

International Journal of Food Engineering

Volume 3, Issue 3

2007

Article 7

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Abstract

The influence of different cooling techniques (dry ice/ice packs) and storage temperature ($-2^{\circ}\text{C}/3^{\circ}\text{C}$) to prolong the shelf life of Arctic charr (*Salvelinus alpinus*) fillets were evaluated by sensory analysis, physical methods, chemical and microbial analysis. The effects of storage temperature were stronger than of different cooling agents. Superchilling (-2°C) of fillets packed with dry ice resulted in 6 days extension of shelf life compared to chilling (3°C). The use of dry ice parallel to superchilling prolonged shelf life for 1 day compared to fillets stored with ice packs. No negative effects on quality of the fillets were detected that could be linked to cell destruction caused by partial freezing or to sour taste, caused by absorption of CO_2 gas in fish flesh.

KEYWORDS: superchilling, dry ice, ice packs, shelf life, charr

*The authors gratefully acknowledge the financial support of the United Nations University-Fisheries Training Programme fund and of ISAGA Company.

1. INTRODUCTION

Shelf life of fish and fishery products is an important factor in the seafood industry. Shelf life extension of fish and fishery products allows the processors to plan and control their processing and marketing in long term as well as giving opportunities to change transport modes. Handling practices and storage conditions are the most important factors that affect the shelf life of fish and fishery products (Doyle, 1995; Huss, 1995). Rapid cooling and maintenance of low temperature from catch to consumer is essential to obtain desired shelf life of seafood. Temperature fluctuations during processing, storage, transportation and retail may have great effects on quality and shelf life.

Different types of cooling agents and packaging methods can be used to minimise effects of temperature fluctuations. For example, dry ice and ice packs have been recommended for transport of fresh fish and seafood by cargo (Terchunian & others, 1999).

Superchilling (0 to -4°C) has been found promising for extension of shelf life (Huss, 1995). It is based on lowering temperature close to freezing point of the fish, which depends on water content and soluble substances of fish (Rahman, 1995). At these temperatures, approximately 30-50% the water in the product is frozen. Different cooling agents or methods can be used for superchilling, like superchilled brine, slurry or liquid ice (small ice crystals in superchilled brine) and dry ice (CO₂ in solid form), depending on fish species, processing stages and products. Accurate control of the ambient temperature is very important during superchilling since the ratio of ice in the muscle and risk of negative effects of ice crystals depends on it.

Scientists do not fully agree on the effects of superchilling on shelf life and quality of products (Boyd *et al.*, 1992; Lee & Toledo, 1984; Reed *et al.*, 1983; Nowlan *et al.*, 1975). Differences in their studies between fish species, chilling methods, storage temperatures and variables analysed might partly be the explanation. However, following conclusion can be stated. The growth rate of spoilage bacteria is reduced by superchilling resulting in extended shelf life (Huss, 1995). The rate of bacterial spoilage at -2°C is only 64% of that at 0°C (Doyle, 1995) showing the potential advantage of superchilling. The disadvantage of superchilling is that the quality of the products may be negatively affected due to slow formation of ice crystals and increased enzyme activity in the temperature range of -1 to -6°C (Robinson, 1985; Sikorski *et al.*, 1976; Love & Robertson, 1968). Higher enzyme activity may be due to increased ionic strength as water freezes out and improved access of enzymes to substrates caused by freeze damage of cell membranes. This may lead to irreversible negative changes in the muscle like increased drip, protein denaturation and oxidation, affecting texture, juiciness, flavor and odor of the products (Huss, 1995; Fennema *et al.*, 1973).

Combined effects of superchilling and CO₂ on the spoilage microflora of fish may be reached by the use of dry ice and control of the storage conditions. The effects of CO₂ on storage life have mainly been studied in MAP (Modified Atmosphere Packaging) where all or some of O₂ is replaced by CO₂ and N₂ to extend shelf life (Stamment *et al.*, 1990). The preservation effect of CO₂ is complex and not only due to replacement of oxygen or lowering of pH when CO₂ dissolves in the muscle. The activity mechanisms on microorganisms has been suggested to be caused by to alteration of cell membranes, decrease or inhibition of enzyme reactions, penetration of bacterial membranes leading to intracellular pH changes and direct changes in physio-chemical properties of proteins (Sivertsvik *et al.*, 2002). The achievable extension of shelf life depends on species, fat content, initial microbial population, gas mixture, and most importantly, storage temperature.

The choice of cooling agents and packaging methods depends on species and characteristics of the products, storage conditions and means of transport from producers to markets. Each method has its advantage and disadvantage and further studies are needed to find out appropriate storage condition for different products. The objectives of this research were to study the effects of different cooling agents (dry ice/ice packs) and storage temperatures (chilling/superchilling) on quality and shelf life of Arctic charr (*Salvelinus alpinus*) fillets.

2. MATERIAL AND METHODS

2.1. Raw material

The Arctic charr (*Salvelinus alpinus*) was farmed in Northeast of Iceland. The age of the fish at slaughtering were 2 years and the average weight 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. At arrival the next day, the temperature in the boxes was 0.1-0.2°C.

2.2. Storage experiments

The Arctic charr fillets were randomly divided into 6 groups that were packed into styrofoam boxes, 3 kg in each, with 150 g of dry ice (99% CO₂) or ice packs as top layer. The ice packs contained about 0.7% of Favor Pac 300 (Degussa Standort Krefeld, Stockhausen GmbH, Krefeld, Germany). Control groups were packed without cooling agent. After that, the boxes were stored at controlled

storage temperature at 3°C or -2°C. Additionally, one group was packed with 150 g of ice packs and stored at -24°C over night (16 h) followed by storage at 3°C (Table 1).

Table 1. Experimental groups were packed with dry ice and ice packs then stored at chilling temperature (3°C) and superchilling temperature (-2°C).

Group	Cooling agents	Quantity of cooling agents	Storage temperature (°C)	Code
1	Ice packs (IP)	150g	+3 (C)	IP150C
4	Dry ice (DI)	150g	+3 (C)	DI150C
7	Ice packs (IP)	150g	-2 (S)	IP150S
8	Dry ice (DI)	150g	-2 (S)	DI150S
9	None (C)	0	+3 (C)	CC
10	None (C)	0	-2 (S)	CS
11	Ice packs (IP)	150g	Stored at -24°C for 16 hours, then at +3°C	FZ

Three fillets in each box were marked with numbered plastic tag and weighted before and after storage and cooking for measurement of drip loss and cooking yield.

2.3. Sampling

Sampling was carried out at arrival of the fillets (day 0) and after 3, 6, 9, 13 and 16 days of storage for evaluation of drip loss, cooking yield, water content, water holding capacity (WHC), color, pH, total viable counts (TVC) and counts of H₂S-producing bacteria (at 15°C). Additionally, protein and fat content was evaluated on day 0 and TVB-N, TMA and TBAR on days 0, 6, 13 and 16.

On each sampling day, six fillets were taken randomly from each group for sensory analysis and three were taken for microbial and chemical analyses. A piece of about 5-6 cm was cut from the middle of the fillets for determination of

water holding capacity, water content and pH. Three marked fillets were used for cooking yield measurement, and other three fillets were used for color measurement.

2.4. Temperature measurements

The temperature was recorded with 15 min intervals, by data loggers (Optic StowAway Tidbit®, Onset Computer Corporation, Massachusetts, USA) placed in the bottom of each box.

2.5. Water holding capacity (WHC)

The samples (n=3) were prepared by chopping them in a mixer (Braun electronic, type 4262, Kronberg, Germany) for 10-15 seconds. Approximately 2 g of minced muscle was weighed accurately and immediately centrifuged at $210 \times g$ (1500 rpm) for 5 min; at a temperature of 2 to 5°C (Eide *et al.*, 1982). The weight loss after centrifugation was divided by the moisture content of the fillet and expressed as % WHC.

2.6. Drip and cooking yield

Drip (%) was determined as the loss in weight of the three marked fillets during storage after 1 hour at 20°C. Total cooking yield was determined as the loss in weight during cooking of the fillets. 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 and W_{raw} was the weight of raw sample before cooking.

2.7. Proximate analysis and pH

Protein content in the minced muscle was determined by the Kjeldahl method (ISO, 1997). Salt content was determined using a potentiometric method (AOAC, 1995). Fat content was determined by the AOCS Soxhlet method Ba 3-38 (AOCS, 1998) using petroleum ether (Bp. 40-60°C) for extraction. Water content (g/100 g) was calculated as the loss in weight during drying at 105°C for 4 h (ISO,

1983). The pH of the minced muscle was measured using a puncture, combination electrode (SE 104, Mettler Toledo, Greifensee, Switzerland) connected to a pH meter (Knick-Portamess 913 pH, Berlin, Germany). The electrode was dipped into minced muscle at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

2.8. TVB-N and TMA

Total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) were determined using steam distillation in the minced shrimp tissue, followed by titration method. The TVB-N analysis was performed through direct distillation into boric acid using a Kjeldahl-type distillatory (Struer TVB-N). The acid was titrated with diluted H_2SO_4 solution. To determine TMA, the same method as for TVB-N was used, except that 20 ml of 35% formaldehyde were added to the distillation flask to block the primary and secondary amines (Malle & Poumeyrol, 1989).

2.9. Thiobarbituric acid (TBAR) content

TBAR were determined by a modified version (Sørensen & Jørgensen, 1996) of the extraction method described by Vyncke (1970, 1975) with 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. TBAR, expressed as μmol malondialdehyde per kilogram of sample (μmol MDA/kg), was calculated using malondialdehyd-bis-(diethyl acetate) as standard.

2.10. 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 4 days. Dilutions were done in Maximum Recovery Diluent (MRD) from Oxoid.

2.11. Sensory analyses

Quantitative Descriptive Analysis (QDA), introduced by Stone (1992), was used to evaluate cooked samples of Arctic charr fillets. An unstructured scale (0-100%) was used on a list of words describing odor and flavor, like characteristic Arctic charr, metallic and oily.

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 odors, in use of scales, and in development and use descriptors. The members of the panel were familiar with the QDA method and experienced in sensory analysis of Arctic charr. Each panelist evaluated the samples in two sessions each day of the sensory evaluation.

2.12. Data analysis

Statistical analysis was performed by Microsoft Excel 2000 (Microsoft Inc, Redmond, Wash., U.S.A.) and (factorial analysis) NCSS 2000 (NCSS, UTAH, USA). Excel was used to calculate means and standard deviations for all multiple measurements and to generate graphs. Multivariate analysis of the data (Principal component analysis) was performed in the statistical program Unscrambler (Version 7.5, CAMO ASA, Oslo, Norway).

3. RESULTS AND DISCUSSION

3.1. Temperature profiles

The use of dry ice resulted in a higher cooling rate of the fillets than ice packs and in lower temperature after cooling than was obtained with ice packs (Figure 1). The temperature recorded in the bottom of the boxes had reached about -0.5°C after 15 minutes when dry ice was used but after 78 hours when ice packs were used. However, a longer time was needed for lowering of temperature in fillets in the center of the boxes.

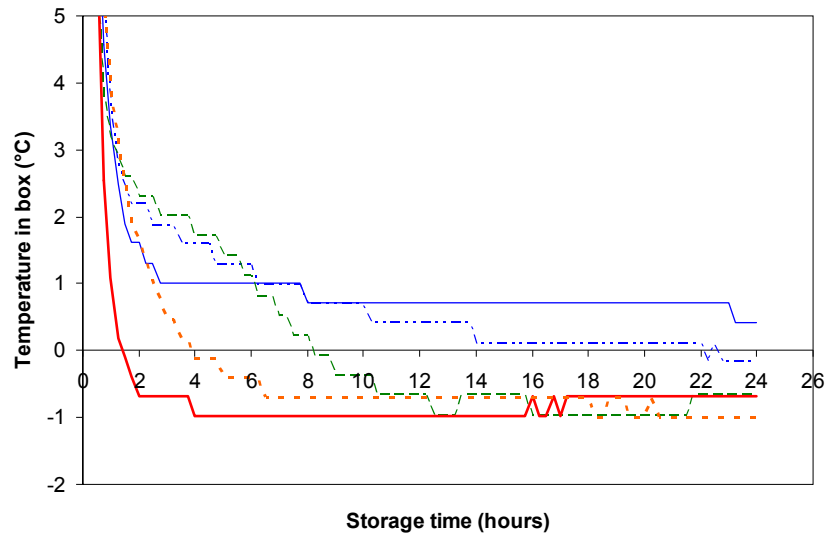


Figure 1. Temperature profiles during the first 24 hours, in bottom of boxes with fillets packed with 150 g of dry ice (DI), 150 g of ice packs (IP) and then stored chilled (C = +3°C) or superchilled (S = -2°C). Additionally, one group was stored over night at -24°C with ice packs (FZ) and than at 3°C (C).

The ice packs were more effective in maintaining low temperature in the boxes, during storage at 3°C over 14 days (Figure 2). After about 3.5 days, the temperature in boxes with ice packs started to rise from about 0.5°C to reach equilibrium with the environment at 3°C. On the other hand, temperature in boxes with dry ice, started to increase rapidly after about 1.5 days.

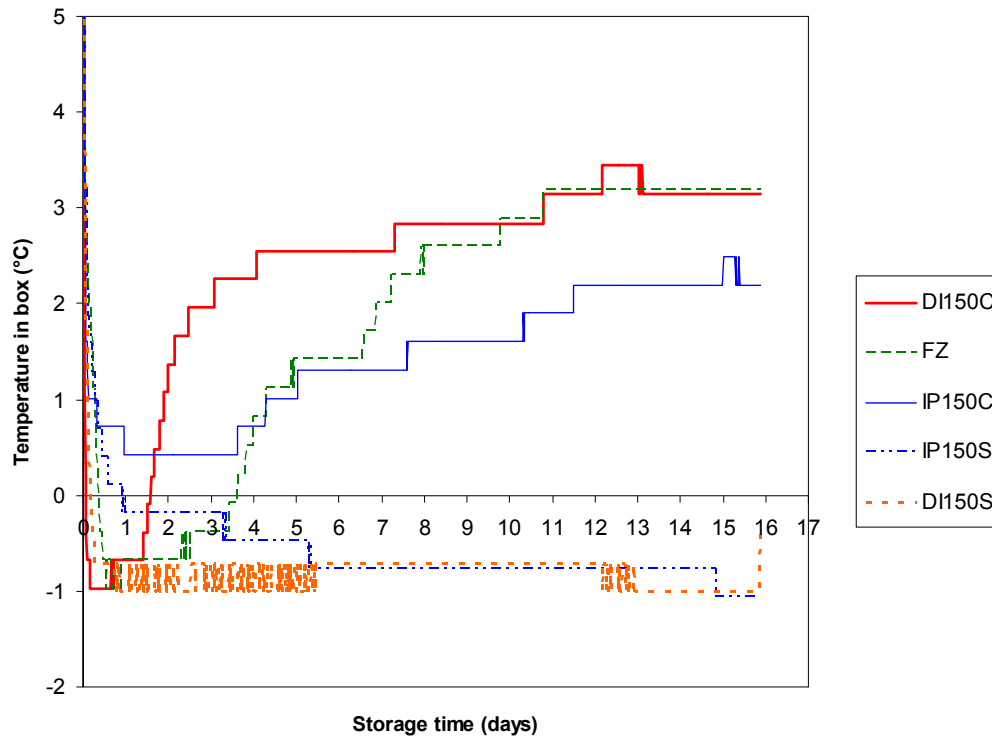


Figure 2. Temperature profiles recorded for 2 weeks, in bottom of boxes with fillets packed with 150 g of dry ice (DI), 150 g of ice packs (IP) and then stored chilled (C = +3°C) or superchilled (S = -2°C). Additionally, one group was stored over night at -24°C with ice packs (FZ) and than at +3°C (C).

The high cooling rate obtained with dry ice was explained by its high cooling capacity. The latent heat of sublimation of dry ice has been known to be about $6,030 \pm 5$ cal/mol which can remove three times the quantity of heat removed by water ice (Sasi *et al.*, 2000). However, sublimation of dry ice happened quickly and the cold CO₂ gas leaked out of the boxes resulting in the rapid increase in temperature.

The principle of chilling with ice packs was similar to flake ice, it maintained temperature of the sample lower than 1°C for about 5 days because of its slow melting. Freezing overnight (FZ) reduced temperature in the boxes to -1°C, but the cooling rate was low. Since the boxes contained ice packs, the temperature of FZ was maintained lower than 1°C for 5 days as in other boxes stored at 3°C with ice packs.

3.2. Change in water content

The water content of fillets in all samples varied between sampling days but in overall it increased slightly with storage time. The water content of fillets in superchilled samples varied more than in chilled samples during the storage period (Figure 3). The reason may have been condensation of water in fillet surfaces at superchilling temperature (-1°C) as found in studies on superchilling of cod fillets (*Gadus morhua*) and winter flounder fillets (*Pseudopleuronectes americanus*) by CO₂ snow (LeBlanc & LeBlanc, 1992).

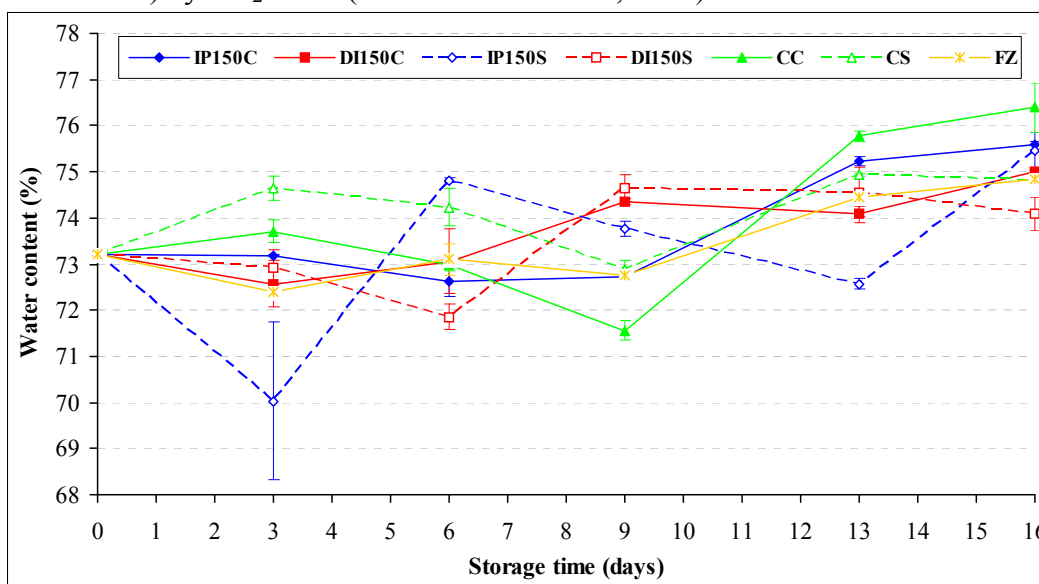


Figure 3. The water content of pooled samples (n=3) from fillets which were packed with 150 g of dry ice (DI), 150 g of ice packs (IP) or without cooling agent as control (C) and then stored chilled (C = +3°C) or superchilled (S = -2°C). Additionally, one group was stored over night at -24°C with ice packs (FZ) and than at +3°C (C).

3.3. Drip loss

It was expected to find higher drip in superchilled fillets as a result of ice formation during superchilling but drip loss was not affected by type of cooling agent or storage temperature. However, the variation of drip in each group was high and made interpretation of the data more difficult. The timing of weighing after storage was regarded as critical. Weighing immediately after removing boxes from the cooling chambers would have resulted in higher weight of the

partly frozen fillets than after temperature had increased above 0°C. To reduce that risk, the fillets were weighted after 1 hour at 20°C

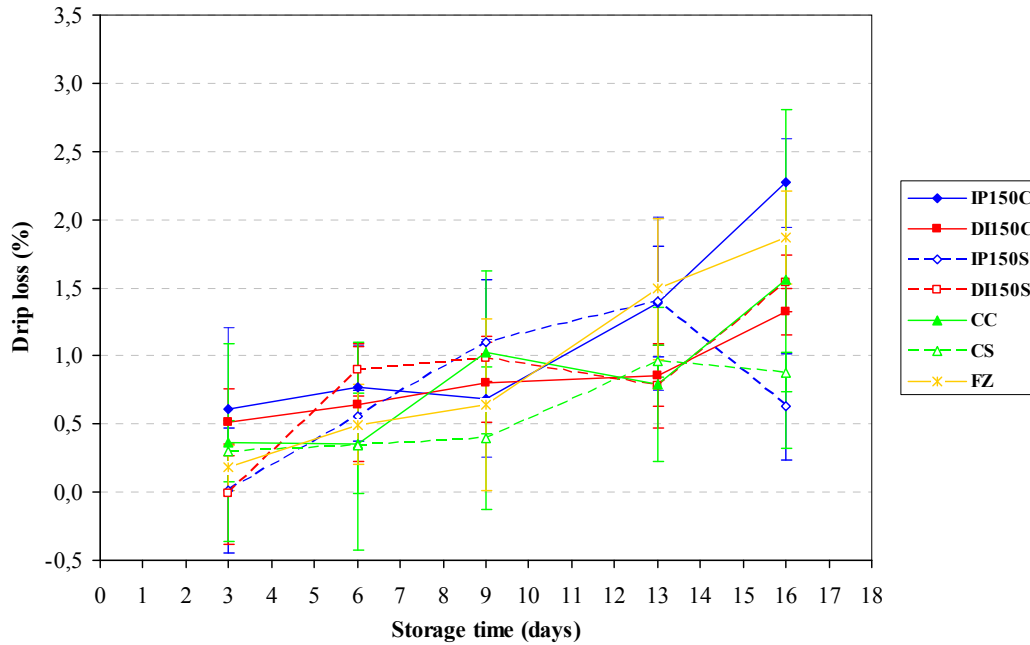


Figure 4. Drip loss of fillets (n = 3) packed with 150 g of dry ice (DI), 150 g of ice packs (IP) or without cooling agent as control (C) and then stored chilled (C = +3°C) or superchilled (S = -2°C). Additionally, one group was stored over night at -24°C with ice packs (FZ) and than at +3°C (C).

Drip tended to increase with storage time (Figure 4) which could be explained by breakdown of the muscle by bacteria and enzymes (Huss, 1995). This was supported by the R-coefficient calculated between drip and TVC (0.75) and TVB-N (0.68) showed that these variables were positively correlated (Table 2).

Table 2. Correlation between measured responses from day 3 to day 16

	TCY	CY	Drip	TVC (log)	H ₂ S (log)	pH	WHC	Water r	TBA	TVB-N
TCY	1,00									
CY	0,95	1,00								
Drip	-0,74	-0,50	1,00							
TVC (log)	-0,51	-0,34	0,75	1,00						
H ₂ S (log)	-0,46	-0,30	0,65	0,91	1,00					
pH	0,07	0,07	-0,21	-0,29	-0,26	1,00				
WHC	-0,46	-0,32	0,56	0,39	0,44	0,28	1,00			
Water	-0,41	-0,30	0,50	0,55	0,52	-0,01	0,46	1,00		
TBA	-0,27	-0,13	0,41	0,73	0,71	0,32	0,59	0,67	1,00	
TVB-N	-0,63	-0,51	0,68	0,72	0,87	-0,02	0,65	0,66	0,57	1,00

3.4. Water Holding Capacity

Factorial analysis showed that water holding capacity (WHC) increased with storage time. It tended to be higher in superchilled than in chilled products and when dry ice was used. However, fluctuations in values between groups and sampling points made a visual comparison on the x-y scatter plot difficult (Figure 5).

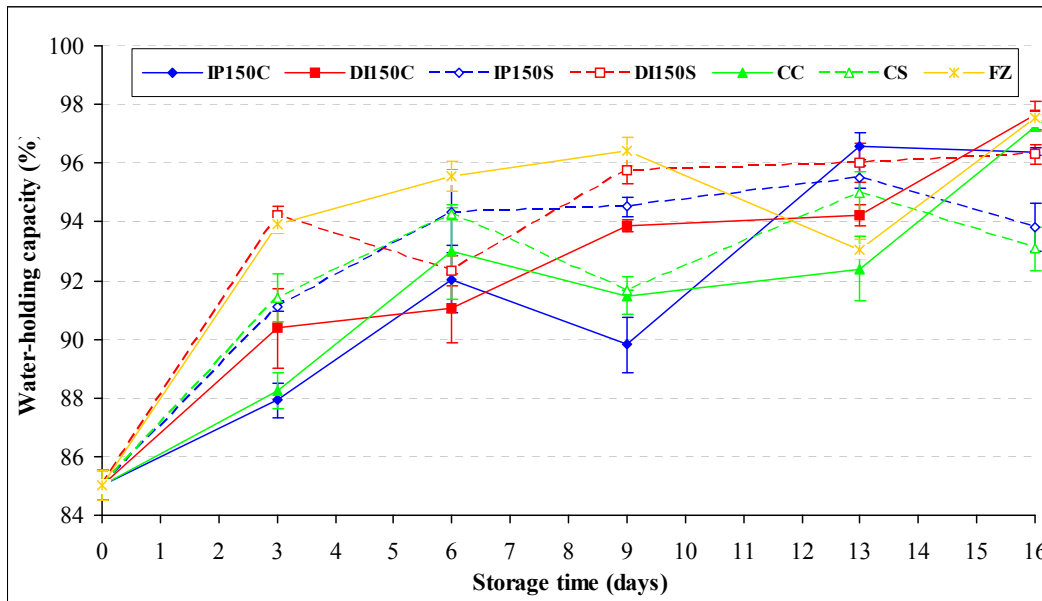


Figure 5. Water-holding capacity of fillets (n = 3) packed with 150 g of dry ice (DI), 150 g of ice packs (IP) or without cooling agent as control (C) and then stored chilled (C = +3°C) or superchilled (S = -2°C). Additionally, one group was stored over night at -24°C with ice packs (FZ) and than at +3°C (C).

Drip and WHC were positively correlated ($R = 0.56$) and increases in WHC may partly have been because higher ratio of the loosely bound water was released as drip with time. Increases in WHC with storage time may also have been due to proteolytic activity in the muscle during storage but that could not be confirmed in this study. Kristensen and Purslow (2001) stated that increasing WHC of pork muscle during storage could be explained by proteolysis of cytoskeletal proteins which affected connections and structure of the muscle. Olsson *et al.* (2003) who studied changes in WHC in halibut muscle suggested that proteolytic activity originating from bacteria may have resulted in increased WHC during storage. It has been stated that the rate of several enzymatic reactions in muscle foods is greater in the critical temperature zone -1°C to -6°C than above freezing point (Sikorski & Kolakowski, 2000) and it is possible that higher WHC in superchilled samples was due to that.

It was expected to find lower WHC in superchilled samples because of partial freezing but the opposite was found as discussed above. The explanation might have been that the freezing point of the charr was lower, about -1°C to -2.2°C (Rahman, 1995), than the storage temperature (-1°C). That decreased the risk of freeze damage of muscle cells.

3.5. Total cooking yield and cooking yield

Storage temperature did not have significant effects ($p > 0.05$) on cooking yield. However, control fillets and fillets packed with dry ice tended to have higher cooking yield after superchilling compared to chilling but temperature had less effects when ice packs were used (Figure 6). The reason may have been that the ice packs maintained low temperature for longer time than dry ice and therefore the storage temperature was not as critical.

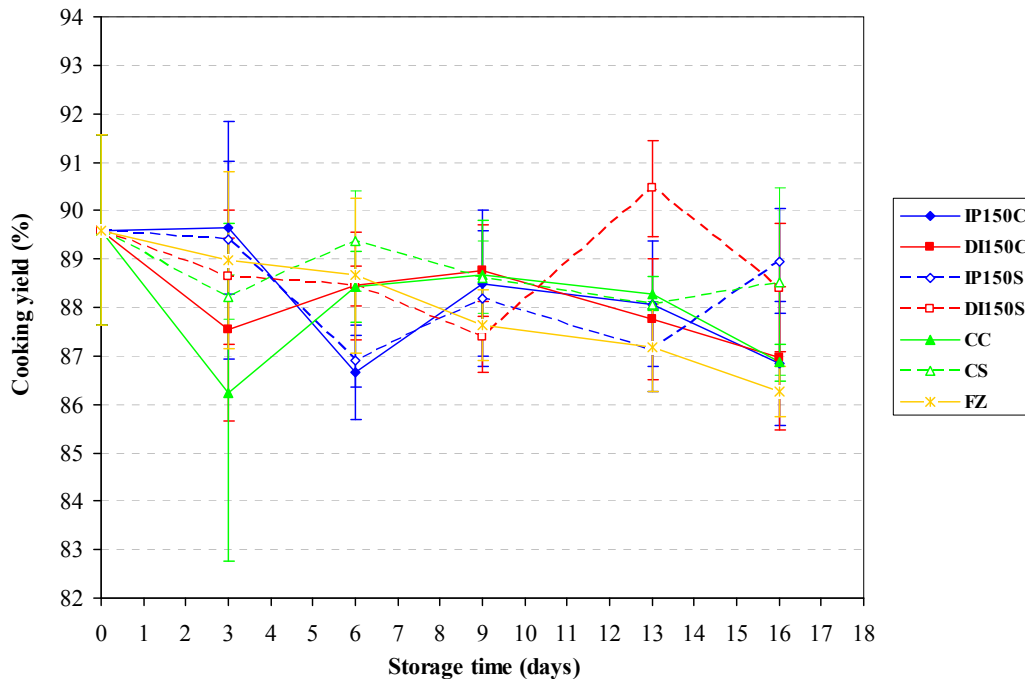


Figure 6. Cooking yield of fillets of fillets ($n = 3$) packed with 150 g of dry ice (DI), 150 g of ice packs (IP) or without cooling agent as control (C) and then stored chilled ($C = +3^{\circ}\text{C}$) or superchilled ($S = -2^{\circ}\text{C}$). Additionally, one group was stored over night at -24°C with ice packs (FZ) and than at $+3^{\circ}\text{C}$ (C).

Storage time had stronger effects than temperature ($p=0.08$ vs $p=0.18$) on total cooking yield, that is where the final weight after cooking was compared to initial weight before storage (Figure 7). It decreased more rapidly with storage time than cooking yield and had a higher negative correlation to TVC and TVB-N than cooking yield (Table 2). This indicated that increased degradation of the muscle with storage time had negative effects on water holding properties of the muscle during heating. The standard deviation of samples was higher for both

factors which might partly be explained by variation in size of the fillets which affected the heat and mass transfer during cooking.

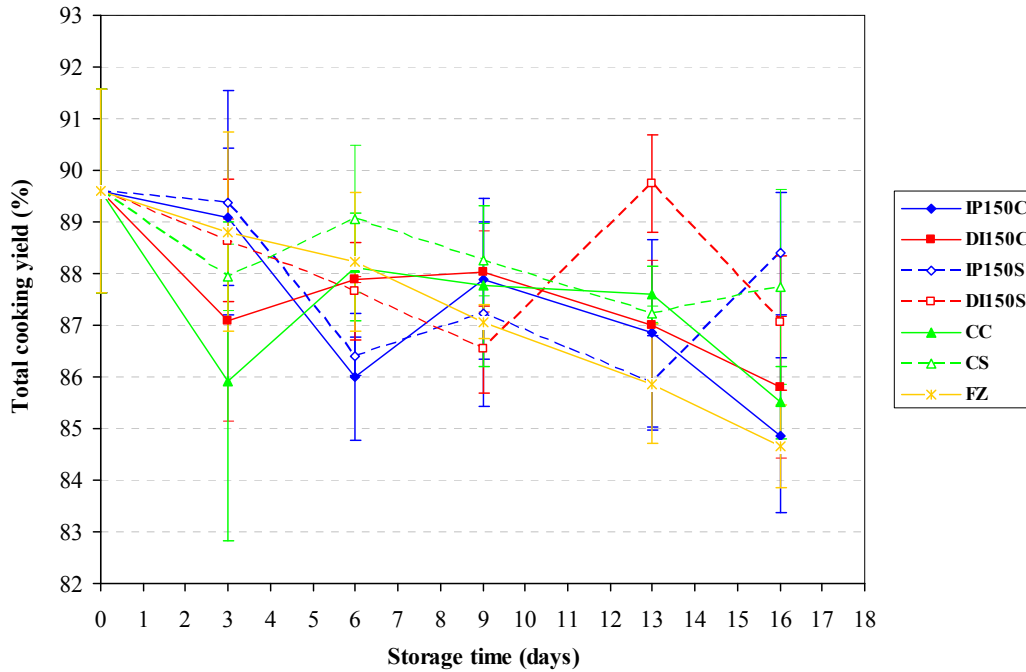


Figure 7. Total cooking yield of fillets ($n = 3$) packed with 150 g of dry ice (DI), 150 g of ice packs (IP) or without cooling agent as control (C) and then stored chilled ($C = +3^{\circ}\text{C}$) or superchilled ($S = -2^{\circ}\text{C}$). Additionally, one group was stored over night at -24°C with ice packs (FZ) and then at $+3^{\circ}\text{C}$ (C).

3.6. Microbial analysis

Total viable count (TVC) of fillets on day 0 was rather high (3.6×10^6) compared to the values obtained after storage, which could not be explained. The effects of storage temperature were strong in control groups where a faster microbial growth was observed in chilled than in superchilled samples. In other groups, the effects seemed to be significant on day 9, 13 and 16 (Figure 8). Comparison of the effects of ice packs and dry ice, showed that packaging with dry ice resulted in slower microbial growth rate than ice packs, which could be explained by higher cooling rate and effects of CO_2 released from sublimation of dry ice during storage.

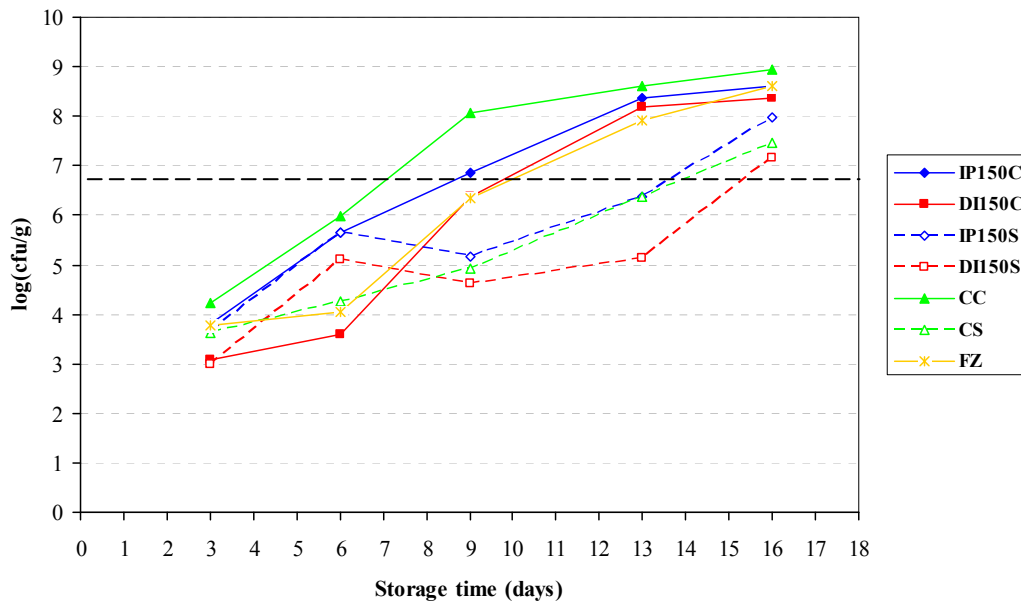


Figure 8. Changes in TVC of pooled samples (n=3) from fillets which were packed with 150 g of dry ice (DI), 150 g of ice packs (IP) or without cooling agent as control (C) and then stored chilled (C = +3°C) or superchilled (S = -2°C). Additionally, one group was stored overnight at -24°C with ice packs (FZ) and than at +3°C (C).

Using 10^7 cfu/g as a limit for spoiled fish (Gram & Dalgaard, 2002) showed that CC had reached that level after 7.5 days of storage, IP150C after 9.5 days and FZ and DI150C after 10.5 days. Superchilling resulted in longer shelf life than chilling. The TVC of IP150S and CS reached the level of 10^7 cfu/g after 14.5 days and 15 days respectively. According to these results, a combination of packaging with dry ice and storage in superchilled condition (-2 °C) are suggest to be the most promising methods for extension of shelf life. Use of dry ice will result in more rapid cooling rate than obtained with ice packs but ice packs can maintain lower temperature for longer time.

The number of H₂S producing bacteria in superchilled samples increased at lower rate than in chilled samples with the same cooling agent. Packaging with dry ice delayed growth of H₂S producing bacteria compared to using ice packs. These effects where seen over the whole storage period when fillets were stored superchilled but only during the first 6 days when fillets were stored chilled. The highest growth rate was observed in the control sample (Table 3). The growth rate of H₂S producing bacteria in fillets stored frozen overnight, was similar to

superchilled samples (IP150S, DI150S) during first 6 days of storage but increased more rapidly after that.

Table 3. Changes in H₂S producing bacteria (cfu/g) of pooled samples (n=3) from fillets which were packed with 150 g of dry ice (DI), 150 g of ice packs (IP) or without cooling agent as control (C) and then stored chilled (C = +3°C) or superchilled (S = -2°C). Additionally, one group was stored over night at -24°C with ice packs (FZ) and than at +3°C (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

Superchilled samples did not exceed the limit H₂S producing bacteria during the 16 days storage period but the reference value was 10⁶ cfu/g (Gram & Dalgaard, 2002). Chilled samples exceeded that limit on day 13 but FZ not until after day 16. At that point significant formation of volatile sulphur-containing compounds could be expected leading to sensorially evident spoilage (Gram & Huss, 1996).

3.7. Change in pH

The pH of all samples increased slightly during the first 3 days of storage (Figure 9) but no special trend could be linked to either storage temperature or cooling agent. This supported the results obtained in a study on salmon fillets where the effects of MAP and superchilling were investigated (Sivertsvik *et al.*, 2003). However, absorption of CO₂ in fish muscle may result in decreased pH (Sivertsvik *et al.*, 2002) although not observed in this study.

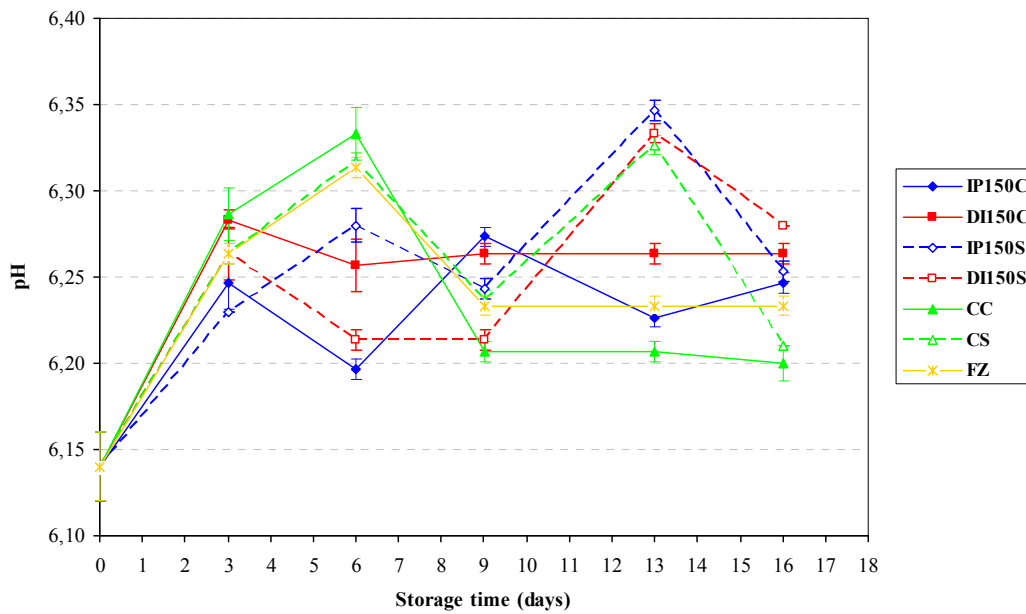


Figure 9. The pH of pooled samples (n=3) from fillets which were packed with 150 g of dry ice (DI), 150 g of ice packs (IP) or without cooling agent as control (C) and then stored chilled (C = +3°C) or superchilled (S = -2°C). Additionally, one group was stored over night at -24°C with ice packs (FZ) and than at +3°C (C).

3.8. Total volatile base nitrogen (TVB-N) content

Total volatile base nitrogen (TVB-N) content of fillets in all samples increased during first 6 days of storage. From day 6 to day 13 of storage time, the TVB-N content of chilled samples (IP150C, DI150C) increased continuously meanwhile the TVB-N content of superchilled samples (IP150S, DI150S) and FZ did not increase. During the last 3 days of the storage period, the TVB-N content of all samples increased, but the TVB-content was about 8 points higher in chilled samples than superchilled at the end of the storage (Figure 10).

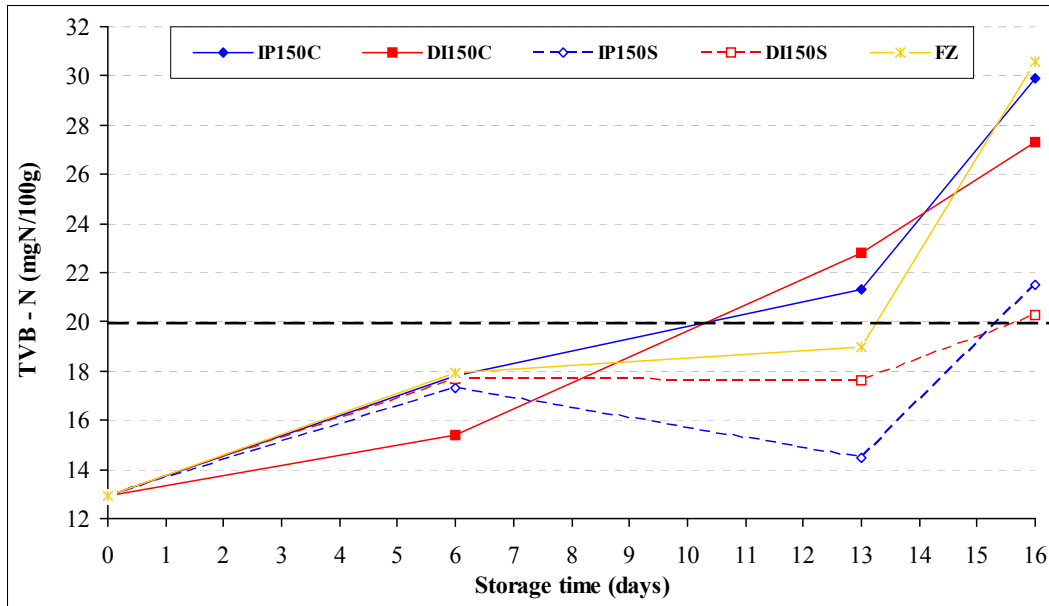


Figure 10. Changes in TVB-N of pooled samples (n=3) from fillets which were packed with 150 g of dry ice (DI), 150 g of ice packs (IP) or without cooling agent as control (C) and then stored chilled (C = +3°C) or superchilled (S = -2°C). Additionally, one group was stored over night at -24°C with ice packs (FZ) and than at +3°C (C).

The limiting level for rejection of TVB-N content is 20 mgN/100g flesh fish (Connell, 1995). TVB-N content of flesh fish in IP150C and DI150C reached level of 20 mgN/100g after 10 days of storage, in FZ reached level of 20 mgN/100g after 13 days of storage, in IP150S reached level of 20 mgN/100g after 15 days of storage and DI150S reached level of 20 mgN/100g on day 16 of storage (Figure 10). The result showed that packaging with dry ice had stronger effects when fillets were stored chilled (3°C) than superchilled (-2°C). Freezing overnight was more effective than chilling, because it resulted in superchilling (<0°C) first 3.5 days of storage.

A positive correlation (Table 2) was observed between TVB-N and TVC (R=0.72) and TVB-N and H₂S producing bacteria (R=0.87) as expected since TVB-N is mainly produced by bacterial decomposition of the muscle. The TVB-N was regarded as a significant quality indicator but the use of it to evaluate fish freshness has been questioned in some studies. For example for trout where values over 18 days period ranges from 18 to 26 mg/100 g (Chytiri *et al.*, 2004). However, the starting value was rather high, about 22 mg/100g. The effectiveness

of TVB-N for evaluation of freshness may possible vary with species, condition of the raw material, storage methods and time.

3.9. Trimethylamine (TMA) content

TMA content of fish flesh in all samples did not increased during the first 6 days of storage (Table 4). The storage temperature had stronger effects on TMA changes than cooling agent. After 6 days of storage the TMA started to increase in chilled samples and increased rapidly during the last 3 days of storage to values of 4.7 to 5.8 mg N/100g. On the contrary, TMA content of superchilled samples, IP150S reached a value of 1.5 mg N/100g after 16 days of storage but TMA content of DI150S was still less than 0.3 mg N/100g flesh on last day.

Table 4. Changes in TMA content (mgN/100g) of pooled samples (n=3) from fillets which were packed with 150 g of dry ice (DI), 150 g of ice packs (IP) and then stored chilled (C = +3°C) or superchilled (S = -2°C). Additionally, one group was stored over night at -24°C with ice packs (FZ) and than at +3°C (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

These values are considered low compared to the limit for TMA which is around 10 mg N/100g. This value has been suggested by Teskeredzic and Pfeifer (1987) as upper acceptable limit of TMA for human consumption in cultured brackish water rainbow trout. However, they were similar with TMA level of 1.8-2.0mg N/100g flesh in aquacultured fresh water rainbow trout reported by Chytiri *et al.* (2004). The explanation may be that trimethylamine oxide (TMAO) content of fresh water fish is lower than TMAO content of brackish water fish and marine fish (30-130 mmol TMAO/kg muscle) (Takeuchi *et al.*, 2003; Seibel, 2002).

3.10. Change in thiobarbituric acid (TBAR) content

During storage, lipid oxidation of fillets was found significant. TBAR content of fish flesh in all samples increased with storage time from initial value of 1.9 µmol/kg. After day 6 of storage the TBAR content was in the range of 2.9 -3.4 µmol/kg. After that it increased rapidly to 6-9 µmol/kg, but the lowest TBAR content was observed in superchilled fillets packed with dry ice both on day 13 (6 µmol/kg) and 16 (7 µmol/kg) (Figure 11).

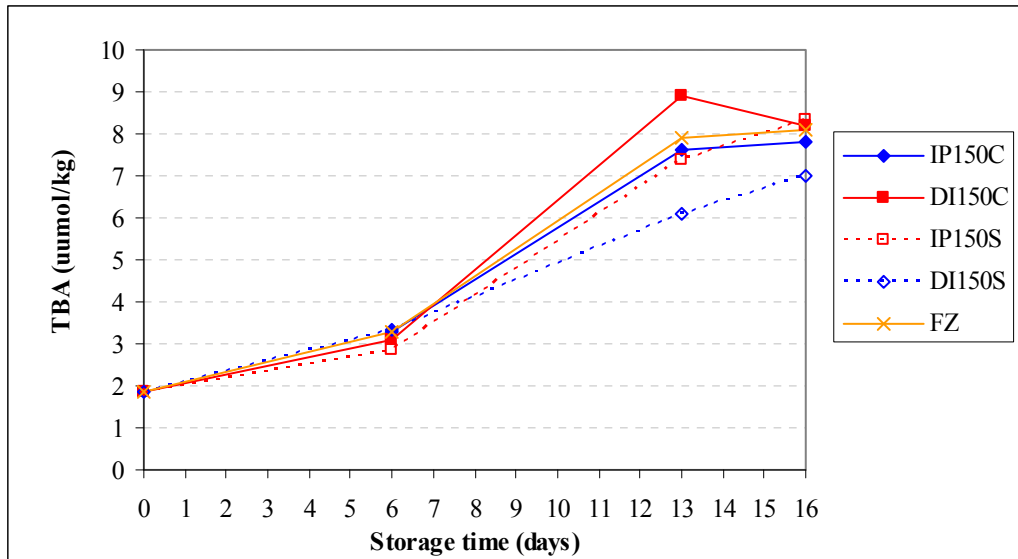


Figure 11. Changes in TBA of pooled samples (n=3) from fillets which were packed with 150 g of dry ice (DI), 150 g of ice packs (IP) or without cooling agent as control (C) and then stored chilled (C = +3°C) or superchilled (S = -2°C). Additionally, one group was stored over night at -24°C with ice packs (FZ) and than at +3°C (C).

Values observed in our experiment remained well below the limit for TBAR in cod 19 µmol/kg (Connell, 1995) but end values were higher than 6 µmol/kg which has been proposed as limit for "excellent" mackerel (Ke *et al.*, 1976). Limits for charr were not found in other references but as discussed in results for sensory analysis, samples were not rejected because of rancid odor and flavor. Multivariate analysis showed that values TBAR content and these sensory descriptors were correlated (Figure 12) but the correlation coefficient (R) was about 0.5. It is suggested that the TBAR content had not reached critical levels with regard to shelf life.

Studies of aquacultured trout fillets stored in ice, showed that TBAR increased from initial value of 10.4 to 19.4 µmol/kg after 18 days of storage (Chytiri *et al.*, 2004). The value obtained for the raw material was rather high compare to charr which is rather high compared to what was obtained for charr in our trial. Andersen *et al.* (1990) who studies quality changes of vacuum packed wild salmon during storage for 6 months at -17°C found that TBAR increased during storage from 2.8 µmol/kg malonaldehyde/kg to 12.5 µmol/kg for light-protected packages, and to 17.6 µmol/kg for packages exposed to fluorescent light.

TBAR content is used widely as an indicator of degree of lipid oxidation, i.e. to measure secondary products during lipid oxidation. However, scientist do not fully agree on usefulness of TBAR to evaluate quality which may vary with fish species, fat content and probably storage conditions and others methods used for purpose of multivariate analysis. The secondary products formed are not end-products of lipid oxidation and may react further with other components of the fish (Auburg, 1993). It must also be kept in mind that many variations for performing the TBAR test have been developed, which makes comparison TBAR values between different studies complicated. Correction for water content were carried out in the modified version used in our trial.

3.11. Sensory changes

The type of cooling agent or storage temperature during 13 days (of 16 in total), did no affect changes in sensory attributes significantly ($p>0.05$). This supports previous results where sensory quality has been similar between fish which was packed with dry ice and water ice (Jeyasekaran *et al.*, 2004; Sasi *et al.*, 2003; Sasi *et al.*, 2000; LeBlanc & LeBlanc, 1992). However, principal component analysis showed that fillets packed with ice packes tended to be softer, juicer and more sour during the first days of storage which might indicate that the type of cooling agent affected the spoilage pattern in different ways. As dicussed before. rancid odor and rancid flavor detected in the cooked fillets of all samples did not reach limits for edible shelf life during the storage period and were correlated with TBAR content in the fillets. Sour flavour and TVC values were positively correlated which indicated increases in sour flavour and odor were due to microbial spoilage of the fillets. Storage temperature seemed to be more effective during the last days of storage where chilled samples had a higher spoilage rate then superchilled samples (Figure 12).

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