% CO_2/30% Ar/10% N_2.

4.1.6 Headspace gas composition and the gas to fish ratio

Composition of modified atmosphere in each group at the beginning of the trial is shown in Table 6. After 6 days of storage, the headspace CO_2 concentration in lower and higher CO_2 packs decreased by 10.7±0.5% and 12.8±0.4%, respectively (Figure 5). The Ar proportion

increased by 10.3±1.3% (mean value) in respective packages while N_2 increased by 3.0% and 9.6% in M4 and M2, respectively, after 6-day storage. On the other hand, oxygen levels had increased in all packages on day 6, i.e. by 0.9±0.1% in average. The concentration of each gas remained fairly stable on day 11 in all MAP groups. Reduction in oxygen levels was not observed on day 11, corresponding to the little growth of aerobic bacteria in these packs. The quantity of CO_2 in the gas mixture and the ratio of gas: fish generally influence the effectiveness of MA packaging. In this study, the gas: fish ratio ranged from 2.2-2.5 and there was no significant difference between ratio in all groups ($p>0.05$).

Table 6. Initial gas content in packages containing redfish fillets

Packages	Gas Measurements			
	CO_2	O_2	Ar	N_2
M1	39.30	0.04	60.66	
M1	39.70	0.04	60.26	
M2	41.10	0.05		58.85
M2	40.90	0.06		59.05
M2	40.30	0.04		59.66

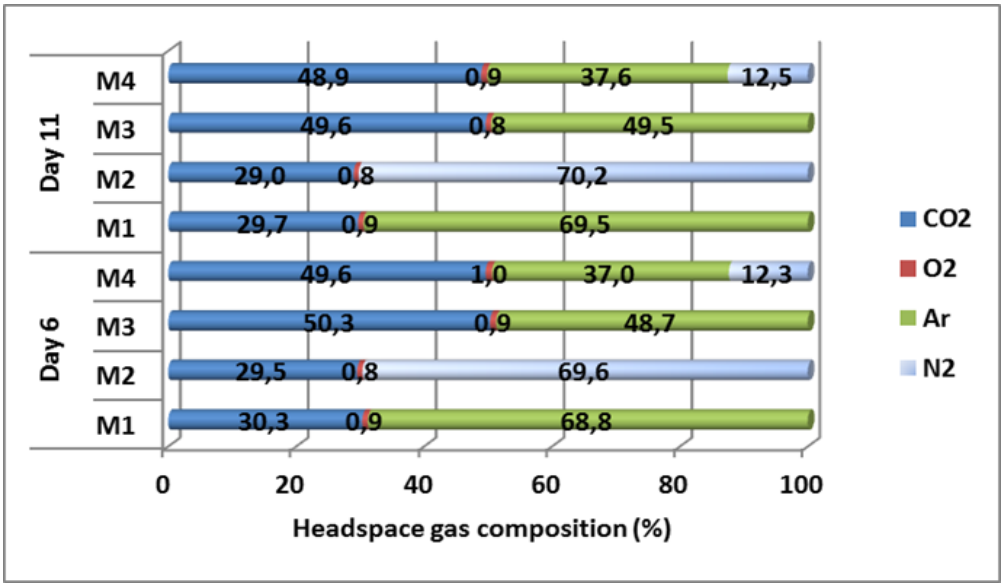

Figure 5. Mean headspace gas composition in different packages of redfish fillets stored for 11 days at -0.4°C (n=3); M1=40% CO_2/60% Ar; M2=40% CO_2/60% N_2; M3=60% CO_2/40% Ar; M4=60% CO_2/30% Ar/10% N_2.

4.1.7 Formation of TVB-N/TMA

As illustrated in Figure 6, TMA was not detected on days 0 and 6, but measurements on day 11 were highest in vacuum packs followed by air-stored fish (6.1 and 2.9 mg N/100g, respectively). There were very small variances between MAP groups (0.5-0.8 mg N/100g). TVB-N content on day 0 (10.2±1.5 mg N/100 g) and day 6 (9.5±0.6 mg N/100 g) was similar in all groups. There was an increase in TVB-N concentration at day 11 in both air and vacuum packages, reaching 16.3 mg N/100g. Conversely, in MAP packs, the levels were similar to day 0 especially in M2 and M4.

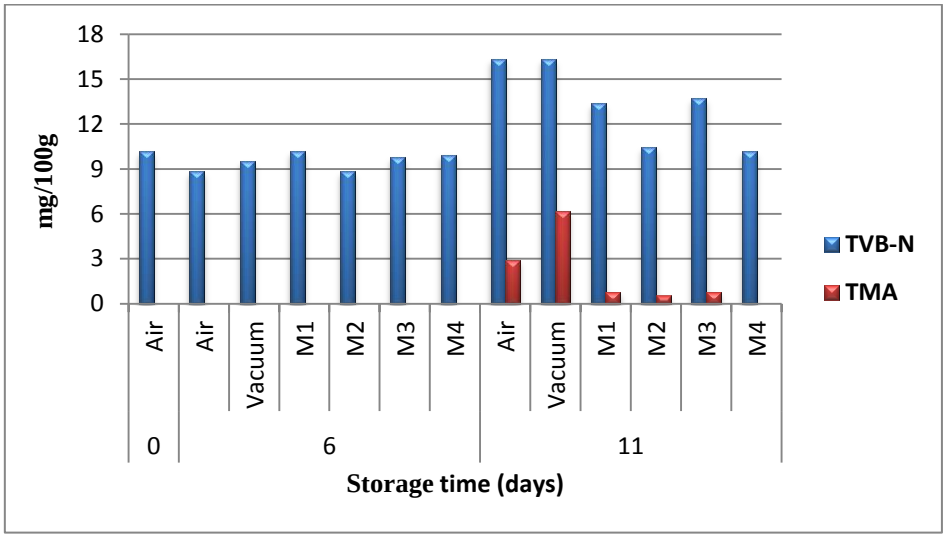

Figure 6. TVB-N and TMA content in redfish fillets stored under different atmospheric conditions at -0.4°C for 11 days; M1=40% CO_2/60% Ar; M2=40% CO_2/60% N_2; M3=60% CO_2/40% Ar; M4=60% CO_2/30% Ar/10% N_2.

4.2 Shelf life study

4.2.1 Temperature monitoring

Fish fillets were stored at -1°C to mimic sea freight transportation for 6 days followed by storage in common storage rooms at distributor/destination at 2°C. Figure 7 shows the temperature changes taking place in each group, monitoring up to 3 packages per group. To begin with, the mean temperature of the raw material obtained was 2.4±1.0°C with lowest temperature and least deviations measured in the M2 packages (Table 7). The mean temperature of the cold storage over the 16-day period was 0.7 ± 1.4 °C. The temperature of packaged products was high at the beginning but -0.5°C was reached after ~8 h. The fluctuations observed in ambient temperature throughout the storage period, were imitated in the products. When the temperature rose to 2°C, the fillets took ~73 h for the fillets to approach 2°C.

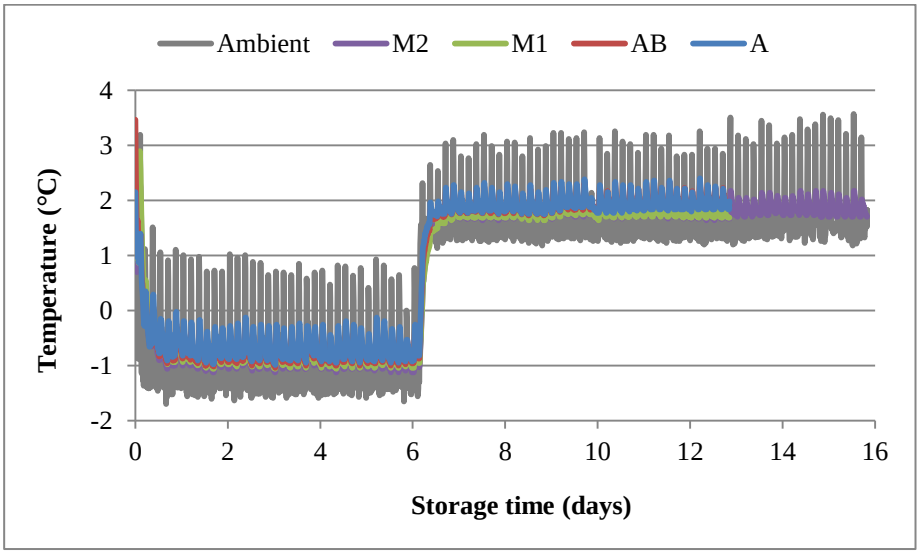

Figure 7. Temperature profile of redfish fillets packaged differently and stored for a 16-day period; M1=40%CO_2/60% Ar; M2=40% CO_2/60% N_2; M3=60% CO_2/40% Ar; A-Air bled AB-Air bled.

Table 7. Mean initial, superchilled and cold storage temperature for redfish fillets packaged differently and stored for a 16-day period. A= unbled fillets stored in air; AB= bled fillets stored in air; M1=40% CO2/60% Ar; M2=40% CO2/60% N_2; M3=60% CO_2/40% Ar; M4=60% CO_2/30% Ar/10% N_2.

	A	AB	M1	M2	All products	Ambient
Initial T (°C)	2.2±1.0	3.5±0.5	2.1±1.0	1.6±0.0	2.4±1.0	
Superchilled storage T (°C)	-0.6±0.4	-0.7±0.4	-0.7±0.5	-0.8±0.5	-0.7±0.4	-1.0±0.7
Cold storage T (°C)	1.9±0.3	1.9±0.3	1.7±0.3	1.7±0.3	1.8±0.3	1.7±0.4

4.2.2 Headspace gas composition and the gas to fish ratio

The initial gas composition sought was M1=40% CO_2/60% Ar; M2=40% CO_2/60% N_2 and the initial values measured are shown in Figure 8. After 6 days, the CO_2 concentration in the headspace had decreased by 14.82±1.45% and 15.38±0.39% in M1 and M2 packs, respectively. An increase was noted in O_2 of 1.35±0.37% and 1.10±0.38% in parallel to 13.47±1.31% Ar and 14.30±0.37% N_2 in M1 and M2, respectively. There were very slight changes in the gas composition during the remainder of the storage period. The gas to fish ratio was calculated from all sampled packs and there were no significant differences in the ratios obtained among the treatments ($p>0.05$). The gas:fish ratio ranged from 1.0 to 1.6% (mean ratio=1.3±0.1%).

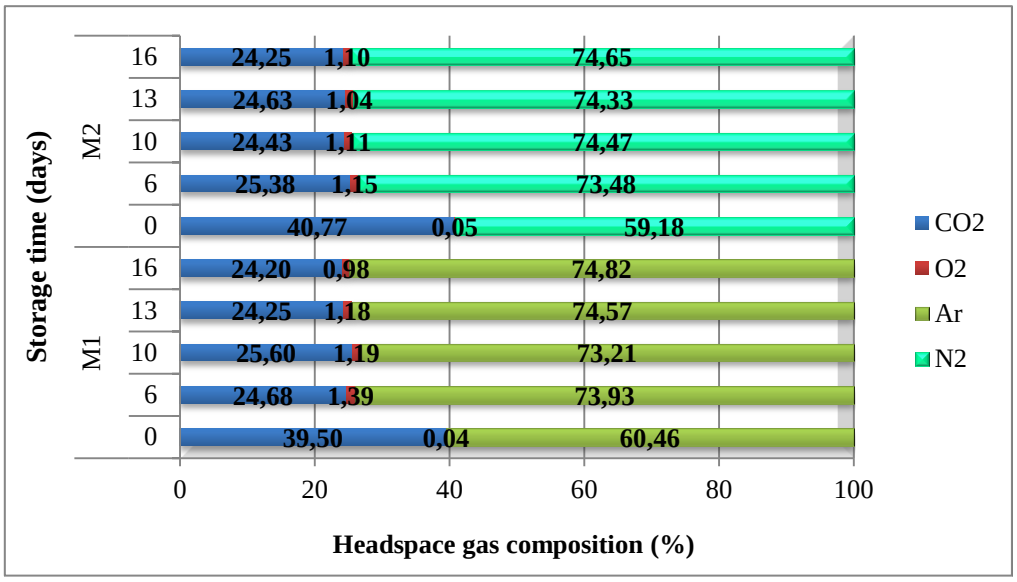

Figure 8. Headspace gas composition in different packages of redfish fillets stored at -1°C for 6 days and 2°C afterwards; M1=40% CO_2/60% Ar; M2=40% CO_2/60% N_2.

4.2.3 Freshness deterioration and shelf life determination

The initial freshness of the raw material was good where the unbled fillets used in MAP and air (A) packs had a mean score of 8.3 early post-processing in comparison to 8.7 for bled fillets (AB), being insignificantly higher ($p=0.060$) (Figure 9). The freshness period for unbled products stored in air ended by day 6 while bled fillets had the highest score of 7.3. Freshness was lost in the AB group after 8 days post-packaging, while in the MAP groups freshness decrease was evident on day 6 but was maintained at a score of 7.1 until day 10

after which further deterioration took place. Comparison of the MAP groups showed similar sensory quality as illustrated by the Torry score.

On day 13 the MAP products were still above the rejection point and their freshness score was significantly higher than that for air-stored fillets ($p<0.05$), which were below the sensory rejection limit. Based on the freshness deterioration slope, the shelf life for air-storage and MAP products is estimated to be 11 and 14 days, respectively.

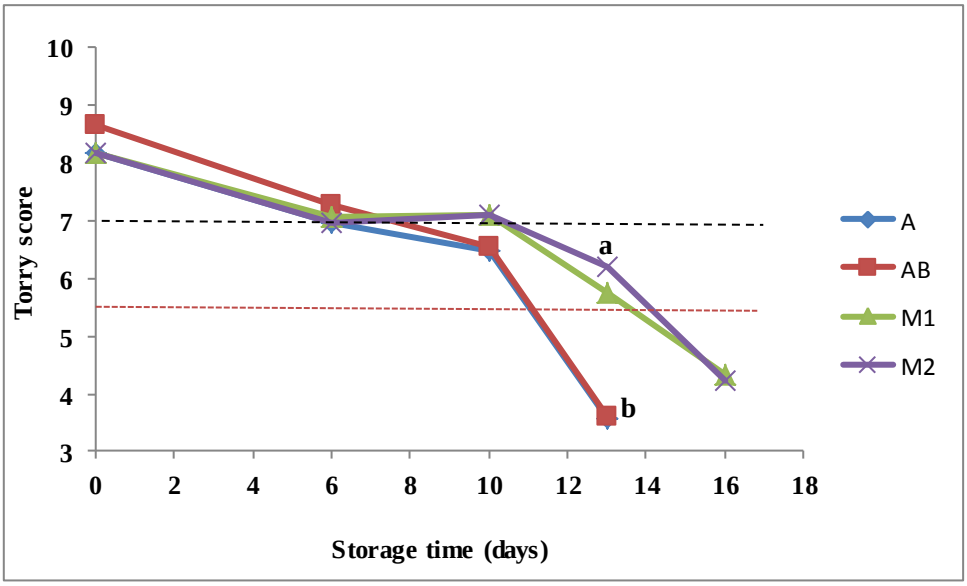

Figure 9. Mean Torry freshness score for cooked redfish fillets packaged differently and stored at -1°C for 6 days and 2°C afterwards. Torry score of 7 is limit for freshness period and score of 5.5 is unfit for consumption (shelf life cut off point). A=Air storage-unbled fish; AB=Air storage-bled fish; M1=40% CO_2/60% Ar; M2=40% CO_2/60% N_2. Symbols (a-b) indicate a significant difference between the group means ($p<0.05$).

4.2.4 Drip loss and pH

Drip loss ranged from 2 to 7% during the storage period. The fillets exposed to air had the least drip loss and gas-packed fillets had higher drip loss (Figure 10). On day 10, drip in M1 (5.5±0.7%) was significantly higher than in the air groups ($p<0.05$), but differed only slightly from M2 for which there was no difference with the air groups. Again, on day 13 the drip loss was significantly higher in M1 than in the A group, while the other groups did not differ significantly. At the end of storage on day 16, similar drip losses were measured in the MAP groups.

The initial pH of the raw material was almost 6.8. Gas packaging caused a decrease in flesh pH, which was significantly lower in MAP than in air groups at some sampling points ($p<0.05$) (Figure 10). After 6 days pH changes were significantly lower (~0.15 units) in M2 than air groups ($p<0.05$). On day 10, the pH decreased in all groups. The pH increased significantly during the remaining storage period. There was no difference between the air groups on day 13 but M1 was significantly higher than A fish.

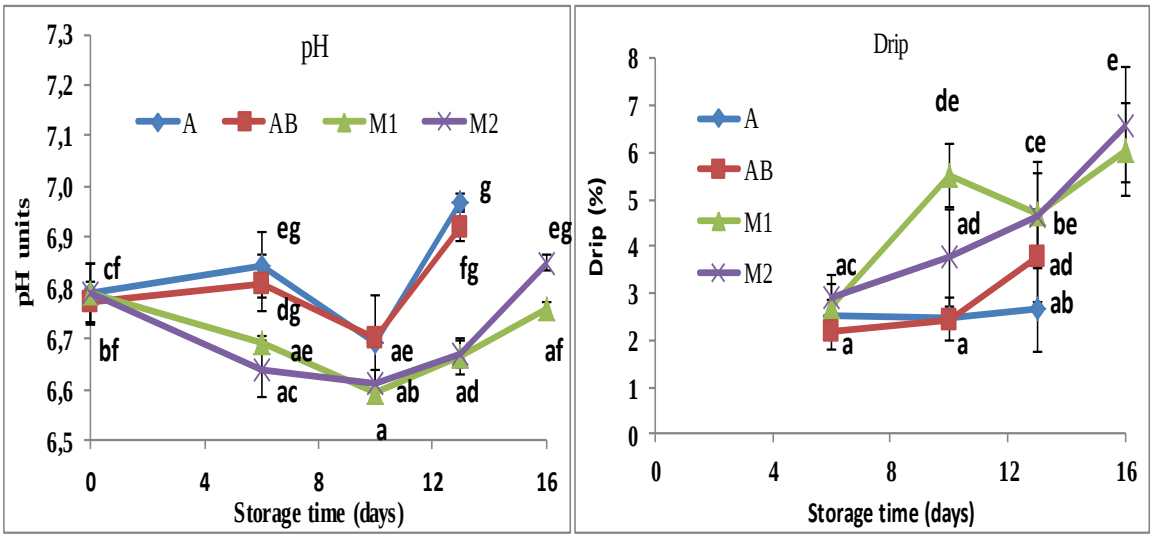

Figure 10. Mean pH changes (n=2) and drip loss (%) (n=4) of raw redfish fillets packaged differently and stored at -1°C for 6 days and 2°C afterwards. Error bars indicate standard deviation. Symbols (a-g) show the significant difference between the group means ($p<0.05$); A=Air storage-unbled fish; AB=Air storage-bled fish; M1=40% CO_2/60% Ar; M2=40% CO_2/60% N_2.

4.2.5 Texture of cooked fillets

Cooked fillets from M2 were significantly tougher than air-stored (A) fillets on day 6 ($p<0.05$). On day 13 there was greater variation between all of the groups in texture parameters, like tenderness and juiciness. Air-stored fillets (A and AB) were significantly drier and tougher than the MAP groups, and M2 was also significantly tenderer than M1. However, lesser differences among the MAP groups were observed on day 16.

4.2.6 Microbiological analyses

Figure 11 illustrates the microbial growth (TVC, H_2S-producers, pseudomonads, *P. phosphoreum*) in the different treatments over the storage period. Bleeding had no effect in the delay of overall psychrotrophic microbial growth (TVC). The growth of TVC on LH and IA media was similar and there were no significant differences between the counts obtained ($p>0.05$). For this reason, only LH counts are shown. The initial load was slightly higher in bled fillets than in unbled ones. There was a rapid rate of growth in the air-stored packages while a lag phase was observed in the MAP groups during the superchilled period). However, an increase in temperature to 2°C on day 6 resulted in proliferation in both air-stored and MAP packages but at a slower rate in the later. The microbial growth was significantly higher in air than in MAP groups throughout storage.

The H_2S-producers were initially just at or close to the detection level (log 1.3 CFU/g) in the unbled fillets used and slightly higher in the bled fillets (log 2.0 CFU/g). Throughout storage, the lowest levels were seen in the MAP groups while significantly higher levels were found in AB than A ($p<0.05$). Superchilled temperature delayed growth in MAP groups but after chilled temperature was obtained, growth increased to some extent. In comparison, there was greater proliferation of H_2S-producers in the air groups and, by day 13, the difference in numbers was 1000-fold in unbled fish. Pseudomonads growth rate was slower in MAP products than those stored in air. An increase in temperature led to rapid increase in groups A

and AB but a gradual increase in MAP. Again, on day 13, the difference in numbers was 1000-fold between air-stored and MAP fish.

The growth of *P. phosphoreum* was slower in bled fish (AB) compared to the other fish treatments and the other SSO. Initial growth was impaired by low temperature where levels were slightly lower than initially or the same, as demonstrated by the extended lag phase in all treatments for the 6-day superchilled storage. Exponential growth became highest in unbled, air-stored fillets after the temperature increased but was slowest in bled fillets. The difference in *P. phosphoreum* levels in air packs compared to MAP fish was less compared to the other microorganisms. *P. phosphoreum* levels were lower than for H_2S-producers and pseudomonads in air-stored products but were higher in the MA packages at end of storage.

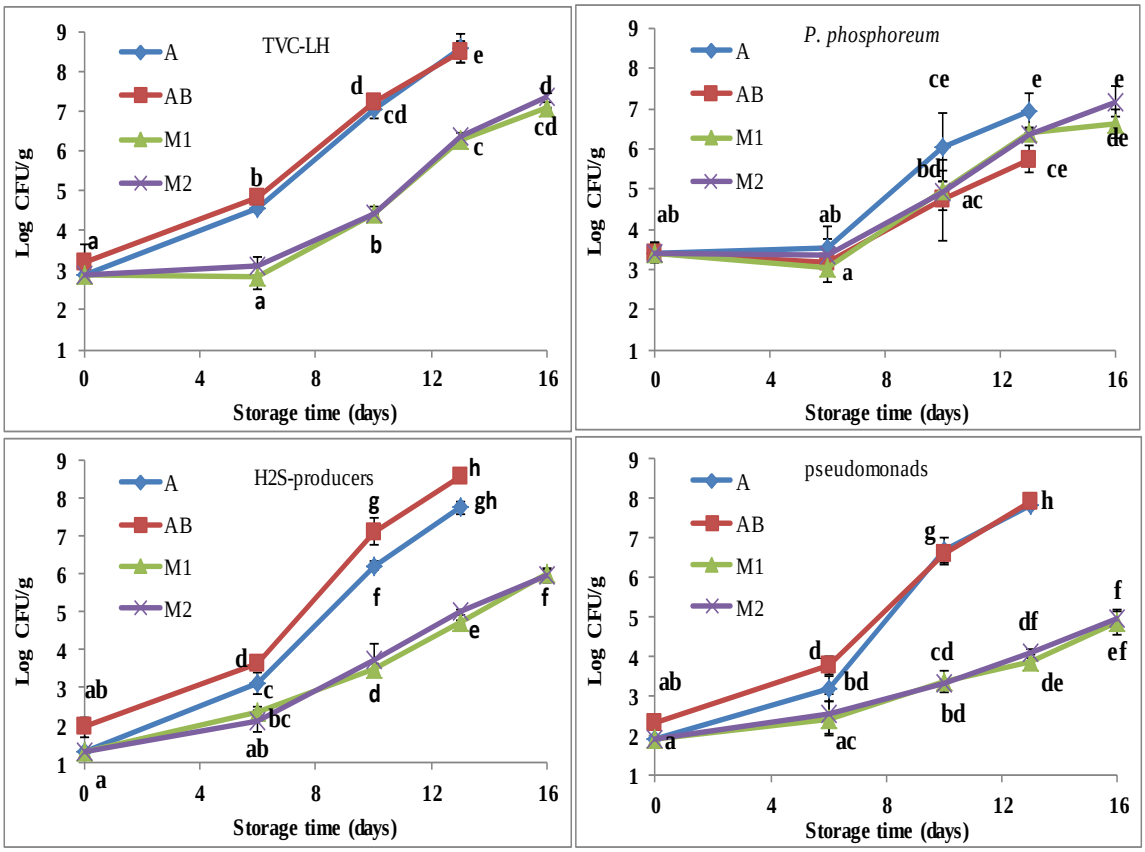

Figure 11. Microbial profile (TVC, H_2S-producers, pseudomonads, *P. phosphoreum*) of raw redfish fillets packaged differently and stored at -1°C for 6 days and 2°C afterwards (n=2). Error bars indicate standard deviation. Symbols (a-h) signify a significant difference between the group means; M1=40% CO_2/60% Ar; M2=40% CO_2/60% N_2; A=Air storage-unbled; AB=Air storage-bled.

Facultative anaerobes, lactic acid bacteria and *B. thermosphacta*, were evaluated in the later stage of storage (Figure 12). The growth of both organisms was significantly greater in the air packs than in MAP fish but LAB counts were most often observed to be at higher levels than *B. thermosphacta*. The bled fillets supported higher levels of both organisms, while M1 contained higher levels among the MAP groups. *B. thermosphacta* developed late in M2 fish, being detected on day 13. Presence of *Clostridium perfringens* was not detected in MAP fish at late storage time (<100 CFU/g at days 10-16).

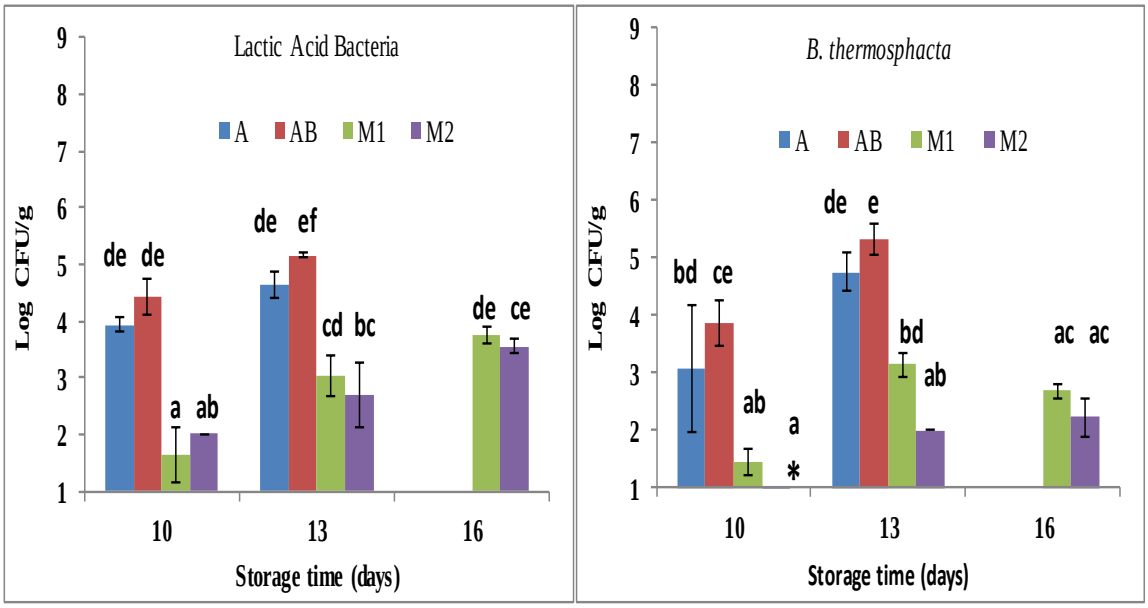

Figure 12. Profile of LAB and *B. thermosphacta* in raw redfish fillets packaged differently and stored at -1°C for 6 days and 2°C afterwards (n=2). Error bars indicate standard deviation. Symbols (a-f) signify a significant difference between the group means; M1=40% CO_2/60% Ar; M2=40% CO_2/60% N_2; A=Air storage-unbled; AB=Air storage-bled. The asterisk indicates that the count was below detection level (log 1.3 CFU/g).

4.2.7 TVB-N/TMA

As shown in Figure 13, the formation of both TVB-N and TMA was directly proportional to each other. Levels of TVB-N were slightly lower in MA treated and A (unbled) fillets than in AB (bled) fish during the first 6 days of storage. As storage progressed, a significant increase in these volatile bases was detected in air-stored fillets, which eventually had the shorter shelf life. There was no significant difference between MAP treatments, except on day 16 where TVB-N levels in M2 were higher ($p<0.05$). No significant change in TMA was observed in first 10 days in all groups. Nevertheless on day 13, levels in air-stored groups were significantly higher than in MAP groups. Bled fillets had a lower TMA content than air-stored unbled fish while M1, which contained Ar, slowed down the production of TMA more effectively than M2, containing N_2, approaching the end of the storage period.

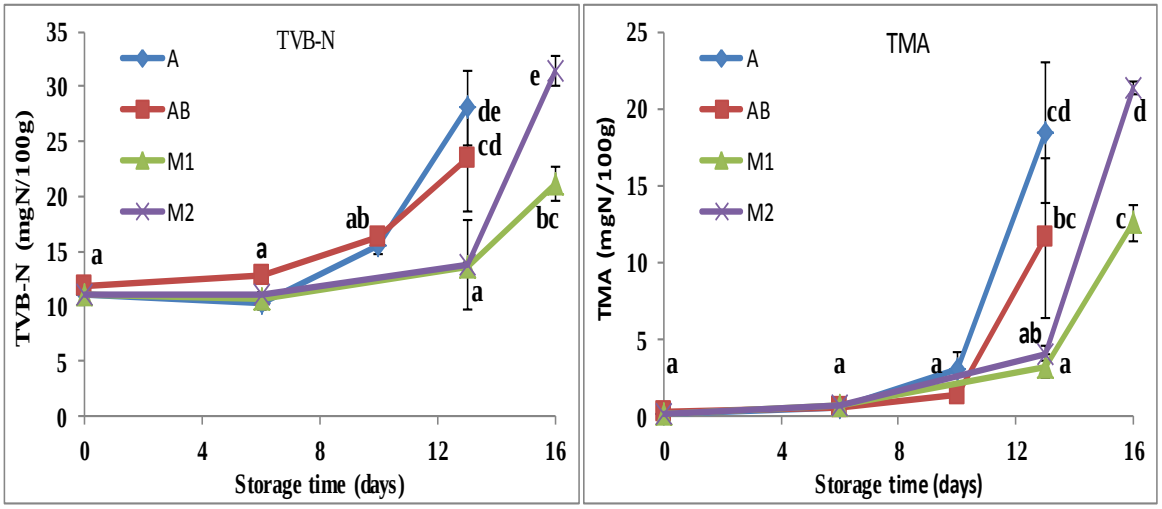

Figure 13. TVB-N and TMA content in raw redfish fillets packaged differently and stored at -1°C for 6 days and 2°C afterwards (n=2). Error bars indicate standard deviation. Symbols (a-e) signify a significant difference between the group means.

4.3 Principal Components Analysis

Figure 14 presents the principal component analysis (PCA) done to evaluate different variables (Torry, drip loss, TVB-N, TMA, pH, TVC, H2S–producers, pseudomonads and *P. phosphoreum* counts) and the treatment samples assessed during storage. The first principal component (PC1) explains 70% of the variation between the treatment samples and describes from left to right the changes observed in Torry freshness scores (from high quality to low quality) in concordance to the evolving spoilage indicators towards the left, with increasing levels of TVB-N and TMA content, and microbial counts as storage time progressed. The second principal component (PC2) accounts for 16% of the variation and differentiates between higher drip (upper position) and higher pH (lower position), the most influencing variables of PC2. Nevertheless, *P. phosphoreum* (upper position) and pseudomonads (lower position) counts account for some variation observed in PC2.

Variables accounting for LAB, *Brochothrix thermosphacta* counts and gas:fish ratio was not included in this analysis because they had little effect on the overall variation observed and were not considered as important. The PCA biplot shows high Torry (freshness) score corresponds to similar high quality in all groups on day 0 and MAP groups on day 6 with low levels of spoilage indicators. As time progressed and high levels of microbes and metabolites were formed, spoilage increased. As pH decreased, the drip loss increased, and growth of *P. phosphoreum*, as the main spoilage microorganisms in MAP groups on day 13-16, was favoured. In comparison, increased pH and low drip loss occurred in air storage where pseudomonads proliferation took place. As storage time progressed, the status of deterioration in treatment samples can be compared between air-stored and MAP fish (air-d6 = MAP d10; air-d10 = MAP d13; and air d13 = MAP d16), reflecting the slower spoilage process in MAP fillets.

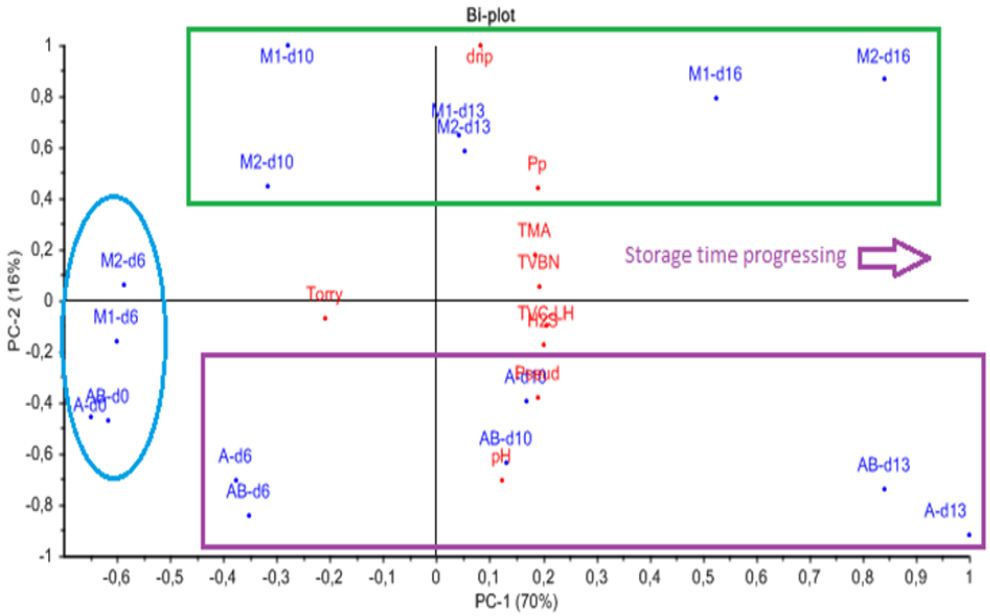

Figure 14. Principal components analysis (PCA) of measured sensory (Torry), physicochemical (TVB-N, TMA, drip loss, pH) and microbial (TVC-LH, pseudomonads, H_2S-producers, *P. phosphoreum*) data of raw redfish fillets packaged differently and stored at -1°C for 6 days and 2°C afterwards during the shelf life study; M1=40% CO_2/60% Ar; M2=40% CO_2/60% N_2; A=Air storage-unbled; AB=Air storage-bled (d0, d6, d10, d13, d16 represent sampling days).

5 DISCUSSION

5.1 Pre-trial

The pre-trial evaluated different atmospheric conditions during a short storage period at a steady temperature (-0.4°C), aiming for the selection of a packaging condition that can extend the freshness of deskinned redfish fillets without negatively affecting other quality and sensory attributes of importance, e.g. appearance and texture. Four MAP conditions (M1 to M4) were compared to air (A) and vacuum (VP) storage. The gas mixtures selected aimed to evaluate the use of Ar to replace partly or totally N_2 and to assess different concentrations of Ar (30-40-60%) and CO_2 (40-60%). The concentration of CO_2 is one of the parameters of importance in MAP, as it is the gas providing mostly the antimicrobial activity generally observed but an increasing concentration may result in texture and appearance defects (Lauzon *et al.* 2002, Davis 1993, Soccol and Oetterer 2003). Another parameter linked to these effects if the gas to fish ratio, as a high ratio will result in greater gas volume influencing the fish products.

Temperature monitoring of fish products and ambient condition revealed the high initial product temperature, which was probably due to delay at processing, during shipping and handling at room temperature during preparation and packaging (Lauzon *et al.* 2011). The initial high temperatures observed in ambient conditions were due to the sensitivity of the cooling chamber after its opening on packaging day where any slight temperature increase triggered cooling. Temperature in the products had a similar pattern to the ambient although not as responsive. Vacuum-packed fillets were most sensitive in responding to the changes in

ambient temperature since they had the highest product temperature while the MA packages overall maintained the lowest temperatures over the storage period in particular M4.

Headspace gas measurement in MA packs at the beginning of experiment showed that 0.04% oxygen was left after the bags were sealed. Oxygen dissolved in the fish muscle was displaced by CO_2 followed by CO_2 uptake in the muscle of the fish as evidenced by its reduced proportion in the headspace few days post-packaging. In order for the gas uptake to be adequate for retardation of microbial growth, the ratio of the gas: food product may need to be twice the volume of the food, although CO_2 is dissolved and absorbed in the surface during storage (McMillin 2008, Masniyom 2011). Although, that finding was reported for meat, similar ratio was attempted in this pre-trial (2.2-2.5) and absorption was obvious when the CO_2 composition in the packages had decreased at early storage time. As a result carbonic acid is formed which lowered pH values in the MAP packs. At low superchilling temperature, the increased activity of CO_2 in MAP, may be due to greater aqueous and lipid solubility.

The differences in texture observed among the treatments were explained by the drip loss measured in the MAP groups as well as their lower pH, likely to enhance protein denaturation and cause more water loss from the fillet, hence resulting in increased toughness and dryness. The water holding capacity of fish muscle is weakened at lower pH, which accounts for the greater exudate loss resulting in tough and dry textures (Lauzon *et al.* 2002, Masniyom 2011, Soccol and Oetterer 2003). This corresponds to the drip loss being higher in MAP and VP in this pre-trial. Similar CO_2 uptake (%) and pH drop were observed in redfish fillets for the lower (40%) and higher (60%) CO_2 treatments. Nevertheless, 60% CO_2 treatments resulted in enhanced drip loss, dryness and toughness in fillets compared to 40% CO_2, which may be explained by the greater concentration absorbed by the muscle.

Freshness deterioration was fastest in vacuum packs, followed by air-stored fish, but slowest in 40% CO_2 treatments. Shelf life of air-stored fish was 11 days, but 13 days for the 60% CO_2 treatments while vacuum-packed fillets had a shorter storage life (probably 9-10 days). The shelf life of the 40% CO_2 treatments was greater than 13 days. MAP treatments with 40% CO_2 apparently slightly extended the freshness period (Torry score >7), while a shelf life extension of several days was achieved in comparison to air and vacuum storage. This can be explained by fact that MAP had the advantage of retarding further development of the aerobic spoilage microflora that became established on the fillets post-processing. In vacuum-packed fish, the more rapid deterioration is explained by the higher TMA formation under anaerobic storage, induced by rapid growth of H_2S-producing bacteria and *P. phosphoreum* (Matamoros *et al.* 2006). This could be expected since TMA is a microbial metabolite produced from TMAO in marine fish, occurring at a faster rate under low oxygen tension (Huss 1972). This suggests that VP is not suitable for long-term storage of redfish fillets.

The three bacterial groups (H_2S-producing bacteria, *P. phosphoreum* and pseudomonads) monitored are important spoilage bacteria in fresh cold water marine fish stored aerobically in chilled storage while pseudomonads grow slowly under low oxygen conditions (Reynisson *et al.* 2010, Olafsdottir *et al.* 2006b, Gram and Dalgaard 2002). H_2S-producing bacteria counted may not only be *S. putrefaciens* or *S. putrefaciens*-like organisms, although this has often been shown to be the case with fresh fish stored on ice (Emborg *et al.* 2002). Vogel *et al*. 2005 identified the main H_2S-producing organism as *S. baltica* in Danish marine fish. The most dominant spoilage organisms at the end of storage were pseudomonads sand *P. phosphoreum*. The fastest growing SSO was *P. phosphoreum* in all treatments; however, a slower growth was observed in MAP than air-stored fish, explaining the slower deterioration

process observed in MAP fish. Gas composition of CO_2 (40-60%) had though a similar effect on *P. phosphoreum* growth.

The reason why shelf life extension of 60% CO_2-packed fillets was lesser than in 40% CO_2 treatments could be explained by the more extensive texture problems and related protein denaturation (Lauzon *et al.* 2002, Masniyom 2011). H_2S-producing bacteria were not prevailing SSO. They thrived best in air followed by vacuum packaging and least in the MAP groups. Previous research has observed that a slower growth rate in *S. putrefaciens* (H_2S-producing bacteria) with an increasing CO_2 concentration (Debevere and Boskou 1996, Dalgaard 2006). Indeed, our results pointed to the slightly slower development of H_2S-producing bacteria under 60% than 40% CO_2 as storage progressed.

MAP groups containing N_2 showed no changes in TVB-N concentrations during the first 11 days of storage and TMA development happened faster in vacuum. *P. phosphoreum* is a facultative anaerobe able to use TMAO as an electron acceptor and reduces it to TMA and levels of this SSO were highest at the end of storage. The microbial data did not indicate which gas mixture is better. Even though the use of MAP storage contributed to a shelf life extension, sensory quality parameters of texture, such as toughness and dryness, were affected. The CO_2 concentration influenced these defects, but they were still detectable at 40% CO_2 with a gas to fish ratio of 2.2-2.5. Reduction of this ratio below a value of 2 could result in lesser texture problems. This was verified during the main shelf life study. Thus two gas mixtures, 40% CO_2:60% Ar and 40% CO_2:60% N_2, were selected for the main shelf life study based on the freshness and texture data as well as drip loss. Further, the sensory scores obtained when the judges assessed the fillets were inconsistent because of the quality differences (bruises and red spots), and changes as time progressed, in the raw material obtained in the beginning of the experiment. As a result, a comparative study on the effect of bleeding on freshness and quality deterioration in redfish fillets was included in the shelf life study.

5.2 Main Study

The main study evaluated different atmospheric conditions selected from the pre-trial during the storage period at (-1°C) for 6 days followed by cold storage (+2°C) afterwards to mimic transportation of products to the export markets. Two packing conditions (M1 and M2) were compared - no bleeding (A) and bleeding in air (AB) on extending freshness of deskinned redfish fillets without negatively affecting other quality and sensory attributes of importance. The gas mixtures selected aimed to evaluate the effectiveness of same volumes Ar and N_2 in MAP and the gas to fish ratio, was lowered to improve textural problems.

Superchilling retarded the growth rate of microbes which rapidly increased under chilled storage in all groups and prevented the growth for *C. perfringens*, food safety hazard (De Jong *et al.* 2003). *P. phosphoreum* proliferation was influenced by temperature, being generally the most sensitive to superchilling conditions and was the most responsive to increasing temperature among SSO evaluated, as reported by Olafsdottir *et al.* (2006b). Initial microbial contamination of bled fillets was slightly higher compared to unbled fillets, which could be explained by processing and handling contamination (Huss 1995). The initial quality of redfish fillets impacted on the length of the freshness period of MAP products and subsequent extension of shelf life.

Bleeding had no impact on initial microbial quality of fillets and was ineffective in retarding the growth of bacteria thereafter, except for *P. phosphoreum*. In fact, growth of *P.*

phosphoreum in bled, air-stored fillets was comparable to the slower growth observed in MAP (unbled) fillets. The higher microbial load and higher initial temperature in AB should result in faster spoilage than A. This was not really observed, because the freshness period was longer for AB and the microbial rate was not faster either (slope was parallel to unbled air-stored fish or often even as time progressed). Literature reports that blood is enriched with nutrients which can induce the proliferation of bacteria in fish and residual blood is one of the main factors inducing the development of undesirable colour of fish flesh during ice storage (Maqsood and Benjakul 2011). Therefore, bleeding should retard some microbial growth by devoiding nutrients and reduce yellowing and red spots (Olsen 2011).

Iron is an important micronutrient used by bacteria and is essential for their metabolism. It is available from haem and blood, and is required as a cofactor for a large number of enzymes and iron-containing proteins (Rachid and Ahmed 2005). Interestingly, pseudomonads are able to bind iron in an iron-low environment and compete with other bacteria and grow better. H_2S-producing bacteria however grew very well in the AB fish, but these may be other bacteria than *S. putrefaciens*, a species reported to be inhibited in presence of pseudomonads (Gram and Melchiorsen 1996). On the other hand, growth of *P. phosphoreum* was delayed in bled fish, which has not been reported yet to our knowledge. Competition of this SSO with H_2S-producing bacteria and pseudomonads was therefore evidenced in bled fillets. It can be suggested that bleeding redfish may extend both the freshness period and shelf life of MAP fillets.

Packaging in an atmosphere of >2% O_2 and storage close to 0°C provide adequate safety against growth and toxin production of pathogenic strains of *Clostridium* spp (Arritt *et al.* 2007). Despite the higher mean product temperature and lower O_2 levels in the MAP treatments investigated during the main study, presence of *C. perfringens* was not detected before the products were spoiled (day 14) or few days later (day 16). LAB and *B. thermosphacta* were detected at the later stage of chilled storage but were not the dominant species, therefore playing only a minor role in spoilage. The later had just reached detectable levels in M2 fish the day prior to end of shelf life and levels were lower than LAB which indicates that N_2 and low temperature were more effective in controlling *B. thermosphacta* growth than Ar (M1). Recently, *B. thermosphacta* were shown to be unable to compete with LAB under similar conditions (Doulgeraki *et al.* 2012).

M1 containing Ar slowed down the production of TVB-N more effectively than M2 containing N_2, but only towards the end of shelf life. This difference may be due to the much higher solubility of Ar in water (Randell *et al.* 1997) and possibly some antimicrobial properties towards TMA-producers. However, this had little effect on maintaining redfish freshness better between the MAP treatments. The TVB-N consumption limit for redfish fillets (*Sebastes* sp.) is 25 mg N/100g (Regulation (EC) No.1022/2008). This level was not quite reached at sensory rejection (incipient spoilage) but was exceeded with extended storage time for air-stored and M2 fish. TMA formation was slower, which is promising for MAP bled fillets due to the slow development of *P. phosphoreum* but increased the pH with the rise in metabolites (Debevere and Boskou 1996, Ruiz-Capillas and Moral 2001).

The unbled fillets used in air and MAP storage had freshness period of 6 days, in comparison to 7-8 days in bled fillets. Bleeding extended the freshness period by almost 2 days but did extend shelf life of the fillets, as summarised in Table 8. On the other, MAP storage maintained the freshness of unbled fillets just above the freshness limit for an additional 4 days. MAP therefore provided some freshness extension, which could be enhanced if bled fillets were MA-packed. Overall, the shelf life extension resulting from MAP (4 days) was

equal to the freshness extension observed (4 days) due to significant difference between Ar and N_2. Other sensory characteristics, such as texture were compromised but the lower gas: fish ratio solved the textural problems in Jan. trial. Randell *et al.* (1997) also found that Ar-containing gas mixtures did not increase the shelf life during storage of trout fillets at 2°C when compared to CO_2-N_2 mixtures, and the Ar levels applied performed similarly based on the sensory and microbiological analyses performed. The upper limit of TVC (t°C) for fish to be safe for consumption is 10^7 CFU/g or log 7 CFU/g (EC 2005). This level was not quite reached at sensory rejection (incipient spoilage) but was exceeded with extended storage time for air-stored and M2 fish.

Table 8. Levels of microbiological and chemical indicators of spoilage for differently treated redfish fillets at the end of shelf life according to the Torry scheme. A=Air storage-unbled fish; AB=Air storage-bled fish; M1=40% CO_2/60% Ar; M2=40% CO_2/60% N_2.

Parameters	A	AB	M1	M2
Freshness period/ remaining shelf life	6;5	8; 3	11; 3	11; 3
Sensory shelf life (days)	11	11	14	14
TVC (log CFU/g)	7.5	7.5	6.5	6.5
H_2S-producers (log CFU/g)	6.5	7.5	5.0	5.0
Pseudomonads (log CFU/g)	7.0	7.0	4.0	4.0
P. phosphoreum (log CFU/g)	6.5	5.2	6.5	6.5
LAB (log CFU/g)			3.2	3.0
B. thermosphacta (log CFU/g)			3.0	2.2
TVB-N (mg N/100g)	20	15-20	15-20	20
TMA (mg N/100g)	0.5	2.0	3.0	13.0
pH	6.8	6.8	6.7	6.7
Drip loss (%)	2-3	3	5	5

6 CONCLUSION

There was no significant difference in the quality of fillets stored in 60% Ar or N_2, both gas mixtures including 40% CO_2 resulted in the same shelf life. Ar is more expensive than N_2 which does not justify the use of argon-containing gas mixtures as commercially viable in the packaging of redfish fillets. The lower gas to fish ratio solved the textural problems encountered during the pre-trial. It is therefore important to carefully design a packaging solution by taking into account the fish bulk quantity, optimal pack size, space available for shipping and related costs. Bleeding extended the freshness period slightly but did not extend the overall shelf life of fillets. Further, the initial colour was better which will make packaged fillets more attractive to the consumer. It means that by bleeding fish properly, an extension of the freshness period and shelf life of redfish fillets can be obtained by reducing *P. phosphoreum* growth rate and TMA production, especially if MAP is used where other spoilage bacteria are delayed. Yellowing of flesh was not detected in the main study and may be explained by the selection of high quality unbled fillets.

Chemical and physical changes still occurred with delayed growth of SSO under MA and superchilled conditions. Therefore, quality deterioration of fish cannot be evaluated by only chemical and microbiological methods. TVB-N and TMA measurements provided no information about quality changes during the first few days of storage. However, the quality

loss was estimated by these tests only at the later stage of storage. Torry scheme was used to determine freshness deterioration and sensory shelf life and the correlation of this parameter to various chemical and microbiological indicators measured was done to properly determine a measure of the progress of deterioration; i.e. increased proliferation of microbes and related metabolites production compared to decrease in sensory quality (indirectly proportional).

Storage temperature is important since it affects the lag stage duration, the maximum growth rate and final cell numbers. Superchilling was able to delay microbial growth, especially for *P. phosphoreum* in the early stages of storage. Processing companies and retailers must ensure limits for storage temperature are maintained during transport to reduce quality deterioration and extend the shelf life of products, since some microbes are psychrotolerant and can grow fast under abused temperatures.

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APPENDIX

Appendix I

Torry freshness scheme.

Score	Odour	Flavour
10	Initially weak odour of boiled cod liver, fresh oil, starchy	Boiled cod liver, watery, metallic.
9	Shellfish, seaweed, boiled meat, oil, cod liver	Oily, boiled cod liver, sweet, meaty characteristic.
8	Loss of odour, neutral odour	Sweet/ characteristic flavours but reduced in intensity.
7	Woodshavings, woodsap, vanillin	Neutral
6	Condensed milk, boiled potato	Insipid
5	Milk jug odours, boiled clothes- like	Slight sourness, trace of "off"-flavours, rancid
4	Lactic acid, sour milk TMA	Slight bitterness, sour, "off"-flavours, TMA, rancid
3	Lower fatty acids (eg acetic or butyric acid) composed grass, soapy, turnipy, tallowy	Strong bitter, rubber, slight sulphide, rancid

Appendix II

QDA attributes for cooked redfish samples.

	sensory attribute	short name	scale	definition
	TEXTURE			
1	soft	T-soft	firm ‖ soft	Softness in first bite
2	juicy	T-juicy	dry ‖ juicy	Dry: draws liquid from mouth. Juicy: releases liquid when chewn
3	tender	T-tender	tough ‖ tender	Tenderness when chewn
4	mushy	T-mushy	none ‖ much	Mushy, porridge like texture
5	meaty mouthfeel	T-meaty	none ‖ much	Reminds of meat texture, rough fibers
6	sticky	T-sticky	none ‖ much	Glues together teeth when biting the fish.

Appendix III

Statistical analysis of mean microbial counts and physical parameters during the pre-trial of differently packaged redfish fillets. One-way ANOVA and Tukey-Kramer Multiple-Comparison Test or Kruskal-Wallis Multiple-Comparison Z-Value Test for drip loss.

Day	Group	TVC-LH		TVC-IA		H2S-IA		Pseud.		Pp		Drip loss		pH	
0	A	5.1	ab	5.0	a	2.3	ab	4.0	a	<1.3	a			6.76	cd
6	A	5.7	b	5.5	a	3.9	ab	5.4	cd	4.2	bc	1.5	a	6.83	d
	V	5.7	ab	5.5	a	3.2	ab	5.0	bc	4.8	bc	4.2	be	6.82	d
	M1	5.1	b	5.0	a	3.0	ab	4.2	b	1.3	ab	2.7	ac	6.55	ab
	M2	5.0	b	5.1	a	3.0	ab	4.3	b	<1.3	a	2.9	ad	6.55	ab
	M3	5.1	b	5.2	a	2.9	ab	4.2	ab	<1.3	a	3.9	ae	6.51	ab
	M4	5.1	b	5.0	a	2.7	ab	4.4	ab	1.5	ab	4.0	ae	6.49	a
11	A	7.3	c	7.5	b	5.0	b	7.1	d	6.7	ce	2.1	ab	6.76	cd
	V	6.7	c	6.7	b	4.9	b	6.4	d	6.6	ce	5.3	df	6.81	d
	M1	4.7	a	4.8	a	3.6	b	4.3	ab	4.5	bc	6.1	ef	6.57	ab
	M2	5.2	ab	4.9	a	3.8	a	4.5	ac	4.7	bc	5.0	cf	6.62	ac
	M3	5.5	ab	5.2	a	3.1	ab	4.1	b	4.4	bc	7.4	f	6.57	ab
	M4	5.0	ab	5.0	a	3.2	ab	4.0	a	4.6	bc	7.3	f	6.67	bd
p value		*0.000*		*0.000*		*0.000*		*0.000*		*0.000*		*0.000*		*0.000*	

Different letters indicate a significant difference among treatment means within a column ($p<0.05$

Appendix IV

Statistical analysis (ANOVA, and Duncan´s Multiple-Comparison Test) of mean Torry freshness score.

Day	A	AB	M1	M2	p-value
0	8,2	8,7			0,060
6	7,0	7,3	7,1	7,0	0,800
10	6,5	6,5	7,1	7,1	0,066
13	3,6 **b**	3,7 **b**	5,8 **a**	6,3 **a**	0,000
16			4,3	4,2	0,628

Different letters indicate a significant difference among treatment means within a column ($p<0.05$).

Appendix V

Statistical analysis (ANOVA, and Tukey-Kramer Multiple-Comparison Test) of mean microbial counts, values of physical and chemical parameters during the shelf life study.

Day	Pack	TVC-LH	TVC-IA	H2S	Pseud	Pp	pH	TVB-N	TMA	Drip	LAB	B. Th
0	A	2.9 a	2.6 a	1.3 a	1.9 a	3.4 ab	6.79 cf	10.96 a	0.14 a			
	AB	3.2 a	3.3 ab	2.0 ab	2.3 ab	3.4 ab	6.77 bf	11.89 a	0.2 a			
6	A	4.5 b	3.9 bc	3.1 cd	3.2 bd	3.5 ab	6.85 eg	10.25 a	0.51 a	2.5 a		
	AB	4.8 b	4.4 c	3.6 d	3.8 d	3.2 ab	6.81 dg	12.74 a	0.53 a	2.06 a		
	M1	2.8 a	2.9 a	2.4 bc	2.4 ac	3.1 a	6.69 ae	10.74 a	0.69 a	2.7 ac		
	M2	3.1 a	2.9 a	2.1 ab	2.6 ac	3.4 ab	6.64 ac	10.95 a	0.67 a	2.91 ac		
10	A	7.0 cd	6.9 e	6.2 f	6.7 g	6.0 ce	6.69 ae	15.5 ab	3.05 a	2.46 a	3.9 de	3.1 bd
	AB	7.3 d	7.2 e	7.1 g	6.6 g	4.7 ac	6.7 ae	16.29 ab	1.34 a	2.41 a	4.4 de	3.9 ce
	M1	4.4 b	4.3 c	3.5 d	3.4 cd	5.0 bd	6.6 a			5.51 de	1.7 a	1.5 ab
	M2	4.4 b	4.3 c	3.7 d	3.3 bd	4.9 bd	6.61 ab			3.76 ad	2.0 ab	<1.3 a
13	A	8.6 e	8.2 f	7.8 gh	7.8 h	6.9 e	6.97 g	28.05 de	18.46 cd	2.69 ab	4.6 de	4.8 de
	AB	8.5 e	8.7 f	8.6 h	7.9 h	5.7 ce	6.92 fg	23.38 cd	11.6 bc	3.80 ad	5.2 ef	5.3 e
	M1	6.3 cd	5.5 d	4.7 e	3.9 de	6.4 ce	6.67 ad	13.49 a	3.21 a	4.7 ce	3.0 cd	3.2 bd
	M2	6.4 cd	5.9 d	5.0 e	4.1 df	6.3 ce	6.67 ad	13.76 a	4.06 ab	4.66 be	2.7 bc	1.5 ab
16	M1	7.1 d	6.7 e	6.0 f	4.9 ef	6.6 de	6.76 af	21.1 bc	12.56 c	6.06 e	3.7 ce	2.7 ac
	M2	7.4 d	7.0 e	6.0 f	5.0 f	7.2 e	6.85 eg	31.39 e	21.37 d	6.59 e	3.6 ce	2.2 ac
p value		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Different letters indicate a significant difference among treatment means within a column ($p<0.05$).