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SILLQUID - Can a Microwave Quick Tool Predict Quality and Origin of Herring

- SILLQUID - a fast and non-destructive MW-spectroscopy method
- Testing the MW-tool for prediction of origin, compositional and quality parameters of herring material
- The promising ability of the MW-tool to predict fat content of pelagic fish



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SILLQUID - Can a Microwave Quick Tool Predict Quality and Origin of Herring?



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Title: SILLQUID - Can a Microwave Quick Tool Predict Quality and Origin of Herring		
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Abstract: There is a great need within the fish industry for quick tools to measure origin, fat content and general quality right after landing. In SILLQUID, the fast and non-destructive method MW-spectroscopy, developed in two recent EU-projects, was tested for prediction of origin, compositional and quality parameters of a unique herring material collected and analyzed during NI-project 02106. Through participation of three pelagic companies, the MW-tool was also tested during industrial production of herring and mackerel fillets. The most important result from this project has been the promising ability of the MW-tool to predict fat content of pelagic fish, at least up to fat levels of 18% (herring) or 25% (mackerel). Most of the information in the MW-spectra about fat content was located either in the “rising edge” of the curve, or at the maximum of the curves. There was also a weak correlation to season of catch, storage time in refrigerated sea water (RSW)-tanks, pre-freezing and storage time in a salt brine. The correlation to all other studied biological and chemical/physical parameters has been low. We believe that the results from the industrial trials would have been even more clear if the collected material had had a wider spread of fat content, if the industrial partners would have had the possibility to practice more on how to carry out the measurements, and if the newest version of the MW-tool would have come in earlier in the project. An important practical outcome has been the need to communicate instructions for the MW-tool in a very clear way, preferably with pictures.		
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Executive summary

The main objectives of this study were to:

- verify whether the fast and non-destructive method MW-spectroscopy, developed in two recent EU-projects, could be used to predict catching area, fat content, lipid oxidation level and certain other parameters of a unique herring material collected and analyzed during NI-project 02106.
- obtain "hands-on" input regarding the applicability of the MW-spectroscopy tool in production through the industrial participation in this project.

This study has met these aims by:

- showing that the MW-tool when used on two types of herring materials (from NI-project 02106 and collected during the industrial trials) appears promising in detecting the fat content of herring and mackerel. Depending on the material used, the tool has shown some limitations above very high levels of fat.
- showing that a certain correlation exists also to catching season, storage time in refrigerated sea water (RSW) tanks and pre-freezing, while no correlation exists to other biological and chemical/physical parameters investigated
- showing that most of the information in the MW-spectra has been situated in the "rising edge" or peak of the curve.
- giving practical input on what is needed for the MW-measurements to be properly carried out within industry. Extremely detailed instructions are needed, preferably with pictures.

Method/implementation

Samples

Three types of herring and mackerel samples have been used: (i) whole fish, (ii) butterfly fillets and (iii) minced fillets. The first set of sample material used (herring) was collected during the NI-project 02106. This material had been collected in Sweden, Iceland and Norway and also during different seasons and years. Some of the material had been stored frozen for up to 18 months in -20°C and then stored until analyses at -80°C. The second set of samples (herring and mackerel) was collected during the current project by our three collaborating industries; Paul Mattsson AB (Ellös, Sweden), Atlantic Pelagic (Austevoll, Norway) and Sildarvinslann (Iceland) during 2008 and 2009.

MW-measurements

MW-measurements have been done with three versions of the Sequid equipment in order to find correlations to catching season, geographic location, storage time, chemical characteristics, microstructure and sensory properties. These versions are: (i) the Time domain reflection (TDR) – Instrument, Prototype TDR **1st Generation**, CAU Kiel (P1), (ii) the TDR Sensor, Prototype hand **3rd Generation** Sequid GmbH (P3) and (iii) the **RFQ Scan 3.0**, hand held, Sequid GmbH.

Chemical and sensory measurements

The main chemical parameter against which the MW-data have been correlated is fat content, which has been analyzed by solvent extraction or indirectly by measurements of dry matter. A few attempts have been made to correlate the MW-data also to n-3 PUFA content, microstructure, lipid oxidation products and sensory properties. The n-3 PUFA were analyzed by gas chromatography (GC) coupled to FID-detection. Microstructure was analyzed using Nile Red and Oil Red O together with microscopic analyses. Lipid oxidation products (PV and TBARS) were analyzed using the colorimetric methods. Sensory analyses were done according to Nielsen et al. (2005).

Concrete results and conclusions

The most important result from this project has been the promising ability of the MW-tool to predict fat content of pre-frozen pelagic fish, at least up to fat levels of 18% (herring) or 25% (mackerel). This ability was seen both from measurements of the herring material collected in NI-project 02106 and the new herring and mackerel materials collected by the industry participants in this project. Most of the information in the MW-spectra about fat content was located either in the “rising edge” of the curve, or at the maximum of the curves. There was also a weak correlation to season of catch, but most likely this was also a result of the seasonal differences in fat content. It should be stressed that it is not known at this point whether it was the actual fat or the water of the muscle that responded in the MW-measurements. Trials done at Paul Mattsson AB revealed that the MW-tool also has some capacity to predict the time herring has been stored in refrigerated sea water (RSW)-tanks, and also, whether herring has been pre-frozen or not. From studies made in Sildarvinnsland, a weak correlation could also be made between MW-signals and time in salt brine. The correlation to all other studied biological and chemical/physical parameters has been low.

In general we believe that the results from the industrial trials would have been more clear if the collected material had had a wider spread of fat content, and if the industrial partners would have had the possibility to practice more on how to carry out the measurements. In relation to the latter, an important outcome has been the need to communicate instructions for the MW-tool in a very clear way, preferably with pictures. Particularly the way the probe is pressed onto the sample surface seems to affect the signals. This applies both to the force of pressing, and the way by which the probe is moved over the sample surface to make replicate measurements (in a circle, in a line, long or short distances). It was however clear that the newest version of the MW-equipment (RFQ Scan 3.0) that was used in the end of the project allowed for much more reproducible measurements.

Recommended future work

We recommend that future work is carried out in this area in order to further confirm the applicability of the MW-tool for quick fat analyses within the fish industry. Efforts should however be made to collect a fish material with a wider spread in fat content than the one used here. In addition, a detailed study should be done to pin-point the best location of the fish to make the measurements on (skin side/meat side, where on the fish) and also to find out how *long* distance the probe should be moved between each measurement. Further, a more detailed instruction sheet illustrated with pictures should be produced to help users to carry out measurements in an optimal way. We believe that the results from SILLQUID would have been stronger if the measurements would have been done in a more reproducible way among all partners. A major improvement would have been if the output from the tool would be a number instead of a scan. It would then have been easier to see directly whether the measurement was successful or not, e.g. in terms of contact between probe and fish material.

Table of Contents

1. Introduction	3
2. MW-measurements done on the herring material from project NIC-project 02106 “Improved Quality of Herring for Humans” (steps i-iv)	4
2.1 <i>Microwave spectroscopy (MW) measurements</i>	4
2.2 <i>Chemical and sensory measurements</i>	6
2.3 <i>Multivariate analysis</i>	6
2.4 <i>Results and Discussion</i>	6
2.4.1 <i>Treatment of MW data from measurements on skin side of fillets (performed by DTU Foods)</i>	6
2.4.2 <i>PCA-results overview regarding correlation of MW-signals to catching place and storage time</i>	7
2.4.3. <i>PLS-results overview</i>	8
2.4.4 <i>Treatment of MW data from measurements of minced herring (performed by DTU Foods)</i>	9
2.4.5 <i>Treatment of MW data from measurements on mince and fillet (performed by SIK)</i>	10
2.4.6 <i>Treatment of MW data (performed by Sequid GmbH) from MW-measurements on skin-side of herring fillets (performed by SIK)</i>	12
3. Extra compositional analyses done on herring from NIC-project 02106 (step v)	13
4. Measurements done on herring and mackerel collected by the industry participants of SILLQUID (steps vi-vii)	15
4.1 Sildarvinnslan, Iceland- Study of the correlation to fat content and brine storage	15
4.1.1 <i>Procedure</i>	15
4.1.2. <i>Results</i>	17
4.1.2.1 <i>Fat content</i>	17
4.1.2.2 <i>Time in salt brine</i>	19
4.2 Atlantic Pelagic, Norway - Study of the correlation to fat content	20
4.2.1 <i>Procedure</i>	20
4.2.2 <i>Results</i>	20

4.3 Paul Mattson AB, Sweden –Study of the correlation to fat content, RSW-storage and pre-freezing	23
4.3.1 Procedure	23
5. Conclusions	28
6. References	29

SILLQUID - Can a Microwave Quick Tool Predict Quality and Origin of Herring?

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1. Introduction

By law citizens are entitled to correct information about their foods, including species, geographical origin and production method. To fulfil this, the fishing sector and fish processing industry must have full access to such information, something which requires efficient methodology for identification. Potential methods for identification today could be analyses of trace elements, DNA, protein and Magnetic Resonance analyses, all which either will be expensive or time consuming. New quick methods would thus be most welcome. Another area of great importance at the fish market and within the fish industry is quick quality control of both fresh and frozen fish. Among parameters that are of particular interest to follow are level of rancidity, fat content, and of course the long chain omega-3 fatty acid content. The latter has in the last few years been an important basis for health claim labelling of seafood products, provided that the product contain a certain level of omega-3. For many years, development of quick and non-destructive quality control tools has therefore been a methodology challenge over the years. Examples of methods that have been tested with limited success are different versions of the Torry freshness meter.

During two recent EU projects, microwave dielectric “spectroscopy” (MW-spectroscopy) was applied in an entirely new approach to study whether dielectrical measurement of fish could be used to follow quality. In the first project (“Added Water”) (Kent et al. 2000), this quick and non-destructive technique coupled with methods of multivariate statistical analysis was able to distinguish different aspects of quality in seafood products e.g. whether raw materials had been frozen and how well they had been stored. In the second project (“SEQUID”) (Kent et al 2005), prediction of different variables associated with loss of quality of both fresh and frozen stored fish was evaluated. In the NI-project 02106 “Improved Quality of Herring for Humans”, a large amount of unique herring samples with different seasonal and geographic background were collected, stored, and deeply analyzed for both chemical, physical and sensory parameters by the different partners. By coupling the “Added water” and “SEQUID” outcome with the samples and data set of NI-project 02106, a fantastic opportunity was provided to evaluate whether it was possible to use the MW-spectroscopy technique in order to predict: (i) the biological origin (geographic location, season, year) of the herring used in NI-project project 02106, and (ii) some of the chemical/physical/sensory data obtained on the herring used in NI-project 02106. The evaluation required that MW-measurements of the remaining collected herring material from project 02106 were carried out, and that multivariate analyses of the new MW-data, in combination with the already existing data on the herring material, was performed. Further, a few new compositional analyses of the collected herring material may be needed to improve or deepen potential correlations found.

Based on this, the main object of this project has been to verify whether the fast and non-destructive method MW-spectroscopy can be used to predict catching area, fat content, lipid oxidation level and certain other parameters of a unique herring material collected and analyzed during NI-project 02106. Based on the industrial participation of this project, an additional object has been to obtain "hands-on" input regarding the applicability of the MW-spectroscopy tool in production.

The project has been carried out in 8 main steps focusing on: (i) MW-analyses of minced herring reference samples from project 02106 (stored at -80°C), (ii) MW-analyses of minced samples stored at -20°C for various amounts of time, (iii) MW-analyses of the skin side of whole herring fillets, both reference samples and stored samples, (iv) multivariate data analyses of the MW-data together with the existing chemistry/sensory/physical data of the herring, (v) extra compositional analyses of the herring material collected during project 02106 (vi) limited testing of the MW-equipment by the various industrial partners, (vii) multivariate analyses of the industrial trials and (viii) reporting.

The long term objective of the project has been to increase the efficiency of the Nordic fishing industry in obtaining quick data on origin and quality of fish. This will then hopefully lead to an optimized production of well-labelled fish products that are appealing to the consumer, and thus, can be used as a part of a healthy life style.

2. MW-measurements done on the herring material from project NIC-project 02106 “Improved Quality of Herring for Humans” (steps i-iv)

2.1 Microwave spectroscopy (MW) measurements

Three types of MW measurement experiments were carried out at SIK on samples collected during the NIC-project 02106 “Improved Quality of Herring for Humans”. See **Figure 1** for details about these samples, and **Figure 2** for illustration of the different MW-instrument prototypes used.

1: Skin side of fillets measured with two types of MW-devices: Network analyser (NWA) and the first generation prototype (P1) developed during the EU- project SEQUID (samples from 31 sampling times, 5 individual fillets from each sampling (i.e. 155 samples) 3-5 measurement points on each fillet (i.e. 681 measurements)).

2: Meat side of fillets with a further developed third generation (P3) prototype developed by SEQUID GMBH (samples from 31 sampling times, 5 individual fillets from each sampling, (i.e. 155 samples), 5 measurement points on each fillet (i.e. 775 measurements)).

3: Minced samples with two types of instruments: Network analyser (NWA) and the first prototype (P1) developed during the EU- project SEQUID (37 samples, 5 measurement point on each sample, i.e. 185 measurements).

All measurements were made on thawed samples.

Catching places and time

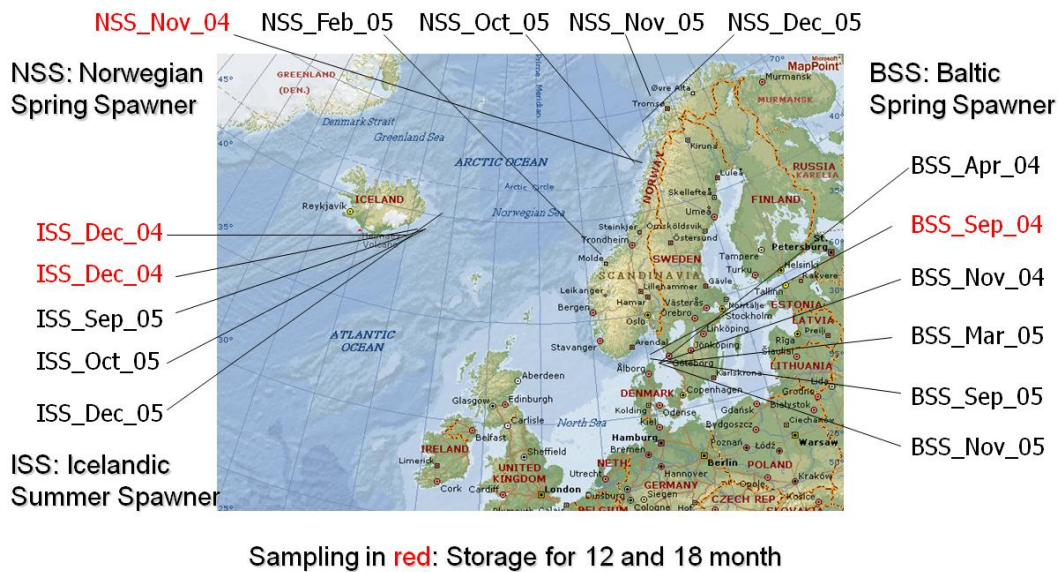


Figure 1: Overview over all samples from the NI-project 02106 used in SILLQUID. Samples in black were stored for 0 or 6 month in -20°C , samples in red were stored for 0, 6, 12 and 18 month in -20°C . After finalized storage, samples were kept in -80°C until measuring with the MW-equipment.



Figure 2 Three versions of MW-measurement equipment used in the project; from left to right; (i) the TDR – Instrument, Prototype TDR 1st Generation, CAU Kiel (P1), (ii) the TDR Sensor, Prototype hand 3rd Generation SEQUID GmbH (P3) and (iii) the RFQ Scan 3.0, hand held, SEQUID GmbH (TDR- Time domain reflection)

2.2 Chemical and sensory measurements

The main chemical parameter against which the MW-data have been correlated is fat content. Fat content in the herring has been analyzed either by the method of Lee *et al.* (1996) as modified by Undeland *et al.* (2002) or indirectly through dry matter analyses. A few attempts have been made to correlate the MW-data also to n-3 PUFA content, microstructure, lipid oxidation products and sensory properties. The n-3 PUFA were analyzed by gas chromatography (GC) coupled to FID-detection according to Olsen *et al.* (2004). Microstructure was analyzed by cutting 10 µm thick samples from the muscle of herring. Sections were mounted on glass slides and stained using Nile Red and Oil Red O (Catalano and Lillie, 1975). The samples were examined in an optical microscope, Leica DM RA2 at 100 x magnification and the space (area) between the fibers were measured as a ratio (%) of the determined area in the muscle. Lipid oxidation products (PV and TBARS) were analyzed using the colorimetric methods of Undeland *et al.* (2002) and Lemon (1975). Sensory analyses were done according to Nielsen *et al.* (2005).

2.3 Multivariate analysis

Correlation analyses between the MW spectroscopy data and already existing data have been carried out by SIK, DTU Foods and Sequid GmbH. The aim of these multivariate data analysis has been to investigate relations between MW measurement and nine variables in the NIC 02106 data set:

- Geographical position (place of catch)
- Season of catch
- Storage time at -20°C
- Fat content
- Fat composition (n-3 PUFA)
- Microstructure
- Lipid oxidation level
- Sensory properties

2.4 Results and Discussion

2.4.1 Treatment of MW data from measurements on skin side of fillets (performed by DTU Foods)

The MW measurement curves obtained from measurement on skin side of fillets from NIC project 02106 samples were evaluated to identify which information the curves contain. Data analysis was done on an average of five measurements from one sample. It was assumed that most of the information is located in the rising part of the MW curve (**Fig 3**). Therefore first and second derivatives were used beside the original data. As the MW-curves also are out of phase and therefore not aligned, an alignment of the first derivative was tried as a pre-processing step to see if this phenomenon contains some valid information or if it is just an instrumental “artefact”. Furthermore the first derivative and aligned data were normalised using a standard normal variate (SNV) transform to see if the height of the first derivative peak, contains relevant information (**Table 1**).

Table 1 Naming of MW data set used in the report in relation data used

Data name	Data used
X1	Original data
X2	First derivatives
X3	Second derivatives
X2A	First derivatives aligned
X2AN	First derivatives aligned and SNV-normalised

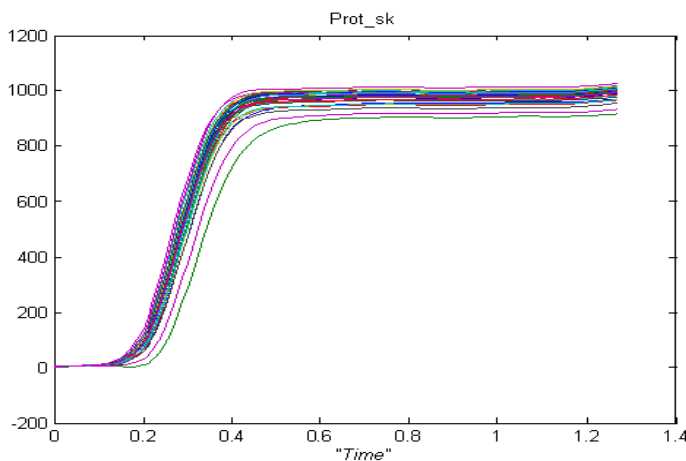


Figure 3: MW measurement curve from measurement on skin side of fillet

2.4.2 PCA-results overview regarding correlation of MW-signals to catching place and storage time

The X1-model was dominated by the single sample (BSS_Apr04) whose curve was shifted the most in position from the main bunch of curves. It is a typical “outlier”, but even after removing this sample from the analysis (and a few others that turned up as possible outliers), the rest of the samples (although well distributed over the scores plot) do not group systematically according to neither place of catch nor storage time. The same conclusions could be drawn from results with X2 and X3 as data. When using X2A or X2AN, the same sample (BSS_Apr04) still may be classified as an outlier, although the alignment has somewhat helped (**Figure 4**). These data also did not group systematically according to catching place or storage time.

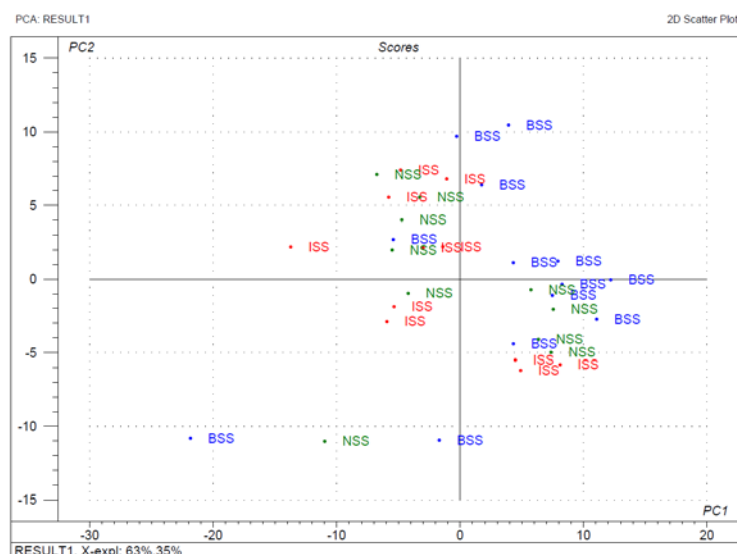


Figure 4: PCA model showing correlation between MW data X2A obtained from MW-measurements on skin side of herring fillets from project NIC 02106 and place of catch as well as storage time

2.4.3. PLS-results overview

Preliminary MW-measurements made on pure oil and water showed large differences between these two samples. This may indicate that the MW-signal possibly contains information about the lipid content of the samples. To explore this, PLS-models were calculated using the various signals as X and lipid content (NSS-samples) as y. All data sets were used in the calculations. It was found that X3 and X2A (**Figure 5**) gave marginally better calibrations than the other MW datasets. As can be seen from **Figure 5**, there is some information on the lipid content in the measured curves. The most profound PCA-outlier was the sample with lowest lipid content (BSS_Apr04). It should be noted though that due to the well-known strong negative correlation between lipid and water content of fish muscle (also observed in the present sample set) the information on lipid content may be indirectly stemming from a relation between the MW signal and the water content.

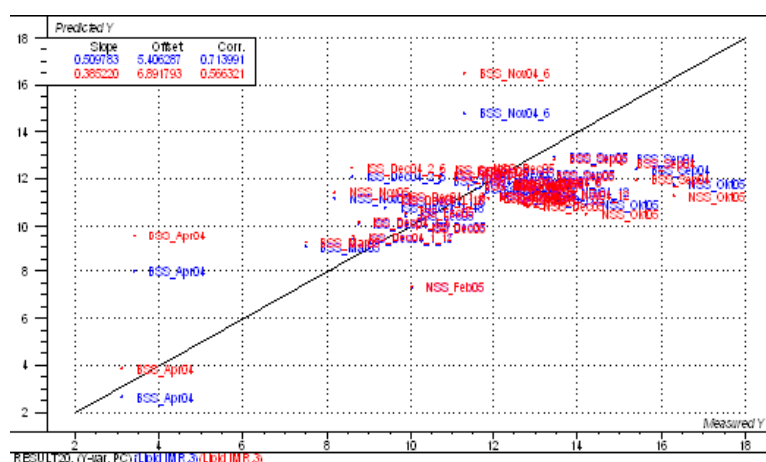


Figure 5: PLS model using X2A MW data as X and lipid content as Y

2.4.4 Treatment of MW data from measurements of minced herring (performed by DTU Foods)

A similar analysis as the one shown above was carried out on the data set obtained from measurements of minced samples. A PCA analysis on X1 showed a weak grouping according to time of catch for the BSS samples (**Figure 6**) with catches in February and April in one end and September in the other end. No grouping of other design parameters (storage time, catching season, year) from the NIC 02106 data set was found. Similar result was obtained using the X2 and X3 data.

A PLS regression model with the X2 data as X and dry matter content as Y gave a similar score plot as the PCA models, showing that the main difference in the MW data was explained by differences in dry matter content, i.e. water and/or lipid content. The high negative correlation between water and lipid content that was observed from the PLS analysis of data from skin side fillet was also found in the data set from minced fillets. Thus it is impossible to conclude whether it is water or lipid that is actually determined by MW-probe.

A PLS regression analysis with X2 data as X and total n-3 PUFA content as Y was also carried out. However, it was not possible to obtain a valid PLS model, and it can therefore be concluded that there is no correlation between n-3 PUFA and MW measurements in samples that have been frozen.

Correlation between MW measurements and microstructure, lipid oxidation and sensory variables was also investigated. However there are no features in the MW-measurements useful for prediction of microstructure, lipid oxidation or generalised sensory variables (called “T1” and “T2”) in samples that have been frozen. This also applies to measurements on the skin-side of fillets.



Figure 6: PCA model showing correlation between time of catch and MW-data X1 obtained from measurements made on minced herring from project NIC 02106.

2.4.5 Treatment of MW data from measurements on mince and fillet (performed by SIK)

The relation between catching place (geographical prediction) of herring from NI-project 02106 and MW measurements made on minces and on the skin side of fillets, respectively, was also investigated by multivariate data analysis at SIK using single measurements as data. In accordance with the data analysis made at DTU Foods, a PCA analysis showed no relation between catching place and MW data (**Figure 7**).

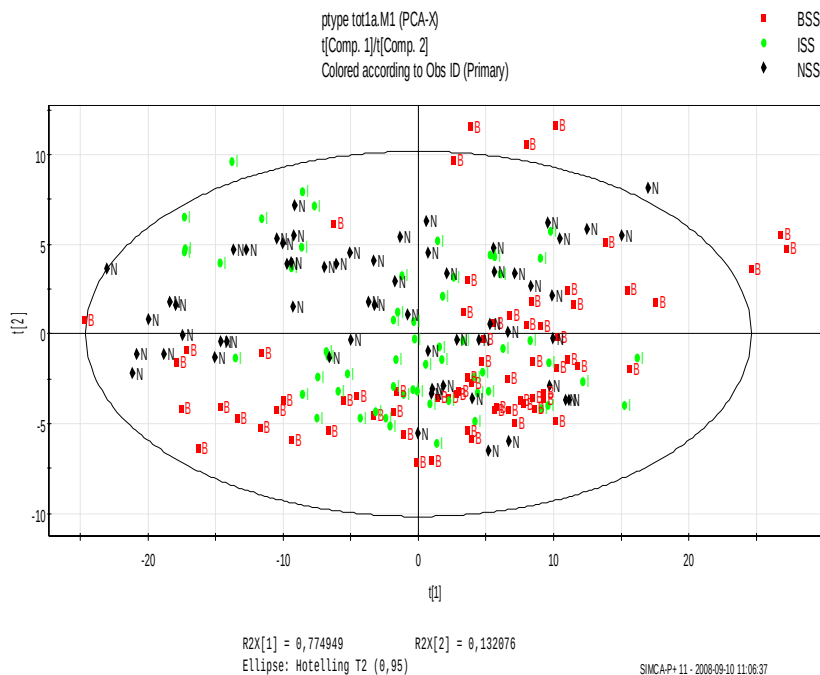


Figure 7. PCA analysis of MW measurements made on minced herring samples from different catching places.

However, analysis of the relation between catching time (seasonal prediction) and MW data showed a weak grouping of samples according to season (**Figure 8**). This finding agreed with the results from DTU Foods, and was probably determined by fat content which changes during season.

The MW-measurements carried out on the skin side of fillets using the first MW-instrument prototype (P1) gave no relation to storage time at -20°C for the different herring samples. (**Figure 9**). The same results were seen for the measurements carried out on meat side with the third prototype (P3).

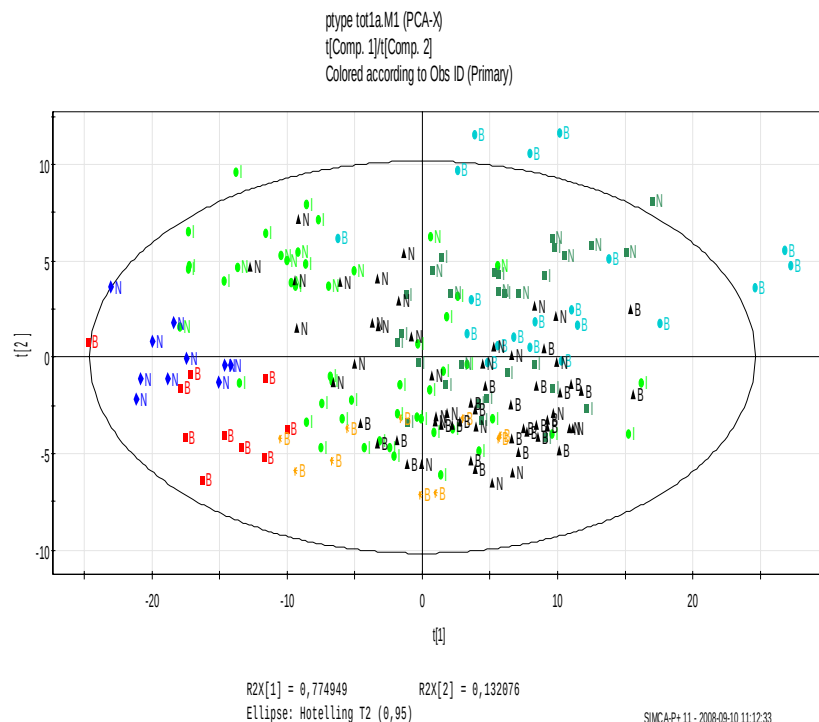


Figure 8. PCA analysis of MW measurement on minced reference samples of herring from different catching times. **Feb=Blue, Mar=Orange, Apr=Red, Sep=Turquoise, Oct=Dark green, Nov=Black, Dec=Light green.** B= Baltic, N= Norwegian coast, I= Icelandic coast

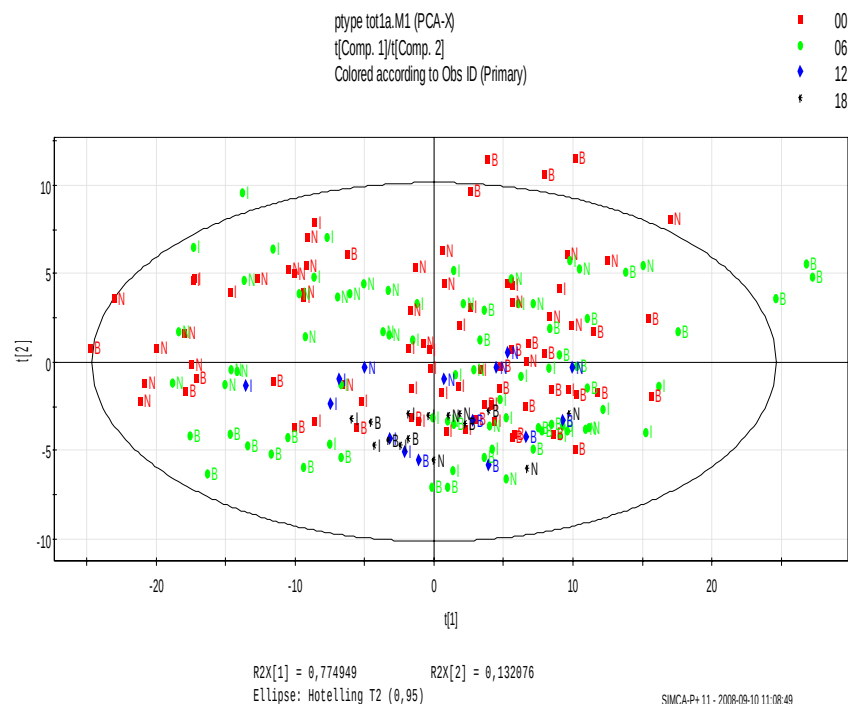


Figure 9: PCA analysis of MW measurement on minced herring samples with different storage time in freezer at -20 °C.

2.4.6 Treatment of MW data (performed by Sequid GmbH) from MW-measurements on skin-side of herring fillets (performed by SIK)

Sequid GmbH measurements and analyses of data with the purpose of developing an algorithm showed a good possibility to group the samples according to storage condition, i.e., -80°C or -20°C followed by -80°C (**Figure 10**). This means that it was possible to distinguish between reference samples and stored samples with developed lipid oxidation and microstructure changes.

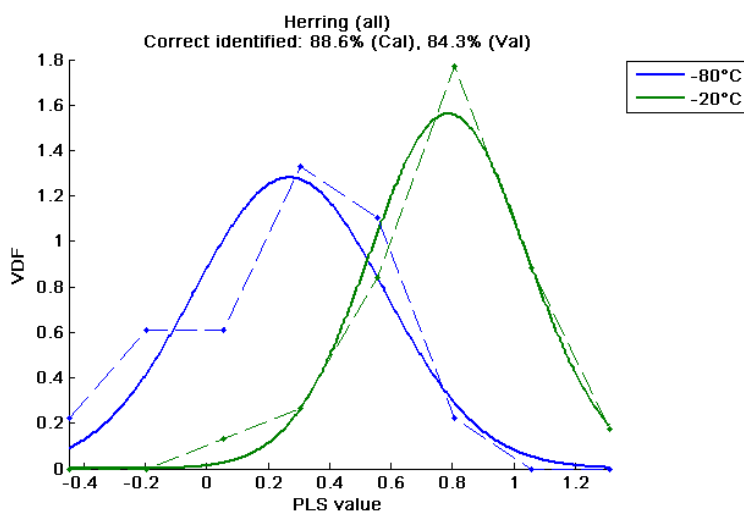


Figure 10 Grouping of herring stored at -20°C for 6, 12 or 18 months (green curve) and herring only stored at -80°C (blue curve). Mw-measurements were done on the fillet side with SEQUID GMBH prototype (P3) and analysed by Sequid GmbH.

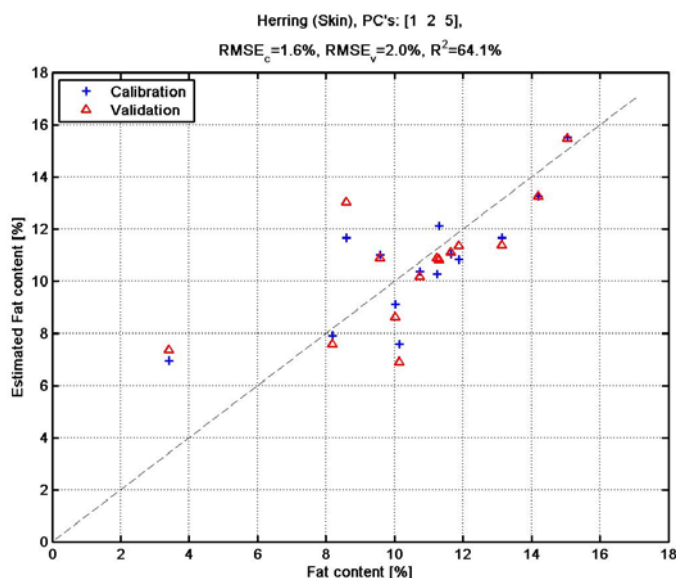


Figure 11. Estimated fat content from skin-side measurements of herring samples from the old NICE project, measured with P1 and analysed by Sequid GmbH.

A weak correlation between fat content and MW-spectroscopy could be seen using PLS-analysis (**Figure 11**). It was found that most information within the MW-responses was located in the “rising edge” of the curve.

No correlation was found between the time the herring had been held in RSW tanks before freezing, and the MW-spectroscopy signals obtained from analyses of the thawed herring after storage in -80°C (**Figure 12**).

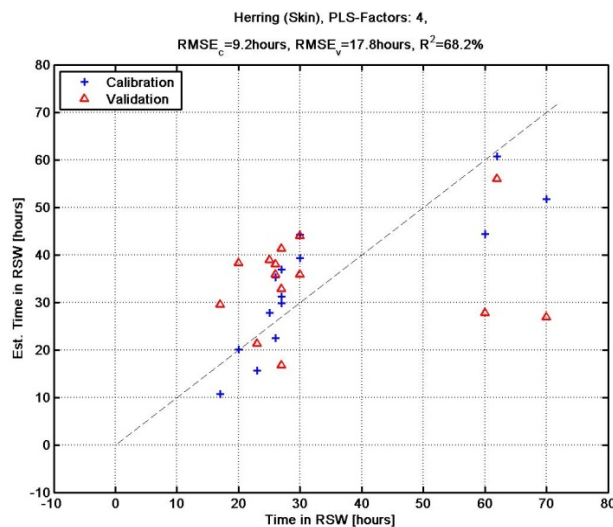


Figure 12. Correlation between estimated time in RSW tanks before freezing of herring from the old NICE project, and MW-signals obtained from measuring the skin side of this herring with the P1 equipment. Multivariate analyses were done by Sequid GmbH.

3. Extra compositional analyses done on herring from NIC-project 02106 (step v)

Chalmers has analyzed ascorbic acid in the liquid fraction (press juice) of the majority of herring lots that were collected during project 02106. This is since ascorbic acid is an important water soluble antioxidant. As expected, all stored batches contained much less ascorbic acid than the reference samples. It was also interesting to notify that levels of ascorbic acid tended to be lower during spring than fall/winter in all of the three herring batches (BSS, NSS and ISS). Similar observations were previously made in a PhD-project at Chalmers (Thippeswamy Sannaveerappa), but its full impact on stability against lipid oxidation has not yet been clarified.

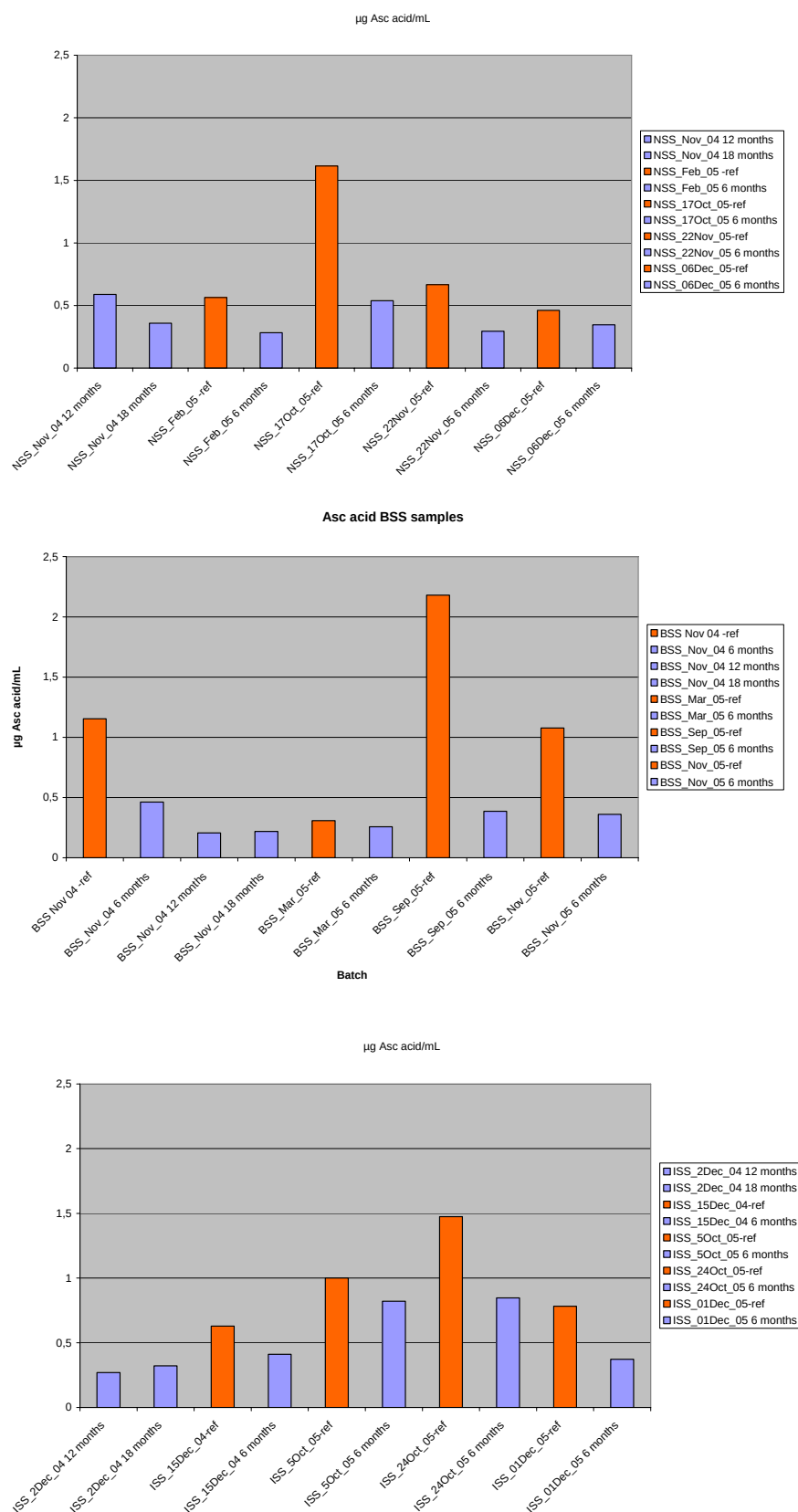


Figure 13; Ascorbic acid levels (µg/mL press juice) in the different batches of BSS, NSS and ISS herring. Orange bars are reference samples, and violet bars stored samples.

4. Measurements done on herring and mackerel collected by the industry participants of SILLQUID (steps vi-vii)

MW measurements have been carried out at Paul Mattson AB, Ellös Sweden, at Seastar International AS, Austevoll Norway and at Matis on samples From SildarvinnsIan, Iceland. The samples have been fresh herring, fresh mackerel and pre-frozen, then thawed, herring. A general procedure was worked out for how to carry out the measurements at each industry;

- Select 5-10 fishes/fillets at each measurement point
- Prepare the measurement series by putting in the sample numbers in the MW-computer program.
- Put the fish samples in a row so that each one gets a certain number
- Mark 5-10 plastic bags with the sample numbers
- Put the sample on a styro-foam plate with the head facing the right (avoid warming up the sample with your hands).
- Make the measurements with the MW-prototype 5 times on each sample on an area under the dorsal fin, but above the belly lining.
- Measure and notify the temperature of the sample
- Repeat this procedure on the next individual
- Put the sample in the respective plastic bag. Remove as much air as possible, and put the samples in the freezer for further analyses e.g. of fat.

4.1 SildarvinnsIan, Iceland- Study of the correlation to fat content and brine storage

4.1.1 Procedure

MW-measurements were performed on pre-frozen herring with three levels of fat content (low fat (~3%), medium fat (~7%) and high fat (~16%)) (**Table 2**). In total 30 fishes were analyzed with the MW-tool according to procedures as given above; 5 replicate measurements on the whole fish, and also on the meat-side of butterfly fillets. The 5 replicate measurements were done by moving the probe a small distance towards the tail. Lipid and dry matter were chemically analysed on the same material why the individual samples used for MW-measurements were stored in a freezer at -80°C.

Table 2. Fat content and size of thawed Herring from Matis/SildarvinnsIan.

Herring Thawed - Fat studie			
Samples	Min fat (g/100g)	Max fat (g/100g)	Size (g)
30	2,9	15,6	150-250

Also 30 frozen herrings were thawed and stored in 18% salt solution at -2°C during 3, 7 and 10 days (**Table 3**). MW-measurements were done on these fishes (5 replicate measurements on the whole fish, and on meat-side of butterfly fillets), and then, total volatile nitrogen compound (TVN) were measured for each fillet. The individual samples used for MW-measurements were then stored in a freezer at -80°C.

Table 3. Total volatile nitrogen (TVN) content and size of thawed Herring from Matis/Sildarvinnsan.

Herring Thawed - Brine studie				
Days in brine	Samples	Min TVN mgN/100g	Max TVN mgN/100g	Size (g)
3	10	11	16	220-320
7	10	12	16	190-280
10	10	10	16	190-300
	<u>30</u>			

The frozen herring material collected by Sildarvinnsan was also subjected to MW-analyses done by Sequid GmbH in February 2010 using a new version of the MW-tool (RFQ-Scan 3.0). The aim was to test the correlation to fat content which had been analyzed on these samples. These MW-analyses were carried out in Germany according to the following:

- 30 Samples of herring were used
- Fat range: 2.9% - 10.6% and 1 sample (M-2009-00400, Nr.25) with 15.6%
- 2 samples (Nr. 8/25) were rejected, because of erroneous measurements. This yielded an amount of 28 samples
- Remark: The samples were very small, frayed out and partially in pieces
- Data analysis 1
- The measured time domain curves were evaluated by performing a principal component analysis. The samples were classified using the first 2 principal components. The classification was defined by a fat content of 6.5%
- The percentage of correctly identified samples was computed
- Remark: The choice of the threshold was not based on practical requirements, but on the given statistic of 28 samples. The constraints were similar size of classification groups and the
- 75% were correctly identified at a threshold of 6.5%
- Data analysis 2
- The measured time domain curves were evaluated by performing a principal component analysis. The fat content of the samples was predicted by applying a multiple linear regression of the principal component 1 and 2 versus the fat content.
- The validation results were obtained by a full internal cross validation, due to the small number of samples

4.1.2. Results

4.1.2.1 Fat content

It was difficult to get useful calibration between the MW-signals and the fat content. The most promising dataset was surprisingly the one comprising the last of the five measurements on each sample (named measurement E). There was a substantial correlation between the maximum of the smoothed values and the fat content, as well as between the maximum of the first derivative curves and fat content (**Table 4**). Likewise, PCR gave similar correlations between predicted and measured fat (**Figure 14 and 15**), although this was not cross-validated. The different transformed data set gave similar results, the relationships are however not convincing.

Table 4. Correlation coefficient between fat content and maximum value of the curves measured on the flesh or skin side of herring

Curve set	Smoothed curves		First derivative	
	Flesh	Skin	Flesh	Skin
A	0,238	0,243	0,268	0,249
B	0,245	0,105	0,196	0,124
C	0,157	0,160	0,079	0,183
D	0,196	0,160	0,111	0,169
E	0,595	0,253	0,585	0,242
Mean	0,393		0,438	
U-K	0,198		0,175	
U-K and E	0,356		0,341	

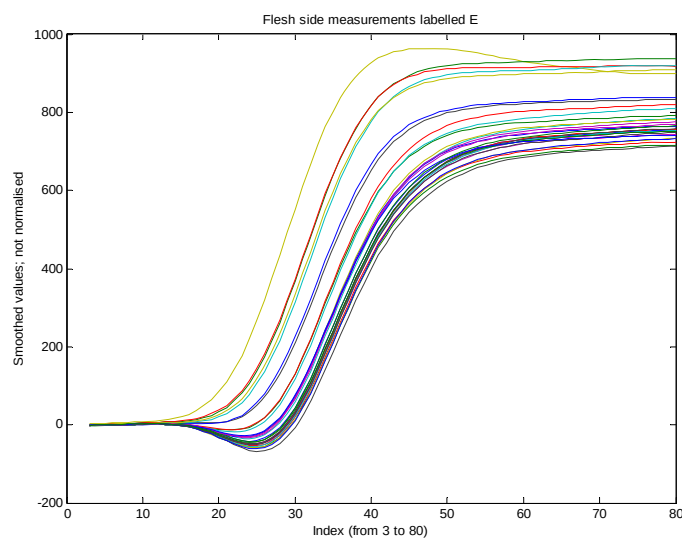


Figure 14. Signals from flesh side MW measurements (X1) of herring labelled E (i.e. the last out of 5 measurements).

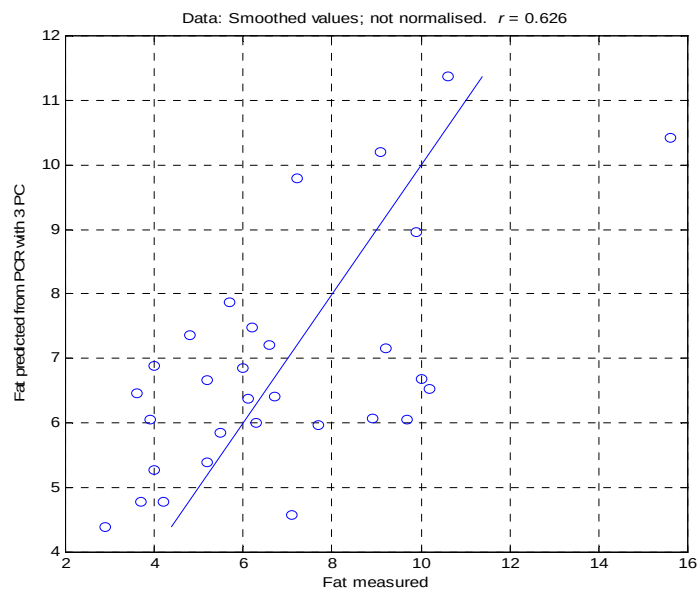


Figure 15. Measured fat vs. predicted fat from a PCR with 3 principal components based on X1 MW data.

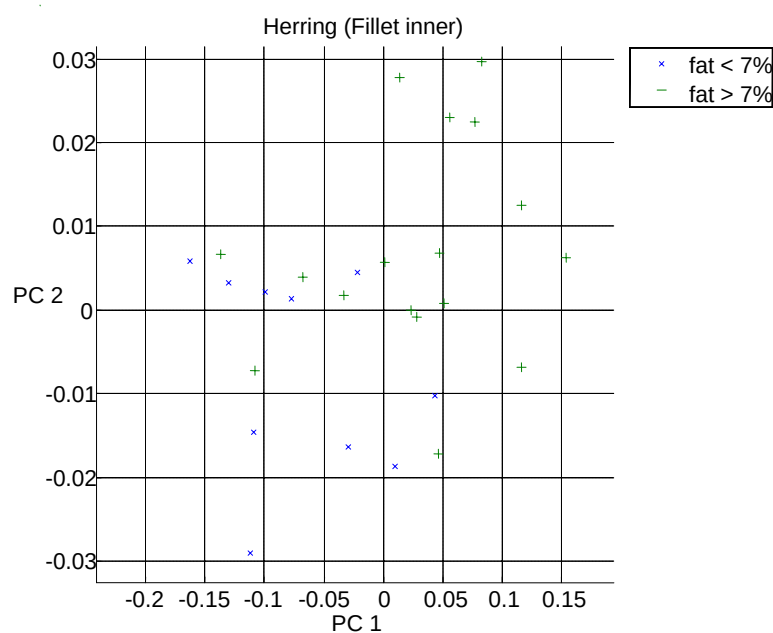


Figure 16. Classification of the samples into two levels of fat content by using two principal components calculated from MW-signals obtained with the RFQ-Scan 3.0 on double frozen herring

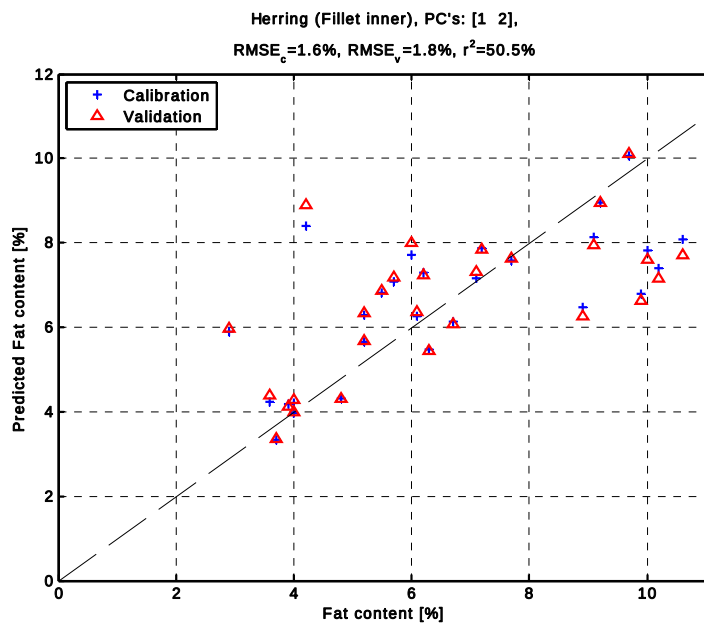


Figure 17. Measured fat vs. predicted fat from a PCR-analysis with 5 principal components based on measurements of double frozen herring using the RFQ-Scan 3.0.

The results from the analyses done by Sequid GmbH using model RFQ-Scan 3.0 showed that a certain classification between very lean and medium fat fish could be done. However, there appeared to be a threshold already around 7% fat. Correlation analyses between predicted and real fat content data showed an r^2 -value of 50.5%. Unfortunately the herring used in this trial were very small, frayed out and partially in pieces. This was probably since it had been frozen twice, transported between Iceland and Germany and then thawed out.

4.1.2.2 Time in salt brine

Principal component analysis (PCA) of a data matrix containing the 5th MW-measurement on the skin-side of each sample showed that the signals contain information about the storage time in salt brine.

The second principal component scores belonging to samples that were salted for 3, 7 or 11 days were significantly different ($p < 0.0001$) when tested by parametric ANOVA and by the non-parametric Kruskal-Wallis test. Post tests showed that it was the 3 day samples that differed significantly from the rest. This result applied to the raw data as well as to first and second derivative curves.

4.2 Seastar International AS, Norway - Study of the correlation to fat content

4.2.1 Procedure

A total of 90 herring samples were collected from 7 different catches during the period of November 15 and December 5 2008. Following processing into butterfly fillets, all herring samples were measured on the right hand part of the meat side, beneath the back fin. Five replicate measurements were done on each individual herring. Similar measurements were done on 165 samples of mackerel collected from 7 different catches during the period of October 10 and November 24, 2008. Sections representative to the measured fillet area were then cut out, frozen with minimum of surrounding air, and sent for chemical analyses of fat content. Left hand fillets were then frozen in vacuum for tentative later tests of effects of freezing on MW-readings in collaboration with Sequid GmbH.

4.2.2 Results

The range of fat content of the herring was 12-26 g/100g (Table 5). Multivariate analyses revealed that it seems to be possible to make a correlation between fat content and the MW-signal from meat side measurements up to at fat content of around 18-20%. Above this level, one can only say that the herring has a fat content >20g/100g. The same seemed to be valid for the Mackerel data set (fat range 16-35 g/100g, Table 6), but with a threshold around 25% fat.

Table 5. Fat content and size of the herring samples caught in Norway 2008.

Herring								
Date of catch	Samples	Min fat (g/100g)	Max fat (g/100g)	100-250	200-300	Size 300+	350+	450+
15-nov	10	12	18		x	x		
27-nov	10	16	26			x	x	
28-nov	15	15	24			x	x	x
28-nov	15	17	25			x	x	x
29-nov	15	16	25			x	x	x
04-dec	10	18	26			x	x	
05-dec	15	12	26	x			x	x
90								

Table 6. Fat content and size of the mackerel samples caught in Norway 2008.

Mackerel									
Date of catch	samples	Min fat (g/100g)	Max fat (g/100g)	S2 100-250	S4 200-400	S5 300-500	S6 400-600	G5 500+	G6 600+
10-okt	25	missing data			x	x	x	x	x
15-okt	15	23	34		x	x	x		
17-okt	25	26	35		x	x	x	x	x
20-okt	25	26	34		x	x	x	x	x
01-nov	25	27	34		x	x	x	x	x
05-nov	25	16	32	x	x	x	x		x
24-nov	25	17	32	x	x	x	x		x
165									

It should be stressed that the fish from Seastar International AS differed both in size and in fat content. This is illustrated with the mackerel data set in Figure 18. Fat content and size were positively correlated.

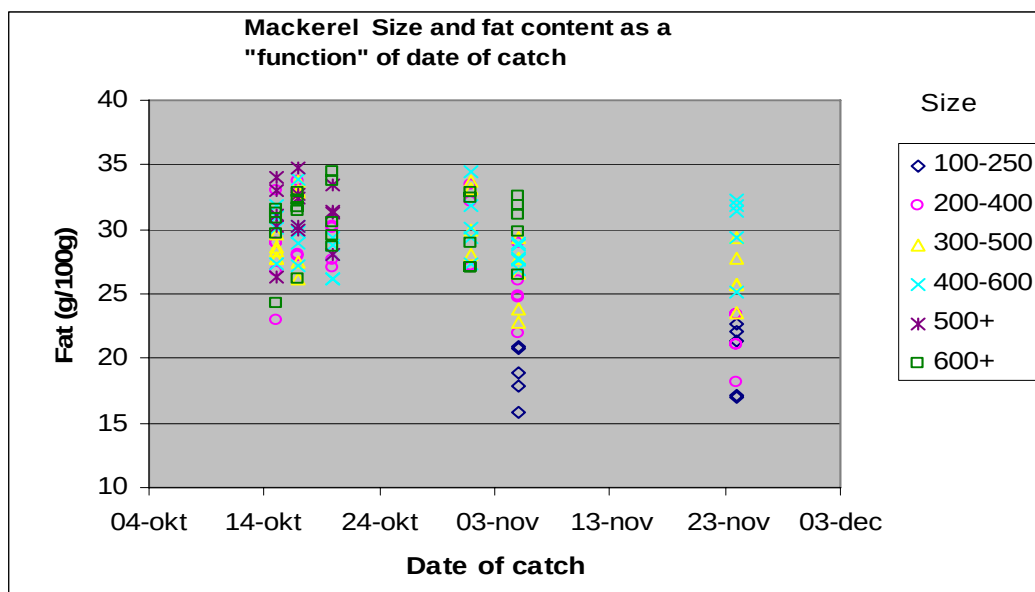


Figure 18. Overview of the Mackerel material showing date of catch, size and fat content

The MW measurement curves were studied to see if there were any obvious outliers. A first threshold test was also used to get rid of a systematic error occurring during the measurement. In nearly all measurements, the fifth replicate (E) was not representative, which is interesting in relation to the Icelandic results where the opposite was seen. A possible reason is that the samples had been taken away from the probe too early, since the curves looks more like measuring air (**Figure 19**). All the obvious outliers were deleted from the data sets before the first calculations of correlation between MW-measurement and fat content were carried out.

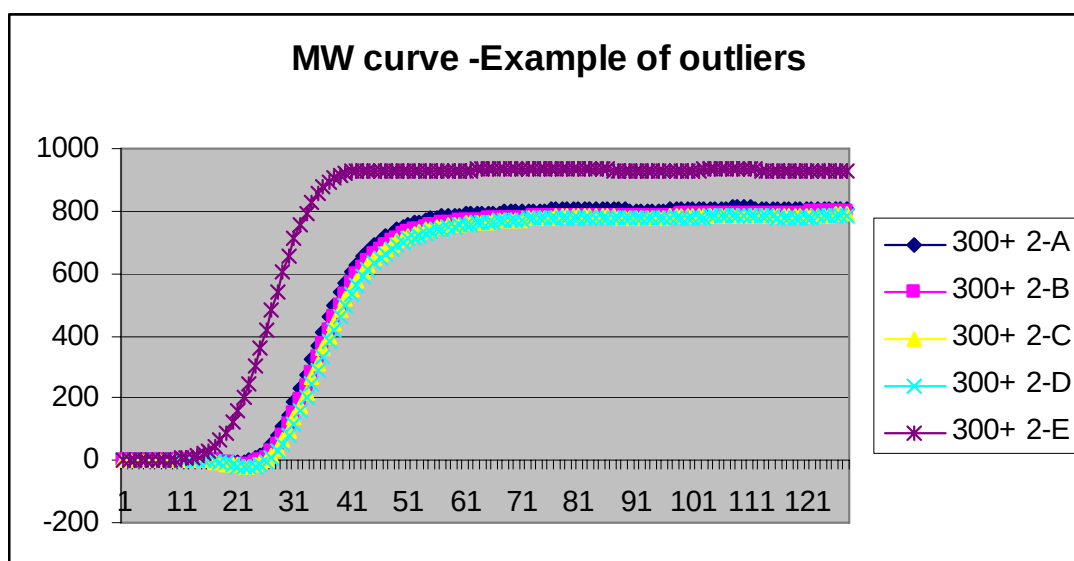


Figure 19. Example of MW-spectra with outliers (measurements E) using P1.

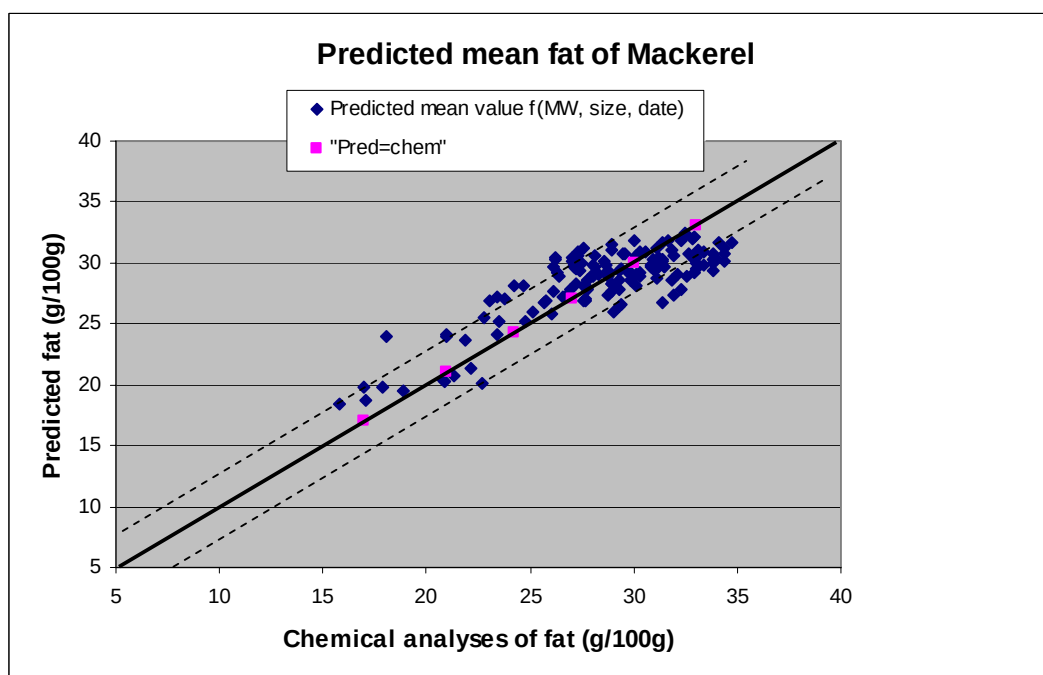


Figure 20. Estimated fat content in fresh mackerel collected and measured by Seastar International in October – November 2008 using the MW-tool P1. PLS-analyses were carried out by SIK

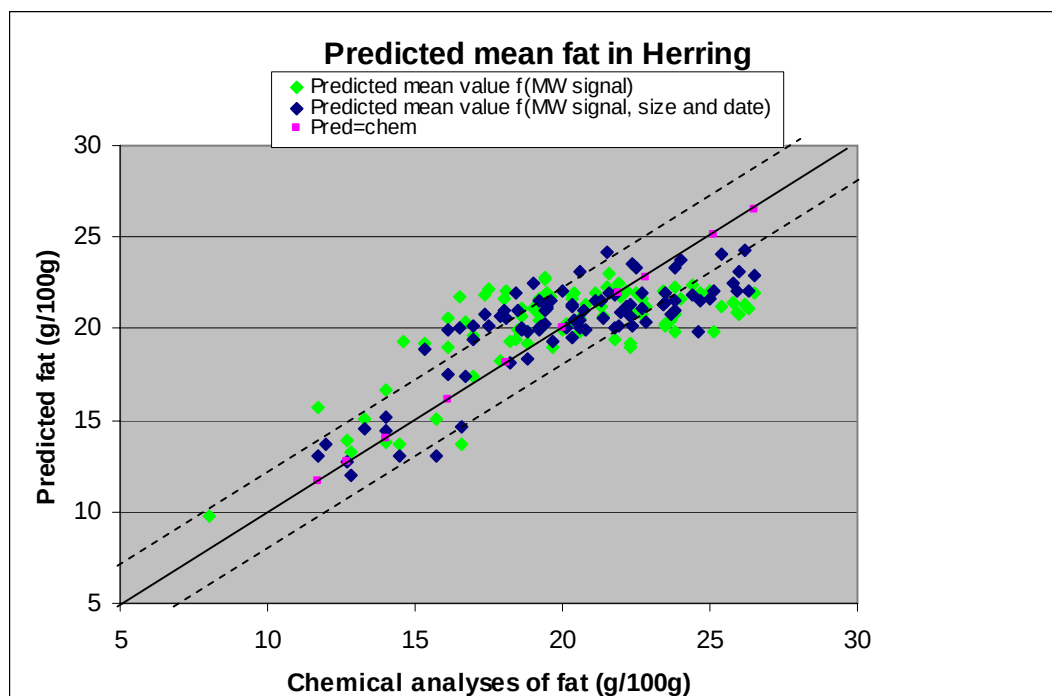


Figure 21. Estimated fat content in fresh herring collected by Seastar International in November- December 2008. MW-measurements were done with the P1-probe. PLS analyses were done by SIK.

The results indicate that the best range for MW-spectroscopy to predict fat content of herring and mackerel is up to 18 and 25%, respectively (**Figure 20 and 21**).

4.3 Paul Mattson AB, Sweden –Study of the correlation to fat content, RSW-storage and pre-freezing

4.3.1 Procedure

The aim of the trials in Sweden was to use the MW-tool to measure: (i) fat content, (ii) post mortem storage time in the RSW-tanks and (iii) pre-freezing of herring.

4.3.1.1 Procedure for trials focusing on fat content

Groups of fish (6-10 fish in each group) that visually appeared to differ in fat content were picked out during April and October 2009. MW-measurements were done on each fish (5 replicate measurements by moving the probe in a circle on the whole fish, on meat-side of butterfly fillets and on mince) according to procedures as given above. The minces used were then stored in the freezer to analyze lipid/dry matter. The trials in October were done in collaboration with both Sequid GmbH and SIK. All measurements in October were performed with the newest equipment (RFQ Scan 3.0) and mostly on size 0 herring (~200g). See Table 7 for a summary over the samples used.

Table 7. Fat content, catching date and measuring date for herring from Paul Mattson AB collected 2008 and 2009

Herring					
	Date of catch	Measuring date	Samples	Fat* (g/100g)	Size (g)
2008	17-sep				
First MW Prototype					
2009	16-mar		6	6,7 *	30
	16-mar		6	8,0*	60
	16-mar		6	12,8*	110
* Fat value calculated from Dry matter analyses on samples from a mixture of 2-4 samples					
2009					
SEQUID RFQ-Scan 3.0					
Frozen	8-Oct	14- Oct	10	17,9-20,5	200
Fresh	13-Oct	14- Oct	10		200
Fresh	14-Oct	15- Oct	10	15-22	200
** Fat value calculated from Dry matter analyses on individual samples					
RSW	12-Oct	14- Oct	5		200
RSW	12-Oct	15- Oct	5	14,5-21,5	200
RSW	12-Oct	15- Oct	10		100
			68		



Figure 22. To the left, herring in ordinary RSW vessels at Paul Mattson AB, to the right samples taken.

4.3.1.2 Procedure for trials focusing on storage time in refrigerated seawater (RSW) tanks

Freshly caught herring was sampled and then stored in nets in RSW-tanks at Paul Mattsson AB up to almost three days (60h) (**Figure 23**). Five to 10 fish were sampled for MW-analyses at each time point tested. Samples used were then frozen for further analyses of lipid/dry matter.



Figure 23: Small scale RSW-storage of herring at Paul Mattsson AB

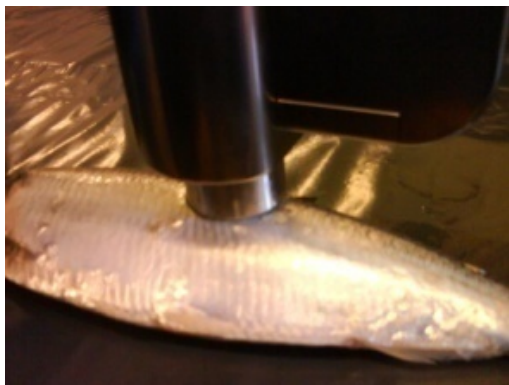


Figure 24: MW-measurements on RSW-stored herring at Paul Mattsson AB

4.3.1.3 Procedure for predicting if herring had been frozen

Freshly caught herring was sampled and then frozen at Paul Mattsson AB for up to six days. Samples were then thawed at low temperature and 10 herrings were sampled for MW-analyses according to the procedure above with the RFQ Scan3.0. Samples used were then frozen again for further analyses of lipid/dry matter.

4.3.2 Results

4.3.2.1 Fat content

Results from the MW-analyses done at Paul Mattsson AB in October 2009 (**Figures 25-27**) showed a weak correlation between fat content and the MW-signal.

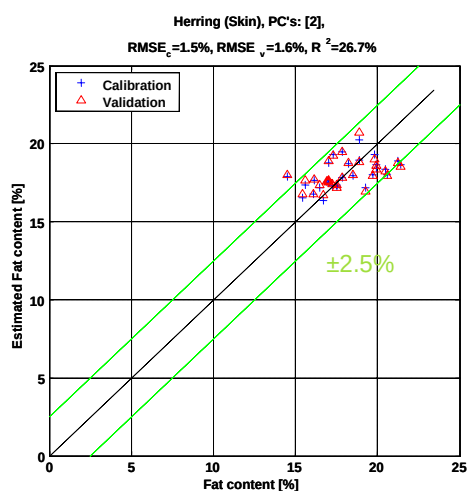


Figure 25. Prediction of fat content in fresh herring measured with RFQ-Scan 3.0 on skin-side.

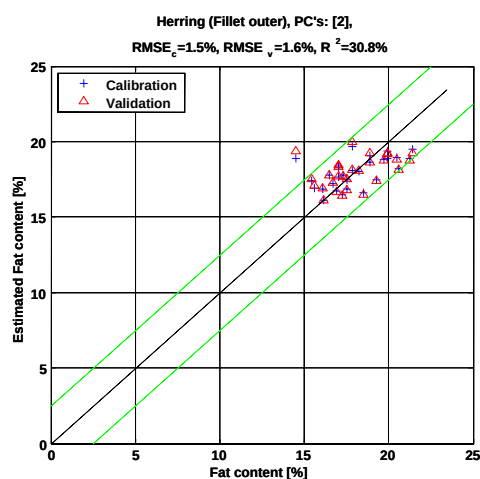


Figure 26. Prediction of fat content in fresh herring, measured with RFQ-Scan 3.0 on flesh-side

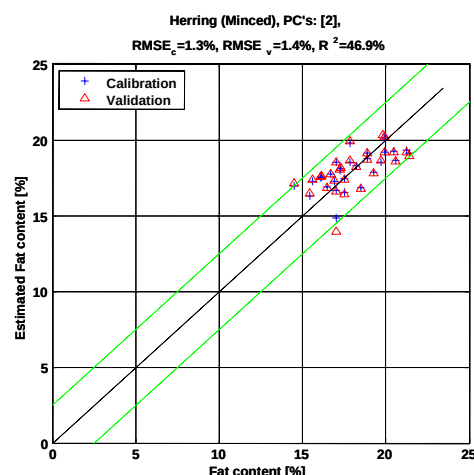


Figure 27. Prediction of fat content in fresh herring measured with RFQ-Scan 3.0 on minced samples.

However, the range of fat content of the fish used in this study was very narrow, 14-21 g fat/100g, and was not representative for the range that could actually be seen in herring over the year (1-30 g/100g). It should be stressed that the fat content was indirectly calculated from dry matter analyses.

4.3.2.2 RSW storage

Regarding correlation to RSW storage (12-60h), the outcome was promising (**Figure 28**). It was possible to classify herring into “short” RSW-storage (≤ 12 -15 h) and “long” RSW-storage (> 40 h). However, this finding has to be investigated further to see if it is possible to strengthen the fish industry with an objective tool to classify incoming deliveries in different quality categories.

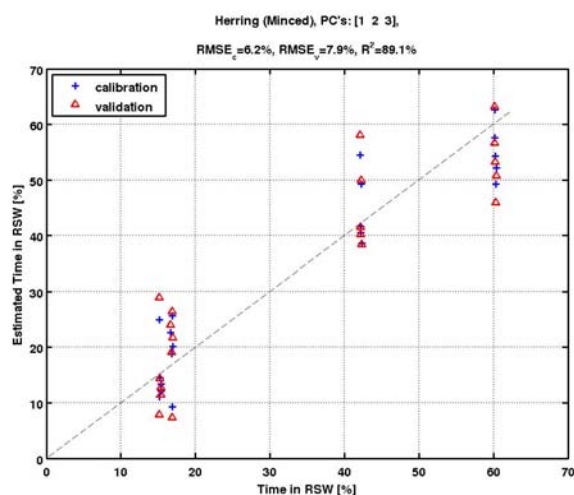


Figure 28. Prediction of time in RSW (hours) measured with RFQ-Scan 3.0

4.3.2.3 Freezing of herring

Results from the MW-analyses done at Paul Mattsson AB in October 2009 showed a good possibility to classify fresh from pre-frozen herring by the MW-signal.

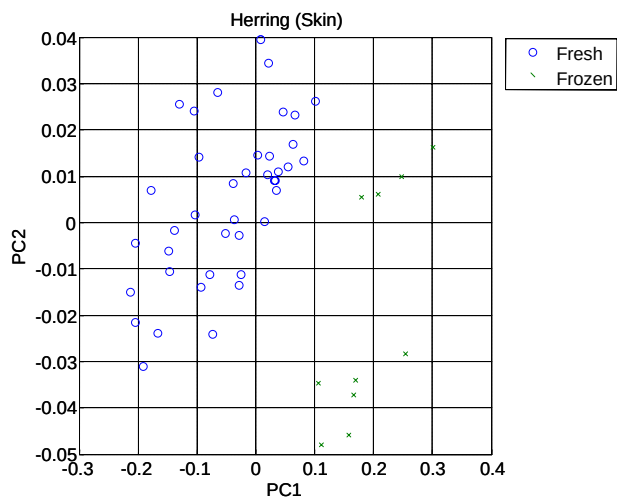


Figure 29. Grouping of fresh and frozen herring after measurements with RFQ Scan 3.0 and data analysis performed by SEQUID GmbH

4.4 General comments from industrial trials

The industrial partners at Atlantic Pelagic working with the first prototype (P1) liked the instrument and their impression was “very nice and fun to work with it”. Since this was the first generation instrument, and since it was not calibrated for fat content, the measurement signals could only be followed by looking at curves coming up on the computer screen (see e.g. **figure 19**). At Paul Mattsson AB, measurements were performed both with the first prototype (P1) and with the latest version of a commercial instrument (RFQ Scan 3.0). The differences between these two instruments, e.g., the pressing of the samples up against a probe vs. holding the probe over the samples and pressing it down, was experienced as a major improvement. Together with the finding that there is a very promising correlation between the MW-signals and fat content, these impressions make the instrument highly interesting as a time-saving alternative to the time consuming dry matter analyses that are currently made.

In order to improve the possibility to make correct MW-analyses within the industry, it was concluded that the instructions for how to use the MW-prototype should have been even more detailed, preferable with pictures and better handling advices. Unfortunately some of the measurements have had to be excluded due to bad contact between probe and fish or due to too fast removal of the fish samples. Discussions have been held in the project on what is the best location of the fish to make the measurements. Also, the question about how long distance the probe should be moved between each measurement came up. Measurements have not been done in the same way at all 3 industries. For example in Paul Mattsson AB, measurements were done in a circle. In Iceland they were done horizontally. During the newest trials done in Germany, the measurements were done in a “gliding mode”. We are sure that these different ways of measurement have influenced the outcome of the results in the project SILLQUID.

5. Conclusions

The most important result from this project has been the promising ability of the MW-tool to predict fat content of pre-frozen pelagic fish, at least up to fat levels of 18% (herring) or 25% (mackerel). This ability was seen both from measurements of the herring material collected in NI-project 02106 and the new herring and mackerel materials collected by the industry participants in this project. Most of the information in the MW-spectra about fat content was located either in the “rising edge” of the curve, or at the maximum of the curves. There was also a weak correlation to season of catch, but most likely this was also a result of the seasonal differences in fat content. It should be stressed that it is not known at this point whether it was the actual fat or the water of the muscle that responded in the MW-measurements. Trials done at Paul Mattsson AB revealed that the MW-tool also has some capacity to predict the time herring has been stored in refrigerated sea water (RSW)-tanks, and also, whether herring has been pre-frozen or not. From studies made in Sildarvinnsland, a weak correlation could also be made between MW-signals and time in a salt brine. The correlation to all other studied biological and chemical/physical parameters in the frozen herring has been low.

In general we believe that the results from the industrial trials would have been more clear if the collected material had had a wider spread of fat content, and if the industrial partners would have had the possibility to practice more on how to carry out the measurements. In relation to the latter, an important outcome has been the need to communicate instructions for the MW-tool in a very clear way, preferably with pictures. Particularly the way the probe is pressed onto the sample surface seems to affect the signals. This applies both to the force of pressing, and the way by which the probe is moved over the sample surface to make replicate measurements (in a circle, in a line, long or short distances). The latter has become less of a problem with the new hand-held instrument (RFQ Scan 3.0) which gives a more constant pressure by its own weight.

6. References

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