Skýrsluágrip Rannsóknastofnunar fiskiðnaðarins

Icelandic Fisheries Laboratories Report Summary

Titill:	Frozen herring as a raw material for spice-salting	
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Ágrip á íslensku:	Síld veidd í október 1995 var annars vegar kryddsöltuð fersk og hins vegar kryddsöltuð eftir 32 daga frystigeymslu við -24°C. Síldin var að öðru leyti meðhöndluð á sama máta. Eftir söltun voru þrjár tunnur af frystu þíddu síldinni sendar á rannsóknarstofur í Evrópu (Rf, FF, Norconserv, FRCF og HIFI) ásamt einni tunnu af fersksaltaðri síld. RIVO-DLO tók einnig þátt í rannsókninni með mælingum á útdregnum sýnum. Síldin var geymd við 5°C og sýni tekin eftir 4, 16 og 26 vikur frá söltun. Sýni voru tekin af ferksöltuðu síldinni eftir 21 viku. Á verkunartímanum var fylgst með skynmatsbreytingum, efnabreytingum (heildar efnasamsetningu, TCA leysanlegu köfnunarefni, breytingum á smápeptíðum, α-amino köfnunarefni, fríum amínósýrum, ATP niðurbrotsefnum og almennri og sérhæfðri próteinsundrandi virkni), breytingum á áferð (metnar með tækjum) litabreytingum og orkumatsbreytingum. Skynmatsniðurstöður allra rannsóknarstofa sýndu að frysta þídda síldin verkaðist á sambærilegan máta og fersksaltaða síldin. Áferðarmat með tækjum sýndi að frysta þídda síldin meyrnaði hraðar en fersksaltaða síldin. Mun hraðara niðurbrot á próteinum í formi TCA leysanlegs köfnunarefnis, α-amino köfnunarefnis, fríum amínósýrum og breytingum á smápeptíðum átti sér stað í frystu þíddu síldinni. Mun meiri almenn og sérhæfð próteinsundrandi virkni fannst í frystu þíddu síldinni í samanburði við ferksöltuðu síldina. Enginn litarmunur fannst á milli ferksöltuðu og frystu þíddu síldarinnar. Í heild sýndu niðurstöður að kryddsöltuð fryst þídd síld verkaðist á sambærilegan máta og ferksöltuð síld en mun hraðar.	
Lykilorð á íslensku:	Síld, verkun, söltun, fersk frosin	
Summary in English:	In this study one batch of herring (*Clupea harengus*) caught in October 1995 was on the one hand spice-salted fresh and on the other spice-salted after 32 days freezer storage at -24C. Both herring groups were processed and handled in the same manner. After salting three barrels of the previously frozen herring and one barrel of fresh salted herring were sent to the participating laboratories in Iceland, Denmark, Norway, Germany and England. RIVO-DLO in the Netherlands participated also by analysing extracts. The herring was kept at 5±1°C and sampled after 4, 16 and 26 weeks from salting. The fresh salted herring was sampled after 21 weeks storage. Ripening was evaluated by sensory evaluation, chemical analysis (proximate composition, TCA soluble nitrogen, peptide fingerprinting, α-amino nitrogen, free amino acids, ATP breakdown compounds and general and specific proteolytic activity), instrumental texture measurements, colour analysis and differential scanning calorimetry. The sensory evaluation results showed that the previously frozen herring ripened in a similar manner to that of the fresh herring but faster. The laboratories obtained the same trend for the samples but there was a variation in actual values. Instrumental texture analysis showed faster tenderising in the previously frozen herring. The formation of breakdown products (TCA soluble nitrogen, peptide fingerprinting, α-amino nitrogen, free amino acids) was much faster in the previously frozen herring. Both general and specific proteolytic activity was much higher in the previously frozen herring. No difference was detected in the colour of samples or in thermoanalytical behaviour of pyloric caeca between fresh and previously frozen herring. The results as a whole show that spice-salted frozen thawed herring ripens in a similar manner to that of fresh salted but much faster.	
English keywords:	Herring, ripening, salting, frozen, fresh	

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1. INTRODUCTION

The salting of fresh herring is a common practice in many European countries. Frozen herring is normally not used for salting; it is generally believed within the salt-herring industry that freezing the raw material renders it unsuitable for salting. Frozen thawed herring is said to ripen in a different manner to that of fresh and does not obtain the characteristic ripened taste (Pálsson, 1981). In recent years the supply of herring, mainly from the Atlanto-Scandic stock, has increased thus leading to considerable amounts of herring being supplied as whole frozen fish. Eastern European countries buy frozen whole herring and part of it appears to be salted (Podeszewski and Iwaszkiewicz, 1972). The question then arises can thawed frozen herring be used for salting and if so, does it ripen in a similar manner to that of fresh herring? There is considerable interest within the herring industry on using previously frozen herring for salting as such raw material may offer new opportunities in production and/or products.

Little research appears to have been carried out on the suitability of salting frozen thawed herring e.g. in regard to ripening, quality and storage life. One trial has been carried out in Iceland and the main finding was that the herring ripened in a similar manner to that of fresh but rancidity appeared to limit the storage life of both raw material and the products (Stefánsson, 1992). Research has also been carried out in Portugal on the suitability of using frozen thawed sardines for anchovy production (Mendes *et al.*, 1994). It has been reported that the penetratibility of fish muscle changes after freezing and subsequent defrosting thus accelerating the salting in process of herring by a factor of 1.2 to 1.3 (Shenderyuk and Bykowski, 1990).

The purpose of the research was to ascertain that thawed frozen herring ripened during salting as determined by sensory evaluation, chemical, biochemical and physical methods. This was accomplished in a collaborative trial between 6 of the participating research laboratories (Iceland, Denmark, Norway, England, Germany and The Netherlands) within the AIR project “Enzymatic ripening of pelagic fish species” (AIR 2 CT 93 1141) and thus in a controlled innovative process applying part of the acquired knowledge from the project.

2. MATERIALS AND METHODS

2.1. Salting

The herring *(Clupea harengus)* used in this experiment was caught to the south east of Iceland in October 1995. The herring was from the Icelandic summer spawning stock. The herring was landed and processed at Borgey fish processing plant in south east Iceland. The herring had been kept on ice for approximately 2 days before salting

and been size graded. The size 3/500 was used (300 to 500 herring in each 100 kg portion).

Fresh herring. The major part of the herring was beheaded in machines leaving all the intestines in the fish and salted in a standard industrial manner using the following recipe: 7,9 kg salt, 3,7 kg sugar and 390 g of spices (containing $NaNO_3$) per 54,5 kg herring. Twenty one (60 litre) barrels were used. The herring was mixed with the dry materials and put into the barrels. The herring was allowed to settle overnight in the barrels before filling up with 20° brine. The barrels were divided randomly into 3 groups (7 barrels in each) and stored at 0°C, 5°C and 10°C, respectively. During the first 8 weeks the barrels were rolled each week to ensure an even salt uptake and good ripening. The fresh salted herring was used by the Icelandic Fisheries Laboratories, IFL, in a trial to estimate the effect of temperature on ripening.

Frozen thawed herring. The rest of the herring raw material (650 kg) was wrapped in polyethylene films (9 kg quantities) and blockfrozen in cardboard boxes using plate freezers. The herring blocks were kept in freezer storage for 32 days at -24°C. After this period the herring was taken out of the freezer, unwrapped and thawed in running water (5-10°C) overnight. After thawing the herring was beheaded by hand (leaving all the intestines in the fish) and salted using exactly the same procedure and recipe as for the fresh salted herring. Due to the use of smaller barrels (30 litre instead of 60 litre) the recipe was adjusted accordingly: 27,5 kg herring (headless), 4,0 kg salt (large crystals), 1,8 kg sugar, 200 g spice (containing $NaNO_3$). Altogether 15 barrels were salted. The dry materials were mixed together shortly before salting. The herring was mixed thoroughly with the dry materials and put into the barrels. The herring was allowed to settle overnight before filling up with 20° saltbrine. After salting the herring was stored at 5±1°C.

Three herring barrels were sent to each of the participating laboratories in Norway (Torstein Skåre and Sissel Olsen, Norconserv, Stavanger), Denmark (Henrik H. Nielsen, Danish Institute for Fisheries Research, FF, Lyngby), Germany (Jørg Oehlenschläger and Reinhard Schubring, Institute for Biochemistry and Technology, FRCF, Hamburg) and England (Simon Derrick and Bill Horner, Humberside International Fisheries Institute, HIFI, Hull) along with a small container of freshly salted herring (approximately 5 kg); the remaining three barrels were stored at IFL. RIVO-DLO in Ijmuiden (Joop Luten), The Netherlands participated in the trial by analysing samples prepared by Norconserv. The laboratories stored the samples at 5°C and rolled the barrels once every week during the first weeks of salting. The outline of the trial can be seen in Figure 1.

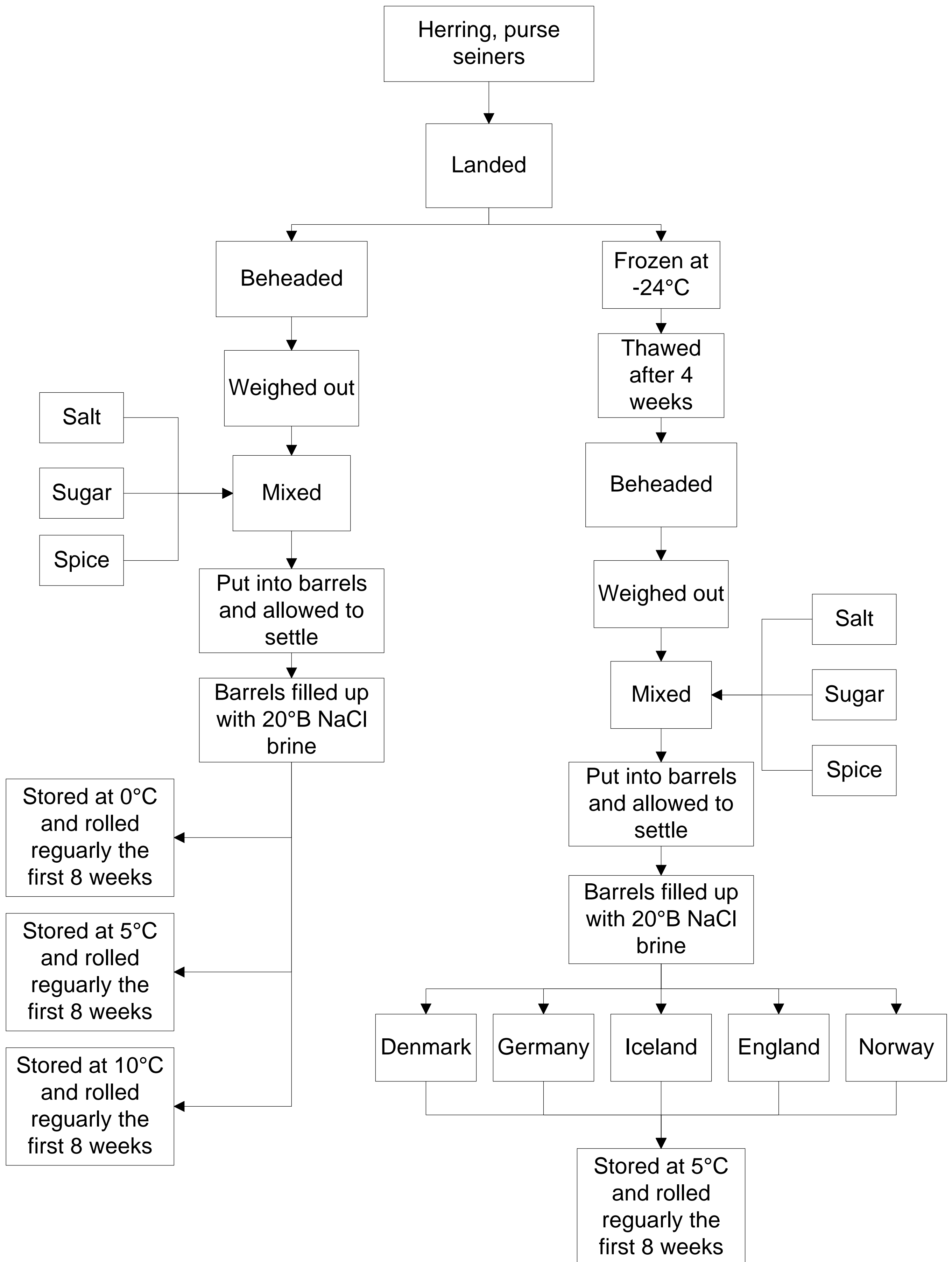


Figure 1. Outline of the trial

2.2. Sampling and preparation of samples

Sampling of brine and herring was carried out in week 4, 16 and 26. The fresh salted herring was sampled at the second sampling point, that is after 21 weeks salt storage. At IFL in Iceland the fresh salted herring kept at 5°C was sampled at 4, 8, 13, 16, 20, 24 and 31 weeks. Fresh salted herring sampled at 8 and 16 weeks was also sent to FF in Denmark for comparison purposes. At each sampling point a new barrel was used. Before sampling the barrel was turned over 3 to 4 times before being opened. After opening, the temperature of brine was determined using a thermometer and the brine strength using a densitometer. Samples were taken in triplicate from brine (3 x 250 mls) and herring (3 x 5 herring for sensory analysis and 3 x 5 herring for other determinations). The herring was always taken from within the depth of the barrel. The brine was filtrated before carrying out measurements. The herring was washed, filleted and skinned by hand. Fillets or fillet pieces were used for sensory and texture analysis but for chemical and biochemical analysis a mince prepared from the fillets was used. Most analyses (e.g. sensory analysis) were carried out within the sampling week; in the cases where this was not possible the samples (mince or brine) were frozen and kept at -24°C until the measurements could be carried out. Samples for biochemical determination (muscle extract and brine for enzyme activity analysis) were stored at -85°C until the measurements could be carried out. Extracts stored at -85 °C were thawed as fast as possible under hot water and put on ice immediately after thawing. Frozen mince was taken out of the freezer and kept at room temperature until it started to thaw; then a sample was taken for measurements.

2.3. Measurements

2.3.1. Sensory analysis. Sensory analysis was carried out by IFL, FRCF, Norconserv and HIFI. At IFL the analysis was carried out using a trained in house panel. The panel consisted of ten people who had previously been selected by a procedure similar to the one described by Meilgaard *et al.* (1991) and trained in the evaluation of fish freshness using the Torry scale. The panellists were then specifically trained in descriptive analysis of spice-salted herring and had received at least 80 hours of training. Eight attributes for taste were used: ripened, raw, malty/creamy, stock fish, salty, sweet, spicy and aftertaste. Ripened taste in spice-salted herring can be described as a creamy/malty taste commonly associated with high fat cream, soft creamy caramel and malt bread. Stock fish taste is commonly associated with the taste of dried ocean catfish. Raw taste in spice-salted herring can be described as the taste of fresh herring commonly associated with the taste of raw (fatty) fish and blood. Each taste attribute was evaluated on an intensity scale anchored at both ends (0 - none to 100 - strong). Three attributes for texture were used: softness, watery and toughness. Softness was defined as the initial force

required to compress the sample between the molars. Softness was evaluated on a 100 point scale anchored by the opposites very firm to very soft. Watery was defined as the amount of moisture released on the initial bite of the sample. Watery was evaluated on a 100 point scale anchored by the opposites not watery to very watery. The panellists were trained in the use of the profile scheme by evaluating spice-salted herring at varying stages of ripening, including extreme samples (no ripening and fully ripened). Training was considered adequate when panellists came to a consensus on the intensity of each attribute. For the purpose of this work a consensus was considered the mean value ±10 points (on a 100 point scale). Retraining was carried out every four weeks. Panellists evaluated each sample, in triplicate, in separate booths under normal light in IFL´s sensory evaluation laboratory using a computerised system (Hypersense, IFL, Iceland) for direct recording of data. Only two samples were evaluated in each session and only two sessions were carried out each day. The panellists used water (10°C) and unsalted crackers for palate cleaning between samples.

At Norconserv samples for sensory analysis were prepared in a different manner to that of IFL. Prior to sensory analysis, the herring was washed, filleted, skinned, packed in glass jars with brine (about 50 % sugar and 10 % acetic acid) and stored at 4°C. A sensory panel consisting of 9 panellists evaluated the samples with respect to taste and texture (ripened taste, malty, salty, sweet, spicy, aftertaste and smoothness, softness, and tenderness respectively) using intensity scores from 1 (none) to 7 (maximum). The different samples from each sampling month were assigned random three digit numbers and presented to the panel in different combinations during a period of 2 weeks (5 samples per session). The panel was trained before the assessments, using commercially available Norwegian spice-salted herring products and raw herring, stock fish and malt extract. The latter were used to introduce the extreme taste of some attributes.

At FRCF the herring was filleted by hand and skinned, placed on a dish and allowed to stand until ambient temperature was reached. The samples were presented to the panellists without further treatment (no desalting, no watering etc.). The panel consisted of 8 members trained in testing lightly to heavily salted herring products. The herring samples were presented to the panel as three individual samples. The panel members did not know until the end of the experiment that the three samples presented in a session were in fact identical. The samples were judged as independent samples. The panel members used for the assessment a sensory scheme developed specially for testing salted herring products. This scheme (see Appendix) is based on a Quality Descriptive Analysis method (QDA). In the scheme the assessors had to make a mark on a scale ranging from 0 to 100 for each individual descriptor. 0 on the scale means that the descriptor is present in its lowest intensity or absent. 100 means that the descriptor is present at its maximum intensity. The panel

members were allowed to test the three samples offered to them singly or together. The results obtained were calculated using the program STATISTICA, (Statsoft, Tulsa, USA). The results obtained at one of the three sampling dates were used as the basis for the calculations. That means that for a single descriptor a total of 24 (8 panel members x 3 scores) values could be used.

2.3.2. TCA soluble nitrogen. IFL and FF carried out analysis of TCA soluble nitrogenous compounds. Protein in muscle and brine was precipitated by adding 10% TCA (trichloroacetic acid). After filtration the amount of low-weight nitrogen compounds soluble in TCA were measured by the semi-macro Kjeltec method (See 2.3.9). Both total nitrogen and TCA-soluble nitrogen compounds were calculated as protein content by multiplying the measured nitrogen with the factor 6.25. TCA index was calculated as the amount of TCA soluble protein in percentages of the total protein content.

2.3.3. Peptide fingerprints. FF analyzed the changes in the pattern of low-molecular weight peptides in brine and muscle during ripening by reversed phased HPLC. 500 ml of 10 % TCA were added to 500 ml of brine and muscle extract; protein was allowed to precipitate in 30 minutes at room temperature and then centrifuged. The supernatant was filtered through a 0.45 mm filter into a HPLC vial and analyzed on a HPLC system (LKB/Pharmacia, Sweden) using a C_2/C_{18} column (Superpac, Pep-s, 5 mm, Pharmacia) and an ethanol gradient. The mobile phases consisted of HPLC grade water containing 0.1% trifluoroacetic acid and 0.02% triethylamine (mobile phase A, pH approx. 2) and ethanol containing 0.1% trifluoroacetic acid and 0.02% triethylamine (mobile phase B).

2.3.4. α-Amino nitrogen. RIVO-DLO carried out analysis of α-amino nitrogen. 25 g of herring was homogenised with 50 ml water for 30 seconds (Ultra Turrax). After filtration, centrifugation and dilution, a buffer solution (pH 8.2) containing 0.1 % (w/v) 2, 4, 6-trinitrobenzene sulfonic acid was added. The solution was placed in a water bath (40 °C) for 2 hours. After adding hydrochloric acid the absorption was measured at 420 nm. Quantification took place by using a calibration curve of DL-alanine.

2.3.5. Free amino acids. Norconserv and RIVO-DLO analysed free amino acids (FAA) in herring samples. At Norconserv the analysis was carried out after extracting the FAA in 10% trichloroacetic acid (TCA) according to the PICO-TAG method (manual) of Waters (1990). Briefly, a sample (3 ± 0.005 g of herring mince) was accurately weighed in a centrifuge tube and extracted with 50 ml of an aqueous solution of TCA (10%) using an Ultra Turrax. Methionine sulfone (1 mg/ml) was added as an internal standard. After extraction for 10 minutes at room temperature, the homogenate was cooled to 4 ^{0}C to precipitate proteins and centrifuged at 3000 rpm for 15 min. An aliquot of the supernatant (2.5 ml) was extracted 2 times with ether (2.5 ml) to remove TCA and lipids. The water phase containing the free amino acids was ultrafiltered (< 0.45 μm), and an aliquot (100μl) was evaporated in a reagent tube (50 x 6.5 mm) in a vacuum station.

The derivatisation was carried out in the following manner: The sample was dissolved in a mixture of methanol : 1M Sodium acetate : trimethylamine, TEA (2:2:1 - 50 ml) and evaporated in a vacuum-station to reach a suitable pH for the free amino acids in sample and standard. A freshly prepared reaction mixture of methanol : TEA : Water : Phenylisothiocyanate (PITC, Edmans degradation reagent) (7:1:1:1 - 100 ml) was then added to the dried samples, and allowed to react for 20 minutes at room temperature. The sample was subsequently vacuum dried (~1hr) to remove all PITC, and then dissolved in 200 ml diluent (PICO-TAG). The phenylthiocarbamates of the free amino acids were analysed by reverse phase HPLC according to the Waters PICO-TAG manual for free amino acid analysis, using a PICO-TAG column (3.9 x 30 cm; Waters, 1990) and Sodium-acetate (pH 6.4) / Acetonitrile / Methanol gradient elution (Eluent 1 & 2; Waters, 1990). Determinations of the free amino acids in samples (containing Methionine sulfone as internal standard) were calculated according to an external standard mixture of free amino acids (Sigma). The following chromatographic equipment (Shimadzu)was used: LC 9A Liquid Chromatograph, SPD-M6A Photodiode Array UV-Vis detector, CTO 10A Column oven (at 47 ^{0}C) and DGU-4A Degasser.

At RIVO-DLO the following procedure was used for the analysis: In order to extract the free amino acids from herring and brine, 36 ml of a 1% (w/v) picric acid solution was added to 10.0 g of sample. The sample was homogenised for 30 s with an Ultra Turrax and stirred for 2 hours. The solution was filtered and 1.0 ml of the filtrate was diluted to 10 ml with dilution buffer. After adding an internal standard and pre-column derivatization with fluoraldehyde reagent, the samples were analysed by reversed phase HPLC (Perkin Elmer) and the fluorescence detected using a high speed Ultrasphere C18 column (75 x 4.6 mm, particle size 3 µm, Beckman Alltech). The mobile phases consisted of 0.09 M sodium acetate (pH 7.2) containing 285 ml methanol and 15 ml tetrahydofurane (mobile phase A). Mobile phase B consisted of methanol. The flow was 1.7 ml/min. The following linear gradient was applied to separate the free amino acids:

Time (min.)	% mobile phase A	% mobile phase B
1	100	0
5	86	14
10	86	14
15	50	50
15	50	50
10	25	75
4	25	75
4	0	100
7	0	100

2.3.6. Proteolytic activity in muscle and brine. All participants except RIVO-DLO carried out analyses of general proteolytic activity (GPA) in muscle and brine. The activity was determined at pH 8.0 and 25°C using azocasein as substrate. The incubation time was 24 hours for both muscle and brine. This method is a modified

method to that of Sarath *et al.* (1989). In case of proteolytic activity in the sample material, the protein substrate is cleaved into low-molecular weight peptides and amino acids containing an azo group which is monitored in a spectrophotometer at 450 nm.

2.3.7. Endo- and exopeptidase activity in muscle and brine. FF carried out endo- and exopeptidase assays with specific synthetic substrates containing p-nitroanilide. The following synthetic substrates were used. For the determination of endopeptidase activity: Benzoyl-L-arginine-p-Nitroanalide (trypsin activity), Succinyl-L-alanine-L-alanine-L-proline-L-phenylalanine-p-Nitroanalide (chymotrypsin activity), Succinyl-L-alanine-L-alanine-L-alanine-p-Nitroanalide (elastase activity). For the determination of exopeptidase activity: Leucine-L-p-Nitroanalide (leucine aminopeptidase activity), Alanine-L-p-Nitroanalide (alanine aminopeptidase activity). All substrates were dissolved in 0.1 M Tris-HCl pH 8.0 (25°C). Activity were measured spectrophotometrically as an increase in absorbance at 405 nm in microplates using a Multiskan plus microplate reader (Lab Biosystems, Finland).

10 μl of sample was pipetted into each microplate well and 250 μl of substrate was added with a 8-channel multichannel pipette. Measurement was done at time zero and every 10 to 15 minutes for a period of 60 to 90 minutes. The plates were incubated at 24±0.3°C between each measurement. Absorbance values were registered by a computer and absorbance change per minute was calculated. Activity concentration (U/ml = μmole/min/ml) was calculated from the absorbance changes per min using the equation:

$$A = \varepsilon / S \times n \qquad \text{(Equation 1)}$$

where ε is the extinction coefficient of the chromophoric substrate **S** is the cross-sectional area perpendicular to the light path of the well and **n** is mole hydrolysed substrate. Equation 1 can be rewritten to $A = \varepsilon \times n/V \times l$ which is identical with Lamberts-Beers law: $A = \varepsilon \times c \times l$ wherefrom U/ml is calculated. A qualitative visual evaluation of the wells was made after 24 hours to confirm the activities measured.

2.3.8. Texture measurements. FRCF carried out measurement of texture properties (Texture Profile Analysis) on both raw material and salted herring samples using a TA.XT2 Texture Analyser (Stable Micro Systems, Haslemere, England) equipped with a flat cylindrical plunger (5 cm diameter). The samples were compressed twice to 80% of its total height with a constant speed of 0,8 mms^{-1}. All measurements were repeated at least ten times. The tissue samples were prepared by cutting out discs with a diameter of 1,5 cm using a cork borer. The texture attributes chewiness, gumminess, springiness, cohesiveness and adhesiveness are dimensionless but the unit for hardness is N. For the investigation of the maximum shear force the Texture Analyser was equipped with a Warner Bratzler cell. Pieces of fillets measuring 25 mm x 25 mm were used (Schubring, 1997 a,b; Schubring and Oehlenschläger, 1997).

2.3.9. Proximate analysis. Proximate analysis was carried out by IFL. The salt content of muscle was determined by the method of Volhard (AOAC 937.09.,1990).The moisture content was determined by mixing samples with sand and drying at 105°C for 4 hours (AOCS, Ba 2a-38, 1989). Weight loss was taken as water content. Fat content was determined by the method of Soxhlet (AOAC 960.39., 1990). The nitrogen content was measured with a semi-macro Kjeltec method, ISO 5983-1979 (Digestion System 40, 1016 digester, Tecator, England).

FF analysed the protein content by the Kjeldahl method, dry matter (in muscle and brine) by drying overnight at 105°C and using the remaining weight as dry matter. Ash (in muscle and brine) was determined by the removal of all organic matter of the dried samples at 600°C for 16 hours. The ash content was used as an indirect measurement of salt content.

2.3.10. pH, brine strength and temperature. All participants except RIVO DLO measured the temperature and the strength of brine and the pH of muscle and brine. pH was determined by the use of a combination electrode at room temperature. Measurements were carried out directly on brine and on a suspension of minced muscle and distilled water (20:80 w:w). A Baume density meter was used to determine the density of the brine.

2.3.11. ATP breakdown products. RIVO-DLO carried out evaluation on the content of ATP breakdown products in herring muscle. In order to extract the ATP-breakdown products from the sample, 80 ml of 5 % (w/v) of a trichloroacetic acid solution was added to 20.0 g of sample. The sample was homogenised for 20 s with an Ultra Turrax. After filtration the solution was neutralised, diluted and filtered prior to reversed phase ion-pair HPLC analysis with UV-detection (Perkin Elmer). HPLC analysis was performed on a Hypersil ODS column (25 x 0.46 cm, 5 μm, Chrompack). The mobile phase consisted of a 0.1 M potassium dihydrogen phosphate solution with 1.44 mM tetrabutylammonium hydrogensulphate as the ionpairing reagent (pH 7.0) containing 104 ml methanol. The flow rate was 1.0 ml and an isocratic elution was used to separate the ATP-breakdown products. Detection took place at 255 nm.

2.3.12. Colour measurements. FRCF carried out measurement of the colour on both intact muscle (meat side of the upper part of the fillet) and the homogenate derived from skinned fillet after 20 sec mincing using a tristimulus colorimeter Chroma Meter CR 300 (Minolta, Ahrensburg, Germany). In the CIELab system L* denotes lightness on a 0 to 100 scale from black to white; a*, (+) red or (-) green; and b*, (+) yellow or (-) blue. ΔE the colour difference denotes the square root from ($\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}$). Measurements were repeated fifteen times and nine times, respectively (Schubring, 1997 a,b).

2.3.13. Differential scanning calorimetry. FRCF performed a differential scanning calorimetry of pyloric caeca using a Perkin-Elmer DSC-7 (Überlingen, Germany) fitted

with Colora-cooling equipment (Kryo-Thermostat WK 5, Colora GmbH, Lorch, Germany) at ambient temperature. The instrument was calibrated for temperature and enthalpy using indium and naphthalene as standards. The pieces of pyloric caeca (15 ± 3 mg) were weighed accurately (to 0.1 mg) into 30 μl aluminium pans and sealed. At least triplicate samples were heated from 25 to 95 °C at a scanning rate of 10 K min^{-1}, with an empty sealed pan as a reference. The transition temperatures (T_{max}) were recorded. The transition enthalpy (ΔH) was determined from the peak area and expressed in Jg^{-1} sample material (Schubring, 1997 a,b).

2.3.14. Data analysis. Principal component analysis (PCA) was performed on sensory analysis results and free amino acid data. Sensory attributes were standardised to equal variance and mean centred prior to PCA. Unscrambler Version 6.0 software package (Camo AS, Trondheim, Norway) was used for PCA.

Table 8 in Appendix gives an overview of the measurement plan.

3. RESULTS AND DISCUSSION

3.1. Temperature, brine density and pH

Results for temperature and brine strength determination can be found in Table 1 along with pH values in brine and muscle. The temperature was 3.7°C in the cooler at HIFI and the temperature varied between 3.5-5°C in the barrels during the trial. HIFI reported that the salt strength varied between 8.1-8.2% in the brine.

Table 1. Temperature, brine density and pH.

Temperature (°C)

Weeks	0	4	16	26
FF		6	6	5,5
IFL		6	5	5
Norcon		5	6	6

Muscle: pH

weeks	0	4	16	26
FF	6,73	6,44	6,21	6,21
IFL	6,7	6,3	6,3	6,3
Norcon		6,19	6,07	6,12
HIFI	6,69	6,36	6,22	6,26
Germany	6,67	6,2	6,22	6,01

Brine: pH

weeks	0	4	16	26
FF		5,8	5,8	5,9
IFL		5,81	5,84	5,84
Norcon		5,74	5,73	5,76
HIFI		5,79	5,71	5,75
Germany	pH was between 5,73 and 5,83			

Salt strength (°Baume)

weeks	0	4	16	26
FF		25	24,5	26,5
IFL		25	23	23
Norcon		21	21	22
Germany		23	22	22

These results are not important for following the ripening during the salting; however if the storage temperature had been too high or too low at any of participating laboratories that could have led to faster or slower ripening. Similarly the brine strength determination showed how well and even the salting was - a too low value would indicate that the barrels had not been tended properly. However the salt (NaCl) content in the brine should be around 24% but not 8% as the results from HIFI indicate.

The pH of brine was between 5.7 and 5.8 throughout the storage period. The pH of herring muscle was initially about 6.7 but after salting the pH was lowered considerably. Most laboratories measured the pH of muscle in the region 6.4-6.2 during the trial period.

In general the results showed fairly similar values, which indicated that the transport, storage and handling of the herring was similar at the laboratories. Therefore it may be assumed that the samples were similar between the countries.

3.2. Sensory evaluation

The sensory evaluation results can be found in Figures 2-7. The sensory evaluation results showed that the frozen thawed herring ripened; it obtained a ripened taste (Figure 2) and the raw taste disappeared quickly during the salting (Figure 3). The taste characteristics were typical for spice-salted herring with a dominant malty/creamy taste and a fairly low intensity of stockfish character (Figures 4 and 5). In comparison the spice-salted herring prepared from the frozen material appeared to obtain more quickly a high intensity of the ripened taste than the freshly salted herring (Figure 2) thus indicating faster ripening. The intensity of the ripened taste appeared to be similar in the fresh and previously frozen herring in the latter stages of the period (from week 24 onwards).

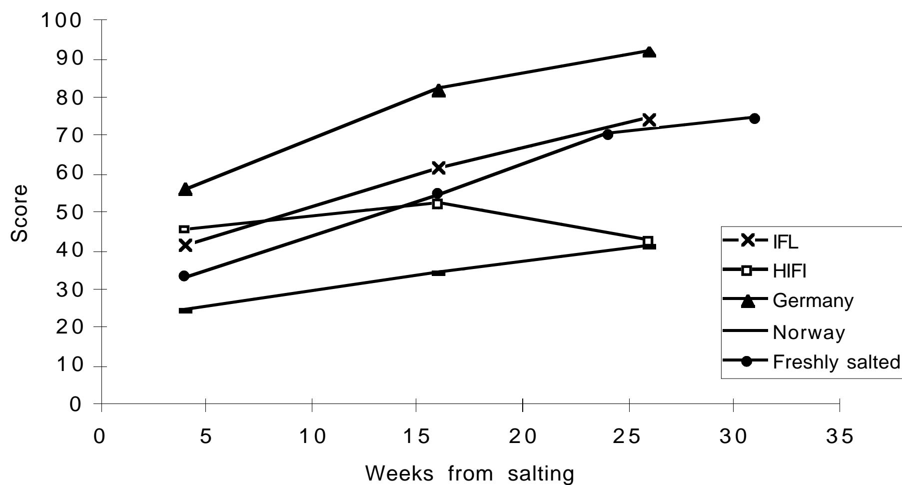


Figure 2. Ripened taste in spice-salted herring stored at 5°C. IFL, frozen thawed herring; HIFI, frozen thawed herring; Germany, frozen thawed herring; Norway, frozen thawed herring.

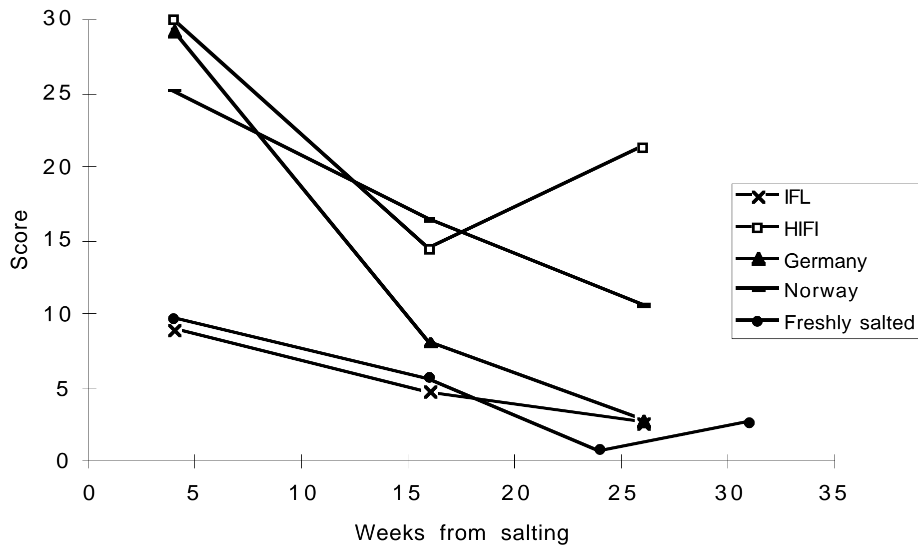


Figure 3. Raw taste in spice-salted herring stored at 5°C. IFL, frozen thawed herring; HIFI, frozen thawed herring; Germany, frozen thawed herring; Norway, frozen thawed herring.

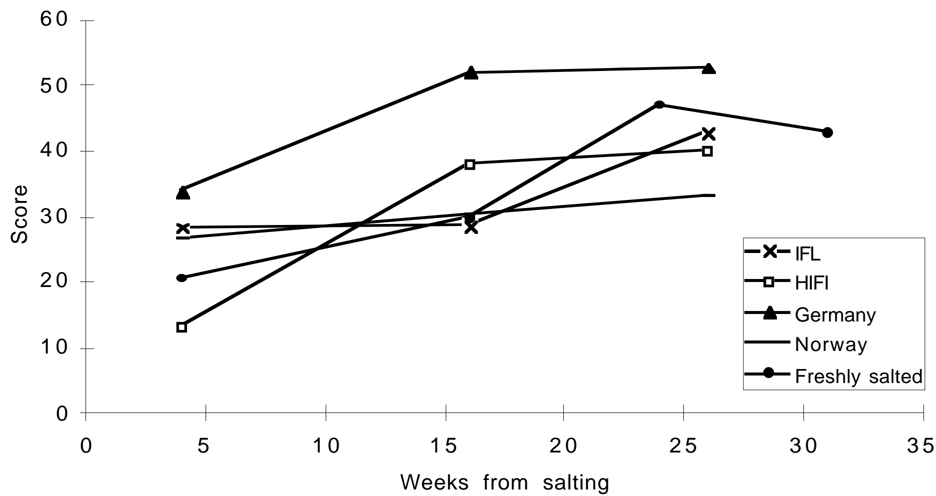


Figure 4. Malty/creamy taste in spice-salted herring stored at 5°C. IFL, frozen thawed herring; HIFI, frozen thawed herring; Germany, frozen thawed herring; Norway, frozen thawed herring.

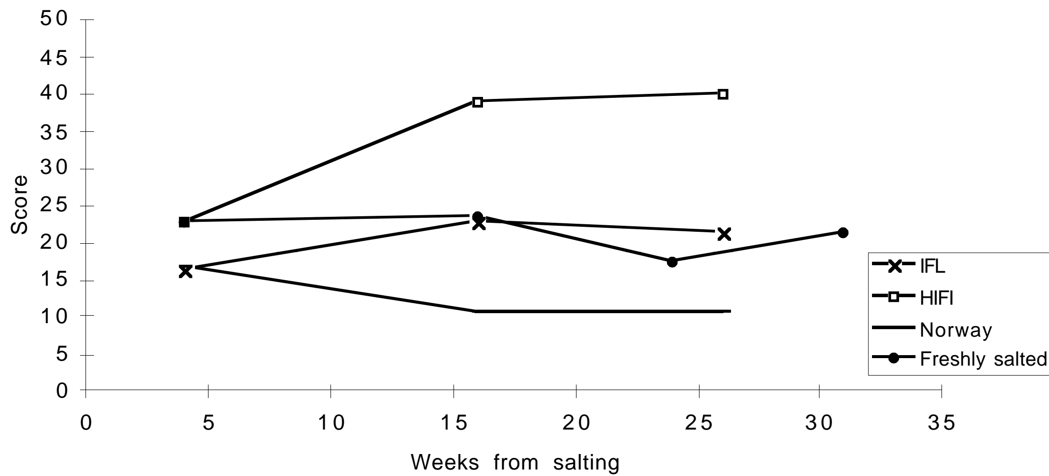


Figure 5. Stockfish taste in spice-salted herring stored at 5°C. IFL, frozen thawed herring; HIFI, frozen thawed herring; Norway, frozen thawed herring.

The frozen herring appeared to become quickly soft and watery (Figures 6 and 7) indicating again a faster ripening. After approximately 20 weeks salt storage the freshly salted herring appeared to have similar intensity in softness and watery as the frozen thawed herring.

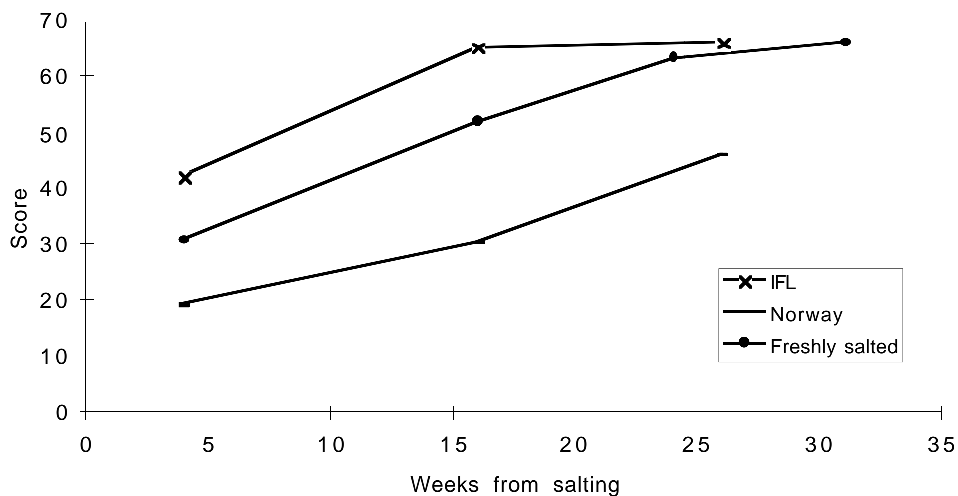


Figure 6. Softness in spice-salted herring stored at 5°C. IFL, frozen thawed herring; Norway, frozen thawed herring.

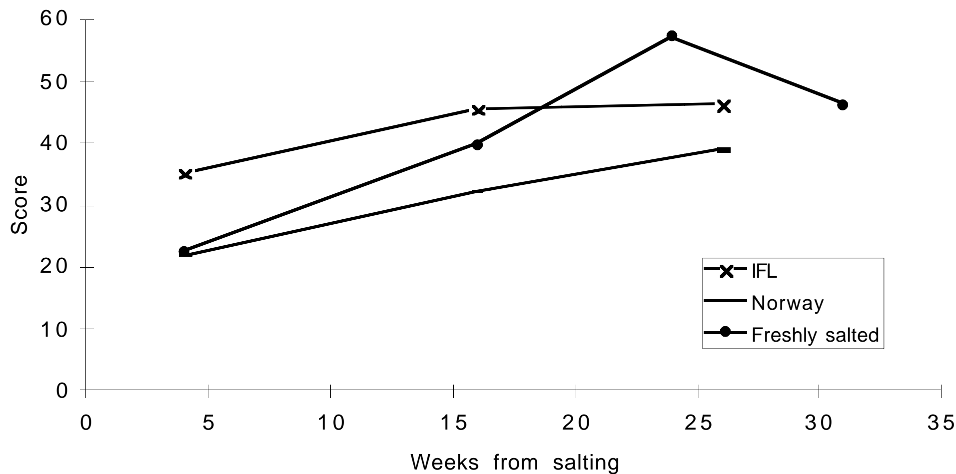


Figure 7. Softness in spice-salted herring stored at 5°C. IFL, frozen thawed herring; Norway, frozen thawed herring.

In this trial four of the participants carried out sensory analysis; the results showed a variation between the laboratories. If for instance the ripened taste is used as an example the highest intensity was always obtained by FRCF but the lowest by Norconserv. All participants however obtained an increasing intensity in ripened taste with storage time (Figure 2). The laboratories seemed to obtain the same trend for the samples but there was little or no consensus on the intensity of the attributes. It should be kept in mind that no training or calibration of the methodology was carried out before the trial. Therefore very little can be said about the results as a whole; each panel appeared to be fairly consistent in its grading but the results did not reflect variation in the samples, but rather variation due to lack of training between the panels. It should also be kept in mind that the sensory evaluation methods differed somewhat between the laboratories; the attributes were not clearly defined so that all panellists had a similar understanding, the scales varied and various attributes were evaluated especially for texture. In order to obtain comparable results between different panels in a trial like this a great deal of work has to be carried out in training, e.g. in choosing attributes that all are familiar with, defining them and coming to a common consensus on the intensity of each attribute for samples of varying ripening degrees. Unfortunately there simply wasn't time enough to carry out this harmonisation work.

3.3. Breakdown products

The changes in (soluble) nitrogenous compounds can be found in Figure 8. The results showed a much faster formation of TCA soluble nitrogen containing compounds in the muscle and brine of frozen/thawed spice-salted herring than encountered in the fresh salted herring.

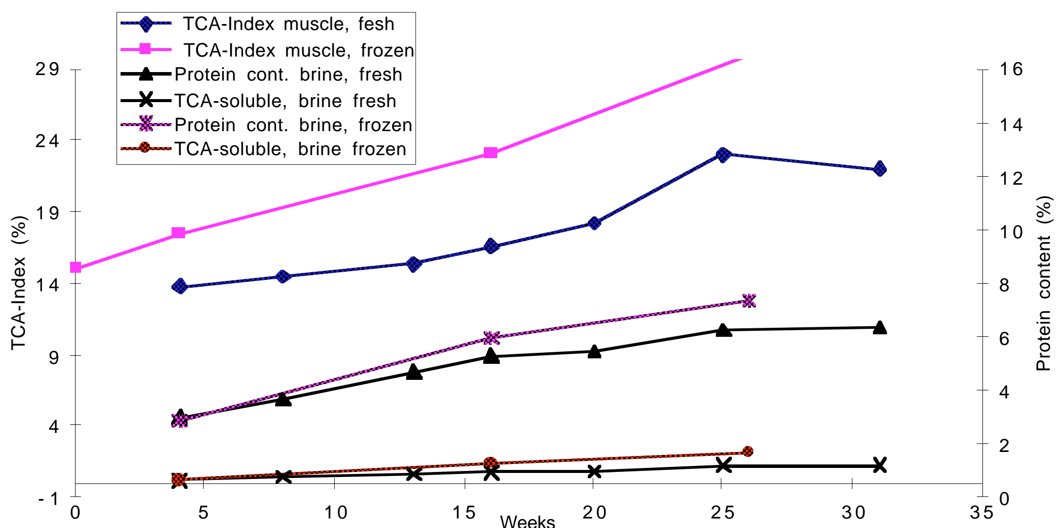


Figure 8. TCA index and protein content in spice-salted herring and brine stored at 5°C. Measurements carried out at IFL.

Similar results were found at FF in Denmark (Table 2). A much faster increase of low molecular weight nitrogen containing compounds was observed in the frozen/thawed spice-salted herring than encountered in the fresh salted herring.

Table 2. TCA index in muscle and brine in spice-salted herring and brine stored at 5°C. Measurements carried out at FF.

TCA muscle

Frozen		Not-frozen	
Weeks	TCA	Weeks	TCA
0	16.7±0.3	0	16.7±0.3
4	17.6±0.4	8	15.0
16	29.4±0.3	16	17.9
26	35.2±0.2	21	18.1±0.4

TCA brine

Frozen		Not-frozen	
Weeks	TCA	Weeks	TCA
4	72.0±1.0	8	68.5
16	86.5±1.2	16	63.3
26	85.1±0.6	21	75.6±0.5

Peptide fingerprints profile of muscle and brine extracts can be found in Figures 9 and 10. The results showed a higher increase of the compounds detected at 280 nm in both brine and muscle from frozen/thawed herring in comparison with fresh spice-salted herring. The difference was especially noticeable for the brine (Figure 10). It is interesting to note that the degradation pattern was similar in the previously frozen

and freshly salted herring both for brine and muscle. The same peaks were found although their intensity was not the same. This indicated that similar breakdown products were being formed or disappearing in both groups but the rate was much faster in the previously frozen material. These results are in agreement with the changes in soluble nitrogenous compounds (Figure 8 and Table 2) and thus again indicate a faster ripening in the previously frozen material in comparison with the fresh salted herring.

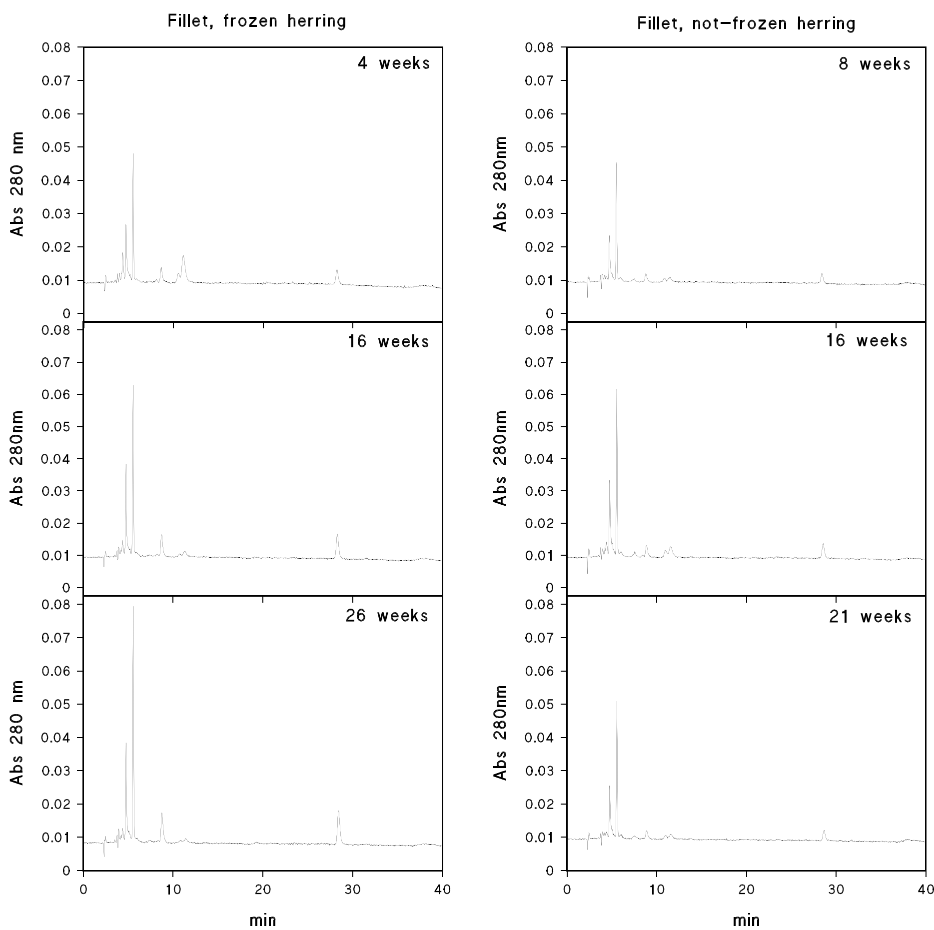


Figure 9. Reversed phase HPLC profile of TCA soluble nitrogenous compounds in spice-salted herring muscle stored at 5°C.

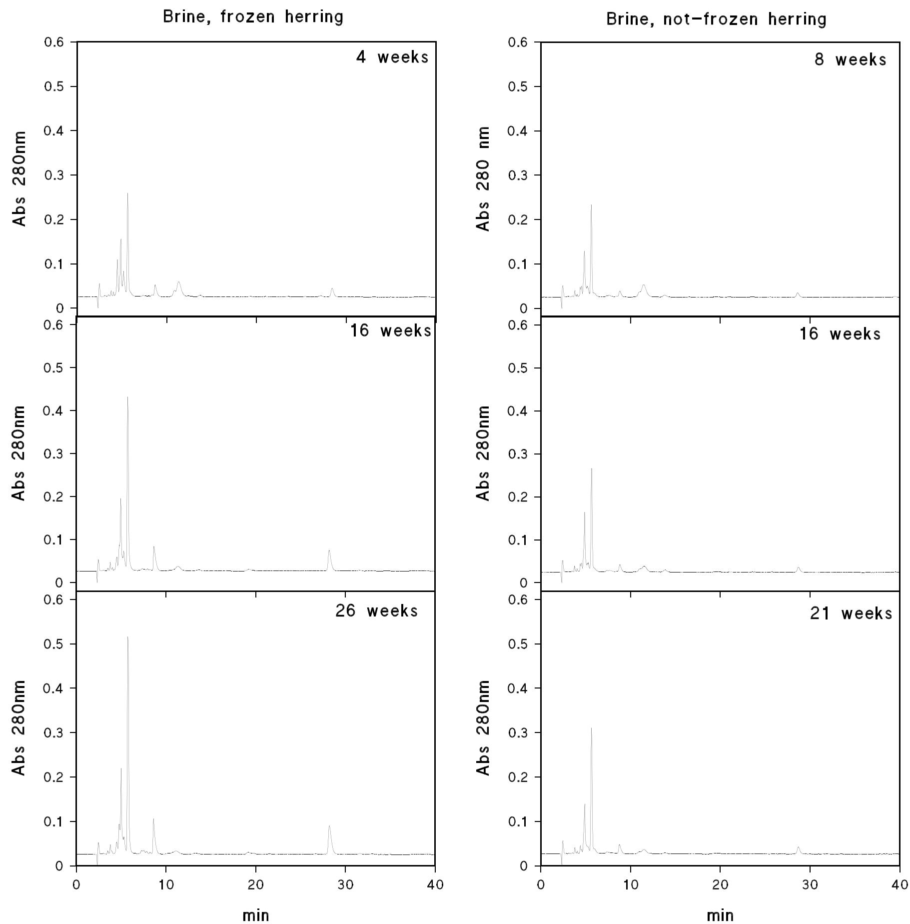


Figure 10. Reversed phase HPLC profile of TCA soluble nitrogenous compounds in brine of spice-salted herring stored at 5°C.

On a similar note the content of alfa amino nitrogen in muscle (Figure 11) and the total content of free amino acids in muscle (Figure 12) and brine (Figure 13) was found to be considerable higher in the previously frozen raw material in comparison with the fresh spice-salted herring. The FAA results from Norconserv and RIVO-DLO can be found in Tables 12 and 14, respectively, in the Appendix.

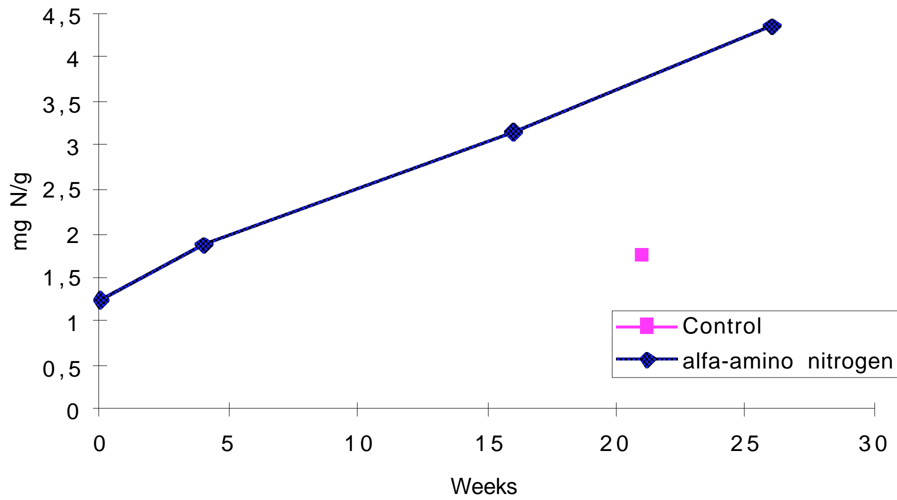


Figure 11. Alfa amino nitrogen in spice-salted herring at 5°C. Control, fresh salted herring; alfa amino nitrogen, frozen thawed herring

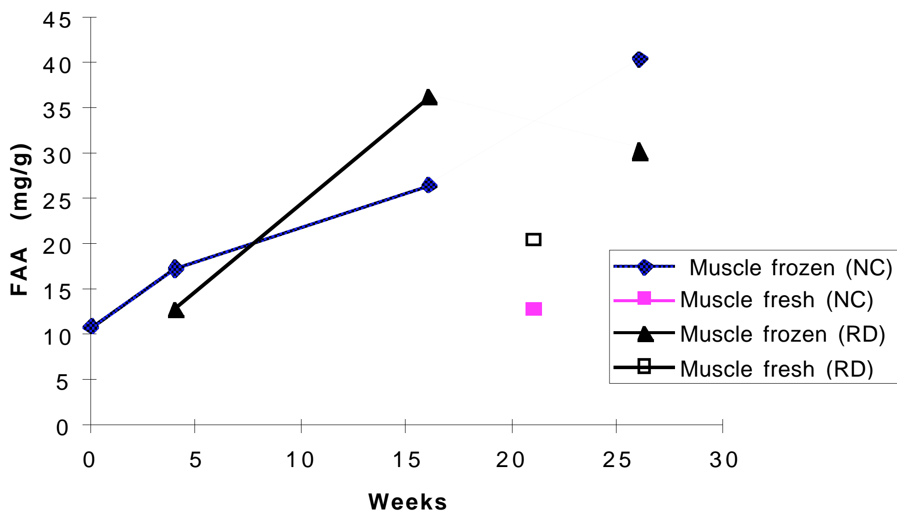


Figure 12. Total content of FAA in the muscle of spice-salted herring stored at 5°C. NC, results from Norconserv; RD, results from RIVO-DLO.

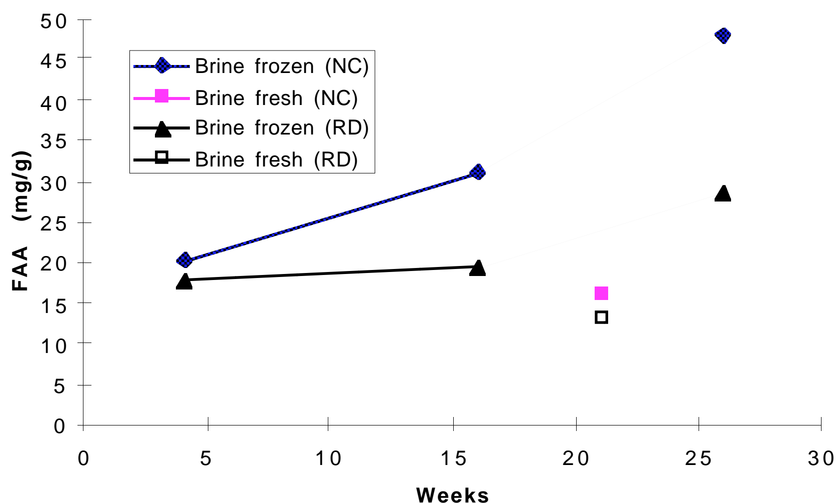
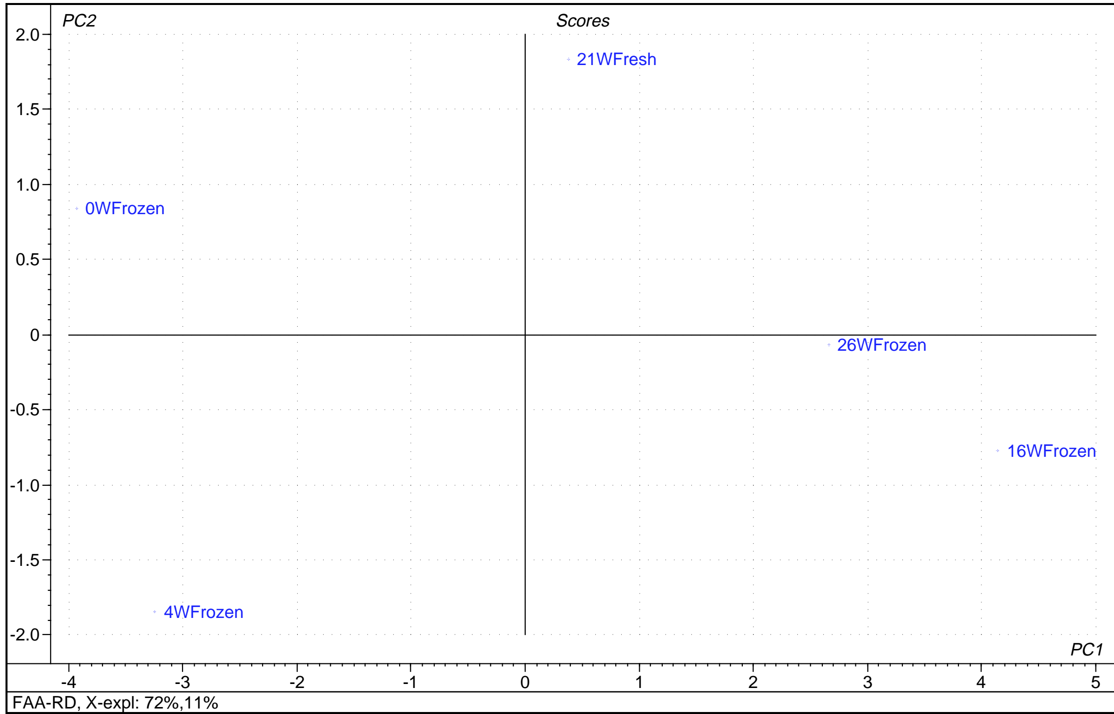


Figure 13. Total content of FAA in the brine of spice-salted herring stored at 5°C. NC, results from Norconserv; RD, results from RIVO-DLO.

Principal component analysis (PCA) of the free amino acid data from Rivo and Norconserv also indicated a faster ripening in the previously frozen material (Figures 14 and 15). Although there are noticeable differences in the content of individual amino acids between the laboratories (Tables 12 and 14 in Appendix) both observed a general increase in the content of free amino acids with ripening time (Clusters on PC1; Figs. 14 and 15). In the clusters the following amino acids were found by both laboratories: serine, valine, glutamic acid, methionine, lysine, aspartic acid, threonine, tyrosine and leucine. It is interesting to note that some of these free amino acids (ser, val, glu, asp and thr) have been reported to increase in fermented and ripened fish products (Kiesvaara, 1975; Mendes *et al*, 1994; Skåra and Olsen, 1994). The results again indicate a faster ripening in the previously frozen herring in comparison with the freshly salted herring. There is however not enough data to indicate whether there are differences in the free amino acid pattern between the fresh and frozen raw materials.



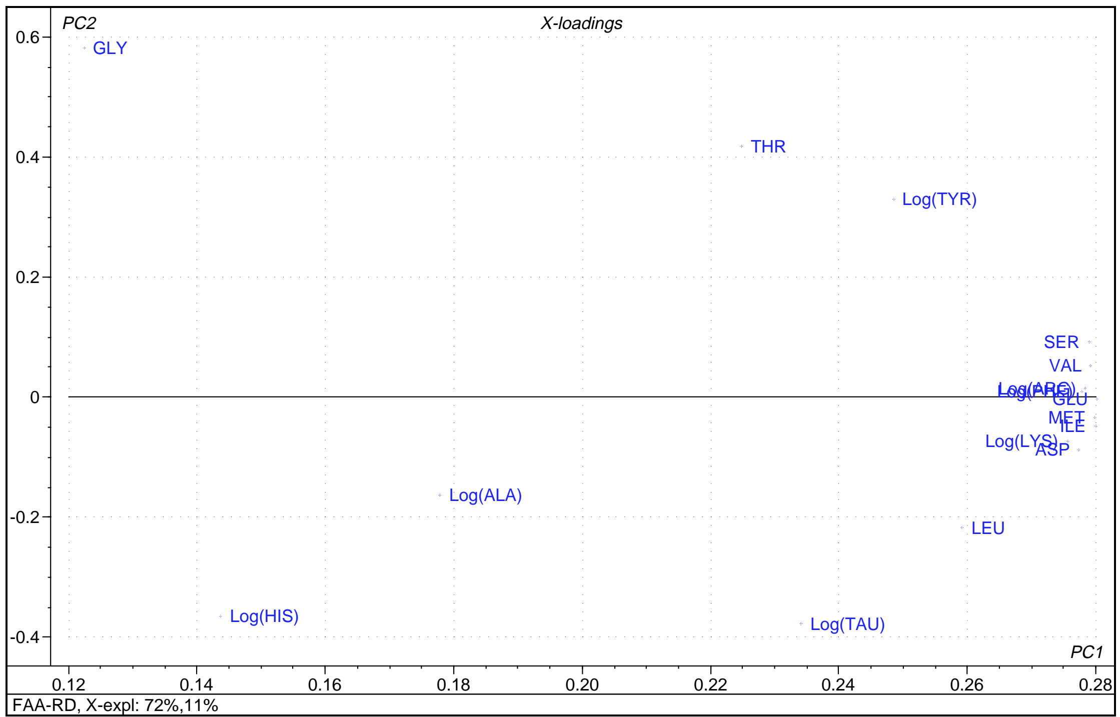
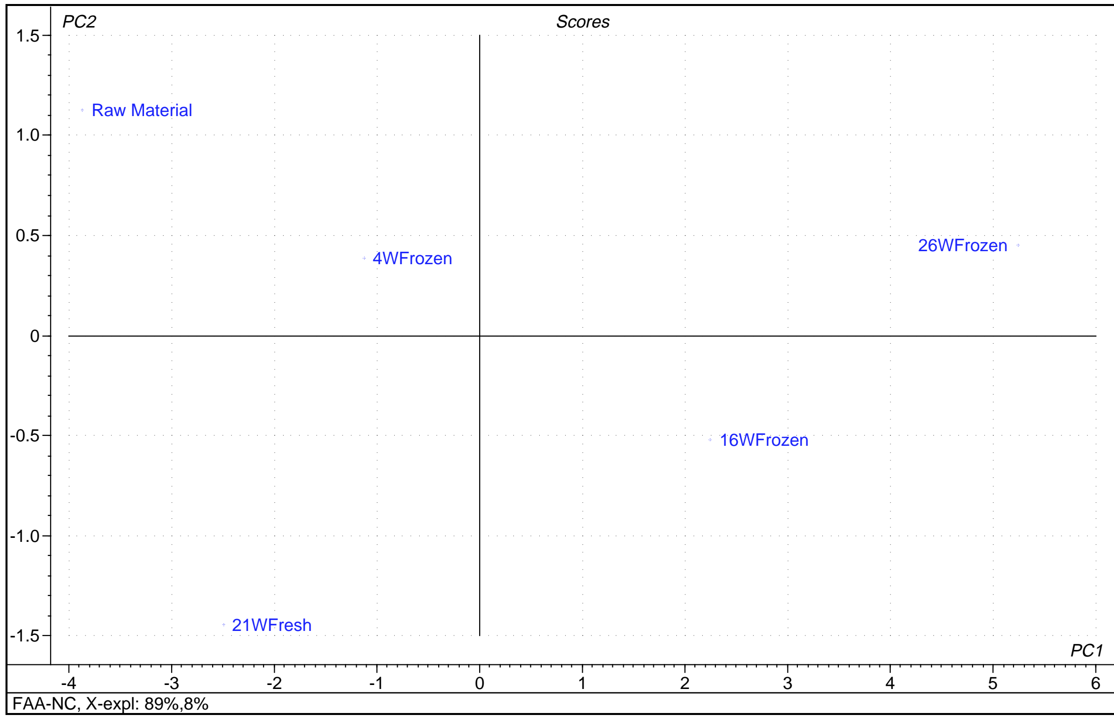


Figure 14. PCA of FAA data from RIVO-DLO.



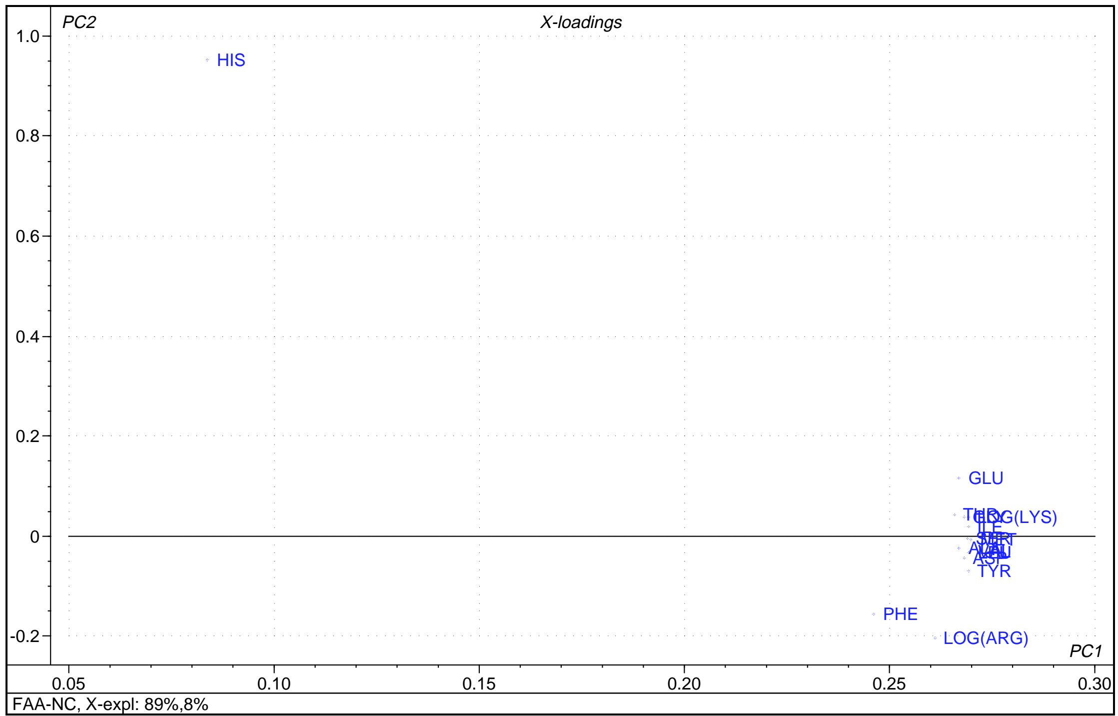


Figure 15. PCA of FAA data from Norconserv.

The content of ATP breakdown products can be found in Table 3. The content of IMP decreased in the herring immediately after salting but interestingly, the content appeared to increase again during the ripening. IMP may possibly contribute to the taste of ripened herring products in a synergistic manner with some FAA (especially glutamic acid; Stefánsson and Guðmundsdóttir, 1995).

Table 3. ATP breakdown products and K-value in herring stored at 5°C.

	[ATP] (μmol/g)	[ADP] (μmol/g)	[AMP] (μmol/g)	[IMP] (μmol/g)	[HxR] (μmol/g)	[Hx] (μmol/g)	K-value (%)
Frozen herring week 4	< 0.2	0.35 ± 0.02	< 0.03	1.15 ± 0.08	11.9 ± 0.8	6.6 ± 0.3	92.5 ± 0.1
Frozen herring week 16	< 0.2	0.14 ± 0.07	< 0.03	1.5 ± 0.1	2.8 ± 0.2	13.7 ± 0.7	90.9 ± 0.2
Frozen herring week 26	< 0.2	< 0.05	0.06 ± 0.00	2.42 ± 0.05	2.7 ± 0.1	12.6 ± 0.2	86.2 ± 0.4
Fresh herring week 21	< 0.2	< 0.05	< 0.03	1.3 ± 0.2	1.7 ± 0.3	11 ± 2	91.0 ± 0.2
Raw material	< 0.2	0.48 ± 0.06	< 0.03	3.6 ± 0.4	6.6 ± 0.4	3.8 ± 0.2	72 ± 1

3.4. Enzymatic activity

The general proteolytic activity (GPA) in spice-salted herring muscle and brine can be found in Figures 16 and 17. It is interesting to note a much higher GPA in the previously frozen material in comparison with the fresh salted herring. The high enzyme activity in the previously frozen material is in agreement with the observed rapid increase in soluble nitogenous compounds, peptide fingerprints, alfa amino acid and total content of amino acids.

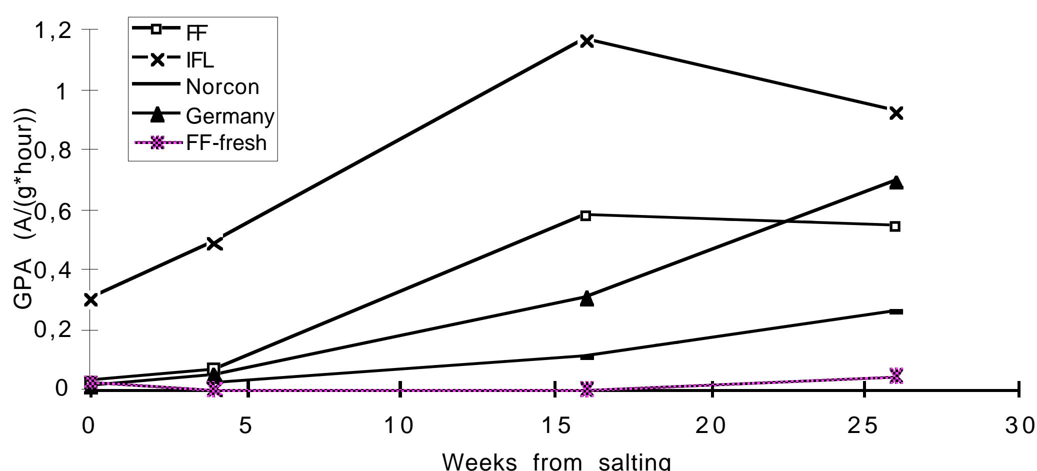


Figure 16. General proteolytic activity in spice-salted herring muscle at 5°C. FF, frozen thawed herring; IFL, frozen thawed herring; Norcon, frozen thawed herring; Germany, frozen thawed herring; FF-fresh, fresh salted herring.

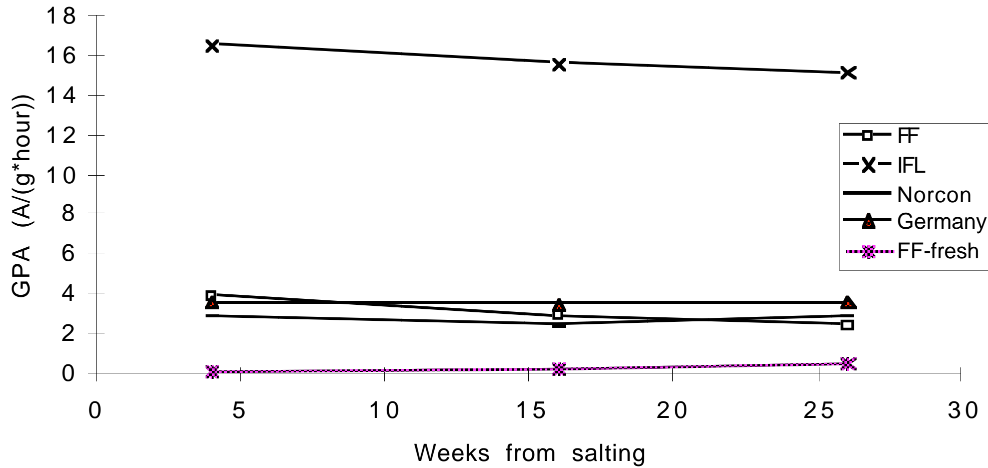


Figure 17. General proteolytic activity in brine of spice-salted herring at 5°C. FF, frozen thawed herring; IFL, frozen thawed herring; Norcon, frozen thawed herring; Germany, frozen thawed herring; FF-fresh, fresh salted herring.

Similar results for GPA were obtained by the participating laboratories with the exception of IFL. IFL obtained systematic higher values at all times in comparison with the other laboratories. No explanation can be found for this but interestingly enough the same trend was observed in a recent collaboration between IFL and FF (Stefánsson *et al.*, 1995). The results from HIFI were not directly comparable to the other results due to different incubation times.

Specific enzyme activity in muscle and brine for both fresh and previously frozen raw material can be found in Table 4.

Table 4. Specific proteolytic activity in musle and brine of herring stored at 5°C.

SPA muscle (x 100)

Frozen						Not-frozen					
Weeks	TS	CT	EL	AAP	LAP	Weeks	TS	CT	EL	AAP	LAP
0	-	0.15±0.02	0.22±0.04	0.64±0.04	0.38±0.06	0	-	0.15±0.02	0.22±0.04	0.64±0.04	0.38±0.06
4	-	0.13±0.06	-	1.5±0.1	0.75±0.05	8	-	-	-	0.42	0.25
16	0.28±0.1	3.34±0.01	0.036	0.93±0.2	0.29±0.08	16	-	-	-	0.44	0.24
26	0.54	3.94	0.046	0.98	0.27	21	-	0.14	-	0.47±0.05	0.16±0.05

SPA brine (x 100)

Frozen						Not-frozen					
Weeks	TS	CT	EL	AAP	LAP	Weeks	TS	CT	EL	AAP	LAP
4	4.9±0.1	13.6±2.5	-	23.9±0.9	6.4±0.1	8	-	0.26	-	8.4	3.5
16	5.5±0.03	46±0.3	1.1±0.01	21.8±0.5	5.9±0.2	16	-	0.48	-	10.2	4.1
26	5.2±0.03	51.5±0.2	1.1±0.07	22.0±0.3	6.0±"0.2	21	-	25.7±0.2	-	13.1±0.3	3.7±0.1

The results show that no trypsin (TS) and elastase (EL) activity was detected in the muscle or brine from fresh salted herring whereas considerable activity was found both in muscle and brine from the previously frozen spice-salted herring (Table 4). The same was observed for chymotrypsin (CT), alanine aminopeptidase (AAP) and leucine aminopeptidase (LAP): the acitivity was low or absent in the muscle and brine of fresh salted herring but considerably higher in the muscle and brine from the previously frozen raw material (Table 4). It is likely that the high general and specific enzyme activity found in the previously frozen raw material in comparison with the fresh salted herring explains the observed faster rate of ripening.

3.5. Instrumental texture measurements

Instrumental texture measurements on fresh and previously frozen spice-salted herring can be seen in Table 5. The results showed that the hardness increased at salting but after salting the hardness decreased steadily. This behaviour seemed to be almost comparable with that of fresh moderately and lightly salted herring products, caught in the North and Baltic Sea, which showed after a short increase, caused by salting, a more or less steady decrease in hardness during prolonged ripening. Heavily salted herring on the other hand showed a continuous increase of hardness (Schubring and Oehlenschläger, 1997). Interestingly enough the hardness of fresh spice-salted herring samples measured after 21 weeks of ripening were almost comparable to values found for frozen thawed herring kept for 16 weeks, thus again indicating a faster ripening for the previously frozen material.

Table 5. Texture measurements on spice-salted herring stored at 5°C. TPA, Texture Profile Analysis

Texture (TPA)

		hardness (N)	gumminess	chewiness	springiness	cohesiveness	adhesiveness
Raw	0 weeks(n=15)	21,55±5,63	3,19±1,15	0,73±0,34	0,22±0,04	0,15±0,02	-0,34 ±0,08
Fresh	21 weeks (n=11)	42,77±6,94	7,72±1,72	2,59±0,81	0,34±0,08	0,18±0,02	-0,59 ±0,29
Frozen	4 weeks (n=15)	47,67±7,31	9,83±2,02	5,08±1,49	0,52±0,12	0,21±0,02	-0,50 ±0,17
Frozen	16 weeks (n=15)	42,91±8,39	7,91±2,24	3,12±1,34	0,39±0,09	0,18±0,02	-0,73 ±0,28
Frozen	26 weeks (n=15)	27,77±4,84	3,98±1,13	1,26±0,62	0,30±0,09	0,14±0,02	-0,93 ±0,18

Texture (Warner-Bratzler shear force (N))

		transversal to the backbone	longitudinal to the backbone
Raw	0 weeks (n=13)	14,23±2,94	5,33±0,96
Fresh	21 weeks (n=10)	21,20±2,30	11,70±1,17
Frozen	4 weeks (n=11)	22,25±3,73	10,59±2,18
Frozen	16 weeks (n=12)	17,36±1,77	8,40±1,62
Frozen	26 weeks (n=11)	15,93±1,93	8,06±1,24

Almost the same tendencies as discussed above were observed for the other texture parameters. The values of springiness, chewiness, gumminess, and cohesiveness were after week 4 higher in comparison with the unsalted raw material. The ripening of frozen-thawed spice-salted herring could be followed by a continuous decrease of the values of the above mentioned texture attributes. All texture attributes measured on frozen-thawed herring after week 16 were almost comparable with those of freshly salted herring measured after 21 weeks storage time. The adhesiveness seems to be strongly influenced by salting and ripening. Compared with the raw material a continuous increase was observed (Table 5).

The shear force values confirmed the increase of tenderness during ripening (Table 5). The results show that the shear force measured on the freshly-salted sample after week 21 was comparable with the value of frozen-thawed herring measured after week 4. This indicates faster tenderising during ripening of the frozen-thawed spice-salted herring in comparison with the fresh salted. The observed differences in shear force values depend on the direction of shearing and indicate the anisotropic character of the musculature as well as that of fish as a mammal (Schubring and Oehlenschläger, 1997; Lepetit and Culioli, 1994).

3.6. Colour

Results for colour measurements can be found in Table 6.

Table 6. Colour measurements on spice-salted herring stored at 5°C.

		fillet	fillet	fillet	homogenate	homogenate	homogenate
		(fillet-n=15; homogenate-n=9)					
		L*	a*	b*	L*	a*	b*
Raw	0 weeks	48,66±2,00	2,67±0,59	7,00±1,14	59,48±0,09	6,06±0,02	12,60±0,21
Fresh	21 weeks	47,17±2,00	4,59±1,96	4,39±0,85	57,36±0,67	7,64±0,31	10,18±0,09
Frozen	4 weeks	37,80±1,51	3,52±1,11	2,82±1,39	46,73±0,34	7,36±0,19	9,57±0,05
Frozen	16 weeks	43,11±1,85	3,27±1,21	4,24±1,11	56,89±0,15	6,23±0,12	10,80±0,09
Frozen	26 weeks	44,83±1,59	2,43±0,78	4,06±0,75	61,68±0,13	5,60±0,21	11,70±0,58

After a sharp decrease in lightness immediately after spice-salting an almost continuous increase was observed during ripening, possibly caused by bleaching through the brine (Table 6). The differences between fresh and frozen-thawed salted herring products were negligible. In general, it could be stated that homogenisation is connected with a remarkable increase in lightness as well as much less standard deviation.

While the redness seemed to decrease, the yellowness increased slightly during ripening, especially in the homogenised samples (Table 6). The higher values in redness could indicate that it is more difficult to bleach out the red colour from freshly salted herring products than frozen-thawed samples.

The tendencies seen in the results for frozen-thawed samples are very similar to results obtained during ripening of salted Baltic herring; the only exception is redness (Schubring and Oehlenschläger, 1997).

3.7. Differential scanning calorimetry

Result of differential scanning calorimetry of pyloric caeca from raw, fresh and frozen thawed spice-salted herring can be found in table 7.

Table 7. Thermoanalytical behaviour of pyloric caeca in herring.

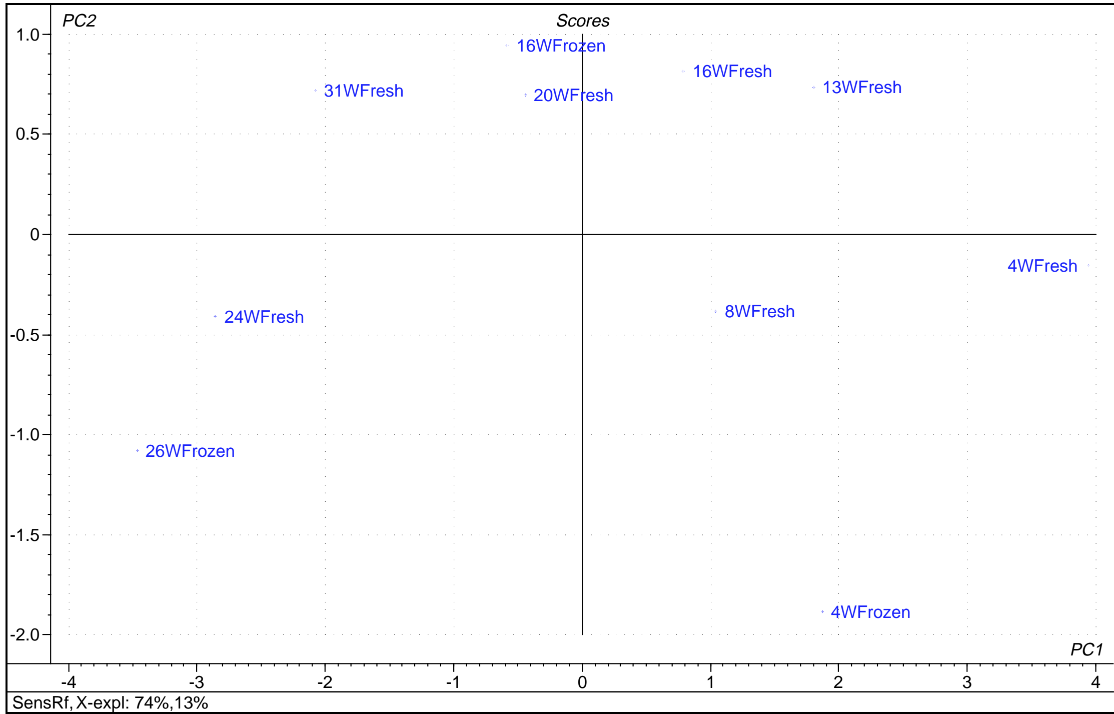
			transition temperature T_{max} (°C)	transition enthalpy (J/g)
Raw	0 weeks	(n=4)	49,05±0,64	0,30±0,13
Fresh	21 weeks	(n=4)	54,10±0,83	0,16±0,03
Frozen	4 weeks	(n=6)	55,85±0,60	0,18±0,07
Frozen	16 weeks	(n=6)	56,25±0,92	0,13±0,04
Frozen	26 weeks	(n=7)	58,16±2,07	0,09±0,04

Salting and ripening seemed to be connected with a rise of the thermal stability of the pyloric caeca tissue as indicated by an increase of the transition temperature compared with the unsalted herring (Table 7). This is opposite to observations on Baltic and North Sea herring where, after a sharp increase of the transition temperature caused by salting, a continuous decrease was observed (Schubring and Oehlenschläger, 1997). On the other hand the decreasing transition enthalpy during

ripening was also observed on the above mentioned different herring stocks; the only exception was the high enthalpy of the raw material. The results of differential scanning calorimetry in connection with ripening have been discussed more fully by Schubring (1997a ,b). No explanation for the differences in thermoanalytical behaviour observed between freshly and frozen thawed Icelandic herring could be found.

3.8. Other analysis

All other results including the primary data from the individual laboratories can be found in the Appendix. PCA was carried out on IFL's sensory results of freshly spice salted herring stored at 5°C and the frozen/thawed herring (Figure 18) and Norconserv's sensory data (Figure 19). The analysis showed very similar results: Faster ripening was found at both laboratories in the previously frozen herring (a few weeks difference). The analysis also showed that during ripening the raw taste decreased and the parameters ripening, tenderness (smoothness), softness, and watery increased. The latter parameters were the main variables in PC1 whereas stock fish (dryfish) was the main variable in PC2. A difference was seen in the results for the laboratories as malty taste fell in with the PC1 variables at IFL but under the PC2 variables at Norconserv.



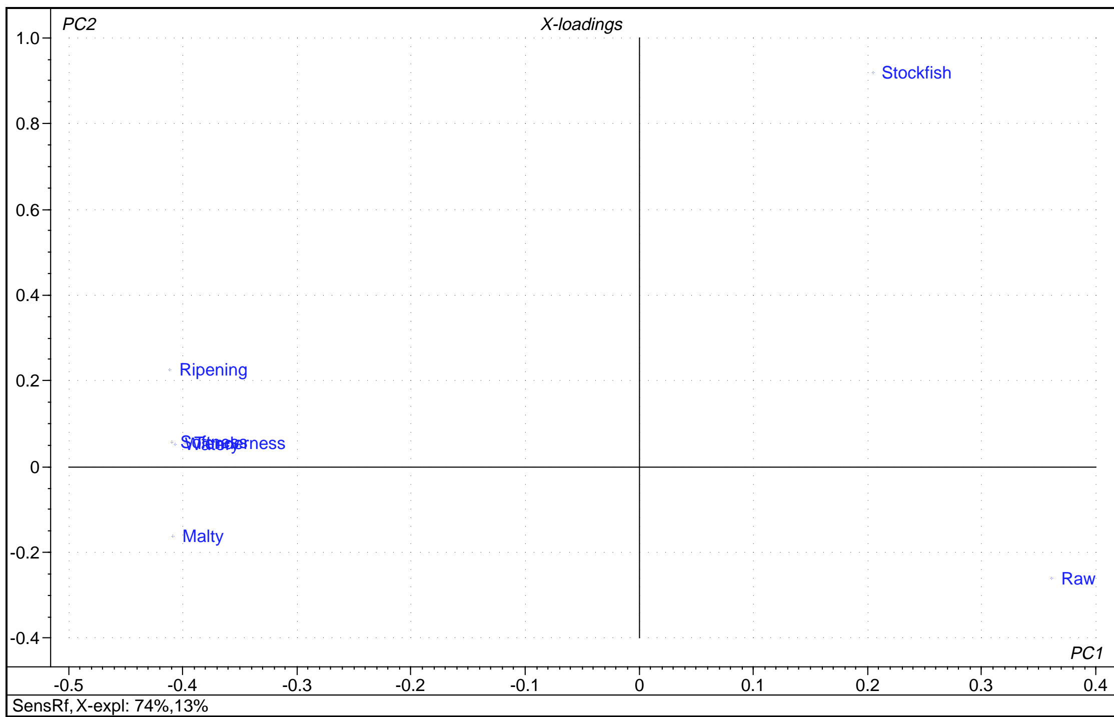
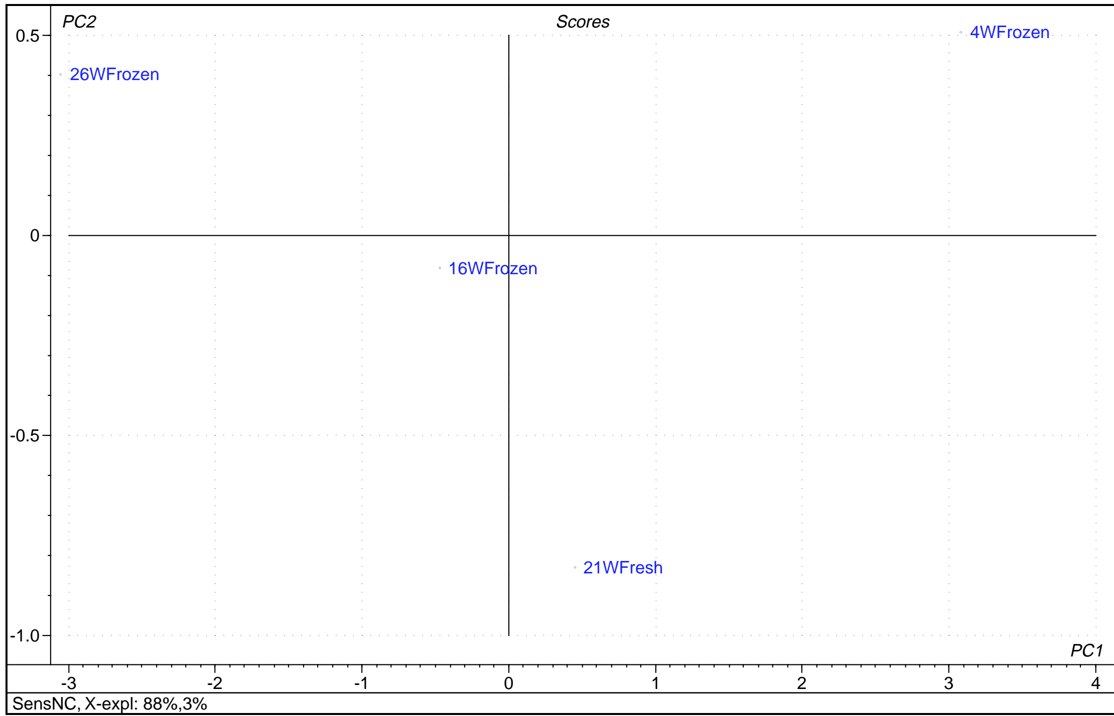


Figure 18. PCA of sensory evaluation data from IFL.



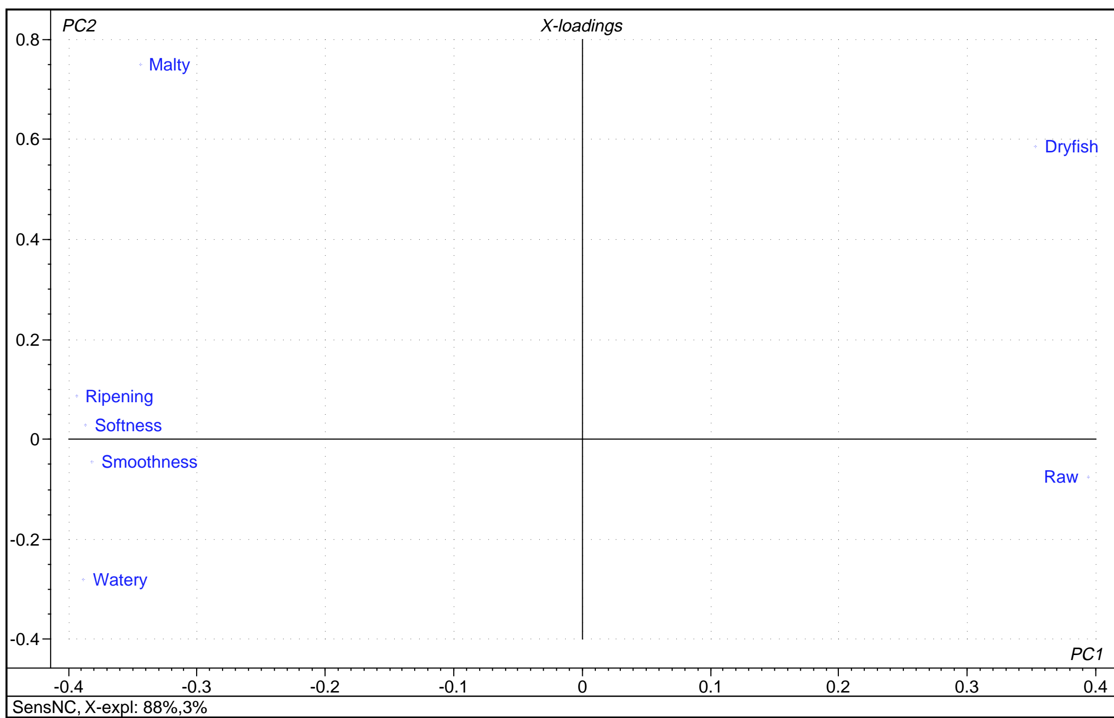


Figure 19. PCA of sensory evaluation data from Norconserv.

4. CONCLUSION

It can be concluded from this trial that spice-salted frozen/thawed herring ripens in a similar manner to that of fresh herring. The ripened taste formed quickly in the frozen/thawed herring although, at the latter stages of ripening, the intensity of the taste was at similar level to that found in fresh salted herring. The taste characteristics of frozen/thawed herring were typical for spice-salted herring with an intense malty/creamy taste. Colour analysis showed also negligible differences between fresh and previously frozen spice-salted herring.

The general and specific proteolytic enzyme activity was found to be much higher in the previously frozen herring resulting in a fast breakdown of macromolecules such as proteins. This was indicated by a rapid increase in soluble nitrogenous componds, peptide like compounds, alfa amino nitrogen and the content of free amino acids. It is likely that the freezing and thawing process activated proteolytic enzymes thus causing the observed changes. The freezing and thawing process is also known to affect the tissue structure e.g. in cell rupture and protein denaturation and thus possibly making the tissues more susceptible for degraditon. The increased proteolytic breakdown could also explain the observed texture changes as the frozen/thawed herring became quickly soft and watery in comparison with the fresh salted raw material. Instrumental texture analysis, both TPA and shear force measurements, confirmed the sensory results - the frozen/thawed herring became more quickly tenderised in comparison with the fresh salted herring. It can therefore be concluded that the frozen/thawed herring ripened faster than fresh herring; the sensory data and instrumental texture analysis indicated that the difference in ripening speed was about 4 weeks.

Further work is required e.g. on the effect of freezer storage, freezing and thawing methods on the raw material before salting and also on the quality and storage life of the salted products.

5. ACKNOWLEDGEMENT

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7. APPENDIX

Table 8. Outline of the measurement plan

Measurement plan

Participant	Rf	FF	FRFC	Norc	HIFI	Rivo*
Method						
Temperature of brine	X	X	X	X	X	
Brine strength	X	X	X	X	X	
pH in muscle	X	X	X	X	X	
pH in brine	X	X	X	X	X	
Sensory analysis on fillets	X		X	X	X	
TCA in muscle		X				
TCA in brine		X				
α-amino nitrogen in muscle						X
Protein in muscle	X	X				
Protein in brine	X	X				
Water in muscle	X					
Salt in muscle	X					
Fat content in muscle	X					
Proteol. activity muscle	X	X	X	X	X	
Proteol. activity brine	X	X	X	X	X	
Proteol. activity pyl. caeca						
Specific enzyme act. muscle		X				
Specific enzyme act brine		X				
Free amino acids in muscle				X		X
Free amino acids in brine				X		X
Peptide fingerprinting		X				
Texture			X			
Colour			X			
DSC measurem. in pyl. caeca			X			
Peroxide and TBA					X	
GC analysis of lipids and vol.					X	
Amine determination					X	
ATP breakdown products						X

*Rivo DLO would like to obtain an extract for analysis from Norconserv

Table 9. Raw data from FF.

Frozen					Not-frozen				
Weeks	$Temp_{brine}$	$Baume_{brine}$	pH_{muscle}	pH_{brine}	Weeks	$Temp_{brine}$	$Baume_{brine}$	pH_{muscle}	pH_{brine}
0	-	-	6.7	-	0	-	-	6.7	-
4	6.0	25	6.3	5.8	8	-	-	6.7	-
16	6.0	24.5	6.3	5.8	16	-	-	6.5	-
26	5.5	26.5	6.3	5.9	21	6.0	22.5	6.6	6.1

Protein muscle

Frozen		Not-frozen	
Weeks	Protein	Weeks	Protein
0	16.7±0.3	0	16.7±0.3
4	18.3±0.4	8	18.7
16	16.4±0.4	16	18.0
26	16.3±0.3	21	13.6±0.2

Protein brine

Frozen		Not-frozen	
Weeks	Protein (%)	Weeks	Protein (%)
4	4.8±0.04	8	4.9
16	6.5±0.1	16	6.3
26	8.3±0.1	21	4.3±0.05

Dry matter muscle

Frozen		Not-frozen	
Weeks	Dry matter (%)	Weeks	Dry matter (%)
0	32.3±1.0	0	32.3±1.0
4	-	8	46.0
16	46.7±1.1	16	47.1
26	47.7±0.7	21	43.8±0.6

Ash muscle

Frozen		Not-frozen	
Weeks	Ash (%)	Weeks	Protein
0	1.2±0.1	0	1.2±0.1
4	-	8	11.3
16	11.1±0.2	16	12.3
26	11.4±0.1	21	11.6±0.1

Table 10. Raw data from IFL.

Sensory evaluation

Week	Ripening	St dev	Stock fish	St dev	Malty	St dev	Rawness	St dev	Watery	St dev	Softness	St dev	Tenderness	St dev
4	33,4	0,5	22,8	4,4	20,4	0,9	9,7	5,1	22,7	2,8	31,1	7,1	40,7	4
8	44,3	6,2	19,5	2,9	29,8	4,7	3,2	1,6	35,8	5,4	46,8	8,6	45,4	4
13	51,6	5	23,6	4,5	25,9	9,2	6	0,5	36,1	3	42,7	3,2	42,7	2,3
16	54,7	3,3	23,5	3	29,8	2,7	5,6	5,5	39,5	4,8	51,9	11,2	52,9	6,2
20	60,3	7,9	22	4,4	34,9	8,7	2,6	2	42,1	6,7	55,2	5,8	58,7	4,2
24	70,1	3,3	17,4	9,4	47	10,7	0,7	0,3	57,1	4,5	63,4	6,5	64,9	6
31	74,7	5,9	21,4	3,6	42,9	6	2,6	0,7	46,2	6	66,4	1,6	70,4	3,3

Frozen/thawed

Week	Ripening	St dev	Stockfish	St dev	Malty	St dev	Rawness	St dev	Watery	St dev	Softness	St dev	Tenderness	St dev
4	41,7	6,2	16,3	1	28,2	6	8,9	3,9	35,1	2,7	42	1,4	44,9	3,8
16	62	2	22,7	4,4	28,5	2,3	4,7	2,6	45,6	1,5	65,5	1,1	67,4	0,6
26	74,7	5,9	21,4	3,6	42,9	6	2,6	0,7	46,2	6	66,4	1,6	70,4	3,3

Chemical results

% Protein content of fillets (KS)					
Week	Value 1	Value 2	Value 3	Mean	Stdev.
4	20,39	20,12	20,18	20,23	0,14
16	18,89	19,07	18,93	18,96	0,09
26	18,42	18,43	18,41	18,42	0,01

% TCA content of fillets (KS)					
Week	Value 1	Value 2	Value 3	Mean	Stdev.
4	0,97	0,97	0,97	0,97	0,00
16	1,20	1,20	1,19	1,20	0,01
26	1,52	1,52	1,52	1,52	0,00

% water content of fillets (KS)					
Week	Value 1	Value 2	Value 3	Mean	Stdev.
4	53,31	54,78	55,02	54,37	0,93
16	54,17	54,02	54,22	54,14	0,10
26	54,19	54,00	54,13	54,11	0,10

% salt content of fillets (KS)					
Week	Value 1	Value 2	Value 3	Mean	Stdev.
4	10,03	10,00	10,07	10,03	0,04
16	11,91	11,88	11,84	11,88	0,04
26	11,43	11,49	11,59	11,50	0,08

Table 10. Raw data from IFL cont'd

% fat content of fillets (KS)					
Week	Value 1	Value 2	Value 3	Mean	Stdev.
4	12,38	12,27	12,25	12,30	0,07
16	10,72	11,06	10,69	10,82	0,21
26	11,66	12,12	11,80	11,86	0,24

Composition of whole headless herring before salting
Sample: Size 3-500

	Value 1	Value 2	Value 3	Mean	Stdev.
% Protein					
% Water	63,23	63,7	63,56	63,50	0,24
% Salt	0,29	0,29	0,29	0,29	0,00
% Fat	18,43	18,07	18,79	18,43	0,36
pH	6,95				

Composition of herring fillets before salting

	Value 1	Value 2	Value 3	Mean	Stdev.
% Protein					
% Water	67,86	67,25	67,38	67,50	0,32
% Salt	0,29	0,29	0,29	0,29	0,00
% Fat	12,23	12,64	12,74	12,54	0,27
%TCA	0,8	0,8	0,8	0,80	0,00

pH in KS fillets

Week	pH
0	6,73
4	6,44
16	6,21
26	6,21

pH in KS brine

Week	pH	Temp. °C	Salt °B
4	5,81	6,0	25,0
16	5,84	5	23
26	5,84	5	23

Table 11. Raw data from Norconserv.

Chemical results

Dato	Week 4	std	week 16	std	Week 26	std
Temp.brine	5,00		6,00		6,00	0,00
Dencity-In	1,16		1,18	0,00	1,18	0,00
Salt / Bo	19.99/19.5		22,60	0,00	22,60	0,00
Dencity-w/v	1,17		1,16		1,17	0,00
Salt / Bo	20.52/20		20,52		21,53	0,45
pH-muscle	6,19	0,01	6,07	0,03	6,12	0,01
pH-brine	5,74	0,02	5,73	0,03	5,76	0,01
GPA-muscle	0,02	0,01	0,10	0,04	0,26	0,04
GPA-brine	2,92	0,19	2,50	0,05	2,96	0,01

Table 12. FAA data from Norconserv.

Content of free amino acids in herring muscle (mean values in µg/g)

		Frozen			Fresh
	Week 0	Week 4	Week 16	Week 26	Week 21
ASP	42	362	949	1646	299
GLU	1093	1843	2153	3395	1170
SER	196	645	1230	2057	509
HIS	2167	1807	1988	2209	781
ASN	0	8	296	434	75
GLY	740	1074	1330	1892	838
*TEU	2798	2871	2413	2614	1880
THR	213	513	1049	1799	429
ALA	907	1633	2367	3747	1509
ARG	132	513	1558	2719	424
*METSO2	667	642	667	667	667
PRO	126	329	444	653	228
TYR	85	415	752	1121	307
VAL	181	703	1299	2357	570
MET	93	501	885	1527	334
CYS2	217	319	501	518	332
ILE	88	605	905	1558	308
LEU	168	985	2178	3681	781
PHE	48	340	588	958	228
TRP	392	93	243	460	87
LYS	703	1209	2713	4633	908
SUM	11056	17408	26508	40643	12883

Table 12. FAA data from Norconserv cont'd.

Content of free amino acids in brine (mean values in µg/g)

	Frozen			Fresh
	Week 4	Week16	Week26	Week 21
ASP	468	1125	1999	384
GLU	1791	2495	4012	1440
SER	776	1448	2461	650
HIS	1659	2410	2572	1076
ASN	56	262	409	87
GLY	1458	1677	2357	1156
*TEU	3979	3220	3325	2572
THR	583	1280	2149	553
ALA	2008	2969	4766	1938
ARG	600	1709	3091	532
*METSO2	642	667	667	667
PRO	433	589	839	309
TYR	432	753	1143	308
VAL	815	1520	2821	713
MET	516	968	1710	404
CYS2	449	499	766	356
ILE	712	1036	1921	383
LEU	1136	2357	4222	936
PHE	409	572	950	236
TRP	83	174	336	377
LYS	1255	3218	5664	1179
SUM	20260	31184	48180	16429

Table 13. Raw data from FRCF.

	4th week		week 16		Week 26	
	Arithmetic means	Standard deviations	Arithmetic means	Standard deviations	Arithmetic means	Standard deviations
ripe	56,33	19,62	81,92	15,37	92,52	6,37
malty	33,70	27,99	52,46	28,10	55,70	29,52
raw	29,30	25,07	8,08	11,21	2,67	2,09
sweet	24,19	24,08	30,38	27,31	37,85	30,61
rancid	1,30	0,60	1,63	0,86	1,48	0,63
aftertaste	31,67	38,39	19,08	29,35	20,52	33,89
succulent	43,19	28,05	65,61	18,64	75,81	18,60
fatty mouth	36,19	24,09	66,04	22,44	68,67	21,48
soft	39,89	19,60	64,00	21,17	80,41	15,89
hard	35,78	21,55	16,92	14,97	5,48	5,92
tender	32,89	22,94	61,13	24,24	80,81	18,96
dry	26,30	24,18	14,79	23,01	4,22	4,79
tough	23,85	20,19	7,92	8,97	2,89	2,90

Table 13. Raw data from FRCF cont'd.

	Weeks		
	4	16	26
Temperature in brine	3,5	5	3
density g/ml	1,187	1,1804	1,1819
pH muscle	6,2	6,22	6,01
pH brine	5,73	5,83	5,71
GPA muscle	0,045±0,02	0,305±0,099	0,689±0,1
GPA brine	3,66±0,01	3,59±0,029	3,66±0,022
GPA pyloric caeca	14,97±2,46	7,3±0,94	5,93±0,83

Table 14. FAA data from RIVO-DLO.

Content of free amino acids in herring samples from Norconserv
(mean and standard deviation)

	Icelandic herring frozen-thawed	Icelandic herring from frozen	Frozen herring experiment
	week 4	week 16	week 26
[ASP] (µg/g)	566 ± 142	2230 ± 67	2114 ± 190
[GLU] (µg/g)	773 ± 170	2752 ± 55	2630 ± 237
[SER] (µg/g)	440 ± 70	1551 ± 31	1391 ± 153
[HIS] (µg/g)	2218 ± 44	3565 ± 71	2003 ± 160
[GLY] (µg/g)	377 ± 41	948 ± 28	666 ± 73
[THR] (µg/g)	302 ± 63	938 ± 38	901 ± 81
[ARG] (µg/g)	658 ± 53	2052 ± 103	2084 ± 188
[TAU] (µg/g)	1820 ± 182	2836 ± 28	1553 ± 202

Content of free amino acids in herring samples from Norconserv
(mean and standard deviation)

	Icelandic herring frozen-thawed	Icelandic herring from frozen	Frozen herring experiment
	week 4	week 16	week 26
[ALA] (µg/g)	766 ± 69	2370 ± 47	2019 ± 40
[TYR] (µg/g)	475 ± 48	1573 ± 47	1199 ± 168
[MET] (µg/g)	419 ± 71	1517 ± 61	1405 ± 211
[VAL] (µg/g)	548 ± 88	3565 ± 71	1826 ± 164
[PHE] (µg/g)	544 ± 87	1821 ± 36	1696 ± 136
[ILE] (µg/g)	344 ± 93	1539 ± 31	1479 ± 74
[LEU] (µg/g)	798 ± 168	2820 ± 56	2708 ± 217
[LYS] (µg/g)	1912 ± 918	5886 ± 24	4667 ± 327

Table 14. FAA data from RIVO-DLO cont'd.

Content of free amino acids in herring samples from Norconserv
(mean and standard deviation)

	Control ripening Icelandic herring	Raw material
[ASP] (μg/g)	1139 ± 126	201 ± 32
[GLU] (μg/g)	1779 ± 285	502 ± 35
[SER] (μg/g)	1102 ± 110	529 ± 16
[HIS] (μg/g)	1848 ± 351	2063 ± 21
[GLY] (μg/g)	1138 ± 125	881 ± 70
[THR] (μg/g)	952 ± 133	635 ± 70
[ARG] (μg/g)	1193 ± 95	617 ± 173
[TAU] (μg/g)	2028 ± 385	1428 ± 100

Content of free amino acids in herring samples from Norconserv
(mean and standard deviation)

	Control ripening Icelandic herring	Raw material
[ALA] (μg/g)	2174 ± 87	1145 ± 80
[TYR] (μg/g)	934 ± 56	369 ± 77
[MET] (μg/g)	1117 ± 56	261 ± 13
[VAL] (μg/g)	1185 ± 47	383 ± 15
[PHE] (μg/g)	1090 ± 55	298 ± 63
[ILE] (μg/g)	353 ± 85	20 ± 7
[LEU] (μg/g)	1390 ± 83	468 ± 28
[LYS] (μg/g)	981 ± 304	411 ± 70

Content of free amino acids in brine from Norconserv
(mean and standard deviation)

	Icelandic herring frozen-thawed	Icelandic herring from frozen	Frozen herring experiment
	week 4	week 16	week 26
[ASP] (μg/g)	1062 ± 53	1259 ± 126	2069 ± 248
[GLU] (μg/g)	1163 ± 47	1542 ± 154	2622 ± 341
[SER] (μg/g)	932 ± 37	878 ± 44	1256 ± 126
[HIS] (μg/g)	2210 ± 133	2188 ± 263	2719 ± 326
[GLY] (μg/g)	1169 ± 47	750 ± 53	889 ± 71
[THR] (μg/g)	1006 ± 40	807 ± 32	1242 ± 335
[ARG] (μg/g)	1111 ± 22	1084 ± 108	1572 ± 865
[TAU] (μg/g)	1581 ± 237	1886 ± 283	1799 ± 144

Table 15. Raw data from HIFI.

BIOCHEMISTRY DATA

Muscle

		n	Sample	Frozen Fresh	Frozen Spice/salt	Frozen Spice/salt	Fresh Spice/salt	Frozen Spice/salt
Ripening Time	(weeks)			0	4	16	23	26
% Moisture		6	mean	68,88	51,74	54,03	57,39	54,67
			std dev	0,01	0,73	1,20	0,96	0,57
pH		3	mean	6,69	6,36	6,22	6,46	6,26
			std dev	0,05	0,73	0,01	0,01	0,02
% Salt	(w/w)	6	mean	0,47	10,84	13,08	13,10	12,83
			std dev	0,05	0,15	0,05	0,11	0,17
FAN		6	mean	43,91	104,88	153,22	45,26	245,37
			std dev	1,71	1,76	4,45	0,75	7,27
TNBS		6	mean	62,27	136,52	195,71	64,46	328,38
			std dev	1,16	10,54	4,02	1,01	4,12
% Lipid	(w/w)	3	mean	11,52	13,12	10,90	14,83	14,03
			std dev	0,94	1,12	2,09	1,53	0,33
Peroxide Value	mEq/kg oil	3	mean	67,00	45,39	34,50	31,72	28,93
			std dev	5,00	10,17	9,41	2,71	1,27
TBARS		3	mean	1,57	0,00	1,74	5,43	1,90
			std dev	1,57	0,00	0,23	2,64	2,00
Protein	(mg/ml)	9	mean	10,19	12,13	10,79	7,25	6,50
			std dev	0,69	0,37	0,33	0,71	0,37
GPA- pH6.0	Ab/h/g fish	6	mean	0,00	0,02	0,04	0,02	0,07
			std dev	0,01	0,00	0,01	0,00	0,01
	Ab/h/mg Protein	6	mean	0,00	0,00	0,00	0,00	0,00
			std dev	0,00	0,00	0,00	0,00	0,00

Table 15. Raw data from HIFI cont'd.

BIOCHEMISTRY DATA

Brines

		n	Sample	Frozen Fresh	Frozen Spice/salt	Frozen Spice/salt	Frozen Spice/salt	Frozen Spice/salt
Ripening Time				0	4	16	23	26
pH		3	mean		5,79	5,71	5,94	5,75
			std dev		0,01	0,01	0,00	0,00
Ab		3	mean		0,22	0,23	0,14	0,21
			std dev		0,01	0,01	0,00	0,01
Salt	(g/ml)	6	mean		0,82	0,85	0,82	0,81
			std dev		0,02	0,01	0,10	0,01
Protein	(mg/ml)	9	mean		7,83	12,04	13,13	12,58
			std dev		0,06	0,06	0,10	0,36
GPA- pH 6.0	Ab/h/ml brine	6	mean		0,16	0,14	0,00	0,15
			std dev		0,01	0,00	0,00	0,01
	Ab/h/mg Protein	6	mean		0,02	0,01	0,00	0,01
			std dev		0,00	0,00	0,00	0,00

SENSORY ANALYSIS

Flavours

Sample		Frozen Fresh	Frozen Spice/salt	Frozen Spice/salt	Fresh Spice/salt	Frozen Spice/salt
Ripening time		0	4	16	23	26
Ripened	mean		4,57	5,22	5,44	4,25
	std dev		2,44	2,49	2,24	3,41
Dried Cod	mean		2,29	3,89	3,44	4,00
	std dev		1,38	2,32	2,30	3,63
Malt	mean		1,29	3,78	2,89	4,00
	std dev		1,25	1,09	1,54	2,00
Raw Herring	mean		3,00	1,44	1,44	2,13
	std dev		2,65	1,13	1,24	1,81
Fruit	mean		2,43	4,33	2,89	4,86
	std dev		1,99	1,73	1,54	1,95
Pepper	mean		2,29	2,89	3,22	2,63
	std dev		1,50	2,76	2,22	1,77
Rancid	mean		1,83	1,00	1,44	2,63
	std dev		1,83	1,73	1,74	2,62
Off Flavour	mean		2,83	0,00	0,71	2,88
	std dev		2,64	0,00	1,50	2,85

Table 15. Raw data from HIFI cont'd.

Texture

		Frozen	Frozen	Frozen	Fresh	Frozen
Sample		Fresh	Spice/salt	Spice/salt	Spice/salt	Spice/salt
Ripening time		0	4	16	23	26
Viscosity	mean		4,00	6,44	4,44	7,88
	std dev		2,90	2,40	1,94	1,81
Elasticity	mean		7,17	2,89	4,00	2,38
	std dev		1,17	1,83	2,06	2,20
Cohesiveness	mean		7,50	2,33	5,22	2,25
	std dev		1,87	0,87	1,99	2,49
Succulence	mean		3,00	5,44	5,67	6,63
	std dev		1,90	1,94	1,32	2,62
Toughness	mean		2,50	5,44	3,67	8,00
	std dev		0,84	2,24	1,41	1,31

TEXTURE ANALYSIS

All results obtained from analysis of 5 fish (2 fillets) ie. 10 replicate analysis
ELASTICITY

Sample	Frozen Fresh	Frozen Spice/salt	Frozen Spice/salt	Fresh Spice/salt	Frozen Spice/salt
Ripening time	0	4	16	23	26
X	*	1,703438	1,618745	**	1,472742
X Std Err.		0,017438	0,004448		0,005021
a		0,588475	0,619308		0,680247
C		1,389356	1,080151		0,934008
C Std Err.		0,791204	0,198185		0,220984

*) Method and results not comparable with non-cured fish
**) Insufficient fish in sample to conduct Texture analysis

Table 15. Raw data from HIFI cont'd.

TEXTURE ANALYSIS

TPA

Sample		Frozen Fresh	Frozen Spice/salt	Frozen Spice/salt	Fresh Spice/salt	Frozen Spice/salt
Ripening time		0	4	16	23	26
Springiness	Mean	*		0,21798	**	0,197444
	Std			0,102706		0,052311
Cohesiveness	Mean			0,3018		0,268444
	Std			0,094748		0,036003
Chewiness	Mean			1,412		1,005444
	Std			0,45015		0,491431
Gumminess	Mean			5,348		4,8655
	Std			1,131355		1,719405
Initial Modulus	Mean			3001800		2182200
	Std			1701653		2061757
Hardness	Mean			16,2905		17,80433
	Std			3,24066		6,716629

*) Method and results not comparable with non-cured fish

**) Insufficient fish in sample to conduct Texture analysis

Figure 20. Sensory scheme used at FRCF.

Sensorische Analyse von Salzfischerzeugnissen

Projekt: Enzymatische Reifung von pelagischen Fischarten

Code-Nr.:__________________ Prüfer:________________ Datum:__________

Bitte charakterisieren Sie die Ihrer Meinung nach zutreffende Ausprägung des Merkmals durch ein Kreuz (x) auf der Linie

Geruch/Geschmack (Aroma)

Reif (Parmaschinken, Käse, Sardellen):

....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....

0 100

Malzig (Malzextrakt, -bonbon, -bier):

....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....

0 100

Roh (roher Fisch, Blutgeschmack):

....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....

0 100

Süß (überreif):

....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....

0 100

Ranzig (alter Lebertran):

....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....

0 100

Nachgeschmack (länger verbleibender Eindruck nach Ausspeien der Probe): *

....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....

0 100

Textur

Saftig (Verbleiben des Wassers in der Probe auch bei längerem Kauen):

....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....

0 100

Fettig (angenehm fettiges Mundgefühl):

....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....

0 100

Weich (irreversible Verformung der Probe):

....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....

0 100

Hart (Widerstand der Probe beim ersten Zubeißen):

....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....

0 100

Zart (Zergehen auf der Zunge beim Druck gegen den Gaumen):

....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....

0 100

Trocken (Gefühl am Mund, am Gaumen nach längerem Kauen):

....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....

0 100

Zäh (Gefügeeindruck nach längerem Kauen):

....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....,....

0 100

*: Wie beschreiben Sie den 'Nachgeschmack'?