TraceMyFISH

Traceability and Quality Monitoring throughout the Fish Value Chain

D6.3 – Second pilot evaluation & KPI assessment

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DELIVERABLE TITLE	Second pilot evaluation & KPI assessment
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ACRONYMS LIST

TMF	Trace My Fish
VIDEOM	Videometer
TVC	Total Viable Count
TVB-N	Total volative base nitrogen

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EXECUTIVE SUMMARY

This deliverable provides an overview of results of experimental work performed in three different pilots aiming to cover the three different seafood value chains within the project. Each pilot partner (AUA, NTNU, UoI and MATIS), individually, designed experiments relevant to their value chain taking into consideration results of D2.3 and discussion with stakeholders and VIDEOM provided sensors and support. The collected data serves as inputs for further use and analysis, i.e., machine learning model training and deployment.

Detailed results of the pilots will be presented in scientific publications to ensure the findings can be distributed to stakeholders effectively. The overview provided in the deliverable shows that the evaluated equipment, the VideometerLab and VideometerLite prototypes showed great potential for evaluation of freshness, both of whole fish and fillets, and could be used for detection of fillet flawes or parasites with good accuracy. The applications of these devices within the seafood value chains provide new possibilities for traceability and non-destructive methods for quality control. The devices are built for by-line use by quality control personell, in the fish market or wherever relevant stakeholders desire a way to further monitor or evaluate their samples and with relatively quick imaging time and good range of wavelengths it provides that. For some specific functions the devices could be further developed based on results from the pilots or further add-ons developed to ease imaging of large samples but for most the devices performed well.

1 INTRODUCTION

The context of WP6 was on setting a wide range of objectives, considered as the backbone towards the successful development of the whole TMF system; i.e., sensor to be employed, development of the required algorithms for quality and safety assessment with prediction capabilities along the product's chain of processing and distribution and traceability as was discussed in detail in D6.2. Further, the WP hosts the data collection experiments planned and results of those pilots will be presented in the deliverable.

The experimental procedures (please also refer to D2.3 and D6.2 for more details and customized protocols for each pilot) were designed to ensure the real food chain is simulated, but under more controlled conditions.

2 THE ATLANTIC SALMON PILOT

The following section summarizes key results from the experiments conducted in the Atlantic salmon pilot. The aim of the Atlantic salmon pilot was to evaluate the potential of using multispectral imaging from Videometer prototypes to detect quality changes along the Atlantic salmon value chain. To achieve this goal, experiments were performed to simulate different scenarios of unwanted incidents that can happen in the different steps from farming to value-added product. These incidents include

1) ***Issues related to pre-slaughtering stress and fish handling***
2) ***Issues related to melanin spots.***
3) ***Issues related to loss of freshness and microbial spoilage on Whole salmon.***
4) ***Issues related to loss of freshness and microbial spoilage on Salmon fillets.***
5) ***Comparison of VideometerLite prototype 1 and 2.***

For imaging, the Videometer prototypes VideometerLite v.1, VideometerLite v.2 and VideometerLab were applied. Extraction of the resulting imaging data was done through nCDA transformation and segmentation in the VideometerLab Software. Then, one way ANOVA of and Pearson correlational coefficients between reflection from imaging data and change in quality factors were calculated in SPSS statistics. The general experimental setup is showed in Figure 1.

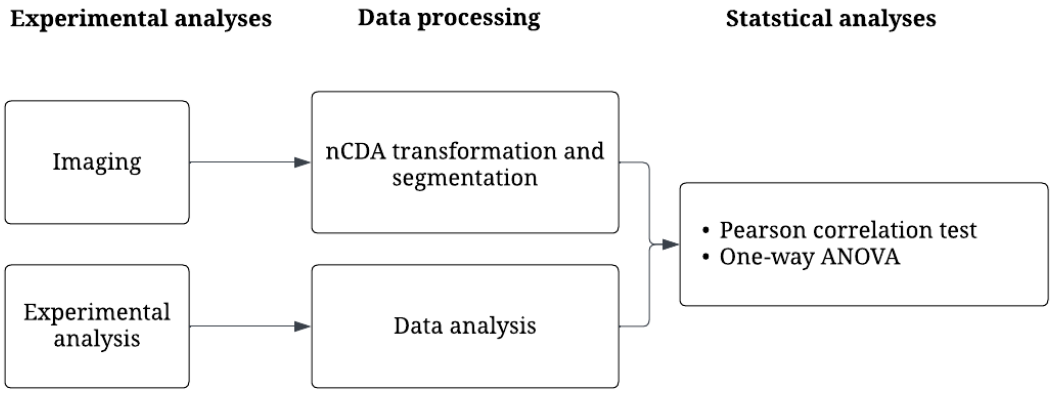


Figure 1 General experimental setup for Atlantic salmon pilot

2.1 EXPERIMENT DESCRIPTIONS AND KEY RESULTS

2.1.1 Issues related to stress

To simulate stress and rough handling in the Atlantic Salmon value chain, a stress experiment was performed over an intensive period of 14 days, having unstressed fish as controls (Figure 2). The design was then used to evaluate the potential of VideometerLite and VideometerLab2 to evaluate textural and colorimetric parameters. To validate the results, multispectral data from the Videometer devices

was compared to data from traditional methodologies measuring textural (penetration test) and colorimetric properties (DigiEye imaging, SalmoFan and muscle pigment concentration.

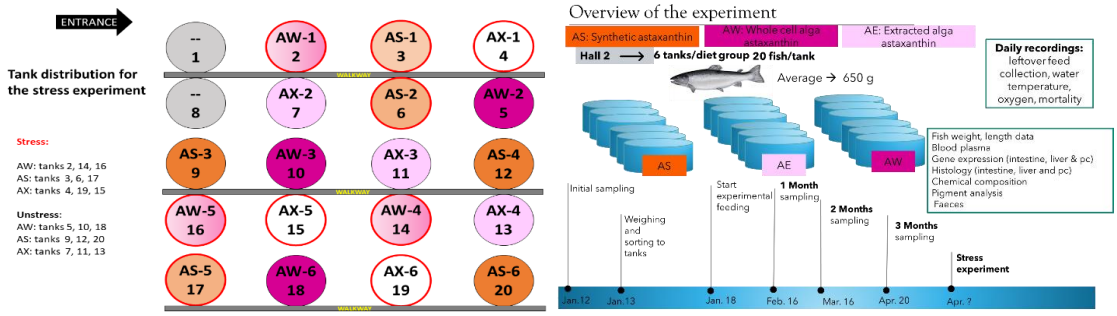


Figure 2 The experimental design of which the fish was used to study the potential of Videometer devices to measure Atlantic salmon's textural and colorimetric properties.

The fillets were grouped into soft, average and firm fillet based on the F60-value (F60<12N, F60=12-15N and F60>15N for soft, average and firm, respectively). No significant difference between groups (Figure 3, ANOVA, P=0.0129-0.661) was found when considering each of the single wavelengths. However, a significant main effect was observed when combining all wavelengths from the reflection data of the fillet surface. Soft fillets gave in average lower reflection than average which was lower than firm fillets (Figure 3, GLM, $P_{firmness}$ = 0.047). Although the observed differences were small, they were significant and indicate a relationship between firmness and fillet surface reflection that should be further investigated.

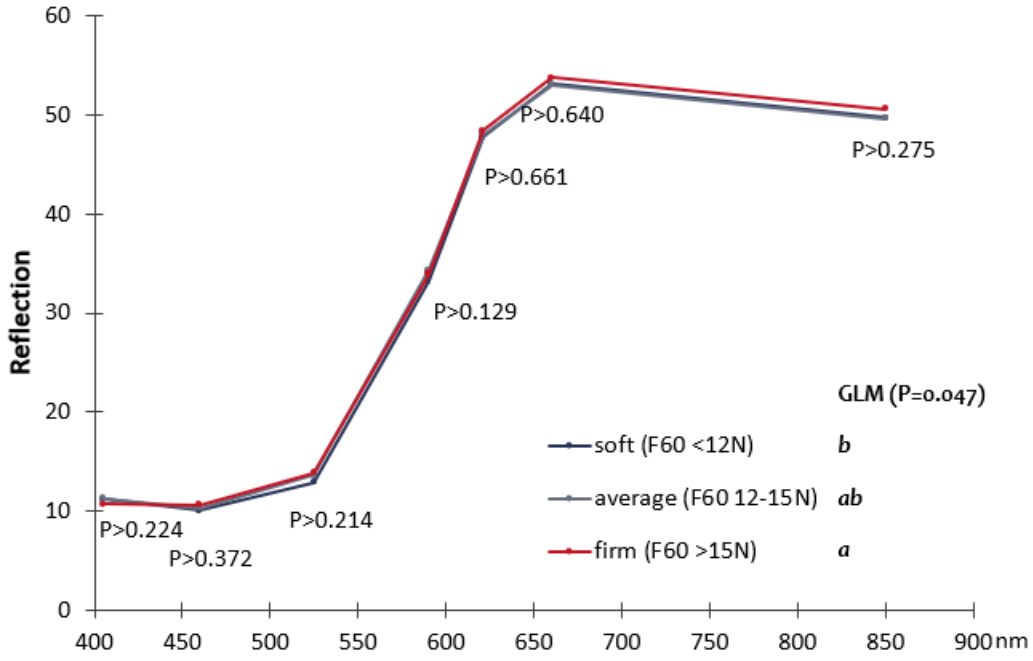


Figure 3. Reflection properties of soft (F60<12N), average (F60, 12-15N), and firm (F60>15N) fillets measured with the VideometerLite prototype. Annotated p-values were calculated by one-way ANOVA, whereas the main effect of fillet firmness was calculated by GLM.

2.1.2 Issues related to melanin spots

Melanin spots are visualized as grey to black pigments, often located in the myocomata and the myosepts. Three experiments related to melanin spots were performed. In the first experiment, reflectance data from multispectral images of ten salmon fillets with melanin spots were compared to a reference tissue. In the second experiment, colorimetric properties (LCH) were investigated. Lastly, in the third experiment an imaging protocol allowing for light pollution was compared to a protocol in controlled, imaging-friendly surroundings.

Images of melanized tissues showed significantly lower reflection (P<0.001) in the green/red part of the visible spectrum (VIS 525-700 nm) and the near-infrared spectrum (NIR, 700-970 nm) than the reference tissue. Moreover, the reflection also varied between individual melanin spots, showing a significant variance in the intensity of the melanized tissue among the fillets sampled for analyses.

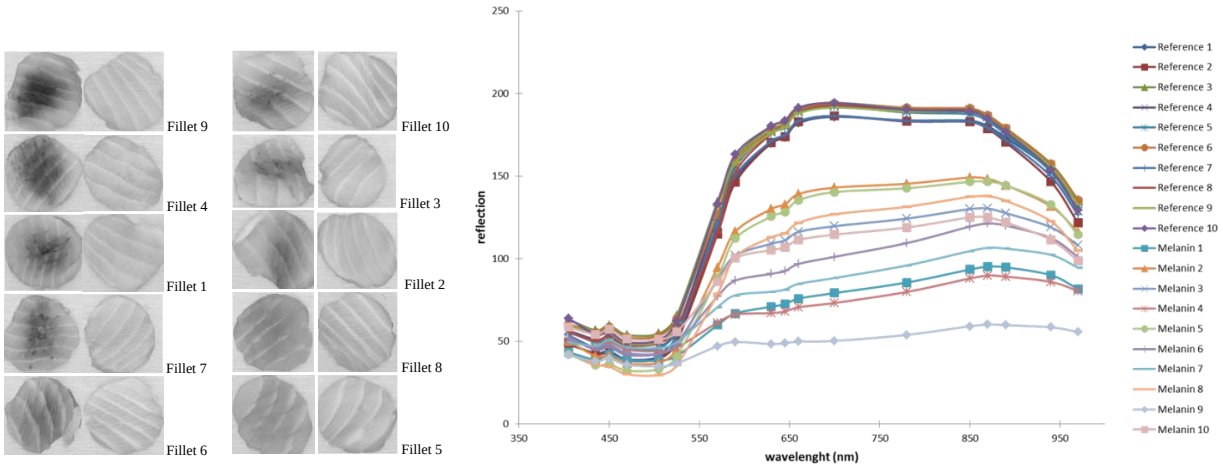


Figure 4 NIR images of salmon fillets sampled for analyses ranged after increased light reflection (decreased melanin intensity) at 870 nm. All images were captured in a VideometerLab2 multispectral system (Videometer A/S, Herlev, Denmark).

The color analysis showed that the melanized tissues were on average significantly darker (lower *L*) and showed a decreased colour saturation (*C*) and Hue-angel (*H*) compared to the reference tissue (P <0.008).

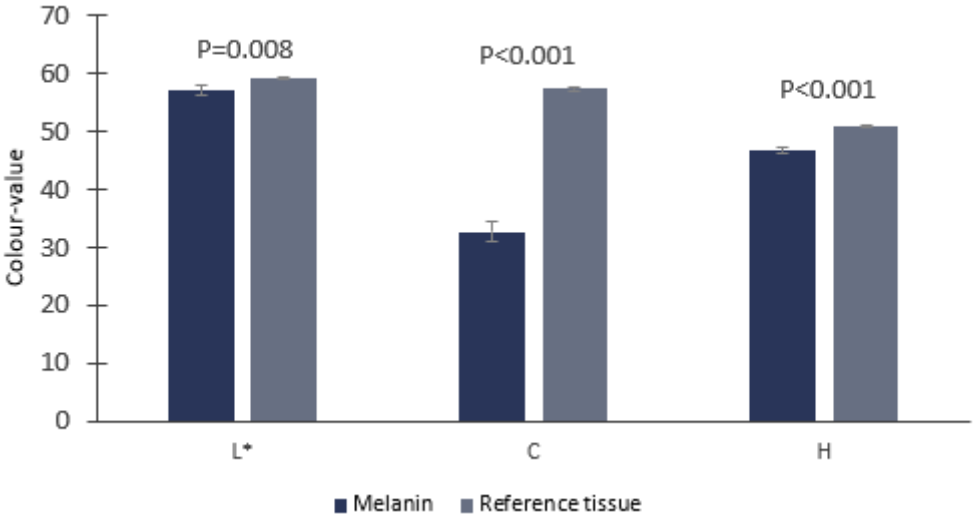


Figure 5. Colorimetric differences between melanized and reference tissue as measured with the VideometerLite v.1. prototype. Statistical differences were found by One-Way ANOVA. Error bars represent one standard deviation.

In the experiment where a controlled protocol was compared to the protocol allowing for light pollution, the results showed that both protocols gave images that were able to distinguish melanized fillets from reference tissue. However, reflection data from the controlled protocol gave clearer results, as there were found significant differences ($p<0.001$) for all the investigated wavelengths, while the protocol allowing for light pollution gave significant differences for only three of the seven wavelengths.

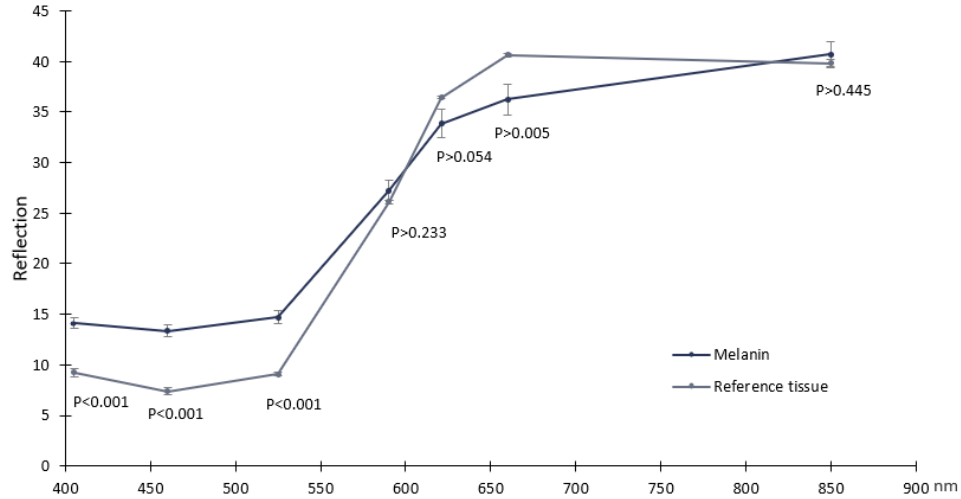


Figure 6. Reflection properties of melanized and reference tissue obtained from images captured with the VideometerLite prototype on whole fillets (a protocol that issues related to light pollution and stability). Significant differences between groups were found using One-Way ANOVA. Error bars represent one standard deviation. The α-level was set to 5 %.

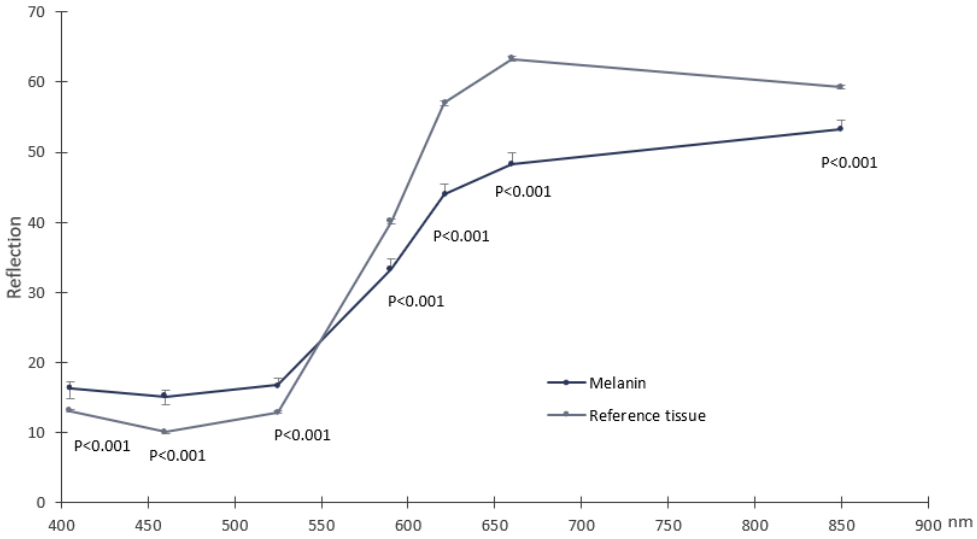


Figure 7. Reflection properties of melanized and reference tissue obtained from images captured with VideometerLite prototype on fillet portions (a protocol that was designed to optimize the imaging capturing conditions). Significant differences between groups were found using One-Way ANOVA. Error bars represent one standard deviation. The α-level was set to 5 %.

2.1.3 Issues related to loss of freshness and microbial spoilage on whole salmon

The aim of the experiments related to loss of freshness and microbial spoilage in whole Atlantic salmon was to assess multispectral imaging data obtained with VideometerLite and VideometerLab to sensorial and microbial analyses from whole head-on-gutted Atlantic salmon. The experiment was divided into two trials, where, in the first trial, imaging was performed on ten fish by VideometerLite v.1 and VideometerLab, and Quality Index Method (QIM) was performed as sensorial analysis during an experimental period of 21 days (Figure 8). The second trial included imaging of 20 fish by VideometerLite v.1 along with QIM and microbial analyses for aerobic psychotrophic plate count (APPC), aerobic mesophilic plate count (AMPC), pseudomonas spp. (Psd) and lactic acid bacteria (LAB) during 17 days, of which the experimental setup is showed in Figure 9. Imaging in both trials were

conducted on the eyes, gills and three different regions of the skin. The extracted imaging data was then correlated to the experimental data by Pearsson correlation coefficients using SPSS statistics.

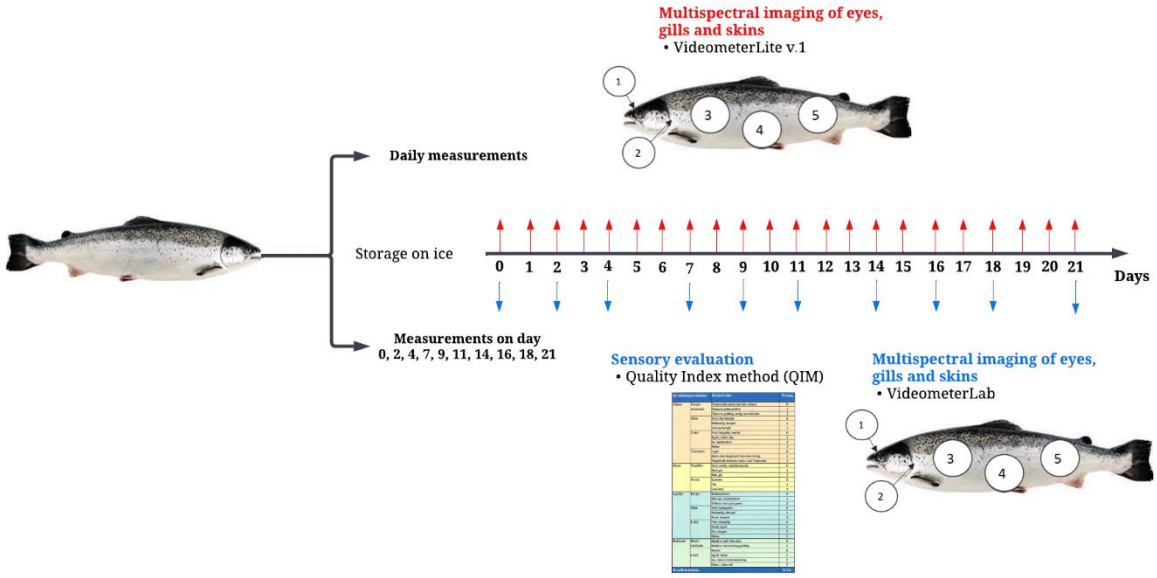


Figure 8. Experimental setup for Issues related to loss of freshness and microbial spoilage for whole head-on-gutted salmon (trial 1).

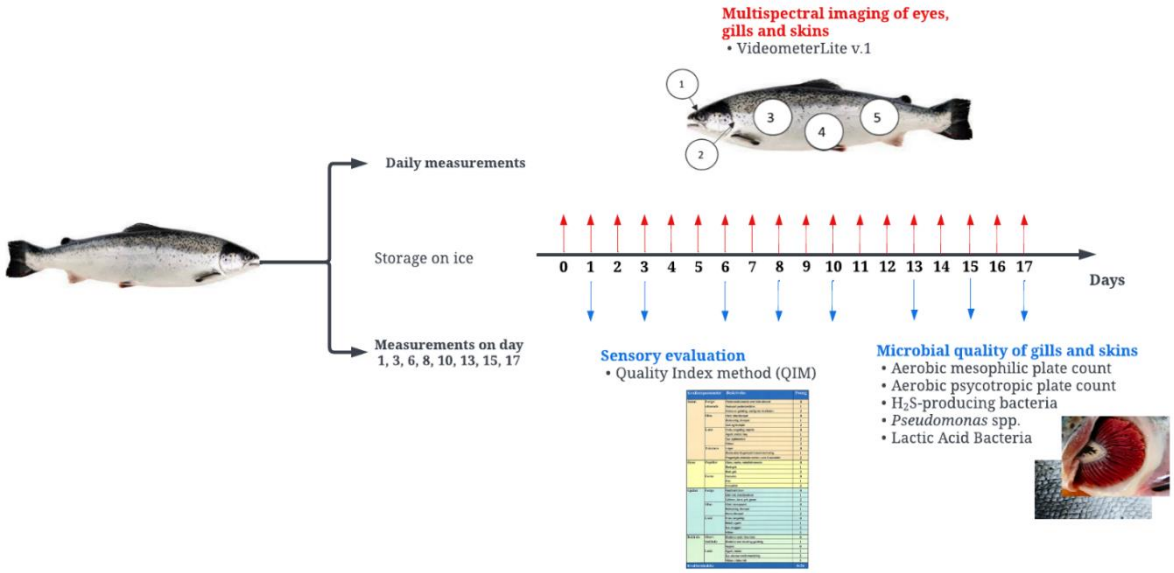


Figure 9. Experimental setup for Issues related to loss of freshness and microbial spoilage for whole head-on-gutted salmon (trial 2).

Figure 10 to 14 present correlation matrices containing correlation coefficients between the change in reflection data from the different wavelengths captured by VideometerLite and development in days, QIM score, and microbial data tested. The white circle shows significant results at the 0.05 level. The correlation coefficients are found between QIM and reflection data from wavelength 405 nm for

region 4 (r= -0.82, p<0.05) and 5 (r= -0.82, p<0.05). For eyes and skin region 3, no significant correlation was shown. However, reflection data from wavelength 850 nm show positive correlation to change in QIM score (r=0.59, p<0.05), growth of aerobic mesophilic bacteria (r=0.73, p<0.05) and H_2S-producing bacteria (r=0.6, p<0.05).

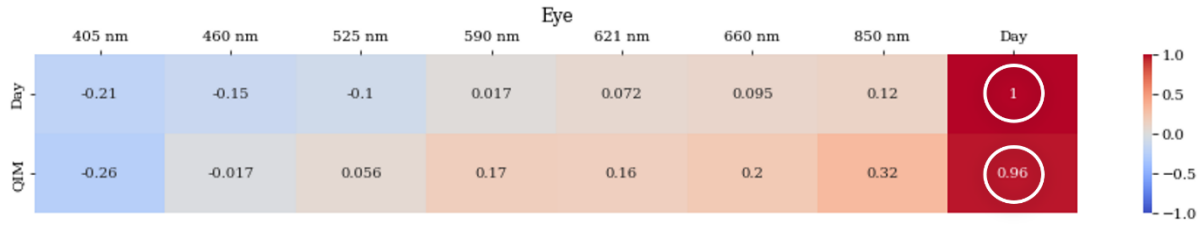


Figure 10. Heatmap with correlation values between reflection from imaging by VideometerLite v.1 and QIM.

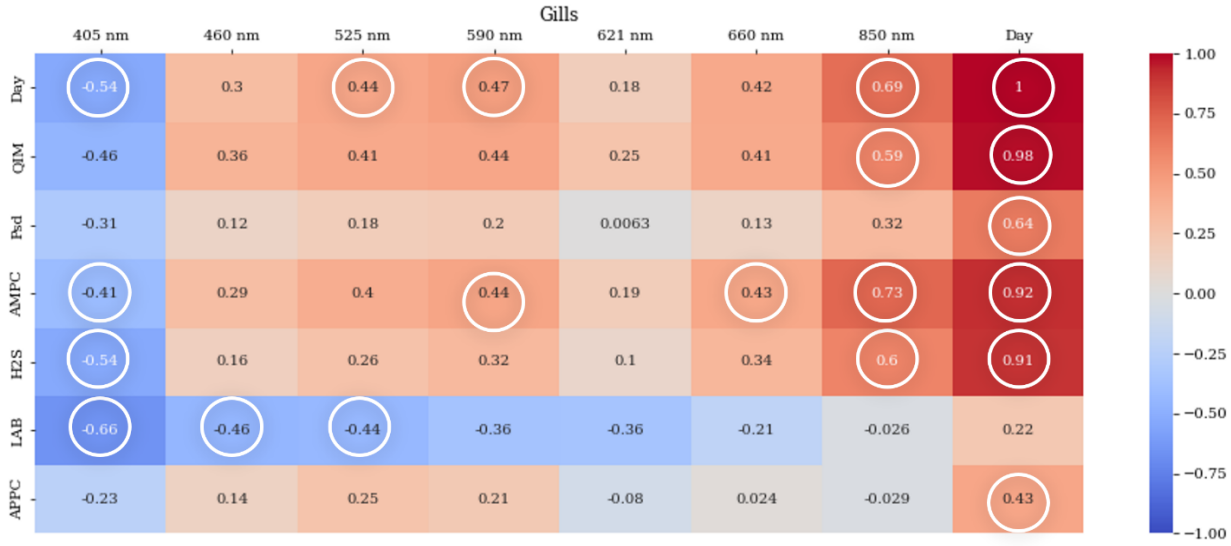


Figure 11 Heatmap with correlation coefficients between reflection from imaging of salmon gills by VideometerLite v.1, QIM and microbial analyses.

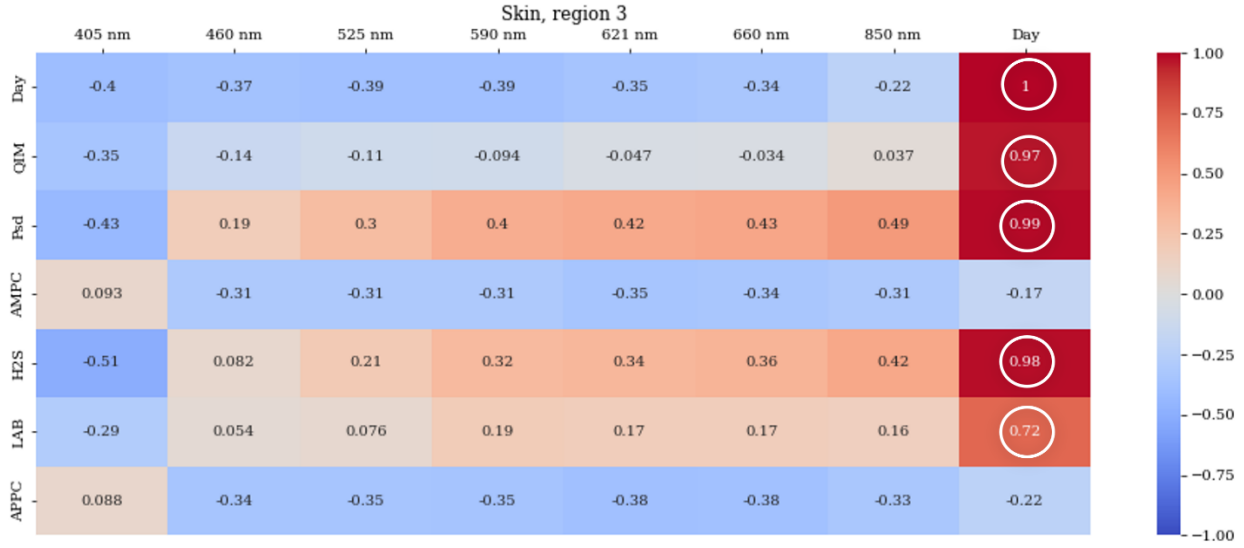


Figure 12 Heatmap with correlation coefficients between reflection from imaging of upper belly of salmon skin by VideometerLite v.1, QIM and microbial analyses.

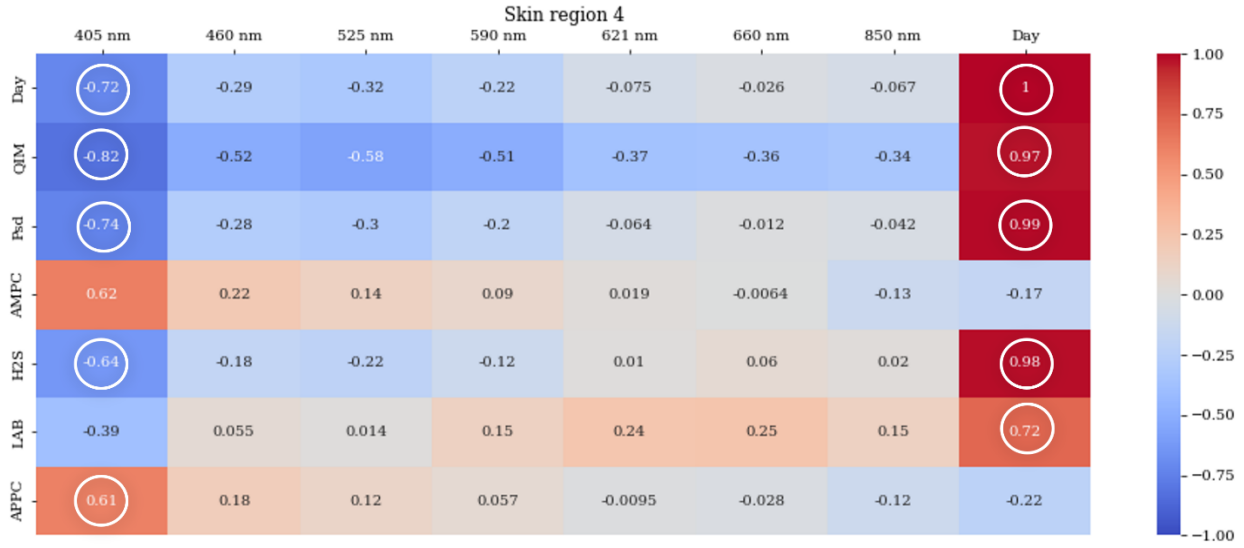


Figure 13 Heatmap with correlation coefficients between reflection from imaging of mid belly area of salmon skin by VideometerLite v.1, QIM and microbial analyses.

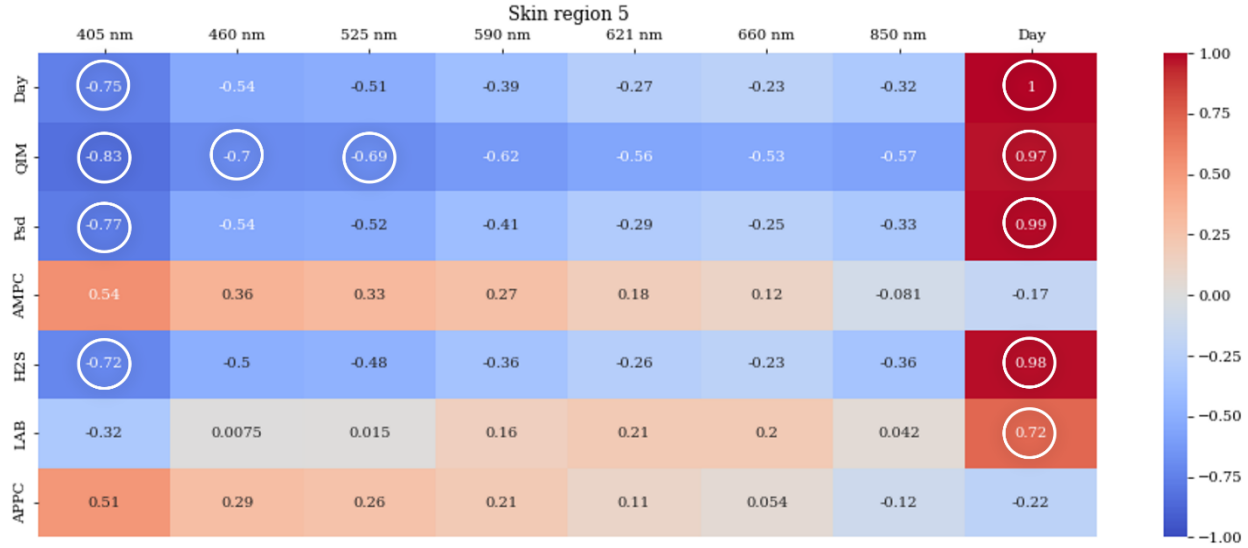


Figure 14 Heatmap with correlation coefficients between reflection from imaging of tail region of salmon skin by VideometerLite v.1, QIM and microbial analyses.

2.1.4 Issues related to loss of freshness and microbial spoilage on salmon fillets

When assessing the freshness of salmon fillets, changes in their physiochemical properties are considered in addition to microbial and sensorial changes. In this experiment, the aim was to link physiochemical parameters (ATP degradation, texture measurement, water holding capacity (WHC) and pH measurements) to imaging data from VideometerLab and VideometerLite v.2. The experiment was performed during a 20-days period, showed in the experimental setup in Figure 15. Then, SPSS statistics was used to determine Pearsson correlation coefficient to analyze the correlation between imaging data and experimental results.

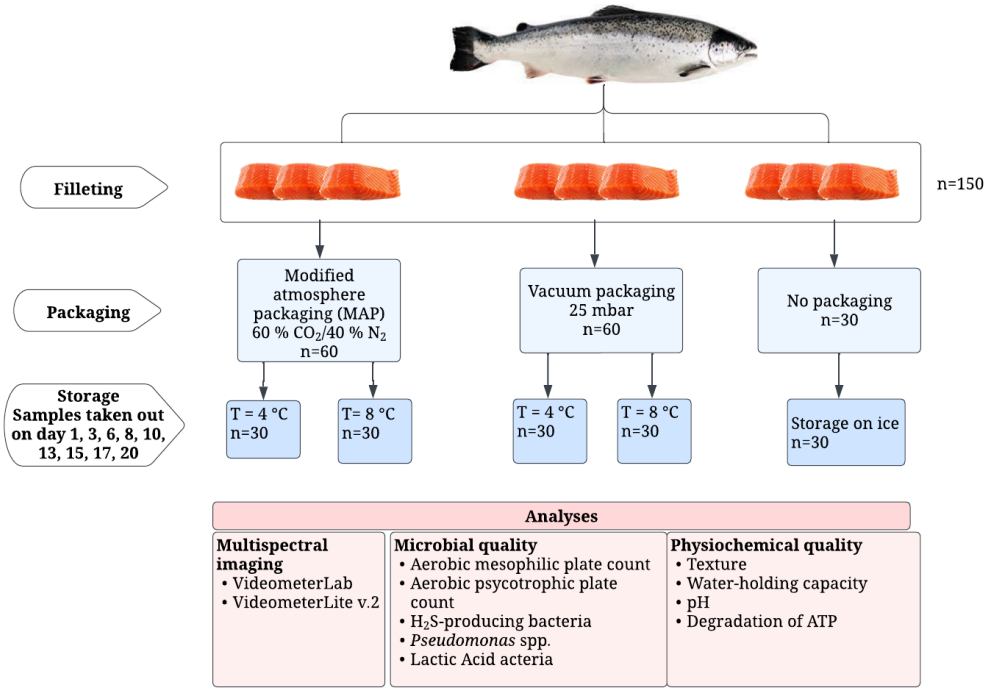


Figure 15. Experimental setup for the experiment related to loss of freshnes and microbial spoilage of salmon fillets.

Figure 16-20 presents heatmaps containing correlation values (r-values) between reflection data from the 13 distinct wavelengths captured by VideometerLite v.2 and the results from microbial and physiochemical analyses. Significant results at 0.05 level are marked with the white circles.

The heatmaps reveal that the highest correlation values are observed when salmon is stored on ice or vacuum packed. Generally, fillets that were stored at lower temperature had stronger correlations between reflection data and various quality attributes. The specific quality attributes that correlate depends on storage conditions.

For salmon fillets stored on ice (Figure 4), the strongest correlations are observed between reflection data obtained from wavelength 365 nm to 405 nm and the growth of *Pseudomonas* spp (r-values ranging from -0.84, all significant at $p<0.05$ level) as well as total aerobic plate count (APPC) (r-values ranging from -0.80 to 0.82 nm, all significant at $p<0.05$).

Salmon fillets packed in modified atmosphere and stored at 4 °C show the strongest correlation values between the texture parameter "Power 60 %" and reflection data obtained from wavelength 590 nm ($r= -0.63$, $p<0.05$) to 850 nm ($r= -0.74$. $p<0.05$). Quality parameters for MAP-packed fillets stored at 8 °C had few significant ($p>0.05$), and no strong r-values with change in reflection data.

For vacuum-packed salmon fillets stored at 4 °C, the strongest correlation values are found between changes in pH and reflection data from wavelengths 630 nm (r= -0.65, p<0.05) and 660 nm (r= -0.64, p<0.05).

In the case of vacuum-packed fillets stored at 8 °C, the strongest correlations are observed between water holding capacity and reflection data from wavelengths 490 nm (r = -0.70, p<0.05) and 515 nm (r= -0.71, p<0.05). Additionally, aerobic mesophilic bacteria had strong correlation to reflection from wavelength 365 (p= 0.61, 0.65 and 0.70 for wavelength 365_1, 365_2 and 365_ 3, respectively, all significant at p<0.05).

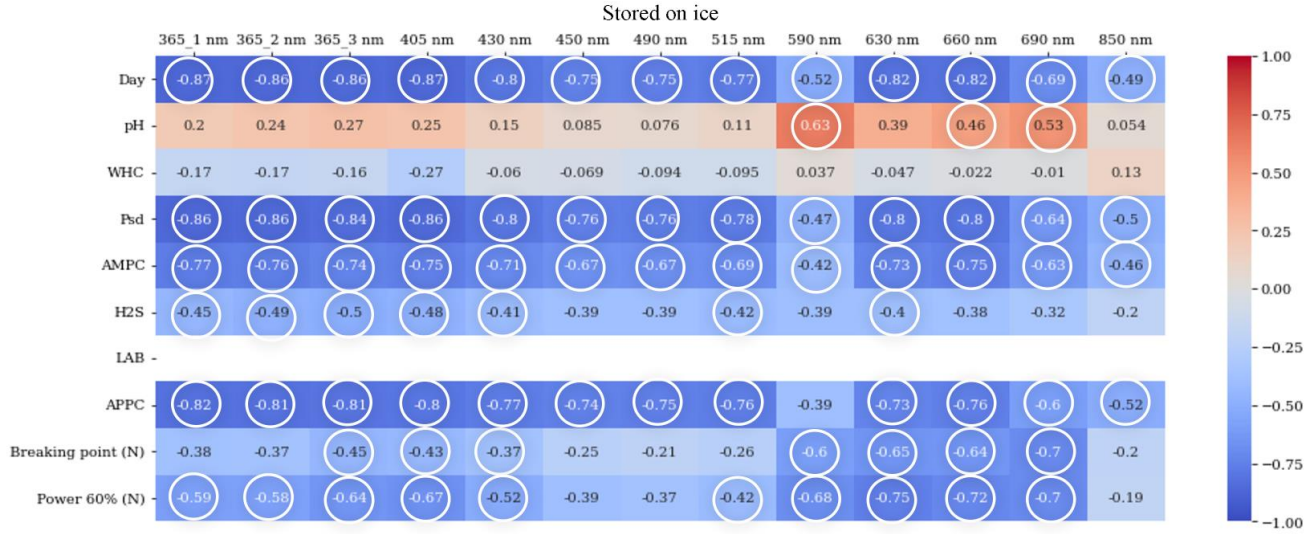


Figure 16 Heatmap with correlation coefficients between reflection from imaging of salmon fillets stored on ice by Videometer v.2 and data from physiochemical and microbial analyses.

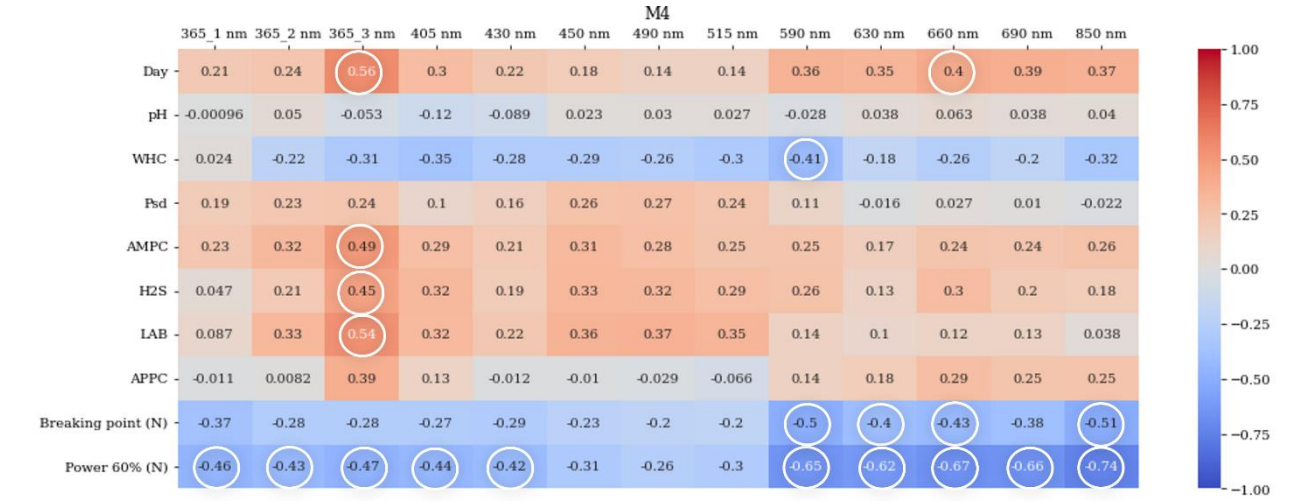


Figure 17 Heatmap with correlation coefficients between reflection from imaging of salmon fillets packed in modified atmosphere and stored at 4 °C by Videometer v.2 and data from physiochemical and microbial analyses.

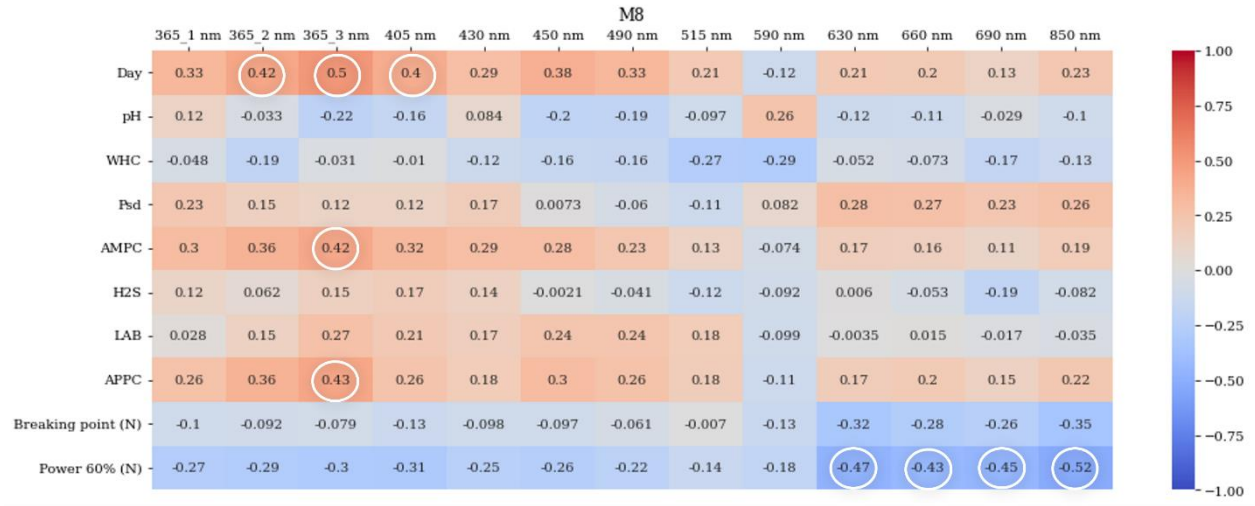


Figure 18 Heatmap with correlation coefficients between reflection from imaging of salmon fillets packed in modified atmosphere and stored at 8 °C by Videometer v.2 and data from physiochemical and microbial analyses.

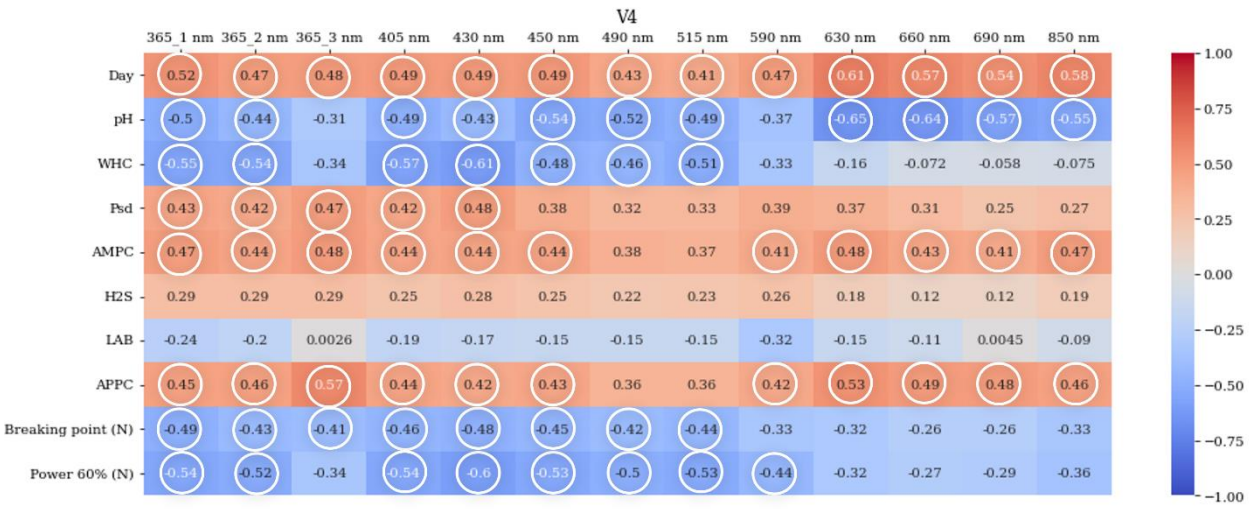


Figure 19 Heatmap with correlation coefficients between reflection from imaging of salmon fillets vacuum packed and stored at 4 °C by Videometer v.2 and data from physiochemical and microbial analyses

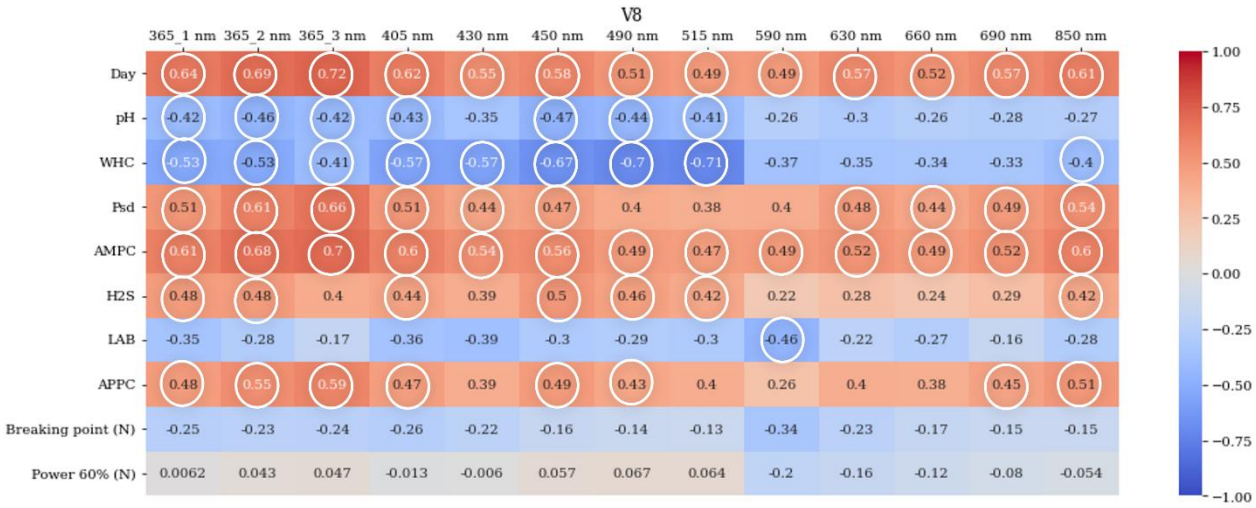


Figure 20 Heatmap with correlation coefficients between reflection from imaging of salmon fillets vacuum packed and stored at 8 °C by Videometer v.2 and data from physiochemical and microbial analyses.

2.1.5 Comparison of VideometerLite prototype v.1 and v.2

A smaller experiment was conducted to examine the differences between VideometerLite prototype v.1 and v.2. The two prototypes were used to capture images of five different salmon fillets throughout a period of 16 days. The reflection data between the images from VideometerLite v.1 and v.2 was compared using ANOVA.

The results showed no significant ($p>0.05$) difference between reflection data from any of the reflection data from the two prototypes (Figure 21). However, by summing the reflection values from all the wavelengths, the prototype v.2 gave significantly ($p<0.001$) lower reflection values than from the prototype v.1.

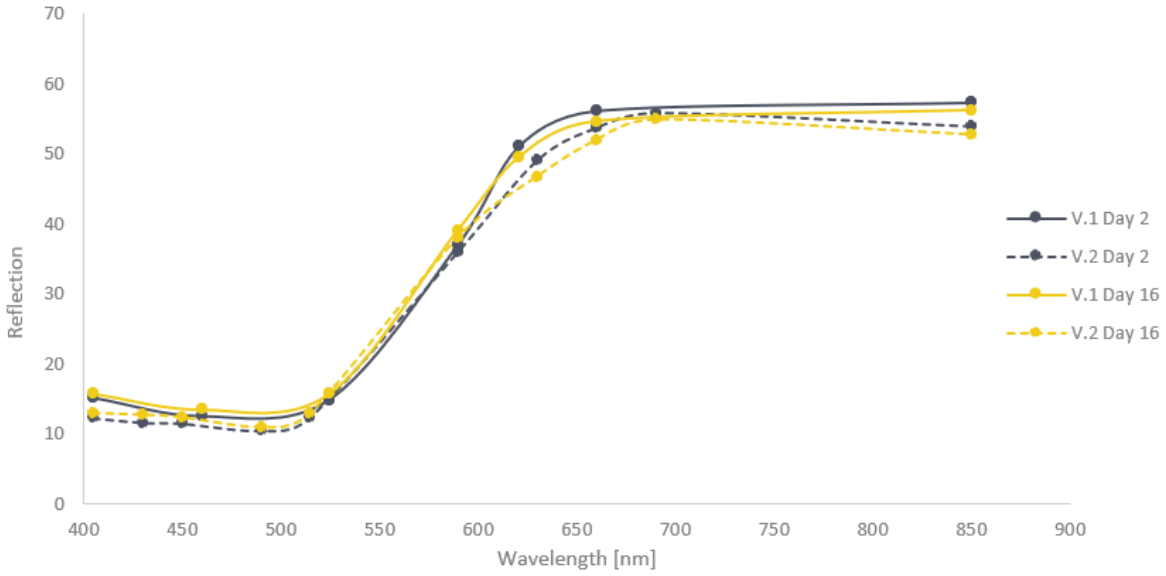


Figure 21. Reflection data from images of salmon fillets captured by VideometerLite v.1 and v.2.

2.2 CONCLUSION

The experiments performed in the Salmon pilot cover various stages in the Atlantic salmon value chain. The results showed that, by utilizing specific wavelengths and areas of the fish, the VideometerLite offers an easy and applicable method for detecting factors indicating quality changes in the stages of the Atlantic salmon production chain. The VideometerLite can detect melanin spots and detect differences between fillet firmnesses. In the whole salmon value chain, reflection data from wavelength 405 nm was most relevant since correlation was observed between the wavelength, microbial spoilage and change in QIM score. For the fillet experiment, the imaging data from VideometerLite showed that, depending on storage conditions, reflection data correlated with different quality changes. To further predict quality changes, the data processing procedures for gills and eyes have potential for refinement to obtain a standardized method for data processing.

2.3 PLAN FOR PUBLICATIONS

Two papers are planned to be submitted during December 2023. The papers are related to the storage experiments and the preliminary titles are "The potential of multispectral imaging for quality monitoring of head-on-gutted Atlantic salmon (*Salmo salar)*" and "Multispectral imaging as a tool for quality assessment in salmon fillet". Suggested journals for publishing are Food control and Food research international.

3 THE ATLANTIC WHITEFISH PILOT

The Atlantic whitefish pilot included evaluation of quality parameters throughout the value chain. These parameters relate to hazards identified and defined in D2.1. The aim of the pilot is to implement VIDEOM analysis into these evaluations making them with the aim of making them more accessible, reliable and traceable. The pilot trials include the whole value chain from whole fish to processing and storage stability relevant to commercial environments. Experiments were performed in a controlled laboratory environment as well as in living lab pilot environments mimicking true industrial conditions. For detailed descriptions of experimental design in the pilot refer to D6.2. The following trials were performed:

1. Assessment of fish freshness through evaluation of eyes, gills and skin (comparison to the Quality Index Method, QIM)
2. Nematode detection and identification.
3. Assessment of texture and freshness of final products

3.1 FRESHNESS ASSESSMENT THROUGH EVALUATION OF EYES, GILLS AND SKIN (QIM)

The appearance of fish changes during storage as it spoils. One of the methods that can be used to evaluate freshness of whole fish is the Quality Index Method (QIM). It entails a physical evaluation by a trained panel, scoring specific characteristics of the fish to procure a Quality Index for the fish. Table 1**Error! Reference source not found.** shows the QIM scale with descriptions of the changes being evaluated. As mentioned, the QIM method requires a trained sensory panel and is therefore not available to the common consumer or whole-sale stakeholder. Providing a fast, and reliable freshness assessment of whole fish would therefore be of great value to these stakeholder groups.

Table 1 The Quality Index Method (QIM) scheme for Atlantic cod (*Gadus morhua*) (Marteinsdóttir et.al., 2001)

Quality parameter		Description	Score
Appearance	Skin	Bright, iridiscent pigmentation	0
		Rather dull, becoming discoloured	1
		Dull	2
	Stiffness	In rigor	0
		Firm elastic	1
		Soft	2
		Very soft	3
Eye	Cornea	Clear	0
		Opalescent	1
		Milky	2
	Form	Convex	0
		Flat, slightly sunken	1
		Sunken, concave	2
	Pupil	Black	0
		Opaque	1
		Grey	2
Gills	Colour	Bright	0
		Less coloured, becoming discoloured	1
		Discoloured, brown spots	0
		Brown, discoloured	3
	Odour	Fresh, seaweedy, metallic	0
		Neutral, grassy, musty	1
		Yeast, bread, beer, sour milk	2
		Acetic acid, sulphuric, very sour	3
	Mucus	Clear	0
		Milky	1
		Milky, dark, opaque	2
Blood	Colour	Red	0
		Dark red	1
		Brown	2
Flesh, fillet	Colour	Translucent, bluish	0
		Waxy, milky	1
		Opaque, yellow, brown spots	2
		Total score	**(0-23)**

A pre-trial was performed in January 2023 to try to evaluate the possible challenges that could arise during imaging and analysing of the images. The results of the preliminary trial revealed a difference in the spectral response of the iris of the Atlantic cod eye throughout storage, mostly on the visual range of the spectra.

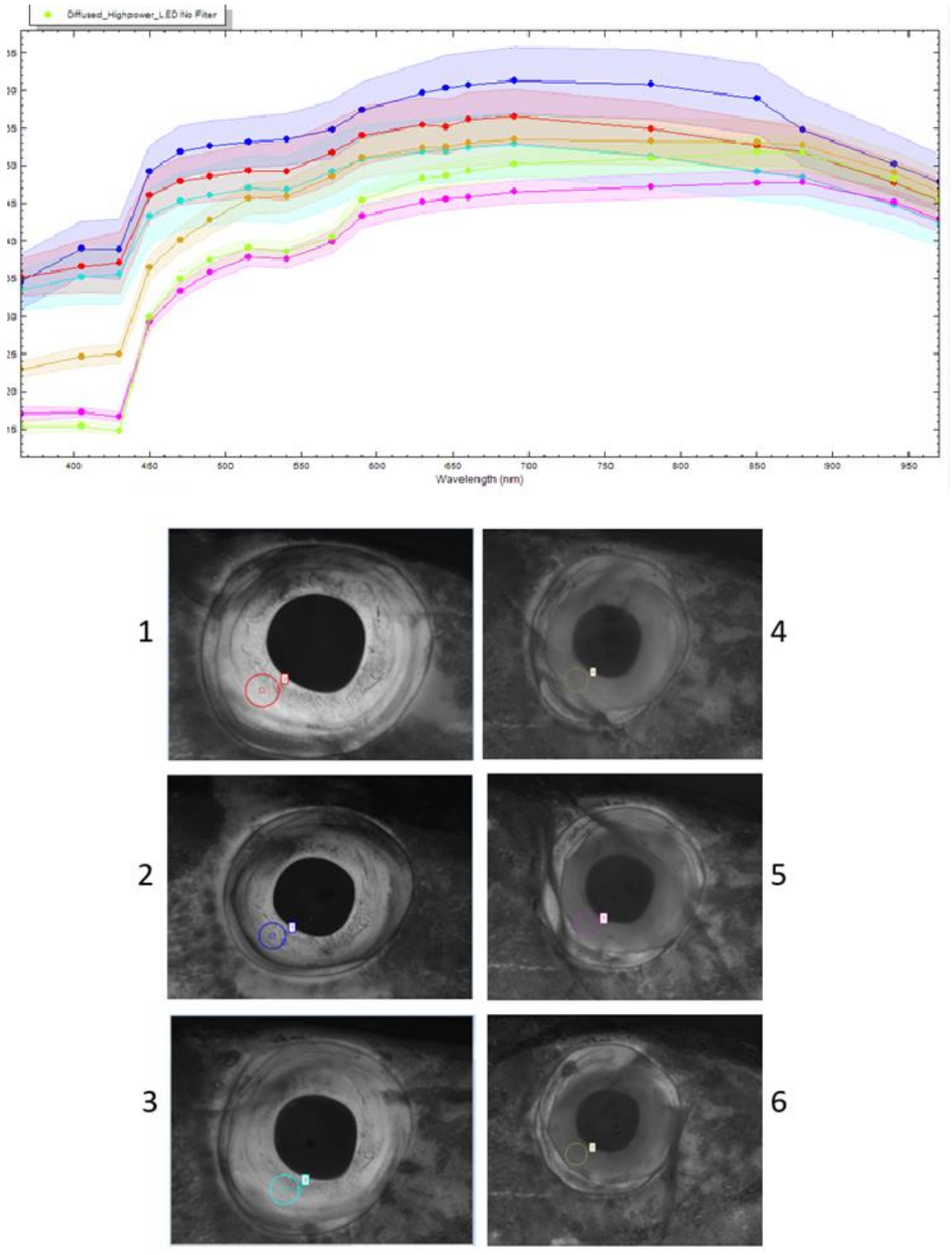


Figure 22 Results of multispectral imaging of a marked area of interest on Atlantic cod (Gadus morhua) eye throughout fresh storage and the areas spectral response.

Following the pre-trial, in June 2023 a large scale trial was performed evaluating multiple fish over the duration of the storage time (16 days) using QIM analysis and imaging using the VideometerLab 4 and VideometerLite 2. Partial Least Square (PLS) regression was performed using Unscramlber (v 11.0) on the spectral data from 4

different chosen areas, the fish eye, gills and 2 parts of the skin (Figure 23). PLS regression was performed following a baseline correction of the spectral data.

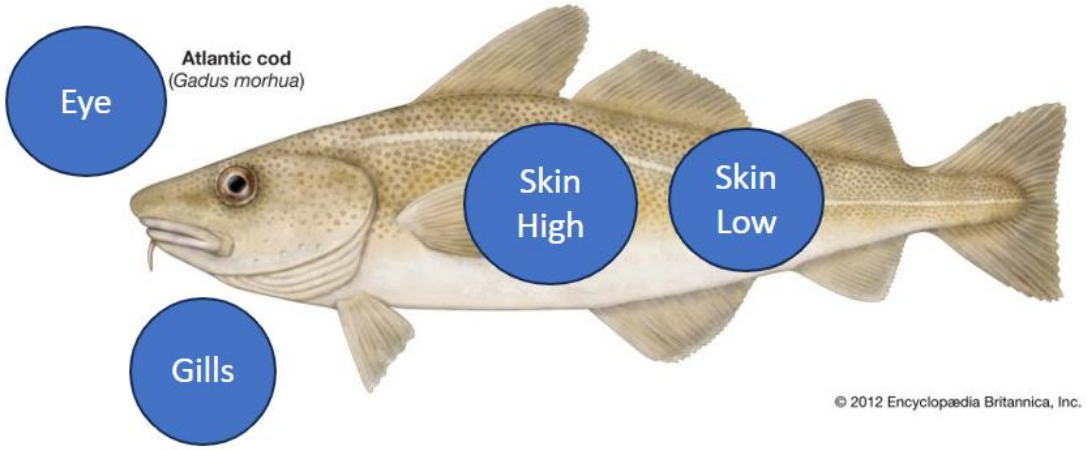


Figure 23 Imaging locations on gutted Atlantic cod during QIM trial

Results of the PLS analysis can be viewed in more detail in the following figures (Figure 24-Figure 31) but for ease of comparison some results are also presented in Table 2. These results show some difference in results between the different Videometer devices though both can provide fairly good predictions of a samples QIM score based on an image. In this analysis only a test set was used, no validation data set is presented in the table but that work will be made available in a pending publication.

Table 2 Results (R^2) of PLS analysis of spectral data from QIM trial

Imaging location	R^2 (VideometerLab 4)	R^2 (VideometerLite 2)
Eyes	0.59	0.77
Gills	0.79	0.76
Skin high	0.74	0.54
Skin low	0.81	0.70

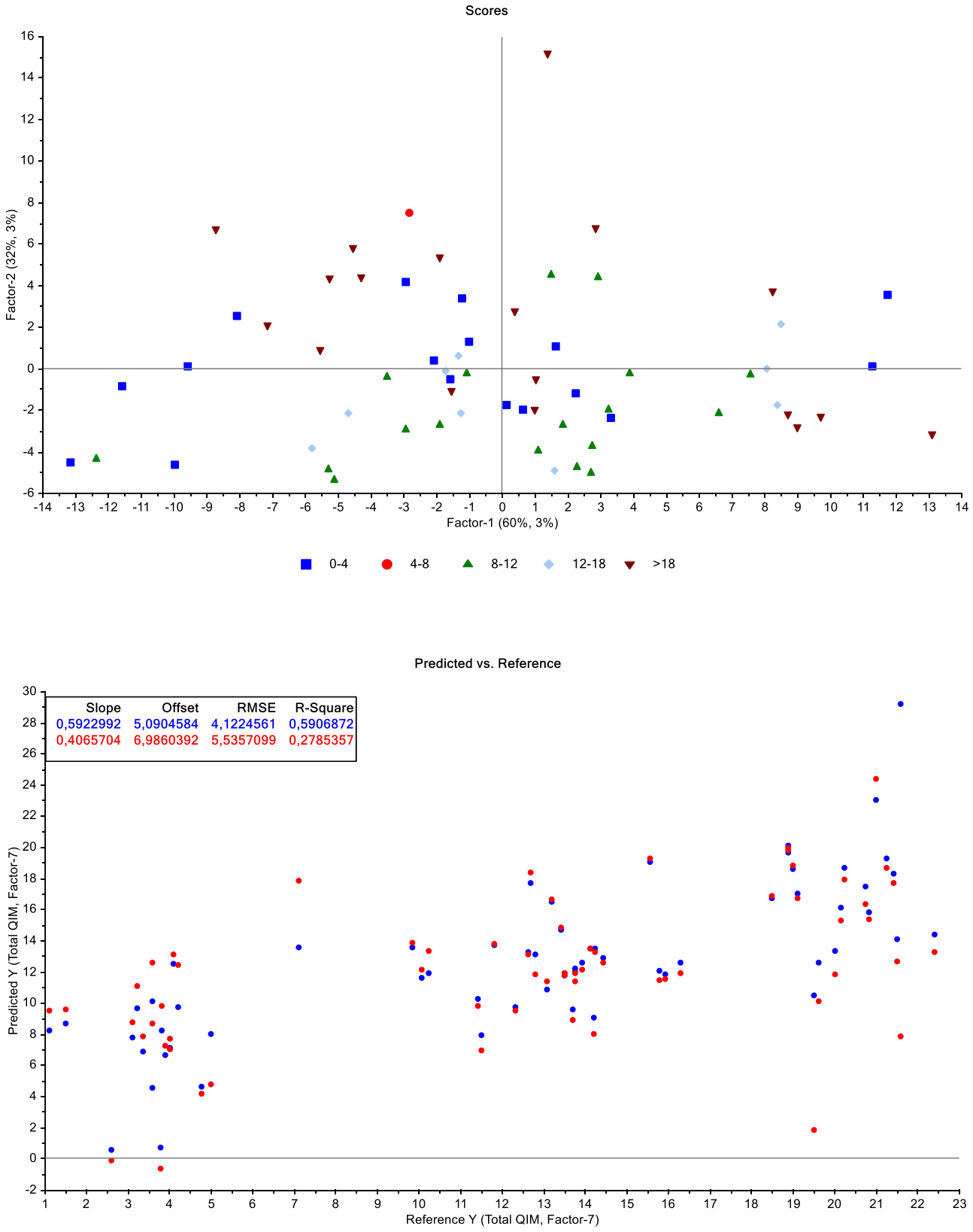


Figure 24 Results of PLS analysis of spectra from images from the VideometerLab 4 of cod eyes for the prediction of samples QIM score

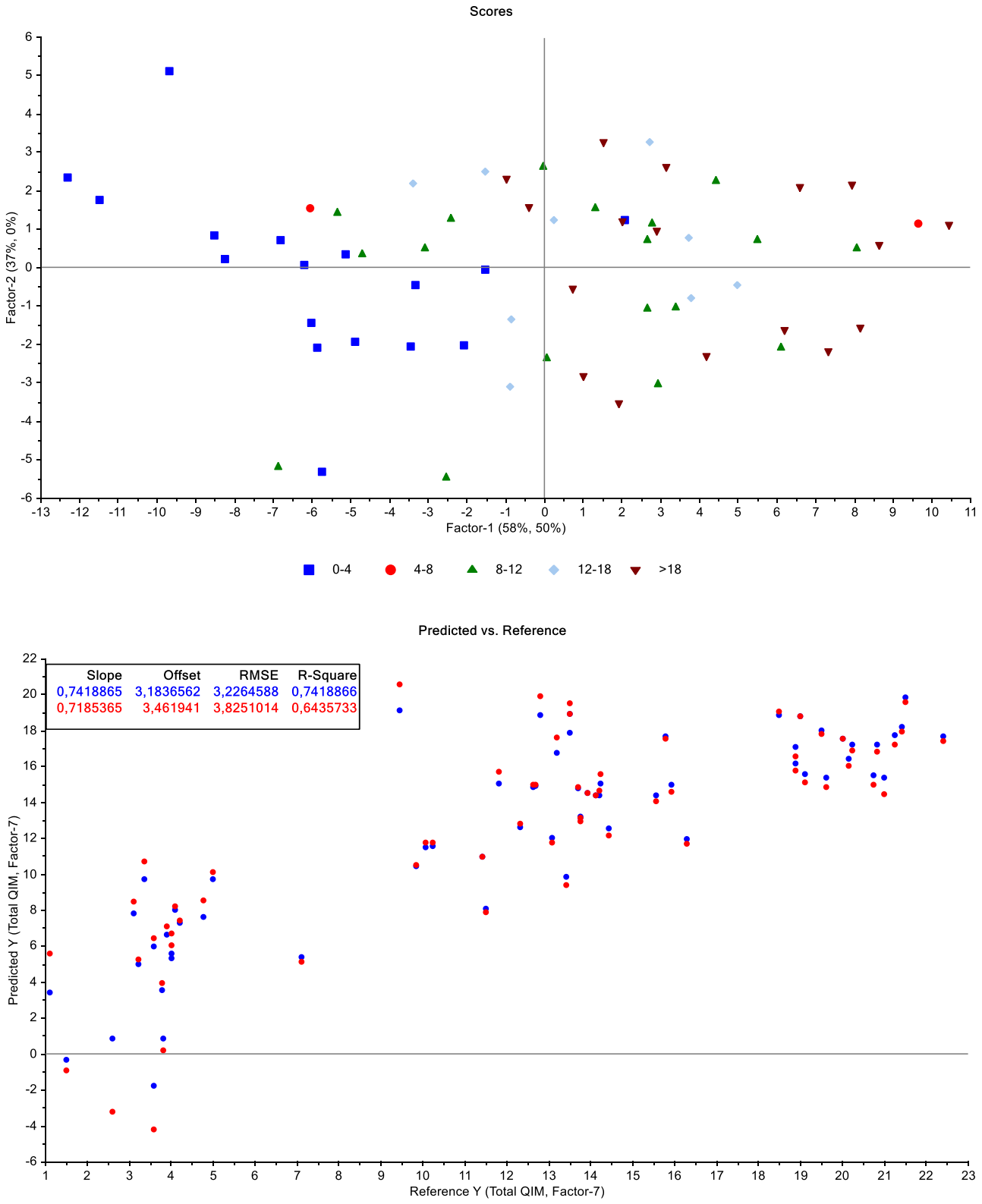


Figure 25 Results of PLS analysis of spectra from images from the VideometerLab 4 of cod skin (high) for the prediction of samples QIM score

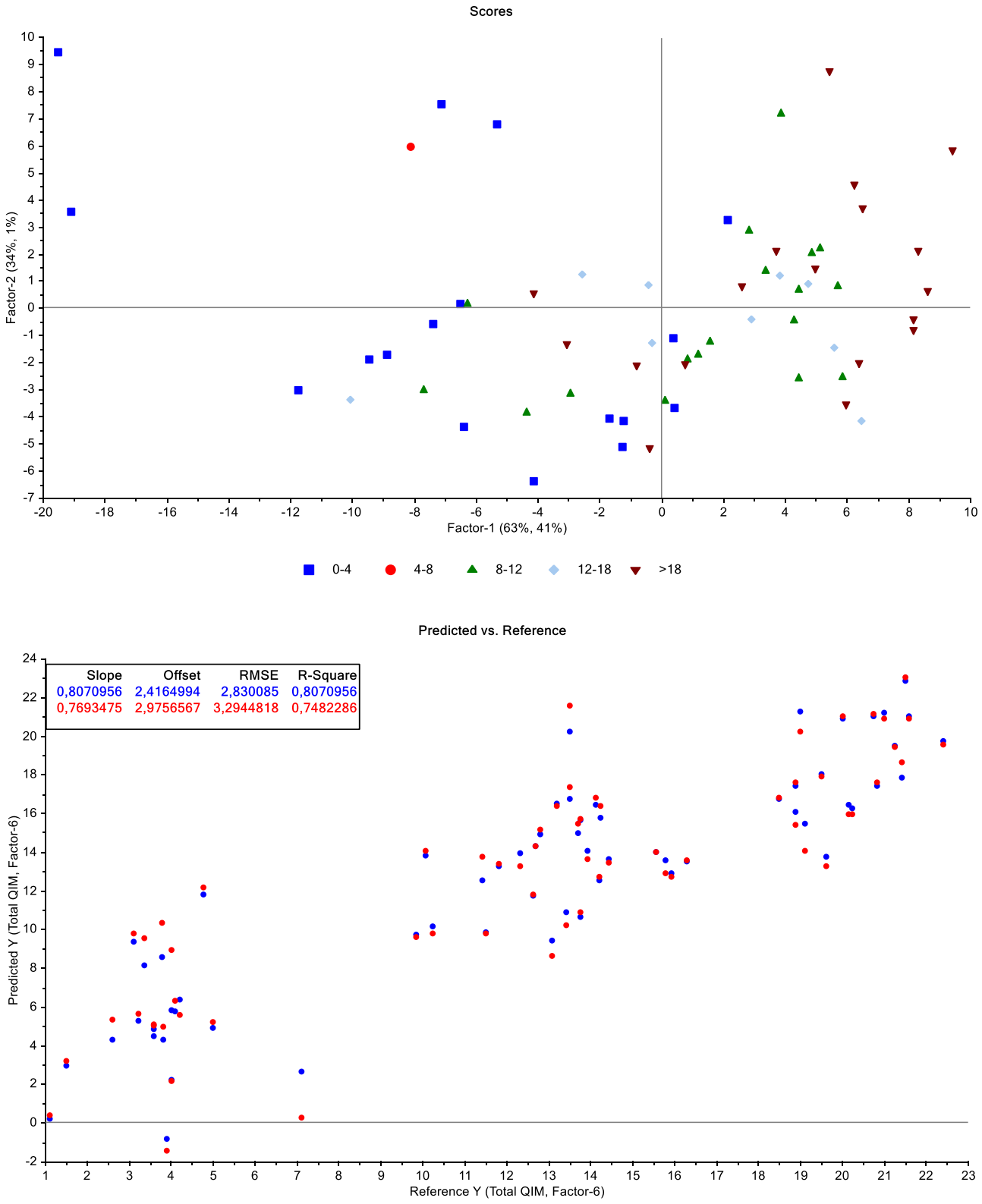
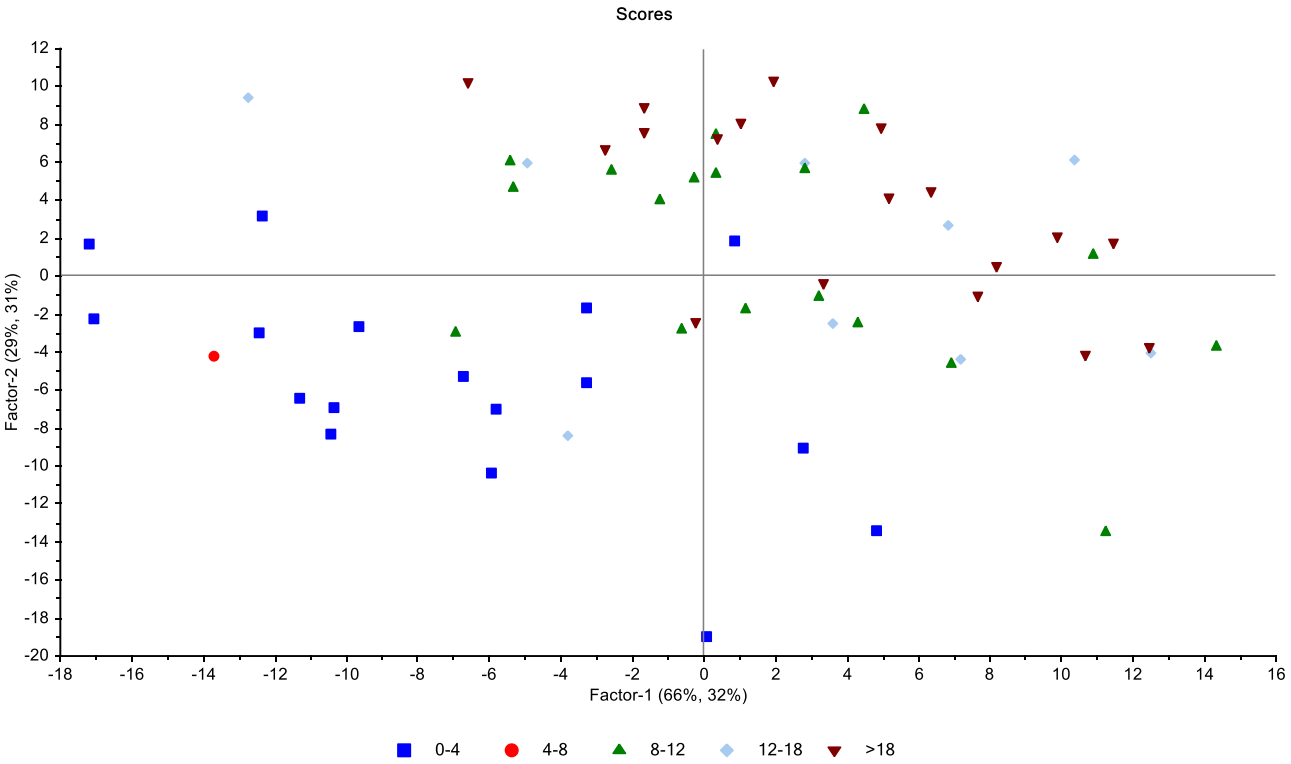


Figure 26 Results of PLS analysis of spectra from images from the VideometerLab 4 of cod skin (low) for the prediction of samples QIM score



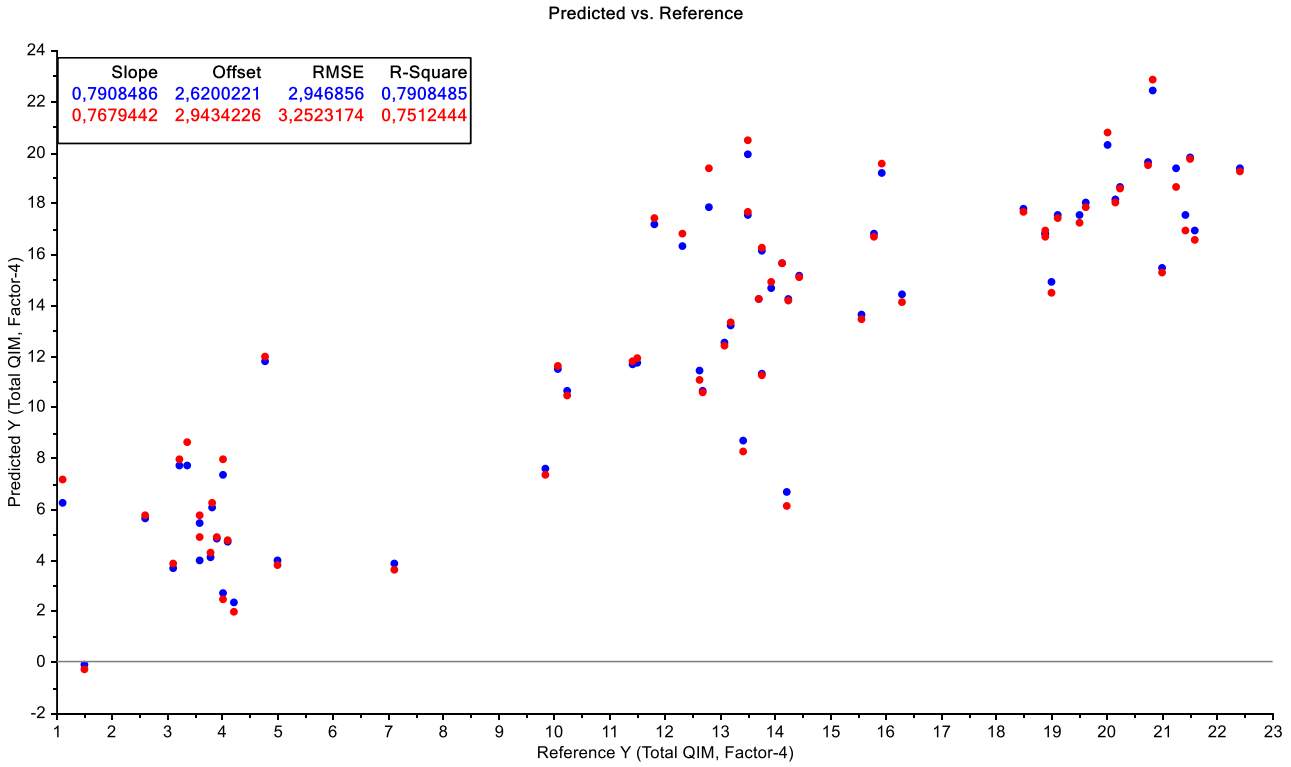


Figure 27 Results of PLS analysis of spectra from images from the VideometerLab 4 of cod gills for the prediction of samples QIM score

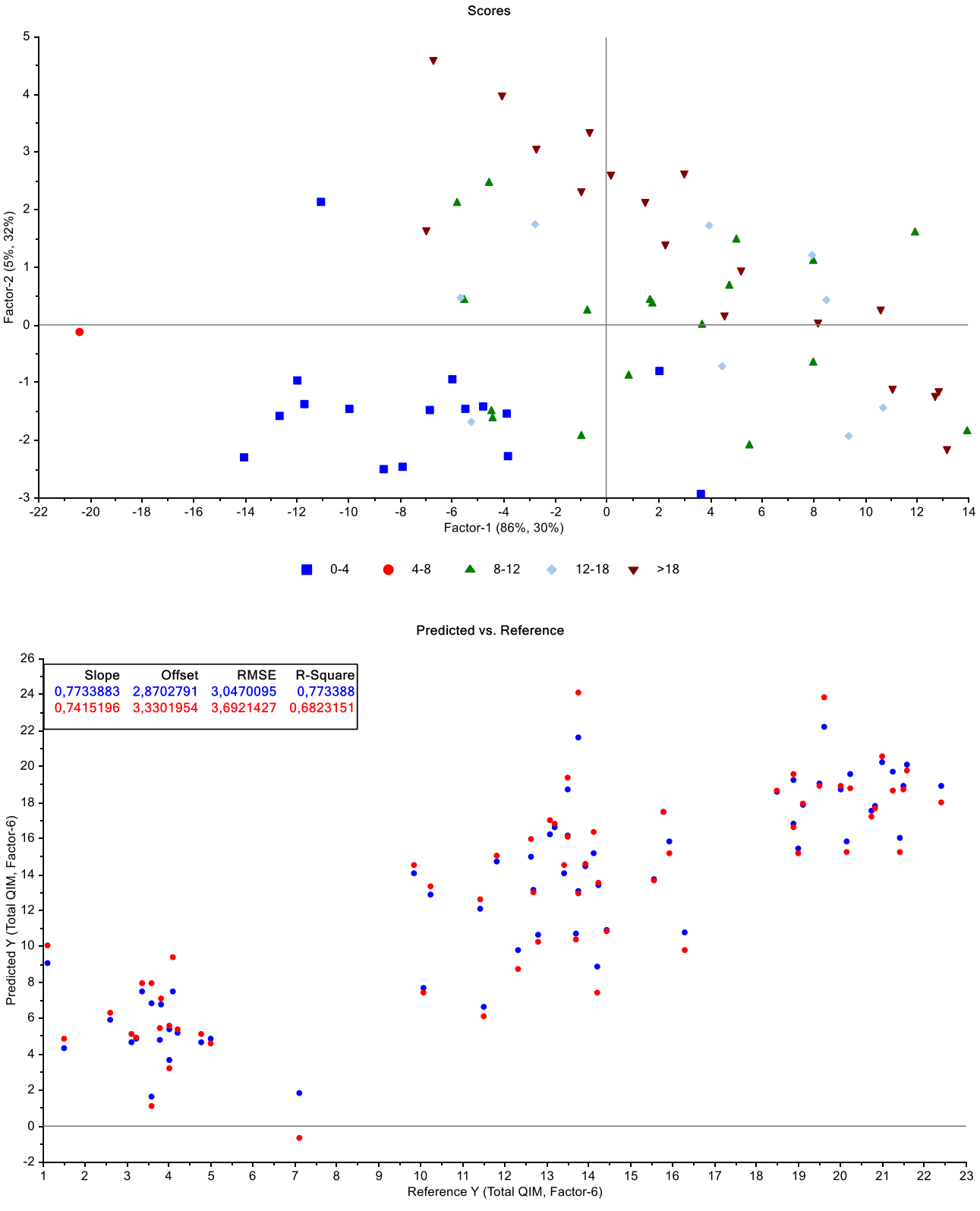


Figure 28 Results of PLS analysis of spectra from images from the VideometerLite 2 of cod eyes for the prediction of samples QIM score

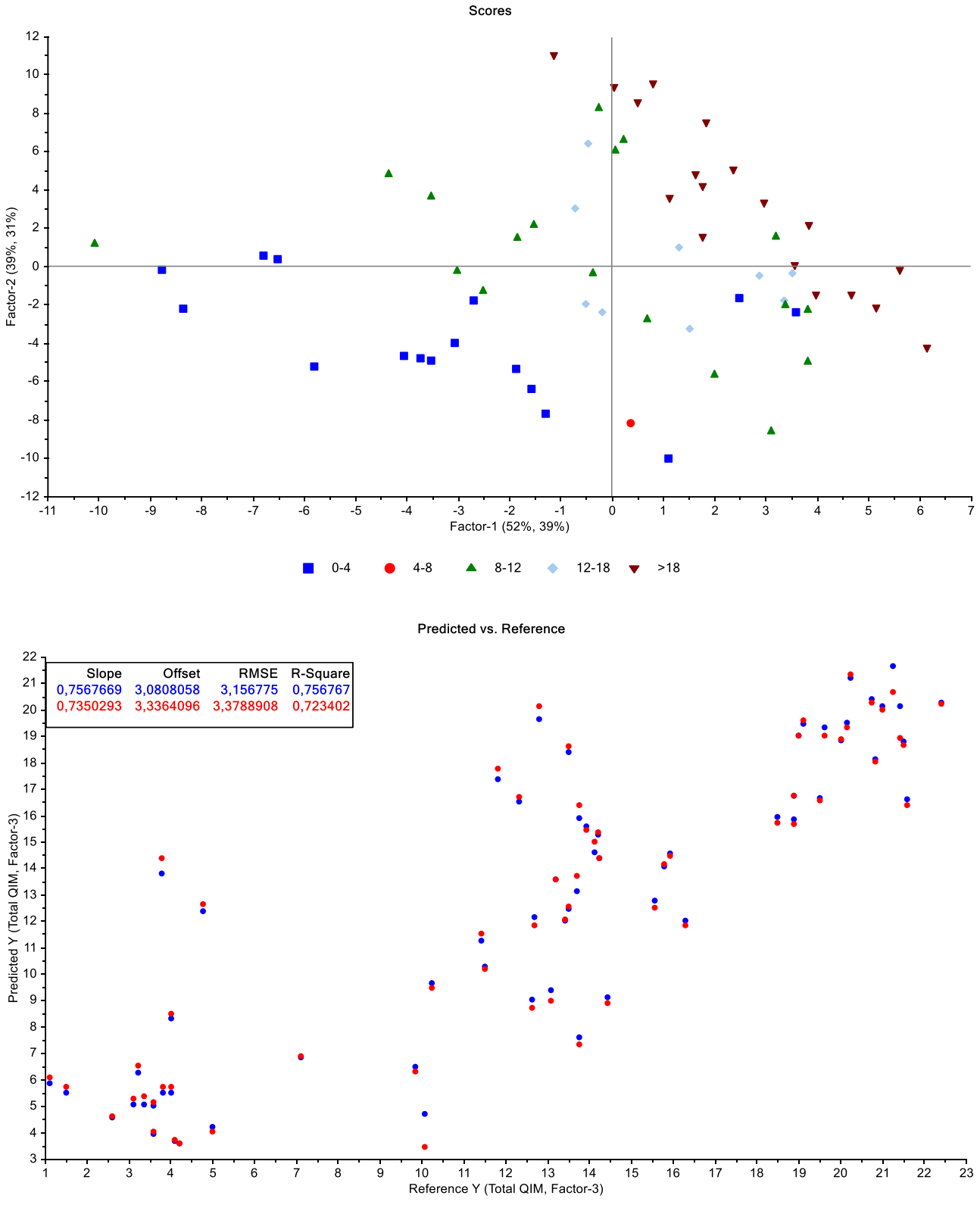


Figure 29 Results of PLS analysis of spectra from images from the VideometerLite 2 of cod gills for the prediction of samples QIM score

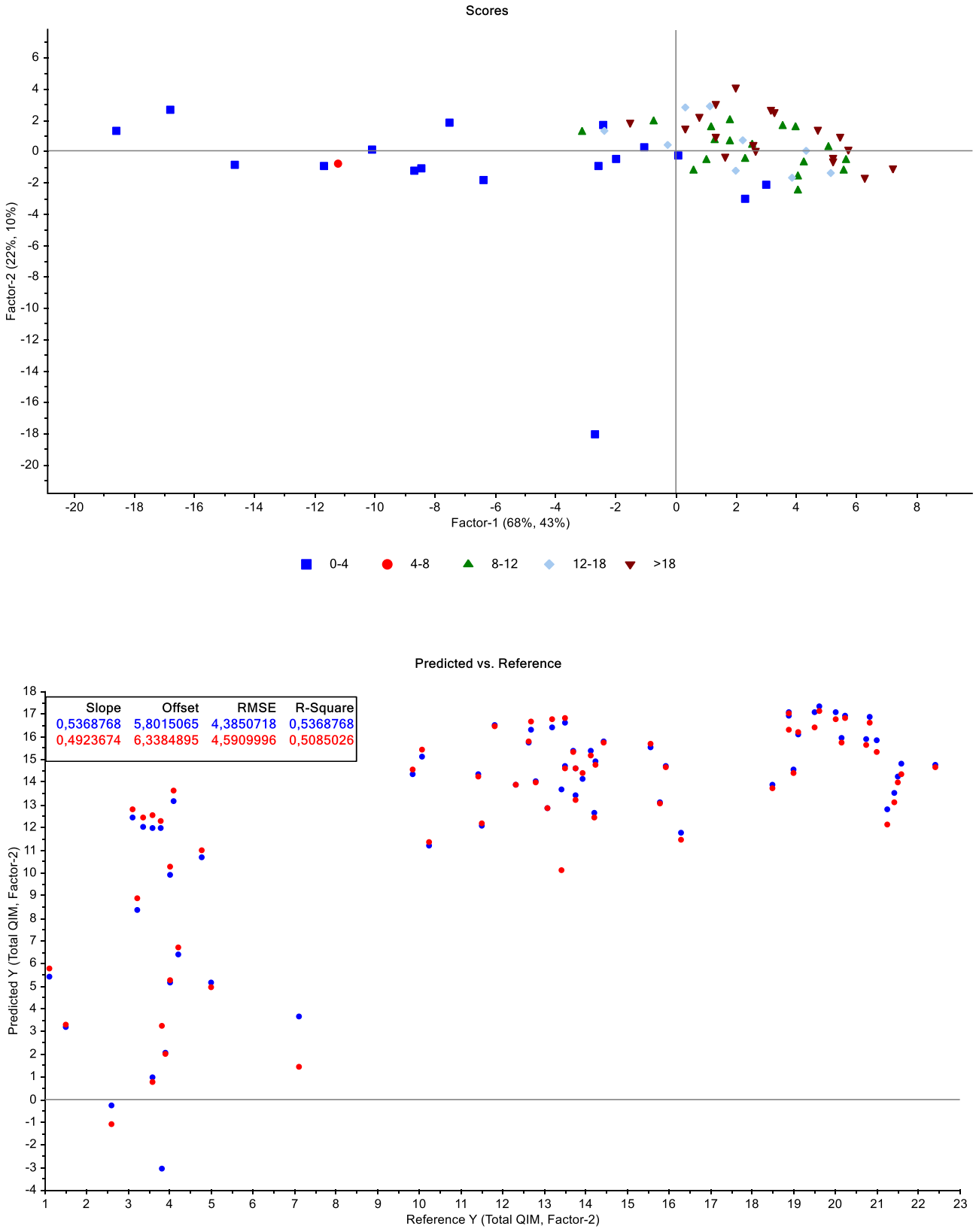


Figure 30 Results of PLS analysis of spectra from images from the VideometerLite 2 of cod skin (high) for the prediction of samples QIM score

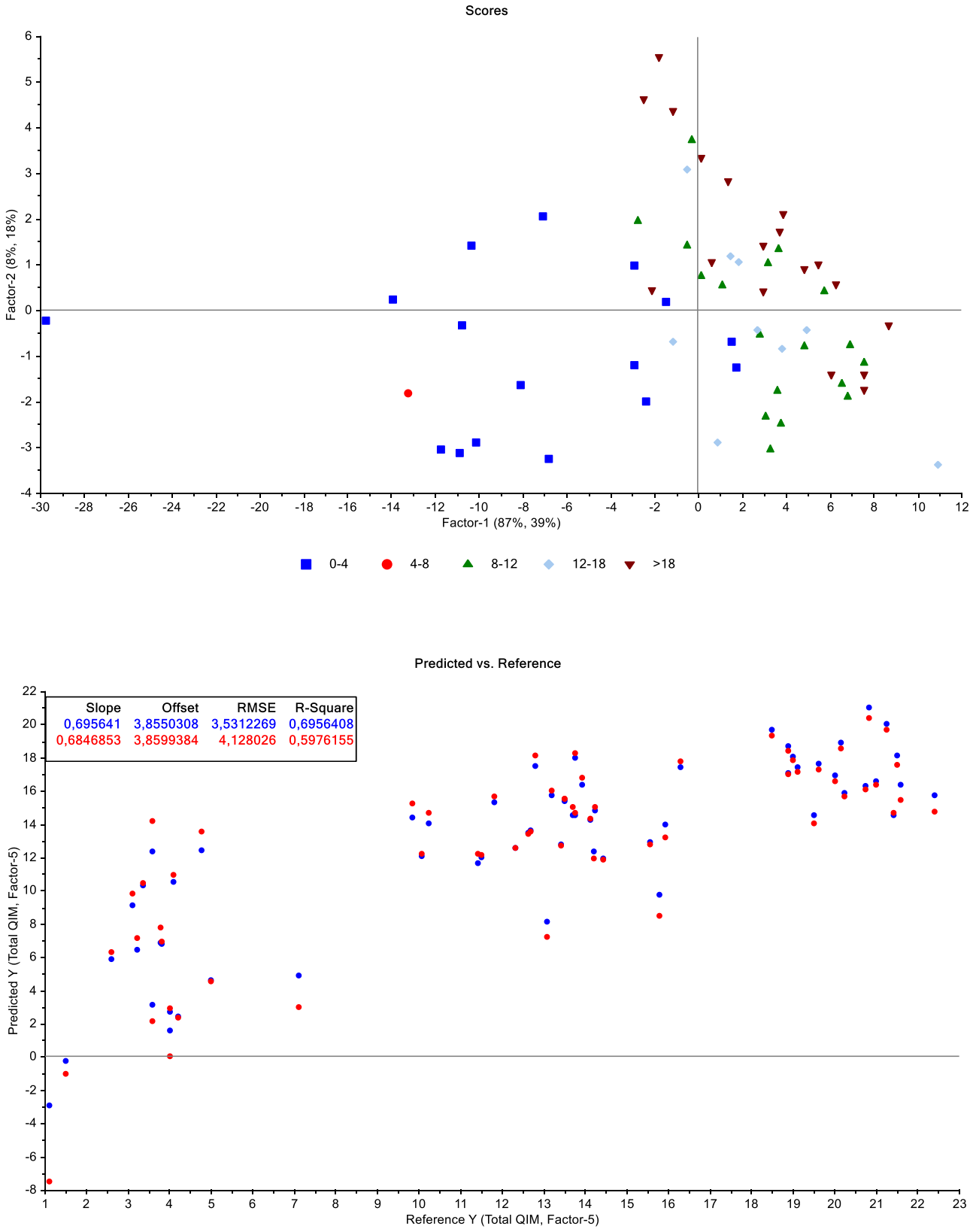


Figure 31 Results of PLS analysis of spectra from images from the VideometerLite 2 of cod skin (low) for the prediction of samples QIM score

3.2 NEMATODE DETECTION

Nematode detection is of great importance in the whitefish processing industry. The parasites can pose a health risk to consumers if fish is not properly cooked. Further, the parasite can have a repelling effect on the consumer both in stores/markets or in the home when the fish is cooked or consumed.

3.2.1 Classification

The primary trials focus was possible detection and an evaluation of the depth at which the worms could be detected into the flesh (Figure 32). These trials, therefore, are considered classification models aiming to classify a piece of fish as having or not having nematodes present excluding information about its or their location. Before imaging the samples the fillets were cut into appropriately sized portions for imaging. Images were procured and analysed to determine accuracy of detection. Classification of nematodes was evaluated using different methodologies (CLIP and Res-Net-50) and CLIP provided higher accuracy in detection and classification of visible nematodes (around 80%).

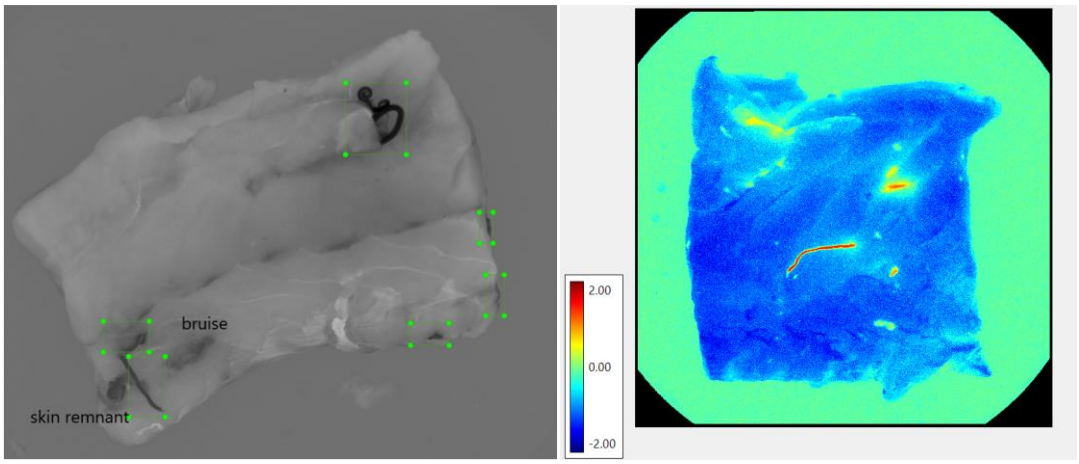


Figure 32 Left: Example of a labelled image with six nematode labels. A skin remnant and a bruise can be seen in the image as well. Right: An nCDA transformation on a random image in the data set. The red colour implies that those pixels are nematodes, and the blue implies that those pixels are fish muscle. Yellow pixels represent neutral areas

Further evaluations of the sensitivity of detection were conducted and the spectral response shows that identifying dark nematodes is possible down to 5-7 mm (Figure 33).

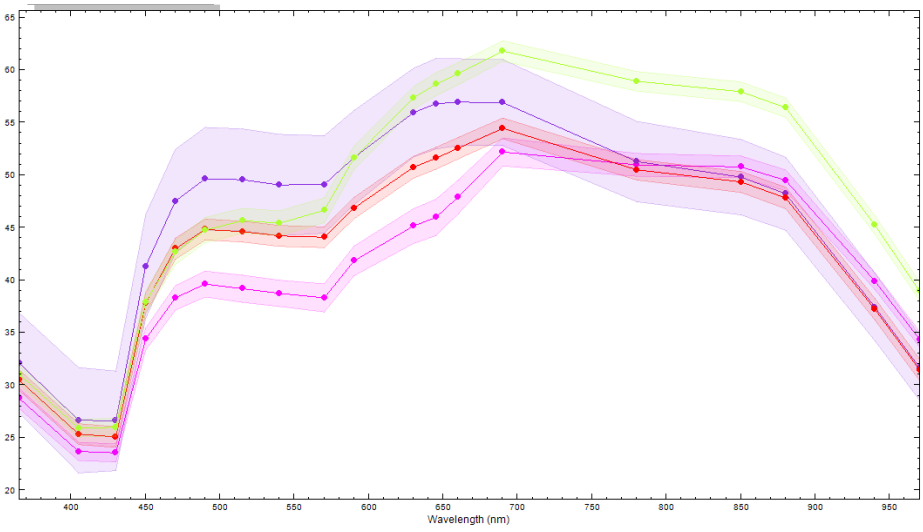


Figure 33 Spectral signature of nematodes of different colour and depth compared to fish muscle. Fish muscle (purple); Dark nematode at 7_1 mm depth (red); Dark nematode at 5_1 mm depth (fuchsia); Light yellow nematode at 5_1 mm (green).

In the white fish value chain, like most others, it is important that when a technology is used to identify hazards or parameters of importance such as nematodes all false responses are problematic but false negative responses provide a greater risk than false positive. Therefore, limiting the likelihood of false negative classifications is of utmost importance. Using the described CLIP model on data collected in the industry trial, the second trial, false negative classification occurred in 8.6% of the images (Figure 34). Ongoing analysis will take this into consideration and aim to limit this even further.

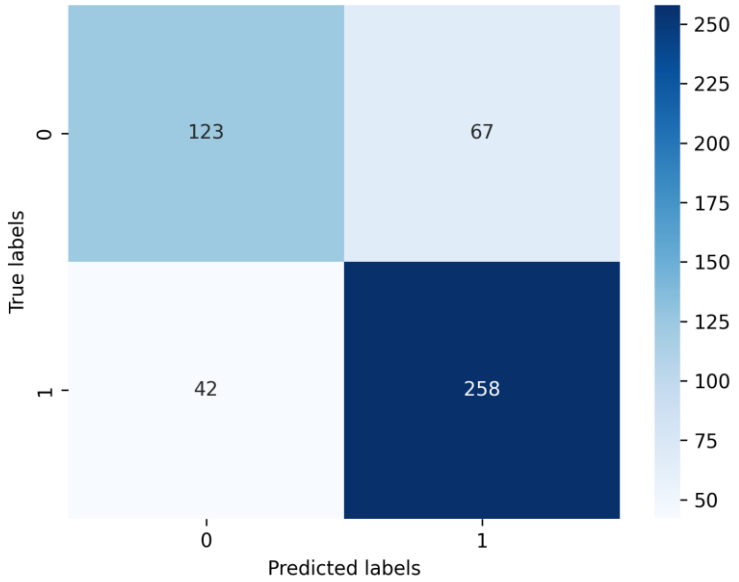


Figure 34 Results of CLIP classification model from second nematode trial. Classification 1 = nematode present, 0 = no nematode present).

3.2.2 Automated detection and location

Further data analysis performed focused on not only classifying images as containing or not containing nematodes but to identify and pinpointing the location of the nematodes, evaluating how many are present in the imaged piece. This analysis was performed on the same dataset described in chapter 3.2.1, consisting of 270 images, from the VideometerLab 4, from 50 different fillets.

Data analysis, again, included normalized Canonical Discriminant Analysis, or nCDA, to distinguish different components within the fish fillets. nCDA is a supervised model based on MSI transformation of the images that determines how to best discriminate between two or more groups of individuals. Two segmentation models using nCDA were made, model A, were nematodes are to be distinguished from fish muscle and blood. Model B, distinguishes skin remnants from every other component present in the fillet, this includes fish muscle, blood, and nematodes. By incorporating multiple segmentation models and exploiting their collective strengths, where individual models may capture certain characteristics within the fish fillet being segmented, the sequence segmentation gives opportunity to be able to detect nematodes. Figure 35 shows on the right an image from the dataset. The blue layer shows predictions from model A, here we can see six areas predicted as nematodes. The orange layer shows predictions from model B, it predicts one area as skin.

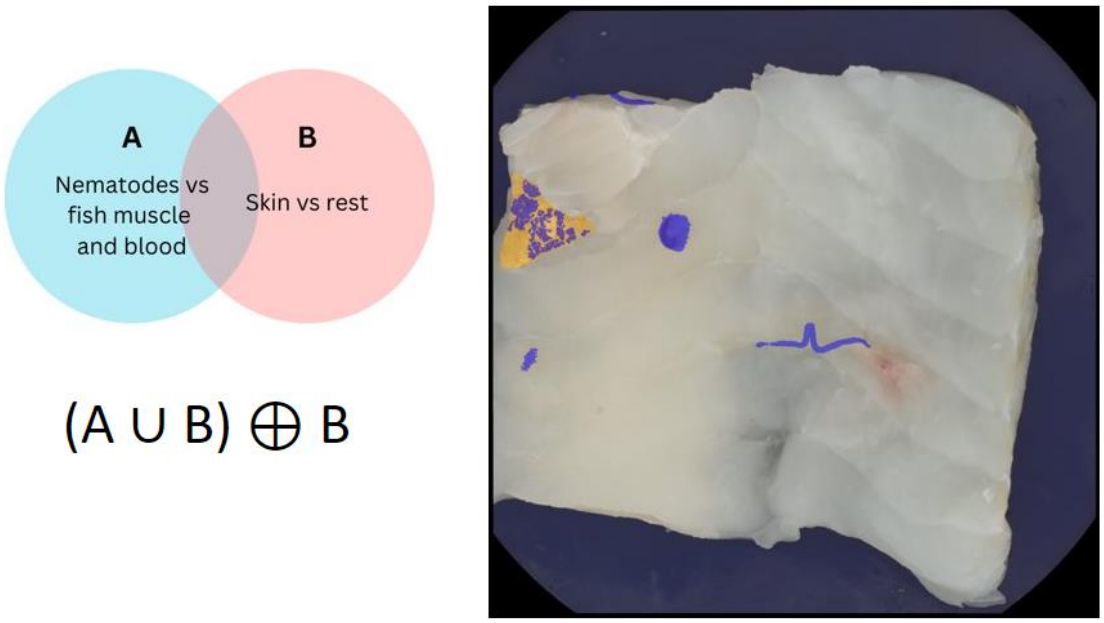


Figure 35 Example of incorporation of the two segmentation models used in the evaluation to identify nematodes in images.

The final prediction of the sequence segmentation model will only include pixels from model A that have not been predicted by model B. In this image, the skin area, where both models have predicted pixels, will be exluded from the final segmentation.

For data labelling, ground truth labelling, of data the Segment Anything (SAM) from Meta AI was used with PCS channels as a blueprint to highlight the nematodes. This provided more precise labelling than could be achived by hand.

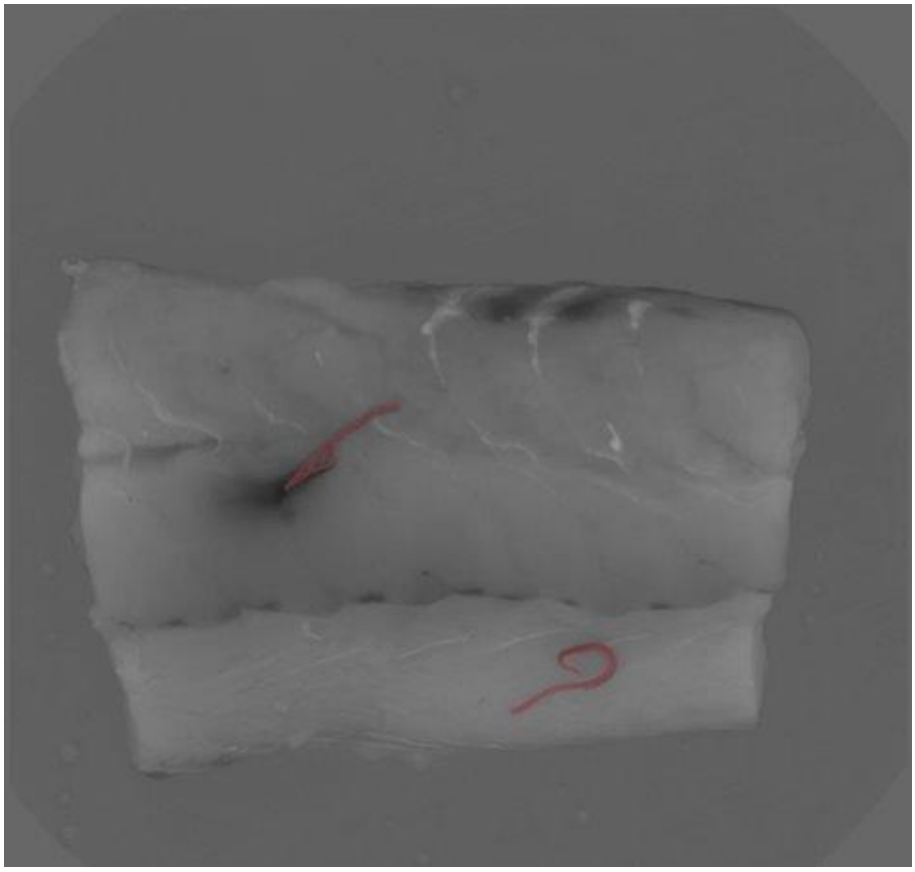


Figure 36 PCA channel image of white fish with nematodes following ground truth labelling with Segment Anything (SAM) from Meta AI

The model constructed was evaluated mainly in two ways:

1) Intersection over Union (IoU)
2) Precision Recall

IoU is a common performance metrics for segmentation tasks. The IoU measures the overlap between the predicted segmentation and ground truth masks. It measures how well the model can separate the objects from their background in an image. This is a pixel based metric, ranging from 0 to 1. The IoU for the train set was 0.56 and for the test set 0.52. This metric provides information on the ratio of pixels correctly identified but it can not provide any information on the amount of nematodes present in an image and further it was clear that in the intersection where nematode meets muscle in the image it had a hard to identifying all the pixels. In praxis this is not problematic since the industry only needs to be able to identify if a nematode is present but not how much of it they are able to identify specifically. Therefore, other metrics were also used.

Precision Recall metric was included following an addition to the model that intended to locate not only pixels belonging to a nematode but to identify how many worms are present in the image and where through analysis of the location and neighbouring area of pixels identified as belonging to a nematode. This provide results for precision and recall for both a test (220 images) and training (50 images) data sets presented in Table 3

Table 3 Precision Recall results for nematode detection trial in the white fish pilot

	Recall (%)	Precision (%)
Train	88.88%	96.96%
Test	92.50%	69.81%

3.3 TEXTURE AND FRESHNESS OF FINAL PRODUCTS

A trial was performed evaluating the ability of VideometerLab 4, VideometerLite 1 and VideometerLite 2 to evaluate freshness or texture of cod portions. Raw material used in the trial was Atlantic cod (*Gadus morhua*) caught in April 2023. Fillets were stored in EPS boxes at 0-2°C and sampled on D0, 1, 2, 6, 8 and 13 after the fish was brought the the research facility. Samples, whole fillets, were portioned to fit the imaging area of the Videometer equipment and images taken (260 images total per instrument). Further, as reference measurements the raw material was analysed for total viable count (TVC), total volatile base nitrogen (TVB-N) and texture (hardness) through a compression test.

Spectral data collected from the different devices was evaluated to determine a need for data preprocessing and it was determined that baseline correction of the spectral data would be beneficial. Freshness was evaluated through TVB-N and TVC analysis of samples and texture through a compression test. Figure 37 to Figure 39 shows results of a PLS regression of spectral data aiming to predict TVC, Figure 40 to Figure 42results for TVB-N and Figure 43 to Figure 45 results for texture. For more ease of comparison between the different devices and their abilities some results have been presented in Table 4.In this case with minimal spectral data pretreatments, baseline correction, a fairly good prediction can be made for TVC and TVB-N of fillet portions using the Videometer Lab 4, less so with the VideometerLite prototypes but the VideometerLite 2 prototype though an ok correlation was noted for both parameters for the VideometerLite 2 and for TVC for VideometerLite 1. It is also clear that the secondary prototype of VideometerLite provide an improvement over the previous one due to e.g. the additional wavelengths it provided and those were beneficial in this application. For prediction of attributes relating to texture the devices did not appear to be successful using this type of data processing and treatments. Further analysis of the data is ongoing to determine if additional pretreatments of the data provide better prediction of textural changes in the fillet portions.

Table 4 Results (R^2) of PLS analysis of spectral data from texture and freshness evaluation of cod portions

Predicted parameters	R^2 (VideometerLab 4)	R^2 (VideometerLite 1)	R^2 (VideometerLite 2)
TVC	0.70	0.52	0.61
TVB-N	0.78	0.31	0.58
Texture	0.27	0.20	0.21

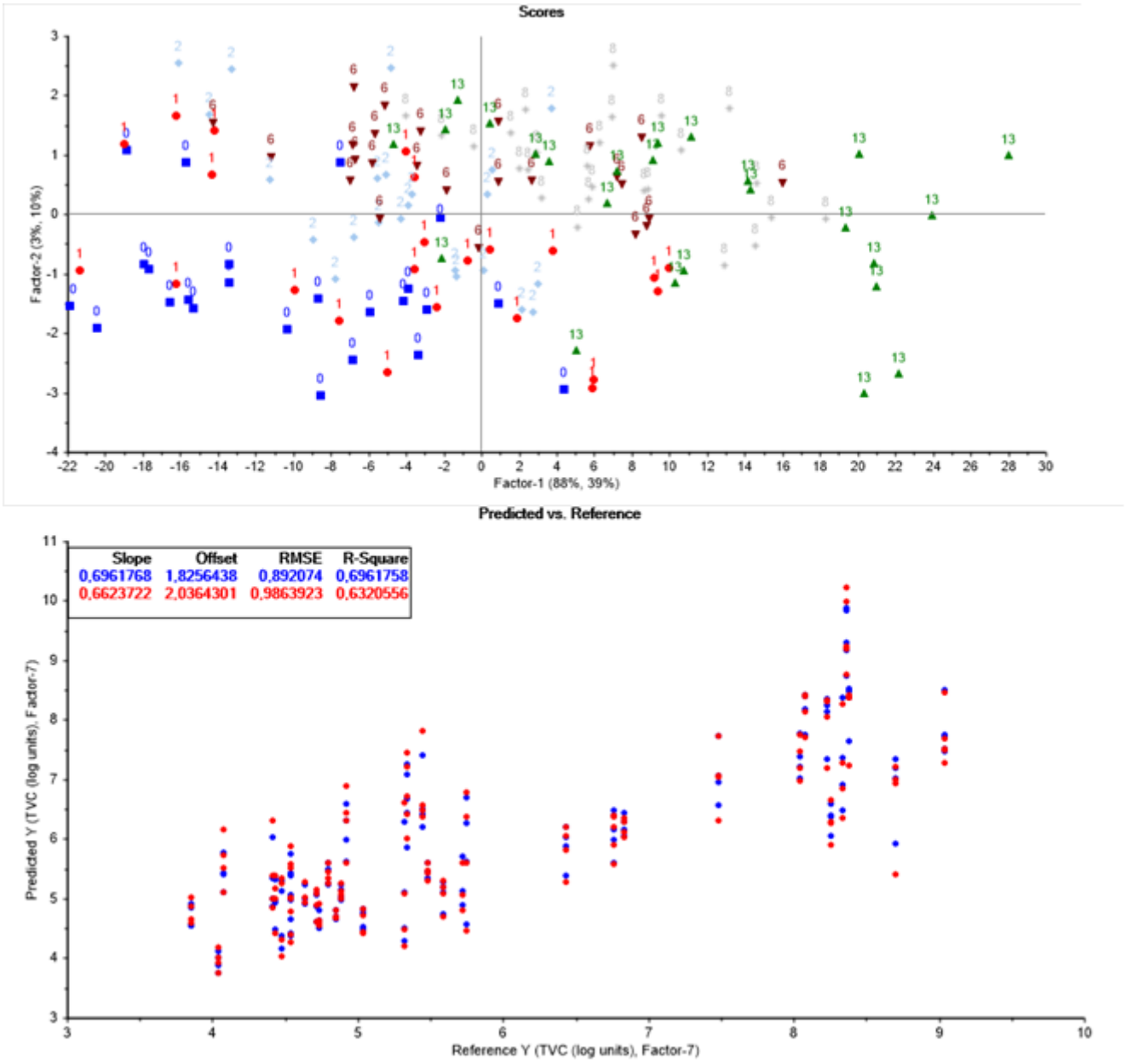


Figure 37 Results of PLS regression of spectral data from the VideometerLab 4 aiming to predict TVC of Atlantic cod fillet portions.

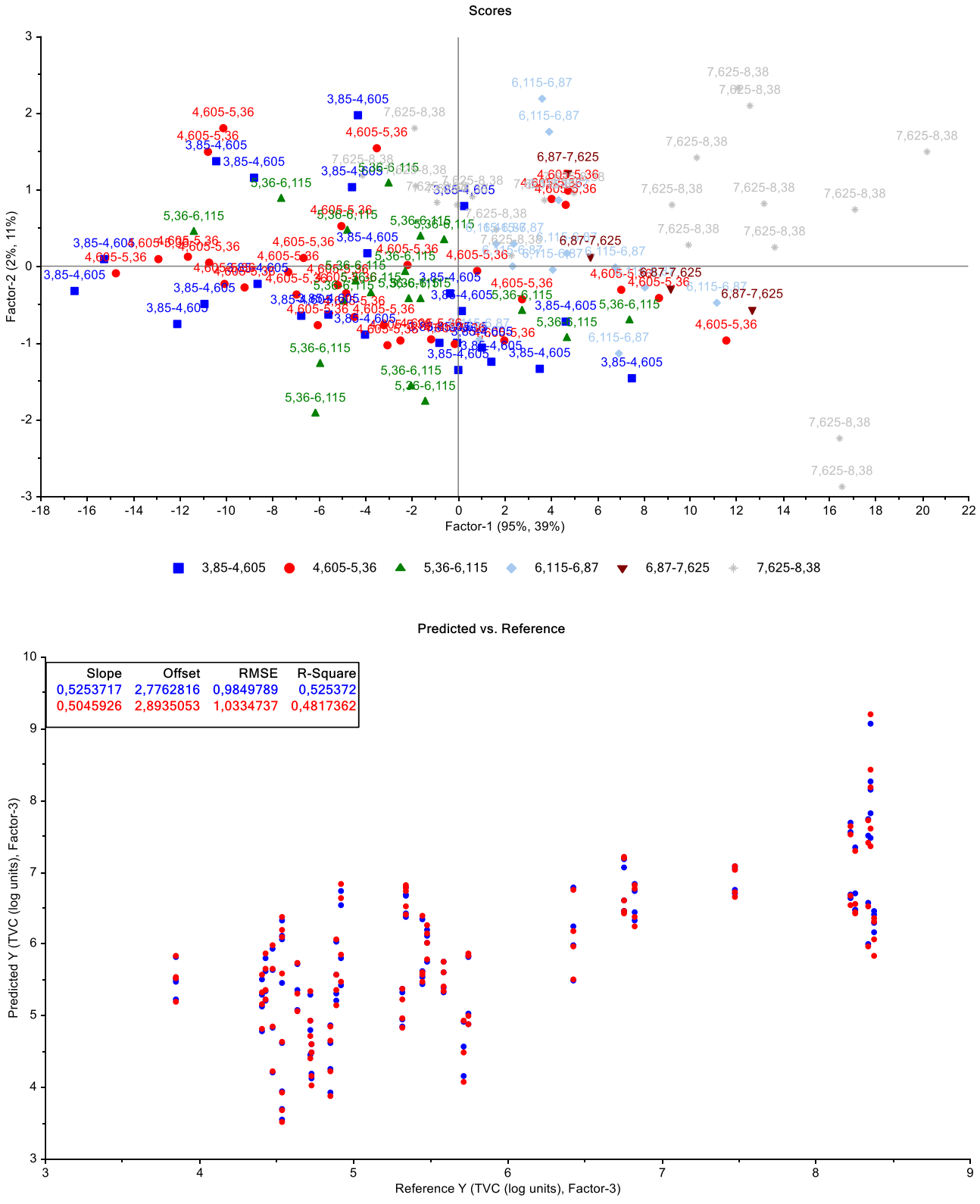


Figure 38 Results of PLS regression of spectral data from the VideometerLite 1 aiming to predict TVC of Atlantic cod fillet portions.

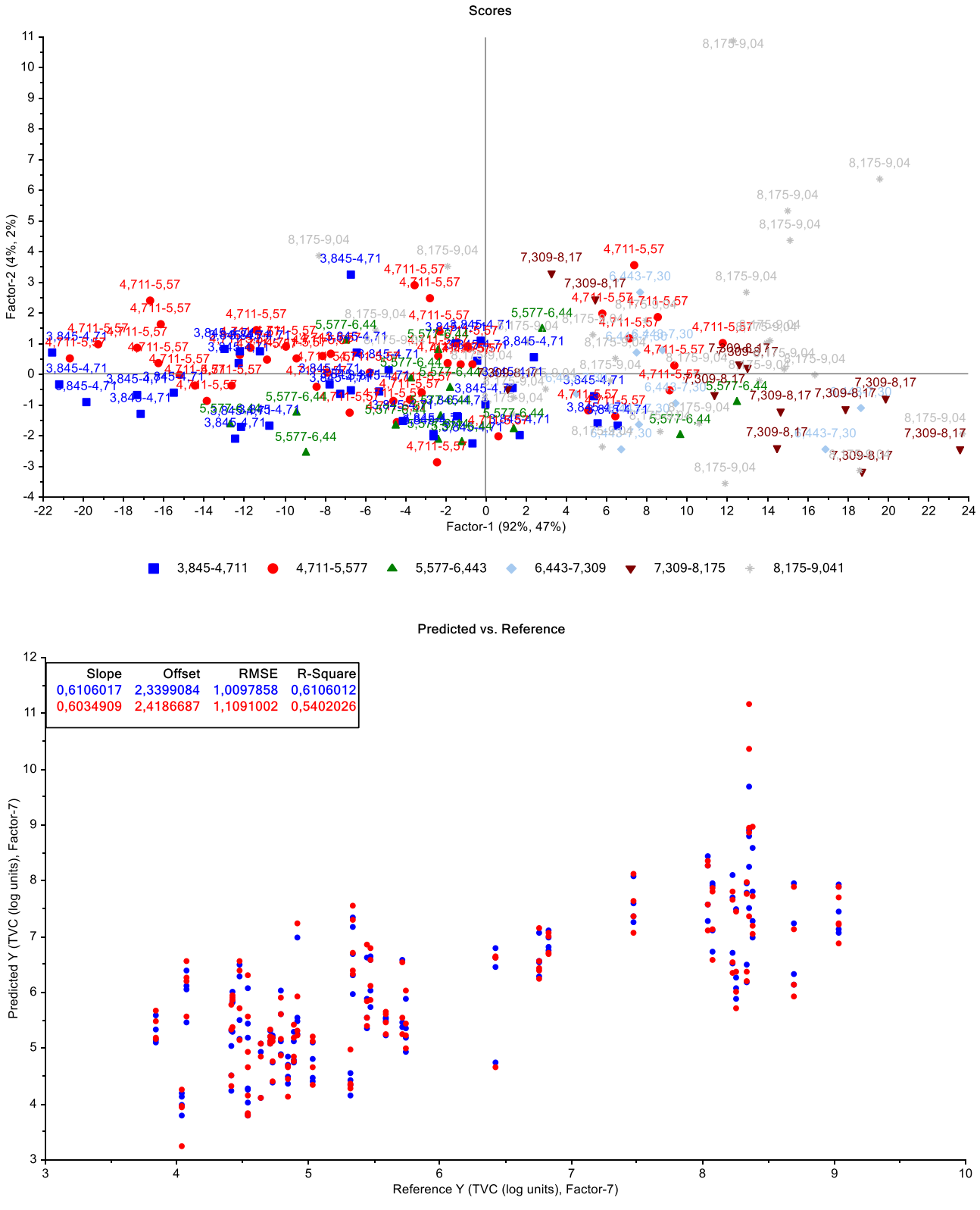


Figure 39 Results of PLS regression of spectral data from the VideometerLite 2 aiming to predict TVC of Atlantic cod fillet portions.

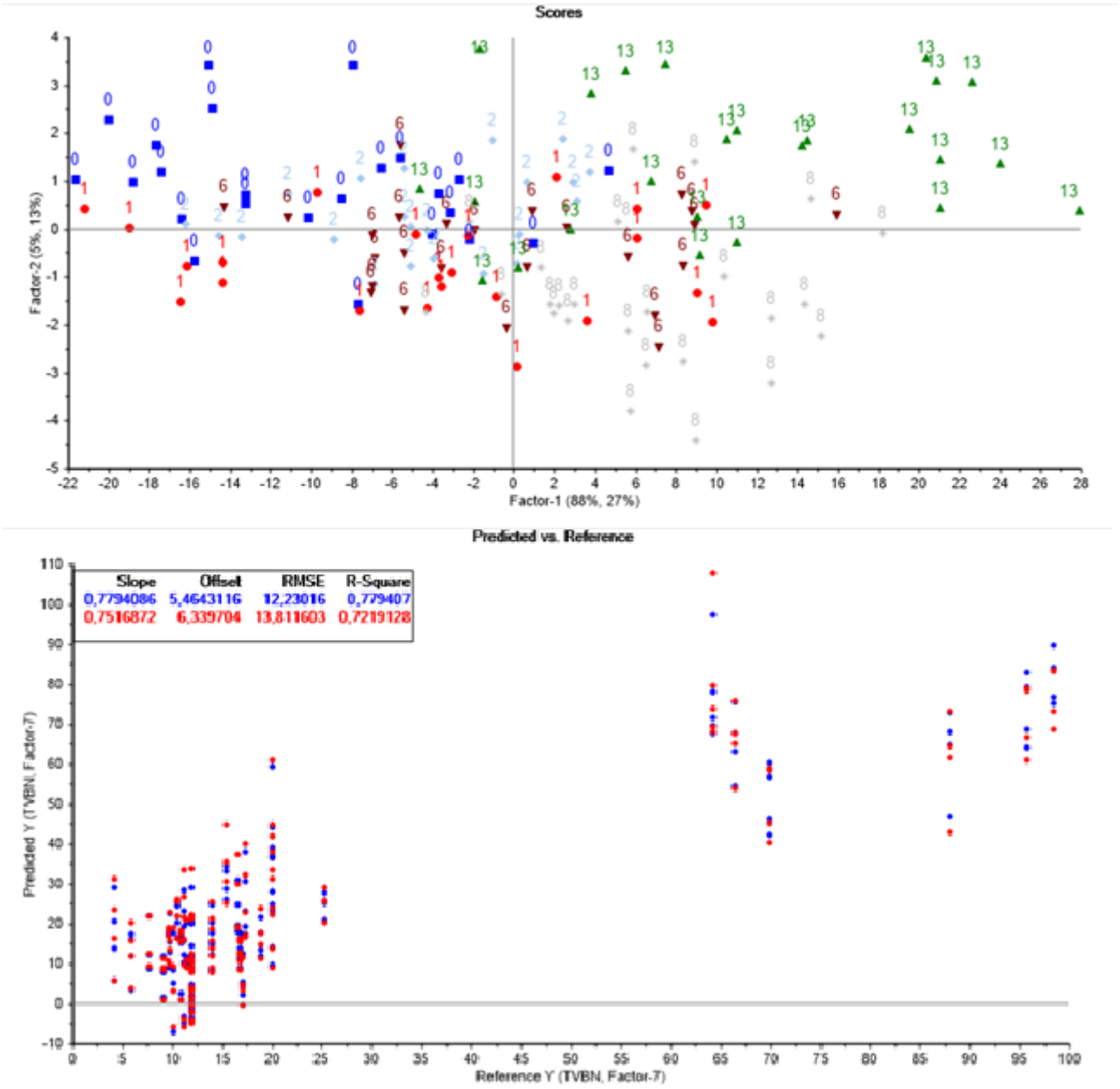


Figure 40 Results of PLS regression of spectral data from the VideometerLab 4 aiming to predict TVB-N of Atlantic cod fillet portions.

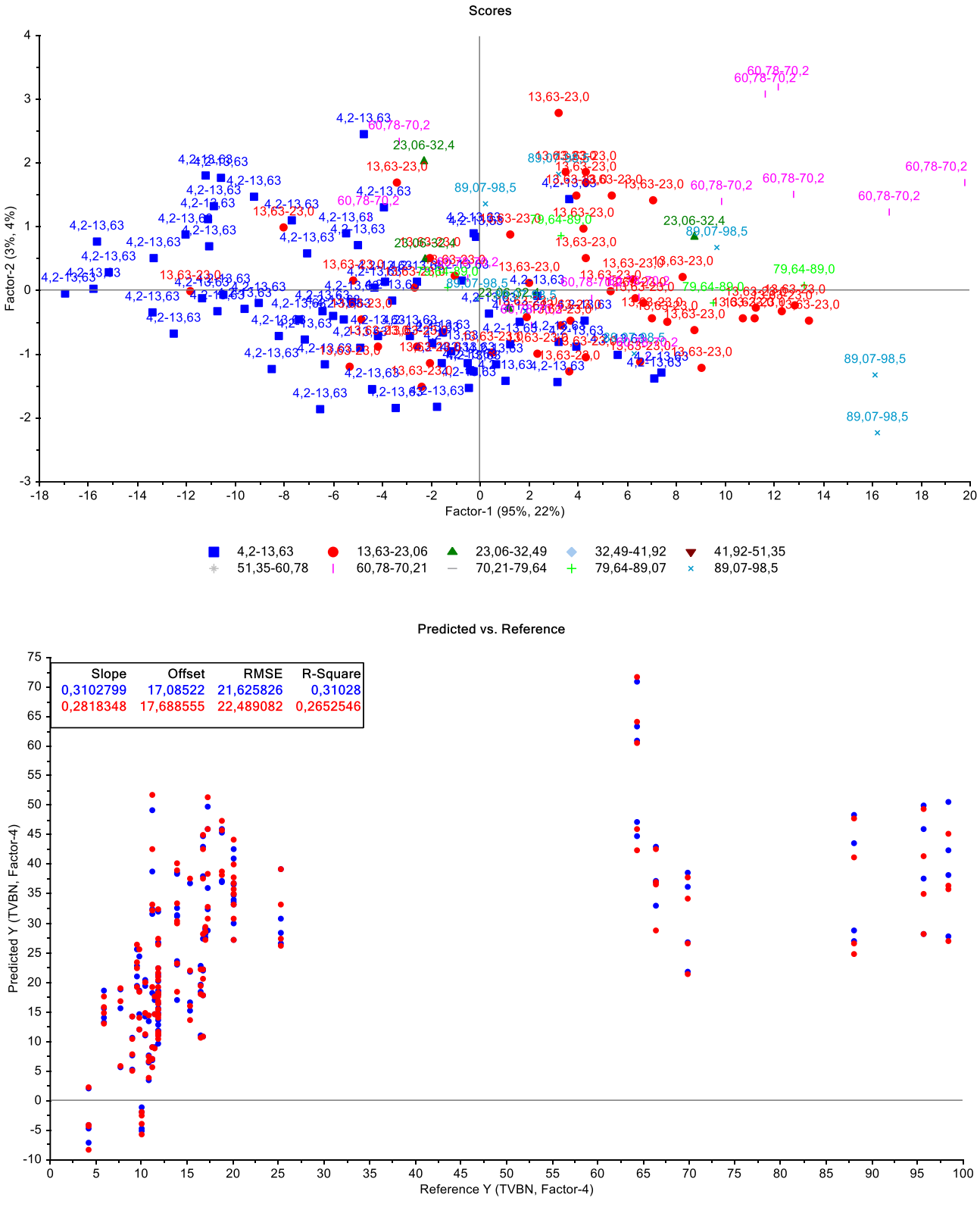


Figure 41 Results of PLS regression of spectral data from the VideometerLite 1 aiming to predict TVB-N of Atlantic cod fillet portions.

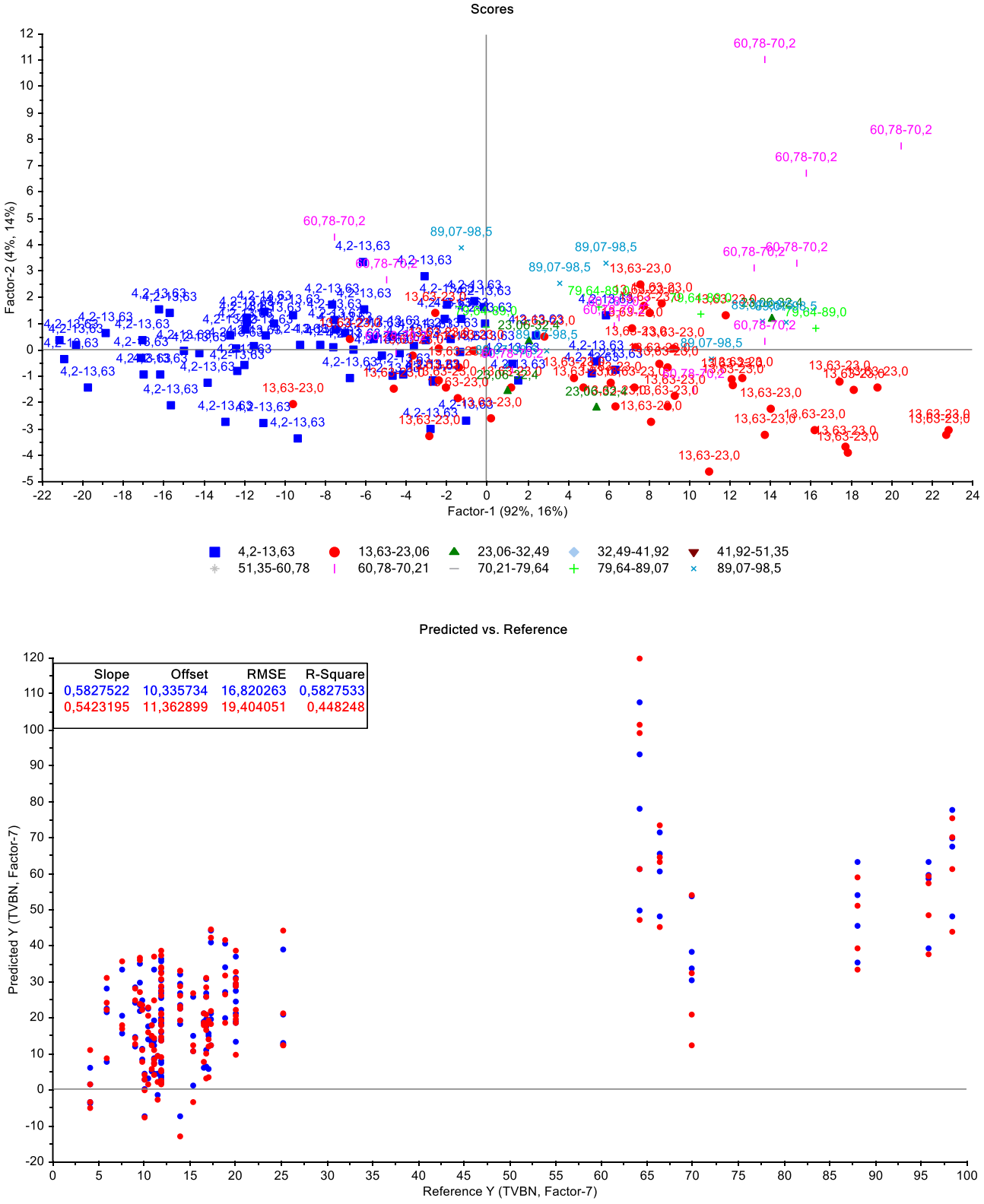


Figure 42 Results of PLS regression of spectral data from the VideometerLite 2 aiming to predict TVB-N of Atlantic cod fillet portions.

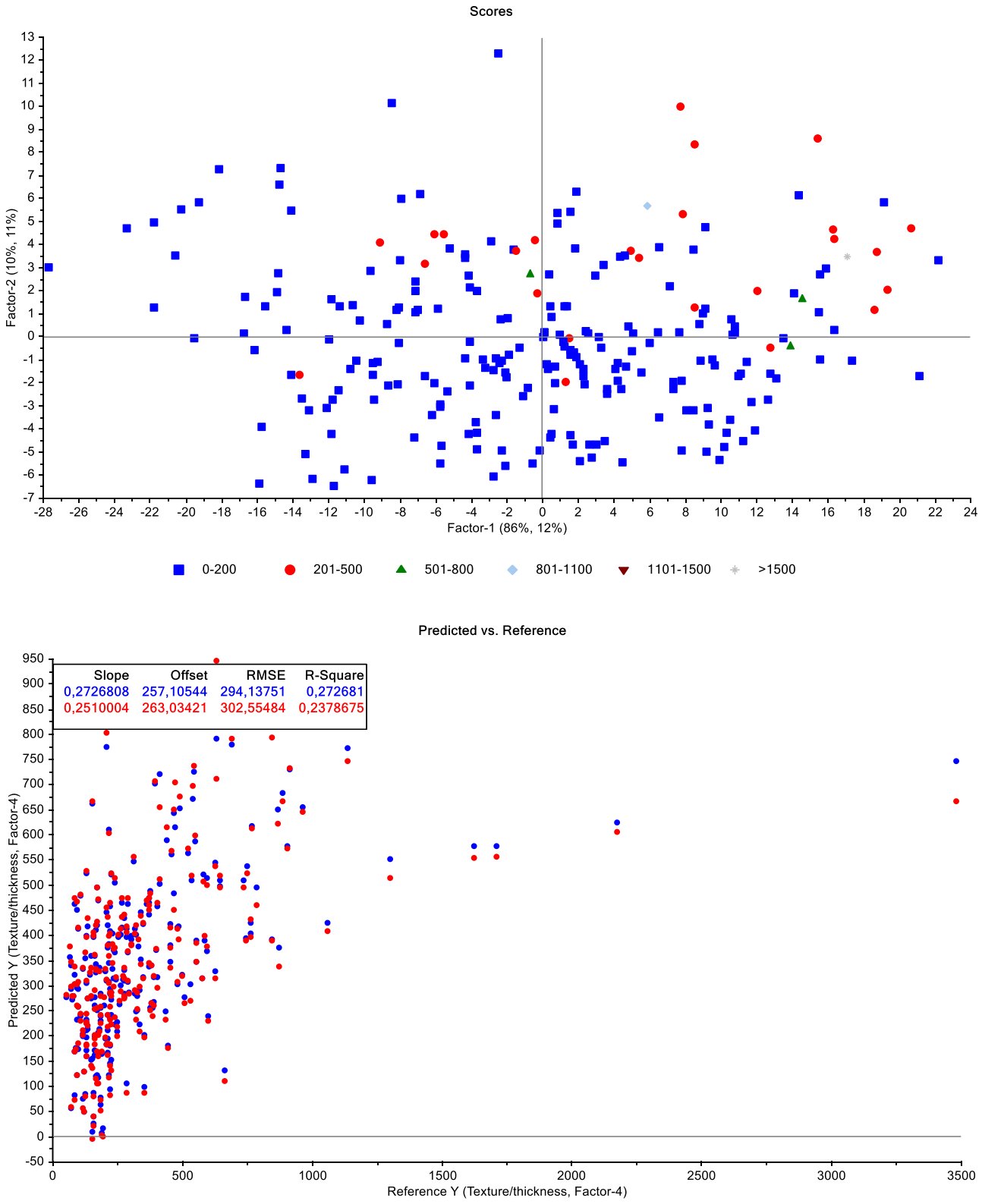


Figure 43 Results of PLS regression of spectral data from the VideometerLab 4 aiming to predict texture (hardness) of Atlantic cod fillet portions.

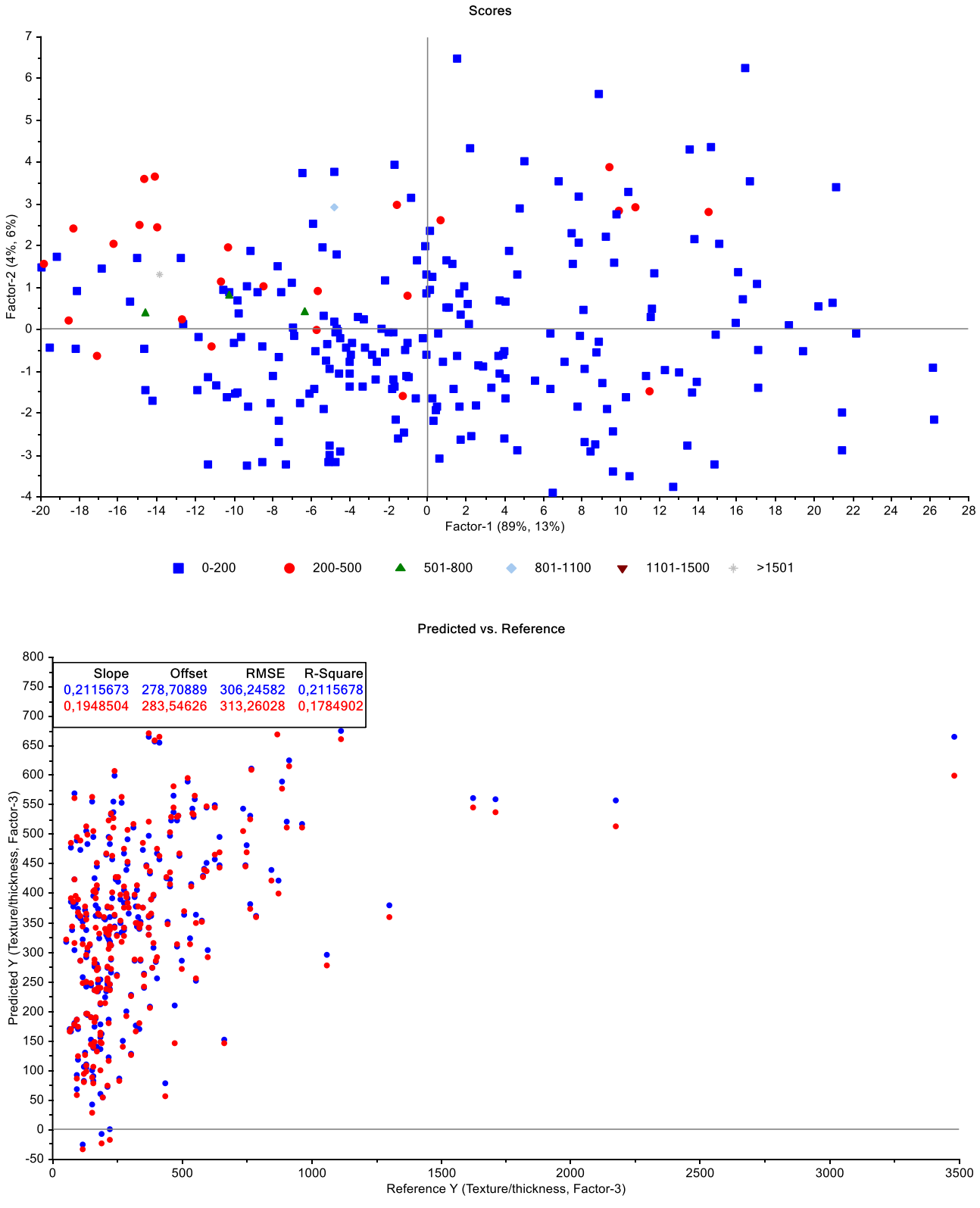


Figure 44 Results of PLS regression of spectral data from the VideometerLite 2 aiming to predict texture (hardness) of Atlantic cod fillet portions.

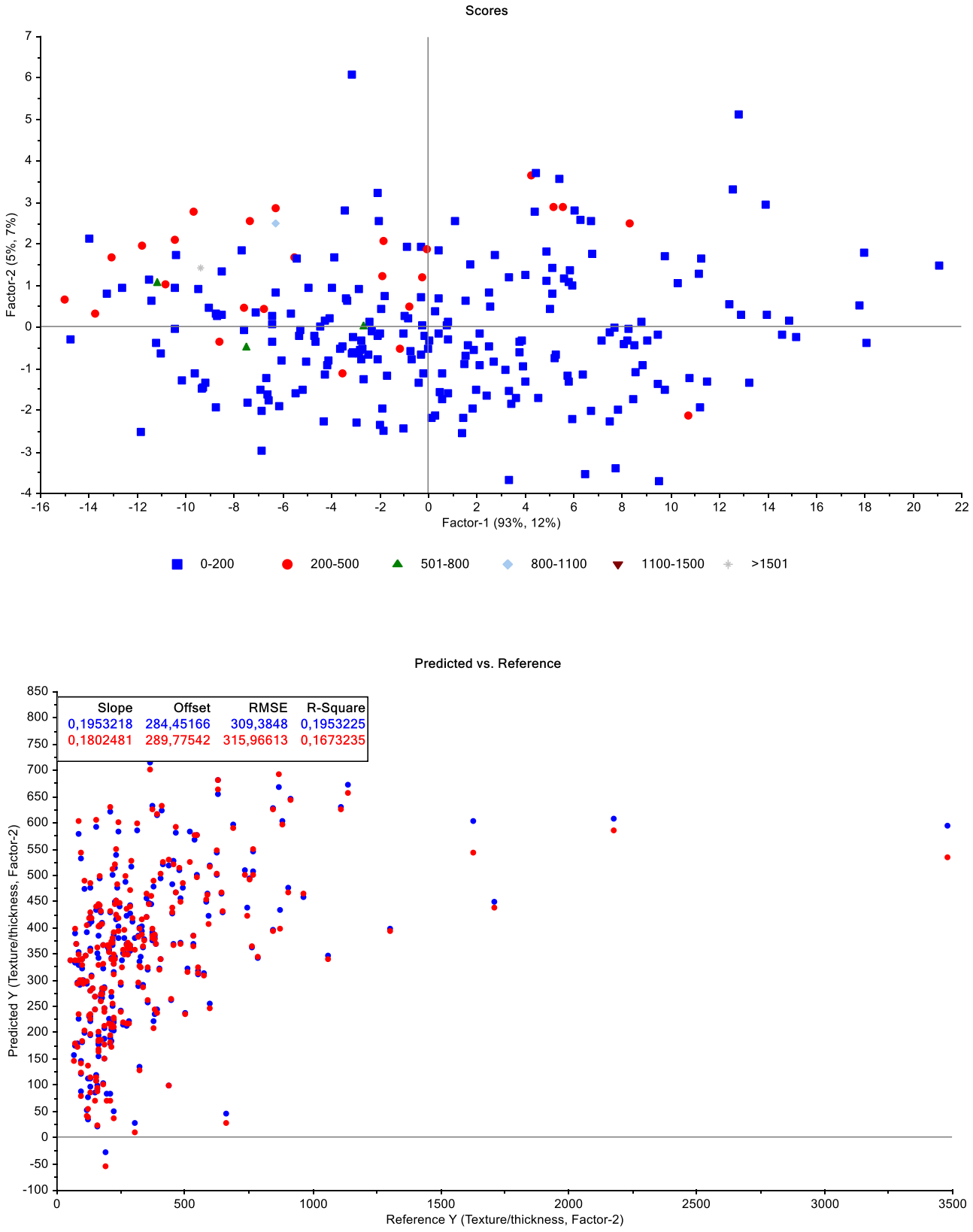


Figure 45 Results of PLS regression of spectral data from the VideometerLite 1 aiming to predict texture (hardness) of Atlantic cod fillet portions.

3.4 CONCLUSION

Results of the whitefish pilot showed great potential for the VideometerLite and VideometerLab devices. The second proved to be well equipped to provide the data needed for nematode identification in fish with good success. Further the VideometerLite 1 and 2 could with fair accuracy predict TVC of fillet portions though the VideometerLab 4 data as better equipped for this and the VideometerLite 2 could further provide adequate predictions for the TVB-N content of the samples as well as the VideometerLab 4. In that case the It was not successfull at prediciting hardness of samples though additional pretreatment of spectral data may provide new opportunities. Finally the VideometerLab 4 and VideoemterLite 2 provided fairly good regression models for prediction of samples QIM scores based on images from different parts of the fish, the best overall results were obtained using images from the skin (low) or gills.

This type of equipment can be applicable in an industry environment. For fast-paced processing plants and high throughput markets the use-cases are mostly related to implication into quality control systems and could be implemented for specific evaluations to perform randomized testing throughout the processing time.

3.5 PLANS FOR PUBLICATIONS

From the whitefish pilot trials 3 publications are pending. First, a publication detailing results of the nematode detection and automation of the data analysis. Second, a publication detailing results of QIM predictions evaluating the best possible practice and suggesting a methodology for the prediction using the VideometerLite and in doing so determining the usability of these devices for the industry dealing with whole gutted fish. Third a manuscript discussing the possibility of using these devices for evaluation of freshness or texture of fillet portions and detailing the differences between the devices and what each one tested provides.

4 THE MEDITERRANEAN SEABREAMA/SEABASS

As reported in the D6.1 and D6.2, seafood quality assessment is among the most significant parts of management systems in Aquaculture, playing a critical role in decision making throughout production and processing.

Activities and experimental plans related to seabream value chain focused on the KPIs that should be achieved for the successful application of the proposed system. In this framework, fish samples from different stages of the supply chain were examined, aiming to the seabream's value chain simulation. Additionally, several quality parameters were tested in an attempt to test the capability of the VideometerLite device to assess the quality and subsequently to contribute to the digitalization of the production process in Aquaculture.

In WP2 and previous Deliverables D2.2 and D2.4 the overall view of the analyses that would be performed during the TMF project was presented while specific requirements of the applied techniques were also provided. Additionally, the value chain of Mediterranean seabream along with the specific hazards related to the production, processing and distribution of seabream/seabass were previously reported.

In this Deliverable, an overview of the analytical procedures followed for the seabream quality assessment is presented, providing key information about the experimental process as well as the main results for each of the tested quality parameters.

Some indicative results from the analysis of data obtained from the Videometer sensors are provided in this deliverable as well. However, a significant amount of data was provided to Scio, for the accomplishment of the WP4.

4.1 MICROBIOLOGICAL QUALITY ASSESSMENT – CORRELATION WITH VIDEOMETERLITE RESULTS

4.1.1 Experimental design

The experimental design has been presented in detail in previous Deliverables. In brief, it was separated in three parts; a. Trial 1 – Analysis of seabream fillets obtained from the Aquaculture, b. Trial 2 – Analysis of seabream fillets obtained from several selling points, c. Trial 3 – Analysis of the same seabream fillets originated from 8 different fish throughout storage at 4 °C. The whole experimental procedure for each sample is illustrated in Figure 46, while the samples that examined in the context of Trial 2, the conditions as well as the analysis, are presented in Figure 47. In total, for the 1st trial, 580 (240 air- and 240 vacuum packaged) samples were analyzed with the VideometerLite. The respective number for the 2nd trial was 252 (140 air- and 112 vacuum packaged samples). The VideometerLite v.2 was also used in the framework of the 1st trial, analyzing 240 seabream samples.

In the 3rd trial, the experimental design was split again in two parts (Figure 48). In the first one, 8 different fish fillets from 3 different selling points were stored aerobically for 9 days at 2 °C. At each sampling point, sample of the same fillet (SF scenario) was used for microbiological (Total Aerobic Counts -TAC) and MSI analysis. In the second part, fillets were obtained from different retail stores and aquaculture companies (n=240) and treated as mentioned above, using at each sampling point a different fish sample (DF scenario). Given the fact that identifying the source of variability and monitoring it, is of critical importance for the success of predictive models, the aim of this study was to monitor the spoilage of an individual, specific fish throughout storage and compare it with the analysis of different fish through microbiological and Multispectral Imaging (MSI) analysis. The specific growth rate (μ_{max}) of total aerobes was estimated for each series of storage. MSI data were used in PLS regression and PLS-DA models to estimate the microbial counts and identify any differences among the two datasets.

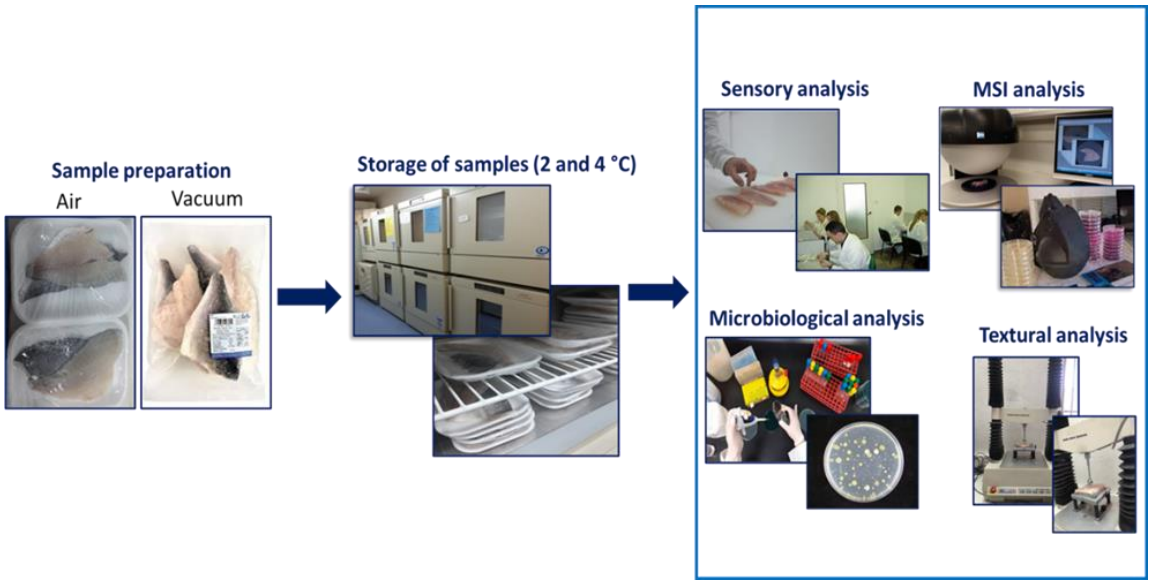


Figure 46 Updated experimental procedure of the first trial.

	AEROBIC PACKAGING - RETAIL											
	Storage temperature											
	2°C	2°C	4°C	4°C	2°C	2°C	2°C	2°C	2°C	4°C	2°C	2°C
	Use-by date											
DAYS OF STORAGE	14/11	14/11	14/11	14/11 Filleted in the lab	14/11	12/11	09/11	10/11	08/11	15/11	Filleted in the lab 10/11	Filleted in the lab 14/11
0	SK	AB			I-LD				P-LD		WF-SK	
1				WF-LD		I-LD	P-LD			P-LD	WF-SK	
2	SK	AB	AB						P-LD	I-LD		
3	-	-	-	-	-	-	-	--	-	-	-	
4	SK	AB		WF-LD				P-LD	P-LD	P-LD	WF-SK	
5						I-LD	I-LD				-	-
6	-	-	-	-	-	-	-	-	-	-	-	-
7	SK	AB	AB		P-LD					I-LD		WF-LD
8				WF-LD							WF-SK	
9	SK	AB								P-LD		
70 fish fillets – 140 MSI samples												

	VACUUM PACKAGING - RETAIL						
	Storage temperature						
	4°C	2°C	2°C	2°C	2°C	2°C	2°C
	Use-by date						
DAYS OF STORAGE	26/10	26/10	25/10	24/10	22/10	20/10	21/10
0	I	T		PR			
1	I	PR			PR		
2	I	T		T			T
3	I	-			T		
-	-	-					
4	I	PR		PR	PR		
5	I	T				T	
6	I	PR	P	T			
7	I	PR, T	T				
56 fillets – 112 MSI samples							

Figure 47 Experimental procedure of the second part

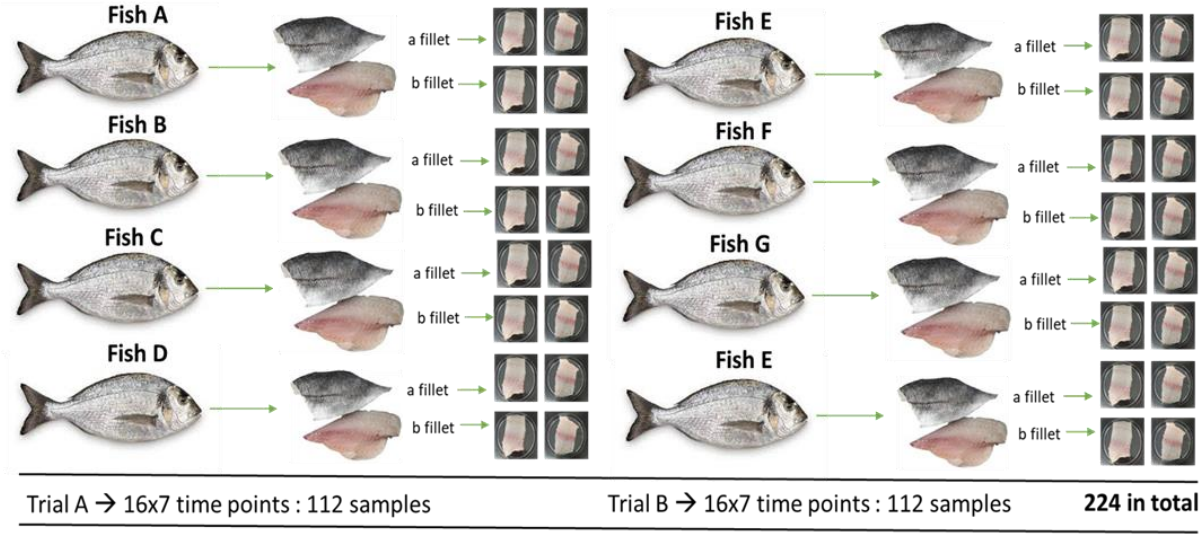


Figure 48 Sample preparation scheme for the third trial.

4.1.2 Main findings

The results of this task concerning the microbiological assessment have been presented in detail in previous Deliverables. In aerobically packaged samples (Trial 1), the microbial profile as well as the growth rate is clearly differentiated between the two packagings. *Pseudomonas* spp. and H2S- producing bacteria are the dominant microbial groups and play a key role not only in spoilage evolution -by microbiological and sensory perspective- but also in spectral analysis, producing significant metabolites. The changes in microbial counts throughout storage are illustrated in Figure 49.

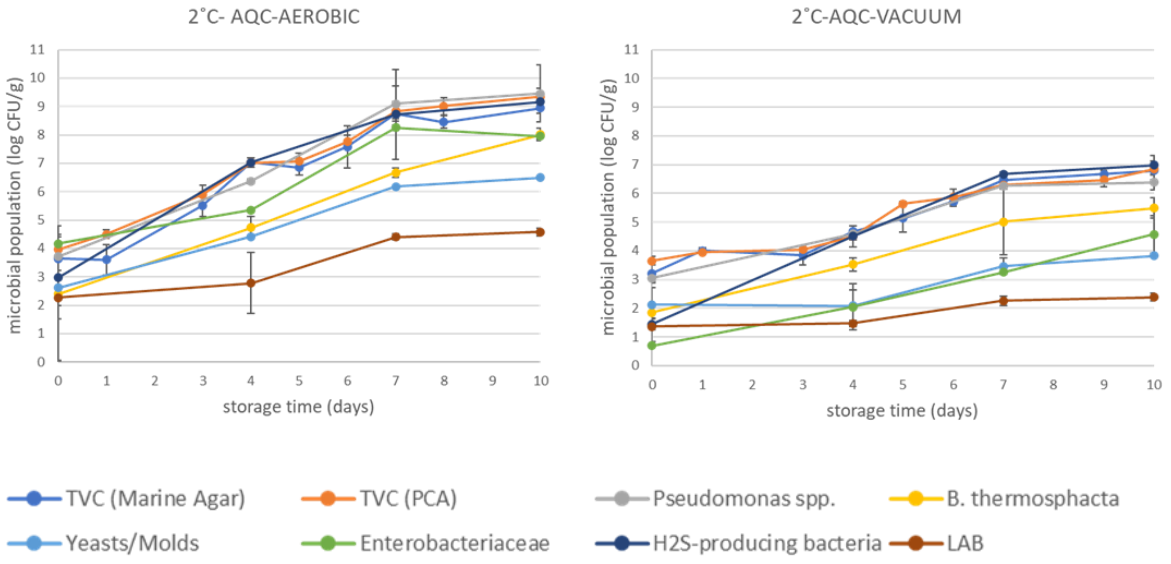


Figure 49 Microbial populations of seabream fillets obtained from the aquaculture throughout storage at 2 °C.

In vacuum packaged samples collected from selling points (Trial 2) the microbial populations until the ''use by'' date was <7.00 log CFU/g for all examined samples, while it is worth mentioning that the microbial counts in some of them was below 5.00 log CFU/g at the use by date, indicating an almost fresh product and highlighting

the need for reevaluation of the way that the use-by date is defined. The pH values of all samples were in the range from 5.86 to 6.33. In air-packaged samples, the initial microbial population of samples from bulk (4.48±0.46 log CFU/g) was higher compared to the initial population of fish fillets from the vacuum packages (3.83±0.72 log CFU/g). The samples stored aerobically had a relatively high population before the th use by date. In the case of higher storage temperature (i.e., 4 °C) microbial counts exceeded 8.00 log CFU/g. The pH ranged from 5.82 to 6.36. In Figure 50, the box plots of microbial population (TVC) for Total aerobic and vacuum packaging on the end of shelf life as proposed by the label (use by date) are shown.

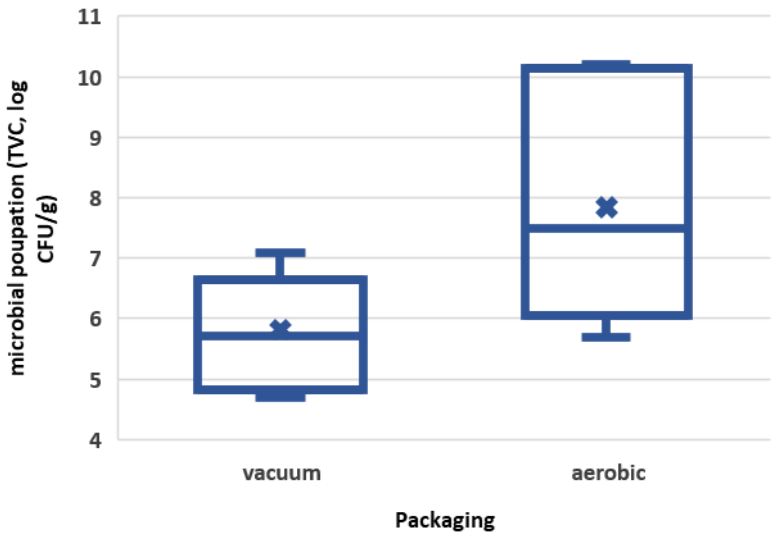


Figure 50 Microbial population (TVC) of fish samples on the end of shelf life (use by date) for samples stored in vacuum packaging (n=9) and in aerobic condition (n=7)

Similar to the microbiological analysis, sensory panel rejected air-packaged samples after 6-7 days of storage while vacuum packaged samples were rejected after 9-10 days of storage. In both cases, the attribute of odour received higher scores compared to overall appearance, indicating that odour is the most characteristic attribute for assessing the freshness of fish. The results of these analysis have been presented in previous Deliverables (6.1, 6.2).

The datasets acquired from the VideometerLite analysis were used for model development (WP4) and the findings are presented in the respective deliverables. However, as Figure 51 shows, small differences can be observed between aerobic and vacuum samples especially in wavelengths higher than 525 nm, and fresh and spoiled flesh samples mainly at 460 and 660 nm.

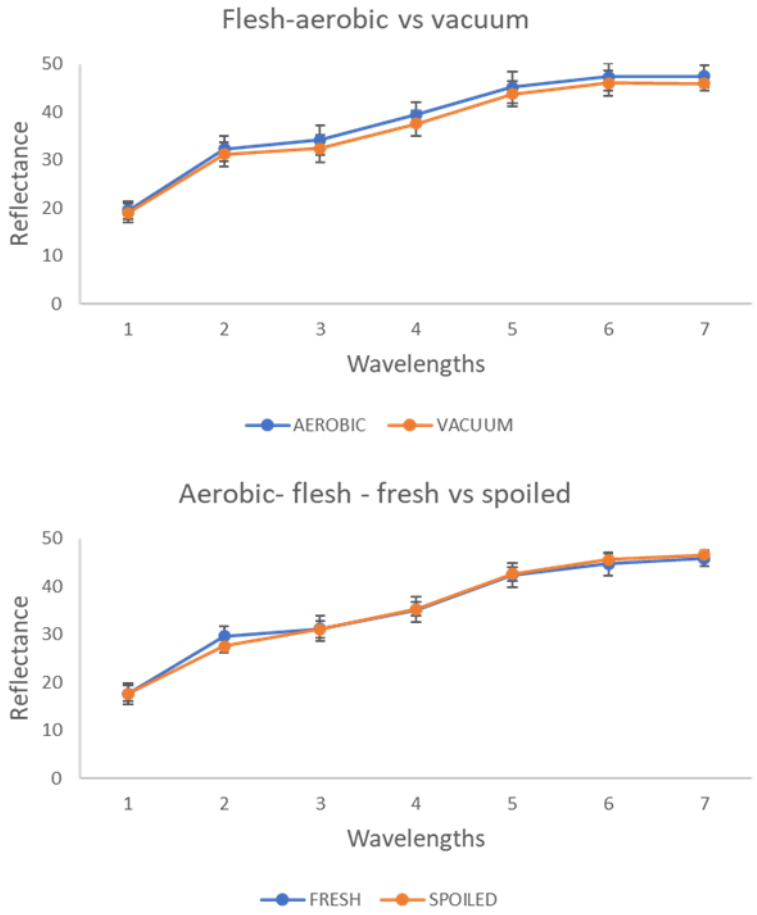


Figure 51 Indicative spectra for the comparison of various types of samples (fresh vs spoiled, skin vs flesh etc). 1;405, 2;460. 3;525, 4;590, 5;621, 6;660, 7;850.

In the 3rd trial, initial populations of different fish samples ranged from 2.4 to 5.2 log CFU/g. The specific growth rates of SF samples were 0.020 to 0.031, while the respective range for DF samples was 0.019 to 0.036, indicating small deviations between the two cases (Table 5). PLS-DA using MSI data revealed differences among SF and DF datasets, as more than 92% of the samples could be grouped at the correct category in model training and 80% in model testing (Table 6). The performance of PLS model for the prediction of microbial counts was better in SF compared to DF scenario based on RMSE (0.91 vs 1.24) and R^2 (0.71 vs 0.65). Features that were more informative for the prediction problem were different among the 2 scenarios (especially in the area between 435 and 470nm) was also differentiated using the two datasets and the combination of them. (Figure 52

Table 5 Specific growth rates of total aerobes in SF and DF scenarios.

	Initial counts (log CFU/g)	μ_{max}		
SAME FISH				
A	4.9	0.02	μ_{max} (average)	0.026±0.004
B	4.7	0.027	Initial counts (average)	4.1±0.79
C	2.5	0.02	max counts	4.9
D	3.4	0.024	min counts	2.5
E	4.2	0.027		
F	4.3	0.031		
G	3.9	0.029		
H	4.6	0.027		
DIFFERENT FISH				
AQ	3.9	0.028	μ_{max} (average)	0.026±0.007
AQ2	4.0	0.036	Initial counts (average)	4.4±0.71
AB	4.8	0.019	max counts	5.4
SK	5.4	0.029	min counts	3.6
LD2	5.0	0.018		
LD	3.6	0.028		

Table 6 Specific growth rates of total aerobes in SF and DF scenarios.

Confusion matrix for the estimation				
from \ to	DF	SF	Total	% correct
DF	193	9	202	95,54%
SF	25	236	261	90,42%
Total	218	245	463	92,66%

Confusion matrix for the validation				
from \ to	DF	SF	Total	% correct
DF	7	2	9	77,78%
SF	2	9	11	81,82%
Total	9	11	20	80,00%

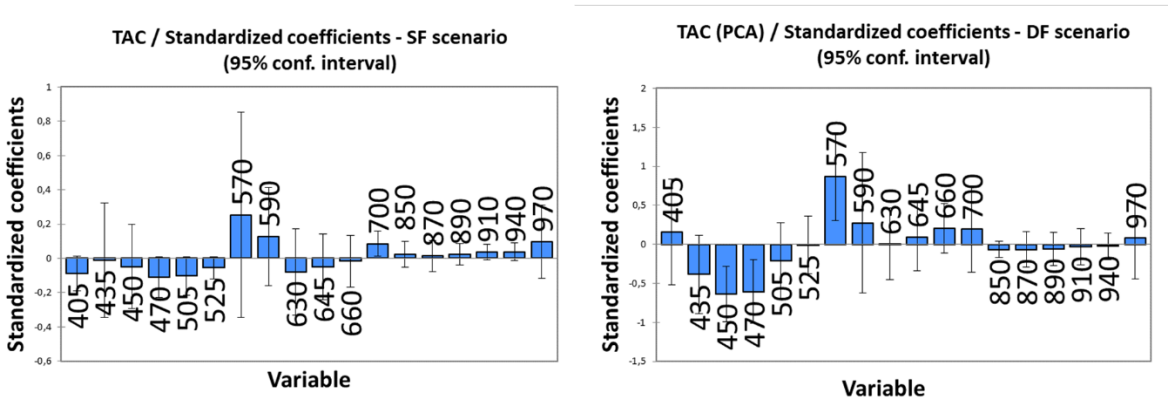


Figure 52 Standardized coefficients in SF and DF scenario

As presented in D3.2, a PLS regression model was applied to estimate the efficiency of the different instruments (VideometerLite 1 and 2) in assessing the microbial counts and consequently the freshness of seabream fillets. The preliminary results show that the VideometerLite second prototype seems to be able to predict more accurately the microbial populations based on R^2 and RMSE values. With regard to the features that were most informative for the prediction, there are slight differences among the most important ones. However, 460, 590,621, 630 and 405 are found to be among the most relevant ones for addressing this problem

4.2 CHANGES IN FISH HEADS/EYES THROUGHOUT STORAGE– ANALYSIS WITH VIDEOMETERLITE

4.2.1 Experimental design

For the evaluation of the quality based on the appearance of seabream eyes, the fish samples were obtained whole, ungutted and stored at 4°C for up to 10 days. At regular intervals two images were acquired for each fish head (i.e., back and front). In parallel to this, microbiological analysis was conducted for the enumeration of total viable counts (TVC - Plate Count Agar, incubation time: 30°C for 2-3 days) as reference indicator of fish quality. In total 252 images were collected from 126 fish heads and the respective microbial population (TVC). Images were acquired using VideometerLab2, VideometerLite and VIdeometerLite2 (Figure 53). The segmentation process is described extensively in D3.2.

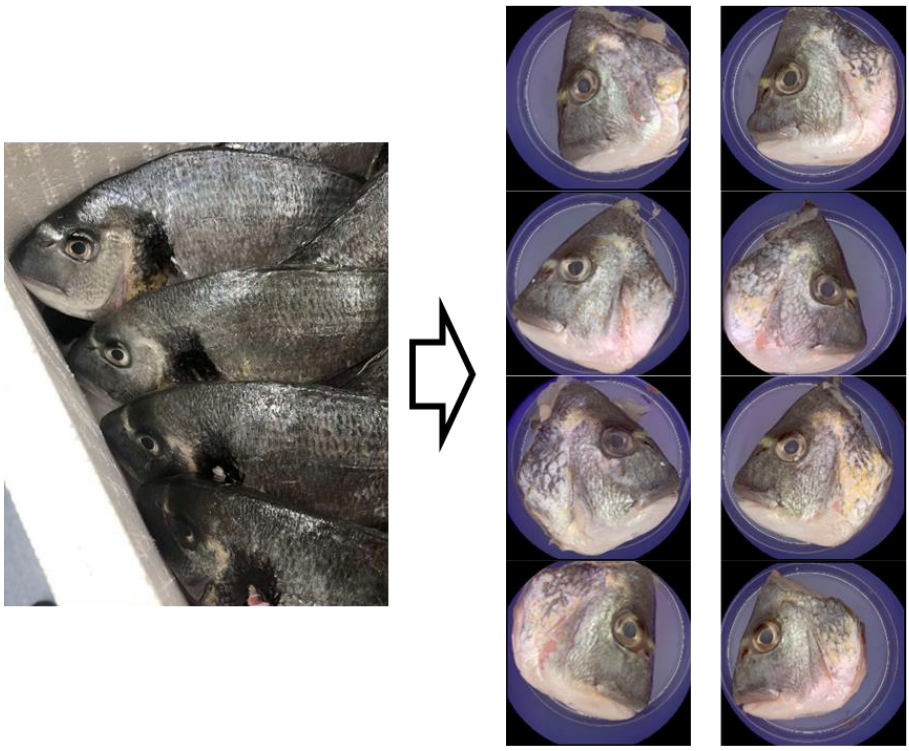


Figure 53 Seabream and example images acquired from VideometerLite

4.2.2 Main findings

In Figure 54 are shown the fisheyes images acquired from four different fish stored for a period of 10d. The images were acquired at eight time points. The different colours correspond to a different day and the respective microbial population of the fish (log CFU/g) is available, which ranged from 3.00 to 10.00 log CFU/g. Comparing images acquired at 0d (green labelled eyes) to 10d (red labelled eyes) shows some differences. The first are clearer and brighter, while the second are dull and milky. The upload of images as blob collection allows the calculation of features related to colour, texture, shape and other. These will be used for the data analysis aiming to the prediction of quality through fish eyes.

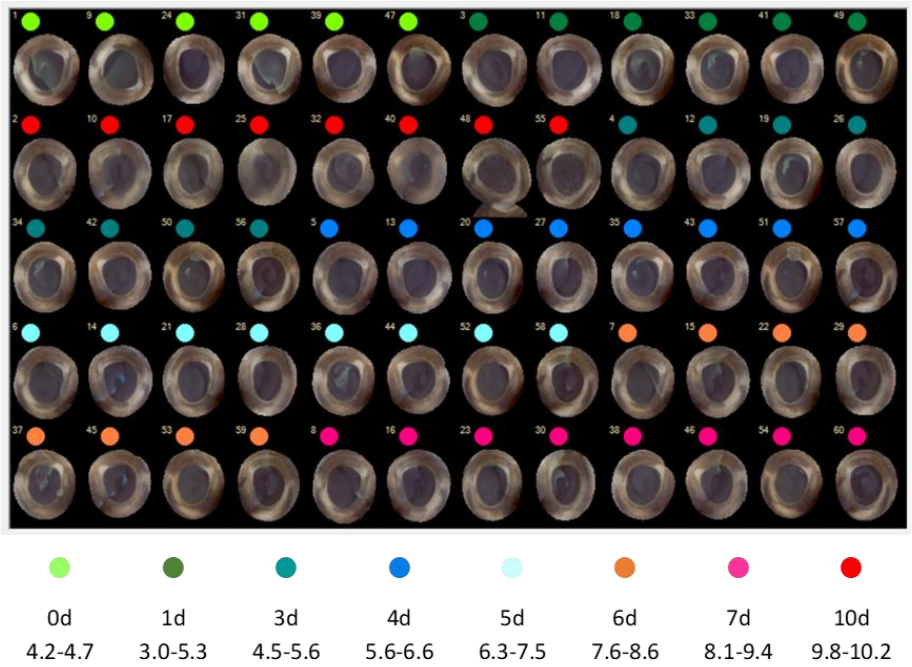


Figure 54 Fish eyes (blob collection) that were uploaded and labelled according to storage day.

4.3 TEXTURE ANALYSIS OF SEABREAM FILLETS

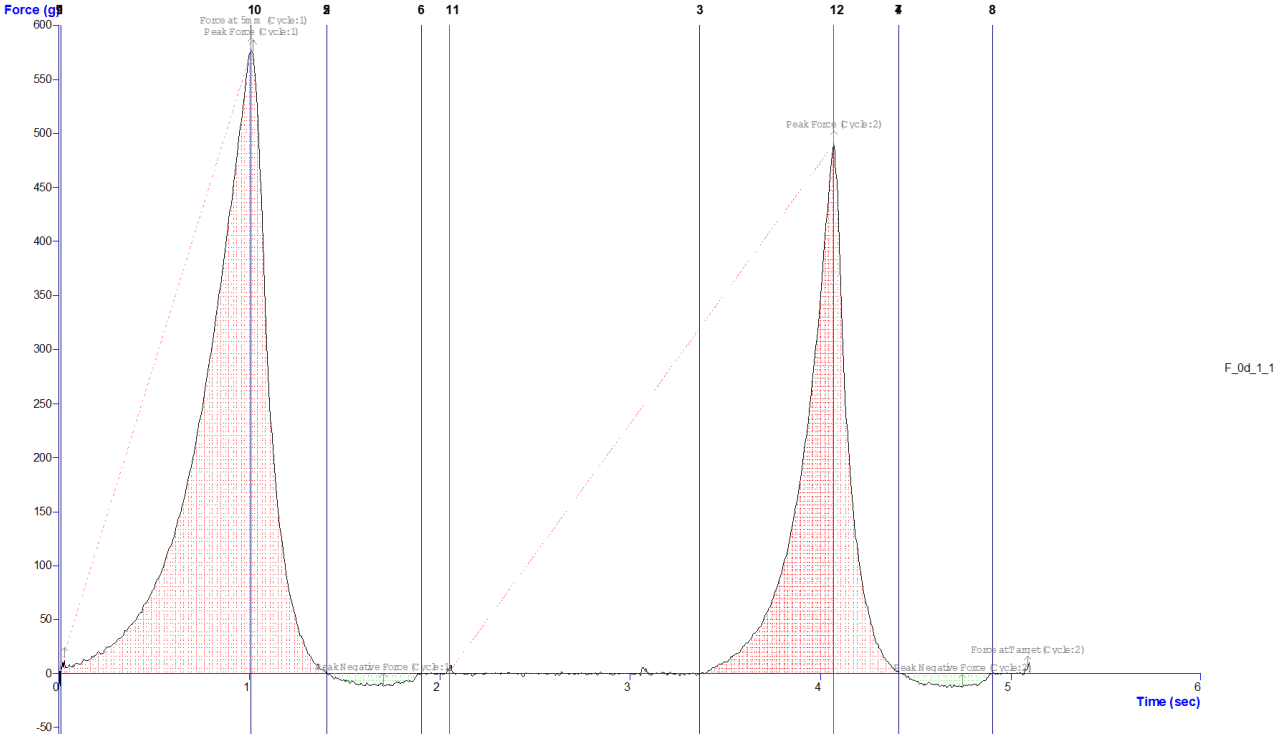
4.3.1 Experimental design

In the framework of Trial 1, apart from microbiological, sensory and multispectral imaging analysis, texture measurements had been also conducted. The texture analysis was performed using an HD-Plus texture analyzer (Stable Micro Pedicels Ltd., Godalming, UK) (Figure 55) and the Exponent Software for the data analysis. The determination of the textural characteristics of fish fillets was conducted with a spherical probe of 2 mm diameter and movement speeds of 5 mm/s during the test, 5 mm/s for the pre-test and 10 mm/s for the post-test. The compression depth was set at 5 mm, the measurement was conducted at the same point in the center of the fillets in each sample and the results were expressed as the maximum recorded force in g.

Figure 55 Analysis of seabream fillets using a HD-Plus texture analyzer (Stable Micro Pedicels Ltd., Godalming, UK)

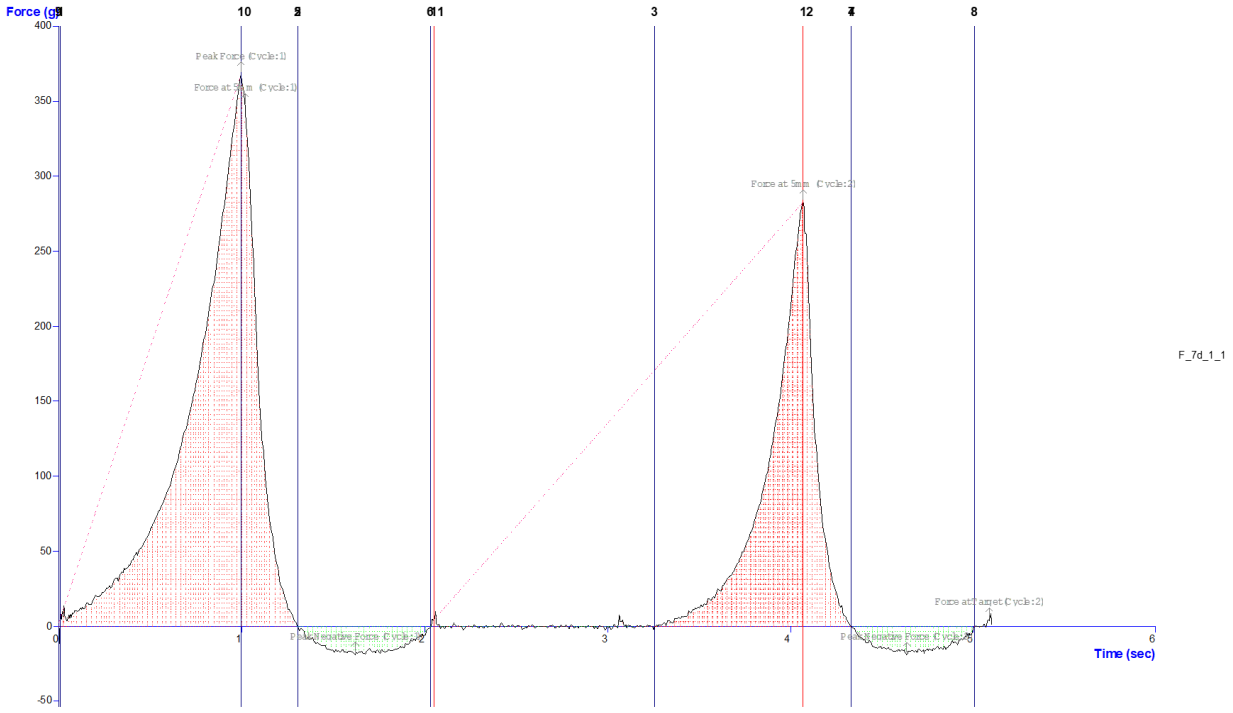
4.3.2 Main findings

In the following Figures (Figure 56 and 57), typical graphs of fresh and spoiled samples as well as the values for typical texture variables, are presented. It is clear that the force that should be applied -for the defined conditions- in fresh samples is significantly higher (576+490 g) compared to this found in the spoiled fillets (368+283 g) indicating the significant changes taking place on the fish flesh during the storage period.



		Peak Positive Force (Cycle: 1)	Peak Positive Force (Cycle: 2)	Peak Negative Force (Cycle: 1)	Peak Negative Force (Cycle: 2)	Peak Positive Distance (Cycle: 1
Start Batch 31-1-23	31-1-23					
F_0d_1_1	31-1-23	576,748	490,202	-12,171	-13,635	5,00

Figure 56 Typical instrumental TPA force–time deformation curve highlighting the area under the curve for the first compression and the area under the curve for the second compression in fresh fillets.



Peak Positive Force (Cycle: 1)	Peak Positive Force (Cycle: 2)	Peak Negative Force (Cycle: 1)	Peak Negative Force (Cycle: 2)	Peak Positive Distance (Cycle: 1
368,609	283,415	-19,045	-19,157	5,00

Figure 57 Typical instrumental TPA force–time deformation curve highlighting the area under the curve for the first compression and the area under the curve for the second compression in spoiled fillet

5 MULTISPECTRAL IMAGING SENSOR EVALUATION

The Videometer Spectral Imaging Technology is very promising and already validated efficiency in prediction of quality and safety technologies in the food domain. It is **non-destructive,** allowing the detection of hazards and quality related issues as well as a product's chemical and physical structure.

Videometer spectral imaging instruments measures more than 12 million individual spectra on a food sample within a few seconds (7-8seconds), in a structure of a data cube, several spectral planes (7 – 20 different wavelengths) where each plane reflects a monochromatic image at a specific wavelength. Every pixel in the image is a spectrum covering UV, visual color, and NIR ranges, including a fluorescence option, and of areas down to 30×30 µm. The analytical power of the technology offers a unique potential for fast characterisation of food integrity in terms of color, surface chemistry, texture, shape, and size without touching the sample and with little or no sample preparation. In the *TraceMyFish* project, Videometer provided two modified versions of the VideometerLite system (VideometerLite - Videometer), shown in Figure 15, that were used to collect data as input for the project iFMS. VideometerLite is a portable and wireless spectral imaging device designed for easy, straightforward, and accurate image analysis. Results presented in this deliverable showed that the application potentials for these devices are many and they can provide opportunities for increased safety, quality monitoring and traceability in the evaluated fish value chains. For some specific evaluations additions or changes could be made to better adjust the imaging protocol to specific products, i.e. larger fish fillets, to limit light pollution but still not requiring the sample be cut up (keeping the method non-destructive) for some applications but for most of the applications evaluated the size was adequate.

Figure 58. VideometerLite, portable handheld multispectral imaging device.

6 CONCLUSIONS AND NEXT STEPS

This deliverable provides an overview of the main results gathered in these studies. It provides comparison between the tested prototypes and laboratory grade equipment as well as a variety of application examples. The developed VideometerLite prototypes proved to have many application potentials throughout the different value chains relating to potential to predict freshness of both whole and filleted fish and detecting hazard or problematic flaws (e.g. nematodes, blood stains). Results of promising application potentials are now being finalized into scientific publications to ensure the information gathered in the project can be disseminated effectively to relevant stakeholders.