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Improved Quality of Herring for Human Consumption

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Ágrip á íslensku:

Síld er ein af mikilvægustu fisktegundum í Norður Atlandshafi og í Eystrasalti. Þrátt fyrir að stór hluti af aflanum fari til manneldis, þá fara um 85% af síld í vinnslu á lýsi og mjöli. Almennur vilji er fyrir því að auka neyslu síldar til manneldis.
Þess var mikilvægt að rannsaka mismunandi þætti sem hafa áhrif á gæði síldar og sérstaklega hvernig þeim er stjórnað af líffræðilegum aðstæðum. Aðlástæðan fyrir gæðavandamálum í síld er hátt innihald efnasambanda sem stuðla að þránun, og hafa áhrif á lit- og áferðarbreytingar, ásamt tapi næringarefna. Betri gæði leiða af sér aukna samkeppni á framleiðslu síldar á Norðurlöndum, ásamt jákvæðu viðhorfi neytenda gagnvart síldarafurðum.

Meginmarkmið verkefnisins var að bæta gæði og magn síldar , til neyslu, með því að rannsaka gæði hráefnisins eftir veiðar. Lögð var áhersla á gæði strax eftir veiðar og gæði hráefnisins eftir mislangan tíma í frosti.
Þættir eins og veiðistaður og veiðitími höfðu ekki áhrif á gæði síldar. Hins vegar hafði geymsla í frosti við -20°C teljandi áhrif á gæði hráefnisins.

Lykilorð á íslensku: *Síld, gæði, þránun, skynmat, myndbygging, áferð.*

Summary in English:

Herring is one of the most important fish species in the North Atlantic and Baltic Sea, with an annual catch exceeding 2 million tonnes. Although a large part of these fish is used for human consumption, as much as 85% of the herring is used for industrial production of fish meal and fish oil. There is a general wish to increase the utilisation of herring for human consumption.

Thus, it was important to study the various parameters which influence the quality of herring, and in particular how these paramenters are controlled by biological factors. A major reason behind quality problems arising during post harvest handling of herring is its high content of compounds that efficiently catalyzes the development of rancidity, pigmentation, texture changes and loss of nutritional value. Improved quality will result in increased competitiveness of the Nordic fish processing industry and would improve the attitude among the consumers towards herring products.

The general objective of the project was to improve the quality and quantity of herring to be used for food production by investigating how natural variation in raw material characteristics affects post harvest quality. Attention was given to the quality immediately after landing and the quality after period of frozen storage.

The results indicated no clear differences in the quality of herring regarding cartching place or season. The frozen storage for a prolonged time had the major influence on the quality of herring fillets.

English keywords: *Herring, quality, lipid oxidation, sensory quality, microstructure, texture.*

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Title: Improved quality of herring for humans

Nordic Innovation Centre (NICe) project number: 02106

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Abstract:

Herring is one of the most important fish species in the North Atlantic and Baltic Sea, with an annual catch exceeding 2 million tonnes. Although a large part of these fish is used for human consumption, as much as 85% of the herring is used for industrial production of fish meal and fish oil. There is a general wish to increase the utilisation of herring for human consumption.

Thus, it was important to study the various parameters which influence the quality of herring, and in particular how these paramenters are controlled by biological factors. A major reason behind quality problems arising during post harvest handling of herring is its high content of compounds that efficiently catalyzes the development of rancidity, pigmentation, texture changes and loss of nutritional value. Improved quality will result in increased competitiveness of the Nordic fish processing industry and would improve the attitude among the consumers towards herring products.

The general objective of the project was to improve the quality and quantity of herring to be used for food production by investigating how natural variation in raw material characteristics affects post harvest quality. Attention was given to the quality immediately after landing and the quality after period of frozen storage.

The results indicated no clear differences in the quality of herring regarding catching place or season. Frozen storage after 12 months at -20°C had a large influence on the lipid oxidation and the sensory quality really started to change, resulting in poor sensory quality of the herring; it became rancid in odour and flavour. Only very small changes occurred in herring frozen at -80°C, even after 18 months of frozen storage. The changes in microstructure resulted in considerable shrinkage of myofibrils and increase in the space between myofibrils for long storage at -20°C.

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Executive summary

The purpose of the project was:

To improve the quality and quantity of herring to be used for food production by investigating how the natural biological variation in raw material characteristics affects the nutritional, structural, chemical and and sensory quality of herring directly after post harvest and frozen storage.

To build mathematical models that can help to predict certain quality characteristics out from biological data and thus, optimize the quality and quantity of herring products and herring products for human consumtion.

The study has achieved this aim by:

Creating a network group for exchange of results between different herring oriented research projects, as well as creating an environment where new ideas can grow.

Working together with other herring oriented research projects within the Nordic countries to improve the knowledge on herring quality.

Analyzing the composition of herring fillets from batches differing in seasonal and geographical characteristics.

Following chemical changes (e.g. lipid oxidation, protein denaturation), sensory quality, structural changes and nutritional value of these batches during post harvest frozen storage.

Statistically treating the data to relate biological and compositional features to sensory, chemical, structural and nutritional changes during storage.

Method

The project was executed in three phases divided into 8 work packages.

Initial phase: A network was created with participants from the industry and from Nordic research institutions. The phase consisted of: Work package 1 (WP 1): Network group; and WP 2: Synergy effects.

Descriptive phase: Biological differences in critical quality parameters and basic mechanisms behind their changes during frozen storage was studied. The workplan was based on input from the other projects. The phase consisted of: WP 3: Sampling and sample verification, WP 4: Composition and nutritional value, WP 5: Texture, WP 6: Lipid oxidation and WP 7: Sensory attributes.

Analytical phase: All data were statistically analysed to establish a relationship between the measured quality parameters and the biological characteristics of the herring. Mathematical models were established to estimate sensorial, structural and nutritional properties of the herring out from selected physical and chemical characteristics.

A sampling procedure for catch, filleting and freezing of herring was established. Sampled herring was machine filleted to butterfly fillets and frozen at -20°C. Samples were stored for 6, 12, and 18 months. Butterfly fillets frozen at -80°C and vacuum packed were used as "fresh herring" samples, because it was not possible to guarantee uniform quality of fresh fillets packed in ice and send it between institutes. The sampling procedure was standardised to the extent for what was practically possible for the participating institutes to carry out. A sampling plan for Norwegian spring spawning herring, Icelandic summer spawning herring and Baltic herring was established. Real samplings were made by IMR, Matis (former IceTec), Sildarvinnslan, Chalmers and Paul Mattsson AB (from the cluster Väst 5 AB) during 2004 where the criteria given by the sampling procedure were followed.

Analysis of all the herring samples was done according to a plan. Samples were distributed between all laboratories. Detailed description of all the analytical methods is presented in the main section of the report.

Concrete results

A homepage (nordicherring.is) was created. All information related to the project are available on this page.

A network for exchange of results and samples between groups working within herring oriented research projects was created in Ålborg in October 2003. As part of WP 1 "Network group" a Nordic meeting on herring "Herring-from sea to product" was held in Hirtshals, Denmark on 14th of April, 2005. More than 50 participants from the Nordic herring industry and Nordic research institutions attended the meeting. The overall aim was to stimulate the exchange of new ideas for future utilization of the herring resource and quality and product innovation on a research oriented level and thereby contributing to the establishment of a network group within Nordic herring research. A total of 10 presentations were given at the meeting, which was arranged together with a Danish research project "Herring - a living resource – a good product" and a EU herring project "Conservation of diversity in an exploited species: spatio-temporal variation in the genetics of herring (*Clupea harengus*) in the North Sea and adjacent areas".

The main results from the sensory analysis of the collected herring samples showed that no clear differences were found in the sensory quality of herring regarding catching place or season. As expected, long frozen storage at -20°C resulted in poor sensory quality of the herring. The herring became rancid both in odour and flavour, but also discoloured and had a high intensity of amine odour and wet dog/sour odour and flavour. Only very small changes occurred in herring frozen at -80°C even after 18 months of frozen storage. A principal component analysis (PCA) of the sensory data defined two generalised sensory variables (the score vectors). The first component was dominated by attributes related to rancidity whereas the second had the largest contribution from colour and texture attributes. These two generalised sensory variables were used when studying the effects on lipid oxidation and microstructure.

No clear differences were found in lipid oxidation of herring regarding catching place or season. Freezer storage time at -20°C had by far the largest influence on the lipid oxidation results. Longer time resulted in increased peroxide value (PV) and thiobarbituric reactive substances (TBARS), as well as loss of α-tocopherol and redness (a*). Chemical oxidation data agreed well with sensory documentation of rancidity, and

also with the protein degradation (e.g. protein solubility). The latter indicates possible lipid-protein interactions during storage. The compositional impact on the oxidation data was evaluated by performing PCA only on samples stored for 6 months. No impact was then seen from lipid content and muscle pH, while salt content, % dark muscle and degree of fillet silvering showed positive correlations to lipid oxidation.

As expected, long freezer storage at -20°C resulted in considerably shrinkage of myofibrils and an increase in the space between myofibrils. These changes in microstructure could neither be related to catching place nor to season. When removing the time effect by looking at samples stored for 6 months only, the fraction of inter-myofibrillar space was related to the amount of expressible water and inversely related to muscle pH and texture.

In conclusion, this project has shown that the geographically diverse stocks of herring that are used by the Nordic herring industry are less diverse than expected when it comes to basic composition, pre-storage quality and post storage quality. Of much higher importance for the various quality parameters was prolonged frozen storage at -20°C. Thus, for any application where a frozen herring material is preferred, the pre-processing storage should be minimized.

As fishing from many of the European fish stocks can not be increased, improved utilization of the available sources are, and will be important. The herring stock has a great potential for a better utilization. By improving the quality, an increased proportion of herring can be marketed at the highest prices; this will improve economic benefits of both the producers in the industry and the fishermen. However, it will in particular benefit the consumer who gets a better and more nutrious food for a low price compared to other animal products. The knowledge created from this project is important for marketing of frozen herring fillets and for the producers of herring products. Also the project has largely increased the theoretical knowledge concerning long-term freezer storage of herring fillets; especially the relations between the structural, nutritional and sensorial quality of herring.

Recommendations for further work

Journal of Agriculture and Food Chemistry has permitted the participants of the project to send 3 scientific papers to them for publication right after each other in the same issue.

A grant proposal for a new 1 year NIC-project was sent to NIC last August together with SIK, Sweden. The new project aims at testing the microwave dielectric spectroscopy technique on collected herring material to evaluate its potential as a quick quality/traceability tool for the industry.

Work-shop is planned to be held in Göteborg, in April 2008 with the industry and other people involved in utilization of herring for food.

Handbook/booklet about composition and characteristics of herring from different stocks.

Disseminations

Project meetings

There were 8 meetings during the project period.

- In Lyngby, Denmark Sept., 11, 2003
- In Lyngby, Denmark Nov., 18, 2003
- In Lyngby, Denmark Feb., 26-27, 2004
- In Käringön/Ellös, Sweden Oct., 25-26, 2004
- In Hirtshals, Denmark March 15, 2005
- In Bergen, Norway Sept., 19-20, 2006
- In Lyngby, Denmark March, 19, 2007
- In Helsingborg, Sweden, July, 2, 2007

Presentations of the Improved quality of herring for humans project.

- Hannes Hafsteinsson (Matis) and others. Presentation of the project at a meeting with Nordic network for exchange of results and ideas in Ålborg, in Denmark in October 2003.
- Hannes Hafsteinsson (Matis). Presentation at NIC workshop about the project and its results, Iceland September 2004.
- Hannes Hafsteinsson (Matis). Presentation of the project and its results at a Nordic Herring meeting in Hirtsals, Denmark in April 2005.
- Hannes Hafsteinsson (Matis). Presentation at internal project meeting in Iceland in May, 2006.
- Hannes Hafsteinsson (Matis). Presentation at NIC workshop about the project and its results in Norway November, 2006.

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1. INTRODUCTION

Herring is one of the most important fish species in the North Atlantic and Baltic Sea, with an annual catch exceeding 2 million tonnes. However, due to failure to comply to the quality requirements necessary for human consumption and/or due to withdrawal of herring from the market because of low prices, as much as 85% goes directly to industrial production of fish oil and fish meal to be incorporated into animal feed. In a period when the stocks of cod and other white fleshed food fishes are drastically declining, this handling of a valuable marine resource is highly questioned. One sector that has expressed a particularly strong interest in better utilisation of herring for food production is the functional food sector. This is because of the high levels of valuable omega-3 fatty acids and antioxidants in the herring tissue (Hølmer 1993; Hamre *et al*., 1997 and Ackman 1980). However, to fully advantage of these nutritional qualities, it is essential to improve the image of herring as a fish for human consumption through development of better quality control system and new process technologies.

A major reason behind quality problems arising during post harvest handling of herring is its high content of compounds that efficiently catalyzes the development of rancidity (Undeland *et al.,* 1988a, 1988b; Undeland *et al.,* 1999; Undeland and Lingnert 1999), pigmentation, texture changes and loss of nutritional value (Bosund 1969; Kolakowska 1981). In addition, the possibilities of controlling these quality degradation reactions are very limited since the chemical composition of herring varies largely with season, catching ground, sexual maturation and feeding pattern (Nielsen *et al*., 2005).

The omega-3 fatty acids in herring are beneficial for human health. On the other hand, polyunsaturated fatty acids are susceptible to oxidation and therefore represent a risk of developing rancid off-flavours (Hølmer 1993; Hultin 1995; Undeland *et al*,. 1998a, 1999). The compositional variations also limit the possibilities of predicting the initial sensory and nutritional quality of the herring which results in a sub-optimized use of herring for various food applications. To date, tools for predicting quality of herring and other wild fish has been limited to certain control of the post harvest systems in order to minimize the quality deterioration during processing, transport and marketing. However, the success of applying e.g. antioxidative strategies has been limited since it is not possible to obtain a pre-set quality on the catch at any time of the year. If extending the

current knowledge on herring quality from a post harvest to a pre harvest perspective, it would be possible to understand how the natural variation e.g. in the content of fat and micronutrients relates to variations in sensory, nutritional and technical properties of herring. Post harvest quality could then be optimized, and the natural compositional variation could be used to target the use of herring/specific herring constituents for various applications. A consistent supply of high-quality herring constituents for food production within and outside the Nordic countries would result in increased competitiveness of the Nordic fish processing industry and would improve the attitude among consumers towards herring.

The general objectives of the project were to improve the quality and quantity of herring to be used for food production by investigating how natural variation in raw material characteristics affects post harvest quality. Attention will be given to the quality immediately after landing and the quality after a period of frozen storage. The interest in utilizing a frozen herring for food production is currently increasing since there is a wish to avoid the large processing peaks in periods when the herring traditionally is considered of high technical quality. There is also a growing market outside Scandinavia for frozen herring. The synergetic effect of the project would be maximized by creating a network for exchange of information and work together with other herring oriented research project and thereby combining the forces and knowledge available in the Nordic countries will be delivered to the industry.

2. MATERIAL & METHODS

2.1 Description of work packages

The project was executed in three phases which were divided into eight different work packages:

1. ***Initial phase.***

   In this phase, a network group for people working with herring was created. The network had participants from the industry and from Nordic research institutes working within the following herring-oriented projects: "Kvalitet fra hav til fat" in Norway, "Herring – A living resource – A good product" in Denmark, "Quality of herring fillets after long time frozen storage" in Iceland. "Optimal storage technology on board purse seiners" - Nordic and "Protein Isolation from herring" - Nordic. The phase consisted of Work package 1 (WP 1): Network group. WP 2: Synergy effects.

2. ***Descriptive phase***.

   During this phase biological differences in critical quality parameters and basic mechanisms behind their changes during frozen storage was studied. Work plan was based on input from the other projects. This phase consisted of: WP 3: Sampling and sample verification; WP 4: Composition and nutritional value; WP 5: Texture; WP 6: Lipid oxidation and WP 7: Sensory attributes.

3. ***Analytical phase.***

   During this phase all data was statistically analysed to establish a relationship between the measured quality parameters and the biological characteristics of the herring. Mathematical model was established to estimate sensory properties of the herring out from selected physical and chemical characteristics. This phase consisted of WP 8: Variation of characteristics.

The following text contains title and objective of each WP.

WP 1: Network group: The objective of this work package was to create a network group for people working with herring from all the Nordic countries. The group met several times during the project period. The participants exchanged results and ideas form ongoing and planned herring projects, with the aim of being a bank of ideas for new research projects within these themes.

WP 2: Synergy: Other currently ongoing herring projects did not provide any information on how biological and chemical parameters related to quality and quality changes of herring during frozen storage. Our project therefore supplemented these projects with valuable information. They also supplemented us with valuable information. Through a collaboration with the other projects, we were able to combine chemical and biological data from frozen herring from the same batches, and thereby increase the knowledge on herring quality.

WP 3: Sampling and sample verification: The objective was to deliver herring samples of documented harvest procedure and live stage prepared and preserved according to the requirements of the work packages 3 to 7. The aim was to collect 5 batches of Norwegian Spring Spawn Herring, 5 batches of the Icelandic Summer Spawning Herring, and 6 batches of the Spring Spawning Baltic Herring.

WP 4: Composition and nutritional value: The objective of this work package was to collect data on the chemical composition, colour, texture, protein functionality, sensory properties and initial levels of e.g. oxidation products of herring fillets from different stocks and live stages.

WP 5: Texture: The goal was to establish the mechanisms behind changes in texture of herring muscle, and develop an understanding of how natural variation in the textural properties of herring could be utilised. This was achieved by studying:

a) The relationship between microstructure and textural properties of herring muscle.

b) The effect of chemical composition of herring flesh, including pH, liquid holding capacity on the textural properties of herring muscle.

c) The variation in textural properties of herring muscle in relation to stock and live stage of the herring.

WP 6: Lipid oxidation: The goal was to link biological variations in the initial content of lipids, pro-oxidants and antioxidants to the oxidative stability of herring during storage. This was achieved by:

a) Collecting data on the content of lipids, pro-oxidants and antioxidants in the different herring raw materials.

b) Following lipid oxidation by chemical, sensory and physical means during frozen storage.

c) Building mathematical models that related the raw material composition to the oxidative stability/end quality.

d) Establish quality criteria for herring to be stored at different sub-zero temperatures.

WP 7: Sensory attributes: The goal was to establish a relationship between sensory attributes of frozen herring from different origin and live stage to lipid quantity/composition, oxidation status, physical texture properties, microstructure and colour. This was achieved by:

a) Development of methods for determining sensory quality for frozen herring.

b) Relate sensory measurements e.g. to lipid quantity, distribution and composition of samples analysed in WP 4.

c) Relate sensory measurements to physical texture properties and influence of microstructure of samples analysed in WP 5.

d) Relate sensory measurements to oxidation and colour of samples analysed in WP 6.

2.2 Sampling of herring

The overall goal of the project, and for each partner, was to be able to measure differences in chemical, physical and sensory characteristics between herring from different seasons and catching area. This required a carefully prepared sampling procedure, that made the differences caused e.g. by different temperatures or post mortem times smaller than the differences caused by geography and season. An important step in this part of the project has therefore been to agree upon every single detail regarding the line of events that takes the herring from the water to the actual analysis to be performed.
A sampling procedure for catch, filleting and freezing was established in the year of 2004. Herring was caught around Iceland (ISS), outside the Norwegian coast (NSS) and in Kattegat/Skagerak (BSS) at various seasons during 2004 and 2005. All catching were done by commercial vessels. This influenced the periods of the catching, especially in Norway and Iceland, where herring was only caught during autumn and winter (Fig. 1).

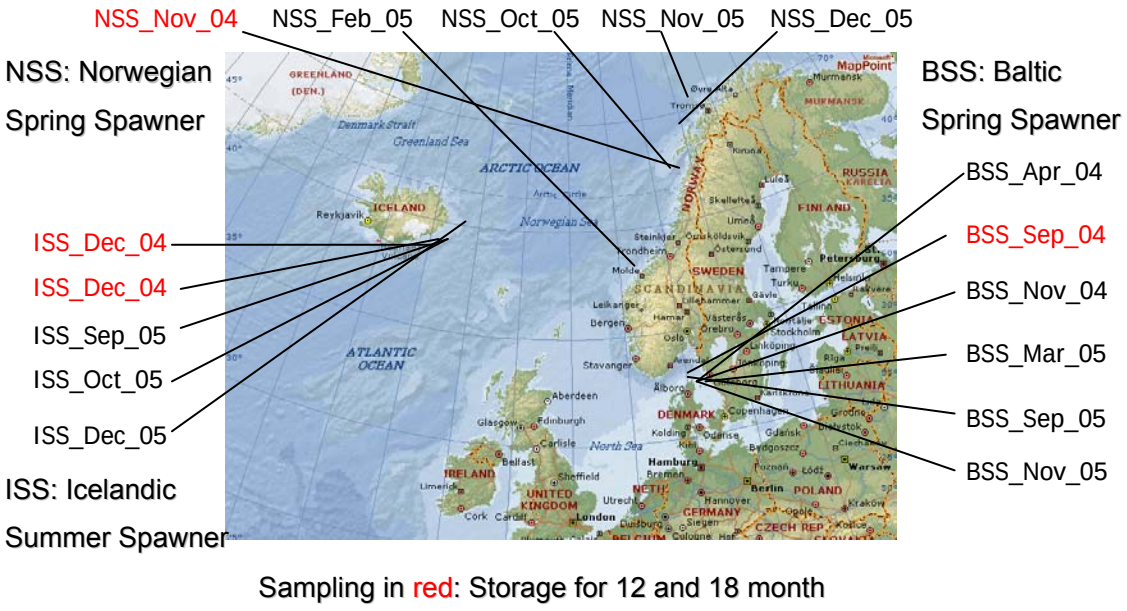


Figure 1. Map of sampling of herring regarding catching place and time.

Herring was kept for 30±3 hours in refrigerated sea water (RSW) after catch and then filleted to butterfly fillets, packed in boxes with six butterfly fillets in each, vacuum-packed, frozen and stored either at -20°C or -80°C. Samples were then stored for 6, 12,

and 18 months. Because it was not possible to guarantee uniform quality of fresh fillets packed in ice and to be sent between institutes, therefore, butterfly fillets frozen at -80°C and vacuum packed were used as control or "fresh herring" samples (Appendix I and Appendix II). The frozen herring was subsequently packed in polystyrene boxes with dry-ice and distributed by courier among the participating institutes. The sampling procedure was standardized to the extent for what was practically possible for the participating institutes to carry out. In February 2004 IMR and Chalmers tested the sampling procedure and subsequently tested the sending procedure of frozen herring between institutes. The results from this sampling were used to set up a harmonized sampling procedure that was possible to perform by all involved sampling partners. A sampling plan (Appendix III) for Norwegian spring spawning herring (NSS), Icelandic summer spawning herring (ISS) and Baltic spring spawning herring (BSS) was then established in 2004. As previous mentioned all samplings were done by commercial vessels and it was therefore not possible for all samplings to keep the 30± hours from catch to freezing. The last three NSS samplings in autumn/winter 2005 in RSW were between 60 and 70 hours. The samplings NSS Feb05 and ISS Oct05 were 17 and 20 hours in RSW, respectively.

DIFRES performed a freezing experiment with herring butterfly fillets in March 2004 (results on homepage). The aim was to investigate the temperature development in frozen butterfly fillets stored at room temperature in polystyrene box with dry ice. Results from the experiment are on the homepage. A thawing procedure was developed (section 2.3) that was based on tight packaging in plastic followed by thawing in running cold water. The almost fully thawed fillets would then be skinned, minced and re-packed.

Sampling was done by Matis (former IceTec), IMR and Chalmers.

2.3 Thawing procedure

From the herring container box, the vacuum bag was removed and the herring fillets were put in a new plastic bag (or two). As much air as possible was taken out before sealing the bag tightly with a rubber band. The fish block was placed in a "dish-wash tub" under cold running water. Weight was put on top of the fish block to keep it under the water

surface. However, the sealed end was kept above the water to avoid leakage. The tub was covered with a black plastic bag, to avoid direct light. After about 45 minutes the herring fillets were thawed. These fillets were placed on a wire mesh to let liquid drip off for 15 minutes (in a refrigerator).

The fillets should be almost soft (some water crystals are OK, but the fillet should not feel hard), before starting to manually remove the skin. If the fillets are too frozen during skinning, more silver and subcutaneous fat will stay on the fillet. The skin might also become torn into pieces. If the fillet is properly thawed, the skinning procedure will be much more reproducible.

Analysis of all the herring samples was done according to a plan that is presented in Appendix IV to the extent that was possible. If analysis was delayed, samples that had been stored at -20°C were vacuum packed and stored at -80°C until analysis could be carried out. Samples were distributed between all laboratories. A list of all the analytical methods used in the project is presented in Appendix V.

2.4 Sensory attributes

Sensory analysis was carried out at DIFRES. Objective descriptive sensory method was used to characterize the sensory quality of the products. This method required a tested and trained panel together with a test room (ISO 8586-1, 1993; ISO 8589, 1988). In the sensory method profiling, a series of words for attributes was developed to describe the sensory characteristics describing appearance, smell, taste and texture (ISO 11035, 1994). Furthermore the panel was trained using a scale describing the intensity of the attributes ensuring that all experimental conditions were represented. Before each sensory session, training sessions of the panel was performed. The samples were served in random order and the sensory attributes were assessed using a 15 cm unstructured line scale.

2.5 Lipid oxidation

Peroxide value was determined by the production of a red thiocyanate complex according to Undeland *et al.* (2002).

The thiobarbituric (TBARS) test was used to measure the secondary lipid oxidation products according to Lemon (1975).

2.6 Microstructure and textural analysis

The microstructure and textural analysis were carried out at Matis. Samples for microstructure analysis were collected from herring pieces using a cork knife, 11 mm in diameter. They were embedded in plastic tubes (Kartell, Novigilo, Italy) containing O.C.T. compound (embedding medium) (Tissue Tek, Sakura, Torrance, CA) and frozen in liquid nitrogen. The frozen specimens were stored at -80°C until cryosectioning and staining. The specimens were sectioned frozen at -27°C in Leica CM1800 cryostat (Leica, Heidelberg, Germany) for transverse cuts, 10 μm thick. Sections were mounted on glass slides and stained using Nile Red and Oil Red O. The stained samples were washed with distilled water, dried at room temperature and mounted (Catalano and Lillie 1975). The samples were examined in an optical microscope, Leica DM RA2 at 100 x magnification and images captured using a Leica DC300F digital camera mounted on the microscope. The space between the myofibrils was measured as a ratio (%) of the determined area in the muscle.

The measurement of textural properties was performed by using compression test on raw herring (Lepetit and Culioli, 1994). The force at strain 80% of initial sample thickness was recorded and stresses calculated (TA.XT2® Texture Analyser). The probe was held for 5 sec. in compression. Flat ended probe with a diameter of 100 mm was used. The probe speed was 150 mm/min. The test was performed in longitudinal configuration.

The measurement of the expressible moisture was performed in the same test as the textural properties. The samples were compressed between layers of weighed Whatman filter papers No. 4. The samples (2.0 cm in diameter and 1.5 cm in thickness) were put between layers of filter papers (Lee and Cung, 1989). After the compression the samples

were removed and the filter papers were reweighed. The moisture was expressed as expressible moisture % of sample weight (EM).

% EM (g fluid/g sample) =

$$\frac{\text{(weight of "wet" filter paper (g))-(weight of filter paper after drying (g))}}{\text{initial weight of sample (g)}} \times 100$$

2.7 Chemical analysis

2.7.1 Dry matter

The analysis of dried matter and ash were carried out at IMR, DIFRES and Chalmers. For the dried material measurement the samples were heated in an oven at 102-105°C for 24 hours. Water corresponds to the weight loss (AOAC, nr.950.46, 1990; Christie *et al*. 1985).

2.7.2 pH measurement

The measurement of pH was carried out at Matis, IMR, DIFRES and Chalmers. At DIFRES, pH was measured directly in mince and fillet equilibrated to room temperature using Metrohm 780 pH meter with a Metrohm combined metro sensor spearhead glass electrode. For Matis, IMR and Chalmers the pH of minced fish was recorded with an Orion combination epoxy Ross® Sure-Flow™ Electrode (Thermo Orion, Beverly, MA, USA) in conjunction with a pH-meter (PHM210 Radiometer Analytical S.A., Villeurbanne, France). Either the measurements were done directly into the fillet with a Hamilton double pour electrode (Hamilton Double Pore, Bonaduz, Switzerland), or one part mince was manually stirred with 9 parts distilled water. The slurry was allowed to equilibrate for 10 min at room temperature before measurement.

2.7.3 Salt content

Determination of salt content was carried out at DIFRES, according to AOAC Official Methods of Analysis (2000).

2.7.4 Protein analysis

Protein determination was carried out at IMR. Method for protein determination was according to Lowry *et al.* (1951). Protein determination was also carried out at DIFRES according to the Kjeldahl method.

Determination of salt soluble proteins was according to Dyer *et al*. (1950) or Kelleher and Hultin (1991). The sample was treated with Dyers buffer (5% NaCl buffered with 0.2 M $NaHCO_3$, pH 7.4), 4.2% LiCl and 0.02 M Li_2CO_3, pH 7.2 and 0.1 M NaOH and centrifuged. The supernatant was analyzed for protein concentration according to the modified Lowry procedure (1951).

Analysis of total extractable heme protein was carried out by Chalmers. Extraction of heme protein from muscle tissue was performed by homogenizing fish mince in cold extraction buffer (10 mM Tris, 1 mM EDTA, 80 mM KCl, pH 8.0), then centrifuged and the supernatant filtered, according to Kranen *et al*. (1999).

The analysis of extractable heme was according to Brown (1961) and Richards and Hultin (2000). The sample was mixed with sodium dithionite and bubbled with CO gas. The absorbance was measured near 418 nm.

2.7.5 Colour measurements

The intensity of the flesh colour was measured by using MiniScan XE plus form Hunterlab using the D65 light source. The instrument records the L* (lightness – intensity of white colour), a* (redness – intensity of red colour) and b* (yellowness – intensity of yellow colour) values.

2.7.6 Total lipids and fatty acid pattern

The analysis of total lipids and fatty acid composition were carried out at IMR. Regarding the fatty acid pattern, the total lipids, the neutral lipids and the polar lipids were analyzed. Total fat for gravimetric fat determination and fatty acid composition analysis was extracted from herring fillet mince using Lee *et al.* (1996) method as modified by Undeland *et al*. (2002). The fatty acid composition was analysed according to Olsen *et al.* (2004).

2.7.7 α-Tocopherol (vitamin E) determination

Tocopherol of the same chloroform fat extract that was used in the PV-measurements was also subjected to α-tocopherol analysis. Tocopherols were separated by high performance liquid chromatography (HPLC) on Kromasil C18 column (150×2.1 mm, 5μm) (Eka Chemicals, Bohus, Sweden). The mobile phase used consisted of 98% methanol (HPLC, Lab-Scan LtD, Dublin, Ireland) in water, and the flow rate was 0.4 ml/min. Peaks were detected with a Shimadzu RF-551 spectrofluorometric detector using an excitation wavelength of 295 nm and emission wavelength of 330 nm. α-tocopherol peaks were quantified against a DL- α-tocopherol standard (Calbiochem, An Affiliate of Merck KgaA Darmstadt, Germany). Results were expressed as mg/kg sample.

2.7.8 Ratio of light and dark muscle

The ratio of light and dark muscle was measured at Matis, IMR and Chalmers. After manual dissection the ratio of light and dark muscle in herring fillets was determined by weight.

2.7.9 Silvering

After skinning, the amount of the "silver surface" staying on the fillet was judged according to a protocol agreed upon in the group. The protocol included a visual grading of the amount silver according to a scale from 1-3 with 1=little silver, 2=medium silver and 3=much silver.

2.7.10 NIR measurements

NIR measurements were carried out at DIFRES according to Nielsen *et al*., 2005. NIR spectra were measured with an InfraProver, II Fourier transform spectrometer (Bran and Luebbe, Germany) using an optical fibre bundle as probe. Fillets were placed on ice in a polystyrene box and measured at three places on the meat side and spectra were sampled at 12 cm^{-1} interval from 4500 to 9996 cm^{-1}.

2.7.11 Sampling and statistics

Sampling of the fillets for chemical analysis was carried out in the following way. Four to six butterfly fillets were thawed, skinned and minced together into a pooled sample. From this pooled sample, 2-4 samples (n) were taken out for the different analyses. The analysis was carried out once or twice. The obtained data sets were compared by multiple comparison ANOVA by Matis using all pair wise comparison by Sigmastat 3.5 (Jandel Scientific Software, Ontario, Canada). Significance of difference was defined at $p < 0.05$. Multivariate data analysis, principal component analysis (PCA) and partial least squares (PLS) regression was performed by The Unscrambler version 9.1, from Camo, Norway. The various measurements on a sample constituted one row in the matrix *X*. Each column in *X* was mean-centred and scaled to unit standard deviation (commonly referred to as 'auto scaling'). For the PLS regression, the *Y* vector was storage time. Models were validated by segment-based cross-validation with samples randomly assigned to one of five segments.

3. RESULTS

3.1 Sensory attributes

The aim of this part was to investigate the influence of catching season and place on sensory quality of the herring after frozen storage. The sensory profiling of the heat

treated samples was performed with assessors, from a trained and tested external panel at DIFRES.

No clear differences were found in the sensory quality of herring regarding catching place or season. As expected, long freezer storage at -20°C resulted in poor sensory quality of the herring. The herring became rancid both in odour and flavour after 6 months of frozen storage at -20°C, but also discoloured and had a high intensity of amine odour and wet dog/sour odour and flavour. Only very small changes occurred in herring frozen at -80°C even after 18 months of frozen storage.

Figure 2 shows a PCA of the sensory profiling after 0, 6, 12 and 18 months of frozen storage at -20°C. The results from the sensory profiling showed that there was only small difference between the samples from IMR stored at -80°C and at -20°C for 6 months. The samples had a rancid odour and taste, more salty taste than the samples from Chalmers and the samples were discoloured. There was a distinct difference between the Chalmers control samples stored at -80°C and -20°C for 6 months. The samples kept at -80°C had an odour of warm milk and a taste of mushroom, sweet, cooked potatoes and they were firm and juicy. The samples stored at -20°C for 6 months had less odour and taste and they were remotely discoloured and less firm. The samples from Matis were grainier after 18 months of storage than the samples from IMR and Chalmers.

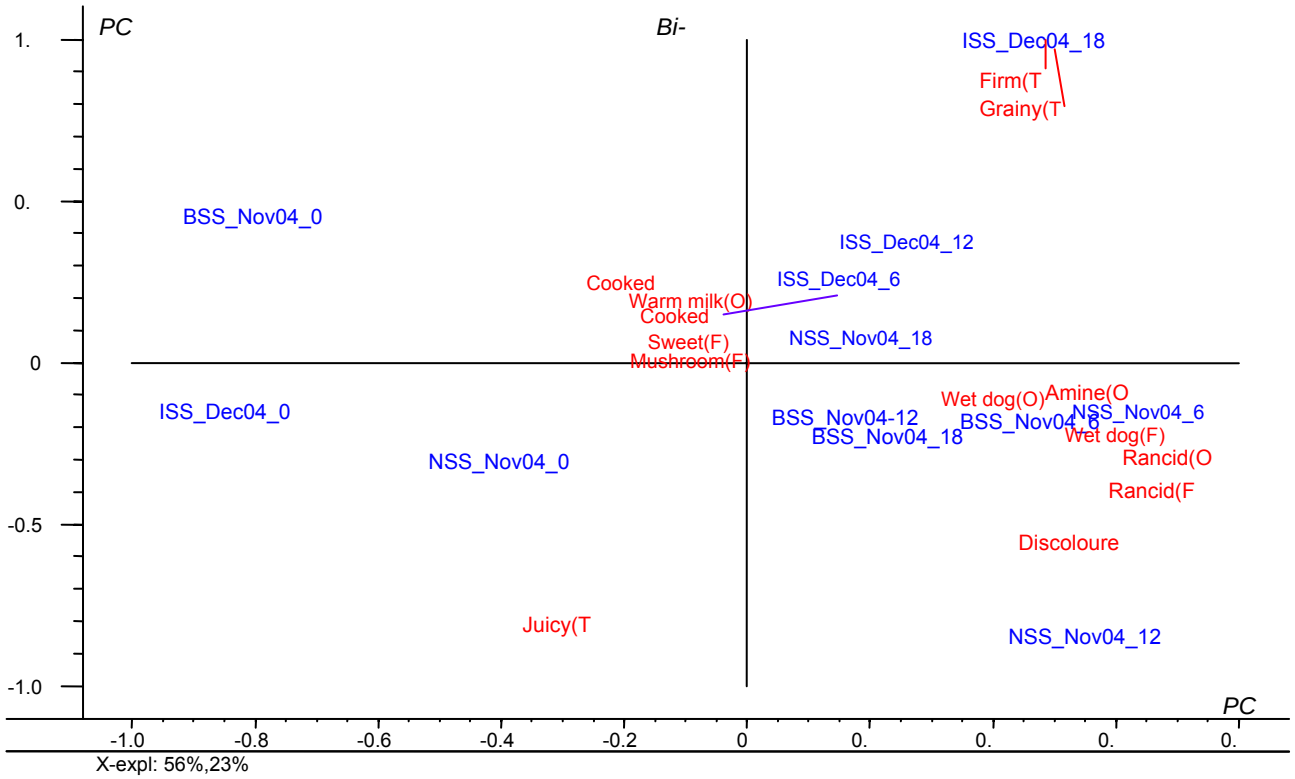


Figure 2. PCA of Sensory profiling of herring after 0, 6, 12 and 18 months storage at -20°C. The sensory parameters describe odour (O), flavour (F), appearance (A) and texture (T).

3.2 Lipid oxidation

The study of lipid oxidation of herring samples was performed by Chalmers. Both primary and secondary oxidation products were measured as peroxide value (PV) and thiobarbituric reactive substances test (TBARS), respectively. Both kinds of products are expressed as µmol/kg mince. The peroxide value in herring increased significantly with storage for all catching places ($p<0.05$). The peroxide values of samples from the Icelandic summer spawning herring (ISS) were in general lower throughout the storage (Fig. 4) than the samples originating from Baltic spring spawning (BSS) (Fig. 3) or Norwegian spring spawning herring (NSS) (Fig. 5). All control samples (stored at -80°C) of NSS herring had slightly higher peroxide values than herring control samples from BSS and ISS, probably due to somewhat longer sampling time. After 6 months storage at -20°C, NSS herring also reached the highest peroxide values, after which the BSS samples were ranked. At one sampling point from each catching place, the storage

periods were extended to 12 and 18 months and the peroxide value continued to increase, again with ISS ranked lower than NSS and BSS.

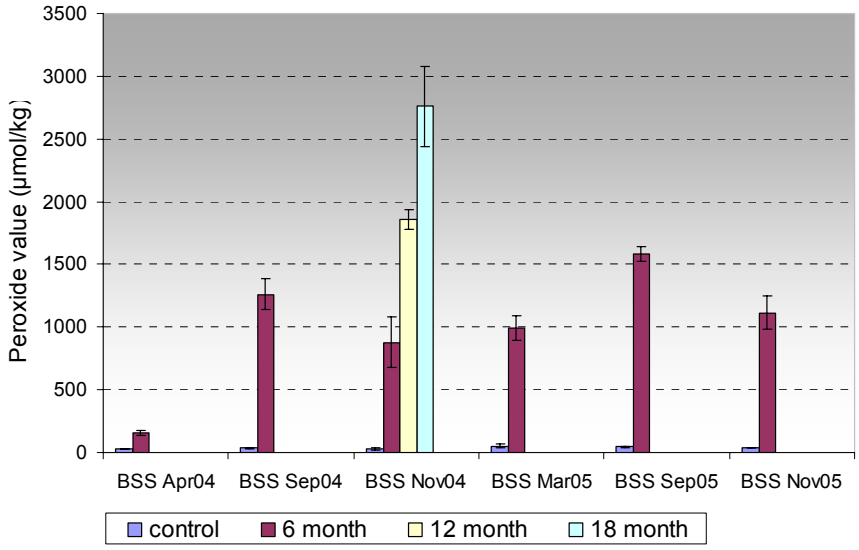


Figure 3. Development of lipid hydroperoxides (PV) in samples from the Baltic spring spawning herring.

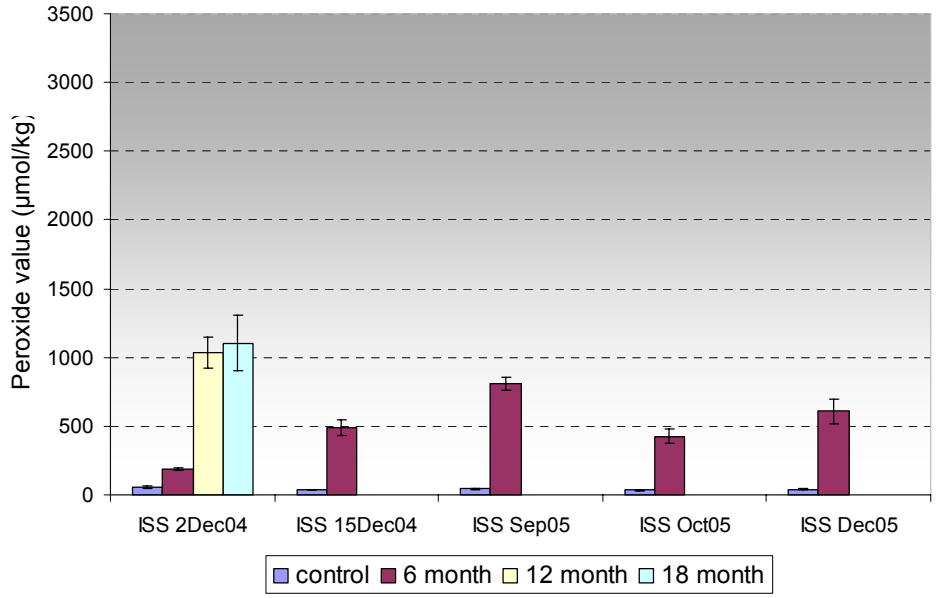


Figure 4. Development of lipid hydroperoxides (PV) in samples from the Icelandic summer spawning herring.

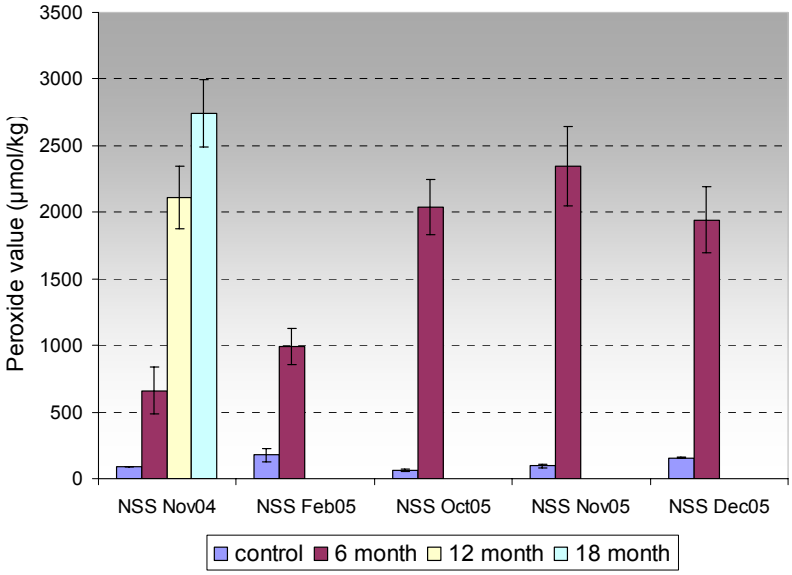


Figure 5. Development of lipid hydroperoxides (PV) in samples from the Norwegian spring spawning herring.

A similar result was achieved with TBARS as was seen from the PV measurements (Fig. 6-8). The TBARS in herring increased significantly ($p<0.05$) with storage time at -20°C for all catching places, with highest value in NSS herring. Lowest TBARS values were in general obtained for ISS herring, followed by BSS herring. With TBARS, one could differentiate control samples from on the one hand NSS and the other ISS and BSS, which could not be done with data from PV measurements.

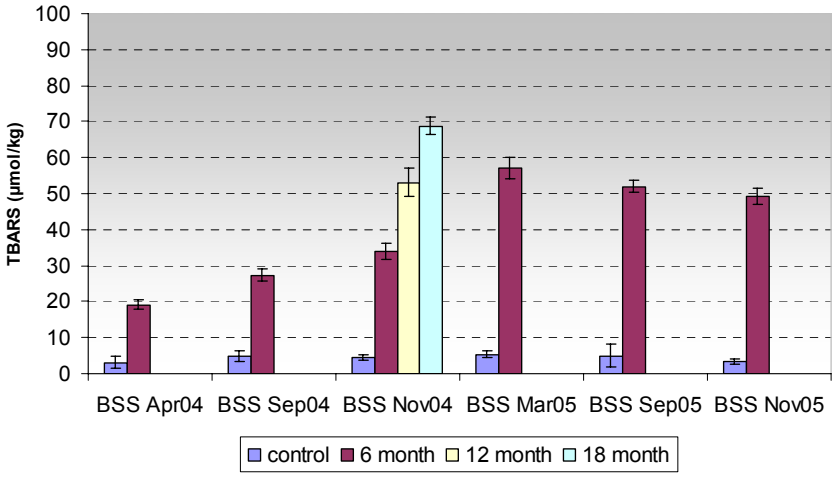


Figure 6. Development of TBARS in samples from the Baltic spring spawning herring.

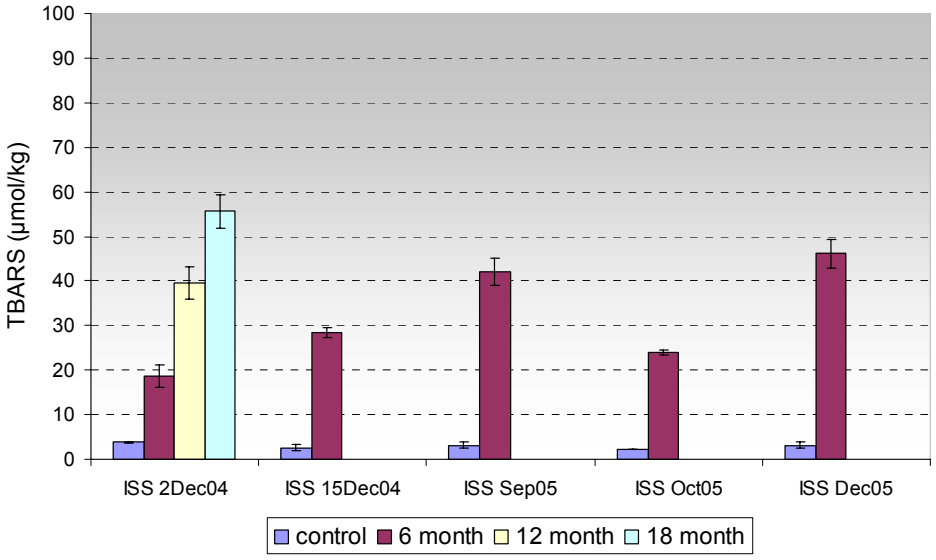


Figure 7. Development of TBARS in samples from the Icelandic summer spawning herring.

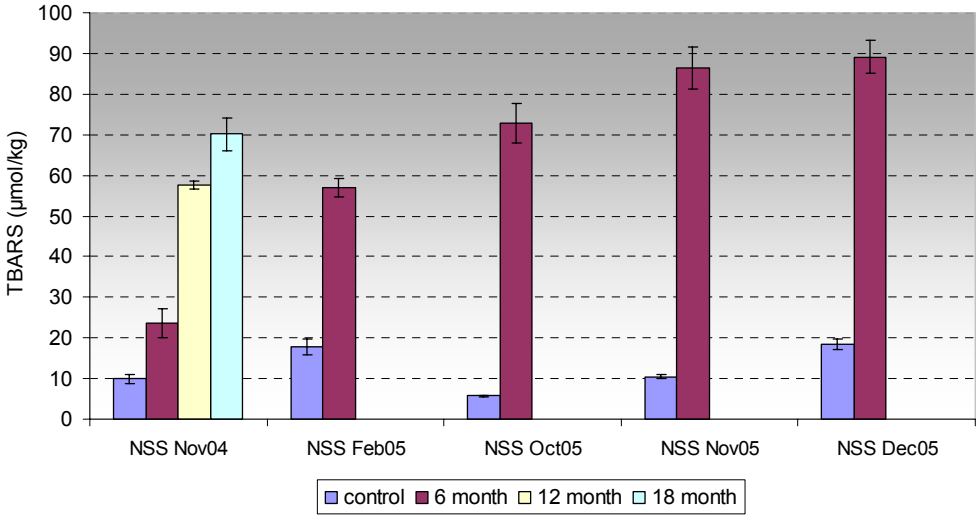


Figure 8. Development of TBARS in samples from the Norwegian spring spawning herring.

3.3 Microstructure and texture properties

The microstructure analysis and texture properties were carried out at Matis. Long frozen storage at -20°C resulted in considerable shrinkage of myofibrils and a resulting increase

in the space between myofibrils. The space between myofibrils in spring spawning Baltic herring fillets increased significantly ($p<0.05$) at all catching seasons as frozen storage time increased (Fig. 9). The same significant difference in space between myofibrils in herring after long frozen storage was obtained in Icelandic summer spawning herring (Fig. 10) and Norwegian spring spawning herring (Fig. 11). These changes in microstructure could neither be related to catching place nor to season.

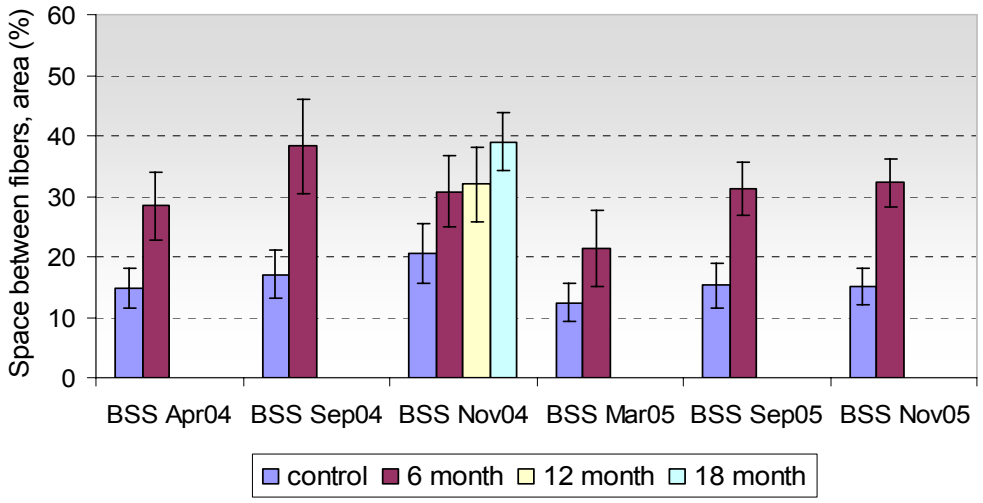


Figure 9. Space between fibers in fillets of Baltic spring spawning herring.

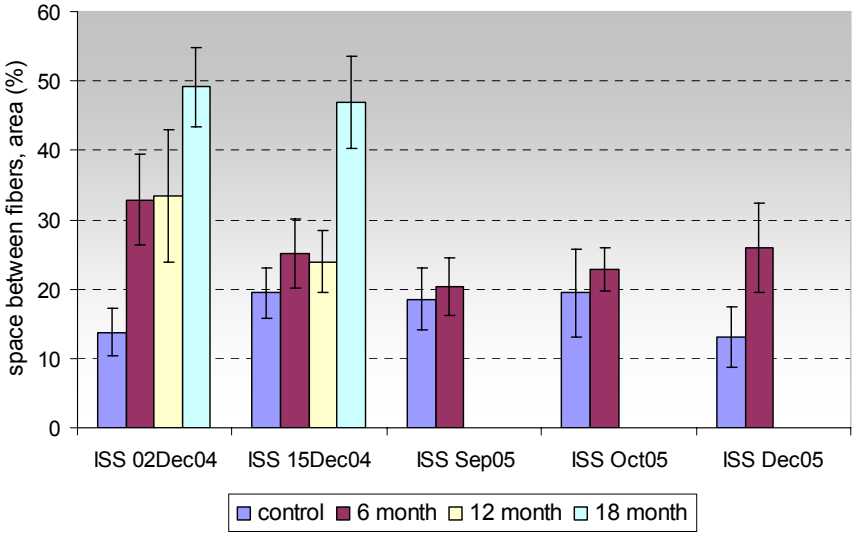


Figure 10. Space between fibers in fillets of Icelandic summer spawning herring.

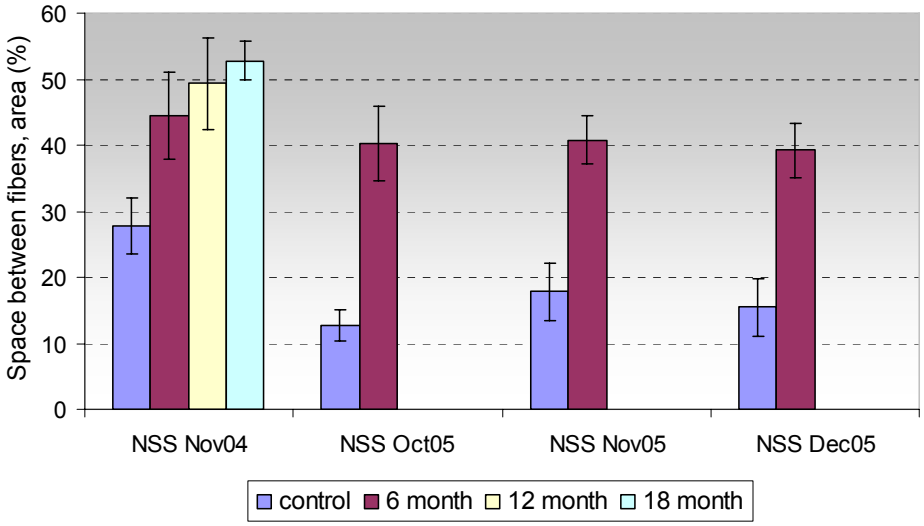
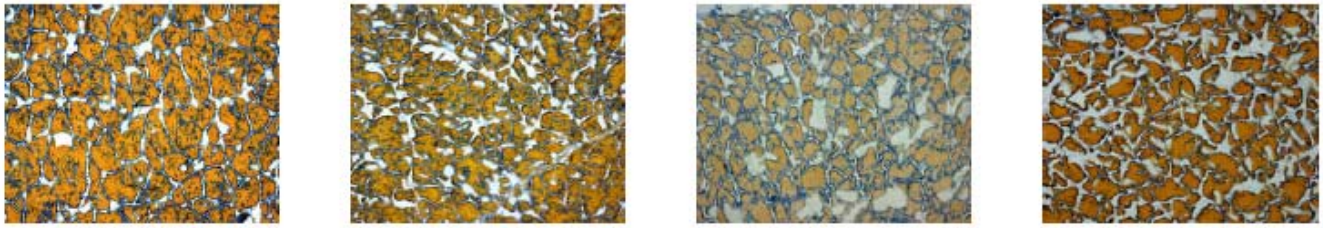
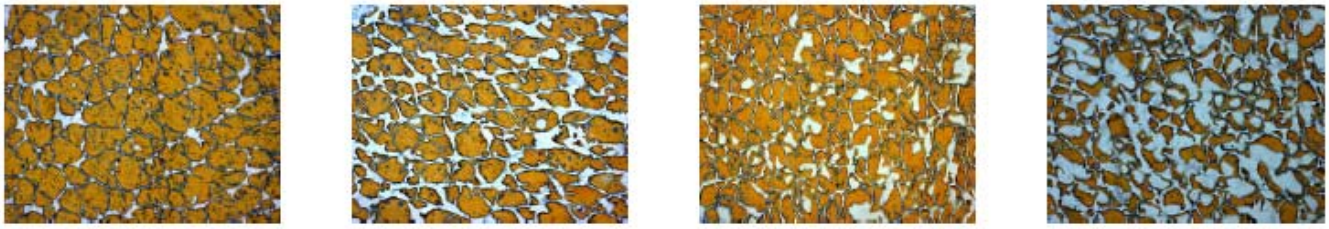


Figure 11. Space between fibers in fillets of Norwegian spring spawning herring.



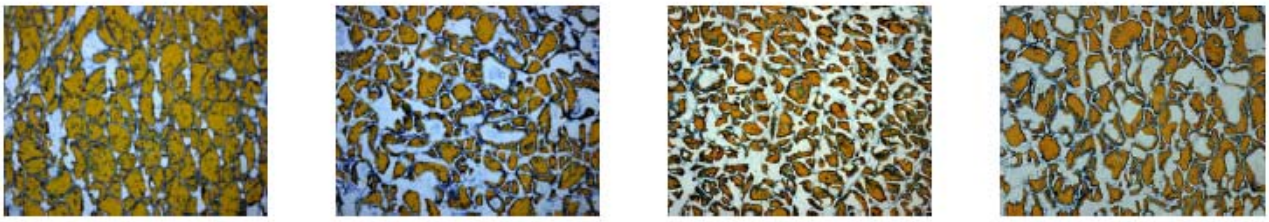
(A) (B) (C) (D)

Figure 12. Microstructure of BSS Nov04. (A) Control stored at -80°C, (B) after 6 months at -20°C, (C) after 12 months at -20°C and (D) after 18 months at -20°C.



(A) (B) (C) (D)

Figure 13. Microstructure of ISS 2Dec04. (A) Control stored at -80°C, (B) after 6 months at -20°C, (C) after 12 months at -20°C and (D) after 18 months at -20°C.



(A) (B) (C) (D)

Figure 14. Microstructure of NSS Nov04. (A) Control stored at -80°C, (B) after 6 months at -20°C, (C) after 12 months at -20°C and (D) after 18 months at -20°C.

Microstructure of control fillets of herring and those that were stored for upto 18 months at -20°C from different catching areas is shown in figures 12-14. The figures indicated that the shrinkage in the myofibrils increased, as the storage time at -20°C increased, resulting in an increased space between the myofibrils. The figures show only the microstructure of herring caught in November 2004 and in early December 2004. The figures of microstructure in herring from other catching times are identical.

Not all the data from textural analysis showed significant differences in hardness in fillets from Baltic spring spawning herring (Fig. 15). It was obvious that hardness increased in fillets of herring caught in November 2004, after frozen storage for 6 and 12 months. But after long frozen storage for 18 months the fillets became significantly ($p<0.05$) softer. Similar results were obtained for Icelandic summer spawning herring (Fig. 16) and Norwegian spring spawning herring (Fig. 17) after long frozen storage.

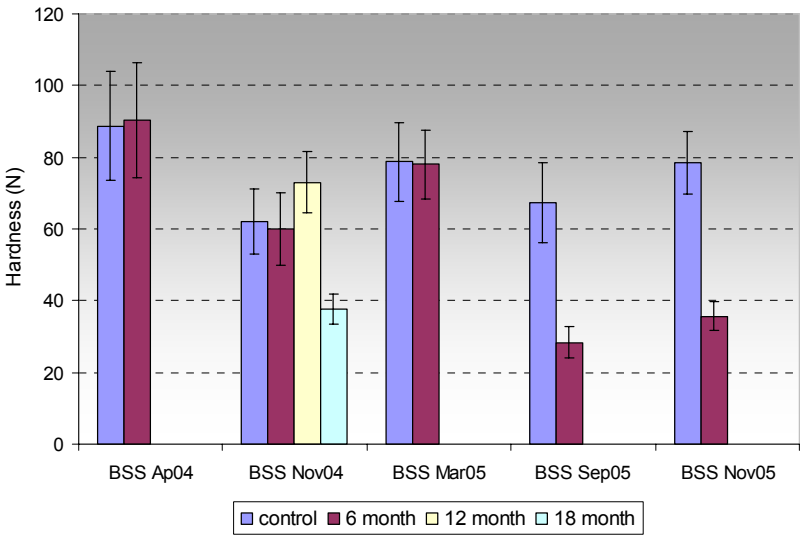


Figure 15. Hardness in fillets of Baltic spring spawning herring.

The fillets of herring from different catching places, caught in 2005 were also significantly ($p<0.05$) softer after frozen storage for 6 months, except for the Baltic spring spawning herring caught in Mars and Norwegian spring spawning herring caught in November, 2005. In most cases the herring was softer as the fat content increased.

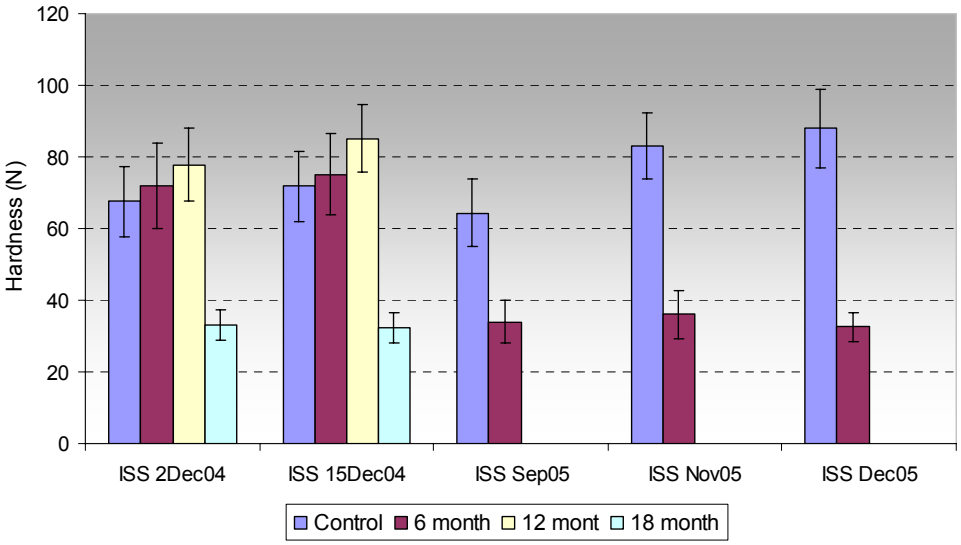


Figure 16. Hardness in fillets of Icelandic summer spawning herring.

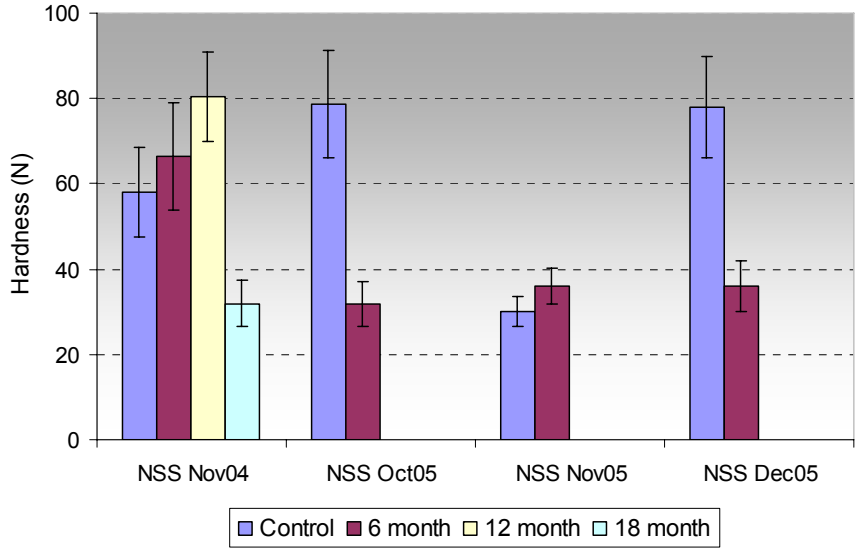


Figure 17. Hardness in fillets of Norwegian spring spawning herring.

The results from the expressible liquid measurements are shown in figures 18-20.

The expressible liquid in the herring fillets increased significantly ($p<0.05$) after frozen storage for 6 months, indicating lower liquid holding capacity in the muscle. The expressible liquid displayed the same trend after frozen storage for 12 and 18 months.

There was a slight difference in expressible liquid between catching places, were expressible liquid in Norwegian spring spawning herring was higher compared to BSS and ISS, especially in herring after 6 month of storage at -20°C, caught in 2005.

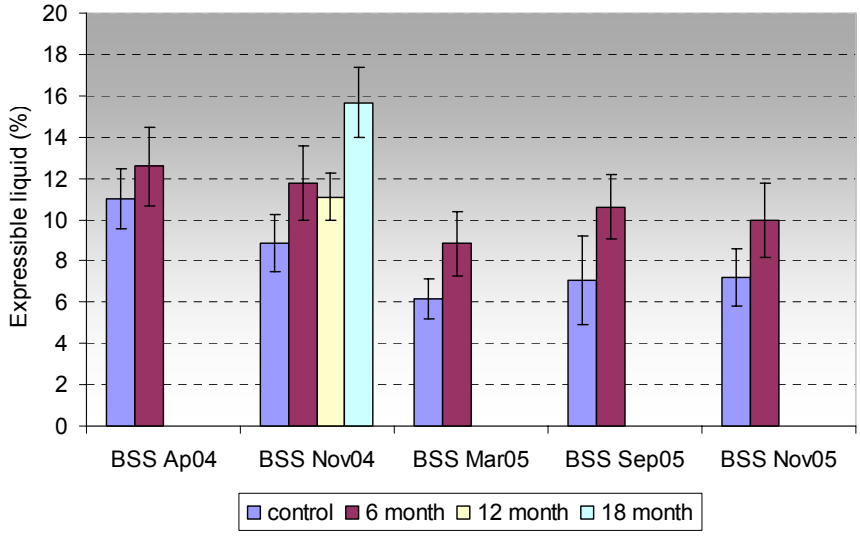


Figure 18. Expressible liquid in fillets of Baltic spring spawning herring.

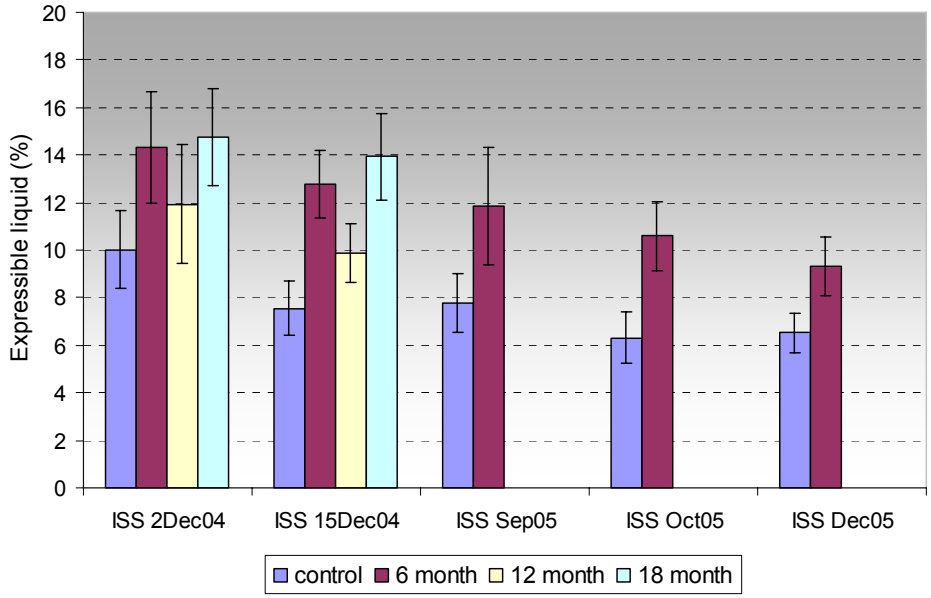


Figure 19. Expressible liquid in fillets of Icelandic summer spawning herring.

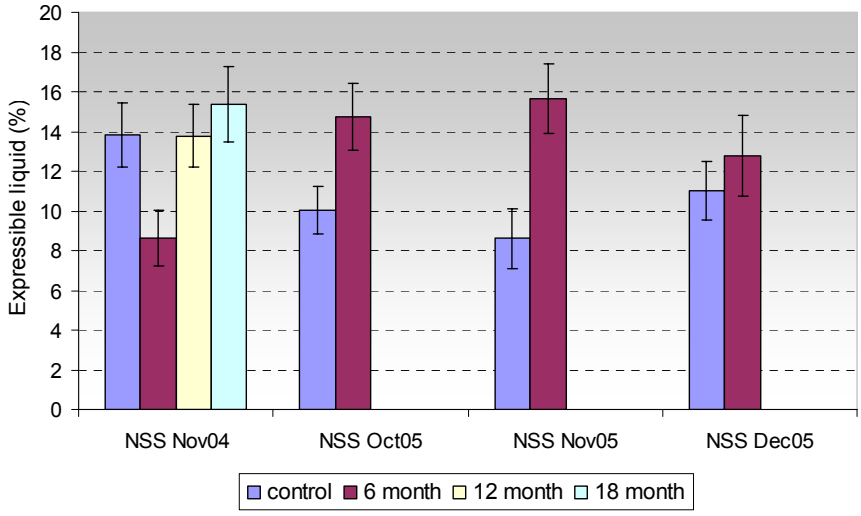


Figure 20. Expressible liquid in fillets of Norwegian spring spawning herring.

3.4 Chemical analysis

Following are the results from chemical analysis and other analysis from all the participants.

3.4.1 Moisture content

The moisture measurements were carried out by IMR, DIFRES and Chalmers.

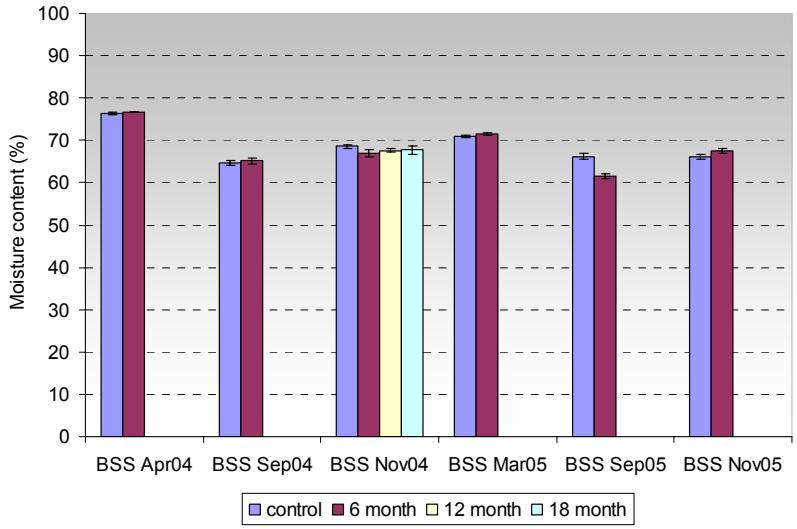


Figure 21. Moisture content in fillets of Baltis spring spawning herring.

The data from each participant were sampled together and the average moisture content can be seen in figure 21-23. The moisture content of ISS herring was constantly around 70% in measured samples, while it varied from from 65-75% in BSS and NSS herring. Generally the moisture content was inversely related to the fat content in herring from each catching place.

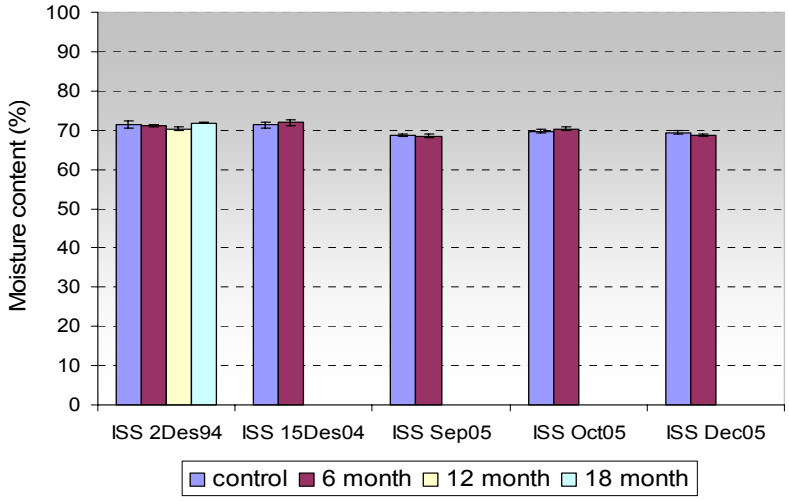


Figure 22. Moisture content in fillets of Icelandic summer spawning herring.

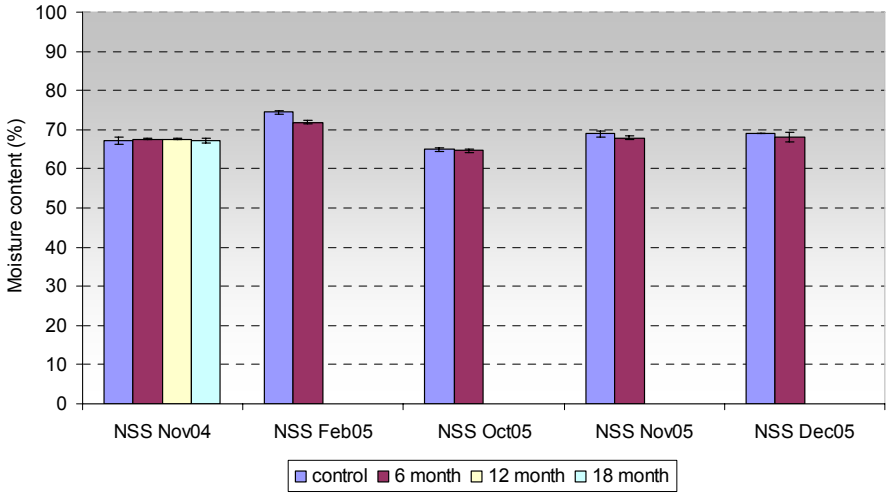


Figure 23. Moisture content in fillets of Norwegian spring spawning herring.

3.4.2 pH measurements

The pH measurements were carried out by Matis, IMR, DIFRES and Chalmers.

The results can be seen in table 1. The pH values obtained in herring from various batches ranged from 6.3 to 6.5. No significant differences ($p>0.05$) in pH were found between batches.

Table 1. Mean value of pH from all the participants.

	BSS		ISS		NSS	
	Mean value	Stdev	Mean value	Stdev	Mean value	Stdev
Control	6,47	0,13	6,49	0,11	6,37	0,09
After 6 months	6,46	0,12	6,45	0,12	6,36	0,09
After 12 months	6,42	0,14	6,53	0,06	6,41	0,06
After 18 months	6,31	0,08	6,4	0,08	6,34	0,06

Table 2. Mean value of pH in different location on the fillet.

	Head	Middle	Tail
BSS	6,63	6,59	6,55
ISS	6,57	6,53	6,50
NSS	6,55	6,53	6,52

IMR measured pH at different locations on herring fillets; near the head, in the middle and at the tail section. The results showed a decrease in pH along the fillet (Table 2) though there was not a significant difference ($p>0.05$) between the locations on the fillet.

3.4.3 Salt content

The measurement of salt content was carried out by DIFRES.

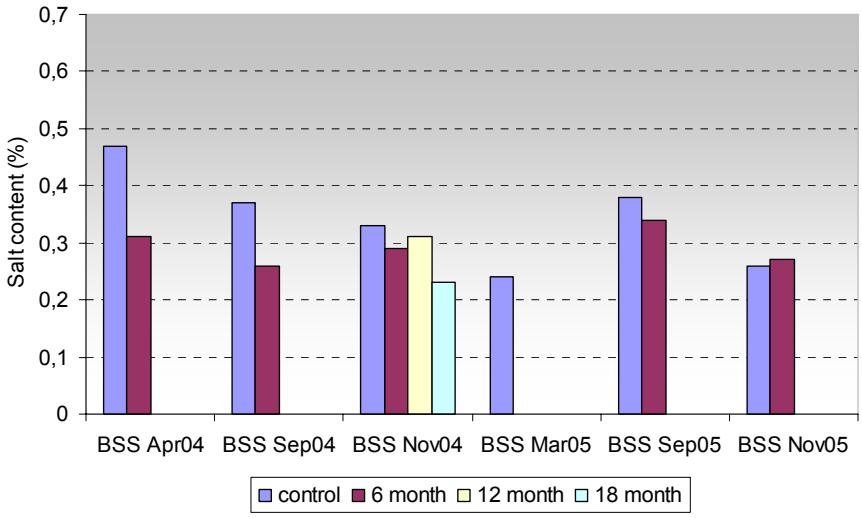


Figure 23. Salt content in fillets of Baltic spring spawning herring.

The salt content in herring from different catch varied slightly, which most likely reflect the time the herring was stored in the RSW-tanks. Also, pressure on the herring during the RSW-storage could play a role. The herring from BSS had the lowest salt content compared to ISS and NSS (Fig. 23-25). A simulation experiment was performed, where the influence of storage time (0, 30, 48 and 72 hours) on quality of herring stored in refrigerated sea water (RSW), were studied. Those results showed that the herring became more salty in flavour as a function of storage time. Unfortunately, not all the herring stayed in RSW after catch in the same amount of time. That could explain some of the variation in salt content in the herring from different catching places and season.

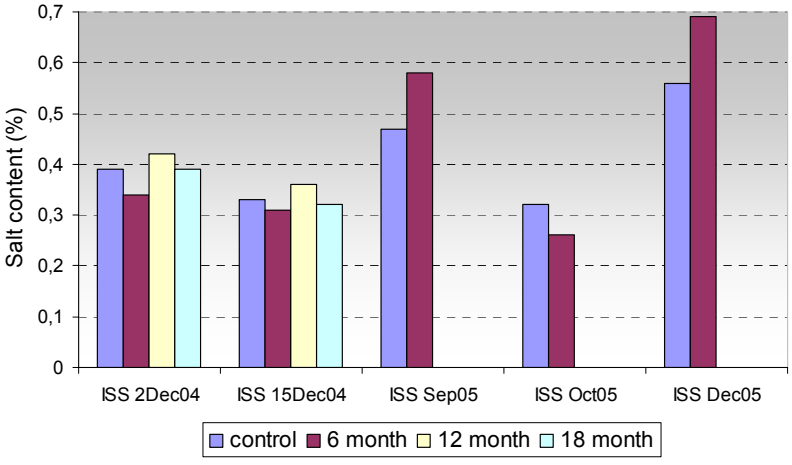


Figure 24. Salt content in fillets of Icelandic summer spawning herring.

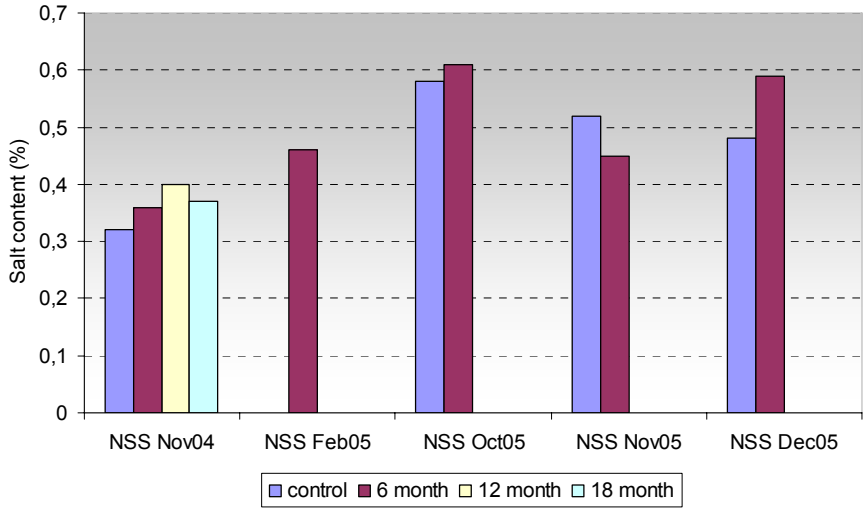


Figure 25. Salt content in fillets of Norwegian spring spawning herring.

3.4.4 Protein analysis

Analysis of protein content and protein solubility in herring was carried out by IMR. Normally the protein content in fish is stable. The average protein content in the control sample of herring between different catching places varied slightly or from 17.9% to 18.9% (Table 3).

Table 3. Average value of protein content in herring from different catching places and seasons.

	Control (mg/gfillet)	6 month (mg/gfillet)	12 month (mg/gfillet)	18 month (mg/gfillet)
BSS Ap04	179,4	171,1	Nm	Nm
BSS Sep04	163,4	181,3	Nm	Nm
BSS Nov04	183,7	187,2	170,3	176,7
BSS Mar05	170,3	166,6	Nm	Nm
BSS Sep05	188,2	169,1	Nm	Nm
BSS Nov05	193,6	178,6	Nm	Nm
ISS 02Dec04	208,6	206,8	187,1	136,9
ISS Sep05	185,4	190,3	Nm	Nm
ISS Oct05	182,5	175,5	Nm	Nm

ISS Dec05	179,3	187,3	Nm	Nm
NSS Nov04	178,3	187,0	191,1	185,5
NSS Feb05	161,3	163,3	Nm	Nm
NSS Oct05	191,2	196,2	Nm	Nm
NSS Nov05	184,3	177,9	Nm	Nm
NSS Dec05	182,9	179,0	Nm	Nm

The average protein solubility in the herring control sample showed the lowest value for the ISS herring or 42.5% compared to 61.6% for BSS and 60.9% for NSS (Table 4). Lower protein solubility indicates that the proteins had changed, e.g. through denaturation, cross-linking or reaction with lipid oxidation products.

Table 4. Average value of protein solubility in fillets of herring caught at diffence catching place and season.

	Control (%of total protein)	6 month (%of total protein)	12 month (%of total protein)	18 month (%of total protein)
BSS Ap04	64,3	39,7	Nm	Nm
BSS Sep04	56,2	39,2	Nm	Nm
BSS Nov04	87,3	58,4	50,4	33,0
BSS Mar05	57,9	40,7	Nm	Nm
BSS Sep05	58,9	48,2	Nm	Nm
BSS Nov05	45,3	41,2	Nm	Nm
ISS 02Dec04	35,7	38,2	35,2	51,1
ISS Sep05	38,8	40,0	Nm	Nm
ISS Oct05	45,0	41,7	Nm	Nm

ISS Dec05	53,3	48,6	Nm	Nm
NSS Nov04	70,7	50,5	39,7	36,7
NSS Feb05	60,7	50,0	Nm	Nm
NSS Oct05	47,5	40,9	Nm	Nm
NSS Nov05	56,2	44,2	Nm	Nm
NSS Dec05	69,5	47,5	Nm	Nm

Analysis of extractable haemoglobin content was carried out by Chalmers. Although it looks like the haemoglobin content decreased significantly with storage (Fig. 26-28), it should be stressed that it was the extractability of haemoglobin from the muscle matrix that was reduced. This could be a result of cross-linking/adherence of the haemoglobin to myofibrillar proteins, possibly after oxidation of the haemoglobin molecule.

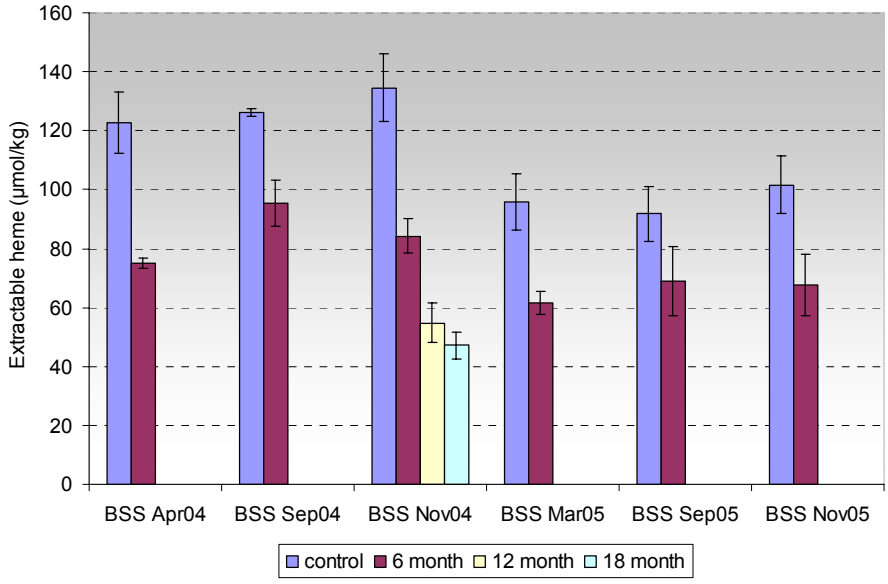


Figure 26. Extractable heme in fillets of Baltic spring spawning herring.

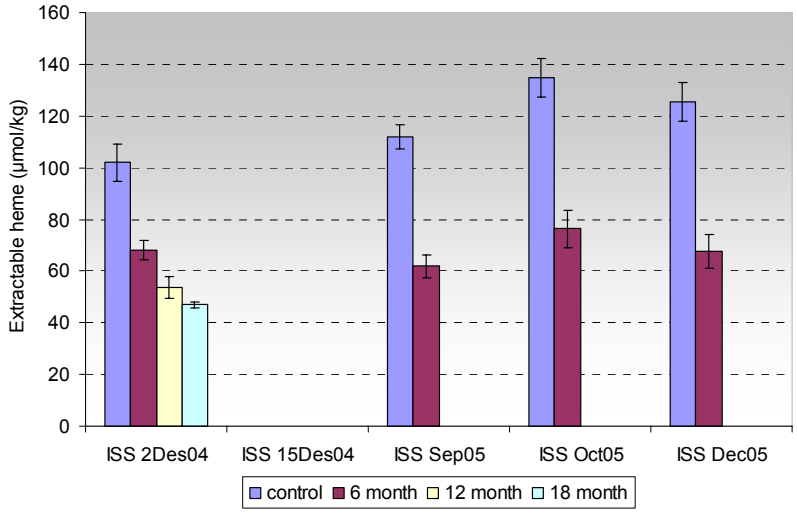


Figure 27. Extractable heme in fillets of Icelandic summer spawning herring.

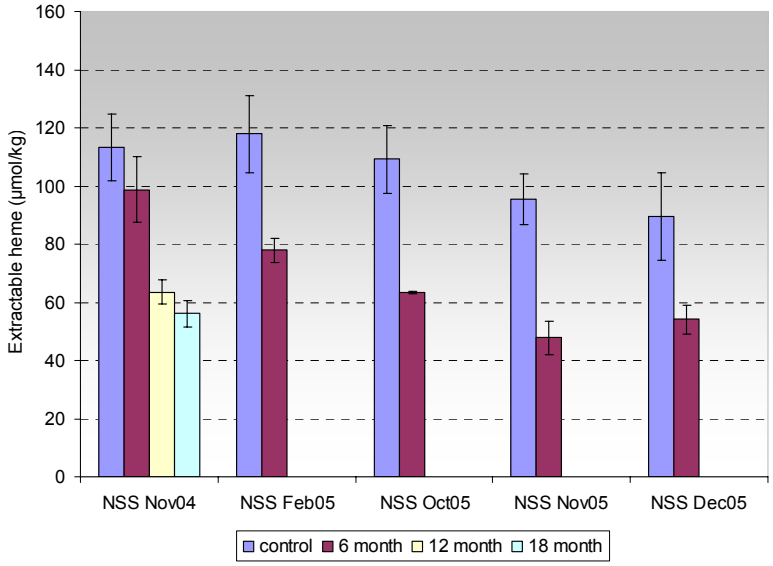


Figure 28. Extractable heme in fillets of Norwegian spring spawning herring.

3.4.5 Colour analysis

The colour measurements were carried out by Matis and IMR. For most samples, the lightness (L*value) in herring fillets increased with longer frozen storage time. This could be a result of a slight dry-out or protein denaturation.

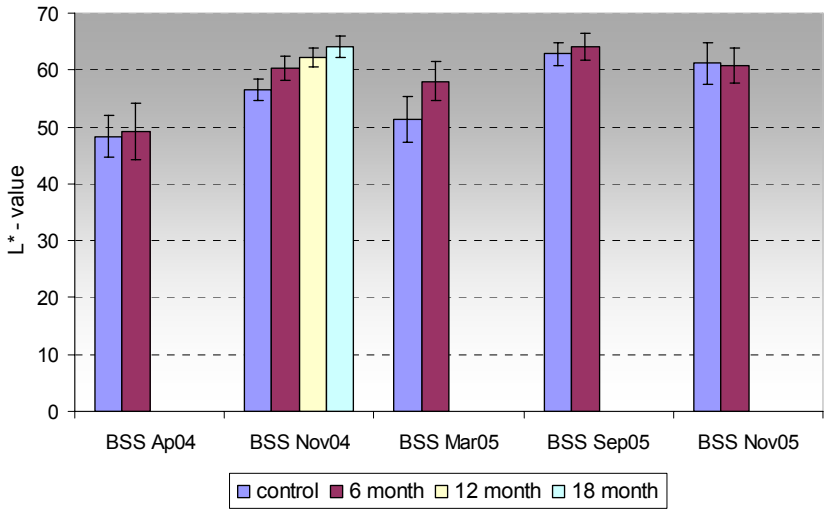


Figure 29. Colour as L-value in fillets of Baltic spring spawning herring.*

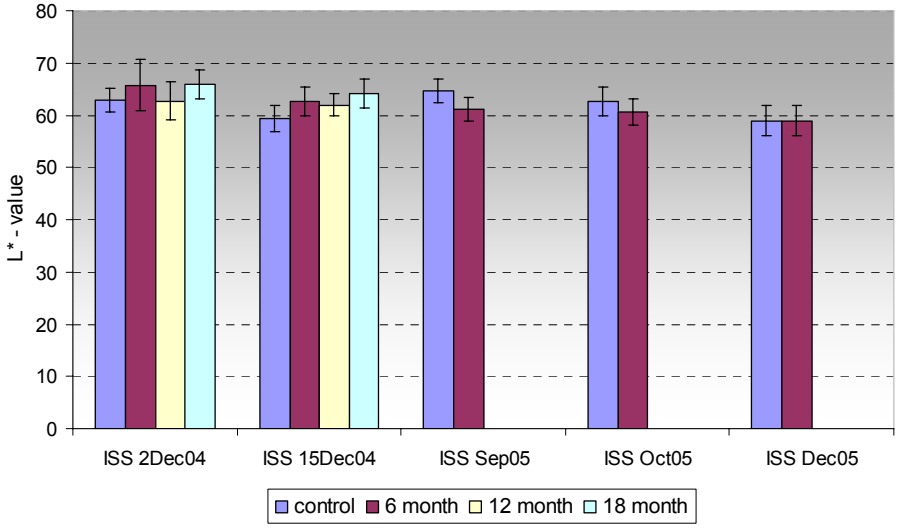


Figure 30. Colour as L-value in fillets of Icelandic summer spawning herring.*

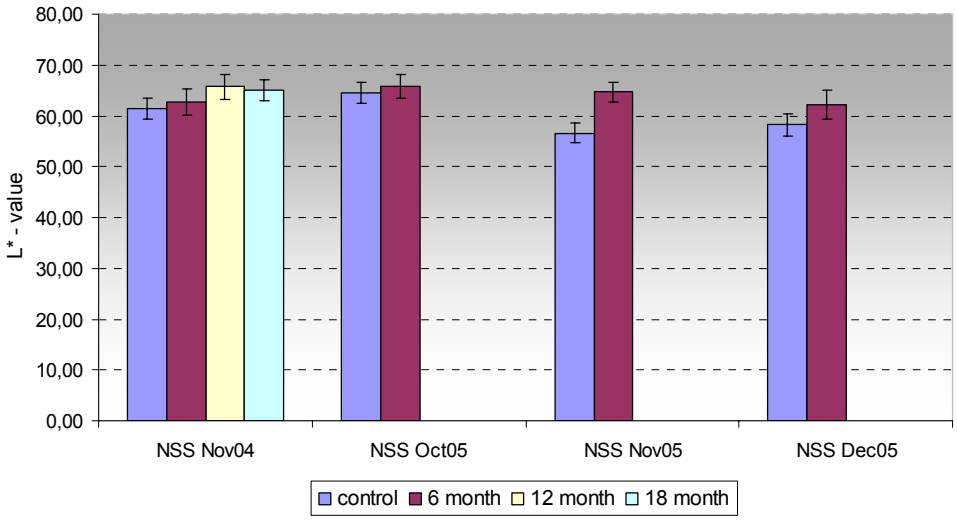


Figure 31. Colour as L-value in fillets of Norwegian spring spawning herring.*

The intensity of red colour decreased dramatically in most BSS herring fillets after frozen storage for 6 months. No significant difference was found due to large variation between the samples (Fig. 32). The same trend was obtained for ISS and NSS herring (Fig. 33 and 34). The ISS sample from Dec 04 behaved very differently which we have no explanation for.

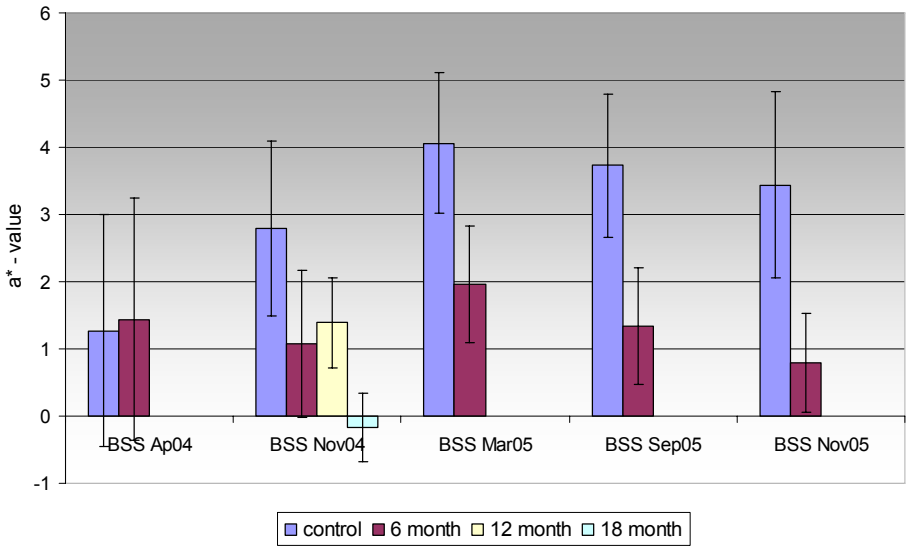


Figure 32. Colour as a-value in fillets of Baltic spring spawning herring.*

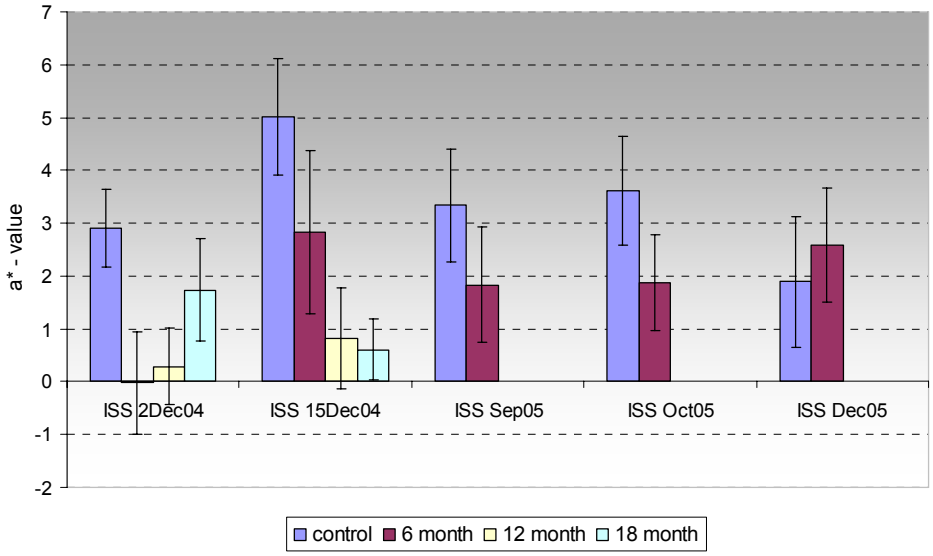


Figure 33. Colour as a-value in fillets of Icelandic summer spawning herring.*

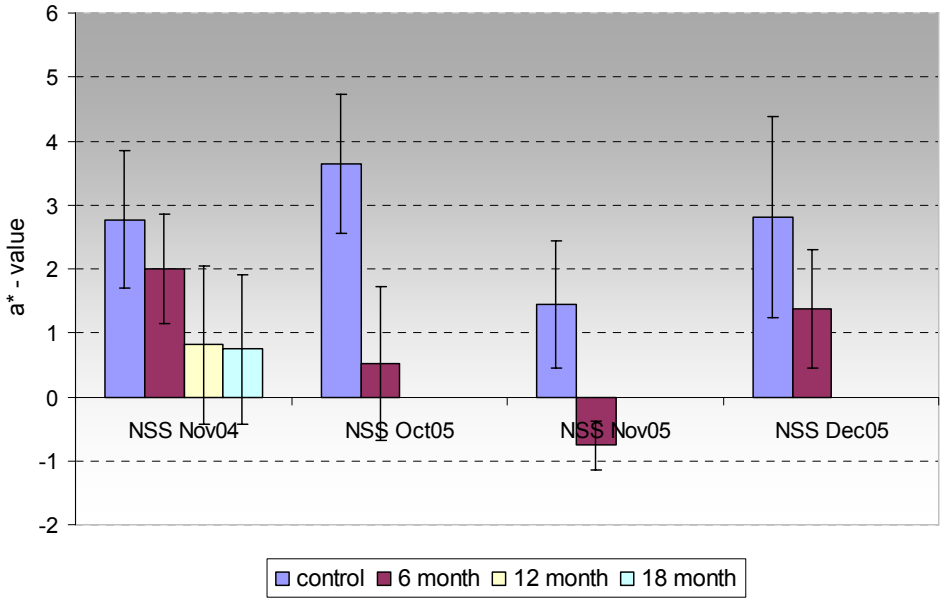


Figure 34. Colour as a-value in fillets of Norwegian spring spawning herring.*

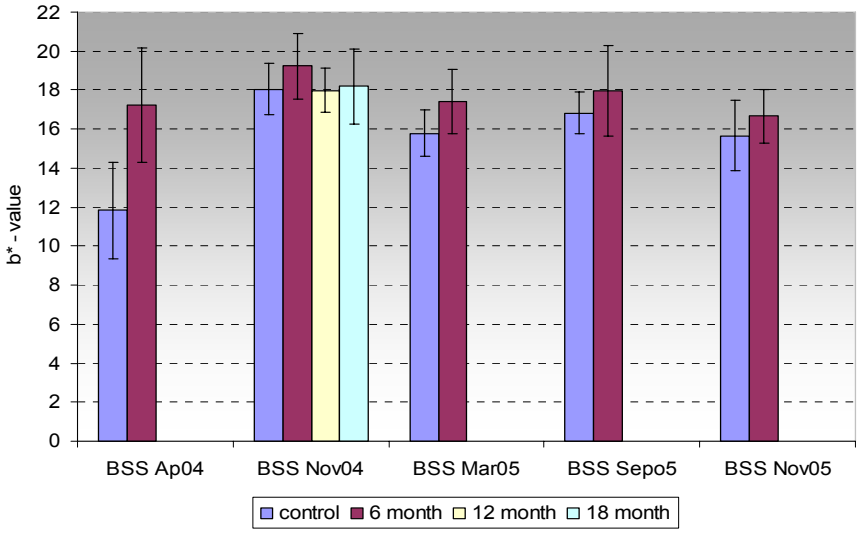


Figure 35. Colour as b-value in fillets of Baltic spring spawning herring.*

The yellowness in BSS herring fillets increased slightly after frozen storage for 6 months, but after 12 and 18 months the difference was negligible (Fig. 35). The same trend was obtained in ISS and NSS herring (Fig. 36 and 37). Increased yellowness probably reflected a formation and cross-linking of so called "Schiff's bases". These bases arise from lipid oxidation products reacting with free amino groups e.g. of proteins.

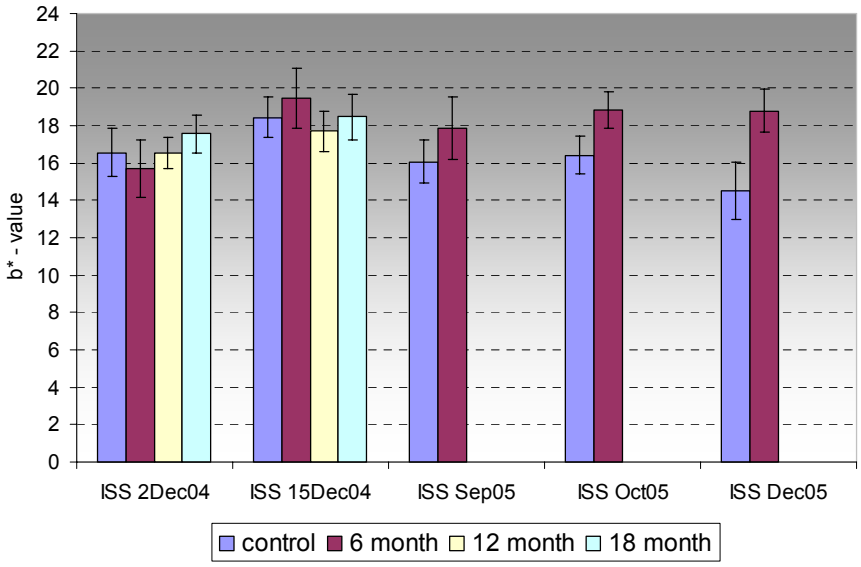


Figure 36. Colour as b-value in fillets of Icelandic summer spawning herring.*

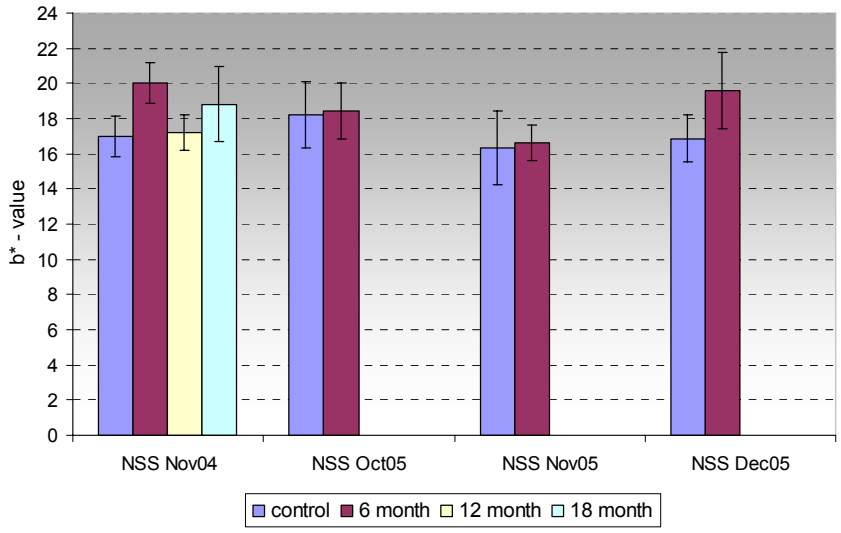


Figure 37. Colour as b-value in fillets of Norwegian spring spawning herring.*

3.4.6 Lipid analysis

Analysis of fat content was carried out by IMR. The results are shown in table 5. It can clearly be seen from these results that the spring spawning herring has the lowest fat content (February and April) comparing with other seasons.

Table 5. Average fat content in fillets of herring caught at different places and seasons.

	Control	6 month	12 month	18 month
BSS Ap04	3,42	3,13		
BSS Sep04	15,06	15,43		
BSS Nov04	11,31	12,13	10,7	11,41
BSS Mar05	7,50	9,32		
BSS Sep05	13,14	13,46		
BSS Nov05	11,89	12,14		
ISS 02Dec04	9,58	8,75	8,64	9,39
ISS Sep05	10,74	12,35		
ISS Oct05	11,24	12,22		
ISS Dec05	10,15	9,87		
NSS Nov04	12,87	12,52	13,02	11,76
NSS Feb05	10,02	7,45		
NSS Oct05	14,21	16,3		
NSS Nov05	8,19	12,24		

NSS Dec05 11,65 12,82

Fatty acid pattern in total fat is shown in Table 6. The dominant fatty acids in total fat were 16:0, 18:1n-9, 20:1n-9, 20:5n-3, 22:1n-11 and 22:6n-3, representing over 8% of the total fat. The fatty acid 18:1n-9 varied in herring from different catching places, where ISS herring contained higher proportion of the acid (~10-13%), compared to BSS and NSS herring (~7-10%). Spring-caught spawning herring (February and April) contained lower n-3 PUFA, compared to other seasons. No obvious changes occurred in n-3 PUFA after different storage time, despite extensive oxidation of lipids. It was interesting to notify that the lipid content did not correlate with lipid oxidation.

Table 6. Fatty acid composition in total fat of herring fillets. Results are shown as % of identified peak area.

	Control sample						After 6 months						After 12 months						After 18 months					
	SFA	MUFA	PUFA	n-3	n-6	n-3/n-6	SFA	MUFA	PUFA	n-3	n-6	n-3/n-6	SFA	MUFA	PUFA	n-3	n-6	n-3/n-6	SFA	MUFA	PUFA	n-3	n-6	n-3/n-6
BSS Ap04	21,5	52,1	26,4	23,4	2,9	7,9	20,3	53,4	26,3	23,1	3,1	7,4												
BSS Sep04	21,4	50,4	28,3	25,5	2,7	9,3	21,9	48,7	29,4	26,3	3,0	8,7												
BSS Nov04	20,6	51,8	27,6	24,7	2,9	8,4	20,7	51,7	27,5	24,4	3,1	7,9	19,3	52,0	28,6	25,4	3,3	7,8	20,7	50,2	29,0	25,8	3,2	8,1
BSS Mar05	20,2	53,6	26,2	22,9	3,2	7,1	22,8	51,3	25,8	21,9	4,0	5,5												
BSS Sep05	18,0	53,9	28,0	25,2	2,8	8,8	20,5	53,3	26,2	23,5	2,7	8,7												
BSS Nov05	17,4	57,2	25,4	22,3	3,1	7,2	21,4	55,1	23,5	20,7	2,8	7,3												
ISS 02Dec04	23,1	50,0	27,1	24,2	2,9	8,4	22,9	53,0	24,0	21,5	2,6	8,3	22,6	51,2	26,2	23,3	2,9	8,0	22,8	52,7	24,4	21,7	2,8	7,7
ISS Sep05	19,9	52,3	27,8	25,6	2,1	12,0	18,3	54,7	26,6	24,2	2,5	9,7												
ISS Oct05	21,0	53,4	25,6	23,2	2,4	9,7	20,0	52,0	28,0	25,6	2,4	10,4												
ISS Dec05	20,4	55,9	23,7	21,1	2,5	8,3	18,4	57,7	23,8	21,4	2,4	9,0												
NSS Nov04	17,4	54,4	28,2	25,6	2,5	10,0	20,4	51,3	28,3	25,5	2,8	9,1	17,9	56,9	25,1	22,8	2,2	10,2	19,3	55,9	24,8	22,3	2,5	8,8
NSS Feb05	18,0	66,0	15,9	13,5	2,4	5,6	20,2	63,0	16,8	14,7	2,1	7,0												
NSS Oct05	17,2	55,2	27,5	25,2	2,4	10,5	16,5	56,1	27,3	25,0	2,3	10,7												
NSS Nov05	22,0	48,8	29,2	26,3	2,8	9,2	24,9	50,5	24,6	22,2	2,4	9,1												
NSS Dec05	23,0	48,7	28,3	25,7	2,5	10,2	19,6	53,5	26,9	24,4	2,4	10,0												

SFA=Saturated fatty acids
MUFA=Monounsaturated fatty acids
PUFA=Polyunsaturated fatty acids

Table 6. Fatty acid composition in the triglyceride (TG) fraction of the herring fat. Results are shown as % of identified peak area.

	Control samples					After 6 months					After 12 months					After 18 months				
	SFA	MUFA	PUFA	n-3	n-6	SFA	MUFA	PUFA	n-3	n-6	SFA	MUFA	PUFA	n-3	n-6	SFA	MUFA	PUFA	n-3	n-6
BSS Ap04	24,6	67,7	7,6	5,2	2,4	22,9	71,2	5,8	3,9	1,9										
BSS Sep04	22,9	57,1	20,0	17,4	2,7	25,3	55,7	19,1	16,4	2,7										
BSS Nov04	23,9	58,1	18,0	15,4	2,6	23,6	58,4	18,0	15,2	2,8	26,5	56,9	16,5	13,8	2,7	22,5	59,1	18,4	15,8	2,6
BSS Mar05	24,1	62,4	13,5	10,8	2,7	26,2	59,4	14,4	11,8	2,6										
BSS Sep05	22,2	61,4	16,4	13,9	2,5	24,5	59,0	16,5	13,7	2,7										
BSS Nov05	22,3	63,0	14,7	12,0	2,6	21,8	61,9	16,3	13,6	2,7										
ISS 02Dec04	26,4	57,0	16,6	14,2	2,4	26,5	59,1	14,4	12,2	2,2	26,1	60,8	13,0	10,7	2,3	26,2	61,0	12,8	10,6	2,2
ISS Sep05	25,0	58,2	16,8	14,8	1.9	26,5	57,9	15,8	13,8	2,0										
ISS Oct05	24,4	60,1	15,6	13,5	2,1	23,6	61,6	14,7	12,7	2,0										
ISS Dec05	24,7	62,8	12,5	10,4	2,1	24,2	63,3	12,5	10,6	1.9										
NSS Nov04	22,5	58,0	19,5	17,0	2,5	23,4	57,5	19,0	16,5	2,5	23,3	56,0	20,7	17,8	2,9	22,8	59,1	18,0	15,6	2,5
NSS Feb05	22,0	68,6	9,4	7,4	2,0	22,4	67,3	10,3	8,3	2,0										
NSS Oct05	26,6	56,6	16,8	14,7	2,12	24,4	57,7	17,9	15,9	2,0										
NSS Nov05	23,6	56,4	19,9	17,3	2,6	24,7	58,7	16,5	14,2	2,4										
NSS Dec05	25,3	57,1	17,5	15,1	2,4	22,6	61,6	15,8	13,5	2,3										

SFA=Saturated fatty acids
MUFA=Monounsaturated fatty acids
PUFA=Polyunsaturated fatty acids

Table 7. Fatty acid composition in the phospholipid (PL) fraction of the herring fat. Results are shown as % of identified peak area.

	Control samples					After 6 months					After 12 months					After 18 months				
	SFA	MUFA	PUFA	n-3	n-6	SFA	MUFA	PUFA	n-3	n-6	SFA	MUFA	PUFA	n-3	n-6	SFA	MUFA	PUFA	n-3	n-6
BSS Ap04	28,2	11,5	60,2	56,6	3,7	27,7	12,1	60,2	55,8	4,4										
BSS Sep04	20,2	16,3	63,5	60,1	3,4	27,0	22,8	50,2	46,4	3,8										
BSS Nov04	26,0	17,6	56,4	52,8	3,6	25,8	22,2	52,0	48,6	3,43	28,2	18,9	53,0	49,4	3,5	25,2	23,4	51,4	47,9	3,5
BSS Mar05	26,1	11,8	62,1	58,3	3,8	25,7	16,5	57,7	54,5	3,2										
BSS Sep05	24,9	19,9	55,2	52,2	3,0	17,7	30,7	51,6	48,2	3,4										
BSS Nov05	27,6	20,5	51,9	47,9	4,0	25,4	20,3	54,3	50,5	3,9										
ISS 02Dec04	24,2	20,5	55,3	52,1	3,2	25,5	23,9	50,6	47,7	3,0	26,8	26,3	46,9	43,6	3,3	21,6	31,9	46,4	42,9	3,5
ISS Sep05	21,6	19,7	58,6	55,1	3,5	21,5	32,2	46,3	44,1	2,2										
ISS Oct05	27,9	24,1	48.0	44,8	3,2	24,5	33,0	42,5	39,8	2,7										
ISS Dec05	28,7	25,9	45,3	42,6	2,7	25,7	25,2	49,1	46,2	2,8										
NSS Nov04	27,4	25,3	47,3	44,2	3,1	27,0	23,3	49,6	46,7	3,0	18,5	29,6	51,9	49,0	2,8	21,4	24,2	54,3	51,0	3,3
NSS Feb05	20,7	18,2	61,1	57,8	3,3	22,4	19,5	58,1	55,0	3,2										
NSS Oct05	23,3	22,8	53,8	50,5	3,3	20,9	24,9	54,2	51,6	2,6										
NSS Nov05	27,4	17,11	55,5	52,6	2,9	25,8	15,0	59,2	56,1	3,11										
NSS Dec05	28,6	24,0	47,4	44,2	3,3	24,6	28,2	47,1	43,9	3,2										

SFA=Saturated fatty acids
MUFA=Monounsaturated fatty acids
PUFA=Polyunsaturated fatty acids

There was a large difference in the relative fatty acid content between the phospholipid (PL) and triglyceride (TG) fractions of the herring fat (Table 6 and 7). The total saturated fatty acids in TG and PL were similar and ~25% of the total fat. The total monounsaturated fatty acids in TG and PL, were 60% and 22%, respectively. The total polyunsaturated fatty acids in TG and PL were 15% and 53%, respectively. Despite extensive oxidation of lipids, no obvious changes were seen in n-3 PUFA content. In the PL, docosahexanoic acid (DHA) made up about 30-40% of the total and eicosapentanoic acid (EPA) contributed to about 11% (data not shown). The dominant fatty acids in TG were monoenes like 20:1 and 22:1 (20-30% of total). These monoenes are of dietary origin which can explain their distribution.

3.4.7 α-Tocopherol (vitamin E) determination

One factor that could play a role for the development of lipid oxidation is the content of α-tocopherol (vitamin E). Therefore, as an attempt to explain observed differences in lipid oxidation, the α-tocopherol content was measured.

Generally, α-tocopherol decreased significantly in herring ($p<0.05$) with storage time for all catching places (Fig.38-40). The results showed that there was no remarkable difference in the amount of α-tocopherol among control samples from the three catcing places when expressed on a wet weight tissue basis. After 6 months storage at -20°C, the α-tocopherol content decreased, and prolonged storage period lowered the amount of α-tocopherol even more.

By comparing the initial α-tocopherol content in control samples (both on a wet weight and lipid basis) with PV and TBARS data after storage, it was not possible to directly correlate a high α-tocopherol content with lower lipid oxidation.

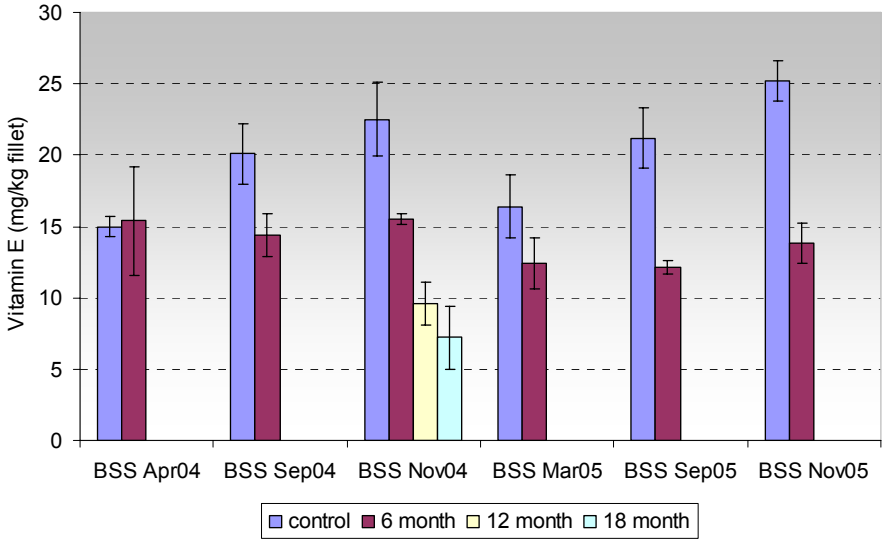


Figure 38. Content of α-tocopherol in samples from the Baltic spring spawning herring.

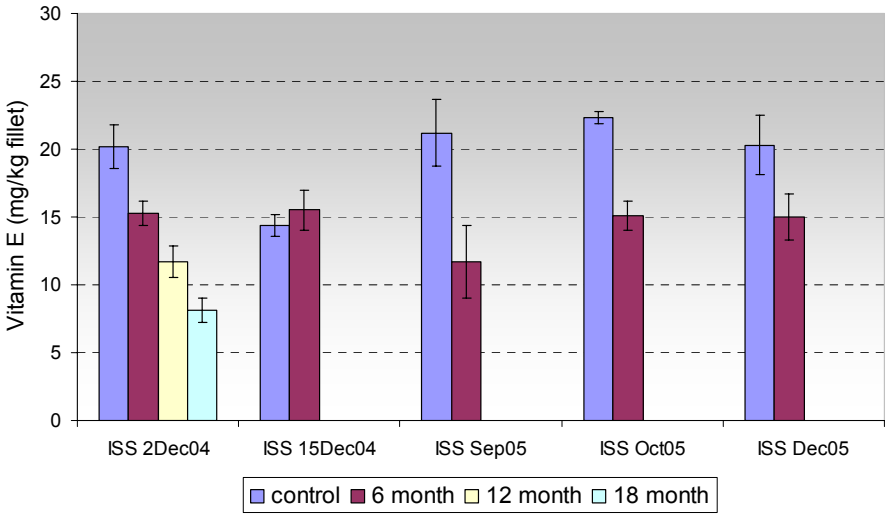


Figure 39. Content of α-tocopherol in samples from the Icelandic summer spawning herring.

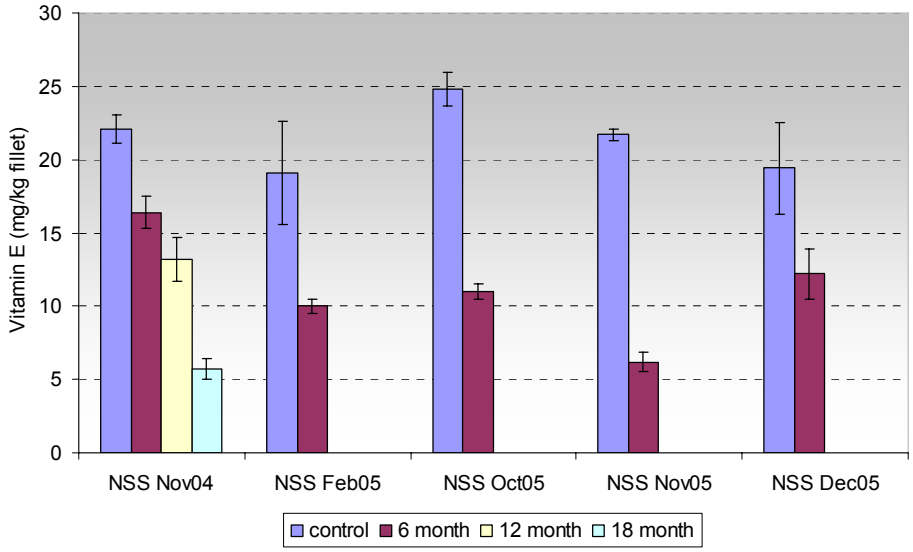


Figure 40. Content of α-tocopherol in samples from the Norwegian spring spawning herring.

3.4.8 Ratio of light and dark muscle

The distribution of light and dark muscle of fillets without skin was determined to see if there was any seasonal and/or catching area variation. The results from the herring samples showed that the ratio was fairly constant over the year and was around 80% light and 20% dark muscle (Table 8). However, from the multivariate data evaluation it seems like the small differences we actually saw played a role for lipid oxidation. The dark muscle is known to be much richer in pro-oxidants and fat than light muscle.

Table 8. The average ratio of light muscle in herring from different catching area.

	BSS	ISS	NSS
Light muscle	79,6%	76,8%	74,2%
Dark muscle	20,4%	23,2%	25,8%

3.4.9 Silvering

Silver was measured as there have been some speculations in previous herring studies that the compounds forming the silver layer (e.g. guanine crystals and melanine) could affect oxidation (Underland, 1998). The degree of silvering left after skinning could also indirectly reflect the size of the subcutaneous fat layer. Usually the silver surface gets more intact if there is a lot of subcutaneous fat, and vice versa. In this project, there were some differences in the degree of silver within the stocks. With univariate evaluations, it was not possible to see whether these differences were systematic. However, after multivariate evaluation of the herring samples stored for 6 months, this parameter correlated positively to higher degree of oxidation. Further analyses are needed for explanations of this phenomenon.

Table 9. Degree of "fillet silvering" in fillets of herring from different catching place and season and after storage.

	Control	6 month	12 month	18 month
BSS Ap04	1,17	1,50	Nm	Nm
BSS Sep04	2,33	2,0	Nm	Nm
BSS Nov04	2,6	1,42	2,0	2,1
BSS Mar05	2,0	2,5	Nm	Nm
BSS Sep05	2,25	2,1	Nm	Nm
BSS Nov05	2,4	2,2	Nm	Nm
ISS 02Dec04	2,2	1,3	Nm	Nm
ISS Sep05	1,7	2,2	Nm	Nm
ISS Oct05	2,1	1,7	Nm	Nm
ISS Dec05	2,2	1,8	Nm	Nm
NSS Nov04	1,7	1,4	2,0	2,2
NSS Feb05	2,8	2,2	Nm	Nm
NSS Oct05	2,5	2,5	Nm	Nm
NSS Nov05	2,0	3,0	Nm	Nm
NSS Dec05	2,1	2,7	Nm	Nm

3.4.10 NIR measurements

Near-infrared (NIR) reflectance measurements on individual fillets from all codes (579 measurements in total) showed small differences according to storage time and to season of catch. By comparing mean spectra it was found, however, that these differences were too small to be of practical importance.

3.5 Multivariate data analysis

In order to describe the main characteristics of the samples and to highlight their main differences, a multivariate statistical procedure, principal component analysis (PCA) was performed on sensory data obtained (Fig. 41).

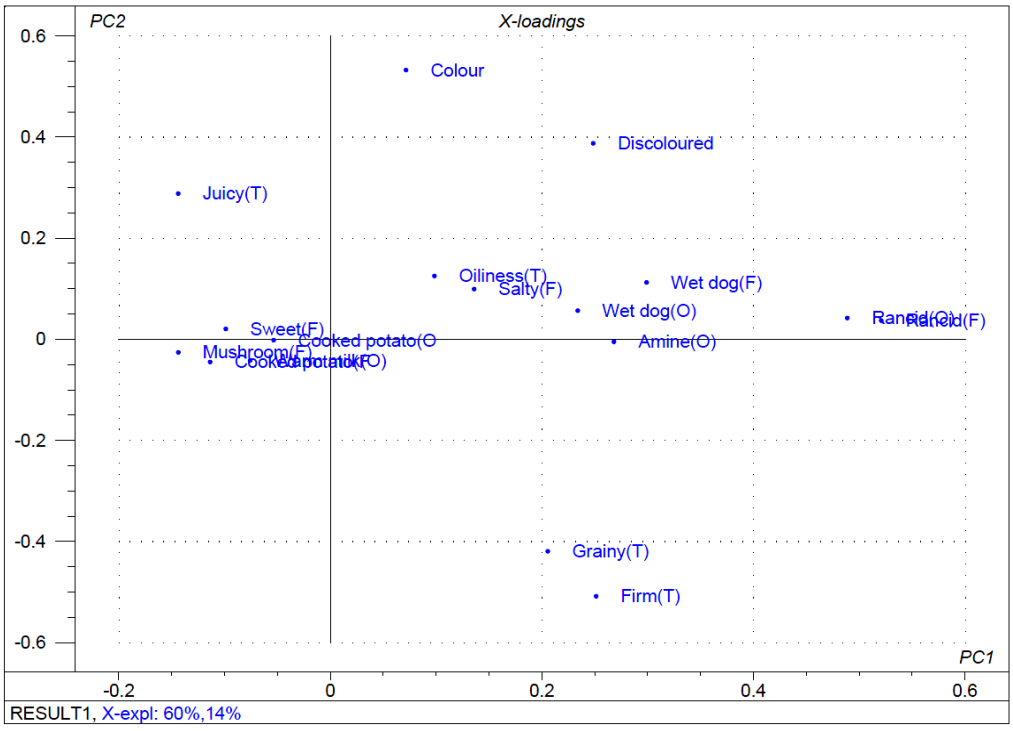


Figure 41. Loading plot for the PCA of the sensory analysis for all samples.

The sensory attributes for smell and taste explained most of the variation in the data set, and were closely aligned along a first principal component (PC1). A second PC was

explained mostly by texture parameters and colour-related differences. Together PC1 and PC2 explained 74% of the variance in the data set.

The principal component analysis of all the sensory data thus defined two generalised sensory variables, T1 and T2 (the score vectors). The first component was dominated by attributes related to rancidity whereas the second had the largest contribution from colour and texture attributes. These two generalised sensory variables were used when studying the effects on lipid oxidation and microstructure.

PLS (partial least squares) regression with place of catch and seasons as Y-variable did not show any predictions ability.

However a PLS regression with storage time as the Y-variable is able to predict storage time very well (Fig. 42 and 43). Both the PLS score plot (Fig. 42) and the predicted vs. measured plot (Fig. 43) also shows clearly that catching time and place did not have any influence as BSS, ISS and NSS at the different storage time are mixed together.

The correlation loadings plot (Fig. 44) show which of the x variables contain valuable information for this prediction. The x variables near the (red) y relate “positively”, i.e. they increase with time and the x-variables opposite y, like vitamin E behave reversely. Variables between inner and outer circles on the plot, are explained between 50% and 100% by the model, i.e. they vary in a systematic pattern with time. The two component models are able to explain 89% of the variation in the y-variable time. As well lipid oxidation as microstructure related variables are important for the PLS model of “Equivalent storage time”, which is the time at -20°C, i.e. it is 0 for the -80°C samples.

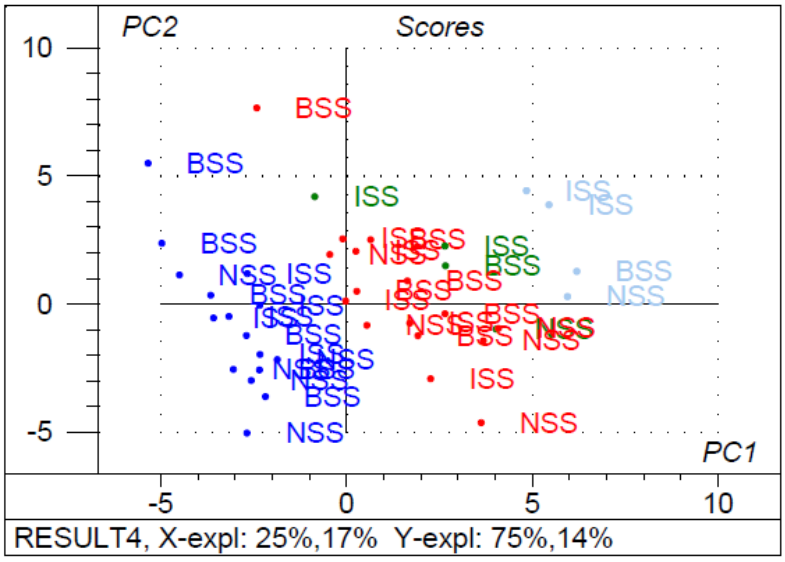


Figure 42. Score plot for the storage time of all samples. Blue=control group, red=6 month, green=12 month and light blue=18 month storage.

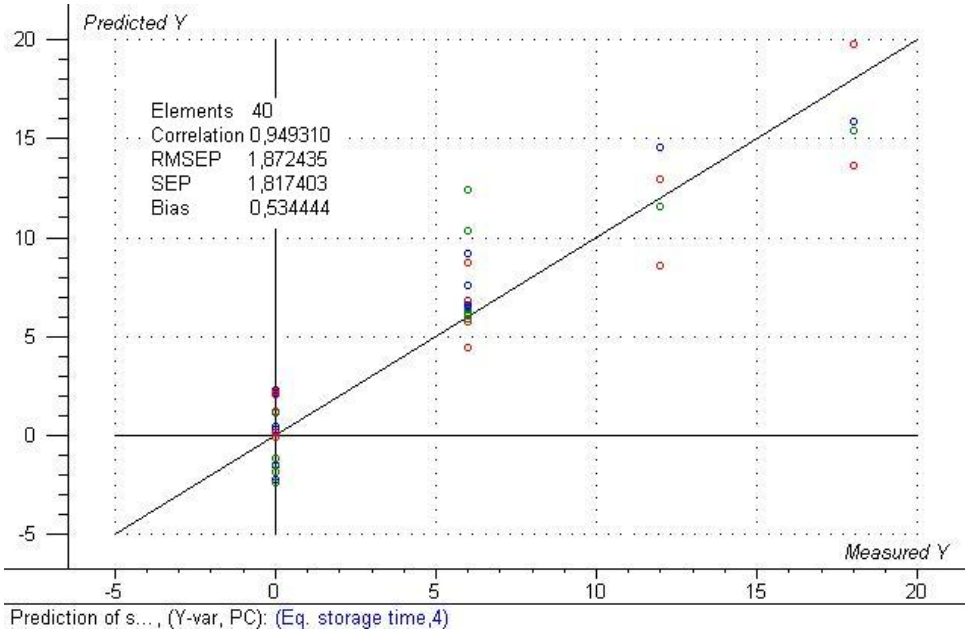


Figure 43. Prediction of storage time vs. measured storage time for all samples.

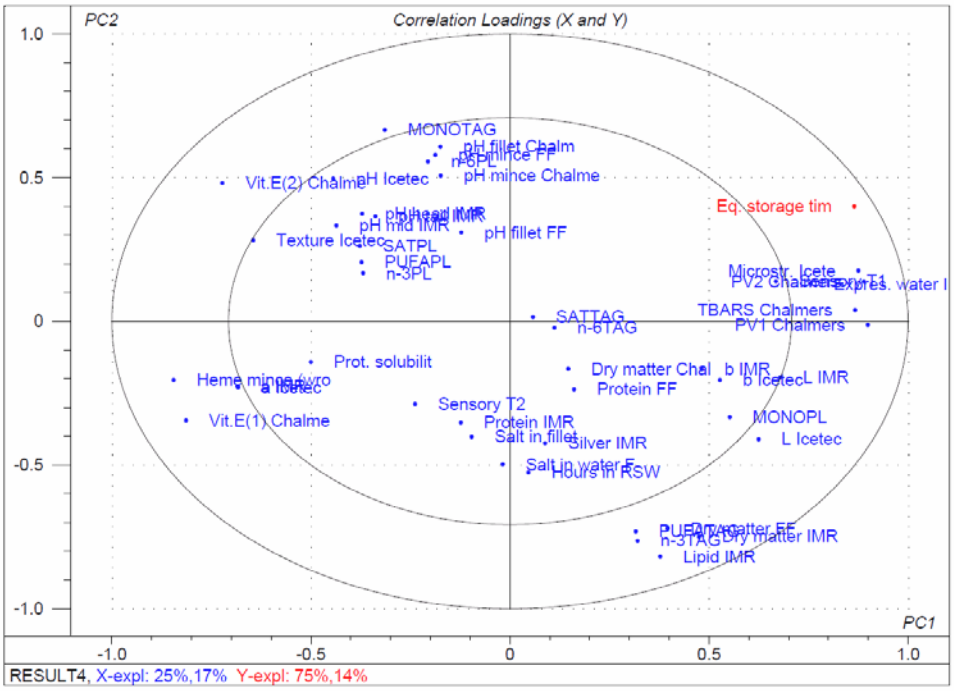


Figure 44. Loading plot for all the data analysis.

A weak relation was seen between higher pH and less oxidation. The same was true for the relation between higher protein solubility and less lipid oxidation. Also, a weak relation was seen between higher degree of silvering and more lipid oxidation. Finally, a weak relation between more light muscle and higher stability was seen.

Storage time at -20°C had a general influence on a number of variables e.g. with increasing values of PV, TBARS and expressible moisture and lower values within protein solubility, vitamin E and colour (a*).

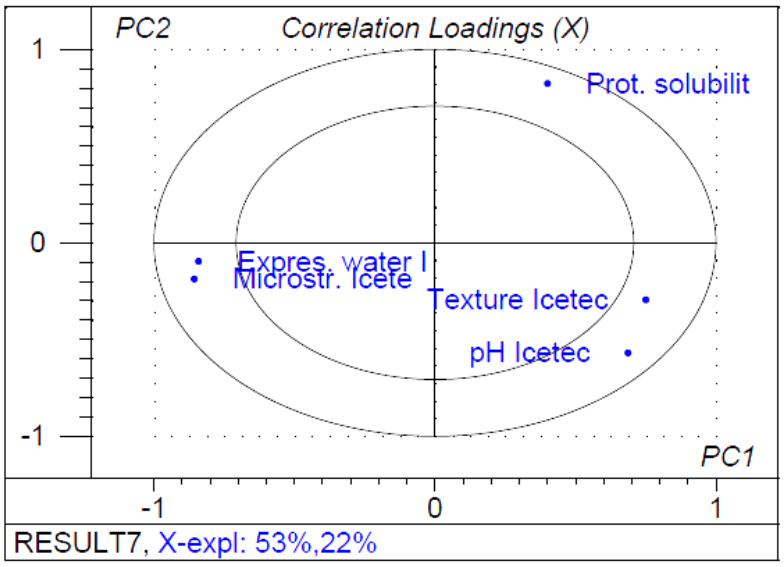


Figure 45. Correlation loading plot for the PCA of the microstructure and the texture analysis for all the samples.

A PCA model of microstructure and texture in relation to pH, expressible water and protein solubility is shown in figure 45. The amount of expressible water is closely related to microstructure (Fig. 45) and is on the first PC inversely to texture which is of course not a surprising result. Protein solubility is also governed by another phenomenon, resulting in a high PC2 (correlation) loading. When removing the time effect by looking at samples stored for 6 months only, the fraction of inter-myofibrillar space was related to the amount of expressible water and inversely related to muscle pH and texture.

When the generalised sensory variables T1 and T2 were included, it was seen that they did not change the model much, i.e. they fit to the general picture. Again no profound effect of neither place or time of catch was observed.

When only taking into consideration the lipid oxidation parameters in the PCA-analysis, the score and loading plots from analyzing all samples (Fig. 46-47) again shows how the samples were grouped mainly according to the storage time at -20°C. Longer storage times resulted in increased PV and TBARS, as well as loss of α-tocopherol and redness (a*). The loading plot shows how chemical oxidation data agreed well with sensory documentation of rancidity, and also fairly well with reduced protein solubility.

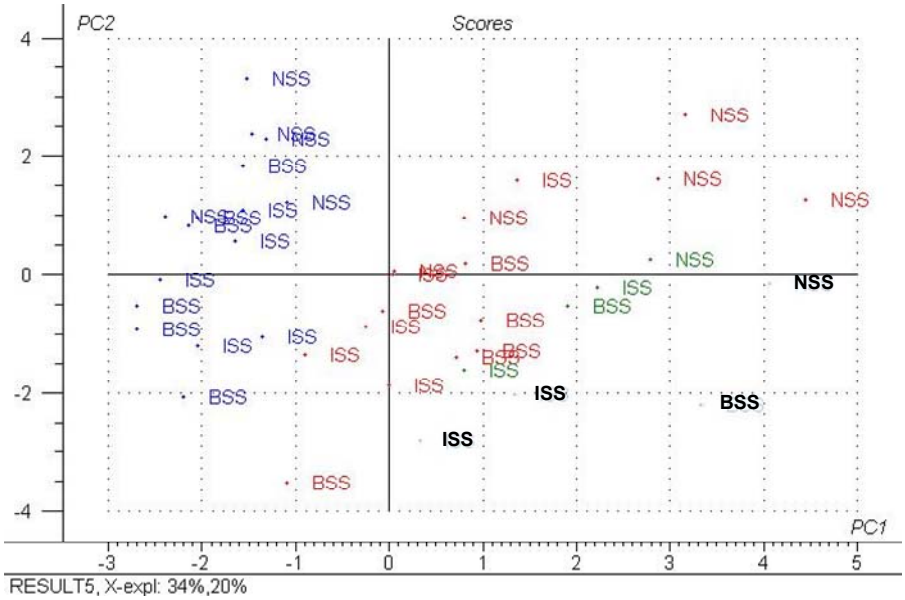


Figure 46. Scoring plot for the PCA analysis, all samples included. (Control, 6 months, 12 months, 18 months).

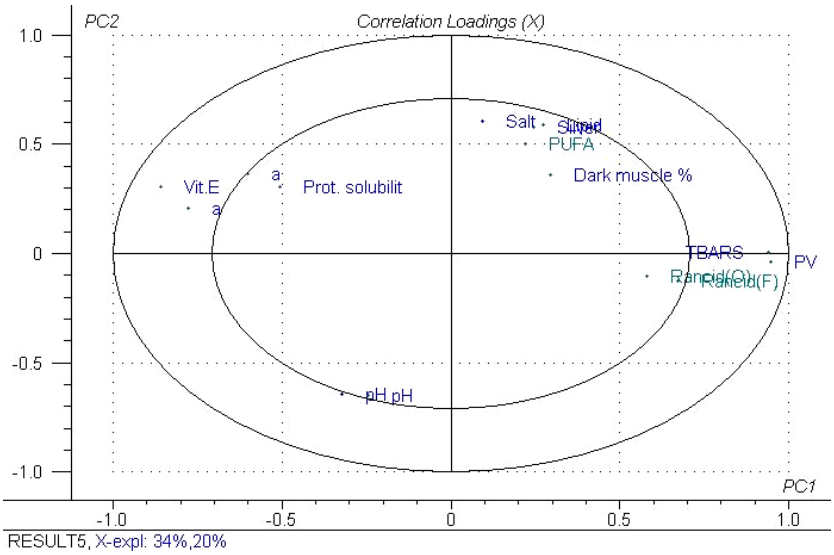


Figure 47. Correlation loading plot. All samples included in the PCA-analysis PUFA and rancid (O &F) passified.

The compositional impact on the oxidation data was evaluated in more detail by performing PCA only on samples stored for 6 months (Fig. 48-49). Almost no impact was then seen from lipid content, lipid composition, and muscle pH, while salt content,

and degree of fillet silvering showed positive correlations to lipid oxidation. Also the % dark muscle showed a fairly strong correlation to oxidation.

It was also interesting to notify that when only 6 months samples were analyzed, a weak grouping of the samples according to their catching place was found. Moving from upper left to lower right, most samples appear in the order NSS-BSS-ISS, which is in accordance with figures 3-8. Thus, the NSS samples were somewhat more oxidised, followed by the BSS and then the ISS samples.

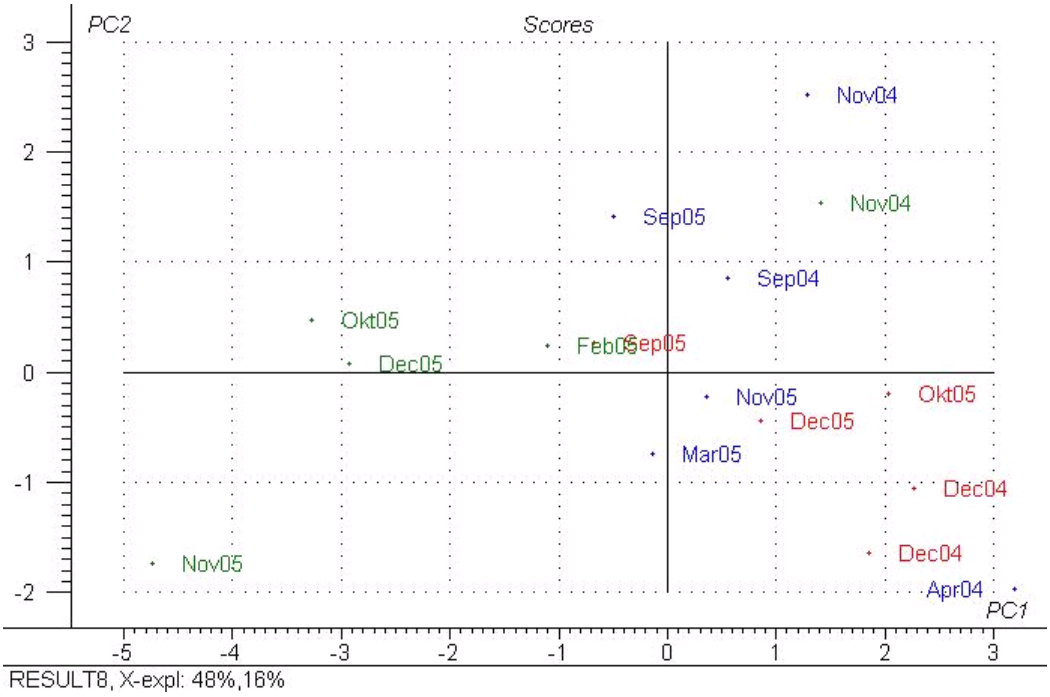


Figure 48. Score plot. Only samples stored 6 months at -20°C included in the PCA-analysis.
NSS: Norwegian Spring Spawner; ISS: Icelandic Summer Spawner; BSS: Baltic Spring Spawner.

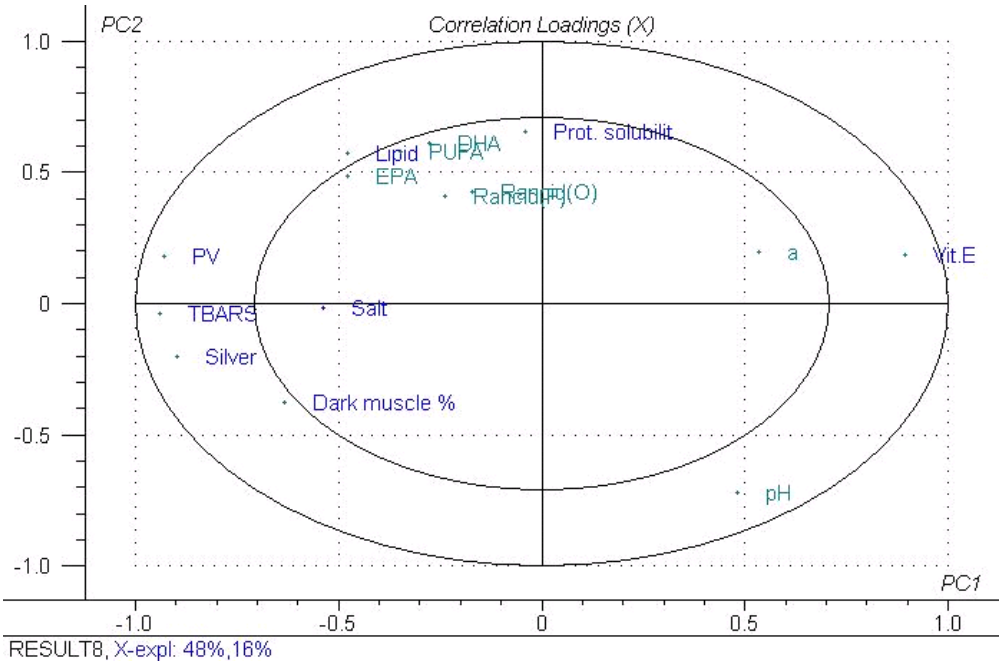


Figure 49. Correlation loadings plot. Only samples stored 6 months at -20°C included in the PCA-analysis.

4. DISCUSSION & CONCLUSIONS

Freezing of herring fillets caused changes in sensory quality and in chemical and textural properties after prolonged frozen storage. However no clear relation was observed between these changes and catching place or season in this study. The herring became rancid both in odour and flavour after 6 months of frozen storage at -20°C. Also the freezing of herring fillets caused changes in colour simultaneously with an increase in lipid oxidation. The fillet became less red, causing a less fresh and more gray appearance. These results were in accordance with results from Hamre *et al.* (2003) where same effects occurred in herring fillets after 30 weeks of frozen storage at -30°C. Herring fillets are rich in polyunsaturated fatty acids, which are very susceptible to peroxidation. In addition, herring contains a high level of catalytic haeme-proteins and has a relatively low *post mortem* muscle pH. Low pH activates haeme as a catalyst of lipid oxidation, and thus, the combination of highly unsaturated fat and active catalysts makes herring very susceptible for lipid oxidation. The primary products of lipid oxidation are the fatty acid hydroperoxide, measured here as peroxide value (PV). Maximum recommended value of PV for human consumption is 10mmol/kg lipid. This is in accordance with observed PV values in frozen herring by Hamre *et al*., (2003). In the present study the maximum value for PV reached 10-18 mmol/kg lipids after 12 months in frozen storage.

Longer frozen time also resulted in increased TBARS, as well as loss of α-tocopherol (vitamin E). α-Tocopherol reacts e.g. with fatty acid peroxide radical, preventing the radical from reacting with new fatty acids and thereby breaking the chain reaction of auto-oxidation.

The herring was kept for 30±3 hours in refrigerated sea water (RSW) after catch. From reviewing a great number of papers, Undeland and Lingnert (1999) reported that prefreezer storage in refrigerated seawater (RSW) compared to storage in air on ice gives better frozen storage stability. They also reported that the frozen storage stability is improved by minimal tissue disruption, removal of oxygen, fast freezing, and low freezer storage temperature reduces rancidity and gives longer shelf life. Thus from a chemical point of view the herring fillets should preferably be frozen sooner than 3 days after catch.

The compositional impact on the oxidation data was evaluated by performing PCA only on samples stored for 6 months. No impact was then seen from lipid content and muscle pH, while salt content, ratio of dark muscle and degree of fillet silvering showed positive correlations to lipid oxidation.

The increase in moisture content and decrease in lipid content in spring-spawning herring from November to March is in accordance with previous studies (Hamre *et al*., 2003; Slotte, 1999) and is a result of the wintering and spawning migration periods, where the herring do not feed but depend on their body energy stores accumulated during spring and summer.

Sigurgisladottir *et al.* (2000) studied the changes in microstructure and texture during smoking of fresh and frozen/thawed Atlantic salmon. The salmon was stored for 1 month at -20°C. They concluded that freezing did affect the muscle structure of the smoked fillets, the fibers shrank and extracellular space increased. In the present study the results of textural measurements in herring varied between the years 2004 and 2005. In the year of 2004 the results indicated an increase in hardness over storage time from 0-12 months but after storage for 18 months the hardness decreased. In 2005 the hardness of the herring fillets decreased after 6 months of storage. The reason is not known, but maybe it is due to the prolonged proteolysis of the protein by catepsin.

However earlier reports have shown that protein denaturation, water loss and toughening of fish flesh are associated with frozen storage (Gill *et al*., 1979; Howgate, 1979; Kreuger and Fenema, 1989: Mackie, 1993; Nilson, 1994). During frozen storage both the myofibrillar proteins and the collagen aggregate inducing a toughening of the muscle (Barroso et al. 1998; Montero and Borderias, 1993). Experiment by Kolakowska *et al*., (1992) on changes in textural properties of Baltic herring during storage on ice caught at different time during the year showed that the herring decreased in elasticity with storage. There was a relationship between the lipid content and hardness in all herring fillets. Fat content decreased with increasing hardness values. This is in accordance with Dunajski (1979) cited by Hyldig and Nielsen (2001), who stated that lipid reduced the structural factors of muscle tissue, lowering its mechanical strength, i.e. fish higher in lipid content were softer in texture. Nielsen *et al.,* (2005) also found correlation between the texture and lipid content in herring fillets. Liquid holding capacity (LHC) and texture are influenced by a variety of factors, e.g. lipid content. There was a linear correlation

between the expressible moisture content and the hardness measurements in herring fillets. Other studies have shown similar trends (Jonsson *et al*. 2001; Nielsen et al., 2005). No obvious changes occurred in n-3 PUFA after different storage time, despite extensive oxidation of lipids.

The main conclusion of the project is that no clear differences were found in sensory quality, lipid oxidation and microstructure of herring on batch level regarding time of catch and catching areas: Kattegat/Skagerak, around Iceland and along the Norwegian coast.

5. ACKNOWLEDGMENTS

The authors would like to give Hannes Hafsteinsson, the coordinator of the project special thanks for starting up and leading this project. Hannes unfortunately past away from his illness last July, and could not see the project finished. The authors at Chalmers would like to thank Nils-Gunnar Carlsson for helping with the vitamin E analysis and the Swedish Institute for Food and Biotechnology (SIK) for lending us their freezing equipment. All the authors thank the employees of the Technological Institute of Iceland (IceTec), Paul Mattson AB, Sildarvinnslan and other institutes and companies for the use of facilities and sampling of herring. At last we would like to thank NIC for their financial support in this project.

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APPENDIX I. SAMPLING PROTOCOL

Cathching method:

Icelandic summer spawners (Matis) and Norwegian spring spawners (IMR) were caught by purse seine. Baltic spring spawners (Chalmers/Paul Mattsson AB) were caught by trawl.

Transportation from catch to landing

Herring is stored in RSW (Refrigerated Sea Water)

Following parameters are recorded:

Catching area

Catching time

Temperature in tanks

At landing.

Herring used: Matis/IMR: 280 g to 350 g; Chalmers 150 to 200g

Temperature in whole fish was recorded – carried out on 10 fish

Landing 30±3 hours after catch:

Herring was machine filleted to butterfly fillets. 50 kg of butterfly fillets were sampled and manually packed in cardboard boxes. Temperature was measured in fillets (10 fillets, measurement in loin part) before packing (See packing procedure) and frozen to –20°C within two hours. Time from sampling to freezing was recorded. Temperature loggers were placed in a box for own use and in boxes that afterwards were sent to collaborating institutes.

Packing procedure:

Butterfly fillets were flattened and packed, meat against skin side in two layers with 3 fillets in each box (box size: 18 cm x 24 cm x 3 cm). Boxes for –20°C were labelled –20°C. Boxes for –80°C were labelled –80°C

Landing earlier than 27 hours after catch:

100 kg herring was sampled after sorting and placed in container with ice and seawater until 30 ±3 hours after catch and was processed as under 2A

Transportation from processing plant to institute

Transportation time: Carton boxes were packed in polystyrene boxes with dry ice or transported by freezing van.

Repacking and storage of reference sample for own use and for collaborators

Boxes for –80°C were vacuum packed and stored at –80°C. Boxes for –20°C were placed in plastic bags and stored at –20°C.

Minimum amount of boxes for the different institutes.

DIFRES: 5 (-20°C) + 5 (-80°). For the September 2004 sampling: 15 (-20°C) + 5 (-80°C)

Matis: 3 (-20°C) + 3 (-80°). For the September 2004 sampling: 9 (-20°C) + 3 (-80°C)

IMR: 2 (-20°C) + 2 (-80°). For the September 2004 sampling: 6 (-20°C) + 2 (-80°C)

Chalmers: 2 (-20°C) + 2 (-80°). For the September 2004 sampling: 6 (-20°C) + 2 (-80°C)

Storage of frozen samples

Fillets at –20°C were stored for 6 months. If fillets were not analysed after 6 months, boxes were vacuum packed and placed at –80°C until analysis.

For September 2004 samplings samples were also stored 12 and 18 months.

Transportation of samples to collaborators

Carton boxes were packed in polystyrene boxes with dry ice and sent immediately with a courier company. At sending message was sent by email to the receiving institute

Thawing of samples

Herring was packed in plastic bags and thawed under running cold water.

APPENDIX II – SAMPLING AND STORAGE INFORMATION SHEET

Sampling code according to sampling plan	
Catching date and hour	
Catching area (latitude and longitude)	
Catching method	
Temperature in tank onboard ship	
Date and hour of landing	
Hours onboard ship	
Temperature in whole fish after loading	
Storage period on land in tank with RSW	
Temperature in whole fish after tank storage	
Average size of whole fish	
Average size of fillets	
Temperature in fillets before packing	
Date and hour of packing	
Freezing procedure	
Recording of temperature during freezing	
Transportation time from freezing equipment to institute,	
Date, transferring minus 20 samples to minus 80	
Date and hour of packing samples for receiving institute	
Institute send to	
Date, hour, receiving samples	
Condition of samples	
File of temperature recording during transportation	
Date, transferring minus 20 samples to minus 80	
Date of thawing samples	
Period of thawing	
PH in fillets	

APPENDIX III – PLAN FOR CATCH OF HERRING FROM THE DIFFERENT STOCKS

The samples are marked with stock_month_year

The background colour indicates the time of the year and the stock.

2004

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Okt	Nov	Dec	
IMR (NSS)		**NSS_26-27Feb04**								**NSS_01Okt04**	**NSS_nov04**	**NSS_Dec04**	NSS (NVG)
Matis (ISS)											**ISS_Okt04**	**ISS_Dec04**	ISS (ISG)
CTH (BSS)		**BSS_16Feb04**		**BSS_13Apr04**					**BSS_09Sep04**		**BSS_04**		BSS (ØFG)

2005

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Okt	Nov	Dec	
IMR (NSS)									**NSS_Sep05**		**NSS_Nov05**	**NSS_Dec05**	NSS (NVG)
Matis (ISS)									**ISS_Sep05**	**ISS_Okt05**		**ISS_Dec05**	ISS (ISG)
CTH (BSS)			**BSS_Mar05**						**BSS_Sep05**			**BSS_Dec05**	BSS (ØFG)

APPENDIX IV – PLAN FOR ANALYSIS OF HERRING FROM THE DIFFERENT STOCKS

The samples are marked with stock_month_year. The background colour indicates the time of the year and the stock.

The red letters is 0-sampels (reference) and storage at -80°C, the green letters are samples storage at -20°C.

For each catch, the 0-sample is stored at -80°C and regarding the sensory measurements it will be analysed together with the samples stored for 6 months at -20°C. September samples 2004 will be stored for 6, 12 and 18 months (md), all other will only be stored for 6 month at -20°C.

	2004										
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Okt	Nov
NSS (NVG)						NSS_Jan-04_Pilot test					
NSS-6md						NSS_Jan-04_Pilot test					
NSS-12md											
NSS-18md											
ISS (ISG)											
ISS-6md											
ISS-12md											
ISS-18md											
NAS (NEG)											
NAS-6md											
NAS-12md											
NAS-18md											
BSS (ØFG)									BSS_Mar_04		
BSS-6md									BSS_Mar_04		
BSS-12md											
BSS-18md											

2005

	Jan	Feb	Mar	Apr	Maj	Jun	Jul	Aug	Sep	Okt	Nov
NSS (NVG)			NSS_Sep_04		NSS_Nov_04	NSS_Dec_04					
NSS-6md			NSS_Sep_04		NSS_Nov_04	NSS_Dec_04					
NSS-12md									NSS_Sep_04		
NSS-18md											
ISS (ISG)			ISS_Sep_04	ISS_Okt_04		ISS_Dec_04					
ISS-6md			ISS_Sep_04	ISS_Okt_04		ISS_Dec_04					
ISS-12md									ISS_Sep_04		
ISS-18md											
NAS (NEG)			NAS_Sep_04								
NAS-6md			NAS_Sep_04								
NAS-12md									NAS_Sep_04		
NAS-18md											
BSS (ØFG)			BSS_Sep_04			BSS_Dec_04			BSS_Mar_05		
BSS-6md			BSS_Sep_04			BSS_Dec_04			BSS_Mar_05		
BSS-12md									BSS_Sep_04		
BSS-18md											

2006

	Jan	Feb	Mar	Apr	Maj	Jun	Jul	Aug	Sep
NSS (NVG)			NSS_Sep_05		NSS_Nov_05	NSS_Dec_05			
NSS-6md			NSS_Sep_05		NSS_Nov_05	NSS_Dec_05			
NSS-12md									
NSS-18md			NSS_Sep_04						
ISS (ISG)			ISS_Sep_05	ISS_Okt_05		ISS_Dec_05			

ISS-6md			ISS_Sep_05	ISS_Okt_05		ISS_Dec_05			
ISS-12md									
ISS-18md			ISS_Sep_04						
NAS (NEG)			NAS_Sep_05						
NAS-6md			NAS_Sep_05						
NAS-12md									
NAS-18md			NAS_Sep_04						
BSS (ØFG)			BSS_Sep_05			BSS_Dec_05			
BSS-6md			BSS_Sep_05			BSS_Dec_05			
BSS-12md									
BSS-18md			BSS_Sep_04						

APPENDIX V – ANALYTICAL METHODS BY LABORATORIES

PARAMETER	Matis Lab. 1	IMR Lab. 2	DIFRES Lab. 3	Chalmers Lab. 4
Photograph	x			
Colour, discoloration	x	x		
Microstructure	x			
Near Infrared Transmission			x	
Peroxid value				x
pH	x	x	x	x
Salt			x	
Sensorial analysis			x	
Texture	x			
TBARS				x
Expressible moisture	x			
Dry Matter		x	x	x
Lipid		x		
Protein		x		
Fatty acid composition		x		
Ratio between dark and light muscle	x	x		x
Protein solubility		x		
Alfa-tocopherol (E-vitamin)				x
NIR-analysis			x	
Haemoglobin concentration				x
Statistical analysis	x	x	x	x