Final report

Validation of multispectral imaging (MSI) technology for food and feed analysis

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Final report: Validation of multispectral imaging (MSI) technology for food and feed analysis

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1. Executive summary

Current testing environments for food and feed samples remain complex using a wide variety of analytical techniques ranging from physio-chemical to biological in nature. Whilst effective, these methods tend to be highly specialised, expensive, invasive, technically demanding, and are often associated with long turn-around times. A recent FSA project examined the applicability of multi-spectral imaging (MSI) as a rapid, multi-analyte, non-targeted and non-invasive screening approach for food and feed analysis (FSA project SEP-EOI-05). The project demonstrated proof-of-principle for the application of MSI methods to screen a broad range of sample types rapidly and inexpensively in the food chain.

The aim of the current project was to provide validated methods for specific prioritised sampling scenarios, guidance on general MSI validation activities, and recommendations on technology transfer and feasibility of developing additional MSI related resources (e.g. an MSI database). The project supported FSA policy by using science and evidence to prioritise an agreed list of sampling scenarios categorised as current and emerging risks. MSI provides a rapid and non-destructive technique for screening foods to ensure they are safe, traceable and properly labelled, further supporting FSA policy and empowering consumers to make informed decisions in relation to food.

An in-depth consultation with the FSA, Defra, AMWG, Public Analysts and literature reviews inclusive of recent RASFFs and the FSA 2016/17 National Coordinated Sampling programme, helped make an informed decision on a priority list of sampling scenarios for further method development and validation. Additional literature reviews and stakeholder consultation helped develop the appropriate sourcing and sample preparation strategy for each sampling scenario.

For each sampling scenario, multiple methods were developed using representative sample and adulterant components, the most promising of which were subject to further method validation. Using Defra guidance on method validation, key performance characteristics were assessed inclusive of precision, analytical sensitivity (limit of detection) and specificity. Seven testing scenarios were

investigated and five validated methods were successfully developed, where the application, scope and key performance characteristics were captured per method. These methods included tests for adulteration in oregano, presence of offal in meat, ground peanut in ground almond, presence of pork in beef products, and presence of almond in commercial paprika samples.

Overall, measurement uncertainty associated with each of the analytical methods (expressed as the coefficient of variation) typically was not larger than 15% at the lower working range of the method. This demonstrated the excellent repeatability of the validated screening methods and the tight precision with which the measurement responses were generated. The exception to this was the validated method for detection of beef heart in beef meat, which showed appreciably more uncertainty towards the limit of detection. The limit of detection, defined as the lowest concentration of adulterant that generated a measurement profile which was significantly different from the 100% pure sample on at least 95% of occasions, varied depending upon the screening method, ranging from <5% adulterant (w/w) to <25% (w/w).

The focus of the current set of methods and associated validation was on the discriminatory potential to identify potential adulterants in samples representative of the market environment. The screening approach was not developed as a fully quantitative method, but clear potential was identified in this area.

A single fully validated method for determination of multiple fish species was not successful, thought mainly due to the broad scope of the method and associated data used to build the models. A recommendation from the current study is to develop and validate a more restricted method which would help realise the excellent discriminatory potential for fish speciation exhibited from the current project.

Written guidance to support general MSI validation studies was provided as part of this project to help facilitate the validation of any generic food sample in an analytical laboratory. Guidance included key features to incorporate, inclusive of initial model building, optimisation and method validation, scope and applicability of methods. Recommendations on transfer of MSI protocols and technology were produced, exploring the transferability of MSI technologies and associated food application protocols to UK analytical laboratories. Finally, recommendations on the feasibility of

developing an MSI database have been provided to facilitate a harmonised approach and access to a common set of food classification models.

The six validated methods generated as part of this project provide evidence of the range of food testing applications for which one MSI instrument is applicable. Whilst the performance of the MSI cannot always reach some of the analytical capabilities demonstrated through molecular biological methods, MSI methods boast a range of other benefits. Methods are rapid, non-destructive, incorporate a large sample size, possess an integrated footprint and afford both non-targeted and multi-analyte analyses, providing an additional method for food testing in the analytical toolbox. It is a recommendation from this study that MSI be utilised as a rapid and robust screening tool as part of a triage system, to identify any potential problems in the UK food chain.

The outputs of this project contribute towards promoting and protecting public health by providing a multi-faceted screening test for food to help ensure it is safe to eat and is what it says it is. This will aid in the traceability of food along the supply chain, ultimately helping empower consumers to make informed choices in relation to food.

In order to capitalise further upon the opportunities provided by MSI approaches for food testing, six areas of further work were identified. These included: 1) A knowledge dissemination event to broaden the impact of this new technology; 2) Evaluation of the transferability and performance compared with alternative imaging technologies; 3) Recommendations for the production of appropriate reference materials and associated imaging profiles on databases; 4) Establishment of online resources to support an accessible and harmonised UK based imaging community; 5) Validation of MSI methods for quantitative determination of food adulterants; 6) Further development of the fish speciation method which demonstrated excellent potential for discrimination.

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3. Glossary

- **%CV** – Coefficient of variation (expressed as a percentage)
- **AMWG** - Authenticity Methodology Working Group
- **CI** – Confidence Interval
- **Defra** – Department for Environment, Food and Rural Affairs
- **FSA** – Food Standards Agency
- **HSI** – Hyper-spectral imaging
- **LOD** – Limit of Detection
- **MSI** – Multi-spectral imaging
- **R2** - coefficient of determination
- **RASFF** - Rapid Alert System for Food and Feed
- **SD** – Standard Deviation
- **w/w** – Weight for weight

4. Aims and objectives

Current testing environments for food and feed samples remain complex using a wide variety of analytical techniques ranging from physio-chemical to biological in nature. Whilst effective, these methods tend to be highly specialised, expensive, invasive, technically demanding, and are often associated with long turn-around times. A recent FSA project examined the applicability of multi-spectral imaging (MSI) as a rapid, multi-analyte, non-targeted and non-invasive screening approach for food and feed analysis (FSA project SEP-EOI-05). The project demonstrated proof-of-principle for the application of MSI methods to screen a broad range of sample types rapidly and inexpensively in the food chain.

The aim of the current project was to provide validated methods for specific prioritised sampling scenarios, guidance on general MSI validation activities, and recommendations on technology transfer and feasibility of developing additional MSI related resources (e.g. an MSI database). The project supported FSA policy by using science and evidence to prioritise an agreed list of sampling scenarios categorised as current and emerging risks. MSI provides a rapid and non-destructive technique for screening foods to ensure they are safe, traceable and properly labelled, further supporting FSA policy and empowering consumers to make informed decisions in relation to food. The project objectives are detailed in Table 1

Table 1. Project objectives

Objective	Details
1	**Full validation study evaluating a panel of priority sampling scenarios selected in consultation with the FSA and other appropriate stakeholders.**
1.1	Project kick-off meeting with the FSA to establish and agree on project details.
1.2	Sourcing and developing (following best practice guidance) agreed priority test models using authenticated materials in collaboration with FSA and other stakeholders.

Objective	Details
1.3	Method validation study evaluating an agreed panel of test materials to establish core performance characteristics and build analytical capabilities.
1.4	Interim report summarising main validation study results and conclusions.
2	**Provision of written guidance to support general MSI validation studies.**
3	**Recommendations on transfer of MSI protocols and technology.**
4	**Examine feasibility of developing an MSI database.**
5	**Provision of final report.**

5. Extent to which the objectives set out in the contract have been met

Objective 1 - Full validation study evaluating a panel of priority sampling scenarios selected in consultation with the FSA and other appropriate stakeholders

Objective 1.1- Project kick-off meeting with the FSA to establish and agree on project details

The project kick-off meeting was successfully held on 16th November 2017 and a priority list of sampling scenarios agreed based on a multifactor scoring system.

Objective 1.2 - Sourcing and developing (following best practice guidance) agreed priority test models

A representative panel of test materials were sourced from stakeholders (Camstar Herbs Ltd., McCormick & Company, Inc., The Bart Ingredients Company Ltd.,) and reputable online suppliers/UK supermarkets (sample authenticity dependent on supplier quality systems, e.g. auditing and testing).

Objective 1.3 - Method validation study evaluating an agreed panel of test materials to establish core performance characteristics and build analytical capabilities

Multiple methods were developed as part of each sampling scenario, the most promising of which were subject to method validation. In total, five of these seven methods were successfully validated.

Objective 1.4 - Interim report summarising main validation study results and conclusions

The interim report was successfully submitted.

Objective 2 - Provision of written guidance to support general MSI validation studies.

Written guidance was provided to help facilitate the validation of any generic food sample in an analytical laboratory using the detailed MSI technology.

Objective 3 - Recommendations on transfer of MSI protocols and technology

A scoping exercise was successfully undertaken to explore the transferability of MSI technologies and associated food application protocols to UK analytical laboratories.

Objective 4 - Examine feasibility of developing an MSI database.

A feasibility study was successfully conducted to investigate the development and maintenance of an MSI-based database/repository of food sample classification and discrimination models.

Objective 5 - Provision of final report.

The final report was successfully submitted to the FSA.

6. Materials and methods

6.1 Objective 1 - Validation study

6.1.1 Objective 1.1 - Project kick-off meeting with the FSA to establish and agree on project details

The project kick-off meeting was held with the FSA and stakeholders to determine project scope and strategy.

6.1.2 Objective 1.2 - Sourcing and developing (following best practice guidance) agreed priority test models using authenticated materials in collaboration with FSA and other stakeholders

A representative panel of test materials were sourced from stakeholders (Camstar Herbs Ltd., McCormick & Company, Inc., The Bart Ingredients Company Ltd.,) and reputable online suppliers/UK supermarkets (sample authenticity dependent on supplier quality systems, e.g. auditing and testing). The sourced materials were selected as to be market representative and capture sample variability from factors such as different suppliers and animals (where appropriate).

6.1.3 Objective 1.3 - Method validation study evaluating an agreed panel of test materials to establish core performance characteristics and build analytical capabilities

6.1.3.1 Multi-spectral imaging system

The VideometerLab 4 (Videometer A/S, Hørsholm, Denmark) is a commercially available multispectral imaging system comprising integrated hardware and software that is capable of determining the spectral (365 nm – 970 nm) and spatial (e.g. morphology) profiles of test materials using reflectance and fluorescent imaging data. The MSI system represents cutting edge technology with intuitive analytical workflows and proven applications within the food testing sector which make it well suited to the current project.

6.1.3.2 Method validation strategy

A preliminary validation strategy was developed that comprised two main phases:

Model development – A broad panel of test scenario specific and related materials were identified and sourced that covered key areas of sample variability such as supplier, variety/cultivar/breed (e.g. different animal/fish) and appropriate processing state. Classification models were developed for the adulterant and bulk materials using the generated MSI datasets and used to characterise the panel of validation materials. The model development process utilised an nMahalanobis-based approach ('Known' vs 'Unknown') and aimed to capture sample type variation, whilst minimising model specificity issues. The resultant methodology was used to determine specificity characteristics and formed the basis for the method validation phase.

Validation – MSI methodologies developed during the method development phase from a representative sample set were used to characterise a restricted panel of test materials containing varying levels of adulterant. The bulk materials were pooled (where possible) during the preparative process in order to effectively challenge the MSI methodologies and ensure that the validation sample set differed from the training set. MSI scans were analysed using a fractional area approach to give an estimate of classified area coverage. This project implemented the national and international best measurement practice guidance for evaluation of performance characteristics during method validation and estimation of measurement uncertainty as jointly laid down by ISO/IEC 17025: 2017 [1], the guide to the expression of uncertainty in measurement [2] and the guidelines for Defra contractors involved in the development and validation of food authenticity assays [3]. Jointly, these guidelines help fulfil suggested analytical requirements as outline in HM Government Elliott Review [4].

Duplicate experiments were performed using experiment specific panels of test materials in order to enhance statistical power. Core method performance characteristics such as precision and Limit of Detection (LOD) were captured as part of the study. The LOD was defined as the lowest concentration of adulterant (e.g. myrtle in a background of oregano) that generated a measurement profile which was significantly different from the 100% pure sample (e.g. oregano) on at least 95% of

occasions.

6.1.3.3 Sampling and Ad-mixture preparation methodology

Test materials used in the development of the methods were sourced so as to be as representative of real-world samples as possible. Every attempt was taken to incorporate key sample variability originating from issues such as plant varietal and production differences by sourcing component materials from multiple sources (where available). Market awareness through consultation with the FSA and key stakeholders was used to make an informed decision on what sample sources to use to encapsulate key variability. The scope of each validated method (the range of samples for which the method performed as fit for purpose) has been clearly identified in the results section.

A proven gravimetric-based approach was chosen to prepare the test materials as this represents standard industry practice. However, it should be noted that components with very different densities will impact on the observable relative surface areas and hence bias any image-based software calculated % adulteration values.

6.1.3.3.1 Oregano herb adulterated with olive/myrtle leaves

Model development/training set – Materials were sourced from leading commercial herb and spice suppliers (including Bart Ingredients Company Ltd, Camstar Herbs Ltd, McCormick & Company Inc.), who provided a range of oregano samples from different batches and processing conditions, as well as targeted adulterants as informed through consultation with the FSA and other project stakeholders. An initial model development/training panel was prepared comprising oregano leaves (different suppliers and process states), selected known potential adulterants (myrtle and olive leaves from multiple suppliers), related species (Cistus, Cretan oregano, marjoram, Mexican oregano and sumac leaves from multiple suppliers where available) and a potential non-food related adulterant (saw dust). Triplicate 5 g sub-samples per sample type were transferred to 90 mm diameter disposable Petri dishes for MSI-based analyses.

Validation – Duplicate panels of test materials were prepared independently. Oregano control materials comprised 100% w/w cistus, 100% Cretan oregano, 100%

w/w marjoram, 100% Mexican oregano, 100% w/w myrtle, 100% w/w olive leaves, 100% w/w oregano (pooled material) and 100% w/w sumac. Adulterant admixtures comprising 75% w/w, 50% w/w, 25% w/w, 10% w/w, 5% w/w, 1% w/w and 0.1% w/w myrtle leaves in a background of oregano were gravimetrically prepared in 90 mm diameter disposable Petri dishes. In addition, a set of independently prepared challenge test admixtures were gravimetrically prepared comprising 30% w/w and 1% w/w olive/myrtle in a background of oregano (pooled material) in 90 mm disposable Petri dishes. The test panel was prepared using triplicate 5 g samples per sample type.

6.1.3.3.2 Beef meat adulteration with offal – undeclared offal

Model development/training set – Materials were sourced from three commercial suppliers (a large UK supermarket, an online organic supplier and a local butchers) who provided a range of whole cuts of meat/offal organs sourced from multiple animals. An initial model development/training panel was prepared based on fresh beef meat cuts, selected known potential adulterants (whole beef heart and whole lamb liver), additional offal adulterants (whole beef liver, and whole chicken liver) and other meat materials (lamb meat for specificity testing purposes) which were sourced from multiple suppliers/animals. These materials were trimmed to remove surface fat/non-muscle, ground using a meat grinder with a 7mm plate (3 repeat passes to ensure homogenisation) and stored on ice/4^{o}C until required. Triplicate 15 g sub-samples per sample type were transferred to 90 mm disposable petri dishes for MSI analysis.

Validation – Duplicate panels of test materials were prepared independently. Fresh ground meat/offal materials prepared as part of the model development process comprising 100% w/w beef (pooled from multiple suppliers); 100% w/w beef heart; 100% w/w beef liver, 100% w/w lamb, 100% w/w lamb liver and 100 % w/w lamb heart were used as the basis for the validation sample panel and associated 100% meat/offal controls. Adulterant admixtures comprising 75% w/w, 50% w/w, 25% w/w, 10% w/w, 5% w/w, 1% w/w and 0.1% w/w ground beef heart in a background of ground beef meat were gravimetrically prepared, mixed using a meat grinder with a 7mm plate (3 repeat passes to ensure homogenisation) and transferred to 90 mm diameter disposable Petri dishes (triplicate 15 g admixtures per sample type). In

addition, a set of independently prepared challenge test admixtures were gravimetrically prepared comprising 30% w/w and 1% w/w ground beef heart/lamb liver in a background of ground beef meat (triplicate 15 g admixtures per sample type).

6.1.3.3.3 Ground almond contaminated/adulterated with ground peanut

Model development/training set - Materials were sourced from multiple commercial nut suppliers to provide multiple whole/ground samples of almond and peanut representative of multiple countries of origin (USA & Spain). An initial model training panel set was prepared comprising ground almond (multiple suppliers), ground related nut species (cashew, hazelnut, walnut) and ground peanut (target adulterant/contaminant from multiple suppliers) in 90 mm diameter disposable Petri dishes (triplicate 15 g admixtures per sample type). Ground almonds/peanuts derived from whole deshelled materials were included in the test panel and ground to the required consistency using a food processor.

Validation - A broad panel of ground nut control materials comprising 100% w/w almond (pooled material); 100% w/w peanut (pooled material); 100% w/w Cashew, 100% w/w Hazelnut and 100% w/w walnut were prepared (triplicate 15 g admixtures per sample type). Adulterant admixtures comprising 75% w/w, 50% w/w, 25% w/w, 10% w/w, 5% w/w, 1% w/w and 0.1% w/w ground peanut in a background of almond were gravimetrically prepared in 90 mm diameter disposable Petri dishes (triplicate 15 g admixtures per sample type). In addition, a set of independently prepared challenge test admixtures were gravimetrically prepared comprising 30% w/w and 1% w/w ground peanut in a background of ground almond in 90 mm disposable petri dishes (triplicate 15 g admixtures per sample type).

6.1.3.3.4 Beef adulterated with pork meat

Model development/training set – Additional meat materials (augmenting those mentioned in the “Beef meat adulteration with offal” model) were sourced from two large UK supermarkets comprising multiple individual packs of whole meat cuts (beef, adulterants and controls) representative of multiple animals. An initial fresh material model development/training panel was prepared based on fresh beef meat cuts, pork meat cuts (selected adulterant), additional potential adulterants (chicken

and turkey breast meat) and other meat materials (lamb meat for specificity testing purposes) which were sourced from multiple suppliers/animals. These materials were trimmed to remove surface fat/non-muscle, ground using a meat grinder with a 7mm plate (3 repeat passes to ensure homogenisation) and stored on ice/4^{o}C until required. Triplicate 15 g sub-samples per sample type were transferred to 90 mm disposable petri dishes for MSI analysis.

Validation – Duplicate panels of test materials were prepared independently. Fresh ground meat materials prepared as part of the model development process comprising 100% w/w beef (pooled from multiple suppliers); 100% w/w pork; 100% w/w chicken, 100% w/w turkey and 100% w/w lamb were used as the basis for the validation sample panel and associated 100% meat controls. Adulterant admixtures comprising 75% w/w, 50% w/w, 25% w/w, 10% w/w, 5% w/w, 1% w/w and 0.1% w/w ground pork meat in a background of ground beef meat were gravimetrically prepared, mixed using a meat grinder with a 7mm plate (3 repeat passes to ensure homogenisation) and transferred to 90 mm diameter disposable Petri dishes (triplicate 15 g admixtures per sample type). In addition, a set of independently prepared challenge test admixtures were gravimetrically prepared comprising 30% w/w and 1% w/w ground pork in a background of ground beef meat (triplicate 15 g admixtures per sample type).

6.1.3.3.5 Paprika adulterated/contaminated with ground almond

Model development/training set - Paprika powder samples representative of multiple types (e.g. paprika, sweet paprika, smoked paprika, different ASTA colour values) were sourced from Bart Ingredients Company Ltd and Camstar Herbs Ltd. Five sets of ground/whole almond samples representative of multiple countries of origin (USA & Spain) were sourced via an online retailer. An initial model training panel set was prepared comprising paprika powder (Sweet paprika, sweet smoked paprika, 75 and 75 ASTA paprika sourced from multiple suppliers), related paprika-type materials (Chilli powder and cayenne pepper), ground almond (target adulterant/contaminant from multiple suppliers) and ground related nut species (cashew, hazelnut, walnut) in 90 mm diameter disposable Petri dishes (triplicate 15 g admixtures per sample type). Ground almonds derived from whole deshelled almonds were included in the test panel and ground to the required consistency

using a food processor.

Validation - A panel of control materials comprising 100% w/w sweet paprika (pooled material); 100% w/w almond (pooled material); 100% w/w peanut, 100% w/w cashew and 100% w/w hazelnut were prepared (triplicate 15 g admixtures per sample type). Adulterant admixtures comprising 75% w/w, 50% w/w, 25% w/w, 10% w/w, 5% w/w, 1% w/w and 0.1% w/w ground almond (pooled material) in a background of a sweet paprika powder (pooled material) were gravimetrically prepared in 90 mm diameter disposable Petri dishes (triplicate 15 g admixtures per sample type). In addition, a set of independently prepared challenge test admixtures were gravimetrically prepared comprising 30% w/w and 1% w/w ground almond (pooled) in a background of sweet paprika powder (pooled) in 90 mm disposable petri dishes (triplicate 15 g admixtures per sample type).

6.1.3.3.6 "White" fish speciation

Model development/training set – The multiple fish species were commercially sourced as fresh UK port landed fish at multiple time points. An initial fresh material model development/training panel was prepared based on a panel of five commonly available white fish species landed fresh at British ports. Fresh cod fillet, haddock fillet, pollack fillet, hake fillet and whiting fillet portions were sourced at multiple time points from the same supplier. These materials were trimmed to roughly 50 – 70 mm x 50 – 70 mm sections and stored on ice/4^{o}C until required. Triplicate trimmed sub-samples per sample type were transferred to 90 mm disposable Petri dishes for MSI analysis.

Validation – Duplicate panels of test materials were prepared independently using fresh fish sourced at different time points. Fresh cod fillet, haddock fillet, pollack fillet, hake fillet and whiting fillet portions (sourced at different time points from the same supplier) were trimmed to roughly 50 – 70 mm x 50 – 70 mm sections and triplicate trimmed sub-samples per sample type were transferred to 90 mm disposable Petri dishes for MSI analysis.

6.1.3.3.7 Meat quality – impact of freeze thawing process on meat characteristics

Model development/training set – Meat samples were sourced as described in the

previous “Beef meat adulteration with offal” and “Beef adulterated with pork meat” models. An initial fresh material model development/training panel was prepared based on fresh beef meat cuts and pork meat cuts which were sourced from multiple suppliers/animals. These materials were trimmed to remove surface fat/non-muscle, ground using a meat grinder with a 7mm plate (3 repeat passes to ensure homogenisation) and stored on ice/4^{o}C until required. Triplicate 15 g sub-samples per sample type were transferred to 90 mm disposable Petri dishes for MSI analysis before being stored at -20^{o}C for a minimum of 48 hours, thawed for approximately 1 hour and rescanned using under the same MSI analysis conditions.

Validation – Duplicate panels of test materials were prepared independently. Fresh ground meat materials prepared as part of the model development process comprising 100 % w/w beef and pork (pooled from multiple suppliers) were used as the basis for the validation sample panel. Triplicate 15 g sub-samples per sample type were transferred to 90 mm disposable Petri dishes for MSI analysis before being stored at -20^{o}C for a minimum of 48 hours, thawed for approximately 1 hour and rescanned under the same MSI analysis conditions.

6.1.3.4 MSI Analysis

Samples were mixed well (where appropriate) to ensure good component distribution, the Petri dish cover removed and placed under the integrating sphere for image capture. Image capture (all 19 wavelengths) was performed using VideometerLab Software Version 3.10.6 (6722) and the default 100 % reflectance light settings without filters.

Image data analysis was performed using VideometerLab Software Version 3.10.6 (6722) to analyse the 100% control materials which were used to generate a normalised Mahalanobis (nM) classification model based on their respective spectral signatures [5, 6]. The model was applied to the panel of test samples, and results returned based on the model’s scoring of how closely the spectral signature of each pixel matched the known control samples.

Automated analysis sessions were developed based on the ‘MSI Area Fraction 3 PlugIn’ algorithm to estimate the area fraction of the sample matching the specified classification model, and hence could be used to very roughly estimate the potential

percentage adulteration levels based on the observable surface area only.

6.1.3.5 Data analyses

Data analyses was performed using the inbuilt functionality provided by Microsoft Excel® 2016 (Microsoft). 95% confidence intervals associated with the data (mean percentage areas) were calculated based on the accepted standard practice of 1.96 multiplied by the standard error of the mean ($s/\sqrt{n}$) of that dataset.

6.1.4 Objective1.4 - Interim report summarising main validation study results and conclusions

An interim report was successfully submitted to the FSA.

6.2 Objective 2 - Written guidance to support general MSI validation studies.

A generic MSI validation strategy was developed based on expertise gained during the method development/validation phase of the project and incorporated key features such as initial model building, optimisation and method validation approaches, and builds in recommendations on generic validation from instrument developers/distributors (e.g. Videometer A/S and Analytik Ltd.).

6.3 Objective 3 - Recommendations on transfer of MSI protocols and technology.

A scoping exercise was conducted to explore the transferability of MSI technologies and associated food application protocols to UK analytical laboratories. Key activities included a review of alternative applicable technologies with applicability to food testing and engagement with instrument manufacturers on the transfer of MSI protocols to ensure that the correct parameters are captured during the validation process.

6.4 Objective 4 - Examine feasibility of developing an MSI

database.

The core strategy investigated the feasibility of the development and maintenance of an MSI-based database/repository of food sample classification and discrimination models. Key areas for discussion have focussed on recommendations and ease of developing, curating and populating a MSI database; and effectively maintaining a cloud based database to ensure that it is secure and curated.

6.5 Objective 5 - Provision of final report.

The final report has been successfully submitted to the FSA.

7. Results

7.1 Objective 1 - Validation study

7.1.1 Objective 1.1 - Project kick-off meeting

The project kick-off meeting was successfully held on 16th November 2017 and a priority list of sampling scenarios agreed based on a multifactor scoring system. This multi-factorial prioritisation scheme was developed to incorporate key stakeholder requirements and employed a ranking system per factor using 1 – 10 scoring system, whereby 10 represents the highest priority. The key ranking factors considered as part of this process included:

- Relative prioritisation level in the FSA national Coordinated Sampling Programme 2016-2017
- Current topical importance
- FSA and Defra strategic importance
- Stakeholder feedback from stakeholders inclusive of AMWG and Public Analysts
- Analytical outcome from the previous FSA/LGC feasibility study (SEP-EOI-05)
- Applicability to MSI
- Ease of sourcing test materials

The identified sampling scenarios were ranked according to their total scoring and the top seven scenarios agreed by project stakeholders as the project test panel (Table 2). Project stakeholders who offered advice on the prevalence of the sampling scenarios included the FSA, Defra, Defra's Authenticity Methodology Working Group (AMWG), Public Analysts, literature reviews, RASFFs, the FSA 2016/17 National Coordinated Sampling program

mme and the FSA Food Crime Unit.

Table 2. Sampling scenario priorities

Priority	Sampling scenario
1	Oregano (olive/myrtle)
2	Meat (offal)
3	Ground Almond (ground peanut)
4	Beef (pork)
5	Paprika (almond)
6	“White” fish speciation
7	Meat quality

The prevalence of gross adulteration found in commercial oregano products has previously been reported by the FSA Food Crime Unit. A consumer watchdog survey in 2016 revealed that just under 25% of commercial oregano samples in UK and Ireland taken as part of this survey had adulteration from olive or myrtle leaves ranging from 21% to 69% (w/w) [7]. Similar prevalence was reported in Australia and Denmark in 2016/2017 [8, 9]. Oregano adulteration was selected as a model system for method development due to it being highlighted as an ongoing food fraud issue and previous good MSI-based analytical performance (FSA project SEP-EOI-05) [10].

The presence of offal in meat samples destined for human consumption was also a high risk as identified through the FSA National Coordinated Sampling programme 2016/17 [11]. Methods exist for identification of offal, but these are often laborious and are not always freely accessible, so a screening technique to augment such testing was regarded as beneficial. The adulteration of skeletal muscle meat with

offal is a long running food fraud issue typified by the presence of offal in meat products. On a related angle, Defra's AMWG and the FSA National Coordinated Sampling programme 2016/17 identified that meat substitution continues to be a topical and real risk in the UK food supply chain. Further methods are needed to screen and monitor for this. The substitution of cheaper meats such as pork/turkey for beef represents a long-standing food fraud issue as well as having cultural implications.

The undeclared presence of allergens in food samples can have serious and life-threatening implications. In 2016 there was a high profile manslaughter case where a UK restaurant owner supplied a takeaway meal to a customer where the almond in the food had been adulterated/substituted with peanut [12]. The selected sampling scenario represents a serious potential health and food labelling issue with recent criminal case examples. Equally well, a high profile incident of almond contamination in paprika was reported in 2017 and became the subject of a UK Government Chemist referee case [13]. The number of high RASFF notifications continues to support the need for additional techniques to screen and test for the presence of allergens.

Whilst not as high priority as the above sampling issues, fish speciation and quality of fish products (e.g. fresh vs. frozen) were also identified as potential problems by the FSA Food Crime Unit, the FSA National Coordinated Sampling programme 2016/17 and the AMWG. The substitution of premium white fish such as cod/haddock with other white fish such as pollack (*Pollack Pollachius*, NE Atlantic Ocean within UK fishing grounds) is a common food fraud issue [14]. Finally, the same concerns on quality of meat products (bio-films, marbling, fresh vs. frozen) were also raised as ongoing issues.

7.1.2 Objective 1.2 - Sourcing and developing agreed priority test models using authenticated materials

A representative panel of test materials were sourced from stakeholders (Camstar Herbs Ltd., McCormick & Company, Inc., The Bart Ingredients Company Ltd.,) and reputable online suppliers/UK supermarkets (sample authenticity dependent on supplier quality systems, for example, auditing and testing).

7.1.3 Objective 1.3 - Method validation study

The method validation strategy described in section 3.1.3.2 “Method validation strategy” was applied to all sampling scenarios, producing results as described below.

7.1.3.1 Oregano herb adulterated with olive/myrtle leaves

7.1.3.1.1 Method Scope

The scope of the method is a qualitative MSI-based screening methodology developed to analyse oregano leaf samples adulterated with myrtle leaves based on the application of classification models built from representative matrices to surface area analyses. Threshold normalisation is employed that is dependent upon control materials that are representative of the test materials.

The method has been validated on a range of oregano materials (different suppliers and process states) using myrtle leaves (multiple suppliers) but it is important that the method is verified as fit for purpose when being applied by another laboratory, as per standard best measurement practice guidance.

7.1.3.1.2 Method Development

Initial method development activities focussed on the spectral profiles associated with the oregano and potential adulterant materials to determine whether a proposed MSI-based approach was achievable. Figure 1 shows a comparison of reflectance spectra for a selection of materials associated with the sampling scenario and highlights that, with the exception of the Petri dish and saw dust sample types, the tested leaf materials exhibit similar reflectance spectra. However, the majority of the spectral profiles show some differences that may translate to workable classification models.

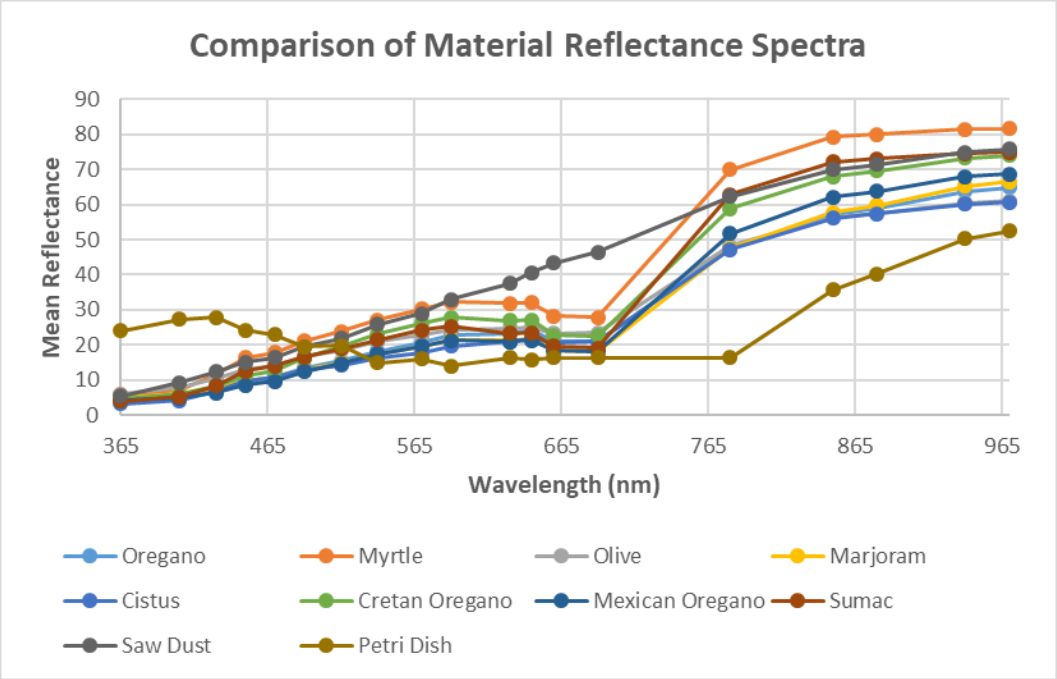


Figure 1. Typical VL4 generated reflectance spectra for a selection of oregano and associated materials.

A representative panel of oregano leaf and associated materials sourced from multiple suppliers, which incorporated core sample variables such as batch-to-batch and process state, was used to develop a multiple classification nMahalanobis model-based oregano adulteration method to analyse test samples for oregano, myrtle and olive material. The method development process was technically challenging due to the close relatedness of the oregano and selected adulterant test materials as highlighted by their spectral profiles (Figure 1).

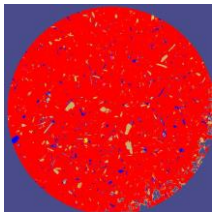
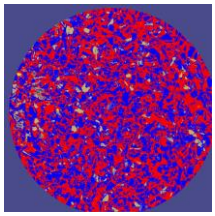
Table 3 shows the relative performance of the MSI methodology against the development sample set and highlighted the potential difficulties in developing a generic oregano model which appears to show poor specificity for some of the species evaluated, e.g. the marjoram sample type has a pooled mean ± 95% confidence interval % model area value of 94.44±1.47 as compared to oregano at 93.48±.3.04. However, the developed oregano model easily differentiates between myrtle and olive sample types which are central to the adulterant classification models.

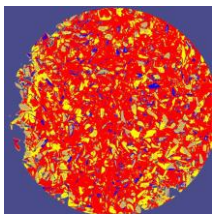
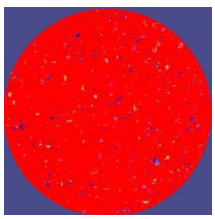
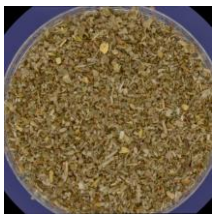
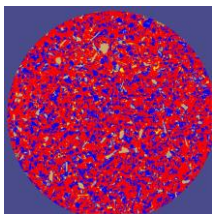
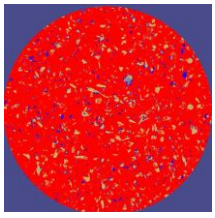
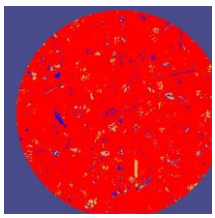
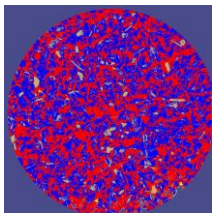
Initial models based on the myrtle and olive reference materials show clear differences between the adulterant and oregano mean % model area values and

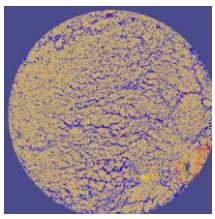
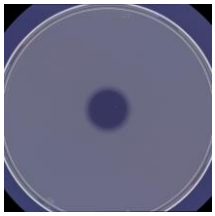
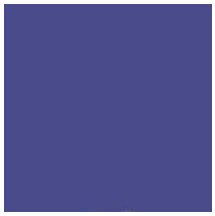
high specificities for their respective target materials (both 93% mean content). The typically high precision associated with the sample replicates supports the general repeatability of the method.

Table 3. MSI analyses of the model building test samples using the oregano adulteration method.

Processed image presented in a false colour format: red areas (oregano sample type), beige areas (general sample type), blue areas (myrtle sample type) and yellow areas (olive sample type). The pooled sample type mean % area matching a specific model based on area fractional calculation (along with the associated 95% confidence interval in parentheses) are shown. Values based on 1 to 15 technical replicates per sample type (N). Please note that the processed image represents a composite rendering from all three sample type models and will preferentially display the model showing the highest mean % content.

Sample Type	N	sRGB Image	Processed Image	Oregano Model – Mean % Area	Myrtle Model - Mean % Area	Olive Model - Mean % Area
Oregano	15			93.48 (3.04)	58.65 (10.50)	41.71 (18.50)
Myrtle	6			43.60 (5.21)	93.29 (1.45)	28.88 (4.37)

Sample Type	N	sRGB Image	Processed Image	Oregano Model – Mean % Area	Myrtle Model - Mean % Area	Olive Model - Mean % Area
Olive	6			79.81 (19.16)	75.38 (26.27)	93.06 (5.43)
Marjoram	9			94.44 (1.47)	79.15 (1.45)	66.45 (5.14)
Cistus	3			60.91 (1.24)	75.71 (1.05)	57.42 (0.64)
Cretan Oregano	3			86.75 (0.90)	74.29 (0.16)	75.56 (0.95)
Mexican Oregano	9			93.10 (1.25)	80.74 (6.29)	83.54 (6.36)
Sumac	3			47.33 (4.67)	90.55 (1.22)	25.64 (2.19)

Sample Type	N	sRGB Image	Processed Image	Oregano Model – Mean % Area	Myrtle Model - Mean % Area	Olive Model - Mean % Area
Saw Dust	3			1.17 (0.75)	18.82 (3.14)	0.43 (0.13)
Petri Dish	1			0.00	0.00	0.00

The method development process necessitated more representative classification models incorporating common sources of sample variability (suppliers and processing) to improve general applicability to real-world samples. However, this meant that the normalised Canonical Discriminant Analysis (nCDA), a “Known vs Known” approach which was employed in the previous FSA MSI project (FSA project SEP-EOI-05, [10]), was not suitable for model building and the more robust normalised Mahalanobis, a “Known vs Unknown” approach, formed the basis for the model building. As a consequence, the model specificity is reduced so that a broader set of set of test samples can be classified, which is shown by relatively high mean % content values associated with non-target materials (**Table 3**).

The typically high backgrounds associated with the results due to the need to develop more robust classification models incorporating core sample variability characteristics necessitated that a simple threshold approach was applied to normalise the dataset. A routinely used technique is to use the standard deviation derived from a control sample with the associated measurement to generate a response baseline that can be used to assign a detection limit [15]. This approach was adapted and applied to the generation of model specific detection thresholds based on the 100% w/w oregano, myrtle and olive sample types and the associated

mean standard deviation applied in a model specific manner.

The oregano model was based on using the 100% w/w oregano control materials as the reference. The threshold to normalise the data was based on the 100% oregano control samples and the assumption that 95% of the data distribution falls within 1.96 standard deviation range from the mean value. Percentage content data for the oregano control generated using the oregano model was used to calculate the mean value and associated standard deviation. 1.96 standard deviation was subtracted from the mean oregano % content value to give the lower range for data and thereby establish the lowest detection threshold level for a sample considered as providing a profile consistent with that of 100% oregano.

To augment this analysis, secondary models based on detection of myrtle and olive type material were developed. A myrtle or olive detection threshold which compensated for the oregano background component within each model was devised based on the assumption that 95% of the data distribution falls within a 1.96 standard deviation range from the mean value. Percentage content data for the 100% oregano sample type generated using the myrtle or olive models were used to calculate the mean oregano background and associated SD. 1.96 SD was applied to the model specific mean oregano background % content value to give the upper range for the background contribution and thereby establish a positive detection threshold level.

Table 4 summarises the model specific threshold normalised data and shows the good specificity (100% detection) of the models to their respective target materials and with no oregano materials detected with the adulterant models. Anticipated specificity issues are observed for the oregano model with the closely related marjoram and Mexican oregano sample types, whilst the potential adulterant models show some cross reactivity with olive, marjoram, sumac and Mexican oregano test materials. However, these specificity issues should not impact on the within scope performance of the methodology.

Table 4. Tabulated data showing the percentage of sample replicates with % model area values passing a model specific threshold setting.

Calculated threshold based on the oregano, myrtle and olive model mean % model

area values for the oregano control and the associated 1.96 standard deviation (assumed to capture 95% of data). Values based on 1 to 15 technical replicates per sample type (N). Model threshold values: 88.21 (Oregano Analysis), 76.83 (Myrtle Analysis) and 73.74 (Olive Analysis)

Sample type	N	% Replicates passing a detection threshold: Oregano Analysis	% Replicates passing a detection threshold: Myrtle Analysis	% Replicates passing a detection threshold: Olive Analysis
Oregano	15	100.0	0.0	0.0
Myrtle	6	0.0	100.0	0.0
Olive	6	50.0	50.0	100.0
Marjoram	9	100.0	100.0	0.0
Cistus	3	0.0	0.0	0.0
Cretan Oregano	3	0.0	0.0	100.0
Mexican Oregano	9	100.0	66.7	100.0
Sumac	3	0.0	100.0	0.0
Saw Dust	3	0.0	0.0	0.0
Petri Dish	1	0.0	0.0	0.0

7.1.3.1.3 Method Validation

Four models were evaluated as part of the method validation exercise in order to prioritise the best performing method(s) for testing for adulteration in oregano samples. These methods were based on classification models of oregano (both a main and alternative method), myrtle leaves and olive leaves.

Following method validation, the alternative oregano classification model was able to successfully identify and confirm the presence of oregano in mixed oregano/myrtle samples containing high oregano levels, but this method was not deemed suitable

for detection of low adulterant levels. The methods based on the myrtle and olive leaf classification models were able to successfully and repeatedly identify and confirm the presence of either myrtle/olive, but only at levels of 50% myrtle and 30% olive respectively. Whilst useful, these methods were deemed best suited to confirm the possible presence of a species once the adulterant had been detected, and only at higher levels. For these reasons, the results from the main oregano classification model only are presented here as the validated method.

The method validation phase focused on a panel of oregano materials including gravimetrically prepared admixtures to validate method performance and determine characteristics including the limit of detection (LOD) and precision. Duplicate experiments were performed comprising 100% w/w controls and admixtures in order to generate a statistically relevant dataset.

As discussed in the method development section, a threshold normalisation approach was applied to the dataset due to the use of more representative and hence less specific classification models. **Table 5** summarises the threshold-based analysis applied to the pooled dataset (2 experimental replicates) and shows that the Oregano model (which is referenced to the 100% oregano control) is capable of successfully detecting the low level adulteration of the oregano sample with olive and myrtle materials as shown by the negative detection result for oregano at the 10% w/w myrtle and 30% w/w olive in oregano admixture materials.

Furthermore, the overall method precision (expressed as a % Coefficient of Variation) was found to be good across the working range of the method varying between 1.45 and 4.53 % CV using this classification model. This suggests that the method variability is low and suitable for analytical applications.

Significant specificity issues were observed for the closely related marjoram and Mexican oregano controls which were classified consistently as having a profile similar to the 100% oregano reference material (100% detection). Within market sourced materials, marjoram and especially Mexican oregano are unlikely to be common adulterants due to geographical restrictions (Mexican oregano) or already a commercially produced herb (marjoram) and not well suited to economically motivated adulteration. The method was therefore considered as demonstrating promising performance characteristics well suited to an initial screening technique for

multiple targets, which is both non-destructive and rapid (less than 1 minute from scan to result).

The approach detailed in the test for oregano adulteration formed the basis for the remaining method validation activities.

Table 5. Tabulated data showing the pooled mean % model area ± 95% confidence interval associated with the oregano classification model and the percentage of sample replicates with % model area values passing a model specific threshold setting.

Calculated threshold based on the oregano model mean % model area values for the oregano control and the associated 1.96 standard deviation (assumed to capture 95% of data). 'CS' prefix refers to challenge sample type. Values based on 2 experiments comprising 3 technical replicates per sample.

Sample	% Model Area: Mean	%Model Area: ± 95% CI	% Replicates passing the detection threshold
100% Oregano	91.97	0.46	100.00
100% Myrtle	50.56	1.83	0.00
100% Olive	64.73	1.18	0.00
75% Myrtle in oregano	68.57	1.60	0.00
50% Myrtle in oregano	80.73	1.16	0.00
25% Myrtle in oregano	86.94	0.81	0.00
10% Myrtle in oregano	88.60	1.03	0.00
5% Myrtle in oregano	90.52	0.73	66.67
1% Myrtle in oregano	91.63	0.48	100.00
0 1% Myrtle in oregano	91.08	0.28	100.00
CS_30% Myrtle in oregano	85.70	1.53	0.00
CS_1% Myrtle in oregano	91.59	0.26	100.00

Sample	% Model Area: Mean	%Model Area: ± 95% CI	% Replicates passing the detection threshold
CS_30% Olive in oregano	89.15	0.65	0.00
CS_1% Olive in oregano	91.62	0.37	100.00
100% Marjoram	95.78	0.43	100.00
100% Mexican	94.31	0.32	100.00
100% Sumac	47.56	1.74	0.00
Petri dish	0.00	0.00	0.00

A limited study was conducted into the quantitative potential of the methodology which was restricted to the myrtle model only (Annex 1 - Oregano Method – Quantitative Modelling).

7.1.3.1.4 Method validation summary

A method was developed and validated for detection of possible adulterants in an oregano sample, using a classification model based on a wide range of different pure oregano samples. Based on the samples used in this controlled experiment, the method was capable of detecting adulteration from myrtle with a detection limit of <10% myrtle in oregano (w/w). The analytical measurement uncertainty associated with the method varied between 1.45 and 4.53% (expressed as a CV) across the working range of the method. The validated method exhibited significant cross reactivity whereby marjoram and Mexican oregano also generated a profile consistent with that of oregano.

7.1.3.2 Beef meat adulteration with offal – undeclared offal

7.1.3.2.1 Method Scope

The scope of the method is a qualitative MSI-based screening methodology developed to analyse ground beef meat samples adulterated with ground beef heart based on the application of a classification model built from representative matrices

to surface area analyses. Threshold normalisation is employed that is dependent upon control materials that are representative of the test materials.

The method has been validated on a range of beef meat and heart samples (multiple suppliers/animals) but it is important that the method is verified as being fit for purpose when applied by another laboratory, as per standard best measurement practice guidance.

7.1.3.2.2 Method Development

Initial method development activities focussed on the spectral profiles associated with the ground beef meat and potential adulterant materials to determine whether a proposed MSI-based approach was achievable. Figure 2 shows a comparison of reflectance spectra for a selection of materials associated with the sampling scenario and highlights that with the exception of the Petri dish, the tested material reflectance spectra appear to group according to material type, e.g. meat, liver or heart. These observed spectral differences in material type are likely translatable to workable classification models.

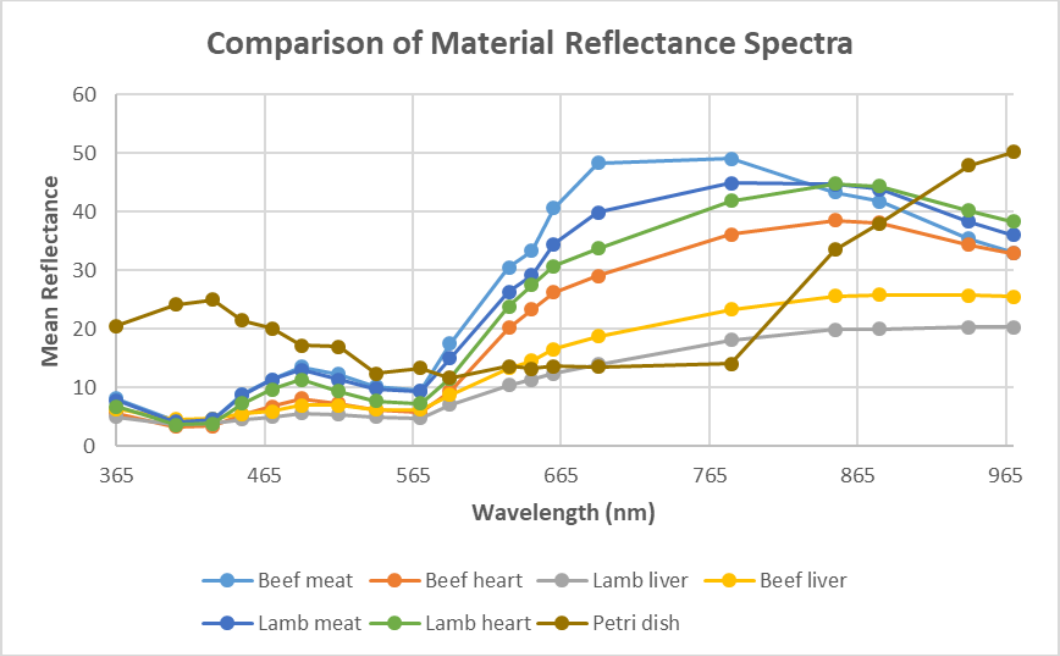


Figure 2. Typical VL4 generated reflectance spectra for ground beef meat and associated ground offal/meat materials

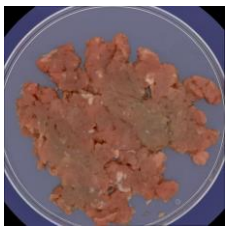
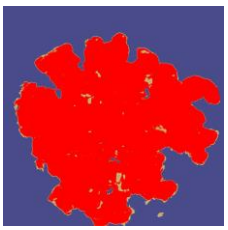
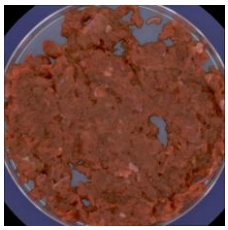
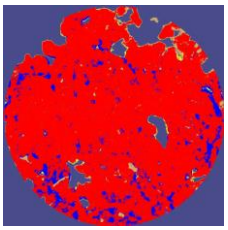
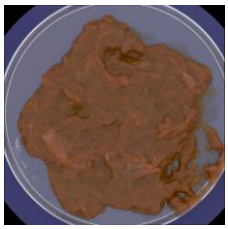
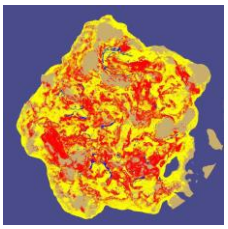
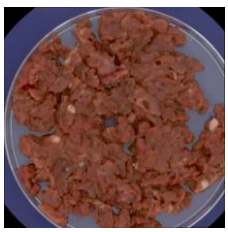
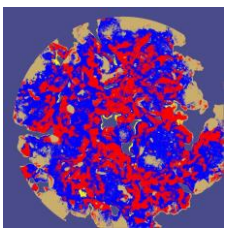
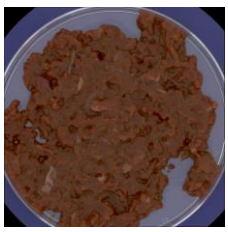
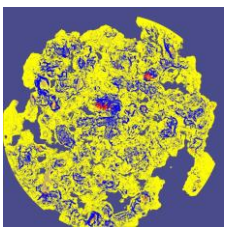
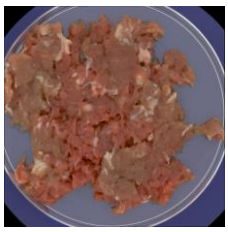
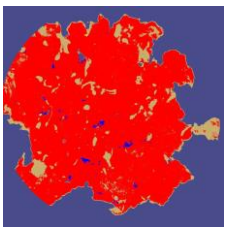
Table 6 shows the relative performance of the MSI methodology against the development sample set and highlights the potential challenges in developing a beef meat model which appears to show poor specificity for some of the key species evaluated. For example, the beef heart sample type has a pooled mean ± 95% confidence interval % model area value of 91.03±2.73 as compared to the target beef meat sample type at 96.74±0.61. However, the developed beef meat model easily differentiates between lamb liver and other offal materials as shown by low mean % area values (0.49 - 26.53 %).

The offal models developed against the beef heart and lamb liver reference materials show clear differences between the beef meat and offal adulterant sample types as demonstrated by low % model area values for beef meat and high values for offal-type materials. For example, the beef heart model analyses of the beef meat and beef heart material shows % model area values of 6.28±4.93 and 93.40±1.98 respectively. The typically tight (low) precision associated with the sample replicates supports the general repeatability of the method.

The threshold normalisation approach developed as part of the meat adulteration method development/validation process was applied to the % model area data based on either using the 100% w/w beef meat control sample as the reference (beef meat model) or compensating for the beef meat background component within the adulterant model (beef heart and lamb liver models).

Table 6. MSI analyses of the model building test samples using the offal adulteration method.

Processed image presented in a false colour format: red areas (beef meat sample type), beige areas (general sample type), blue areas (beef heart sample type) and yellow areas (lamb liver sample type). The pooled sample type mean % area matching a specific model based on area fractional calculation (along with the associated 95% confidence interval in parenthesis) are shown. Values based on 1-6 technical replicates per sample (N). Please note that the processed image represents a composite rendering from all three sample type models and will preferentially display the model showing the highest mean % content.

Sample Type	N	sRGB Image	Processed Image	Beef Meat Model – Mean % Area	Beef Heart Model - Mean % Area	Lamb Liver Model - Mean % Area
Beef Meat	6			96.74 (0.61)	6.28 (4.93)	0.23 (0.20)
Beef Heart	3			91.03 (2.73)	93.40 (1.98)	5.47 (1.57)
Beef Liver	3			26.53 (3.43)	6.49 (1.99)	73.92 (7.76)
Lamb Heart	3			49.06 (16.16)	79.02 (3.86)	4.05 (0.82)
Lamb Liver	3			1.27 (0.66)	24.52 (1.90)	97.10 (0.31)
Lamb Meat	3			89.59 (2.84)	32.36 (9.93)	0.29 (0.03)

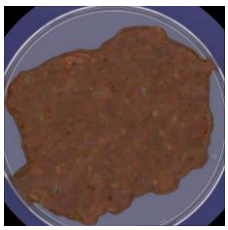
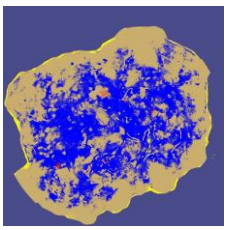
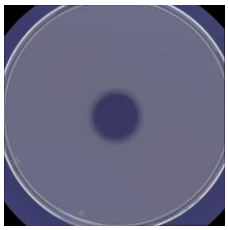
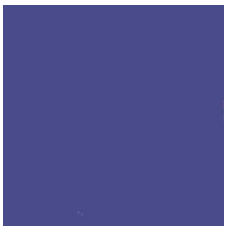
Sample Type	N	sRGB Image	Processed Image	Beef Meat Model – Mean % Area	Beef Heart Model - Mean % Area	Lamb Liver Model - Mean % Area
Chicken Liver	3			0.49 (0.29)	39.71 (1.14)	2.85 (0.85)
Petri Dish	1			0.00	0.00	0.00

Table 7. Tabulated data showing the percentage of sample replicates with % model area values passing a model specific threshold setting.

Calculated threshold based on the beef meat, beef heart and lamb liver model mean % model area values and the associated 1.96 standard deviation (assumed to capture 95% of data). Values based on 1 to 6 technical replicates per sample type (N). Model thresholds values: 95.68 (Beef Meat), 14.82 (Beef Heart) and 0.58 (Lamb Liver).

Sample Type	N	% Replicates passing a detection threshold: Beef Meat Analysis	% Replicates passing a detection threshold: Beef Heart Analysis	% Replicates passing a detection threshold: Lamb Liver Analysis
Beef Meat	6	100.0	0.0	0.0
Beef Heart	3	0.0	100.0	100.0
Beef Liver	3	0.0	0.0	100.0

Sample Type	N	% Replicates passing a detection threshold: Beef Meat Analysis	% Replicates passing a detection threshold: Beef Heart Analysis	% Replicates passing a detection threshold: Lamb Liver Analysis
Lamb Heart	3	0.0	100.0	100.0
Lamb Liver	3	0.0	100.0	100.0
Lamb Meat	3	0.0	100.0	0.0
Chicken Liver	3	0.0	100.0	100.0
Petri Dish	1	0.0	0.0	0.0

Table **7** summarises the specificity performance of the three developed models against the model building test samples. This shows that the beef meat model is specific to the beef meat sample type (100% detection) whilst the beef heart and lamb liver models demonstrate specificity issues with non-target offal materials (although the beef heart model does not detect lamb liver materials). The methods were therefore considered as demonstrating promising performance characteristics suited to initial screening techniques for multiple targets and were subject to further method validation.

7.1.3.2.3 Method Validation

Three models were evaluated as part of the method validation exercise in order to prioritise the best performing method(s) for testing for the presence of offal in beef meat. These methods were based on classification models of beef meat, beef heart and lamb liver. Following method validation the beef meat classification model lacked discriminatory potential, even with samples containing a high adulteration level from offal. The method based on the lamb liver classification model performed better, but had a compromised limit of detection compared to the method based on the beef heart model. For these reasons, the results from the beef heart classification model only are presented here as the validated method.

Table 8. Tabulated data showing the pooled mean % model area ± 95% confidence interval associated with the beef heart classification model and the percentage of sample replicates with % model area values passing a model specific threshold setting.

Calculated threshold based on the beef heart model mean % model area values for the beef meat control and the associated 1.96 standard deviation (assumed to capture 95% of data). 'CS' prefix refers to challenge sample type. Values based on 2 experiments comprising 3 technical replicates per sample.

Samples	% Model Area: Mean	%Model Area :± 95% CI	% Replicates passing the detection threshold
Beef Meat	0.00	0.00	0.00
Beef Heart	91.82	1.14	100.00
Beef Liver	6.49	0.73	100.00
75% Beef Heart in Beef	80.79	3.72	100.00
50% Beef Heart in Beef	46.49	13.82	100.00
25% Beef Heart in Beef	5.23	4.88	100.00
10% Beef Heart in Beef	0.00	0.00	16.67
5% Beef Heart in Beef	0.00	0.00	66.67
1% Beef Heart in Beef	0.00	0.00	33.33
0.1% Beef Heart in Beef	0.00	0.00	50.00
CS_30% Beef Heart in Beef	0.16	0.15	100.00
CS_1% Beef Heart in Beef	0.00	0.00	33.33

CS_30% Lamb Liver in Beef	62.80	5.58	100.00
CS_1% Lamb Liver in Beef	0.00	0.00	33.33
Lamb Meat	40.52	7.51	100.00
Lamb Heart	80.19	1.90	100.00
Lamb Liver	24.21	2.57	100.00
Petri Dish	0.00	0.00	0.00

7.1.3.2.4 Method validation summary

A method was developed and validated for detection of beef heart (offal) in a beef meat background using a classification model based on a beef heart reference material. Based on the samples used in this controlled experiment, the method was capable of detecting the presence of beef heart with a detection limit of <25% beef heart in beef meat (w/w). The analytical measurement uncertainty associated with the method was 116.46% (expressed as a CV) at this level, even though 100% of all test samples were correctly classified. The validated method showed no cross reactivity with the background of beef meat, but did exhibit cross reactivity with other offal samples (beef liver, lamb heart, lamb liver) as well as some cross reactivity with lamb meat.

7.1.3.3 Ground almond contaminated/adulterated with ground peanut

7.1.3.3.1 Method Scope

The scope of the two methods are as qualitative MSI-based screening methodologies which have been developed to analyse ground deshelled almond samples adulterated/contaminated with ground deshelled peanut based on the application of classification models built from representative matrices to surface area analyses.

Threshold normalisation is employed that is dependent upon control materials that

are representative of the test materials. The methods have been validated on a range of ground almond and ground peanut samples (multiple suppliers) but it is important that the methods are verified as being fit for purpose when applied by another laboratory, as per standard best measurement practice guidance.

7.1.3.3.2 Method Development

Initial method development activities focussed on the spectral profiles associated with the ground almond and potential adulterant materials to determine whether a proposed MSI-based approach was achievable. Figure 3 shows a comparison of reflectance spectra for a selection of materials associated with the sampling scenario and highlights differences in reflectance spectra, notably between almond and peanut materials. These observed spectral differences in material type are likely translatable to workable classification models.

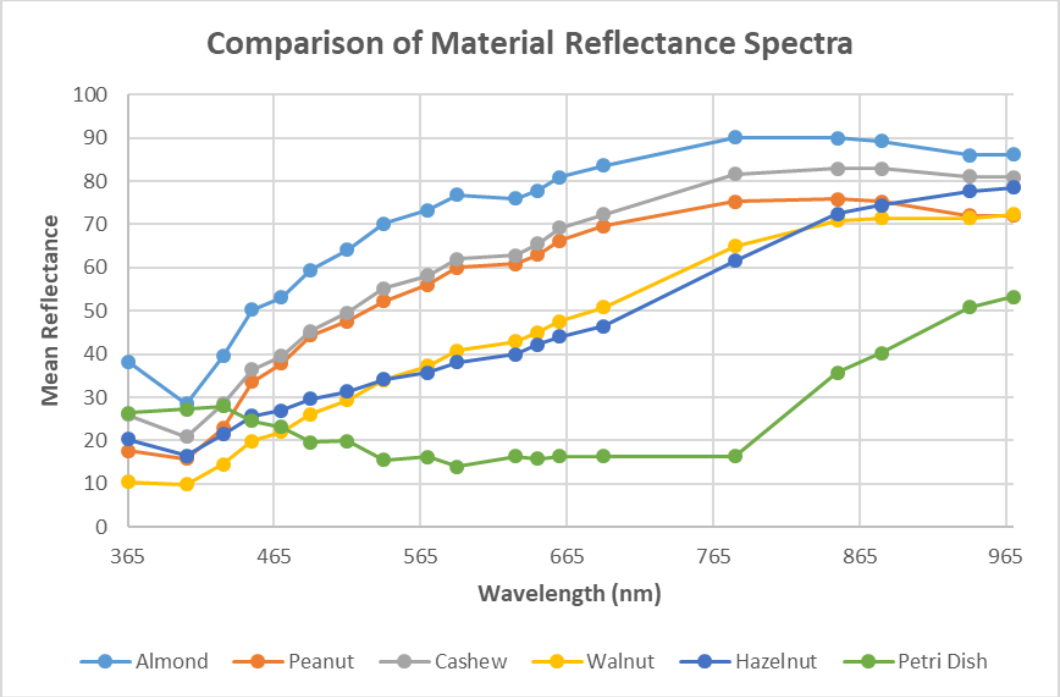


Figure 3. Typical VL4 generated reflectance spectra for ground almond and associated ground nut materials

Table 9 shows the relative performance of the MSI methodology against the development sample set and highlights the good specificity associated with the ground almond model. For example, the peanut sample type has a pooled mean ±

95% confidence interval % model area value of 2.43±1.96 as compared to the target almond sample type at 91.02±6.88.

The adulterant model developed against the peanut reference materials also demonstrate clear differences between the almond and adulterant sample types, as demonstrated by low % model area values for the almond materials and high values for adulterant peanut materials. For example, the peanut model analyses of the almond and peanut material shows % model area values of 8.10±8.93 and 96.09±1.39 respectively. The typically tight (low) precision associated with the sample replicates supports the general repeatability of the method.

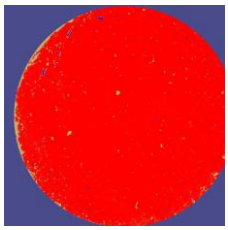
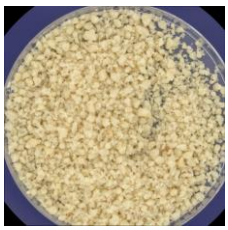
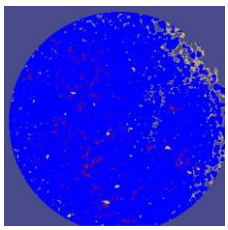
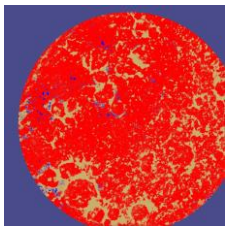
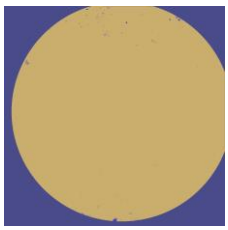
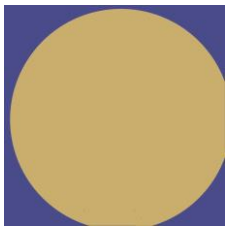
The threshold normalisation approach developed as part of the almond method development/validation process was applied to the % model area data based on either using the 100% w/w almond control sample as the reference (almond model) or compensating for the almond background component within the adulterant model (peanut model).

Table 10 summarises the specificity performance of the two developed models against the model building test samples. The almond model is specific to the almond sample type (100% detection), with some specificity issues associated with the cashew sample type, whilst the peanut model demonstrates good specificity characteristics as shown by targeted detection of the peanut sample type (100% detection). The methods were therefore considered as demonstrating promising performance characteristics suited to initial screening techniques for multiple targets, and were subject to further method validation.

Table 9. MSI analyses of the model building test samples using the almond adulteration method.

Processed image presented in a false colour format: red areas (almond sample type), beige areas (general sample type) and blue areas (peanut sample type). The pooled sample type mean % area matching a specific model based on area fractional calculation (along with the associated 95 % confidence interval in parentheses) are shown. Values based on 1-15 technical replicates per sample (N). Please note that the processed image represents a composite rendering from both

sample type models and will preferentially display the model showing the highest mean % content.

Sample Type	N	sRGB Image	Processed Image	Almond Model – Mean % Area	Peanut Model - Mean % Area
Almond	15			91.02 (6.88)	8.10 (8.93)
Peanut	9			2.43 (1.96)	96.09 (1.39)
Cashew	3			75.12 (4.18)	2.69 (0.36)
Walnut	3			0.00 (0.00)	0.29 (0.23)
Hazelnut	3			0.00	0.00

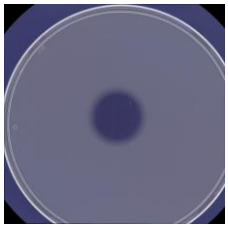
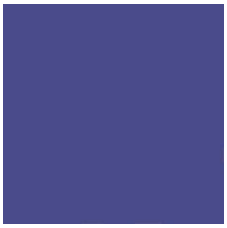
Sample Type	N	sRGB Image	Processed Image	Almond Model – Mean % Area	Peanut Model - Mean % Area
Petri Dish	1			0.00	0.00

Table 10. Tabulated data showing the percentage of sample replicates with % model area values passing a model specific threshold setting.

Calculated threshold based on the almond and peanut model mean % model area values and the associated 1.96 standard deviation (assumed to capture 95% of data). Values based on 1 to 15 technical replicates per sample type (N). Model thresholds values: 79.11 (Almond) and 23.56 (Peanut).

Sample Types	N	% Replicates passing a detection threshold: Almond Analysis	% Replicates passing a detection threshold: Peanut Analysis
Almond	15	100.0	0.0
Peanut	9	0.0	100.0
Cashew	3	33.3	0.0
Walnut	3	0.0	0.0

Hazelnut	3	0.0	0.0
Petri Dish	1	0.0	0.0

7.1.3.3.3 Method Validation

Two models were evaluated as part of the method validation exercise in order to test their fitness for purpose for assessing ground almond samples for the possible presence of ground peanut. These methods used classification models based on ground almond and ground peanut. The method based on the ground almond classification model performed well and is reported below. The method based on the ground peanut classification model outperformed expectations significantly, raising doubts as to whether the performance was correct or an artefact as a result of the experimental conditions used. However, the level of replication used in the validation provided supporting evidence as to this good performance of the ground peanut classification model, so the method validation results are also reported here.

Table 11. Tabulated data showing the pooled mean % model area ± 95% confidence interval associated with the ground almond classification model and the percentage of sample replicates with % model area values passing a model specific threshold setting.

Calculated threshold based on the almond model mean % model area values for the almond control and the associated 1.96 standard deviation (assumed to capture 95% of data). ‘CS’ prefix refers to challenge sample type. Values based on 2 experiments comprising 3 technical replicates per sample.

Samples	% Model Area: Mean	%Model Area: ± 95% CI	% Replicates passing a detection threshold:
100% Almond Mix	94.34	2.64	100.00
100% Peanut Mix	2.86	0.46	0.00

Samples	% Model Area: Mean	%Model Area: ± 95% CI	% Replicates passing a detection threshold:
75% Peanut in Almond	26.46	1.21	0.00
50% Peanut in Almond	62.56	2.99	0.00
25% Peanut in Almond	79.75	2.40	0.00
10% Peanut in Almond	90.97	2.76	83.33
5% Peanut in Almond	92.41	1.50	66.67
1% Peanut in Almond	93.59	1.85	50.00
0 1% Peanut in Almond	95.03	1.32	83.33
100% Cashew	77.83	3.42	0.00
100% Hazelnut	0.00	0.00	0.00
100% Walnut	0.00	0.00	0.00
CS_1% Peanut in Almond	91.63	2.95	66.67
CS_30% Peanut in Almond	84.05	1.76	16.67
Petri Dish	0.00	0.00	0.00

Table 12. Tabulated data showing the pooled mean % model area ± 95% confidence interval associated with the ground peanut classification model and the percentage of sample replicates with % model area values passing a model specific threshold setting.

Calculated threshold based on the peanut model mean % model area values for the almond control and the associated 1.96 standard deviation (assumed to capture 95% of data). ‘CS’ prefix refers to challenge sample type. Values based on 2 experiments comprising 3 technical replicates per sample.

Samples	% Model Area: Mean	%Model Area: ± 95% CI	% Replicates passing a detection threshold:
100% Almond Mix	8.54	1.32	0.00
100% Peanut Mix	96.74	0.47	100.00
75% Peanut in Almond	97.04	0.40	100.00
50% Peanut in Almond	82.72	1.44	100.00
25% Peanut in Almond	51.35	7.38	100.00
10% Peanut in Almond	19.73	1.07	100.00
5% Peanut in Almond	18.93	2.14	100.00
1% Peanut in Almond	14.71	2.71	100.00
0.1% Peanut in Almond	14.09	1.38	100.00
100% Cashew	8.18	2.34	16.67
100% Hazelnut	0.00	0.00	0.00
100% Walnut	0.38	0.15	0.00
CS_1% Peanut in Almond	13.99	3.93	83.33
CS_30% Peanut in Almond	59.23	1.75	100.00
Petri Dish	0.00	0.00	0.00

7.1.3.3.4 Method Validation Summary

Two alternative but complimentary methods were validated for analysis of ground almond adulterated with ground peanut.

A method was developed and validated for detection of ground peanut in ground almond using a classification model based on ground 100% almond reference material. Based on the samples used in this controlled experiment, the method was capable of detecting the presence of ground peanut with a detection limit of <25% ground peanut in ground almond (w/w). The analytical measurement uncertainty

associated with the method was 3.76% (expressed as a CV) at this level. The validated method showed no cross reactivity with ground samples derived from cashew, hazelnut or walnut.

The second method was developed and validated for detection of ground peanut in ground almond using a classification model based on ground 100% peanut reference material. Based on the samples used in this controlled experiment, the method was capable of detecting the presence of ground peanut with a detection limit of <5% ground peanut in ground almond (w/w) (based on the “Test_1% Peanut in Almond” test sample as not being detected as having peanut present on ≥95% of occasions). The analytical measurement uncertainty associated with the method was 14.14% (expressed as a CV) at this level. The validated method showed some cross reactivity with ground samples derived from cashew nuts and therefore may not be able to distinguish between the presence of adulterant peanut and cashew.

As previously mentioned, the performance of the method based on the ground peanut classification model exceeded all expectations. Whilst the validation data supported this, it is important that the performance of this method is further verified when transferred to other laboratories, as per standard laboratory best measurement practice when implementing a new method.

7.1.3.4 Beef adulterated with pork meat

7.1.3.4.1 Method Scope

The scope of the method is a qualitative MSI-based screening methodology developed to analyse ground beef meat samples adulterated with ground pork meat based on the application of classification models built from representative matrices to surface area analyses.

Threshold normalisation is employed that is dependent upon control materials that are representative of the test materials. The method has been validated on a range of samples derived from fresh beef and meat cuts (sourced from multiple suppliers/animals), trimmed to remove intra-muscular fat and then ground. It is important that the method is verified as being fit for purpose when applied by another laboratory, as per standard best measurement practice guidance.

7.1.3.4.2 Method Development

Initial method development activities focussed on the spectral profiles associated with the ground beef meat and potential meat adulterant materials to determine whether a proposed MSI-based approach was achievable. Figure 4 shows a comparison of reflectance spectra for a selection of materials associated with the sampling scenario and highlights differences in reflectance spectra, notably between the ground beef and ground pork materials. These observed spectral differences in material type are likely translatable to workable classification models.

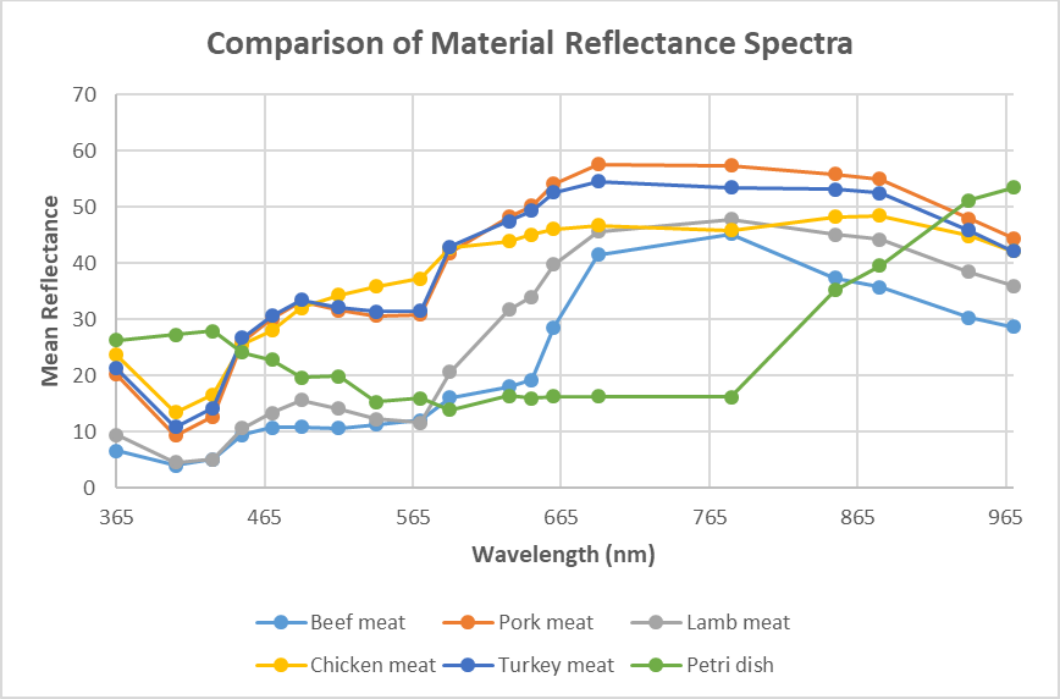


Figure 4. Typical VL4 generated reflectance spectra for ground beef meat and associated ground meat materials

Table 13 shows the relative performance of the MSI methodology against the development sample set and highlights the good specificity associated with the ground beef model. For example, the pork meat sample type has a pooled mean ± 95% confidence interval % model area value of 0.00 as compared to the target beef meat sample type at 94.26±1.12.

The adulterant model developed against the pork meat reference materials also demonstrates clear differences between the beef meat and adulterant sample types,

as demonstrated by low % model area values for the beef meat materials and high values for adulterant meat materials. For example, the pork meat model analyses of the beef meat and pork meat material shows % model area values of 0.02±0.04 and 94.48±1.42 respectively .The turkey meat sample type response was found to be higher than other meat materials, but is still sufficiently different to pork. The typically tight (low) precision associated with the sample replicates supports the general repeatability of the method.

The threshold normalisation approach developed as part of the beef method development/validation process was applied to the % model area data based on either using the 100% w/w beef meat control sample as the reference (beef meat model) or compensating for the beef meat background component within the adulterant model (pork meat model).

Table 14 summarises the specificity performance of the two developed models against the model building test samples. This shows that the beef meat model is specific to the beef meat sample type (100% detection) whilst the pork meat model demonstrates relatively poor specificity characteristics as shown by the detection of all adulterant type materials (50-100% detection). The method based on the beef meat model was therefore considered as demonstrating promising performance characteristics suited to an initial screening technique for multiple targets, and was subject to further method validation.

Table 13. MSI analyses of the model building test samples using the beef adulteration method.

Processed image presented in a false colour format: red areas (beef meat sample type), beige areas (general sample type) and blue areas (pork meat sample type). The pooled sample type mean % area matching a specific model based on area fractional calculation (along with the associated 95 % confidence interval in parentheses) area shown. Values based on 1-6 technical replicates per sample (N). Please note that the processed image represents a composite rendering from both sample type models and will preferentially display the model showing the highest mean % content.

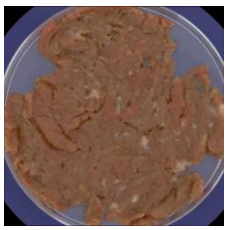
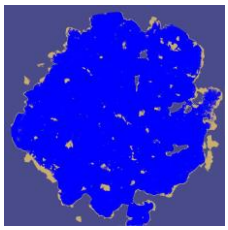
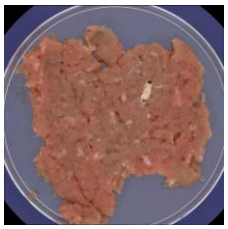
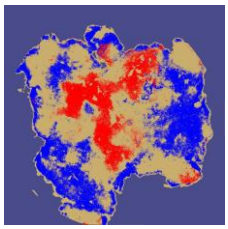
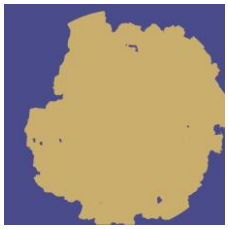
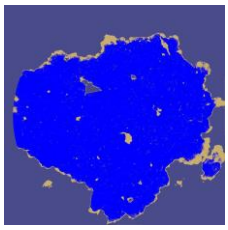
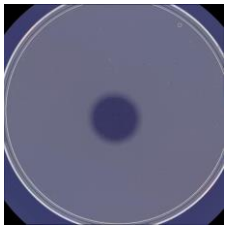
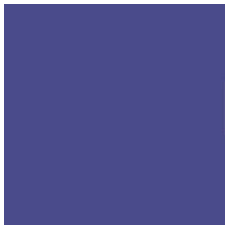
Sample Type	N	sRGB Image	Processed Image	Beef Meat Model – Mean % Area	Pork Meat Model - Mean % Area
Beef Meat	6			94.26 (1.12)	0.02 (0.04)
Pork Meat	6			0.00	94.48 (1.42)
Lamb Meat	6			6.62 (8.47)	24.61 (11.54)
Chicken Meat	6			0.00 (0.00)	1.91 (2.37)
Turkey Meat	6			0.00	87.85 (4.49)
Petri Dish	1			0.00	0.00

Table 14. Tabulated data showing the percentage of sample replicates with %

model area values passing a model specific threshold setting.

Calculated threshold based on the beef and pork meat model mean % model area values and the associated 3 standard deviation (assumed to capture 99.7% of data). Values based on 6 technical replicates per sample type except for the Petri Dish sample type at 1 technical replicate. Model thresholds values: 91.30 (Beef) and 0.13 (Pork).

Sample Type	% Replicates passing a detection threshold: Beef Analysis	% Replicates passing a detection threshold: Pork Analysis
Beef	100.0	0.0
Pork	0.0	100.0
Lamb	0.0	100.0
Chicken	0.0	50.0
Turkey	0.0	100.0
Petri Dish	0.0	0.0

7.1.3.4.3 Method Validation

Two models were evaluated as part of the method validation exercise in order to prioritise the best performing method(s) for testing for the presence of pork meat in a background of beef meat. These methods were based on classification models of beef meat and pork meat respectively. Following method validation, the pork meat classification model was shown to have a compromised limit of detection as well as exhibiting significant cross reactivity with other common meat species including lamb, chicken and turkey. For these reasons, the results from the beef meat classification model only are presented here as the validated method (Table 15).

Table 15. Tabulated data showing the pooled mean % model area ± 95%

confidence interval associated with the beef meat classification model and the percentage of sample replicates with % model area values passing a model specific threshold setting.

Calculated threshold based on the beef meat model mean % model area values for the beef meat control and the associated 1.96 standard deviation (assumed to capture 95% of data). 'CS' prefix refers to challenge sample type. Values based on 2 experiments comprising 3 technical replicates per sample.

Sample	Mean	± 95% CI	% Replicates passing the detection threshold
100% Beef	95.14	0.85	100.00
100% Pork	0.00	0.00	0.00
75% Pork in Beef	49.35	2.92	0.00
50% Pork in Beef	84.87	2.18	0.00
25% Pork in Beef	90.49	1.01	0.00
10% Pork in Beef	93.15	1.74	66.67
5% Pork in Beef	95.87	0.56	100.00
1% Pork in Beef	94.72	0.89	66.67
0.1% Pork in Beef	93.58	0.77	50.00
100% Lamb	14.10	7.91	0.00
100% Chicken	0.00	0.00	0.00
100% Turkey	0.00	0.00	0.00
CS_1% Pork in Beef	94.13	1.36	83.33
CS_30% Pork in Beef	91.45	1.70	16.67
Petri Dish	0.00	0.00	0.00

7.1.3.4.4 Method Validation Summary

A method was developed and validated for detection of pork meat in beef meat using a classification model based on a 100% beef meat reference material. Based on the samples used in this controlled experiment, the method was capable of detecting the presence of pork meat with a detection limit of <25% pork meat in beef meat (w/w). The analytical measurement uncertainty associated with the method varied between 1.40 and 7.39% (expressed as a CV) across the working range of the method. The validated method showed no cross reactivity with other common meat materials including lamb, chicken and turkey.

7.1.3.5 Paprika adulterated/contaminated with ground almond

7.1.3.5.1 Method Scope

The scope of the method is a qualitative MSI-based screening methodology developed to analyse paprika powder samples adulterated/contaminated with ground deshelled almond based on the application of classification models built from representative matrices to surface area analyses.

Threshold normalisation employed is dependent upon control materials that are representative of the test materials. The method has been validated on a mixed paprika sample (Sweet paprika, sweet smoked paprika, 75 and 75 ASTA paprika sourced from multiple suppliers) and ground almond (multiple suppliers), but it is important that the method is verified as being fit for purpose when applied by another laboratory, as per standard best measurement practice guidance.

7.1.3.5.2 Method Development

Initial method development activities focussed on the spectral profiles associated with the paprika and potential adulterant materials to determine whether a proposed MSI-based approach was achievable. Figure 5 shows a comparison of reflectance spectra for a selection of materials associated with the sampling scenario and highlights differences in reflectance spectra, with good spectral separation between the paprika and nut sample types. These observed spectral differences in material type are likely translatable to workable classification models.

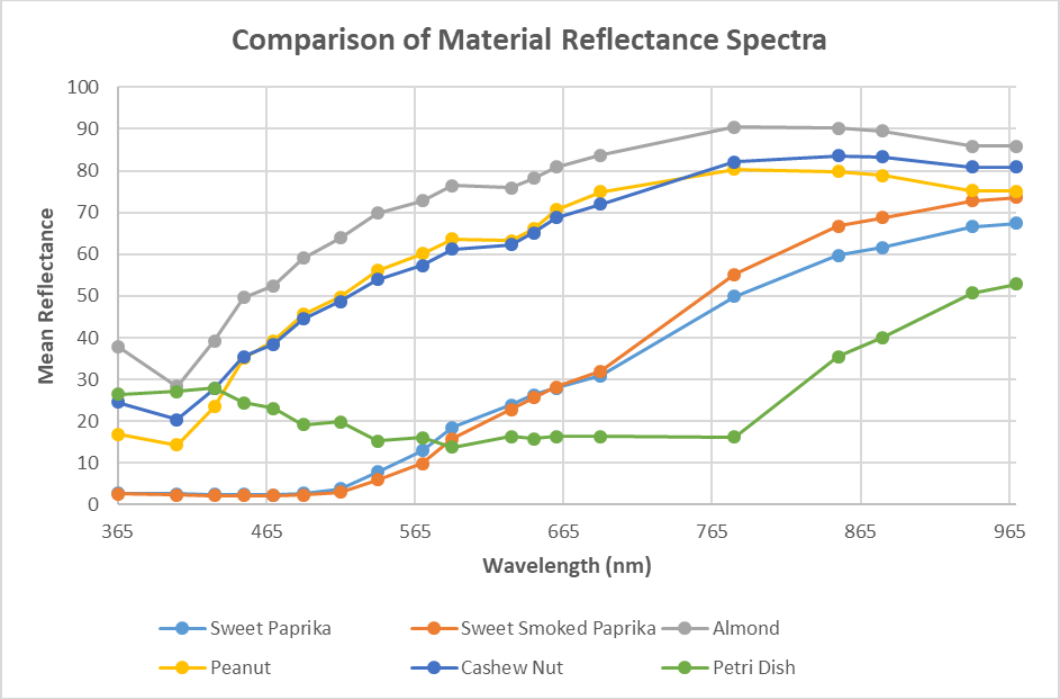


Figure 5. Typical VL4 generated reflectance spectra for paprika and nut type materials

Table 16 shows the relative performance of the MSI methodology against the development sample set and highlights the good specificity associated with the paprika model. For example, the almond sample type has a pooled mean ± 95% confidence interval % model area value of 0.00 as compared to the target paprika sample type at 98.83±0.82. Paprika related materials such as cayenne pepper and chilli also provided strong measurement responses.

The adulterant model developed against the almond reference materials also demonstrates clear differences between the paprika and adulterant sample types, as demonstrated by low % model area values for the paprika materials and high values for the almond, cashew and peanut adulterant nut materials. For example, the almond model analyses of the paprika and almond materials shows % model area values of 0.00 and 96.85±2.79 respectively. The cashew and peanut sample type response was found to be higher than other nut materials, but still sufficiently different to almond. The typically tight (low) precision associated with the sample replicates supports the general repeatability of the method.

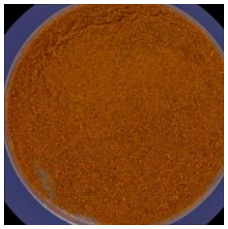
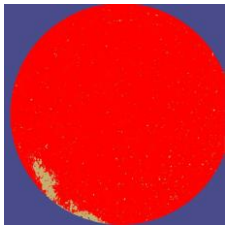
The threshold normalisation approach developed as part of the paprika method development/validation process was applied to the % model area data based on

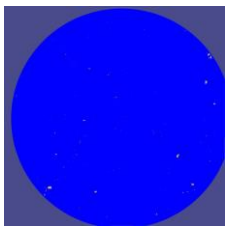
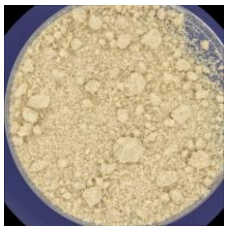
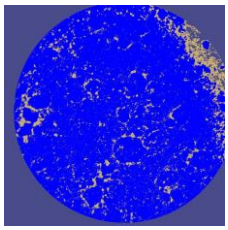
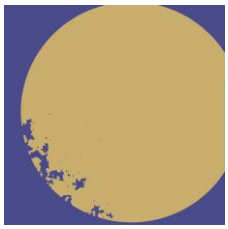
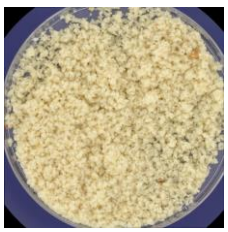
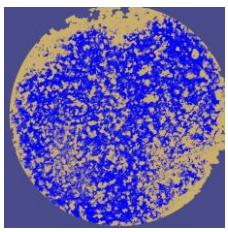
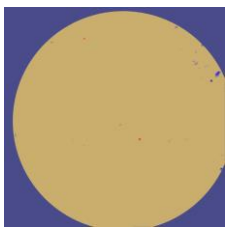
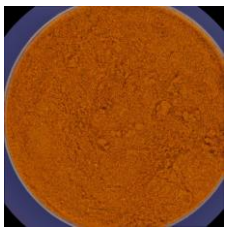
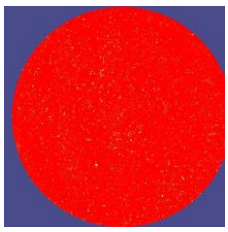
either using the 100% w/w paprika control sample as the reference (paprika model) or compensating for the paprika background component within the adulterant model (almond model).

Table 17 summarises the specificity performance of the two developed models against the model building test samples. This shows that the paprika model is specific to the paprika sample type (100% detection) whilst the almond model demonstrates relatively poor specificity characteristics as shown by the detection of all the tested nut adulterant type materials (100% detection). The method based on the paprika model was therefore considered as demonstrating promising performance characteristics suited to an initial screening technique for multiple targets, and was subject to further method validation.

Table 16. MSI analyses of the model building test samples using the paprika adulteration method.

Processed image presented in a false colour format: red areas (paprika sample type), beige areas (general sample type) and blue areas (almond sample type). The pooled sample type mean % area matching a specific model based on area fractional calculation (along with the associated 95% confidence interval in parentheses) are shown. Values based on 1-24 technical replicates per sample (N). Please note that the processed image represents a composite rendering from both sample type models and will preferentially display the model showing the highest mean % content.

Sample Type	N	sRGB Image	Processed Image	Paprika Model – Mean % Area	Almond Model - Mean % Area
Paprika	24			98.83 (0.82)	0.00

Sample Type	N	sRGB Image	Processed Image	Paprika Model – Mean % Area	Almond Model - Mean % Area
Almond	12			0.00	96.85 (2.79)
Cashew	3			0.00	91.89 (3.08)
Hazelnut	3			0.01 (0.01)	0.00 (0.00)
Peanut	3			0.00	49.29 (1.36)
Walnut	3			0.02 (0.00)	0.06 (0.05)
Cayenne Pepper	3			95.11 (1.41)	0.00

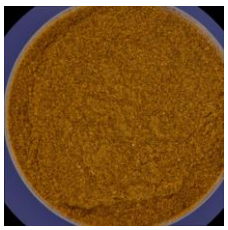
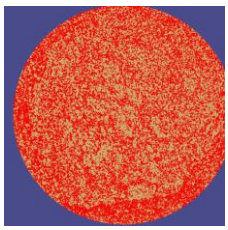
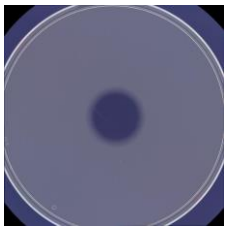
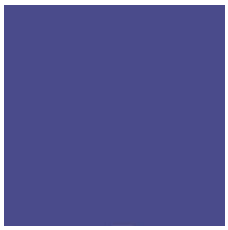
Sample Type	N	sRGB Image	Processed Image	Paprika Model – Mean % Area	Almond Model - Mean % Area
Chilli	3			51.39 (0.93)	0.00
Petri Dish	1			0.00	0.00

Table 17. Tabulated data showing the percentage of sample replicates with % model area values passing a model specific threshold setting.

Calculated threshold based on the paprika and almond model mean % model area values and the associated 1.96 standard deviation (assumed to capture 95% of data). Values based on 1 to 24 technical replicates per sample type (N). Model thresholds values: 91.30 (paprika) and 0.13 (almond).

Sample Types	N	%Replicates passing a detection threshold: Paprika Analysis	%Replicates passing a detection threshold: Almond Analysis
Paprika	24	100.0	0.0
Almond	12	0.0	100.0
Cashew	3	0.0	100.0
Hazelnut	3	0.0	100.0
Peanut	3	0.0	100.0

Walnut	3	0.0	100.0
Cayenne	3	0.0	0.0
Chilli	3	0.0	0.0
Petri Dish	1	0.0	0.0

7.1.3.5.3 Method Validation

Two models were evaluated as part of the method validation exercise in order to prioritise the best performing method(s) for testing for the almond adulteration/contamination in ground paprika samples. These methods were based on classification models of ground paprika and ground almond samples respectively. Following method validation the almond classification model was shown to demonstrate a reduced discriminatory power even when paprika samples had high levels of almond present, as well as the method demonstrating cross reactivity with cashew, hazelnut and peanut. For these reasons, the results from the paprika classification model only are presented here as the validated method (**Table 18**).

Table 18. Tabulated data showing the pooled mean % model area ± 95% confidence interval associated with the paprika classification model and the percentage of sample replicates with % model area values passing a model specific threshold setting.

Calculated threshold based on the paprika model mean % model area values for the paprika control and the associated 1.96 standard deviation (assumed to capture 95% of data). 'CS' prefix refers to challenge sample type. Values based on 2 experiments comprising 3 technical replicates per sample.

Sample Types	%Model Area: Mean	% Model Area: ± 95% CI	% Replicates passing the detection threshold
100% Paprika Mix	99.18	0.16	100.00
100% Almond Mix	0.00	0.00	0.00

Sample Types	%Model Area: Mean	% Model Area: ± 95% CI	% Replicates passing the detection threshold
75% Almond in Paprika	19.90	2.12	0.00
50% Almond in Paprika	72.11	1.90	0.00
25% Almond in Paprika	92.92	0.98	0.00
10% Almond in Paprika	97.66	0.10	0.00
5% Almond in Paprika	98.45	0.43	33.33
1% Almond in Paprika	98.82	0.49	66.67
0.1% Almond in Paprika	99.14	0.44	83.33
100% Cashew	0.00	0.00	0.00
100% Hazelnut	0.00	0.00	0.00
100% Peanut	0.00	0.00	0.00
CS_1% Almond in Paprika	98.82	0.75	83.33
CS_30% Almond in Paprika	90.96	0.98	0.00
Petri Dish	0.00	0.16	0.00

7.1.3.5.4 Method Validation Summary

A method was developed and validated for detection of ground almond in ground paprika using a classification model based on a ground 100% paprika reference material. Based on the samples used in this controlled experiment, the method was capable of detecting the presence of ground almond with a detection limit of <10% almond in paprika (w/w). The analytical measurement uncertainty associated with the method varied between 0.13 and 13.35% (expressed as a CV) across the working range of the method. The validated method showed no cross reactivity with other common nut materials including cashew, hazelnut and peanut and is therefore

unlikely to show a measurement response when in the presence of these nut materials.

The presence of any significant amount of ground almond material in a background of paprika is likely to cause a heterogeneous sample granularity to form. This is because the powdered paprika material is likely to stick and coat the more oily almond material. It is therefore advisable to thoroughly mix the test sample prior to analyses to ensure a more even distribution of adulterant/contaminant almond material which will be recognised by the MSI method.

7.1.3.6 "White" fish speciation

7.1.3.6.1 Method Scope

The MSI-based screening methodology was developed with the aim to assign species identity to fresh whole white fish flesh samples based on a relative ranking approach using classification model specific surface area analyses. The method was tested based on fresh fish landed at British ports sourced at multiple time points from the same supplier. The method was developed with the aim to identify five fish species (haddock, cod, pollack, whiting and hake) sourced from UK-landed materials.

7.1.3.6.2 Method Development

Initial method development activities focussed on the spectral profiles associated with the selected fish species to determine whether a proposed MSI-based approach was achievable. Figure 6 shows a comparison of reflectance spectra for a selection of materials associated with the sampling scenario and highlights some differences in reflectance spectra. These observed spectral differences in material type are likely translatable to workable classification models.

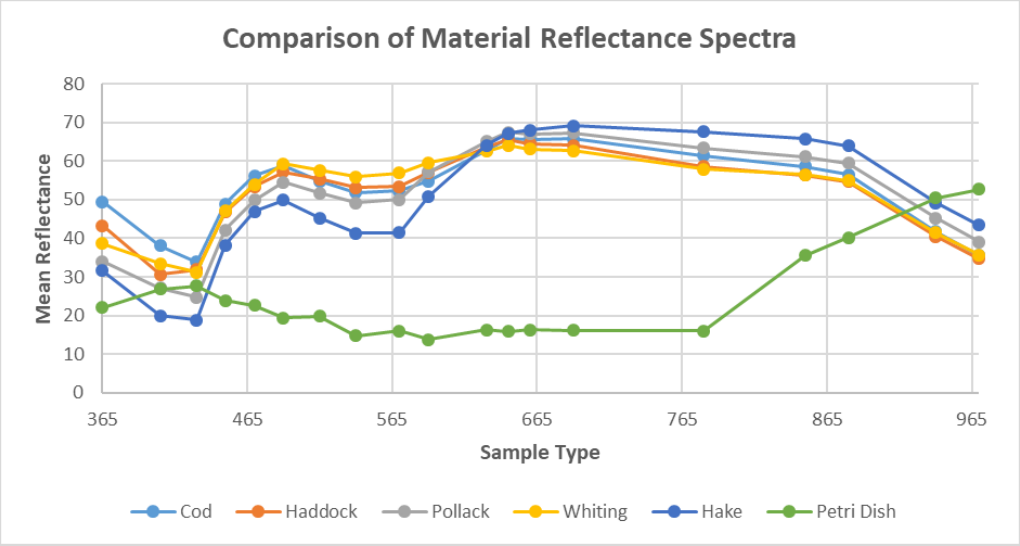


Figure 6. Typical VL4 generated reflectance spectra for the selected fish species

Table 19. MSI analyses of the model building test samples using the fresh fish speciation method.

Processed image presented in a false colour format: red areas (haddock sample type), beige areas (general sample type), blue areas (cod sample type), yellow areas (hake sample type), pink areas (pollack sample type) and green areas (whiting sample type). The pooled sample type mean % area matching a specific model based on area fractional calculation (along with the associated 95 % confidence interval in parentheses) are shown. Values based on 2 to 9 technical replicates per sample (N). Please note that the processed image represents a composite rendering from all five sample type models and will preferentially display the model showing the highest mean % area.

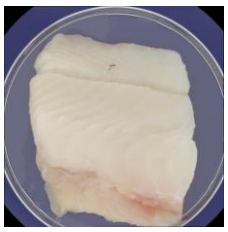
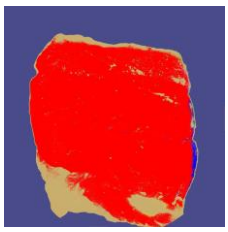
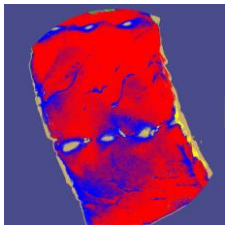
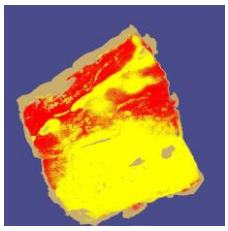
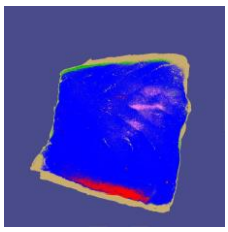
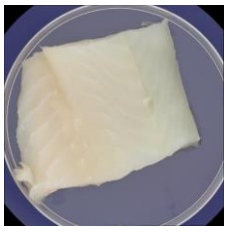
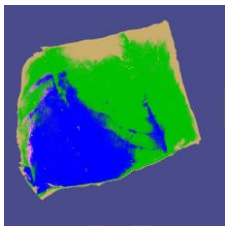
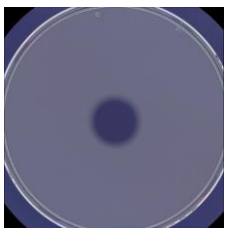
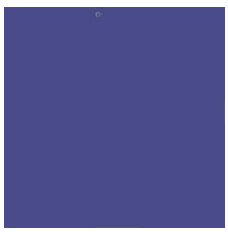
Sample Type	N	sRGB Image	Process Image	%Mean: Haddock	% Mean: Cod	% Mean: Hake	% Mean: Pollack	% Mean: Whiting
Haddock	9			87.13 (5.79)	64.90 (15.69)	57.04 (24.85)	48.19 (13.90)	41.80 (19.38)
Cod	9			25.84 (31.44)	89.50 (4.74)	28.96 (37.03)	21.70 (13.87)	57.85 (18.50)
Hake	9			21.61 (12.71)	21.16 (30.90)	83.12 (3.64)	12.28 (22.35)	11.59 (17.42)
Pollack	9			51.55 (38.20)	63.08 (20.27)	43.67 (39.18)	84.16 (6.09)	65.26 (20.79)
Whiting	9			34.81 (27.25)	64.35 (15.48)	11.63 (16.44)	48.15 (21.30)	83.84 (7.60)
Petri Dish	2			0.00	0.00	0.00	0.00	0.00

Table **19** shows the relative performance of the MSI methodology against the development sample set and highlights the good specificity associated with the all the fish models, as shown by the high pooled mean % model area values associated with the model target materials (>83 %). The associated precision was found to be poor (high) which is likely due to natural sample variability and suggests that the measurement uncertainty associated with this method is larger than observed in previous methods.

The standard threshold normalisation approach was not applied to this method as it had been developed to be a multi-screening approach based on the relative performance of each classification model when applied to a particular test sample. Therefore, a simple screening approach was employed based on fish specific models and the calculated % model areas ranked by value per sample to determine putative identity.

Table 20. Fish speciation method specificity results.

Training dataset (9 replicates per fish type) screened using fish specific models and the individual model specific % areas ranked by value to determine putative identity.

Sample Type	% of samples correctly identified
Haddock	100.00
Cod	100.00
Hake	100.00
Pollack	77.78
Whiting	100
Petri Dish	Not Detected

Table 20 summarises the specificity performance of the developed models against the model building test samples and shows that all the fish samples were correctly identified (100% detection) with the exception of the pollack sample type which showed lower specificity (77.78% detection). The methods were therefore

considered as demonstrating promising performance characteristics suited to initial screening techniques for multiple targets, and were subject to further method validation.

7.1.3.6.3 Method Validation

The fish speciation methodology incorporating multi-species models was evaluated to determine fitness for purpose. The discriminatory power of the method was found to be variable (**Table 21** and **Table 22**) with species specific biases which did not fit the basic criteria for a validated fish speciation method.

Table 21. Tabulated data showing the Pooled Mean % model area and associated data for each sample type. Values based on 2 experiments comprising 3 technical replicates per sample.

Sample	Pooled Mean % Model Area	Pooled Mean % Model Area	Pooled Mean % Model Area	Pooled Mean % Model Area	Pooled Mean % Model Area
Haddock	70.42	54.89	12.55	39.81	57.54
Cod	28.18	82.55	4.14	29.30	67.25
Hake	72.82	66.68	51.58	54.74	53.60
Pollack	17.09	18.72	0.50	27.30	26.33
Whiting	31.24	44.06	6.93	33.52	58.03
Petri Dish	0.00	0.00	0.00	0.00	0.00

Sample	Pooled 95% CI	Pooled 95% CI	Pooled 95% CI	Pooled 95% CI	Pooled 95% CI
Haddock	10.75	7.17	12.29	16.19	31.23
Cod	24.24	3.21	4.68	18.89	15.94

Sample	Pooled 95% CI	Pooled 95% CI	Pooled 95% CI	Pooled 95% CI	Pooled 95% CI
Hake	13.27	11.31	15.50	17.96	21.45
Pollack	14.36	25.48	0.28	20.50	20.66
Whiting	14.59	20.02	5.60	19.92	14.35
Petri Dish	0.00	0.00	0.00	0.00	0.00

Table 22. Fish speciation method specificity results. Validation dataset (based on 2 experiments comprising 3 technical replicates per sample.) screened using fish specific models and the individual model specific % areas ranked by value to determine putative identity.

Fish Type	Correct Identification: A	Correct Identification: B	% of replicates correctly identified per sample type
Haddock	No	No	16.67
Haddock	No	Yes	16.67
Haddock	No	No	16.67
Cod	No	No	50.00
Cod	No	Yes	50.00
Cod	Yes	Yes	50.00
Hake	No	No	0.00
Hake	No	No	0.00
Hake	No	No	0.00
Pollack	Yes	Yes	50.00
Pollack	Yes	No	50.00
Pollack	No	No	50.00

Fish Type	Correct Identification: A	Correct Identification: B	% of replicates correctly identified per sample type
Whiting	Yes	Yes	100.00
Whiting	Yes	Yes	100.00
Whiting	Yes	Yes	100.00

7.1.3.6.4 Method Validation Summary

Whilst the method development approach based on a training set of different fish samples exhibited good potential for fish speciation, the subsequent method validation was not successful (**Table 21** and **Table 22**). The analytical variability associated with the spectral profiles for the test samples used in the validation exercise was too large to provide sufficient discriminatory potential for all five fish species in one test (Table 21). For this reason, the method is not recommended as being successfully validated at this stage.

The discriminatory potential of the model is dependent upon the scope of the dataset used to build the model. In the examples provided here, the scope of the method was developed as being very general to try to afford discrimination between five different white fish species commonly used in the UK. The dataset used for the initial method development was more restricted but demonstrated excellent discriminatory potential. This study is still therefore supportive of refining and developing the model further in order to fully capitalise upon the MSI's discriminatory potential for fish speciation. For example, should the analytical question be revised to "*Is this cod or has this been substituted for with pollack*?" where the MSI is just trying to differentiate between two white fish species (Table 21), then there is a much stronger likelihood of a method being successful.

7.1.3.7 Meat quality – impact of freeze thawing process on meat characteristics

7.1.3.7.1 Method Scope

The MSI-based screening methodology was developed to discriminate between

fresh or freeze-thawed ground pork or beef meat samples based on a threshold approach using classification model specific surface area analyses. The method was tested based on fresh beef and meat cuts (sourced from multiple suppliers/animals), trimmed to remove intra-muscular fat and then ground.

7.1.3.7.2 Method Development

Initial method development activities focussed on the spectral profiles associated with the fresh versus thawed meat materials to determine whether a proposed MSI-based approach was achievable. Figure 7 shows a comparison of reflectance spectra for a selection of materials associated with the sampling scenario and highlights limited differences in reflectance spectra based on relative reflectance changes. These limited spectral differences indicate that the model development process was likely to be challenging.

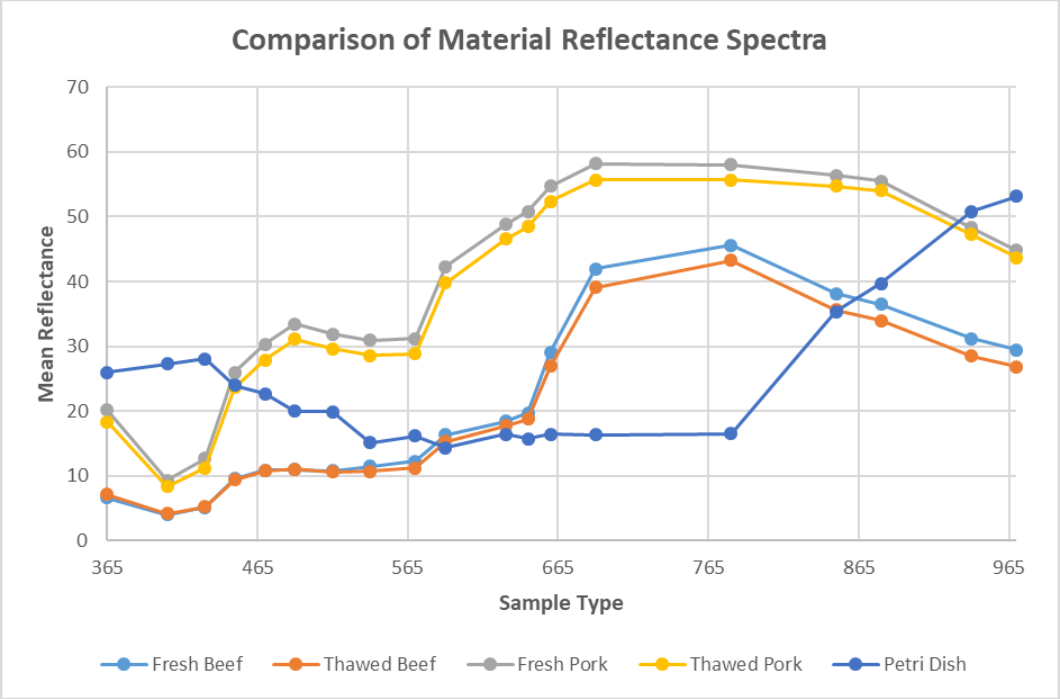


Figure 7. Typical VL4 generated reflectance spectra for fresh and freeze/thawed meat materials

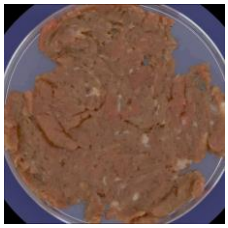
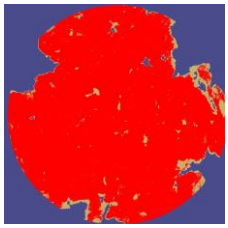
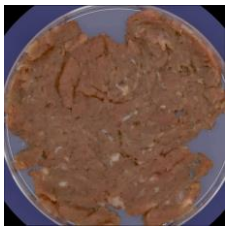
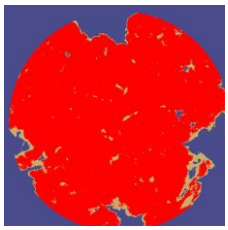
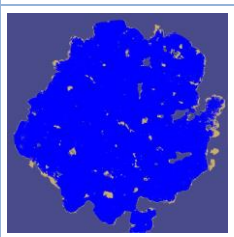
Table 23 shows the relative performance of the MSI methodology against the development sample set and highlights the poor discriminatory potential observed for all the developed classification models. For example, the fresh beef meat model

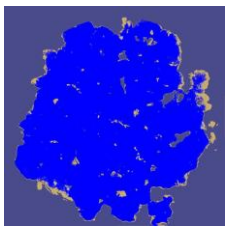
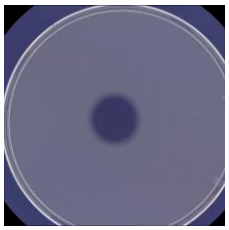
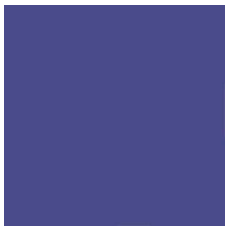
analyses of the fresh beef meat and thawed beef meat materials shows % model area values of 93.20±1.62 and 93.82±1.61 respectively. The method does demonstrate good (low) levels of precision as observed with other meat–based MSI methods.

Further method validation work was not performed due to the poor discriminatory potential of the models. Whilst spectral differences are observed between the two material states, these are not sufficient to build fit for purpose models to discriminate fresh versus thawed meat under these circumstances.

Table 23. MSI analyses of the model building test samples using the fresh/thawed meat quality method.

Processed image presented in a false colour format: red areas (fresh beef sample type), beige areas (general sample type), blue areas (fresh pork sample type), pink areas (thawed beef sample type) and pale green areas (thawed pork sample type). The pooled sample type mean % area matching a specific model based on area fractional calculation along with the associated 95 % confidence interval is shown. Values based on 2 to 6 technical replicates per sample (n). Please note that the processed image represents a composite rendering from all four sample type models and will preferentially display the model showing the highest mean % model area.

Sample Type	N	sRGB Image	Processed Image	Mean %: Fresh Beef	Mean %: Thawed Beef	Mean %: Fresh Pork	Mean %: Thawed Pork
Fresh Beef	6			93.20 (1.62)	89.97 (2.18)	0.02 (0.04)	0.01 (0.02)
Thawed Beef	6			93.82 (1.61)	93.07 (1.68)	0.00 (0.00)	0.00 (0.00)
Fresh Pork	6			0.00	0.00	96.01 (0.78)	94.30 (1.04)

Sample Type	N	sRGB Image	Processed Image	Mean %: Fresh Beef	Mean %: Thawed Beef	Mean %: Fresh Pork	Mean %: Thawed Pork
Thawed Pork	6			0.00	0.00	95.61 (1.24)	94.59 (1.29)
Petri Dish	2			0.00	0.00	0.00	0.00

7.1.3.7.3 Method Validation

An initial training set of a panel of fish samples was used to provide proof of principle of an MSI model for the differentiation of fresh and thawed fish samples. The results of this initial investigation were reported to the FSA as part of the Interim report (Task 1.4) associated with the current project.

Following further discussions the method was then adapted to test if it could be used to detect fresh and thawed samples in the more topical area of meat quality. Unfortunately, the models developed as part of this initial development exercise showed lack of any discriminatory potential within a species to differentiate between fresh and thawed tissue samples. The beef and pork models developed as part of this work could clearly differentiate between the beef and pork species used in the training data set, but this was not the purpose of the current validation.

7.1.3.7.4 Method Validation Summary

The poor discriminatory potential to differentiate between fresh and thawed meat materials within a species, as demonstrated by the overlapping spectral profiles (**Figure 7** and **Table 23**), precluded the method from further successful method

validation. For this reason, no successfully validated method for testing for fresh/thawed meat samples was provided at this stage.

7.1.4 Objective 1.4 - Interim report

The interim report was successfully submitted to the FSA.

7.2 Objective 2 - Written guidance to support general MSI validation studies.

7.2.1 Introduction

Multispectral imaging represents a novel technology with a broad range of analytical capabilities (e.g. non-targeted and multi-analyte functionalities) that are well suited to being applied to screening activities. Central to the application of MSI to food testing is appropriately validated methods that encompass the scope and analytical details. Developing fit for purpose validated MSI-based methods requires guidance and support. General MSI validation activities require a multi-step-wise approach which comprise processes such as method development and validation.

7.2.2 Scope

The following guidance is intended to support suitably trained personnel to facilitate the validation of a MSI compatible food sample in an analytical laboratory using the VideometerLab 4 MSI platform (Videometer A/S, Denmark) with associated VideometerLab software. The guidance is focussed on a simple ‘Area Fraction’-based approach as this provides good analytical flexibility and facilitates rapid method development. It should be noted that fine powders and liquid sample types are not recommended test analytes due to sensor limitations (fine powders), and the impact of light transmission within the medium and associated surface reflectance issues (liquids).

Please note that the following guidance was developed using the VideometerLab 4 instrument and VideometerLab Version 3.10.6 (6722) software (Videometer A/S, Denmark) and that future software versions may change/rename the software tools available to the user.

7.2.3 Initial Assessment of Analytical Requirements

A thorough understanding of the analytical requirements should be performed prior to any developmental work so that challenges can be identified and resourcing prioritised. This assessment should include:

- Providing a clear rationale for the analytical test and associated minimum performance requirements, e.g. specificity and sensitivity, single or multi-analyte method.
- A short literature study to determine whether spectral imaging-based approaches have been successfully applied to the test material/s or to a similar matrix.
- Investigating the availability of appropriate reference/control materials to facilitate standardisation/harmonisation.

7.2.4 Method Development

7.2.4.1 Sourcing materials, sampling and sample preparation strategy

- The developer should identify and source a range of test materials that capture key anticipated sample variability characteristics such as supplier, country of origin, processing and varietal (plant-based)/breed (animal-based) parameters. The materials should be representative of the test analyte and include related materials in order to characterise specificity. The selected panel of materials may be authenticated as required, e.g. if the supplier does not adhere to industry standard traceability systems.

7.2.4.2 Classification Model Building

- Initial scans of a representative panel of samples should be performed using the VideometerLab 4 system to determine the relative spectral profiles. The recommended approach comprises the following steps:
    a. Per sample, undertake a single full scan (all 19 bands) without filters (no band pass filters) at 100% light settings. The Light Setting may need to be optimised in the event that significant image saturation is observed and this optimised setting applied to all test scans.

b. Use the ‘Region of Interest’ tools to select representative areas to generate associated spectra.

c. The specific material spectra can be compared using the ‘Spectrum Measurement’ tool and used to determine whether sufficient spectral differences are available to develop a working MSI method.

- Assuming that sufficient spectral differences are observed between the analyte and associated materials, then the method development process should proceed as planned. In the event that limited/no spectral differences are observed, the developer may proceed with the understanding that the development process may not be successful.
- The developer should aim to develop classification models based on the following approach:

    a. Highlighting key areas of variability across multiple samples and incorporating into target specific Transformation files based on the ‘nMahalanobis‘ methodology which is a ‘Known vs Unknown’ approach and is recommended due the inherent flexibility of this type of analyses. During the Transformation development process, the developer should ensure that when applied to a subset of control samples, the anticipated discriminatory results are observed before continuing to the next step.

    b. Utilising representative Transformation files to build Segmentation files. These Segmentation files should be developed using an appropriate segmentation settings strategy. A recommended approach is to use the ‘Simple Threshold’ approach which is widely applicable to general area analyses without a requirement for blob analyses. During the Segmentation development process, the developer should ensure that when applied to a subset of control samples, the anticipated discriminatory results are observed before continuing to the next step.

- An appropriate number of separate classification models (Segmentations) should be generated covering the required analytes and the global background (e.g. plastic petri dish and blue sampling disc).

7.2.4.3 Session Development and Method Assessment

- The required segmentation files should be built into a Session file using the Session Manager incorporating the following features:
    a. Utilising the ‘MSI Area Fraction 3’ PlugIn Algorithm with the target analyte and background segmentation files.
    b. Sample area matching the segmentation model is presented as a % Area Fraction.
    c. The Session Recipe can be used with pre-scanned or fresh samples.
- The generated Session Recipe should be applied to the training MSI scans to confirm that the classification models work effectively and any specificity issues detailed. The minimum acceptable performance criteria should include a clear difference (dependent upon the analysis approach applied) between the test analytes and related materials and good repeatability, e.g. <25% CV.

7.2.5 Method Validation

A suitable method validation strategy is required to ensure that the developed methodology is fit for purpose by thorough demonstration of appropriate performance characteristics, e.g. specificity, sensitivity and repeatability. Comparison with a gold standard approach can be beneficial in benchmarking analytical performance. The following guidance is focussed on a single laboratory method validation with a range of control materials and gravimetrically prepared adulterated samples.

7.2.5.1 Method Validation Strategy

- Utilising expertise and the Session method (incorporating the classification models) built during the method development stage, the developer should formulate a clear and well-structured strategy that encompasses core areas such as sampling, sample preparation and analysis.

7.2.5.2 Sampling and Sample Preparation Strategy

- The results from the method development stage should inform the developer on the selection of an appropriate subset of 100% w/w control sample types

comprising the target analyte/s (e.g. food sample and adulterant) and non-target control samples.

- Bulk materials sufficient for the preparation of the planned set of gravimetrically prepared admixtures should be prepared and mixed/homogenised well.
- The sample preparation strategy developed during the method development stage should be adapted to this phase and incorporate the following key attributes:
    a. Gravimetrically prepared admixtures representative of the expected adulteration range which for simplicity could be standardised as 75%, 50%, 25%, 10%, 5%, 1% and 0.1% w/w adulterant in the target sample. The selected range should cover potential trace and gross adulteration levels.
    b. The use of 90 mm diameter single use Petri dishes are the recommended sample container due to the large sampling area and compatibility with VL4 instrument
    c. A consistent sample size which will vary dependent on the test materials, but is likely to range between 5 – 25g per petri dish.
    d. A suitable level of replication (minimum 3 technical replicates) should be incorporated so that sample variability can be assessed.
    e. Storage during and after sample preparation should be appropriate to the selected test materials, e.g. chilled, dark, air-tight.
    f. An empty petri dish should be included in the sample set to act as a background control.
- A minimum of two independent experiments (dependent on available materials and resourcing) should be prepared such that repeatability estimates can be determined.

7.2.5.3 Session Analyses

- In addition to using the VideometerLab software, the developer may wish to run the Session Recipe using the standalone VideometerSession software. This is limited to running the Session Recipes only and thereby minimise any user interaction with the development tools. The developed Session Recipe provides the developer with the flexibility to either directly scan and analyse the test samples or to analyse stored MSI images (.hips files).
- The developer should set the Light and Filter Setting to the parameters used in the method development process and either (i) independently scan the samples for later analyses using the Session Recipe, or (ii) use the Session Recipe for real-time sample imaging and data analyses.
- The Session generated dataset should then be processed using a software package such as Microsoft® Excel® 2016 (Microsoft Corporation) and key performance characteristics determined which should include:
    a. Specificity – assessing whether the developed methodology is specific (within scope) to the target material. This may involve applying a background threshold detection approach to % Area Fraction data which frequently shows limited specificity issues due to the nature of the robust classification models developed.
    b. Sensitivity – classically expressed as the lowest level at which the adulterant is no longer reliably detected (>95% confidence), termed the Limit of Detection (LOD).
    c. Repeatability – a method repeatability estimate such as percentage coefficient of variation (% CV) should be determined, e.g. a well performing analytical method should be <25% CV.
    d. Measurement uncertainty – an appropriately generated repeatability estimate gives a good idea of the key measurement uncertainty associated with a method.

- The collated performance characteristics should then be considered to determine whether the method is fit for purpose. Table 24 summarises suggested minimum key performance requirements.

Table 24. Example performance criteria associated with MSI based approaches to qualify a method towards being fit for purpose.

Performance Characteristic	Requirement
Specificity	Within method scope, no non-target materials are detected
Sensitivity	Appropriate limit of detection (LOD) is method dependent, ideally should be towards levels of EMA for a screening approach for example, 10% (w/w) or less
Repeatability	<25% CV or equivalent

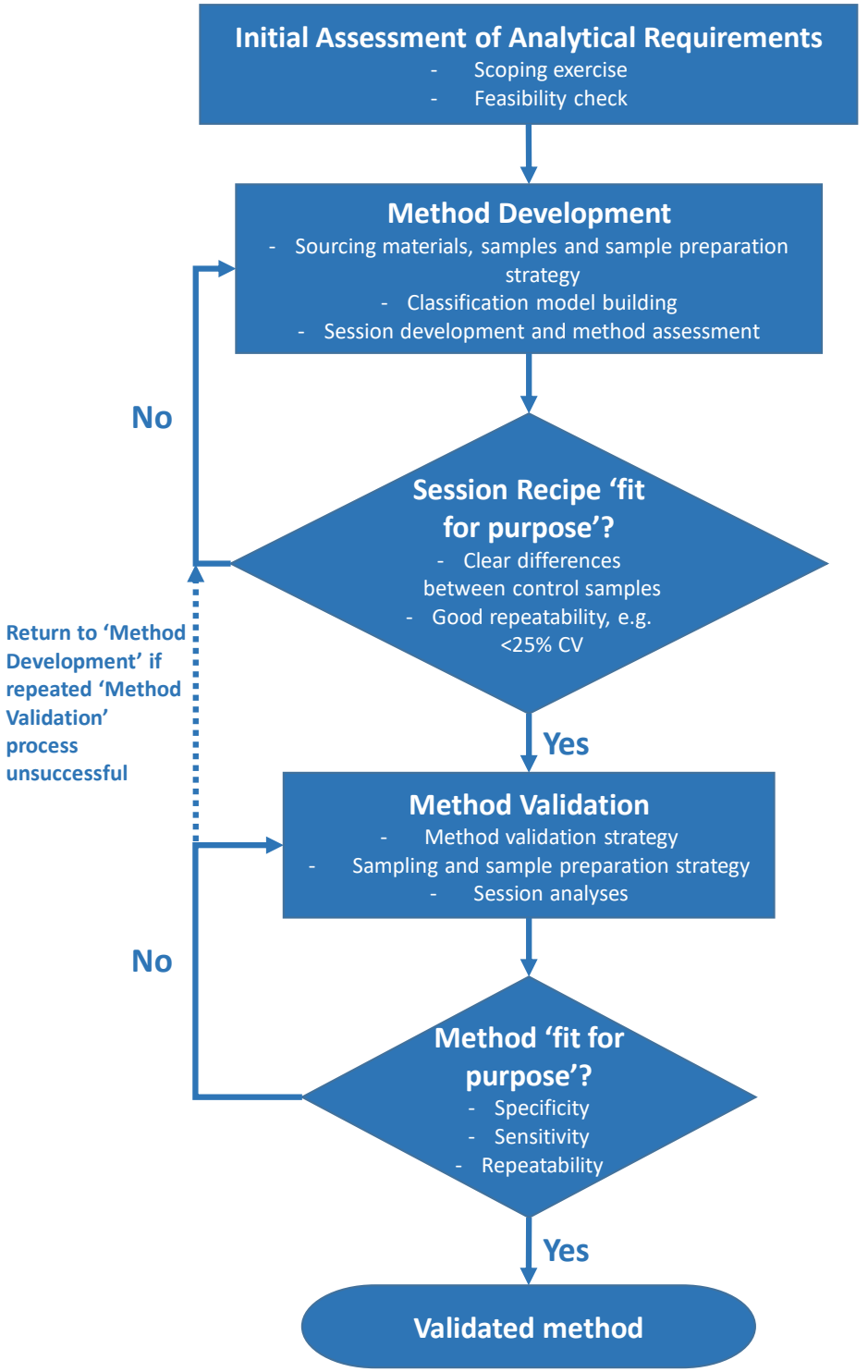


Figure 8. Work flow diagram summarising the MSI Method Validation process

7.2.6 Discussion

The proposed guidance (workflow summarised in Figure 8) has been developed to support the validation of any generic food sample in an analytical laboratory using the VideometerLab 4 platform. The guidance provides a trained developer with the basic strategy to develop and validate an appropriate food testing methodology. Key processes are covered that will help reduce development/validation timeframes and support the harmonisation/standardisation of MSI-based methods.

7.3 Objective 3 - Recommendations on transfer of MSI protocols and technology.

7.3.1 Introduction

Multi-spectral imaging represents a novel analytical technology with proven applicability within the foods testing sector which includes areas such as the analysis of fruits [16, 17] and vegetables [18] and increasing application to seafood [19, 20] and meat materials [21, 22]. The technology differs from standard spectroscopic approaches such as Near Infrared (NIR) and Raman in that rather than generating continuous spectra over defined ranges, MSI combines specific spectral bands with spatial data over a large sampling area. This key difference necessitates the use of different data processing and analytical workflows to that employed with the well understood spectroscopic field and thereby presents a challenge to transferability within these technology areas.

A scoping exercise was undertaken that explored the transferability of MSI technologies and associated protocols to UK analytical laboratories. This exercise included a brief review of the capabilities of other food sector compatible spectral imaging instrumentation currently on the market, provision of recommendations on the use of MSI for food authenticity testing, and an investigation of the suitability of spectral imaging point of test devices to augment existing laboratory-based analytical methodologies.

7.3.2 Spectral Imaging Technologies

A limited review of spectral imaging technologies (**Table 25**) with applicability to food testing was conducted that identified a number of commercially available devices suitable for point of test/contact applications based on either multi-spectral imaging (MSI) or hyper-spectral imaging techniques (HSI).

Table 25. Selected spectral imaging instruments with foods analysis applicability

Detail	Instrument: HinaLea 4200	Instrument: Specim IQ	Instrument: VideometerLab 4
Manufacturer	HinaLea Imaging	Specim, Spectral Imaging Ltd.	Videometer A/S
Website (Developer/ Distributor)	https://hinaleaimaging.com/model-4200-wide-field-hyperspectral-camera/	https://www.specim.fi/downloads/iq/manual/software/iq/topics/specim-iq-introduction.html	https://videometer.com/Products/VideometerLab
Specifications & Features	•Weight – 2 kg •Dimensions (LxWxH) – 310 x 80 x 80 mm •Up to 600 spectral bands •< 5 minutes time to result	•Weight – 1.3 kg •Dimensions (LxWxH) – 207 x 91.2 x 74 mm •Integrated, line scan camera •< 5 minutes time to result	•Weight – 14.1 kg •Dimensions (LxWxH) – 585 x 420 x 590 mm •Integrated platform (hardware & software) •< 5 minutes time to result
Approx. list price	£12,000	£15,500	£50,000
Technological basis	Hyper-Spectral Imaging	Hyper-Spectral Imaging	Multi-Spectral Imaging
Spectral range	400 – 1000 nm	400 – 1000 nm	365 – 970 nm
Potential food applications	•Wide range including contaminants, food quality, analyses of fruits	•Wide range including contaminants, food quality, analyses of fruits	•Wide range including contaminants, food quality, analyses of fruits

Detail	Instrument: HinaLea 4200	Instrument: Specim IQ	Instrument: VideometerLab 4
	and vegetables, analyses of meats/sea foods	and vegetables, analyses of meats/sea foods	and vegetables, analyses of meats/sea foods
Potential advantages of the instrument	•Relatively cheap •Portable system with PC	•Relatively cheap •Portable system	•Integrated platform •Desktop portable
Potential limitations of the instrument	•Requires light source, halogen recommended •Not an integrated platform requiring image analyses suite	•Limited on board processing •Requires light source, halogen recommended	•Limited portability •Relatively expensive

MSI involves the analysis of reflected light for a small number (typically 3 to 15) of spectral bands, which may be distributed across the electromagnetic spectrum. In comparison, HSI analyses reflected light spectra for a large number of contiguous spectral bands, (typically 100 or more) [23]. Both spectral imaging techniques are interchangeable, with only the MSI-based approach being limited by the number of spectral bands that can be analysed in a single pass.

The review highlighted the developing nature of the sector as shown by the limited commercial availability of spectral imaging instruments with desirable characteristics such as portability, ease of use and food testing applicability. The HinaLea 4200 (HinaLea Imaging) and Specim IQ (Specim, Spectral Imaging Ltd.) devices are representative of HSI instruments and employ line scanning-based approaches to build up their spectral images. In comparison, the VideometerLab 4 (Videometer A/S) instrument utilises an integration sphere with illumination provided by 19 fixed wavelength LEDs to generate spectral images.

The instrument platforms generates ‘hips’ data cube files that can be analysed using

analytical packages such as MATLAB® (The MathWorks, Inc.). However, these approaches are not as user friendly as using fully integrated software tools such as those associated with the VideometerLab 4 platform (VideometerLab software) which can automate complex analytical methodologies and standardise analyses which is crucial to broadening the uptake of MSI technologies within the wider testing community.

7.3.3 Point-of-test (POT) suitability

The three highlighted spectral imaging instruments (**Table 25**) are suitable for a variety of POT applications. Whilst the HinaLea 4200 (HinaLea Imaging) and Specim IQ (Specim, Spectral Imaging Ltd.) systems are easily portable, they both require support infrastructure that includes a fixed light source to standardise image capture, device/camera mount and associated computer to control the camera (HinaLea 4200) or analyse imaging data (HinaLea 4200, Specim IQ). The handheld Specim IQ system possesses standalone capabilities which include on board image capture and limited data processing which makes it particularly well suited to POT situations. In comparison, the VideometerLab 4 (Videometer A/S) is a fully integrated desktop portable platform designed to minimise sampling and analytical variability, e.g. standardised analytical workflows.

Spectral imaging devices are well suited to augment existing laboratory-based analytical methodologies by providing simple and non-destructive frontend screening to help identify potentially problematic test samples and direct those samples towards laboratory-based methods. The relative portability and lack of sample processing associated with the highlighted instruments means that they can be deployed in non-laboratory environments such as a port or factory for rapid sample screening for multiple analytes (method dependent). This triage approach better targets valuable laboratory resources and enables a greater number of samples to be integrated into the overall testing process.

7.3.4 Transfer of MSI protocols and technology

The transfer of MSI protocols and technology is dependent on instrument, software and data compatibility. MSI instruments fundamentally differ from spectroscopic

systems in the way data is generated and processed, e.g. spectral profiles versus spectral images (combined spectral and spatial data), which limits transferability between spectroscopic and spectral imaging technologies.

Indeed, recent studies have shown that the area of concept of operations (CONOPS) are bespoke to specific instruments (including separate imaging instruments) within the broad envelope of those technologies classified as point of contact instruments [24]. This is where the fitness for purpose of an instrument is very much influenced by the end-user requirements of the technology and application, where a “one size fits all” strategy is rarely effective.

At the raw data level, typical MSI instruments generate spectral image files that can be analysed using spectral imaging software packages such as ENVI® (Harris Geospatial Solutions, Inc.) or via MATLAB® (The MathWorks, Inc.). The ability to analyse spectral image files using industry standard software packages highlights the inherent transferability of the technology.

Generally applicable across all analytical instrumentation is the concept of limited transferability of techniques. This is relatively ubiquitous across any type of analytical technology to a certain level, whereupon protocols and techniques have to be changed and optimised before they can be considered as fit for purpose, even when transferring between related technology areas (e.g. real-time PCR instruments). MSI imaging protocols are no exception, often being instrument dependent. There is therefore a requirement for the developer to standardise to a particular camera system/technical approach and the development of instrument specific software packages. Hence, instrument specific protocols are typically not transferable between systems due to incompatibility, but the broader methodology can be adapted to alternative instruments.

The simplest approach to facilitate general transferability is to standardise on a fit for purpose MSI system that demonstrates the required analytical and performance characteristics. The obvious benefit of this approach is that validated analytical workflows can be developed and quickly transferred to the wider analytical community. The VideometerLab 4 system provides a potential route to harmonised MSI-based analyses as the developer has designed an integrated platform with built-in software and hardware backwards compatibility. Session files are available which

automate the image acquisition/processing process and incorporate the required classification/analysis models for a given method. These files are less than 1 MB and well suited to distribution electronically through file transfer or via a cloud-based system.

MSI transferability is also dependent upon adequate training and support resources to help upskill analysts and provide guidance. Support resources could also include a curated repository of validated methods which would provide the UK analytical community with rapid and easy access to appropriate methodologies.

7.3.5 Recommendations on the use of MSI for food authenticity testing

The application of MSI to food authenticity testing has been shown to be successful when applied to appropriate testing scenarios. The following recommendations are aimed at improving technology transferability and general accessibility to MSI-based food testing methods:

1) Establishing a virtual network to encourage collaboration within the area and provide an MSI analytical community;
2) The provision of guidance and training resources to the analytical community through appropriate dissemination routes, e.g. web-based, interactive training events;
3) Standardising MSI testing on a single platform demonstrated to be fit for purpose' and well suited to a broad range of analytical challenges;
4) Developing a centralised curated MSI database comprising reference methods with the associated reference scans/instrument protocols generated using authenticated materials and where appropriate value assigned using gold standard approaches.

7.4 Objective 4 - Examine feasibility of developing an MSI database

7.4.1 Introduction

In common with spectroscopic techniques, multi-spectral imaging-based methods are well suited to the application of databases holding reference data. MSI methodologies generate a variety of outputs amenable to electronic storage on a database platform such as spectral image files, classification models (e.g. segmentation files) and associated analytical method files (e.g. Session Recipe). The development of an MSI database would facilitate the cataloguing of core reference images/associated data and provide a networked resource (local or web-based) to support analysts and method developers. Donarski et al., (2019) provides general guidance on developing and curating food authenticity databases [25].

The following study investigates the feasibility of developing and maintaining an MSI-based database/repository of food sample classification and discrimination models.

7.4.2 MSI Database Development

Rationale – Developing an MSI database would build upon current and previously funded research within the area by providing a central repository for MSI data and associated information which is accessible to the wider analytical community and supports UK analytical science within the food authenticity sector.

Database platform selection - Modern database platforms such as MySQL (Oracle Corporation) and Microsoft SQL Server (Microsoft Corporation) are scalable relational database management systems that are capable of storing and managing a wide variety of data. MySQL (https://www.mysql.com/) is a widely used and supported open source platform that can be deployed locally or through cloud-based computing services such as Amazon Web Services and would be a good candidate platform to develop a MSI database.

Database fields – A standardised minimum set of information is required to adequately capture the MSI methodology. The MSI-analytical process can be broken down into core areas such as material details, sample preparation and sample

analysis, which can be further refined in order to build large relational metadata sets. In addition, to process related information, key method files such as classification models, instrument settings, instrument analytical methods, spectral images and results can also be stored within the database. Routinely storing spectral imaging files may be problematic due to the large file size (typically ~350 MB for the VideometerLab 4 system) which will impose potential limitations when these files are shared externally.

Database design – Database platforms provide various tools to help the developer build a functional database based on a series of connected tables populated with appropriate fields. Professional database development services are also available to reduce the risks associated with in-house development.

Database population – Crucial to the success of an MSI database is the validity of the data and any reference materials. The developer must ensure that reference materials (food stuff and adulterant) used to generate spectral images are appropriately authenticated. In addition a broad cross section of reference materials should be sourced that are representative of the target and potential adulterant materials.

Database hosting – The MSI database can be hosted on local servers using appropriate computing resources (e.g. standalone server). A locally hosted approach provides the developer with a well understood development route that benefits from data security and easier upkeep. However, local servers can have higher associated costs and scaling issues. In comparison, a centrally held database delivered through cloud-based servers (e.g. Amazon Web Services or Microsoft Azure) is typically cheaper upfront and easier to scale. This approach does suffer from potential issues such as security concerns and network reliability/latency.

Database accessibility – Ideally, access to the database should be controlled in order that authorised organisations/individuals are able to utilise the database resource without providing potential intelligence to food fraudsters. In addition, different contribution levels should be considered to allow data uploads and or curation.

7.4.3 MSI Database Maintenance

Internal database maintenance – Routine database maintenance should be undertaken regularly using the appropriate database tools.

Database curation – Effective curation is essential to the long term usefulness of the database by refreshing the selection of available reference materials so that contemporary spectral images/data representative of different sources are available to the analyst/method developer. This can be achieved through dedicated database curation/validation studies or via the wider MSI community whereby contributions are sought and then validated prior to incorporation into the database.

7.4.4 Discussion and Recommendations

The presented feasibility study provides guidance on the steps and options available to a developer wishing to provide and maintain an MSI database. The availability of an appropriate curated MSI database comprising reference methodologies with the associated reference spectral data would support the uptake of MSI technologies and facilitate general standardisation/harmonisation. The following recommendations are aimed at supporting the development of such an MSI database:

1) A feasibility project to develop a locally hosted pilot database that incorporates the core aspects discussed in the feasibility assessment and to trial approaches to identify and implement the most effective database development strategies. The project should aim to develop a scalable and functional database with a user friendly web-based interface that can be accessed securely through a local network or via the World Wide Web;
2) A follow-on project utilising the outputs from the database feasibility project to transfer the pilot database and associated development strategies to a suitable hosting platform, for example, hosted by the FSA. Appropriate resourcing will be fundamental in enabling the delivery of a fully functioning MSI database that can be utilised by stakeholders within the UK food testing community.

7.5 Objective 5 - Provision of final report

This final report has been successfully submitted which includes details on relevant validated methods and recommendations on general guidance to support MSI development and deployment as a screening tool for food analysis.

7.6 MSI model availability

The successfully validated methods developed as part of the current study are based on optimisation of models built during the method development phase using Session recipes compatible with the Videometer VL4 multi-spectral imaging instrument. Copies of these session scripts have been supplied to the FSA for secure keeping as an output from this project, such that the models can be provided to those stakeholders who wish to access the methods and replicate the results, following relevant requests. LGC and the FSA can be contacted in order to request a copy of the relevant MSI session recipes developed as part of this project for food testing purposes.

8. Discussion

Food safety is of great public concern, and the occurrence of food-related illnesses or injury can have a large economic and health impact. The publication of the Elliot review [26] has highlighted this high level of concern which now exists with respect to apparent prevalence of food fraud and food crime within the EU. Of the seven recommendations made in the review, significant weight was placed on the need for government to invest in research and development for authenticity testing in order to maintain both consumer confidence, and make food crime as difficult to commit as possible. Consequently, the need for the development of sensitive, faster, cheaper, and more reliable methods for the analysis of food and feed produce has become of paramount importance.

Whilst the concept of multi-spectral imaging for the analysis of biological materials is not new, the improvements in the technology in recent years and the increase in its affordability mean that the application of multi-spectral imagining for food authenticity

and safety testing is now a reality. This is reinforced through a growing number of scientific publications within the sector [27-32] describing the application of MSI to food and feed testing in areas such as the adulteration of cereal grains; speciation and quality of nuts, fruits, meat, fish, seafood, vegetables and eggs; and the detection of GMO grain.

This report presents evidence for the applicability of using multi-spectral imaging as a rapid screening tool for analysis of priority sampling issues as identified through a range of stakeholder consultations (FSA, Defra, AMWG, RASFFs, Public Analysts and the FSA National Coordinated Sampling Programme 2016/17). MSI was successfully applied to topical issues inclusive of the adulteration of oregano (herbs & spices) with myrtle leaves (as described in the FSA's Food Crime Unit annual strategic assessment report 2016), and the adulteration of ground almond with ground peanut (a known allergen that requires labelling according the relevant EU Directive). The importance of this area was reinforced by a high profile case where a restaurant owner was convicted of manslaughter by willingly providing a meal to a customer which had almond substituted with ground-nuts (including peanut) when the customer had ordered a nut-free meal [33].

The applicability of MSI is dependent on the spectral and physical properties of the component materials, and the image analyses methodologies applied. The test scenarios explored in this study covered a wide variety of challenging test materials with differing physiochemical characteristics. The results clearly showed that the MSI methodologies applied were capable of detecting and differentiating across a range of different samples and test components.

As part of this study, six validated methods were developed. Typically, measurement uncertainty associated with each of the analytical methods (expressed as the coefficient of variation) did not exceed 15% at the lower working range of each method. This demonstrated the excellent repeatability of the validated methods and the tight precision with which the measurement responses were generated. The limit of detection, defined as the lowest concentration of adulterant that generated a measurement profile which was significantly different from the 100% pure sample, varied depending upon the sampling scenario. This typically varied from <25% to <5% adulterant (w/w).

The analytical power (for example, analytical sensitivity) associated with the validated MSI methods cannot compete with the analytical specifications of the more established molecular biology technologies such as real-time PCR. However, as a cost effective simple screening approach and a “first line of defence” in the food supply chain, the MSI approach does not have to. Traditional molecular biology based analyses are typically destructive and can take hours (or even days) to produce a result. This has obvious cost implications in terms of the necessary staff time of a skilled analyst to produce the result, as well as potential incurred costs for storage/holding of a suspect food shipment/consignment until the result is known. A result using MSI can be provided within a minute. The benefits of the MSI approach include rapidity (as little as 20 seconds to generate a result in optimal circumstances), cost effectiveness, non-destructive nature and non-targeted multi-analyte capabilities, which support MSI being used as a screening approach as part of triage system. Should an issue be found whilst using the MSI screening approach (i.e. something in a sample has been identified that should not necessarily be there), the sample can be submitted for further analysis using the approved confirmatory approach. Should the threshold for further action not be triggered, then resources (costs, time, etc.,) have been saved by not having to apply a more complex analytical procedure to determine if an issue existed with the sample.

The successful utility and application of MSI to food testing is dependent upon the model which has been developed to afford discrimination, and hence upon the exact analytical question that is being asked in relation to food testing. In the current study, the aim was to develop MSI screening methods to facilitate identification of potential contaminants in topical food sampling scenarios. A representative range of samples were taken for each sampling scenario, being as close as possible to the market situation within the confines of a controlled experiment. For example, in the oregano method, images of oregano samples from multiple varieties, cultivars and treatments were used to build a spectral profile of “oregano”. The broadening of this sample set to incorporate so many different types of oregano will also, by necessity, increase the variability of the oregano dataset. This may result in the model having less discriminatory power compared to a more simplistic model based on just one type of oregano sample. In some instances, the oregano model in particular, this has also

meant that the model is now so broad that it also recognises closely related materials (e.g. sumac and Mexican oregano) as having very similar spectral profiles. Whilst the results in this report have demonstrated the applicability of using the method as a simple screening approach to identify potential adulteration, the method and associated model can be further refined depending upon the analytical question being asked. For example, if the exact type/state/cultivar of oregano was known in a sample, a more discriminating model could be generated using a more restrictive data set, which would permit a greater level of discrimination as well as generating data for potential quantitative purposes.

Associated with refinement of what the analytical question is and the broadness of the dataset used to build up a discriminatory model, is the potential to use MSI in a more quantitative fashion to estimate the approximate percentage adulteration that may be present in a sample. This can be based on the percentage area fractionation of the test sample image which does not meet the spectral profile of the pure sample. The methods and models detailed in this report have been based on taking multiple images from a range of sample types in order to try to mimic typical species variability in the market place with typical adulterants. The discriminatory potential of the methods and models could be further improved should the question become "*Is there evidence of any presence of adulterant species A in the background of species B?*". Despite the broad datasets generated for the methods described in the current study, evidence has been presented for the quantitative potential of using MSI as a screening tool. Examples are provided in Annex I and also in the various tables e.g. Table 5 shows that the "10% myrtle in oregano" test sample provides a measurement response of 88.60 % (w/w) oregano, which is close to the ideal percentage area of 90% oregano if 10% of the sample is myrtle in origin. Should MSI be used for quantitative purposes, it is a recommendation from this study that further research in this area be conducted.

9. Main implications of the findings

The results of the project have the potential to have an economic and operational impact through providing shorter timeframes for screening samples and reducing associated test costs. The project supports FSA policy by using science and

evidence to prioritise an agreed list of sampling scenarios seen as new and emerging risks. Using MSI as a screening approach provides a rapid and non-destructive technique for screening foods to ensure they are safe, traceable and properly labelled, further empowering consumers to make informed decisions in relation to food. The multi-analyte nature of MSI lends itself well to providing support for the FSA research policy of allergen analysis (e.g. almond in paprika), as well as providing direct support for the FSA research priorities of using innovation in food regulation and helping assure food safety and standards.

The results of this study demonstrate the range of food testing applications for which one MSI instrument is applicable, as illustrated through the range of validated methods provided. Whereas traditional molecular biology based analyses are typically destructive and can take hours (or even days) to produce a result, a result using MSI can be provided in a few seconds. The MSI approach, being rapid, non-destructive, incorporating a large sample size, having an integrated footprint and affording both non-targeted and multi-analyte analyses, provides an additional method for food testing in the analytical toolbox. Its application includes tests for food quality, safety, authenticity and adulteration. The performance and rapid turnaround time of a multi-spectral imaging approach lends itself well as a robust screening tool, as part of a triage system, to identify if a potential problem exists with a food sample. Should an issue be identified, the sample can then be submitted for confirmatory analysis using the relevant approach in a laboratory based environment.

The outputs of this project contribute towards promoting and protecting public health by providing a multi-faceted screening test for food to help ensure it is safe to eat and is what it says it is. This will aid in the traceability of food along the supply chain, ultimately helping empower consumers to make informed choices in relation to food. The application of MSI as a screening tool has implications in terms of testing for food safety and standards and demonstrates application of innovative tools in line with recognised FSA research priorities.

10. Possible future work

In order to fully exploit the potential behind using the MSI as a multi-analyte, non-

targeted, and non-destructive screening approach, the following five areas for future work should be considered:

1) **Knowledge exchange and dissemination: interactive workshop on imaging approaches for food testing**

   It is proposed that a dissemination activity involving a hands-on workshop is developed. This would involve inviting Public Analysts and UK industry to a one-day interactive workshop where the results of the project will be disseminated, the imaging device will be demonstrated with an example method validated from the project, participants can test out the system themselves and opportunities will be given to ask questions about the technology and its application. This dissemination activity will raise awareness of the utility of imaging technologies to help combat food fraud and test food for its quality and authenticity, as well as promote the transferability of the protocols and technology.

   A further option would be to combine/replace the workshop with a GoToMeeting in order to broaden the uptake and accessible in line with government guidance in observing social distancing during the COVID-19 pandemic. Such a workshop would maximise the impact of the project as well as the likelihood of uptake of the instrumentation by the UK Official Control Network and other UK based industry.

2) **Evaluate the transferability of methods and comparability with alternative imaging technologies**

   A set of six validated methods specific to individual sampling scenarios for the multispectral imaging device have been provided as part of the current project. Since the project inception, there are a number of other imaging technologies which have reached near market readiness for food testing, further reinforcing the benefits of this FSA project. It would be beneficial to do a cross-platform comparison with other available instrumentation, in order to characterise the transferability of the validated methods and the concept/rationale behind these protocols. At the same time, the performance of alternative instrumentation can be evaluated compared to the original multispectral imaging device using a common set of food samples provided from the current FS301017 project, which will have been prepared to the highest levels of quality and have been checked

beforehand for their representativeness of the UK market situation. The outputs from this work would allow interested parties (e.g. FSA, Public Analysts, etc.) to objectively compare the performance of alternative imaging instruments and their utility for food testing applications and tailor these to their specific laboratory requirements.

3) **Examine the feasibility of producing Reference Materials and associated imaging profiles**

The efficacy of any analytical approach (e.g. genomic or proteomic strategies) is dependent upon the availability of suitable reference materials or databases. Imaging technologies are no different – reference materials and/or databases containing verified image data will help enable the full utilisation of this new and emerging technique for food testing with confidence. The materials produced from the previous suggestion for future work (Transferability of methods and comparability with alternative imaging technologies) will be subject to a scientifically controlled time course experiment to characterise the stability of the materials and how their integrity varies according to time, temperature, light, humidity, etc. The output from this will be the provision of a small selected panel of characterised reference materials and associated imaging/spectral data which can be used as stable positive controls to assess the performance of imaging devices for the future.

4) **Establishment of online resources to support UK based imaging community**

In order to create, improve and maintain UK analytical expertise in imaging technologies for food authenticity testing, it is important to establish an active and engaging analytical community. Such a community can share ideas, queries, experiences and expertise on the use of imaging devices. It is proposed that the pre-existing and well established Food Authenticity Network website (which much of the UK food related analytical community is part of) be used to facilitate and host a dedicated service for the imaging community. A regular newsletter will be produced to highlight key aspects associated with imaging work. The community

will be hosted by LGC who will provide a regularly updated FAQ in relation to the use of imaging technologies, as well as respond to bespoke and specific queries raised by members of the imaging community. Additionally, web links and reports will be provided. A help facility and portal will also be provided on the FAN for these purposes, as well as an additional option to provide a service for uploading and downloading image files. Both low level and high level technical support (the latter with prior agreement from the relevant manufacturer/supplier) could also be catered for within the imaging community.

5) **Evaluate the quantitative potential associated with multi-spectral imaging methods**

The current project has provided validated protocols for a number of different sampling scenarios, aimed at detecting topical adulterants often associated with those samples. In addition, to maximise the applicability of those methods, steps were taken to ensure the models were built on a broad range of sample types where possible, taking into account different varieties, cultivars, treatments, etc. Whilst this inclusive dataset has the advantage of maximising the market representativeness of the methods used, the model based on a larger dataset will typically have more variability associated with it. The quantitative potential associated with the MSI approach has been clearly demonstrated as part of the current project, both in the quantitative instrument measurement responses associated with different adulterant levels (e.g. Table 4) as well as a statistical correlation shown in Annex I. The refinement of the model as applied to specific datasets, for example as advised by FSA/Defra on particular sampling situations of adulterant A in a background of species B, would improve both the discriminatory power and the quantitative estimates of any MSI method. It is therefore a recommendation of this project that further consideration be given to explore the quantitative potential behind this methodology.

6) **Further development work to capitalise upon potential for fish speciation analysis**

The initial method development work to discriminate between five different white fish species commonly used in the UK demonstrated excellent potential. However, a validated method was not successfully developed, thought to be

mainly due to the breadth of the model used to try to differentiate between five different fish species which incorporated a lot of variability. The method still demonstrates excellent potential for fish speciation using a more focused analytical question such as "*Has this cod been substituted with pollack*?", which is both a more topical question in line with current evidence for substitution as well as using a more restrictive data set coupled with a model using a higher discriminatory potential. It is a recommendation from this study that additional developmental work be conducted in order to fully capitalise upon the discriminatory potential of an MSI approach for fish speciation.

11. Action resulting from the research (for example, IP, Knowledge Exchange)

Peer reviewed papers under consideration dependent upon resourcing.

12. Acknowledgments

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14. Annex 1

14.1 Oregano Method – Quantitative Modelling

Further work was undertaken to investigate the quantitative potential of an MSI method for assessing adulteration in oregano by building a simple estimation model utilising linear regression. The myrtle model specific threshold-based results from the method validation dataset (Experiment A only) was used to generate a calibration curve of the assigned versus observed % myrtle model area (Figure 1.) with the following performance metrics:

Metric	Experiment A
R^2	0.964
Slope	0.224
Intercept	69.896

Figure 1 and the associated metrics demonstrate a strong correlation (R^2>0.95) between the assigned and observed values, inferring the data is suitable for further quantitative analyses. Regression analysis was used to estimate the calculated mean % content for the 30% w/w myrtle in oregano test sample which was determined as 27% and shows good agreement with the assigned value. This demonstrates the promising quantitative potential of the methodology which warrants further investigation.

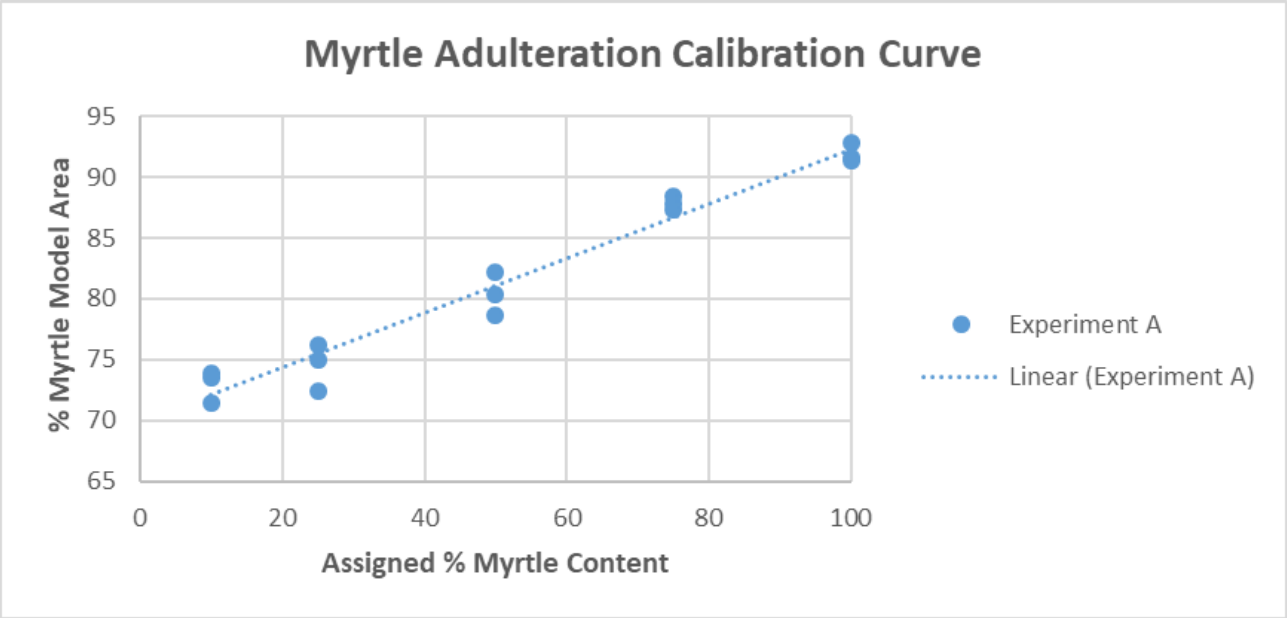

Figure 1. Myrtle adulteration calibration curve comparing the % assigned with % model area, Each calibration point represented by 3 replicate measurements.