Project Acronym:	PhasmaFOOD
Grant Agreement number:	732541 (H2020-ICT-2016-1 - RIA)
Project Full Title:	Portable photonic miniaturised smart system for on-the-spot food quality sensing

DELIVERABLE

Deliverable Number	**D3.1**
Deliverable Name	**Feasibility results and use case benchmarking-v1**
Dissemination level	Public
Type of Document	Report
Contractual date of delivery	30th September 2017
Deliverable Leader	AUA
Status & version	V4.0
WP / Task responsible	WP3 / T3.1, 3.2 and 3.3 - AUA
Keywords:	alcoholic beverages, fish, fraud, fruits, grains, image analysis, meat, milk, mycotoxins, nuts, oil, spectrum analysis, spoilage, vegetables
Abstract (few lines):	The PhasmaFOOD project aims to develop a miniaturized, multi-parameter and programmable sensing node for (i) detection of mycotoxins, (ii) detection of food spoilage and prediction of shelf life; and (iii) detection of food fraud and adulteration. In this report, a feasibility assessment of the above mentioned (sub-) use cases is initially displayed. D3.1 is the first report of results on real-life samples in a series of successive reports (D3.2 - M18 and D3.7 - M27) leading to a final feasibility assessment of the proposed use cases. In order to do so, this deliverable describes the standard operating procedures for the PhasmaFOOD VIS and NIR sensors, sampling strategies, data assessment and conclusions until M9 of the project. For the

	mycotoxin use case, first results on sub-use cases maize flour and almonds are reported, whilst for the spoilage use case, extensive experimental data are produced for fish and rocket salad. The NIR sensor is tested for general food samples and oils' mixtures for feasibility in the food fraud use case.

Deliverable Leader:	George-John E. Nychas (AUA)
Contributors:	AUA, CNR, DLO, IPMS
Reviewers:	IPMS, WINGS
Approved by:	INTRA

Document History			
Version	**Date**	**Contributor(s)**	**Description**
0.1	30/06/2017	AUA, CNR, DLO, IPMS	Early version for internal review
0.2	02/08/2017	AUA, CNR, DLO, IPMS	Second version for internal review
1.0	01/09/2017	AUA, CNR, DLO, IPMS	First version for consortium review
2.0	13/09/2017	AUA, CNR, DLO, IPMS	Second version for consortium review
3.0	18/09/2017	AUA, CNR, DLO, IPMS	Third version-Submitted for internal review (reviewers: IPMS, WINGS)
4.0	28/09/2017	AUA, CNR, DLO, IPMS	Final version

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

Table of Contents

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

Definitions, Acronyms and Abbreviations

Acronym	Title
CO	Confidential, only for members of the consortium (including Commission Services)
CR	Change Request
D	Demonstrator
DL	Deliverable Leader
DM	Dissemination Manager
DMS	Document Management System
DoA	Description of Action
Dx	Deliverable (where x defines the deliverable identification number e.g. D1.1.1)
EIM	Exploitation Innovation Manager
EU	European Union
FM	Financial Manager
MSx	project Milestone (where x defines a project milestone e.g. MS3)
Mx	Month (where x defines a project month e.g. M10)
NIR	Near-infrared
O	Other
P	Prototype
PC	Project Coordinator
PM	partner Project Manager
PO	Project Officer
PP	Restricted to other program participants (including the Commission Services)
PU	Public
QA	Quality Assurance
QAP	Quality Assurance Plan
QFD	Quality Function Deployment
QM	Quality Manager
R	Report
RE	Restricted to a group specified by the consortium (including Commission Services)
STM	Scientific and Technical Manager
TL	Task Leader
UV	Ultraviolet
VIS	visible
WP	Work Package
WPL	Work Package Leader
WPS	Work Package Structure

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

1 Executive summary

The PhasmaFOOD project aims to develop a multifunctional optics-based miniaturized sensor for fast characterization of foods by industry and consumers. The PhasmaFOOD scanner will be smart-phone operated and consist of a visible (VIS) and near-infrared (NIR) spectrometer and a board-level VIS camera. Specifically, WP3 is focusing on assessing the performance of the individual micro-devices (sensors) on three use cases, namely (i) mycotoxin detection, (ii) food spoilage detection and shelf-life prediction, and (iii) food fraud detection. This assessment will be performed prior to the delivery of the fully integrated prototype device by (a) benchmarking individual food sub-use cases, (b) developing smart signal processing in tandem with chemometrics, (c) building a basic reference database for each sub-use case backed up by chemical and/or other fingerprinting reference methods, (d) validating the established reference database, and finally (e) developing smart data correlation algorithms between the tested micro-devices prior to full integration.

In this first version of reports dealing with the deliverable *'Feasibility results and use case benchmarking*', the initial results of the feasibility of the individual PhasmaFOOD sensors on the different target foods as well as benchmarking of the individual food sub-use cases are reported. Specifically, in this deliverable, measurement strategies, planning, sensors' standard operating procedures, sampling strategies, reference methods and chemometric protocols are elaborated upon during the first nine months of activity in the project.

This comprises first VIS measurements of mycotoxins in maize flours and almonds for the first use case. In the use case of spoilage and shelf-life prediction, extensive data are reported on fish and vegetable (rocket salad) spoilage, using the PhasmaFOOD VIS and NIR spectrometer sensors as well as relatively more well established spectral techniques (vibrational spectroscopy and surface chemistry) as a comparison. Finally, for the third use case on food fraud, the sampling and measurement strategy is discussed, while preliminary measurements on general food samples and oils' mixtures also are presented.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

2 Introduction

The need for the development of analytical techniques and/or instruments capable of providing credible estimates of food safety and quality in a timely, rapid and non-invasive manner has been well acknowledged during the last decade. Such a need has provided the trigger for truly ambitious research attempts aiming at the development of sensors that will be able to accurately describe various characteristics or properties of food products that delineate their safety and/or quality status. Spectroscopy and imaging technologies hold a prominent position among the approaches that have been evaluated and utilized for the purpose of food sensor development, demonstrating a promising potential with regard to the assessment of various aspects pertinent to food protection.

Therefore, the aim of PhasmaFOOD project is to develop a multifunctional optical sensing node for food applications that will be ultimately operated by consumers. The scanner array consists of a visible (VIS) and near-infrared (NIR) spectrometer and a board level camera (CMOS) and will be smartphone operated. In this report, first feasibility assessment of the three use cases on which the PhasmaFOOD sensor will be initially tested are reported: (I) mycotoxin detection, (II) food spoilage detection and shelf-life prediction and (III) food fraud detection. An extensive description of each use case is contained in Deliverable Report D1.1 – Use case description and validation plan. Each use case consists of different target foods and, therefore, different experimental approaches and criteria for feasibility are used for each use case. This report (D3.1) is the first report of results on real-life samples in a series of successive reports (D3.2 - M18 and D3.7 - M27) leading to a final feasibility assessment of the proposed use cases.

2.1 State of the art

2.1.1 Use case 1: detection of mycotoxins in food products

Mycotoxins are toxic secondary metabolites produced by certain species of fungi mainly in contaminated grains (Neme and Mohammed, 2017). The presence of mycotoxins in animal feed and the food supply chain constitutes a significant global food safety issue (Zachariasova et al., 2014). Indeed, consumption of food products contaminated with mycotoxins has been associated with adverse health effects, ranging from transient symptoms such as nausea and vomiting to long-term genotoxicity and carcinogenicity (Borchers et al., 2017). One of the challenges in the detection of mycotoxins in food lies in the high cost, time and labour requirements of well-established analytical methods such as thin layer chromatography and liquid chromatography mass spectrometry (Miedaner et al., 2015).

Various alternative approaches have been recently assessed for their potential utilization in mycotoxins detection, including the use of biosensors, electrochemical-based sensing

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

platforms, Fourier transform infrared (FTIR) spectroscopy, hyperspectral imaging and complementary metal–oxide semiconductor (CMOS) sensor (Chauhan et al., 2016; Hernández et al., 2017; Kos et al., 2016; Orlov et al., 2017; Sharma et al., 2017; Toro et al., 2016; Wang et al., 2015). Nonetheless, given that most of the analytical spectroscopic techniques that have been individually evaluated only allow for the detection of rather high concentrations of mycotoxins, the conjunctional approach of the PhasmaFOOD project is anticipated to result in more robust data, potentially allowing for a higher accessibility of detection methods throughout the food supply chain. In this context, the application of the PhasmaFOOD sensor is expected to constitute an alternative means of mycotoxins detection, in a time-efficient and non-destructive to food samples manner. The different sub-use cases considered in the PhasmaFOOD project are:

1. **Maize (flour)**. AF B1, total AFs and DON
2. **Milk powder**. AF M1 and M2
3. **Paprika powder**. AF B1 and total AFs
4. **Tree nuts (homogenised or whole nuts)**. AF B1 and total AFs

2.1.2 Use case 2: detection of early sign of spoilage, spoilage and shelf-life estimation in meat, fish, fruit and vegetables.

Food quality, although constituting a central theme in food science research, has been considered as a term not easily definable scientifically and that it comprises many different aspects, with the latter being subject to constant changes. It has been opined that *"food quality represents the sum of all properties and assessable attributes of a food item"*, and that this is accomplished through three categories of quality: sensory value, suitability value and health value (Leitzmann, 1993). In the context of a holistic assessment of food quality, several additional (to the aforementioned) categories of quality have been also taken into account, including notional, cultural, political and ecological values of food (Leitzmann, 1993). Indeed, it is widely accepted that the consumers' perception regarding food quality is a very important parameter when assessing food spoilage and shelf-life. Food spoilage, a complex ecological phenomenon which is underlain mainly by the biochemical activity of microorganisms, is related mainly to the sensoric and suitability values of food quality. The food quality changes composing spoilage are related to the metabolic activity of certain groups of microorganisms, referred to as "specific spoilage organisms", and the type and availability of the required energy substrates in foods (Hamad, 2012; Koutsoumanis and Nychas, 2000). Although numerous methods (organoleptic, microbiological or physico-chemical) have been developed for the purpose of food quality assessment (Ellis and Goodacre, 2001; Karoui and De Baerdemaeker, 2007), the majority of them are time-consuming, labour-intensive, destructive, and provide

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

retrospective information. Hence, various novel analytical approaches have been recently evaluated and proposed for the non-destructive and rapid assessment of food spoilage.

Examples of such promising approaches include enzymatic reactor systems, sensor arrays (e.g. electronic noses), spectroscopy methods (e.g. vibrational, NMR or mass spectroscopy techniques), as well as imaging technology approaches (Dufour, 2011; Papadopoulou et al., 2013; Porep et al., 2015; Xiong et al., 2015). By means of combining VIS, NIR and CMOS images, the PhasmaFOOD sensor is expected to be effective in estimating spoilage and shelf-life of a fresh product. Still, since food spoilage is a rather complex ecological phenomenon, it should be kept in mind that spoilage prediction can be a fairly difficult task, also in the PhasmaFOOD project. One has to take into account biochemical activity of specific groups of microorganisms (strongly associated with the shelf-life of various food products), the evolution of these specific groups of microorganisms evaluated in conjunction with the physical and sensory changes of the food product (e.g. colour, appearance, odour) *etc.* The different sub-use cases considered in the PhasmaFOOD project are 'fresh' products which are highly perishable and may pose a high risk towards consumers for foodborne infection or intoxication:

1. Meat
2. Fish
3. Fruit and vegetables

2.1.3 Use case 3: food fraud

Food fraud is a collective term referring to the *"deliberate substitution, addition, tampering or misrepresentation of food, food ingredients or packaging, or false or misleading statements made about a product for economic gain"*, as defined by the United States Pharmacopeia Convention (USP Pharmacopeia Convention Food Fraud Database). In Europe, consumers are protected by EC Regulation No. 178/2002, underpinning the concept of informed consumer choice in the purchase of food. Unfortunately, the number of food adulteration and fraud cases being unravelled in several EU member states is rising. Since the conventional laboratory analysis methods for detecting fraud/adulteration are laborious and expensive, the need for a smart non-invasive, rapid and, ideally, hand-held device is eminent. In this framework, various analytical technologies have been recently assessed for their efficacy in detecting food fraud/adulteration and, thus, for their potential value in food authentication. Such analytical technologies include visible/near-infrared spectroscopy, UV-VIS spectroscopy, use of compact digital camera as well as image analysis approaches, while examples of food authentication applications being evaluated include edible oil composition monitoring, detection of minced meat adulteration and fresh/frozen-thawed fish discrimination (Fernandes et al., 2013; Ottavian et al., 2014; Ropodi et al., 2015, 2016, 2017).

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

Overall, the smart sensor-based system which is planned to be developed within the PhasmaFOOD project, is anticipated to allow for the accurate assessment of all the aforementioned food protection aspects through the utilization of relevant spectral and/or imaging data, and as such to be of great value for practical application throughout the food supply chain (food manufacturers, retailers, food service, consumers). The different sub-use cases considered are:

1. Milk powder
2. Meat
3. Alcoholic beverages
4. Edible oils

2.2 Description of micro-sensors

2.2.1 NIR microspectrometer

The PhasmaFOOD smart sensing system will integrate a miniaturised NIR spectrometer to cover the wavelength range from 1000 to 1900 nm (Fig. 1). The device was developed by partner IPMS, with a size of the sensor head of 16 × 17 × 12 mm. The central active component inside this device is a miniature optical grating, which oscillates resonantly at a frequency of ~ 100 Hz, driven by electrostatic forces from a comb-like structure. This micro-electromechanical (MEMS) component is fabricated in the IPMS clean room (Pügner et al., 2016). The assembly of the spectrometer also takes place at IPMS. IPMS provides this spectrometer as a demonstrator kit complete with read-out electronics but without housing, as this is part of the integration work of WPs 2 and 5. Once the miniaturized NIR spectrometer is integrated into the PhasmaFOOD device, it will be available for testing in WP3. Further technical details are elaborated in Deliverable Report D2.2.

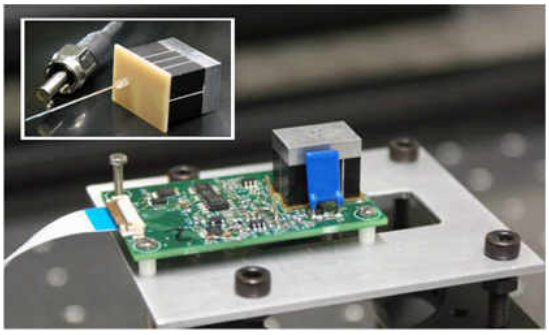

Figure 1: MEMS, miniaturized version of the NIR spectrometer developed at IPMS.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

In the meantime, for laboratory testing in the early stages of WP3, a more robust solution was found, namely the NIR spectrometer SGS1900 (Fig. 2). This device incorporates the same technology and measuring principle as the above miniaturized version and is commercially available from Hiperscan GmbH, a spin-off of IPMS. For the purposes of WP3, in order to test the general applicability of the sensing method for the three PhasmaFOOD use-cases, an SGS1900 instrument was provided by IPMS as a free loan to PhasmaFOOD partners.

Figure 2: The NIR spectrometer SGS1900 used for testing of the general applicability of the sensing method for the PhasmaFOOD use cases.

The size of the SGS1900 housing is 105 x 80 x 86 mm. It comprises a MEMS-based scanning grating for spectral dispersion, and an uncooled InGaAs diode for detection. A detailed data sheet is available online (HiperScan). A halogen light source provided illumination (in-house constructed by IPMS) was coupled via SMA 905 connector into an Ocean optics Y-shaped fibre bundle (QR400-ANGLE-VIS). The fibre bundle comprises a probe tip with a window, which is set at 30° to the front face of the fibres. During measurement, this window is placed in direct contact with the sample. Light from the halogen source is fed to the sample and the diffuse reflectance from the sample is collected back into a 400 µm core optical fibre in the centre of the fibre bundle. This fibre then transmits the collected light to the SGS1900 spectrometer via SMA connector (Fig. 3). The software "Quickstep" (version 0.99 by Hiperscan GmbH) serves as operation software.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

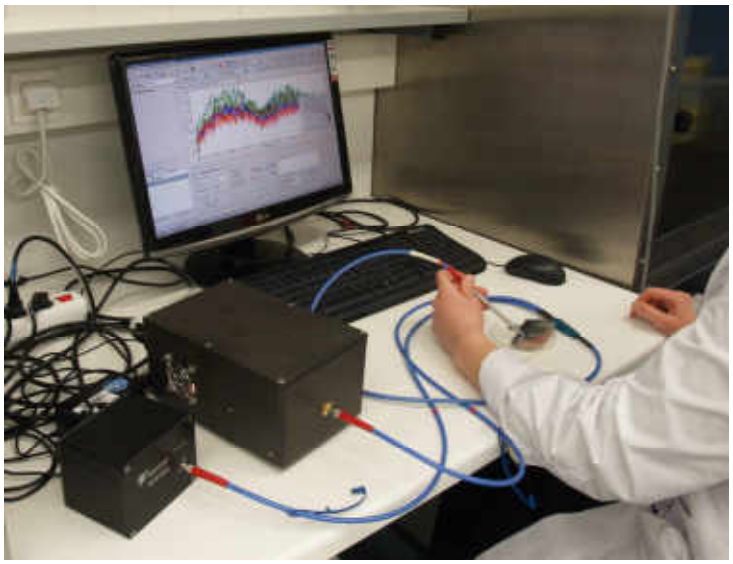

Figure 3: Experimental set up of the NIR spectrometer compartments.

2.2.2 UV-VIS spectrometer

The UV-VIS spectrometer used in the PhasmaFOOD sensor is the Hamamatsu C12880MA. The device has a spectral range from 340 to 850 nm (larger than other models), high sensitivity (Conversione Efficiency 50 µs/e-), compact dimensions (20 mm × 15 mm × 10 mm) and spectral resolution of 15 nm. In our specific application, it is employed to detect the fluorescence signal of aflatoxins and for visible range spectroscopy in the two other use cases, so only the visible range is exploited. A UV filter with 400 nm cutoff wavelength is introduced in front of the spectrometer aperture to avoid that the UV illumination used to excite the fluorescence could saturate the detected signal. Such a filter will also be integrated into the PhasmaFOOD sensing prototype, into the parallel beam section between sample and UV-VIS spectrometer. In the first phase of the project, the evaluation board from Hamamatsu has been used to drive the UV-VIS sensor. A custom developed board is under development by the project partners CNR and WINGS.

Figure 4: Hamamatsu C12880MA UV-VIS spectrometer.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

2.2.3 CMOS camera

A miniature CMOS camera from Ximea (MU9PC-MH model) has been chosen to be integrated in the PhasmaFOOD sensor (Fig. 5). The CMOS camera measures 15 mm × 15 × 8 mm and has a resolution of 5 mega pixels (2592 × 1944 pixels), which to the best of our knowledge fits the sensing requirements of the use cases (D1.1) and hardware requirements (D1.2). The Ximea camera has high definition, high sensitivity, low crosstalk and low noise image capture capability in an ultra-small and lightweight design in the visible range. The CMOS camera will help the user to identify the region of interest on the food samples in for example identification of microbial spots in the use case on spoilage and shelf life prediction. In addition, the camera may be employed to perform multispectral imaging by using Red-Green-Blue (RGB) imaging analysis and possibly LEDs of different colors in order to get complementary information on the targeted use-case.

Figure 5: The Ximea MU9PC-MH camera with a 20 Eurocent coin for size comparison.

2.3 WP3 and D3.1 scope, strategy and planning

The goal of this WP is to test the individual use cases as described in D1.1 for feasibility. This includes first activities on practical application of the PhasmaFOOD micro-sensors on realistic samples, exploration of the methods for smart signal processing, chemometrics, development and adaptation of algorithms for decision making and initial validation of the spectral databases belonging to each individual (sub-) use case. Furthermore, data-fusion strategies are explored to fully exploit the advantage of using multiple spectral sensors and to accurately estimate the feasibility of the use cases as they were proposed in D1.1. In this deliverable, a focus has been placed on reporting initial exploration of feasibility of the use cases: detection of mycotoxins in grains and nuts: Aflatoxin detection (T3.1), detection of early sign of spoilage, spoilage and shelf-life estimation in fruits, vegetables, meat and fish (T3.2) and detection of food fraud: adulteration of alcoholic beverages, oil, milk powder and meat (T3.3).

The results presented in this report were obtained by using the individual UV-VIS, NIR and (if applicable) the CMOS camera sensors in the period M1-M9. This strategy has been adapted to

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

already gain insight in the practical application of the sensors before completion of the first PhasmaFOOD prototype. Furthermore, the findings in this report provide feed-back on the design of the integrated PhasmaFOOD instrument and the timely delivery of spectral data-bases which are extensive enough to provide TLR4 validated results before the end of the project.

Within this reporting period (till M9), activities have been concerned with writing standard operating procedures (SOPs) for standardized operation of the micro-sensors in each laboratory where feasibility testing is performed (CNR, AUA and DLO). Furthermore, for the period M1-M12, a planning has been produced for the circulation of the micro-sensors between the involved laboratories, taking into consideration the time required to perform measurements for feasibility estimation. Most sensor time in Year 1 has been allocated for T3.2 as experiments on spoilage are lengthy (ca. M5-M8), whilst for T3.1 (M5, M11) and T3.3 (M11 and M12) most samples can be acquired and stored until sensors are available. Therefore, in this deliverable, first results on mycotoxins (maize flour and almonds) are reported, while for T3.2 already more extensive data on spoilage of fish and rocket salad are available. The measurement and sampling strategies for T3.3 are elaborated upon in D3.1, some initial preliminary in nature measurements using the NIR sensor are presented, whereas additional results are expected in M11 and M12.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

3 Materials and methods

3.1 SOPs for micro-sensor operation

3.1.1 NIR spectrometer

In this section, the operation procedure for the Hiperscan SGS1900 NIR spectrometer (Serial Number W1141 157) is described. The NIR micro-spectrometer will follow a similar operation procedure albeit details like the software structure may differ. The steps for operating the SGS1900 spectrometer include:

0. Prior to operation: Install the NIR acquisition software on your lab computer. In order to run it, you require the MONO version 2.6.4 (or older). IPMS provides access to both programs through a content server during the period of the PhasmaFOOD project.
1. Setting up: Connect the SGS1900 spectrometer to grid power (220 V) via the included power adapter. The power status light of the SGS1900 should light up green. Connect the SGS1900 spectrometer to your laboratory computer via the included micro-USB-to-USB cable. Switch on your lab computer. Start the NIR acquisition software. The USB status light of the SGS1900 should pulse in green during data transfers while the instrument is being initialized. Once the instrument is properly initialized, you will see its registration number in the "Device" window.
   Inspect the two ends of the fiber bundle – one end comprises six fiber s in a ring, the other end comprises a single central fiber. Connect the single- fiber end to the SGS1900 spectrometer by plugging it into the SMA connector and fixing the screw. Connect the six-fiber end to the halogen light source in the same way. Connect the light source to grid power and switch it on by pressing the black switch at the back of the housing. Looking into the angled window of the fiber probe, you should now see a ring of six fibers lighting up. Wait for 5 minutes for the lamp to warm up.
2. Set number of averages: In the NIR acquisition software ("Properties" window), select the number of averages. If this window does not show upon starting the software, you can open it by choosing the "View" tab in the menu bar, then "Window layout" and then selecting "Property editor". A high number of averages results in longer measurement times and in reduced data noise. A typical number is 1000 averages. Above 2000 averages, no further reduction of noise is expected. You may change the number of averages by typing a number into the "Averages" window and pressing "Apply". The software will send a reminder message to renew the dark and background references once the number of averages has changed.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

3. Dark intensity reference (after each change of acquisition parameters): Make sure that the SGS1900 spectrometer does not receive light by disconnecting the fiber from the SGS1900 or by pointing the fiber probe towards dark empty space. In the software, press the red button next to "Dark intensity". Once the measurement is done, confirm the setting by pressing "Apply". The dark intensity measurement is required to correct for the baseline noise of each measurement. Data can later be exported in a format that has this baseline correction automatically included.
4. White background reference (after each change of acquisition parameters): Connect the fiber bundle to SGS1900 spectrometer and halogen lamp as described above. Make sure that the fiber probe is clean and dry. Leave the light on, hold the fiber probe so that it touches a surface suitable as a white reference (a PTFE target or any other white reference target suitable for NIR). In the software, press the red button next to "Background". The spectrometer should now record the spectrum of the halogen lamp which is scattered back from the reference target. This measurement is saved internally in the NIR acquisition software. Once the measurement is done, confirm the setting by pressing "Apply". The white background reference measurement is required to correct for the lamp spectrum and calculate extinction/absorbance from the raw data.
   Note: Dark intensity and background lamp spectrum may change slightly over time. Therefore, each time the spectrometer is switched on, these references must be recorded again. After that, press "Apply" and you're ready to start measuring. If you are measuring in long sessions, it is advisable to record new references approx. every 2 hours.
5. Measurement – setup: Measurements with the y-shaped fiber bundle are done in direct contact with the sample. The tip of the fiber probe is cut at an angle of 30° in order to avoid direct reflexes from the sample. Only diffuse scattered light is collected, which contains information about the NIR absorption spectrum of the sample. Hold the fiber probe so that the window at the tip of the probe and the sample surface are in full contact, i.e. parallel.
   Note: This measurement geometry is suitable for samples that absorb or scatter light in the NIR spectral range. Transparent samples cannot be investigated this way. Intermediate cases may arise when samples are physically thin such that the NIR lamp light is partly transmitted through them. In these cases, please fold the sample over or stack several samples to increase the thickness. A sufficient thickness is reached when no NIR lamp light is observed through the back of the sample. If this precaution is not followed, the physical background of the sample will influence the measurement.
6. Measurement – data acquisition: In the software, press the red button to acquire a spectrum. Hold the fiber tip still during acquisition. Several spectra may be acquired in sequence, e.g. to observe the variation across a single sample. For a quick overview of the measured data, the acquisition software opens a tab with the spectrum diagram. Via the "View" tab in the main menu, the "Plot type" view mode in this window can be

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

changed between intensity/absorbance/SNV and other modes. Under "Plot type" you can select your preferred option. Default is an intensity plot corrected for the dark intensity. For evaluation, Absorbance is preferred.

7. Saving data:
   Option 1: Select the "File" tab in the main menu, then select "Export". The acquisition software will export all data from the currently viewed window in the currently used View option into a single *.csv file. Caution: Please select Intensity mode before exporting data. The acquisition software has a known bug in the export of absorbance values. The dark counts offset is automatically subtracted in the saved intensity values. The white reference spectrum required to calculate absorbance is not saved automatically but is only stored in the software while running the spectrometer. In order to calculate absorbance later during data analysis, the white lamp spectrum must be recorded like a regular spectrum in the same diagram window. Tip: Measurements can be grouped into sets by opening new diagram tabs, e.g. a new tab for each sample or session.
   Option 2: Select the "Extensions" tab in the main menu, then, "Acquisition", then select "Save after acquisition". You will be asked for the file name. Please use a file name in this format: "<sampleinfo>_01". The software will then save all spectra in the currently viewed diagram window in subsequently numbered (02, 03, etc. …) individual *.csv files. These files contain a header with meta information like instrument settings and dark counts, one column for x-axis (wavelength in nm), one column for intensity values (raw, dark counts NOT subtracted), a third column for absorbance values (dark counts subtracted and white reference spectrum taken into account). No bugs are known for this absorbance calculation, i.e. the absorbance spectra can be trusted and directly used in analysis.
   It is recommended to use Option 2 for saving data as all meta-information and raw data are automatically kept.
8. Shutting down: Close the acquisition software. Switch off the lamp. Disconnect all electrical and optical connections of the lamp and SGS1900 spectrometer. Clean the fiber probe window and put the plastic caps on the fiber ends/connectors of the spectrometer, lamp and probe.
9. Cleaning: If necessary, in order to avoid contamination between measurements or samples, the front window of the fiber probe can be cleaned by wiping with a simple tissue containing ethanol, isopropanol or DI water. Please do not use any other solvent (especially no acetone) or anything abrasive for cleaning. Please also do not dip the fiber probe into any solvent for a longer time to avoid solvent entering the housing. Dipping into liquids for a short time (minutes) is allowed, as long as the rim of the probe stays above the liquid. The probe window should be cleaned and dried directly after the end of the measurements, before the instrument is put away for the night. Simple optical

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

inspection by eye can tell whether the probe window needs cleaning between measurements.

3.1.2 UV-VIS spectrometer

The list of the possible steps for the operating procedure of the UV-VIS spectrometer is described below. Please note again that the spectrometer is protected with a UV excitation filter during measurements. Therefore, strictly only VIS spectra will be obtained throughout the PhasmaFOOD project. This does not affect the measuring procedure as it simply leads to zero signal over the blocking range of the UV filter. Measured spectral data are considered valid in the visible spectral range of the Hamamatsu spectrometer, i.e. between 400 nm and 850 nm. Please note, that this restriction does not apply to the light sources, which will illuminate in both, UV and VIS spectral ranges as the UV light is required to excited VIS fluorescence from the sample. The following measuring procedure is based on the first integrated design of UV-VIS spectrometer and lighting system (UV and white LEDs) (Fig. 6).

1. Power on the device (lights OFF).
2. Launch acquisition software.
3. From acquisition software, tool -> set parameters (integration time= for acquisition in white light reflectance mode, according to use case, usually hundreds of microseconds, number of average counts usually 10; for acquisition in fluorescence mode, integration time in the range of hundreds of milliseconds and 3 average counts).
4. Dark calibration
    a. While the lights are off, orientate the instrument toward a dark surface (the optical spot) and keeping the device under a darkening hood start dark mode to perform dark measurement and automatic storage.
5. White light reflectance mode (at the beginning of a new session or every day)
    a. turn VIS light ON
    b. Wait 15-20 minutes (this step depends on the white LED characteristics)
    c. Point the acquisition window toward a white reference (if not available white paper could be used)
    d. Start Reference mode and perform acquisition of the white reference.
    e. Put the acquisition window in front of the sample.
6. Measurement mode (performed for each single sample measurement)

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

a. Measurement mode and perform acquisition on the sample (different areas, and different samples). Of course, acquisition parameters must be kept constant throughout the measurement session.

b. The software for each measurement will give 3 files (*.csv format) as output: sample1.dark, sample1.reference, sample1 (if not changed during the measurement session, dark and reference file will be the same for all samples).

c. Data normalization and classification will be performed using the developed data analysis models (that could be different for each case study) considering also the dark and reference measurements.

7. 7. At the end of measurement session, perform measurement of the reference (using measurement mode).

8. Close measurement software

   a. Switch off illumination

   b. Switch off device

For the **Fluorescence mode**

1-to 3 as above

4. Dark calibration

   a. Direct the instrument toward a dark surface (the optical spot) and keeping the device under a darkening hood and Start dark mode (UV lights off) to perform dark measurement and automatic storage.

5. **UV light-induced Fluorescence mode (At the beginning of a new session or every day)**

   a. Turn UV light ON

   b. Point the acquisition window toward a non-fluorescent reflective reference standard (black plastic reference)

   c. Start Reference mode and perform acquisition.

6. Start Measurement mode and perform acquisition on the sample (different areas, and different samples). Of course, acquisition parameters must be kept constant throughout the measurement session. As already indicated, the software for each measurement will give 3 files (*.csv format) as output: sample1.dark, sample1.reference, sample1 (if not changed during the measurement session, dark and reference file will be the same for all samples).

7. At the end of measurement session, perform measurement of the reference (using measurement mode).

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

8. Close measurement software

    a. Switch off illumination

    b. Switch off device

3.1.3 CMOS camera

CMOS camera will work in two main modalities:

1. White light imaging.
2. Multi-channel imaging.

Complete operating procedures for calibration and image analysis will be defined according to use cases and upon completion of feasibility tests.

3.2 Detection of mycotoxins

In order to verify the feasibility of mycotoxins detection by fluorescence and VIS reflectance spectroscopy, several tests have been performed on samples prepared by ISPA-CNR at different aflatoxin contamination levels. The samples have been prepared by the inoculation technique described below and the corresponding contamination levels were in the range of parts per million (ppm). In this way, the ability of the VIS spectrometer to detect the presence of the contaminants could be assessed. The samples consisted of maize flour and almond flour both with different granularity. A reference sample for maize flour from Trilogy Lab was also used and differences between non-contaminated and contaminated sample spectra were recorded. More accurate calibration of the detectors will be performed in the successive project phases.

3.2.1 Maize flour

3.2.1.1 Materials

Production of maize kernels contaminated with aflatoxins

Maize kernels were inoculated with a strain of *Aspergillus flavus* that produces aflatoxins B_1 and B_2. In particular, maize kernels were subjected to a mild sterilization by immersion for 2 min in a solution of 1% sodium hypochlorite with manual agitation. Subsequently, kernels were washed with sterile water and then further surface-disinfected with 70% ethanol for 1 min. Kernels were then transferred, under sterile conditions, in sterile flasks and portions of 1 kg of maize were treated with 300 ml of a conidial suspension containing 3.3×10^5 conidia/ml of *A. flavus*.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

The conidial suspension was obtained from *A. flavus* inoculated on plates of potato dextrose agar (PDA) and grown for 4 days in the dark. Fungal conidia were collected from the surface of plates by using a sterile spatula and sterile water. Appropriate dilutions with sterile water were made to obtain a final concentration of 3.3×10^5 conidia/ml. After inoculation, flasks were manually stirred to uniformly distribute the conidial suspension on kernels' surface and closed with a stopper of raw cotton and a sheet of aluminum foil to avoid excessive water evaporation. The flasks were incubated for 4 days at 28 °C in the dark. After incubation, maize kernels were dried at 40 °C for 48 h. Then three portions of maize kernels (10 g each) were randomly collected from each batch, content of flask, and analyzed for their aflatoxin content.

Determination of AFB_1 and AFB_2

Five grams of dry ground sample were extracted with 50 ml of extraction mixture of acetone/water (85:15 v/v) by sonication for 30 min. The extract was filtered through a filter paper and 0.1 ml was diluted with 6 ml of MeCN/H_2O (30:70 v/v), stirred, filtered through a 0.45 µm PTFE and 0.1 ml was injected into the HPLC system (corresponding to 0.17 mg sample).

HPLC-FLD equipment and conditions

The HPLC-FLD analyses were performed with an Agilent 1260 consisting of a binary pump, an autosampler with a 100 µl loop, a fluorescence detector fixed at 365 nm λex and 435 nm λem and a thermostatic oven set at 30 °C. The column used was a 150 mm × 4.6 mm i.d., 3 µm, Luna PFP (2) (pentafluorophenyl-propyl) with a 3 mm i.d., 0.45 µm pore size guard. The chromatographic separation was performed in the isocratic condition using a mixture of MeCN/H_2O (30:70 v/v) at flow rate of 0.8 mL/min. A photochemical postcolumn derivatization UVE was used to enhance the fluorescence of AFB_1 and AFB_2.

Granularity separation

Different granularities have been separated with a sieve including different meshes (2 mm / 1 mm / 500 µm and 300 µm) and then considered separately. For example, 1mm refers to flour with dimensions > 500 µm but <1000 µm.

Certified reference material

Naturally contaminated (aflatoxin B_1 18.8 µg/kg aflatoxin B_2 0.9 µg/kg aflatoxinG1 2.4 µg/kg aflatoxin G2 ND (µg/kg) deossinivalenol 2.6 mg/kg ochratoxin A 4.0 µg/kg T-2 Toxin 263.7 µg/kg HT-2 Toxin 523.3 µg/kg zearalenon 352.0 µg/kg fumonisin B1 28.3 mg/kg fumonisin B2

7.1 mg/kg fumonisin B3 1.7 mg/kg) and non-contaminated control maize flour samples purchased from Trilogy Analytical laboratory were also used for feasibility tests.

3.2.1.2 Experimental design

The maize flour sample measurement set-up consists of a 100mm diameter Petri dish in which the maize flour is poured. Measurements at five different spatial positions over the sample have been performed, and in each position five replicates have been acquired and averaged. A preprocessing procedure including averaging and normalization steps was applied during data processing. VIS spectrometer is positioned on top of the Petri dish and light is shielded by a dark cover. Fluorescence measurements and VIS spectroscopy measurements have been performed with this setting.

The analysed maize flour samples consisted of three different levels of Aflatoxin B_1 contamination (No cont= no aflatoxin contamination, Low cont= 23,3 µg/g, High cont= 96,5 µg/g) obtained by inoculation with the fungi *A. flavus*. Preliminary measurements have also been performed on maize flour reference material from Trilogy Lab contained in the Petri dish (18.8 µg/kg B1 aflatoxin). Also in this case, measurements at different positions have been acquired.

Figure 6: Example of experimental setting for fluorescence measurements.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

Figure 7: Example of experimental setting for visible reflectance measurements.

3.2.1.3 Implementation of sensors (food science laboratory)

Fluorescence measurements and VIS spectroscopy measurements have been performed according to the SOP described in section 3.1.2. With regard to the fluorescence measurements, the integration time has been set at 100 ms, while a plastic black reference has been used as a dark reference. In the case of VIS spectroscopy, the integration time has been set at 400 μs, while a plastic black reference and white paper has been used as dark reference and white reference material, respectively.

3.2.1.4 Other experimental procedures

In our preliminary experiments, samples have been also characterized with standard techniques like high-performance liquid chromatography (HPLC) for validation purposes.

3.2.2 Milk powder

Preliminary tests have not been yet performed but will be performed in the course of WP3. The expected measurement set-up will be similar to the ones used in grain and almond flour.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

3.2.3 Paprika powder

Preliminary tests have not been yet performed but will be performed in the course of WP3. The expected measurement set-up will be similar to the ones used in grain and almond flour.

3.2.4 Tree nuts

3.2.4.1 Materials

Production of almonds contaminated with aflatoxins

Shelled almonds (cs. Genco) were inoculated with a strain of *Aspergillus flavus* that produces aflatoxins B_1 and B_2. In particular the almonds were subjected to a mild sterilization by immersion for 2 min in a solution of 1% sodium hypochlorite with manual agitation. Subsequently, almonds were washed with sterile water and then further surface-disinfected with 70% ethanol for 1 min. Almonds were then transferred, under sterile conditions, in sterile flasks and aliquots of 1 kg of almonds was treated with 300 mL of a conidial suspension containing 3.3 × 105 conidia/mL of *A. flavus*. The conidial suspension was obtained from *A. flavus* inoculated on plates of potato dextrose agar (PDA) and grown for 4 days in the dark. Fungal conidia were collected from the surface of plates by using a sterile spatula and sterile water. Appropriate dilutions with sterile water were made to obtain a final concentration of 3.3 × 105 conidia/mL. After inoculation, flasks were manually stirred to uniformly distribute the conidial suspension on almond's surface and closed with a stopper of raw cotton and a sheet of aluminum foil to avoid excessive water evaporation. The flasks were incubated for 4 days at 28 °C in the dark. After incubation, almonds were dried at 40 °C for 48 h. Then three aliquots of almonds (10 g each) were randomly collected from each batch, content of flask, and analyzed for their aflatoxin content (see maize preparation).

3.2.4.2 Experimental design

The experimental design for tree nuts is similar as for the maize flour with some minor modifications. For almond, four different levels of contamination have been considered: no contamination, low contamination, medium contamination and high contamination corresponding to 0, 20, 11, 4 and 7,9 µg/g, respectively. In this case, contamination has also been obtained by inoculation with *A. flavus*. Grained almond flour has been separated according to particles' dimensions with the help of mechanical sieves with sizes >2 mm / 2 mm / 1 mm and 500 µm. Depending on contamination level, not all dimensions are available (contamination caused damage in the texture of the sample matrix).

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

3.2.4.3 Implementation of sensors (food science laboratory)

Fluorescence measurements and VIS spectroscopy measurements have been performed according to the SOP described in section 3.1.2. In the case of fluorescence measurements, the integration time has been set at 100 ms, while a plastic black reference has been used as a dark reference. In the case of VIS spectroscopy, the integration time has been set at 400 μs, whereas a plastic black reference and a white paper has been used as a dark reference and a white reference material, respectively.

3.2.4.4 Other experimental procedures

Determination of AFB_1 and AFB_2

Five grams of dry ground sample were extracted with 50 ml of extraction mixture of acetone/water (85:15 v/v) by sonication for 30 min. The extract was filtered through a filter paper and 0.1 ml was diluted with 6 ml of $MeCN/H_2O$ (30:70 v/v), stirred, filtered through a 0.45 μm PTFE and 0.1 ml was injected into the HPLC system (corresponding to 0.17 mg sample).

HPLC-FLD equipment and conditions

The HPLC-FLD analyses were performed with an Agilent 1260 consisting of a binary pump, an autosampler with a 100 μl loop, a fluorescence detector fixed at 365 nm λex and 435 nm λem and a thermostatic oven set at 30 °C. The column used was a 150 mm × 4.6 mm i.d., 3 μm, Luna PFP (2) (pentafluorophenyl-propyl) with a 3 mm i.d., 0.45 μm pore size guard. The chromatographic separation was performed in the isocratic condition using a mixture of $MeCN/H_2O$ (30:70 v/v) at flow rate of 0.8 mL/min. A photochemical postcolumn derivatization UVE was used to enhance the fluorescence of AFB_1 and AFB_2.

3.3 Detection of food spoilage and shelf-life prediction

3.3.1 Meat

Preliminary tests are in progress and will be reported in the further course of WP3 deliverable reports.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

3.3.2 Fish

3.3.2.1 Experimental design

The experimental procedure designed and performed under this section aimed at the monitoring and evaluation of the spoilage of gilthead sea bream (*Sparus aurata* L.) during aerobic storage under different isothermal conditions. For this purpose, aquacultured ungutted fish, within two days from harvest, were packaged in styrofoam trays wrapped by cling film and stored at 0, 4 and 8°C in high-precision (±0.5°C) programmable incubators (MIR-153, Sanyo Electric Co., Osaka, Japan) for a maximum time period of 11 days. At regular time intervals during storage, depending on the storage temperature, duplicate fish samples (originating from different fish) were subjected to the following analyses/measurements:

- Microbiological analyses (using reference methods for determining the microbial spoilage of fish)
- Measurements of pH
- Sensory evaluation
- Multispectral image (MSI) acquisition
- NIR spectroscopy measurements (NIR spectrometer developed by IPMS)
- Fourier transform infrared (FTIR) spectroscopy measurements

A schematic of the applied experimental analyses is provided in Figure 9. The spectroscopy (FTIR, NIR) measurements as well as the MSI acquisition were performed both on the skin and flesh side of the fish samples. The FTIR spectroscopy measurements and the MSI acquisition were performed as additional advanced spectroscopy methods, with the ultimate goal of serving as point of reference for the presented PhasmaFOOD scanners in relation to the spoilage status of the food samples analyzed.

Two independent experiments (i.e. different time instances and different fish batches) were conducted, and a total of 158 fish were analyzed.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

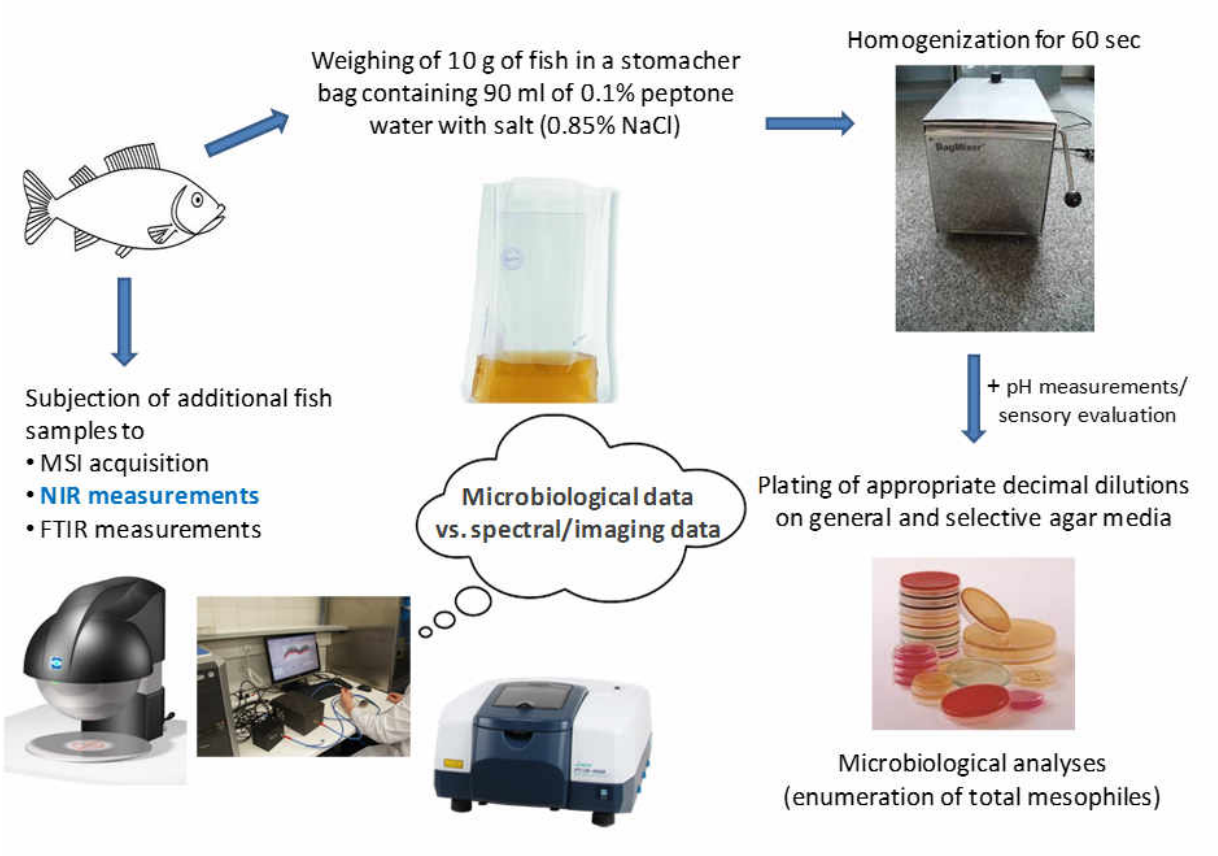

Figure 8: Experimental analyses for the evaluation of the feasibility of the application of the NIR spectrometer for the detection of fish spoilage.

3.3.2.2 Implementation of sensors (food science laboratory)

The **NIR spectrometer** evaluated in the PhasmaFOOD project was used in the fish spoilage experiments following the standard operating procedure (SOP) described below.

1. The spectrometer is turned on and the Quickstep software is initialized.
2. The following selections are made from the menu bar: View → Window layout → Property Editor.
3. The number of averages is selected: this number may range from 1 to 2000; herein, an average number of 1000 was selected and applied by pressing "Apply".
4. The dark background (**dark reference**) was set by shutting the spectrometer's entrance using the yellow plastic cover provided and by pressing the red button next to "Dark intensity"; once the measurement was completed, "Apply" was pressed.
5. The **white reference** was set according to the following steps:
    - The Y-shaped fiber was connected to the spectrometer (center fiber) and to a halogen lamp (hexagonal fiber set).

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

- The halogen lamp was switched on and left on for 5 min for warming up purposes; the lamp is properly connected when a circle of six little lights appears at the tip of the fiber probe.
- The fiber probe was held against a surface that is suitable as a white reference (i.e. folded white paper) and when full contact was ensured, the red button next to "Background" was pressed.
- Once the measurement was completed, the button "Apply" was pressed for confirmation.
- The acquired and applied measurement corresponds to the spectrum of the lamp which is scattered back from the white target, and is being saved internally in the Quickstep software; however, a separate recording (saving) as a spectrum is advisable.

6. The food sample measurements were taken and recorded according to the following steps:
   - The fiber probe was held against the tested food sample at the same tilted angle in which the tip of the probe is cut, so that the window at the tip and the sample surface were in full contact (i.e. in parallel).
   - Measurements were taken from at least 10 different spots on the sample following a raster pattern.
   - The Quickstep software allows the user to view the acquired data in various modes (formats). The default view mode is the "Intensity" which provides an intensity plot corrected for the dark intensity. Nonetheless, for evaluation and analysis purposes, the "Absorbance" mode is often preferable. In the latter mode, the measurement is also corrected for the white reference. In the present experimental procedure, the absorbance mode was selected from the menu bar following the route "View → Type of diagram → Absorbance".
   - The acquired measurements (spectral data) were saved selecting from the menu bar the route "File → Export → Save as" and by choosing either the *.csv file format.

Since the object of the applied spectral measurements is food, it is really important that the NIR micro-sensor, used as described above, is also thoroughly cleaned, sanitized and dried between measurements (as well as when measurements are completed). This is so critical for the specific application for two main reasons: (i) the tested food samples are and should remain independent, meaning that no transfer of components or contaminants from one sample to the other should take place; and (ii) food residues (containing water, proteins and fat) should be effectively removed from the fibre probe to avoid interference with the acquired measurements. The sanitation standard operating procedure (**SSOP**) improvised and applied for the NIR micro-sensor in the experimental procedures of the PhasmaFOOD project consisted of the following steps:

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

- The fiber probe was soaked in diluted (in water) dishwashing product for a few seconds.
- The fiber probe was rinsed with ample deionized water using a wash bottle to make sure that the applied cleaning agent was effectively removed.
- The fiber probe was sanitized by being rinsed with 100% (absolute) ethanol (which evaporates quickly and also allows for the efficient removal of all excess water), and then wiped gently with tissue paper.
- CAUTION:
  - No hard solvent (e.g., acetone, acids or any other abrasive substances) should be used for the purpose of cleaning and/or disinfection.
  - Care should be taken to remove all cleaning/sanitation agents from the fiber probe before the next measurement, since each substance (including water) has its own spectrum and may interfere with the measurements reducing their accuracy.

3.3.2.3 Other experimental procedures

Microbiological analyses

Ten grams from each fish were transferred aseptically to a 400-ml sterile stomacher bag (Seward Medical, London, United Kingdom) containing 90 ml of sterilized peptone saline diluent (0.1% w/v peptone, 0.85% w/v sodium chloride), and were homogenized for 60 sec (Lab Blender 400, Seward Medical). For the enumeration of total mesophiles, appropriate serial decimal dilutions in peptone saline diluent were surface plated on tryptic glucose yeast agar (Biolife, Milan, Italy), and colonies were counted after incubation of plates at 30°C for 72 h. Furthermore, at some of the sampling intervals (i.e. seven at 0°C, five at 4°C and four at 8°C), the fish samples also were tested for the determination of the populations of *Pseudomonas* spp., *Brochothrix thermosphacta* and lactic acid bacteria (LAB). *Pseudomonas* spp. were enumerated by surface plating on Pseudomonas agar base supplemented with CFC (Cephalothin, Fucidin, Cetrimide) Selective Supplement (Lab M Limited) after incubation of plates at 25°C for 48 h. *Br. thermosphacta* was enumerated by surface plating on streptomycin-thallous acetate-actidione (STAA) agar (Biolife) after incubation of plates at 25°C 72 h. LAB were enumerated by pour plating in de Man Rogosa and Sharpe (MRS) agar (Biolife) after incubation of plates at 30°C for 5 days. The obtained microbiological data were converted to log (colony forming units) per gram of fish (log CFU/g). Upon completion of the microbiological analyses, the pH values of the fish samples also were measured using a digital pH meter (RL150, Russell pH, Cork, Ireland) with a glass electrode (Metrohm AG, Herisau, Switzerland). Finally, each fish sample was evaluated with regard to its sensory attributes of appearance and odour. Specifically, each attribute was scored on a three-point hedonic scale where: 1=fresh; 2=marginal; and 3=unacceptable. A score of 1.5 was used to characterize a sample as semi-fresh and was regarded as the first indication of change from that of typical fresh fish (i.e. clear

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

eyes, odour slightly changed, but still acceptable by the consumer). Scores above 2 rendered the product spoiled and were regarded as indicative of the end of the product's shelf life.

Multispectral image (MSI) acquisition

Images from each fish sample were acquired using the VideometerLab system, originally developed by the Technical University of Denmark (Carstensen and Hansen, 2003) and commercialized by "Videometer A/S" (http://www.videometer.com). This instrument, which has been described in detail elsewhere (Panagou et al., 2014; Ropodi et al., 2015), acquires multispectral images in 18 different, non-uniformly distributed wavelengths ranging from UV (405 nm) to short wave NIR (970 nm). Prior to image acquisition, the system was subjected to a light set up procedure known as "autolight" and calibrated radiometrically and geometrically as previously described (Ropodi et al., 2015). Each fish sample was placed in a Petri dish which was then was placed (without the plate's lid) inside an Ulbricht sphere in which the camera is top-mounted, and the corresponding multispectral image of the product's surface (both of the skin and flesh side) was taken.

FTIR spectroscopy

FTIR spectral data were collected using a ZnSe 45° HATR (Horizontal Attenuated Total Reflectance) crystal (PIKE Technologies, Madison, Wisconsin, United States), and an FT/IR-6200 JASCO spectrometer (Jasco Corp., Tokyo, Japan) equipped with a standard sample chamber, a TGS detector and a Ge/KBr beamsplitter. A small portion from each fish sample (both from the skin and the flesh side) was transferred in the crystal plate and covered with a small piece of aluminum foil, and then pressed with a gripper in order for the best possible contact with the crystal to be achieved. The crystal used has a refractive index of 2.4 and a depth of penetration of 2.0 μm at 1000 cm^{-1}. Using the Spectra Manager™ CFR software version 2 (Jasco Corp.), spectra were collected over the wavenumber range of 4000 to 400 cm^{-1}, by accumulating 100 scans with a resolution of 4 cm^{-1} and a total integration time of 2 min. Prior to the measurements of the tested samples, reference spectra were acquired using the cleaned blank (no added fish sample) crystal. After each measurement, the crystal's surface was cleaned, first with detergent and distilled water and then with analytical grade acetone, and dried using lint-free tissue. The FTIR spectra that were ultimately used in further analyses were in the approximate wavenumber range of 3100 to 2700 and 1800 to 900 cm^{-1}.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

3.3.3 Fruit and vegetables

3.3.3.1 Experimental design

The experimental procedure designed under this section aimed at the monitoring and evaluation of the spoilage of rocket salad (*Eruca sativa* L.) during aerobic storage under different temperature conditions. For this purpose, fresh, washed and cut (ready-to-eat) rocket salads were provided by a local manufacturer within one day from production. The salads were stored in their original (commercial) packages under isothermal conditions (i.e. 4, 8 and 12°C), as well as at dynamic storage conditions with periodic changes from 4 to 12°C (i.e. 8 h at 4°C, 8 h at 8°C and 8 h at 12°C). At regular time intervals during storage, depending on the storage temperature, duplicate rocket samples (originating from different salad packages) were subjected to the following analyses/measurements:

- Microbiological analyses (using reference methods for determining the microbial spoilage of fresh produce commodities)
- Measurements of pH
- Sensory evaluation
- Multispectral image (MSI) acquisition
- VIS spectrometry measurements (UV-VIS spectrometer developed by CNR)
- NIR spectroscopy measurements (NIR spectrometer developed by IPMS)
- Fourier transform infrared (FTIR) spectroscopy measurements

A schematic of the applied experimental analyses is provided in Figure 9. As mentioned previously, measurements taken with a vibrational spectroscopy instrument (e.g., FTIR spectrometer) and surface chemistry (e.g., MSI) data acquisition were performed as additional advanced metabolomics/fingerprinting methods, with the ultimate goal of serving as point of reference for the presented PhasmaFOOD scanners in relation to the spoilage status of the food samples analyzed. Two independent experiments (i.e. different time instances and different salad batches) were conducted.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

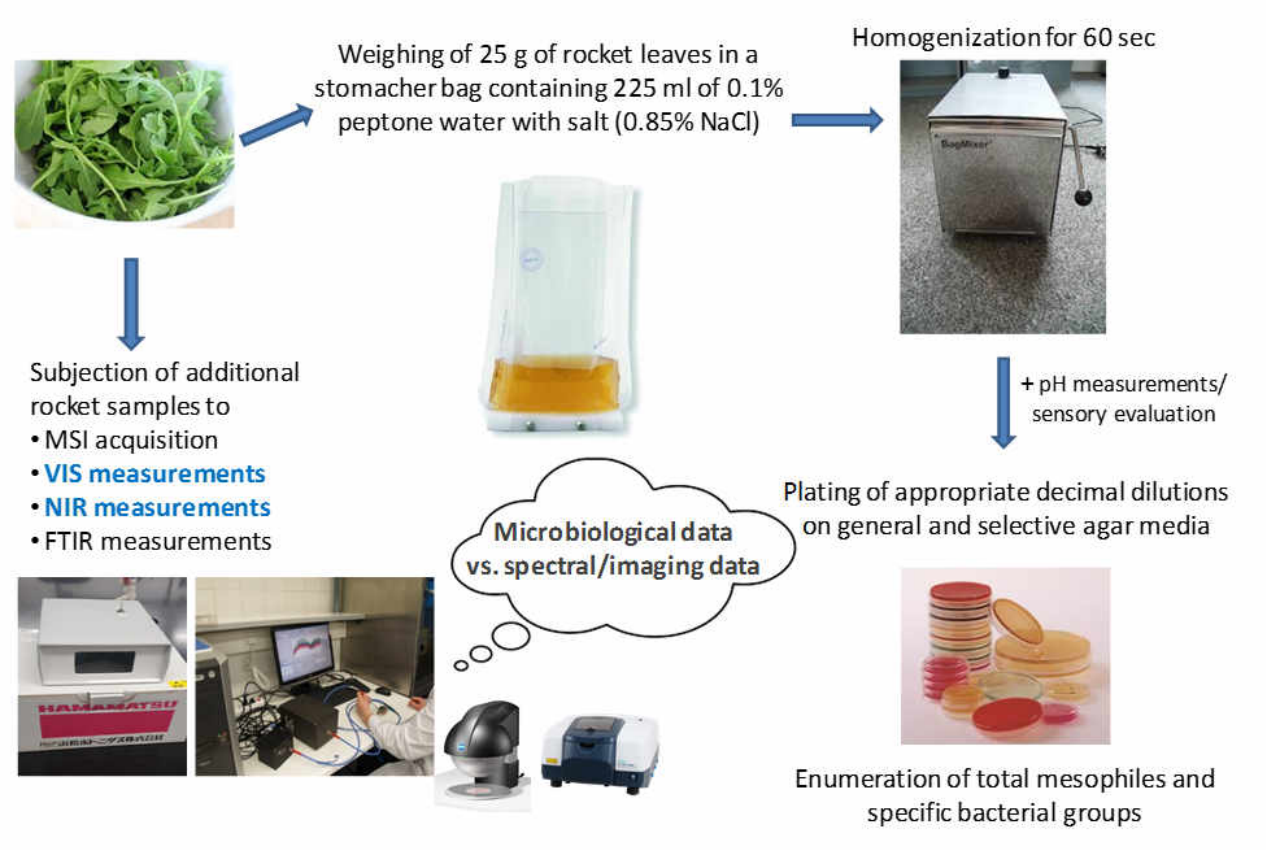

Figure 9: Experimental analyses for the evaluation of the feasibility of the application of the UV-VIS and NIR spectrometers for the detection of vegetable (rocket salad) spoilage.

3.3.3.2 Implementation of sensors

The **NIR spectrometer** evaluated in the PhasmaFOOD project was used in the rocket spoilage experiments following the SOP described above (section 3.3.2.2) with two modifications aiming at the optimization of the spectra acquisition procedure:

- The recorded data were saved using Option 2 as described in section 3.1.1. Alternatively, and for comparison purposes, the recorded could be saved as "Raw intensity" values using Option 1, and then corrected (by subtracting) for the dark intensity. From the dark corrected data, absorbance can be calculated according to the following equation:

  Absorbance = -ln [(white reference-measured spectrum)/white reference]

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

- The cleaning and sanitation of the fibre probe (SSOP described in 3.3.2.2) was performed using tissue paper soaked in water/ethanol (instead of using a wash bottle).

The **UV-VIS spectrometer** evaluated in the PhasmaFOOD project was used in the rocket spoilage experiments following the SOP described below.

1. Turn on the device (without turning on the lamp).
2. Turn on the computer and launch the software.
3. In the software do the following: Tool → Set parameter and enter the following settings:
   - Scan count: 10
   - Average count: 1
   - Integration time: **250 μs (for acquisition in visible)** or 200000 μs (for acquisition in fluorescence mode)
   - Capture Mode: SPECIFIED NUMBER
   - Trigger Polarity: Rising
   - Trigger Output: OFF

   Then press "SET".
4. Place the sensor's box inside a darkening hood (or just cover it with a dark-colored cover) and with the lamp still OFF, start the "**Dark mode**". There is no need to save the dark background acquisition.
5. Turn ON the VIS lamp and wait (for warming up purposes) for 15-20 min.
6. Place the sensor's box on the top of a folded sheet of white paper (used as white reference), start the "**Reference mode**" and perform acquisition of the white reference. Make sure that the acquisition window of the sensor's box is always on top of the area of interest, and the sensor is covered during spectra acquisition (including the white reference) with dark-colored cover. Again, there is no need to save the white reference acquisition.
7. Place the sensor's box (acquisition window) on top the food sample that needs to be measured. The food sample should be preferably in a Petri dish, covering the greatest possible surface of the dish) and measurements should be performed directly on the food sample without any cover (Petri dish lid or cling film) interfering.
8. Cover again the sensor's box with a dark-colored cover and Start "**Measurement mode**" to perform spectra acquisition of the food sample. Multiple measurements (at least 10) should be taken corresponding to as much different as possible areas/points of the tested food sample. Keep the same acquisition parameters for all measurements taken at the same time. After a measurement is completed, save it as a *.csv file.
9. A white reference acquisition is good to also be performed at the end of the measurements sessions to check the stability of the lamp by comparing it the white

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

reference acquired at the beginning of the measurements. For this purpose, using the "Measurement mode", take one measurement of the white paper that was used (at the beginning) as a white reference, and without changing any of the pre-existing settings.

10. When measurements are completed, the software will automatically save three files (for each measurement): one for the dark background, one for the white reference and one for the sample measurement. The dark and reference files will be the same for all samples measured within the same measurement session.
11. Close measurement software → Turn off illumination → Turn off device (by disconnecting the corresponding cable).

3.3.3.3 Other experimental procedures

Microbiological analyses

Twenty-five grams from each salad sample (i.e. commercial package) were transferred aseptically to a 400-ml sterile stomacher bag containing 225 ml of sterilized peptone saline diluent (0.1% w/v peptone, 0.85% w/v sodium chloride), and were homogenized for 60 sec. For the enumeration of total mesophiles, appropriate serial decimal dilutions in peptone saline diluent were surface plated on tryptic glucose yeast agar, and colonies were counted after incubation of plates at 25°C for 72 h. In addition, *Pseudomonas* spp. populations were enumerated, as previously described (section 3.3.2.3), by surface plating on CFC agar, and total populations of yeasts and moulds were determined by surface plating on Rose Bengal Chloramphenicol agar (Lab M Limited) after incubation of the plates at 25°C for 5-7 days. Furthermore, at some of the sampling intervals (i.e. six at 4 and 8°C, four at 12°C, and six at dynamic temperature conditions) , the rocket salad samples also were tested for the determination of the populations of lactic LAB (as previously described, section 3.3.2.3), Enterobacteriaceae by pour plating in Violet Red Bile Glucose (VRBG) agar (Biolife) after incubation of plates at 37°C for 24 h, and *Staphylococcus* spp. by surface plating on Baird-Parker agar after incubation of the plates at 37°C for 24 h. The obtained microbiological data were converted to log CFU/g. Upon completion of the microbiological analyses, the pH values of the rocket salad samples also were measured using a digital pH meter with a glass electrode. Finally, each rocket salad sample was evaluated with regard to its sensory attributes of appearance (colour) and odour, using the procedures described previously (section 3.3.2.3).

Multispectral image (MSI) acquisition and FTIR spectroscopy

Multispectral imaging and FTIR spectral data were collected for the rocket salad samples by using an appropriate amount of whole and cut leaves, respectively, according to the procedures described previously (section 3.3.2.3).

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

3.4 Detection of food fraud

3.4.1 Milk powder

3.4.1.1 Experimental design

Twenty-five skim milk powders (SMP) were obtained from routine controls (residue samples) which had passed the routine controls as described in EC regulation 273/2008 on public intervention for butter and SMP. Milk powder adulteration experiments were divided in two groups: (I) safety related experiments using nitrogen enhancers and (II) non-hazardous low-value fillers.

For the safety related issues, the following nitrogen enhancers were considered: melamin, ammonium chloride, caprolactam, diammonium phosphate and polyvinylpyrrolidone. All chemicals will be purchased in pure or technical grades of purity. Nitrogen enhancers will be dry blended[1] in a full factorial design in concentrations of 0.1 to 4.5 % (% w/w), which results in an apparent raise of protein concentration of 0.1 to 2.0 % (% w/w). For the non-hazardous fillers, plant protein isolates (PPI), starch, acid whey (AW), buttermilk powder (BP) and maltodextrin (MD) were considered. Again dry blending will be applied in a similar design as for the safety related nitrogen enhancer, but now in a concentration range of 2.5 to 25 % (%w/w).

3.4.1.2 Implementation of sensors (food science laboratory)

Preliminary tests have not been performed yet. All PhasmaFOOD sensors will be tested.

3.4.1.3 Other experimental procedures

All SMPs were analyzed for dry matter, fat content, protein content, acid whey content, buttermilk content and carbohydrate content according to the accompanying ISO methods stated in EC regulation 273/2008.

[1] It is imperative to realize that SMP can be measured in dry form or in reconstituted form. Karunathilaka et al. (2017) utilised Raman spectroscopy to classify non-adulterated milk powder and melamine adulterated milk powders, made using dry or wet blending methods. Utilising PCA to analyse their spectra, the authors noted that dry blended adulterated samples were more difficult to differentiate from authentic samples, and that replicates were not tightly clustered. Possible differences arising from the use of dry blending versus wet blending have also previously been mentioned by Capuano et al. (2015). In this sub-use case, dry blending will be used for reasons of practicality in sample preparation.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

3.4.2 Meat

Preliminary tests have not been performed yet and the experimental design is under discussion.

3.4.3 Alcoholic beverages

3.4.3.1 Experimental design

Approximately fifty distilled spirit samples are obtained from various liquor shops in The Netherlands from various categories: bourbon, Dutch gin (light, young and old), gin, grain wine, grappa, whiskey and vodka. This sub-use case is divided in assessing (I) dilution (i.e. prediction of ethanol content), (II) presence of technical alcohols (i.e. methanol) and (III) counterfeit products. During this first feasibility test the PhasmaFOOD sensors will be tested for dilution (range 15 – 50 % alcohol by volume (abv)) and presence of methanol (range 1 – 20 % abv), in a factorial sampling design. The production of counterfeit samples for 'white' spirits like vodka, (Dutch) gin by water/ethanol mixtures will be attempted when acceptable results for dilution and technical alcohols are obtained.

3.4.3.2 Implementation of sensors (food science laboratory)

Preliminary tests have not been performed yet. All PhasmaFOOD sensors will be tested.

3.4.3.3 Other experimental procedures

The alcoholic strength and determination of methanol will be executed according to EC regulation 2870/2000 on the reference methods for the analysis of spirit drinks.

3.4.4 Edible oils

3.4.4.1 Experimental design

The initial experimental approach will be to determine to what extend the PhasmaFOOD sensors are capable in distinguishing different types of edible oil from each other. In total approximately fifty authentic edible oils will be collected from various suppliers: extra virgin olive oil, refined olive oil, sunflower oil, rapeseed oil, arachide oil, walnut oil, hazelnut oil,

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

coconut oil, frying oil and rice oil. Determination of 'dilution' of extra virgin olive oils will be performed when there is clarity on which types of oils the PhasmaFOOD sensors can distinguish from each other. In section 5.3, IPMS has performed a pilot experiment using the NIR sensor and mixtures of extra virgin olive oil and sunflower oil. The results are obtained via NIR transmission measurements and demonstrate the general applicability of NIR spectroscopy to fraud detection of edible oils. For the PhasmaFOOD sensing prototype, a transflection geometry will be used during measurements. Liquid, partially transparent samples require a special sample container in order to be scanned in transflection. The identification of a suitable concept for such a sample holder is an additional planned outcome of the tests in WP3/use case 3.

3.4.4.2 Implementation of sensors (food science laboratory)

Preliminary tests have not been performed yet. All PhasmaFOOD sensors will be tested.

3.4.4.3 Other experimental procedures

The authenticity of the oils and fats have been established by gas chromatography of fatty acid methyl esters (ISO 12966-4:2015) and subsequent comparison to the DLO in-house database containing several hundred authentic oil samples.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

4 Data analysis

The data processing could be divided in two main parts. The first part will be devoted to acquire the measurements from different sensor systems (NIR spectrometer, UV-VIS spectrometer and CMOS camera) while the second will be mainly composed of the following steps:

1. Data Preprocessing: data compression (for the sensor required, noise removal, data normalization for de-correlation and to have the same range of values for each of the sensors (this can guarantee stable convergence of the downstream developed models).

2. Feature selection: identification for each case study (exploiting the information that we have obtained from the preliminary measurements) of the most significant features for each sensor device. In this way, it is not necessary to declare *a priori* which kind of sensors will be used for the specific case study.

3. Classification/identification/regression: In this step, using the most significant features as input for ad hoc use case model will be applied to the data in order to have as output a sample prediction.

4.1 Mycotoxin detection

At the present stage of tests, detection of mycotoxins (particularly aflatoxin B1) has relied mainly on fluorescence emission spectra. Visible reflectance data have been recorded and will be considered in multivariate analysis.

4.2 Food spoilage detection and shelf-life prediction

4.2.1 Meat

No data analysis has been performed yet but will be performed and reported in the further course of WP3.

4.2.2 Fish

From the first fish fillets experiment (pilot data acquisition) the collected data are presented next in brief, for all available, at the time, sensors.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

4.2.2.1 NIR spectrometer

A total of 161 files were collected, with each one of them referring to:

- A specific storage temperature (i.e. 0, 4 and 8°C),
- Sampling time (depending on the temperature) and
- Flesh and skin fish samples
- Two replicates in each case

Each file contains 901 wavenumbers (from 1000 to 1900), absorbance values for 10 to 12 different sampling sites on the sample (in columns).

For the skin samples from all storage temperatures (0, 4, 8°C), several pre-processing normalization and standardization techniques were tested in terms of model (regression model) efficiency and generalization properties, apart from using the raw values. Namely, z-score standardization and Standard Normal Variate (SNV) and center-and-scale normalization were employed. Next, using in serial 2 out of the three storage temperatures a Partial Least Square Regression (PLSR) model was trained and built. In this way, three models were developed and tested for their performance/efficiency in predicting total mesophiles values of unknown new samples. For each model we also performed feature selection on the basis of the number of PLSR components used as predictors, which was defined each time as the number that is adequate to explain (represent) at least 90% of the total variance of the training datasets. The training procedure was done by 10-fold cross validation for model parameters definition and optimization and with five Monte Carlo repetitions for model validation.

4.2.2.2 Multispectral imaging (MSI) sensor (VideometerLab system)

The collected data consist of:

- Four *.xlsx files: each file accounts for the two replicates and the two surfaces (skin, flesh) at 0 hour. The data consist of the mean reflectance values (only the region of interest, i.e. fish surface) at each wavelength (18 wavelengths in total) and the corresponding standard deviations.
- Twelve *.xlsx files: each file accounts for the two replicates, the two surfaces (skin, flesh) and the three different storage temperature. The data consist of the mean reflectance values (only the region of interest) at each wavelength (18 wavelengths) and the corresponding standard deviations, one row for each time sampling point.
- One *.xlsx file with 3 sheets, where each sheet accounts for a storage temperature (i.e. 0, 4 and 8°C). Each sheet holds the microbial counts (Total mesophiles) at the specific time intervals of sampling.

A repository for the raw MSI data, including 316 images has been created

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

After the multispectral acquisition, the images were pre-processed so as to detect the region of interest, i.e. only fish surface, excluding any background areas (as shown in Fig. 10). The segmentation was performed with software provided by the sensor, which in brief, maximizes the contrast between the sample material and irrelevant objects, in order to enable a threshold operation coupled with Canonical Discriminant Analysis (CDA) (a supervised transformation building method) so as to divide the image into regions of interest. Next and at each spectral band (specifically 18 bands from 405 to 970 nm) a mean reflectance value and the corresponding standard deviation were computed and used as inputs (in total 18 mean reflectance values and the 18 corresponding standard deviations) in model building and testing. Using the skin samples collected from the three storage temperatures (0, 4, and 8^{o}C), and similarly to the case of the NIR sensor, several pre-processing normalization and standardization techniques were tested in terms of model (regression model) efficiency and generalization properties, apart from using the raw values. For each model we also performed feature selection on the basis of the number of PLSR components used as predictors, which was defined each time as the number that is adequate to explain (represent) at least 90% of the total variance of the training datasets. Next, using in serial 1 out of the three storage temperatures a PLSR model was trained and built. In this way, three models were developed and tested for their performance/efficiency in predicting total mesophiles values of unknown new samples (every time at the remaining two different storage temperatures). The training procedure was performed by 10-fold cross validation for model parameters definition and optimization and five Monte Carlo repetitions for model validation.

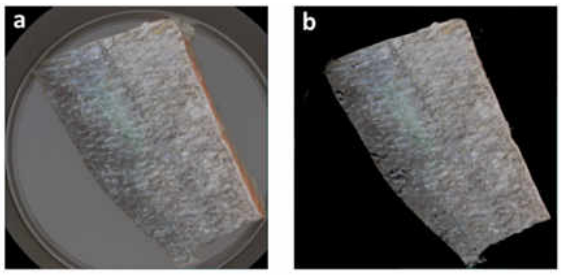

Figure 10: Pseudo RGB images of the MSI images as obtained by the VideometerLab sensor: original image with surrounding (a) and segmented image showing only the region of interest (i.e. fish surface) (b).

4.2.2.3 FTIR spectroscopy

The collected data consist of:

- Four *.xlsx files: each one contains samples (two replicate samples), with sampling at several time points (depending on the storage temperature). For each sample the whole

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

FTIR spectrum (i.e. 4000 to 400 cm^{-1}) was collected. Two of the *.xlsx files refer to fish skin and two of them to fish flesh samples.

- One *.xlsx file with three sheets, with each sheet accounting for a storage temperature (i.e. 0, 4 and 8°C). Each sheet holds the microbial counts (total mesophiles) at the specific time intervals of sampling.

The selected FTIR spectra were subjected to second derivative transformation based on the Savitzky Golay algorithm (9 smoothing points, 2^{nd} degree polynomial). Then, a PLSR model was developed using FTIR spectra (in the wavenumber ranges of 3100 to 2700 and 1800-900 cm^{-1}) as input variables and total mesophiles (log CFU/g) as output variables, with the number of significant latent variables being determined based on the results of leave-one-out cross-validation. The developed model was trained using the spectral/microbiological data corresponding to the storage temperatures of 0 and 8°C (both replicates) and tested (validated) using the spectral/microbiological data corresponding to 4°C (both replicates). A total of 96 and 62 fish samples were analysed for the purpose of model training and testing, respectively.

4.2.3 Fruit and vegetables

The analysis of the data derived from the experiments on rocket salad spoilage is in progress.

4.3 Food fraud detection

Preliminary tests have not been performed yet, except for a small pilot on extra virgin olive oil/sunflower oil mixtures. For this pilot, chemometrics were not applied yet.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

5 Results and Discussion

5.1 Feasibility of micro-sensor application for detection of mycotoxins

5.1.1 Maize flour

The first tests were performed on maize samples at three different levels of Aflatoxin B1 contamination (No cont= no aflatoxin contamination, Low cont= 23,3 µg/g, High cont= 96,5 µg/g) obtained by inoculation with the fungi *Aspergillus flavus*. The results shown hereafter are relative to the 1000 µm granularity sample, but the same measurements have been performed on 2000 µm granularity, with similar results. Each plot has been averaged over five measurements in different positions across the sample, where each measurement is the mean of five spectra. Preprocessing procedures included background subtraction and normalization to the maximum peak (excitation reflectance peak) in the case of normalized fluorescence signal, and background subtraction followed by normalization to the white reference in the case of visible reflectance.

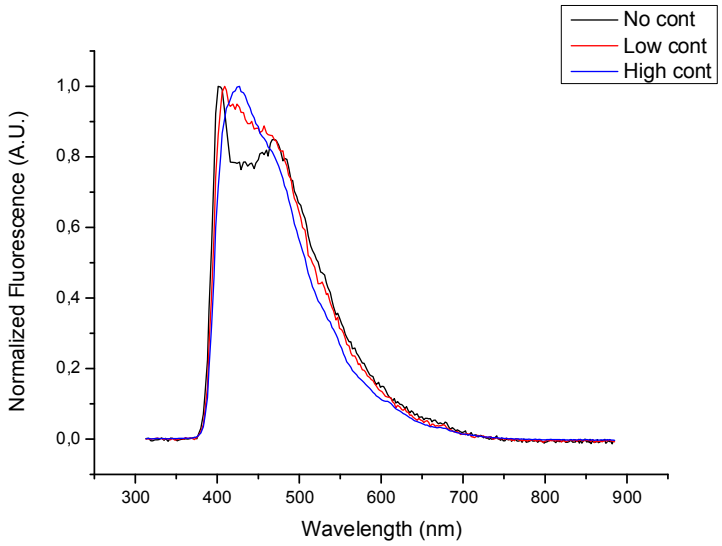

Figure 11: Normalized fluorescence intensity plot for 1000 µm dimension maize flour from not contaminated, low and high contamination samples. The fluorescence emission peaked at 430 nm is due to the presence of Aflatoxin B1, and can be clearly seen in the case of high and low contamination. On the contrary, no emission is observed for the non contaminated sample in that spectral region while the emission at about 500 nm is relative to intrinsic fluorescence of maize.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

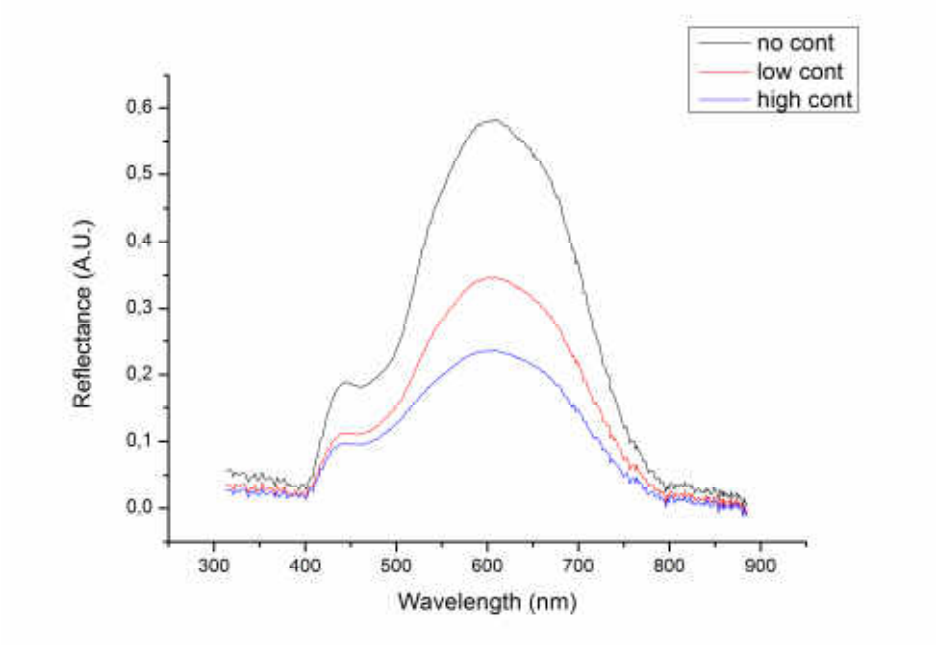

Figure 12: Normalized visible reflectance intensity plot for 1000 µm dimension maize flour form not contaminated, low and high contamination samples.

Other tests were performed on a reference sample characterized by the presence of several contaminants, among which aflatoxin B1 (18,8 µg/kg), aflatoxin B2 (0,9 µg/kg), aflatoxin G1 (2,4 µg/kg), together with ochratoxin A and several species of fumonisins. The acquisition procedures and parameters were the same as for the contaminated samples.

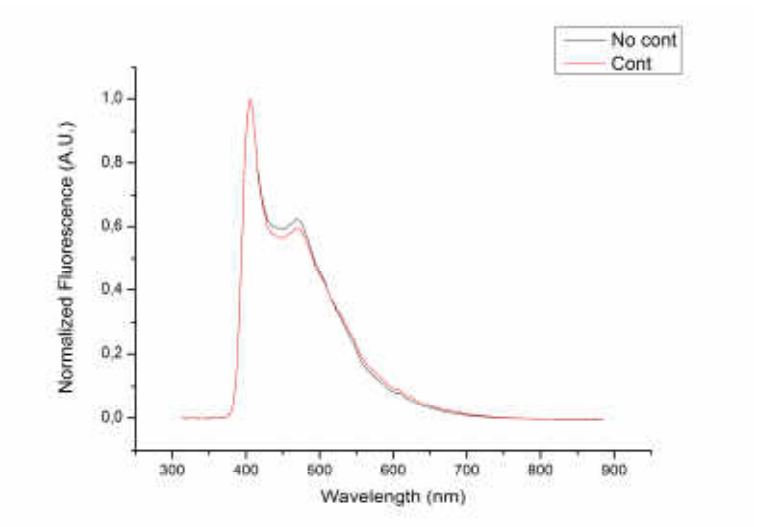

Figure 13: Normalized fluorescence intensity plot of Trilogy maize flour for not contaminated and naturally contaminated reference samples.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

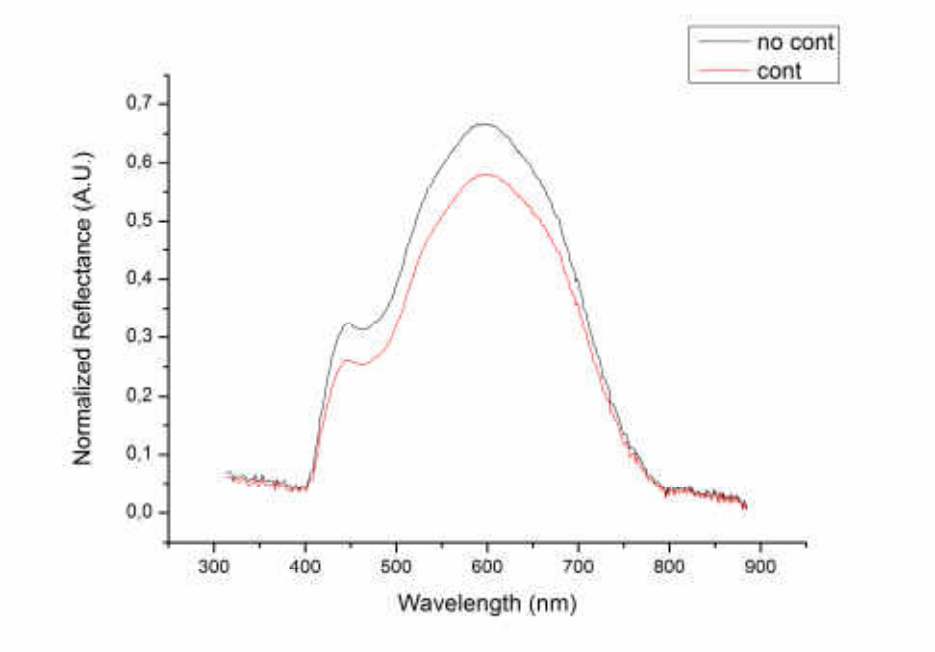

Figure 14: Normalized visible reflectance intensity plot of Trilogy maize flour of not contaminated and contaminated reference samples.

5.1.2 Milk powder

Preliminary tests have not been performed yet.

5.1.3 Paprika powder

Preliminary tests have not been performed yet.

5.1.4 Tree nuts

The tests were performed on artificially (in the laboratory) contaminated (i.e. inoculation with *A. flavus*) almond samples at four different levels of Aflatoxin B1 contamination (No cont= no aflatoxin contamination, Low cont= 7.9 µg/g, medium cont= 11.4 µg/g High cont= 20 µg/g). The plots are relative to the 1000 µm granularity sample, but the same measurements have been performed on 2000 µm granularity and >2000 µm granularity, with similar results. Each plot has been averaged over five measurements in different positions across the sample, where each measurement is the mean of five spectra.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

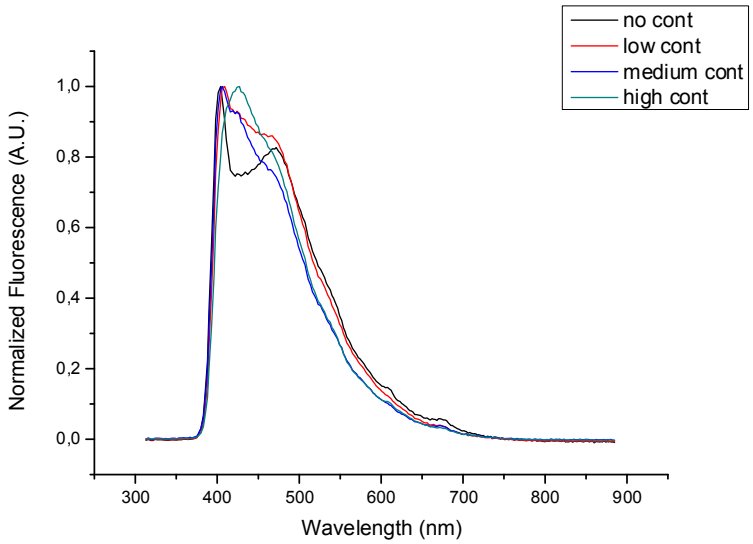

Figure 15: Normalized fluorescence intensity plot for 1000 µm dimension almond flour showing emission patterns for no contamination, low contamination, medium contamination and high contamination samples. The fluorescence emission peaked at 430 nm is due to the presence of Aflatoxin B1, and is observed in case of high/medium/low contamination. No emission is observed for the non contaminated sample in that spectral region, while the emission at about 500 nm is relative to intrinsic fluorescence of almond.

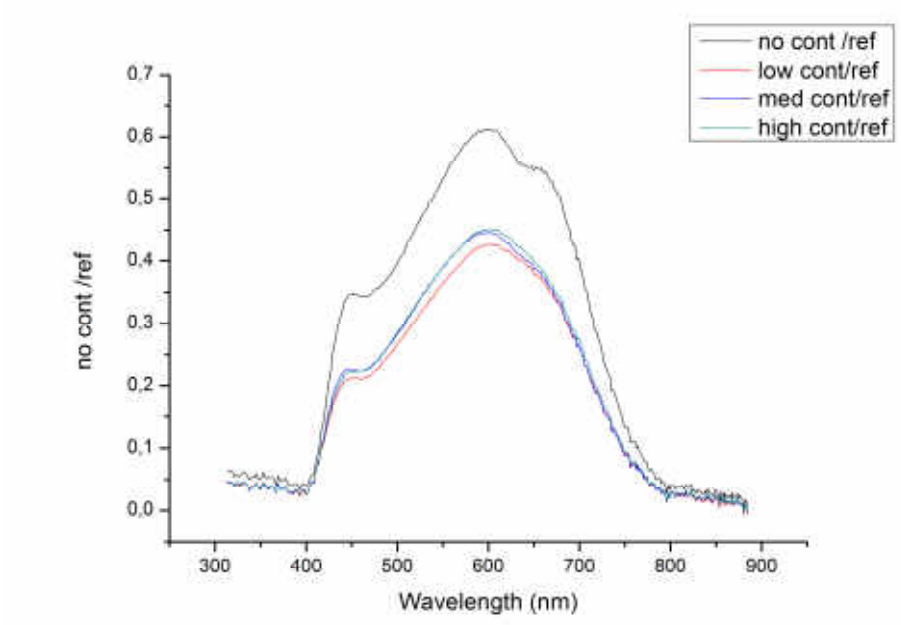

Figure 16: Normalized reflectance intensity plot of 1000 µm dimension almond flour from not contaminated sample and low, medium and high contamination samples.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

5.1.5 Evaluation of fluorescence measurement results

During the measurements, we realized that the non-contaminated maize flour and also the almond flour show an intrinsic fluorescence. We could find confirmation of this behaviour in the literature (Zandomeneghi, 1999).

The results show that in the range of concentration of aflatoxin contamination obtained with the inoculation procedure (µg/g i.e. ppm), the presence of aflatoxin is easily detectable since fluorescence spectrum shows a marked fluorescence emission signal peaked around 430 nm as expected from the scientific literature (Espinosa-Calderon, 2011), both in maize and in almond samples. In both cases in fact, is easy to distinguish the emission due to aflatoxin presence from auto-fluorescence of the matrix (both in maize and almond, peaked at wavelength > 500 nm). In this range of concentrations, is furthermore possible to observe a relationship between aflatoxin concentration and fluorescence spectral features (peak position and spectral shape) that, upon increase of dataset can lead to set up a calibration curve. It is also worth to note that visible reflectance evidences a marked decrease in the intensity of the normalized spectrum. In the range of contamination of the Trilogy reference sample (µg/kg, i.e. ppb), the fluorescence emission peak at 430 nm with the current acquisition parameters is not detectable, while visible reflectance shows a decrease in the intensity signal.

5.2 Feasibility of micro-sensor application for detection of spoilage

5.2.1 Meat

Preliminary tests have not been performed yet.

5.2.2 Fish

5.2.2.1 Microbiology and sensory data

The results regarding the evolution of microbial populations (total mesophiles and selected specific microbial groups) during storage of fish under different isothermal conditions are presented in Figures 17-19. As expected, the higher the storage temperature the higher the growth rate of the microbial groups tested, and hence, the faster the spoilage of fish, both from a microbiological (Figs. 17-19) and a sensorial (Fig. 20) perspective. Moreover, although tested only sporadically during storage, it becomes evident that *Pseudomonas* spp. constitute the dominant spoilage microflora (i.e. specific spoilage organisms) of fish during aerobic storage at all tested temperatures.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

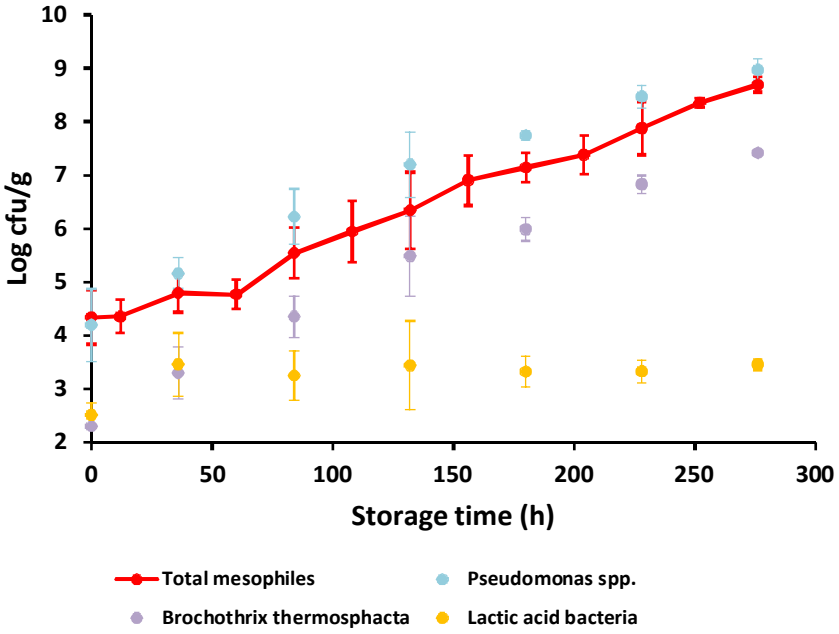

Figure 17: Microbial populations during aerobic storage of gilthead sea bream (*Sparus aurata* L.) at 0°C.

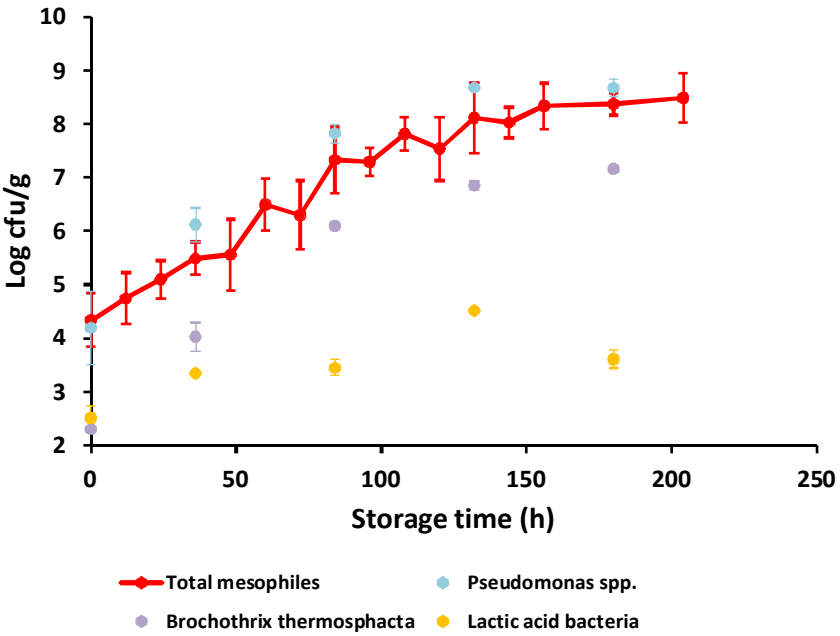

Figure 18: Microbial populations during aerobic storage of gilthead sea bream (*Sparus aurata* L.) at 4°C.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

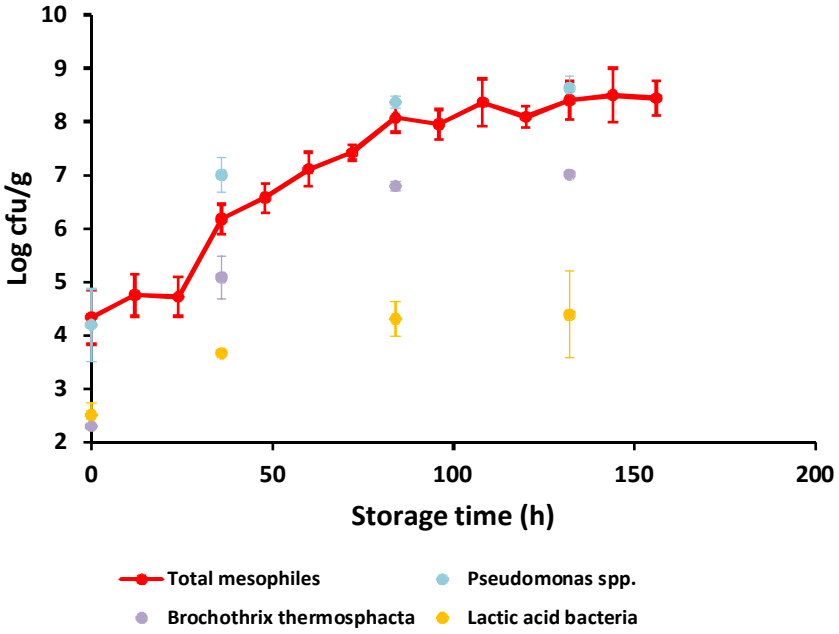

Figure 19: Microbial populations during aerobic storage of gilthead sea bream (*Sparus aurata* L.) at 8°C.

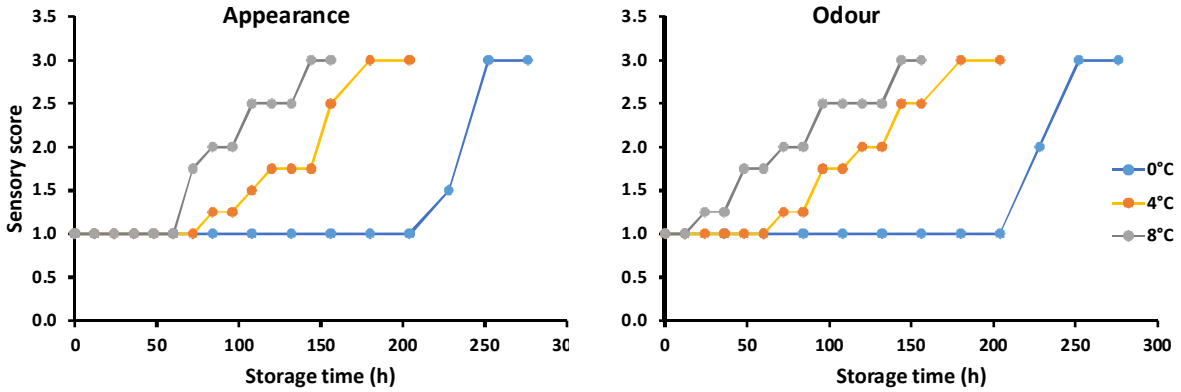

Figure 20: Sensory evaluation of gilthead sea bream (*Sparus aurata* L.) during aerobic storage at different temperatures.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

5.2.2.2 NIR spectrometer

As discussed in section 4.2.2, the PLSR modeling resulted in three different models for the case of skin fish fillet samples. Each model was trained with two out of three storage temperatures and tested on the third. In terms of data preprocessing, the best efficiency was yielded in the case of no preprocessing being applied. Thus, the models were built using the raw absorbance values. Next, the results on the basis of total mesophiles prediction for each model (on the dataset not used for training) are presented. The predicted and measured total mesophiles values were fitted with linear regression $y = p_1 x + p_1$ so as to see if a one-to-one correspondence exists (Table 1).

- *Model 1* (Fig. 21 a, b): eight selected components (accounting for variance explained > 90%) for data at 0 and 4°C, test on data at 8°C.
- *Model 2* (Fig. 21 c, d): eight selected components (accounting for variance explained > 90%) for data at 0 and 8°C, test on data at 4°C.
- *Model 3* (Fig. 21 e, f): eight selected components (accounting for variance explained > 90%) for data at 4 and 8°C, test on data at 0°C.

Table 1: Linear regression fit properties. Parameters' values and goodness of fit.

	p1	p2	R-square	RMSE
Model 1	0.41	3.39	0.17	1.35
Model 2	0.32	4.56	0.15	1.11
Model 3	0.32	5.08	0.16	1.07

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

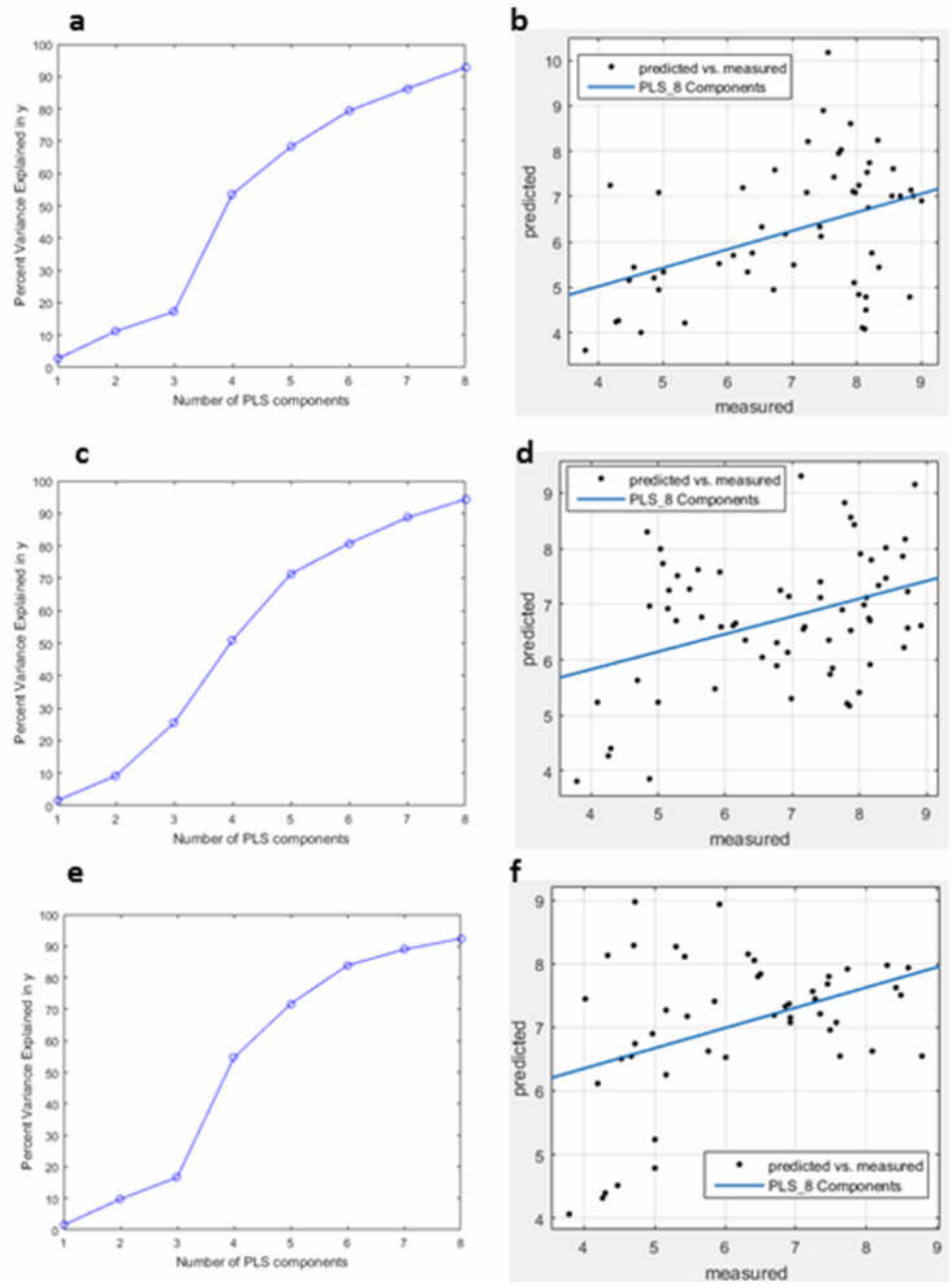

Figure 21: Models' prediction evaluation on the test set: Cumulative variance explained by the selected number of PLSR predictors for dataset at 8°C as test data (a); Linear regression fit of predicted vs. measured total mesophiles values for dataset at 8°C as test data (b); Cumulative variance explained by the selected number of PLSR predictors for dataset at 4°C as test data (c); Linear regression fit of predicted vs. measured total mesophiles values for dataset at 4°C as test data (d); Cumulative variance explained by the selected number of PLSR predictors for dataset at 0°C as test data (e); Linear regression fit of predicted vs. measured total mesophiles values for dataset at 0°C as test data (f).

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

The outcome of the aforementioned data analysis for the NIR sensor suggests that the use of prediction models should be further elaborated. The limitation in the anticipated reliability of the models can be imputed to several reasons that do not include the efficiency of the sensor to capture the underlying (spoilage) phenomenon. Since the acquired data of NIR correspond to the spectrum range of 1000 to 1900 nm, i.e. beyond the visible (VIS) and up to short wavelength infrared (IR), the outcome is expected to be promising. One recognized (and solved issue) is the "bug" in the NIR sensor accompanying software for absorbance calculation – which from now on will be computed manually; a second recognized concern has been the light power of the fibre, which has also been resolved by tripling its power. All adaptations were performed prior to the next experimental procedure. At this point, we must stress out the importance of the parallel use of additional advanced spectroscopy methods (i.e. MSI and FTIR) that allowed the assessment of the aforementioned technical shortcomings.

5.2.2.3 Multispectral imaging (MSI) sensor (VideometerLab system)

The modelling procedure described in section 4.2.2 was also applied here. The PLSR modeling resulted in three different models for the case of skin fish fillet samples. Each model was trained with one out of the three storage temperatures and tested on the remaining two. In terms of data preprocessing, the best efficiency was yielded in the case of no preprocessing being applied. Thus, the models were built using the mean and standard deviation values of the raw reflectance values. Next, the results on the basis of total mesophiles prediction for each model (on the dataset not used for training) are presented. The predicted and measured total mesohiles values were fitted with linear regression $y = p_1 x + p_2$ so as to see if a one-to-one correspondence exists (Table 2).

Model 1 (Fig. 22 a, b): five selected components (accounting for variance explained > 90%) for data at 0°C, test on data at 4 and 8°C.
Model 2 (Fig. 22 c, d): five selected components (accounting for variance explained > 90%) for data at 4°C, test on data at 0 and 8°C.
Model 3 (Fig. 22 e, f): five selected components (accounting for variance explained > 90%) for data at 8°C, test on data at 0 and 4°C.

The outcome of the analysis for the MSI sensor suggests that the prediction models are able to provide high prediction efficacy for all the temperatures, showing that the models are more or less irrelevant to the storage temperature. For all models we can see that the predicted total mesophiles values exhibit a one-to-one relationship to the measured ones (Table 2). The value of p_1, representing the slope of the fitted line, is close to 1 and the bias/offset parameter p_2 is relatively low, especially in the case of model 3. Additionally, the measures of "goodness of fit", RMSE and R-square prove that the fitting is robust and reliable. In conclusion, MSI can adequately provide information on spoilage, even with a small number of input variables (i.e.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

18 mean and 18 standard deviation values). Such a high efficacy of this particular sensor may be related to the large sample surface of sampling.

Table 2: Linear regression fit properties. Parameters' values and goodness of fit.

	p1	p2	R-square	RMSE
Model 1	0.71	1.19	0.89	0.34
Model 2	0.93	0.58	0.94	0.35
Model 3	1.05	0.04	0.94	0.40

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

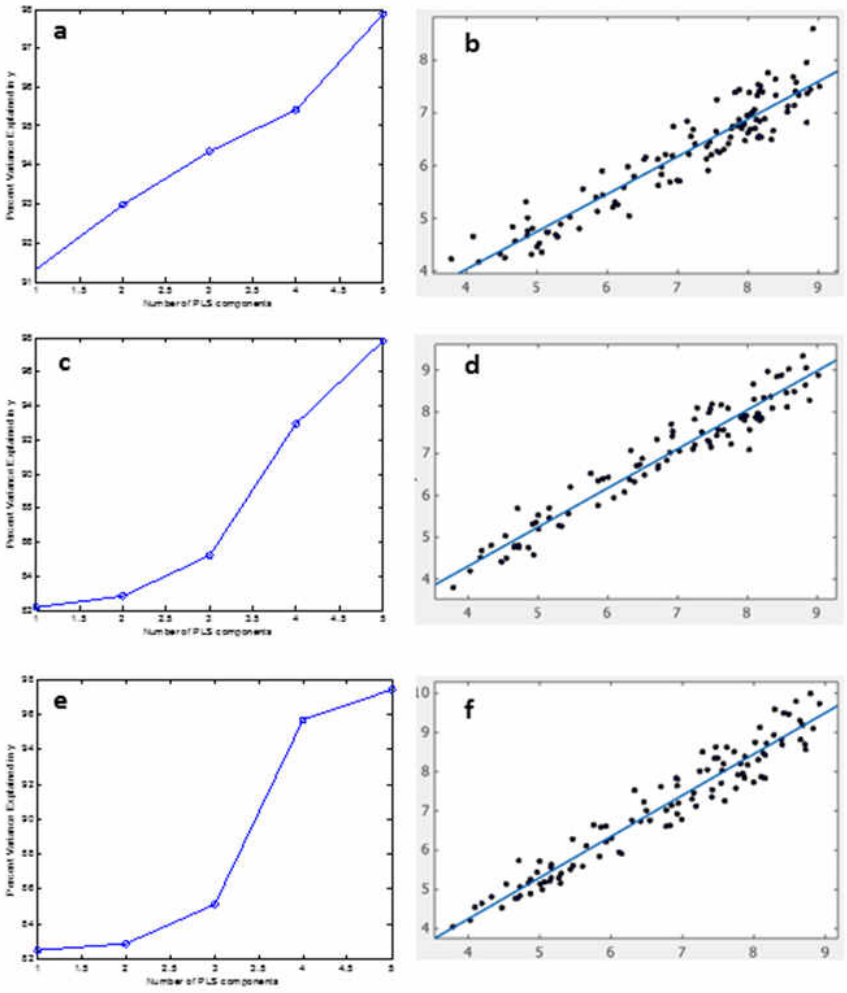

Figure 22: Models' prediction evaluation on the test set: Cumulative variance explained by the selected number of PLSR predictors for datasets at 4 and 8°C as test data (a); Linear regression fit of predicted vs. measured total mesophiles values for dataset at 4 and 8°C as test data (b); Cumulative variance explained by the selected number of PLSR predictors for dataset at 0 and 8°C as test data (c); Linear regression fit of predicted vs. measured total mesophiles values for dataset at 0 and 8°C as test data (d); Cumulative variance explained by the selected number of PLSR predictors for dataset at 0 and 4°C as test data (e); Linear regression fit of predicted vs. measured total mesophiles values for dataset at 0 and 4°C as test data (f).

5.2.2.4 FTIR spectroscopy

Two representative FTIR spectra (in the wave number range of 4000 to 800 cm^{-1}) corresponding to fresh (0 h) and spoiled (144 h of storage at 8°C) fish skin samples are shown in Figure 23. Further analyses, as mentioned previously, were conducted on selected wavenumber ranges (i.e. 3100 to 2700 and 1800-900 cm^{-1}), which are regarded as corresponding to a chemometric "fingerprint" with regard to fish spoilage.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

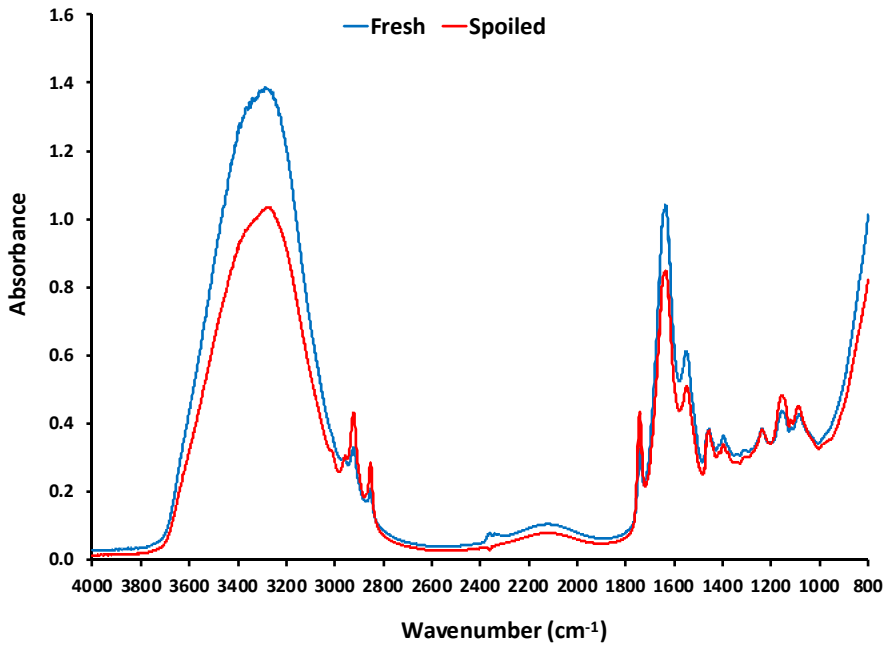

Figure 23: Representative FTIR spectra of fresh and spoiled fish skin samples.

Based on the results of the analysis of the FTIR data, the developed model appears to be capable of providing considerably high efficacy in predicting microbial (i.e. total mesophiles) populations (Fig. 24), and even more importantly, in a temperature-independent manner. Indeed, the developed PLSR model was trained on data (spectral/microbiological) corresponding to 0 and 8°C and tested (validated) against data corresponding to 4°C, and exhibited good performance, with the estimated values of the bias factor and accuracy factor being 1.00 and 1.08, respectively. Furthermore, the slope of the fitted line was close to 1 (i.e. 0.94), whereas the offset parameter was relatively low (i.e. 0.46). Finally, the measures of "goodness of fit", RMSE and R-square were 0.62 and 0.79, respectively, demonstrating that that the fitting was sufficiently robust and reliable

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

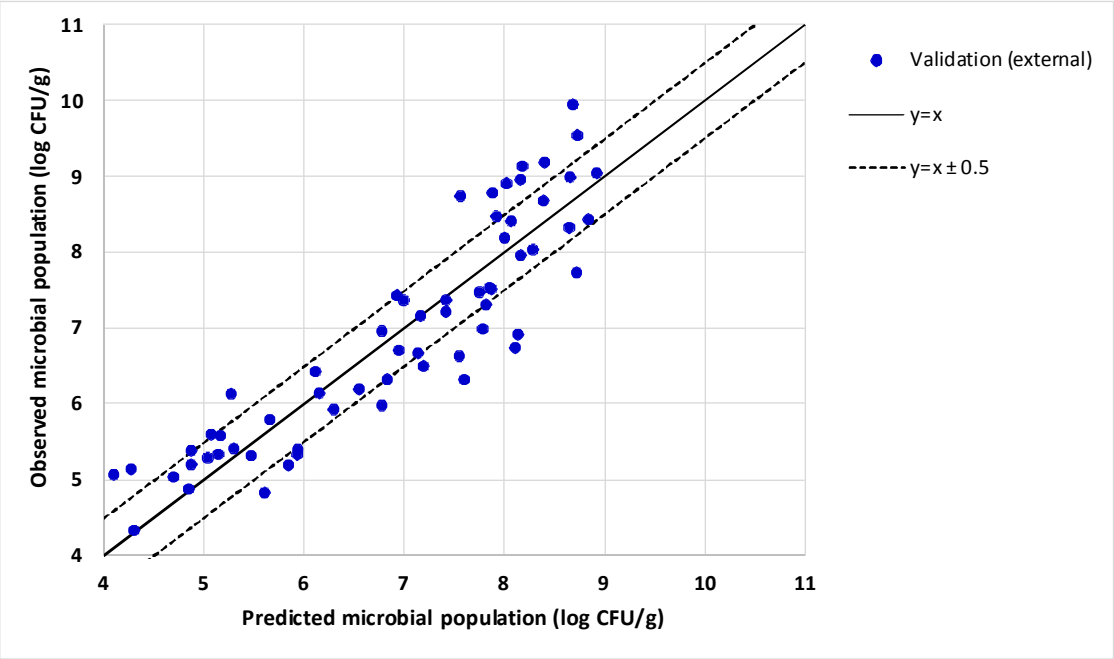

Figure 24: Observed and predicted by the PLSR model population of total mesophiles based on FTIR spectral data.

5.2.3 Fruit and vegetables

The analysis of the data and the interpretation of the results derived from the experiments on rocket salad spoilage are in progress.

5.3 Feasibility of micro-sensor application for detection of fraud

5.3.1 General food test

In order to make the other partners familiar with the capabilities of the NIR spectrometer, IPMS has carried out a trial to demonstrate the NIR signals of several different foods measured in transflection geometry. This trial is mainly related to use case 3 but will also be instructive to the other use cases.

Purpose of the general food test. These measurements were done to illustrate the properties, potential and pitfalls of NIR measurements as well as demonstrating typical NIR spectra obtained with the SGS1900 NIR spectrometer.

Samples of the general food test. The samples are commercially available foods, namely:

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

Wheat flour / Soy flour / Sugar / Milk powder / Leibniz biscuits / Palm fat (Palmin) / Baking fat (Biskin) / Raw cured ham / Raw potato / Raw apple

The raw fruit were cut and the inside was measured. The ham samples were folded over until the thickness was large enough to prevent (by scatter or absorption) NIR light from transmitting through the sample. All other samples were measured as purchased.

Detection equipment for the general food test. For the measurements, we used the same Hiperscan SGS1900 NIR spectrometer that is also being used in the other use case trials. Also, the light source is the same, see Section 2.2.1. Measurements were done in transflection geometry using the reflection fibre probe that was employed in the fish and rocket measurements of use case 2. Dark and white background references were recorded in accordance with the protocol elaborated in section 3.1.1. Then the tip of the probe was placed in direct contact with the sample and the NIR spectrum was measured. Each sample was measured at least 10 times in different places to evaluate the importance of inhomogeneity across the sample.

Measurements of the general food test. The measurements reveal the variability between samples and within the individual samples. Figure 25 shows the NIR spectra of soy flour. The values were calculated from the raw NIR intensity spectra according to the formula with which absorbance is normally calculated. Please note that in this case, the calculation yields a sum of both absorbance and scatter, i.e. extinction values. The scatter component contributes to the spectral background while the absorbance component is revealed in the band structure of the spectrum. Soy flour contains protein as well as fat, carbohydrates and water as the main ingredients. In figure REF, one can clearly distinguish three bands at 1200, 1480, 1560 and beyond 1700 nm, which are associated with the typical band regions of either of these ingredients. For instance, the C-H groups of fatty acids typically respond in the regions of 1200 and 1720 nm. The variability between different measurement spots is comparably small, as expected from a powdery homogeneous sample. It arises mainly as an offset, which must be attributed to differences in the scattering properties of the sample, which may vary from one spot to the other.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

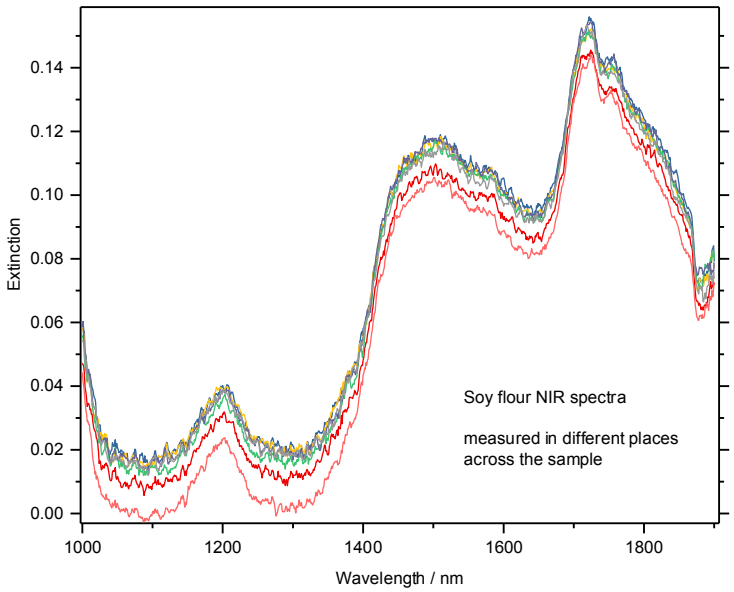

Figure 25: NIR spectra of soy flour. Please note that extinction is shown, as the sum of absorbance and scatter.

The next sample under investigation is an apple, the spectra are shown in Figure 26. Here, the most prominent absorption band is that of water noticeable as a great peak around 1450 nm. Furthermore, the overall intensity scattering back to the spectrometer is reduced as compared to the soy flour sample. For soy flour, the background of the extinction spectra occasionally dips back to 0.0, meaning that 100% of the incoupled light is fed back to the spectrometer. For the apple, this value is closer to 0.8, equivalent to only 16 % of the incoupled light that reaches the spectrometer. This is due to the reduced scattering cross section of the apple tissue as compared to the soy flour, which essentially consists only of small scattering particles. The apple consists of larger segments characterised by similar optical properties such that scattering occurs less frequently. Also, as a natural unprocessed fruit, an apple is more inhomogeneous that soy which has been homogenised by a milling process. Therefore, the variability between apple spectra is greater. Finally, due to the reduced scattering and large concentration of water (above 50%), the NIR signal may even become unusable: This effect occurs for two spectra in the spectral region of the water absorption band.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

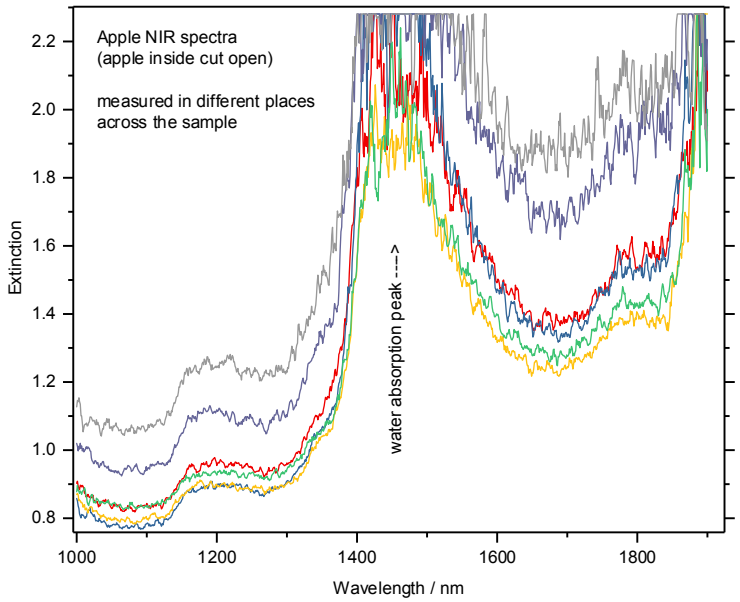

Figure 26: NIR spectra of an apple. Please note that extinction is shown, as the sum of absorbance and scatter.

The third sample investigated is palm fat, as shown in Figure 27. Naturally, the absorption bands associated with fatty acids stand out at 1200 and 1700-1770 nm. The absorption band at 1400-1450 nm may be attributed to a small concentration of water but no reference analysis has been carried out to verify its presence. The variability across the palm fat sample lies between that of the apple and soy flour. Again, it is attributed to different scattering properties across the sample as it only appears in the form of an offset – the absorption bands are largely invariant in shape and size. Furthermore, the overall scattering cross section of the palm fat sample also lies between that of apple and soy flour, which is expected from their visible appearances. In the visible spectrum, the surface of soy flour is lighter than that of palm fat, which is in turn lighter than that of the apple inside.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

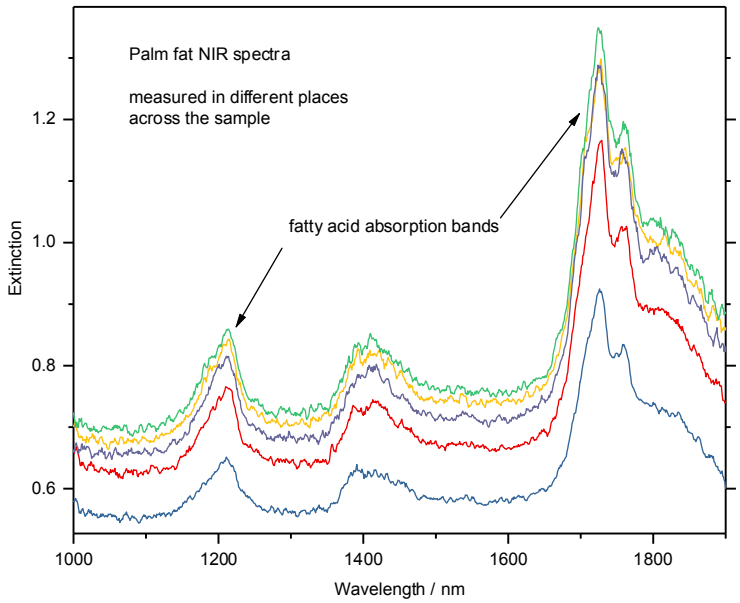

Figure 27: NIR spectra of palm fat. Please note that extinction is shown, as the sum of absorbance and scatter.

Next, we compare the NIR spectra directly between the different food types. Figure 28 shows NIR raw intensity spectra, one for each type of sample investigated. Please note that the spectral shape is dominated by the spectrum of the NIR lamp. However, small dips due to distinct absorption bands can clearly be distinguished, e.g. in the spectra of fat samples or sugar. Most of all, Figure 28 serves to illustrate how the scattering properties differ for different types of food. Please note that the dark background has not been subtracted from these raw data. It lies around 0.27. Accordingly, almost no light returns from a potato, while the sugar sample is scattering an order of magnitude more strongly. This is how the NIR spectra will look like before any processing is done.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

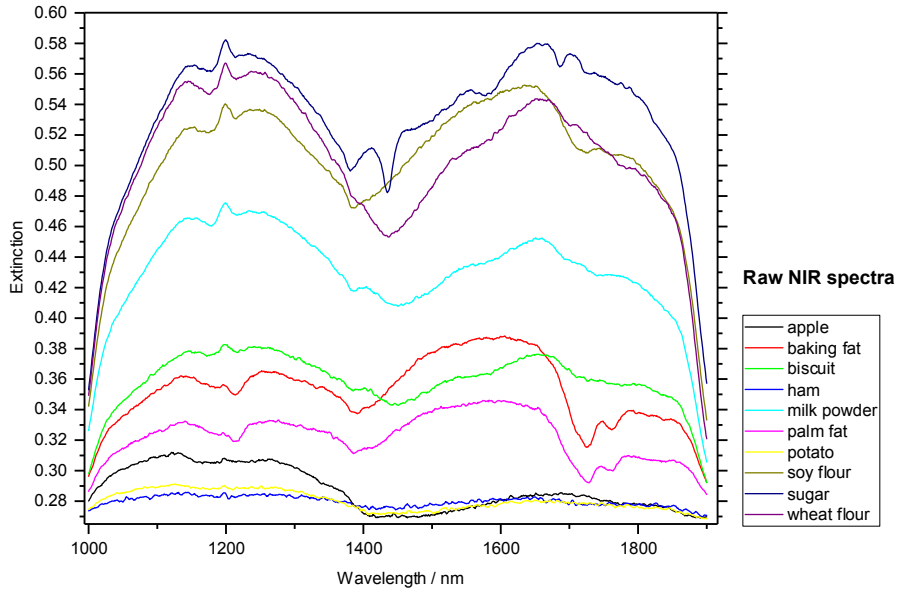

Figure 28: Raw NIR intensity spectra from various food samples.

Prior to analysis in any of the use cases, the dark background must be subtracted from these spectra. Furthermore, for chemometrical analysis, absorbance A (or as explained above, extinction) must be calculated for each wavelength of the spectrum according to: $A = - \lg (I / I_0)$, where I is the intensity signal measured by the spectrometer and I_0 is the intensity signal of the reference lamp spectrum. Figure 29 shows extinction spectra, one for each food type.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

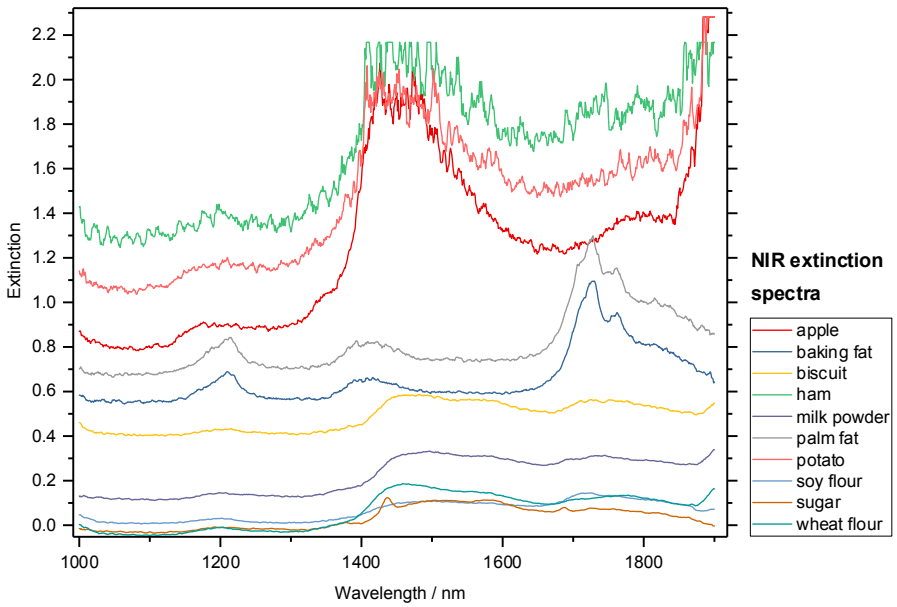

Figure 29: NIR extinction spectra of various food samples.

Here, the absorption bands of the food ingredients appear clearer but another type of background offset (other than the dark instrument background) is still present: scatter. As opposed to the visible, in the NIR spectral region, the spectral dependency of scattered light is almost negligible. Therefore, it may be regarded as a sample-dependent but constant offset contribution to the spectrum. If this contribution is not employed in analysis, it can be eliminated, e.g. using the common strategy of calculating derivative spectra. An example is shown in Figure 30. Here, the first derivative of a biscuit, soy flour and wheat flour spectrum can be directly compared. In more subtle cases, one would not resort to single spectra but instead average over the variety of spectra of a sample. However, in this case, the spectral contributions are very distinct so that one can attempt an interpretation from single spectra. Apparently, the biscuit spectrum follows that of wheat flour – not of soy flour. Therefore, it can be concluded that a large concentration of wheat flour is present in the biscuit, in accordance with the ingredients list on the pack.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

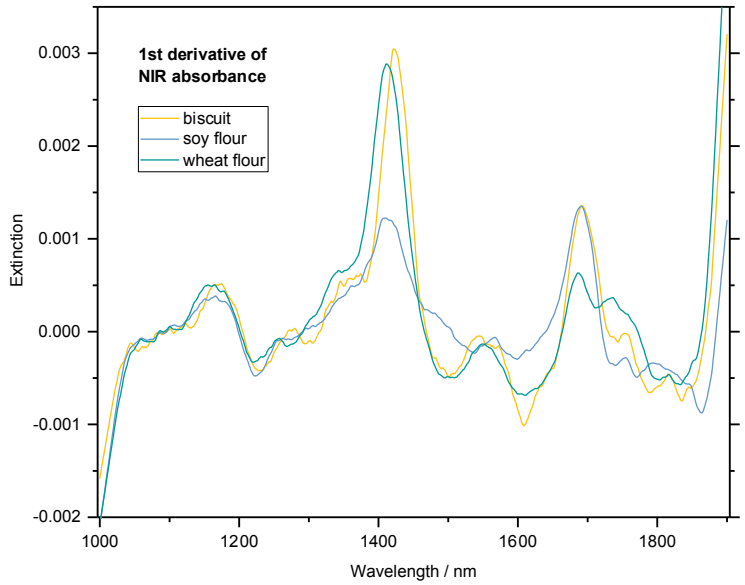

Figure 30: Derivative spectra of NIR extinction spectra of a biscuit, soy flour and wheat flour.

Summary and outlook of the general food test. Apparently, the NIR spectra vary greatly between different food samples. This is the strength PhasmaFOOD will be playing on. The absorption bands of fatty acids and water are the most prominent examples. However, care must be taken during both measurements and analysis in order to obtain valid data:

1. NIR information is contained only in the light fraction that is diffusely scattered from the sample. The geometrical setup of the PhasmaFOOD sensing device is designed to implement that.
2. The scattering cross section of each sample carries a spectral dependency. For strongly scattering samples, this scattering dependency may dominate the entire NIR spectrum. As a rule-of-thumb, visibly light samples scatter more strongly than dark samples. A sample may be inhomogeneous in this respect such that a meaningful NIR calibration becomes impossible. Therefore, it is recommended to eliminate the influence of the scatter background through data preprocessing strategies such as calculating the first derivative of absorbance spectra. Prior to calibration.
3. Even samples that show little visible inhomogeneity (such as powders) will exhibit a statistical range of NIR spectra. During calibrations, this must be taken into account via recording a representative number of spectra per each sample. This is already being implemented in the measurements of fish and rocket in use case 2.

In summary, NIR spectroscopy with a scanning grating spectrometer produces data that are characteristic for the main ingredients of a food sample.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

5.3.2 Milk powder

Preliminary tests have not been performed yet.

5.3.3 Meat

Preliminary tests have not been performed yet.

5.3.4 Alcoholic beverages

Preliminary tests have not been performed yet.

5.3.5 Edible oils

Purpose of the edible oil test. The purpose of this trial was not to authenticate the individual brands or ingredients but, instead, to demonstrate the capability of NIR spectroscopy with a scanning grating NIR spectrometer of identifying the fractions of two different oils in a binary mixture, and of determining the detection limit.

Samples edible oil test. Samples are mixtures (by weight) of sunflower (Brand: "Ja!") and olive oil (Brand: "Primadonna extra virgin"), made on-site at the IPMS spectroscopy laboratory from commercially available brands of edible oil.

Detection equipment edible oils test. For the measurements, we used the same Hiperscan SGS1900 NIR spectrometer that is also being used in the other use case trials. Also, the light source is the same. Measurement is performed in transmission geometry using a glass transmission cell of 10 mm path length.

Measurements edible oils test. Figure 31 shows the absorbance spectra of the sunflower/olive oil mixtures. Absorption was calculated like above, taking into account the dark counts of the NIR spectrometer and the lamp spectrum as a white reference. A slight trend is observed in the absorbance spectra from samples with low olive oil content to samples with high olive oil content. However, the baseline variation partly masks this trend so that these spectra cannot be used for a calibration of the olive oil content in the sample. As elaborated above, this baseline originates from the scattering properties of the samples. Scatter is reduced during these measurements due to the use of transmission geometry and the low number of particles in the oil samples. However, even its reduced occurrence is sufficient to modulate the absorption features and prevent calibration.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

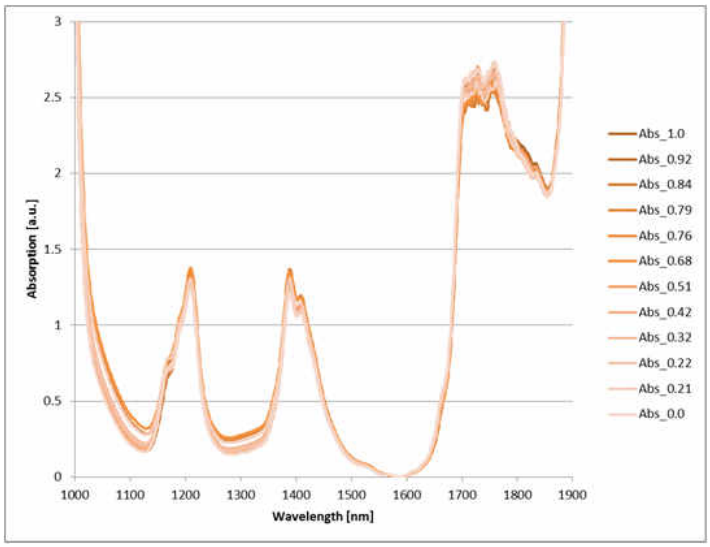

Figure 31: Absorbance spectra of sunflower-olive oil mixtures. The legend indicates the content of olive oil in the sample, between 1.0 (100 %) and 0.0 (0 %).

Therefore, the first derivative of the absorbance spectra is calculated, after a 43-point polynomial smoothing of the data. Figure 32 shows the results as complete spectra while figure REF shows an enlarged section for better clarity. These data can already be used for a rudimentary calibration. In order to test which spectral region is best suited for the calibration, the values of the derivative spectra at individual wavelengths are now plotted against the nominal olive oil content, see Figure 32. All of these plots show a monotonous dependency on olive oil content suitable for linear fitting. This fitting is done for 4 wavelengths, i.e. the peaks at 1156, 1168, 1654 and 1663 nm. The best regression is obtained for 1663 nm. The linear fit calibrates the value of the derivative spectrum at 1663 nm against the nominal olive oil content with a standard deviation of 3.8 % olive oil in sunflower oil, which is the accuracy of the analysis method. One can further refine the analysis by using average values over a larger spectral region, by scaling up the number of samples (here: only 12) or by using chemometrical analysis methods.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

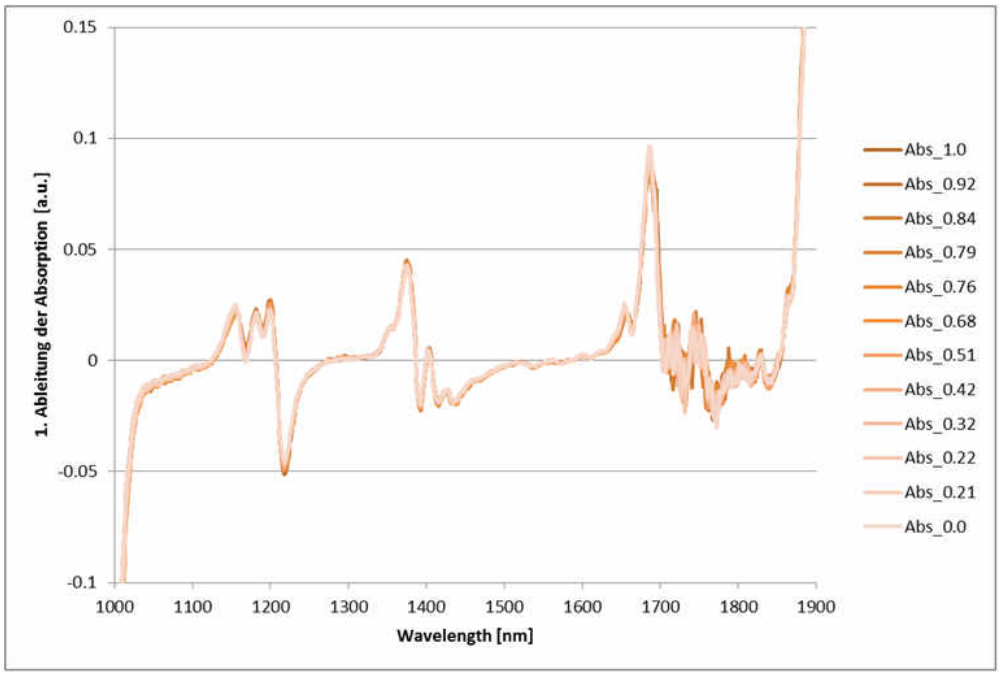

Figure 32: First derivative of the absorbance spectra of sunflower-olive oil mixtures of figure 31. The legend indicates the content of olive oil in the sample, between 1.0 (100 %) and 0.0 (0 %).

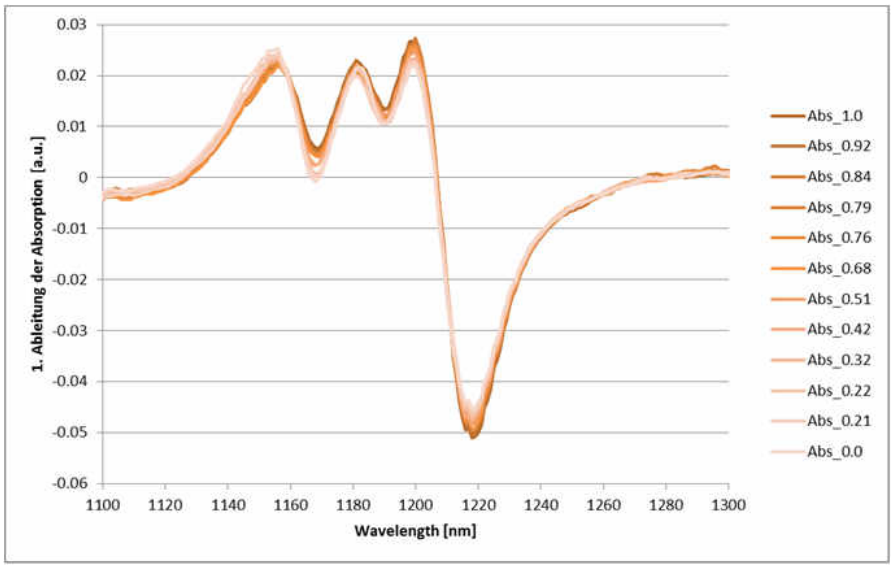

Figure 33: Enlarged section of figure 32. The legend indicates the content of olive oil in the sample, between 1.0 (100 %) and 0.0 (0 %).

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

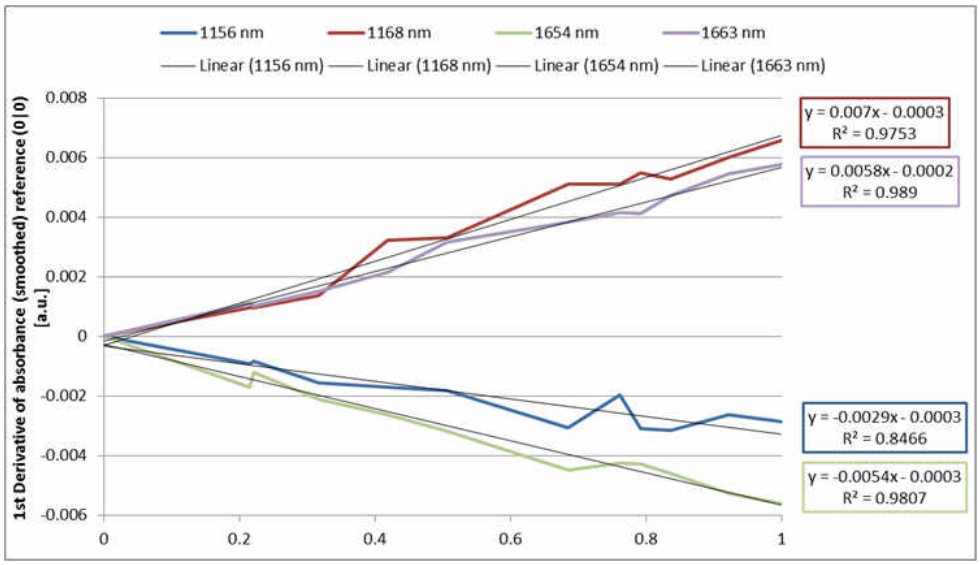

Figure 34: Plot of the values of the derivative spectra at fixed, single wavelengths (see legend above the graph) against olive oil content. The grey lines depict linear fits of these plots and the insets show the fit parameters.

Summary edible oils test. The contents of a binary mixture of edible oils can be detected with an accuracy of 3.8 weight %. This result was obtained using a very simple analysis based on single values of first derivatives of absorbance spectra. With very little extra effort such as averaging over wavelength bands and measuring more samples, the accuracy of this "calibration" can be improved further. Therefore, NIR spectroscopy is suitable for detecting food fraud in this case. For more complex sample compositions, a chemometrical analysis, e.g. a principal components analysis and partial least square regression, can be employed in T3.3.

Outlook edible oils test. The measurements of the PhasmaFOOD sensing device will be carried out in transflection mode instead of transmission. Transflection measurements are suitable for the investigation of opaque samples, whose surface is able to scatter NIR light. Here, the light enters the sample, where it is partly absorbed depending on the absorption and scattering properties of the sample. Any light that is then scattered back towards the detector carries spectral information about the chemical composition of the sample, which can be made visible using the NIR spectrometer detector.

The liquids used here, i.e. edible oils, do not contain a sufficient amount of particles to scatter NIR light. Light will enter the oil sample, be partly absorbed according to the absorption bands of the oil, while the remaining light will be transmitted through the sample. In order to direct this light back to the detector, the oil (or any other sample with comparable optical properties) must be placed in a sample holder that exhibits a diffuse reflecting surface behind the sample.

It will be the task of WP3 to identify the optimal material for such a reflector and the optimal depth of the sample container.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

6 Conclusions and outlook

The present D3.1 report, being the first in a series of successive reports, is a compilation of all collected information and experimental data until M9 of the PhasmaFOOD project, allowing for an initial feasibility assessment of the proposed use cases (i.e., detection of mycotoxins, spoilage and fraud). Overall, in the framework of D3.1, elaboration upon planning, sampling and measurement strategies, standard operating procedures of the VIS and NIR sensors, as well as reference methods and chemometric protocols has been provided. More specifically, the information presented in this deliverable is pertinent to (i) first results on mycotoxins' detection in the sub-use cases of maize flour and tree nuts (almonds) using the VIS sensor; (ii) extensive experimental data reporting along with initial data analysis approaches on detection of spoilage of the sub-use cases of fish and vegetables (i.e. rocket salad) using the NIR and VIS sensors; and (iii) preliminary measurements of general food samples using the NIR sensor, as well as results of a pilot experiment using mixtures of extra virgin olive oil and sunflower oil, demonstrating the general applicability of NIR spectroscopy to food fraud detection.

The forthcoming research goals that, in the context of WP3, are anticipated to be completed and reported in the further course of deliverable reports (D3.2 – M18 and D3.7 – M27), involve the finalization of sampling/measurement strategies, protocols and operating procedures, the continuation and evolution of data analysis for the already collected data, as well as the initiation and completion of experiments for the rest of the sub-use cases that have not been investigated yet. Specifically, complete operating procedures for calibration and image analysis for the utilization of the CMOS camera in the proposed use cases will be defined and the corresponding feasibility tests will be completed and presented. With reference to use case 1 (detection of mycotoxins), preliminary tests in the sub-use cases of milk powder and paprika powder, with the expected measurement set-up being similar to the one used in grain (i.e. maize) and almond flour, are planned to be conducted. Regarding use case 2 (detection of spoilage and prediction of shelf life), next objectives include analysis of data derived from the rocket salad experiments (described in the present deliverable report), completion of preliminary tests in meat, and design and conductance of experiments involving additional sub-use cases, with the latter being meat (ground pork) and fruit (ready-to-eat pineapple) products. Finally, with regard to use case 3 (detection of food fraud), in addition to the provided in this report pilot measurements in edible oils, which are expected to be substantiated with additional experimental data, preliminary experiments in skim milk powder, meat and alcoholic beverages (distilled spirit samples) will be performed using all PhasmaFOOD sensors.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

7 References

Borchers, A. T., Chang, C., & Gershwin, M. E. (2017). Mold and human health: a reality check. *Clinical Reviews in Allergy & Immunology, 52*, 305-322.

Carstensen, J. M., & Hansen, J. F. (2003). An apparatus and a method of recording an image of an object. Patent family EP1051660.

Capuano, E., Boerrigter-Eenling, R., Koot, A., van Ruth, S. M., (2015). Targeted and untargeted detection of skim milk powder adulteration by near-infrared spectroscopy. *Food Analytical Methods*, *8*, 2125-2134.

Chauhan, R., Singh, J., Sachdev, T., Basu, T., & Malhotra, B. D. (2016). Recent advances in mycotoxins detection. *Biosensors and Bioelectronics, 81*, 532-545.

COMMISSION REGULATION (EC) No 273/2008 of 5 March 2008 laying down detailed rules for the application of Council Regulation (EC) No 1255/1999 as regards methods for the analysis and quality evaluation of milk and milk products.

COMMISSION REGULATION (EC) No 2870/2000 of 19 December 2000 laying down Community reference methods for the analysis of spirits drinks.

Dufour, E. (2011). Recent advances in the analysis of dairy product quality using methods based on the interactions of light with matter. *International Journal of Dairy Technology, 64*, 153-165.

Ellis, D. I., & Goodacre, R. (2001). Rapid and quantitative detection of the microbial spoilage of muscle foods: current status and future trends. *Trends in Food Science and Technology, 12*, 414-424.

Espinosa-Calderon, A., Contreras-Medina, L. M., Munoz-Huerta, R. F., Millan-Almaraz, J. R., Gonzalez, R. G. G., & Torres-Pacheco, I. (2011). Methods for Detection and Quantification of Aflatoxins. In I. Torres-Pacheco (Ed.), Aflatoxins: Detection, Measurement and Control, 109-128. Rijeka: InTechOpen.

Fernandes, J.K., Umebara, T., Lenzi, M. K., & Alves, E. T. D. (2013). Image analysis for composition monitoring. Commercial blends of olive and soybean oil. *Acta Scientarum-Technology, 35*, 317-324.

Hamad, S. H. (2012). Factors affecting the growth of microorganisms in food. In R. Bhat, A. Karim Alias, & G. Paliyath (Eds.), Progress in food preservation (pp. 405-427). Oxford: Wiley-Blackwell.

Hernández, D. B., Mishra, R. K., Muñoz, R., & Marty, J. M. (2017). Low cost optical device for detection of fluorescence from OchratoxinA using a CMOS sensor. *Sensors and Actuators B, 246*, 606-614.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

Hiperscan GmbH, https://www.hiperscan.com/de/sgs1900

ISO 12966-4:2015(en) Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters — Part 4: Determination by capillary gas chromatography

Karoui, R., & De Baerdemaeker, J. (2007). A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. *Food Chemistry, 102*, 621-640.

Karunathilaka, S. R., Farris, S., Mossoba, M. M., Moore, J. C., & Yakes, B. J. (2017). Non-targeted detection of milk powder adulteration using Raman spectroscopy and chemometrics: melamine case study. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment, 34*, 170-182.

Kos, G., Sieger, M., McMullin, D., Zahradnik, C., Sulyok, M., Öner, T., Mizaikoff, B., & Krska, R. (2016). A novel chemometric classification for FTIR spectra of mycotoxin-contaminated maize and peanuts at regulatory limits. *Food Additives & Contaminants: Part*, *33*, 1596-1607.

Koutsoumanis, K., & Nychas, G.-J. E. (2000). Application of a systematic experimental procedure to develop a microbial model for rapid fish shelf-life predictions. *International Journal of Food Microbiology, 60*, 171-184.

Leitzmann, C. (1993). Food quality-definition and holistic view. In H. Sommer, B. Petersen, & P. v. Wittke (Eds.), Safeguarding Food Quality (pp. 3-15). Berlin, Heidelberg: Springer-Verlag.

Miedaner, T., Han, S., Kessel, B., Ouzunova, M., Schrag, T., Utz, F. H., & Melchinger, A. E. (2015). Prediction of deoxynivalenol and zearalenone concentrations in *Fusarium graminearum* inoculated backcross populations of maize by symptom rating and near-infrared spectroscopy. *Plant Breeding*, *134*, 529-534.

Nemme, K., Mohammed, A. (2017). Mycotoxin occurrence in grains and the role of postharvest management as a mitigation strategies. A review. *Food Control, 78*, 412-425.

Orlov, A. V., Burenin, A. G., Massarskaya, N. G., Betin, A. V., Nikitin, M. P., & Nikitin, P. I. (2017). Highly reproducible and sensitive detection of mycotoxins by label-free biosensors. *Sensors and Actuators B, 246*, 1080-1084.

Ottavian, M., Fasolato, L., Serva, L., Facco, P., Massimiliano, B. (2014). *Food and Bioprocess Technology, 7*, 1025-1036.

Panagou, E. Z., Papadopoulou, O., Carstensen, J. M., & Nychas, G.-J. E. (2014). Potential of multispectral imaging technology for rapid and non-destructive determination of the microbiological quality of beef filets during aerobic storage. *International Journal of Food Microbiology, 174*, 1-11.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

Papadopoulou, O. S., Panagou, E. Z., Mohareb, F., & Nychas, G.-J. E. (2013). Sensory and microbiological quality assessment of beef fillets using a portable electronic nose in tandem with support vector machine analysis. *Food Research International, 50*, 241–249.

Porep, J. U., Kammerer, D.R., & Carle, R. (2015). On-line application of near infrared (NIR) spectroscopy in food production. *Trends in Food Science and Technology, 46*, 211-230.

Pügner, T., Knobbe, J., & Grüger, H. (2016). Near infrared grating spectrometer for mobile phone applications. *Applied Spectroscopy, 70*, 734-745.

Ropodi, A. I., Panagou, E. Z., & Nychas, G.-J. E. (2016). Data mining derived from food analyses using non-invasive/non-destructive analytical techniques; determination of food authenticity, quality & safety in tandem with computer science disciplines. *Trends in Food Science and Technology, 50*, 11-25.

Ropodi, A. I., Panagou, E. Z., & Nychas, G.-J. E. (2017). Multispectral imaging (MSI): A promising method for the detection of minced beef adulteration with horsemeat. *Food Control, 73*, 57-63.

Ropodi, A. I., Pavlidis, D. E., Mohareb, F., Panagou, E. Z., & Nychas, G.-J. E. (2015a). Multispectral Image Analysis approach to detect adulteration of beef and pork in raw meats. *Food Research International, 67*, 12–18.

Sharma A., Goud, K.Y., Hayat, A., Bhand, S., & Marty, J. L. (2017). Recent advances in electrochemical-based sensing platforms for aflatoxins detection. Chemosensors, 5, 1; doi:10.3390/chemosensors5010001.

Toro, P. C. D., Arévalo, F. J., Fumero, M. V., Zon, M. A., & Fernández, H. (2016). Very sensitive electrochemical sensor for moniliformin detection in maize samples. *Sensors and Actuators B, 225*, 384-390.

USP Pharmacopeial Convention Food Fraud Database. *https://www.foodfraud.org/*.

Wang, W., Ni, X., Lawrence, K. C., Yoon, S.-C., Heitschmidt, G. W., & Feldner, P. (2015). Feasibility of detecting Aflatoxin B1 in single maize kernels using hyperspectral imaging. *Journal of Food Engineering, 166*, 182-192.

Ximea, https://www.ximea.com/en/products/application-specific-cameras/subminiature-usb-cameras/mu9pc-mh.

Xiong, Z., Xie, A., Sun, D.-W., Zeng, X.-A., & Liu, D. (2015). Applications of hyperspectral imaging in chicken meat safety and quality detection and evaluation: A review. *Critical Reviews in Food Science and Nutrition, 55*, 1287-1301.

Zachariasova, M., Dzuman, Z., Veprikova, Z., Hajkova, K., Jiru, M., Vaclavikova, M., Zachariasova, A., Pospichalova, M., Florian, M., & Hajslova, J. (2014). Occurrence of multiple mycotoxins in

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.

European feedingstuffs, assessment of dietary intake by farm animals. *Animal Feed Science and Technology, 193*, 124-140.

Zandomeneghi, M., (1999). Fluorescence of cereal flours. *Journal of Agricultural and Food Chemistry 47,*878-882.

Project Title: PhasmaFOOD
Contract No. 732541
Project Coordinator: INTRASOFT International S.A.