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Shelf life of radio-frequency and conventionally heated frozen cod and salmon fillets

Radio-Frequency Heating Technology for Minimally Processed Fish Products; RF-Fish
EU project number: QLK1-CT-2001-01788

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Skýrsluágrip Rannsóknastofnunar fiskiðnaðarins
Icelandic Fisheries Laboratories Report Summary

Titill / Title	*Shelf life of radio-frequency and conventional heated frozen cod and salmon fillets*		
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Ágrip á íslensku:	Skýrsla þessi er samantekt rannsókna í verkefninu "Radio-Frequency Heating Technology for Minimally Processed Fish Products" árin 2004 og 2005. Framkvæmdar voru geymsluþolstilraunir við -24°C á soðnum (radio frequency heated (RF) og conventionally autoclave heated (CON)) og ferskum, frystum (IQF) þorski og laxi. Öll sýnin voru í loftdregnum umbúðum. Eftir 0, 3, 6, 9 mánuði (þorskur) og 0, 3, 6 og 8 mánuði (lax) voru sýnin þiðið og soðin/upphituð fyrir skynmat og mælingar á áferð, vatnsheldni og þránun. Ekki var mikill munur á áhrifum geymslutímans á sýnahópa. Ferskleikaeinkunn lækkaði með tíma fyrir þorskinn og var um 6,5 eftir 9 mánuði, áferð breyttist lítið en vatnsheldni var hærri í þorski eftir 3-9 mánuði í geymslu. Í laxinu breyttust jákvæði matsþættir lítið en neikvæðir, jarð-, súr og þráalykt, jukust heldur með geymslutíma. Bragð og áferð breyttist lítið. Eftir 8 og 9 mánaða geymslu höfðu sýnin ekki náð lokum geymsluþols. Fryst (IQF) sýni voru flögukenndari og höfðu minni vatnsheldni og mýkt samanborið við upphituð sýni (RF og CON).		
Lykilorð á íslensku:	*RF-hitun, geymsluþol, frystigeymsla, skynmat, áferð, vatnsheldni, þorskur, lax.*		
Summary in English:	This report is a part of the EU-project "Radio-Frequency Heating Technology for Minimally Processed Fish Products" and combines studies from 2004 to 2005. Quality and shelf life during frozen storage (-24°C) of pre-heated (radio-frequency and conventionally autoclave heated) cod and salmon loins and fresh frozen loins (IQF) was compared. All samples were vacuum packed. Samples were thawed and re-heated/cooked after 0, 3, 6, 9 months (cod) and 0, 3, 6, 8 months (salmon) and evaluated with sensory evaluation. Texture, water holding capacity and oxidation was measured. Freshness of cod samples decreased somewhat, texture did not change much but water holding capacity was higher in cod samples after 3-9 months of frozen storage. Positive sensory attributes of salmon did not change much through the storage, but negative attributes, such as sour and rancid increased with the storage time. The samples had not reached the end of shelf life after the 8 and 9 months of frozen storage. IQF samples were flakier and had less water holding capacity and were less soft compared to pre-heated samples (RF and CON).		
English keywords:	*Radio-Frequency heating, shelf life, frozen storage, sensory evaluation, texture, water holding capacity, cod, salmon*		

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1. INTRODUCTION

The experiments described in this report were carried out as a part of the EU project "Radio-Frequency Heating Technology for Minimally Processed Fish Products" (EU project number: QLK1-CT-2001-01788).

In the first part of this project a comparison of quality measurements of fresh and thawed cod fillets was done. Sensory analysis, texture measurements and measurement of water holding capacity in addition to microbial counts were all methods that gave valuable information about the quality (Sveinsdottir et al, 2003b).

The same methods were used in the second part of the project, which was comparison of four different treatments of cod and salmon fillets; fresh packed, frozen, radio-frequency heated and conventionally heated fillets. In this comparison fresh and frozen/thawed samples were cooked and compared to re-heated samples of radio-frequency heated and conventionally heated fillets. The comparison showed that fresh and frozen/thawed samples received higher freshness scores. Rancid flavour was detected in the re-heated salmon fillets and liquid formation was observed in the re-heated samples. The results in the second part indicated that the use of fresh raw material of good quality for the production of heated products was very important (Thorkelsdottir et al, 2004).

The next step in the project was to perform shelf life studies of the heated products (cod and salmon), during frozen and chilled storage. Shelf life of fresh fish is short, only 15 days for whole cod stored in ice (Martinsdottir, 2001) and 20 days for whole salmon stored in ice (Sveinsdottir et al, 2003a). According to information from fish processing companies (HB-Grandi, Akranes, Iceland), storage time of fresh packed fillets is often regarded to be 4-7 days. According to the same source, storage time of fresh frozen cod fillets is usually regarded to be 1-2 years, but most commonly set at 18-24 months, depending on the country exported to. Shelf life of frozen salmon is much shorter than for lean fish such as cod. Storage study at IFL indicated that 4 months was the maximum shelf life for for frozen salmon fillets stored at -26°C (Þórisson and Bragadottir, 1992). Whole frozen salmon can be stored longer, Refsgaard et al (1998) reported only minor

sensory changes in whole salmon during 8 months of storage at -30°C. However, Þórisson and Bragadottir (1992) found that 8 months was the maximum shelf life of whole frozen salmon at -26°C as thereafter changes occurred in colour and flavour.

Pre-cooked products are well known and pre-cooking may be used to extend shelf life. Processing methods, storage conditions such as temperature and packaging material are important factors in keeping the shelf life as long as possible. Odour, flavour, appearance and texture will be affected; resulting in more poor quality product.
In a shelf life study of sous vide salmon (Conzález-Fandos et al, 2005) fish processed at 65°C had shelf life about 21 days when stored at 2°C, which was extended up to 45 days when product was processed at 90°C.
Expected storage time of pre-cooked cod produced at Fjordkokken are 33 days kept at 0-4°C and 26 days for salmon at same condition. Maximum six months are expected storage time for frozen pre-cooked salmon and 10 months for pre-cooked cod, though not recommended (information from Fjordkokken AS, Varhaug, Norway).

In this study the main objective was to estimate the quality and shelf life of frozen cod and salmon fillets after radio-frequency (RF) and conventional (CON) heating during storage at -24°C, in comparison to traditionally individually quickly frozen products. Measurement were done by sensory evaluation, texture measurement with Texture Analyser, Water Holding Capacity (WHC), cook-out %, water and fat content, pH, Formaldehyde (FA) and Thiobarbituric reactive substances (TBARS) contents and Peroxide value.

2. MATERIAL & METHODS

Sample preparation

Cod (*Gadus morhua*):

Raw material (Icelandic wild cod (*Gadus morhua*)) was collected by the fish processing company HB-Grandi (Akranes, Iceland) in May 2004. After catch, the cod was stored whole in ice for 2-3 days until it was filleted, deskinned and trimmed, loin parts (140±10g) were cut from the fillets, packed in vacuum pack as in previous trials (Sveinsdottir et al, 2003b) and stored at 0-1°C.

The day of packing, the cod samples were packed with ice mats in polystyrene boxes and transported to NORCONSERV (Stavanger, Norway) for conventional heating and to Fraunhofen IVV (Freising, Germany) for RF heating. The samples were heated at 75°C 4 days after packing and stored at -24°C until transported to IFL (Icelandic Fisheries Laboratories, Reykjavik, Iceland), in polystyrene boxes. At IFL the samples were analysed after 0, 3, 6 and 9 months of frozen storage at -24°C. Samples were thawed at 4°C for 24 hours before measurements.

Salmon *(Salmo salar)*:

Raw material (Norwegian farmed salmon (*Salmo salar*)) was collected by FK (Fjordkjokken AS, Varhaug, Norway) in August 2004. Loin parts (140±10g) were cut from the fillets, packed in vacuum pack as in previous trials (Sveinsdottir et al, 2003b) and stored at 0-1°C.

The day of packing, the salmon samples were packed with ice mats in polystyrene boxes and transported to NORCONSERV for conventional heating and to Fraunhofen IVV for RF-heating. The samples were heated at 75°C 4 days after packing and stored at -24°C until transported to IFL, in polystyrene boxes. At IFL the samples were analysed after 0, 3, 6 and 8 months of frozen storage at -24°C. Samples were thawed at 4°C for 24 hours before measurements.

Sensory evaluation of cooked and re-heated fillets

The sensory evaluation of freshness of cooked and reheated cod was done using the Torry scheme giving scores from 10 (very fresh) to 3 (Shewan et al, 1953). In addition, the Quantitative Descriptive Analysis (QDA) method (introduced by Stone and Sidel, 1985) was used to assess the cooked samples. The method assumes detailed description of a product, such as odour, flavour, appearance and texture. However, in this project the focus was only on texture for cod and therefore an unstructured scale (0-100) was used for a list of words describing texture i.e. flakes, softness, juiciness and tenderness.

Cooked and reheated salmon was evaluated by the QDA method (introduced by Stone and Sidel, 1985). Unstructured scale (0-100%) was used on a list of fifteen sensory descriptors to describe odour and flavour intensity and texture attributes of cooked salmon. Attributes were both positive and negative to evaluate the freshness of samples. Odour attributes evaluated were characteristic odour, seaweed-sea, liver-oil, earthy, sour and rancid odour. Flavour attributes evaluated were characteristic flavour, metal, oil, sweet, earthy, sour and rancid flavour. Texture attributes were flakes, softness, juiciness and tenderness.

Twelve panellists of the Icelandic Fisheries Laboratories sensory panel participated in the sensory evaluation. They were all trained according to international standards (ISO, 1993); including detection and recognition of tastes and odours, training in the use of scales, and in the development and use of descriptors. The members of the panel were familiar with the Torry and QDA method and experienced in sensory analysis of cod and salmon.

The samples were heated at 95-100°C in a pre-warmed oven (Convostherm, Convostar, Germany) with air circulation and steam for 9-10 minutes in the vacuum packs. Core temperature in samples was in the range of 2 to 4°C when put into the oven and around 70°C after heating. Four samples were collect from each loin. The size of each sample was ca. 1-2 cm in width, and 4-6 cm in length. The samples were placed in aluminium boxes (5 cm in width x 8 cm in length x 4 cm in height) and closed with plastic covers before served for the sensory panel. Each sample was coded with a composite of 3

numbers that did not indicate the storage time or any other information. Each panellist evaluated 3 samples in each session and each sample was evaluated in duplicate.
All sample observations were conducted according to international standards (ISO, 1988).

Texture measurements

Texture of cooked samples was measured using the Texture Profile Analysis test (TPA). Three cooked loins of cod and salmon were measured from each treatment. The texture analyser used was the TA.XT2i Stable Micro Systems (Stable Micro Systems Ltd., Godalming, England).

The force - time curve was analysed to determine three texture parameters: *Hardness*: The maximum force (N) at certain adjusted deformation. *Cohesiveness*: Amount (%) of displacement before the sample brakes (strength of inner bonds). *Resilience*: The capability (%) of a strained body to recover after deformation caused by compressive stress.

The SMS probe and setting for the TPA test were: Aluminium Compression plate, 100 diameter (P/100). Pre test speed 2.0 mm/s; speed in sample 0.8 mm/s. Strain (distance) 80%. Load Cell Capacity (kg) 25. During a TPA test the sample was compressed two times in a reciprocating motion that was supposed to imitate the action of the jaw.

The samples (loins) were cooked in the vacuum packs in a steam oven (95-100°C) for 9-10 minutes in a Convostar oven (Convotherm, Elektrogeräte GmbH, Eglfing, Germany) and put on ice, and stored in 0-2°C refrigerator for minimum 2 hours before measured. Each loin sample was cut into two up to four 2.5 x 2.5 cm cubes, depending on sample size, and measured. The reported TPA force for each sample was the average value of these 2-4 measurements. All fish pieces and prepared samples were stored on plastic film on ice until measured.

Cook-out

Evaluation of cook-out was performed by steam cooking/heating the vacuum packed loins (n=3) at 95-100°C for 9-10 minutes in a Convostar oven (Convotherm, Elektrogeräte GmbH, Eglfing, Germany). Core temperature in samples was in the range of 2 to 4°C when put into the oven. The loins were cooled on ice for 15 min prior to weighting. The total weight of the vacuum packed loins was recorded, then the package was cut open and the cooked-out liquid pored away. Then weight of the fish and packing material was recorded and finally only the packaging material was weighted. The values obtained were used to calculate the cook-out, which was expressed as percent of the weight lost due to cooking.

$$\text{Cook-out \%} = 100 \times \frac{\textit{Weight of sample in packaging} - (\textit{Weight of drained sample} + \textit{packing material})}{\textit{Weight of sample} - \textit{packing material}}$$

Water holding capacity (WHC)

The analysis of WHC was based on method described by Børresen (1980) but was modified by reducing the speed from 1500 g`s to 500x g`s. Cooked samples (n=3) were stirred with a spatula to homogenise the sample. Approximately 2 g of the muscle were weighted accurately into a test tube with known weight and centrifuged (SS-34 rotor; Sorvall RC-5B, Du Pont, Delaware, USA) at 530g for 5 min; with temperature maintained at 2 to 5 °C. Two parallels were used for each sample. After centrifugation, the total weight of each test tube and sample was recorded and used to calculate sample weight after centrifugation. WHC was calculated as percentage remaining water of initial water in sample:

WHC (%) = $(v_1-\Delta r)/(100-\Delta r)* 100\%$

v_1 = % water in sample before centrifugation

v_1 = (Weight before drying-Weight after drying) / (Weight before drying) * 100%

Δr= Weight before centrifugation - Weight after centrifugation / (Weight before centrifugation) * 100%

Water content, fat content and pH

Water content (g/100g) was calculated as the loss in weight, during drying at 105 °C for 4 hours (ISO, 1983). Fat content was determined by the AOCS Soxhlet method Ba 3-38

(AOCS, 1998) using petroleum ether (Bp. 40-60 °C) for extraction. The pH was measured before WHC-analysis by inserting a combination electrode (SE 104, Mettler Toledo GmbH, Greifensee, Switzerland) directly into the samples. The electrode was connected to a portable pH meter (Portamess 913 pH, Knick, Berlin, Germany).

Peroxide value

Extraction of lipids was carried out by chloroform/methanol extraction system based on the method of Bligh and Dyer (Bligh and Dyer, 1959) with some modifications (Hanson and Olley, 1963) and with butylated hydroxytoluene (BHT) admixed into all solvents (50-100 mg/L). The following determinations on the lipid fraction were performed after evaporation (Büchi, Switzerland) at 37 °C under vacuum. Peroxide value (meq/kg lipid) of the extracted lipids was measured by iodometric titration according to AOAC official method 965.33 (AOAC, 1990).

Thiobarbituric reactive substances (TBARS)

TBARS were determined by a modified version (Sørensen and Jørgensen, 1996) of the extraction method described by Vyncke (1970, 1975) with few modifications. The sample size was reduced to 15 g and homogenized with 30 mL of 7.5% trichloroacetic acid solution containing 0.1% of both propyl gallate and EDTA. The absorbance of samples and standards were measured at 530 nm. TBARS, expressed as µmol malondialdehyde per kilogram of sample (µmol MDA/kg), was calculated using malondialdehyde-bis-(diethyl acetate) as standard.

Formaldehyde (FA) content

Samples were prepared with addition of phosphoric acid and distillation of formaldehyde and then react with cromotropicacid. Absorbance were measured at 530 nm. (Z.Anal. Chem. 1937).

Data analysis

Statistical analysis was performed by Microsoft Excel 8.00 (Microsoft Inc, Redmond, USA) and NCSS 2000 (NCSS, Utah, USA). Student's t-test, ANOVA and Duncan's test were performed to analyse if samples were statistically different. Multivariate

comparison of the different attributes measured was carried out in the statistical programme Unscrambler ®, Version 6.1 (CAMO, Trondheim, Norway), with principal component analysis (PCA). Before the analysis, variables were scaled. Each element in the matrix was multiplied with the inverse of the standard deviation of the corresponding variable if the variables had different ranges. By doing this, each variable has the same variance. The significance level was $p < 0.05$.

3. RESULTS AND DISCUSSION

EXPERIMENT 1; SHELF LIFE OF individually quick frozen COD (IQF), CONVENTIONALLY (CON) AND RADIO-FREQUENCY (RF) HEATED COD STORED AT -24°C

Sensory evaluation

Samples were evaluated every three months from beginning of frozen storage until nine months of frozen storage with sensory evaluation. The results are shown in table 1 and Figure 1 and 2.

Table 1. Average sensory scores of cod samples as evaluated by the sensory panel. Different superscripted letters indicate difference within each row ($p<0.05$). CON = conventionally heated, IQF = individually quick frozen, RF = radio-frequency heated, M = storage months at -24°C.

Sample name	Torry freshness score	Flakes	Softness	Juiciness	Tenderness
CON 0M	$7,5^{ace}$	46	55	38	53
IQF 0M	$8,1^{a}$	61^{acf}	48^{ce}	43	50
RF 0M	$7,7^{abe}$	40^{e}	61^{acd}	41	58
CON 3M	$7,3^{adfg}$	44^{bde}	54^{cd}	33	44^{bc}
IQF 3M	$7,3^{adfg}$	64^{a}	43^{ce}	43	42^{cd}
RF 3M	7^{begh}	40^{e}	72^{a}	44	64^{ab}
CON 6M	$6,6^{cfgh}$	41^{e}	47^{ce}	38	44^{bc}
IQF 6M	$6,6^{gh}$	61^{ab}	43^{df}	42	47^{bc}
RF 6M	$6,4^{gh}$	36^{e}	62	41	53
CON 9M	$6,2^{h}$	40^{ef}	57	35	56
IQF 9M	$6,3^{h}$	52	45^{ce}	41	42^{cd}
RF 9M	$6,5^{fh}$	41^{e}	70^{abd}	42	69^{a}

Freshness decreased with the storage time. After nine months of frozen storage the samples were still acceptable with regard to freshness, slightly above the score 6. At IFL, the average score of 5.5 on the Torry freshness score sheet has been regarded as the limit for shelf life. The sample groups were not different with regard to freshness within each storage time evaluated.

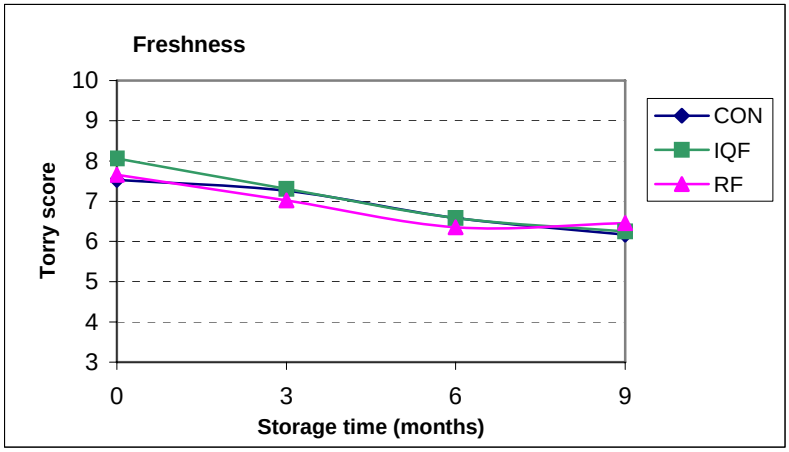

Figure 1. Average Torry freshness scores of cooked/re-heated cod; IQF (Individually Quick Frozen)-cod, RF (Radio-Frequency heated)-cod and CON (Conventionally heated)-cod after 0-9 months of frozen storage at -24°C.

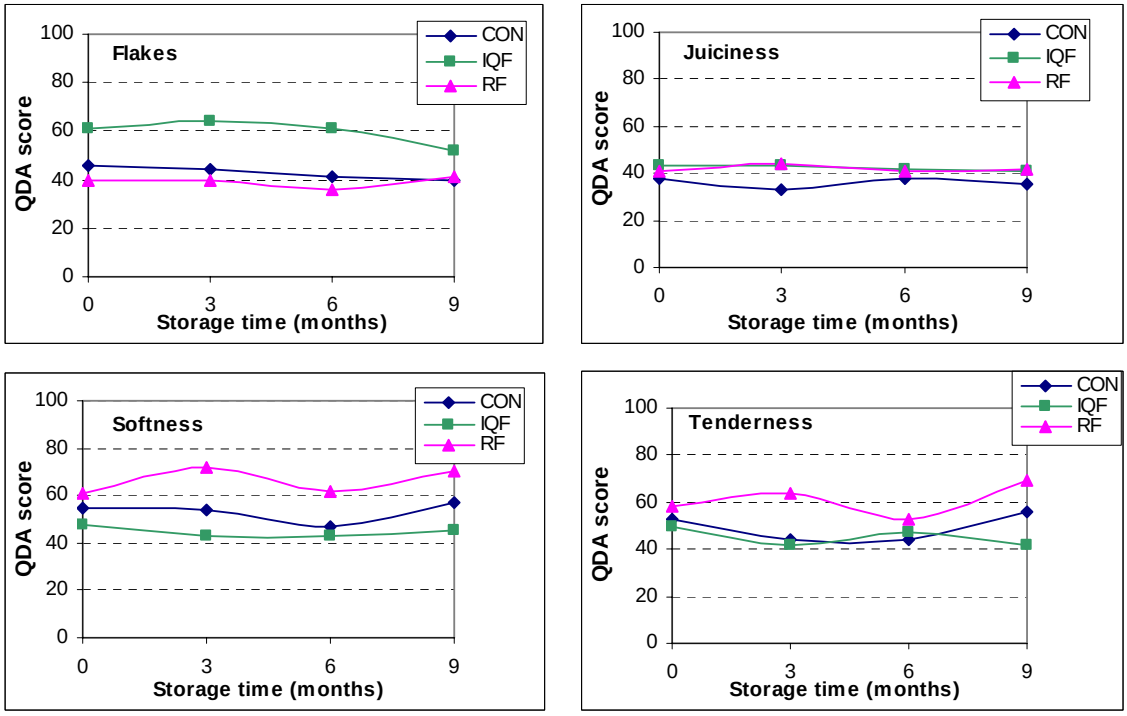

Figure 2. Average QDA (texture) scores of cooked/re-heated cod; IQF (Individually Quick Frozen)-cod, RF (Radio-Frequency heated)-cod and CON (Conventionally heated)-cod after 0-9 months of frozen storage at -24°C.

Flakiness did not change through the storage time, but the IQF samples were flakier compared to RF and CON samples. Softness and tenderness did not change much through the storage time, but RF samples were more soft and tender compared to CON and IQF

samples. All samples received rather low scores for juiciness through the storage time (Figure 2).

Instrumental texture measurements

The measured averages for hardness of cooked and reheated cod are shown in Figure 3 and the values for cohesiveness and resilience of the same samples are shown in Figures 4 and 5.

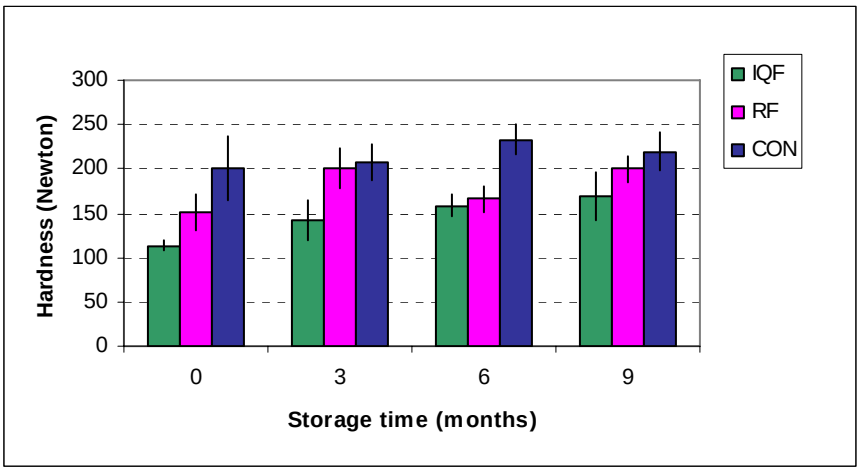

Figure 3. Hardness measurement of cooked/re-heated cod; IQF (Individually Quick Frozen)-cod, RF (Radio-Frequency heated)-cod and CON (Conventionally heated)-cod after 0-9 months of frozen storage at -24°C.

As can be expected the trend for hardness as seen in Figure 3 is to increase with extended frozen storage. The IQF samples received significantly lower values for hardness than RF and CON after 0 and 3 months but after 6 months CON is significantly higher in hardness than IQF and RF which have at this time very close values. After 9 months of frozen storage CON is significantly higher in hardness than IQF. IQF cod is therefore lower in hardness during the 9 months of frozen storage compared to reheated RF and CON cooked cod. It is possible that the IQF showed lower values in the instrumental measurement than RF and CON as IQF was found significantly flakier. The IQF samples tended to slide more apart during the instrumental measurement and received therefore lower values.

Compared to CON cooked cod, the hardness of RF cooked cod is significantly lower after 0 and 6 months of frozen storage but the trend is though that CON is higher in hardness compared to RF in all the measuring points. Same trend was observed for chilled CON and RF samples were compared earlier in the project (Thorkelsdottir et al, 2004).

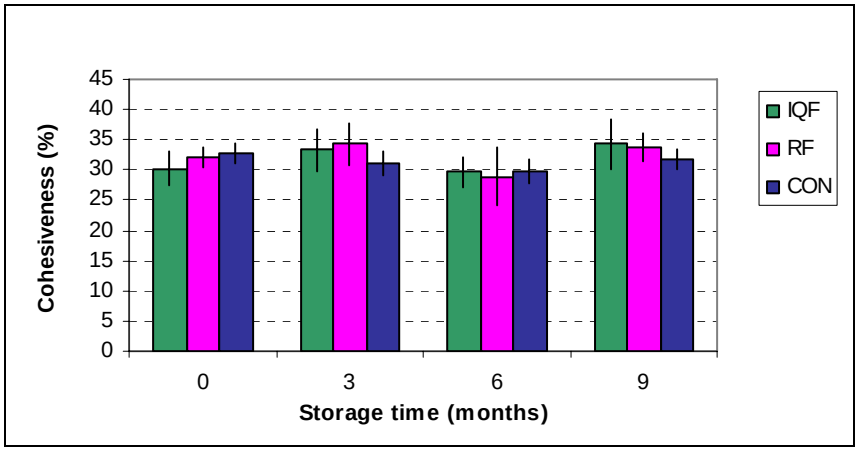

Figure 4. Cohesiveness of cooked/re-heated cod; IQF (Individually Quick Frozen)-cod, RF (Radio-Frequency heated)-cod and CON (Conventionally heated)-cod after 0-9 months of frozen storage at -24°C.

The values for cohesiveness (Figure 4) were not different with time or between treatments during the 9 months of frozen storage.

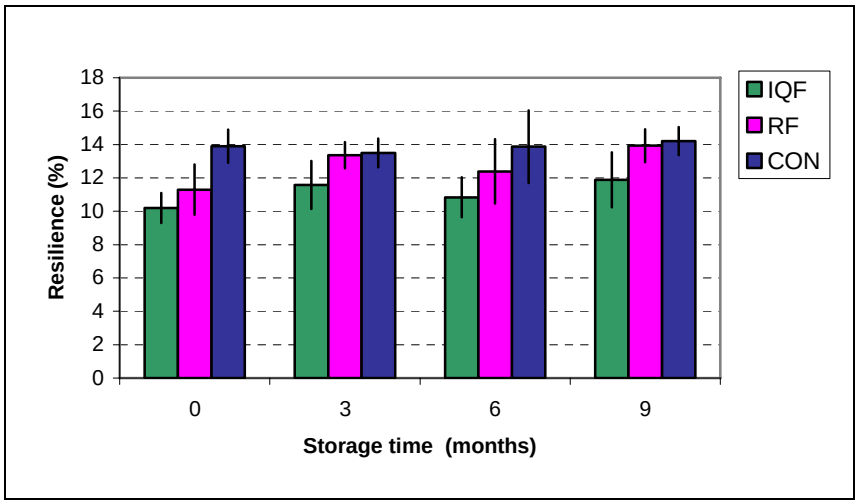

Figure 5. Resilience of cooked/re-heated cod; IQF (Individually Quick Frozen)-cod, RF (Radio-Frequency heated)-cod and CON (Conventionally heated)-cod after 0-9 months of frozen storage at -24°C.

IQF is significantly less resilient (Figure 5) in all measuring points during the frozen storage compared to RF and CON. RF is significantly lower in resilience than CON after 0 and 6 months but have almost the same value after 3 and 9 months of frozen storage. Chilled RF heated cod has also been found to be less resilient than chilled CON heated cod (Thorkelsdottir et al, 2004).

Cook-out and water holding capacity (WHC)

Cook-out in cod samples was not significantly affected by type of heat treatment or by double heating as could be seen by comparing the CON and RF samples to IQF samples which were only single cooked at IFL before sensory analyses. On the other hand, WHC was both affected by heat treatment and time. CON samples had higher WHC compared to RF samples, but IQF samples had the lowest WHC. The WHC tended to be higher after 3 to 9 months of frozen storage than at the beginning of the storage time (Figure 6). In trial II (Thorkelsdottir et al, 2004), it was observed that cook-out was higher in CON heated samples than in IQF and RF-heated samples. On the contrary, no significant effects of heating method on WHC were observed. It must be mentioned, that samples were only analysed once by Thorkelsdotter et al (2004) but not 4 times over 9 months as in this experiment.

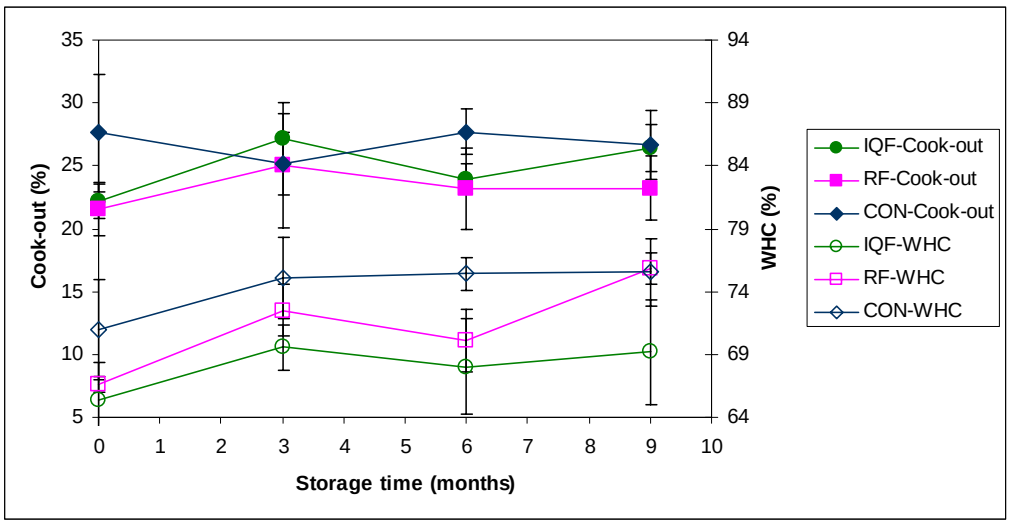

Figure 6. Cook out and WHC (%) of cooked/re-heated cod; IQF (Individually Quick Frozen)-cod, RF (Radio-Frequency heated)-cod and CON (Conventionally heated)-cod after 0-9 months of frozen storage at -24°C.

Water content and pH

Effects of type of heat treatment or time on water content were not significant in cod samples stored frozen from 0 to 9 months (Figure 7). This was expected with regard to the results for cook-out which was similar for all groups.

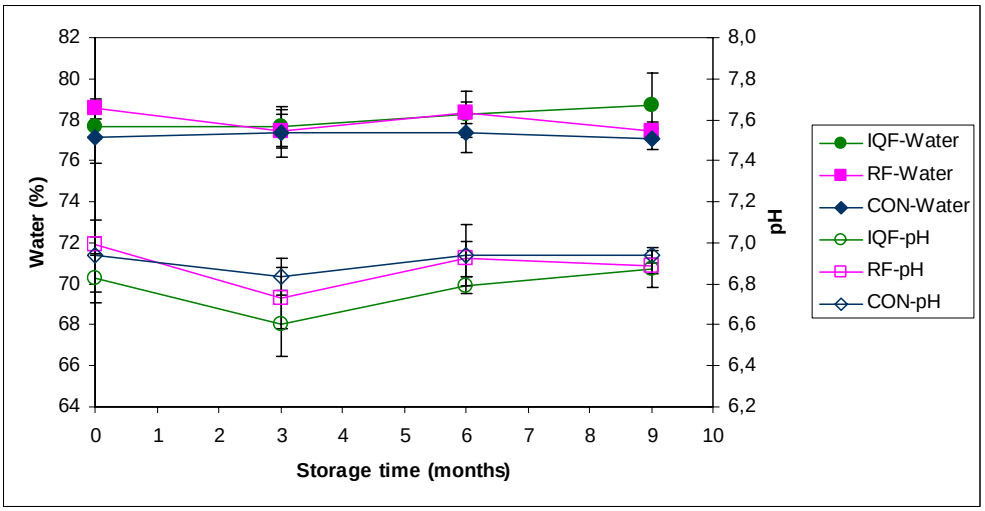

Figure 7. Water and pH of cooked/re-heated cod; IQF (Individually Quick Frozen)-cod, RF (Radio-Frequency heated)-cod and CON (Conventionally heated)-cod after 0-9 months of frozen storage at -24°C.

The pH tended to be lower in IQF samples than in CON and RF (Figure 7) as in trial II (Thorkelsdottir et al, 2004). However, differences between heat treatments were not significant.

Formaldehyde (FA) and thiobarbituric reactive substances (TBARS)

The formaldehyde was only determined after 6 months of frozen storage and was 112 µg/g in CON heated samples compared to 62 µg/g in RF and 53 µg/g in IQF samples. These values were relatively low compared to studies on raw cod. However, cooking may have increased binding of formaldehyde in the muscle, resulting in lower values then in raw fish (Rehbein, 1987). Leblanc et al (1987) showed that the formaldehyde content in frozen cod fillets stored at -22 °C, increased over 3 months period from about 1 to 4 mg/kg, whereas values of about 20 mg/kg and 2.6 mg/kg where observed after 3 months at -12 °C and -30 °C, respectively (Leblanc et al, 1988). In another study, the formaldehyde content of the raw material stored for 48 hours on ice was about 1.2 mg/kg but increased to 1.8 mg/kg after 6 months storage at -30 °C (Leblanc et al, 1987).

The TBARS was similar in all groups but decreased from 2.2 - 2.8 μmol/kg after 6 months to 1.3 - 1.5 μmol/kg after 9 months. Aubourg and Medina (1999) who studied lipid deterioration of cod over 12 months of frozen storage, reported values of 1.5 μmol/kg at the beginning of the storage, the highest values 8.4 μmol/kg was obtained after 5 months. However, lower values, 1 to 3.9 μmol/kg, were observed after 7, 9 and 12 months.

Values observed in our experiment remained well below the limit for TBARS in raw cod 19 μmol/kg (Connell, 1975). Scientist do not fully agree on usefulness of TBARS to evaluate quality, but it is used widely as an indicator of degree of lipid oxidation, i.e. to measure secondary products. However, these products are not end-products of lipid oxidation and may react further with other components of the fish (Auburg, 1993). The effectiveness of using TBARS may vary with fish species and experimental design, i.e. when and if TBARS is analysed over certain period of time and in relation with other methods.

What also makes the interpretation of TBARS and formaldehyde difficult is that these factors are in our experiment analysed in cooked samples and may as such only function as reference values for later studies since little or no experience has been gained with analyses on cooked fish.

Comparison of measurements

Results from all methods were averaged over sample groups and compared in a PCA plot (Figure 8).

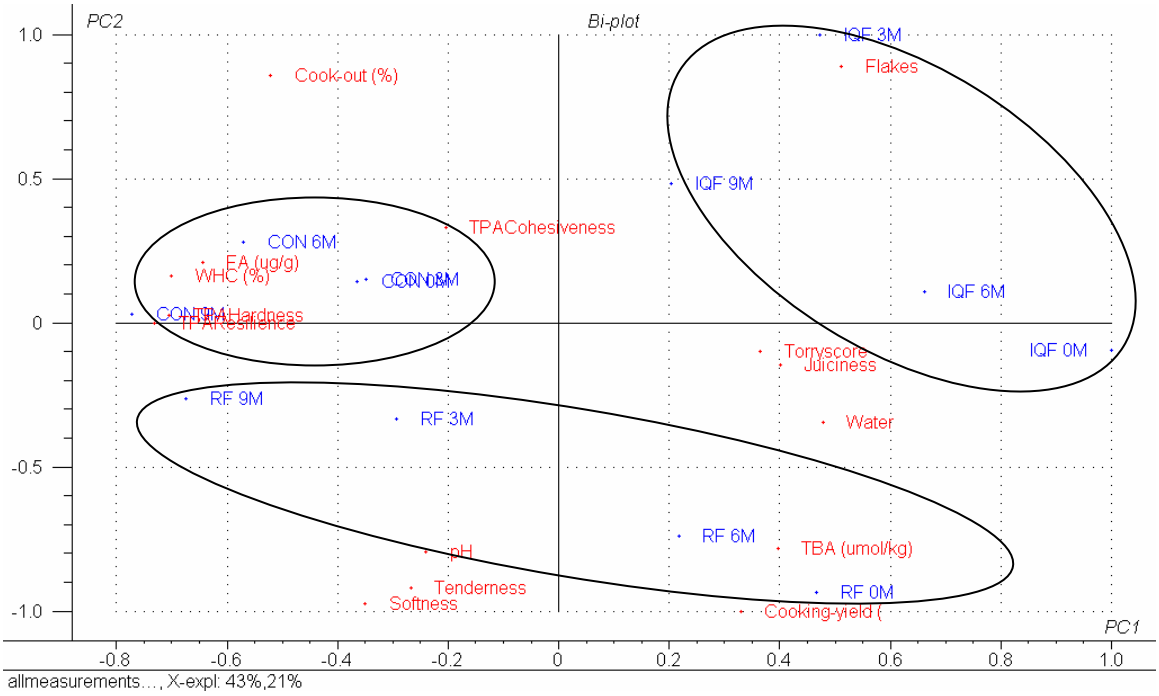

Figure 8. Scores and loadings (Bi-plot) of all measured parameters and sample groups, PC1 vs. PC2.; IQF (Individually Quick Frozen)-cod, RF (Radio-Frequency heated)-cod and CON (Conventionally heated)-cod after0-9 months of frozen storage at -24°C. (TBA= TBARS)

The sample groups are separated in the PCA plot, IQF samples with high values of flakiness and low values of e.g. WHC and instrumental texture parameters. CON samples on the other hand were related to high WHC and instrumental texture parameters. RF samples were more related to softness and tenderness.

EXPERIMENT 2; SHELF LIFE OF INDIVIDUALLY QUICK FROZEN (IQF), CONVENTIONALLY (CON) AND RADIO-FREQUENCY (RF) HEATED SALMON STORED AT -24°C

Sensory evaluation

Samples were evaluated every three months from beginning of frozen storage up to eight months of frozen storage with sensory evaluation. The results are shown in table 2 and Figures 9 and 10. PCA of the salmon samples and sensory attributes is shown in Figure 11.

Table 2. Average sensory scores of salmon samples as evaluated by the sensory panel. Different superscripted letters indicate difference within a row ($p<0.05$). CON = conventionally heated, IQF = individually quick frozen, RF = radio-frequency heated, M = storage months at -24°C, o = odour, f = flavour.

Sample name	characteristic (o)	seaweed-sea (o)	liver-oil (o)	earthy (o)	sour (o)	rancid (o)	Flakes	Softness	Juiciness	Tenderness	characteristic (f)	metal (f)	oil (f)	sweet (f)	earthy (f)	sour (f)	rancid (f)
CON 0M	54	27	30	21	7	7	36	65[a]	52	64	55	26	38	30	23	7	9
IQF 0M	54	28	30	20	10	6	52	54	57	63	52	25	47	29	18	9	10
RF 0M	60[a]	36	35	16[a]	2	0	39	62[a]	58	66	62[a]	25	38	30	18[a]	2	3
CON 3M	55	21	24	22	5	9	43	62[a]	56	64	56	30	38	25	28	7	10
IQF 3M	48	20	22	32	15	13	54	50	53	55	47	26	38	26	37[b]	13	13
RF 3M	53	22	27	29	4	8	43	65[a]	60	68[a]	54	27	36	28	32	8	10
CON 6M	50	24	26	25	7	7	39	57	45	58	52	27	28	21	26	10	10
IQF 6M	40[b]	22	18	25	14	10	54	43[b]	49	53	47	20	34	20	34	16	16
RF 6M	44	18	20	24	9	7	41	51	47	55	50	19	29	22	24	11	10
CON 8M	51	31	25	35[b]	7	10	46	57	43	50[b]	47	24	33	15	32	6	11
IQF 8M	41	31	30	33	11	9	52	52	50	55	42[b]	30	41	18	36	15	17
RF 8M	50	25	32	31	5	6	45	64[a]	57	65	54	27	41	17	34	5	14

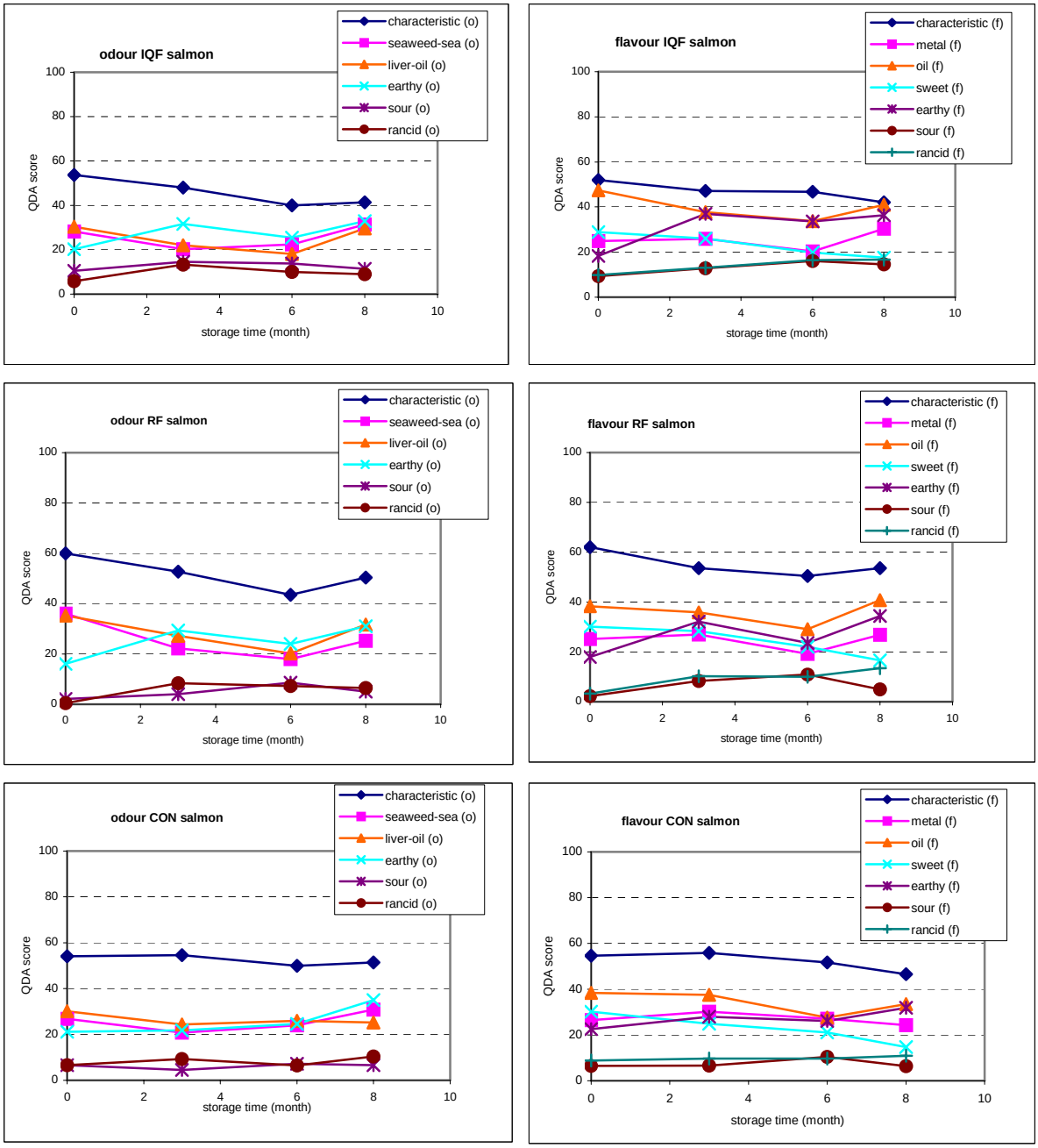

Figure 9. Average QDA (odour and flavour) scores of cooked/re-heated salmon; IQF (Individually Quick Frozen)-salmon, RF (Radio-Frequency heated)-salmon and CON (Conventionally heated)-salmon after 0-8 months of frozen storage at -24°C.

The three sample groups IQF and RF and CON were not different within each sampling day with regard to sensory evaluation (table 2).

The sensory attributes evaluated did not change much during eight months of frozen storage at -24°C (Figure 9). No significant changes were in the positive attributes

seaweed-sea odour, liver-oil odour and flavour, metal flavour and sweet flavour. Characteristic odour and flavour reduced a little. There was a little increase in the negative attribute earthy. Sour and rancid did not change during the storage time but from the first sampling day, IQF samples received higher scores for these attributes compared to RF and CON samples. The scores for those attributes were very close to 20, which has been considered as the limits for acceptable sensory quality for salmon (Sveinsdottir et al, 2002) after 8 months of frozen storage, in accordance to what has previously been reported by others (Þórisson and Bragadottir, 1992).

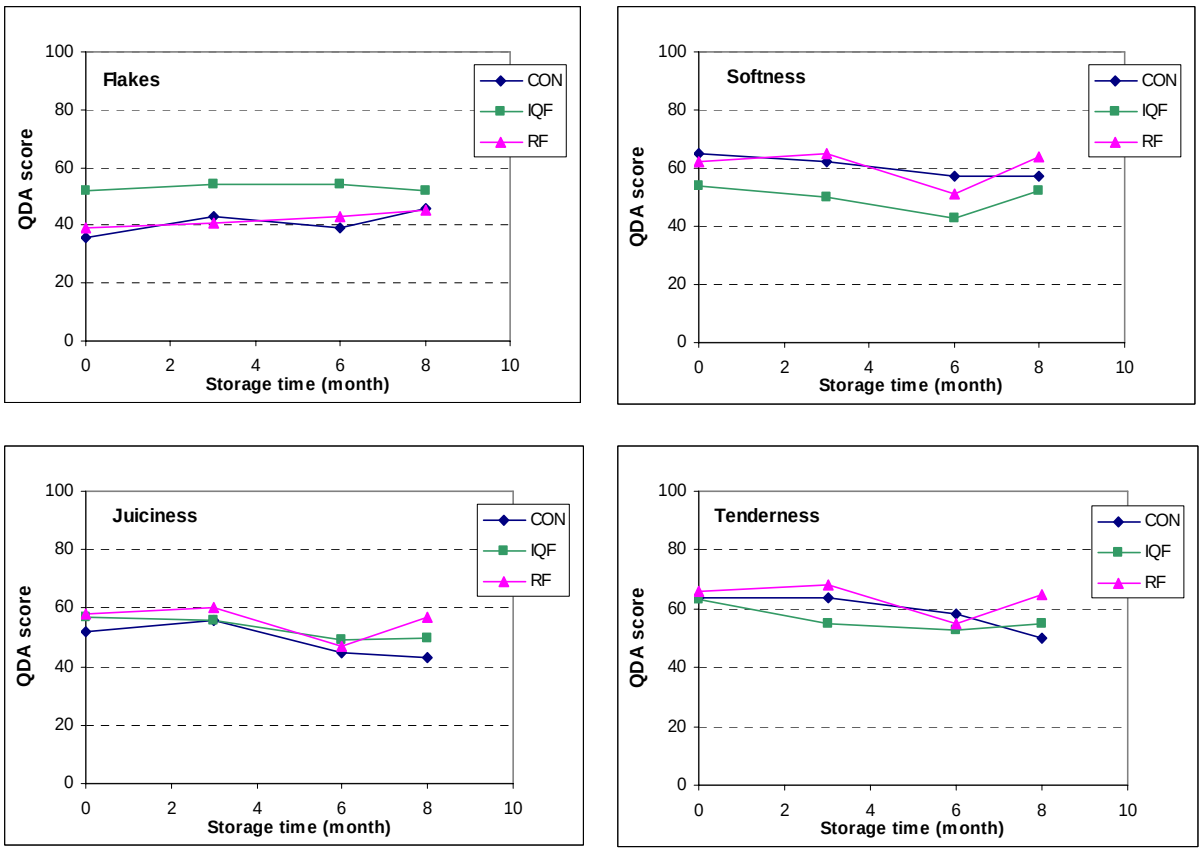

Figure 10. Average QDA texture scores of cooked/re-heated salmon; IQF (Individually Quick Frozen)-salmon, RF (Radio-Frequency heated)-salmon and CON (Conventionally heated)-salmon after 0-8 months of frozen storage at -24°C.

RF and CON heated samples were softer than IQF samples through the storage time (Figure 10). Juiciness and tenderness did not change much. IQF samples appeared to be flakier compared to RF and CON through the storage time samples, though the difference was not significant.

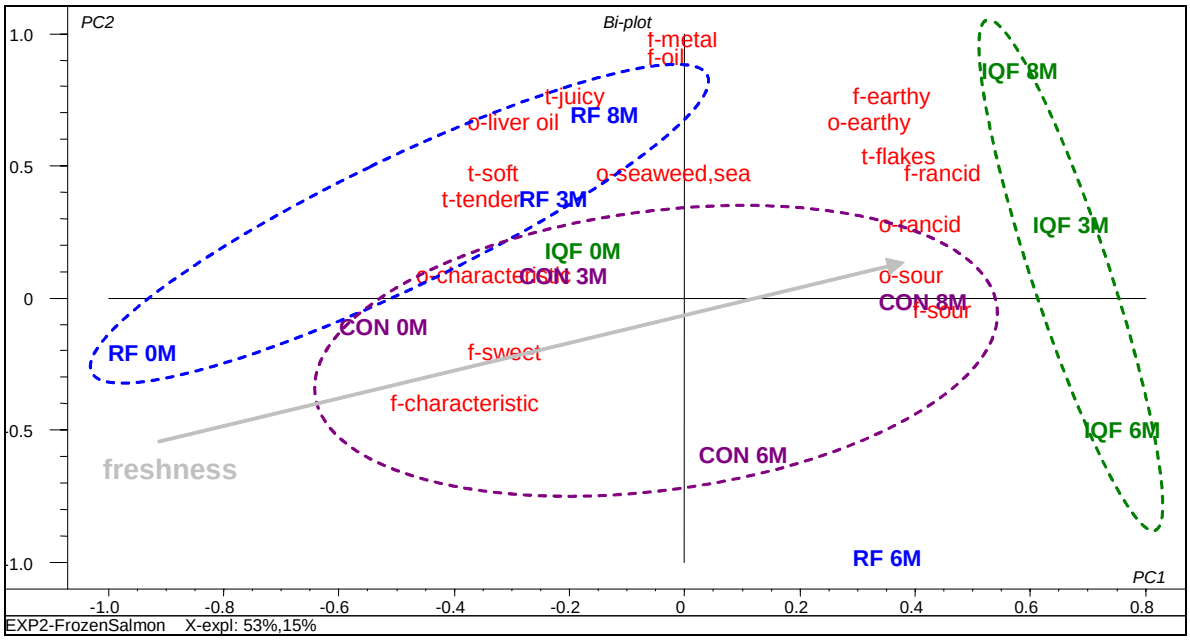

Figure 11. PCA of average QDA scores of cooked/re-heated salmon; IQF (Individually Quick Frozen)-salmon, RF (Radio-Frequency heated)-salmon and CON (Conventionally heated)-salmon after 0-8 months of frozen storage at -24°C. M = months of frozen storage, o = odour, f = flavour, t = texture.

Figure 11 shows an overview of the evaluated salmon samples and the sensory attributes evaluated. The PC1 axis, explaining 53% of the variation between the samples, mainly represents storage time with the positive (freshness) sensory attributes located in the left side and the negative (storage, spoilage) sensory attributes to the right. After 3, 6 and 8 months of storage, the IQF samples differ from the RF and CON samples as the IQF samples had higher scores of negative sensory attributes such as rancid, sour and earthy odour and flavour. In addition the IQF samples were flakier. Some separation can be seen in the PC2 axis (explaining 15% of the variation between the samples) between the RF and CON sample groups, indicating that the RF samples might be more juicy, soft and tender compared to the CON samples.

Instrumental texture measurements

The results from the instrumental texture measurement on hardness, cohesiveness and resilience are shown in Figures 12-14.

It was very difficult to measure the texture of the cooked salmon samples. When they were compressed the samples tended to slide (flake) apart invalidating the measurements. This difficulty in measuring the salmon induced very high standard deviation.

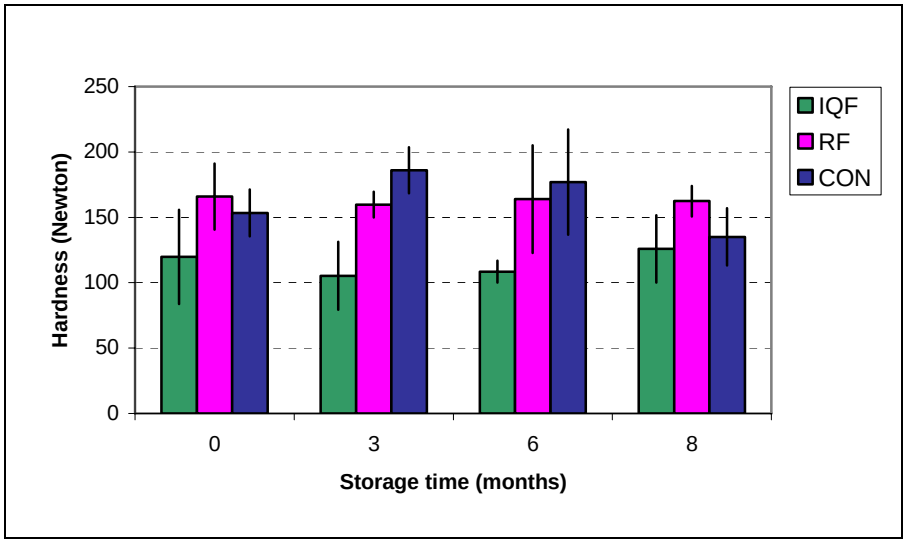

Figure 12. Hardness measurement of cooked/re-heated salmon; IQF (Individually Quick Frozen)-salmon, RF (Radio-Frequency heated)-salmon and CON (Conventionally heated)-salmon after 0-8 months of frozen storage at -24°C.

IQF had significantly lower values for hardness compared to RF and CON. This could be due to that the IQF samples were not precooked before frozen storage as the RF and CON samples and reheating seems to add to the firmness of the samples.

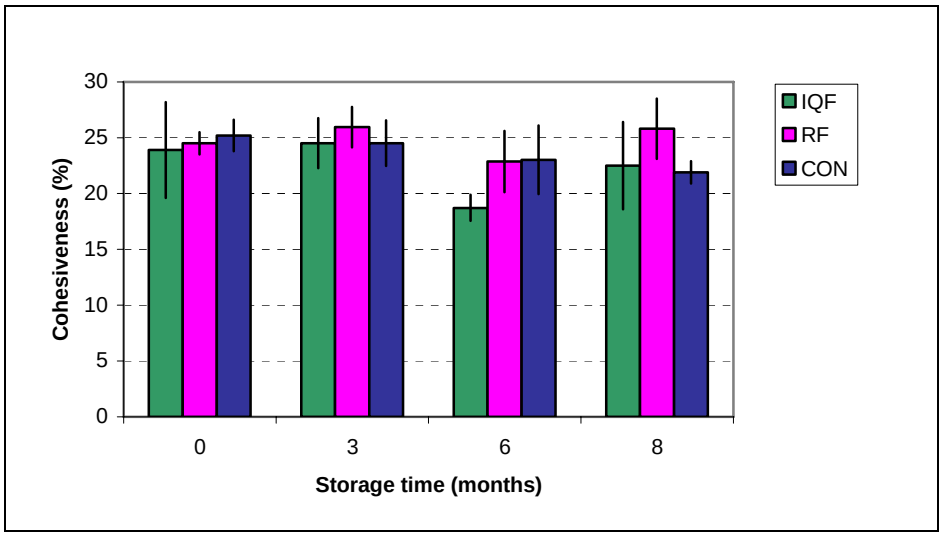

Figure 13. Cohesiveness of cooked/re-heated salmon; IQF (Individually Quick Frozen)-salmon, RF (Radio-Frequency heated)-salmon and CON (Conventionally heated)-salmon after 0-8 months of frozen storage at -24°C.

There is not a significant difference between treatments for the cohesiveness (Figure 13). There is however a tendency for the cohesiveness to decrease with storage time.

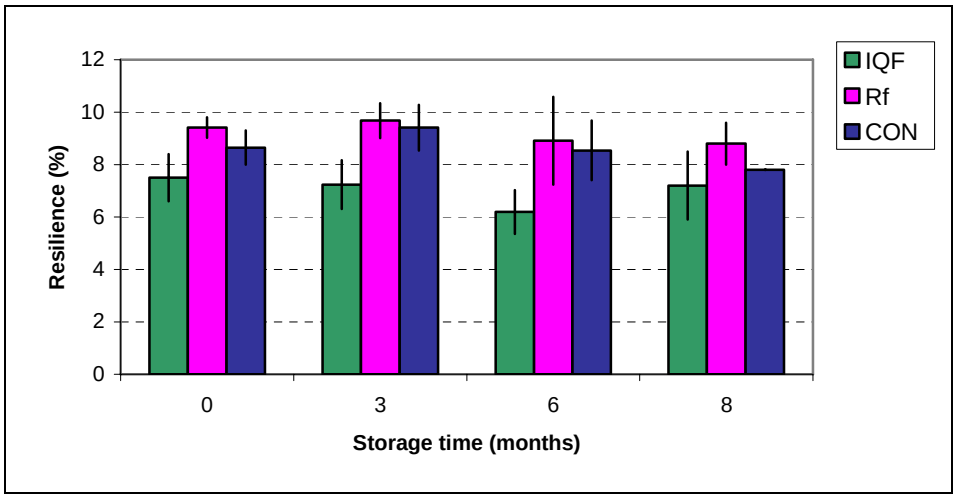

Figure 14. Resilience of cooked/re-heated salmon; IQF (Individually Quick Frozen)-salmon, RF (Radio-Frequency heated)-salmon and CON (Conventionally heated)-salmon after 0-8 months of frozen storage at -24°C. Calculated resilience (%) values with standard deviation from the time –force curve of TPA measurement.

IQF samples were significantly less resilient compared to RF and CON. Re-heating salmon may therefore increase resilience which indicates a tougher product. There is a

trend all through the storage time that the RF salmon is more resilient than the CON salmon but the difference is not significant.

Cook-out and water holding capacity (WHC)

The effects of reheating were significant with regard to cook-out as could been seen when comparing single heated IQF samples to reheated RF and CON samples. Cook-out of IQF samples was lower than in RF and CON heated samples at all sampling points and after 3 to 8 months it was higher in CON samples than in RF samples (Figure 15). It increased in CON samples with time but the variation was less in RF and IQF samples. The results confirmed what was observed in trial II (Thorkelsdottir et al, 2004), where cook-out tended to increase in the following order; IQF<Rf<CON.

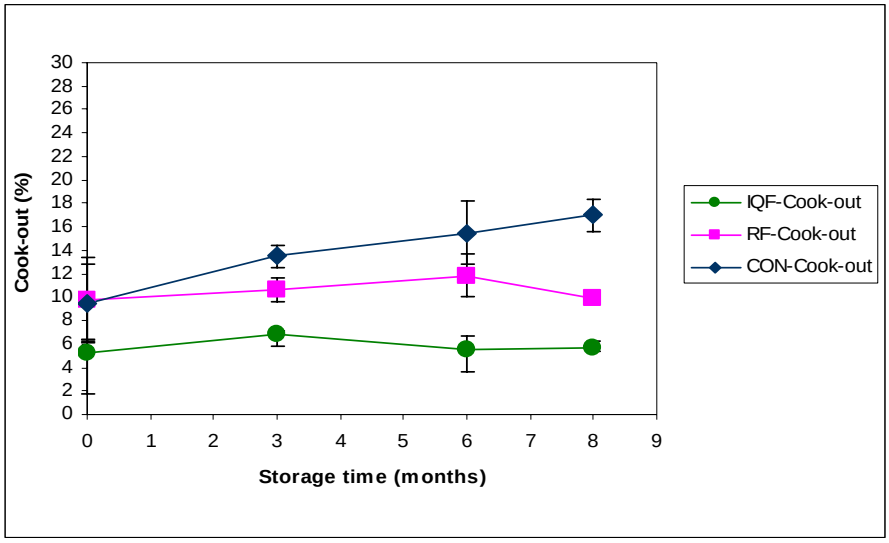

Figure 15. Cook-out (%) of cooked/re-heated salmon (n=3); IQF (Individually Quick Frozen)-salmon, RF (Radio-Frequency heated)-salmon and CON (Conventionally heated)-salmon after 0-8 months of frozen storage at -24°C.

Variation within each group was high which made it hard to evaluated effects of storage time but WHC tended to be higher after 3-9 months of frozen storage than at the beginning of the storage time (Figure 16). At the beginning of the storage, WHC was similar in all groups after 3 and 6 months in increased in the following order; IQF<CON<Rf, and CON<IQF<Rf after 9 months of frozen storage. In trial II (Thorkelsdottir et al, 2004), WHC was not significantly affected by heated treatment although it was slightly lower in IQF samples like in this experiment.

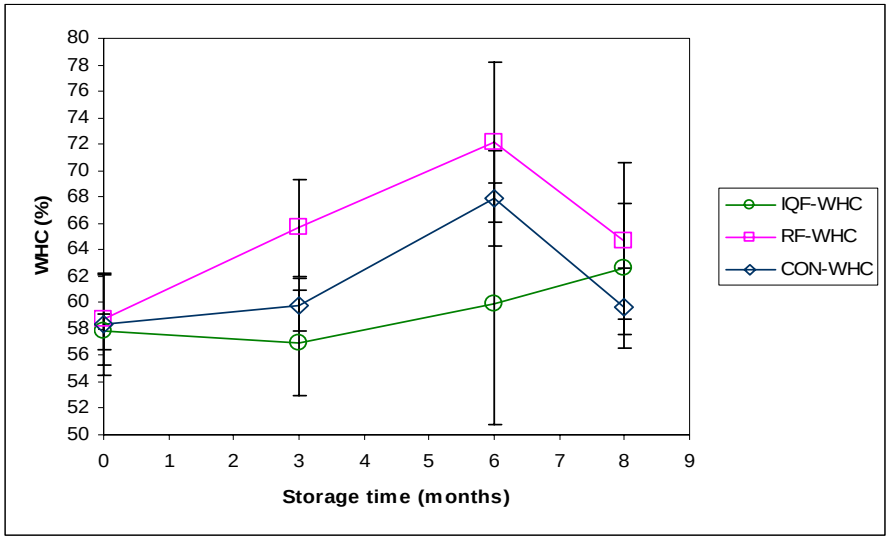

Figure 16. WHC (%) of cooked/re-heated salmon (n=3); IQF (Individually Quick Frozen)-salmon, RF (Radio-Frequency heated)-salmon and CON (Conventionally heated)-salmon after 0-8 months of frozen storage at -24°C.

Water, fat content and pH

Effects of type of heat treatment and time on water and fat content were not significant in salmon samples stored frozen from 0 to 8 months (Figure 17 and 18). The pH similar in all groups and the effects of storage time were not significant (Table 4).

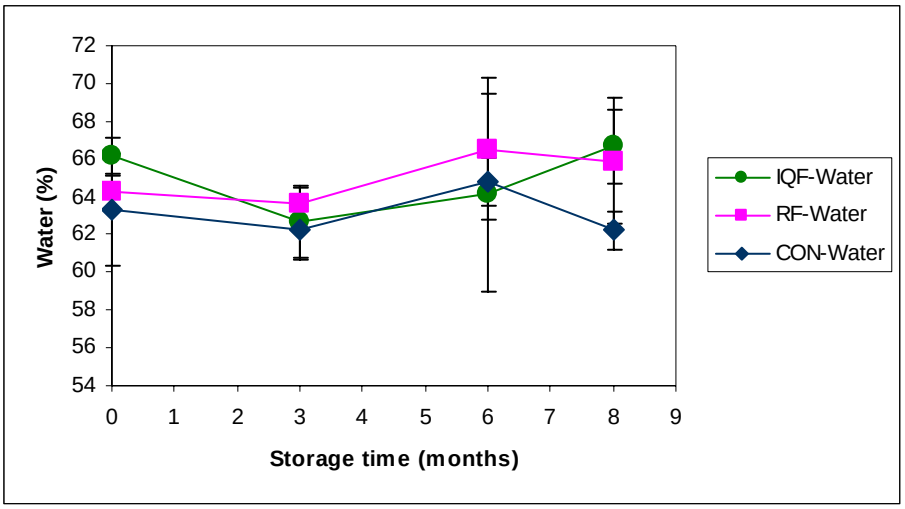

Figure 17. Water content (%) of cooked/re-heated salmon (n=3); IQF (Individually Quick Frozen)-salmon, RF (Radio-Frequency heated)-salmon and CON (Conventionally heated)-salmon after 0-8 months of frozen storage at -24°C.

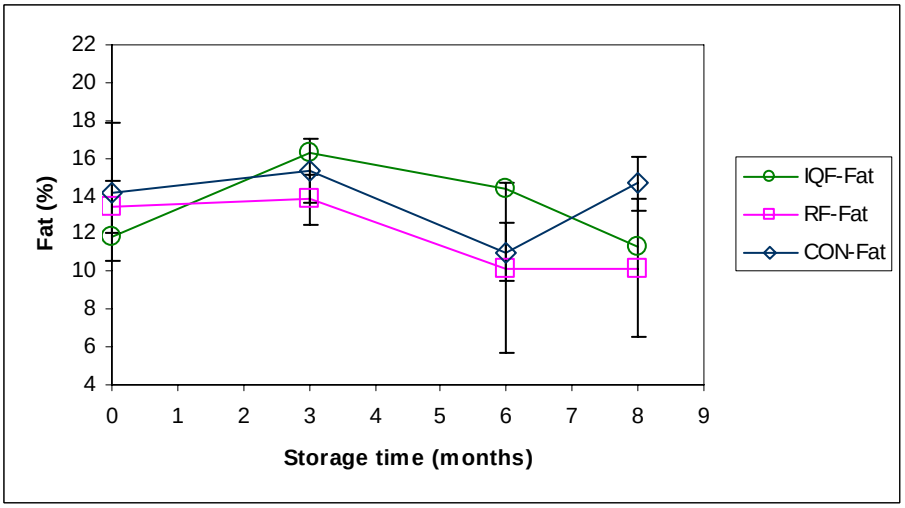

Figure 18. Fat content (%) of cooked/re-heated salmon (n=3); IQF (Individually Quick Frozen)-salmon, RF (Radio-Frequency heated)-salmon and CON (Conventionally heated)-salmon after 0-8 months of frozen storage at -24°C.

Table 4. pH of precooked samples (n=3) which were reheated after 0-8 months of frozen storage at -24°C (IQF=Individually quick frozen, RF =Radio-Frequency heated before freezing and CON = Conventionally heated before freezing)

Storage (months)	0			3			6			8		
IQF	6,37	±	0,00	6,33	±	0,03	6,34	±	0,02	6,36	±	0,01
RF	6,39	±	0,05	6,36	±	0,03	6,40	±	0,04	6,38	±	0,02
CON	6,37	±	0,07	6,35	±	0,02	6,39	±	0,01	6,40	±	0,05

Peroxide value (PV) and thiobarbituric reactive substances (TBARS)

Peroxide value determined after 6 and 8 months, was higher in RF and CON samples than in IQF samples (Figure 19). The PV content (4.5-7.9 meq. peroxide/kg) indicated that primary oxidation products were present. As an example for fresh salmon it can be mentioned that Fagan et al (1998) found that PV in fresh salmon was 1.6 meq. peroxide/kg. As an example, rancidity index for Atlantic mackerel based on PV has been set as PV<2 for acceptable quality. The fat content of mackerel in spring may be in the range of 5-11% but in the range of 11-20% in autumn (Ke et al, 1976). The fat content of the farmed salmon used in this trial was in the range of 10-16%.

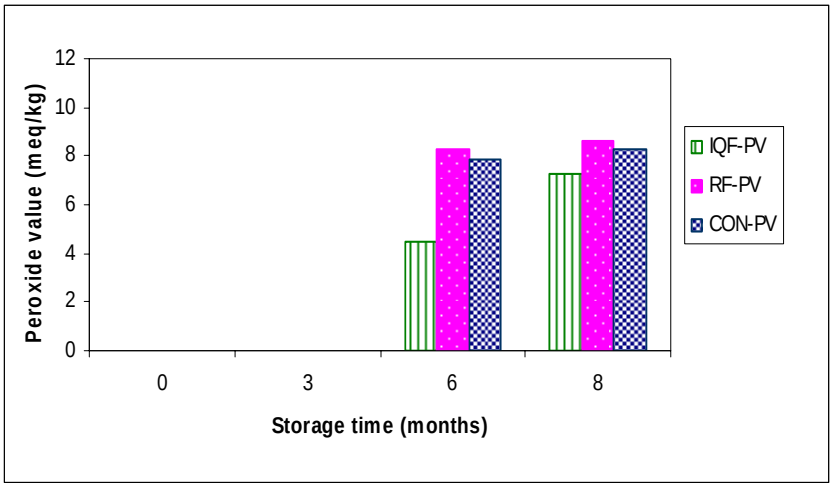

Figure 19. Peroxide value (PV) of cooked/re-heated salmon (pooled samples (n=3)); IQF (Individually Quick Frozen)-salmon, RF (Radio-Frequency heated)-salmon and CON (Conventionally heated)-salmon after 6 and 8 months of frozen storage at -24°C.

After 6 months of frozen storage, the TBARS was similar in IQF and RF samples but higher in CON heated samples. It increased further in the CON sample group from 24.8 after 6 months of frozen storage to 30.8 μmol/kg after 8 months. The TBARS of RF heated samples remained similar, 20.6 to 21.4 μmol/kg after 6 and 8 months respectively but decreased in IQF samples from 19.5 to 13.8 μmol/kg (Figure 20).

Andersen et al (1990) studied quality changes of vacuum packed wild salmon during 6 months storage at -17 °C and found that TBARS increased during storage from 2.8 μmol/kg to 12.5 μmol/kg for light-protected packages, and to 17.6 μmol/kg for packages

exposed to fluorescent light. The values obtained in our study were relatively high compared to this, but the samples were cooked which limits the comparison to other studies were raw fish was used for analyses.

Many factors may affect TBARS like species, evisceration, fat content and fatty acid composition, (Ruiz-Capillas et al, 2001) which is probably the reason that acceptable limits cannot be easily set. It is known that the extent of lipid oxidation is increased after cooking. The process probably disrupts the muscle membrane system, thereby exposing the lipid components to oxygen and other reaction catalysts such as iron (Hardy, 1980; Flick et al, 1992; Mielche and Bertelsen, 1994; Undeland et al, 1998).

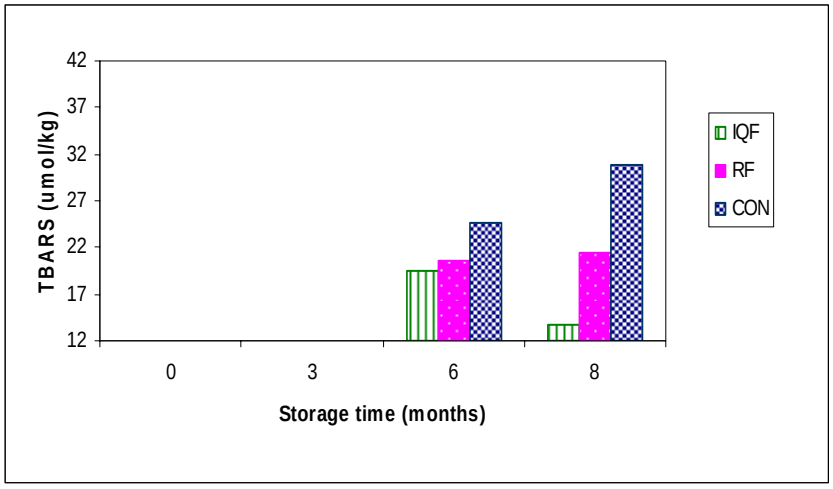

Figure 20. TBARS and PV of cooked/re-heated salmon (pooled samples (n=3)); IQF (Individually Quick Frozen)-salmon, RF (Radio-Frequency heated)-salmon and CON (Conventionally heated)-salmon after 6 and 8 months of frozen storage at -24°C.

Comparison of methods

Results from all methods were averaged over sample groups and compared in a PCA plot (Figure 21).

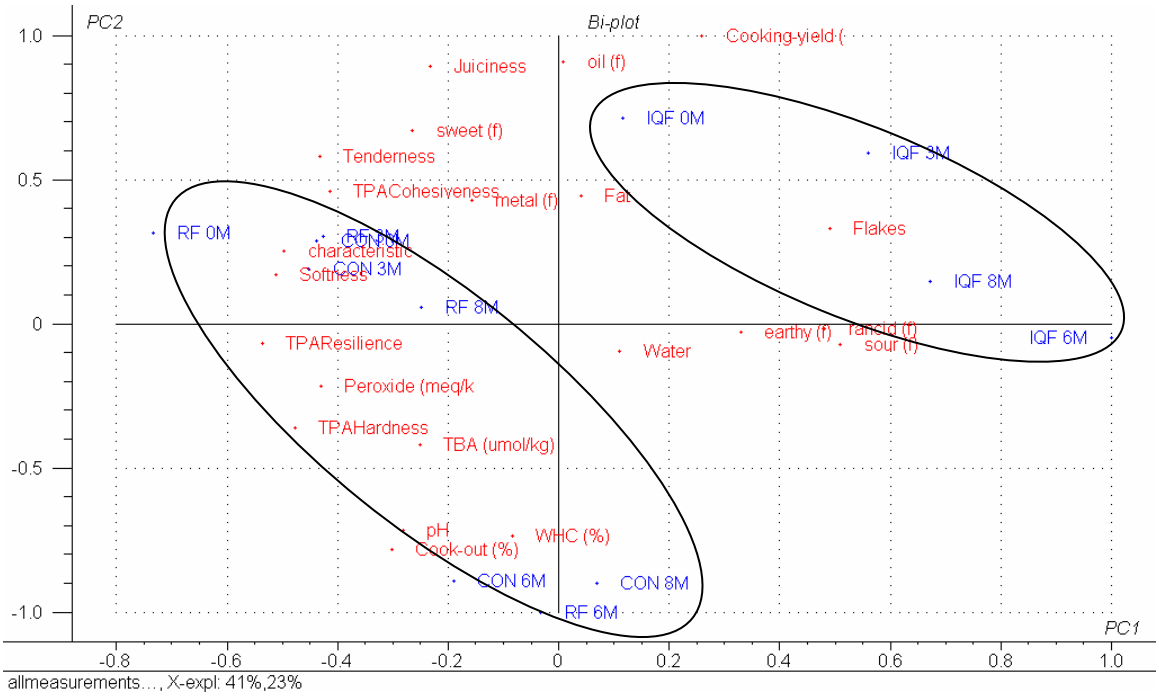

Figure 21. Scores and loadings (Bi-plot) of all measured parameters and sample groups, PC1 vs. PC2.; IQF (Individually Quick Frozen)-salmon, RF (Radio-Frequency heated)-salmon and CON (Conventionally heated)-salmon after0-8 months of frozen storage at -24°C. (TBA = TBARS)

The salmon sample groups are not as clearly separated in the PCA plot (Figure 21) as the cod sample groups (Figure 8), but can be divided into IQF and the precooked sample groups RF and CON. IQF salmon samples are grouped with high values of flakiness and low values of e.g. WHC and instrumental texture parameters. IQF samples are also related to higher values of the negative sensory attributes rancid and sour. CON and RF samples on the other hand are related to high values of WHC and instrumental texture parameters.

4. CONCLUSIONS

In this report comparison of individually quick frozen, radio-frequency heated at 75°C and conventionally heated at 75°C samples of cod and salmon were done during 8-9 months at -24°C storage. Effect of storage time and effect of treatments were investigated.

During nine months storage at -24°C, no significant differences were observed between the three different cod products with regard to freshness at each sampling day. However, the freshness decreased significantly during the storage time but after the nine months of storage it was still acceptable and the cod products had not reached the end of shelf life. The texture of the cod samples did not change much through the storage time but was different within products groups. IQF cod was flakier than the reheated sample, which indicated that the reheated samples did not keep the normal form of cooked fish where it brakes into flakes. RF samples were softer and more tender compared to IQF and CON samples but the sample groups were not different with regard to juiciness.

Overall the TPA texture measurements on cod showed increasing hardness during frozen storage. Hardness and resilience measured with texture analyser was higher in reheated cod than cooked IQF samples but cohesiveness was similar in all samples. No difference was observed with storage time. CON samples tended to have higher values for hardness and resilience compared to RF but the difference was not determinant in the measuring points during 9 months of frozen storage.

WHC in cod samples was affected by product treatment and time. It was lowest in IQF samples and higher after 3 to 9 month in storage. It might have been expected to see higher water holding capacity in IQF samples as those samples had only been heated once, compared to the double heating of CON and RF samples. It is suggested that freezing of IQF samples before cooking may have affected the results. CON and RF samples were prepared from chilled fish and then frozen after cooking whereas IQF samples were stored frozen and then cooked before sensory analysis at IFL.

Cook-out and WHC could not be used to reflect relevant sensory parameters such as juiciness, but were positively correlated each other.

The pH was lower in IQF cod samples than CON and RF and it was measured lower after three months than at other sampling days.

Measurement of formaldehyde and TBARS in cod samples were carried out during the second half of the shelf life experiment due to unexpected early loss of freshness. Formaldehyde values were rather high and CON samples might have been considered of unacceptable quality. Values of TBARS observed in the experiment remained below the given limits for TBARS in cod.

Storage time affected freshness of all cod sample groups in a similar way. Sample groups were mainly different due to different processing treatments and in general were IQF heated samples significantly different from reheated RF and CON heated samples. IQF samples were flakier, were measured lower in hardness and resilience, water holding capacity (WHC) and pH. CON and RF heated samples were softer and more tender than IQF samples. CON samples had higher WHC compared to RF samples, but RF samples were slightly more soft and tender compared to CON.

Variation between the salmon samples was mainly caused by storage time according to multivariate analysis of sensory data. After eight months in frozen storage, characteristic odour and flavour reduced a little, but earthy flavour increased somewhat. Higher scores were given for rancid and sour flavour during evaluation of IQF samples compared to RF and CON samples and were close to the average score of 20 which has been used as the limit of consumption, or end of shelf life for those negative attributes during freshness evaluation of salmon. Texture was in general not significantly affected by the storage time, but tenderness had negative correlation to storage time. Sensory attributes were correlated to storage time of salmon samples, as the positive sensory parameters characteristic odour and flavour and sweet flavour decreased, but the negative sensory parameters earthy odour and flavour and rancid flavour increased with the storage time.

Hardness and resilience measured with the texture analyser were lower in IQF salmon samples compared to reheated samples but cohesiveness was not different between products, which was similar to what was observed for the cod samples. Reheating seemed to add to the firmness of the samples and increases its resilience, which indicated that it resulted in a tougher product.

IQF salmon samples had less cook-out than RF and CON. The cook-out increased in CON samples during the storage but did not change in RF samples. IQF had lower WHC than RF. This was similar for pH as IQF had lower pH than RF samples.

Peroxide value in salmon measured after six and eight months indicated that primary oxidation products were present.

The main conclusion of this experiment is that there is a different between individually quick frozen samples and frozen radio frequently heated and conventional autoclave heated samples, especially with regard to texture. However, the profile of measured and evaluated parameters was rather similar during the eight and nine month of storage and samples were still acceptable at the end though freshness was reduced.

5. ACKNOWLEDGEMENTS

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