Final report: Application of multispectral imaging (MSI) to food and feed sampling and analysis

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1 Executive summary

Effective sampling and analysis is an important aspect of official food and feed controls. Traditionally, there are a number of physio-chemical and biological methods available for monitoring food and feed, but many of these techniques are expensive, invasive, technically demanding, and are often associated with long turn-around times.

Multispectral imaging (MSI) represents an innovative and non-invasive technique combining both imaging and spectral technologies. It typically uses multiple wavelengths from the UV through to the near infra-red spectrum, permitting fast and accurate determination of surface colour, texture and potential chemical composition. MSI has a number of advantages compared with traditional molecular biology approaches for food sample analysis, including rapidity, lower costs, and the non-targeted multi-analyte nature of sample analysis. Such performance characteristics may make it suitable for efficient sample screening, helping augment or provide alternatives to pre-existing traditional analytical approaches.

This preliminary project was aimed at providing demonstrable evidence of the proof-of-principle practical implementation of MSI to a range of topical authenticity, quality and safety issues associated with food and feed sampling and analysis in the UK. In terms of cost effectiveness, ease of use and rapidity, the following table summarises a review of some of the key comparative characteristics from a range of instrumentation used in the modern analytical laboratory (indicative values only):

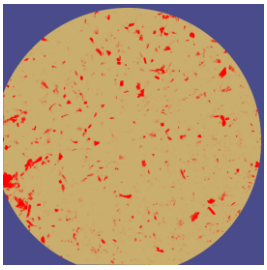
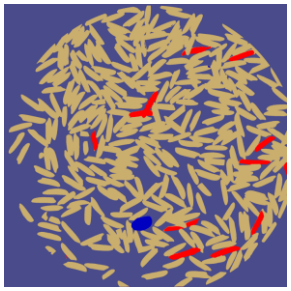
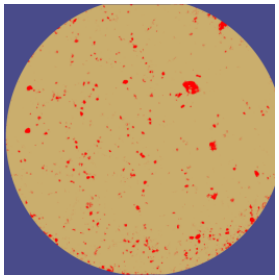
Parameter	Technology			
	MSI	**Real-time PCR**	**Mass Spec**	**ELISA**
Analyte	Multiple	DNA/RNA	Protein	Protein
Typical sample throughput	Up to 30 per hour	Up to 96/384 reactions per run	Up to 20 samples	Up to 96 samples
Sample preparation required	No	Yes	Yes	Yes
Typical workflow	• MSI scan • Data analysis	• Sample preparation • DNA/RNA extraction • RT-PCR required for RNA • qPCR test • Data analysis	• Sample preparation • Protein extraction • Peptide purification and preparation • MS test • Data analysis	• Sample preparation • ELISA test • Data analysis
Typical total analyses time (including extraction & analysis)	< 10mins	Up to 9 hours	Days	Up to 4 hours
Analyst skill level	Low/medium	Medium	High	Medium
Indicative per sample reagent/consumable costings	~£0.25	~£6	~£200	~£10

On face value, MSI has advantages over other analytical instrumentation in terms of limited specialist training required to operate, rapid sample turnaround times, and lower

costs.

Following consultation with the FSA, a priority list of sampling scenarios to use with the study was finalised. This list was largely steered and showed synergy with the FSA 2016/2017 National Coordinated Sampling Programme. The performance capabilities of MSI were evaluated in relation to a panel of representative test samples.

Figure to show key project highlights using MSI:

Visible spectrum	Multispectral imaging
 Oregano adulterated with olive leaves	 Adulterant olive leaves highlighted in red
 Basmati rice adulterated with “plastic rice” and “foreign body” gravel	 “Plastic rice” highlighted in red and “foreign body” gravel in dark blue
 Ground almond adulterated with ground peanut	 Adulterant ground peanut highlighted in red

Test sample adulterant levels were prepared and selected on a gravimetric w/w basis (0 %, 0.5 %, 1 %, 5 %, 10 %, 25 % and 50 % w/w where possible) to represent a range of levels that may be experienced when screening samples.

Multispectral imaging showed very good differentiation with promising quantitative capability in relation to analysis of **oregano herb adulterated with olive leaves**. The Limit of Detection (LOD) was estimated as the lowest adulterant tier level which could still be detected and was different from the non-adulterated control on at least 95% of occasions.

For the oregano model, the LOD was estimated as ≤ 0.5 % w/w with a coefficient of variation (CV) of 18 % at the 0.5% w/w level. This LOD appears fit for purpose when taking into account published threshold levels which state up to 1 % (ISO specifications) [1] or 2 % (European Pharmacopeia) [2] for impurities in oregano samples are tolerable. Quantitative data showed good proportionality to the assigned levels across the entire sample range. Furthermore, MSI was capable of simultaneously differentiating between oregano leaves and oregano sticks/stems within the same sample, demonstrating its applicability for quality testing and use as a non-targeted multi-analyte tool. The applicability of using MSI in this area is further highlighted in the FSA's Food Crime Unit report (2016), which stated that around 25% of oregano samples taken as a sampling exercise earlier this year contained myrtle and olive leaves, and these levels ranged from 21 % to 69 %.

The particulate nature of grain, seed and leaf material make such samples ideally suited for discrimination. MSI was used to test for **Basmati rice adulterated with plastic rice and gravel impurities**. MSI easily differentiated between basmati rice, adulterant "plastic rice" and gravel impurity types with good quantitative potential. An LOD ≤ 1 % w/w for both plastic rice and gravel impurities, with CV's of 8 % and 18 % respectively at the 1% w/w level, was achieved. These results further demonstrate the non-targeted and multi-analyte capability of MSI by simultaneously identifying and classifying adulterant "plastic rice" and the gravel "foreign body" simultaneously within the same sample during the same analysis.

MSI was applied to additional sampling scenarios and demonstrated good analytical potential. For **beef meat blended with pork meat**, an LOD ≤ 5 % w/w was achievable, with a CV of 25 % at the 5% w/w level. Adulterated minced meat samples may provide further discriminatory potential for MSI to capitalise upon compared to blended meat samples, because of the additional physical characteristics and structure afforded by the mincing process. For **undeclared offal** (beef meat adulterated with beef heart (blended)) an LOD ≤ 10 % w/w was achieved with a high CV of 40 % at the 10% w/w level.

In relation to detection of allergens, MSI also demonstrated good discriminatory capability and quantitative potential. For **cumin adulterated with ground almond shell**, an LOD of ≤ 5 % w/w was achieved with a CV of only 4 % at the 5 % w/w level. **Cumin adulterated with mahaleb** gave the same LOD estimate but with poorer precision (CV of 60 % at the 5% w/w level). These sampling scenarios demonstrate the applicability of MSI for screening in the topical issues of almond/mahaleb found in cumin and paprika spice mixes, which were subject to a well-publicised and costly withdrawal from the international market in 2015. For **almond adulterated with peanut**, MSI was capable of achieving an LOD of ≤ 5 % w/w with a CV of 25 % at the 5% w/w level. Although visually similar within the visible spectrum, the components of almond and peanut powder have a distinct and unique spectral profile which can easily be differentiated with MSI. This application is also of topical importance as a UK restaurant owner was recently convicted for the manslaughter of a customer by using groundnut powder (containing peanuts) substituted for almond powder in a takeaway meal. For **wheat flour adulterated with peanut flour**, MSI achieved an approximate LOD of ≤ 10 % w/w with a poor CV of 64 % at the 10% w/w level. The high LOD associated with this adulteration model may be due to the fine particulate size of the wheat and peanut flours exceeding the resolution of the image capture system and resulting in the wheat flour masking the spectral characteristics of

peanut flour until a compositional threshold has been reached.

MSI was applied to food supplements of dried skimmed **milk powder adulterated with melamine** (1,3,5-Triazine-2,4,6-triamine). The results demonstrated the potential for further developmental opportunities of MSI within this area because of the inherent high reflectance properties of the powders investigated, resulting in pixel saturation, and the fine grain size which exceeded the resolution of the image capture system. However, alternative analyses conducted using mean component spectral data showed a clear association between melamine content across a panel of wavelengths that should be further evaluated and exploited as part of a follow on project.

A summary of the estimated limits of sensitivity and associated CV's for the samples described above is presented in the following table:

Sampling Scenario Sample (adulterant)	LOD (w/w)	CV	Notes
Oregano (olive leaves)	≤ 0.5%	18%	Documented issue in 2016 FSA report
Rice (Plastic rice)	≤ 1%	8%	Multi-analyte capability
Rice (gravel)	≤ 1%	18%	Multi-analyte capability
Beef (pork)	≤ 5%	25%	Complex blended sample
Beef (offal)	≤ 10%	40%	Complex blended sample
Ground cumin (ground almond shell)	≤ 5%	4%	Particulate nature
Ground cumin (ground mahaleb)	≤ 5%	60%	Particulate nature
Ground almond (ground peanut)	≤ 5%	25%	Particulate nature
Wheat flour (peanut flour)	≤ 10%	64%	Wheat flour coats peanut oil

An additional priority on the sampling scenario list was that of **arsenic contamination in rice**. Preliminarily analysis of the provided samples was found to be inconclusive due to the absence of multiple arsenic levels within the same rice varietal samples, thereby precluding a proper scientific evaluation.

Based on this preliminary study, MSI has demonstrated good potential to be used as a screening method for a range of issues, and has additional functionality when an adulterant or contaminant is present above a certain threshold level. Clearly MSI will not be applicable as a screening approach for every conceivable sampling situation (food authenticity, adulteration, quality and safety testing), and the added advantage of the MSI's quantitative capability would benefit from further characterisation. Full method validation on selected sampling scenarios and evaluation of the quantitative accuracy of MSI would facilitate an objective judgement of its fitness for purpose and applicability on a sample case by case basis.

Centered on the priority list provided by the FSA 2016/2017 National Coordinated Sampling Programme, the novel application of MSI showed good levels of sensitivity and precision (an estimated LOD of ≤5% and associated CV ≤25%) in the following sampling scenarios: oregano leaves adulterated with olive leaves; Basmati rice adulterated with plastic rice; Basmati rice with impurities (gravel); beef adulterated with pork (as a blend); cumin adulterated with almond; and almond adulterated with peanut. Future work should focus on full method validation of these sampling scenarios further. Given the pioneering

work of using MSI and the preliminary nature of this proof-of-principle project, the applicability of using MSI as a screening approach to other sampling scenarios should also be investigated. Examples include oregano adulterated with myrtle leaves, and meat species adulterated with other meat species (e.g. burgers, mince meat, etc.). There is also scope to further refine the method to test its applicability on appropriate rice samples with differening levels of arsenic, redesign a model based on the mean spectral data to determine different levels of melamine in milk powder, and investigate different sample mixtuires of offal in meat.

As demonstrated using these models, MSI can be used as an automated, rapid, non-destructive, quantitative technique for testing for adulteration, authenticity and quality of food and their ingredients. A simple but effective screen of a sample can be conducted within 20 seconds, results obtained, and a judgement made on the nature of a sample, based on a simple work-flow using an integrated device. The technique is a non-targeted approach (allowing the determination of “unknown” ingredients present in a sample e.g. presence of macro-filth, insect parts and other undesirables, grit, chaff, foreign bodies, contaminants, impurities, etc.) as well as exhibiting a true multi-analyte approach. This multi-analyte nature means that only one instrument is required to perform a wide range of applications. These characteristics, coupled with the requirement for only limited specialist training, and the non-proprietary nature of the method, make MSI a very attractive approach for use by UK stakeholders for the rapid and cost-efficient monitoring and testing of the food and feed supply chain. The affordability and transferability of the approach across MSI instruments will make the purchase of such a technology as a rapid screening tool an attractive proposition to both UK industry and control authorities alike. MSI offers the potential to significantly reduce both the timeframes currently required for testing samples and the incumbent cost burden to UK Government and commercial testing laboratories.

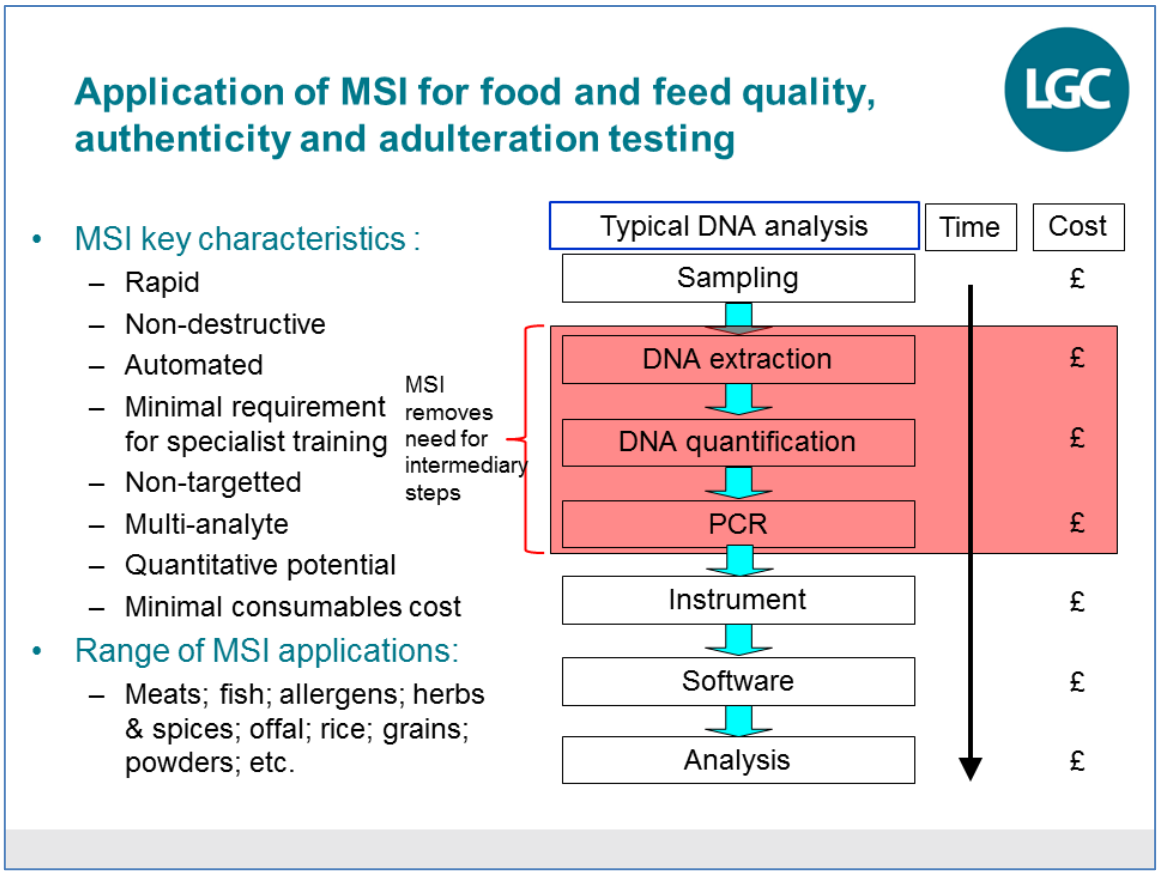
This technological development helps reinforce the overarching aims of the FSA Strategic Plan, lending itself well to supporting the initiative of making food safe to eat, and protecting consumers’ interests in relation to food labelling and quality.

We have made a series of recommendations for the full utilisation of this technology as a result of this proof of principle study:

- Full method validation to evaluate performance characteristics (e.g. trueness, precision, sensitivity, specificity, robustness and measurement uncertainty) based on priority samples and samples seen as successful in this preliminary study (e.g. allergens in herbs & spices and ready meals; meat speciation; offal in meats) in consultation with expert guidance from the FSA. Ensure that representative and authenticated materials and adulterants are sourced;
- Provision of guidance for validating the use of MSI on any sample. A protocol or SOP could be written to guide the analyst in assessing general and bespoke performance characteristics of MSI and what quality metrics and performance criteria need to be fulfilled in order to provide evidence that the method is fit for purpose for any given sample. This will increase the scope, applicability and utilisation of MSI by UK analytical labs
- Experimental evaluation alongside other analytical approaches in current use (e.g. real-time PCR) in terms of time, costs, expertise and accuracy;

- Evaluate the applicability of MSI to other samples it is uniquely suited towards (e.g. vegetable quality, fish speciation and quality, and grain quality [*Fusarium* infection]);
- Assess the transferability and commutability of the approach and provide recommendations on the deployment and applicability of MSI instrumentation for UK Official Control Laboratories, as well as optimal routes for dissemination and knowledge transfer of the new technology.

Graphical Executive summary:



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2 Introduction

Increasing consumer demand for premium quality food produce at a reduced cost has resulted in the food industry being faced with a spectrum of challenges including the maintenance of high quality standards and assurances of food safety. For the producer and consumer, the grading of food for quality (e.g. fruit ripeness, meat marbling, etc.) and safety (e.g. absence of pathogens and chemical residues) has become increasingly important, particularly following the number of recent high profile incidents that have been publicised in the media e.g. 2013 EU horse meat adulteration of beef and other meat products [3] and the 2008 Chinese melamine addition to milk powder investigation [4]. As a result of the increasing consumer pressure that has arisen following these incidents, and the governmental recommendations made in the recent Elliot report into the integrity of existing food support networks [5], the determination of food safety and authenticity has become a major issue in the food industry, especially for meat and meat based products.

Spectral imaging is a well-established technology that utilises the unique spectral characteristics of a sample to identify and quantify components based on non-destructive image analyses. Spectral imaging analyses have been widely applied to the quality and safety evaluation of food produce [6]. Spectral imaging was originally applied to areas such as meat quality testing [7], but has more recently been successfully used within meat authentication applications [8-12]. However, conventional spectroscopic techniques only provide spectral information with respect to a whole sample, which lacks the spatial (physical co-ordinate) component provided by machine vision systems. More recently, a new and innovative approach, termed multispectral imaging (MSI) has been gaining in popularity. MSI uses the simultaneous measurement of both reflected light across a large number of wavelengths, from ultraviolet (220 nm) to far infra-red (12,500 nm), plus an order of spatial information. Therefore, spectral imaging technology may offer an alternative method for determining the quality, safety and authenticity of food products in a rapid and non-destructive manner.

3 Project scope

This FSA preliminary project aimed to provide actionable evidence to the proof-of-principle practical implementation of MSI to topical authenticity, quality and safety issues associated with food and feed sampling and analysis in the UK. Key objectives (Table 1) included the identification and prioritisation of core UK authenticity, quality and safety issues associated with the sampling and analysis of food and feed through discussions with stakeholders, sourcing and developing a range of appropriate test materials, and evaluating the performance capabilities of MSI to characterise the panel of representative test samples.

Table 1. Table showing key project deliverables

Objective Number	Objective Description
1	**Phase 1** Identify a priority list of food and feed sample testing scenarios, regarding authenticity, quality and safety issues associated with sampling and analysis of food and feed in the UK. This will be achieved through an in-depth literature review and UK stakeholder engagement.
2	Source and provide relevant food and feed samples that are representative of the testing scenarios identified as part of the priority list from Objective 1.
3	Initial study: Evaluate the capability of MSI to assess the top three testing scenarios in the priority list of food and feed sample issues, and provide interim report to FSA.
4	**Phase 2** Full study: Evaluate the capability of MSI to assess remaining testing scenarios in the priority list of food and feed samples, representative of different levels of adulteration, quality and safety issues.
5	Provide a final report on the demonstrable application of MSI inclusive of recommendations on how UK stakeholders can implement the technology.

4 Priority list of food and feed sampling scenarios

4.1 Sampling and reporting

Building upon the previous nationwide sampling programme [13], the Draft 2016/17 National Co-ordinated Sampling Programme [14] helped provide a list of prioritised sampling scenarios for initial evaluation under the project. The draft sampling programme (Table 2) highlights multiple areas such as meat speciation and allergens which are suited to MSI-based testing. Existing European wide reporting systems such as the Rapid Alert System for Food and Feed (RASFF) support the proposed sampling list and highlight priority areas such as aflatoxins [15]. Figure 1 shows the Top 10 UK RASFF Notifications for the 2010-2015 period and illustrated that aflatoxins and high metal content were common notification areas which may benefit from MSI-based approaches. In addition, many of the RASFF notification areas are covered by the FSA Co-ordinated Sampling Programme which supports the project prioritisation strategy.

4.2 Objectives 1 & 2 - Sampling priorities review and sample sourcing assessment

The draft FSA sampling list provided key guidance in prioritising project resourcing. The individual priorities were assessed for technical and sample suitability for spectral imaging analyses in order to provide a ‘Sampling Decision Matrix’ to inform the decision process (Table 3). Appendix 1 provides a summary of the full review and decision process across the FSA sampling list and additional potential priorities for consideration. The preliminary assessment highlighted the ‘Allergens and gluten’ and ‘Meat species’ sampling scenarios as high priority candidates to be evaluated during the project.

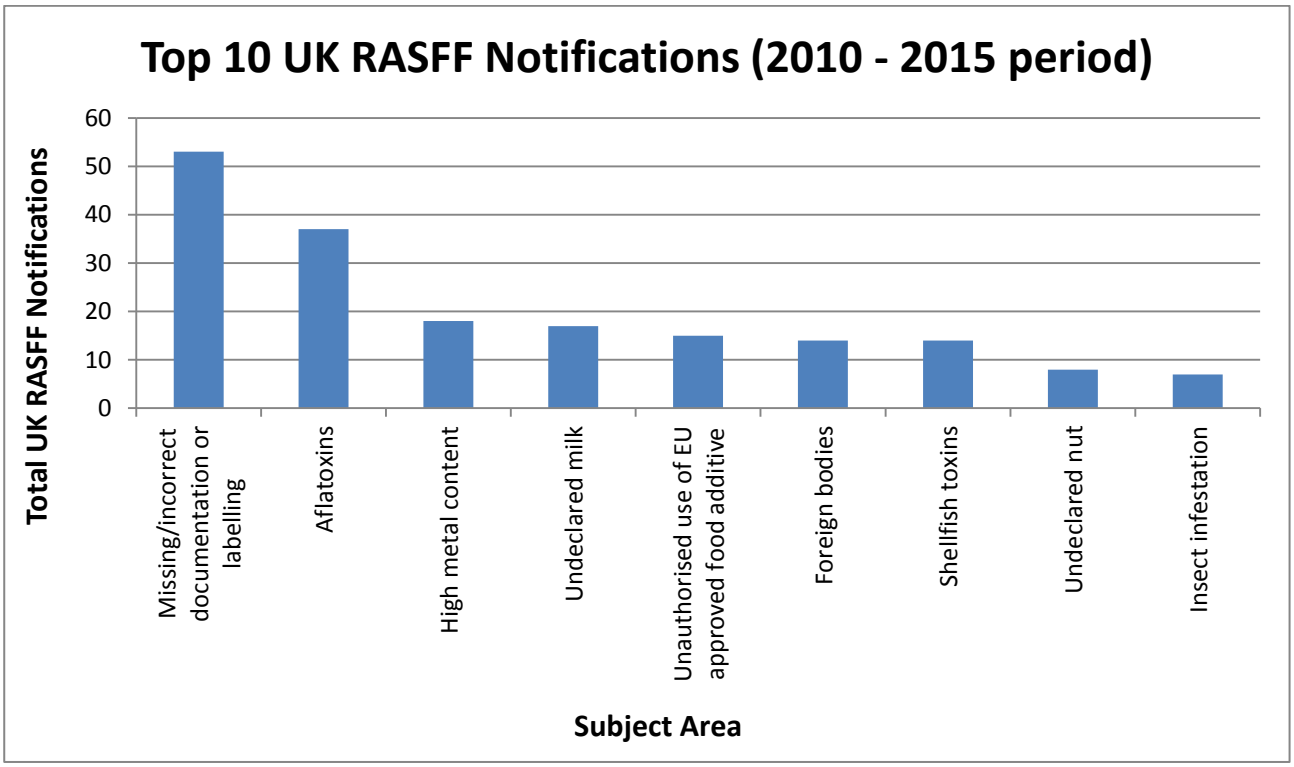


Figure 1. Top 10 UK RASFF notifications over the 2010 to 2015 period. Data sourced from RASFF portal (http://ec.europa.eu/food/safety/rasff/index_en.htm)

Table 2. Draft list of sampling priorities from the draft national coordinated sampling programme 2016/17. 'Level 1' denotes highest priority

Priority Level	Sampling Type	Description
1	Country of origin labelling	Country of origin labelling of fresh and frozen lamb, goat, pig and poultry. Label/traceability check only to check that recent legislation is being followed/raise awareness.
2	Allergens and gluten	Allergens and gluten in non-prepacked meals provided by food service/caterers. Specific allergen free meals to be requested and tested. To assess compliance and raise awareness.
3	Cyanide in bitter apricot kernels	Cyanide in bitter apricot kernels, powder and spreads. These are marketed to a vulnerable group and there are considerable safety concerns. The data is needed to refine risk assessments and inform the EU negotiations to set levels.
4	Meat species	Meat species in a range of meat preparations, products and meals. Substitution continues to be a problem. This project will follow on from the 15/16 project, but will be targeted using the results from the 15/16 project.
5	Adulteration of wines and spirits	Adulteration of wines and spirits. A continuing problem with potential consumer safety implications
6	Food supplements	Unauthorised health claims, illegal substances, and ingredient levels incorrect. This will follow on from the 15/16 project, but will be targeted using the results from the 15/16 project.
7	Fusarium toxin in maize and maize products	High levels of toxin have been found in maize grown in European countries following adverse weather. This is to monitor compliance with legal limits.
8	Acrylamide in various food products	Focussing on chips from chip shops and crisps cooked on food service premises. To assess business compliance and to inform ongoing policy development in Europe.
9	Arsenic and inorganic arsenic in rice and rice products	Including gluten free and products for infants, plus in UK produced fruits and vegetables, cereals and milk and dairy. To check compliance with maximum levels being introduced on 1 January 2016 for rice and rice products and to inform future risk management/dietary exposure.
10	Erucic acid in mustard oil, mustard oil blends, sesame and rapeseed oil	There have been problems with these oils exceeding the permitted level of erucic acid. Some mustard oil is sold for external use only – but often sold alongside foods.
11	Plastic rice	Rice' made with potato and sweet potato held together with industrial synthetic resins that are potentially harmful to health has been on the market in China for a few years now. There is intelligence that it is reaching other Asian markets, including India, Indonesia and Vietnam. Now that it is no longer a purely Chinese issue, we need to check that it is not entering the UK market.

Table 2 Continued. Draft list of sampling priorities from the draft national coordinated sampling programme 2016/17. 'Level 1' denotes highest priority

Priority Level	Sampling Type	Description
12	Accuracy of net weight/count declarations for frozen prawns and seafood	We are aware of industry data that suggests that there is significant poor and misleading practice. The proportion of ice glaze to product appears to be growing (not illegal in itself).
13	Various mycotoxins	Sterigmatocystin in rice and oat products, aflatoxin B1 and total aflatoxin in fresh and dried chillies and mycotoxins in flour and bread products from Poland
14	Heavy metals suite	Cadmium, lead, mercury and nickel in a wide range of foods, including fruit, vegetables, fungi, legumes, nuts, oilseeds, dairy, cereals, fish, tea, herbs, liver and kidney. This will check compliance with maximum levels for cadmium, lead and mercury and provide data on nickel to support ongoing European discussions.
15	Dioxins and PCBs in organic and free range eggs	Small/medium sized commercial producers. This is to look at non-compliance with legal limits that may be due to localised environmental contamination.
16	Undeclared offal	In retail/wholesale meat products such as burgers, minced meat and other meat products. This is about fraud and misdescription, which may be of particular concern to consumers who avoid eating particular species. (Note that this priority will only be undertaken on successful roll out of the method to PA labs.)
17	Dyes (crystal violet, leucocrystal violet, malachite green, leucomalachite green) and their metabolites in imported farmed fish	From South East Asia (particularly Vietnam). The precise detail will be decided on the basis of the outcome of the 15/16 sampling results.
18	Pesticide residues	In imported okra and beans.
19	Fish substitution	Of fresh, frozen fish and fish products. This continues to be a high risk area. Sampling will be targeted using the results of the 15/16 sampling programme.
20	Undeclared and under-declared water in fresh, chilled and frozen chicken	That looks like a cut, joint, slice etc of pure meat, and undeclared/misdeclared added proteins of different animal origin. Sampling will be targeted using the results of the 15/16 sampling programme.
21	Irradiated food	To check that rules are being followed in relation to both what can be irradiated and labelling requirements. Herbs and spices, noodle meals, food supplements and soft fruits.
22	Iodine levels in seaweed	Including seaweed used as salt replacers. This is to collect baseline data about iodine levels, which have potential consumer safety implications.

Table 3. Sampling decision matrix based on the Draft list of sampling priorities for the draft national coordinated sampling programme 2016/17. Overall Prioritisation Rating: 1-3 = Low, 4-6 = Medium, 7-9 = High

Draft FSA Priority Level	Sampling Type	Ease of Sourcing Materials	Sourcing Rating	MSI Suitability	Suitability Rating	Combined Rating	Overall Prioritisation Rating
1	Country of origin labelling	Difficult	1	Low	1	1	Low
2	Allergens and gluten	Easy	3	High	3	**9**	**High**
3	Cyanide in bitter apricot kernels	Medium	2	Medium	2	4	Medium
4	Meat species	Easy	3	High	3	**9**	**High**
5	Adulteration of wines and spirits	Easy	3	Medium	2	**6**	**Medium**
6	Food supplements	Easy	3	Medium	2	**6**	**Medium**
7	Fusarium toxin in maize and maize products	Medium	2	High	3	**6**	**Medium**
8	Acrylamide in various food products	Medium	2	Medium	2	4	Medium
9	Arsenic and inorganic arsenic in rice and rice products	Medium	2	Medium	2	4	Medium
10	Erucic acid in mustard oil, mustard oil blends, sesame and rapeseed oil	Difficult	1	Low	1	1	Low
11	Plastic rice	Difficult	1	High	3	3	Low
12	Accuracy of net weight/count declarations for frozen prawns and seafood	Difficult	1	Low	1	1	Low
13	Various mycotoxins	Medium	2	High	3	**6**	**Medium**
14	Heavy metals suite	Difficult	1	Low	1	1	Low
15	Dioxins and PCBs in organic and free range eggs	Difficult	1	Low	1	1	Low
16	Undeclared offal	Easy	3	Low	1	3	Low
17	Dyes (crystal violet, leucocrystal violet, malachite green, leucomalachite green) and their metabolites in imported farmed fish	Difficult	1	Medium	2	2	Low
18	Pesticide residues	Difficult	1	Medium	2	2	Low
19	Fish substitution	Easy	3	Medium	2	**6**	**Medium**
20	Undeclared and under-declared water in fresh, chilled and frozen chicken	Difficult	1	Low	1	1	Low
21	Irradiated food	Difficult	1	Low	1	1	Low
22	Iodine levels in seaweed	Difficult	1	Low	1	1	Low

4.3 Project priority list

Following discussions with the FSA a sampling scenarios priority list (Table 4) was finalised based on the results from Table 3, that formed the basis for the subsequent experimental evaluation work. The top sampling scenarios comprised allergens/gluten, meat speciation and melamine in milk powder.

Table 4. FSA agreed sampling scenarios priority list

Overall Ranking	Sampling scenarios	Details
Top 3		
1	Allergens and gluten (e.g. peanut in flour; almond in paprika; mahaleb in cumin)	Unlabelled food allergens represent a high risk to susceptible individuals.
2	Meat species (e.g. beef, pork, lamb, horse)	Meat adulteration/contamination represents a labelling and religious/societal issue.
3	Melamine powder in milk powder	Melamine is a toxic potential adulterant used to artificially improve the apparent protein content of food stuffs such as health supplements.
Other scenarios for feasibility study		
4	Undeclared offal (e.g. beef heart vs. beef muscle)	The term 'offal' includes non-meat animal by-products such as heart, kidney and lungs, and requires appropriate labelling should such components be included in any foodstuffs.
5	Arsenic and inorganic arsenic in rice and rice products	Arsenic is selectively accumulated in rice and represents a potential toxic/carcinogenic risk to consumers with routine consumption.
6	Foreign bodies (e.g. impurities in wheat, spelt, barley; contaminants in cereals) to include oregano leaves, macrofilth, shell/kernal	Food contaminants require appropriate quality control processes to ensure the purity and safety of foodstuffs.
7	Cyanide in bitter apricot kernels	Bitter apricot cultivars present a potential risk to consumers due to the elevated levels of the naturally occurring cyanogenic glycoside, amygdalin which is enzymatically converted to cyanide.
8	Fusarium toxin in maize and maize products	Fusarium species can infect maize crops/products and under the right conditions produce mycotoxins (secondary metabolites) which are toxic to humans and animals, causing issues such as kidney failure and cancer.
9	Plastic rice (depending on availability of authentic samples)	The identification of fake rice, termed 'plastic rice' (formed using potato starch and plasticisers), represents a novel potential health hazard and fraudulent scenario to regulators and consumers.

5 Objectives 3 and 4 - Evaluate the capability of MSI to assess testing scenarios in the priority list of food and feed samples

The purpose of Objective 3 was to investigate the top three prioritised sampling scenarios in order to optimise analytical processes and prioritise the subsequent full study. Objective 4 was the evaluation of up to seven more sampling scenarios from the prioritised list. For the purposes of brevity in this final report, the results from all ten sampling scenarios from Objectives 3 and 4 are presented together below.

5.1 Materials and methods

5.1.1 Multispectral 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.

5.1.2 Materials

A representative panel of test materials were sourced from research organisations, reputable online suppliers/UK supermarkets (sample authenticity dependent on supplier quality systems, e.g. auditing and testing) and scientific reagent suppliers.

5.1.3 Ad-mixture preparation methodology

A gravimetric-based approach comprising test materials generated using percentage weight per weight (% w/w) components, 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, e.g. high density materials such as stone will have a smaller volume than lighter density materials such as rice which will affect any observable surface area-based observations.

5.1.4 Foreign bodies (e.g. impurities in wheat, spelt, barley; contaminants in cereals)

a) Oregano herb adulterated with olive leaves: Olive leaves were ground using an IKA Tube Mill (IKA, Germany) and the resultant material sieved to remove powdered components. A broad panel of oregano adulteration admixtures comprising 100 % w/w oregano; 100% w/w ground olive leaves; 50 % w/w, 25 % w/w, 10 % w/w, 5 % w/w, 1% w/w and 0.5 % w/w ground olive leaves in a background of oregano were gravimetrically prepared in 90 mm diameter disposable Petri dishes (triplicate 10 g admixtures per sample type).
b) Basmati rice containing fine gravel impurities: Sample preparation and analysis combined with the plastic rice test scenario.

5.1.5 Plastic rice

a) Basmati rice adulterated with 'plastic rice' and gravel impurities: Artificial rice grains were prepared using Parafilm M (Bemis, Neenah, USA) formed into ~2 mm diameter plastic roll which was cut into ~5 – 10 mm lengths. A broad panel of basmati rice adulteration/contamination admixtures were gravimetrically prepared in 90 mm diameter disposable Petri dishes (triplicate 5 g admixtures per sample type) comprising:
    - 100 % w/w basmati rice; 100 % w/w 'plastic rice'; 100 % w/w gravel (2 – 4 mm size)
    - 50 % w/w, 25 % w/w, 10 % w/w, 5 % w/w and 1% w/w 'plastic rice' or gravel in a background of basmati rice
    - 25% w/w (50% total), 12.5 % w/w (25 % total), 5 % w/w (10 % total) and 2.5 % w/w (5 % total) 'plastic rice' and gravel in a background of basmati rice

5.1.6 Meat species

a) Beef meat blended with pork meat: Beef and pork cuts were trimmed to remove surface fat/non-muscle and homogenised to a paste using a food processor. A broad panel of blended meat adulteration admixtures comprising 100 % w/w beef meat; 100 % w/w pork meat; 50 % w/w, 25 % w/w, 10 % w/w, 5 % w/w, 1 % w/w and 0.5 % w/w pork meat blended into a background of beef meat were gravimetrically prepared and transferred to 90 mm diameter disposable Petri dishes (triplicate 25 g admixtures per sample type).

5.1.7 Undeclared offal

a) Beef meat adulterated with beef heart: Beef cuts were trimmed to remove surface fat/non-muscle and homogenised to a paste using a food processor. A broad panel of beef meat adulteration admixtures comprising 100 % w/w beef meat; 100% w/w beef heart; 50 % w/w, 25 % w/w, 10 % w/w, 5 % w/w, 1 % w/w and 0.5 % w/w beef heart in a background of beef meat were gravimetrically prepared and transferred to 90 mm diameter disposable Petri dishes (triplicate 25 g admixtures per sample type).

5.1.8 Allergens and gluten

a) Cumin adulteration with ground almond shell: Whole almonds were deshelled and the collected shells ground using an IKA Tube Mill (IKA, Germany). The ground almond was then sieved using a 500 μM metal sieve to remove any large granular material. A broad panel of paprika adulteration admixtures comprising 100 % w/w ground cumin; 100 % w/w ground almond shells; 50 % w/w, 25 % w/w, 10 % w/w, 5 % w/w, 1% w/w and 0.5 % w/w ground almond shell in a background of ground cumin were gravimetrically prepared in 90 mm diameter disposable Petri dishes (triplicate 10 g admixtures per sample type).
b) Cumin adulterated with mahaleb: A broad panel of cumin adulteration admixtures comprising 100 % w/w ground cumin; 100 % w/w ground mahaleb (*Prunus mahaleb* seeds); 50 % w/w, 25 % w/w, 10 % w/w, 5 % w/w, 1% w/w and 0.5 % w/w ground mahaleb in a background of ground cumin were gravimetrically prepared in 90 mm diameter disposable Petri dishes (triplicate 10 g admixtures per sample type).

c) Almond adulterated with peanut: Deshelled almonds and peanuts were ground using an IKA Tube Mill (IKA, Germany). A broad panel of almond adulteration admixtures comprising 100 % w/w ground almond; 100 % w/w peanut; 50 % w/w, 25 % w/w, 10 % w/w, 5 % w/w, 1 % w/w and 0.5 % w/w ground peanut in a background of ground almond were gravimetrically prepared in 90 mm diameter disposable Petri dishes (triplicate 10 g admixtures per sample type).

d) Wheat flour adulterated with defatted peanut flour: A broad panel of wheat flour adulteration admixtures comprising 100 % w/w wheat flour; 100% w/w peanut flour; 50 % w/w, 25 % w/w, 10 % w/w, 5 % w/w, 1 % w/w and 0.5 % w/w peanut flour in a background of wheat flour were gravimetrically prepared in 90 mm diameter disposable Petri dishes (triplicate 20 g admixtures per sample type).

5.1.9 Food supplements

a) Dried skimmed milk powder adulterated with melamine (1,3,5-Triazine-2,4,6-triamine): A broad panel of skimmed milk powder adulteration admixtures comprising 100 % w/w dried skimmed milk powder; 100 % w/w melamine (99 %, Acros Organics); 50 % w/w, 25 % w/w, 10 % w/w, 5 % w/w, 1 % w/w and 0.5 % w/w melamine in a background of dried skimmed milk powder were gravimetrically prepared (50 g admixtures per sample type) and transferred to Videometer A/S (Hørsholm, Denmark) for analysis.

5.1.10 Arsenic and inorganic arsenic in rice and rice products

a) Arsenic contamination in rice: A panel of rice flours (Table 5) were provided by P S Analytical Ltd (Orpington, UK) containing a range of arsenic levels (4 ppb – 433 ppb total As). Due to sample size limitations, single 7.5 g portions were transferred to a 90 mm diameter disposable Petri dish for analysis.

Table 5. Details of rice flour samples provided by P S Analytical Ltd (Orpington, UK) showing a range of Arsenic levels

Name	Country	Colour	Inorganic As (ppb)	Total As (ppb)	Relative As Level
Wild rice	Unknown	Black	6	7	Low
Madagascar, red rice	Madagascar	Red	2	4	Low
Acquerello, Superfino Carnaroli,	Italy	White	60.1	83	Medium
Shinode Reis USA	USA	White	83.3	115	Medium
Riso Venere	Italy	Black	207	242	High
Riz rouge	France	Red	349	422	High

5.2 MSI Analysis

Admixtures were mixed well to ensure good component distribution, the Petri dish cover removed and placed under the integrating sphere for image capture. Image capture (all wavelengths) was performed using VideometerLab Software Version 3.0.28.3833 and the default 100 % reflectance light settings unless image saturation necessitated an optimised light setting which was then applied across all samples within the specific test scenario.

Image data analysis was performed using VideometerLab Software Version 3.0.28.3833 to analyse the 100 % control materials which were used to generate a ‘known vs known’ normalised Canonical Discriminant Analysis (nCDA) transformational model based on their respective spectral signatures. The resultant 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. Where possible, further analyses were performed using the ‘MSI Area Fraction 3 PlugIn’ data processing tool to estimate the area fraction of the sample matching a chosen model, e.g. the adulterant, and hence could be used to very roughly estimate the potential percentage adulteration levels based on surface area only.

In the event that standard analyses could not be performed due to poor discrimination, the resultant spectral data was analysed using the ‘General Statistics - Statistics PlugIn’ data processing tool to determine mean component spectral data across the whole target region.

5.3 Results

5.3.1 Foreign bodies (e.g. impurities in wheat, spelt, barley; contaminants in cereals)

a) Oregano herb adulterated with olive leaves

The results (Table 6) show that the applied MSI methodology is capable of successfully differentiating between ground oregano leaves and adulterant ground olive leaves with good quantitative potential. A rough limit of detection can be applied to this approach (estimated as the lowest w/w level that gave at least a 95 % chance of detecting a measurement response from the adulterant whose signal was different from the blank control) and equated to ≤ 0.5 % w/w with a coefficient of variation (CV) of 18 % at the 0.5% w/w level.

Table 6. MSI analyses of the oregano adulterated with olive leaves test samples. Processed image presented in a false colour format: red areas (olive sample type), beige areas (oregano sample type) and blue areas (background). The mean estimated % content based on area fractional calculation along with the associated 95 % confidence interval is shown. Values based on three technical replicates per sample.

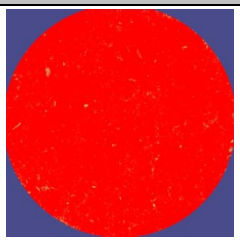
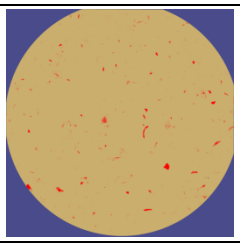
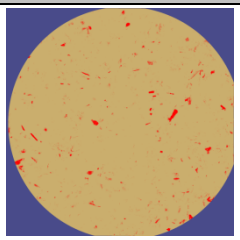
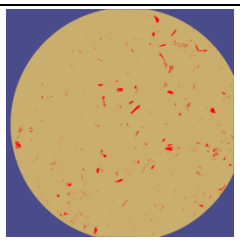
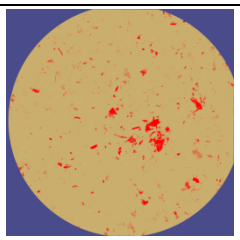
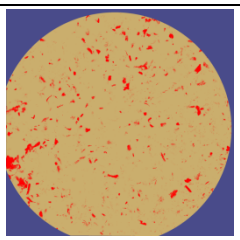
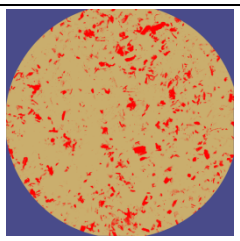
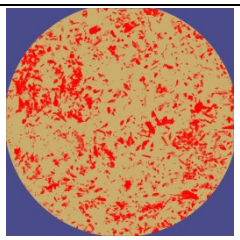
Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model	% Coefficient of Variation
100 % w/w olive leaves			97.0 ± 0.7	0.6
100 % w/w oregano			1.0 ± 0.3	25.1

Table 6 Continued.

Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model	% Coefficient of Variation
0.5 % w/w olive leaves in oregano			1.9 ± 0.4	18.0
1 % w/w olive leaves in oregano			2.3 ± 0.9	36.1
5 % w/w olive leaves in oregano			3.2 ± 1.0	26.3
10 % w/w olive leaves in oregano			4.6 ± 0.3	6.0
25 % w/w olive leaves in oregano			13.4 ± 3.4	22.7
50 % w/w olive leaves in oregano			22.8 ± 2.3	8.7

b) Basmati rice contaminated with fine gravel impurities

The evaluation work was combined with ‘plastic rice’ test scenario and will be discussed collectively.

5.3.2 Plastic rice

a) Basmati rice adulterated with ‘plastic rice’ and contaminated with fine gravel impurities

The particulate nature of grain, seed and leaf material make such samples ideally suited for discrimination using multispectral imaging. The results (Table 7) show that the developed MSI methodology is capable of successfully differentiating between basmati rice, the plastic rice adulterant and gravel impurity types with good quantitative potential. The estimated LOD using this model was ≤ 1 % w/w for both non-basmati rice components.

The associated quantitative data is based on visible surface area and therefore under/over estimates contributions derived from components with very different material densities to the background sample material, e.g. gravel shows a lower % composition level than the equivalent mass of plastic rice when compared with the basmati rice background. The quantitative data shows good levels of precision for a novel methodology as characterised by an estimated CV of 8 % (plastic rice) and 18 % (gravel) at the 1 % w/w level. The higher individual % CV values are correlated with low level MSI mis-classification artefacts when applied to admixtures not containing the target analyte, e.g. plastic rice or gravel.

These results demonstrate the clear non-targeted and multi-analyte capability of MSI in which the MSI methodology correctly identified and classified the adulterant “plastic rice” grains and identified the gravel “foreign body” with the same sample using the same analysis. This showcases the rapidity and cost-effectiveness of such a multi-analyte approach.

Table 7. MSI analyses of the basmati rice adulterated with 'plastic rice' and contaminated with fine gravel test samples. Processed image presented in a false colour format: red areas (plastic rice sample type), dark blue areas (gravel sample type), beige areas (basmati rice sample type) and lighter blue areas (background). The mean estimated % content based on area fractional calculation along with the associated 95 % confidence interval is shown. Values based on three technical replicates per sample.

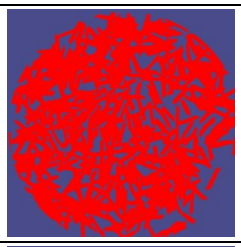
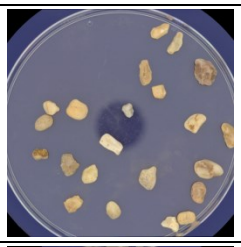
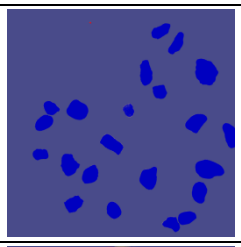
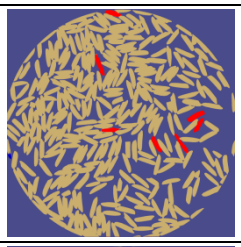
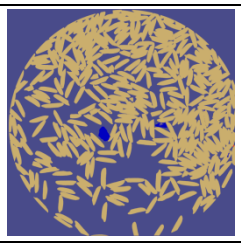
Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model		% Coefficient of Variation	
			'Plastic Rice'	Gravel	'Plastic Rice'	Gravel
100 % w/w Basmati rice			0.0 ± 0.0	0.0 ± 0.0	79.1	61.7
100 % w/w 'plastic rice'			100.0 ± 0.0	0.0 ± 0.0	0.0	79.2
100 % w/w gravel			0.0 ± 0.0	98.4 ± 0.3	16.0	0.2
1 % w/w 'plastic rice' in basmati rice			2.0 ± 0.2	0.1 ± 0.0	7.9	32.3
1 % w/w gravel in basmati rice			0.0 ± 0.0	0.6 ± 0.1	4.8	17.8

Table 7 Continued.

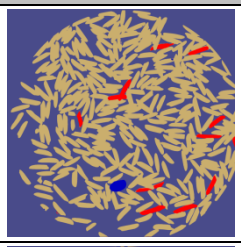
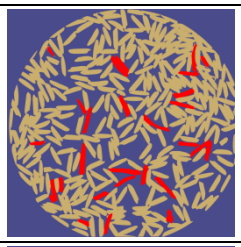
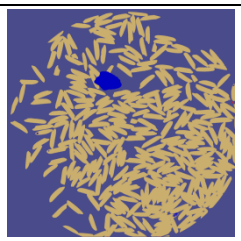
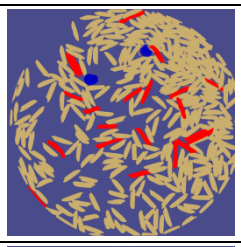
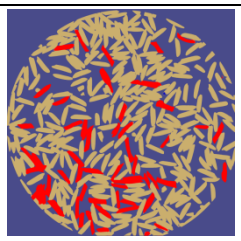
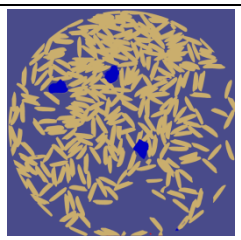
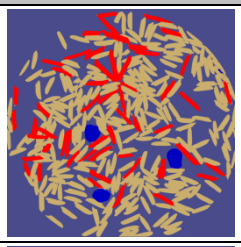
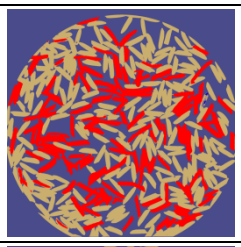
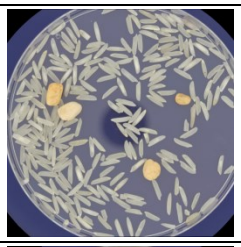
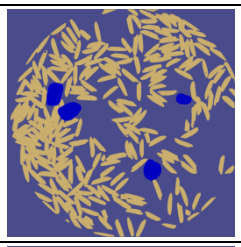
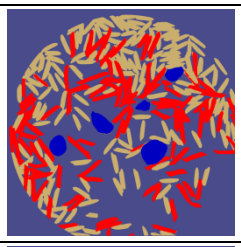
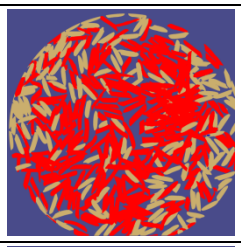
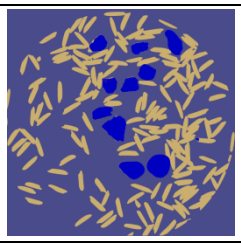
Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model		% Coefficient of Variation	
			'Plastic Rice'	Gravel	'Plastic Rice'	Gravel
2.5 % w/w gravel and 2.5 % w/w 'plastic rice, in basmati rice			4.0 ± 0.4	0.6 ± 0.1	7.9	10.5
5 % w/w 'plastic rice' in basmati rice			9.1 ± 0.2	0.0 ± 0.0	1.7	72.0
5 % w/w gravel in basmati rice1			0.0 ± 0.0	1.2 ± 0.4	39.7	26.9
5 % w/w gravel and 5 % w/w 'plastic rice' in basmati rice			8.4 ± 0.6	1.2 ± 0.1	6.6	10.1
10 % w/w 'plastic rice' in basmati rice			16.6 ± 0.8	0.0 ± 0.0	4.5	19.5
10 % w/w gravel in basmati rice			0.0 ± 0.0	2.6 ± 0.1	56.5	4.2

Table 7 Continued.

Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model		% Coefficient of Variation	
			'Plastic Rice'	Gravel	'Plastic Rice'	Gravel
12.5 % w/w gravel and 12.5 % w/w 'plastic rice' in basmati rice			20.4 ± 0.5	2.9 ± 0.3	2.0	9.2
25 % w/w 'plastic rice' in basmati rice			36.3 ± 0.2	0.0 ± 0.0	0.5	98.8
25 % w/w gravel in basmati rice			0.0 ± 0.0	6.8 ± 1.2	43.7	15.9
25 % w/w gravel and 25 % w/w 'plastic rice' in basmati rice			41.9 ± 0.7	6.6 ± 0.8	1.4	11.2
50 % w/w 'plastic rice' in basmati rice			65.2 ± 1.3	0.0 ± 0.0	1.8	74.0
50 % w/w gravel in basmati rice			0.4 ± 0.2	17.3 ± 1.0	49.0	5.1

5.3.3 Meat species

a) Beef meat blended with pork meat

Table 8 summarises the application of MSI to the detection of component meat species within finely ground beef and pork meat test samples which had been blended together. The LOD was estimated as ≤ 5 % w/w based on blended meat material with a CV of 25 % at the 5% w/w level. In addition, quantitative information can be determined that whilst underestimating % adulteration levels gives an indication of the relative levels.

The quantitative potential of the approach may have been limited by the sample prepration method employed to generate the meat samples which involved homogenising meat admixtures using a food blender to form a fine paste. The similarity in meat spectral profiles combined with the finely blended nature of the test materials likely reduced the discriminatory potential of the approach. The use of adulterated minced meat samples may improve the discriminatory potential of the MSI-based approach by reducing overall homogeneity and therby enhancing any tissue specific physiochemical characterisitcs.

Table 8. MSI analyses of the beef meat blended with pork meat test samples. Processed image presented in a false colour format: red areas (pork sample type), beige areas (beef sample type) and blue areas (background). The mean estimated % content based on area fractional calculation along with the associated 95 % confidence interval is shown. Values based on three technical replicates per sample.

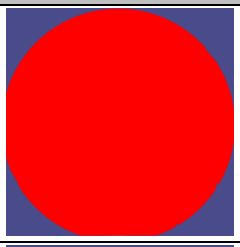
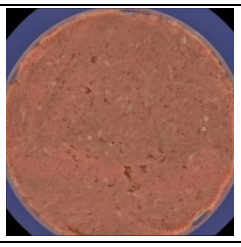
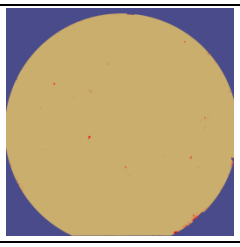
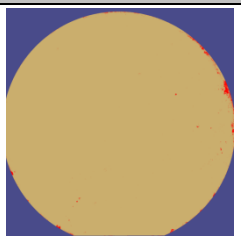
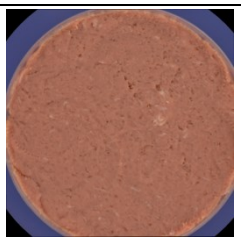
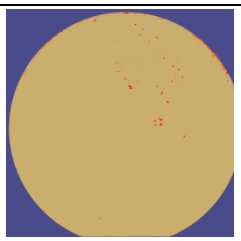
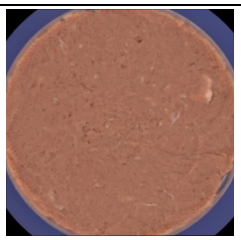
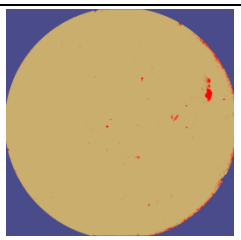
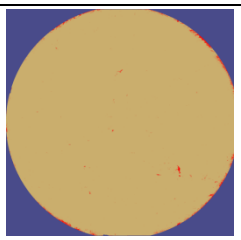
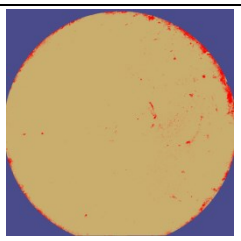
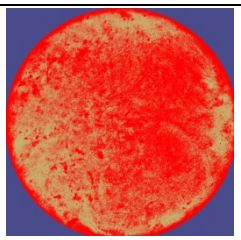
Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model	% Coefficient of Variation
100 % w/w pork			100.0 ± 0.0	0.0
100 % w/w beef			0.2 ± 0.1	25.4

Table 8 Continued.

Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model	% Coefficient of Variation
0.5 % w/w pork in beef			0.4 ± 0.3	58.4
1 % w/w pork in beef			0.2 ± 0.2	61.9
5 % w/w pork in beef			0.6 ± 0.2	25.0
10 % w/w pork in beef			0.6 ± 0.1	18.3
25 % w/w pork in beef			2.2 ± 0.1	3.7
50 % w/w pork in beef			47.6 ± 10.9	20.2

5.3.4 Undeclared offal

a) Beef meat adulterated with beef heart

The results (Table 9) show that the applied MSI methodology is capable of successfully differentiating between finely ground beef meat blended with the finely ground beef heart adulterant with limited quantitative potential. The estimated LOD was ≤ 10 % w/w with a CV of 40 % at the 10% w/w level. As previously discussed, the sample preparation approach employed generated a meat paste which reduced overall sample complexity and minimised the scope for generating discriminatory models which is shown by the gross adulteration levels required for robust detection.

Table 9. MSI analyses of the beef meat adulterated with beef heart test samples. Processed image presented in a false colour format: red areas (beef heart sample type), beige areas (beef meat sample type) and blue areas (background). The mean estimated % content based on area fractional calculation along with the associated 95 % confidence interval is shown. Values based on three technical replicates per sample.

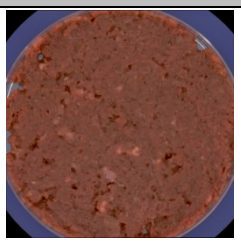
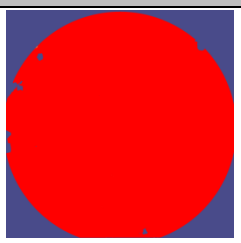
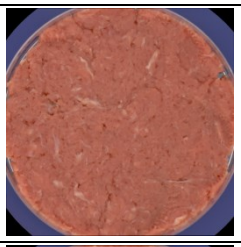
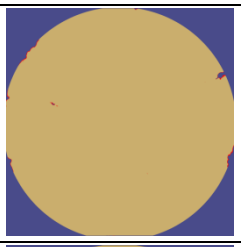
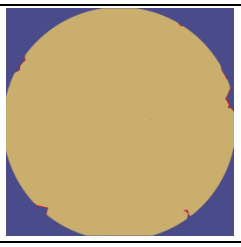
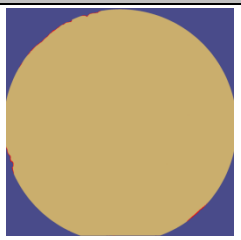
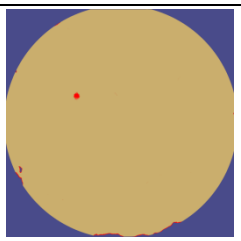
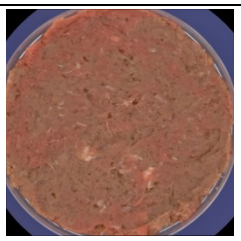
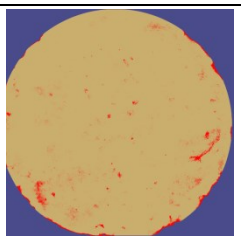
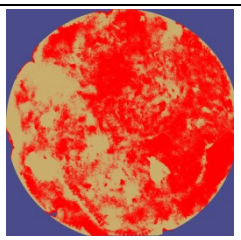
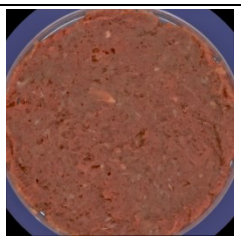
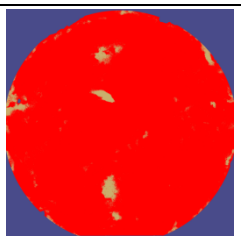
Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model	% Coefficient of Variation
100 % w/w beef heart			100.0 ± 0.1	0.1
100 % w/w beef meat			0.1 ± 0.1	70.6
0.5 % w/w beef heart in beef meat			0.1 ± 0.0	28.1

Table 9 Continued.

Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model	% Coefficient of Variation
1 % w/w beef heart in beef meat			0.2 ± 0.1	26.8
5 % w/w beef heart in beef meat			0.2 ± 0.0	22.1
10 % w/w beef heart in beef meat			2.3 ± 1.0	39.7
25 % w/w beef heart in beef meat			51.4 ± 8.5	14.6
50 % w/w beef heart in beef meat			97.1 ± 0.9	0.9

5.3.5 Allergens and gluten

a) Cumin adulterated with ground almond shell

The results (Table 10) clearly show that the MSI methodology used is capable of differentiating between cumin spice and the ground almond shell adulterant. The LOD was

estimated as ≤ 5 % w/w with a very precise CV of 4 % at the 5% w/w level. Quantitative information can be determined, that whilst underestimating % adulteration levels, gives a good indication of the relative levels. Further refinement to the model used should improve the diagnostic potential of this methodology.

In addition to standard allergen testing, these results highlight the applicability of MSI to the detection and estimation of impurities (foreign bodies) within a test sample as the ground almond shell is not a recognised food component.

Table 10. MSI analyses of cumin adulterated with almond shells test samples. Processed image presented in a false colour format: red areas (almond sample type), beige areas (cumin sample type) and blue areas (background).). Statistical analyses derived from 3x MSI scans per sample type (1x MSI scan per test sample). Accuracy shown as the 95 % confidence interval. Estimated % content based on area fractional calculation.

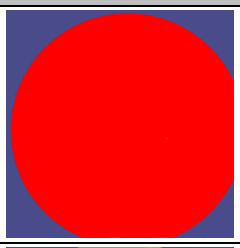
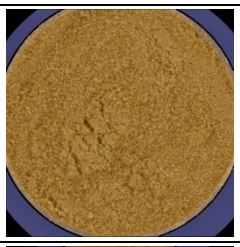
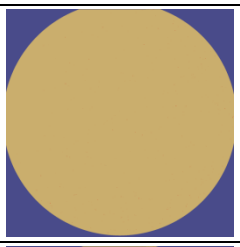
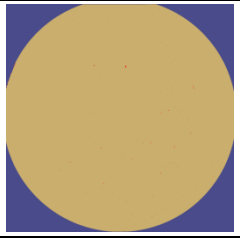
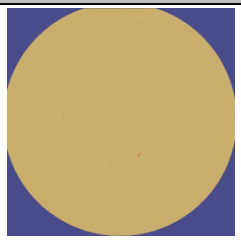
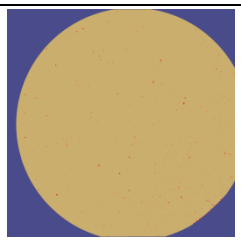
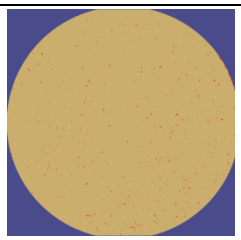
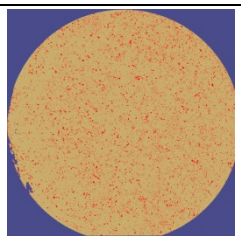
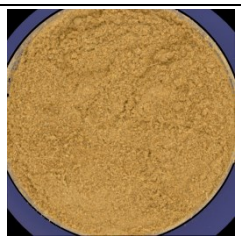
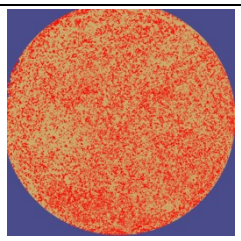
Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model	% Coefficient of Variation
100 % w/w ground almond shells			99.6 ± 0.7	0.6
100 % w/w Cumin			0.1 ± 0.0	57.9
0.5 % w/w ground almond shells in cumin			0.0 ± 0.0	19.6

Table 10 continued.

Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model	% Coefficient of Variation
1% w/w ground almond shells in cumin			0.1 ± 0.1	61.1
5 % w/w ground almond shells in cumin			0.2 ± 0.0	3.8
10 % w/w ground almond shells in cumin			0.4 ± 0.0	7.2
25 % w/w ground almond shells in cumin			5.2 ± 1.7	28.9
50 % w/w ground almond shells in cumin			28.8 ± 7.1	21.7

b) Cumin adulterated with mahaleb

The results (Table 11) show that the applied MSI methodology is capable of differentiating between cumin spice and the mahaleb adulterant with poor quantitative potential. The estimated LOD was ≤ 5 % w/w with a CV of 60 % at the 5% w/w level. The test scenario

exhibits restricted analytical performance due to the limited spectral differences between the component materials. Further work is required in order to investigate alternative approaches to improve the discriminatory potential of the MSI approach with respect to this test scenario.

Table 11. MSI analyses of the cumin adulterated with mahaleb test samples. Processed image presented in a false colour format: red areas (mahaleb sample type), beige areas (cumin sample type) and blue areas (background). The mean estimated % content based on area fractional calculation along with the associated 95% confidence interval is shown. Values based on three technical replicates per sample.

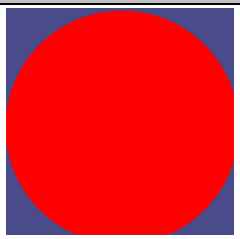
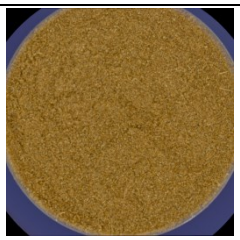
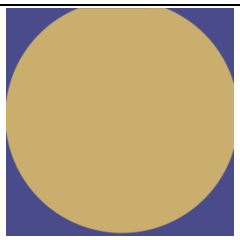
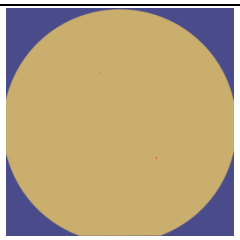
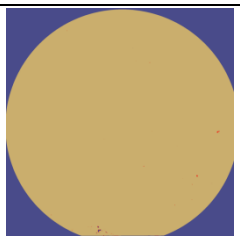
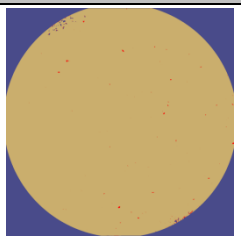
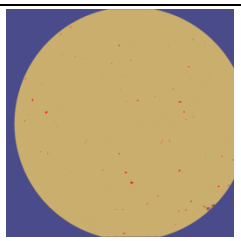
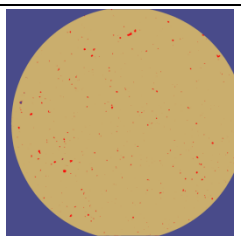
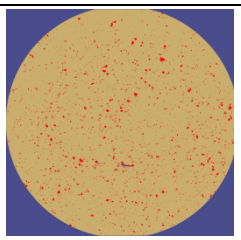
Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model	% Coefficient of Variation
100 % w/w mahaleb			100.0 ± 0.0	0.0
100 % w/w cumin			0.0 ± 0.0	75.9
0.5 % w/w mahaleb in cumin			0.0 ± 0.0	57.7
1 % w/w mahaleb in cumin			0.0 ± 0.0	48.1

Table 11 continued.

Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model	% Coefficient of Variation
5 % w/w mahaleb in cumin			0.1 ± 0.0	45.9
10 % w/w mahaleb in cumin			0.1 ± 0.0	3.1
25 % w/w mahaleb in cumin			0.5 ± 0.1	11.1
50 % w/w mahaleb in cumin			4.2 ± 1.3	26.7

c) Almond adulterated with peanut

The results (Table 12) show that the applied MSI methodology is capable of differentiating between ground almond and the ground peanut adulterant with limited quantitative potential at the low level adulteration range. Although visually similar within the visible spectrum, both components have a distinct and unique spectral profile when assessed using multispectral imaging. The LOD is estimated as ≤ 5 % w/w with a CV of 25 % at the 5% w/w level.

Table 12. MSI analyses of the almond adulterated with peanut test samples. Processed image presented in a false colour format: red areas (peanut sample type), beige areas (almond sample type) and blue areas (background). The mean estimated % content based on area fractional calculation along with the associated 95 % confidence interval is shown. Values based on three technical replicates per sample.

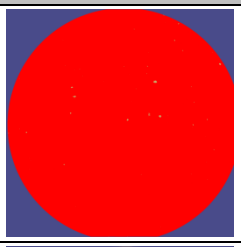
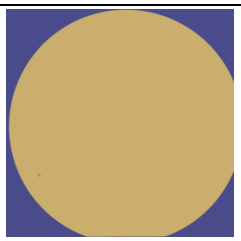
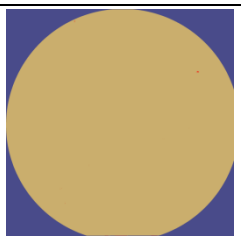
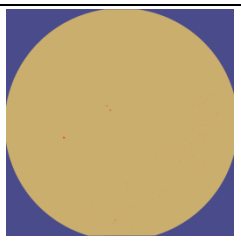
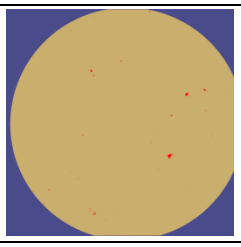
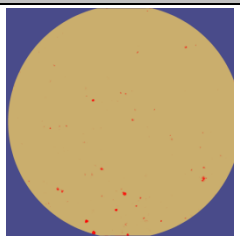
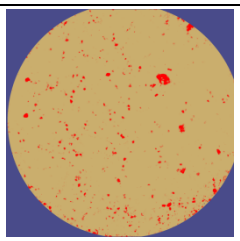
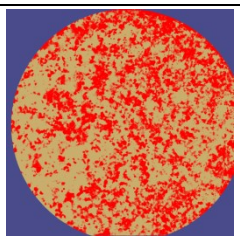
Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model	% Coefficient of Variation
100 % w/w ground peanut			99.7 ± 0.3	0.3
100 % w/w ground almond			0.0 ± 0.0	79.2
0.5 % w/w ground peanut in almond			0.0 ± 0.0	58.1
1 % w/w ground peanut in almond			0.0 ± 0.0	86.0
5 % w/w ground peanut in almond			0.1 ± 0.0	25.1

Table 12 continued.

Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model	% Coefficient of Variation
10 % w/w ground peanut in almond			0.3 ± 0.2	57.8
25 % w/w ground peanut in almond			1.8 ± 0.7	34.4
50 % w/w ground peanut in almond			33.2 ± 4.2	11.2

d) Wheat flour adulterated with peanut flour

The results (Table 13) show that the applied MSI methodology is capable of differentiating between wheat flour and the peanut flour adulterant with limited quantitative potential. The LOD was estimated as ≤ 10 % w/w with a CV of 64 % at the 10% w/w level. The detection sensitivity and quantitative capabilities of this method are likely to have been limited by the the fine particulate size associated with the wheat and peanut flours which exceeds the resolution of the image capture system. Therefore, mixed flour test materials will show poor discriminatory results as the ground almond component will typically mask the spectral signature associated with the peanut adulterant until a compositional threshold level has been reached. This hypothesis is supported by the high adulteration levels (≥ 25 %) required for robust detection and associated adulteration over estimates.

Table 13. MSI analyses of the wheat flour adulterated with peanut flour test samples. Processed image presented in a false colour format: red areas (peanut sample type), beige areas (wheat sample type) and blue areas (background). The mean estimated % content based on area fractional calculation along with the associated 95 % confidence interval is shown. Values based on three technical replicates per sample.

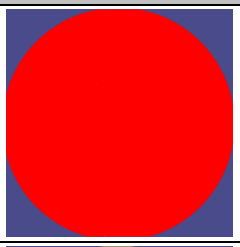
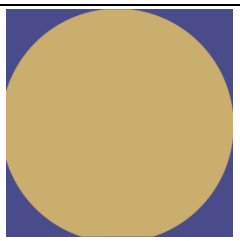
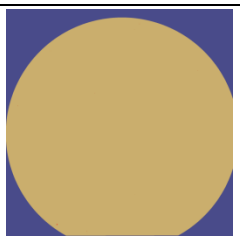
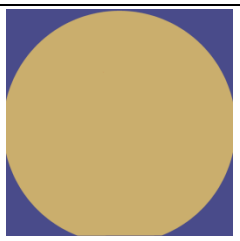
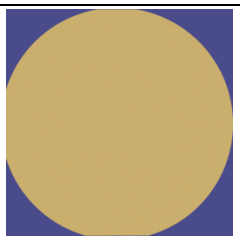
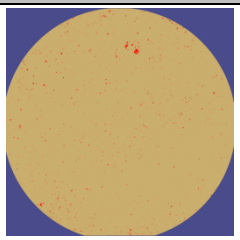
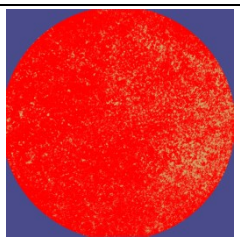
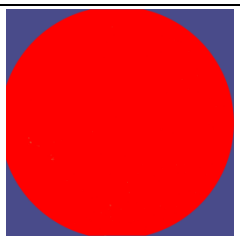
Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model	% Coefficient of Variation
100 % w/w peanut flour			100.0 ± 0.0	0.0
100 % w/w wheat flour			0.0 ± 0.0	79.8
0.5 % w/w peanut flour in wheat flour			0.0 ± 0.0	31.2
1 % w/w peanut flour in wheat flour			0.0 ± 0.0	107.0
5 % w/w peanut flour in wheat flour			0.0 ± 0.0	20.8

Table 13 Continued.

Sample	sRGB Image	Processed Image	Mean Estimated % Area Most Closely Matching "Adulterant" Model	% Coefficient of Variation
10 % w/w peanut flour in wheat flour			0.8 ± 0.5	63.7
25 % w/w peanut flour in wheat flour			81.7 ± 5.0	6.1
50 % w/w peanut flour in wheat flour			100.0 ± 0.0	0.0

e) Discussion

The unreported presence of allergenic components such as nuts represents a serious threat to a susceptible individual's health and wellbeing. The allergen test scenarios explored under the remit of this project demonstrate the clear applicability of MSI for allergen detection and potential quantitative capabilities.

Further refinement to the analytical models used should improve the diagnostic potential of this methodology. Routinely screening high risk foodstuffs with MSI-based approaches could reduce the incidence of allergic reactions and increase overall consumer confidence.

5.3.6 Food supplements

a) Dried skimmed milk powder adulterated with melamine (1,3,5-Triazine-2,4,6-triamine)

The MSI-based analysis of dried skimmed milk powder adulterated with melamine was found to be particularly challenging due to the inherent high reflectance properties of the powders investigated, resulting in pixel saturation, and the fine grain size which exceeds the resolution of the image capture system. Therefore, whilst the 100 % control milk powder and melamine samples could be clearly differentiated spectrally, mixtures could not be analysed using the standard normalised Canonical Discriminant Analysis (nCDA)-based method.

Further analyses were conducted based on the mean component spectral data across the whole target region (Figure 2) to evaluate the impact of melamine on sample spectral characteristics. Figure 2 shows that there is an association between the % w/w melamine content and reflectance across the MSI wavelengths which allow the test admixtures to be differentiated. This may allow MSI to be used to differentiate between samples of milk powder which had different amounts of melamine present in them based on the reflectance across different wavelengths.

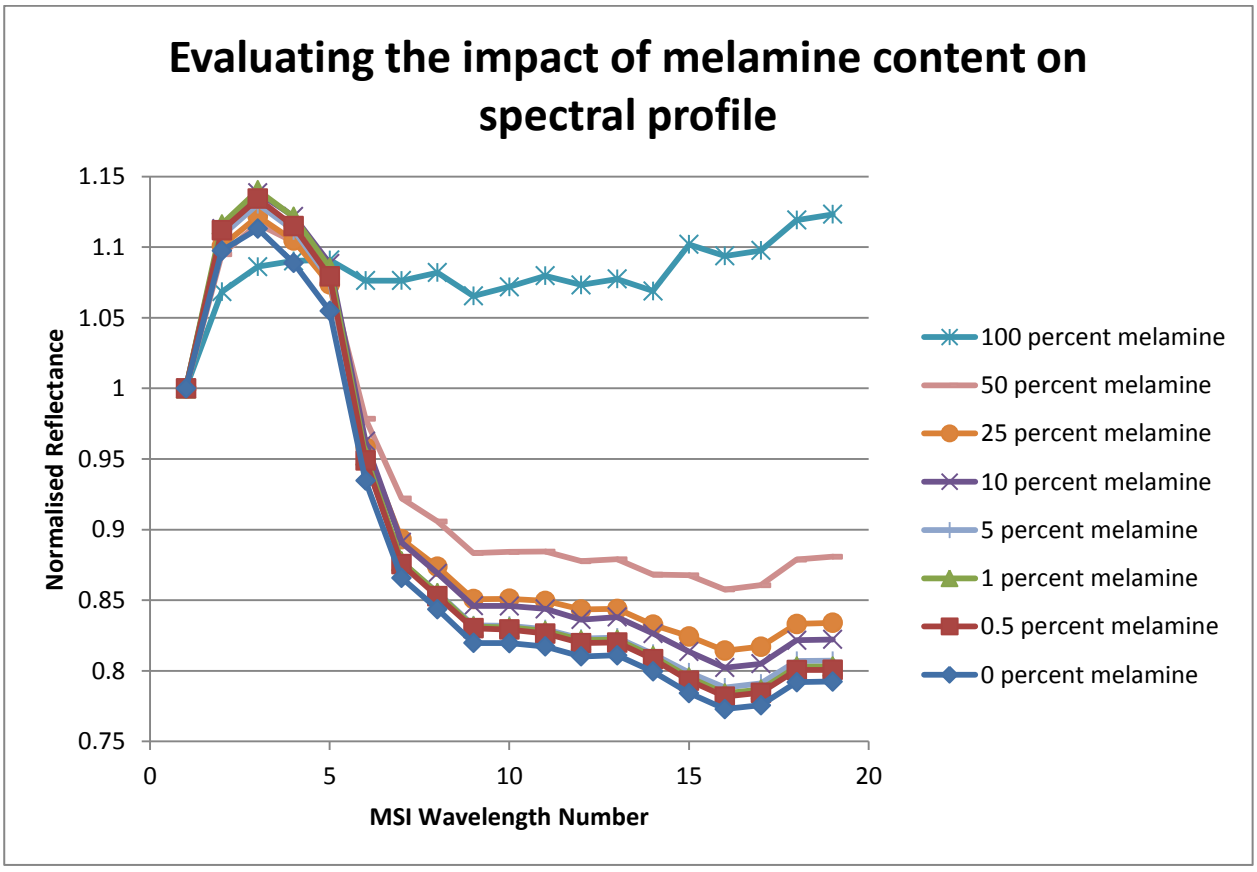


Figure 2. Graph showing MSI spectral profiles generated from single MSI scans per melamine in milk powder sample type normalised to band 1 reflectance values

5.3.7 Arsenic and inorganic arsenic in rice and rice products

a) Arsenic contamination in rice

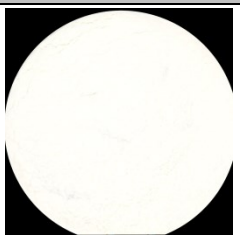
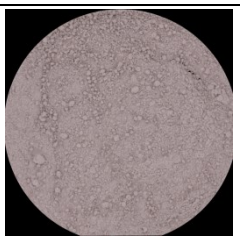
Evaluating the applicability of MSI to analysing arsenic contamination in rice is dependent on the presence of detectable unique spectral characteristics indicative of arsenic content. Unfortunately, test samples with characterised and confirmed different arsenic content within the same rice species were extremely difficult to source from any provider as part of this project. The actual test samples provided for this project comprised multiple varieties of different base colours (Table 14) which prevented the development of any MSI-based discriminatory models for arsenic alone. Figure 3 shows the normalised spectral profiles generated by the rice materials and highlighted the absence of features related to arsenic content whilst the estimated arsenic level groupings (low, medium and high) were clearly

associated with the general rice colour only.

In order to fully explore the applicability of using MSI for evaluating arsenic concentration in rice, sourcing different samples of known arsenic concentration from the sample rice variety is a prerequisite. Based on the scope and utilisation of MSI we believe that the technology is better suited to looking at other food testing applications, but until applicable rice samples can be sourced, this has yet to be proven.

Table 14. MSI analyses of the rice powders containing a range of arsenic concentrations.

Sample	sRGB Image	Relative Arsenic Level
Wild Rice		Low
Madagascar		Low
Acquerello		Medium

Sample	sRGB Image	Relative Arsenic Level
Shinode Reis		Medium
Riso Venere		High
Riz rouge		High

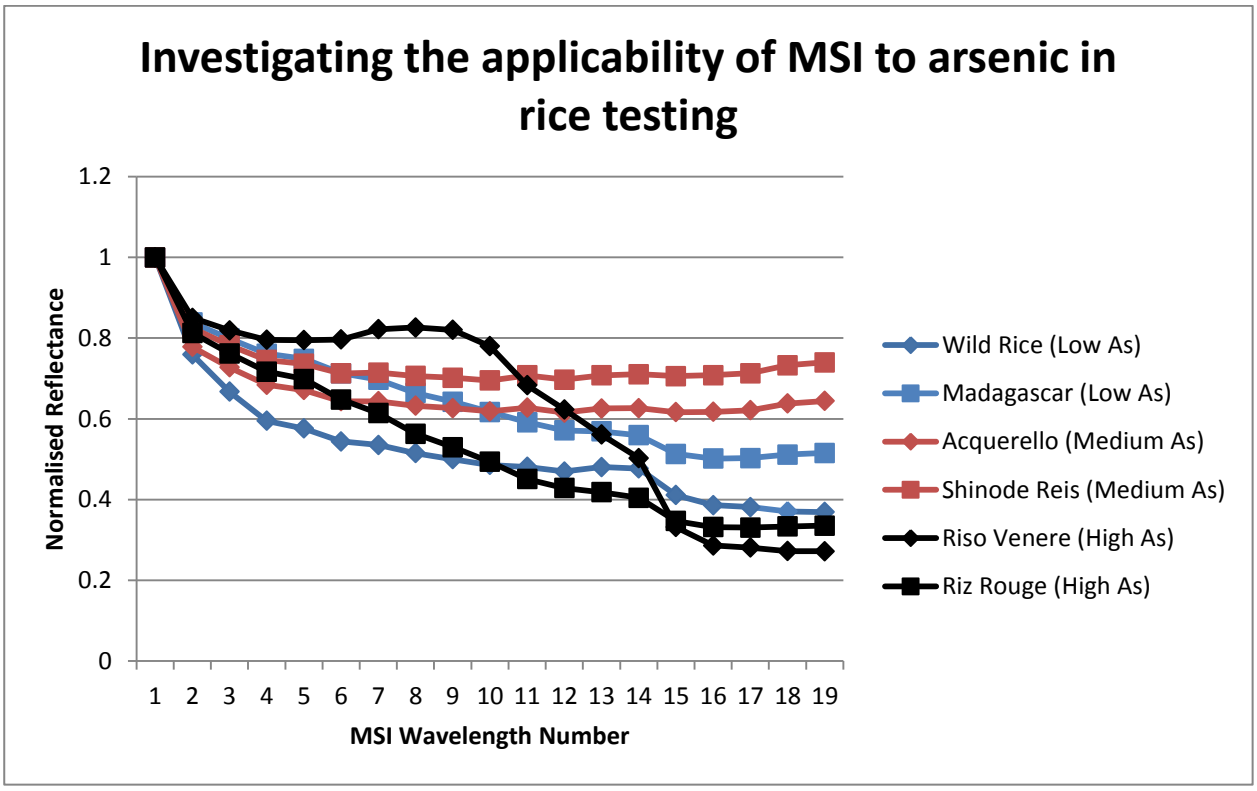


Figure 3. Graph showing MSI spectral profiles generated from single MSI scans per arsenic in rice powder sample type normalised to band 1 reflectance values

5.4 Conclusions

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 covered a wide variety of challenging test materials with differing physiochemical characteristics. The results clearly show that the MSI methodologies applied were capable of detecting and differentiating between the majority of test components and provided preliminary quantitative data.

A summary of the estimated limits of sensitivity and associated CV's for the samples assessed in this project is presented in the following table:

Sampling Scenario Sample (adulterant)	LOD (w/w)	CV	Notes
Oregano (olive leaves)	≤ 0.5%	18%	Documented issue in FSA 2016 report
Rice (Plastic rice)	≤ 1%	8%	Multi-analyte capability
Rice (gravel)	≤ 1%	18%	Multi-analyte capability
Beef (pork)	≤ 5%	25%	Complex blended sample
Beef (offal)	≤ 10%	40%	Complex blended sample
Ground cumin (ground almond shell)	≤ 5%	4%	Particulate nature
Ground cumin (ground mahaleb)	≤ 5%	60%	Particulate nature
Ground almond (ground peanut)	≤ 5%	25%	Particulate nature
Wheat flour (peanut flour)	≤ 10%	64%	Wheat flour coats peanut oil

The oregano adulteration test scenario was found to perform particularly well as demonstrated by trace level detection capabilities (≤ 0.5 % w/w) and reasonable preliminary quantitative performance, e.g. 1 % w/w olive leaves in oregano leaves admixture experimentally reported as 2.3 % ± 0.9 %. The estimated LOD appears fit for purpose based on published threshold levels for impurities of up to 1 % (ISO Specifications) or 2 % (European Pharmacopeia) for oregano samples. The quantitative data, whilst typically under estimating the adulterant content, shows good proportionality to the assigned levels across the entire sample range.

The applicability of MSI to new and emerging food threats was clearly demonstrated by the ease in which MSI could identify 'plastic rice' contaminants. MSI is uniquely positioned to classify components based on their spectral signatures which should differ significantly

between standard rice grains and ‘plastic rice’ due to chemical composition. This test scenario also showcased the multi-analyte capabilities of MSI when applied to a single test sample, wherein a single analysis could detect and partially quantify the rice grain, ‘plastic rice’ and gravel components.

MSI showed applicability in detection of challenging meat blends, and could detect ≤ 5 % w/w of pork meat blended into beef meat with reasonable precision. The applicability of MSI to detect undeclared offal was also investigated, with a detection limit of ≤ 10 % but with a high CV of 40 % at the 10% w/w level. In relation to detection of allergens, MSI could detect cumin adulterated with ground almond shell, with a detection limit of ≤ 5 % w/w with very tight precision as demonstrated by a CV of only 4 % at the 5% w/w level. Cumin adulterated with mahaleb gave the same LOD estimate but with poorer precision (CV of 60 %). For almond adulterated with peanut, MSI was capable of achieving a detection limit of ≤ 5 % w/w with a CV of 25 % at the 5% w/w level. These examples are of topical importance as a UK restaurant owner was recently convicted for the manslaughter of a customer by using groundnut powder (containing peanuts) substituted for almond powder in a takeaway meal.

Test scenarios based on fine grain materials such as melamine in milk powder showed discriminatory problems arising from the inherent high reflectance properties of the powders investigated, resulting in pixel saturation, and the fine grain size which exceeds the resolution of the image capture system. Whilst the standard nCDA-based analysis showed limited applicability under these scenarios, a global analytical approach can be applied that utilises the mean component spectral data to successfully differentiate between adulteration levels, and highlighted the analytical flexibility of MSI.

Based on this preliminary study, MSI has demonstrated good potential to be used as a screening method for a range of issues, and has additional functionality when an adulterant or contaminant is present above a certain threshold level. Clearly MSI will not be applicable as a screening approach for every conceivable sampling situation (food authenticity, adulteration, quality and safety testing), and the added advantage of the MSI’s quantitative capability would benefit from further characterisation. Full method validation on selected sampling scenarios and evaluation of the quantitative accuracy of MSI would

facilitate an objective judgement of its fitness for purpose and applicability on a sample case by case basis.

This feasibility study clearly supports the application of MSI to food-based testing and the need for future evaluation work to assess core performance characteristics and establish validated diagnostic tests. Based on the priority list provided by the FSA 2016/2017 National Coordinated Sampling Programme, the novel application of MSI showed good levels of sensitivity and precision (an estimated LOD of ≤5% and associated CV ≤25%) in the following sampling scenarios: oregano leaves adulterated with olive leaves; Basmati rice adulterated with plastic rice; Basmati rice with impurities (gravel); beef adulterated with pork (as a blend); cumin adulterated with almond; and almond adulterated with peanut. Future work should focus on full method validation of these sampling scenarios further.

Given the pioneering work of using MSI, the preliminary nature of this proof-of-principle project, and the steer of which samples to initially evaluate based on the sample list from the FSA 2016/2017 National Coordinated Sampling Programme, the applicability of using MSI as a screening approach to other sampling scenarios should also be investigated. For example, according to an FSA Food Crime – annual strategic assessment report (2016), oregano leaves have also been adulterated with myrtle leaves, which should be examined further. The sample of beef adulterated with pork investigated as part of the current project was based on a very challenging homogenised blended sample matrix, and meat samples subject to a different processing nature (e.g. in burgers, as mince, etc.) arguably may have greater discriminatory potential due to other surface characteristics that reflectance patterns can capitalise upon. Through consultation with Defra's AMWG and other UK stakeholders, and referral to the published literature, MSI also has the potential to be applied to additional important food stuffs which are either not mentioned in the FSA 2016/2017 National Coordinated Sampling Programme or were beyond the resource of the current project. These include adulteration of cereal grains, cereal grain spoilage through fusarium infection, speciation and quality of nuts, fruits, meat marbling, fish (e.g. bacterial biofilms and parasitic infestation), seafood, vegetables and eggs; and the detection of GMO grain.

Finally, whereas the application of MSI was not totally successful as a discriminatory

screening approach in a handful of sampling scenarios from the FSA 2016/2017 National Coordinated Sampling Programme as part of this initial study, its value in these areas should not be discounted completely. For example, with the sampling scenario of offal (beef heart) in meats (beef meat), a highly homogenised and blended sample matrix was investigated. If the meat and offal were to be of a different processed nature, e.g. as unprocessed samples mixed together, as mince etc., the particulate nature of such a sample may make it easier to generate unique reflectance patterns of the meat and offal, facilitating better levels of sensitivity and precision. Equally well, the technical application of MSI to the provided arsenic in rice samples did not fail. Rather this issue was associated with the confounding effect of only different varieties of rice being sourced with different arsenic concentrations, making any meaningful differentiation based on arsenic concertation alone impossible. Future work would be to source samples from the same variety which had different levels of arsenic concentration present, and then to subject these to the MSI approach. Lastly, the reflectance properties of melamine and milk powder were very similar, precluding differentiation between the two based on the normal algorithm and model used for spectral properties alone. However, further examination showed there was a good association between the mean component spectral data across the whole target region and the amount of melamine present in the milk powder samples. Such a model could be further investigated and refined to incorporate it as an algorithm for more routine testing, given additional funding to help investigate this important area.

6 Analytical technology comparison

Food diagnostics is underpinned by a variety of technologies ranging from molecular biology (e.g. PCR) to mass spectrometry with differing analytical workflows and cost/time profiles. Table **15** compares a selection of analytical technologies for food analyses and highlights the core features and approximate indicative costs associated with each. MSI benefits from a number of important advantages compared to competitor technologies, which includes a low test cost base due to limited use of consumables, multi-analyte capability, simple workflow and short total analyses time arising from the lack of complex sample preparation/purification and analysis steps. MSI represents an emerging analytical

technology within the food sector with the potential to augment or even replace multiple current tests with a single flexible platform.

Table 15. Comparison of analytical platforms for foods analyses inclusive of approximate indicative costs.

Parameter	Technology					
	MSI	**qPCR**	**ddPCR**	**MS**	**NGS**	**ELISA**
Details	Multispectral imaging	qPCR technology Real-time	qPCR technology End-point	Peptide mass fingerprints	Massively parallel sequencing	ELISA plate-based
Example Instrument/System	VideometerLab4 (Videometer)	AB 7900HT Fast Real Time PCR System (Life Technologies)	QX200 Droplet Digital PCR System (Bio-Rad)	MALDi-TOF/MS and nano-LC MS/MS	Illumina MiSeq	Tecan Infinite® 200 PRO
Analyte	Multiple	DNA/RNA	DNA/RNA	Protein	DNA/RNA	Protein
Typical sample throughput	Up to 30 per hour	Up to 96/384 reactions per run	Up to 96 reactions per run	Up to 20 samples	1 sample (without multiplex sequencing)	Up to 96 samples
Sample preparation required	No	Yes	Yes	Yes	Yes	Yes
Typical workflow	• MSI scan • Data analysis	• Sample preparation • DNA/RNA extraction • RT-PCR required for RNA • qPCR test • Data analysis	• Sample preparation • DNA/RNA extraction • RT-PCR required for RNA • dPCR test • Data analysis	• Sample preparation • Protein extraction • Peptide purification and preparation • MS test • Data analysis	• Sample preparation • DNA/RNA extraction • Library preparation • NGS run • Data analysis/ bioinformatics	• Sample preparation • ELISA test • Data analysis
Typical total analyses time (including extraction & analysis)	<10 mins	Up to 9 hours	Up to 13 hours	Days	Days	Up to 4 hours
Analyst skill level	Low/medium	Medium	Medium	High	High	Medium
Approx. per sample reagent/consumable costings	~£0.25	~£6	~£9	~£200	~£1,200 (less with multiplex sequencing)	~£10

7 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 recent publication of the Elliot review [5] 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 report, 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 multispectral 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 [16-21] 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.

In addition to characterising food/feed materials, the analytical flexibility of MSI could be applied to related food fraud/security issues such as documentation and labelling which was highlighted by the high levels of RASFF notifications in this area (Figure 1). Spectral imaging is capable of analysing and determining the authenticity of documents and labelling through the detection/lack of expected security features and spectral characteristics invisible to the human eye. Whilst this potential application was not explored within the scope of the project, an integrated approach comprising the food/feed product and associated documentation/packaging would improve the detection and control of fraudulent activities.

This report presents evidence for the applicability of using multispectral imaging (MSI) as a rapid screening tool for analysis of priority sampling issues as identified by the FSA draft

list of sampling priorities for the National Coordinated Sampling Programme 2016/17. MSI was successfully applied to the topical issues of the adulteration of oregano (herbs & spices) with olive leaves (as described in the FSA's Food Crime Unit annual strategic assessment report 2016), the adulteration of ground almond with ground peanut (a known allergen that requires labelling according the relevant EU Directive) and "Plastic Rice" (adulteration and detection of undesirables). The importance of this area was reinforced by a recent case where a restaurant owner was convicted of manslaughter by willingly providing a meal to a customer which had almond powder substituted with ground-nuts (including peanut) when the customer had ordered a nut-free meal) [22];

As part of the overall project, multispectral imaging has also been successfully applied to other sampling scenarios inclusive of ground almond in paprika, ground mahaleb in cumin, pork meat blended with beef meat, and undeclared offal in beef. These sampling scenarios represent topical and important examples referred to in the FSA draft list of sampling priorities for the National Coordinated Sampling Programme 2016/17. The application of multispectral imaging to these important sampling priorities, coupled with its rapid, automated, non-destructive, non-targeted, multi-analyte nature, clearly demonstrate the need to further validate this technology so that it can be used as a routine screening method (both by enforcement labs and industry) as a cost-effective tool to test food quality, authenticity and ingredient adulteration. The non-proprietary nature, small capital expenditure, limited requirement for specialist training and quantitative potential of multispectral imaging instrumentation means that the acquisition and use of such a system is a realistic and likely probability for food and feed testing laboratories. This also compares well with other applications for food analysis, for example Fourier Transform Infrared Spectroscopy (FTIR), where FTIR often still requires sample grinding, a complex workflow, further manipulation of sample data and importing that data into a third party statistical software to allow interpretation of the results.

The original FSA Research Specification document stated that new methods (or new applications of existing methods) to help fill gaps in the UK's capability for optimising food and feed sampling and analysis, were a priority. Additionally, ideas on novel and more cost effective approaches were being sought. The nature of MSI means that it can be considered as a true non-targeted, multi-analyte screening approach (compared with

protein and DNA approaches that are analyte specific per assay), meaning that MSI can be quickly applied in a variety of situations and to a wide scope of food and feed samples. It can be used to determine and identify the presence of either unlabelled ingredients (authenticity) or the presence of undesirable substances (quality, health and safety) in food and feed, and has the potential to do this in well under one minute after non-invasively scanning a sample. This fits the remit mentioned in the original FSA Research Specification that explicitly listed the potential use of approaches for screening for multiple analytes (making more efficient use of the samples), and non-targeted analysis (not looking for a specific analyte) as well as the use of rapid methods. In addition, the non-destructive nature of MSI leaves the tested sample suitable for follow-on analyses if required, which represents a significant benefit to the analytical laboratory, e.g. matched sample test results, reduced sample requirements, retrospective non-MSI testing as required.

The results of the project have the potential to have an economic and operational impact through providing shorter timeframes for screening samples and reducing testing costs. Furthermore, recent improvements in technology and software means that MSI instrumentation can now be miniaturised and lends itself well to point of test devices.

8 Recommendations

From this FSA Proof-of-Principle project, there is evidence to suggest that MSI could be used as a rapid, automated and cost-effective screening tool to help augment pre-existing approaches for food authenticity, adulteration and quality testing, by a range of UK laboratories. We have made a series of recommendations for the full utilisation of this technology as follows:

- **Conduct a full validation exercise on sampling scenarios which are seen as priority issues, in consultation with expert guidance from the FSA.**
  This would capture the performance characteristics of the method (e.g. trueness, precision, sensitivity, specificity, robustness and measurement uncertainty) based on using representative and authenticated reference materials and adulterants. The

acquisition of authenticated reference materials (e.g. CRMs or materials authenticated via DNA sequencing) is a fundamental part of providing confidence in the results of any analytical test, and such materials will be sought as part of any follow on project. An evaluation of other representative varieties/cultivars should also be conducted, in order to build up a spectral identity database of authenticated samples and to determine any base spectra that are representative of a key species in general. As part of any validation exercise, the use of MSI for food testing should also be compared to other appropriate analytical tests currently used for food authenticity testing (e.g. real-time PCR or DNA sequencing) in terms of representativeness of results, quantitative capability, time and cost efficiency.

- **Full method validation on sample specific scenarios.**
  This preliminary project has shown clear proof of principle of the applicability of MSI as a screening approach to the following sampling scenarios mentioned in the FSA 2016/2017 National Coordinated Sampling Programme: oregano leaves adulterated with olive leaves; Basmati rice adulterated with plastic rice; Basmati rice with impurities (gravel); beef adulterated with pork (as a blend); cumin adulterated with almond; and almond adulterated with peanut. It is a recommendation from this project that full method validation of these sampling scenarios be investigated using appropriate samples representative of the UK market situation, in order to fully qualify the fitness for purpose of MSI and further demonstrate utilisation of the technique for potential UK testing and control laboratories. Following successful validation, it would be a recommendation to transfer the technology to appropriate UK analytical laboratories.
- **Provision of guidance for validating the use of MSI on any sample**
  Whilst this preliminary project has highlighted the applicability of MSI for specific sampling scenarios included in the FSA 2016/2017 National Coordinated Sampling Programme, guidance should also be given on how to validate the use of MSI for any general sampling situation, irrespective of the food being analysed. This would increase the scope, applicability and utilisation of MSI, and would act as guidance for those laboratories (e.g. enforcement labs) wishing to apply MSI to samples they routinely investigate. An SOP or protocol could be provided on what general and

bespoke performance characteristics would need to be evaluated as part of the method validation, and what quality metrics and performance criteria need to be fulfilled in order to provide evidence that the method is fit for purpose.

- **Evaluate the applicability of MSI to other important and topical sample and testing scenarios to which MSI is uniquely suited.**
  The evaluation of other priority testing scenarios following consultation with government and industry stakeholders. Examples include vegetable quality, fish speciation and quality (e.g. biofilms and parasitic infestation), and grain quality (e.g. fusarium infection).
- **Provide optimal routes for dissemination and knowledge transfer of the new technology and food and feed specific application protocols to stakeholders (Public Analysts and Industry).**
  In consultation with the FSA, dissemination activities should include Knowledge Transfer events (one day meetings), electronic seminars on the Food Authenticity Network, presentations at conferences, peer reviewed publications and guidance notes, on-site visits to interested stakeholder laboratories to provide be-spoke advice tailored to a laboratories requirements, and an international collaborative trial of applicable methods.
- **Conduct a scoping exercise to examine the feasibility of transferring multispectral food application protocols and acquisition of MSI instrumentation to analytical laboratories (Public Analysts and Industry).**
  This should include an evaluation of the commutability of the approach and recommendations made on the deployment and applicability of MSI instrumentation for UK Official Control Laboratories. An evaluation of the capabilities of other MSI instrumentation currently on the market should be made, and the range of wavelengths they use will form the basis of being able to determine the commutability of MSI food authenticity protocols based on key discriminatory wavelengths and base spectral profiles. Hand held and point of test devices using MSI are becoming increasingly common, and the transferability of key lab based analytical protocols onto point of test devices should be made.
- **Develop and maintain a database of classification and discrimination models**

as well as a repository of MSI applications to specific food samples.
Such a database should be centrally maintained, provided on a server, or be Cloud based, to help facilitate rapid access and deployment of a multi-analyte screening approaches using MSI. “Session recipes” (protocols based on specific food applications) should also be developed to provide a harmonised approach to sample analysis and interpretation. The feasibility of transferring the approaches to other MSI instruments should be examined, taking into account the analysis of wavelengths that allow optimal discrimination and to determine mean component spectral data across target regions, as an aid to examining the commutability of the methods onto different machines. There is also a need to both develop and maintain a database of applications of MSI to specific food samples to help facilitate promotion of the technology.

- **Evaluate the application of MSI to the detection of fraudulent documentation and packaging.**
  Provide recommendations to stakeholders on the application of MSI to fraud control (e.g. security features, workflow) and the development of a database, with the collaboration of industry and enforcement communities, for reference purposes.

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10 Appendix