Traceability and Quality Monitoring throughout the Fish Value Chain

D6.2 – First pilot evaluation & KPI assessment

DELIVERABLE NUMBER	D6.2
DELIVERABLE TITLE	First pilot evaluation & KPI assessment
RESPONSIBLE AUTHOR	Hildur Inga Sveinsdóttir (Matis)

TraceMyFish is part of the ERA-NET Cofund BlueBio with funding provided by national sources [i.e., General Secretariat for Research and Innovation in Greece, Research Council of Norway, Innovation Fund Denmark and Icelandic Centre for Research in Iceland] and co-funding by the European Union's Horizon 2020 research and innovation program, Grant Agreement number 817992.

PROJECT ACRONYM	TraceMyFish
PROJECT FULL NAME	Traceability and Quality Monitoring throughout the Fish Value Chain
STARTING DATE (DUR.)	01/11/2021 (24 months)
ENDING DATE	31/10/2023
COORDINATOR	Panagiotis Zervas
COORDINATOR EMAIL	panagiotis@scio.systems
WORKPACKAGE N. | TITLE	WP6 | Piloting and Evaluation
WORKPACKAGE LEADER	AUA
RESPONSIBLE AUTHOR	Hildur Inga Sveinsdóttir
RESPONSIBLE AUTHOR EMAIL	hilduringa@matis.is
DATE OF DELIVERY (CONTRACTUAL)	28/02/2023
DATE OF DELIVERY (SUBMITTED)	03/05/2023
VERSION | STATUS	1.0 | Final
NATURE	Report
DISSEMINATION LEVEL	Private
AUTHORS (PARTNER)	Hildur Inga Sveinsdóttir (Matis), María Guðjónsdóttir (UoI), Anastasia Lytou (AUA), Jørgen Lerfall (NTNU), Anita Nordeng Jakobsen (NTNU)
CONTRIBUTORS	Andrea Rakel Sigurðardóttir (UoI), Lemonia Fengou (AUA), Panagiotis Tsakanikas (AUA), George-John Nychas (AUA), Sine Moen Kobbenes (NTNU), Marcus Hoff Hansen (NTNU)
REVIEWER	Panagiotis Zervas (SCiO)

VERSION	MODIFICATION(S)	DATE	AUTHOR(S)
0.1	ToC and initial draft	20.03.2023	Hildur Inga Sveinsdóttir (Matis)
0.4	Section 4 input	04.04.2023	Anastasia Lytou (AUA)
0.7	Section 2 input	18.04.2023	Jørgen Lerfall (NTNU), Anita Nordeng Jakobsen (NTNU)
0.9	Section 3 input	02.05.2023	Hildur Inga Sveinsdóttir (Matis), María Guðjónsdóttir (UoI) Andrea Rakel Sigurðardóttir (UoI)
1.0	Internal Review and Editing	03.05.2023	Panagiotis Zervas (SCiO)

PARTICIPANTS		CONTACT PERSON
SCiO P.C. (SCiO, Greece) **Coordinator**		Panagiotis Zervas E-mail: panagiotis@scio.systems
Department of Food Science and Human Nutrition, Agricultural University of Athens (AUA, Greece)		George-John Nychas E-mail: gjn@aua.gr
Department of Biotechnology and Food, Norwegian University of Science and Technology Science (NTNU, Norway)		Jørgen Lerfall E-mail: jorgen.lerfall@ntnu.no
Videometer A/S (VIDEOM, Denmark)		Nette Schultz E-mail: NS@videometer.com
Faculty of Food Science and Nutrition, University of Iceland (UoI, Iceland)		Maria Guðjónsdóttir E-mail: mariagu@hi.is
Matis (MATIS, Iceland)		Hildur Inga Sveinsdóttir E-mail: hilduringa@matis.is

ACRONYMS LIST

TMF	Trace My Fish
VIDEOM	Videometer
TVC	Total Viable Count

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EXECUTIVE SUMMARY

This deliverable provides a preliminary report on the first's pilot experimental design and on the acquired data. In addition, a first evaluation of the VIDEOM multispectral imaging sensor in terms of its application on the specific types of fish and on its actual application along the fish chain and the identified critical points in this chain (functionality, operational conditions etc.). Thus, towards this scope and in the general concept of WP6, the corresponding partners (AUA, NTNU, UoI and MATIS), define and set up the experimental conditions of each of the three pilots and acquire the measurement using the VIDEOM provided sensor. The collected data serves as inputs for further use and analysis, i.e., machine learning model training and deployment.

In Task 6.2, which this deliverable accounts for, partners will connect spectroscopic imaging data with reference measurements and analysis using traditional physicochemical and/or microbiological methods throughout the fish value chains, specifically the value chains of 3 types of fish: Atlantic salmon, Atlantic whitefish, and Mediterranean seabream/seabass. The experiments planned and described in D2.3 and D6.1 aim to tackle the most relevant quality and safety parameters mentioned in stakeholder interviews and further to provide added traceability of the product throughout the process in question. The experiments set out to provide assessment of relative KPIs identified for each value chain. That includes;

1) the availability of critical targets/sites in chain, which are ensured by chain simulation for the 3 fish types integrating within product variability.
2) Verification of the mitigation in quality/safety, authenticity, traceability and also in terms of waste via the acquired along the chain data enabling early alerts.
3) The successful demonstration of the whole closed system in terms of accuracy, quality, traceability and mitigation verification.

Through the work in the task the TraceMyFish systems functionality and performance was and will continue to be evaluated. The outcomes of the testing will be documented, and recommendations for improvement will be provided to the technical partners. Furthermore, the progress of the improvement will be monitored. This deliverable, 6.2, provides a preliminary evaluation of the pilots and assessment of KPIs to be updated through deliverable 6.3.

1 INTRODUCTION

The context of WP6 is focus 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. The first objective is the most obvious one, accounting to data acquisition per se at several food chain sites (either in real life or within the lab with conditions mimicking the real-life ones). In addition to this first objective is the standardization of the data acquisition method along with the operational functionality of the sensor. The latter will provide insights and the relative experience towards the designing of a more efficient and hands on walkthrough for the end-users to follow, concerning each instance of the whole food chain of at least the considered in the project types of fishes. Next, another objective is to benchmark each individual use case, inf terms of fish type, originated from the three geographical areas considered (Norway, Iceland and Greece). This benchmarking is crucial in order to gain insights for the sensor related advantages and/or shortcomings, along with the developed hazard detection/prediction approaches and also for the traceability system. So, this specific objective would ensure the feasibility validation for all the components of the developed "closed" monitoring system TMF, throughout the food chain. Another objective is the creation of a fish samples related database, where the data are acquired at several stages of the chain and "simulates" the whole journey. This information is very crucial for robust model building and traceability issues due to the large variability in samples and sample conditions along the food chain that will incorporate. This is further enhanced by the fact that those data will be accompanied by the "true" values of the samples properties as they are accompanied by traditional microbiological analyses from the laboratories and also under controlled treatment. Finally, the last objective is to perform, on the field evaluation and validation of the TMF system in terms of origin, detection and prediction of eminent hazardous situations. The specifics of the KPIs that will support the successful application of the system vary between the value chains but they are all based on the following;

1) the availability of critical targets/sites in the value chain, which are ensured by chain simulation for the 3 fish value chains integrating the within product variability.
2) verification of the mitigation in quality/safety, authenticity, traceability and waste via the acquired along-the-chain data enabling early alerts.
3) the successful demonstration of the whole closed system in terms of accuracy, quality, traceability and mitigation verification.

The experimental procedures (please also refer to D2.3 for more details and customized protocols for each pilot) were designed to ensure the real food chain is simulated, but under more controlled conditions. This will help us trace any inconsistencies during the analysis leading to "bizarre" prediction results. All involved partners have and will simultaneously and independently for each of the three pilots, differentiated by the level of partners' location (i.e., Greece, Iceland and Norway) and the corresponding types of fish (Atlantic salmon, Atlantic whitefish, and Mediterranean seabream/seabass).

2 THE ATLANTIC SALMON PILOT

The Atlantic salmon value chain, defined in D2.2 *User requirement specification (v2.0)*, includes all steps from farming to convenient, consumer-friendly, value-added retail salmon products. Unwanted incidents and hazards along the value chain were identified in D2.2 and are the basis for the pilot design presented in D2.4 *Pilot Design and pilot plan (v2.0)*. Data acquisition has mainly been conducted from laboratory-designed experiments simulating different scenarios of unwanted incidents in the salmon value chain. Unwanted incidents include:

1) Issues related to pre-slaughtering stress and fish handling.
2) Issues related to melanin spots.
3) Issues related to poor bleeding and residual blood.
4) Issues related to loss of freshness and microbial spoilage.

Seafood quality assessment is a significant part of management systems in Aquaculture since it plays a critical role in decision-making regarding several actions in aquaculture activities. In previous Deliverables, D2.2 and D2.4, the overall view of the analyses planned, and the specific requirements of applied techniques during the TMF project were presented. In this Deliverable, the whole analytical procedure for the Atlantic salmon quality assessment is described in detail, and some preliminary results are provided. The analysis of data obtained from the VideometerLab2 and VideometerLite prototype is still ongoing, and the findings of these analyses will be presented in the following deliverables.

2.1 EXPERIMENTAL DESIGN

2.1.1 Issues related to pre-slaughtering stress and fish handling

Stress and rough handling of fish affect textural and colorimetric properties. Fish from an ongoing project funded by the research council of Norway (project 321586) was used to obtain fish with a texture gradient related to stress and rough handling. After conducting a feeding trial with three experimental fish feed, a stress experiment was performed over an intensive period of 14 days, having unstressed fish as controls (Figure 1). The design was then used to evaluate the potential of VideomterLite and abVideometerLAB2 to evaluate textural- and colorimetric parameters. The results were validated by comparing multispectral data from the Videometer devices with data from traditional methodologies measuring textural (penetration test) and colorimetric properties (DigiEye imaging, SalmoFan, and muscle pigment concentrations). All measurements were performed on the Norwegian quality cut of fresh fillets at day one postmortem and after six days of refrigerated storage. In D6.2, we present the potential of the VideometerLite prototype to distinguish between soft and firm fillets. Other results obtained from this trial will be published in the final Pilot Evaluation & KPI report (to be published October 2023), or through other channels.

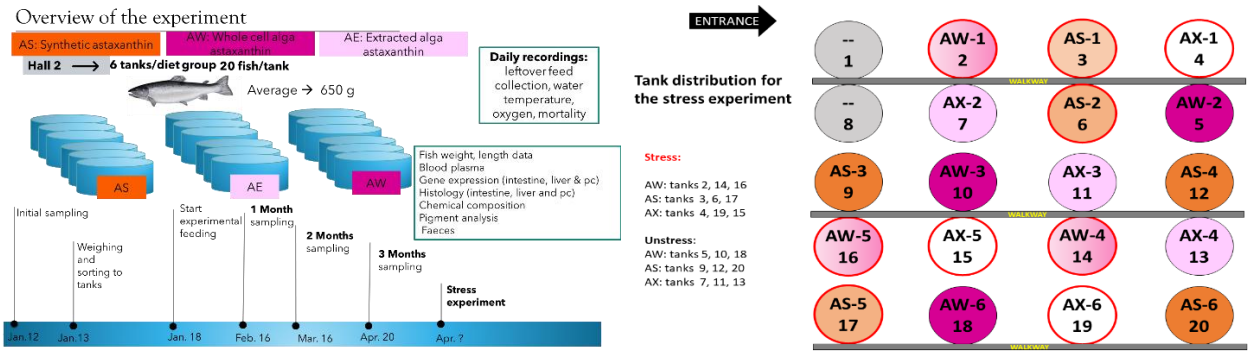


Figure 1. The experimental design of which the fish was used to study the potential of Videometer devices to measure Atlantic salmon's textural and colorimetric properties.

2.1.2 Issues related to melanin spots

Downgraded fillets (fillets containing melanin) were obtained from a nearby salmon processing plant to obtain multispectral data of fillets showing melanin spots. The experiment was conducted twice, of which the first was evaluated using abVideometerLAB2, whereas VideometerLite, abVideometerLAB2, and DigiEye were used to obtain data in the second one. In both experiments, images of the melanin spot were captured, and a nearby area was used as the reference tissue (Figure 2). The fillets used in these experiments were selected to give a visual gradient melanin score from light grey to dark black. In trial 1, ten fillets were used, whereas 20 was used in the second experiment. In D6.2, colorimetric and reflective data obtained from the VideometerLite prototype is reported, as well as an evaluation of two different imaging capturing protocols.

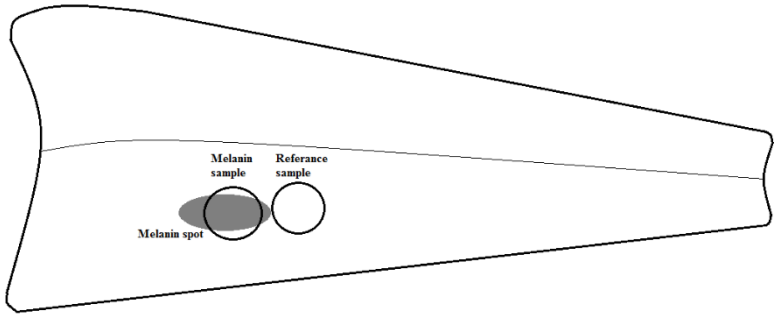


Figure 2. Schematic illustration of sampling locations on fillets containing melanin spots.

2.1.3 Issus related poor bleeding and residual blood

Poor bleeding happens, and residual blood in the salmon fillets is a concern for the salmon processing industry. An experiment is designed with different bleeding protocols to obtain fish with a muscle blood gradient (Figure 3). The worst-case scenario will be unbleed gutted fish, filleted, with no further handling to remove the blood. The other groups will consist of gill-cutted gutted fish bled in a make-shift Chilled Seawater System (CSW) for either 15, 30, 45, 60, or 90 min before filleting. The sample size will be a minimum of five individuals, giving ten fillets per group, of which each experimental group has different residual blood levels. Additionally, fillets will be vacuum packaged and stored for 21 days to realize muscle blood to the fillet surface for improved calculations. The experiment will be conducted in June 2023 at the NTNUs salmon research farm in Romsdalsfjorden, Norway. We will use the VideomterLite prototype to measure residual fillet blood on-site and repeat the measurements after arrival at NTNU's laboratory on day one post-bleeding. When measurements are repeated, the multispectral blood data will be compared to the visual blood score and DigiEye measurements. No results from this trial will be presented in D6.2.

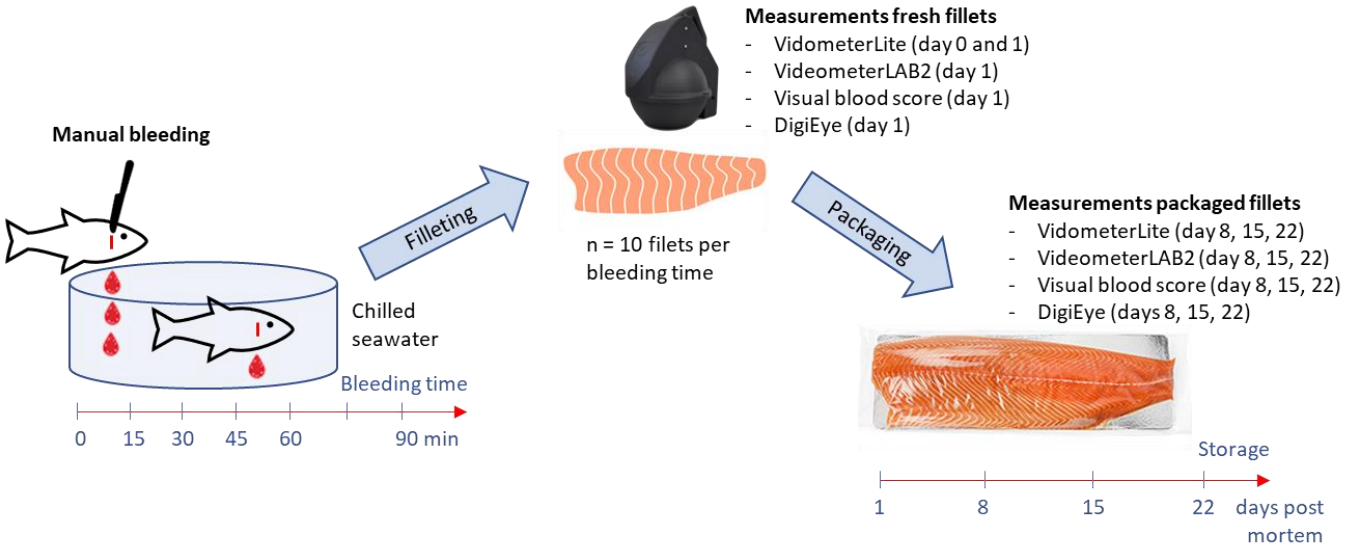


Figure 3 The Experimental design of which the fish was used to study the potential of Videometer devices to measure residual blood in Atlantic salmon filets.

2.1.4 Issues related to loss of freshness and microbial spoilage

Salmon shelf life highly depends on distribution- and storage conditions such as temperature, packaging atmosphere, and degree of processing (whole fish versus fillets). Two laboratory experiments were designed to follow quality changes of whole salmon and salmon fillets during cold storage.

2.1.4.1 Storage trial of whole Atlantic salmon on ice

The first experiment (Figure 4) was designed to test the potential of the VideometerLite and VideometerLab2 to evaluate quality changes (freshness and microbial spoilage) as a function of storage time for raw, farmed Head-On-Gutted (HOG) salmon stored on ice. Atlantic salmon was slaughtered at a nearby slaughterhouse in Mid-Norway and immediately transported on ice in polystyrene (EPS) boxes to NTNU, arriving at the laboratory five hours post-slaughter.

Images of eyes, gills, and skin obtained by the VideometerLite prototype and LAB2 were compared to the traditional Quality Index Method (QIM) scheme for raw, farmed HOG salmon in two independent experiments. A total sample size of ten individuals was used. The second experiment included microbial analysis of total aerobic and specific spoilage microorganisms (Figure 4 and Table 1). A cloth was used to wipe off the skin of one side of each fish sample. After sampling, the cloth was reintroduced into its original plastic bag and immediately analyzed. Cloth samples were properly diluted with peptone water before plating.

QIM scores and microbial counts on skin and gills were correlated against imaging data obtained from the Videometer devices to understand better the potential of the VideometerLite prototype for measuring the loss of freshness and spoilage of HOG salmon as an alternative to the QIM evaluation currently used along the Atlantic salmon value chain. In D6.2, reflective data from skin and gills obtained from the VideometerLite prototype is reported, and correlated against QIM, and microbial growth. Detailed results will be presented in the final Pilot Evaluation & KPI report (to be published October 2023) or through other channels.

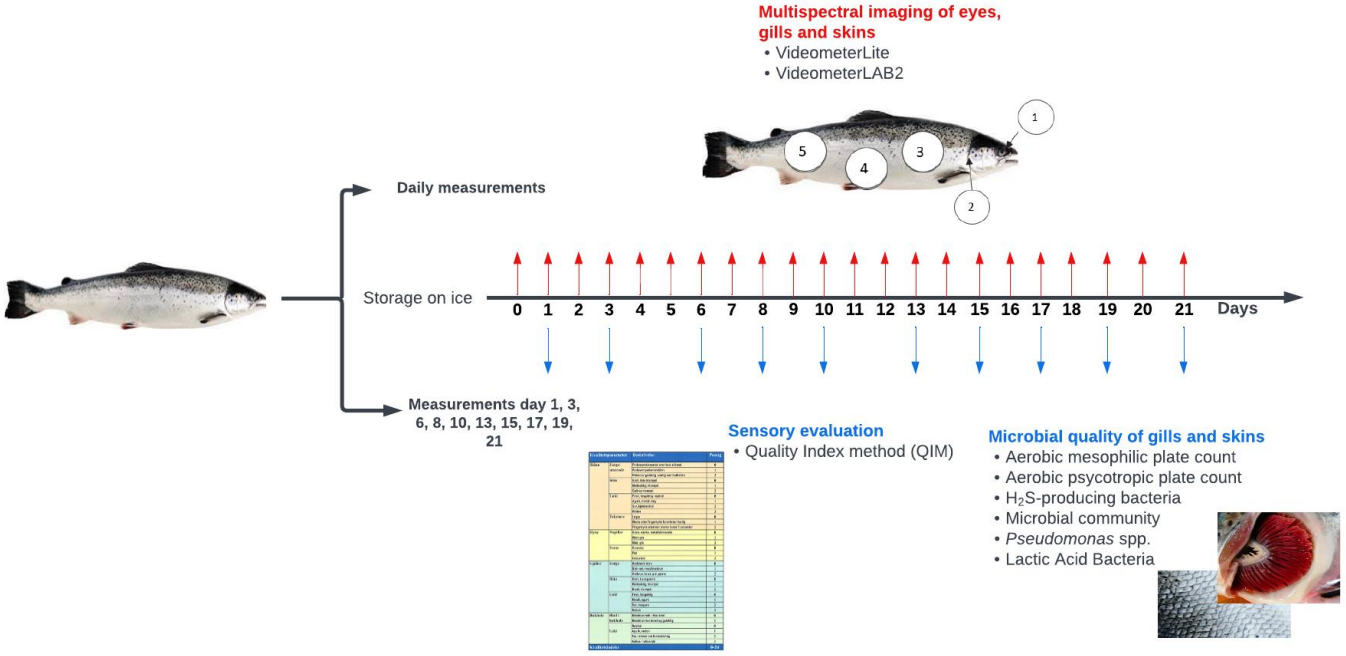


Figure 4 The Experimental design of the fish was used to study the potential of Videometer devices to evaluate quality changes (freshness and microbial spoilage) of HOG salmon stored on ice as a function of storage time.

Table 1 Culture media, target microorganisms, and incubation conditions for the microbial analysis

Culture media	Target microorganisms	Incubation conditions
Iron agar	Aerobic mesophilic bacteria and H_2S-producing bacteria	22°C for three days
Long and Hammer agar	Psychrotrophic aerobic bacteria	15°C for five days
Man, Rogosa and Sharp agar	Lactic Acid Bacteria	25°C for 5 days
Pseudomonas Agar	*Pseudomonas* spp.	25°C for 2 days

Physiochemical analyses: The salmon fillet's textural, colorimetric, and water-holding properties will be evaluated as affected by storage time. These data will be obtained using a texture analyser, imaging, and in-house methodology for water holding capacity (based on centrifugation), as well as VideometerLite and Videometer Lab.

2.2 DATA ACQUISITION AND DESCRIPTION

Images captured in the beforementioned experiments consist of a large amount of data that must be analysed to extract the information of interest to validate the different issues that were raised in D2.2 *User requirement specification (v2.0)*. Image analyses were performed using segmentation where the image transformation was fixed to fit the specific tissue analysed. Therefore, different transformations were used for *i.e.*, the fish skin, the melanized tissue, eyes, gills, and the muscle. Using the muscle as an example, a transformation removing the background and the connective tissue was used.

Obtained data was first examined to verify the potential of using the chosen devices and their setup to accurately predict the identified KPIs in the salmon value chain. An example of a dataset obtained from the VideometerLite prototype is shown in Figure 5 (data obtained from studying melanin spots).

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	**Videometer Results**															
2																
3	**Average**									**Stdev**						
4	Range	Violett	Blue	Cyan	Amber	Red	Red	NIR		Violett	Blue	Cyan	Amber	Red	Red	NIR
5	nm	405	460	525	590	621	660	850		405	460	525	590	621	660	850
6	NQCPS-01	14.20	9.73	12.34	40.11	57.44	63.44	57.80		2.35	2.08	2.25	3.07	2.70	2.72	2.20
7	NQCPS-02	12.71	9.25	11.80	38.31	55.93	63.48	59.11		2.30	2.07	2.26	3.77	3.81	3.76	3.07
8	NQCPS-03	13.40	10.48	12.89	39.07	56.36	63.11	58.30		3.74	3.54	3.40	4.26	4.27	4.63	3.53
9	NQCPS-04	12.49	8.94	11.56	37.35	55.00	61.17	58.08		2.09	1.91	2.08	3.28	3.66	4.10	3.22
10	NQCPS-05	13.36	9.54	11.75	37.15	55.21	61.62	56.75		3.08	2.63	2.68	4.00	3.64	3.75	2.99
11	NQCPS-06	13.45	9.60	12.30	39.37	58.29	65.76	61.17		2.84	2.49	2.57	3.89	3.77	4.17	3.23
12	NQCPS-07	13.95	11.00	13.45	38.95	56.40	63.66	60.29		2.88	2.87	2.83	3.71	4.87	4.82	3.72
13	NQCPS-08	15.26	11.39	14.63	43.94	60.13	65.44	59.94		2.64	2.39	2.48	3.55	4.06	4.41	3.45
14	NQCPS-09	14.49	11.15	13.56	37.46	54.34	60.22	57.29		3.21	2.96	3.11	4.22	4.25	4.30	3.52
15	NQCPS-10	14.54	11.53	14.24	39.12	56.65	63.05	58.96		2.46	2.41	2.39	3.38	3.83	4.34	3.43
16	NQCPS-11	14.09	10.75	14.06	42.40	59.67	66.16	61.09		3.00	2.63	2.73	4.12	4.65	5.14	3.97
17	NQCPS-12	13.92	10.99	13.36	38.87	58.37	65.53	60.91		2.53	2.41	2.48	3.70	3.25	3.02	2.55
18	NQCPS-13	14.99	11.04	13.54	39.82	56.26	63.45	59.34		3.15	2.91	2.93	3.99	4.92	4.94	3.70
19	NQCPS-14	14.49	10.27	12.98	41.89	60.63	66.68	61.43		2.69	2.39	2.46	3.47	3.07	3.15	2.57
20	NQCPS-15	14.28	10.12	12.63	37.68	54.89	61.77	57.73		2.77	2.29	2.37	3.23	2.95	2.72	2.17

Figure 5 An example of a dataset obtained from the VideometerLite prototype that will be used for the prediction.

2.2.1 Issues related to pre-slaughtering stress and fish handling

The experimental design (shown in Figure 1) gave us a fillet firmness (force, N at 60% compression, F60) gradient ranging from 9.7 to 21.0 N and a surface breaking force (B_f) gradient from 9.9 to 26.4 N. The average firmness and B_f were 13.7 ± 2.5 N and 14.3 ± 3.2 N, respectively (n=40). The likelihood distribution of the two investigated parameters were as shown in Figure 6.

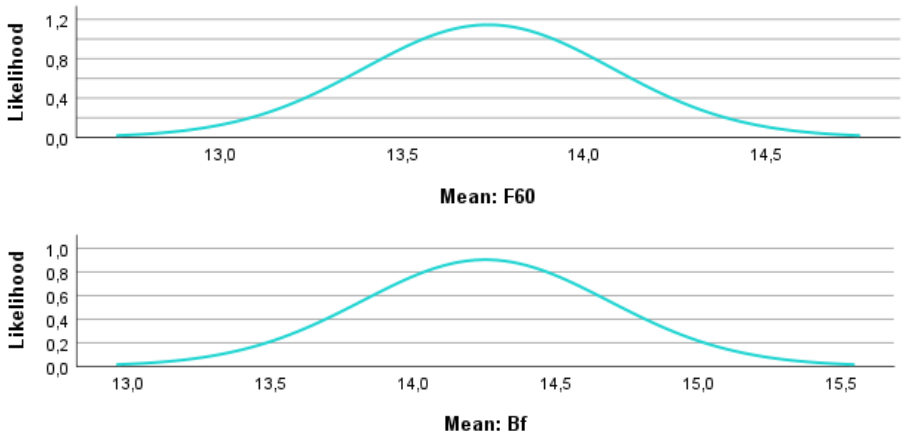


Figure 6 The likelihood distribution in fillet firmness, N at 60% compression (F60) and the surface breaking force (Bf) in N of the experimental fish.

To investigate the potential of the VideometerLite prototype to identify fillets with poor texture, images were captured two- and eight-days post slaughtering and acquisition data obtained from the images were correlated against the F60 and B_f values. Unfortunately, no correlations were found between the investigated wavelengths (405, 460, 525, 590, 621, 660, and 850nm), and the textural parameters measured (F60 and B_f). The obtained person correlation (r) ranged from -0.259 to 0.121 with p-values higher than 0.059 for the Bf, and from -0.149 to 0.204 with p-values higher than 0.139 for the F60 value, *i.e.*, no observed relationship between

any of the wavelengths given by the VideometerLite prototype, and the fillet firmness nor the surface breaking force.

To further test the possibility to distinguish between soft and firm fillets, we decided to group them related to their F60 value. By this grouping, we ended with three groups represented soft (F60 < 12N), average (F60 between 12-15 N) and firm fillets (F60 > 15 N). Although no single wavelength differences between groups (Figure 7, ANOVA, P = 0.0129-0.661), a significant main effect was observed when considering all wavelengths giving on average soft fillets lower reflection than firm fillets placing the average firm fillets in between (Figure 7, GLM, $P_{firmnes}$ = 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.

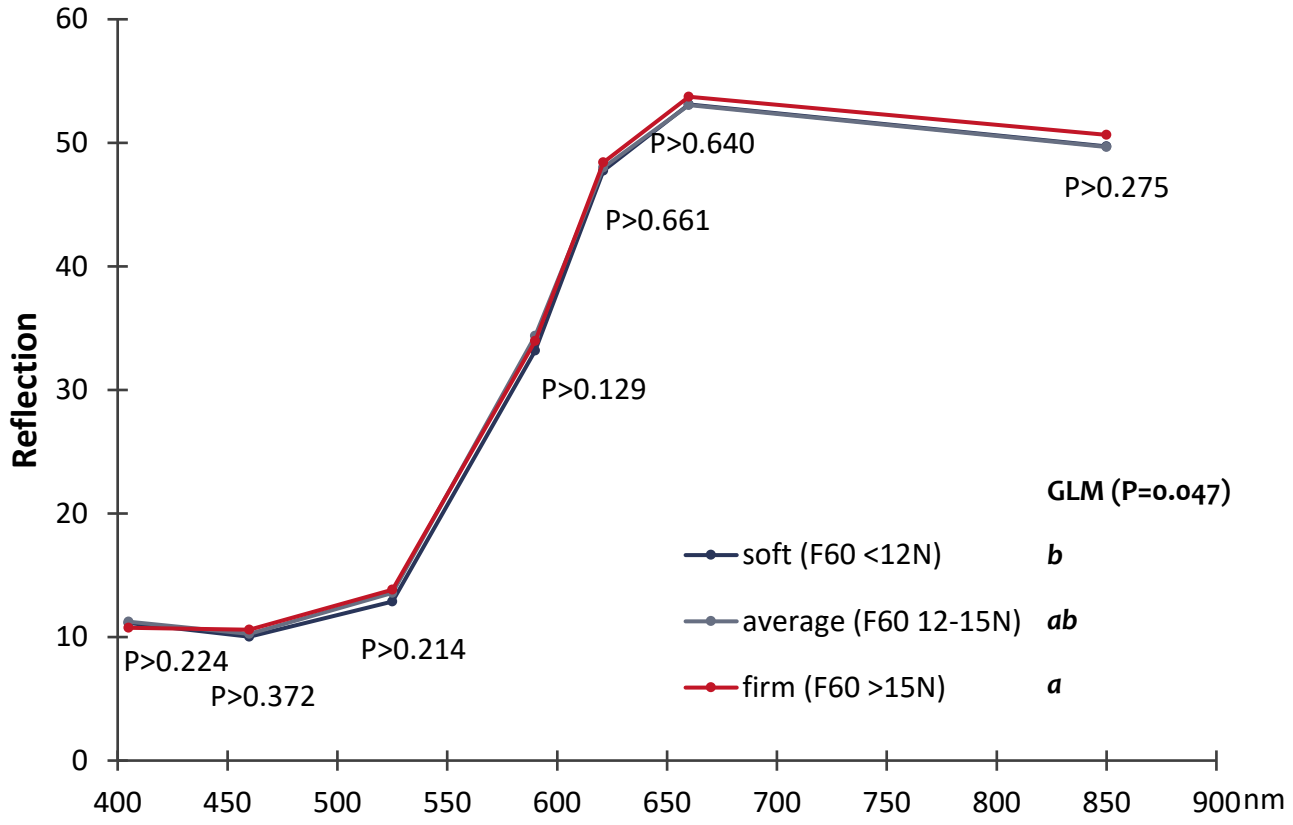


Figure 7 Reflection properties of soft (F60<12N), average (F60, 12-15N), and firm (F60>15N) fillets measured with the VideometerLite prototype. Annotated p-values were calculated by one-way ANOVA, whereas the main effect of fillet firmness was calculated by GLM. Significant differences between groups were found using Tukeys comparison test and are as lower-case letters (ab) on the right side of the group legends. The α-level was set to 5 %.

2.2.2 Issues related to melanin spots

2.2.2.1 *Investigating melanin spots in salmon fillets using VideometerLAB2*

Although the amount of data is large, only a limited amount is analysed. However, a summary is given on the use of abvideometerLab2 in analysing fillet melanin. In this experiment, ten fillets with melanin spots were selected (Figure 8). Visual evaluation of the salmon fillets sampled for analyses showed a melanin score of 3.05 ± 1.22 (ranging from 1-4) according to the standard procedure worked out by a group of scientists and quality managers (Mørkøre, 2012). In eight of the fillets, melanized focal changes were in the belly region, whereas two fillets had focal changes in the dorsal muscle.

Multispectral imaging (abVideometerLab2) of melanized tissue showed a significantly lower reflection of light ($P < 0.001$) in the green/red part of the visible spectrum (VIS, 525-700 nm) and the near-infrared spectrum (NIR, 700-970 nm) as compared to the reference tissue (Figure 9). 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.

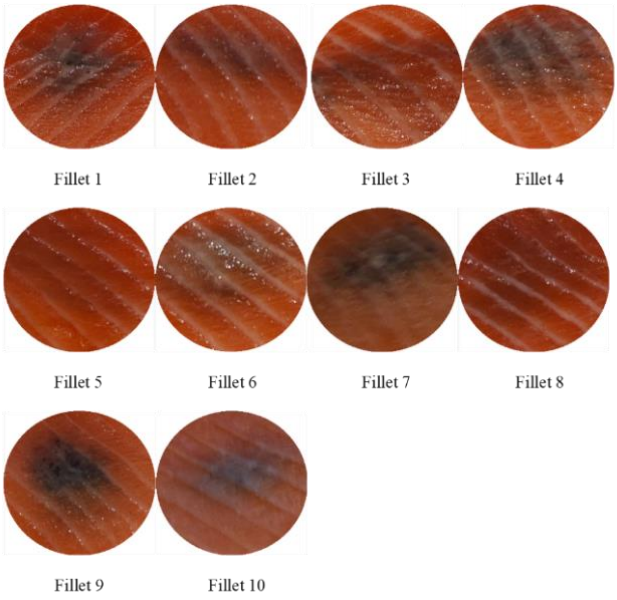


Figure 8 Melanin spots sampled for analyses.

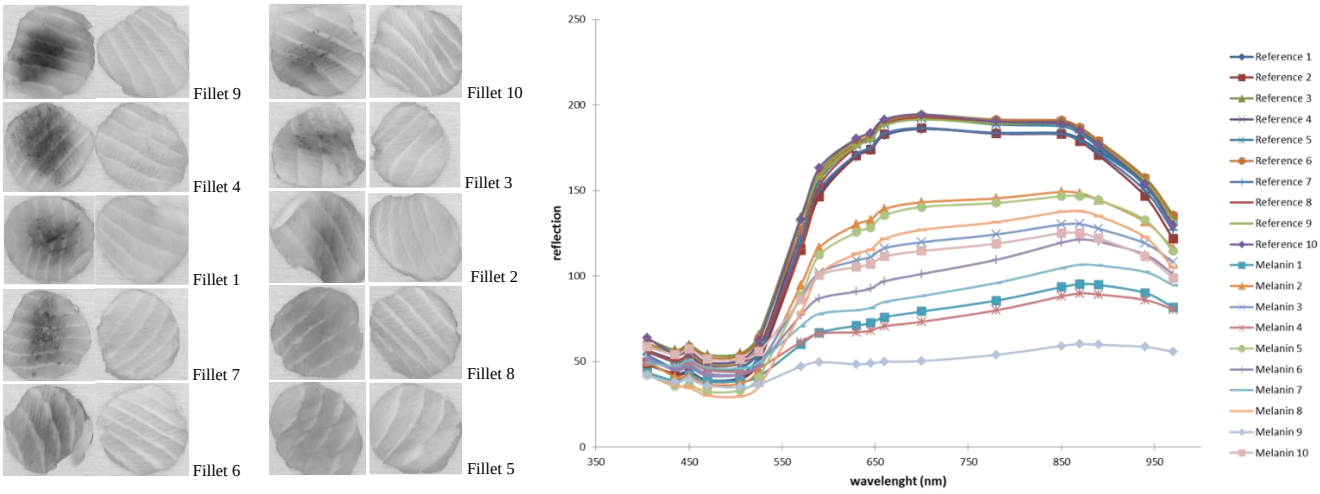


Figure 9 NIR images of salmon fillets sampled for analyses ranged after increased light reflection (decreased melanin intensity) at 870 nm. All images were captured in a VideometerLab2 multispectral system (Videometer A/S, HerlevHerlev, Denmark).

2.2.2.2 *Investigating melanin spots in salmon fillets using the VideometerLite prototype*

Melanin spots are visualized as gray to black pigments, often located in the myocomata and the myosepts. As an initial measure, the colorimetric properties (*LCH*) of the selected ROIs were investigated (Figure 10). 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 ($P < 0.008$).

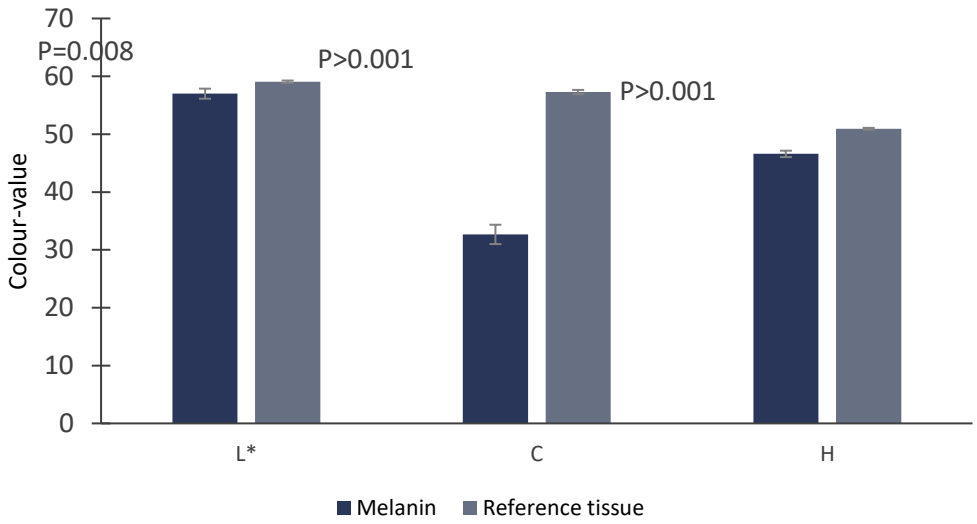


Figure 10 Colorimetric differences between melanized and reference tissue as measured with the Videometerlite prototype. Statistical differences were found by One-Way ANOVA. Error bars represent one standard deviation.

By looking into the reflective properties at each of the wavelengths provided by the VideometerLite prototype for the melanized and the reference tissue, a clear pattern distinguishes the spots and the reference tissue. To verify the image capturing protocol two strategies were tested. Firstly, images were captured of whole fillets whereas the second protocol included portioning to avoid light pollution and instabilities when capturing the images. Although disturbance with both light, and movements, the results identified several wavelengths to distinguish between the melanized and the reference tissue without cutting the fillets into pieces. Based on the results, four wavelengths (405, 460, 525, and 660) stands out with a potential to identify melanized tissue of whole fillets (Figure 11).

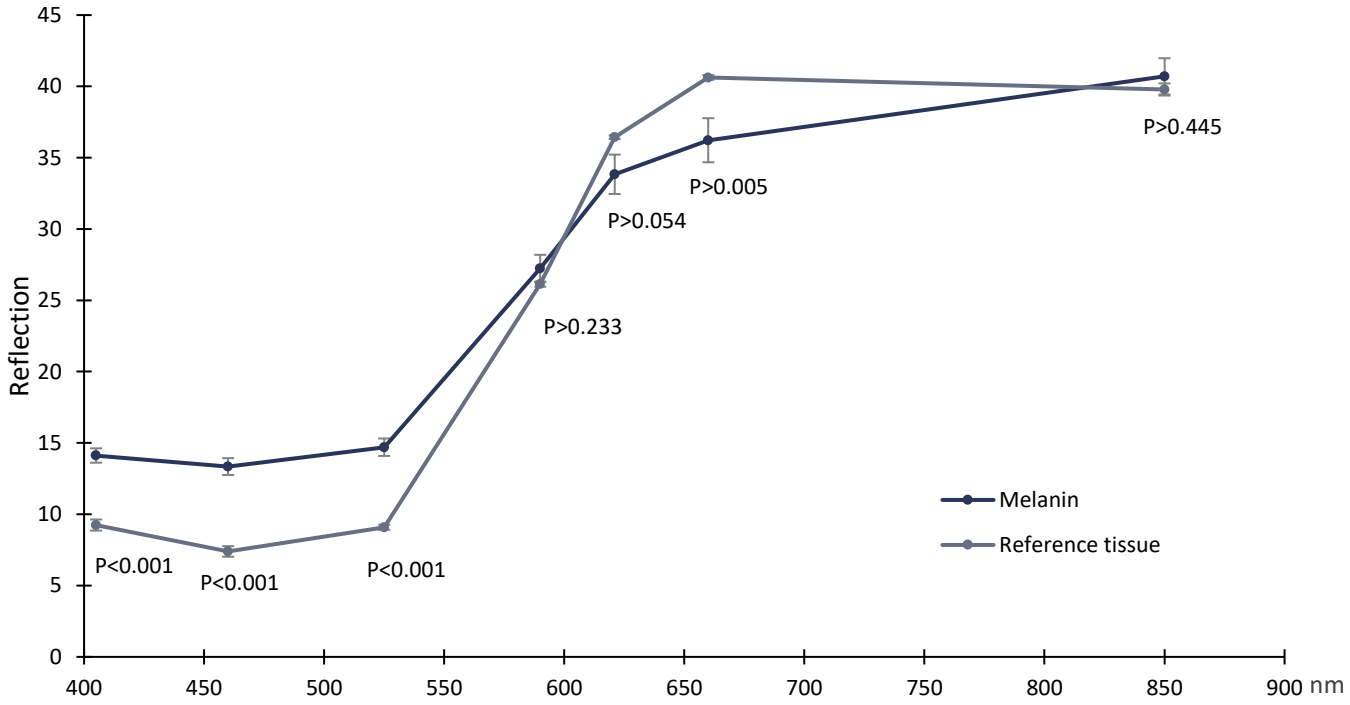


Figure 11 Reflection properties of melanized and reference tissue obtained from images captured with the VideometerLite prototype on whole fillets (a protocol that issues related to light pollution and stability). Significant differences between groups were found using One-Way ANOVA. Error bars represent one standard deviation. The α-level was set to 5 %.

To investigate the potential of the VideometerLite prototype to distinguish between the melanized and the reference tissue under optimized condition images of fillet portions fitted to the diameter of the VideometerLite capturing area. In this experiment, the same samples were used as for the whole-fish experiment (Figure 11) to secure comparable results. The results obtained from the second experiment performed under controlled conditions, verified the potential of identifying melanin spots on whole fillets. However, the pattern found was clearer and gave significant differences between the melanized and the reference tissue at all investigated wavelengths (Figure 12).

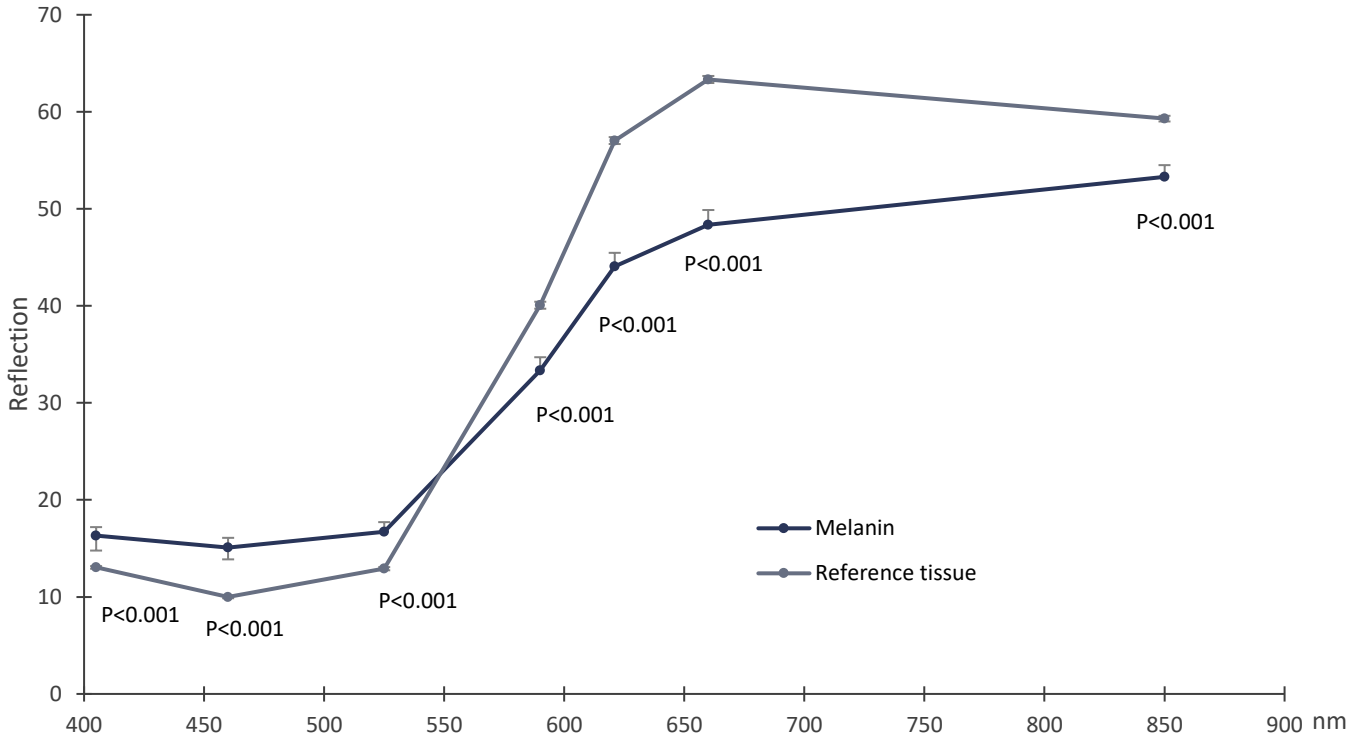


Figure 12 Reflection properties of melanized and reference tissue obtained from images captured with VideometerLite prototype on fillet portions (a protocol that was designed to optimize the imaging capturing conditions). Significant differences between groups were found using One-Way ANOVA. Error bars represent one standard deviation. The α-level was set to 5 %.

2.2.3 Issus related poor bleeding and residual blood

The planned experiment to investigate the potential of the VideometerLite prototype to detect residual fillet blood (Figure 3) has been postponing until June 2023. Results from this trial will be presented in the Final Pilot Evaluation & KPI report (to be published October 2023).

2.2.4 Issus related to microbial spoilage and loss of freshness and physiochemical quality

2.2.4.1 *Storage trial of whole Atlantic salmon on ice*

This experiment assessed the potential of VideometerLite and VideometerLab2 to measure quality changes of whole Atlantic salmon during ice storage for 17 days. Traditional QIM and microbial analysis were used to assess salmon freshness and microbial spoilage. The fish was assessed as fresh (QIM score< 5), mature (5<QIM score <15) or spoiled (QIM score>15) on days 1-6, 8-15 and after storage day 15, respectively.

Evaluation of the fish skins potential to be used as an indicator of loss of freshness and salmon spoilage:

Initial average total aerobic and psychrotrophic counts per skin surface were 3.8 and 4.1 CFU per skin, respectively. Potential spoilage bacteria of *Pseudomonas*, H2S-producing bacteria, were also detected on day 1, with counts above log 3 CFU/skin (Figure 13). The microbial counts for all tested parameters, except lactic acid bacteria, increased steadily until day 17 and correlated with the increase in QIM score (r=0.941-0.974, p<0.001).

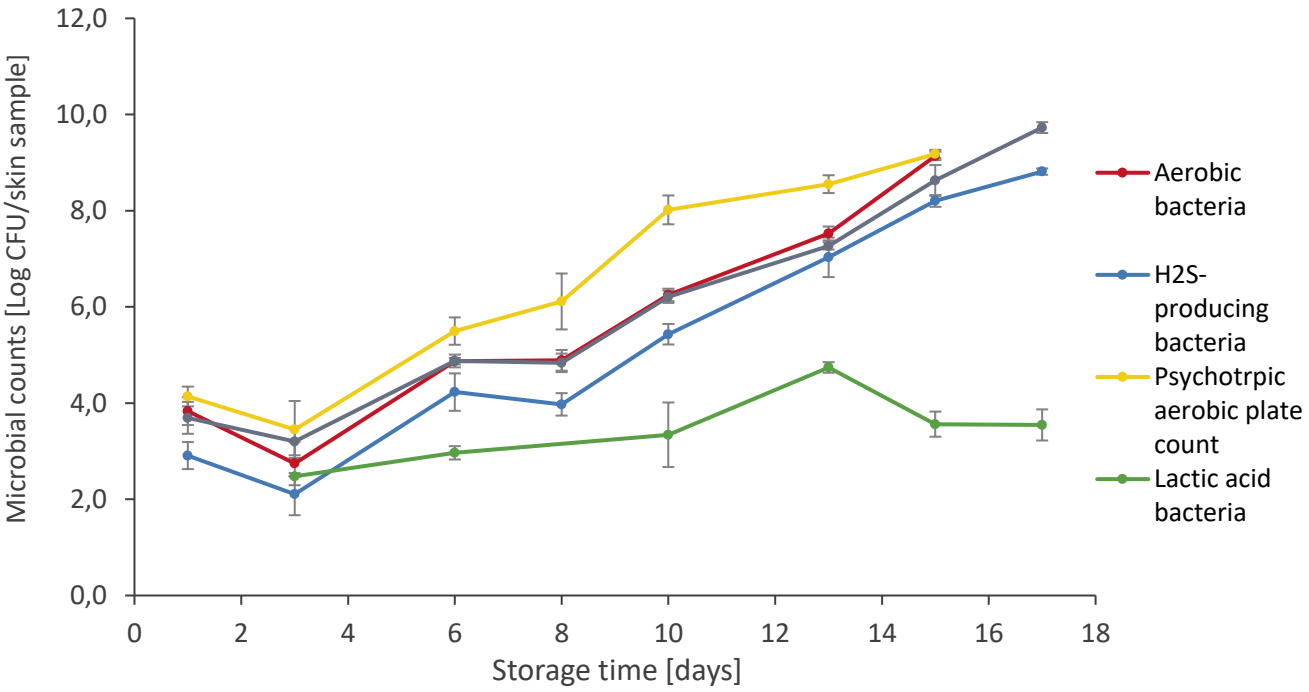


Figure 13 Microbial counts of the skin of Head-On-Gutted (HOG) salmon stored on ice. Error bars represent one standard error (n = 3)

Figure 14 shows skin reflections of fresh, mature, and spoiled salmon skin at seven wavelengths (405 nm, 460 nm, 525 nm, 590nm, 621 nm, 660 nm, 850 nm). The images are from three different skin regions, as illustrated in Figure 4. Reflections of fresh skin were significantly higher ($p<0.05$) than from spoiled skin for all wavelengths and skin regions tested (Figure 14 A-C), except for wavelength 850 nm in skin region 3 (Figure 14 A). The decrease in reflection from skin region 4 and 5 from day 1 to day 17 showed moderate negative correlation with the increase in QIM score for all wavelengths ($r=-0.792$- -0.385, $p<0.05$). The strongest correlation for both regions was found at 405 nm. For region 3, the reflection had low or no correlation with the QIM score ($r=-0.368$- -0002, $p=0.021$-0.988).

No significant differences between mature and spoiled skin reflections were observed for region 3. In region 4 (Figure 14 B), the reflections of fresh, mature, and spoiled skin were all significantly different at wavelengths 405 nm, 460 nm, 525 nm, and 590 nm. Region 4, which is the fish belly, is described in the QIM manual changing from pearl shine to yellowish during storage.

In skin region 5 (Figure 14 C), the reflection of fresh, mature, and spoiled skin was all significantly different at wavelengths 460 nm and 590 nm, whereas wavelengths the wavelengths 405 nm and 525 separated spoiled skin from the others. The fresh skin was significantly different from mature and spoiled skin at 621 nm, 660 nm, and 850 nm. Moreover, the absolute delta values observed between each category were lower in region 5 than at the wavelengths separating the categories of skin in region 4 (Figure 14 B).

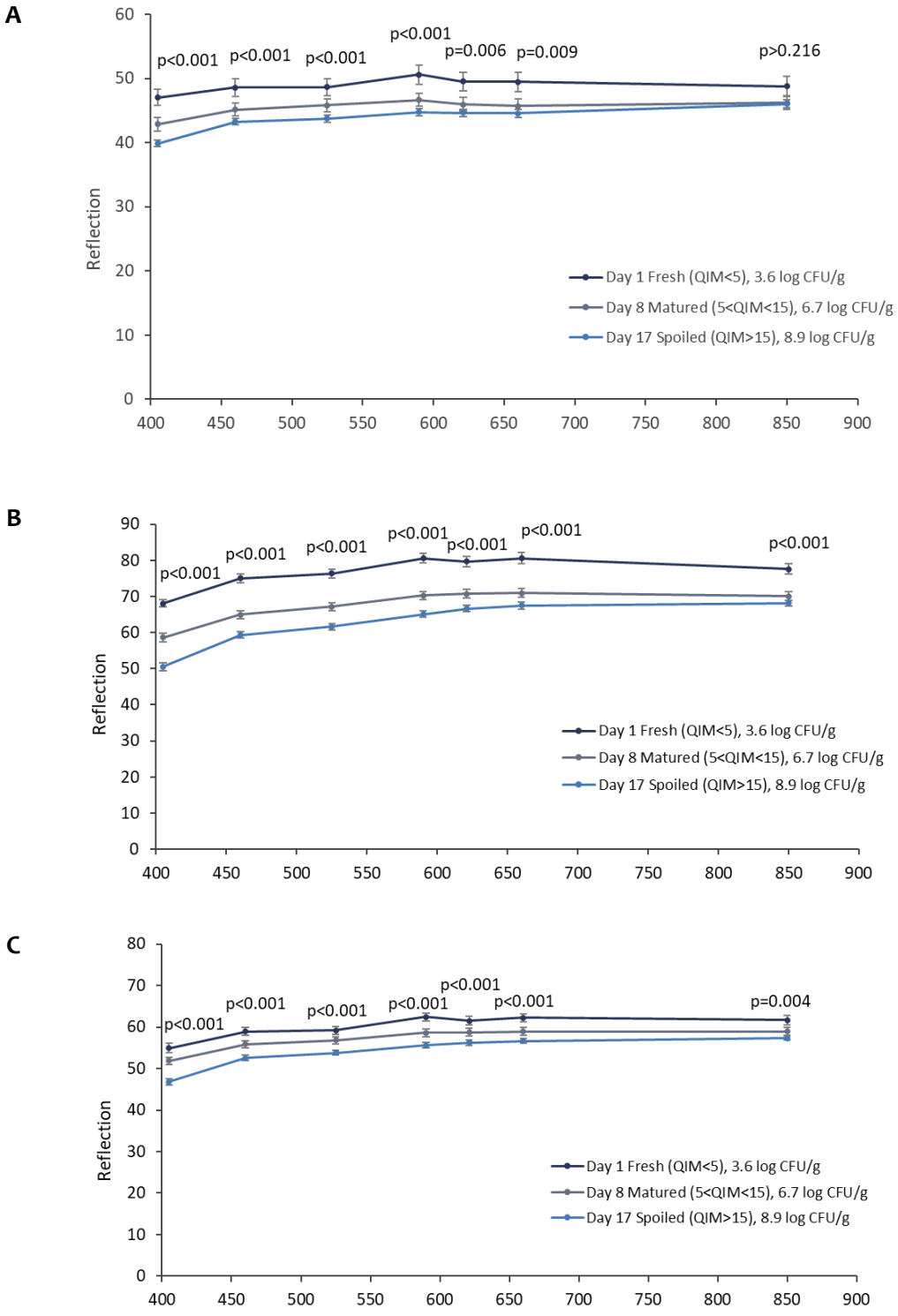


Figure 14 Reflectance from images of the skin of Head-On-Gutted (HOG) salmon stored on ice obtained from the VideometerLite prototype. A represent images captured at the dorsal region behind the gills, B the belly, and C, the dorsal part of the Norwegian Quality Cut (NQC). Bars represent one standard deviation (n = 10).

Evaluation of the fish gills potential to be used as an indicator of loss of freshness and salmon spoilage:

Initial average total aerobic and psychrotrophic counts of gills were 3.8 and 3.6 CFU per g, respectively. Low Pseudomonas and H2S-producing bacteria counts were detected (<2.6 CFU/g) Figure 15). The microbial counts for all tested parameters, except lactic acid bacteria, increased until day 17 and correlated with the increase in QIM score (r=0.812-0.920, p<0.05).

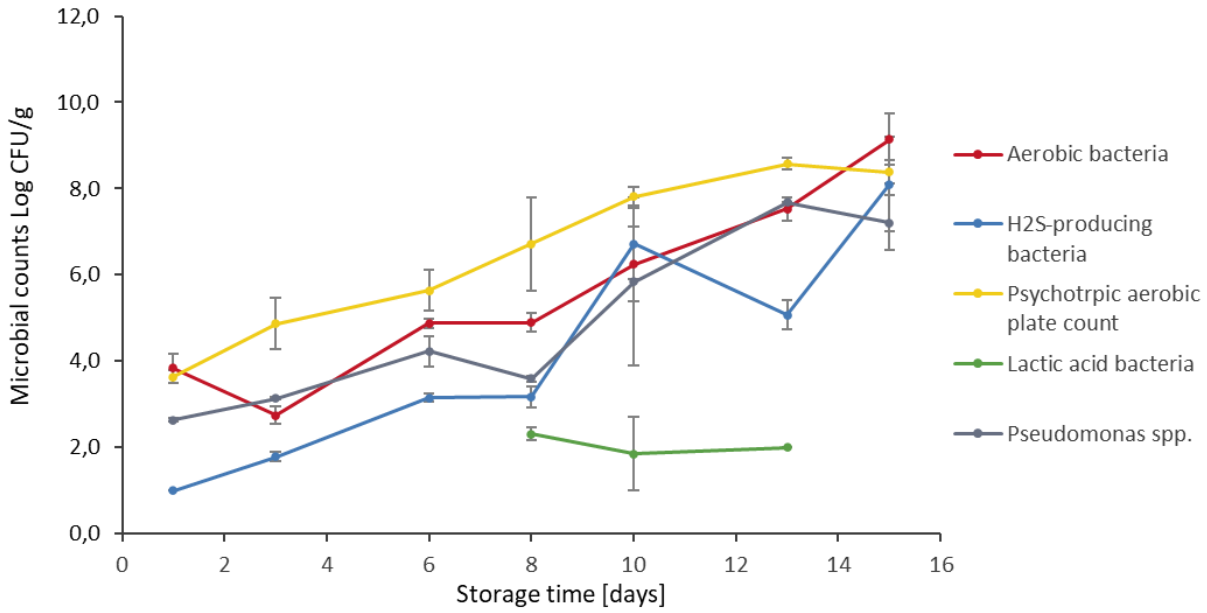


Figure 15 Microbial counts of gills of Head-On-Gutted (HOG) salmon stored on ice. Bars represent one standard error (n = 3)

Figure 16 shows reflections of gills from fresh, mature, and spoiled salmon at seven wavelengths (405 nm, 460 nm, 525 nm, 590nm, 621 nm, 660 nm, 850 nm). Significant differences were only detected between the groups at 850 nm, where spoiled gills showed higher reflectance than those analysed fresh and mature.

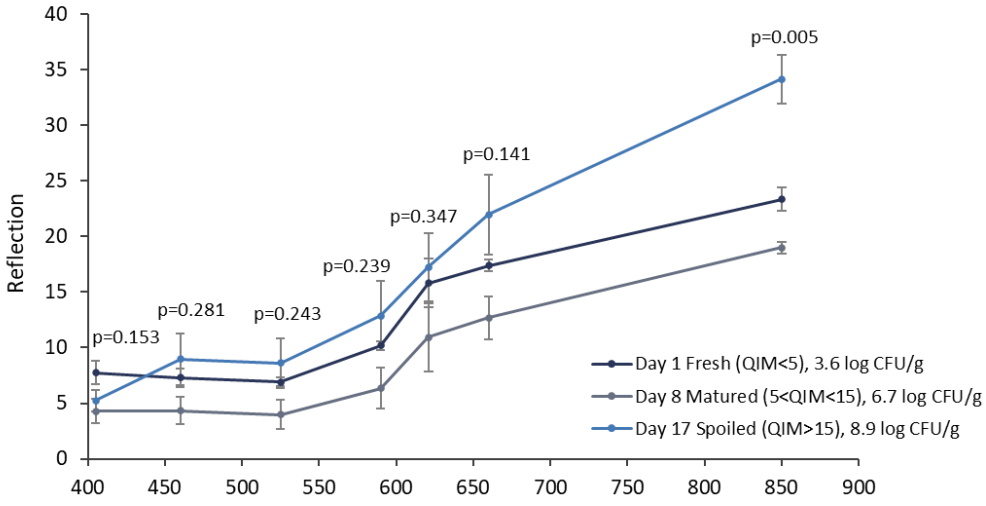


Figure 16 Comparison of reflectance from gills of fresh, mature, and spoiled Head-On-Gutted (HOG) salmon stored on ice obtained from the VideometerLite prototype. Bars represent one standard deviation (n = 20)

Issues related to the loss of quality of salmon fillets at different storage conditions will be conducted in May-June 2023. Data from this trial (as presented in chapter 2.1.1) will be shown in the final pilot evaluation & KPI report (to be published October 2023).

2.3 KPI ASSESSMENT

The *Key performance indicators* (KPIs) obtain through the presented results are several promising areas of use for the VideometerLite prototype in the evaluation of issues in the Atlantic salmon value chain described in *D2.2 - User* requirement specification (v2.0). Some specific findings to be highlighted are:

- There is a weak relationship between surface reflectance and fillet firmness.
- Colorimetric properties can be verified.
- Melanin spots can easily be detected.
- It is possible to distinguish between fresh, matured, and spoiled fish by analysing the fish skin.
- It is possible to identify spoiled fish based on the reflectance of the fish gills.

More results on other factors such as pigment concentration, muscle blood content, etc., and their evaluation as KPIs will be given the final pilot evaluation & KPI report (to be published October 2023).

3 THE ATLANTIC WHITEFISH PILOT

The Atlantic whitefish pilot includes 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 will be performed in a controlled laboratory as well as in living lab pilot environments mimicking true industrial conditions. The following trials will be 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. Monitoring of blood spots and poor bleeding
4. 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. Figure 17 shows the QIM scale and example photos showing some of the visual changes that occur during storage and are evaluated in the QIM method. As mentioned, the QIM method requires a trained 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.

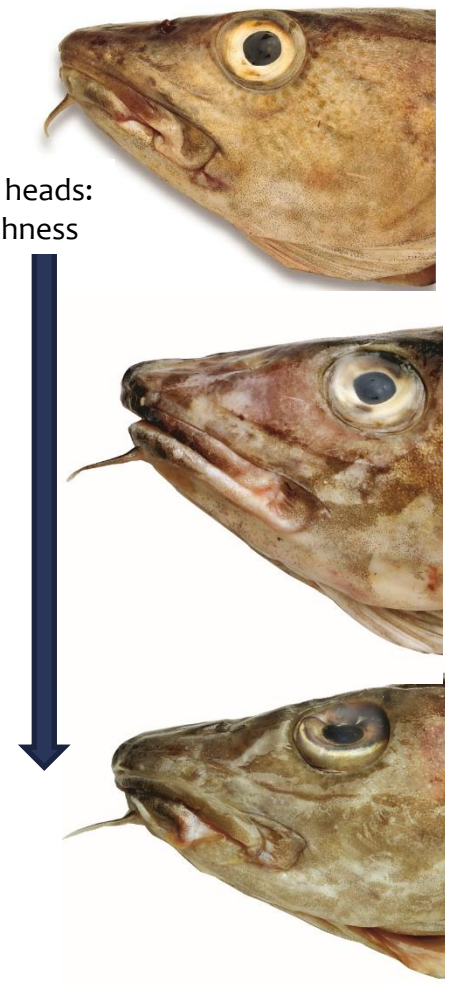
A

Quality Index Method (QIM) scheme for cod and haddock

Quality parameter		Description	Score
Appearance	Skin	Bright, iridescent pigmentation	0
		Rather dull, becoming discoloured	1
		Dull	2
	Stiffness	In rigor	0
		Firm, elastic	1
		Soft	2
		Very soft	3
Eyes	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	2
		Brown, discoloured	3
	Smell	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
Flesh, fillets	Colour	Translucent, bluish	0
		Waxy, milky	1
		Opaque, yellow, brown spots	2
Blood	Colour	Red	0
		Dark red	1
		Brown	2
Quality Index			0-23

B

Cod heads:
Freshness



C

Cod gills:
Freshness

Figure 17 A) The Quality Index Method (QIM) scale form. B) Photos of Atlantic cod (Gadus morhua) heads of decreasing freshness. C) Photos of Atlantic cod (Gadus morhua) gills of decreasing freshness. The Index for and images acquired from results of the project "Introduction of Quality Index method (QIM) in the European Fishery Chain" funded by the European Commission.

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 pre-trial showed the best way to perform the imaging was to image the eyes by placing the devices directly onto the surface of the eyes rather than taking the images at a fixed height or using additional material to try to limit additional light pollution from entering the dome of the equipment (Figure 18). This was determined through evaluating the spectral response of chosen areas of the images. With larger samples this is important to evaluate to ensure the VideometerLite can be used as a non-destructive solution for larger samples as well as smaller.

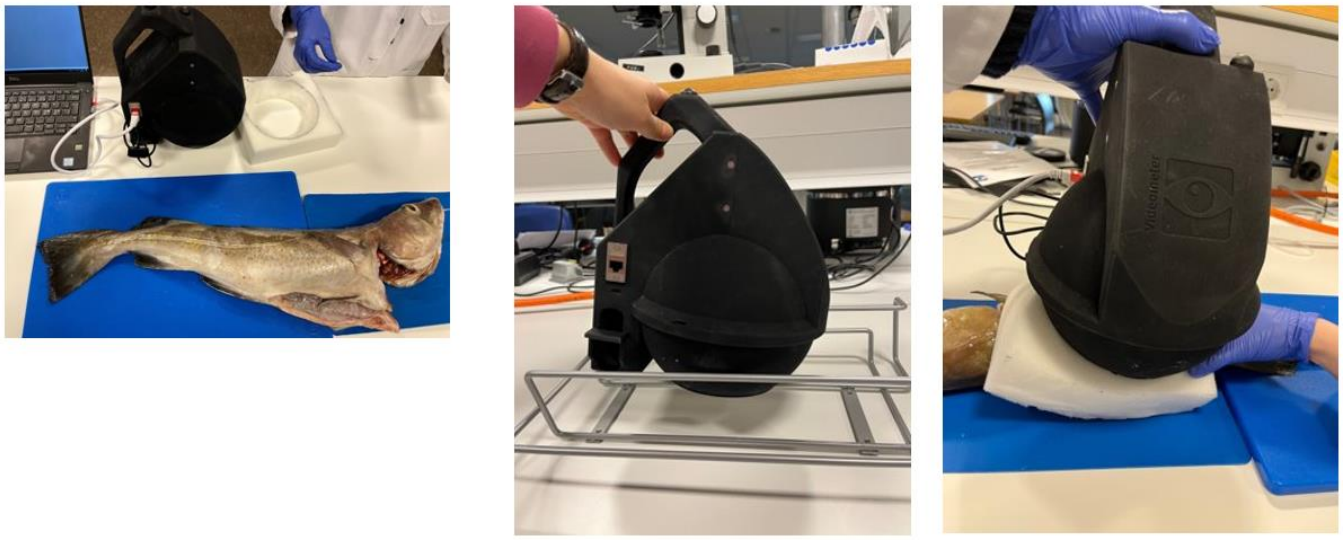


Figure 18 The setup of preliminary trials for imaging of cod eyes using the VideometerLite.

Further, 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.

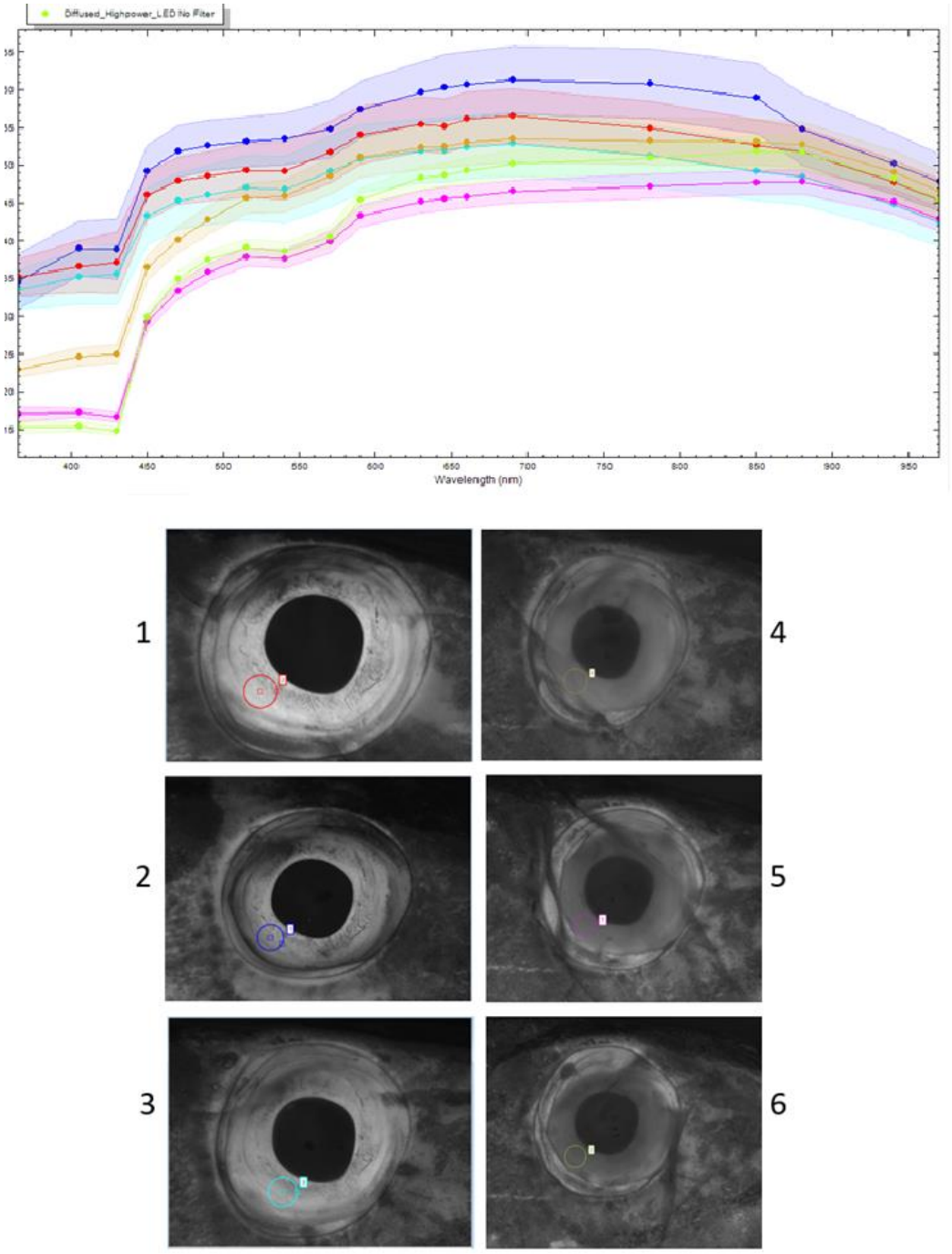


Figure 19 Results of multispectral imaging of a marked area of interest on Atlantic cod (Gadus morhua) eye throughout fresh storage and the areas spectral response.

In June 2023 a full trial will be performed comparing images and data collected in this manner (from VideometerLite and VideometerLab) to results of a full evaluation of freshness by trained sensory panellists using the QIM score and relevant microbiological and chemical analysis. Data will be evaluated to determine how effectively the technological solutions can determine freshness. The results will be presented in deliverable 6.3.

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. Two trials have been performed to evaluate the feasibility of using VIDEOM spectral imaging solutions for nematode detection, one using only VideometerLab and the other VideometerLab and VideometerLite.

The primary trials focus was possible detection and an evaluation of the depth at which the worms could be detected into the flesh (Figure 20). 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%).

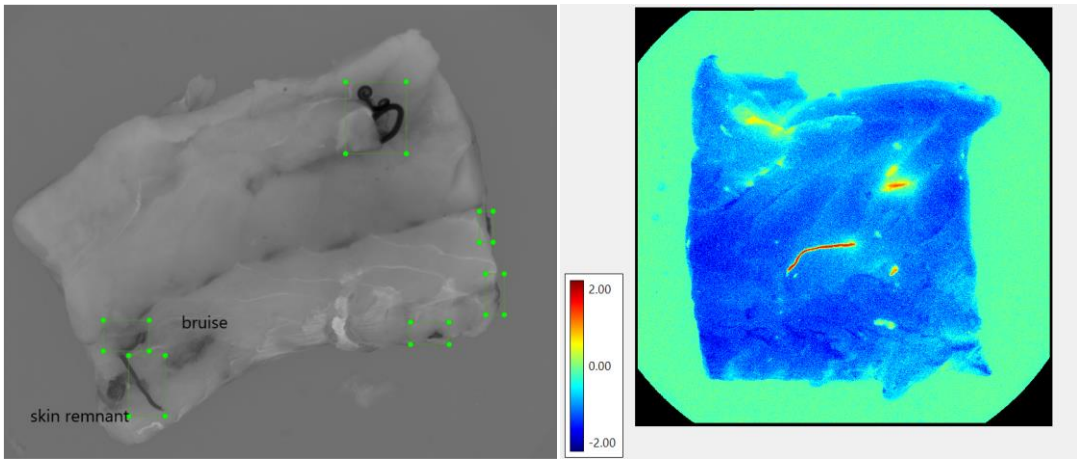


Figure 20 Left: Example of a labelled image with six nematode labels. A skin remnant and a bruise can be seen in the image as well. Right: An nCDA transformation on a random image in the data set. The red colour implies that those pixels are nematodes, and the blue implies that those pixels are fish 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 21).

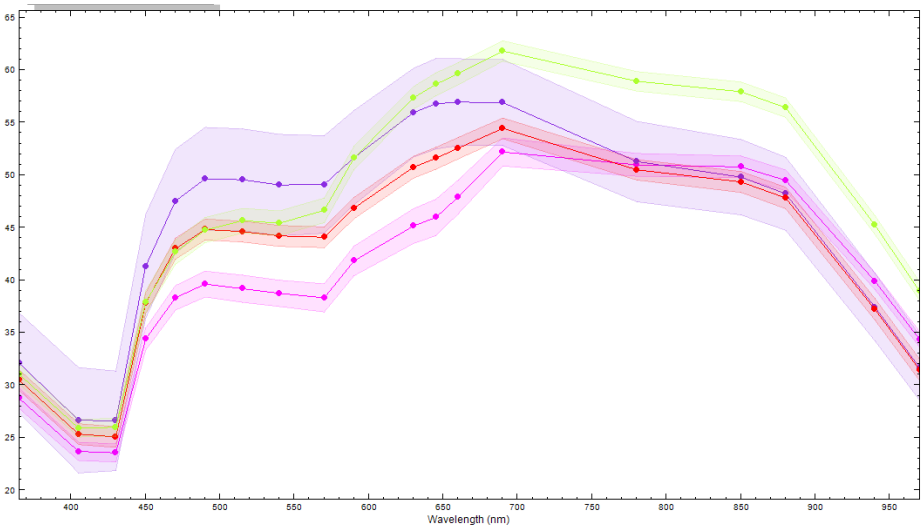


Figure 21 Spectral signature of nematodes of different colour and depth compared to fish muscle. Fish muscle (purple); Dark nematode at 7_1 mm depth (red); Dark nematode at 5_1 mm depth (fuchsia); Light yellow nematode at 5_1 mm (green).

The second trial performed in this part of the pilot aimed to compare the VideometerLab and VideometerLite capabilities to determine how those solutions compare to the industry standard method used today, candling (a manual removal of nematodes on a candling table illuminating the fillets from below). In this trial the fillet samples were kept whole to mimic industrial conditions and images collected using both devices. Further, samples were treated by a trained employee of the fish processing company Vísir which specialises in candling and the process was monitored and a video recorded.

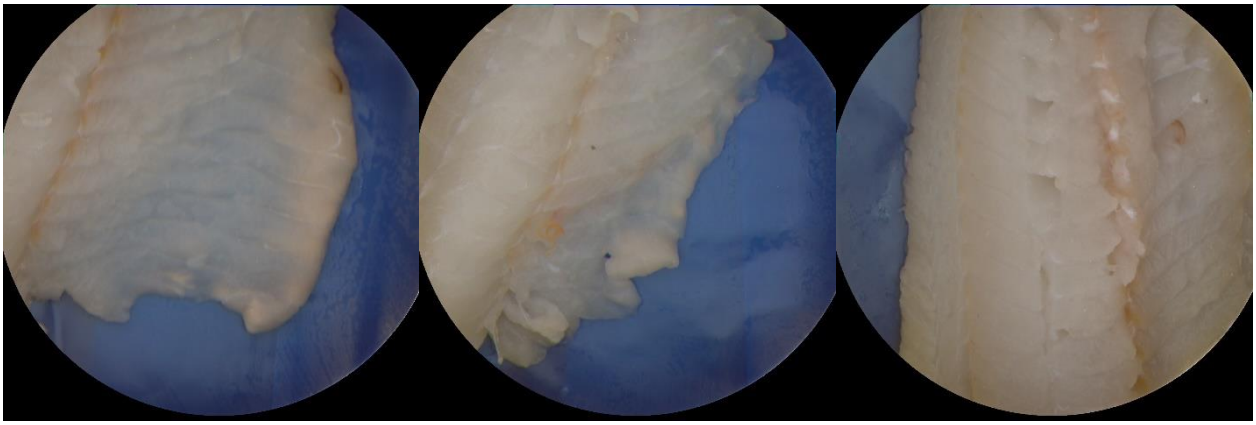


Figure 22 Images from the VideometerLite captured in the second nematode detection trial.

In the second trial it was necessary to adapt the imaging method to ensure fillets could be kept whole. Therefore, images were taken at a fixed height with an open dome. Data analysis is ongoing and a similar method has been used to process the data from the second trial as the first.

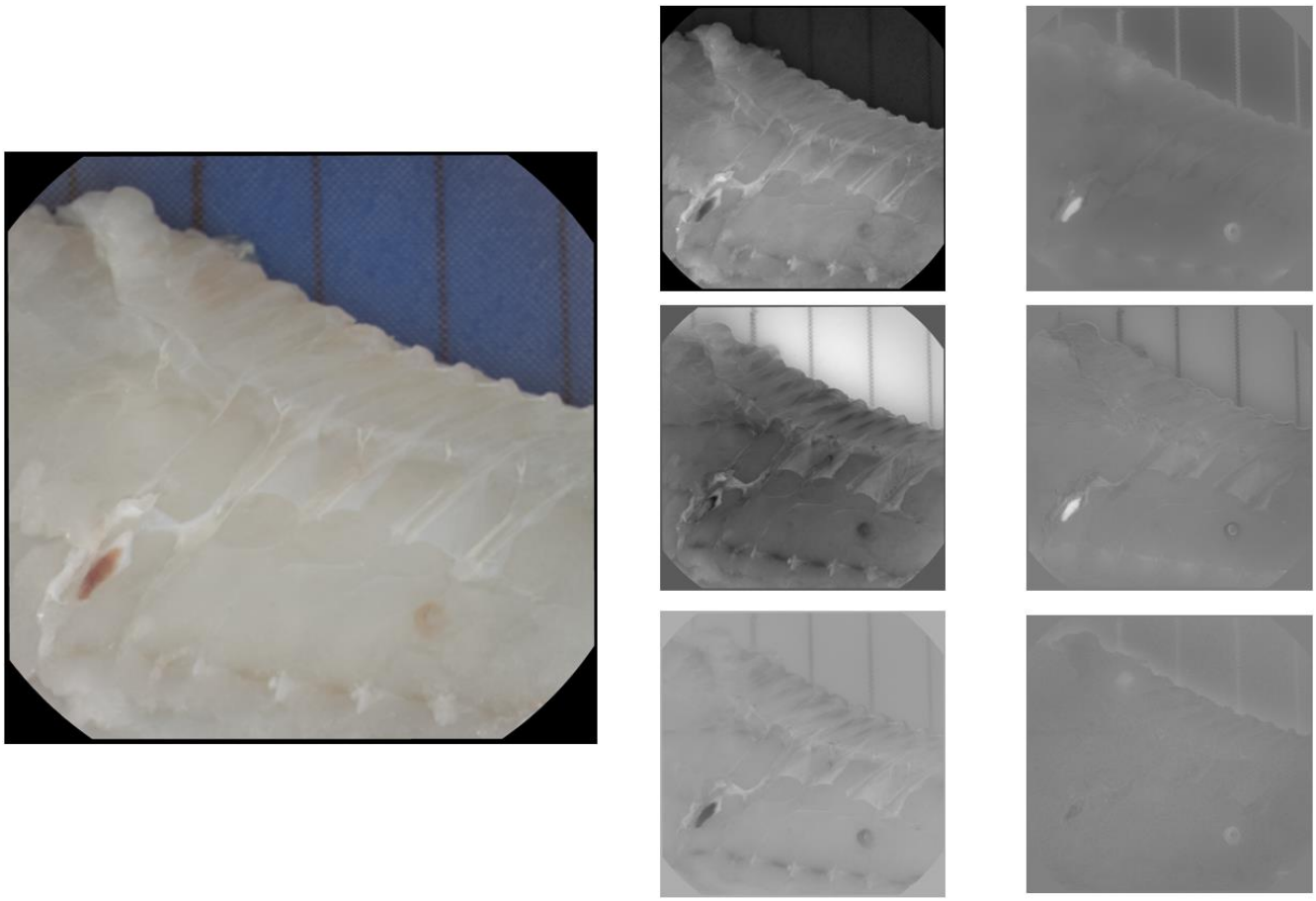


Figure 23 PCA images of Atlantic cod muscle with nematodes.

Preliminary results of the VideometerLab images show similar ability for detection when the dome was open at a fixed height as in the first trial where the dome of the VideometerLab was closed. Final results will be presented in deliverable 6.3 comparing the capabilities of the VideometerLite and VideometerLab as well as the effects of using different analysis methodologies and strategies being evaluated at this time.

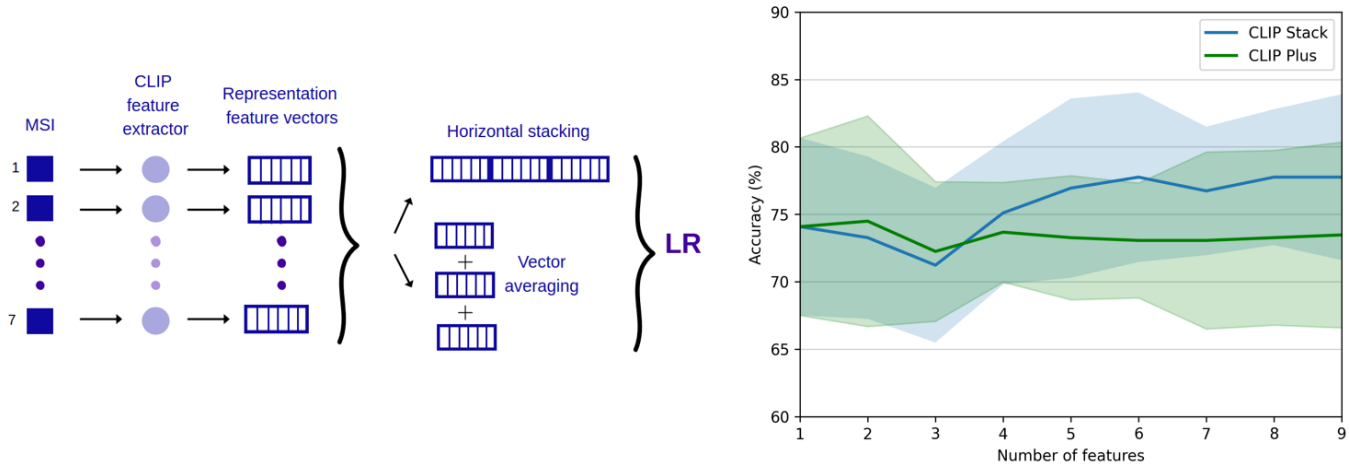


Figure 24 Classification accuracy of nematodes using CLIP.

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 25). Ongoing analysis will take this into consideration and aim to limit this even further.

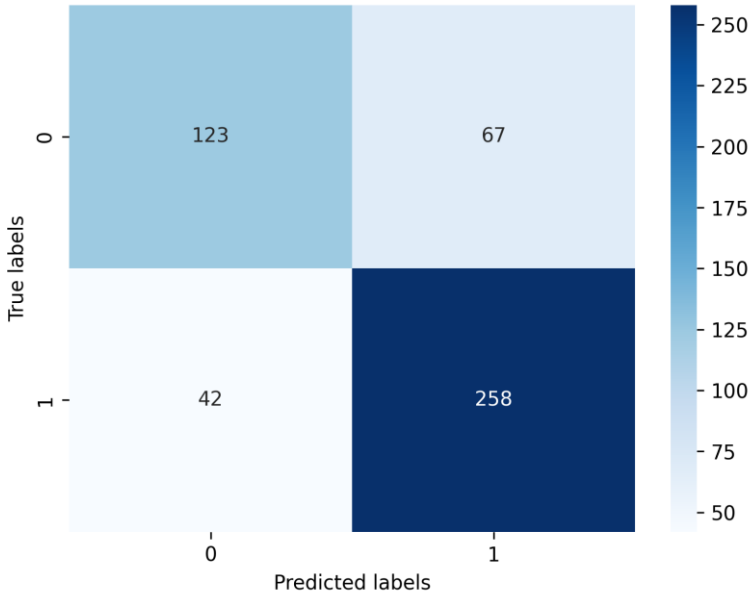


Figure 25 Results of CLIP classification model from second nematode trial. Classification 1 = nematode present, 0 = no nematode present).

3.3 MONITORING OF BLOOD SPOTS AND POOR BLEEDING

Poor bleeding of whitefish can cause issues relating to visual quality (blood spots), possibly shorten shelf life and cause issues in production of final products such as salting. A trial has been performed comparing imaging data collected from saithe or pollock (a white fish commonly caught around Iceland). Fish of different sizes (2-5 kg) had been bled for different amount of time (15-25 min) were used. The sample were processed, and images collected using both VideometerLab and VideometerLite. Data analysis is ongoing and the final results will be presented in deliverable 6.3.

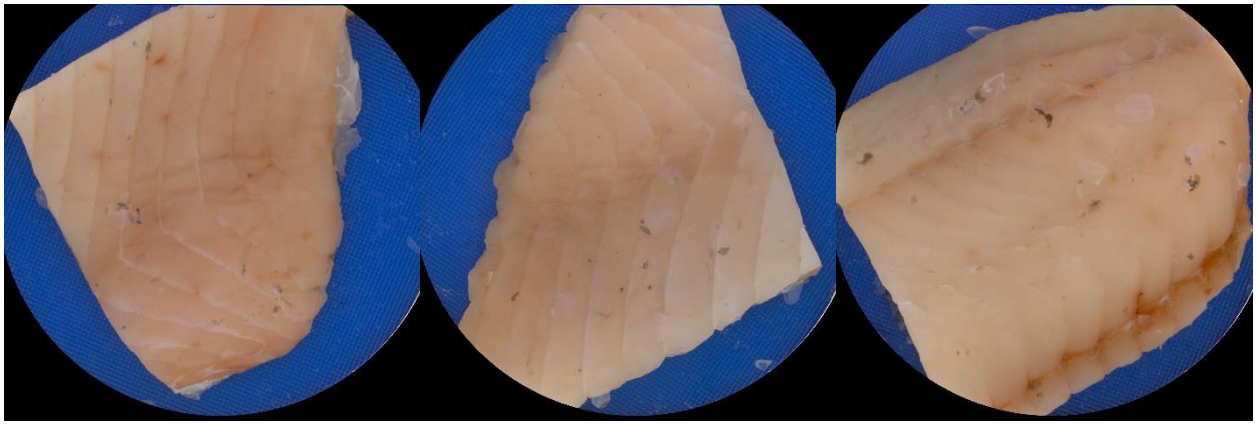


Figure 26 Images from the VideometerLite captured in the preliminary bleeding and blood spot detection trial.

3.4 TEXTURE AND FRESHNESS OF FINAL PRODUCTS

Laboratory experiments are being performed to evaluate possibilities of using the VideometerLite and/or VideometerLab to determine the texture or freshness of cod fillets during storage. Samples were collected in April 2023 and are being evaluated using the VIDEOM imaging equipment and for reference texture analysis and microbiological analysis is being performed to determine freshness. All results will be available by mid- May 2023 and final results presented in deliverable 6.3.

4 THE MEDITERRANEAN SEABREAM/SEABASS

As reported in the D6.1, seafood quality assessment is considered to be a significant part of management systems in Aquaculture, since it plays a critical role in decision making regarding several actions in aquaculture activities.

Activities and experimental plans related to seabream value chain were focused to the KPIs that should be achieved for the successful application of the proposed system. These are; 1) the availability of critical targets/sites in chain, which are ensured by chain simulation for the 3 fish types integrating the within product variability; 2) verification of the mitigation in quality/safety, authenticity, traceability and also in terms of waste via the acquired along the chain data enabling early alerts; and 3) the successful demonstration of the whole closed system in terms of accuracy, quality, traceability and mitigation verification.

In WP2 and previous Deliverables D2.2 and D2.4 the overall updated 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 specific hazards related to the production, processing and distribution of seabream/seabass were previously reported.
In this Deliverable, the whole analytical procedure for the seabream quality assessment is described in detail and some preliminary results are provided.

The analysis of data obtained from the VideometerLab2 and VideometerLite is still ongoing and the findings of these analyses will be presented in the following deliverables. However, a significant amount of data was provided to Scio, for the accomplishment of the WP4

4.1 EXPERIMENTAL DESIGN

Sample collection

The experimental design was separated in three parts as described below.

Trial 1 – Analysis of seabream fillets obtained from several selling points

The first one was related to the analysis of seabream fillets obtained from several selling points (Figure 27). In this context, packaged and non-packaged samples with different use-by-dates, from different retail stores and different brands were collected. Moreover, samples that were being sold filleted ready-to-cook and samples that were filleted at the lab were also analysed. The collected samples were subsequently stored at different temperatures (2 and 4 °C) for specific time intervals (2-3 days after the expiration date of the sample) so as to create samples of different level of freshness (fresh, semi-fresh and spoiled samples). Fifty-six (56) of these fillets were packaged in MAP conditions while 70 of them were aerobically packaged. At each sampling point (time point), the filles were subjected to microbiological, sensory and Multispectral Imaging analysis (2 samples per fillet – 252 samples in total). The experimental procedure is illustrated in Figure 28.

Figure 27 Indicative samples tested at the first trial for seabream quality assessment.

	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							

Letters correspond to different selling points

Microbiological analysis

Multispectral Imaging Analysis - VideometerLite

Sensory analysis

Figure 28 Experimental procedure of the first part

Trial 2 – Analysis of seabream fillets obtained from the Aquaculture

The second part includes the analysis of samples that were obtained directly from the facilities of Avramar, the aquaculture company collaborating with AUA in the framework of the TMF project (Figure 29). In this case, both aerobically and vacuum packaged samples stored at 2 and 4 °C were tested. In this trial several parameters related to seabream quality were investigated, including microbiological and sensory analysis (presented previously in D6.1) as well as analysis of seabream texture (more details will be presented in the final Deliverable of the WP6) and fish eye colour, throughout storage and shelf life. All the samples were also subjected to Multispectral Imaging analysis using VideometerLite1, analysing 480 flesh samples in total (240 aerobically and 240 vacuum packaged) and VideometerLite2 (280 samples in total). Apart from the flesh, skin samples were also analysed in both replications of this trial (Figure 30).

Figure 29 Samples obtained from the Aquaculture (Avramar)

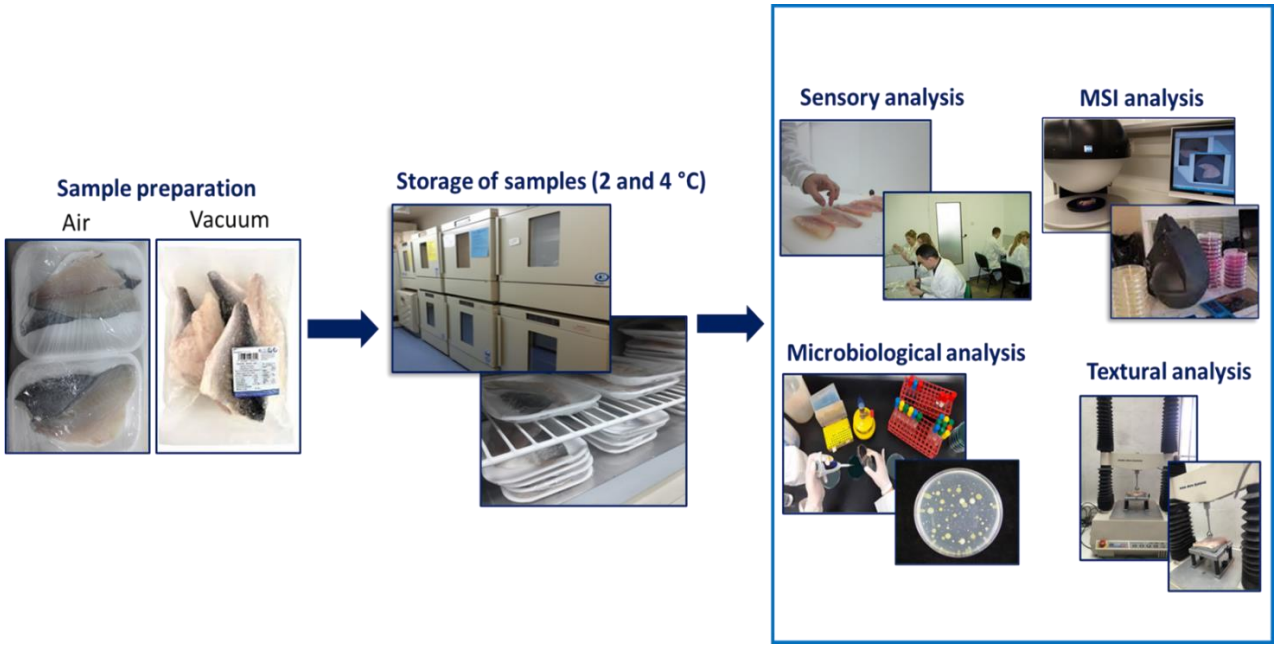


Figure 30 Updated experimental procedure of the second trial.

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 31).

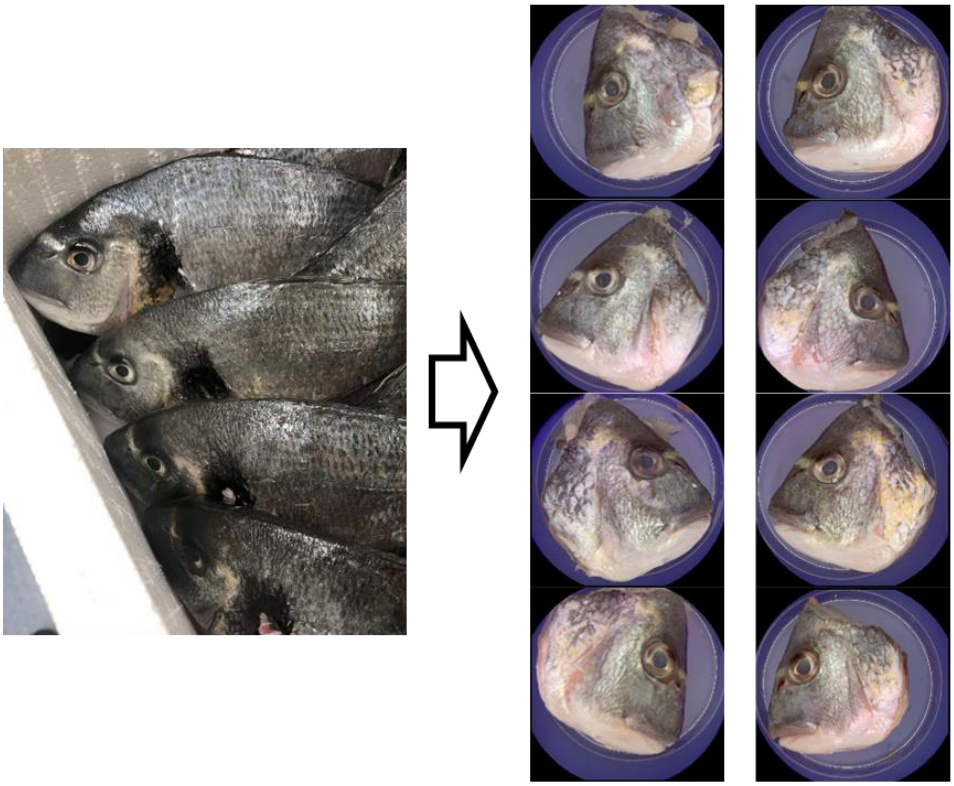


Figure 31 Seabream and example images acquired from VideometerLite

Trial 3 – Analysis of same seabream fillets originated from 8 different fish throughout storage at 4°C

The third part included the analysis of 8 specific fish samples (16 fillets). Two parts of each fillet were cut (the one close to the head and the other close to the tail of the fish), placed into petri dishes and stored at 4 °C for 8 days (Figure 32). MSI analysis of the same sample was taking place every day along with microbiological analysis of each fillet by using the remained fillet (after removing the 2 parts for the MSI analysis). For this scenario, 224 MSI samples (flesh) were analysed in total.

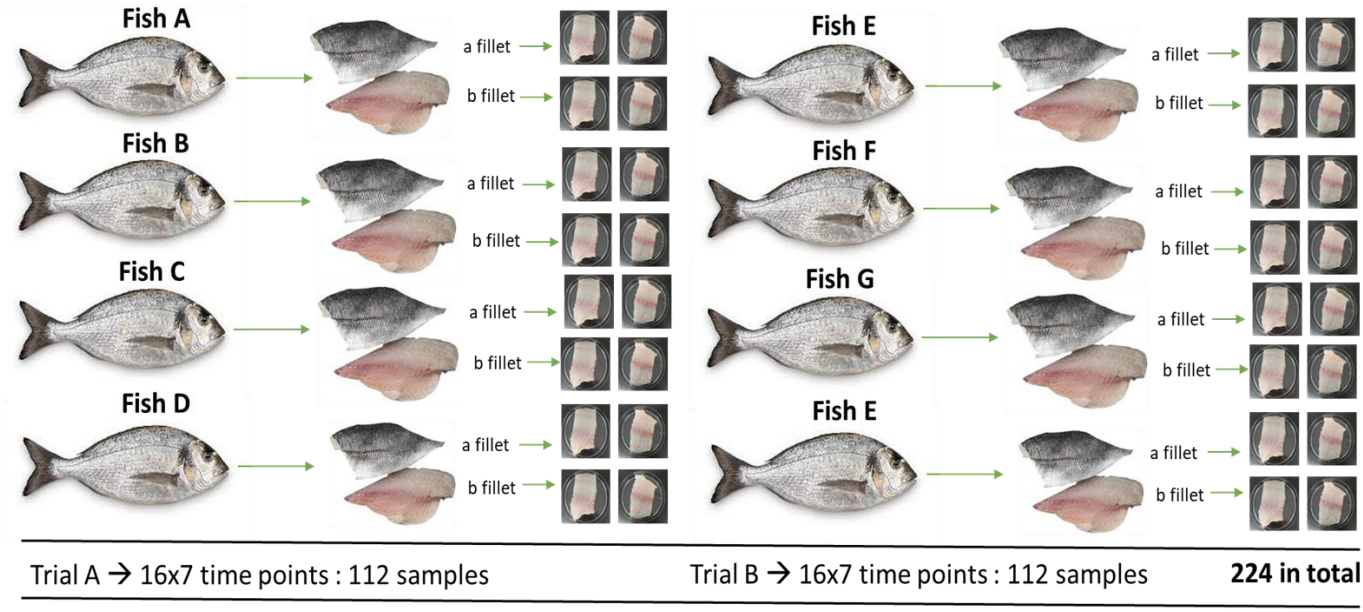


Figure 32 Sample preparation scheme for the third trial.

4.2 DATA ACQUISITION AND DESCRIPTION

A part of the results of the aforementioned analysis is presented below, while an extended version of the results will be described in detail in the following deliverable.

4.2.1 Trial 1

In Table 2 the results of TVC (MA) for the fish fillets from four different retail markets (RT1, RT2, RT3, RT4) are presented. The fish fillets were vacuum packaged containing two fillets each and packages from six different batches were purchased (different use by date). The packages were distributed in a period from 0 to 8d and in two different storage temperatures simulating storage in a consumer's refrigerator or retail (i.e., 2 and 4°C). The microbial population until use by date was <7.08 log CFU/g for all examined samples. The pH values of all samples were in the range from 5.86 to 6.33.

Table 2 Microbial population, TVC (log CFU/g), of the vacuum packaged fish fillets. The orange shaded cells is the time point of use by date.

Storage temperature	4°C	2°C					
Batch (same use by date)	1	1	2	3	4	5	6
Sampling day	Microbial population (log CFU/g)						
0	RT1 3.04 3.04	RT2 4.74 5.05		RT3 3.63 3.62			
1	RT1 3.18 2.98	RT3 2.65 2.00			RT3 2.98 2.85		
2	RT1	RT2					RT2

	3.70 2.70	3.54					5.69 5.72
3	RT1 4.00 3.70				RT2 6.60		
4							
5	RT1 5.52 5.82	RT3 4.50 4.34		RT3 4.70 4.74	RT3 7.48 7.45		
6	RT1 5.83 6.52	RT2 5.23 5.01				RT2 6.88 6.00	
7	RT1 6.27 6.67	RT3 4.90 7.08	RT4 6.57 6.45	RT2 5.22 5.24			
8	RT1 7.04 7.11	RT3 8.10 7.91 RT2 6.85 8.42	RT2 6.74 6.95				

Microbiological analysis

Aerobically stored samples were also investigated. For that purpose, samples from five supermarkets were collected. The samples were either purchased from retail markets in packages or on bulk stored on ice. Subsequently, they were packaged aerobically and stored under refrigeration conditions.

In Table 3 the microbial population of the purchased fish fillets in the different time points is shown. In general, 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 high population before the time point use by date. In the case of higher storage temperature (i.e., 4°C) microbial population reached higher than 8.00 log CFU/g. The pH ranged from 5.82 to 6.36.

Table 3 Microbial population, TVC (log CFU/g), of the fish fillets stored under aerobic conditions. The orange shaded cells is the time point of use by date.

Storage temperature	2°C					4°C	2°C		4°C
Batch (same use by date)	1	2	3	4	5	6	7	8	9
Sampling day	Microbial population (log CFU/g)								
0	RT1 3.79 3.76						4.86 4.74	3.82 4.50	
1		RT1 6.93 5.80	RT2 6.35			RT2 5.73 5.72			
2									
3					RT2 8.44 7.59	RT1 6.13 5.90	7.03 7.12	6.26 6.26	7.72 7.56
4				RT2 7.38	RT2 8.60 8.35	RT2 7.28 6.88	7.94 8.03	7.45 7.42	
5		RT1 5.70 6.06	RT1 9.18 8.39						
6									
7	RT2 7.49 7.43					RT1 8.74 8.20	7.75 8.13	8.63 7.52	8.59 8.57
8									
9						RT2 8.48 9.04	8.03 7.88	7.90 7.90	

In Table 4 the results of microbial analysis for 8 fish, which were filleted in the laboratory and stored aerobically are presented. Also, two marinated vacuum packages were microbiologically analyzed. The TVC was less than 6.5 log CFU/g even after the use by date time point, in contrary to the fish fillets where the microbial population was high (>7.5 log CFU/g). The pH values for marinated samples were in the range from 5.97 to 6.07 and the fish fillets from 5.91 to 6.45.

Table 4 Microbial population, TVC (log CFU/g), in the laboratory filleted fish and vacuum packaged marinated samples. The orange shaded cells is the time point of use by date.

Storage temperature	4°C	2°C		
Retail market	RT1	RT2	RT1	RT3 (Marinated samples)
Sampling day	Microbial population (log CFU/g)			
0	4.26 3.74	3.94 3.80		
1		3.98 5.11		6.12 5.54
2				
3	7.98 7.86			
4		6.30 9.03		5.00 6.15
5				
6				
7	10.21 10.13		7.85	
8		8.87		

In Figure 33, 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.

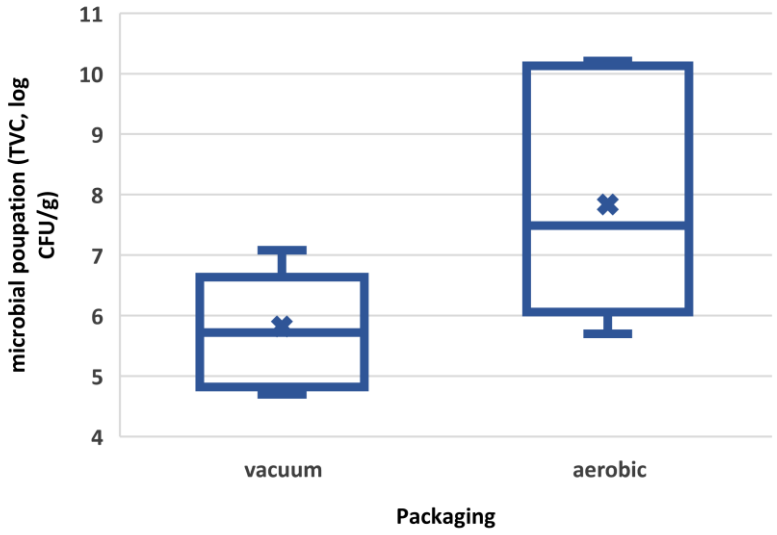


Figure 33 Microbial population (TVC) of fish samples on the end of shelf life (use by date) for samples stored in vacuum packaging (n=9) and in aerobic condition (n=7)

4.2.2 Trial 2

Microbiological analysis

In Figure 34Figure 34, the total viable counts as well as the populations of specific spoilage microorganisms in air and vacuum packaged seabream fillets obtained directly from the Aquaculture site, are presented. The microbial profile as well as the growth rate is clearly differentiated between the two packaging. *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.

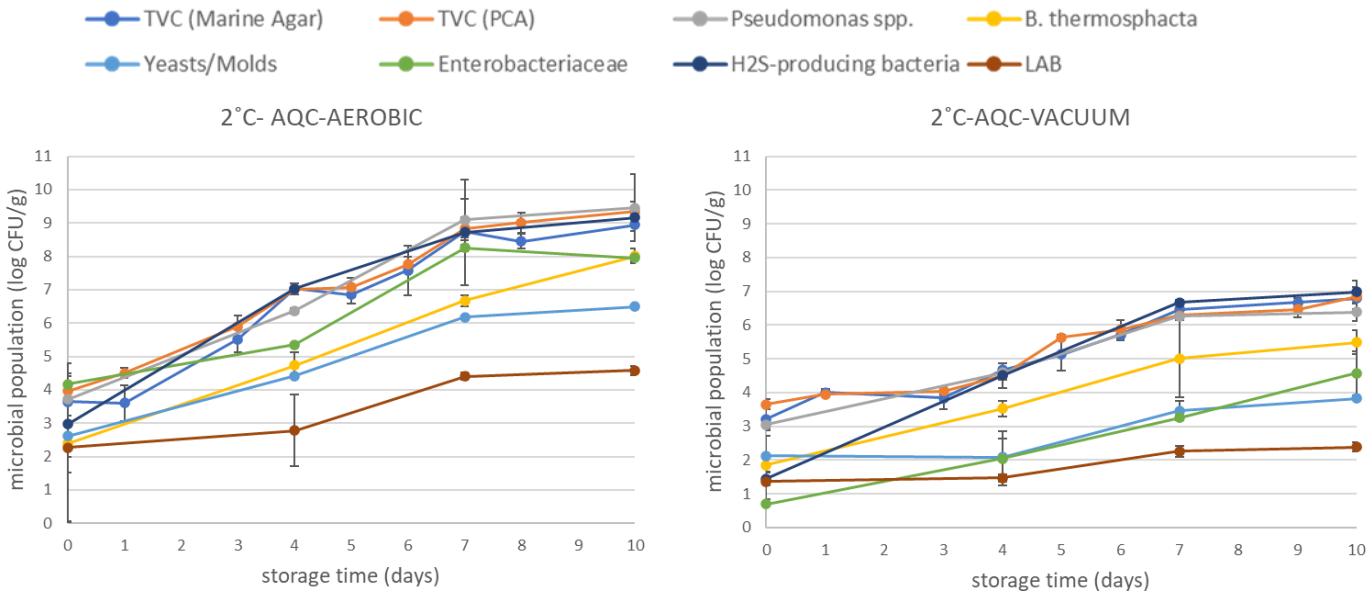


Figure 34 Microbial populations of seabream fillets obtained from the aquaculture throughout storage at 2 ˚C.

Sensory analysis

Similarly 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 (Figure 35). In both cases, the attribute of odour had higher scores compared to overall appearance, indicating that odour is the most characteristic attribute for assessing the freshness of fish.

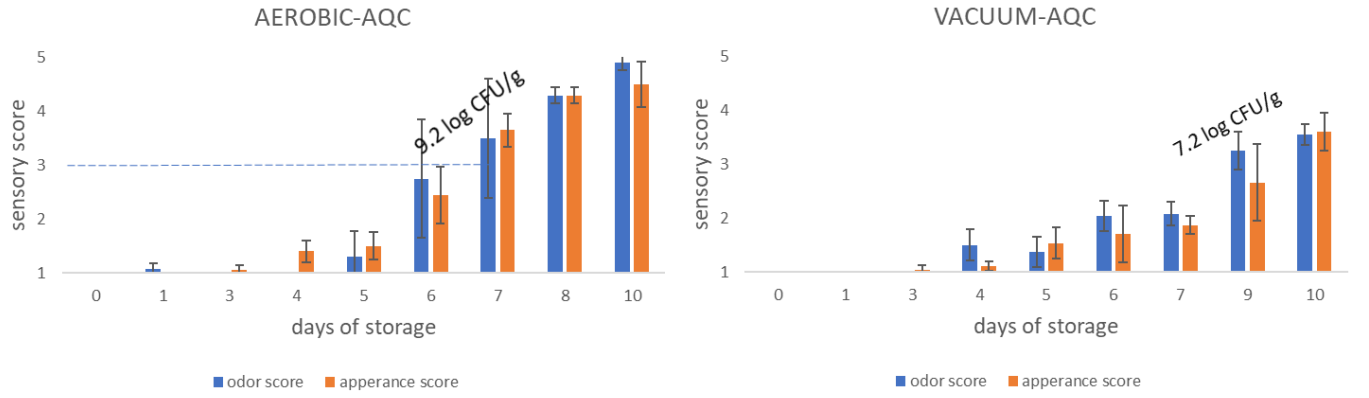


Figure 35 Sensory scores of aerobically and vacuum packaged seabream fillets obtained from the Aquaculture site throughout storage at 4 °C.

Multispectral Imaging Analysis

In Figure 36, small differences can be observed between aerobic and vacuum samples especially in wavelengths higher than 525 nm. Additionally, the spectrum of skin samples is not of high quality since no differences are observed for different wavenumbers while high standard deviations have been calculated as well/In the case of AUA experimental design, four different images per fish fillet were acquired corresponding to the flesh (2 samples) and the skin (2 samples) of the fillet (Figure 36).

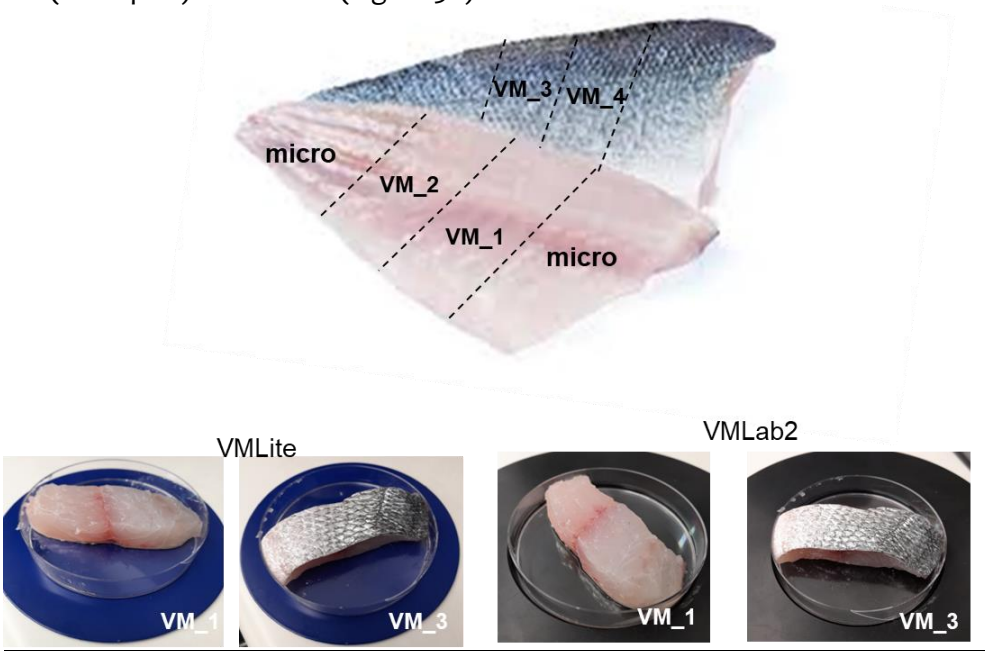


Figure 36 Samples used for microbiological analysis, VideometerLab and VideometerLite.

In Figure 36, 4 indicative images captured throughout the experiments in both instruments are shown. Pre-processing of data, using the VideometerLab software is still in progress, while several datasets (such as this in Figure 37) will be obtained and further used for the models' development and validation.

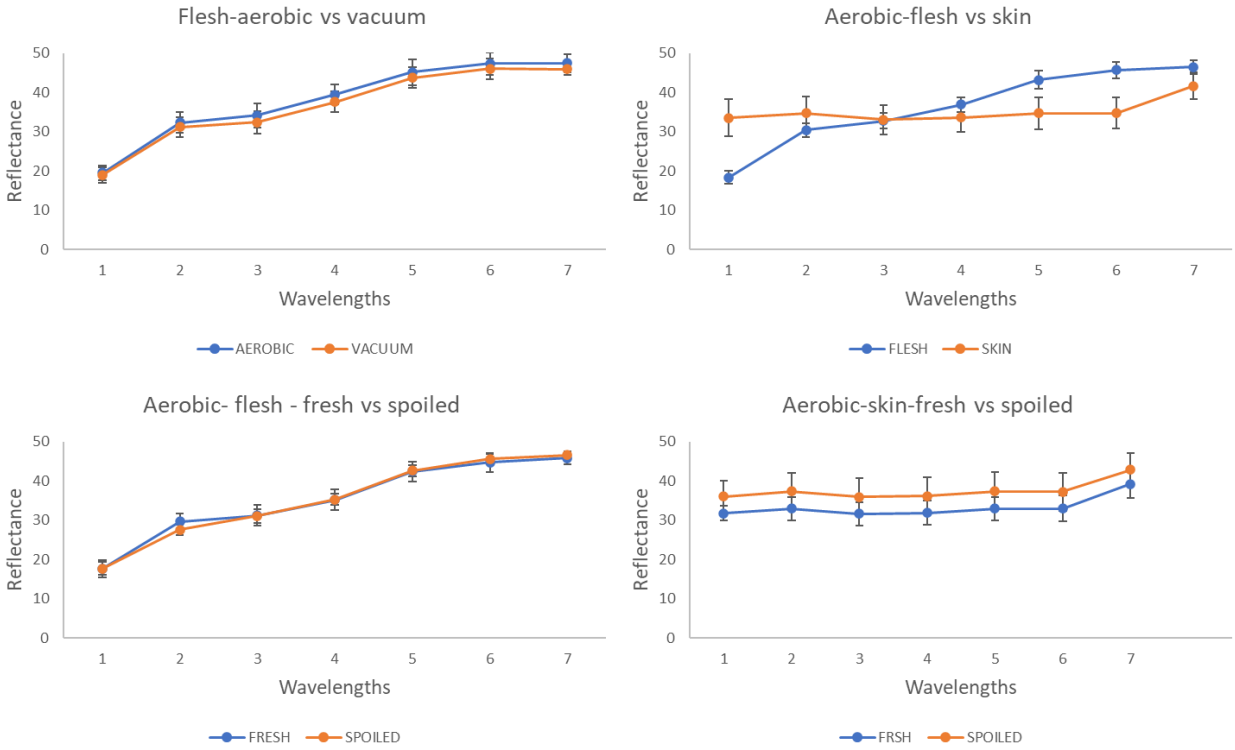


Figure 37 Indicative spectra for the comparison of various types of samples (fresh vs spoiled, skin vs flesh etc). 1;405, 2;460. 3;525, 4;590, 5;621, 6;660, 7;850

Eye color/appearance

Before the data analysis a pre-processing step is needed to make a mask allowing the isolation of the segment of the image that contains only the information of the eye of the fish. For that purpose, the VideometerLab software v. 3.24.34 (13095) was used. In Figure 38 is shown the effective segmentation of the image using a binary masking image.

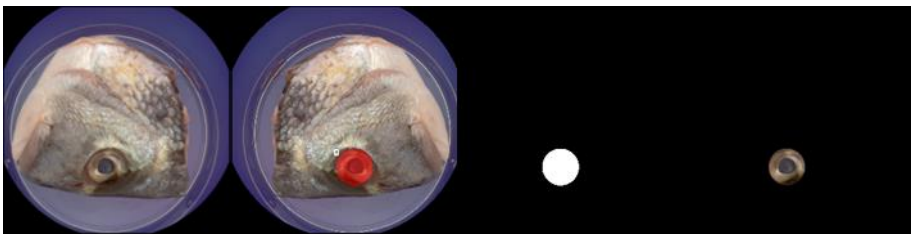


Figure 38 Image segmentation using a binary masking image

Although this type of segmentation is efficient, it would be time consuming since it should be applied on each image separately. Another attempt was done, of which the workflow is shown in Figure 39. A quite good segmentation was achieved in 50% of the samples while 50% of the images were not segmented well.

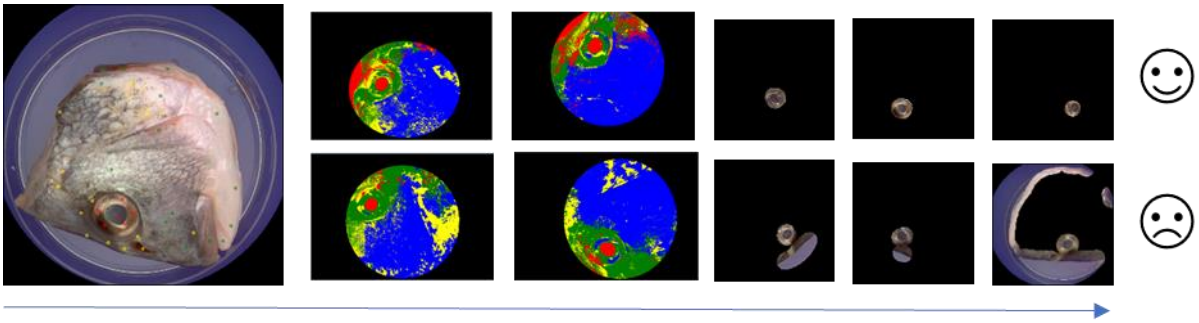


Figure 39 Pixel classifier segmentation was applied to divide foreground class (fish eye-black part) from the background (all the other classes) and followed by morphological filtering.

The difficulty on segmenting only the fish eye could be explained from the similarities in colours on the head of the fish and in the eye. In Figure 40the spectra of some regions on the fish head and on the eye of the fish and how mean±stdev are overlapping are shown.

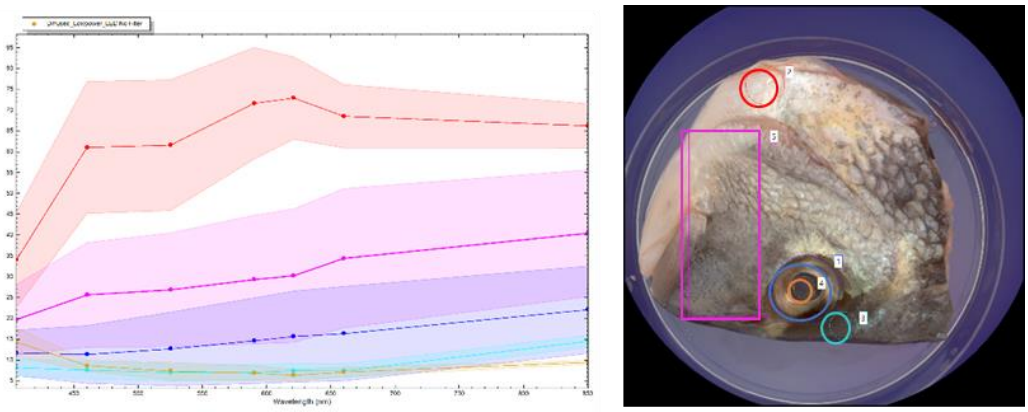


Figure 40 Fish head spectra vs fish eye spectra

4.2.3 Trial 3

Microbiological analysis

Total viable counts of fish that were analysed every day (the same fish) and obtained from fish markets and directly from the Aquaculture are presented in Figure 41. A extensive data analysis and explanation for this Task will be provided in the final Deliverable of WP6.

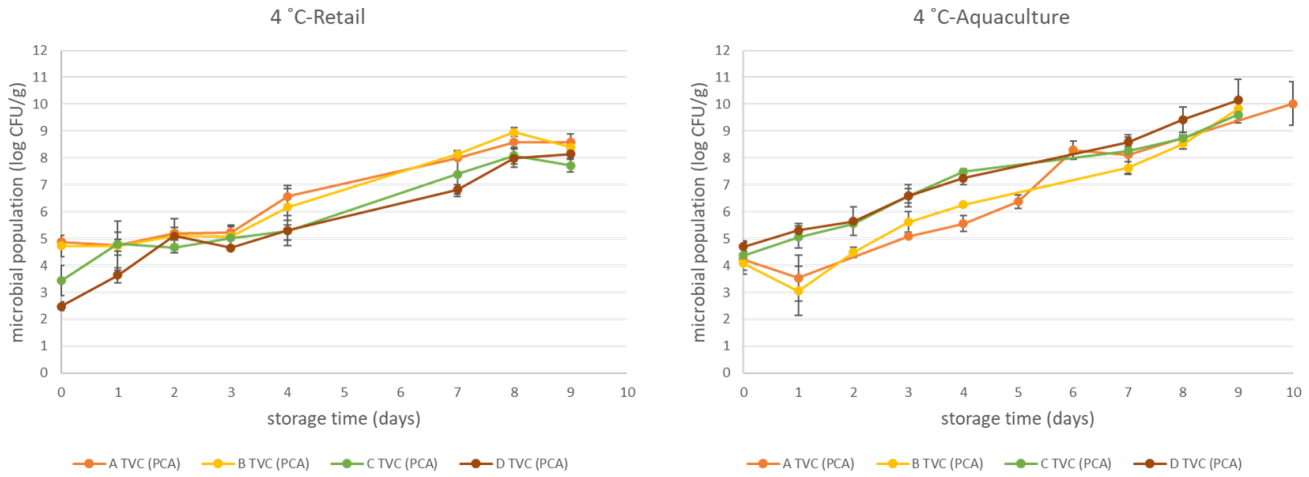


Figure 41 Microbial populations of seabream fillets obtained from retail stores throughout storage at 4 °C

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 a modified version of the VideometerLite system (VideometerLite - Videometer), shown in Figure 15, that has been 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.

Figure 42. VideometerLite, portable handheld multispectral imaging device.

With its state-of-the-art technology, the instrument allows for the determination of colour, texture, and surface chemical composition of up to 100x100 mm of sample size. Using strobed LED systems, VideometerLite efficiently combines the measurements of seven wavelengths into a single spectral image, where each pixel corresponds to a different reflectance spectrum, wavelength range [405-850 nm] – nonuniformly distributed. Thus it includes both visual and NIR wavelengths for a precise, accurate, and thorough quality inspection foods.

6 CONCLUSIONS AND NEXT STEPS

This deliverable provides a preliminary report on the first's pilot experimental design and on the acquired data. Results from pilot experiments show great potential for the technology for various applications and, further, provide important feedback into its development. The applicability of this type of device differs between the three tested fish value chains due to the large variability between. However, the explorative setup of the project provides an opportunity to investigate some extreme possible applications of the the Videometer technology within these value chains.

This technology shows promise in determining freshness of seabream, identifying hazards such as nematodes in white fish and quality parameters such as melanin and blood spots in salmon etc.

The most promising applications, as assessed by the described pilots, will be investigated further and validated further during the coming months. The obtained results on the most promising applications will be further communicated in Deliverable 6.3.