TraceMyFISH

Traceability and Quality Monitoring throughout the Fish Value Chain

D6.1 – Pilot Execution Report

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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 will serve as inputs for further use and analysis, i.e. machine learning model training and deployment.

In Task 6.1, to which this deliverable accounts for, AUA, NTNU, UoI and MATIS will perform measurements with the provided by VIDEOM sensor on domestic fish samples, specifically 3 types of fish: Atlantic salmon, Atlantic whitefish, and Mediterranean seabream/seabass. The measurements are performed in a way that simulates the real food chain so as to evaluate the sensor in tandem with the hazard/quality detection algorithms that will be developed according to the initial experiment design for the 3 pilots as outlined in D2.3. Initially, a first round of measurements has been applied in order to ensure and compensate any field variations reflected on the data.

Those data will further be used for the training of the prediction/detection models to be developed. Then, additional measurements will be performed for system validation purposes along the whole food chain, resulting in the creation of a database, so as to reproduce the results and compare the final algorithms and analytics approaches in order to ensure the efficiency and robustness in terms of future application and traceability of any erroneous predictions. It should be mentioned that all measurements are backed up by simultaneously acquired reference measurements coming from conventional/traditional microbiological and/or chemical methods. Those reference measurements serve as the "ground truth" for system validation and evaluation in Task6.2 with the participation of VIDEOM, where the evaluation will be on a specific KPIs set; the accuracy on the determination of sample properties like colour, texture, surface chemistry, contamination, oxidation, species and/or parasites, all this in conjunction to the "ground truth" as provided by lab analyses; Also, the traceability efficiency will be validated across the food chain, knowing the "real" sample properties.

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 of 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, in 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 experimental procedure (please also refer to D2.3 for more details and customized protocols for each pilot) should be designed in such a way that the real food chain is simulated, but under more controlled conditions. This will help us to trace any inconsistencies that may occur during the analysis leading to "bizarre" prediction results. Multifactorial experiments (FT×T×P) that will represent the different stages across the food chain, has been designed and conducted in order to provide real-like but at the same time "controlled conditions" for data acquisition. Thus, in a nutshell, the fish (Factor FT–Fish Type) will be stored at different temperatures (Factor T) and if applicable, under two different packaging conditions (Factor P). Also, the storage and transportation should also be simulated as if the fishes are transported along the different chain types, mainly via the Temperature factor and additional storage times. The measurements will be performed across the whole food chain, as defined, initially under simulated conditions so as to reach the feasibility results required for the sensor, while on a second phase the models can be validated in real life scenarios. All involved partners work 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). The expected outcomes include: 1) data collection and 2) an operational system specifications and operational requirements definition. Along with the quality/safety indicators, the location and treatments like storage.

2 PILOT EXPERIMENTAL DESIGN AND DATA ACQUISITION

2.1 THE ATLANTIC SALMON PILOT

The Atlantic value chain, defined in D2.1, 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 D2.1 and are the basis for the pilot design presented in D2.3. Data acquisition will mainly be conducted from laboratory-designed experiments simulating different scenarios of unwanted incidents in the salmon value chain. If possible to organise, a pilot following the cold chain of fillet transportation from the slaughterhouse in Norway to the European market (e.g. France) by trucks will be conducted. Unwanted incidences includes:

1) Issues related to stress and handling
2) Issues related to melanin spots
3) Issues related to poor bleeding
4) Issues related to broken cold chain

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, D2.1 and D2.3, 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 is still ongoing, and the findings of these analyses will be presented in the following deliverables.

2.1.1 Experimental Design

Issues related to stress and handling:

To obtain fish with a texture gradient related to stress and rough handling, we used fish from an ongoing project funded by the research council of Norway (project 321586). 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 VideometerLAB to measure textural- and colorimetric parameters. The results were validated by comparing multispectral data obtained from the Videometer devices with data obtained from traditional methodologies measuring textural properties (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, as well as after six days refrigerated storage:

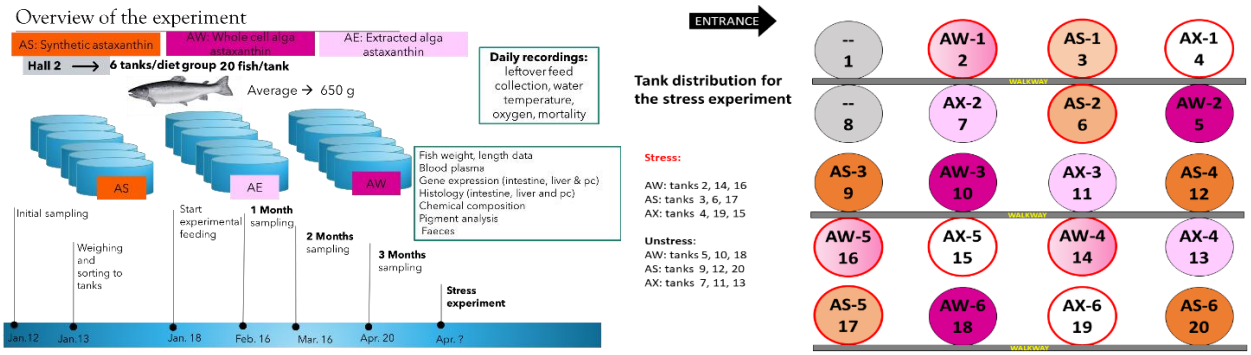


Figure 1. The experimental design of which the fish was used to study the potential of Videometer devices to measure the textural and colorimetric properties of Atlantic salmon.

Issues related to melanin spots:

Downgraded fillets (fillets containing melanin) were obtained from a nearby salmon processing plant to obtain multispectral data from fillets showing melanin spots. The experiment was conducted twice, of which the first was evaluated using VideometerLAB2, whereas VideometerLite, VideometerLAB2, and DigiEye were used to obtain data in the second one. In both experiments, images of the melanin spot itself were captured, and a nearby area was used as a reference (Figure 2). Fillets used in these experiments were selected to give a visual gradient melanin score from light grey to dark black.

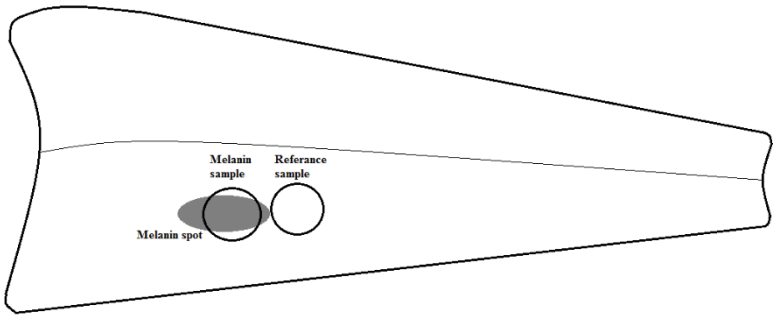


Figure 2. Schematic illustration of sampling locations on fillets containing melanin spots.

Issues related to poor bleeding:

Poor bleeding happens, and residual blood in the salmon fillets is a concern for the salmon processing industry. We designed an experiment with different bleeding protocols to obtain fish with a muscle blood gradient. 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 for either 15, 30, 45, 60, or 90 min in a make-shift Chilled Seawater System (CSW) before filleting. The sample size will be a minimum of six individuals, giving 12 fillets per group, of which each experimental group has different residual blood levels. The experiment is planned to be conducted in early January 2023 at the NTNUs salmon research farm in Romsdalsfjorden, Norway. We will use the VideomterLite to measure residual fillet blood on-site and repeat those measurements in 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.

Issues related to a broken cold chain:

Laboratory experiments will be conducted to simulate different incidents related to a broken cold chain for salmon products packed and distributed under different conditions (whole fish on ice, vacuum -and modified atmosphere packed). Atlantic salmon will be slaughtered at a nearby slaughterhouse and transported to NTNU for further processing/packaging. To ensure the similar chemical composition of the experimental portions, the back- and mid-loin running from the gills to the Norwegian quality cut (NQC) will be used to prepare uniform experimental samples that will be randomly in the planned storage trails.

One experiment will be designed as a challenge study, where salmon fillets are inoculated with known spoilage bacteria (e.g., *Pseudomonas*, *Photobacterium*), and quality parameters will be assessed during storage at different storage conditions (vacuum -and modified atmosphere packed). Un-inoculated salmon will be applied as a control group in all storage conditions.

Another experiment will be designed to analyse quality changes as a function of storage time for the whole salmon stored on ice. The sample size will be a minimum of six individuals. The traditional Quality Index Method (QIM) scheme for raw, farmed Atlantic salmon will be compared to results obtained by VideometerLab2 and VideometerLite. Traditional QIM includes analysis of colour/appearance of skin folds, mucus, and smell of skin;

colour and form of eyes; cut surface and blood in the abdomen, membrane on the inside of abdomen and odour of abdomen; colour/appearance of mucus and odour of gills; and texture (elasticity) of flesh.

A third experiment will simulate the expected and worst-case temperature scenario in the cold chain of fillet transportation from the slaughterhouse in Norway to the European market (e.g., France) by trucks.

Microbiological- and physiochemical quality parameters will be evaluated using VideometerLab2, VideometerLite, and traditional wet-chemistry and microbiological methodologies to monitor quality changes during storage time (6-10 times) in all experiments.

Microbiological analysis: The microbiological profile of the samples will be analysed using a variety of agars (Table 1). Molecular analysis of microbial communities will be applied if required.

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

Culture media	Target microorganisms	Incubation conditions
Iron agar	Aerobic bacteria (APC) and H_2S-producing bacteria	22°C for 3 days
Long and Hammer agar	Psychrotrophic aerobic plate count	15°C for 6 days
STAA	*B. thermosphacta*	25°C for 2 days
Man, Rogosa and Sharp agar	Lactic Acid Bacteria	25°C for 5 days
Pseudomonas Agar + CFC (centrimide,fucidin,cephaloridine)	*Pseudomonas* spp.	25°C for 2 days
Violet Red Bile Dextrose Agar (VRBG)	Enterobacteriaceae	37°C for 24 hours
Brilliance Listeria agar with Brilliance Listeria Supplement	*Listeria* spp. (and *Listeria monocytogenes*)	37°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 VideometerLAB.

Sensory evaluation: In some experiments, simple sensory evaluation of salmon samples will be conducted by a semi-trained panel of 6-8 members. The freshness of the samples will be evaluated using a 5-point scale (1 - like very much, 2 - like moderately, 3 - neither like nor dislike, 4 - dislike moderately, 5 - dislike very much).

The experiments will be conducted in Q1-Q2 in 2023 in NTNU's laboratory.

2.1.2 Data acquisition and description

Many images of analytical data will be collected and analysed as part of the beforementioned experimental setups. Obtained data will first be analyzed to verify the potential of using the chosen devices and their setup to accurately predict the identified hazards in the salmon value chain. An example of a dataset obtained from VideometerLite can be shown in Figure 3 (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 3. An example of a dataset from VideometerLite2 will be used for the prediction.

Preliminary results from a previous experiment investigating melanin spots in salmon fillets

Although the amount of data is large, only a limited amount is analysed. However, a summary is given on the use of videometerLAB2 in analysing fillet melanin.

In the first experiment, ten fillets with melanin spots were selected (Figure 4). Visual evaluation of the salmon fillets sampled for analyses shows an average ± SD total melanin score of 3.05 ± 1.22 (ranging from 1-4). In 8 of the fillets, melanised focal changes were located in the belly region, whereas two fillets had focal changes in the dorsal muscle.

Multispectral imaging (VideometerLAB2) of melanised tissue shows 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 samples (Figure 5). Moreover, the reflection also varied between individual melanin spots, showing significant differences in the intensity of the melanised tissue from the other fillets sampled for analyses.

In upcoming deliverables, detailed results from all conducted and planned experiments will be given.

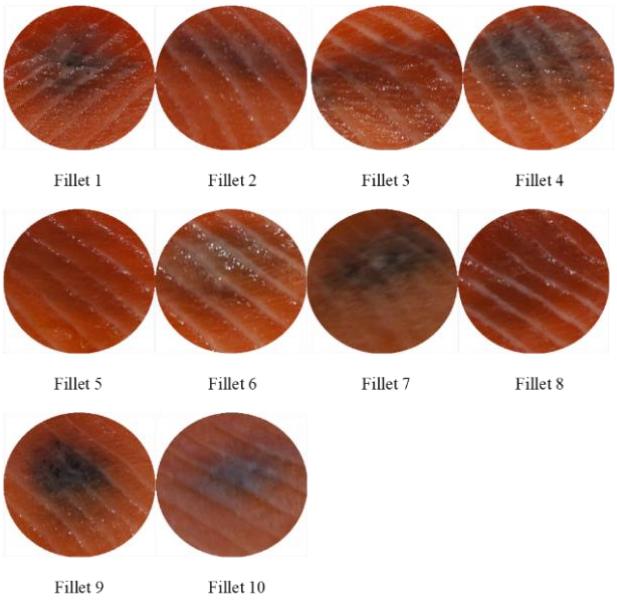


Figure 4. Melanin spots sampled for analyses. The spots were located in the belly region of ten different salmon fillets.

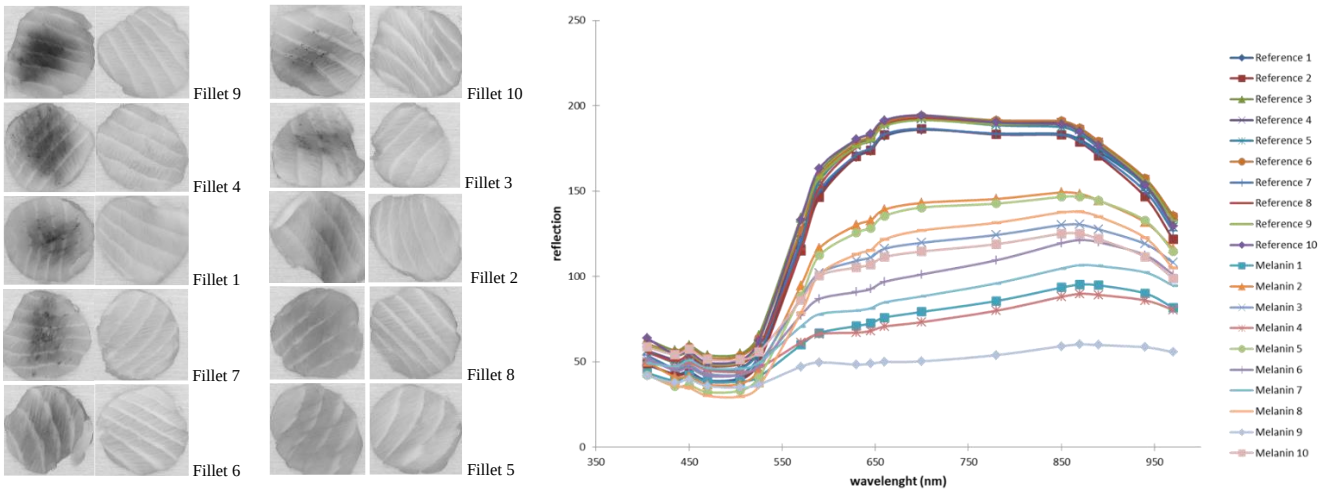


Figure 5. NIR images of salmon fillets sampled for analyses ranged after increased light reflection (decreased melanin intensity) at 870 nm. All images were captured in a VideometerLab2 multispectral system (Videometer A/S, Hørsholm, Denmark).

2.2 THE ATLANTIC WHITEFISH PILOT

The Atlantic whitefish pilot includes evaluations of diverse quality parameters, some which are already evaluated to some extent throughout the value chain, while others will be included in coming trials. The quality parameters that will be covered within the Atlantic whitefish pilot mainly relate to potential hazards, such as parasite detection and identification, effects of poor bleeding, and shelflife and freshness identification. These parameters were identified and defined in D2.1. The aim of the pilot is to implement the VIDEOM tools and assessing their precision in evaluating the defined quality parameters, with the aim of making quality assessment within the whitefish value chain more accessible, reliable, and traceable. The pilot trials include the whole value chain from whole fish onboard the fishing vessels, to processing and storage stability relevant to commercial environments. Experiments will be performed in a controlled laboratory and pilot environments mimicking true industrial conditions. The following trials will be performed:

1. Assessment of fish freshness through eye and gill evaluation
2. Nematode detection and identification
3. Monitoring of blood spots and poor bleeding effects
4. Shelf life/freshness of final products

2.2.1 Experimental Design

Freshness through eye and gill evaluation

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 6 shows the QIM scale and example photos showing some of the visual changes that occur during storage and are evaluated in the QIM method.

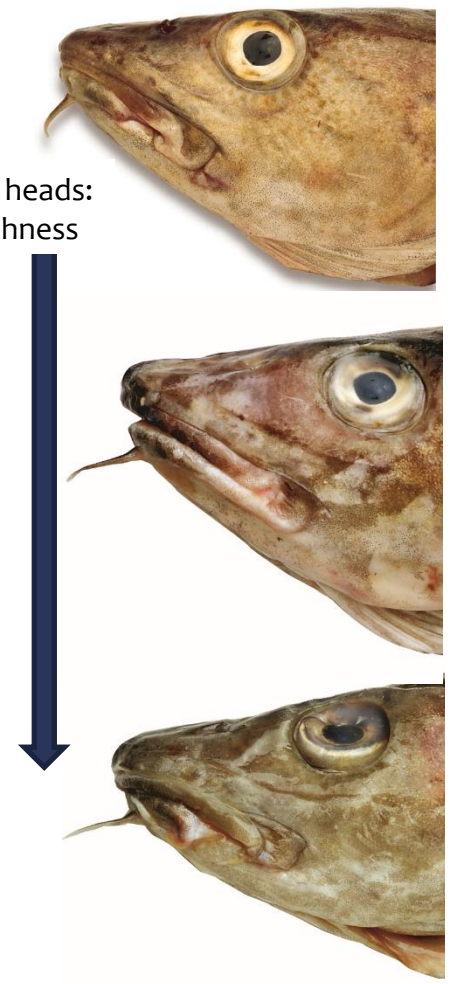
A

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 6 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.

Heads of whitefish will be collected, stored, and evaluated regularly throughout the storage time using both a trained QIM panel and the VIODEM equipment. Data will be evaluated to determine how effectively the technological solutions can determine freshness and how they compare to traditional methods.

Nematode detection and identification

Nematode detection is of great importance in 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 if it appears on the plate. Two trials have been performed to evaluate the feasibility of using spectral imaging VIDEOM solutions for nematode detection, one using only the VideometerLab instrument, while the other trial applied both the VideometerLab and VideometerLite technologies.

The primary trials focus was possible detection and an evaluation of the depth at which the worms could be detected into the flesh (Figure 7). Before imaging the fillets were cut into appropriately sized portions. 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%).

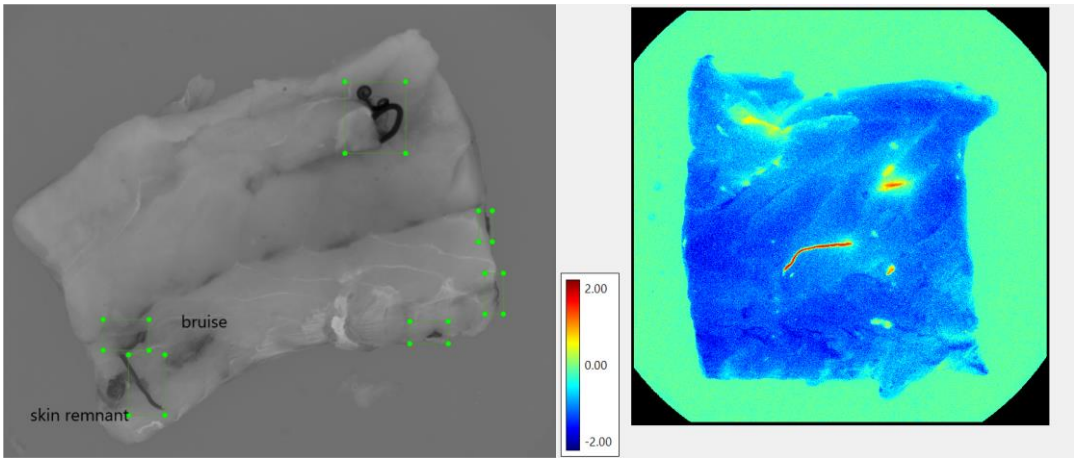


Figure 7 Left: Example of a labelled image with six nematode labels. A skin remnant and a bruise can be seen in the image as well. Right: An nCDA transformation on a random image in the data set. The red colour implies that those pixels are nematodes, and the blue implies that those pixels are fish muscle. 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 8).

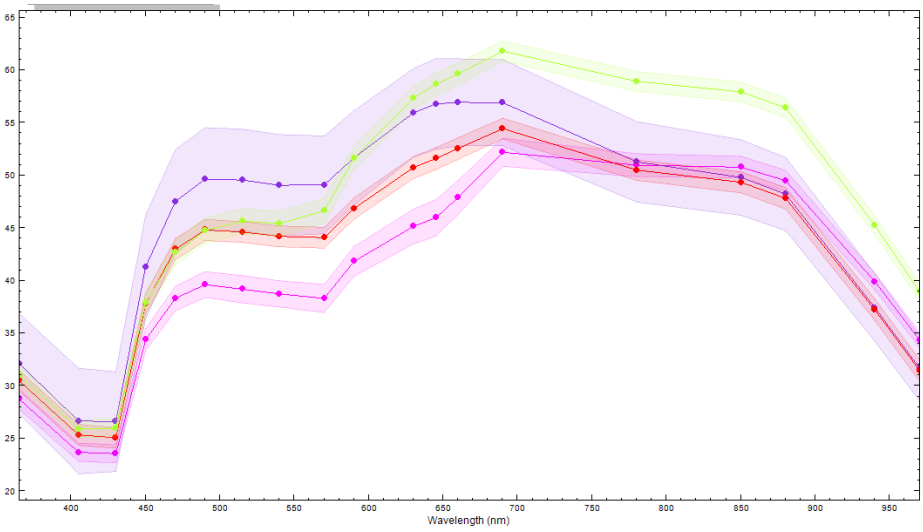


Figure 8 Spectral signature of nematodes of different colour and depth compared to fish muscle. Fish muscle (purple); Dark nematode at 7_1 mm depth (red); Dark nematode at 5_1 mm depth (fuchsia); Light yellow nematode at 5_1 mm (green).

The second trial performed in this part of the pilot aimed to compare the VideometerLab capabilities and VideometerLite, and determine how those solutions compare to the industry standard method used today, so called 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 were 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. Data analysis is currently ongoing.

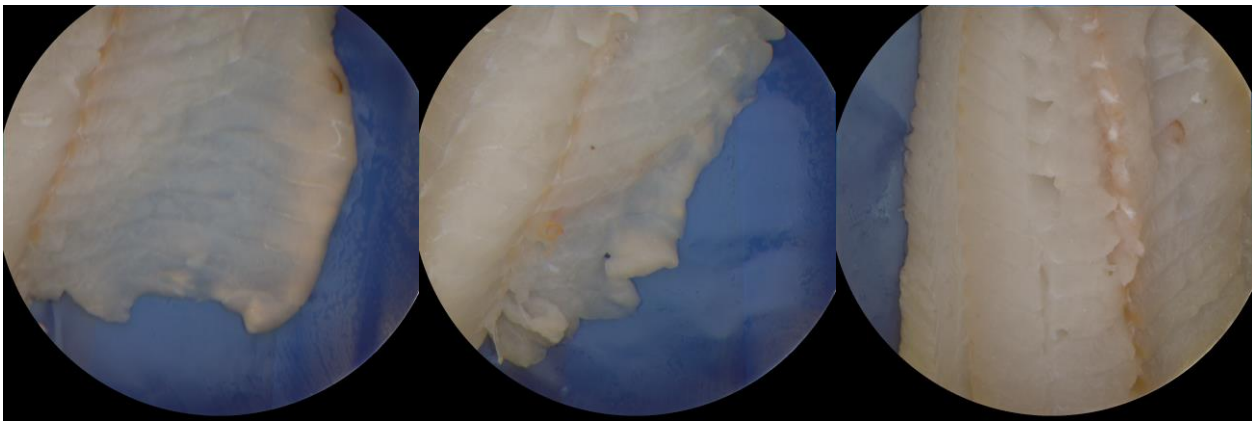


Figure 9 Images from the VideometerLite captured in the second nematode detection trial.

Monitoring of blood spots and effects of poor bleeding

Poor bleeding of whitefish can cause issues relating to visual quality (blood spots), gaping, and possibly shorten shelf life and cause issues in production of final products such as salting or drying. A trial, similar to that described in 2.1.1 will be performed using whitefish. A comparison of fish ranging from un-bled to well bled will be evaluated, and relevant analysis performed. This trial is planned for spring 2023.

A preliminary study was performed using saithe in November 2022 to evaluate suitable imaging methodologies and relevant image analysis (Figure 10). In the preliminary study saithe of two different size

categories, bled for 15 or 25 min, respectively, were collected and filleted. The samples were processed, and images collected using both the VideometerLab and VideometerLite instruments. Data analysis is ongoing, and results will be used to finalise the experimental design of the main trial planned for spring of 2023.

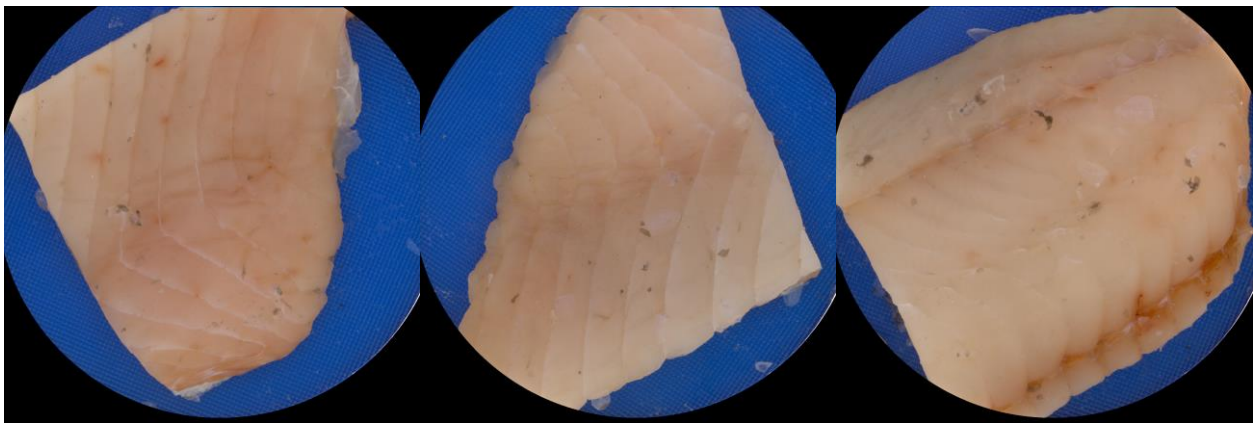


Figure 10 Images from the VideometerLite captured in the preliminary bleeding and blood spot detection trial.

Shelf life/freshness of final products

Laboratory experiments will be performed to evaluate shelf life of whitefish products and relevant analysis performed to determine shelf life and freshness of products. The trial will take into consideration the design of the trial described in 2.1.1 relating to the storage and broken cold chain to enable comparison between the different samples. Traditional chemical methods will be used as well as sensory evaluation.

2.2.2 Data acquisition and description

The results of the aforementioned analysis will be presented in detail in following deliverables. All data will be collected and shared with the TraceMyFish partners for use in the pilot setup and evaluation as needed.

2.3 THE MEDITERRANEAN SEABREAM/SEABASS

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.
In WP2 and previous Deliverables D2.1 and D2.3 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 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.

2.3.1 Experimental Design

Sample collection

The experimental design was separated in three parts. The first one was related to the analysis of seabream fillets obtained from several selling points (Figure 11). 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-four (54) of these fillets were packaged in MAP conditions while 74 of them were aerobically packaged. At each sampling point (time point), the filles were subjected to microbiological, sensory and Multispectral Imaging analysis. The experimental procedure is illustrated in Figure 12.

Figure 11. Indicative samples tested at the first trials for seabream quality assessment.

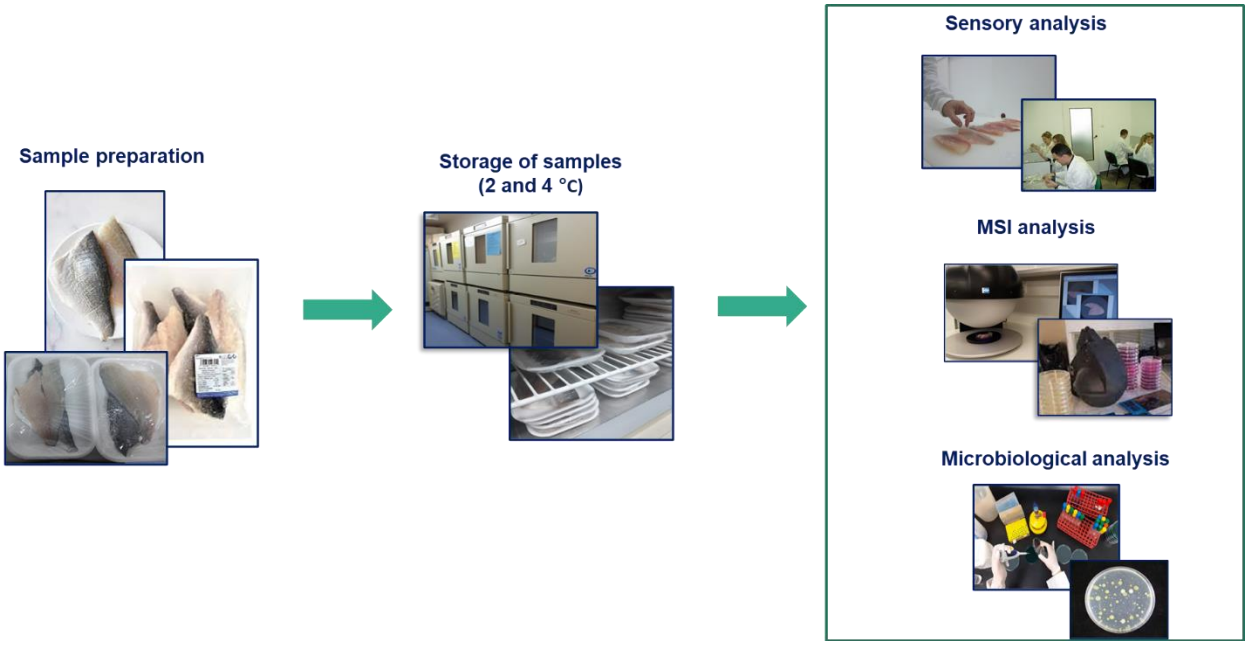


Figure 12. Experimental procedure of the first part.

The second part included the analysis of 4 specific fish samples (8 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 2 °C for 8 days (Figure 13). 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, 128 MSI samples (flesh) were analysed in total.

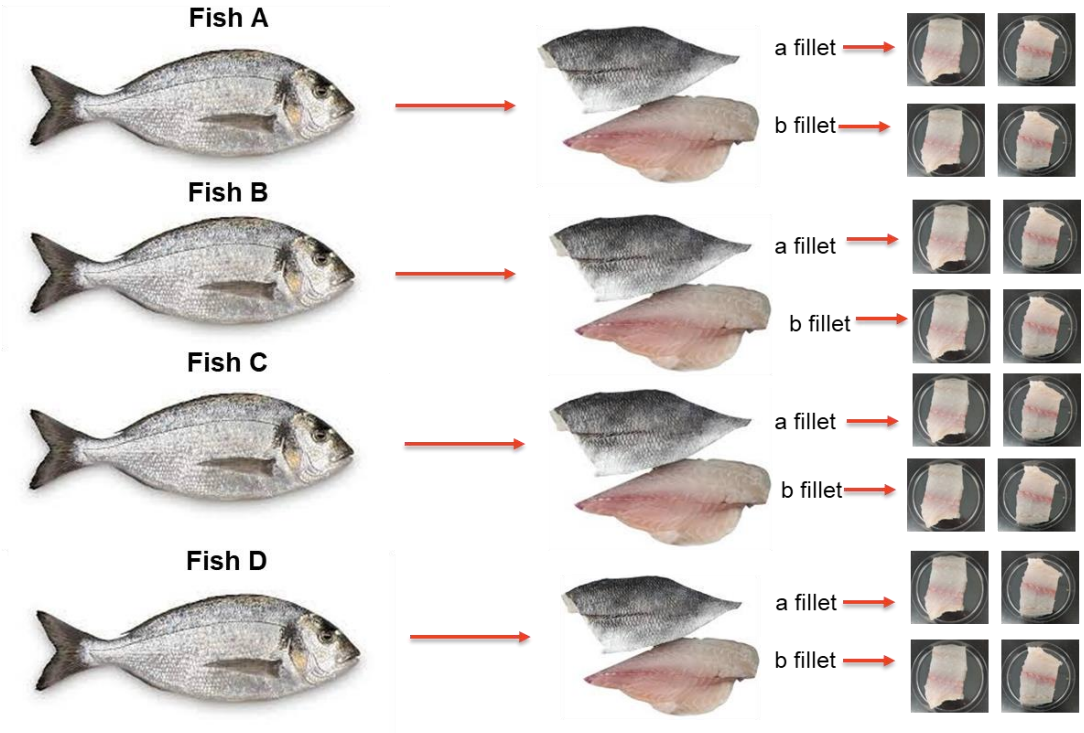


Figure 13. Sample preparation for the second trial.

The third part that will be completed the following months, includes the analysis of samples that will be obtained directly from the facilities of Avramar, the aquaculture company collaborating with AUA in the framework of the TMF project.

Microbiological analysis

Samples were analyzed at regular time intervals (at 0-the day of arrival at the laboratory and at certain days of storage) for monitoring the spoilage microorganisms' evolution. A number of nutrient media was used at the first sampling (day 0) for the determination of the products' microbial profile including two (2) non selective media for the estimation of the total mesophilic microbial population and ten (10) for the estimation of specific spoilage or pathogenic microorganisms (Table 2). Fish samples were weighed aseptically, added to maximum recovery diluent (MRD) and homogenized in a stomacher (Lab Blender 400, Seward Medical, London, UK) for 120s at room temperature. Serial decimal dilutions were prepared in MRD solution and duplicate 1- or 0.1-ml aliquots of appropriate dilutions were plated on the appropriate media.

Table 2. Microorganisms, culture media and the relative incubation conditions examined for the determination of seaweeds microbial profile.

Nutrient media	**Target microorganisms**	**Incubation conditions**
Plate Count Agar – PCA	Total viable counts	30°C for 2-3 days
Marine Agar	Total viable counts	30°C for 2-3 days
STAA	*B. thermosphacta*	25°C for 2 days
MRS Agar	Lactic Acid Bacteria	30°C for 3 days
Pseudomonas Agar + CFC (centrimide,fucidin,cephaloridine)	*Pseudomonas* spp.	25°C for 2 days
Violet Red Bile Dextrose Agar (VRBG)	Enterobacteriaceae	37°C for 24 hours
Iron Agar	Sulphur producing bacteria	25°C for 3 days
TCBS Agar	*Vibrio* spp.	30°C for 2-3 days
XLD Agar	*Salmonella*	37°C for 24 hours
ALOA Agar + PALCAM Agar	*Listeria* spp. (and *Listeria monocytogenes*)	37°C for 2 days
TBX Agar	*Escherichia coli*	30°C for 2 days
Rose Bengal Chroramphenicol Agar (RBC)	Yeast and molds	25°C for 3-4 days

Sensory analysis

The fish fillets were organoleptically tested for the attributes of odour and overall appearance. For this reason, a 6-member panel was used evaluating the freshness of the samples using a 5-point scale (1 - like very much, 2 - like moderately, 3 - neither like nor dislike, 4 - dislike moderately, 5 - dislike very much).

2.3.2 Data acquisition and description

The results of the aforementioned analysis will be presented in detail in the following deliverables. Next, some indicative, preliminary findings are given.

Microbiological analysis

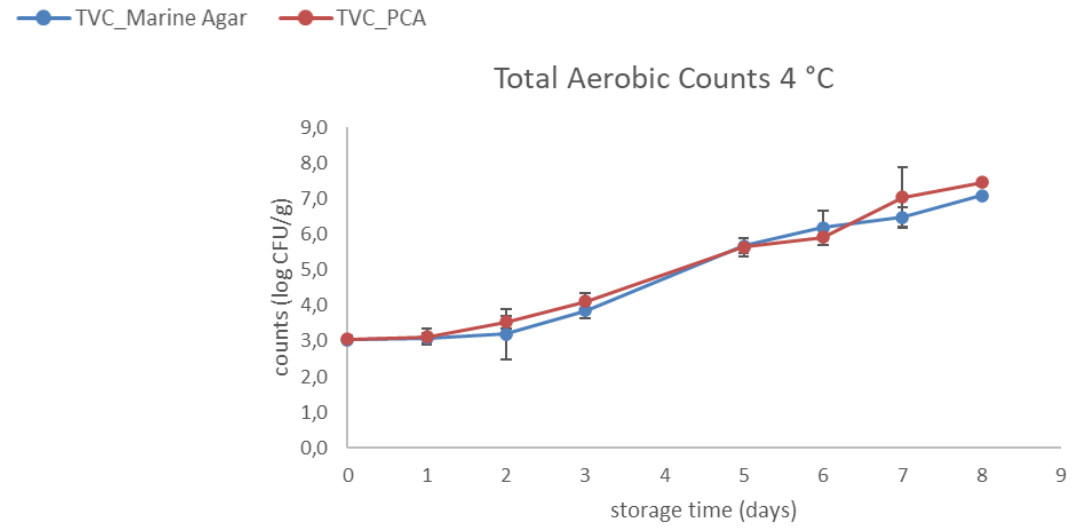


Figure 14. Total aerobic counts of seabream fillets obtained from retail stores throughout storage at 4 °C.

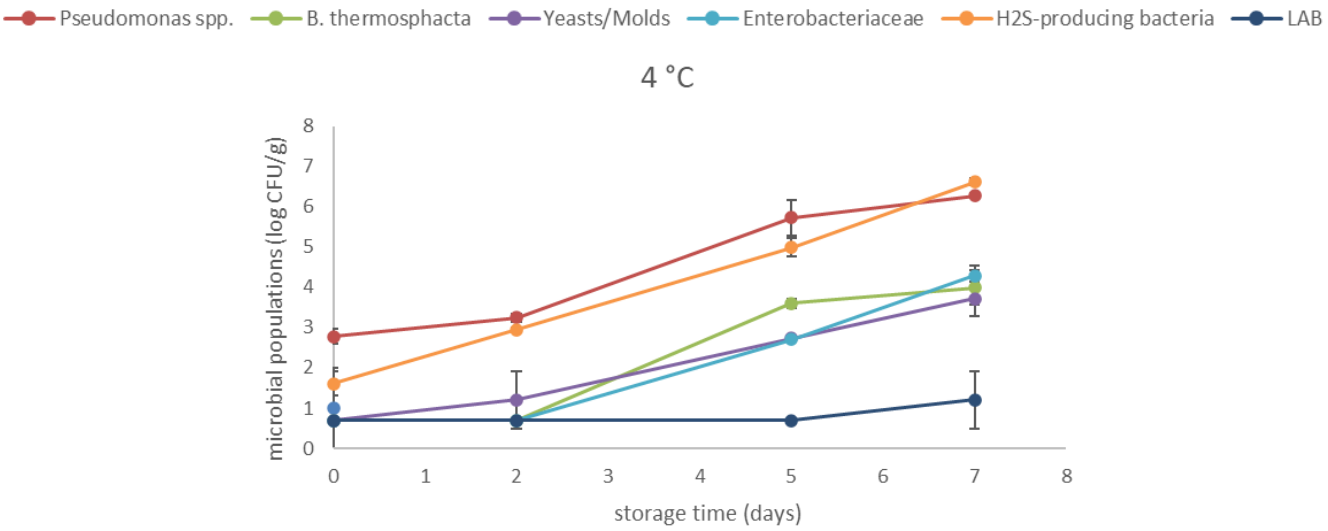


Figure 15. Microbial populations of seabream fillets obtained from retail stores throughout storage at 4 °C.

Sensory analysis

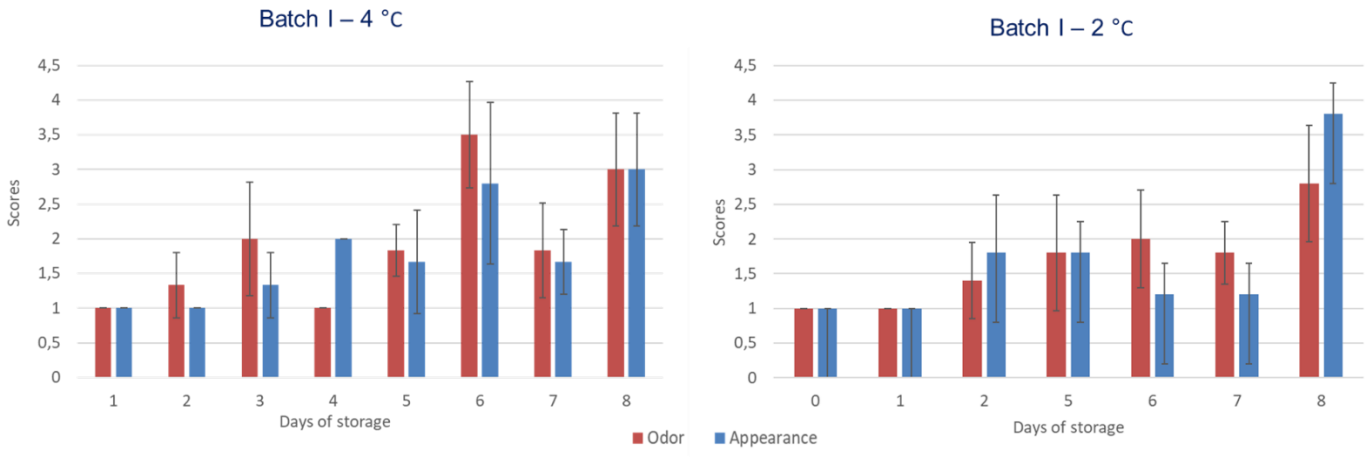


Figure 16. Sensory scores of seabream fillets obtained from retail stores throughout storage at 4 and 2 °C.

Multispectral Imaging Analysis

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 17).

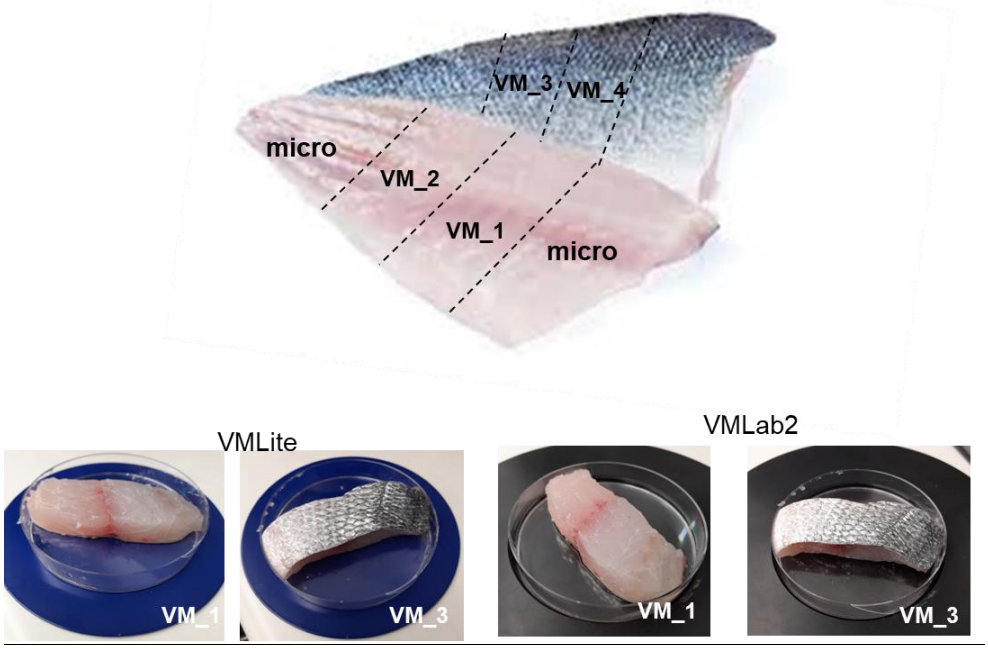


Figure 17. Samples used for microbiological analysis, VideometerLab and VideometerLite.

In Figure 17, 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 18) will be obtained and further used for the models' development and validation.

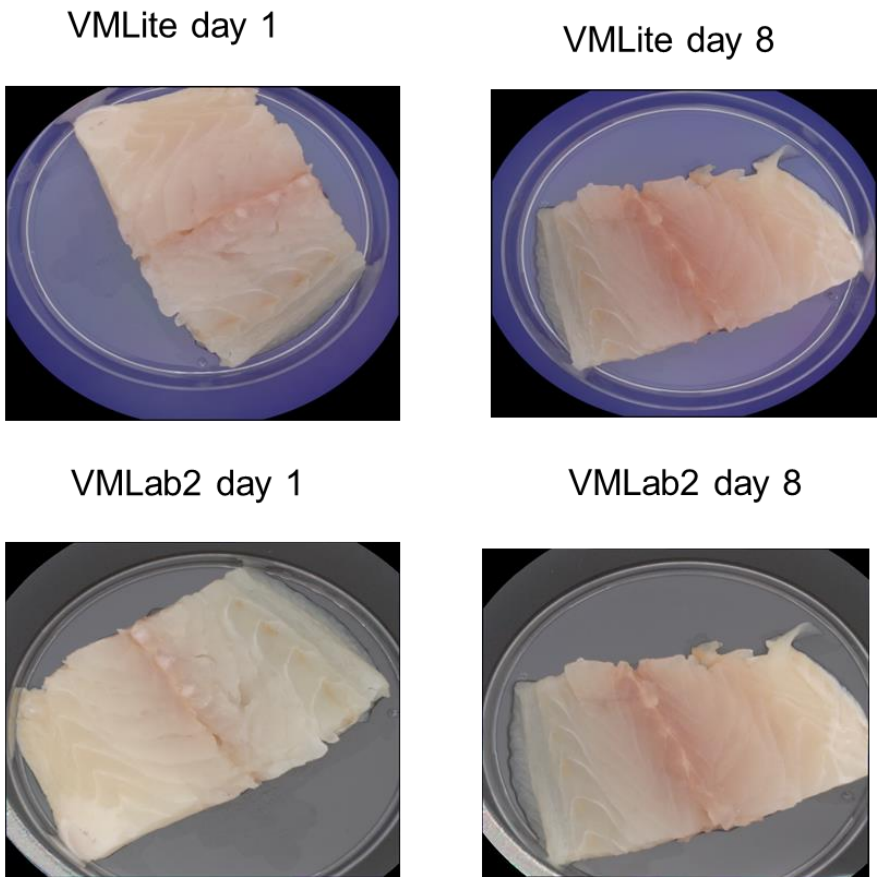
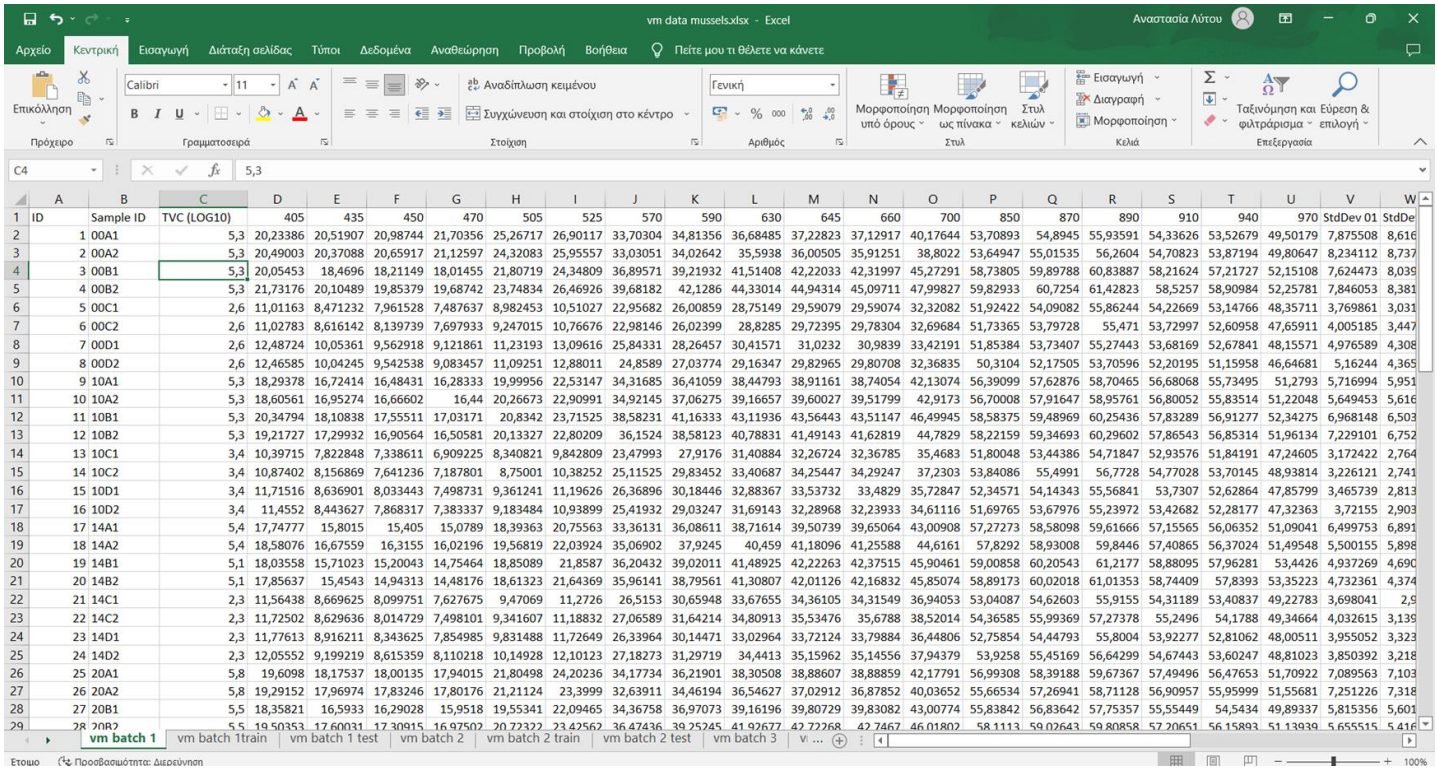


Figure 18. Indicative images captured with VideometerLab2 and VideometerLite.



vm data mussels.xlsx - Excel

ID	Sample ID	TVC (LOG10)	405	435	450	470	505	525	570	590	630	645	660	700	850	870	890	910	940	970	StdDev 01	StdDe
1	00A1	5,3	20,23386	20,51907	20,98744	21,70356	25,26717	26,90117	33,70304	34,81356	36,68485	37,22823	37,12917	40,17644	53,70893	54,8945	55,93591	54,33626	53,52679	49,50179	7,875508	8,616
2	00A2	5,3	20,49003	20,37088	20,65917	21,12597	24,32083	25,95557	33,03051	34,02642	35,5938	36,00505	35,91251	38,8022	53,64947	55,01535	56,2604	54,70823	53,87194	49,80647	8,234112	8,737
3	00B1	5,3	20,05453	18,4696	18,21149	18,01455	21,80719	24,34809	36,89571	39,21932	41,51408	42,22033	42,31997	45,27291	58,73805	59,89788	60,83887	58,21624	57,21727	52,15108	7,624473	8,039
4	00B2	5,3	21,73176	20,10489	19,85379	19,68742	23,74834	26,46926	39,68182	42,1286	44,33014	44,94314	45,09711	47,99827	59,82933	60,7254	61,42823	58,5257	58,90984	52,25781	7,846053	8,381
5	00C1	2,6	11,01163	8,471232	7,961528	7,487637	8,982453	10,51027	22,95682	26,00859	28,75149	29,59079	29,59074	32,32082	51,92422	54,09082	55,86244	54,22669	53,14766	48,35711	3,769861	3,031
6	00C2	2,6	11,02783	8,616142	8,139739	7,697933	9,247015	10,76676	22,98146	26,02399	28,8285	29,72395	29,78304	32,69684	51,73365	53,79728	55,471	53,72997	52,60958	47,65911	4,005185	3,447
7	00D1	2,6	12,48724	10,05361	9,562918	9,121861	11,23193	13,09616	25,84331	28,26457	30,41571	31,0232	30,9839	33,42191	51,85384	53,73407	55,27443	53,68169	52,67841	48,15571	4,976589	4,308
8	00D2	2,6	12,46585	10,04245	9,542538	9,083457	11,09251	12,88011	24,8589	27,03774	29,16347	29,82965	29,80708	32,36835	50,3104	52,17505	53,70596	52,20195	51,15958	46,64681	5,16244	4,365
9	10A1	5,3	18,29378	16,72414	16,48431	16,28333	19,99956	22,53147	34,31685	36,41059	38,44793	38,91161	38,74054	42,13074	56,39099	57,62876	58,70465	56,68068	55,73495	51,2793	5,716994	5,951
10	10A2	5,3	18,60561	16,95274	16,66602	16,44	20,26673	22,90991	34,92145	37,06275	39,16657	39,60027	39,51799	42,9173	56,70008	57,91647	58,95761	56,80052	55,83514	51,22048	5,649453	5,616
11	10B1	5,3	20,34794	18,10838	17,55511	17,03171	20,8342	23,71525	38,58231	41,16333	43,11936	43,56443	43,51147	46,49945	58,58375	59,48969	60,25436	57,83289	56,91277	52,34275	6,968148	6,503
12	10B2	5,3	19,21727	17,29932	16,90564	16,50581	20,13327	22,80209	36,1524	38,58123	40,78831	41,49143	41,62819	44,7829	58,22159	59,34693	60,29602	57,86543	56,85314	51,96134	7,229101	6,752
13	10C1	3,4	10,39715	7,822848	7,338611	6,909225	8,340821	9,842809	23,47993	27,9176	31,40884	32,26724	32,36785	35,4683	51,80048	53,44386	54,71847	52,93576	51,84191	47,24605	3,172422	2,764
14	10C2	3,4	10,87402	8,156869	7,641236	7,187801	8,75001	10,38252	25,11525	29,83452	33,40687	34,25447	34,29247	37,2303	53,84086	55,4991	56,7728	54,77028	53,70145	48,93814	3,226121	2,741
15	10D1	3,4	11,71516	8,636901	8,033443	7,498731	9,361241	11,19626	26,36896	30,18446	32,88367	33,53732	33,4829	35,72847	52,34571	54,14343	55,56841	53,7307	52,62864	47,85799	3,465739	2,813
16	10D2	3,4	11,4552	8,443627	7,868317	7,383337	9,183484	10,93899	25,41932	29,03247	31,69143	32,28968	32,23933	34,61116	51,69765	53,67976	55,23972	53,42682	52,28177	47,32363	3,72155	2,903
17	14A1	5,4	17,74777	15,8015	15,405	15,0789	18,39363	20,75563	33,36131	36,08611	38,71614	39,50739	39,65064	43,00908	57,27273	58,58098	59,61666	57,15565	56,06352	51,09041	6,499753	6,891
18	14A2	5,4	18,58076	16,67559	16,3155	16,02196	19,56819	22,03924	35,06902	37,9245	40,459	41,18096	41,25588	44,6161	57,8292	58,93008	59,8446	57,40865	56,37024	51,49548	5,500155	5,898
19	14B1	5,1	18,03558	15,71023	15,20043	14,75464	18,85089	21,8587	36,20432	39,02011	41,48925	42,22263	42,37515	45,90461	59,00858	60,20543	61,2177	58,88095	57,96281	53,4426	4,937269	4,690
20	14B2	5,1	17,85637	15,4543	14,94313	14,48176	18,61323	21,64369	35,96141	38,79561	41,30807	42,01126	42,16832	45,85074	58,89173	60,02018	61,01353	58,74409	57,8393	53,35223	4,732361	4,374
21	14C1	2,3	11,56438	8,669625	8,099751	7,627675	9,47069	11,2726	26,5153	30,65948	33,67655	34,36105	34,31549	36,94053	53,04087	54,62603	55,9155	54,31189	53,40837	49,22783	3,698041	2,9
22	14C2	2,3	11,72502	8,629636	8,014729	7,498101	9,341607	11,18832	27,06589	31,64214	34,80913	35,53476	35,6788	38,52014	54,36585	55,99369	57,27378	55,2496	54,1788	49,34664	4,032615	3,139
23	14D1	2,3	11,77613	8,916211	8,343625	7,854985	9,831488	11,72649	26,33964	30,14471	33,02964	33,72124	33,79884	36,44806	52,75854	54,44793	55,8004	53,92277	52,81062	48,00511	3,955052	3,323
24	14D2	2,3	12,05552	9,199219	8,615359	8,110218	10,14928	12,10123	27,18273	31,29719	34,4413	35,15962	35,14556	37,94379	53,9258	55,45169	56,64299	54,67443	53,60247	48,81023	3,850392	3,218
25	20A1	5,8	19,6098	18,17537	18,00135	17,94015	21,80498	24,20236	34,17734	36,21901	38,30508	38,88607	38,88859	42,17791	56,99308	58,39188	59,67367	57,49496	56,47653	51,70922	7,089563	7,103
26	20A2	5,8	19,29152	17,96974	17,83246	17,80176	21,21124	23,3999	32,63911	34,46194	36,54627	37,02912	36,87852	40,03652	55,66534	57,26941	58,71128	56,90957	55,95999	51,55681	7,251226	7,318
27	20B1	5,5	18,35821	16,5933	16,29028	15,9518	19,55341	22,09465	34,36758	36,97073	39,16196	39,80729	39,83082	43,00774	55,83842	56,83642	57,75357	55,55449	54,5434	49,89337	5,815356	5,601
28	20B2	5,5	19,50353	17,60031	17,30915	16,97502	20,72322	23,42562	36,47436	39,25245	41,92677	42,72268	42,7467	46,01802	58,1113	59,02643	59,80858	57,20651	56,15893	51,13939	5,655515	5,416

Figure 19. An example of a dataset from VideometerLab2 that will be used for the models development.

3 MULTISPECTRAL IMAGING SENSOR EVALUATION

3.1 OUTLINE OF VIDEOM MULTISPECTRAL IMAGING SENSOR

The Videometer Spectral Imaging Technology is one 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 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 65×65 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 will provide a modified version of the VideometerLite system (VideometerLite - Videometer), shown in Figure 20, that will be 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 20. 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.

4 CONCLUSIONS

Herein, we present the actions performed with regard to pilots execution based on the D2.1 and D2.3 where the pilots' experimental design and needs have been defined. TraceMyFish involved partners, namely AUA, NTNU, MATIS and UOI proceeded with data acquisition under several different scenarios of VideoMeterLite system applications concerning quality, microbiological assessment and other aspects/properties (e.g. nematode detection and evaluation of the depth that can be detected) showcasing the quality and safety deterioration of the fish products under investigation as they considered within the TraceMyFish context. The work has been extended (with regard to D2.3) to additional interesting for the industry aspects that emerged during the experimentation in a research form of study and under the evaluation of the detection power of the data acquisition system used, i.e. VideoMeterLite. Large volume of data have been acquired while more data will continue to be accumulated during the pilots. So far, just a preliminary evaluation for all use cases (the three considered fish value chains, namely 1) the Atlantic salmon value chain, 2) the Atlantic whitefish value chain, and 3) the Mediterranean seabream/seabass value chain) has been performed since data acquisition is currently performed and more data are needed in order to extract robust and firm results. Having said that, they system, acquisition, experimental design, user experience concerning the employment of the sensor and corresponding/expected results have shown promising indications on the basis of addressing the needs and requirements of the fish value chain actors with the main objective to be as close to reality (samples traveling along the food chain) in terms of conditions and possibly emerging hazards.