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**Contribution to product specification** 

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

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Ágrip á íslensku:	vörulýsingu, það er hver Skýrslan er hluti af ve <i>Minimally Processed Fish</i> yfir þá vinnu sem Rannsó komið að. Lokið var við skilgreininga við skilgreiningu á efna- o á að vinna með ferskt hrá	nig sýnin sem nota á í t rkefninu <i>Radio-Frequency</i> <i>Products</i> sem styrkt er af iknastofnun fiskiðnaðarins ur á þeim þáttum sem sýnin g eðlisfræðilegum breytum	ður varðandi ákvörðun um ilrauninni skulu framleidd. <i>Heating Technology for</i> Evrópusambandinu, og nær (Rf) og HB-Grandi hf hafa áttu að uppfylla og var lokið í hráefninu. Lögð er áhersla metið skv. QIM aðferðinni. heta eftir suðu sýnanna.	
Lykilorð á íslensku:	Radio frequency, hitun soðinn lax	n, áferð, vatnsheldni, sk	ynmat, soðinn þorskur,	
Summary in English:	<ul> <li>Sootm tax</li> <li>This report summaries the work done by The Icelandic Fisheries Laboratories (IFL) and by HB-Grandi hf (HARALD) regarding product definition. This report is part of the EU project <i>Radio-Frequency Heating Technology for Minimally Processed Fish Products</i>.</li> <li>The characterisation of fish material, development of experimental model food and packages has been finished. It is important to use only fresh raw material and that all raw material should be evaluated by the QIM method. It was also decided what parameter and methods should be used when evaluating the cooked samples.</li> </ul>			
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# **TABLE OF CONTENTS**

INTRODUCTION	3
RAW MATERIAL, MODEL FOOD AND PACKAGE DEVELOPMENT	3
Objectives	3
CHARACTERISATION OF FISH MATERIAL.	3
DEVELOPMENT OF EXPERIMENTAL MODEL FOOD	5
DEVELOPMENT OF EXPERIMENTAL MODEL FOOD	6
SPECIFICATION FOR SAMPLES – MODEL PRODUCT:	6
FISH COOKING KINETICS	8
Objectives	8
MEASUREMENT OF WATER BINDING AND TEXTURE	8
MICROBIOLOGICAL SAFETY	9
OBJECTIVES	9
MICROBIOLOGICAL EVALUATION OF EXPERIMENTAL PROGRAMME	9
CONCLUSION	12
REFERENCES	13

## **INTRODUCTION**

IFL, HARALD and other partners worked on product definition to assure a specific and uniform level for the raw material for the cod and salmon to be used in the project RF-heating. It was also decided to use certain measuring and evaluations methods to evaluate the samples that were sent between partners. In this report there is a description of the raw material and brief description of evaluation methods. More detailed description of methods of various measurements are to be found in IFL reports "Qualuty evaluation of heated samples and chilled storage shelf life report" (Deliverables 6.1 and 6.2)

## **RAW MATERIAL, MODEL FOOD AND PACKAGE DEVELOPMENT**

#### **Objectives**

Characterisation of fish raw material in terms of physical, chemical, and microbiological properties. Setting up quality standards for selection of fish material for heating experiments. Development of model recipes and of model packages for heating experiments.

#### Characterisation of fish material.

It is well known that seasonal changes in fish species varies and effects parameters such as fat, water and protein content. For cod, sea temperature is believed to be the most effective parameter on the growth. Researches on cod living in Icelandic waters have shown that the rate of growth is reduced in cold waters. The availability of food also affects the growth rate of cod.

The average chemical and nutritional composition of cod have been published (Table 1). Results from experiments done at IFL have showed similar results. However, it should be considered that chemical composition as well as physical parameters like texture could vary according to seasonal changes as well as to fishing grounds.

Chemical and nutritional composition of cod (per 100 g of fish meat)		D	
		Е	1,10
Energy	326 KJ	B1	0,03
Protein	18,1g	B2	0,03
Fat	0,5g	Folasin	16µ
Saturated fatty acids	0,1g	С	0
Unsaturated fatty acid	0,3g	Mineral	
Cholesterol	58mg	Calcium	7m
Carbohydrate	0g	Sodium	118r
Vitamin		Potassium	332r
A	1µg	Iron	0,17

Table 1. Official chemical and nutritional composition of cod (Reykdal 1996).

#### **Microbiological specification**

IFL has given microbiological guidelines for frozen fish and frozen lobster. Those guidelines are used as general rules during microbiological evaluation of fish. The guidelines used at IFL are shown in table 2.

Table 2. Microbiological guidelines for frozen fish and frozen lobster given by IFL.

	Good	Fair	Poor
Plate count/g 35°C	<100.000	100.000-200.000	>200.000
Plate count/g 30°C	<150.000	150.000-350.000	>350.000
Plate count/g 22°C	<250.000	250.000-500.000	>500.000
Total coliforms,	<100	100-200	>200
MPN/g			
Faecal coliforms,	< 0.3	0.3-4	>4
MPN/g			
Staphylococcus	<10	10-100	>100
aureus/g			
<i>Listeria</i> in 25g	-		+

Those guidelines were approved in Reykjavík, 30. of October 1998.

It was recommended by IFL that guidelines in table 2 should be used during quality evaluation of raw material for use in this project and only raw material rated as good was used.

#### Sensory methods to ensure quality standards of raw material

The whole fish was evaluated with the QIM – method, so it was not necessary to do other tests on the fillets except microbiological evaluation, as it was necessary to know the initial count of bacteria before transport of samples to FhG/IVV and Norconserv for cooking.

The Quality Index method is a seafood freshness quality grading system, which is used to assess fish freshness in a rapid and reliable way. QIM is based upon a scheme originally developed by the Tasmanian Food Research Unit (Bremner 1985).

#### **Development of experimental model food**

Wild captured cod can vary very much in size and quality, so it is necessary to evaluate what kind of raw material is the best for this project. To minimize the variance in captured fish it has been decided to use cod that is not older than 3 days from catch and the fish shall be evaluated according to the QIM-method and the QIM score shall not exceed the equivalence of 3 days storing time on ice.

IFL in cooperation with HARALD suggested portion sizes for the cod after evaluating available raw material and popular portion sizes for the European market. It is always a compromise between the raw material available and what the market wants when it comes to decide sizes of portions. The most common size of cod that comes into production at HARALD is 1,5 - 2,2 kg and that gives according to average yield in production, fillets that are 300-500 g trimmed skinless and boneless.

According to market information on portion sizes and HARALD experience, cod portion sizes between 120 and 180 g are the most popular ones. Fillets are usually divided into three types of portions that are loins (the thickest part), centre cut and tail (Figure 1). The most requested and also the most valuable part of the fillet is the loin part, and as the aim is to develop a method to produce valuable products it was decided to use only the thickest part of the fillet. The loin portion is also the most uniform part in thickness and as that parameter could influence the evaluation of the process that also favoured the decision to use cod loins.

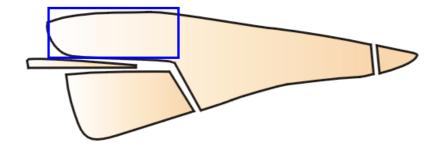


Figure 1. The frame shows the loin part of a fillet.

As wild captured fish can vary a lot it is difficult to have very strict limits for all the different parameters. According to measurements 140g cod portions cut from fillets weighing 300-450 g would be  $12 \text{ cm} \pm 2 \text{ cm}$  in length, 4-6 cm in width and be 2,5-3,5 cm thick. That size of portion will also fit very well into the moulds of the vacuum packing machine that is available at HARALD.

The salmon is more uniform in size as only farmed fish will be used. FK is already using 140g portions in there production so a standard product from their production will be used as it fits well to the dimensions of the packaging size that will be used.

## **Development of experimental model food**

#### **Specification for samples – model product:**

#### **Raw material**

Fresh cod, winter and summer catch, not more than 3 days on ice after catch according QIM evaluation method.

Farmed salmon not more than 3 days on ice after slaughtering according to QIM evaluation method.

## **Fillets and portions**

All bones, blood spots and parasites shall be removed and the fillet shall be trimmed according to figure 2 and only the loin part of cod shall be used for the model product.



Figure 2. The model product, vacuum packed cod loin and salmon portion

Loins cod parameters and salmon portion parameters

- ✓ Weight 140 g +/-10g
- ✓ Length 12 +/- 2 cm
- ✓ Width 4-6 cm
- $\checkmark$  Thickness 2,5-3,5 cm

## Packaging

Packages will be of the evacuated flexible pouch form. And it was decided after some research that the standard packaging film used at FK fits well with respect to resistance to RF exposure, temperature resistance, mechanical resistance of film and of seals, barrier properties, flexibility, and quick-freeze stability. The moulds in the vacuum packing machine at HARALD give single portion packaging with the outer dimension of 92 x 298 mm. Similar size is used at FK.



Figure 3. The dimensions of packed model product 92 x 298 mm.

## Thawing and cooking test

HARALD did some tests to answer some questions about thawing and cooking of vacuum packed portion they used a portion cut from minced cod block for the testing.



Figure 4. Thawed vacuum-packed minced portion.

Vacuum packed frozen minced cod portion did not loose shape after defrosting as can be seen on figure 4.

During cooking the vacuum pack started to float so it is necessary to take that into account when designing the experimental equipment.

## **FISH COOKING KINETICS**

#### **Objectives**

Provide kinetic data on texture changes, cook out, biochemical changes, colour and sensory changes under rapid heating conditions and compare to conventional heating. Calculation of a cook value for fish under rapid heating that will be used in the set-up of an optimum-heating regime for the minimally processed fish products.

## Measurement of water binding and texture

Water binding and texture are the most obvious and interesting quality parameters influenced by heating. When evaluating the samples throughout the project IFL did measurement on cookout, water holding capacity and texture, the results are to find in reports "Quality evaluation of heated samples and chilled storage shelf life" and "Frozen storage shelf life" (Deliverables 6.1 and 6.2)

The main objectives were to evaluate methods that could be used to monitor changes in physical parameters such as texture, water holding capacity and sensorial parameters.

The instrumental texture measurements may be reduced to TPA measurements of cooked fish, as this project deals mainly with cooked fish. The Cutting force did not give useful information about the product, gave high standard deviation and was difficult to perform.

The main conclusions were that the WHC showed some correlation to several texture parameters, firm-soft and tough-tender, evaluated with sensory evaluation, hardness measured by TPA both raw and cooked samples and cohesiveness measured by TPA of cooked samples.

All the methods described above give valuable information on the product. The raw material should be evaluated by the QIM method as it is very accurate and gives useful information about freshness of the raw material. Sensory evaluation of cooked fish should be included as it gives the best indication on the consumers experience and valuation of food. Water holding capacity (WHC) is an important quality parameter that affects both consumer satisfaction and economically elements. It should be one of the parameters used to describe overall quality of cooked samples and raw samples.

## MICROBIOLOGICAL SAFETY

#### Objectives

Practical demonstration of microbiological safety of the process by collecting data on inactivation during heating experiments and by performing microbiological challenge tests. Use of mathematical modelling and prediction in order to enhance significance of experimental validation.

#### Microbiological evaluation of experimental programme

IFL and Norconserv had a telephone meeting to coordinate the methods for microbiological measurements methods during the project. Following are the standard methods used at IFL:

The basic methodology used at Icelandic Fisheries Laboratories is according to the Compendium of Methods for the Microbiological Examination of Foods published by the American Public Health Association (APHA-1992). The methods used for individual tests are briefly described below.

*Total Plate Counts*. In the Reykjavík laboratory these are done with a Spiral plater on Plate Count Agar with 0.5% NaCl. Incubation temperatures are either 35 or 30°C. Incubation time is 48 hours. Occasionally, counts at 22°C (72 hours) are used for psychrotrophic bacteria. At the branch laboratories, the conventional "pour-plate" method is used.

*Total and faecal coliforms.* Most probable number (MPN) method is used. Preenrichment is in LST broth (35°C for 24/48 hours) and confirmation tests are done in BGLB broth for total coliforms (35°C for 48 hours) and in EC broth for faecal coliforms (44.5°C for 24 hours). Confirmation test for *Escherichia coli* is done by the MUG method (44.5°C for 24 hours).

*Staphylococcus aureus*. The isolation medium used is Staphylococcus med. no. 110 with egg yolk added. Incubation temp. is 35°C for 72 hours. Typical colonies are tested for coagulase. The staphyslide test is also sometimes used for confirmation.

*Salmonella*. First enrichment is in Lactose broth (35°C for 24 hours). Second enrichment is in Selinite broth and tetrathionate broth (35°C for 24 hours). From these broths we streak onto two solid media: BG agar and BS agar (35°C for 24/48 hours). Typical colonies (2-4 or as needed) are inoculated into TSI-agar and LI-agar (35°C for 24 hours). Finally we test for urease-production. Species identification is carried out at the University Hospital by serological methods.

*Listeria*. The methodology for Listeria is based on information from U.S. Department of Agriculture (USDA-FSIS, 1989), the APHA (1992) and others. Enrichment broth is UVM modified Listeria broth (30°C for 24 hours). Then we inoculate into Fraser broth (35°C for up to 40 hours). Growth from black tubes is streaked onto Modified Oxford Agar (MOX) (35°C for 48 hours). Confirmation tests are done on 5 colonies

and include Gram-staining, catalase and motility. Species identification includes haemolysis on Blood agar and testing on API Listeria (System for the identification of Listeria, bioMérieux SA/France).

*Sulphite-reducing clostridia*. MPN method is used. The medium of choice is Differential Reinforsed Clostridial Medium (DRCM) (incubated at 30°C for at least 96 hours). Prior to inoculation, the sample is "pasteurized" at 75°C for 30 min to kill vegetative cells.

Yeasts and moulds. The isolation medium used is Dichloran Rose-Bengal Chloramphenicol Agar (DRCB-Agar). urface plating is used. Plates are incubated at 22°C for 120 hours.

*Enterococci*. The medium KF streptococcal agar is used (pour-plate method). Plates are incubated at 35°C for 48 hours) and typical colonies counted.

*Enterobacteriaceae*. The medium Violet Red Bile Glucose Agar (VRBGA) is used (pour-plate method with overlay). Plates are incubated for 24 hours at 35°C and typical colonies counted. Oxidase test is used for conformation.

IFL carried out some preliminary experiment to see if it was possible to homogenize the whole fish portion in a laboratory stomacher; homogenisator. E.g. with 140 g fish and 140 g dilution liquid.

IFL obtained representative samples of unfrozen cod pieces from HARALD. IFL tried some sizes of pieces, cut and uncut and varying time in the stomacher and found out that the following works:

Place 150 g uncut piece in a filter stomacher bag (IFL uses bags from Bagsystem Line, Breveté, France). Add 150 g of buffer. Place in the stomacher and blend for 2 min. After that a 1/10 dilution can be made in the following way: Notice that 2 ml of this mixture gives 1 g fish. From the filtered section of the bag pipette 22 ml (equals 11 g fish) into a new filter bag and add 88 g buffer. This should give 1/10 dilution. For plate counts on Plate

Count Agar with 0.5% NaCl by the spread plate technique divide 1 ml onto 3 plates (1/10). After that make tenfold dilutions as needed. Incubate at 22°C for 3 days. For Bacillus spore count heat 10 ml of the 1/10 mixture at 75°C for 30 min. Use the pour-plate technique and the same agar as above. Incubate at  $35^{\circ}$ C for 2 days.

Regarding the spread plate method 1 ml of the 1/10 dilution (ca. 0.33ml/plate) should be divide onto 3 plates and then sum up the no. of colonies on the 3 plates, thus giving total no. of colonies in 1 ml of 1/10. Duplicate plates should be use for other dilutions (2x0.1ml of 1/10 dilution on 2 plates, count both plates and divide by two etc.). IFL will also use 2 plate per dilution in the pour-plate method. There it is possible to put 1 ml on each empty plate.

## CONCLUSION

The product definition as described above was used through the whole process of producing samples. The samples of salmon portions were produced according to standard specification at FK, but less salt used than usual, and the cod loins that were produced at HARALD had no added salt and were produced according to above mentioned specifications.

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