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	**D1.1**
Deliverable Name	**Use case description & validation plan**
Dissemination level	Public *Disclaimer:* An extended deliverable version is submitted to EC to also include confidential level information.
Type of Document	Desk Study
Contractual date of delivery	31st May 2017
Deliverable Leader	DLO
Status & version	Final – version 5.0
WP / Task responsible	WP 1 / T1.1 – DLO (Yannick Weesepoel)
Keywords:	Use case, Mycotoxins, Food Spoilage, Food Fraud, Validation
Abstract (few lines):	The PhasmaFOOD project aims to develop a multifunctional spectral sensing device for food applications to be operated by untrained persons. In this report, the target foods are discussed in the use cases of (I) mycotoxins, (II) food spoilage and shelf-life prediction and (III) food fraud. Furthermore, the system level requirements and sensing requirements (measurement range, sensitivity, accuracy) for each target food are defined. Smart chemometric algorithms and data fusion methodologies are proposed. Finally, general sample plans and validation strategies for assessing the performance of the use case spectral databases of the PhasmaFOOD device are described.

Deliverable Leader:	Yannick Weesepoel (DLO)

Contributors:	DLO, INTRA-LU, WINGS[1], VLF, IPMS, AUA, FUB, CNR
Reviewers:	WINGS[1] and AUA
Approved by:	George Koutalieris (INTRA)

Executive Summary

The PhasmaFOOD sensor is a multi-sensor miniaturized optical device for fast characterization of foods by untrained people. The scanner array consists of a visible and near-infrared (NIR) spectrometer and an on-board level camera and will be smartphone operated. In this report, the three use cases on which the PhasmaFOOD sensor will be initially tested are described: (I) mycotoxin detection, (II) food spoilage and shelf-life prediction and (III) food fraud. Each use case consists of different target foods and therefore different system level requirements and sensing requirements (e.g. measurement range, sensitivity, accuracy) were specified. Furthermore, for each case operational constraints, like food packaging were considered.

The first case on mycotoxins will focus on the presence of aflatoxins and (when applicable) deoxynivalenol in maize flour, skimmed milk powder, paprika powder and tree nuts. The next case focuses on spoilage and shelf-life estimation of fruits, vegetables meat and fish. The last use case on food fraud will cover skimmed milk powder, meat, olive oils and other edible oils and alcoholic beverages. The limits of detection for the use cases are generally based on either EU legislation or technical feasibility as described in peer reviewed scientific papers.

In the last part of the report, smart chemometric algorithms and data fusion strategies were proposed. Next to that, general sample plans and validation strategies are discussed, together with the construction of a spectral database which will be able to operate at technology readiness level 4 (interlaboratory performance). Statistical approaches towards validation are proposed for performance quantification of the chemometric models and spectral databases of the individual use cases and target foods.

[1] Paraskevas Bourgos, Panagiotis Vlacheas, Dimitris Kelaidonis

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

Document History			
Version	**Date**	**Contributor(s)**	**Description**
0.1	28/03/2017	AUA, CNR, DLO	Early version for internal review
0.4	31/03/2017	AUA, CNR, DLO	Version for consortium review
0.6	01/05/2017	AUA, CNR, IPMS, VLF, WINGS, DLO	Version for consortium review
0.9	15/5/2017	AUA, INTRA, WINGS	Final revisions
1.0	19/5/2017	DLO, UTOV, AUA	Version for CL
2.0	31/5/2017	All	Final version for submission

Table of Contents

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

Definitions, Acronyms and Abbreviations

Acronym	Title
abv	alcohol by volume
AF	aflatoxin
ANN	artificial neural networks
CMOS	complementary metal-oxide semiconductor
CVA	canonical variate analysis
Dx.x	deliverable x.x
DON	deoxynivalenol
EU	European Union
EC reg.	European Commission regulation
FN	false negative
FP	false positive
FTIR	Fourier transform infrared
GA	genetic algorithm
GP	genetic programming
HCA	Hierarchical cluster analysis
LED	light emitting diode
LOD	limit of detection
MIR	mid-infrared
MLR	multiple linear regression
MRL	maximum residue limit
NIR	near-infrared
nm	nanometre
PCA	principal component analysis
PCR	principal component regression
PLS-DA	partial lease squares discriminant analysis
PLSR	partial lease squares regression
SVM	support vector machine
TN	true negative
TP	true positive

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

TRL	technology readiness level
WPx	work package x
UV-Vis	ultra-violet and visible

Definitions

System level requirements

The set of statements that identify the PhasmaFOOD system functions, characteristics and constraints for the specific case study. The system function statements indicate the feedback the PhasmaFOOD system should be able to deliver to the end-user concerning the food product. The characteristic statements indicate how the PhasmaFOOD system will be operated and the constraint statements indicate the limitations of the application or the detection limits of the PhasmaFOOD sensors.

Sensing requirements

The measurement range, sensitivity and accuracy required from the PhasmaFOOD sensors in order to differentiate with acceptable low false positive and false negative rates the minimum requirements set in the system level requirements.

- *Measurement range definition: The spectral range of the spectral sensor used*
- *Resolution: the distance between two adjacent variables of the sensor used*
- *Target detectivity: the maximum difference between the actual value (which must be measured by a primary or good secondary standard) and the indicated value at the output of the sensor.*

Performance criteria (Validation)

Performance of the chemometric model upon validation.

- *True positive (TP): Sample is correctly identified as being ' positive' (e.g. truly authentic)*
- *True negative (TN): Sample is correctly identified as being ' negative' (e.g. truly counterfeit)*
- *False positive (FP): Sample is incorrectly identified as being 'positive' (e.g. incorrectly assigned as authentic).*
- *False negative (FN): Sample is incorrectly identified as being 'negative' (e.g. incorrectly assigned as counterfeit).*
- *Sensitivity: The proportion of positives that are correctly identified as such To be calculated as number of TP / (number TP + number of FN).*
- *Selectivity or Specificity: the proportion of negatives that are correctly identified as such. To be calculated as number of TN / (number of TN + number of FP).*
- *Efficiency: The ability to correctly identify TP and TN samples versus the total assigned samples. To be calculated as (number TP + number TN)/(number of TP + number of TN + number of FP + number of FN)*
- *% Reliability: Percentage of the cases in which a TP or TN answer is provided. To be calculated as 100-%FP-%FN*

1 Introduction

1.1 PhasmaFOOD sensor

The aim of PhasmaFOOD project is to develop a multifunctional optical sensing node for food applications that will be ultimately operated by consumers. The PhasmaFOOD project will start with the definition of the use cases (this deliverable), hardware and software requirements (D1.2) and business analysis (D1.3). The PhasmaFOOD sensing device will be smartphone operated with communication with the spectral database residing in the PhasmaFOOD cloud platform. The power of the PhasmaFOOD sensor will be based on the smart correlation of multiple heterogeneous sensors, enabling the device to be a universally applicable scanner integrating:

- Vis-microspectrometer (Hamamatsu C12880MA, range 340-850 nm)
- NIR-microspectrometer (IPMS MEMS NIR-spectrometer, range 1000-1900 nm)
- On-board CMOS camera (Raspberry Pi Infrared Camera Module, range visible to 880 nm)

The Vis and NIR microspectrometers will serve as the actual chemical sensors of the PhasmaFOOD sensor, whilst the CMOS camera will deliver information on parameters concerning the validity of the measurement. The camera sensor can determine if the illumination of the sample is correct, if the sample is positioned correctly, if a sample is homogeneous or heterogeneous and perform colour measurements of the sample to support the Vis-microspectrometer. For the purpose of sample illumination, different miniature light sources such as LEDs and tungsten lamps will be applied. These specifications are further elaborated upon in D1.2.

In order to test the performance of the (combination of) sensors on a technology readiness level (TRL) of 4 (interlaboratory deployment), a number of versatile use cases were worked out in the PhasmaFOOD project proposal:

- Use case 1: detection of mycotoxins in food products
- Use case 2: detection of early sign of spoilage, spoilage and shelf-life estimation in meat, fish and fruits and vegetables
- Use case 3: detection of food fraud.

In this deliverable we further define the scope of the use cases and their target food products (subcases) and provide an overview of the target operational specifications and system level requirements. These targets will be translated into the system specifications and design during the subsequent tasks. This report also explains the strategy planned to be embraced in order to evaluate (I) the performance of the different use cases (and subcases), and (II) the potential of the applications' further development to TRL 5 level (relevant environment deployment).

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

1.2 Configuration of the PhasmaFOOD sensor

The PhasmaFOOD sensor will have to deal with a versatile array of food matrices like powders, solid materials and transparent liquids. Different food matrices require different measurement strategies. For solid or powdered foods, measurements need to be performed in diffuse reflectance modus, whilst for transparent liquids transflectance measurements are more effective. Furthermore, environment light and focus distance may need to be dealt with or may need to be standardized. Therefore, as a conceptual design, a number of accessories and designs to facilitate spectroscopic measurements are proposed in D1.2 (Fig. 1).

A 'spacer' accessory or 'distance adaptor' may assist in standardizing the distance to the sample and to avoid excessive stray light in the PhasmaFOOD sensors. Furthermore, it prevents direct contact of the sensing head with polluting substances like powders and unhygienic surfaces. A 'sample holder' accessory serves the same purpose as the spacer. The end-user can put a sample in a separate standardized container when measuring conditions or space limitations do now allow for a good on-the-spot measurement. In order to optimize the efficiency of light collection and, thereby, sensitivity, the sensor-sample distance will be kept small. For liquids, the accessories may serve a number of purposes: (I) it serves as a water-proof cover for the PhasmaFOOD sensor to be directly applied in liquids as the reflecting surface is composed of a white 99% diffuse reflecting surface in order to properly diffuse the incoming light towards the sensor detectors. A disadvantage of applying directly in liquids is that the liquid accessory will have to be washed. Therefore, the second (II) application of the liquid accessory may be to serve as a pipette holder. Standardized disposable pipettes may be used to take samples and to apply the pipette in the liquid holder. In this way, the distance from the optical head to the sample is standardized and measurements can be performed hygienically.

End users will be informed through the PhasmaFOOD mobile application when and how to utilize PhasmaFOOD accessories for the sensing device. Mobile application (iOS and Android) will provide calibration interface where user can specify a type of food being scanned and type of measurement (type of measurement dictated by realized use cases). Based on the selection, the user will be instructed on how to properly utilize provided accessories. Furthermore, the mobile applications and the PhasmaFOOD cloud platform will provide interface for process calibration and validation experiments. Within WP4, the PhasmaFOOD mobile apps will be prepared in two versions. The first version of the mobile apps (proof of concept) will be used for internal testing, data acquisition, calibration and validation of use cases. It will provide valuable inputs for development of mobile apps for end users with engaging GUI providing all the necessary information and instructions. The cloud platform will build on top of data sets gathered during the use case experiments. It will feature a web dashboard which will be used during use case

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

validation and system calibration. It will help interpret use case experimental results and data and provide inputs for further developments and calibrations.

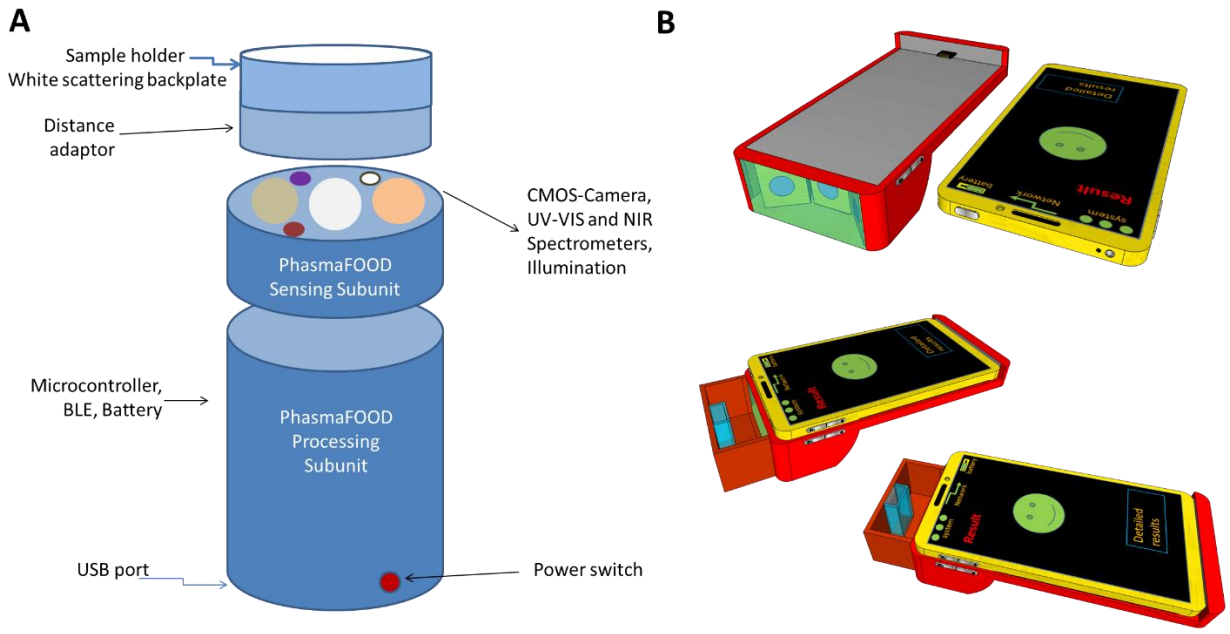

Figure 1. Proposed PhasmaFOOD designs (A) wireless sensor design and (B) physical connected sensor, top: design for solids, bottom: design for transparent liquids.

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

2 Detection of mycotoxins in foods

2.1 Need and Justification of the case

Mycotoxins are naturally produced toxic metabolites of fungi found in food such as cereals, grains and nuts. The presence of mycotoxins, focusing in this case of aflatoxins (AFs) and deoxynivalenol and its acetylated derivatives (DON), in the food supply chain are significant food safety issues for regulatory agencies worldwide. The severity of adverse effects caused by these mycotoxins range from long term effects such as genotoxicity and carcinogenicity (AFs) to more transient effects such as short-term nausea and vomiting (DON). In this use case, AFs B1, B2, G1 and G2 are considered, however the focus will be mainly on AF B1 and the total concentration of AFs (sum of B1, B2, G1 and G2). This is because AF B1 is regulated specifically in EC reg. 1881/2006 from the total AFs concentration (Table 1) [1]. Furthermore, in this use case, AF metabolites will be investigated using the PhasmaFOOD sensor. These metabolites are produced by cattle ingesting AF contaminated feed. During digestion of the feed, AFs B1 and B2 are bio-converted to AFs M1 and M2 which can be excreted in the animals milk and ultimately end up in the human food chain.

The European Union (EU) has the most rigorous regulations concerning mycotoxins in food worldwide, as defined in EC reg. 1881/2006. For example, the EU has established the maximum residue levels (MRLs) of AFs in cereals, peanuts and dried fruits for direct human consumption at 4 μg/kg for total aflatoxins, whilst in the USA a maximum level of 20 μg/kg has been established by the Food and Drug Administration [2]. Due to the strict EU regulations and great consumer interest in these compounds, several research and industrial projects have been devoted to the study of the AFs presence in various foods. For DON also strict EU regulations apply, but MRLs are substantially higher than that for AFs (Table 2).

One of the challenges in the detection of mycotoxins in food lies in the high cost, time and labour requirements of current analytical methods such as thin layer chromatography and liquid chromatography mass spectrometry [3, 4]. The application of the PhasmaFOOD sensor is a potential alternative method for the detection of mycotoxins, and has the advantages of being fast and non-destructive to samples [5]. Still the application of a single analytic spectroscopic technique only allows rather high concentrations of mycotoxins to be positively detected [6, 7, 8, 9]. Therefore, the combinatorial approach of the PhasmaFOOD sensor may result in lower limits of detection or more robust results. This rather simple click-and-scan principle of the PhasmaFOOD device could serve to quickly scan food batches for individual customers, for quality control of incoming goods in retail or for quality control in the food industry. While

Table 1. Comparison of AF MRLs (in μg/kg) for food products in the EU (EC reg. 1881/2006), Codex Alimentarius (Codex Stan 193-1995) and the USA [1, 10, 11].

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

Food category	EU		Codex	USA
	AFB1	AF(total)	AF(total)	AF(total)
Peanuts (for further processing)	8	15	15	-
Peanuts (RTE)	2	4	-	20
Almonds and pistachios (for further processing)	12	15	15	-
Almonds and pistachios (RTE)	8	10	10	20
Hazelnuts and Brazil nuts (for further processing)	8	15	15	-
Hazelnuts and Brazil nuts (RTE)	5	10	10	20
Tree nuts other than those above (for further processing)	5	10	-	-
Tree nuts other than those above (RTE)	2	4	-	20
Dried figs	6	10	10	20
Dried fruit other than dried figs (for further processing)	5	10	-	-
Dried fruit other than dried figs (RTE)	2	4	-	20
Maize and rice (for further processing)	5	10	-	-
Cereal based foods for infants and young children	0.1	-	-	20
Cereals and cereal products other than those above	2	4	-	20
Infant dietary foods for special medical purposes	0.1	-	-	20

RTE: Ready to eat, - indicates limit not specifically mentioned.

Table 2. Comparison of DON MRLs (in µg/kg) in food products in the EU (EC reg. 1881/2006), Codex Alimentarius (Codex Stan 193-1995) and the USA [1, 10, 11].

Food category	EU	Codex	USA
Cereal based foods for infants and young children	200	200	-
Unprocessed wheat and oats	1750	2000	-
Unprocessed maize (other than maize for wet milling)	1750	2000	-
Unprocessed cereals other than those above	1250	2000	-
Cereal flours (other than maize) for direct human consumption	750	1000	1000#
Pasta	750	-	-
Bread, pastries, biscuits, cereal snacks and breakfast cereals	500	-	-

- For wheat products only, - indicates limit not specifically mentioned.

PhasmaFOOD focuses on addressing the former two aspects, the latter field of application will also benefit from a general proof of principle of the proposed combined measuring approach.

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

Therefore, in this use case study, the feasibility for a PhasmaFOOD spectral application of four product types are investigated concerning the presence of AFs B1, total AFs, AF metabolites and DON and its derivatives.

2.2 Target food products for this case

The use case on mycotoxins focuses on a number of (processed) food products that may contain the toxins of interest. Although plenty of products are available in these product groups, it is not simple to acquire substantial amounts of samples which are contaminated with the mycotoxins of interest. Furthermore, for chemometric calibration of the individual PhasmaFOOD micro-sensors, samples with various concentrations covering ranges below and above the (MRLs) need to be included in the spectral database. Therefore, target products were chosen of which certified reference material is available to perform initial testing and calibration of the sensors:

1. **Maize (flower)**. AF B1, total AFs and DON
2. **Skimmed 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

In EC reg. 1881/2006 AF B1 is specifically of interest due to its high toxicity, and has its own specific MRL value for each product group (Table 1). Also, the total AFs, comprising of the sum of AFs B1, B2, G1 and G2, have specific MRL values for each product case. DON and its acetylated derivatives were also grouped together for specific product groups (Table 2) and will be considered for maize (flower).

2.3 Operational specifications

2.3.1 System level requirements

The PhasmaFOOD sensor should operate within MRLs accepted in the EU. International maximum limits for contaminants in food have been established by the Codex Alimentarius Commission (Tables 1 and 2). Although countries are not legally obliged to adopt these limits, many countries do take them as a reference. Regulatory limits for food contaminants are based on the outcomes of risk assessment, which take into consideration the toxicity of the contaminant as well as the population's dietary intake of the compound. As dietary intakes among different populations can be expected to vary, different countries may impose varying legal maximum limits based on the outcomes of their respective risk assessments.

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

Table 1 compares the maximum limits for aflatoxins in food established by the Codex Alimentarius Commission, the USA and the EU. In general, the EU has specified different maximum limits for various products, Codex has adopted similar maximum limits for AFs total in a few products such nuts, and the USA has established higher guidance limits at 20 μg/kg for all food product categories. Table 2 compares the maximum limits for DON in the same jurisdictions. In both Tables 1 and 2, it can be seen that the lowest maximum limits for AFs and DON are applied to food categories for infants and young children. This is due to the increased sensitivity of this age group to these natural toxins arising from their relatively low body weight. For all other food products intended for consumption by the general population, the most stringent aflatoxin limits of 2 μg/kg AFB1, 4 μg/kg AF(total), and 750 μg/kg DON have been applied by the EU.

Taking into account the MRLs displayed in Table 1 and 2, the following system level requirements for the PhasmaFOOD scanner may be established:

Functions: For all four products, a clear feedback should be provided to the end-user on whether a product is safe to eat or not. This decision may be made on the basis of the lowest MRLs set for that product category and may be based on a specific type of AF (like AFB1) or DON, total AF or total mycotoxin content. The PhasmaFOOD mobile applications will be the main interface towards the end user. Mobile app GUI will provide end user with interface to select mycotoxin measurement. Next, end user will be prompted to specify type of food (from the selected 4 types) being scanned. After that, the end user will be instructed on how to utilize the scanner and provided accessorise to properly conduct measurement. Results will be presented and interpreted to the end user. The mobile app will provide additional information, statistics, regulatory aspects which will help end user to interpret obtained results and get familiar with the meaning of false negatives which may occur.

Characteristics: The application should be operated in diffuse reflectance and/or fluorescence modus. The products to be tested appear as powders (tree nuts potentially as whole nuts) and are contained in either transparent or light-shaded containers. The end-user can then perform measurements by using a ‘spacer’ to directly place the sensing device onto the sample or by transferring powder to a sample holder (Fig. 1). Both methods provide sufficient possibility for standardisation and protection form environmental influences. Due to the powdered nature of the samples, a protective, cleanable and suitably transparent window is recommended to be placed between the sensors and the samples, in order to avoid contamination between measurements. For the tree nuts, a similar approach may be followed. The challenge here will be to address the potentially inhomogeneous surface of the product. Possible solutions are for example performance of multiple measurements by guidance of the on-board camera. The PhasmaFOOD mobile application will guide the end user during the measurements. As AFs and DON represent food safety risks, a disclaimer should be displayed (through the PhasmaFOOD mobile apps) on the potential risks of false negatives.

Constraints: As all four selected products have different MRLs, the PhasmaFOOD spectral database should be considering the following LODs in μg/kg:

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

1. **Maize (flour)**: AF B1 – 5, total AFs – 10, DON – 1750
2. **Skimmed milk powder**: Total AFs M – 0.05
3. **Paprika powder**: AF B1 – 5, total AFs - 10
4. **Tree nuts (homogenised or whole nuts)**: AFB1 – 2 to 12, total AFs - 4 to 15 (depending on the type of tree nut chosen)

For tree nuts, whole nuts can be considered when suitable reference materials are available. These materials may be artificially produced by one of the PhasmaFOOD partners, as most reference samples comprise of homogenised materials.

2.3.2 Sensing requirements

Visible spectroscopic techniques: Some aflatoxin producing fungi including *Aspergillus flavus* or *A. parasiticus*, produce bright greenish yellow fluorescence (BGYF) when inspected under UV light (365 nm). This characteristic has been utilised in the presumptive BGYF test (AACCI Method 45-15.01), which has been used in corn as well as figs. However, because the fluorescence is not caused by the aflatoxins directly, but rather, the interaction between kojic acid from the fungi and plant peroxidase [12], caution is required when interpreting results. Ono et al. (2010) compared the BGYF test against thin-layer chromatography and spectrofluorimetry methods for the detection of aflatoxins in corn, and found that BGYF showed 20% false-negative results [13]. In addition, two other factors may influence the outcome of the BGYF test. Firstly, the fluorescence is not stable and disappears in 4 to 6 weeks after continuous exposure to visible or ultraviolet radiation, while the aflatoxin remains. As a result, the BGYF test is only suitable for fresh samples. Secondly, a positive outcome of a BGYF test does not absolutely indicate the presence of aflatoxins, since non-toxicgenic fungi as well as other naturally present compounds may also cause BGY fluorescence [14].

Infrared spectroscopic techniques: Mycotoxins can be directly analysed in food matrices by IR based methods, however in an uncontrolled environment LODs increased rapidly [15, 16]. In controlled environments, AFs and DON can be determined at the legal limits set in the EU legislation [6]. Contamination of crops by toxigenic fungi could be indirectly detected through the investigation of spectral bands related to proteins and carbohydrates [5, 6].

Imaging: Identification of the presence of toxigenic fungi may be performed by means of imaging of contaminated spots on heterogeneous materials. Still it should be noted that the absence of toxigenic fungi cannot be presumed to be an indication of the absence of mycotoxins since toxins can last on substrate well after the mycelium has disappeared. Also the amount of toxigenic fungi is not representative for the amount of mycotoxins produced, as this is dependent on the nutritional and environmental conditions for the fungus. Furthermore, the formation of mycotoxins can continue during storage without the presence of the fungus. Therefore, the imaging component can only be used as a potential indicator for the presence of fungi.

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

Hyperspectral imaging was successfully used to identify AFB1 on maize kernels to levels as low as 10 μg/kg [7, 9]. For DON also multiple studies have been performed on using hyperspectral imaging, with altering success rate [16]. While hyperspectral imaging is not foreseen within the PhasmaFOOD approach, the validation tests will address the synergies arising from the combined use of a vis camera, a Vis spectrometer, and a NIR spectrometer.

Sensor combination and data fusion: All three PhasmaFOOD sensors will provide specific information, all containing unique features on either presence of AFs and DON, presence of fungi or both. Combining the strengths of the three sensors will increase the possibility that the PhasmaFOOD sensor will be able to sense at MRL levels for both AFs and DON in a more robust way.

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

3 Detection of early sign of spoilage, spoilage and shelf-life estimation in fruits, vegetables, meat and fish

3.1 Need and Justification of the case

Food spoilage is a complex ecological phenomenon, underlain mainly by the biochemical activity of microorganisms. The food quality changes composing spoilage are related to the metabolic activity of certain groups of microorganisms, referred to as "ephemeral spoilage organisms", and the type and availability of the required energy substrates in foods [17, 18]. Although numerous methods (organoleptic, microbiological or physico-chemical) have been developed for the purpose of food quality assessment [19, 20], the majority of them are time-consuming, labour-intensive, destructive, and provide 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 [21-24]. In this context, the development of a smart sensor-based system which, by utilizing relevant spectral and/or imaging data, will allow for food spoilage detection and shelf-life estimation is expected to be of great value throughout the food supply chain (food manufacturers, retailers, food service, consumers).

3.2 Target food products for this case

1. **Meat.** Fresh meat and poultry constitute food commodities of high biological value and economic significance. At the same time, however, due to their intrinsic characteristics (i.e. high water content and pH value of 5.5-6.5), these commodities favour microbial growth, and are thus highly prone to spoilage. With the importance of spoilage detection and shelf-life estimation being apparent for this food category, this subcase will involve the study of minced meat and/or fillets (e.g., beef, pork, chicken). In this context, the spoilage status of the aforementioned food products will be monitored during storage at different temperatures and packaging conditions (i.e. atmospheres).

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

2. **Fish.** Due to their high protein and polyunsaturated fatty acids contents, fish is regarded a food of exceptionally high nutritional value. Nonetheless, similarly to what is the case for meat, fish is a highly perishable food commodity, with its shelf-life being dramatically limited by enzymatic activity, chemical reactions and bacterial growth and metabolism. In this subcase, fish spoilage and shelf-life will be assessed and characterized as a function of storage conditions (i.e. temperature and atmosphere), and the product that will be studied is fresh whole bream (*Sparus aurata*).

3. **Fruits and vegetables.** The available research regarding the potential use of spectral/imaging data for the purpose of spoilage detection of fresh produce items is scarce. For this reason, this subcase will focus on evaluating the application potential of such data in spoilage monitoring and shelf-life estimation of (I) ready-to-eat packaged salad and (II) whole fruit (e.g. tomatoes) and under various isothermal conditions.

3.3 Operational specifications

3.3.1 System level requirements

Since food spoilage is a rather complex ecological phenomenon, with numerous physiological, biochemical, microbial and sensory attributes contributing to its manifestation, utilization of specific spoilage levels and deciding upon requirements of spoilage prediction are fairly difficult tasks. Nonetheless, it is widely accepted that the consumers' perception regarding food quality is a very important parameter when assessing food spoilage and shelf-life. Taking this into account, and also considering that the biochemical activity of specific groups of microorganisms is strongly associated with the shelf-life of various food products, knowledge of the interactions between these specific groups of microorganisms and the sensory quality attributes of food (e.g., colour, appearance, odour, texture and taste) is a high-priority objective.

In this context, the PhasmaFOOD approach will utilize reference sets of samples corresponding to the aforementioned subcases (i.e. meat, fish, fruits and vegetables) with various kinds of spoilage (i.e. spoilage attributed to various groups of microorganisms depending on the raw material, its natural microbial composition and the applied packaging and storage (temperature) conditions) and spoilage levels (i.e. microbial concentrations). With regard to spoilage microbial levels and their correlation with sensory quality and shelf-life, all the studied food categories are generally regarded as spoiled at total microbial populations (total viable counts, TVC) of 10^7 to 10^8 colony forming units (cfu) per gram of food (cfu/g). However, in terms of detection of early signs of spoilage (i.e. incipient spoilage), the corresponding concentrations are expected to be at lower levels, depending on the food type, but these lower levels need to be somehow correlated to another spoilage criterion, such as sensory attributes' deterioration. Indeed, as provided by research data, the joined evaluation of sensory analysis and microbiological data delineate a TVC

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

(mainly consisting of bacteria) of 7.0 and 5.5-6.0 log cfu/g as a spoilage level of relevance with regard to the end of the 'sensory' shelf-life of fish and meat, respectively [25, 26]. With regard to fruits and vegetables, the latter limit may be even lower. Certainly, fresh produce items and particularly fresh-cut (minimally processed) products are highly perishable, with microbial populations (consisting mainly of yeasts) higher than 5 log cfu/g being associated with off-odours in these products [27, 28]. Hence, in this use case of the PhasmaFOOD project, various kinds and levels of spoilage (including the aforementioned limits) will be studied and used for the calibration of the individual micro-sensors, aiming at the development of a database which will, ultimately, allow for their validation (feasibility study assessing the effectiveness of these micro-sensors in detecting early signs of spoilage of meat, fish and fresh produce). In this framework, the developed spoilage models are expected to be used for the prediction of the shelf-life of the studied products, providing also input for smart data correlation of the PhasmaFOOD sensor output (Section 4.2).

The PhasmaFOOD approach, through the involvement of micro-sensors and their utilization in food spoilage prediction and shelf-life estimation, presents several advantages compared to approaches utilizing traditional methods. Such advantages include its simple (in terms of application) and non-invasive character, its time-efficiency as well its potential to be used throughout the food supply chain. However, the enormous amount of data anticipated to be generated by the technology outlined in the PhasmaFOOD approach can be very difficult to interpret without the employment of appropriate data analysis methods. The acquired data are expected to be complex and, thus, their analysis demands a multi-disciplinary approach.

In all the aforementioned target food products, the main physical limitation that needs to be considered is sample presentation and type of packaging material. Indeed, fresh meat, fish and produce items sold in supermarkets are often covered by transparent membrane on top of a plastic or a styrofoam container, and in some cases (e.g. minimally processed, ready-to-eat fresh-cut salads) are packaged in bags under modified atmospheres. This means that the end-user may be expected to perform a scan through the packaging material in such types of products. At the same time, however, the same products can be found without specific packaging in the food supply chain (e.g., in open markets), indicating that both sets of acquired spectral data (i.e. with and without packaging) are expected to be of value for the purpose of spoilage detection and shelf-life estimation. Therefore, the system level requirements for all the three aforementioned subcases are the following:

a. Functions: Feedback on food spoilage and shelf-life related issues. The feedback, measurement results and their interpretation will be provided through the PhasmaFOOD mobile apps. Also, the mobile app will provide end user with means to calibrate the measurement process (1. select this particular measurement group; 2. specify food type from provided options; 3. specify scanning conditions i.e. with or without packaging).

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

b. Characteristics: The application should be operated in diffuse reflectance modus. Measurements can be performed either by direct contact of the PhasmaFOOD sensor with the food item (meat, fish, produce), simulating the case of products being sold in open markets (e.g., grocery markets or butcher shops), or by applying the scanner on the top of the existing package. For measuring directly on the food product, a spacer should be used to standardize the distance between food and sensors, minimize environmental (light) influences, as well as for the purpose of maintaining hygienic practices. In the case of measuring on the top of packaging, the potential effects of various packaging materials on the accuracy of the sensor's measurements need to be taken into account. End user will be instructed on how to proceed with measurements through the PhasmaFOOD mobile app.

c. Constraints: The applied method concerns meat, fish and fresh produce (fruits and vegetables) items, typically stored at refrigeration temperatures (0-8 ^{o}C) and under various atmospheres (air, vacuum, modified atmospheres). Furthermore, the end-users will also use the PhasmaFOOD scanner at room temperature (20-25 ^{o}C), which will need to be accounted for in spectral database building and validation. Potential temperature abuse, however, should also be taken into account due to the high likelihood of its occurrence in the food supply chain (farm-to-fork approach), and particularly at the retail and consumer (i.e. domestic) levels.

3.3.2 Sensing requirements for each specific case

1. **Meat**
    a. Visible spectroscopic techniques: UV-Vis reflectance spectrum analysis has been evaluated as an effective means of monitoring ATP and/or microbial plate count of meat. For instance, the second derivative of raw reflectance spectra of pork samples (lean part of pork loin), measured from 240 to 540 nm, gave a high correlation with viable cell count, with the estimated determination coefficient being 0.83 at 318 nm [29]. Overall, the wavelength UV-Vis range of importance for meat spoilage is 240-1200 nm.
    b. Infrared spectroscopic techniques: As indicated by the findings of numerous studies assessing the association of infrared spectral data with meat spoilage detection and shelf-life estimation, the wavelength range of significance for this food commodity is 400-2500 nm. More specifically, with regard to FTIR, the areas that have been studied are located in the following two wavelength ranges: 1000-1200 nm and 1500 - 2500 nm. Of this range, the PhasmaFOOD

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

sensors cover the wide portions between 400 and 800 nm, as well as 1000 to 1900 nm. It will be the task of the PhasmaFOOD validation to perform a feasibility study for this sensor combination.

c. Imaging: With regard to multispectral and hyperspectral imaging, the wavenumber range of interest in terms of meat spoilage is 405-970 nm. With reference to hyperspectral imaging, the corresponding and most commonly studied range is 400–1000 nm, while the development of some emerging approaches has also been assessed in the range of 900-1700 nm. While hyperspectral imaging is not foreseen within the PhasmaFOOD approach, the validation tests will address the synergies arising from the combined use of a Vis and NIR spectrometer and a CMOS camera.

2. **Fish**

a. Visible spectroscopic techniques: Visible reflectance spectroscopic measurements have been evaluated with regard to their potential utilization in monitoring the volatile nitrogen compounds generated during fish spoilage [30]. In general, the wavenumber range that has been evaluated in association with fish spoilage is 250-600 nm (eye assessment).

b. Infrared spectroscopic techniques: The infrared wavelength range of significance for study of fish is the same as the one for meat i.e., 400-2500 nm. Similarly, the FTIR areas of interest are located in the following two wavenumber ranges: 1000-1200 nm and 1500 - 2500 nm. Of this range, the PhasmaFOOD sensors cover the wide portions between 400 and 800 nm, as well as 1000 to 1900 nm. It will be the task of the PhasmaFOOD validation to perform a feasibility study for this sensor combination.

c. Imaging: With regard to multispectral imaging, the wavenumber range of interest in terms of fish spoilage is 405-970 nm. With reference to hyperspectral imaging, the corresponding and most commonly studied range is 400-1758 nm. While hyperspectral imaging is not foreseen within the PhasmaFOOD approach, the validation tests will address the synergies arising from the combined use of a Vis and NIR spectrometer and a CMOS camera.

3. **Fruits and vegetables**

a. Visible spectroscopic techniques: The application of protocols utilizing enzymatic methods with UV-Vis spectroscopy have allowed for the assessment of metabolites produced by spoilage-causing bacteria in fresh produce items[31]. Overall, the UV-Vis wavelength range that has been studied for the purpose of assessing the spoilage of fruits and vegetables is 190–1100 nm.

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

b. Infrared spectroscopic techniques: Similarly to the values reported above, the infrared wavelength range of significance for produce items is 400-2500 nm, while the FTIR areas studied are located in the following two wavenumber ranges: 1000-1200 nm and 1500 - 2500 nm. Of this range, the PhasmaFOOD sensors cover the wide portions between 400 and 800 nm, as well as 1000 to 1900 nm. It will be the task of the PhasmaFOOD validation to perform a feasibility study for this sensor combination.
c. Imaging: With regard to multispectral imaging, the wavenumber range that has been studied for the purpose of fruits and vegetables spoilage is 405-970 nm. With reference to hyperspectral imaging, the corresponding and most commonly studied range is 400-1100 nm. While hyperspectral imaging is not foreseen within the PhasmaFOOD approach, the validation tests will address the synergies arising from the combined use of a Vis and NIR spectrometer and a CMOS camera.

Sensor combination and data fusion

The available data regarding data fusion specifically for the purpose of spoilage detection and/or shelf-life estimation for the aforementioned food commodities are scarce. To our knowledge, only a recent study reports on the intelligent evaluation of total volatile basic nitrogen content in chicken meat via the development of a multiple level data fusion model, combining an odour sensor and a highly advanced optical sensor [32]. Based on the available research data, both the infrared sensor and the imaging system are expected to contribute significantly to the credible detection of food spoilage and the accurate estimation of the shelf-life of all the described food commodities (subcases). The potential involvement in such predictions of the visible sensor presents high scientific interest, while data fusion between the most promising individual sensors is anticipated to result in lower detection limits and more accurate predictions.

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

4 Detection of food fraud: Adulteration of milk powder, meat, alcoholic beverages and edible oils

4.1 Need and Justification of the case

Food fraud is a collective term that covers 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 [33]. In Europe, consumers are protected by EC Regulation No. 178/2002, underpinning the concept of informed consumer choice in the purchase of food [34]. Unfortunately, the number of food adulteration and fraud cases being unravelled in several EU member states is rising. As food fraud, in most cases, does not comprise a safety risk for consumers and laboratory analysis methods for detecting fraud are laborious and expensive, the need for a smart non-invasive hand-held device is eminent for consumers. In this way, consumers can be assisted in making a choice on the spot before purchasing a product. Also, as the PhasmaFOOD scanner will be connected to the internet and has location services, local/national competent authorities may monitor scanning results to have a fast response to provide information to the public or constrain a product or commodity when a number of alarming outcomes are found.

4.2 Target food products for this case

The food fraud use case is comprised of four different subcases covering a variety of high-value and high-risk food (raw) materials common subject to adulteration or complete counterfeiting. Furthermore, the cases are chosen on the basis of their matrix properties (e.g. liquids, solids, wet solids) in order to investigate the versatility of the PhasmaFOOD scanner. Each subcase will deal with a number of adulteration issues commonly encountered for the specific food commodity, for example dilution, complete counterfeiting or addition of additives with food safety hazards.

1. **Milk powder.** Milk powder adulteration can be divided in two groups: (I) safety related (e.g. nitrogen replaces like melamine and its analogues) and (II) non-hazardous low-value fillers (e.g. acid whey, maltodextrin, buttermilk powders).
2. **Meat.** In the case of meat, the food fraud issues that will be addressed for raw minced meat and products thereof (e.g., meatballs, burgers) are : (I) adulteration of pork and beef

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

into raw minced meat samples and (II) adulteration of horse and beef into raw minced meat samples.

3. **Alcoholic beverages.** Fraud with alcoholic beverages is generally recognised as either fraud with low-alcohol fermented products (e.g. beer, wine) or high-alcohol distilled products (whiskey, vodka). This subcase will focus on distilled spirits, as they have the highest tax value and adulteration is a relatively simple exercise. The subcase is comprised of investigating authentic, adulterated and counterfeited spirits by either (I) dilution of the original product by a water-ethanol mixture, (II) dilution of the original product by a water-technical alcohol mixture, which might pose a safety risk (methanol) and (III) complete counterfeiting of the product by mixtures of water – ethanol or other technical alcohols and colorants / fragrances.

4. **Edible oils.** Oil fraud is generally divided in two separate branches: (I) olive oil fraud and (II) fraud with other edible oils. Both cases are investigated for the following parameters:

    a. Olive oil: Mislabelling, counterfeiting of adulteration of extra virgin olive oil by inferior quality olive oil (refined olive oil, added dyes, pomace oil, sunflower oil). Furthermore, dilution of high quality oils is often done by addition of hazelnut oil. Although this may be a high-value additive, it is extremely hard to detect due to its chemical similarity with olive oil [35].
    b. Other oils: Adulteration of sunflower oil by animal fat (e.g. refined chicken fat), vegetable fat (reused oils, inferior quality oils) and/or hazardous pollutions (mineral oils, diesel, motor oils).

4.3 Operational specifications

4.3.1 System level requirements

The target food products in the food fraud case require a versatile set of system level requirements, as the food matrices involved are either powders and wet-solid materials (diffuse reflectance measurements) or liquid materials (transflectance measurements with substances of varying refractive index). Furthermore it is not always desirable that the product packaging is opened in order to make a correct analysis of the product, especially with fresh products like meat and products prone to oxidation like oils. Still, spectroscopic (physical) limitations have to be considered in finding a compromise between sample presentation and violation of the packaging material. Food fraud may also comprise a food safety risk, for example the presence of methanol in alcoholic beverages and presence of melamine in milk powder. Therefore, the system level requirements for this case are complex and will have multiple constrains for application by consumers.

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

For example, fresh meat and meat products sold in supermarkets are often covered by transparent thin membrane on top of a plastic or a styrofoam container. Therefore, the scanner should enable the end-user to perform a scan on top of the packaging material. The influence of the packaging material on the measurement results will be relatively simple to standardise as the amount of different meterials used by manufacturers for covering fresh produce will be limited. Still, in order to standardise the measurement distance to the meat will be challenging. The package material for alcoholic beverages and oils are not as uniform as for meat, as bottles can be made from glass and plastics and appear in different colours and shapes. There are simply too many variations in packaging materials to account for in a spectra database. Furthermore, scanning directly on a bottle containing a transparent liquid will not result in an optimal measurement, as little light will be reflected to the detectors of the optical sensing units. Operating in transflectance modus is, therefore, the more sensible choice.

The constraints of each sub-case mainly concern the limit of detection of adulterants. Although it is difficult to predict what these limits will be for the PhasmaFOOD scanner, either scientific reports concerning a spectroscopic technique or an EC regulation may provide of the product concerned may be consulted. Therefore, the system level requirements for each subcase are the following:

1. **Skimmed milk powder.**

    a. Functions: Feedback on (I) dilution of the original product by non-hazardous low-value fillers and (II) food safety related issues. The feedback, measurement results and their interpretation will be provided through the PhasmaFOOD mobile apps. Also, the mobile app will provide end user with means to calibrate the measurement process.
    b. Characteristics: The application should be operated in diffuse reflectance modus. Usually milk powder packages are not transparent and therefore a sample should be taken by the end-user. Measurements using the PhasmaFOOD sensor on milk powder may be performed by using a spacer or transferring milk powder to a sample holder (suggestions see Fig. 1). Both methods provide sufficient possibility for standardisation and protection form environmental influences. Due to the powdered nature of the samples, a protective, cleanable and suitably transparent window is recommended to be placed between the sensors and the samples, in order to avoid contamination between measurements.

    c. Constraints: This method concerns commercial skimmed milk powder at room temperature (20-25 ^{o}C). Both types of fraud will be investigated at the appropriate levels. For melamine and its analogues a maximum limit is established at 2.5 mg/kg for food stuffs and 1 mg/kg for infant formulas (EC reg. 594/2012)[36]. For low-value fillers, a minimum range of 2 – 5% (depending on the low-value filler) is estimated to be feasible [37].

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

2. **Meat.**

   a. Functions: Feedback on meat species for aforementioned either case being (I) adulteration of pork and beef into raw minced meat samples, and (II) adulteration of horse and beef into raw minced meat samples. The feedback, measurement results and their interpretation will be provided through the PhasmaFOOD mobile apps. Also, the mobile app will provide end user with means to calibrate the measurement process.

   b. Characteristics: The application should be operated in diffuse reflectance modus. As already indicated above, the application can be developed by measuring on the transparent packaging of a super market product or directly on the meat in case of products from the market or butcher. For packed products, the end-user may operate the PhasmaFOOD scanner by directly applying the scanner on the top-membrane of the package, minimising the distance between the scanner and the meat. For measuring directly on meat, a spacer should be used to standardise the distance between meat and sensors, minimise environmental (light) influences and for hygienic purposes.

   c. Constraints: This method concerns beef and pork or horse minced meat at refrigerator temperatures (4-7 ^{o}C). Adulterations should be detected at levels of 10% proportion in the samples, e.g. 10% proportion step of pork mixing into beef samples and 10% proportion step of horse into beef samples.

3. **Alcoholic beverages**.

   a. Functions: Feedback on (I) dilution of the original product, by indication of alcohol by volume percentage, (II) presence of technical alcohols and (III) indication of the authenticity of the alcoholic beverage. The feedback, measurement results and their interpretation will be provided through the PhasmaFood mobile apps. Also, the mobile app will provide end user with means to calibrate the measurement process (1. select this particular measurement group; 2. specify food type from provided options; 3. specify scanning conditions).

   b. Characteristics: The application should be operated in transflectance mode. This can comprise of either a liquid-proof transflectance adapter to apply the PhasmaFOOD scanner in the liquid itself, an adapter to apply a standardized transparent pipette containing the alcoholic beverage at the PhasmaFOOD sensors or a separate add-on with a mirror surface to manually reflect the PhasmaFOOD diode light to the PhasmaFOOD sensors (See Fig. 1). Liquids concerned are transparent distilled spirits between 20-50% abv at room temperature.

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

c. Constraints: Liquids concerned are transparent distilled spirits between 20-50% abv (alcohol by volume) at room temperature (20-25 °C). The detection limit for deviations in ethanol concentration should 2% abv (e.g. label claim is 40% abv, scanner detection limits between 38-42% abv). Detection of technical alcohols, especially methanol, should be as low as <1% abv levels according to EC reg. 110/2008 [38]. This will most probable be very challenging for the PhasmaFOOD scanner, as detection limits for methanol using visible or near-infrared sensing are typically around 1% abv. However, due to food safety issues with methanol, a disclaimer should be displayed on the potential risks of false negatives.

4. Edible oils.

a. Functions: Feedback on (I) dilution or counterfeiting of extra vierge olive oil with inferior products and (II) adulteration of sunflower oil by either animal fats or hazardous pollutions. The feedback, measurement results and their interpretation will be provided through the PhasmaFOOD mobile apps. Also, the mobile app will provide end user with means to calibrate the measurement process.

b. Characteristics: The application should be operated in transflectance mode. This can comprise of either a liquid-proof transflectance adapter to apply the PhasmaFOOD scanner in the liquid itself, an adapter to apply a standardized transparent pipette containing the alcoholic beverage at the PhasmaFOOD sensors or a separate add-on with a mirror surface to manually reflect the PhasmaFOOD diode light to the PhasmaFOOD sensors (See Fig. 1).

c. Constraints: Oils concerned are olive oils and sunflower oils which are transparent and liquid at room temperature (e.g 20-25 °C). Dilution of extra vierge olive oils and sunflower oil by non-hazardous low-value products should be detectable from 10%. Detection of hazardous pollutions which were (mineral oils, diesel, motor oils) should be as low as in the range of 1 - 1000 mg/kg due to their toxicity [39]. This will be very challenging for the PhasmaFOOD scanner, as detection limits for mineral oils, diesel and motor oils using visible or near-infrared sensor technology may produce too many false negatives. Later, when the PhasmaFOOD scanner is developed beyond TRL4 and brought to the end consumer, it might be necessary to display a legal disclaimer on the potential risks of false negatives. It will be one of the tasks of the PhasmaFOOD validation to quantify this risk and of the PhasmaFOOD business plan to envision the future presentation of the device to the market.

The sensor validation will provide a feasibility test for the abovementioned use cases.

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

4.3.2 Sensing requirements for each specific case

Scientific reports are available for each sub-case concerned and a single spectroscopic sensing technique. Therefore it is possible to estimate which of the sensing components for each of the sub-case problems may be effective and which sensors may work in synergy in order to get better results. Therefore in the text below, scientific references refer to published results and do not reflect the performance of the individual sensing components of the PhasmaFOOD sensor.

1. **Milk powder**
   a. Visible spectroscopic techniques: There are no relevant reports on the usage of (UV-) visible sensors for milk powder authentication
   b. Infrared spectroscopic techniques: NIR is used for detection of low-value additives (acid whey, starch, maltodextrin) as well as hazardous nitrogen enhancers like melamine. For melamine LODs by NIR were reported to be below 1 ppm [37, 40]. The NIR spectrometer used here covers the range from 1000 to 1900 nm.
   c. Imaging: Hyperspectral imaging is used extensively in detection of whey powder, starch, urea and melamine in milk powders. Detection limits of low-value additives have been reported from 2%, whilst for melamine the analytical LOD was found to be below 1 mg/kg. Specific wavelengths for melamine were reported to be 1447 and 1466 nm in hyperspectral imaging [41], [42]. While HIS is not foreseen within the PhasmaFOOD approach, the validation tests will address the synergies arising from the combined use of a CMOS camera, visible spectrometer, and a NIR spectrometer.

Sensor combination and data fusion: It is expected that both the infrared sensor as well as the imaging sensor will be able to identify low-value additives and melamine in an untargeted approach. Although lowering of the limit of detection may not be possible, data fusion will probably result in a more robust model for melamine and low-value additive detection. There are to the best of our knowledge no reports on data fusion of spectroscopic techniques and milk powder available.

2. **Meat**
   a. Visible spectroscopic techniques: UV-Vis sensors have been used in one research for adulteration of beef minced meat with turkey meat [43]. The wavelengths reported range 200–780 nm. As the visible spectrometer used here strictly requires a blocking filter for the excitation light (in order to fulfil the fluorescence measurements in use case 1), only the wavelength range > 400 nm is available here.
   b. Infrared spectroscopic techniques: Multiple reports exist on species detection (e.g. horse meat, turkey meat) in (minced) beef in raw condition as well as processed condition. Detection limits range from 10 – 20% adulteration [44, 45, 43]. Although challenging, untargeted detection of meat adulteration would be of great advantage for the end-user. The NIR spectrometer used here covers the range from 1000 to 1900 nm.

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

c. Imaging: Multispectral imaging was used successfully in identification of adulterants in beef meat (pork and horse meat) [46, 47]. The spectral resolution of the sensors used in the PhasmaFOOD device is far superior to the above. While Multispectral imaging is not foreseen within the PhasmaFOOD approach, the validation tests will address the synergies arising from the combined use of a CMOS camera, a visible spectrometer, and a NIR spectrometer.

Sensor combination and data fusion: It is expected that the imaging system will contribute most to the detection of meat species. The infrared sensor can contribute to species identification and give an additional indication of the moisture and protein content. Therefore, data fusion between the imaging system and infrared sensor will probably result in more robust models and more accurate predictions. There few relevant reports on data fusion specifically for meat, using UV-Vis, NIR and MIR [48] [43].

3. **Alcoholic beverages**
   a. Visible spectroscopic techniques: UV-Vis analysis is used in the 200-450 nm range for identification of complete counterfeited spirits of for example whiskeys and other high value distilled spirits [49, 50]. Furthermore, dilutions or mixtures of different spirits may be identified using UV-Vis. As the visible spectrometer used here strictly requires a blocking filter for the excitation light (in order to fulfil the fluorescence measurements in use case 1), only the wavelength range > 400 nm is available here.
   b. Infrared spectroscopic techniques: NIR is used for detection of diluted spirits by water/ethanol mixtures and for the detection of methanol or other technical alcohols in the 1100 – 2100 nm range [51]. Also FTIR is used for such purposes [52]. The NIR spectrometer used here covers the range from 1000 to 1900 nm.
   c. Imaging: There are no relevant reports on multispectral and hyperspectral imaging of spirit drinks.

Sensor combination and data fusion: It is expected that both the visible and infrared sensor will contribute to identification of counterfeit spirits and the identification of diluted spirits with either water/ethanol or a technical alcohol like methanol. Although little is reported on data fusion, it is reported that fusion of spectral data did improve statistical results on spirit quality controls [53]. Furthermore, multiple data fusion methods with spectroscopics and other types of sensors (gas and liquid sensors) are reported [48].

4. **Edible oils**
   a. Visible spectroscopic techniques: Although no reports exist on the identification of olive oil or sunflower oil in food fraud cases, visible spectroscopy is used in some cases to identify mixtures and quality defects of extra virgin olive oil. The

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

wavelengths reported range from 300 to as far as 1000 nm [54, 55]. As the visible spectrometer used here strictly requires a blocking filter for the excitation light (in order to fulfill the fluorescence measurements in use case 1), only the wavelength range > 400 nm is available here.

b. Infrared spectroscopic techniques: Both NIR and FTIR are utilized frequently for addressing addition of low-value oils in either extra virgin olive oil or other edible oils like sunflower oil. In literature reports, an enormous variation of wavelengths of the infrared spectrum is utilized, depending on the specific goal of the chemometric application [44 56]. The NIR spectrometer used here covers the range from 1000 to 1900 nm.

c. Imaging: There are no relevant reports on multispectral imaging of olive oils or other edible oils. Still hyperspectral imaging systems are used to estimate quality parameters of olive oils samples like acidity, moisture and peroxide values [57].

Sensor combination and data fusion: It is expected that the infrared sensor will be most compatible for identification of diluted extra virgin olive oils and other application like addition of animal fats or pollution with mineral oils of sunflower oil. The visible sensor may provide supportive data in some cases (e.g. color assessment). It is not known what the imaging system may contribute to this application. There have been multiple reports on data fusion for olive oils (for example UV-Vis, NIR and MIR fusion) [48].

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

5 Smart algorithms for analysis of spectral data and data fusion

The operation performance of the PhasmaFOOD scanner highly relies on the application of smart chemometric algorithms for classification or quantification purposes. This multivariate statistics approach accounts for all use cases described in sections 2 – 4. Furthermore, data fusion strategies are discussed in order to improve correct classification of samples and to improve robustness of the smart algorithms and spectral database combination. For both strategies, general guidelines are postulated, as extrapolating detailed multivariate statistic approaches and data fusion strategies are precarious and may limit the degrees of freedom in finding an optimal solution for the PhasmaFOOD scanner.

5.1 Smart algorithms for analysis of spectral data

The PhasmaFOOD approach anticipates and plans smart chemometric algorithms that will allow for the efficient and credible analysis of the acquired data. For the three use cases a similar chemometric approach will be used, meaning that the algorithms proposed (Table 3) and data handling approach are universally applicable for the PhasmaFOOD project. The algorithms will be properly devised and implemented based on existing methods and practices. Partner AUA has recently published a review article that will be used as the baseline for these algorithms [58]. The article compiles available information pertaining to:

1. Data mining and data analysis derived from non-destructive, non-invasive instruments reported in the literature in recent years, regardless of sensor, food type and application. A summary of the (chemometric) methods to be considered is presented in Table 3.

2. The aspects related to the implementation of these techniques to the food industry and other stakeholders, e.g., food managers who are not familiar with these approaches.

Table 3. Overview of smart chemometric algorithms [58].

Method category	Method to be considered

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

Unsupervised methods	Among the unsupervised methods, Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA) have been extensively used in food.
Supervised methods	Depending on the nature of the problem there are discriminant and regression algorithms for qualitative and quantitative approaches, respectively. Discriminant Function Analysis (DFA) or Canonical Variate Analysis (CVA) is a cluster analysis based method that is used to classify individuals into two or more predetermined groups. Linear regression methods are extensively used in food applications, as they are relatively easy to apply. These methods include Multiple Linear Regression (MLR), Principal Component Regression (PCR), Partial Least Squares Regression (PLSR).
Machine learning/evolutionary computation	Recent methods based on more complicated algorithms that involve machine learning and computational intelligence have been introduced. These terms apply to heterogeneous algorithms, such as neural networks, fuzzy systems, rough set, evolutionary computation, swarm intelligence, probabilistic reasoning, multiagent systems, etc. Very common computational intelligence methodologies found in research articles and reviews are Artificial Neural Networks (ANNs), Support Vector Machines (SVMs) and evolutionary-based algorithms, including Genetic Algorithms (GAs) and Genetic Programming (GP). The latter algorithms are used for optimization purposes.

The data analysis for all three cases will be focused in particular on the following four main parts:

1. Data normalization and preprocessing: This step is a crucial part in order to increase as much as possible the signal-noise ratio. To reach this goal different preprocessing will be considered from the averaging to different filters.

2. Classification or regression models: in this phase several classification approaches will be tested based on internal and external databases to select that shows the best performances (Table 3).

3. Feature selection: In this phase, features will be selected, either coming from the Vis or NIR microspectrometer or in combinations thereof (see section 5.2).

4. Model optimization: This last phase will be devoted to the optimization of the measurement protocol. The proposed data processing algorithms are also optimized to reduce requirements in terms of calculus, the amount of data stored and minimize data transfer back and forward to the cloud.

As for the detection of AFs in use case 1, already a more specific approach towards the data analysis can be postulated, as the chemical structure and optical properties of the specific toxins involved are known and can therefore be correlated to reported scientific literature. Most probably, the data analysis will therefore include the following steps:

1. A starting step will be represented by the presence of fluorescence emission in the spectral range of 410-550 nm by excitation at 365 nm excitation (see D1.2) [59].

2. The next step will be the assessment of spectral features suggested in literature with comparison with a reference database [60].

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

3. The possibility for rough classification of the contamination level, will include combined Vis and NIR reflectance spectra analysis using discriminant analysis and partial least squares regression, or their modifications [61]. A reference database also in this case will be built and used as calibration.

4. A possible fourth level will be represented by image analysis using the CMOS camera. Analysis of reference samples will indicate if the data resulting from the camera can be used to contribute to identification of AFs in the proposed sub-cases [62].

5.2 Data fusion

In order to optimally use the data-streams from the PhasmaFOOD individual sensors, smart and flexible data fusion strategies can be applied. Initially, information from the Vis and NIR microspectrometers will be used as sources for data fusion where this is necessary. The CMOS camera data will be initially used for assessing measurement quality (e.g. focusing distance to the sample). However, the proposed strategy for data fusion can be expanded by an unlimited amount of instrumental techniques. The general strategies for data fusion of sensors considering the three use-cases can be carried out at three (arbitrary) levels (Fig 2) [48].

1. **Low level fusion** comprises of straight-forward concatenation of spectral data for each sample versus the variables measured by the spectral sensors. After pre-processing, base-line correction or in somehow standardization of the data, a smart chemometric algorithm can be applied as proposed in section 5.1, which results in a decision model at low level. Low level fusion is the most simple approach to data fusion, but has the disadvantage that high amounts of data need to be processed and transferred.

2. **Mid level fusion** is performed by extracting relevant features from each data matrix resulting from each sensor. These features may comprise of scores from principal component analysis (unsupervised) or partial least squares discriminant analysis (supervised). Although the feature selection will result in significant reduction of calculus, data transfer and noise reduction, the level of complexity in finding the optimal features for fusion for each sensor is challenging. E.g. 'overfitting' by overrepresentation of certain features may lead to erroneous decisions by the model.

3. **High level fusion** uses separate classification or regression models calculated from each spectroscopic sensor. The results of the individual models are then combined in order to form a decision on the presented sample. By using high level fusion, specific particularities of each spectral sensor can be used for enforcing a correct decision. Only a few values

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

from the original data are used and this is favourable for a cloud-based system. A disadvantage is that very accurate data pre-processing and correlation of the smart algorithm with the reference values need to be performed. If this is not preformed correctly, crucial features of the sensor data may be lost resulting in an overfitted or underfitted model.

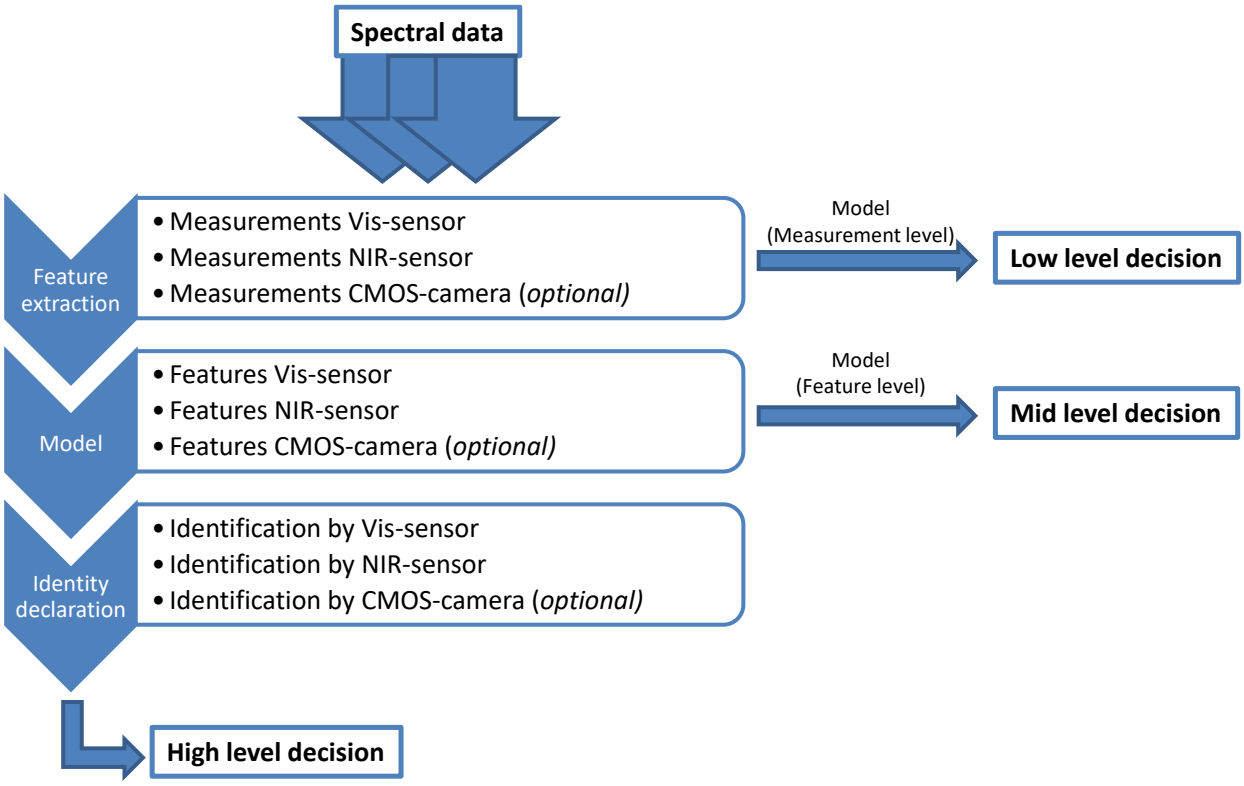

Figure 2. Data fusion strategies at low, mid and high levels. Adapted from Borras *et al* (2015) [48].

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

6 Validation strategy

Next to the correct application of smart chemometric algorithms, validation is required in order to assess the performance of the PhasmaFOOD scanner. This paragraph specifically focuses on the validation strategy to specify standard procedures and samples to ensure comparability of the results amongst all test users and test environments. Also, the validation strategy incorporates the evaluation of the spectral database upon presentation of 'unknown' samples (e.g. samples of a use case which have not been incorporated in the spectral database). The validation strategy is divided in a laboratory validation plan, comprising of different reference sample sets divided over different laboratories, and a validation strategy to obtain a quantitative estimate of the feasibility of the use case. The ultimate goal is to reach a TRL 4 for each use case, representing a broad validation in a laboratory environment.

6.1 Laboratory validation plan

Validation of the spectral databases for each use case is divided in three phases which will be executed in WP3 and WP6 of the PhasmaFOOD project: (I) Lab scale validation – within laboratory validation using laboratory personnel, (II) Model deployment validation – within laboratory validation by a different laboratory staff member and (III) Real deployment – interlaboratory validation by a different laboratory and a different staff member. For all three use cases a similar approach towards reaching a TRL 4 validated model and spectroscopic database can be formulated (Fig. 3). The three laboratory validation steps are combined with the step-wise establishment of the spectra databases which will be used for the deployment of the PhasmaFOOD scanner at TRL 4. After the three steps of validation have been completed, the spectra database containing the smart correlated data should contain all the relevant variation in order to be tested in a relevant environment (e.g., super markets).

To include enough relevant variation in the spectral databases, the collection of reference samples for each specific use case (and subcase) will be different and have to be assessed from case to case. In Table 4 a prospectus is provided for each case in order to cover the specifications set in the use case descriptions. For each individual subcase counts that the sample origin should be versatile, e.g. different producers, different seasonal / year production and different geographical provenance should be present in the sample set. For all cases this can be achieved due to the connections of the different partners in retail business, control authorities and local market and the project will be actively acquiring spectral data over the duration of the project.

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

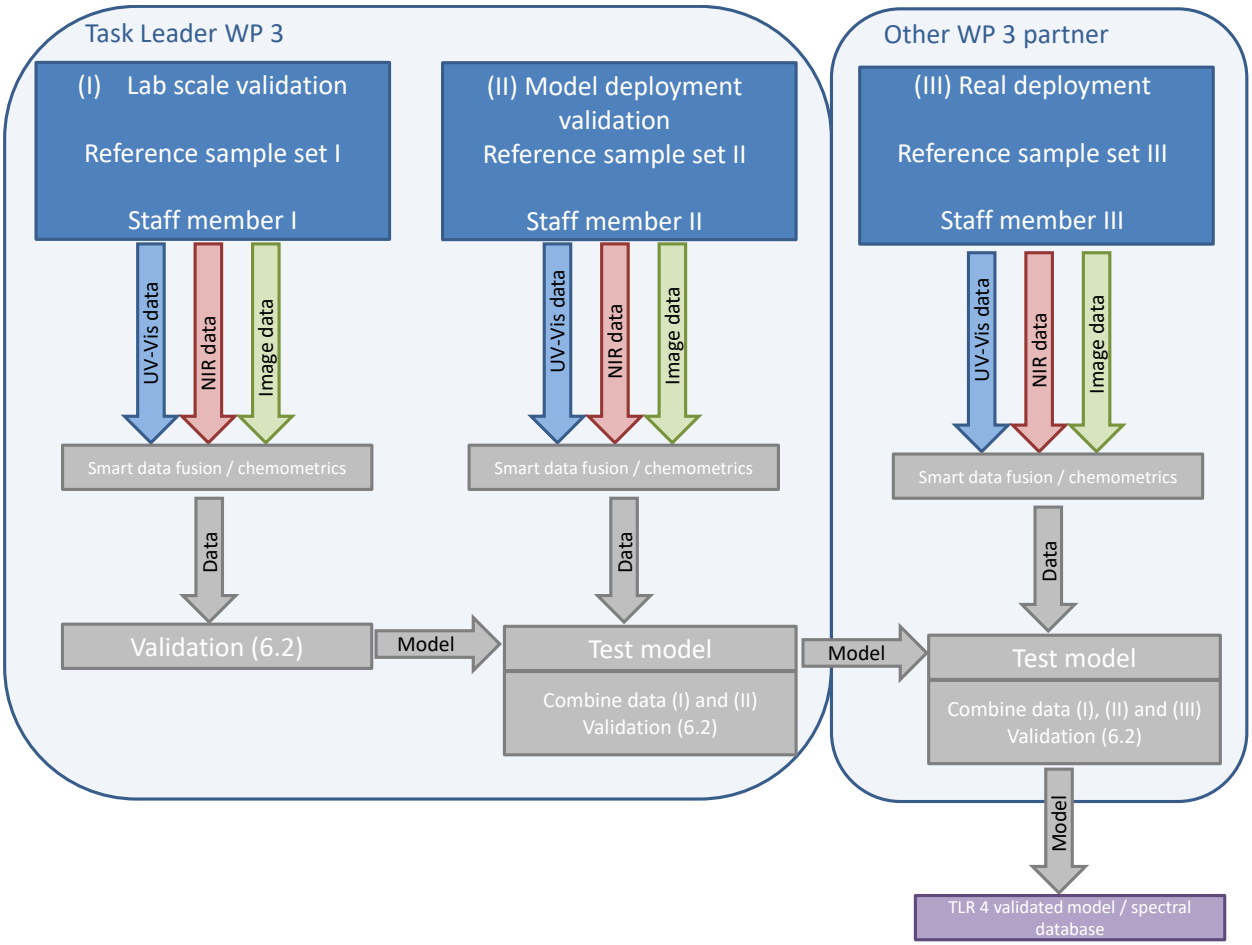

Figure 3. General approach to obtain a TRL 4 validated model and spectra database for the three use cases.

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

Table 4. Sampling strategy and estimation of sample amount for spectral database building and validation, for each validation phase as depicted in Fig. 2.

Use case	Sub-case	Sub-problem	Proposed (Max.) Limits	Range compounds, organisms or adulterantsof interest	No. authentic samples	Sample source	No. adulterated samples	No. adulterants applied	Sample source
Mycotoxin	Maize flour	AF B1	5 µg/kg	0 - 50 µg/kg	25	Retail / controls	25	NA	Ref. materials / controls
		Total Afs	10 µg/kg	0 - 50 µg/kg	25	Retail / controls	25	NA	Ref. materials / controls
		DON	1750 µg/kg	0 - 3000 µg/kg	25	Retail / controls	25	NA	Ref. materials / controls
	Skimmed milk powder	Total Afs M	0.05 µg/kg	0 - 5 µg/kg	25	Retail / controls	25	NA	Ref. materials / controls
	Paprika powder	AF B1	5 µg/kg	0 - 50 µg/kg	25	Retail / controls	25	NA	Ref. materials / controls
		Total Afs	10 µg/kg	0 - 50 µg/kg	25	Retail / controls	25	NA	Ref. materials / controls
	Tree nuts (homogenized or whole)	AF B1	2-20 µg/kg	0 - 50 µg/kg	25	Retail / controls	25	NA	Ref. materials / controls
		Total Afs	2-15 µg/kg	0 - 50 µg/kg	25	Retail / controls	25	NA	Ref. materials / controls
Food Spoilage	Meat	TVC	10^8 cfu/g	10^3-10^8 cfu/g	25	Retail	25	NA	In-house aging
	Fish	TVC	10^8cfu/g	10^3-10^8 cfu/g	25	Wholesale (marine cultured)	25	NA	In-house aging
	Fruits & Vegetables	TVC	10^8 cfu/g	10^3-10^8 cfu/g	25	Retail	25	NA	In-house aging

NA: not applicable

This document is part of a project that has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 732541. It is the property of the PhasmaFOOD consortium and shall not be distributed or reproduced without the formal approval of the PhasmaFOOD Management Committee.

Table 4. (Continued)

Use-Case	Sub-case	Sub-problem	Max. Limits	Range compounds of interest	No. authentic samples	Sample source	No. adulterated samples	No. adulterants applied	Sample source
Food Fraud	Alcoholic beverages	Dilution	2% abv deviation	0 - 50% abv dilution	25	Retail / producers	25	1	In-house preparation
		Tech. alc	1% abv	0 - 25% abv dilution	25	Retail / producers	25	1	In-house preparation
		Counterfeit	NA	Authentic / counterfeit	25	Retail / producers	25	NA	Producers / controls
	Edible oils	Olive oil dilution	10% deviation	0 - 50% dilution	25	Retail / producers	25	5	In-house preparation
		Olive oil counterfeit	NA	Authentic / counterfeit	25	Retail / producers	25	NA	In-house preparation / retailers / controls
		Sunflower oil dilution	10% deviation	0 - 50% dilution	25	Retail / producers	25	5	In-house preparation
		Sunflow oil pollution	<1 %	0 - 10 % pollution	25	Retail / producers	25	5	In-house preparation / controls
	Skimmed milk powder	Milk powder dilution	2.5% deviation	0 - 25% dilution	25	Retail / producers	25	5	In-house preparation / controls
		Nitrogen enhancers	1 μg/kg	0 - 500 μg/kg	25	Retail / producers	25	1	In-house preparation / controls
	Meat	Meat species	5% deviation	0 - 50% deviation	25	Retail / producers	25	3	In-house preparation / controls
		Low-value additives	10% deviation	0 - 25% deviation	25	Retail / producers	25	5	In-house preparation / controls

NA: Not applicable

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

6.2 Limit of detection (LOD) predictions and validation

6.2.1 LOD prediction

LOD predictions are especially important in the use case concerning food fraud. In most of the food fraud cases, the amount of authentic samples versus the amount of adulterants is enormous. It is therefore not possible to produce all authentic/adulterated sample mixtures on a laboratory scale to populate the spectra database for the specific subcase. Therefore, for initial model development, spectral data can be generated by intrapolation of existing results. E.g. a mixture of 7.5% olive pomace oil in extra virgin olive oil can be calculated by proportionally multiplying the UV-Vis or NIR spectrum of 50% olive pomace oil and 100% extra virgin olive oil. Herein, linearity of the data is assumed. After LOD determination by chemometrics of this virtual dataset, the virtual LOD can be verified by actually checking mixtures of olive pomace oil and extra virgin oil close to the virtual LOD.

6.2.2 Validation

The validation of the multivariate classification methods based on the (fused) spectroscopic fingerprint database is of vital importance to assess the performance of the application. The performance of the application is comprised of the rates of true positives, true negatives, false positives and false negatives. Furthermore, parameters of sensitivity, selectivity, efficiency and reliability supply additional information on the potential behaviour of the PhasmaFOOD scanner at the TLR 4 level. The performance parameters can also be adjusted to fit the need of the use-case. For example, in the case of AFs, a high amount of false negatives is not desirable, whilst a higher amount of false positives will only lead to increased laboratory control of the food product.

Validation of multivariate models and spectral databases is extensively described by Alewijn *et al.* for fingerprinting methods and food and feed products and comprises of a number of increasingly stringent performance quantifications [63]:

- Sample re-substitution: Prediction of a sample used to create the chemometric model;
- Permutation test: Prevention of overfitting of the chemometric model by random coincidence;
- Repeatability test: Testing the analytical error of the spectroscopic method and its influence on the models performance;

This document is part of a project that has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 732541. It is the property of the PhasmaFOOD consortium and shall not be distributed or reproduced without the formal approval of the PhasmaFOOD Management Committee.

- Cross validation probability distribution: Testing the predictive capability of the method when confronted with samples which are not contained in the spectral database, but have very similar sample natures;
- External validation probability distribution: Challenging the model with new samples which are as different as possible, but within the intended scope of the application;

Finally, an expanded distribution test can be performed. This involves assessment of the performance of the model when the training and external validation sample sets are combined, e.g. this step results in the final TRL 4 database as shown in Fig. 2.

The PhasmaFOOD cloud platform will host all collected and imported data sets which will be used for model training, validation and calibration. Web dashboard will be developed within WP4. This cloud based dashboard will enable use-case validators, experimenters and lab personnel to manage collected data, format and organize data sets, perform necessary calibrations and visualize statistics and measurement results. This will ensure that use-case experiments are conducted in controlled and managed environments not just in laboratory, but also with respect to handling and formatting collected data.

7 References

1. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance). *http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32006R1881. Last visited: March 30th 2017.*
2. Ricci, F.; Volpe, G.; Micheli, L.; Palleschi, G., A review on novel developments and applications of immunosensors in food analysis. *Anal. Chim. Acta* **2007**, *605*, 111-129.
3. Miedaner, T.; Han, S.; Kessel, B.; Ouzunova, M.; Schrag, T.; Utz, F. H.; Melchinger, A. E., Prediction of deoxynivalenol and zearalenone concentrations in Fusarium graminearum inoculated backcross populations of maize by symptom rating and near-infrared spectroscopy. *Plant Breeding* **2015**, *134*, 529-534.
4. Miedaner, T.; Han, S.; Kessel, B.; Ouzunova, M.; Schrag, T.; Utz, F. H.; Melchinger, A. E., Prediction of deoxynivalenol and zearalenone concentrations in Fusarium graminearum inoculated backcross populations of maize by symptom rating and near-infrared spectroscopy. *Plant Breed* **2015**, *134*, 529-534.
5. McMullin, D.; Mizaikoff, B.; Krska, R., Advancements in IR spectroscopic approaches for the determination of fungal derived contaminations in food crops. *Analytical and Bioanalytical Chemistry* **2015**, *407*, 653-660.
6. Kos, G.; Sieger, M.; McMullin, D.; Zahradnik, C.; Sulyok, M.; Öner, T.; Mizaikoff, B.; Krska, R., A novel chemometric classification for FTIR spectra of mycotoxin-contaminated maize and peanuts at regulatory limits. *Food Additives & Contaminants: Part A* **2016**, *33*, 1596-1607.
7. Wang, W.; Heitschmidt, G. W.; Ni, X.; Windham, W. R.; Hawkins, S.; Chu, X., Identification of aflatoxin B1 on maize kernel surfaces using hyperspectral imaging. *Food Control* **2014**, *42*, 78-86.
8. Dale, L. M.; Thewis, A.; Boudry, C.; Rotar, I.; Dardenne, P.; Baeten, V.; Pierna, J. A. F., Hyperspectral Imaging Applications in Agriculture and Agro-Food Product Quality and Safety Control: A Review. *Applied Spectroscopy Reviews* **2013**, *48*, 142-159.
9. Wang, W.; Ni, X.; Lawrence, K. C.; Yoon, S.-C.; Heitschmidt, G. W.; Feldner, P., Feasibility of detecting Aflatoxin B1 in single maize kernels using hyperspectral imaging. *J. Food Eng.* **2015**, *166*, 182-192.
10. CODEX GENERAL STANDARD FOR CONTAMINANTS AND TOXINS IN FOOD AND FEED (CODEX STAN 193-1995). *http://www.fao.org/fileadmin/user_upload/livestockgov/documents/1_CXS_193e.pdf. Last visited: March 30th 2017.*
11. U.S. Food and Drug Administration. *https://www.fda.gov/. Last visited: March 30th 2017.*
12. Marsh, P. B.; Simpson, M. E., Detection of Aspergillus Flavus and Aflatoxins in Cotton and Corn by Ultraviolet Fluorescence1. *Journal of Environmental Quality* **1984**, *13*, 8-17.
13. Ono, E. Y. S.; Silva, M. d.; Ribeiro, R. M. R.; Ono, M. A.; Hayashi, L.; Garcia, G. T.; Hirooka, E. Y., Comparison of thin-layer chromatography, spectrofluorimetry and bright greenish-yellow fluorescence test for aflatoxin detection in corn. *Brazilian Archives of Biology and Technology* **2010**, *53*, 687-692.
14. Dickens, J. W.; Welty, R. E., Fluorescence in pistachio nuts contaminated with aflatoxin. *Journal of the American Oil Chemists Society* **1975**, *52*, 448-450.
15. Saccon, F. A. M.; Parcey, D.; Paliwal, J.; Sherif, S. S., Assessment of Fusarium and Deoxynivalenol Using Optical Methods. *Food Bioprocess Technol.* **2017**, *10*, 34-50.
16. Smeesters, L.; Meulebroeck, W.; Raeymaekers, S.; Thienpont, H., Non-destructive detection of mycotoxins in maize kernels using diffuse reflectance spectroscopy. *Food Control* **2016**, *70*, 48-57.
17. Hamad, S. H., Factors Affecting the Growth of Microorganisms in Food. In *Progress in Food Preservation*, Wiley-Blackwell: 2012; pp 405-427.
18. Koutsoumanis, K.; Nychas, G. J., Application of a systematic experimental procedure to develop a microbial model for rapid fish shelf life predictions. *International journal of food microbiology* **2000**, *60*, 171-184.
19. Ellis, D. I.; Goodacre, R., Rapid and quantitative detection of the microbial spoilage of muscle foods: current status and future trends. *Trends Food Sci. Technol.* **2001**, *12*, 414-424.
20. Karoui, R.; De Baerdemaeker, J., A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. *Food Chem.* **2007**, *102*, 621-640.
21. Dufour, É., Recent advances in the analysis of dairy product quality using methods based on the interactions of light with matter. *International Journal of Dairy Technology* **2011**, *64*, 153-165.
22. Papadopoulou, O. S.; Panagou, E. Z.; Mohareb, F. R.; Nychas, G.-J. E., Sensory and microbiological quality assessment of beef fillets using a portable electronic nose in tandem with support vector machine analysis. *Food Res. Int.* **2013**, *50*, 241-249.
23. Porep, J. U.; Kammerer, D. R.; Carle, R., On-line application of near infrared (NIR) spectroscopy in food production. *Trends Food Sci. Technol.* **2015**, *46*, 211-230.
24. Xiong, Z.; Xie, A.; Sun, D. W.; Zeng, X. A.; Liu, D., Applications of hyperspectral imaging in chicken meat safety and quality detection and evaluation: a review. *Critical reviews in food science and nutrition* **2015**, *55*, 1287-1301.
25. Koutsoumanis, K.; Giannakourou, M. C.; Taoukis, P. S.; Nychas, G. J. E., Application of shelf life decision system (SLDS) to marine cultured fish quality. *International journal of food microbiology* **2002**, *73*, 375-382.
26. Argyri, A. A.; Jarvis, R. M.; Wedge, D.; Xu, Y.; Panagou, E. Z.; Goodacre, R.; Nychas, G.-J. E., A comparison of Raman and FT-IR spectroscopy for the prediction of meat spoilage. *Food Control* **2013**, *29*, 461-470.
27. Finnegan, E.; O'Beirne, D., Characterising and tracking deterioration patterns of fresh-cut fruit using principal component analysis - Part I. *Postharvest Biology and Technology* **2015**, *100*, 73-80.
28. Paillart, M. J. M.; van der Vossen, J.; Levin, E.; Lommen, E.; Otma, E. C.; Snels, J.; Woltering, E. J., Bacterial population dynamics and sensorial quality loss in modified atmosphere packed fresh-cut iceberg lettuce. *Postharvest Biology and Technology* **2017**, *124*, 91-99.

29. Oshita, S.; Al-Haq, M. I.; Kawagishi, S.; Makino, Y.; Kawagoe, Y.; Ye, X.; Shinozaki, S.; Hiruma, N., Monitoring of ATP and viable cells on meat surface by UV–Vis reflectance spectrum analysis. *J. Food Eng.* **2011**, *107*, 262-267.
30. Byrne, L.; Lau, K. T.; Diamond, D., Monitoring of headspace total volatile basic nitrogen from selected fish species using reflectance spectroscopic measurements of pH sensitive films. *The Analyst* **2002**, *127*, 1338-1341.
31. Farkas, V.; Dalmadi, I., Detection of a metabolite produced by acidophilic spoilage-containing bacteria using different analytical and sensory methods. *ACTA ALIMENTARIA- ACADEMIAE SCIENTIARUM HUNGARICAE* **2013**, *42*, 19-26.
32. Khulal, U.; Zhao, J.; Hu, W.; Chen, Q., Intelligent evaluation of total volatile basic nitrogen (TVB-N) content in chicken meat by an improved multiple level data fusion model. *Sensors and Actuators B: Chemical* **2017**, *238*, 337-345.
33. USP Pharmacopeial Convention Food Fraud Database. https://www.foodfraud.org/. *Last visited: March 30th 2017.*
34. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. http://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX:32002R0178. *Last visited: March 30th 2017.*
35. Flores, G.; Ruiz del Castillo, M. L.; Herraiz, M.; Blanch, G. P., Study of the adulteration of olive oil with hazelnut oil by on-line coupled high performance liquid chromatographic and gas chromatographic analysis of filbertone. *Food Chem.* **2006**, *97*, 742-749.
36. Commission Regulation (EU) No 594/2012 of 5 July 2012 amending Regulation (EC) 1881/2006 as regards the maximum levels of the contaminants ochratoxin A, non dioxin-like PCBs and melamine in foodstuffs Text with EEA relevance. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32012R0594. *Last visited: March 30th 2017.*
37. Capuano, E.; Boerrigter-Eenling, R.; Koot, A.; van Ruth, S. M., Targeted and Untargeted Detection of Skim Milk Powder Adulteration by Near-Infrared Spectroscopy. *Food Anal. Meth.* **2015**, *8*, 2125-2134.
38. Regulation (EC) No 110/2008 of the European Parliament and of the Council of 15 January 2008 on the definition, description, presentation, labelling and the protection of geographical indications of spirit drinks and repealing Council Regulation (EEC) No 1576/89. http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32008R0110. *Last visited: March 30th 2017.*
39. Purcaro, G.; Barp, L.; Moret, S., Determination of hydrocarbon contamination in foods. A review. *Analytical Methods* **2016**, *8*, 5755-5772.
40. Balabin, R. M.; Smirnov, S. V., Melamine detection by mid- and near-infrared (MIR/NIR) spectroscopy: A quick and sensitive method for dairy products analysis including liquid milk, infant formula, and milk powder. *Talanta* **2011**, *85*, 562-568.
41. Forchetti, D. A. P.; Poppi, R. J., Use of NIR hyperspectral imaging and multivariate curve resolution (MCR) for detection and quantification of adulterants in milk powder. *LWT-Food Sci. Technol.* **2017**, *76*, 337-343.
42. Huang, M.; Kim, M. S.; Delwiche, S. R.; Chao, K.; Qin, J. W.; Mo, C.; Esquerre, C.; Zhu, Q. B., Quantitative analysis of melamine in milk powders using near-infrared hyperspectral imaging, and band ratio. *J. Food Eng.* **2016**, *181*, 10-19.
43. Alamprese, C.; Casale, M.; Sinelli, N.; Lanteri, S.; Casiraghi, E., Detection of minced beef adulteration with turkey meat by UV-vis, NIR and MIR spectroscopy. *LWT-Food Sci. Technol.* **2013**, *53*, 225-232.
44. Lohumi, S.; Lee, S.; Lee, H.; Cho, B. K., A review of vibrational spectroscopic techniques for the detection of food authenticity and adulteration. *Trends Food Sci. Technol.* **2015**, *46*, 85-98.
45. Alamprese, C.; Amigo, J. M.; Casiraghi, E.; Engelsen, S. B., Identification and quantification of turkey meat adulteration in fresh, frozen-thawed and cooked minced beef by FT-NIR spectroscopy and chemometrics. *Meat Sci.* **2016**, *121*, 175-181.
46. Ropodi, A. I.; Panagou, E. Z.; Nychas, G. J. E., Multispectral imaging (MSI): A promising method for the detection of minced beef adulteration with horsemeat. *Food Control* **2017**, *73*, 57-63.
47. Ropodi, A. I.; Pavlidis, D. E.; Mohareb, F.; Panagou, E. Z.; Nychas, G. J. E., Multispectral image analysis approach to detect adulteration of beef and pork in raw meats. *Food Res. Int.* **2015**, *67*, 12-18.
48. Borras, E.; Ferre, J.; Boque, R.; Mestres, M.; Acena, L.; Busto, O., Data fusion methodologies for food and beverage authentication and quality assessment - A review. *Anal. Chim. Acta* **2015**, *891*, 1-14.
49. Barbosa-Garcia, O.; Ramos-Ortiz, G.; Maldonado, J. L.; Pichardo-Molina, J. L.; Meneses-Nava, M. A.; Landgrave, J. E. A.; Cervantes-Martinez, J., UV-vis absorption spectroscopy and multivariate analysis as a method to discriminate tequila. *Spectroc. Acta Pt. A-Molec. Biomolec. Spectr.* **2007**, *66*, 129-134.
50. Savchuk, S. A.; Vlasov, V. N.; Appolonova, S. A.; Arbuzov, V. N.; Vedenin, A. N.; Mezinov, A. B.; Grigor'yan, B. R., Application of chromatography and spectrometry to the authentication of alcoholic beverages. *J. Anal. Chem.* **2001**, *56*, 214-231.
51. Pontes, M. J. C.; Santos, S. R. B.; Araujo, M. C. U.; Almeida, L. F.; Lima, R. A. C.; Gaiao, E. N.; Souto, U., Classification of distilled alcoholic beverages and verification of adulteration by near infrared spectrometry. *Food Res. Int.* **2006**, *39*, 182-189.
52. Anjos, O.; Santos, A. J. A.; Estevinho, L. M.; Caldeira, I., FTIR-ATR spectroscopy applied to quality control of grape-derived spirits. *Food Chem.* **2016**, *205*, 28-35.
53. Bernardes, C. D.; Barbeira, P. J. S., Different Chemometric Methods for the Discrimination of Commercial Aged Cachacas. *Food Anal. Meth.* **2016**, *9*, 1053-1059.
54. Aroca-Santos, R.; Cancilla, J. C.; Perez-Perez, A.; Moral, A.; Torrecilla, J. S., Quantifying binary and ternary mixtures of monovarietal extra virgin olive oils with UV-vis absorption and chemometrics. *Sens. Actuator B-Chem.* **2016**, *234*, 115-121.
55. Borras, E.; Ferre, J.; Boque, R.; Mestres, M.; Acena, L.; Calvo, A.; Busto, O., Olive oil sensory defects classification with data fusion of instrumental techniques and multivariate analysis (PLS-DA). *Food Chem.* **2016**, *203*, 314-322.
56. Nunes, C. A., Vibrational spectroscopy and chemometrics to assess authenticity, adulteration and intrinsic quality parameters of edible oils and fats. *Food Res. Int.* **2014**, *60*, 255-261.
57. Gila, D. M. M.; Marchal, P. C.; Garcia, J. G.; Ortega, J. G., On-line system based on hyperspectral information to estimate acidity, moisture and peroxides in olive oil samples. *Comput. Electron. Agric.* **2015**, *116*, 1-7.
58. Ropodi, A. I.; Panagou, E. Z.; Nychas, G. J. E., 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 Food Sci. Technol.* **2016**, *50*, 11-25.
59. Farsaie, A.; McClure, W. F.; Monroe, R. J., DEVELOPMENT OF INDEXES FOR SORTING IRANIAN PISTACHIO NUTS ACCORDING TO FLUORESCENCE. *J. Food Sci.* **1978**, *43*, 1550-1552.

60. Hruska, Z.; Yao, H. B.; Kincaid, R.; Brown, R.; Cleveland, T.; Bhatnagar, D., Fluorescence Excitation-Emission Features of Aflatoxin and Related Secondary Metabolites and Their Application for Rapid Detection of Mycotoxins. *Food Bioprocess Technol.* **2014**, *7*, 1195-1201.
61. Pearson, T. C.; Wicklow, D. T.; Maghirang, E. B.; Xie, F.; Dowell, F. E., Detecting aflatoxin in single corn kernels by transmittance and reflectance spectroscopy. *Trans. ASAE* **2001**, *44*, 1247-1254.
62. Kalkan, H.; Beriat, P.; Yardimci, Y.; Pearson, T. C., Detection of contaminated hazelnuts and ground red chili pepper flakes by multispectral imaging. *Comput. Electron. Agric.* **2011**, *77*, 28-34.
63. Alewijn, M.; van der Voet, H.; van Ruth, S., Validation of multivariate classification methods using analytical fingerprints – concept and case study on organic feed for laying hens. *Journal of Food Composition and Analysis* **2016**, *51*, 15-23.

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