EFFECTS OF DRY ICE AND SUPERCHILLING ON THE QUALITY AND SHELF LIFE OF ARCTIC CHARR (*SALVELINUS ALPINUS*) FILLETS

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ABSTRACT

Arctic charr (*Salvelinus alpinus*) fillets were packed with dry ice or ice packs and stored chilled (3-4°C) and superchilled (-2°C) for up to two weeks. The physical changes (drip loss, cooking yield, water holding capacity and colour), chemical changes (pH, water, trimethylamine, total volatile base and thiobarbituric acid), microbial changes (total viable count and H_2S producing bacteria) and sensory changes were observed on day zero, three, six, nine, 13 and 16 of the storage period. The shelf life of fillets packed with the ratio of 150 g dry ice to 3 kg Arctic charr fillets and stored at superchilling temperature was 16 days. This was six days longer than the shelf life of chilled fillets which were packed with the same ratio of dry ice (10 days) and one day longer than fillets which were packed with ice packs and controlled at superchilling temperature (15 days). There were no detected effects on the quality of fillets caused by cell destruction due to partial freezing or soured taste due to the absorption of CO_2 gas in the fish flesh. This permits transportation of higher quality Arctic charr fillets to distant markets.

Keywords: Arctic charr, dry ice, ice packs, superchilling, chilling, shelf life, drip loss, cooking yield, water holding capacity.

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1 INTRODUCTION

Shelf life of fish and fishery products is a very important consideration in the seafood processing industry. Shelf life extension of fish and fishery products allows the processors to plan and control their processing and marketing in the long term and more transport modes are available to them. Handling practices and storage conditions are the most important factors that affect the shelf life of fish and fishery products. If the fish and fishery products are well handled, the storage conditions (temperature) at which they are stored determines their shelf life.

In marketing, the cost and quantity of fish and fishery products are the two most important factors. Maintained quality and extended shelf life of raw fishery products can be achievedby lowering the storage temperature to superchilling with various types of cooling agents. The choice of cooling agent for superchilling of raw fish and fishery products is very important with regard to shelf life extension and lower transport costs. Transport by flight, in particular, has special requirements forcooling agents. Dry ice and ice packs are recommended for air shipment of fresh fish and seafood to maintain low temperature.

The effects of different storage methods on the extension of the shelf life of different species have been studied and promising results have been found. Superchilling and modified atmosphere packaging (MAP) are often considered. Superchilling is based on lowering temperature close to the freezing point of the fish (0°C to -4°C) using different cooling agents such as refrigerated air, brine, refrigerated sea water (RSW), chilled sea water (CSW), liquid ice and dry ice. At superchilled temperatures, the spoilage bacterial growth is reduced resulting in extended shelf life. In comparison, the effect of MAP on shelf life extension of fish and fishery products is based on the inactivation of spoilage bacteria and the oxidation reaction in anaerobic conditions caused by modified atmosphere at different levels of CO_2 concentration. However, each method has its advantages and disadvantages depending on the characteristics of the products, storage conditions and means of transport. Therefore, studies are needed to find out appropriate storage conditions for each of fishery product.

Vietnam is a seafood export country. The export turnover increased from 971 million US\$ in 1999 to 2.4 billion US\$ in 2004. The shelf life extension of fish and fishery products is therefore of great interest to Vietnamese processors. Although the shelf life extention methods of fish and fishery products, such as superchilling and MAP, have been researched and applied in the world for a long time, in Vietnam these methods have not been researched and applied properly to Vietnamese species. Most processors apply storage methods based on their experience or customers' requirements. Better understanding of the research and application of the shelf life extension methods would offer prospects for quality improvement of fishery products in Vietnam.

The aim of this project is to observe the effects of dry ice and superchilling on the quality and shelf life of fish products in comparison with ice packs, chilling and freezing for the same storage time. Arctic charr (*Salvelinus alpinus*) fillets were used in this trial due to relatively high fat content and colour to simulate changes that could occur in important species for Vietnam such as tuna or basa where deterioration of fat and colour are critical factors.

2 LITERATURE REVIEW

2.1 The Arctic charr

Arctic charr (*Salvelinus alpinus*) are a salmonid species well adapted to the rigorous environment of the most northerly freshwaters. It grows rapidly at cold temperatures and may produce a higher fillet yield than other salmonids such as rainbow trout and Atlantic salmon (Swatland *et al.* 1997).

Most commonly the charr spawns in the autumn, from September to December, but this varies between breeds. There are breeds that spawn in January or February and even in other seasons. Early in the maturation stage the quality of the fish is highly affected. The skin becomes thicker and changes colour, the belly becomes red and the back of the fish darkens, slime production increases, the flesh becomes whiter as colourants go into the skin and eggs, the protein and fat content of the flesh decreases and its consistency becomes watery. The result is a bland-tasting fish and a change in smell (Charrnet-Aquafarmer 2004).

2.1.1 *Chemical composition*

The chemical composition of Arctic charr varies considerably with the season and the breeding cycle. The lipid content of wild Arctic charr muscle is about 1-5% depending upon the season and sex. By the end of winter the male Arctic charr post-spawners have lost 50-55% of the lipid present in the body, whereas female post-spawners have lost over 80% of the body lipid (Jobling *et al.* 1998).

For aqucultured Arctic charr, the dry matter is about 29.16% (water content is about 70%); protein is about 17.16%; lipid is 7.79% (Krasnov *et al.* 1999). The chemical composition of Arctic charr is similar to other salmonid species (Table 1).

Table 1: The chemical composition of some salmonids species (Huss 1995, Mills 2001).

Salmonids species	Water (%)	Protein (%)	Fat (%)
Rainbow trout (*Oncorhynchus mykiss*)	70-80	18-20	0.7-8.3
Salmon (*Salmo salar*)	67-77	21.5	0.3-14.0
Trout (*Salmo trutta*)	70-79	18.8-19.1	1.2-10.8

Astaxanthin is the predominant carotenoid found in wild Arctic charr. The carotenoid concentration in the flesh of aquacultural Arctic charr varies from 0.4 to 7.9 mg/kg. The colour of the flesh depends on genetics, maturation, age and diet (Hatlen *et al.* 1995). The relationship between redness (a* value) and astaxanthin content in the muscle of Arctic charr was established by Olsen and Mortensen (1997) as follows:

$$\text{a* value} = 0.84 + 1.06 \times C_{Ast}$$

Where C_{Ast} is astaxanthin content in mg/kg of fish muscle.

Usually, the customers want to buy the fish with strongly pink flesh.

2.2   Quality changes and spoilage of raw fish and fishery products

Quality changes and spoilage of fish and fishery products result from changes caused by oxidation of lipid and carotenoids, reactions caused by activities of the endogenous enzymes in fish, and metabolic activities of microorganisms.

Enzymatical changes
The most obvious changes in fish are in sensory characteristics. Firstly, complicated biochemical reactions during rigor mortis, cause muscle fibres to shorten and tighten and finally the fish becomes stiff. Rigor mortis has many technological consequences. For example, if the fish is filleted pre-rigor, the length of the fillet may be shortened by 30% after the onset of rigor and the texture of cooked flesh will be very soft and pasty (Huss 1995). Table 2 summarises the enzymes which are involved in fish quality changes.

Table 2: Summary of enzymes, which are involved in fish quality changes (Amaya and Marangoni 2000a, Amaya and Marangoni 2000b, Gill 2000, Haard 2000, Huss 1995, Jiang 2000, Pan and Kuo 2000, Sikorski and Kolakowski 2000, Simpson 2000, Sotelo and Rehbein 2000).

Enzymes	Substrates	Changes
Glycolytic enzymes: • glycogen phosphorylase • glycogen phosphorylase kinase	Glycogen	• Production of lactic acid, pH of tissue drops, denaturalization and loss of water holding capacity in muscle • High temperature rigor may result in gaping
Autolytic enzymes involved in nucleotide breakdown: • ATPase • Myokinase • AMP deaminase • 5' nucleotidase • Nucleotide phosphorylase • Inosine nucleosidase • Xanthine oxidase	ATP ADP AMP IMP Inosine Inosine Xanthine	• Loss of fresh fish flavour, gradual production of bitterness with hypoxanthine (Hx) at later stages. • The formula was developed for fish freshness based upon the changes in the nucleotides (K value or freshness index) as follows: $K\% = \dfrac{[Ino]+[Hx]}{[ATP]+[ADP]+[AMP]+[IMP]+[Ino]+[Hx]} \times 100$
Calpain	Myofibrillar proteins	• Softening of muscle tissue
Cathepsins	Proteins, peptides	• Softening of muscle tissue
Pepsin, chymotrypsin, trypsin, carboxy-peptidases	Proteins, peptides	• Bursting of fish belly (Caused autolysis of visceral cavity)
Collagenases	Connective tissue	• Gaping of fillets • Softening of muscle tissue
Lipases, phospholipases	Lipids, phospholipids	• Formation of off flavour or off odour • Denaturation of myofibrillar caused of changes in texture and water holding capacity of muscle proteins • Destruction of some vitamins and amino acids
Lipoxygenases	PUFA, carotenoids (Astaxanthin, tunaxanthin and β-carotene)	• Formation of the typical oxidative fishy odour • Changes in colour from dark to colourless, faded pink or red colour of skin or flesh fish
TMAO reductase TMAO demethylase	TMAO	• Formation fishy odour (TMA) • Toughening of frozenn storage fish cause of formaldehyde formation.

ATP: Adenosine-5'-triphosphate; ADP: Adenosine diphosphate; AMP: Adenosine monophosphate; IMP: Inosine monophosphate; TMAO: Trimethylamine oxide; PUFA: Polyunsateraturated fatty acid

If the fish is filleted in rigor, the filleting yield will be very poor and the texture of cooked flesh will be tough but not dry. Post-rigor the flesh will become firm, succulent and elastic. The rate in onset and resolution of rigor is variable depending on the species, size of the fish, handling methods and storage temperature (Bykowski and Dutkiewicz 1996, Huss, 1995). Other changes that relate to the appearance, odour, texture and taste of the fish are due to the enzymatic breakdown. These reactions are catalysed by endogenous or bacterial enzymes as summarized in Table 2 above.

The post mortem reduction in the pH of the fish muscle has an effect on the physical properties of the muscle. As the pH drops, the net surface charge on the muscle proteins is reduced, causing them to partially denature and lose some of their water holding capacity. Muscle tissue in the state of rigor mortis loses its moisture when cooked and is particularly unsuitable for further processing, which involves heating, since heat denaturation enhances the water loss. Loss of water has a detrimental effect on the texture of fish muscle. Toughness and water loss during cooking (cooking yield) occur at lower pH levels (Huss 1995).

Enzymatic autolytic changes lead to the formation of the characteristic, sweet, creamy and meaty flavours of fresh fish. These changes do not produce the unpleasant flavours of spoiled fish. However, the products of autolysis are used by bacteria as substrates for metabolism and growth generating the unpleasant flavours of spoiled fish (Whittle *et al.* 1990). Although the enzymatic changes do not cause seafood spoilage directly, they create good conditions for bacterial growth. Therefore, the enzyme activities take part in the spoilage of fish and fishery products.

Bacterial changes
Bacterial changes are the most important cause of fish spoilage. The bacteria present in fish and fishery products come from the fish's living environment or by contamination during handling, processing and storage. Bacterial growth in fish and fishery products has serious consequences such as changes in sensory characteristics and unsuitability for human consumption (Gram and Dalgaard 2002).

The microflora of temperate water fish is dominated by psychrotrophic Gram-negative, rod-shaped bacteria belonging to the genus *Pseudomonos*, *Moraxella*, *Acinetobacter*, *Shewanella*, *Flavobacterium*, *Vibrionaceae* and *Aeroemonadaceae*. The flora on tropical fish often carries a slightly higher load of Gram-positive and enteric bacteria (Gram and Huss 1996). The spoilage bacteria of fresh fish and fishery products are summarized in Table 3 for different aquatic environments.

The total number of organisms vary from 10^2-10^7cfu (colony forming units)/cm^2 on the skin surface and from 10^3-10^9cfu/g on the gills or intestines (Huss 1995). There are no bacteria in the muscles of dead fish. In fish fillets, most of the microbial contamination is found to occur during filleting and subsequent handling prior to packaging (Jay 1996). Opening of the fish muscle by filleting creates conditions for the invasion of bacteria. Thus the spoilage rate of fish fillets is usually higher than that of whole fish during chilled storage (Whittle *et al.* 1990). The experiment of Chytiri *et al.* (2004) also showed that the changes in microbial flora of filleted rainbow trout during iced storage are greater than those in whole rainbow trout. Initial mesophilic viable counts of whole and filleted rainbow trout were 2.5 and 3.8 log cfu/cm^2, respectively on day zero. The mesophilic viable counts reached 7.0 log cfu/cm^2 after

10 days of storage for fillets and after 18 days for whole rainbow trout (Chytiri *et al.* 2004).

Table 3: The specific spoilage bacteria of fresh fish and fishery products (Gram and Huss 1996, Huss 1995).

Atmosphere	Specific spoilage bacteria of fresh fish and fishery products				Tipical spoilage compounds
	Temperate waters		Tropical waters		
	Marine	**Fresh**	**Marine**	**Fresh**	
Aerobic	*S. putrefaciens*		*S. putrefaciens*		TMA, H₂S, CH₃SH, (CH₃)₂S, Hx
	Pseudomonos ssp.	*Pseudomonos* ssp.	*Pseudomonos* ssp.	*Pseudomonos* ssp.	Ketones, aldehydes, esters, non-H₂S sulphides
CO₂	*P. phosphoreum*				TMA, Hx
		Lactic acid bacteria	Lactic acid bacteria		Lactic acid
			TMAO reducing bacteria	TMAO reducing bacteria	TMA

Oxidable changes

Fish fats contain a high proportion of polyunsaturated fatty acids which are easily attacked by atmospheric oxygen, forming unpleasant flavours especially in fatty fish. In chilled fresh fish and even in fish stored at ambient temperatures, lipid oxidation does not seem to be a dominant spoilage process, even though in the later stages of spoilage of fattier species such as trout, sardine, herring and mackerel, rancid flavours affect acceptability (Whittle *et al.* 1990). However, subfreezing or irradiation prior to chilled storage tend to result in oxidative rancidity because the lipid hydrolitic enzymes are more active at subfreezing temperatures (Sikorski and Kolakowski 2000) and oxygen use of bacteria is paused and radical forming precursors are started (Whittle *et al.* 1990).

The partial freezing and irradiating of fish may also motivate astaxanthin oxidation, caused by free-radical mechanisms (Hutchings 1999b). The oxidation of astaxanthin results in discolouration of the fish. This is a quality parameter, where discolouration indicates lower quality. However, the colour varies between individuals.

2.3 Methods for storage and extended shelf life of raw fishery products

Raw fishery products are highly perishable, because of their high water activity (a_w), neutral pH, and the presence of autolytic enzymes. The rate of deterioration is highly dependent on temperature and can be slowed by the use of low storage temperatures such as chilling, superchilling and freezing.

2.3.1 Low temperature preservation of raw fishery products

According to Doyle (1995), "seafood shelf life is a function of temperature". The temperature will control the rate of bacterial spoilage, enzyme activity and oxidation reaction. At low temperatures, the spoilage rate of fish is reduced and the products

remain edible longer. The relative rate of spoilage (RRS) of chilled and superchilled raw fish was described in the square root spoilage model (Huss 1995) as follows:

$$\sqrt{RRS} = 0.1 \times t + 1$$, where t is storage temperature (°C).

Storage of raw fishery products at temperatures between 0°C and -4°C is called "superchilling", "deep chilling" or "partial freezing" (Huss 1995). At these temperatures approximately 30 - 50% of the water in the product is frozen (Figure 1). The freezing point of different species depends on the water content and soluble substances of the fish. The freezing point of salmon is -2.2°C and -1.0°C of trout (Rahman 1995).
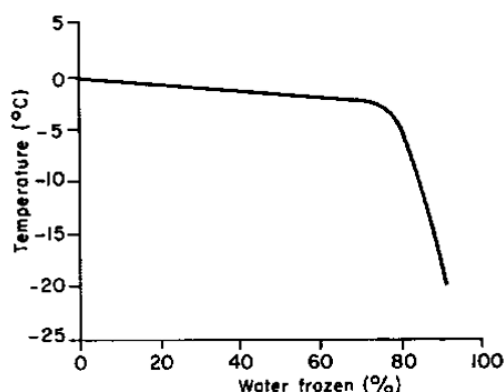
Figure 1: Freezing of cod muscle (Graham *et al.* 1992).

The shelf life of various fish and fishery products can be extended by storage at superchilled temperature. The square root spoilage (RRS) model ($\sqrt{RRS} = 0.1 \times t + 1$) gives a reasonable description of the RRS of superchilled raw fishery products (Figure 2).
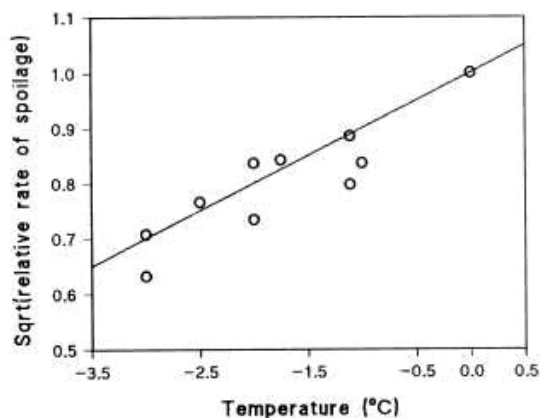
Figure 2: The relative rate of spoilage of superchilled raw fishery products was predicted by the equation $\sqrt{RRS} = 0.1 \times t + 1$ (Huss 1995).

At temperatures below 0°C the growth rate of bacteria is significantly reduced. The rate of bacterial spoilage at -2°C is only 64% of the rate at 0°C (Doyle 1995). This shows the potential advantage of superchilling. However, the results from the study of

Tanaka *et al.* (1999) indicate that bacteria in fish are not killed at -1°C and they can survive for a long time. Therefore, storage of raw fishery products at superchilled temperature cannot prevent spoilage.

The decrease in the spoilage rate of fish at low temperature can be roughly described by the known Arrhenius equation ($k = A * \exp^{(-E_a / R*T)}$ where k is the rate coefficient, A is a constant, E_a is the activation energy, R is the universal gas constant, and T is the temperature in degrees Kelvin). Under superchilled conditions, however, the temperature effect is still comparatively low, while the change in the concentration of soluble substances in the fish flesh is dramatic. At -6°C, about 80% of the tissue water in the muscle of cod turns to ice (Figure 1). Thus the rate of several enzymatic reactions in the muscle foods is greater in the critical temperature zone -1°C to -6°C than above 0°C. For example, in haddock, muscle glycolysis proceeds at -2°C, which is at least twice as fast as at 0°C. The maximum rate is from -3.2°C to -3.7°C. Denaturation of proteins in frozen haddock muscle proceeds at a maximum rate between -2°C and -4°C. The degradation rate of glycogen and organic phosphates in cod muscle increases at temperatures of -1°C to -5°C and the hydrolysed rate of phosphatidylcholine in fish muscle reach a maximum at -5°C (Sikorski and Kolakowski 2000). For this reason, the action of endogenous enzymes in fish muscle at superchilled temperatures has been noted.

Below -5°C, the flesh texture toughens and the myofibrillar protein becomes in extractable (Schultz and Anglemier 1964).

2.3.2   Chilling and superchilling methods

The cooling agents used for the chilling and superchilling of fishery products are fresh water ice, liquid ice, ice packs, gel packs, "Frigido-system", refrigerated sea water (RSW) (Huss 1995), refrigerated air (Kreuzer 1969), chilled sea water (CSW), brine, dry ice (solid CO_2) and liquid nitrogen (LeBlanc and LeBlanc 1992). Each agent has its advantages and disadvantages. For example, advantages of liquid ice are a faster chilling rate as compared with flake ice, deriving from its higher heat exchange capacity, and reduced physical damage. The oxidation and dehydration mechanisms may also be limited by overall coverage of the surface of the products (Pineiro *et al.* 2004). However, disadvantages of this agent are increased nutritional loss and salt absorption.

Cooling agents such as dry ice (CO_2 solid) or liquid nitrogen are quick ways of decreasing product temperature in minimum time. Dry ice is sublimated at -78°C and liquid nitrogen is evaporated at -195.9°C (LeBlanc and LeBlanc 1992). Dry ice has been used for the rapid transport of fresh fishery products by air, as it is weightless and evaporates directly instead of turning to liquid.

2.3.3   The effects of CO_2 and superchilling

Storage of fishery products by using CO_2 solid (CO_2 snow or dry ice) results in a combined effect of low temperature and CO_2 on the spoilage microflora of the fish. The prospect of this method in transportation of high quality fish and fishery products to distant markets and saving is great because of the longer lasting effects of dry ice chilling in comparison with other methods. LeBlanc and LeBlanc (1992) studied the

effects of superchilling with CO_2 snow on the quality of commercially processed cod (*Gadus morhua*) and winter flounder (*Pseudopleuronectes americanus*) fillets. Their observations show that the fillets, which were sprayed with CO_2 snow chilled rapidly and remained at a temperature lower than 0°C without water ice for about three and a half days. The graded quality was not affected but moisture loss and expressible moisture were higher than in the iced fillets. The total volatile base-nitrogen (TVB-N) and trimethylamine-nitrogen (TMA) were lower than in the iced fillets and the shelf life extension was one day.

Modified atmosphere packaging (MAP) is another method where CO_2 is applied to extend the shelf life of fish and fishery products to inhibit bacterial growth and oxidative reactions. The extension of shelf life depends on species fat content, initial microbial population, gas mixture, and most importantly, storage temperature. The shelf life of some fresh fishery products packed under modified atmosphere are shown in Table 4.

Table 4: Shelf life of some fresh fishery products packed under modified atmosphere (Sivertsvik *et al.* 2002).

Type of fresh fishery products	Storage temperature (°C)	Modified atmosphere (%)	Shelf life (days)
Salmon (*S. salar*) fillets	2	60 : 40 : 0 (CO_2 : N_2 : O_2)	17
	2	40 : 60 : 0 (CO_2 : N_2 : O_2)	17
	2	Vacuum	17
	2	Air	11
Trout (*S. gairdneri*) fillets	1.7	80 : 20 : 0 (CO_2 : N_2 : O_2)	20
	1.7	Air	10
Rainbow trout (*O. mykiss*) fillets	2	20 : 80 : 0 (CO_2 : N_2 : O_2)	9
	2	40 : 60 : 0 (CO_2 : N_2 : O_2)	9
	2	Vacuum	6
Cod (*G. morhua*) fillets	2	40 : 60 : 0 (CO_2 : N_2 : O_2)	11
	1	60 : 40 : 0 (CO_2 : N_2 : O_2)	12
	1	Air	9
	0	2 : 98 : 0 (CO_2 : N_2 : O_2)	14
	0	3 : 97 : 0 (CO_2 : N_2 : O_2)	13
	0	29 : 71: 0 (CO_2 : N_2 : O_2)	16
	0	48 : 52 : 0 (CO_2 : N_2 : O_2)	20
	0	97 : 3 : 0 (CO_2 : N_2 : O_2)	15-16
Herring (*C. harengus*) fillets	0	60 : 40 : 0 (CO_2 : N_2 : O_2)	14
	0	Air	12
Mackerel (*S. Scombrus L.*)	-2	100 : 0 : 0 (CO_2 : N_2 : O_2)	> 21
Snapper (*C. auratus*) fillets	3	100 : 0 : 0 (CO_2 : N_2 : O_2)	6-8
	3	Air	3
	-1	100 : 0 :0 (CO_2 : N_2 : O_2)	18
	-1	40 : 60: 0 (CO_2 : N_2 : O_2)	9
	-1	Air	9
Catfish, channel (*I. punctatus*) fillets	2	80 : 20 (CO_2 : Air)	28
Tilapia (*Tilapia ssp.*) fillets	16	75 : 25 : 0 (CO_2 : N_2 : O_2)	9-13
	8	75 : 25 : 0 (CO_2 : N_2 : O_2)	13-16
	4	75 : 25 : 0 (CO_2 : N_2 : O_2)	>25
	16	Air	3-6
	8	Air	6-9
	4	Air	9-13

CO_2 in the storage environment inhibits growth of the normal spoilage flora in the air, such as *Pseudomonas* and *Shewanella putrefaciens*, but other microoganisms, such as *Lactobacillus* spp. and *Photobacterium phosphoreum* were dominant in the spoilage flora (Gram and Huss 1996, Huss, 1995). On the other hand, the possibility that *Clostridium botulinum* type E and non-proteolytic type B strains will grow and produce toxins in a low-oxygen atmosphere at chilled temperatures has caused great concern in studies on MAP storage of seafood (Sivertsvik *et al.* 2002). Therefore, storage temperature is a prerequisite factor to control quality and safety of fish and fishery products. The Arrehenius model for the influence of temperature and CO_2 concentration on the growth of microflora was obtained by Koutsoumanis *et al.* (2000) as follows:

$$\ln(\mu_{\max}) = \ln\left(\mu_{ref} - d_{CO_2} \times [CO_2]\right) + \frac{E_A}{R} \times \left(\frac{1}{T_{ref}} - \frac{1}{T}\right)$$

Where:

$\mu_{\max}$ is the maximum specific bacterial growth rate (in hours^{-1})

T is the absolute temperature (Kelvin)

$[CO_2]$ is the concentration of carbon dioxide in the package (%)

E_A is the activation energy (in kilojoules per mole)

R is the universal gas constant

T_{ref} is the reference temperature (273 K)

μ_{ref} is the $\mu_{\max}$ under reference storage conditions (T_{ref}, $[CO_2]$ = 0) (in hours^{-1})

d_{CO2} is a constant expressing the effect of carbon dioxide on the $\mu_{\max}$

The combined effects of superchilling under modified atmospheres has been researched and applied. It has been shown to increase shelf life and maintain good quality of fish and fishery products. For example, bulk-packaged, superchilled, whole, gutted salmon combined with a high CO_2 atmosphere maintained a high microbiological and sensory quality for more than three weeks (Sivertsvik *et al.* 1999). The shelf life of mackerel fillets which were stored at -2°C in 100% CO_2 was more than 21 days (Hong *et al.* 1996). The superchilled modified atmosphere packaged salmon maintained a good quality, with negligible microbial growth (<1000 cfu/g) for more than 24 days (Sivertsvik *et al.* 2003).

Other studies have shown that the shelf life extentions of fish packaged under MAP is 50% to 400% (Stammen *et al.* 1990). However, often it is only the period of moderate to low quality that has been extended and not the initial period of prime quality (Fletcher and Statham 1988).

3   MATERIAL AND METHODS

3.1   Material

3.1.1   Arctic charr fillets

The Arctic charr (*Salvelinus alpinus*) used for this study was farmed in the northeast of Iceland (Húsavík). The age of the fish at slaughtering was two years and the average weight was 650 g.

After slaughtering, the fish was bled for 20-30 minutes in water and chilled in ice slurry (water mixed with ice). After gutting and filleting, the fillets were packed in 5 kg Styrofoam boxes with 250 g ice packs and transported to the Icelandic fisheries Laboratories (IFL) in Reykjavík. Upon arrival the next day, the temperature in the boxes was 0.1-0.2°C.

3.1.2   Cooling agents

Dry ice
Dry ice (99% CO_2) was supplied by the company Is-Aga, Reykjavík, Iceland.

Ice packs
Ice packs were supplied by the company Tempra in Hafnarfjordur, Iceland.

3.2   Experimental design

The Arctic charr fillets were randomly divided into eight groups that were packed into styrofoam boxes with different ratios of dry ice or ice packs and controlled storage temperatures of 3-4°C or -2°C. The control groups were packed without cooling agent and stored at 3-4°C and -2°C.

Additionally, one group was packed with 150 g of ice packs and stored at -24°C overnight (16 hours) followed by storage at 3-4°C.

In each box were 3 kg of Arctic charr fillets. The three fillets in each box were marked with numbered plastic tages for weighing and measurement of drip loss and cooking yield.

The experimental groups are shown in Table 5.

Table 5: Experimental groups were packed with dry ice and ice packs, then stored at chilling temperature (3-4°C) and superchilling temperature (-2°C).

Group	Cooling agents	Quantity of cooling agents	Storage temp. (°C)	Code
1	Ice pack	150 g	3-4	IP150C
2	Ice pack	300 g	3-4	IP300C
3	Ice pack	450 g	3-4	IP450C
4	Dry ice	150 g	3-4	DI150C
5	Dry ice	300 g	3-4	DI300C
6	Dry ice	450 g	3-4	DI450C
7	Ice pack	150 g	-2	IP150S
8	Dry ice	150 g	-2	DI150S
9	Control	0	3-4	CC
10	Control	0	-2	CS
11	Ice pack	150 g	Stored at -24°C overnight (16 hours) and then at 3-4°C	FZ

3.3 Sampling

Sampling was carried out on the arrival of the fillets (day zero) and after three, six, nine, 13 and 16 days of storage for evaluation of drip loss, cooking yield, water content, water holding capacity (WHC), colour, pH, total viable counts (TVC) and counts of H_2S-producing bacteria (at 15°C). Additionally, protein and fat content was evaluated on day zero and TVB-N, TMA and TBA on days zero, six, 13 and 16. Sensory analysis was carried out on the groups with 150 g of cooling agent (dry ice/ice packs) stored at chilling (3-4°C) and superchilling (-2°C) temperatures.

On each sampling day, six random fillets were used for sensory analysis, three random fillets of each sample were used for microbiology tests and chemical tests, three marked fillets were used for cooking yield measurements, and another three random fillets were used for colour mesurement. Then the central part (part 2, see Figure 3) of each fillet was used for measurement of water holding capacity, water content and pH (Figure 3).

Part 3   **Part 2**   **Part 1**
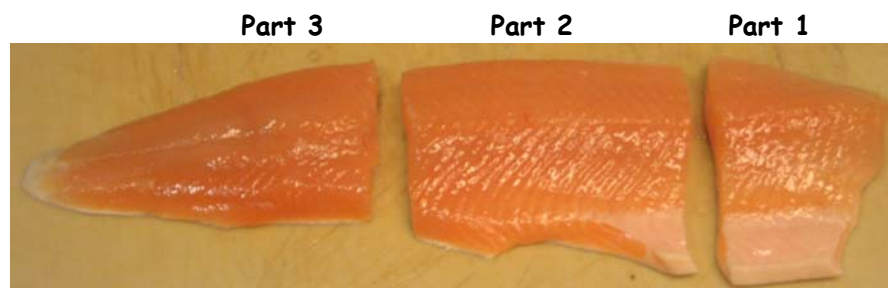
Figure 3: An Arctic charr fillet portioned for sampling for measurement of water holding capacity, water content and pH.

3.4 Temperature measurements

The temperature was recorded with 15 min intervals, by data loggers placed in the top and the bottom of each box. Additionally, the temperature in the centre of the boxes was measured on the sampling day (day three, six, nine, 13 and 16) by a thermometer.

3.5 Methods of analyses

3.5.1 Physical analyses

Water holding capacity (WHC)
Water Holding Capacity (WHC) was determined by a method that is built on a method by Børresen (Eide 1982). The sample glasses were made from plexi-glass and their dimensions were: height 62 mm, inner diameter 19 mm and outer diameter 25 mm. The rotor used was SS-34 for Sorvall centrifuge, type RC-5B (Dupoint, USA).
The samples were centrifuged at 1500 rpm for five minutes in special sample glasses. Samples were prepared by chopping them in a Braun Mixer (Type 4262, Germany) for 10-15 seconds (until homogenous).

The sample glass was weighed empty and then 2 grams of the sample were weighted into the glass. After centrifugation, the sample glass was weighed again with the sample in it minus the loose bounded water.

The Water Holding Capacity (WHC) of the sample was then calculated using the following formula:

$$WHC = \frac{W_1 - \Delta r}{W_1} \times 100 (\%)$$

Where:
    W_1 is the water content of the sample before centrifugation (%).
    Δr is the weight lost by centrifugation (%).

Flesh fish colour
The flesh fish colour was measured with a Minolta Chroma Meter CR-300 (Minolta, Osaka, Japan) using the CIE L*a*b system (Figure 4). L describing lightness (L = 0 for black, L = 100 for white), a describing intensity in red (a*>0), b describing intensity in yellow (b*>0) (Hutchings 1999a).
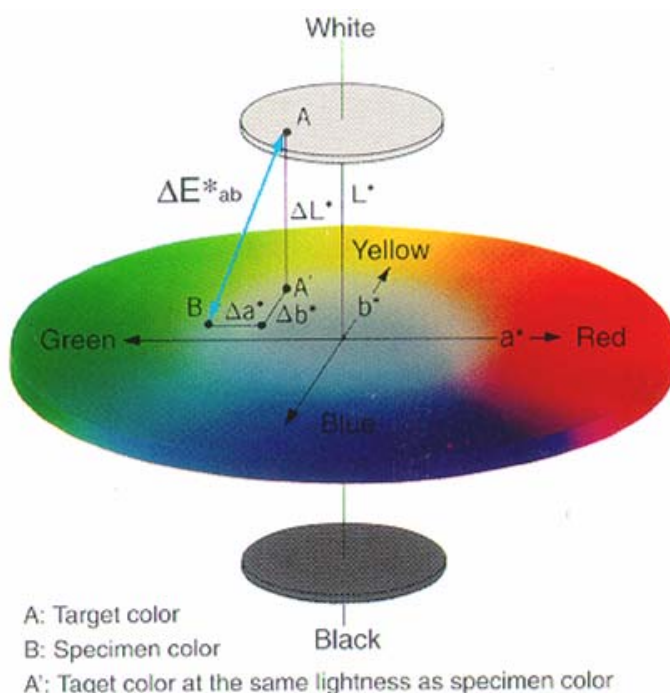
Figure 4: Description of colour measurement using the CIE L*a*b system.

The colour was measured above the lateral line at three positions, close to the head, in the middle of the fillet and close to the tail of each fillet as shown in Figure 5.

Figure 5: The colour measurement sites on the fillet.

Drip loss

Drip loss was defined as the amount of liquid that was lost during storage expressed as a percentage of loss based on the initial sample weight. It was determined by the weight of the fillets before and after storage.

Cooking yield

Cooking yield was defined with regards to the amount of liquid that was lost during cooking. The total cooking yield (TCY) and cooking yield (CY) were calculated as:

$$TCY = \frac{W_{cooked}}{W_{initial}} \times 100(\%)$$

$$CY = \frac{W_{cooked}}{W_{raw}} \times 100(\%)$$

Where:

W_{cooked} was the weight of cooked sample.

$W_{initial}$ was the weight of raw sample before storage packaging.

W_{raw} was the weight of raw sample before cooking.

3.5.2 *Chemical analyses*

Water content
Water content of the fresh fish was determined according to ISO 6496:1999(E). About 5 g of homogenized fillets were placed in an aluminium foil dish which was prepared with a thin layer of sea-sand and a glass rod. The sample was mixed thoroughly with the sand. The glass rod was kept on the dish and then left to dry for $4h \pm 0,1h$ in the oven at 103°C. The dish was removed from the oven and allowed to cool to ambient temperature in the desiccator for about 15 minutes. The water content was calculated by fomula as follows:

$$\frac{m_1 - (m_3 - m_2)}{m_1} \times 100 (\%)$$

Where:
m$_1$ is the mass, in grams, of the test portion.
m$_2$ is the mass, in grams, of the dish, test portion, sand and glass rod.
m$_3$ is the mass, in grams, of the dish, dried test portion, sand and glass rod.

Protein content
Protein content in the fish flesh was determined by the Kjeldahl method (ISO 1997). A sample of 5 g was digested in sulphuric acid in the presence of copper as a catalyst. Thereafter, the sample was placed in a distillation unit, 2400 Kjeltec Auto Sample System. The acid solution was made alkaline by a sodium hydroxide solution. The ammonia was distilled into boric acid and the acid was simultaneously titrated with diluted H_2SO_4. The nitrogen content was multiplied by the factor 6.25 to get the ratio of crude protein.

Fat content
Fat content in the fish flesh was determined by the method of AOCS Official Method Ba-3-38 (1997). The sample was extracted with petroleum ether, boiling range 40-60°C. The extraction apparatus was 2025 Soxtec Avanti Automatic System.

Total volatile basic nitrogen (TVB-N) and Trimethylamine (TMA)
The total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) were determined using steam distillation which was performed as described by Malle and Poumeyrol (1989). Briefly, 200 ml of a 7.5% aqueous trichloroacetic acid solution were added to 100 g of fish muscle and homogenized in a Waring blender. The mixture was filtered through Whatman n[o]3 filter paper.

Steam distillation was performed using a Kjeldahl-type distillator (Struer TVN). 25 ml of filtrate were transferred into a distillation flask followed by 6 ml of 10% NaOH. A beaker containing 10 ml of 4% boric acid and 0.04 ml of methyl red and bromocresol green indicator was placed under the condensor for the titration of ammonia.

Distillation was started and steam distillation continued until a final volume of 50 ml was obtained in the beaker (40 ml of distillate). The boric acid solution turned green when alkalinized by the distilled TVB-N which was titrated with aqueous 0.025 N sulphuric acid solution using a 0.05 ml graduated burette. Complete neutralisation was obtained when the colour turned pink on the addition of a further drop of sulphuric acid. The TVB-N content was calculated by the following fomula:

$$\frac{14mg/mol \times a \times b \times 300}{25ml}(mgN/100g)$$

Where:
    a = ml of sulphuric acid.
    b = nomality of sulphuric acid.

To assess TMA using the same method as for TVB-N but 20 ml of formaldehyde was added to the distillation flask to block the primary and secondary amines.

Thiobarbituric acid (TBA) content
Thiobarbituric reactive substances (TBARS) were determined by the extraction procedure described by Vyncke (1975) with a few modifications. The sample size was reduced to 15 g and homogenized with 30 ml of 7.5% trichloroacetic acid solution containing 0.1% of both propyl gallate and EDTA. The absorbance of samples and standards were measured at 530 nm. TBARS, expressed as µmol malondialdehyde per kilogram of sample (µmol MDA/kg), was calculated using malondialdehyd-bis-(diethyl acetate) as standard.

pH measurement
pH was measured using a calomel electrode (SE 104) pH meter (Knick-Portamess 913 (X) pH meter, Germany, Berlin). Glass calomel electrode was dipped into minced fish flesh at room temperature.

3.5.3   Microbial analyses

Total viable counts and counts of H_2S-producing bacteria were done on Iron agar as described by Gram *et al*. 1987 with the exception that 1% NaCl was used along with surface plating. Plates were incubated at 15°C for four days. Dilutions were done in Maximum Recovery Dilutent (MRD) from Oxoid.

3.5.4   Sensory analyses

Quantitative Descriptive Analysis (QDA), introduced by Stone and Sidel (1998), was used to evaluate cooked samples of Arctic charr fillets. An unstructured scale (0-100%) was used on a list of words describing odour and flavour.

Seven to eight panelists of the Icelandic Fisheries Laboratories' sensory panel participated in the QDA of the cooked Arctic charr. They were all trained according to international standards (ISO 1993), including detection and recognition of tastes and odours, training in the use of scales, and in the development and use of descriptors. The members of the panel were familiar with the QDA method and experienced in the

sensory analysis of Arctic charr. Each panelist evaluated the samples in two sessions for each day of the sensory evaluation.

3.6   Data analysis

Microsoft Excel 2000 was used to calculate means and standard deviations for all multiple measurements and to generate graphs. The predicted shelf life graphs were generated by Seafood Spoilage and Safety Predictor (SSSP) ver. 1.0 (Copyright © 2004 Danish Institute for Fisheries Research).

4   RESULTS

4.1   Chemical composition of Arctic charr fillets

The chemical composition of the fillets was measured on their arrival at the laboratory. The water content was 73.2%, the crude protein content was 19.5%, and the fat content was 9.4%.

4.2   The temperature profiles of the cold storage simulator and cooling room

The temperature profiles of the chilling cabinet (superchilling storage) and cooling room (chilling storage) are shown in Figure 6. The storage temperature of the cooling room and chilling cabinet was rather stable. The cold storage simulator temperature varied from -3.3°C to -1.1°C and the cooling room temperature varied from 2.3°C to 4.6°C (Figure 6). The average temperature throughout the storage period of the cooling room was 3.1±0.3°C and -1.9±0.3°C of the chilled cabinet was.
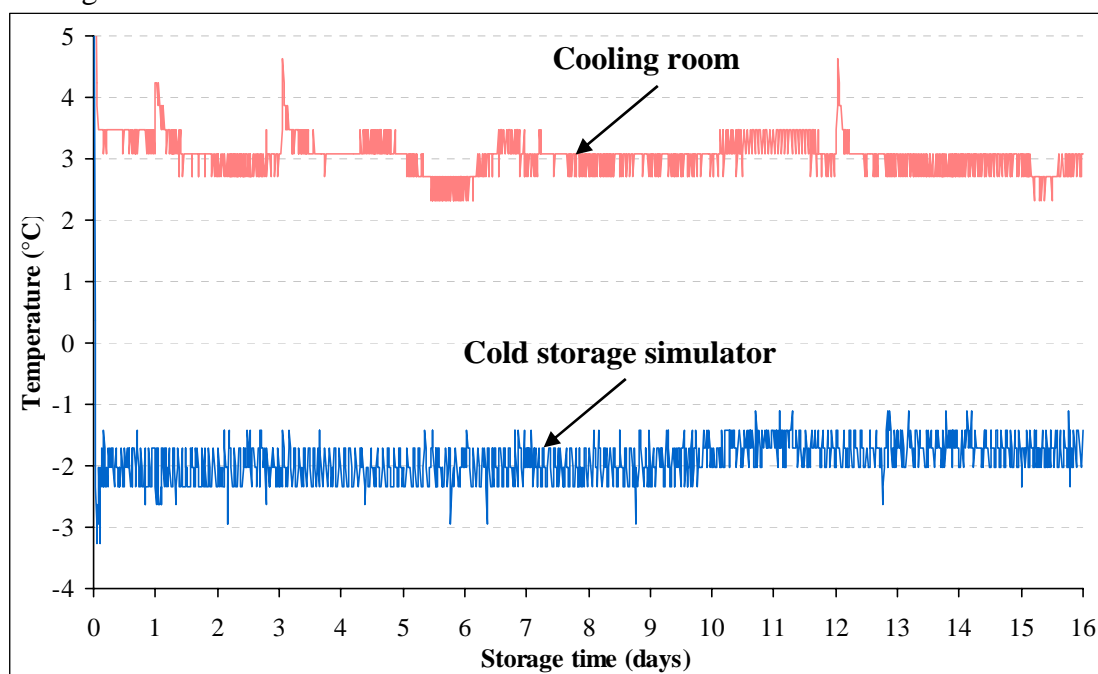
Figure 6: Temperature profiles of the chilling cabinet and cooling room.

4.3   Observed appearance

On day three of storage, ice crystals were observed on the surface of the fillets in group DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C) and DI150S (packed with 150 g dry ice and stored at -2°C). On day six of storage, the fillets in DI450C, IP150S and DI150S still had ice crystals on the surface, but only fillets in the bottom layer in DI300C (Figure 7) had ice crystals.

Observations on day nine showed that ice crystals still covered the whole surface of all fillets in IP150S and DI150S, whereras only little remained on fillets placed in the center of the DI450C box and all crystals had melted in DI300C.

The ice crystals could still be seen in IP150S and DI150S on day 13 but only in DI150S on day 16.

Sample DI150S6

Sample DI300C6

Sample DI450C6

Figure 7: Differences in the appearance of ice crystals between sample DI150S6 (packed with 150 g dry ice and stored at -2°C within six days), sample DI300C6 (packed with 300 g dry ice and stored at 3-4°C within six days) and sample DI450C6 (packed with 450 g dry ice and stored at 3-4°C within six days).

There were differences in appearance of ice crystal shapes between the samples on day six (Figure 7). A lot of the small units of ice crystals were crystalized and disconnected on the surface of the fillets in sample DI150S6. On theother hand, the ice crystals in sample DI450C6 were bigger and clung to the surface of the fillets.

4.4   Effects of chilling and superchilling with different cooling agents on the quality and shelf life of Arctic charr fillets

4.4.1   Temperature profiles of chilled and superchilled fillets during storage

The temperature was recorded with 15 min intervals, by data loggers placed in the top and the bottom of each box. Additionally, the temperature in the centre of the boxes was measured on the sampling day using a thermometer. Figure 8 shows that the temperature was similar in the top and bottom of both IP150C and IP150S. Temperature in the centre of sample IP150C was higher than the temperature in the top and bottom. On the contrary, temperature in the centre of sample IP150S was lower than the temperature in the top and bottom.
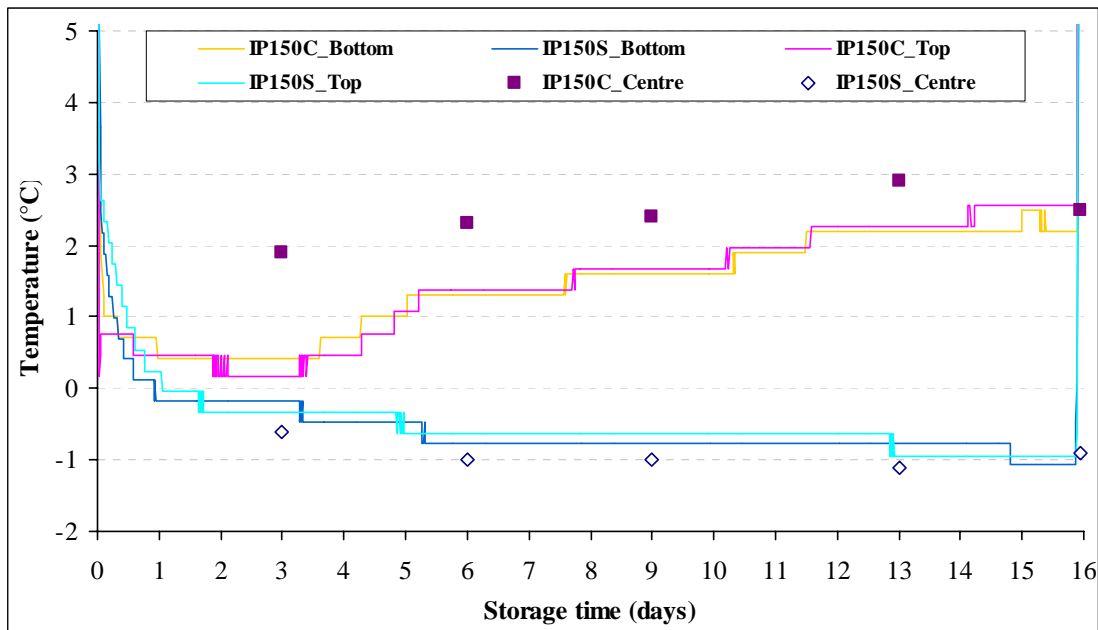
Figure 8: Temperature profiles (lines) in boxes of samples IP150C (packed with 150 g ice packs and stored at 3-4°C) and IP150S (packed with 150 g ice packs and stored at -2°C). Additionally, the temperature in the centre of the boxes was measured on each sampling day (points).

The temperature in the top of boxes DI150C and DI150S dropped very low (DI150C dropped to -4.5°C and DI150S dropped to -8°C) during the first 60 minutes of storage. The temperature in the centre and bottom of these boxes was the same (Figure 9).
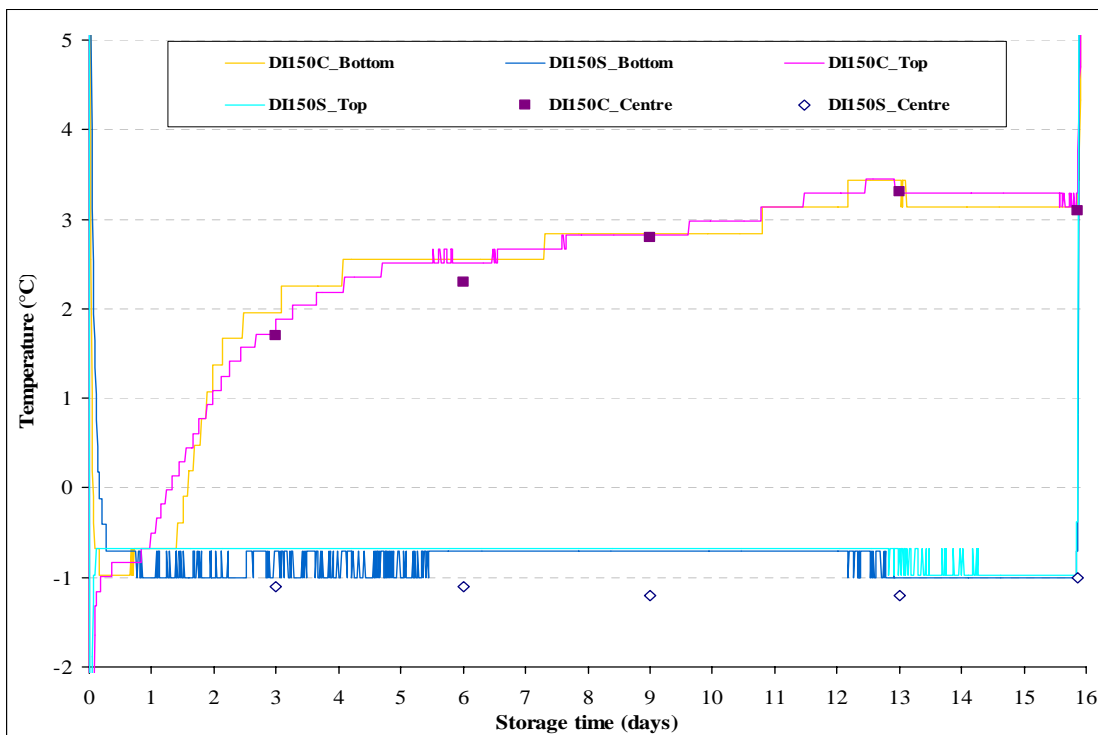
Figure 9: Temperature profiles (lines) in boxes of samples DI150C (packed with 150 g dry ice and stored at 3-4°C) and DI150S (packed with 150 g dry ice and stored at -

2°C). Additionally, the temperature in the centre of the boxes was measured on each sampling day (points).
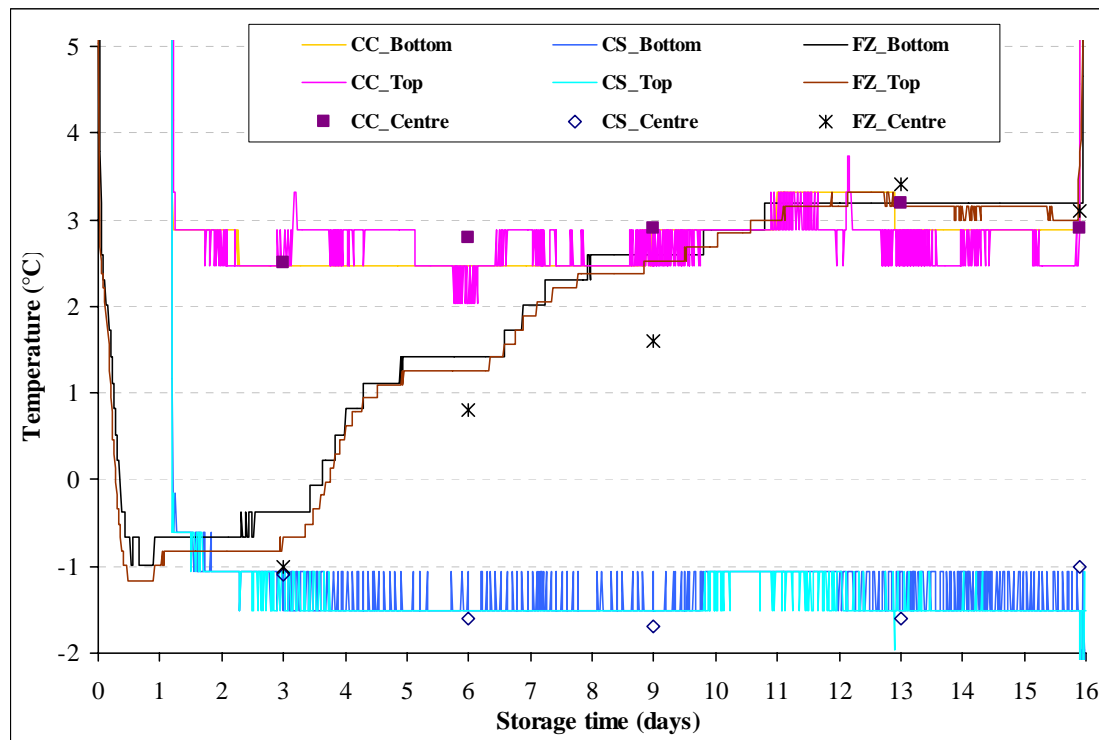
Figure 10: Temperature profiles (lines) in boxes of samples CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C). Additionally, the temperature in the centre of the boxes was measured on each sampling day (points).

The temperature in the centre of sample FZ was lower than the temperature in the top and bottom during the first nine days of storage and was the same after that. The temperature in the top, centre and bottom of samples CC and CS was similar. The temperature in the centre was closer to the temperature in the top than in the bottom (Figure 10).

4.4.2   Physical changes

Drip loss
The total drip loss of fillets in IP150C, IP150S, CC, CS and FZ was similar during the first three days of storage, meanwhile higher drip loss of DI 150S was observed and the fillets in DI150C gained weight (Figure 11). From day three, drip loss increased gradually. Drip loss of superchilled samples seemed to be lower than chilled samples at the end of the storage period.
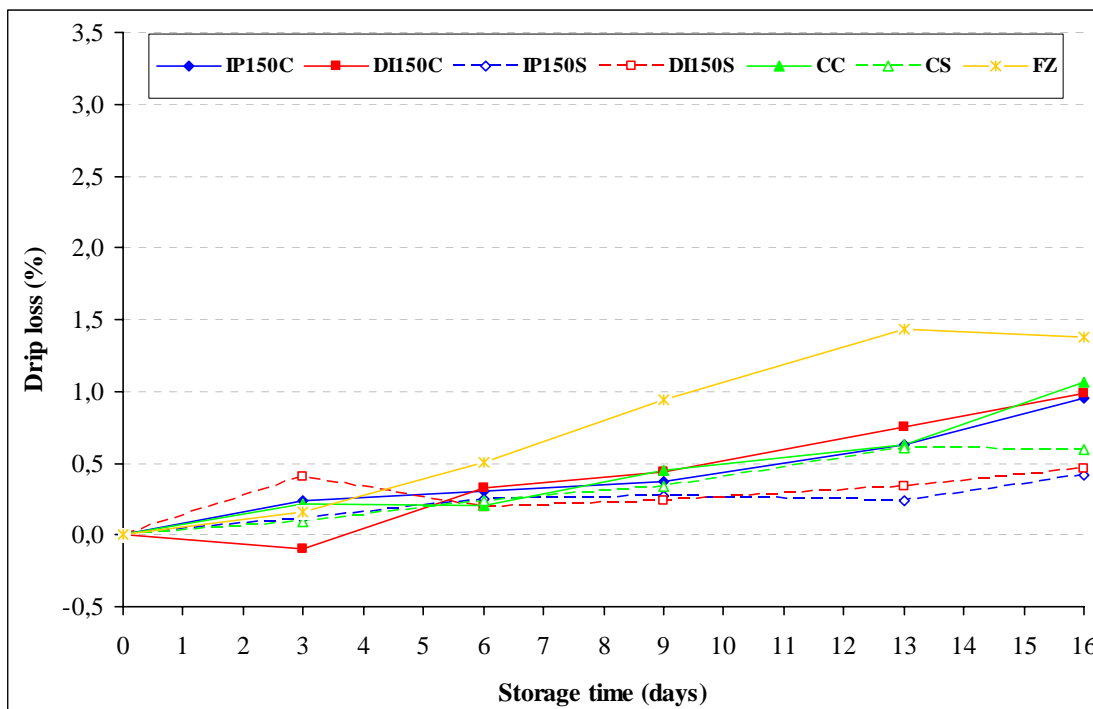
Figure 11: Drip loss of whole samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

Comparison of drip loss of the three fillets weighted individually in each box, showed that drip loss of groups increased gradually with storage time and the variation between groups was lower than when the total batch was weighted (Figure 12).
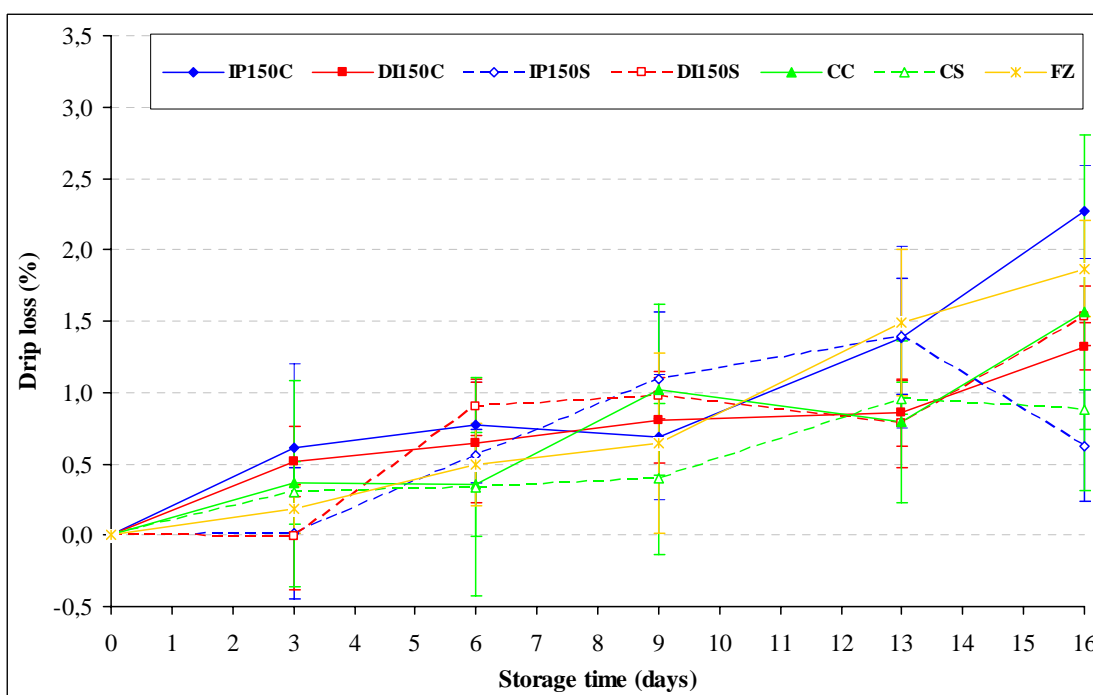
Figure 12: Drip loss of (measured on three fillets after one hour at 20°C) samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

Cooking yield

The total cooking yield (TCY) and cooking yield (CY) of samples IP150C, IP150S, DI150C, CC and CS dropped at the beginning of the storage period, then increased but did not reach the initial yield, and then decreased with storage time. The TCY and CY of sample DI150S increased from day nine to day 13 after having decreased gradually for the first nine days of the storage period.The TCY and CY then dropped on day 16. The TCY and CY of sample FZ decreased with storage time from the beginning (Figures 13 and 14).

Figure 13: Total cooking yield of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).
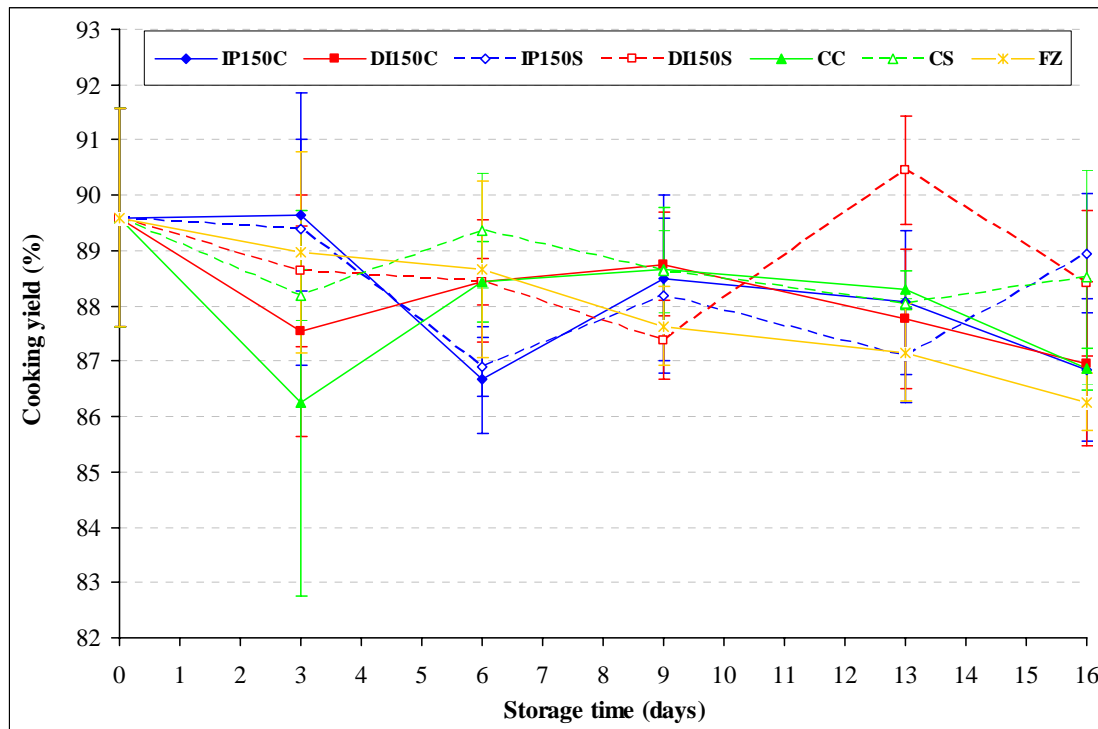
Figure 14: Cooking yield of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

Water Holding Capacity

The water holding capacity (WHC) was 85% on day zero but increased to 88-94% during the first three days and to 91-96% from day three to day six. After that it did not increase and varied between samples. The WHC of superchilled samples and sample FZ seemed to be higher than that of chilled samples (Figure 15).

Figure 15: Water holding capacity of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

Colour

The L* value or lightness of the fillets in samples IP150C, DI150C, IP150S, CC, CS and FZ increased with storage time. The sample DI150S increased in L* value during the first nine days of storage but then decreased at the end of the storage period (Table 6).

Table 6: The L* value or lightness of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

Storage day	IP150C	DI150C	IP150S	DI150S	CC	CS	FZ
0	38.37 ± 2.97	38.37 ± 2.97	38.37 ± 2.97	38.37 ± 2.97	38.37 ± 2.97	38.37 ± 2.97	38.37 ± 2.97
3	39.61 ± 3.76	40.38 ± 2.93	38.53 ± 1.63	40.55 ± 2.20	38.80 ± 3.01	41.94 ± 2.72	37.90 ± 1.01
9	39.91 ± 1.54	40.06 ± 2.02	40.15 ± 1.36	40.65 ± 1.57	39.99 ± 2.29	38.59 ± 1.48	41.39 ± 1.87
13	39.33 ± 1.90	39.76 ± 1.85	38.21 ± 1.23	36.54 ± 1.90	41.89 ± 2.39	41.10 ± 2.66	40.48 ± 2.12
16	42.80 ± 1.09	41.20 ± 2.75	40.09 ± 1.98	37.63 ± 1.33	42.80 ± 3.14	40.20 ± 2.17	40.83 ± 2.20

The a* value describing intensity in red of the fillets in samples IP150C, DI150C, IP150S and DI150S increased during the first 13 days of storage, and then seem to decrease. Samples CC, CS and FZ stayed at their highest value for up to nine days of storage, and then decreased markedly (Table 7).

Table 7: The a* value describing intensity in red of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

Storage day	IP150C	DI150C	IP150S	DI150S	CC	CS	FZ
0	8.26 ± 3.30	8.26 ± 3.30	8.26 ± 3.30	8.26 ± 3.30	8.26 ± 3.30	8.26 ± 3.30	8.26 ± 3.30
3	9.38 ± 2.79	9.95 ± 2.88	12.47 ± 1.73	9.59 ± 2.67	13.10 ± 1.12	7.22 ± 1.81	13.72 ± 0.62
9	11.35 ± 1.19	11.11 ± 2.62	8.29 ± 1.95	8.99 ± 2.99	13.53 ± 1.47	10.83 ± 3.25	10.31 ± 1.79
13	13.24 ± 2.16	10.25 ± 4.40	12.00 ± 1.12	12.23 ± 0.57	6.93 ± 1.02	8.46 ± 1.83	8.58 ± 2.80
16	10.30 ± 2.70	11.06 ± 3.44	11.98 ± 0.77	13.01 ± 2.12	9.16 ± 1.99	10.64 ± 2.69	11.70 ± 1.65

The b* value describing intensity in yellow of the fillets in samples IP150S and DI150S increased with storage time. All the other samples increased during the first nine days of storage, and then decreased at the end of the storage period (Table 8).

Table 8: The b* value describing intensity in yellow of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

Storage day	IP150C	DI150C	IP150S	DI150S	CC	CS	FZ
0	12.31 ± 3.82	12.31 ± 3.82	12.31 ± 3.82	12.31 ± 3.82	12.31 ± 3.82	12.31 ± 3.82	12.31 ± 3.82
3	14.61 ± 2.48	17.55 ± 3.49	20.59 ± 1.82	16.19 ± 3.78	19.67 ± 2.12	11.99 ± 1.77	20.03 ± 2.09
9	19.17 ± 1.15	17.10 ± 3.64	13.55 ± 1.76	15.37 ± 5.13	21.28 ± 1.54	16.23 ± 3.54	18.24 ± 1.76
13	18.36 ± 2.49	16.44 ± 6.47	20.01 ± 1.37	18.09 ± 1.61	10.74 ± 1.00	14.20 ± 2.20	14.69 ± 4.89
16	15.21 ± 3.15	15.91 ± 4.49	20.30 ± 1.44	18.91 ± 1.95	14.81 ± 3.14	17.31 ± 2.35	16.31 ± 1.82

4.4.3   Microbial changes

Changes in total viable count
Total viable count (TVC) of fillets on day zero was rather high (3.6×10^6). This may be because of contamination during sampling. The TVC increased faster in chilled samples than superchilled samples. The exception was sample DI150C, where TVC increased slower than sample DI150S from day three to day six of the storage period. TVC increased at a lower rate in the dry ice samples than in the ice pack samples (Figure 16).
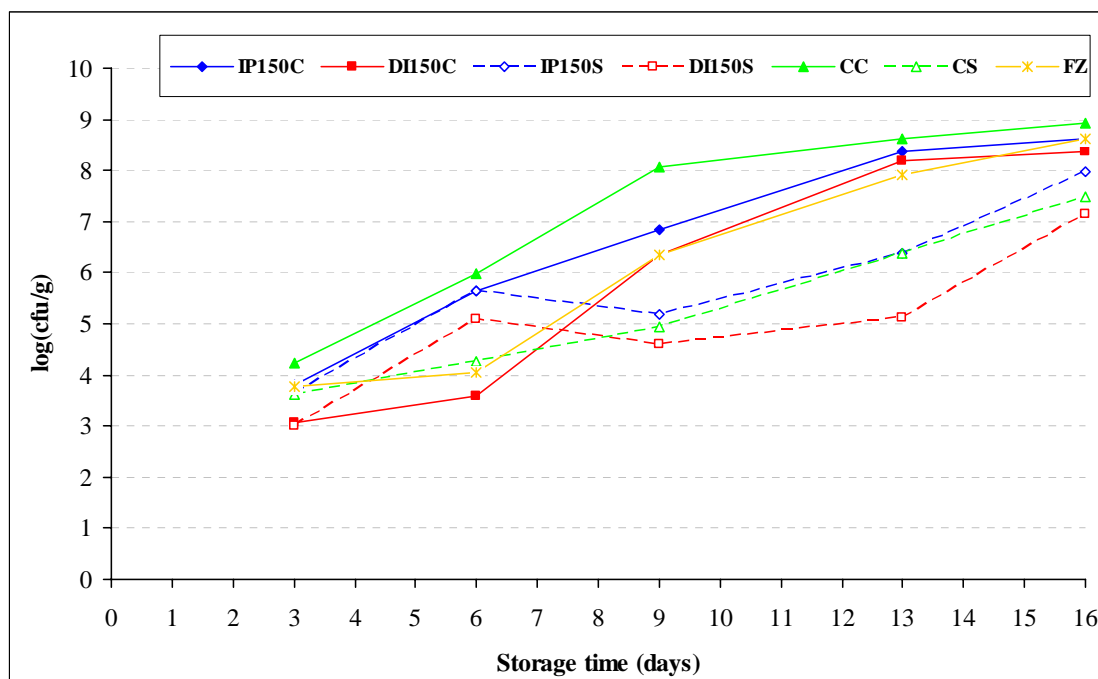
Figure 16: Changes in TVC of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

H_2S producing bacteria

The number of H_2S producing bacteria in superchilled samples increased slower than in chilled samples (Table 9). The use of dry ice delayed growth of H_2S producing bacteria compared to the use of ice packs but the highest growth rate was observed in the control sample. The growth rate of H_2S producing bacteria in fillets frozen overnight was similar to superchilled samples during the first six days of storage but was higher after that.

Table 9: Changes in H_2S producing bacteria (cfu/g) of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

Storage day	IP150C	DI150C	IP150S	DI150S	CC	SC	FZ
0	$<10^3$	$<10^3$	$<10^3$	$<10^3$	$<10^3$	$<10^3$	$<10^3$
3	1.2×10^3	$<10^2$	1.7×10^3	1.0×10^2	1.5×10^3	1.1×10^3	7.0×10^2
6	1.8×10^4	2.0×10^2	1.1×10^3	6.5×10^3	3.5×10^4	1.2×10^4	1.9×10^3
9	8.0×10^4	4.3×10^4	3.0×10^3	7.0×10^2	5.0×10^5	1.5×10^4	1.1×10^4
13	2.0×10^6	1.9×10^6	1.5×10^4	6.3×10^3	4.5×10^7	1.5×10^4	6.0×10^5
16	1.8×10^7	1.5×10^7	2.0×10^5	3.0×10^4	4.0×10^6	3.0×10^4	4.9×10^7

4.4.4 Sensory changes

No significant changes ($p > 0.05$) in odour and flavour were observed during the storage period (Figures 17, 18 and 19).
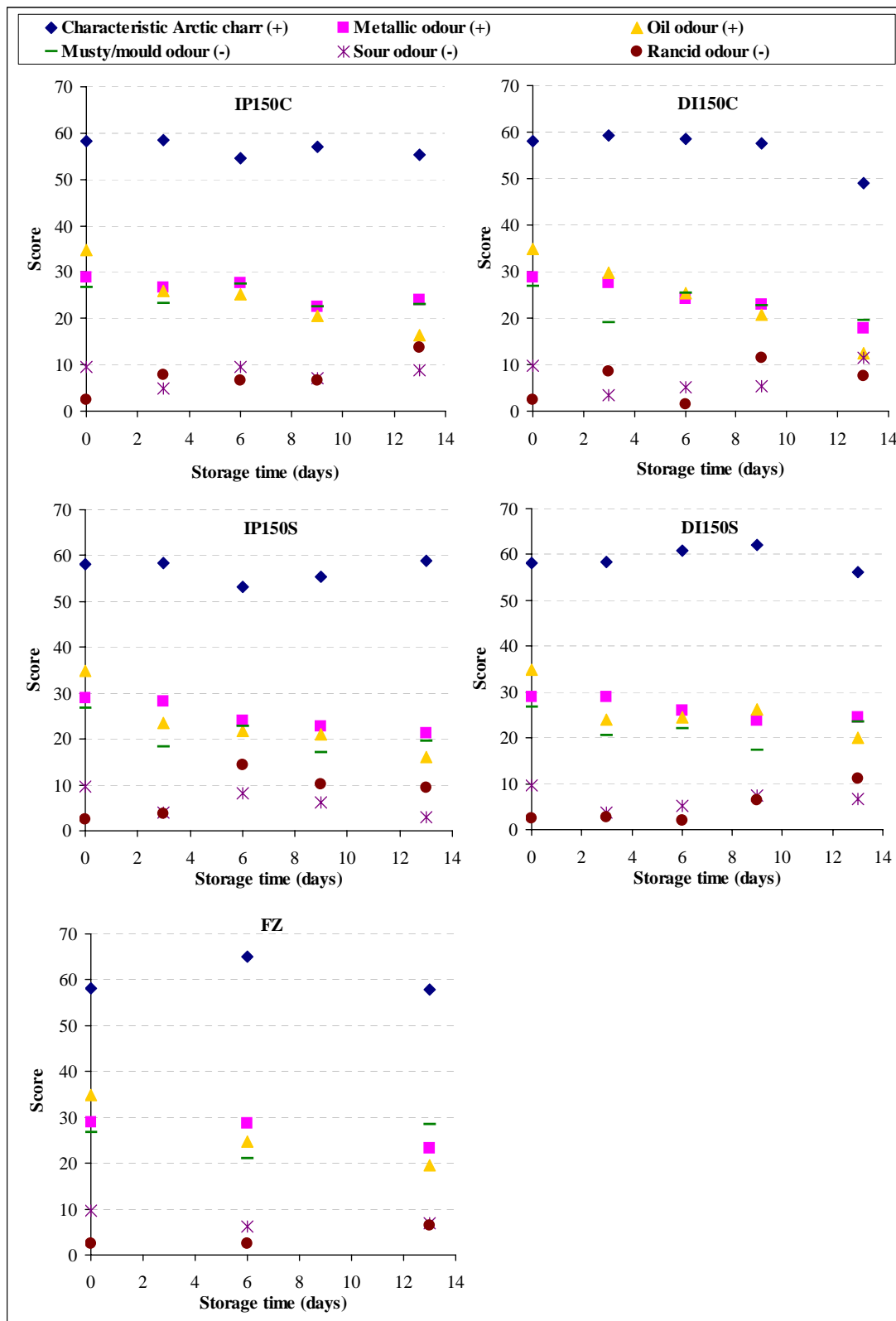
Figure 17: Changes in odour positive attributes (+) and negative attributes (-) of cooked fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).
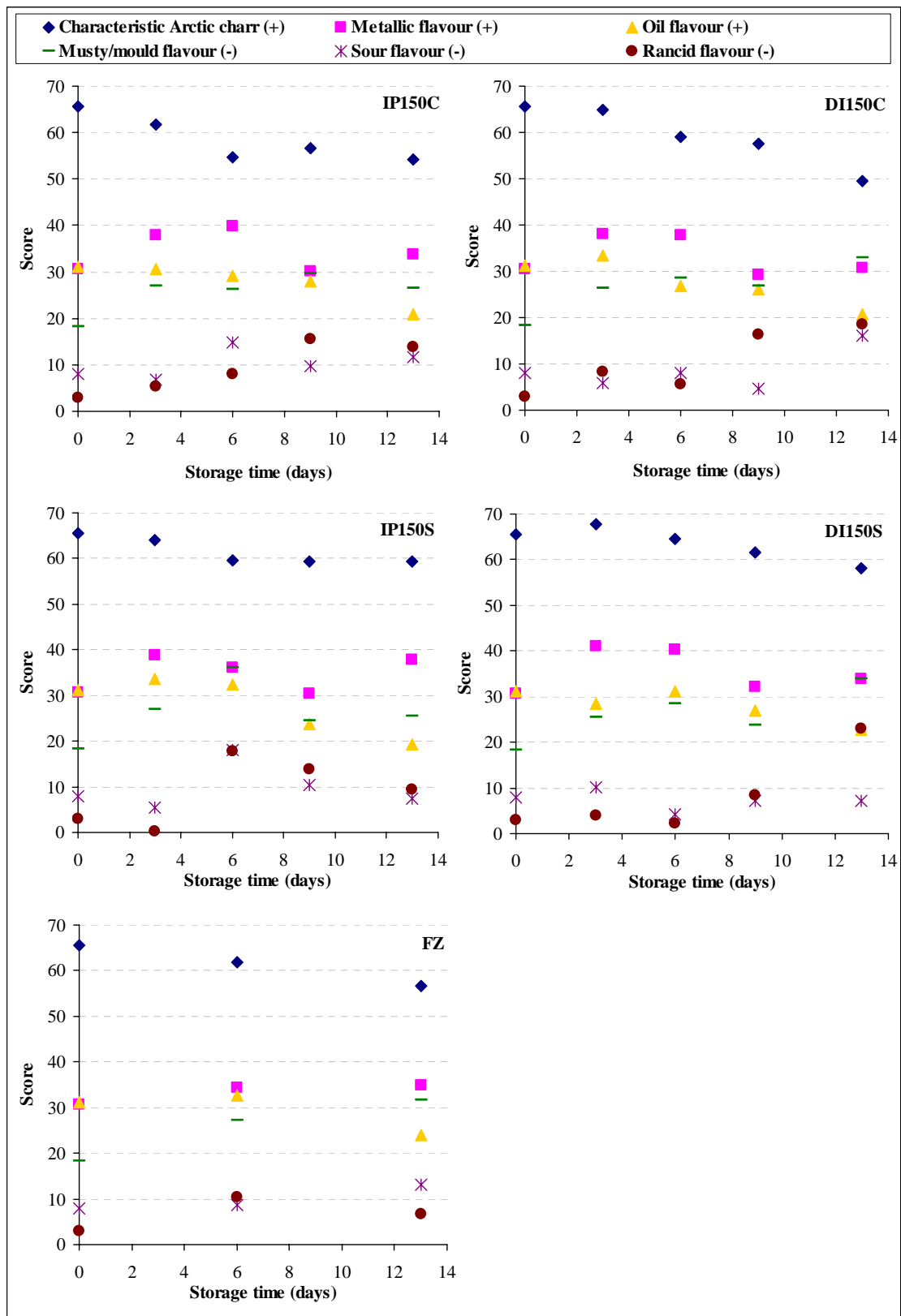
Figure 18: Changes in flavour positive attributes (+) and negative attributes (-) of cooked fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).
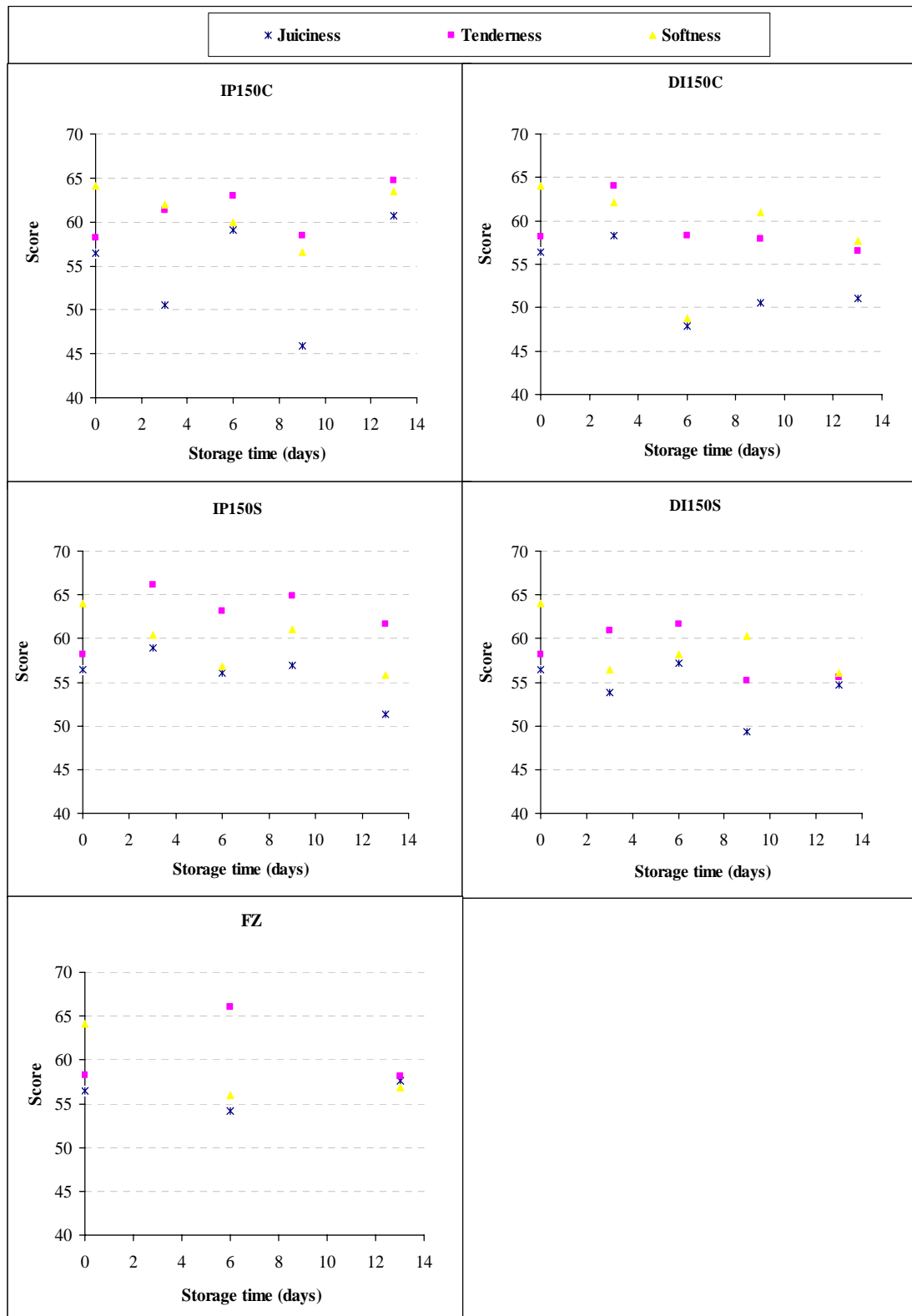
Figure 19: Changes in juiciness, tenderness and softness of cooked fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

4.4.5   Chemical changes

Change in pH
The pH in all groups increased during the first three days of storage (Figure 20), but the pH in superchilled samples increased slower than in chilled samples with the same cooling agent packaging. From day three to day six, the pH in samples which were packed with dry ice (DI150C and DI150S) and sample IP150C decreased slightly meanwhile the pH in the other samples increased continuously up to day six and then dropped on day nine. From day nine to the end of the storage period, pH in chilled samples and sample FZ remained similar. However, the pH in superchilled samples increased from day nine to 13 and then dropped to values similar to those observed in chilled samples on day 16.

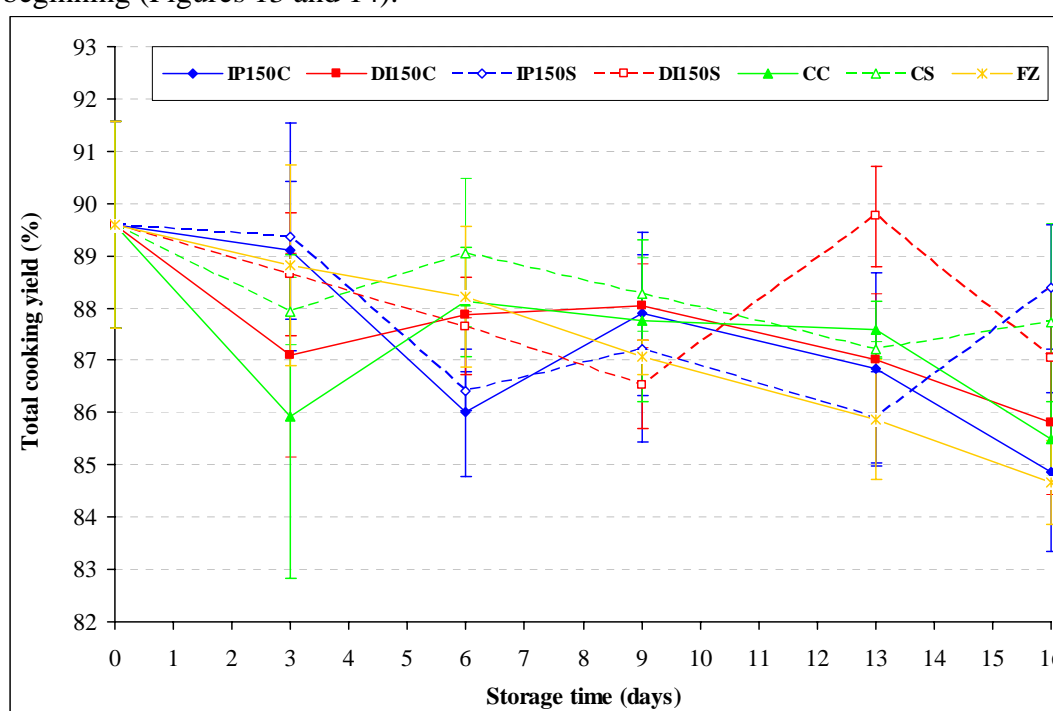Figure 20: The pH value of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

Change in water content
The water content of all samples increased slightly with storage time (Figure 21). The water content of superchilled samples changed more during storage than that of the chilled samples.
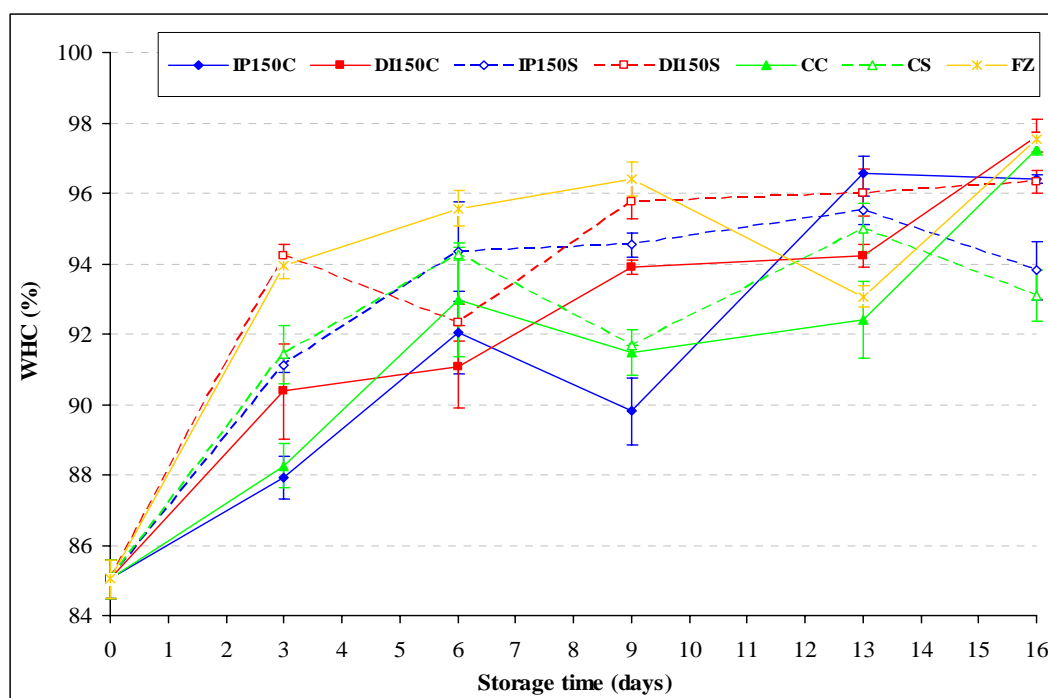
Figure 21: The water content of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

Change in trimethylamine
Changes in the trimethylamine (TMA) content (mgN/100g) of fillets which were packed with 150 g ice packs or dry ice and stored at chilling temperature (3-4°C) or superchilling temperature (-2°C) (Table 10) show that the TMA content of samples IP150C, DI150C and FZ did not change during the first six days of the storage period, increased slightly from day six to day nine of storage and then increased during the last three days of the storage period. The TMA content of sample IP150S did not change during the first 13 days of the storage period and then increased slightly during the last three days of storage. The TMA content of samples DI150S was very low (0.3 mgN/100g) over the entire period of storage.

Table 10: Changes in TMA content (mgN/100g) of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

Storage day	IP150C	DI150C	IP150S	DI150S	FZ
0	< 0,3	< 0,3	< 0,3	< 0,3	< 0,3
6	< 0,3	< 0,3	< 0,3	0,3	< 0,3
13	1,0	3,0	0,3	< 0,3	0,5
16	4,7	5,3	1,5	< 0,3	5,8

Change in total Volatile Base nitrogen and Thiobarbituric acid

Total volatile base nitrogen (TVB-N) content of fillets which were packed with 150 g ice packs or dry ice and stored chilled (3-4°C) or superchilled (-2°C) (Figure 22) increased during the first three days. From day six to day 13 of storage, the TVB-N content of samples IP150C and DI150C increased continuously. Meanwhile, the TVB-N in IP150S, DI150S and FZ remained similar. During the last three days of the storage period, the TVB-N content of all samples increased. Figure 23 also shows that the TBA content of all samples increased with storage time and there was little variation between groups.

Figure 22: Changes in TVB-N and TBA content of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

4.5   Effect of cooling agent on quality and shelf life of Arctic charr fillets

4.5.1   Temperature profiles of chilled and superchilled fillets during storage

Figure 23 shows that the temperature curves in the top and botton of all samples were similar. The temperature in the centre of all samples was higher than the temperature in the top and bottom.

A comparison between the effects of ice packs (Figure 23) and dry ice (Figure 24) on temperature showed that dry ice was more effective in lowering temperature rapidly. However, ice packs remained at a low temperature for a longer period of time.
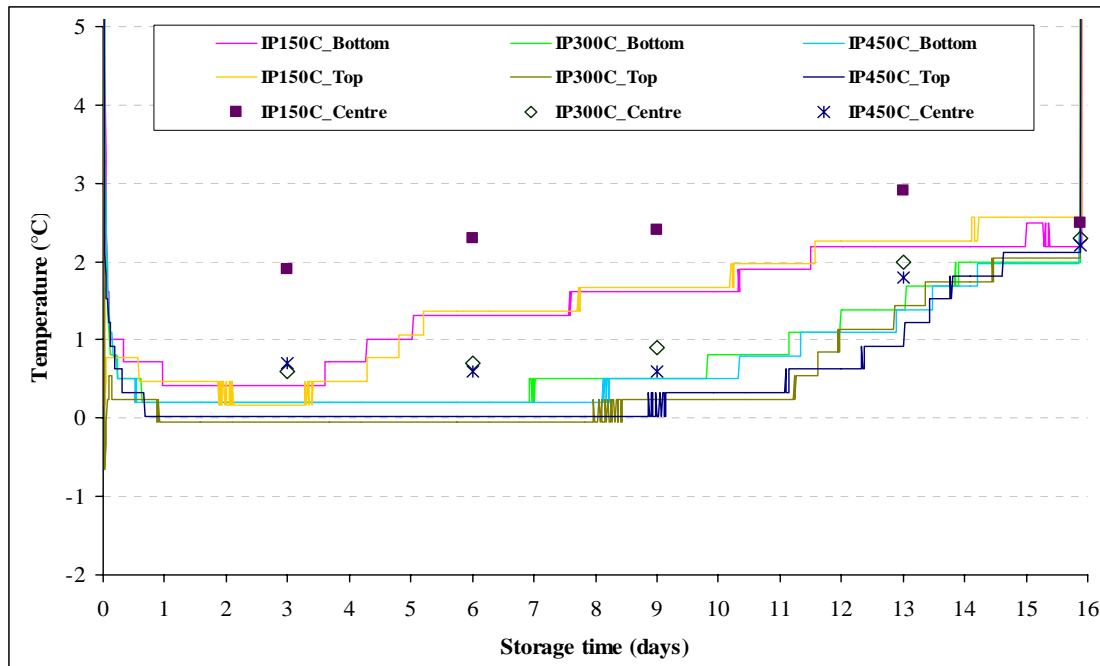
Figure 23: Temperature profiles (lines) in boxes of samples IP150C (packed with 150 g ice packs), IP300C (packed with 300 g ice packs) and IP450C (packed with 450 g ice packs) stored at 3-4°C. Additionally, the temperature in the centre of the boxes was measured on each sampling day (points).
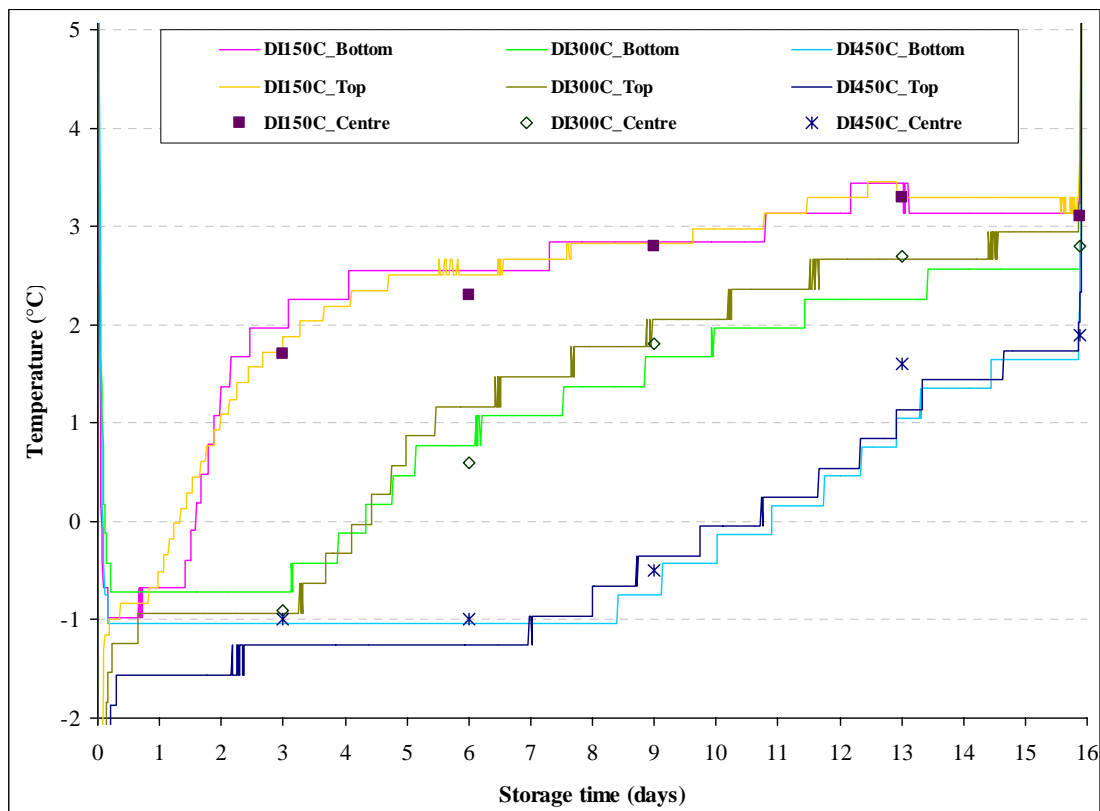
Figure 24: Temperature profiles (lines) in boxes of samples DI150C (packed with 150 g dry ice), DI300C (packed with 300 g dry ice) and DI450C (packed with 450 g dry ice) stored at 3-4°C. Additionally, the temperature in the centre of the boxes was measured on each sampling day (points).

The temperature in the top of boxes DI150C, DI300C and DI450C dropped very low (DI150C dropped to -4.5°C, DI300C dropped to -23.9°C, DI450C dropped to -28.2°C) within 60 minutes at the beginning of storage. The temperature in the top, centre and bottom of these boxes was the same (Figure 24).

4.5.2   Physical changes

Drip loss
Drip loss of fillets in whole samples (Figure 25) and of three fillets in each sample after one hour at 20°C (Figure 26) shows that the drip loss of samples, which were packed with dry ice was lower than in those packed with ice packs with the same ratio of cooling agent during the first six days of storage . From day nine to the end of storage, the drip loss of the samples, which were packed with ice packs increased while the drip loss of the samples, which were packed with dry ice started to increase on day 13 of storage. Drip loss, which was measured based on three fillets in each sample after one hour at 20°C, was higher than in the whole sample.
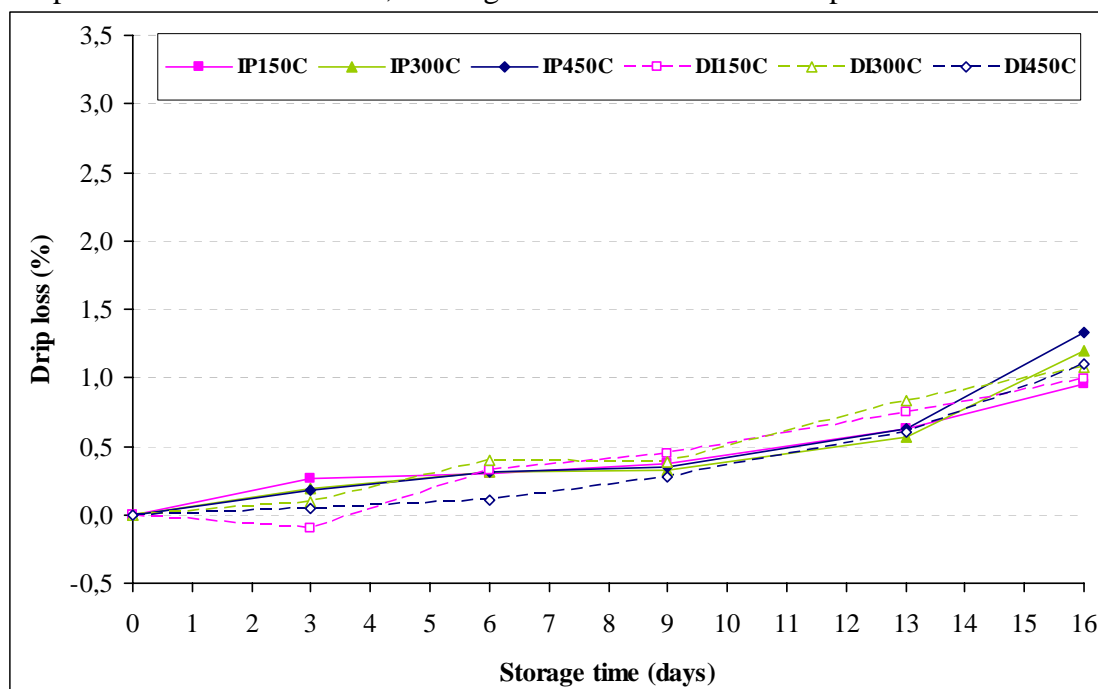
Figure 25: Drip loss of whole samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).
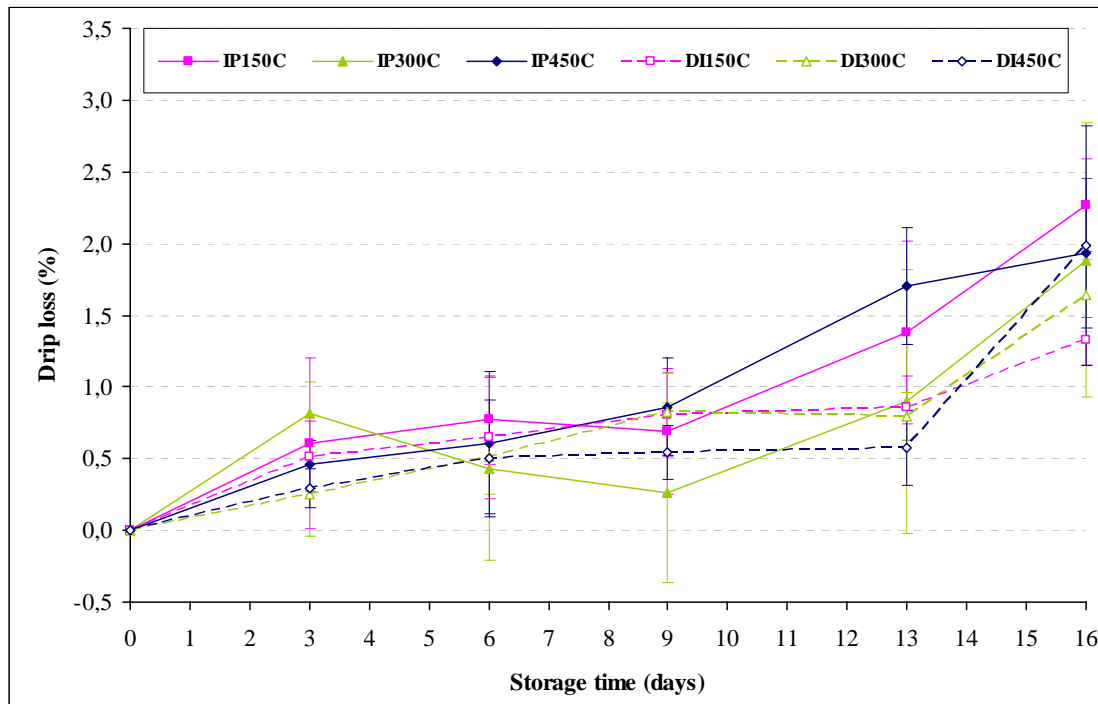
Figure 26: Drip loss (measured on three fillets after one hour at 20°C) in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).

Cooking yield
Total cooking yield (TCY) (Figure 27) and cooking yield (CY) (Figure 28) of the fillets in all samples varied very much during storage. The CY of sample DI150C and samples, which were packed with ice packs decreased slightly with storage time. The CY of samples DI300C and DI450C varied at a higher level than other samples.

Figure 27: Total cooking yield of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).
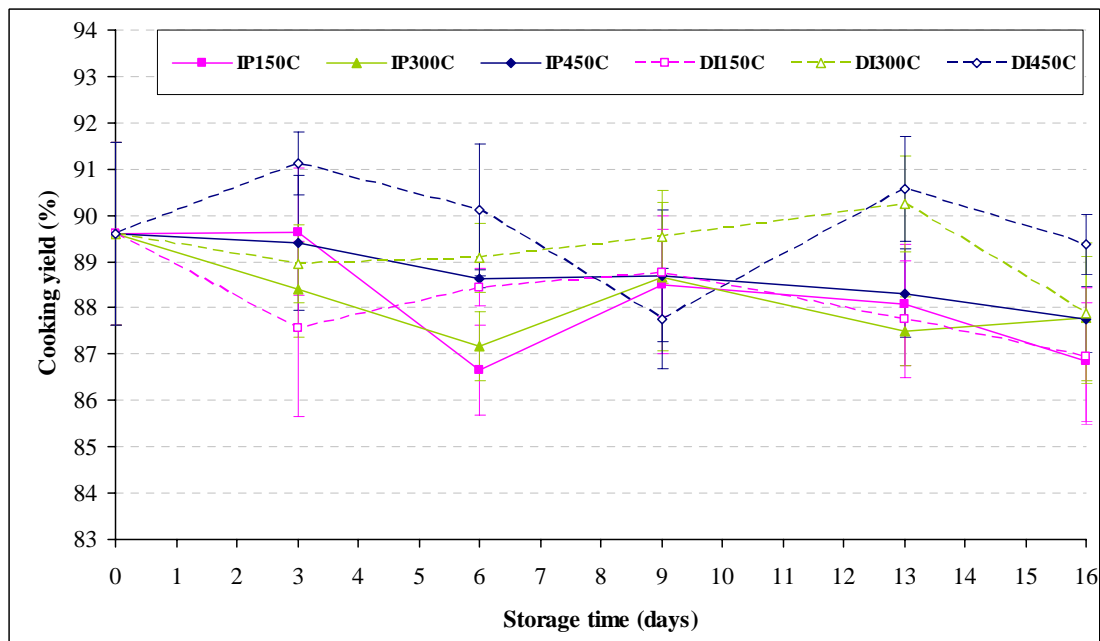
Figure 28: Cooking yield of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).

Water Holding Capacity

The water holding capacity (WHC) of the fillets in all samples seemed to be constant from day six to day nine of storage and then increased at the end of the storage period. The WHC of samples, which were packed with dry ice, was higher than that of those packed with ice packs over the storage period (Figure 29).
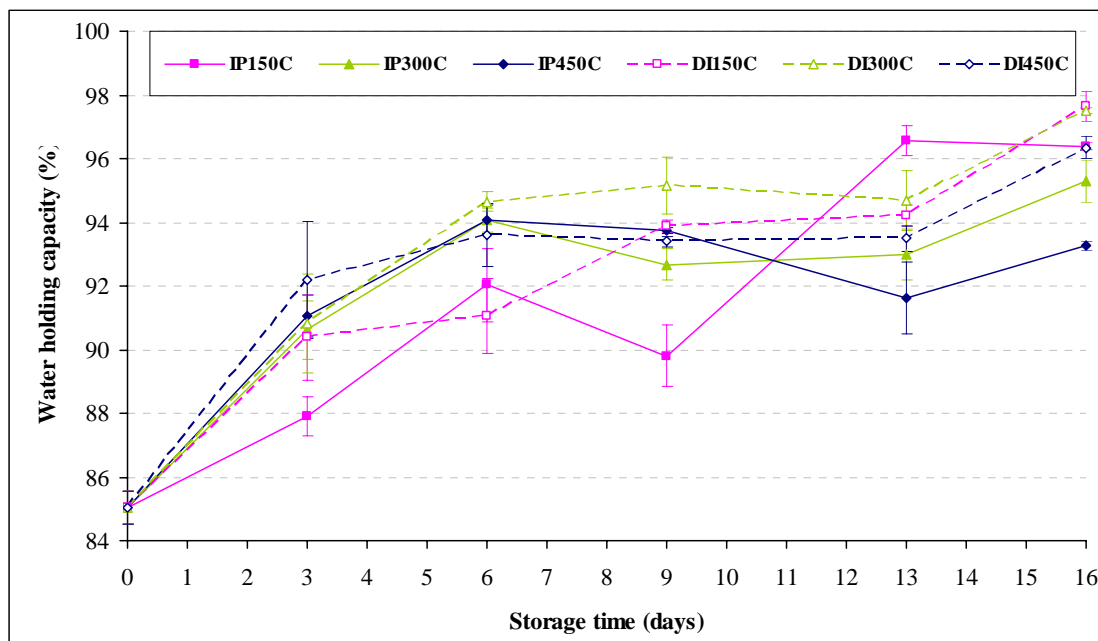
Figure 29: Water holding capacity (WHC) of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).

Colour

There was not much difference in the L* values or lightness of the fillets between samples. The L* value of all samples seems not to have increased during the first 13 days of storage. From day 13 to the end of the storage time, the L value of all samples increased slightly (Table 11).
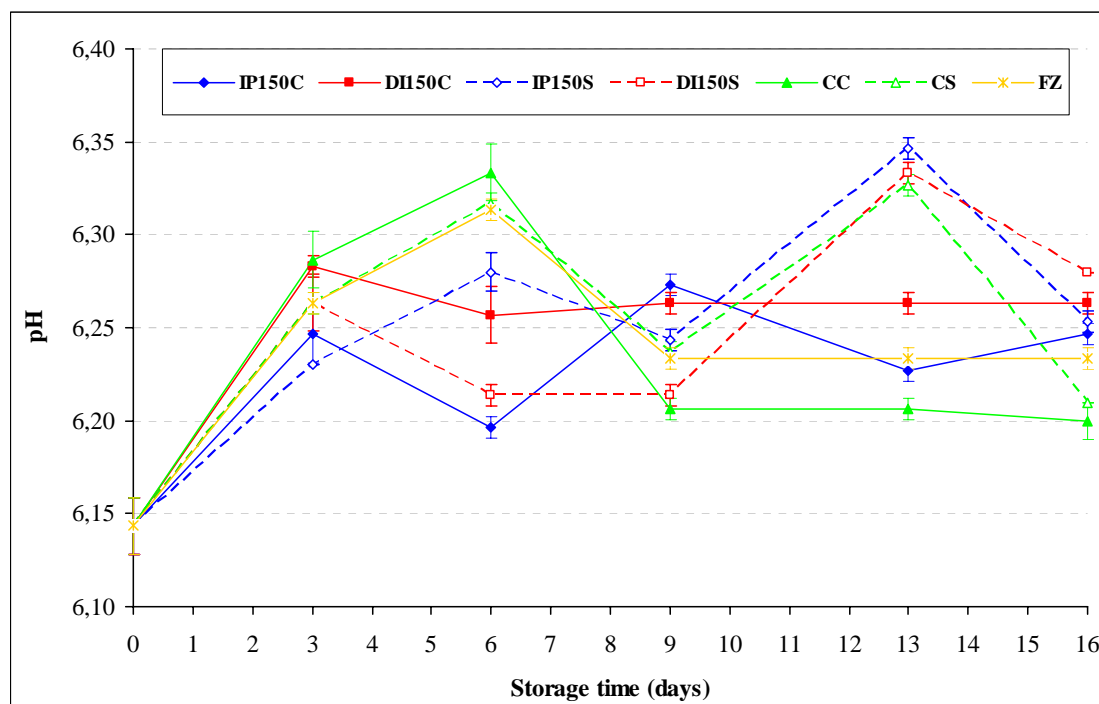
Table 11:   The L* value or lightness of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).

Storage day	IP150C	IP300C	IP450C	DI150C	DI300C	DI450C
0	38.37 ± 2.97	38.37 ± 2.97	38.37 ± 2.97	38.37 ± 2.97	38.37 ± 2.97	38.37 ± 2.97
3	39.61 ± 3.76	38.75 ± 3.01	39.88 ± 4.28	40.38 ± 2.93	41.54 ± 1.27	39.15 ± 1.69
9	39.91 ± 1.54	38.03 ± 2.16	40.30 ± 2.21	40.06 ± 2.02	39.45 ± 1.68	37.32 ± 1.29
13	39.33 ± 1.90	38.05 ± 1.47	38.81 ± 2.36	39.76 ± 1.85	39.43 ± 1.53	38.95 ± 1.65
16	42.80 ± 1.09	39.69 ± 1.25	39.96 ± 1.04	41.20 ± 2.75	41.30 ± 1.29	40.89 ± 2.68

The a* value describing intensity in red of fillets in all samples increased during the first nine days of storage then decreased until the end of the storage time (Table 12).
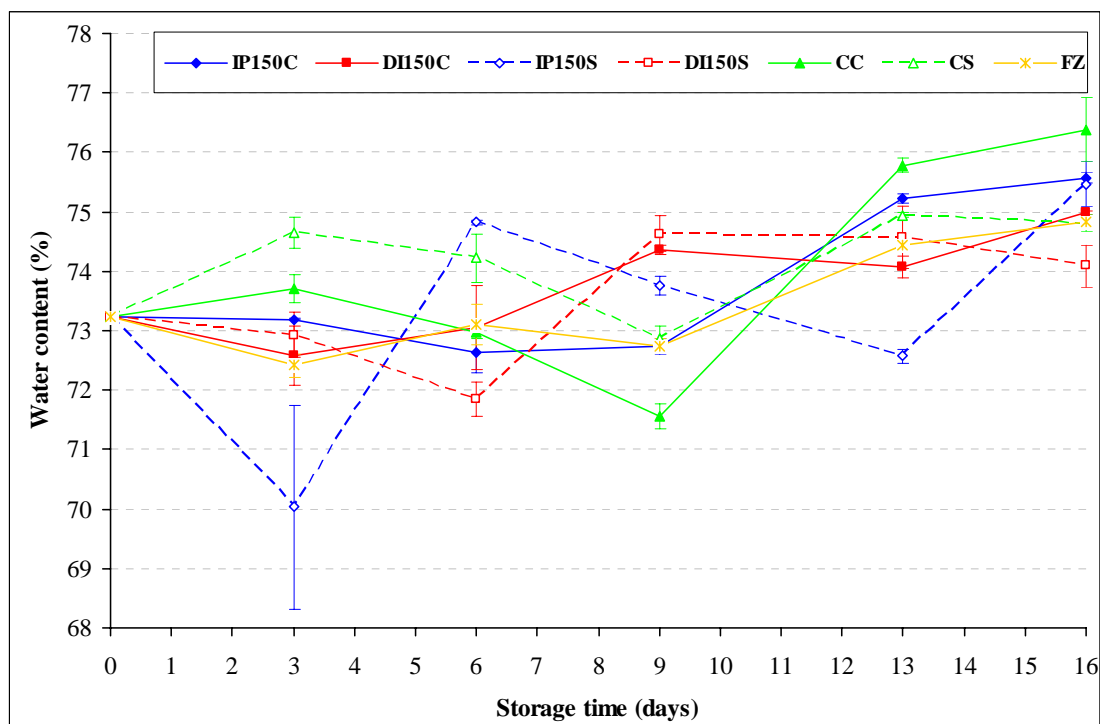
Table 12: The a* value describing intensity in red of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).

Storage day	IP150C	IP300C	IP450C	DI150C	DI300C	DI450C
0	8.26 ± 3.30	8.26 ± 3.30	8.26 ± 3.30	8.26 ± 3.30	8.26 ± 3.30	8.26 ± 3.30
3	9.38 ± 2.79	11.89 ± 4.04	11.48 ± 3.73	9.95 ± 2.88	8.98 ± 1.60	10.10 ± 2.97
9	11.35 ± 1.19	14.31 ± 1.47	10.73 ± 2.38	11.11 ± 2.62	12.80 ± 0.64	14.06 ± 1.64
13	13.24 ± 2.16	13.51 ± 1.08	11.07 ± 2.71	10.25 ± 4.40	9.85 ± 2.82	11.50 ± 2.78
16	10.30 ± 2.70	12.19 ± 1.96	12.33 ± 1.62	11.06 ± 3.44	11.69 ± 1.60	12.40 ± 1.17

The b* value describing intensity in yellow of fillets in all samples increased during the first nine days of storage then decreased until the end of the storage time (Table 13).

Table 13: The b* value describing intensity in yellow of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).

Storage day	IP150C	IP300C	IP450C	DI150C	DI300C	DI450C
0	12.31 ± 3.82	12.31 ± 3.82	12.31 ± 3.82	12.31 ± 3.82	12.31 ± 3.82	12.31 ± 3.82
3	14.61 ± 2.48	17.40 ± 4.67	15.63 ± 4.29	17.55 ± 3.49	13.57 ± 1.63	14.94 ± 3.72
9	19.17 ± 1.15	20.39 ± 1.32	16.04 ± 3.06	17.10 ± 3.64	21.20 ± 1.72	20.21 ± 2.10
13	18.36 ± 2.49	20.32 ± 1.28	15.32 ± 2.88	16.44 ± 6.47	16.42 ± 3.89	17.78 ± 2.90
16	15.21 ± 3.15	17.66 ± 1.75	17.80 ± 2.20	15.91 ± 4.49	18.02 ± 1.27	20.67 ± 2.88

4.5.3   Microbial changes

Changes in total viable count
Total viable count (TVC) of the fillets on day zero was rather high (3.6×10^6). The TVC of the fillets in all samples increased with storage time. The TVC of the samples which were packed with dry ice was lower than of those packed with ice packs. The samples which were packed with a higher ratio of dry ice were lower in TVC at the same day of storage (Figure 30).
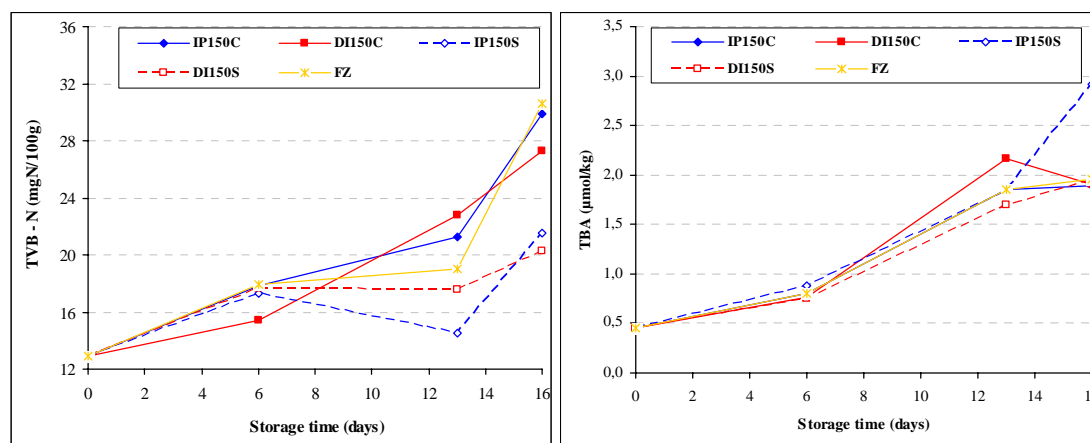
Figure 30: Changes in TVC of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).

H_2S producing bacteria

The number of H_2S producing bacteria of the fillets in the samples, which were packed with dry ice increased slower than in those packed with ice packs with the same cooling agent ratio. The samples, which were packed with a higher ratio of cooling agent had a lower number of H_2S producing bacteria. The number of H_2S producing bacteria in sample DI450C was lower than 10^3 over the storage period (Table 14).

Table 14: Changes in the number of H_2S producing bacteria (cfu/g) of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).

Storage day	IP150C	IP300C	IP450C	DI150C	DI300C	DI450C
0	$<10^3$	$<10^3$	$<10^3$	$<10^3$	$<10^3$	$<10^3$
3	1.2×10^3	2.0×10^2	$<10^2$	$<10^2$	$<10^2$	1.1×10^3
6	1.8×10^4	1.4×10^3	8.0×10^2	2.0×10^2	$<10^2$	3.0×10^2
9	8.0×10^4	2.0×10^4	1.2×10^4	4.3×10^4	7.0×10^3	$<10^3$
13	2.0×10^6	9.0×10^4	2.0×10^5	1.9×10^6	1.9×10^5	$<10^3$
16	1.8×10^7	6.0×10^6	2.0×10^6	1.5×10^7	2.0×10^5	$<10^3$

4.5.4   Chemical changes

pH
The pH value of the fillets in all samples increased during the first three days of storage and then seemed to stagnate (Figure 31).

Figure 31: The pH value of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).

Water content

The water content of the fillets in the samples, which were packed with dry ice seemed to be stable throughout the storage period. The water content of the fillets in the samples, which were packed with ice packs increased slightly at the end of the storage period (Figure 32).
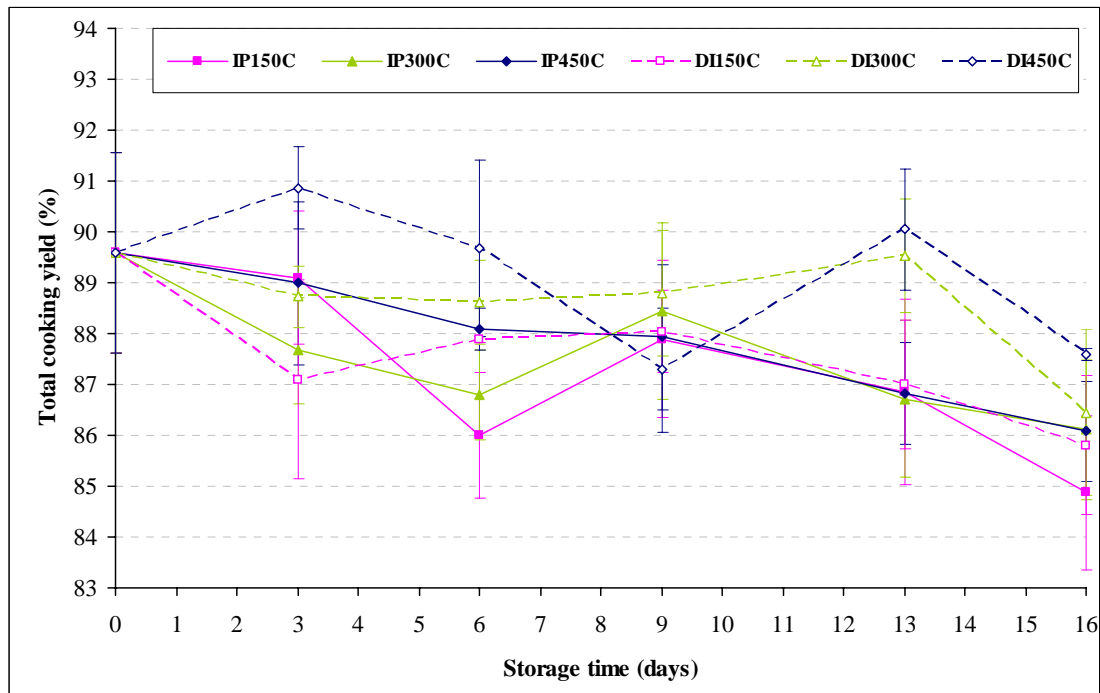

Figure 32: The water content of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).

Trimethylamine

The trimethylamine (TMA) content of the fillets in all samples did not change during the first six days. The TMA then increased throughout the storage period (expect for IP450C). The samples which were packed with a higher ratio of cooling agent were lower in TMA content (Table 15).

Table 15: Changes in TMA content (mg N/100g) of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).

Storage day	IP150C	IP300C	IP450C	DI150C	DI300C	DI450C
0	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
6	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
13	1.0	1.0	1.3	3.0	0.8	< 0.3
16	4.7	2.8	1.1	5.3	2.4	0.5

Total volatile base nitrogen and thiobarbituric acid

Changes in the total volatile base nitrogen (TVB-N) and thiobarbituric acid (TBA) content of the fillets (Figure 33) show that the TVB-N content of sample DI450C did not change for the first 13 days of storage. Meanwhile, the TVB-N content of the other samples increased. During the last three days of the storage period, the TVB-N content of all samples increased. Figure 33 also shows that the TBA content of all samples increased with storage time and did not differ much between the samples.
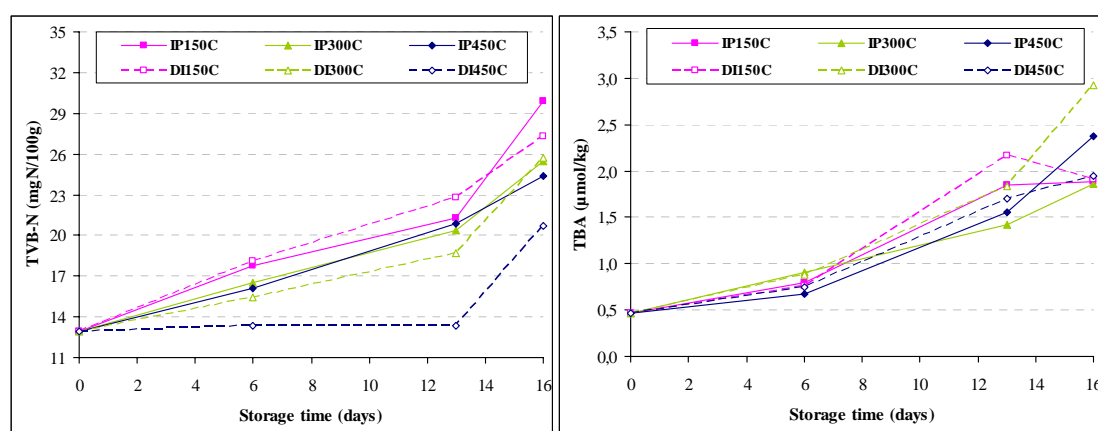
Figure 33: Changes in the total volatile base nitrogen (TVB-N) and thiobarbituric acid (TBA) content of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).

4.6 The relation between total cooking yield, cooking yield and weight of the fillets

The relation between total cooking yield, cooking yield and weight of the fillets in the experiment (Figures 34 and 35) shows that the cooking yield of the fillets increased with weight.
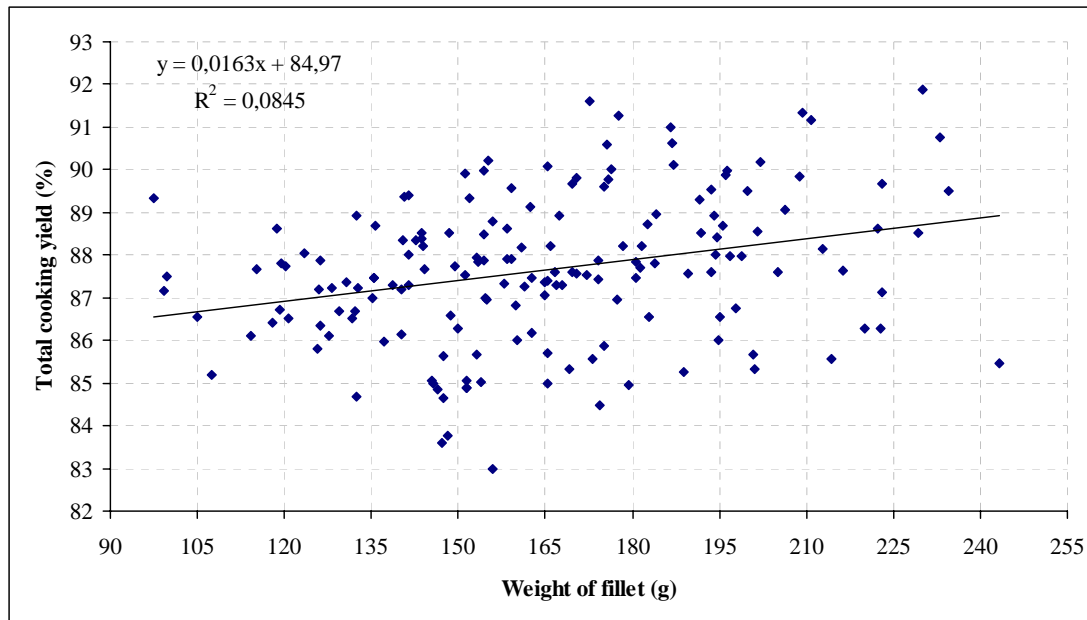
Figure 34: The relationship between the weight of the fillets and total cooking yield in the experiment.
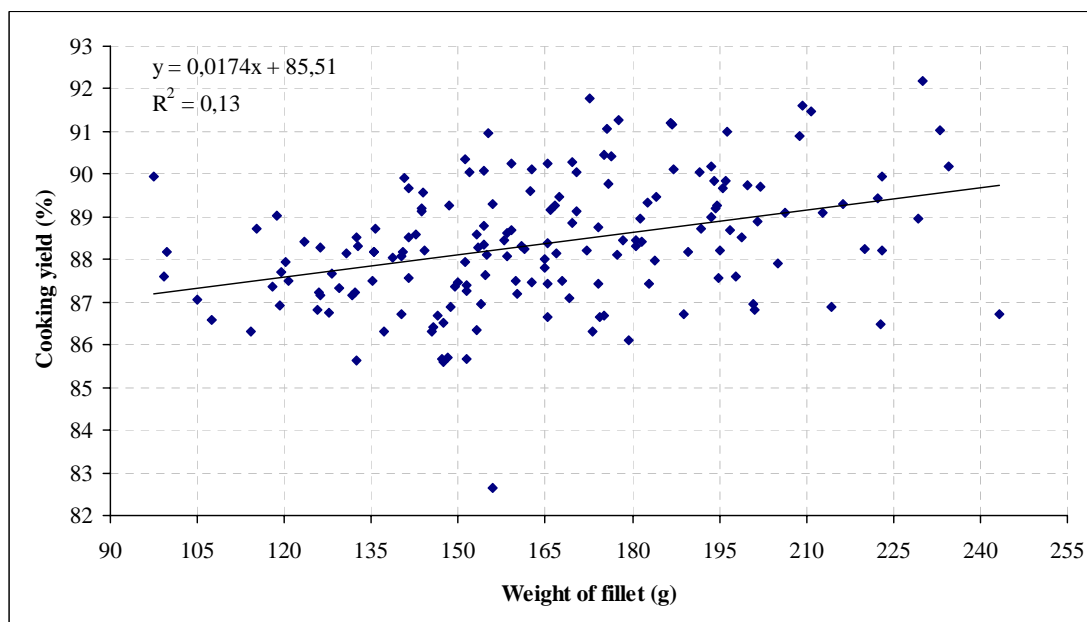
Figure 35: The relationship between the weight of the fillets and cooking yield in the experiment.

4.7   Prediction of shelf life based on the storage temperature profiles using SSSP software

4.7.1   The effect of superchilling with different cooling agents on the predicted shelf life of Arctic charr fillets

Based on the storage temperature profiles of the fillets which were packed with 150 g ice packs and dry ice and stored chilled (3-4°C) and superchilled (-2°C) (Figure 36), the predicted shelf life of sample CC is shortest (about eight and a half days). The predicted shelf life is about 10 days for sample DI150C, 11 days for samples FZ and IP150C , 16 days for samples IP150S and DI150S and more than 16 days for sample CC.
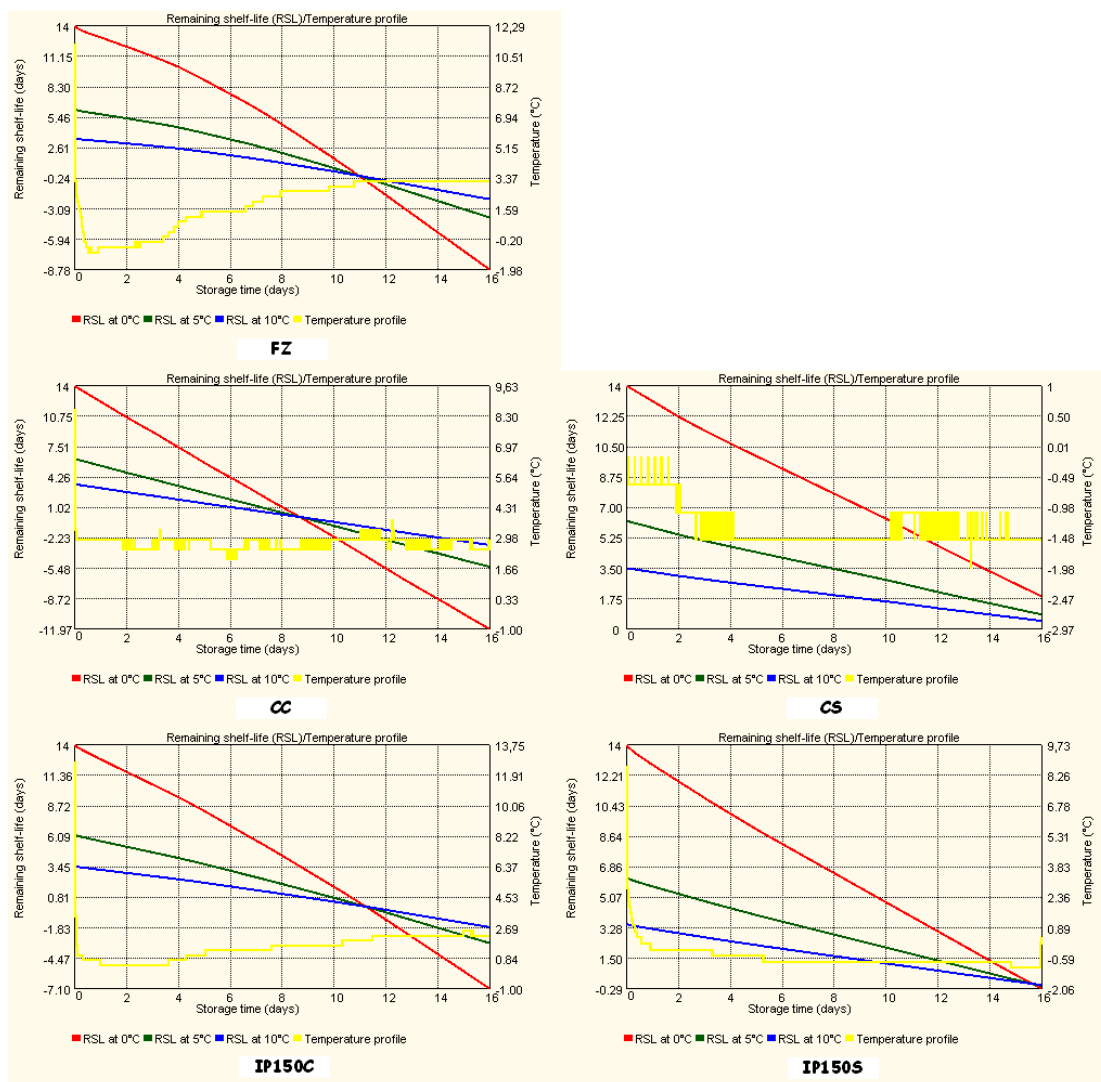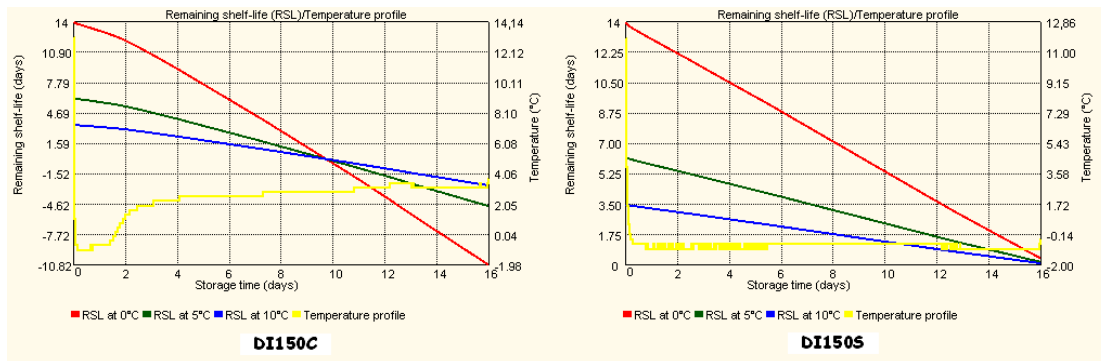
Figure 36: Predicted shelf life (base on storage temperature profiles) of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP150S (packed with 150 g ice packs and stored at -2°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI150S (packed with 150 g dry ice and stored at -2°C), CC (control at 3-4°C), CS (control at -2°C) and FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C).

4.7.2 The effect of cooling agent/fillets ratio on the predicted shelf life of Arctic charr fillets

Based on the storage temperature profiles of the fillets which were packed with different ratios of ice packs or dry ice (Figure 37), the samples which were packed with a higher ratio of cooling agent had longer predicted shelf lives. The shelf life of sample DI450C is longest (about 15 days).
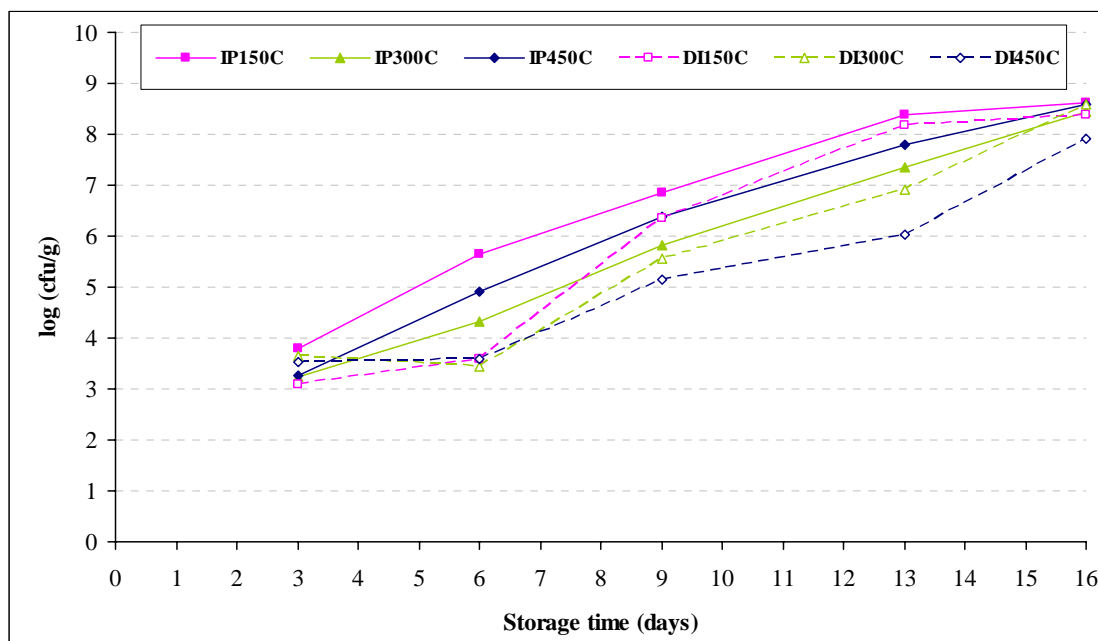
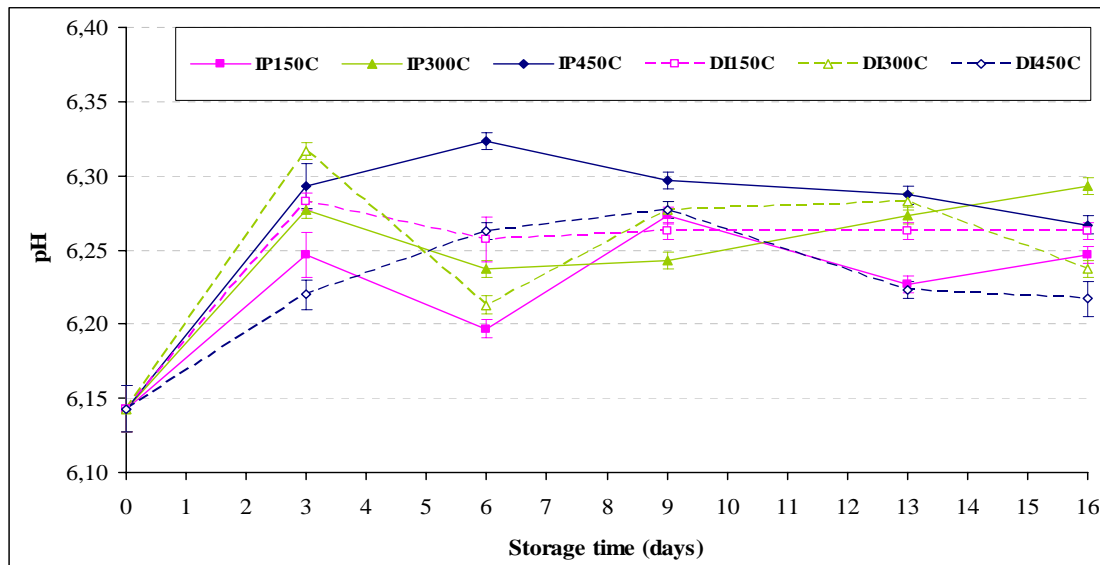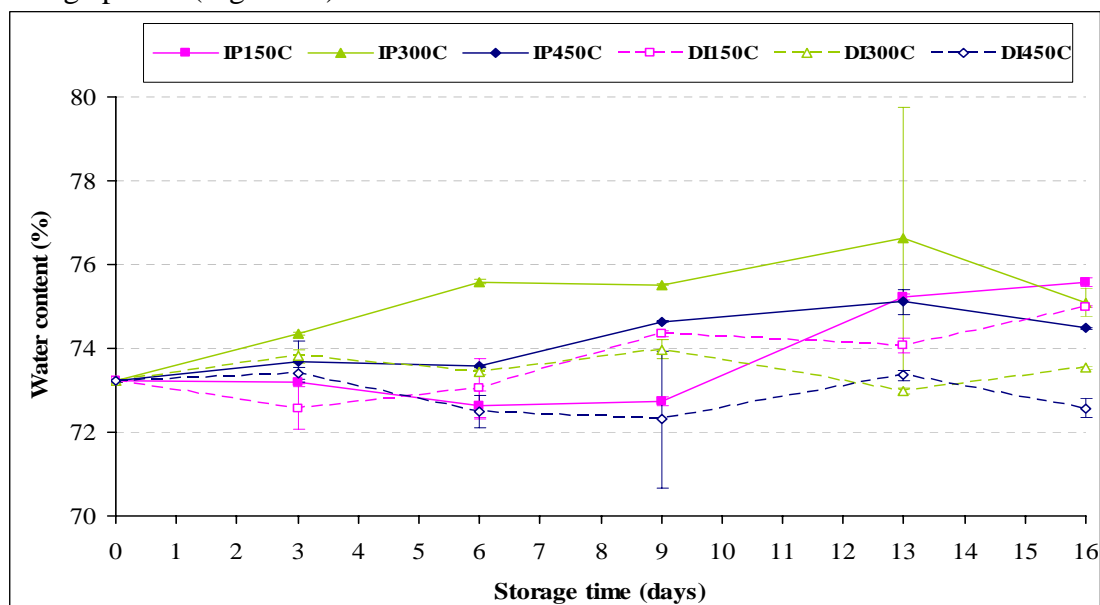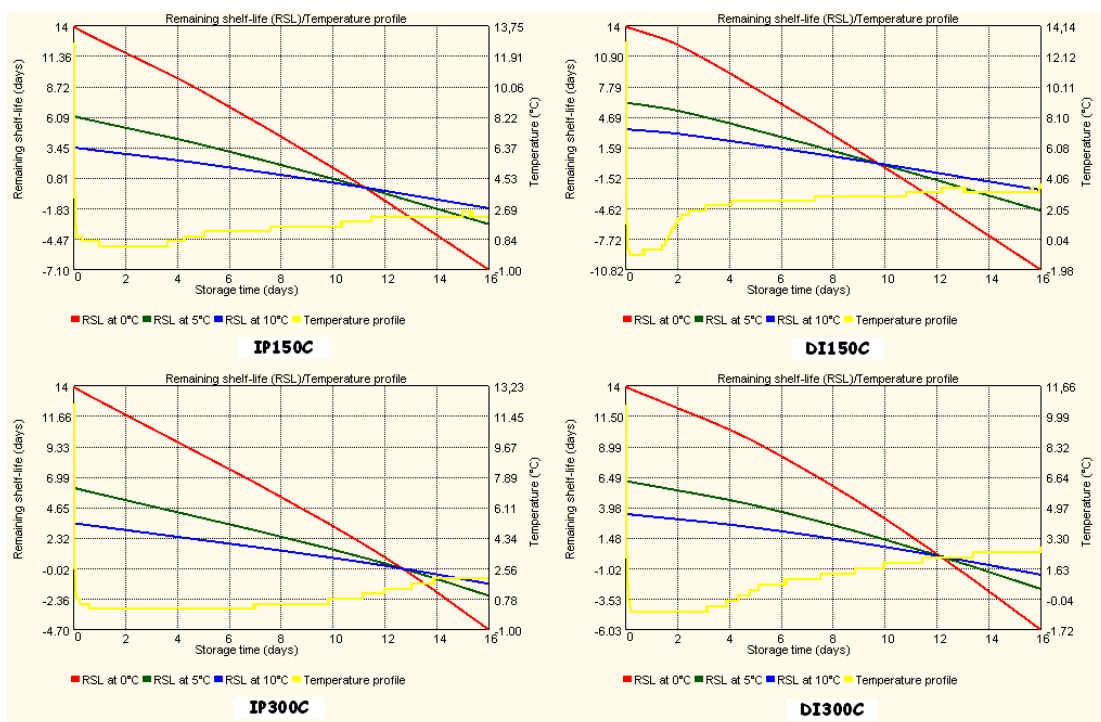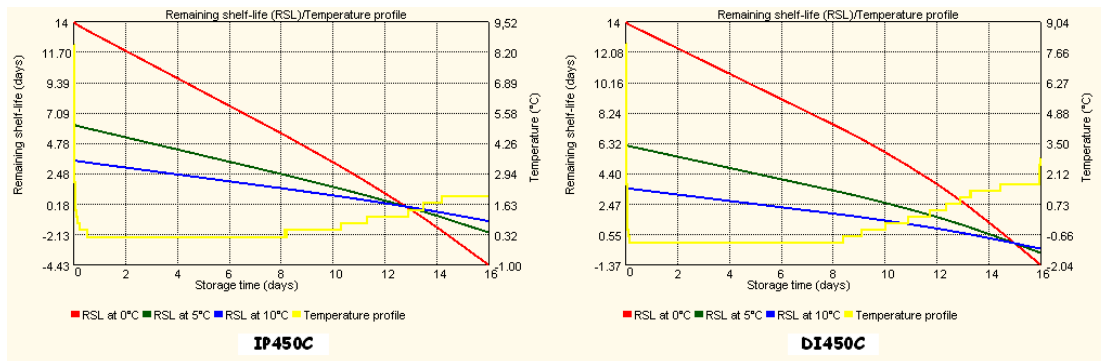Figure 37: Predicted shelf life (based on storage temperature profiles) of the fillets in samples IP150C (packed with 150 g ice packs and stored at 3-4°C), IP300C (packed with 300 g ice packs and stored at 3-4°C), IP450C (packed with 450 g ice packs and stored at 3-4°C), DI150C (packed with 150 g dry ice and stored at 3-4°C), DI300C (packed with 300 g dry ice and stored at 3-4°C), DI450C (packed with 450 g dry ice and stored at 3-4°C).

5   DISCUSSION

5.1   The effect of cooling agents and storage temperature profiles

The temperature of the cooling room and chilling cabinet was rather stable. Therefore, the temperature in the box of each sample was affected by the ratio and type of cooling agent used. Storage temperature is a critical factor which influences the quality and shelf life of fish and fishery products. Temperature fluctuation during storage, transportation and retail greatly affect the quality and shelf life of fish and fishery products.

In chilling storage conditions (3-4°C), the temperature at the bottom of sample DI150C (packed with 150 g dry ice and stored at 3-4°C) dropped to -1°C within four hours and remained sub-zero for about 38 hours. Meanwhile, the temperature at the bottom of sample FZ (packed with 150 g ice packs, frozen overnight and then stored at 3-4°C) decreased to -1°C within 16 hours and was at sub-zero for more than three and a half days. On the contrary, the temperature of sample IP150C (packed with 150 g ice packs and stored at 3-4°C) did not go below 0°C. However, packaging with ice packs maintained the temperature in the box lower than 1°C for about five days, while packaging with dry ice maintained the temperature lower than 1°C for less than two days and less than four days for the freezing overnight sample.

The temperature of sample DI150C dropped to -1°C quickly, because of the large chilling capacity of dry ice (the latent heat of sublimation of dry ice is about 6030±5 cal/mol) which can remove three times the quantity of heat in comparison with water ice (Sasi *et al.* 2000).

However, the temperature of this sample could be maintained at 0°C for only 38 hours because the dry ice had sublimated to CO_2 gas and leaked out. Hence the temperature in the box increased very fast on day two and day three of storage and then slowed down and reached cooling room temperature on day 13. The principle of chilling with water ice packs is similar. It could not make the temperature of sample IP150C lower than 0°C. However, it maintained the temperature of the sample lower than 1°C for about five days because of its slow melting. Freezing overnight could reduce the temperature in the box to -1°C, but temperature reduction was rather slow. Because of packaging with ice packs, the temperature of sample FZ was maintained lower than 1°C for five days as in sample IP150C.

In chilling storage conditions (3-4°C), using dry ice in combination with ice packs for reducing and maintaining low temperatures of fillets might be more effective than using separate cooling agents or combinations of freezing and ice packs.

In superchilling storage conditions (-2°C), packaging with dry ice (DI150S - packed with 150 g dry ice and stored at -2°C) was most effective. It reduced the temperature in sample DI150S to -1°C within 20 hours while the temperature of the control sample (CC) reached -1°C after 37 hours and the temperature of sample IP150S (packed with 150 g ice packs and stored at -2°C) could not be reduce to -1°C until day 13 of storage. Because melting temperature is higher than -1°C, ice packs are not effective in sperchilling storage conditions. Not only couldn't they reduce storage temperature to -1°C but temperature reduction was also slow (Figures 8 and 18).

Reduction of the temperature in the samples increased with the ratio of cooling agent to fillets. Increasing the ratio of ice packs up to 300 g (IP300C) and 450 g (IP450C) only reduced temperature in the box to around 0°C for nearly one day. The temperature reducing rate of two samples IP300C and IP450C was the same, but sample IP450C was maintained at the lowest temperature one day longer than sample IP300C (Figure 23). In this case, dry ice had more advantages than ice packs because of its ability to reduce temperature fast. The temperature at the bottom of sample DI450C, which was packed with 450 g dry ice and stored at 3-4 ° C, was reduced to -1°C within four hours and the temperature was maintained for more than eight days (Figure 24). However, a high ratio of dry ice to fillets (300 g dry ice/3 kg of fillets and 450 g dry ice/3 kg of fillets) lowered the temperature at the top of these samples to less than -20°C within two hours from the beginning of the storage period. With temperature dropping so fast, fillets on the top layer may be frozen (Figure 7) resulting in loss of quality. Therefore, a high ice/fillets ratio is not always appropriate. It would be better to pack fillets with the ratio 150 g dry ice to 3 kg fillets at the beginning and then add 150 g dry ice after one and a half day of storage and 150 g further on the third day of the storage period (Figure 24).

5.2 Effect of chilling and superchilling with different cooling agents on the quality and shelf life of Arctic charr fillets

With regards to the relationship between storage temperature and shelf life, the predicted shelf life of samples IP150C and FZ was one day longer than for sample DI150C and two days longer than the control sample (CC). For samples with the same cooling agent to fillets ratio the superchilled samples had five to six days more shelf life (IP150C and IP150S; DI150C and DI150S). The control sample at superchilling temperature (-2°C) had double the shelf life of the control sample at chilling temperature (3-4°C) (Figure 36). However, in addition to storage temperature, shelf life of fish and fishery products is affected by the amount of CO_2 gas in the storage environment (Koutsoumanis *et al.* 2000) and the reduction rate of the initial temperature.

The shelf life of samples which were packed with ice packs was shorter than the predicted shelf life by about one day. The TVB-N content of sample IP150C reached 20 mgN/100 g flesh fish on day 10 and sample IP150S reached this value on day 15. The limiting level for rejection of TVB-N is 20 mgN/100 g flesh fish (Connell 1995). The shelf life of the samples which were packed with dry ice was approximately as predicted (Figure 36). The TVB-N content of sample DI150C reached 20 mgN/100 g flesh fish on day 10 and sample DI150S reached this value on day 16 (Figure 22). The shelf life of the FZ sample was longer than the prediction by two days (Figure 36) with the TVB-N level reaching 20 mgN/100 g flesh fish on day 13 (Figure 22). This shows that the shelf life was affected by the cooling rate of the fish at the start as well as the time under superchilling conditions.

Trimethylamine (TMA) and thiobarbitutic acid (TBA) are also chemical criteria for the determination of fish shelf life (Connell 1995). The TMA content of all samples (IP150C, DI150C, IP150S, DI150S and FZ) increased very slowly during storage, especially superchilled samples, which had attained a value of 1.5 mg N/100 g flesh (sample IP150S) and less than 0.3 mg N/100 g flesh (sample DI150S) at the end of the

storage period (Table 8). These values are considered low as compared to the value of around 10 mg N/100 g flesh suggested by Teskeredzic and Pfeifer (1987) as upper acceptable limits of TMA in cultured brackish water rainbow trout for human consumption. However, these results are similar with the TMA level of 1.8-2.0mg N/100 g flesh in aquacultured fresh water rainbow trout, as reported by Chytiri *et al.* (2004). This may be explained by a lower level of trimethylamine oxide (TMAO) content in fresh water fish in brackish water fish and marine fish (30-130 mmol TMAO/kg muscle) (Takeuchi *et al.* 2003, Seibel 2002).

During storage, the lipid oxidation of the fillets was found to be significant. The TBA value in the fish flesh increased with storage time. The TBA value of all samples increased after day six of the storage period. However, the maximum TBA value of all samples remained rather low (< 3 µmol/kg fish flesh or < 0.35 µmol/g lipid) and below the level of 1-2 µmol/g lipid at which rancid flavours may become evident in fish (Connell 1995) until end of the storage period (Figure 22). Similar TBA values have been reported for farm-raised European sea bass (Kyrana and Lougovois 2002), pond-rised hybrid striped bass (Boyd *et al.* 1992) and maricultured gilthead sea bream (Kyrana *et al.* 1997). In fact, the rancid odour and rancid flavour detected in the cooked fillets of all samples during storage time suggested edible shelf life (Figures 17 and 18). It may be caused by the immature stage of Arctic charr with a high ratio of free fatty acid (FFA) to total lipid in its muscles. Results from Jobling *et al.* (1998) showed the content of FFA in immature Arctic charr muscle (14-17% total lipid) is higher than in maturing Arctic charr muscle (7-9% total lipid). The rate of lipid oxidation increases with a higher ratio of FFA in fish muscle.

The water content of fillets in all of the samples increased slightly with storage time and reached its highest value at the end of the storage period (Figure 21). The water content of fillets in superchilled samples varied more than in chilled samples during the storage period (Figure 21). This may be caused by the condensation of water in fillet surfaces at superchilling temperature (-1°C) (LeBlanc and LeBlanc 1992).

More often fish spoilage is characterized by off-odours and off-flavours caused by bacteria metabolism (Gram and Huss 1996). When the number of microorganisms grows to higher than 10^7-10^8 cfu/g (Gram and Dalgaard 2002) and/or the number of H_2S producing bacteria exceeds 10^6 cfu/g (Gram and Huss 1996), significant amounts of volatile sulphur-containing compounds are produced and spoilage becomes sensorially evident. Results of this reaseach have shown similar levels (about 10^7 cfu/g) of the total viable count (TVC) for chilled samples (IP150C, DI150C) on day 10 and superchilled samples (IP150S, DI150S and CS) on day 14-16 of the storage period (Figure 16). In this case, dry ice packaging affected the growth rate of bacteria by more rapid cooling of fillets than with ice packs and by the effects of CO_2 on aerobic bacteria (Figures 9 and 16). The TVC of samples FZ and CC reached 10^8 cfu/g and theTVB-N content reached 20 mgN/100 g (sample FZ, Figure 22) (sample CC, Figure 36). However, the number of H_2S producing bacteria in these samples (FZ and CC) was the same with limit 10^6 cfu/g (Gram and Huss 1996) at the day that is suggested as the end of shelf life. The number of H_2S producing bacteria of other samples was lower than this limit (10^6 cfu/g) suggested by Gram and Huss (1996) (Table 7).

Drip loss from the samples increased with storage time and increased with storage temperature during the first three days. This was caused by bacteria and enzymes in the fish which are controlled by temperature (Huss 1995). The drip loss of chilled samples (IP150C and DI150C) was 0.5% higher than of superchilled samples (IP150S and DI150S) on day three (Figure 12). The same was observed in salmon fillets which were packed in modified atmosphere and superchilled (Sivertsvik *et al.* 2003), there was no effect of dissolved CO_2 in the fillets that could decrease the water holding capacity (Figure 15) or decrease the pH value (Figure 20) of the fish flesh. These effects were discussed by Reddy *et al.* (1992).

Total cooking yield (TCY) or cooking yield (CY) are important criteria which have been of some concern to producers as well as consumers. The TCY and CY decreased with storage time because of degradation of organic substances in the flesh fish by enzymes and bacteria. The TCY and CY of superchilled samples were higher than of chilled samples (Figures 13 and 14). However, the results from TCY and CY measurements showed great variance. The TCY and CY not only depended on storage time and temperature, but also on the weight of the fillets. The TCY and CY were higher in heavier fillets (Figures 32 and 33). This is due to differences in heat transfer and mass transfer during cooking.

Changes in myofibrillar proteins which affect the quality of fish muscle have been related to proteolytic activity in the muscle (Benjakul *et al.* 1997, Osatomi *et al.* 1997). These changes could be reflected in water holding capacity (WHC) of fish muscle. The fish muscles' ability to retain water is regarded as an essential quality parameter and a high WHC is of great importance both to the industry and the consumers (Olsson *et al.* 2003). However, the results indicated that the WHC of all samples increased during the storage period (Figure 15). This may be explained by the investigation of Kristensen and Purslow (2001) which shows that increasing WHC of muscles during storage is caused by the extension of proteolysis of cytoskeletal proteins such as vinculin, desmin and talin. This also might explain why the increase in WHC of superchilled samples (IP150S, DI150S, CS and FZ) was higher than of chilled samples (IP150C, DI150C and C) during the first nine days (Figure 15). The rate of several enzymatic reactions in muscle foods is greater in the critical temperature zone -1°C to -6°C than above the freezing point (Sikorski and Kolakowski 2000). Calpain and cathepsin are enzymes involved in the degradation of fish muscles (Jiang 2000, Huss 1995).

Changes in sensory characteristics are caused by physical, chemical and microbial changes in the fish. The positive attributes (+) for odour and flavour of Arctic charr were described as characteristic Arctic charr, metallic and oily on a scale ranging from 0 to 100%. Average scores for most positive odour attributes decreased with storage time (Figure 18). On the contrary, the average scores for most positive flavour attributes did not change during the first nine days for chilled samples (IP150C, DI150C) and up to 13 days for superchilled samples (IP150S, DI150S) and sample FZ (Figure 19). Whilst both negative attributes (-) for odour and flavour of Arctic charr increased with storage time (Figures 18 and 19). The increasing rancid odour and flavour caused lipid oxidation which was discussed in the changes in TBA values above.

Comparison on changes in sensory attributes between the samples which were packed with dry ice (DI150C, DI150S) and ice packs (IP150C, IP150S) did not show any difference. The authors (Jeyasekaran *et al.* 2004, Sasi *et al.* 2003, Sasi *et al.* 2000, LeBlanc and LeBlanc 1992) did not observe any difference in sensory quality between the fish which was packed with dry ice and water ice. Changes in sensory attributes between chilled samples (IP150C, DI150C) and superchilled samples (IP150S, DI150S) at the same storage day were not evident (Figures 17, 18 and 19). Thus Arctic charr fillets which were superchilled at -1°C and/or packed with dry ice (ratio 150 g dry ice per 3 kg of Arctic charr fillets) were not affected on the sensory quality. This can be explained by similar chemical composition to other Salmonid species (trout, salmon), the freezing point of Arctic charr is about -1°C to -2.2°C (Rahman 1995). This temperature is lower than the superchilling temperature of our experiment (-1°C). Therefore, the fish muscles were not damaged because of slow freezing at a lower freezing point temperature.

The results suggest that the storage temperature is more important and superchilled storage of Arctic charr fillets at -1°C (in box temperature) does not lead to excessive lost drip or cooking yield caused by cell destruction due to freezing.

5.3 Effect of the ratio of cooling agent to fillets on the quality and shelf life of Arctic charr fillets

The ratio of cooling agent (ice packs and dry ice) to fillets was expected to influence the quality and shelf life of the fillets by reducing storage temperature and inhibiting bacteria growth by released CO_2 gas.

Cooling agents have an ability to reduce storage temperature of fish by removing heat through melting (ice packs) or sublimation (dry ice). Therefore, the storage temperature in the boxes of samples (IP150C, IP300C, IP450C, DI150C, DI300C and DI450C) decreased with an increasing ratio of cooling agent (Figures 23 and 24). The reduction rate of storage temperature is also very important. Dry ice reduced the temperature in the boxes of samples (DI150C, DI300C, DI450C) to under -1°C within four hours from the beginning of the storage period (Figure 24) while ice packs could not reduce the temperature in the boxes of samples (IP150C, IP300C, IP450C) to below 0°C. The lowest temperature of sample IP150C (0.4°C), of samples IP300C and IP450C (0.2°C) was reached after one day of storage (Figure 23). This is because of the special characteristic of dry ice when it sublimates. Dry ice sublimating can remove three times the quantity of heat compared to water ice melting (Sasi *et al.* 2000). A higher ratio of dry ice maintained the temperature at -1°C for a longer time. The ratio of 450 g dry ice to 3 kg Arctic charr fillets (DI450C) could maintain storage temperature at -1°C about 10 times longer than the ratio of 150 g dry ice to 3 kg Arctic charr fillets (DI150C) and two times longer than the ratio of 300 g dry ice to 3 kg Arctic charr fillets (DI300C) (Figure 24).

Contrary to dry ice, the ratio of 300 g ice packs to 3 kg Arctic charr fillets (IP300C) and the ratio of 450 g ice packs to 3 kg Arctic charr fillets (IP450C) were not different in their temperature profiles (Figure 23). Thus using a higher ratio of 300 g ice packs in this experiment did not effect the storage temperature.

Differences in storage temperature resulted in different shelf lives of the samples. Regarding the relationship between storage temperature and shelf life, the predicted shelf life of samples IP150C and ID150C was less than 11 days and less than 13 days for samples IP300C and IP450C (Figure 37). This prediction corresponded to our observation. The total volatile base nitrogen (TVB-N) content of samples IP150C and ID150C exceeded the level of 20 mgN/100 g fish flesh which was recommended for rejection by Connell (1995) on day 10 and the TVB-N value of samples IP300C and IP450C exceeded this level on day 12 (Figure 33). Furthermore, the total viable count (TVC) of these samples exceeded the level of 10^7cfu/g which was found in spoiled fish (Gram and Dalgaard 2002) at the storage day with the TVB-N content of 20 mgN/100 g. Whilst the predicted shelf lives of samples DI300C and DI450C are 12 days and 15 days respectively (Figure 37). In this case the shelf life prediction was about one day shorter than observed. In fact, the total volatile base nitrogen (TVB-N) content of samples DI300C reached the level of 20 mgN/100 g fish flesh on day 13 and sample DI450C reached this value on day 16 of the storage period (Figure 33). Their TVC had also reached the level of 10^7cfu/g on these days (Figure 30). Thus dry ice not only affected storage temperature, but it also inhibited growth of bacteria because of released CO_2 gas from sublimation. The effect of dry ice on bacterial growth could be recognised through the number of H_2S producing bacteria. A comparison between samples IP150C and DI150C shows that the ratio of 150 g dry ice to 3 kg Arctic charr fillets was only effective during the first six days with a lower number of H_2S producing bacteria than in the ice pack sample with the same ratio (IP150C). The H_2S producing bacteria of sample DI300C was inhibited up to day nine and to the end of the storage period in sample DI450C with a level lower than 10^3cfu/g (Table 12).

Trimethylamine (TMA) and thiobarbituric acid content were also recommended as indicators for evaluation of fish quality, but they did not give clear results in this case. The TMA value of all samples was very low (Table 13) compared with the level 10 mgN/100 g fish flesh which was suggested as an upper acceptable limit of TMA for human consumption in cultured brackish water rainbow trout (Teskeredzic and Pfeifer 1987). The samples which were packed with different ratios of ice packs (IP150C, IP300C, IP450C) did not show differences in TMA content during the first 13 days while the samples which were packed with different ratios of dry ice (ID150C, DI300C, DI450C) showed a difference on day 13 of storage time (Table 13). The TMA content of the samples had increased inversely with the ratio of cooling agent. However, there was not much difference in the TMA content between samples which were packed with dry ice and ice packs at the same ratio. This maybe due to the fact that Arctic charr is a fresh water fish, so the trimethylamine oxide (TMAO) content in its flesh is very low and the degradation of TMAO had not been reflected clearly in this reseach.

Although Arctic charr is a fatty fish (9.4% fat), the TBA value of all samples remained rather low (with a maximum value less than 3 µmol/kg fish flesh or 0.35 µmol/g lipid) and below the level of 1-2 µmol/g lipid at which rancid flavours may become evident in fish (Connell 1995). However, rancid flavour of cooked fillets were recognised at a low level of TBA content (< 3 µmol/kg) (Figure 18). Kyrana *et al.* (1997 and 2002) and Boyd *et al.* (1992) have also reported lower TBA content in other species compared with the level of 1-2 µmol/g lipid. Therefore, this level may not be useful for determining fish shelf life.

The changes in drip loss (Figures 25 and 26), cooking yield (Figures 27 and 28) and water holding capacity (Figure 19) show the advantage of using dry ice for fish storage. The samples which were packed with dry ice (DI150C, DI300C, DI450C) had lower drip loss and higher cooking yield and water holding capacity. However, there was also much difference between the samples depending on theratio of cooling agent.

Changes in colour and pH value of fish flesh are problems that have been of concern when fillets are stored with dry ice. High ratios of dry ice may affect the colour and pH value of fish flesh because of absorbed CO_2 gas on the fillet surfaces and the reaction between CO_2 and organic substances in the fish. However, the results in measured colour and pH values of the samples which were packed with dry ice were not different to the ice pack samples with the same ratio of cooling agent (Figure 31 and Tables 9, 10, 11). Thus the ratios of dry ice which were used in this experiment (150 g, 300 g and 450 g to 3 kg Arctic charr fillets) did not affect the quality of the fillets. On the contrary, the shelf life of the samples packaged with dry ice was one to four days longer than of those packaged with ice packs..

6   CONCLUSION

Storage of Arctic charr fillets using dry ice can reduce temperature in fish boxes to below 0°C as is expected for superchilling storage of fish whereas ice packs could not lower the temperature to below 0°C. The temperature reduction rate of the samples which were packed with dry ice was faster than of fillets packed with ice packs with the same ratio of cooling agent. These advantages can result in extended shelf life and maintain a higher quality of fish during storage and transport to distant markets.

Using higher ratios of dry ice did not give a faster reduction rate of storage temperature, but maintained a lower storage temperature for a longer period of time. Thus the rate of fish spoilage was delayed and the shelf-life extended.

The observation did not detect an affective quality of fillets caused by cell destruction due to partial freezing or caused by soured taste due to the absorption of CO_2 gas in the fish flesh.

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APPENDICES
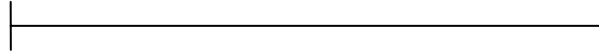**Appendix 1. Quantitative descriptive analysis (QDA) parameters**

Odour

Characteristic for species

Week   Strong

Metallic

Week   Strong

Oil

Week   Strong

Musty/mould

Week   Strong

Sour

Week   Strong

Rancid

Week   Strong

Flavour

Characteristic for species

Week   Strong

Metallic

Week   Strong

Oil

Week   Strong

Musty/mould

Week   Strong

Sour

Week   Strong

Rancid

Week   Strong

Texture

Softness
├──────────────────────────────────────┤
Firm   Soft
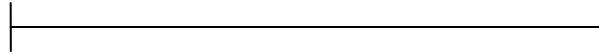
Juiciness
├──────────────────────────────────────┤
Dry   Juicy

Tenderness
├──────────────────────────────────────┤
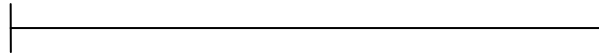Tough   Tender

Appendix 2. The shelf life evaluation and physical changes

The shelf life evaluation regards to TVC, H_2S producing bacteria and TVB-N content of the fillets during storage. The TVB-N content at level of 20 mgN/100g fish flesh which was recommended for rejection by Connell (1995) and the total viable count (TVC) at level of more than 10^6-10^7cfu/g which was found in spoiled fish (Gram, 2002).