Development of the FoodAlert database and piloting Food Fingerprinting

A report prepared for the Food Standards Agency

Project Number: FS204012

Date of Report: 24th March 2013

Prof Chris Elliott, Dr Simon Haughey, Mrs Pamela Galvin-King

Institute for Global Food Security, Queen's University Belfast

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(i) Executive Summary

There are two separate elements to this project. Part A was to illustrate the potential development of a system, ***the FoodAlert Register,*** for prioritising testing for food commodities. This early model was developed using the information that is available from the European Union Rapid Alerts System for Food and Feed (RASFF). From this information, it was possible to construct tables and charts related to (i) country of origin of food commodity associated with a notification and (ii) frequency of notifications associated with a particular food commodity. The analysis of the information from the RASFF was carried out for the notifications from 2008-2011. The development of this model was illustrated using spices and oils/fats commodities and the hazard assessed was related to the inclusion of dyes in these products. As well as the frequency of the notifications, their severity also had to be determined which was achieved by construction of a scoring system. By multiplying the severity score and frequency of the notification, it was then possible to observe if a particular type of alert was increasing or decreasing over a rolling 3 year period as well as ranking this against other types of alerts as part of a priority testing scheme.

Part B of the project was to develop fast, non-destructive, spectroscopic screening tests for the identification of selected adulteration issues. Near Infra-Red and Raman spectroscopies were explored as non-destructive techniques to determine the adulteration of chilli powders with Sudan I dye and the adulteration of olive oils with cheaper sunflower oils. The spectral data that were obtained coupled to chemometric analysis allowed the generation of qualitative and quantitative calibration models that could potentially be used to determine adulteration of these commodities. Examples of quantitative calibration models for Sudan I in chilli powder using NIR and Raman spectral data respectively were as follows: (i) R^2= 0.9936, RMSEC= 0.217% and RMSEP=0.139% using PLS algorithm with SNV, 1st Derivative, Savitzky Golay (9,3) pre-processing using 6 factors; (ii) R^2= 0.9724, RMSEC= 0.437% and RMSEP=0.6% using PCR algorithm with SNV, 1st Derivative, Savitzky Golay (11,4) pre-processing using 10 principal components. Examples of quantitative calibration models for the sunflower oil adulteration of olive oils using NIR and Raman spectral data respectively were as follows: (i) R^2= 0.9972, RMSEC= 2.32% and RMSEP=2.44% using PLS algorithm with MSC, 1st Derivative, Norris derivative filter (5,5) pre-processing using 10 factors; (ii) R^2= 0.9955, RMSEC= 3.1% and RMSEP=3.44% using PLS algorithm with MSC, 1st Derivative, Norris derivative filter (5,5) pre-processing using 10 factors.

In conclusion, this project has demonstrated an example of a process by which a tool to easily and accurately identify the major agents associated with food adulteration/contamination can be employed. The **FoodAlert Register** can also identify trends to allow the prediction of emerging hazards for food commodities. This is however a *'first attempt'* at developing such a tool and future work would include the full development of the Register to cover all food commodities which would allow a hazard based, highly targeted approach to the selection of food types (from specific geographic origins) most likely to be adulterated/contaminated. Thus this tool could be employed to prioritise testing requirements across the UK. The input of more expert opinions to how the scoring system (and the weighting of each score) should be developed is required.

The successful development of NIR and Raman spectroscopy based methods to determine adulteration of food commodities such as spices and oils has highlighted the potential of these types of rapid, low cost non-destructive techniques as a first line defence in food quality and safety monitoring programmes. These spectroscopic techniques could be further used to develop calibrations models for other adulteration issues within the food chain. The oil work gave the most impressive results and should be taken forward, fully developed and validated as a method to be used in the UK to detect adulteration of olive oil.

(ii) Non-technical (laypersons) Executive Summary

There are two parts to this project. Part A was to illustrate the potential development of a system entitled the **FoodAlert Register**, for giving authorities a means of prioritising what testing should be undertaken in foods to ensure the UK consumer is not being exposed to unwanted adulteration or contamination of what we purchase. The model was developed using information freely available from European Union sources (the Rapid Alerts System for Food and Feed). This information comes from testing carried out by all EU member states and the results are used to notify the EU of any undesirable agents that are associated with different types of food commodities coming from around the world. Within this project the collected information has been used to construct a series of tables and charts related to the country of origin of food commodity associated with an agent and the frequency of its occurrence. The information collected over the past 4 years in relation to several food types (spices and oils) and contamination/adulteration was analysed to show how this tool could be developed. As well as the frequency of the notifications, its severity in relation to potential adverse health effects by consumption was attempted to be quantified. To achieve this, a scoring system to determine the potential severity of hazards was then constructed. By multiplying the severity score and frequency related to each notification, it was possible to observe if a particular agent was present and if it was increasing or decreasing over a 3 year rolling period. A very easy to use, fit for purpose model system to prioritise the testing performed on several food types has been constructed to illustrate how a tool could be developed for the most effective use of resources available to the authorities. The full development of this tool to include all food types would be an important step in making the food we eat safer and of a higher quality.

In Part B of the project, several rapid testing methods were also developed to prove how they could be employed as front line screening tools to detect adulteration and gross contamination of our foods. Near Infra-Red and Raman spectroscopies, based on the interaction of matter with light, were explored to determine the adulteration of chilli powders with Sudan I dye and the adulteration of olive oils with cheaper sunflower oils. The information that was obtained coupled to statistical analysis allowed for the generation of procedures that could potentially be used to determine adulteration and gross contamination of these commodities. Using this approach tests were developed which could possibly detect Sudan I adulteration of chilli powders and sunflower oil adulteration of olive oil products.

In conclusion this project has demonstrated the prospective development of an important new tool, the **FoodAlert Register**, which has the potential to be used to predict emerging hazards for food commodities as well as ranking the known hazards for monitoring purposes. The register will require refinements and modifications to make it a robust tool to determine the major hazards that require the attention of industry and authorities.

The development of NIR and Raman spectroscopy based methods to determine adulteration and gross contamination of food commodities such as spices and oils has highlighted the potential of these low cost, rapid, non-destructive techniques as a first line defence in food quality and safety in the UK. Recent events in the UK have shown that new, low cost and rapid means of detecting adulteration of our foods is required.

(iii) Glossary

MSC: Multiplicative Signal Correction pathlength setting uses a mathematical function to compensate for differences in pathlength. This allows you to analyze samples with different pathlengths when it is difficult or impossible to obtain an independent measure of sample pathlength.

NIRS: Near Infrared Spectroscopy

Norris Derivative Filter: Norris derivative smoothing filter is useful for improving the appearance of peaks that are obscured by random noise. It is typically applied to Near-IR spectra If the Norris derivative smoothing filter is selected, the settings for Segment Length and Gap Between Segments determine the degree of smoothing. The Norris derivative filter must be used with first or second derivative spectra. It is often used to enhance a sharp band that is overlapped by another broad band.

OPLS-DA: Orthogonal Partial Least Squares-Discriminant Analysis is similar to PLS-DA but used an extra orthogonal plane when processing the spectral data.

PCA: Principal Component Analysis is an unsupervised statistical approach to qualitative analysis that can be applied to spectral data.

PCR: Principal Component Regression is also a statistical quantitative analysis technique based on the principal component regression algorithm.

PLS: Partial Least Squares is a supervised statistical approach to quantitative analysis based on the partial least squares algorithm and can be applied to spectral data. Multiple regions of the spectrum can be used for the analysis.

PLS-DA: Partial Least Squares-Discriminant Analysis is a supervised statistical approach to qualitative analysis that can be applied to spectral data.

Savitsky-Golay Filter: Savitzky-Golay smoothing filter is useful for improving the appearance of peaks that are obscured by random noise. Within the Savitzky-Golay smoothing filter, the settings for Data Points and Polynomial Order determine the degree of smoothing. Savitzky-Golay smoothing can be applied to the original spectra or to the first or second derivative spectra. To configure typical Savitzky-Golay smoothing, set the Data Format and smoothing parameters as described below:

- **(i)** **Data Format:** Spectrum (Raw Data) for simple smoothing
- **(ii)** **First derivative:** pre-processing for smoothed first derivative algorithm
- **(iii)** **Second derivative:** pre-processing for smoothed 2nd derivative algorithm
- **(iv)** **Data Points:** Set to 7 data points for average smoothing. Increase for more smoothing; decrease for less.
- **(v)** **Polynomial Order:** Set to 3rd order (cubic) polynomial for average smoothing. Higher orders result in less smoothing; lower orders cause more smoothing.

SNV: Standard Normal Variate pathlength setting scales the spectral data in order to compensate for differences in sample path length. This allows you to analyze samples with different effective pathlengths when it is difficult or impossible to obtain an independent measure of sample pathlength. The SNV and MSC are similar, however, MSC calculates an ideal spectrum from the calibration standards and uses it to correct the data, while the SNV correction removes the effects of scattering by normalizing the spectra individually.

PART A

Development of the FoodAlert Register:

A Case Study using Spices and Oils

A1 Introduction

A1.1 Food brought into the UK and enforcement

Local authorities and port health authorities are responsible for the enforcement of food safety and food standard controls on food products in the UK. Outside of port areas, local authorities are responsible for enforcing food safety controls on foodstuffs including food brought into the UK. There is a strong need to support the decision making processes of these authorities with regard to what types of foods present the greatest risks, the countries of origin of a commodity related to a particular notification and the food contaminants that may be present in foods that require the greatest degree of scrutiny.

A key element of the Food Standards Agency's (**FSA**) strategy for 2010–2015 is that imported food is safe to eat. To deliver this outcome port health and local authorities must be provided with the tools to undertake risk based targeted checks at ports and local authority monitoring of foodstuffs brought into the UK throughout the food chain. The present project will illustrate the development strategies that will allow such decisions to be taken using a risk management tool (**FoodAlert Register**). The feasibility of adding this database to the Local Authority Enforcement Monitoring System (LAEMS) will be investigated. The data from this system points to the fact that a significant number of authorities continue to carry out very little, or no enforcement and one of the reasons behind this is the lack of knowledge about how best to target the limited resources available to undertake monitoring activities.

A1.2 Major Hazards from Food brought into the UK

Around half of food consumed in the UK is from countries outside the UK and this proportion is increasing year on year. This statistic gives a good indication of the enormous task facing authorities in relation to trying to ensure food brought into the UK from other member states and from outside the EU is safe for the UK consumer.

Intelligence is made available to the authorities via FSA alerts and there are examples of where such alerts have been extremely helping in targeting the types & origins of foods sampled. However better sources of up to date information need to be collated, analysed and presented in terms that will improve the ability to undertake targeted sampling.

At Queen's University's Centre for Safe, Assured & Traceable Foods (ASSET Centre) such forms of analysis have been on-going since 2009. This has resulted in the compilation of a number of unique data sets

One such important undertaking by the ASSET Centre was the development of a register of imported animal feed materials into the UK and Ireland. This major undertaking analysed all the notifications relating to animal feed materials from the EU Rapid Alerts system (http://ec.europa.eu/food/food/rapidalert/index_en.htm) over a three year period. The data was subjected to an analysis based on the types of commodities, reasons for notifications and its geographic source. Each notification was further subjected to an analysis related to consumption by farm animals and humans and scored accordingly. Thus for each type of feed material brought into the UK an accurate picture of what contaminants are most likely to be found based on the origin of the material and the likelihood of this contamination entering the food supply chain. This database, entitled **FeedAlert Register** is now being implemented by a large number of UK and Irish companies that bring feed materials into the UK to target their monitoring programme for contaminants. The aim of the current project was to illustrate the development of a parallel system to analyse the food hazards using data from the EU Rapid Alerts system to produce a potential tool for authorities to decide on their sampling for foods brought into the UK.

Figure 1 and **Figure 2**, come from the EU Rapid Alerts system (RASFF) which shows the major triggers for alerts associated with herbs and spices and oils and fats respectively.

Figure 1: A summary of the number of RASFF notifications relating to herbs and spices (2010-2011 data set).

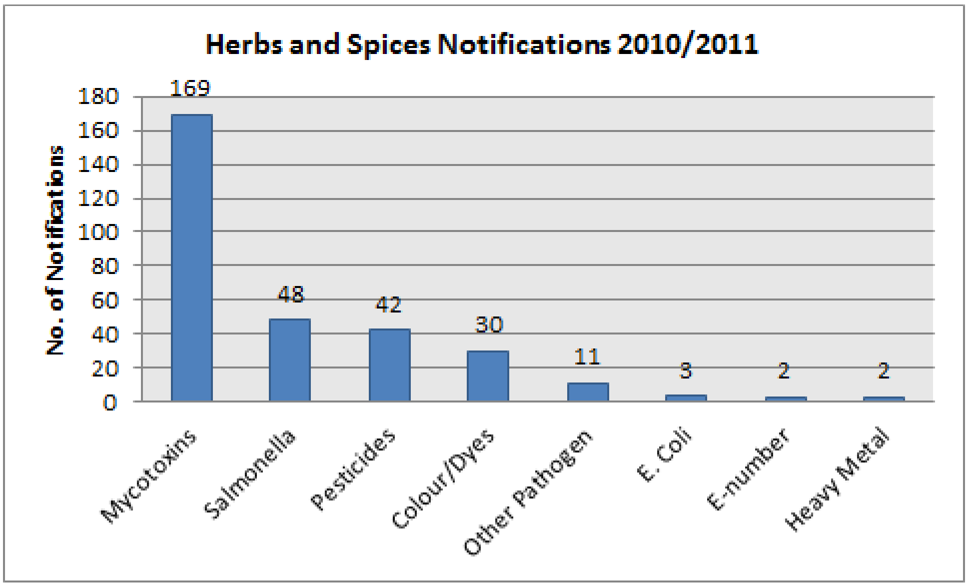
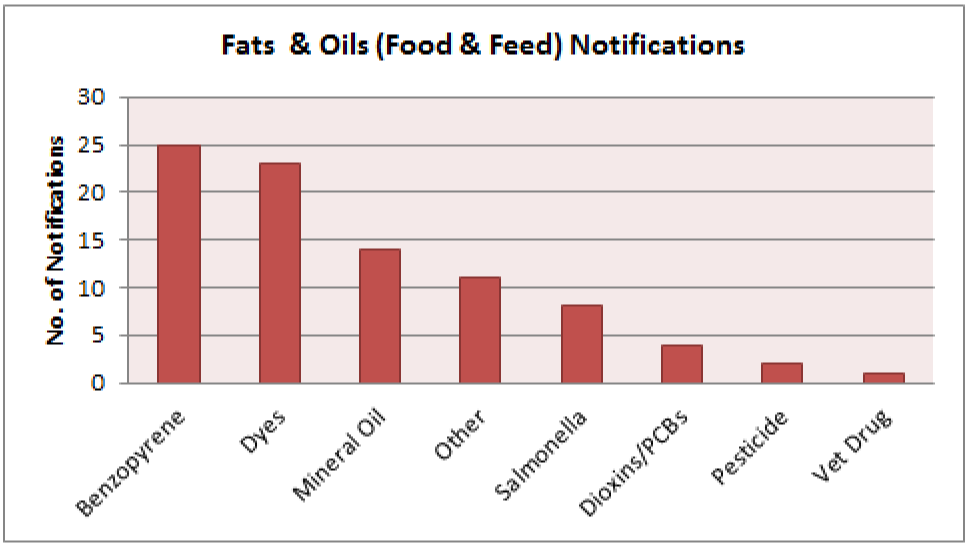


Figure 2: A summary of the number of RASFF notifications relating to fats and oils (2008-2010 data set).



Thus from these datasets it can be seen that herbs and spices and oils and fats play a significant role in food notifications (more than 1 in 10), and that are among the major issues with these food types include mycotoxin contamination and adulteration with illegal colours and dyes in the case of herbs and spices and benzopyrene and dyes in the case of fats and oils.

The focus of one of the research strands in the current project was to determine the feasibility to perform rapid screening of commodities for adulteration/contamination with illegal colours and dyes (see Part B).

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A.1.3 Main evidence gaps being addressed in the current project.

A1.3.1 The decision making process for sampling foods brought into the UK.

It has been outlined already that one of the greatest difficulties in sampling at ports and by local authorities for possible food hazards is what types of foods should actually be sampled and what the most likely contaminants to be present are. This illustration of the development of the **FoodAlert Register** database will provide an important risk management tool for those tasked with sampling. This will be constructed as a simple to use, evidence based system for determining the following:

- Types of foods that are most likely to be contaminated/adulterated.
- Types of contaminants that are the most likely to be present based on notifications
- The countries of origin of foods most often linked to food contamination issues.

Therefore a food from a particular region contaminated with a particular unwanted substance will be assigned a score. An illustration of the development of this scoring system is highlighted in the project.

A1.4 The scientific basis for the project

A1.4.1 The development of the FoodAlert Register database

In order to provide an illustration of the potential database tool that would guide surveillance and enforcement it would be possible to utilize a structured approach comprising: (i) an element that would build together knowledge about past alerts and (ii) another element that would seek to identify potential hazards by combining numerical factors/scoring system (based on assessment of relevant knowledge of substances such as ‘genotoxic’ or regulatory criteria e.g. ‘not authorised’)’. The construction of the database followed the same principles as a database constructed for the ‘Fortress Ireland’ project, i.e. a project aimed at determining the major hazards associated with the importation of animal feed materials onto the Island of Ireland. Data were extracted from the past three year’s information available from RASFF and inserted into a range of spreadsheets. The information was then collated using a number of formulae and a final score assigned for each commodity/agent combination. The scientific rationale used in the construction of this database has been explained to the following organisations and received support:

- Northern Ireland Grain Trade Association
- Department of Agriculture and Rural Development for Northern Ireland
- Food Standards Agency, Northern Ireland

A1.5 Advancement of knowledge in the area in relation to the FSA requirement

The FSA’s Research Requirements Document in relation to ‘ Strategic Challenges on Food Security’ asked for proposals to address the issues relating to how to ensure that imported food is safe to eat, including early identification of emerging hazards’. The illustrated development of the FoodAlert register in this project addresses this important requirement which will, by using an evidence based system, indicate the types of foods that should be sampled most frequently, the most important origins of these foods and the type of contaminants that should be tested for.
This will enhance the ability to ensure food entering the UK is safer than is currently the case by generating a highly novel, innovative strategy that will provide the UK with a substantially improved system for ensuring that emerging hazards are identified quickly and thus ensuring food is safe.

A2.1 Illustrative model: Development of the FoodAlert Register for Spices

In order to demonstrate the potential of this tool, a database has been constructed by QUB detailing the reported spice commodity related notifications generated during 2008, 2009, 2010 and 2011 from the EU Rapid Alerts System for Food & Feed (**RASFF**). The 20 spices highlighted included curry powder, turmeric, chilli powder, saffron, mixed spice and paprika with the full list as detailed in **Table 2**. A total of 521notifications were identified and were assigned to one of the following subheadings: Dyes, Salmonella, Mycotoxins, Pesticides (Illegal), Pesticides (Legal), Heavy Metals, Other Pathogens, Others. As dyes are highlighted in the development of this model, they can be broken down further into individual dyes: Sudan I, Sudan IV, Fast Garnet, E160 B (Annatto/Bixin/Norbixin), E124 (Ponceau 4R), Oil Orange, Methyl Yellow (Butter Yellow), E102 (Tartrazine), E110 (Sunset Yellow), Toluidine Red, E127 (Erythrosine), E129 (Allura Red), Basic Red 46, E100, Rhodamine B, Red Sandalwood, E123 (Amaranth), Orange II. Within the context of this pilot study dyes are being treated as a generic group of hazardous agents. However, should the project move from the development of the illustrative model to a more complete register dyes would be broken down into various categories such as unauthorised and authorised as the associated risks of each of these classes will be substantially different .

The following sections give a breakdown of the data extracted and how the prototype FoodAlert Register was constructed.

A2.1.1 Country of Origin, Hazards and Frequency of Notifications

A2.1.1.1 Country of Origin (excluding transhipment)

From the data in the EU RASFF it is possible to assign the country of origin as well as the associated agent. **Figure 3** shows the country of origin of commodities for countries with notifications related to dyes. This does not include country of transhipment (see **Section A3.1.1.2**). In summary the data show that India and Turkey have the most notifications based on country of origin of the commodity followed by Pakistan with a further 22 countries associated with 1-9 notifications for dyes. The chart in **Figure 4** shows the frequency of the notifications for dyes associated with the country of origin and, based on the data, the highest numbers of alerts are related to Sudan dyes (I & IV).

Figure 3: Frequency of Notifications for Dyes based on Country of Origin

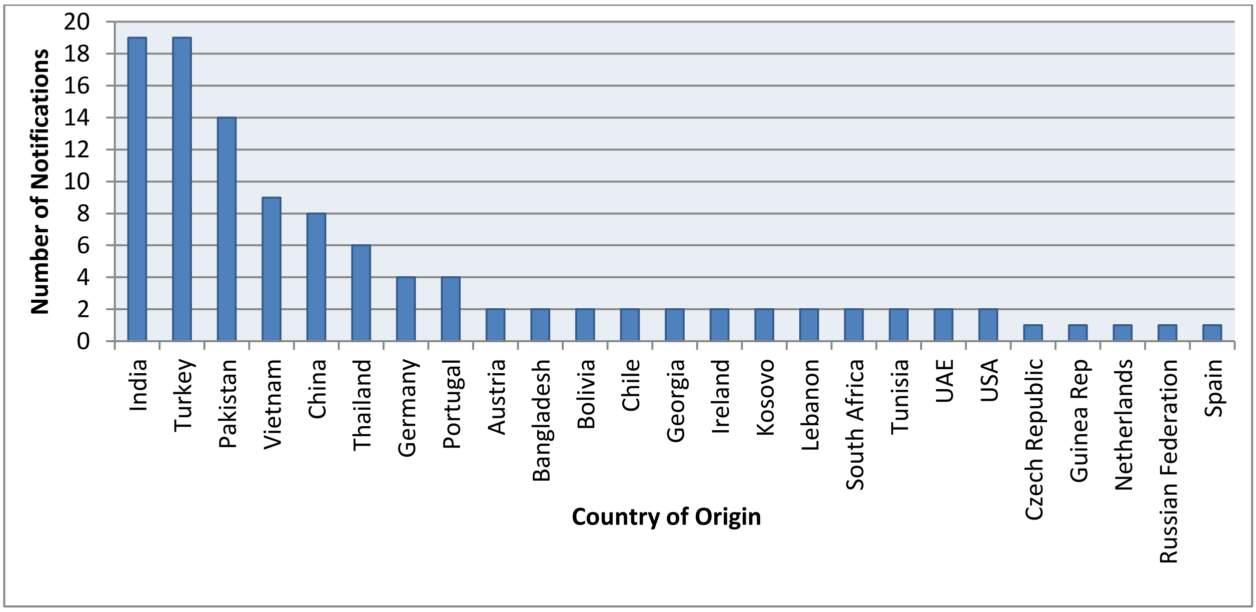
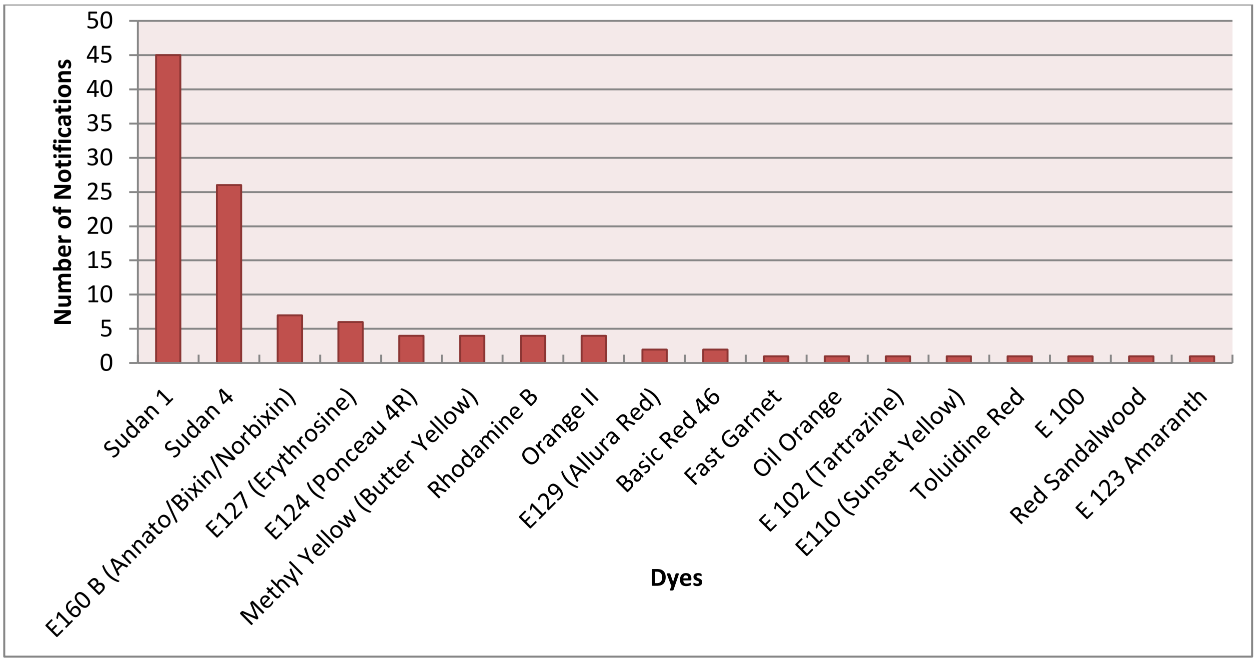


Figure 4: Frequency of Notifications relating to Dyes



By combining the data in **Figure 3** and **Figure 4**, the chart in **Figure 5** shows the country of origin, the number of notifications associated with that country and the dyes identified from the data. Although **Figure 5** looks a little more complex than the other Figures, it clearly shows the frequency of the individual dyes notifications that are related to each country.

Figure 5: Combined data related to country of origin and associated dye

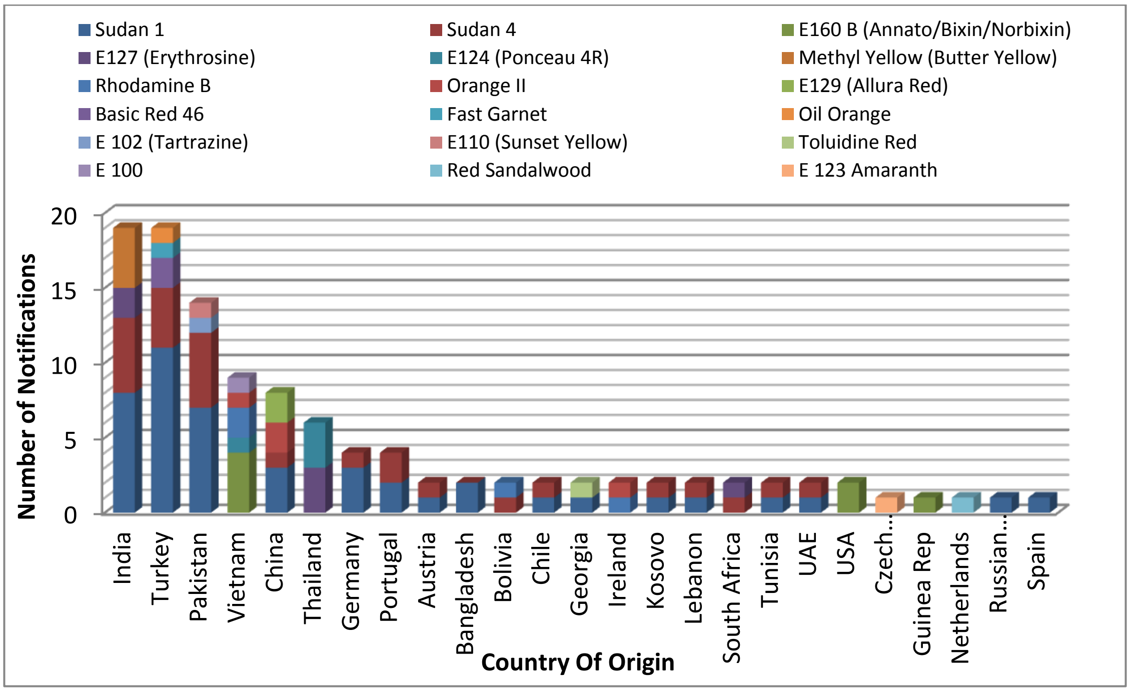


Table 1 shows the notification frequency of each agent for each year based on the associated spice commodity. The data can be broken down further to look at the frequency of notification related to individual dyes as shown in **Table 2,** and to 3 year data sets as shown in **Table 3**. The frequency data were used to assist in the defining of the priority rating of each hazard identified and building the FoodAlert model (**Section 3.1.3**).

A2.1.1.2 Country of Origin (including transhipment)

It is possible that a hazard associated with a particular commodity could occur whilst in transhipment through another country. For this reason the data related to country of transhipment and country of origin in the EU RASFF for each commodity can be included in the FoodAlert Register. **Figure 6** shows the country of origin, including transhipment, of spice commodities. In summary the data shows that there is some difference from Figure 4 above which is mainly an increase in alerts for the European country responsible for the transhipment especially Germany and the Netherlands which are major importers and transhippers of commodities within Europe.

Figure 6: Notifications for Country of Origin of Commodity including transhipment

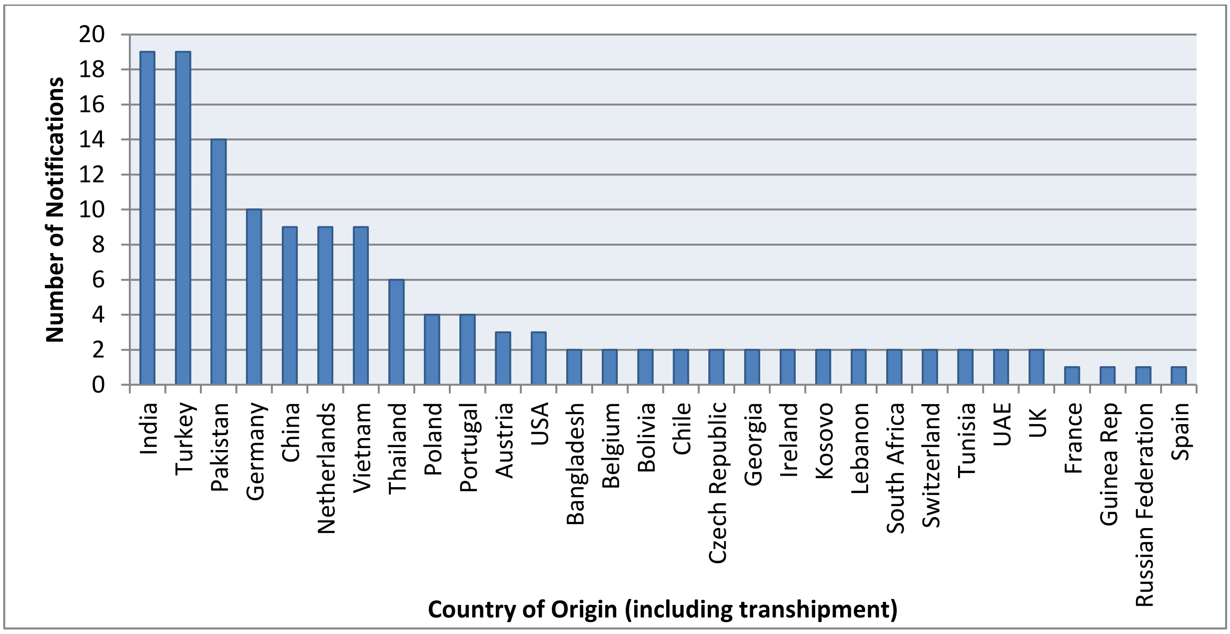


Table 1: Frequency of Notifications for each hazard associated with each spice commodity from the RASFF (2008-2011)

	Dyes				Salmonella				Mycotoxins				Pesticides Illegal				Pesticides Legal				Heavy Metals				Other Pathogens				Others				Total Alerts			
	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008
Curry Powder	3	9	11	5	1	2			1	26	3	3																		1	2	1	5	38	16	9
Curry Leaves													3				41	22	17						1					1			45	23	17	0
Turmeric	2			1		1			2	5	1	1																					4	6	1	2
Chilli Powder	4	7	7	14	1	3		1	22	35	5	13								5					1	2				1		1	28	48	12	34
Saffron	1		2																														1	0	2	0
Adjika	1																																1	0	0	0
Mixed Spice	5	1	3	1	1	1		1	1	1		3																1	2	2	1	1	9	5	4	7
Dried Pepper	1		1	1	1	2	1	5	7	4	6	4		1				2			3				2	1			2	3	5		16	13	13	10
Sumac Spice	3																																3	0	0	0
Red Annatho Seed	2																																2	0	0	0
Kebab Spice	1								2	1		1																					3	1	0	1
Paprika		5	2	2	2			1	3	9	13	1																	1				6	14	15	4
Fennel Seed		1																									1						0	1	1	0
Pickled Ginger			8																														0	0	8	0
Ginger Powder					1				5	8																			1	1	3		7	9	3	0
BBQ Mix			2																														0	0	2	0
Safflower			2																														0	0	2	0
Seasoning				1																									2		1	1	2	0	1	2
Curcuma				2					1	1																							1	1	0	2
Nutmeg								1	14	22	6	4									1								1	1			16	23	6	5
Others					3				1	1	1														1	1			2		1		7	2	2	0
Total Alerts	23	23	38	27	10	9	1	9	59	113	35	30	3	1	0	0	41	24	17	5	4	0	0	0	5	4	1	1	11	10	13	4	156	184	105	76
Totals	111				29				237				4				87				4				11				38				521			

Table 2: Frequency of Notification of individual dyes and spice commodities.

	Sudan 1				Sudan 4				Fast Garnet				E160 B				E124 (Ponceau 4R)				Oil Orange				Methyl Yellow				E102 (Tartrazine)				E110 (Sunset Yellow)				Toluidine Red				E127 (Erthyrosine)				E129 (Allura Red)				Basic Red 46				E100				Rhodamine B				Red Sandalwood				E123 (Amaranth)				Orange II				Total Alerts			
	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008
Curry Powder	1	3	5	2	1	4	4	2					1	1		1										1	2																																																3	9	11	5
Curry Leaves																																																																												0	0	0	0
Turmeric	1			1	1																																																																								2	0	0	1
Chilli Powder	2	2	3	7	2	1	1	3			1			1	1	2				1			1																																			2		1										1			4	7	7	14
Saffron			1				1																														1																																								1	0	2	0
Adjika	1																																																																													1	0	0	0
Mixed Spice	1		2	1	2																						1														1																				1					1							5	1	3	1
Dried Pepper	1			1			1																																																																					1	0	1	1
Sumac Spice	1				1																																												1																												3	0	0	0
Red Annatho Seed																																																					1				1																2	0	0	0
Kebab Spice	1																																																																												1	0	0	0
Paprika		4	1	1		1	1									1																																																													0	5	2	2
Fennel Seed																																										1																																			0	1	0	0
Pickled Ginger																			3																								3				2																												0	0	8	0
Ginger Powder																																																																													0	0	0	0
BBQ Mix																															1				1																																								0	0	2	0
Safflower																																																																									2		0	0	2	0
Seasoning				1																																																																								0	0	0	1
Curcuma				1				1																																																																					0	0	0	2
Nutmeg																																																																													0	0	0	0
Others																																																																													0	0	0	0
Total Alerts	9	9	12	15	7	6	8	6	0	0	1	0	1	2	1	4	0	0	3	1	0	0	1	0	0	1	3	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	3	0	0	0	2	0	1	0	0	0	1	0	0	0	1	2	0	1	1	0	0	0	0	1	0	0	0	1	2	0	23	23	38	27
Totals	45				27					1			8				4				1				4				1				1				1				5				2				1				1				4				1				1				3				111			

Table 3: Frequency of Notifications per Spice Commodity for Dyes in 3 year periods

	Dyes	
	2008-2010	**2009-2011**
Curry Powder	25	23
Curry Leaves	0	0
Turmeric	1	2
Chilli Powder	28	18
Saffron	2	3
Adjika	0	1
Mixed Spice	5	9
Dried Pepper	2	2
Sumac Spice	0	3
Red Annatho Seed	0	2
Kebab Spice	0	1
Paprika	9	7
Fennel Seed	1	1
Pickled Ginger	8	8
Ginger Powder	0	0
BBQ Mix	2	2
Safflower	2	2
Seasoning	1	0
Curcuma	2	0
Nutmeg	0	0
Others	0	0
Total Alerts	88	84

A2.1.3 Hazard Scoring and Priority Status for Testing of Spice Samples

Risk management is the identification, assessment, and prioritization of risks (as defined in ISO 31000). To undertake the priority ranking of each agent identified from the RASFFs, a preliminary assessment of the 'magnitude of impact' was undertaken and a scoring system based on four key areas is illustrated below. The questions in the key areas are as follows:

1. Has the substance been approved or unauthorised?
For approved, score **1**
For an unauthorised, score **5**

2. Is there any scientific evidence of hazard causing acute illness?
For no evidence, score **1**
For evidence of causing acute illness, score **40**

3. Is there evidence of carcinogenicity?
For no evidence, score **1**
For evidence of carcinogenicity, score **10**

4. Is there evidence of Genotoxic/Mutagenic effect?
For no evidence, score **1**
For evidence of Genotoxic/Mutogenic, Effect score **10**

By applying this scoring system to dyes, a score is obtained as shown in **Table 4.** Dyes can be scored as follows: (i) The dyes are unauthorised so this scores 5; (ii) There is some evidence that a small proportion of dyes cause acute illness by digestion and so this score 5; (iii) There is evidence that some dyes are carcinogenic or potentially carcinogenic, so this scores 7; (iv) There is evidence that some dyes are genotoxic/mutagenic or potentially genotoxic/mutagenic, so this scores 7 also. By adding these all together there is a Score (Severity) of 24.

Table 4: Magnitude of Impact Scoring System for Dyes

Magnitude of impact	Approved/ Unauthorised 1 – 5 (a)	Acute Illness 1 – 40 (b)	Carcinogenicity 1 – 10 (c)	Genotoxicity/ Mutagenicity 1 – 10 (d)	Score (Severity) (a+b+c+d)
Dyes	5	5	7	7	24

By analysing the information, the accurate identification of the frequency of the notifications related to the agent over 3 year rolling periods (08-10 & 09-11) and the severity of the associated hazard was determined. In **Table 5** it can be seen that, based on the parameters identified and by multiplying the frequency of notification of the agent by the severity score, a value is obtained for each commodity e.g. curry powder (25x24=600). By using data from 3 year periods in this model it can be observed if dyes are an increasing or decreasing hazard for the particular spices. The full impact of this model would need to be accessed in the context of all the agents identified (Salmonella, Mycotoxins, Heavy Metals, Pesticides and other bacteria) associated with these spice commodities. An example is shown in **Table 6**. Curry powder for 2009-2011 had a score of 552 (24x23) related to dyes. If using this model, agent X gave a score of 900 and agent Y gave a score of 48 then these could be ranked in order of testing priorities as shown with the primary testing for hazard X.

Table 5: Severity x Frequency score for Dyes in Spice Commodities over 3 year periods

	Dyes		
	2008-2010	**2009-2011**	**Hazard: Increasing/Decreasing**
Curry Powder	600	552	↓
Curry Leaves	0	0	—
Turmeric	24	48	↑
Chilli Powder	672	432	↓
Saffron	48	72	↑
Adjika	0	24	↑
Mixed Spice	120	216	↑
Dried Pepper	48	48	
Sumac Spice	0	72	↑
Red Annatho Seed	0	48	↑
Kebab Spice	0	24	↑
Paprika	216	168	↓
Fennel Seed	24	24	—
Pickled Ginger	192	192	—
Ginger Powder	0	0	—
BBQ Mix	48	48	—
Safflower	48	48	—
Seasoning	24	0	↓
Curcuma	48	0	↓
Nutmeg	0	0	—

Table 6: Ranking priorities

Curry Powder	Agent X	Dyes	Agent Y
Severity x Frequency Score	900	552	48
Testing Priority	**Primary**	**Secondary**	**Tertiary**

A2.2 Illustrative model: Development of the FoodAlert Register for Oils/Fats

This has been developed as detailed for the spices in Section A2.1. To illustrate the tool for oils, a database has been constructed by QUB detailing the reported oil/fat commodity related notifications generated during 2008, 2009, 2010 and 2011 from the EU Rapid Alerts System for Food & Feed (**RASFF**). The oil/fat commodities highlighted included oliveoil, palm oil, sunflower oil, rapeseed oil (see **Table 8** for full list). A total of 88 notifications were identified and were assigned to one of the following subheadings as follows: Dyes, Mineral Oil, Benzopyrene, Fraud, Eurcic Acid, Peroxide, Salmonella and Others. As dyes were used in the development of this model, they can be broken down further into individual dyes: Sudan III, Sudan IV, and Sudan Red G. The following sections give a breakdown of the data extracted and how the prototype FoodAlert Register for oils was constructed.

A2.2.1 Country of Origin, Hazards and Frequency of Notifications Hazards

A2.2.1.1 Country of Origin (excluding transhipment)

From the data in the EU RASFF it is possible to assign the country of origin as well as the associated agent. **Figure 7** shows the country of origin of oil/fat commodities for countries with certain hazards identified including dyes. This does not include country of transhipment (see **Section A2.2.1.2**). In summary the data shows that Ukraine has the most notifications based on country of origin of the commodity and associated agent followed by Ghana, Senegal and Nigeria with a further 27 countries associated with 1-4 notifications for hazards related to oils/fats.

The chart in **Figure 8** shows the frequency of the notifications for oils/fats associated with the country of origin, and based on the data the highest numbers of alerts are related to Sudan IV, benzopyrene and mineral oil. (N.B. The “Other” classification in **Figure 8** relates to miscellaneous notifications for oils/fats that are not classified under the current hazard headings).

Figure 7: Notifications for Country of Origin of Oil/Fat Commodity

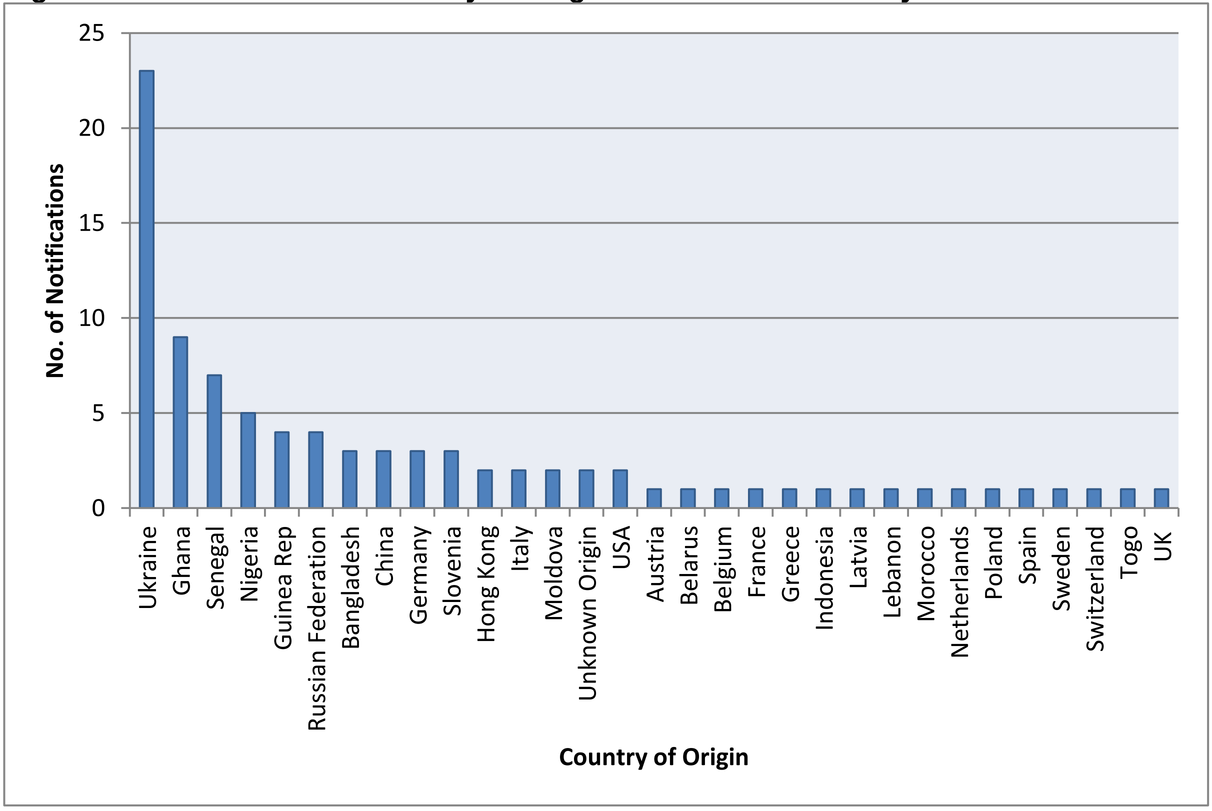
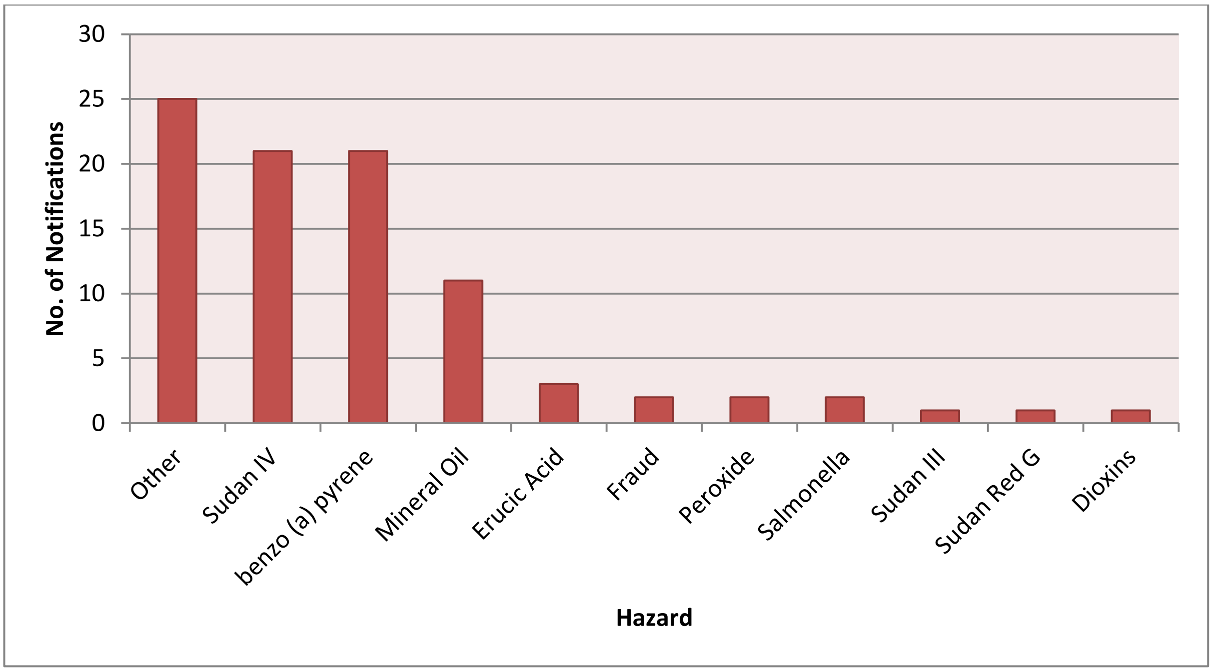


Figure 8: Frequency of Notifications for Oils/Fats



By combining the data in **Figure 7** and **Figure 8**, the chart in **Figure 9** shows the country of origin, the number of notifications associated with that country and the agents identified from the data. Although **Figure 9** appears to be a little more complex than the other Figures, it clearly shows the major agents associated with oil commodities from each country.

Figure 9: Combined data related to country of origin and associated agent

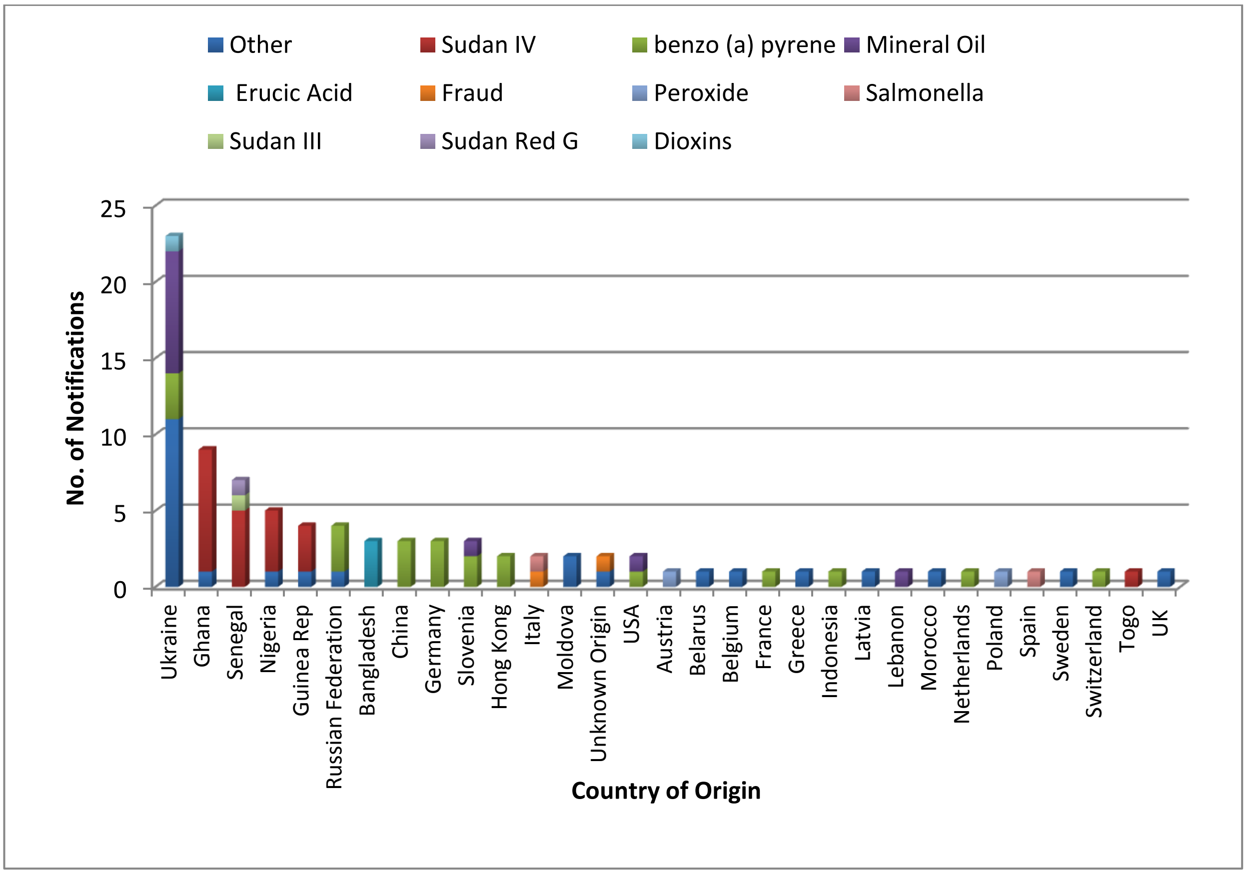


Table 7 shows the notification frequency of each agent for each year based on the associated oil/fat commodity. The data can be broken down further to look at the frequency of notification related to individual dyes as shown in **Table 8** and 3 year rolling data sets as shown in **Table 9**. The frequency data were used to assist in the defining of the priority ranking of the hazard identified and building the FoodAlert model (**Section A2.2.2**).

A2.2.1.2 Country of Origin (including transhipment)

It is possible that the occurrence of an agent associated with a particular commodity could arise during transhipment through another country. For this reason the data related to country of transhipment and country of origin in the EU RASFF for each commodity can be included in the FoodAlert Register. **Figure 10** shows the country of origin, including transhipment, of oil/fat commodities. In summary the data shows that there is not too much difference from **Figure 7** above except for a slight increase in alerts for the European country responsible for the transhipment especially Austria.

Figure 10: Notifications for Country of Origin of Commodity including transhipment

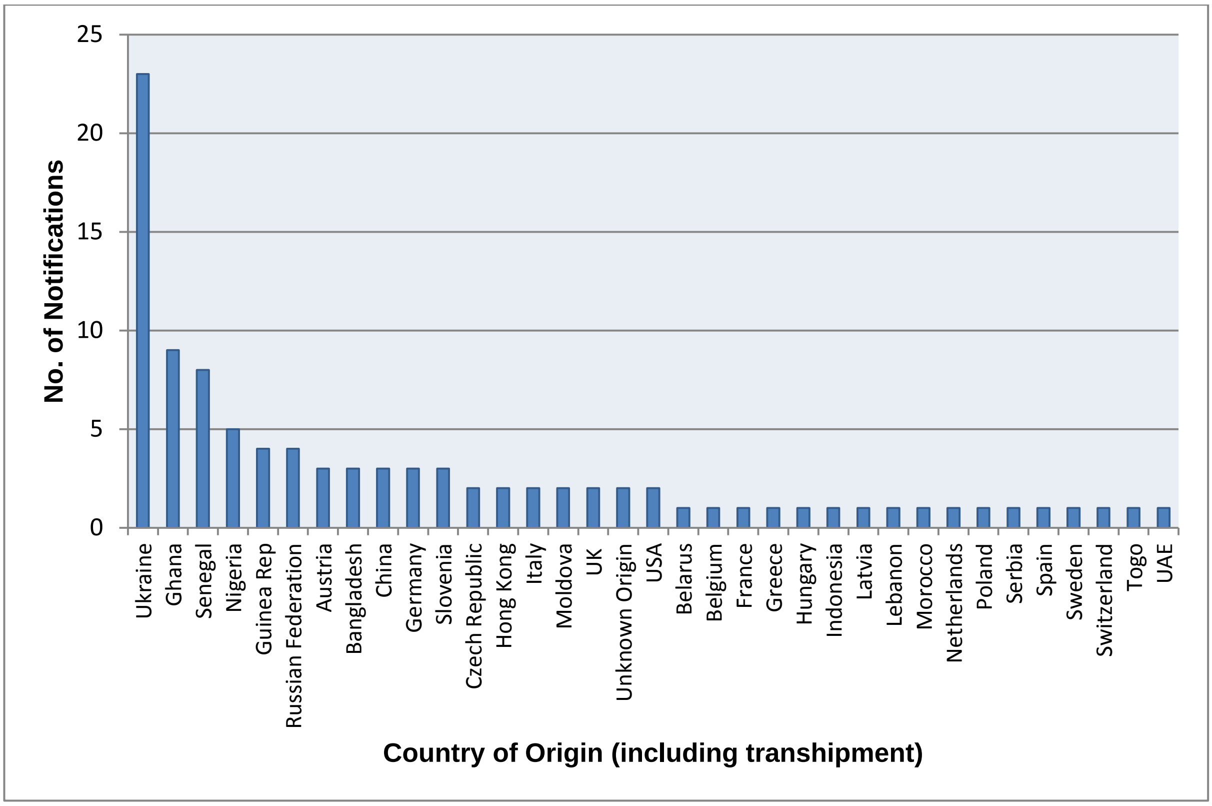


Table 7: Frequency for Notifications for each agent and oil/fat commodity

	Dyes				Mineral Oil				benzo (a) pyrene				Fraud				Erucic Acid				Peroxide				Dioxins				Salmonella				Other				Total Alerts			
	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008
Olive Oil																2																					0	0	0	2
Palm Oil	2	6	7	7	1				1																								1		1	1	5	6	8	8
Sunflower Oil							1	7		1	3																						3	1	5	2	3	2	9	9
Pumpkin Seed Oil									2	2		1																									2	2	0	1
Sea-buckthorn Oil									1			1																									1	0	0	1
Coconut Oil												1																									0	0	0	1
Walnut Oil							1																														0	0	1	0
Mustard Oil																		2	1																		0	2	1	0
Chia Seed Oil																																		1			0	1	0	0
Rapeseed Oil									1	1																								1			1	2	0	0
Soybean Oil																																	2	2			2	2	0	0
Fish Oil																																		1			0	1	0	0
Hot Chilli Flavoured Oil									1																												1	0	0	0
Porcine Lard																						1		1													0	1	0	1
Pork Fat																														2					1		0	2	1	0
Pure Lard																																		1			0	1	0	0
Vegetarian Magarine																																	1				1	0	0	0
Coconut Fat Acid																										1											0	1	0	0
Vegetable Oil									1																												1	0	0	0
Sesame Oil									2																												2	0	0	0
Palm Kernel Oil	1																																				1	0	0	0
Maize Oil							1																														0	0	1	0
Other																																		1			0	1	0	0
Total	3	6	7	7	1	0	3	7	9	4	3	3	0	0	0	2	0	2	1	0	0	1	0	1	0	1	0	0	0	2	0	0	7	8	7	3	20	24	21	23
Total Alerts	23				11				19				2				3				2				1				2				25				88			

Table 8: Frequency of Notifications for oil/fat commodities with individual dyes

	Sudan 3				Sudan 4				Sudan Red G			
	2011	2010	2009	2008	2011	2010	2009	2008	2011	2010	2009	2008
Olive Oil												
Palm Oil		1			2	5	7	6				1
Sunflower Oil												
Pumpkin Seed Oil												
Sea-buckthorn Oil												
Coconut Oil												
Walnut Oil												
Mustard Oil												
Chia Seed Oil												
Rapeseed Oil												
Soybean Oil												
Fish Oil												
Hot Chilli Flavoured Oil												
Porcine Lard												
Pork Fat												
Pure Lard												
Vegetarian Magarine												
Coconut Fat Acid												
Vegetable Oil												
Sesame Oil												
Palm Kernel Oil					1							
Maize Oil												
Other												
Total	0	1	0	0	3	5	7	6	0	0	0	1
Total Alerts	1				21				1			

Table 9: Number of Notifications per Commodity for Dyes in 3 year periods

	Dyes	
	2008-2010	**2009-2011**
Olive Oil	0	0
Palm Oil	20	15
Sunflower Oil	0	0
Pumpkin Seed Oil	0	0
Sea-buckthorn Oil	0	0
Coconut Oil	0	0
Walnut Oil	0	0
Mustard Oil	0	0
Chia Seed Oil	0	0
Rapeseed Oil	0	0
Soybean Oil	0	0
Fish Oil	0	0
Hot Chilli Flavoured Oil	0	0
Porcine Lard	0	0
Pork Fat	0	0
Pure Lard	0	0
Vegetarian Margarine	0	0
Coconut Fat Acid	0	0
Vegetable Oil	0	0
Sesame Oil	0	0
Palm Kernel Oil	0	1
Maize Oil	0	0

A2.2.2 Scoring and Priority Status for Testing of Oil/Fat Samples

The scoring system that has been previously developed (**Section A2.1.3**) was applied to dyes found in oils in the same manner to develop the illustrative model. Severity scores for dyes are as presented in **Table 4**.

Analysing the information within the database, allows for the identification of the frequency of notifications over 3 year rolling periods (2008-2010 and 2009-2011 data) and the severity of the associated agent. In **Table 10** it can be seen that, based on the parameters identified and by multiplying the frequency of notification of each agent by severity score, a value is obtained for each commodity e.g. palm oil (20x24=480). By using data from 3 year periods in this model it can be observed whether dyes are an increasing or decreasing risk for the oil/fat commodities highlighted. Based on this model, it can be noted that although still a hazard for Palm Oil, the risk from dyes has decreased. However for palm kernel oil, dyes as a hazard are an emerging risk and therefore a possibility of increased testing for dyes in this commodity would be recommended. The full impact of this model would need to be accessed in the context of all the hazards (Mineral Oil, Benzopyrene, Dioxins/PCBs, Salmonella, Fraud, Erucic Acid and Peroxide) associated with these oil/fat commodities. An example could be as shown in **Table 11**. Palm Oil for 2009-2011 had a score of 360 (15x24) related to dyes. If using this model, agent X gave a score of 750 and agent Y gave a score of 20 then the hazards could be ranked in order of testing priorities as shown with the primary testing for agent X.

Table 10: Table of Severity x Frequency score for Dyes in Oil/Fat Commodities over 3 year periods

	Dyes		
	2008-2010	**2009-2011**	**Hazard: Increasing/Decreasing**
Olive Oil	0	0	—
Palm Oil	480	360	↓
Sunflower Oil	0	0	—
Pumpkin Seed Oil	0	0	—
Sea-buckthorn Oil	0	0	—
Coconut Oil	0	0	—
Walnut Oil	0	0	—
Mustard Oil	0	0	—
Chia Seed Oil	0	0	—
Rapeseed Oil	0	0	—
Soybean Oil	0	0	—
Fish Oil	0	0	—
Hot Chilli Flavoured Oil	0	0	—
Porcine Lard	0	0	—
Pork Fat	0	0	—
Pure Lard	0	0	—
Vegetarian Margarine	0	0	—
Coconut Fat Acid	0	0	—
Vegetable Oil	0	0	—
Sesame Oil	0	0	—
Palm Kernel Oil	0	24	↑
Maize Oil	0	0	—

Table 11: Ranking priorities

Palm Oil	Agent X	Dyes	Agent Y
Severity x Frequency Score	750	360	20
Testing Priority	Primary	Secondary	Tertiary

A3 Conclusions and Recommendations

- This six month project has illustrated that a tool, **the FoodAlert Register,** was developed for several food commodities and could be potentially used to allow for a risk based sampling and testing scheme to be implemented.
- The EU RASFF system was used as an information source in development of this tool which is a way of using existing available data in a more intelligent manner.
- Not only were existing hazards identified but emerging hazards can also be observed based on the trends analysis.
- The contractor recommends the further and full development of the **FoodAlert Register** to enable the implementation of a UK wide monitoring scheme based on a risk based approach. Such a system will allow for better targeted use of the dwindling resources available to ensure the protection of the UK consumer against purchasing low quality or unsafe foods. The following aspects should be considered in further development of the register:

    - The FoodAlert Register should be expanded to include analysis of individual commodities and hazards rather than the current “generic” format.

    - The type of notifications that are used in the model.

    - A shortcoming of the existing model is that it solely relies on the RASFF notifications. Therefore if other local UK data from testing could be made available and included in the model, a more powerful **FoodAlert** system could be built.

    - Differentiation within the model for compliance issues and health issues related to certain hazards.

    - Undertake a full toxicological assessment of each agent and include this parameter in the register.

    - Further development of the scoring system to include an equation that also takes account of the toxicity of the agent, the volume of the commodity that is brought into the UK and the country of origin of the commodity.

PART B

Development of Food Fingerprinting Methods: A Proof of Concept Study using Near Infrared and Raman Spectroscopies

B1.1 Introduction

In Part B, an additional important element to the project was to perform a pilot study to show the feasibility of providing a tool that can be used to perform rapid scanning of foods entering the UK for a number of identified chemicals. One of the difficulties facing the sampling authorities is the associated costs of the testing to be undertaken and the length of time it takes to get the laboratory to process the sample and deliver the result.

A proof of principle project was undertaken to select a range of foods (spices and oils) and grossly contaminate/adulterate over a range of concentrations. These commodities were analysed by a number of spectroscopic techniques (Near Infrared and RAMAN) to develop ‘fingerprints’ associated with non- contaminated and contaminated commodities. These techniques are rapid, low cost and require minimal sample processing which enables fast and low cost monitoring to be performed.

Calibration models have been developed from the spectral data and chemometric software. These calibration models will be able to be used with unknown samples to determine abnormalities due to changes in the samples molecular fingerprint. It should be noted that the models were developed for gross contamination or adulteration testing and not for the low level contaminant testing at the legislative limit. The latter would need modification of the current techniques which was not feasible due to the time limitations of the current project.

The use of technologies and new techniques to produce ‘fingerprints’ for animal health, animal feeds and human foods are gaining interest in many applications. In the tracing and verification of the origins of foods in and determining the authenticity of food products a range of techniques have been shown to deliver data that can provide evidence based on the molecular structure/composition of the samples being tested. With regards the detection of chemical contaminants in foods, RAMAN has recently been shown to be capable of detecting melamine in milk[1]. In the ASSET Technology Centre at Queen’s, Near Infra-Red (NIR) methods of detecting melamine in soya bean products[2] and waste oils in animal feed oils[3] have been successfully developed and validated. The potential of Raman spectroscopy for the determination of melamine adulteration of animal feed and food stuff has been accessed[4]. FT-Raman coupled with chemometric analysis has been used to discriminate the country of origin in relation to Corsican honey[5] whilst near infrared spectroscopy has been applied to produce a fingerprint for Corsican honey to confirm their claimed provenance[6]. Near infrared transmittance spectroscopy has been used to confirm geographical origin of Ligurian olive oil with the best calibration models correctly predicted the origins of samples in the prediction set up to 92.8 and 81.5% for Ligurian and non-Ligurian olive oil samples respectively[7]. Near infrared spectroscopy has been employed to determine the contamination of the spice paprika with mycotoxins and compared to a HPLC-FD technique[8]. Near infrared spectroscopy has been used to quantify the three main alkaloids in roasted coffee i.e. caffeine, theobromine and theophylline, with this method compared to the commonly used HPLC-MS method[9]. Near infrared spectroscopy has been applied to saffron spice to determine it chemical composition and geographical origin and validated by comparison with UV-Vis and HPLC_DAD measurements[10]. Another technique that has been successfully used to determine adulteration of spices with Sudan I-II-III-IV dyes was UV-Vis spectroscopy (Anibal et al, 2009).

The object was to produce, as a proof of concept, a number of rapid, easy to use food fingerprinting methods to allow the early detection of contaminated foods entering the UK. If successful, these methods can be expanded to cover a very wide range of foods and associated contaminants (chemicals and microbiological threats).

B2.1 Experimental Procedures

B2.1.1 Sample Collection and Preparation

Commercial samples of spices (n=114) and oils (n=49) were purchased at various convenience shops, supermarkets and market places mostly in UK/Ireland in addition to some obtained globally. A number of chilli powders (n=15) were selected and these were spiked with Sudan I dye at various concentrations (5%, 2.5%, 1%, 0.5%, 0.2%, 0.1% and 0%).

Olive oil samples (n=49) were selected and adulterated with cheaper sunflower oil at concentrations (0-100%, in 10% increments). All the adulterated samples were used to obtain spectral fingerprints which were consequently used for to develop qualitative and quantitative calibration models.

B2.1.2 Near-Infrared Spectroscopy

Near-infrared spectra, reflectance mode for spices and transmittance mode for oils, were recorded on an Antaris II FT-NIR (Thermo Fisher Scientific, Dublin, Ireland).

The samples of spices (10 g) were poured onto the sample cup spinner on the Integrating Sphere module of the instrument. All the spectra were computed at 8 cm^{-1} resolution across the spectral range 12000-3800 cm^{-1} and ran in triplicate. Instrument control and initial spectral manipulation were performed with Result Integration software. The spectra were recorded at ambient temperature and a total of 64 scans were acquired for each spectrum.

Samples of the oils (600 µl) were pipetted into sealed-glass vials, 3 replicate spectra of each sample were acquired. All the spectra were computed at 8 cm^{-1} resolution across the spectral range 12000-3800 cm^{-1} with a constant pathlength of 8 mm. Instrument control and initial spectral manipulation were performed with Result Integration software. Oil samples were recorded at ambient room temperature and a total of 16 scans were acquired for each spectrum.

B2.1.3 Raman Spectroscopy

Raman spectra of the spices and oils were recorded using an Advantage 1064 Raman Spectrometer (DeltaNu Inc., Laramie, Wyoming, USA) using the spinning mechanism upgrade which had been a crucial part of the project (see **Section B2.2.1**).

The samples of spices were transferred into small glass vials and placed on the spinning mechanism and the spectra for each sample were acquired with an integration time of 5 seconds. All the spectra were computed at 10 cm^{-1} resolution across the spectral range 200-2000 cm^{-1}. Instrument control was performed using NuSpec software and library development software which was provided with the instrument (DeltaNu Inc., Laramie, Wyoming, USA). The spectra were recorded at ambient temperature.

Samples of the oils (400 µl) were pipetted into glass vials, and the spectra for each sample were acquired with an integration time of 5 seconds. All the samples were computed at 10 cm^{-1} resolution across the spectral range 200–2000 cm^{-1}. Instrument control was performed using NuSpec software and library development software which was provided with the instrument (DeltaNu Inc., Laramie, Wyoming, USA). Oil samples were recorded at ambient room temperature.

B2.1.4 Chemometric Analysis of Spectral Data

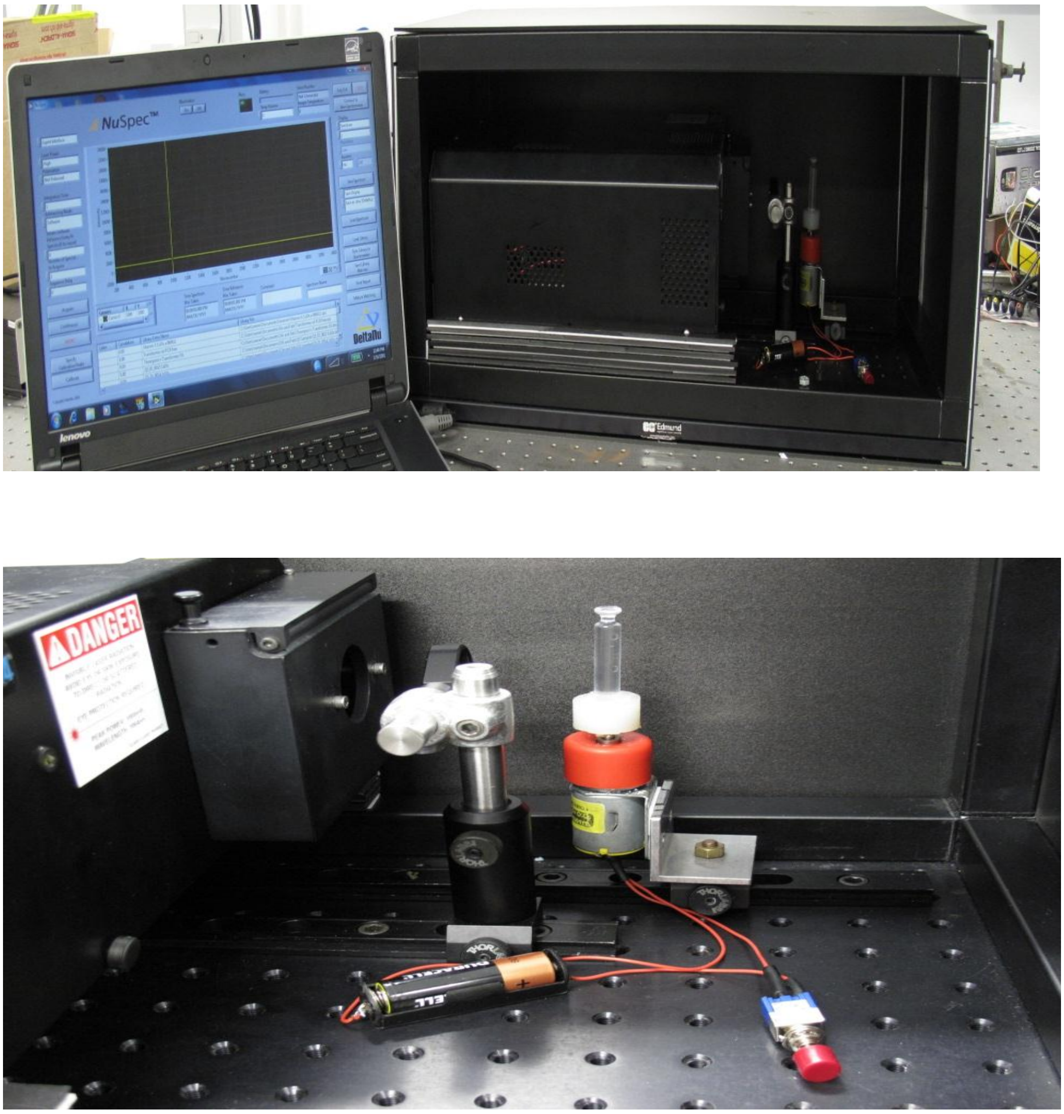
Multivariate analysis was used for quantitative and qualitative analysis of both the NIRS and Raman spectroscopic data. The analysis was carried out using the software package TQ Analyst (Thermo Fisher Scientific, Dublin, Ireland) and SIMCA P+13 (Umetrics, Sweden). Principal Components Analysis (PCA), Partial Least Squares Discriminate Analysis (PLS-DA) and Orthogonal Partial Least Squares-Discriminate Analysis (OPLS-DA) were used as qualitative tests in this investigation. Principal components regression (PCR) and partial least squares (PLS) were used in the current study for quantitative model building. For the quantitative tests the equation selected for each oil was obtained using the lowest standard error of prediction to minimise the of over-fitting when the model accuracy was evaluated. Models were developed using various wavelength ranges: 9000-4500 cm^{-1} (NIRS) and 800-1800 cm^{-1} (Raman).   The data pre-treatments examined were none (raw data), 1st and 2nd derivatives calculated in conjunction with the Savitzky-Golay/ Norris Derivative smoothing methods.

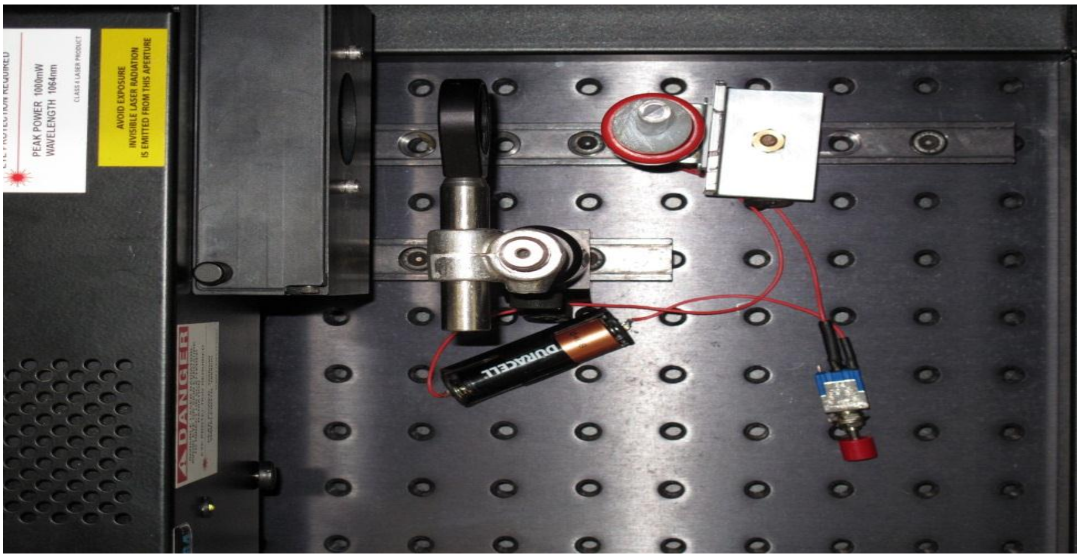
B2.2.1 Upgrade of the Raman Spectrometer

As part of the initiative in the this project, the potential for the upgrade of the Raman spectrometer was explored so that samples could be rotated, hence giving a larger surface area for analysis as well as reducing the problems caused by heterogeneous samples. The Raman instrument has been upgraded with a prototype sample compartment with a spinning mechanism for sample analysis. This entailed the construction of a box with a steel speedframe with black PVC foam walls and removable top, attached to a steel bench plate. The inclusion of a removable top is to facilitate access to the spinner and also to fulfil Health and Safety regulations with regards to use of a Class 3 laser. The spinner was positioned so that a lens external to the Raman instrument could focus the laser beam onto the sample position. Photographs of the upgrade of the sample compartment are shown in Figure 11.

The adaptation of the Raman spectrometer will be fully described in a peer review publication.

Figure 11: Photographs of Upgrade to Raman Instrument





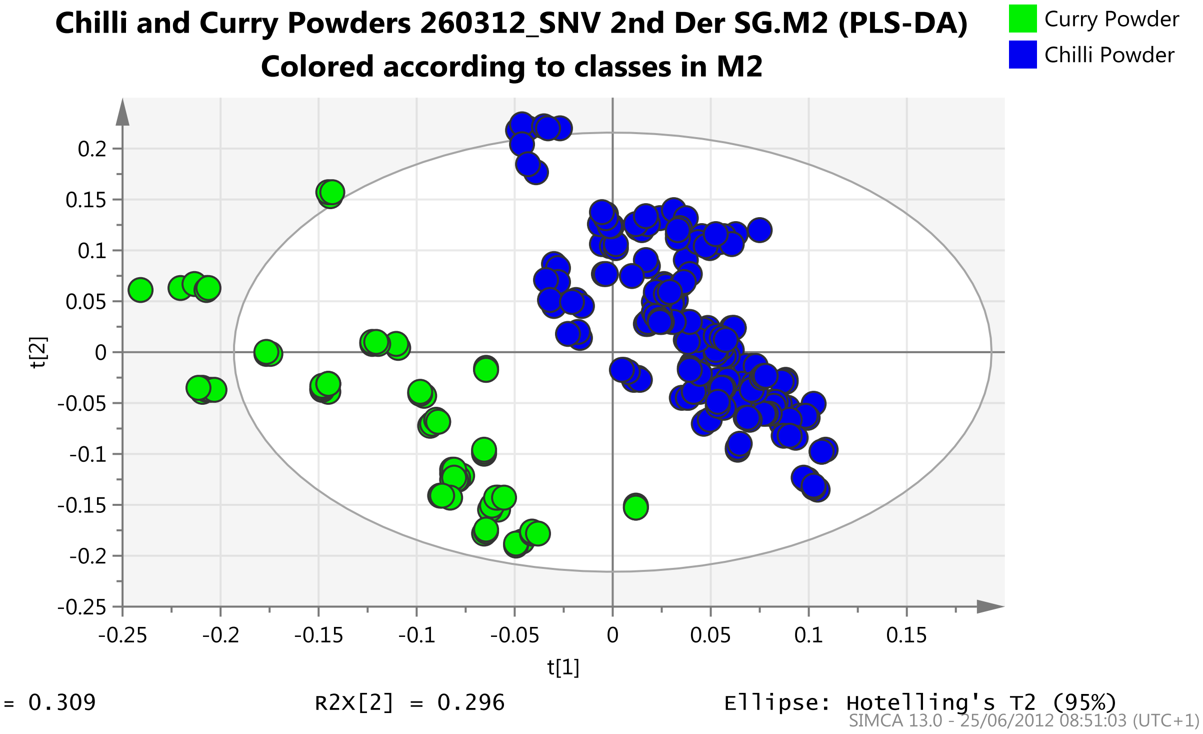
B2.2 Results and Discussion

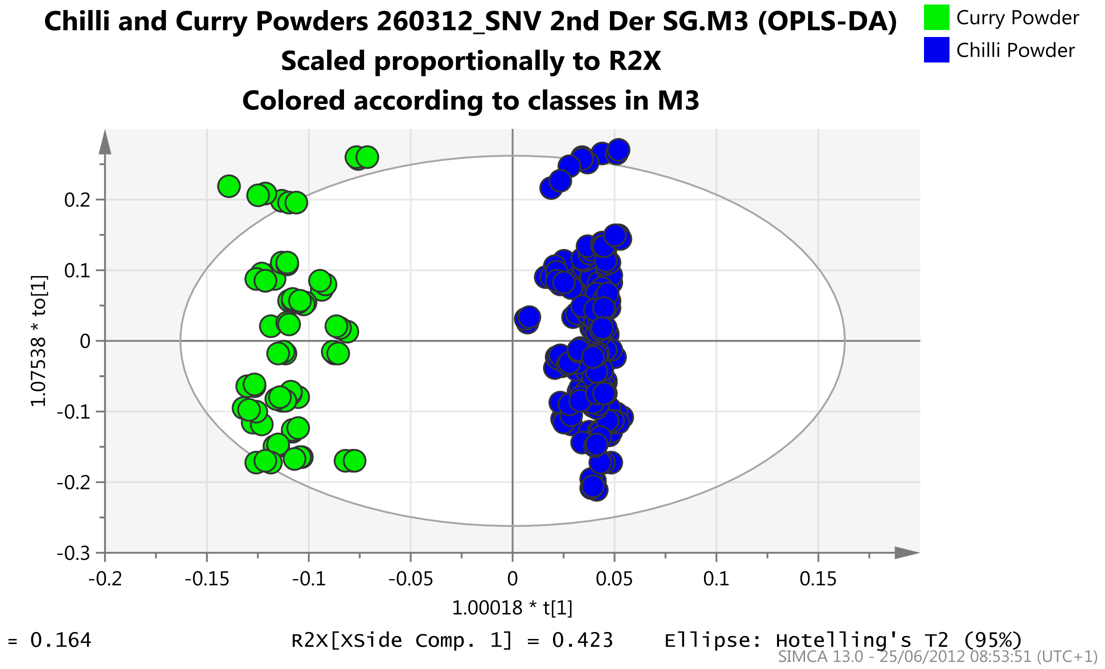
B2.2.1 Chemometric analysis of spectral data of spices

B2.2.1.1 Qualitative Analysis of NIRS Spectral Data of Chilli and Curry Powders

NIRS spectral data were used in conjunction with chemometric algorithms to test whether chilli powders and curry powders could be distinguished. **Figure 12** shows the Partial Least Squares Discriminate Analysis (PLS-DA) and Orthogonal Partial Least Squares Discriminate Analysis (OPLS-DA) of these spices using the spectral data. The data was pre-processed using standard normal variate (SNV), 2nd Derivative algorithm with Savitzky-Golay smoothing (9 and 3). Distinct clusters formed for chilli powders and curry powders as shown by both models

Figure 12: PLS-DA and OPLS-DA calibration models distinguishing curry powders and chilli powders

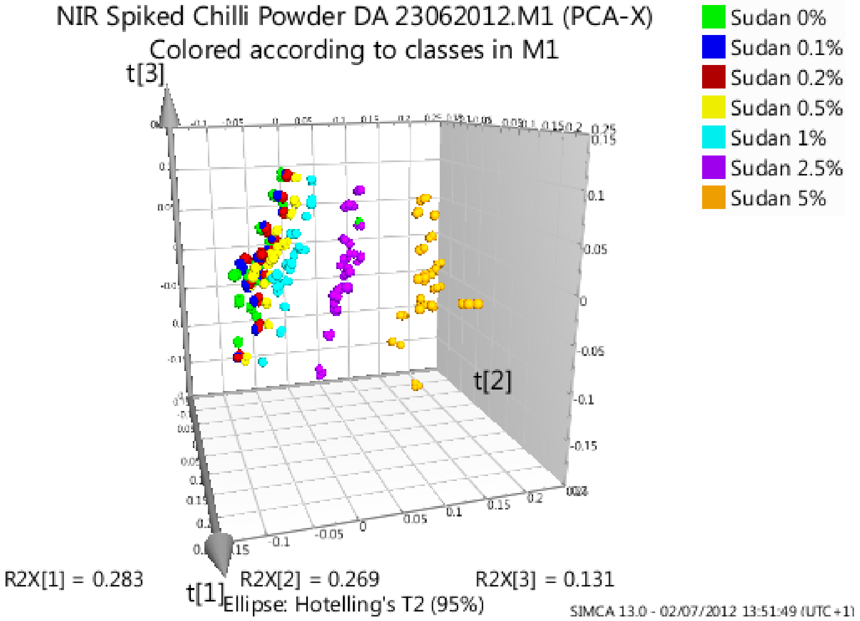




B2.2.1.2 Qualitative and Quantitative Analysis of NIRS Spectral Data of Sudan I spiked Chilli Powders

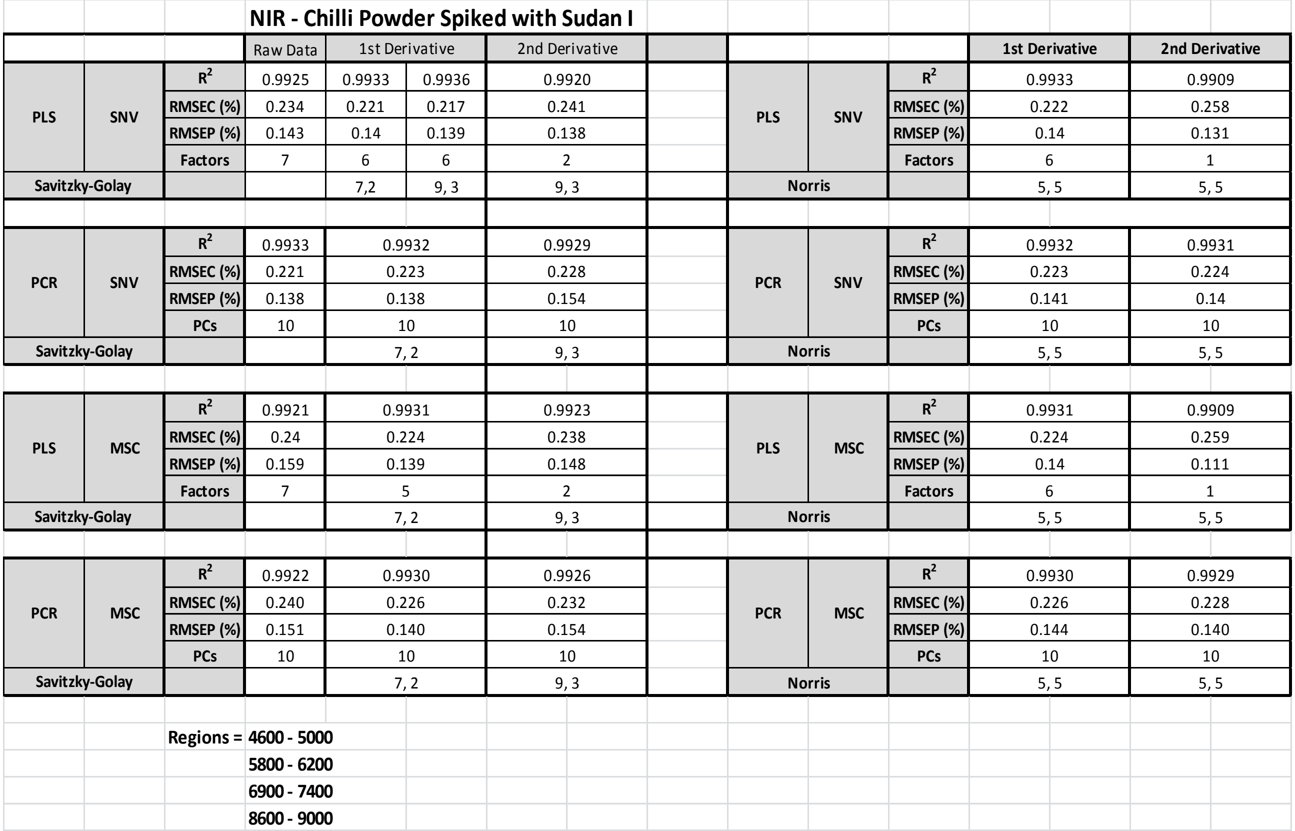
Fifteen different chilli powders were selected for a spiking experiment with Sudan I dye which has been highlighted as a major hazard in the initial FoodAlert register for spices (Part A). The selected chilli powders were spiked at 5%, 2.5%, 1%, 0.5%, 0.2%, 0.1% and 0% with Sudan I. These spiked samples were run on the NIRS instrument. The chemometric software was used to construct a qualitative model using PCA algorithm to see if it was possible to quickly distinguish spiked samples from pure samples. The data was pre-processed using SNV, 2nd Derivative algorithm with Savitzky-Golay smoothing (11 and 2). **Figure 13** shows clusters for each spiked level indicating that it may be possible to use this approach to quickly screen for adulteration/gross contamination.

Figure 13: PCA qualitative calibration model for Sudan I spiked chilli powders



For quantitative analysis the various chemometric parameters tested are highlighted in **Table 12**. The models were assessed and the best models will have a high value for the correlation coefficient (R^2) and low values for the root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) using the quantitative chemometric algorithms, partial least squares (PLS) and principal component regression (PCR). For these models, the error of predication gives an indication of the sensitivity of the test. **Figure 14** shows a typical quantitative calibration model obtained with actual concentration along the x-axis and the NIRS predicted concentration along the y-axis.

Table 12: Quantitative calibration models generated using NIR spectral data and chemometric software



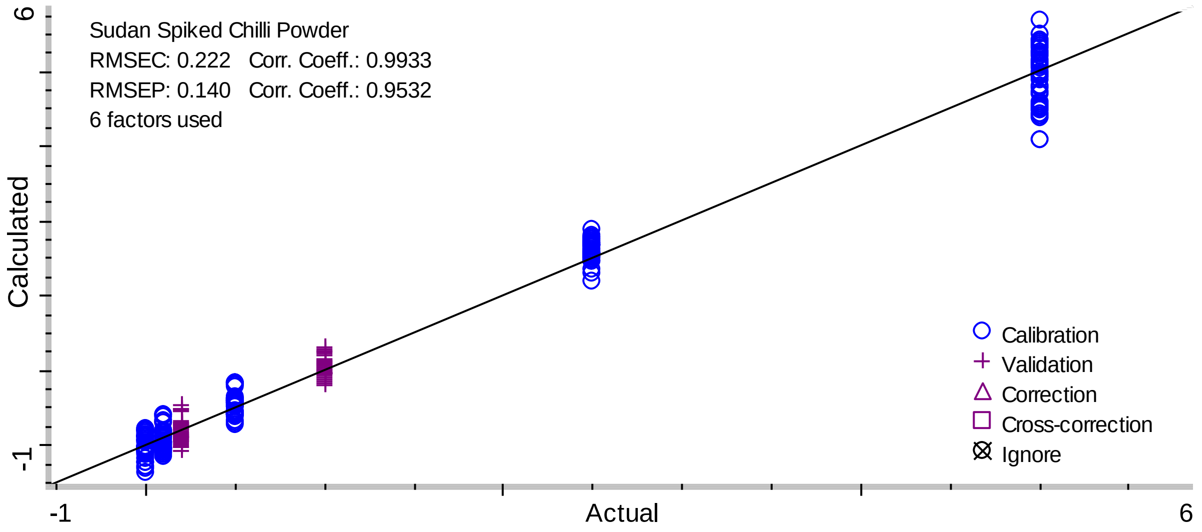
NIR - Chilli Powder Spiked with Sudan I

			Raw Data	1st Derivative		2nd Derivative
PLS	SNV	R^2	0.9925	0.9933	0.9936	0.9920
		RMSEC (%)	0.234	0.221	0.217	0.241
		RMSEP (%)	0.143	0.14	0.139	0.138
		Factors	7	6	6	2
Savitzky-Golay				7,2	9, 3	9, 3
PCR	SNV	R^2	0.9933	0.9932		0.9929
		RMSEC (%)	0.221	0.223		0.228
		RMSEP (%)	0.138	0.138		0.154
		PCs	10	10		10
Savitzky-Golay				7, 2		9, 3
PLS	MSC	R^2	0.9921	0.9931		0.9923
		RMSEC (%)	0.24	0.224		0.238
		RMSEP (%)	0.159	0.139		0.148
		Factors	7	5		2
Savitzky-Golay				7, 2		9, 3
PCR	MSC	R^2	0.9922	0.9930		0.9926
		RMSEC (%)	0.240	0.226		0.232
		RMSEP (%)	0.151	0.140		0.154
		PCs	10	10		10
Savitzky-Golay				7, 2		9, 3

			1st Derivative	2nd Derivative
PLS	SNV	R^2	0.9933	0.9909
		RMSEC (%)	0.222	0.258
		RMSEP (%)	0.14	0.131
		Factors	6	1
Norris			5, 5	5, 5
PCR	SNV	R^2	0.9932	0.9931
		RMSEC (%)	0.223	0.224
		RMSEP (%)	0.141	0.14
		PCs	10	10
Norris			5, 5	5, 5
PLS	MSC	R^2	0.9931	0.9909
		RMSEC (%)	0.224	0.259
		RMSEP (%)	0.14	0.111
		Factors	6	1
Norris			5, 5	5, 5
PCR	MSC	R^2	0.9930	0.9929
		RMSEC (%)	0.226	0.228
		RMSEP (%)	0.144	0.140
		PCs	10	10
Norris			5, 5	5, 5

Regions = **4600 - 5000**
5800 - 6200
6900 - 7400
8600 - 9000

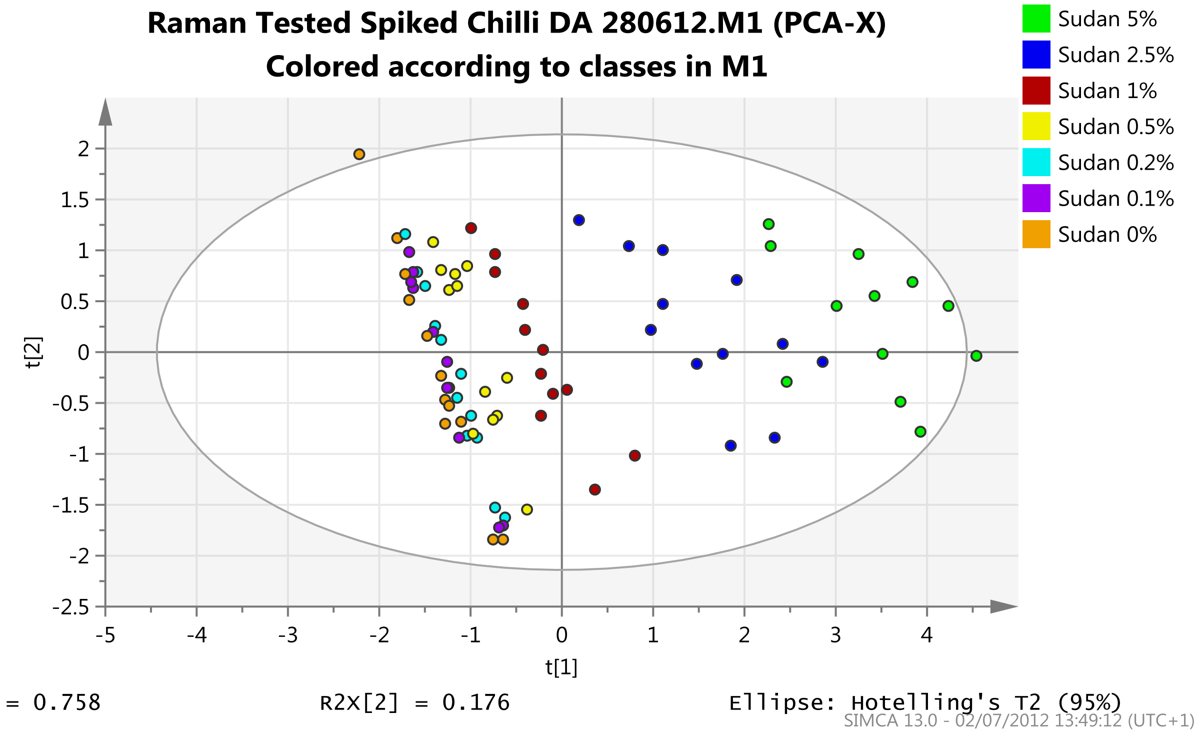
Figure 14: Typical Quantitative Calibration model constructed using NIR data



B2.2.1.3 Qualitative and Quantitative Analysis of Raman Spectral Data of Sudan I spiked Chilli Powders

Fifteen different chilli powders were selected for a spiking experiment with Sudan I dye which has been highlighted as a major hazard in the initial FoodAlert register for spices. The selected chilli powders were spiked at 5%, 2.5%, 1%, 0.5%, 0.2%, 0.1% and 0% with Sudan I. These spiked samples were run on the Raman instrument using the spinning mechanism. The chemometric software was used to construct a qualitative model using the PCA algorithm to see if it was possible to quickly distinguish spiked samples from pure samples. The data was pre-processed using SNV, 1st Derivative algorithm with Savitzky-Golay smoothing (9 and 2). **Figure 15** shows that there are clusters for each spiked level down to between 0.5-1% indicating that it may be possible to use this approach to rapidly screen for adulteration.

Figure 15: PCA qualitative calibration model for Sudan I spiked chilli powders

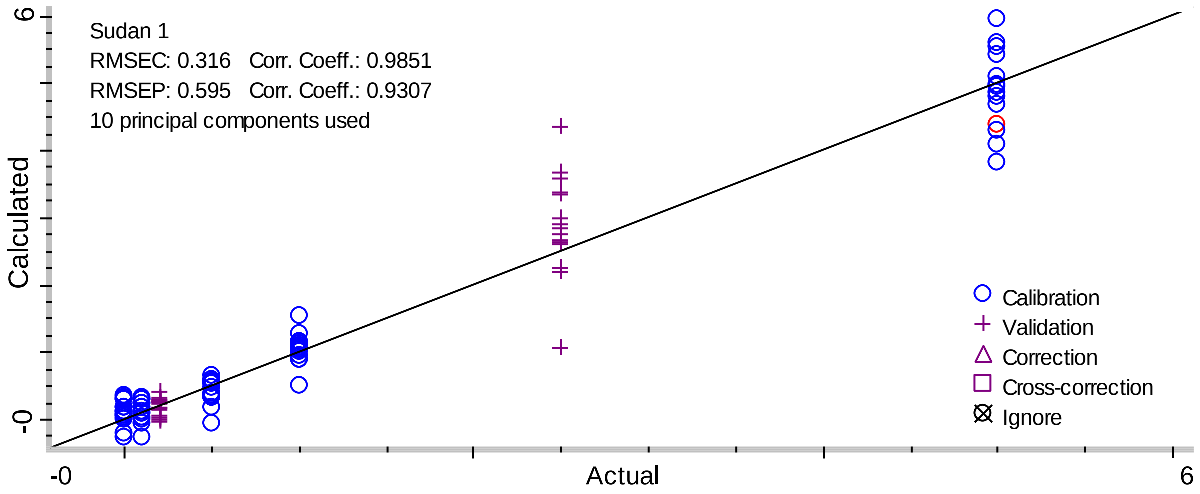


For quantitative analysis the various chemometric parameters tested are highlighted in **Table 13**. The models were assessed and the best models will have a high value for the correlation coefficient (R^2) and low values for RMSEC and RMSEP using the quantitative chemometric algorithms, PLS and PCR. **Figure 16** shows a typical quantitative calibration model obtained with actual concentration along the x-axis and the Raman predicted concentration along the y-axis. From **Table 13** the error of prediction is ~0.6% which is similar to what we can detect qualitatively (**Figure 16**) i.e. 0.5-1%.

Table 13: Quantitative calibration models generated using Raman spectral data and chemometric software

			Raman - Chilli Spiked with Sudan I								
			Raw Data	1st Derivative	2nd Derivative					1st Deriva	2nd Derivative
PLS	**SNV**	R^2	0.957	0.95783	0.96298		**PLS**	**SNV**	R^2	0.95736	0.96032
		RMSEC (%)	0.543	0.538	0.505				RMSEC (%)	0.541	0.523
		RMSEP (%)	0.643	0.624	0.608				RMSEP (%)	0.627	0.612
		Factors	4	3	3				Factors	3	3
Savitzky-Golay				7, 2	9, 3		Norris			5, 5	5, 5
PLS	**MSC**	R^2	0.91771	0.91055	0.91359		**PLS**	**MSC**	R^2	0.91039	0.91023
		RMSEC (%)	0.744	0.774	0.762				RMSEC (%)	0.775	0.776
		RMSEP (%)	0.526	0.547	0.535				RMSEP (%)	0.548	0.545
		Factors	3	2	2				Factors	2	2
Savitzky-Golay				7, 2	9, 3		Norris			5, 5	5, 5
PCR	**SNV**	R^2	0.9576	0.96029	0.96578	0.97241	**PCR**	**SNV**	R^2	0.96087	0.96245
		RMSEC (%)	0.54	0.523	0.486	0.437			RMSEC (%)	0.519	0.509
		RMSEP (%)	0.641	0.648	0.603	0.6			RMSEP (%)	0.655	0.606
		PCs	10	10	10	10			PCs	10	10
Savitzky-Golay				7, 2	9, 3	11, 4	Norris			5, 5	5, 5
PCR	**MSC**	R^2	0.92768	0.93079	0.93374		**PCR**	**MSC**	R^2	0.93155	0.93342
		RMSEC (%)	0.699	0.685	0.671				RMSEC (%)	0.681	0.672
		RMSEP (%)	0.491	0.524	0.457				RMSEP (%)	0.523	0.473
		PCs	9	9	9				PCs	10	10
Savitzky-Golay				7, 2	9, 3		Norris			5, 5	5, 5
	Regions:	1150-1270									
		1300-1530									
		1570-1630									

Figure 16: Typical Quantitative Calibration model constructed using Raman data



B2.2.1.4 Conclusions to Dyes Study

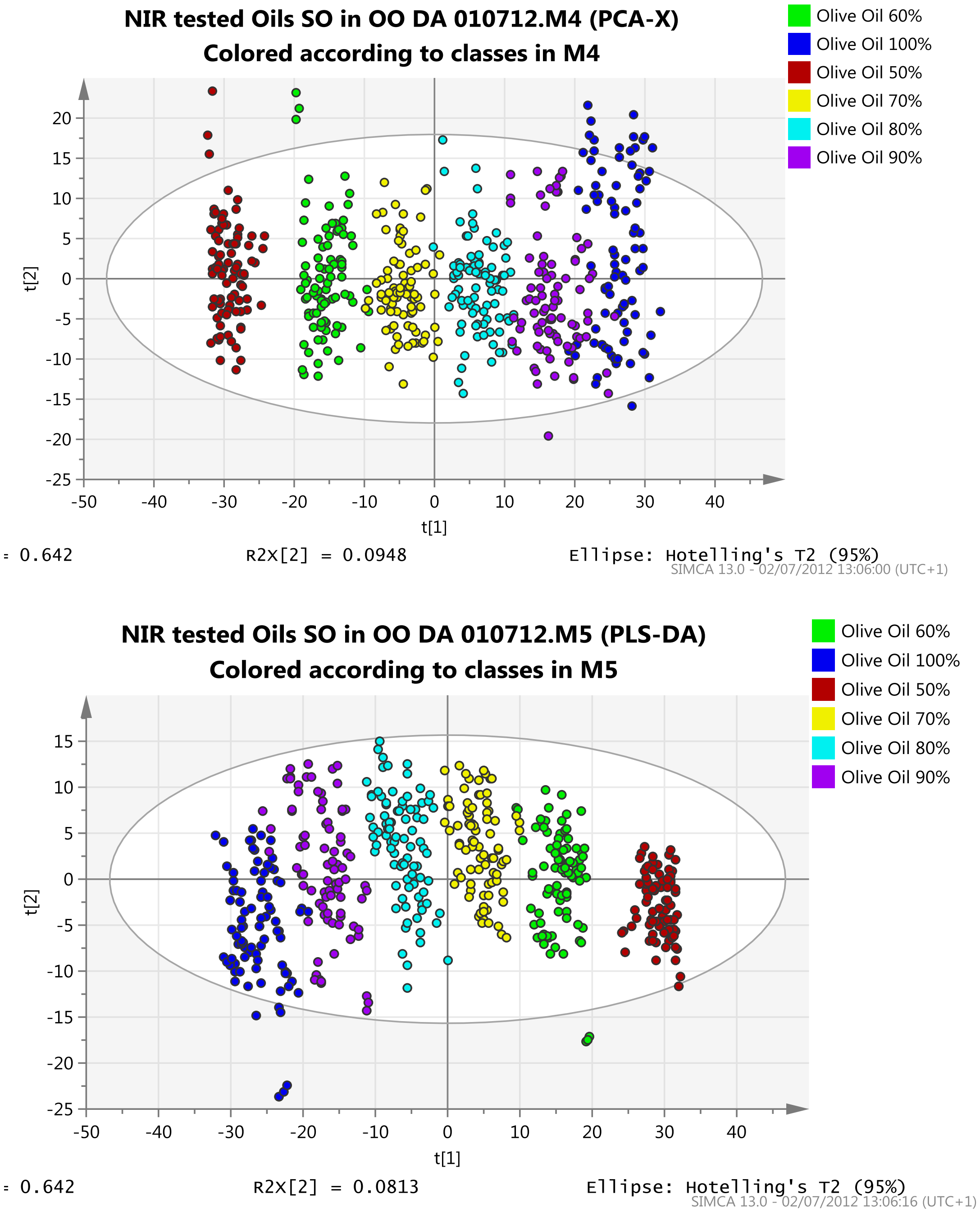
Both NIR and RAMAN have been demonstrated to be capable of detecting the presence of Sudan dyes in a number of spices. The methods were very fast, easy to use and require minimal sample preparation. The sensitivity of the methods has demonstrated that they can detect gross contamination or levels used to adulterate spices. While low level contamination could not be detected these levels have much less of an importance to human health with regards exposure to toxic agents. Thus the tools developed can be considered suitable for the protection of consumers to exposure of potentially toxic levels of chemicals but not suitable to enforce the EU legislation relating to dyes. The ability to take a low cost device such as the RAMAN instrument, modify it and develop a chemometric model to use for rapid detection of dyes in foods is highly innovative.

B2.2.2 Chemometric analysis of spectral data of Oils

B2.2.2.1 Qualitative and Quantitative Analysis of NIRS Spectral Data of Olive Oils adulterated with Sunflower Oil

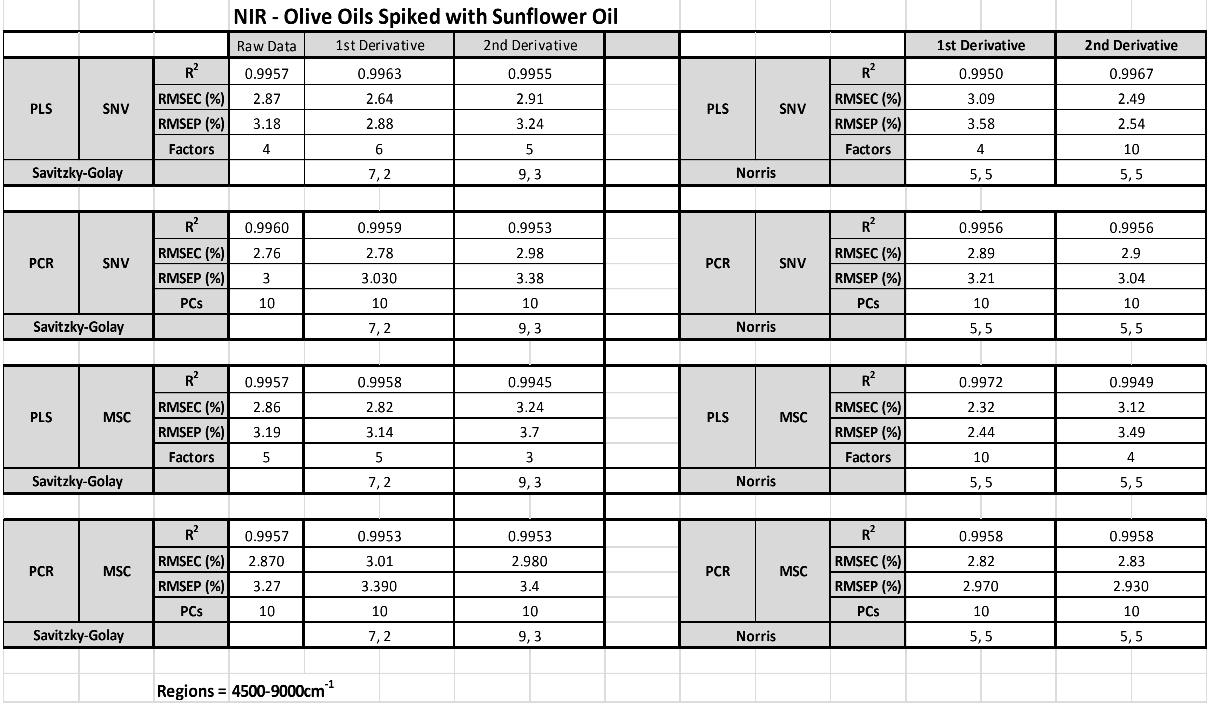
The olive oils were adulterated at 10% incremental levels from 0-100% with sunflower oil. These samples were run on the NIRS instrument using the transmittance mode. Firstly the chemometric software was used to construct a qualitative model using the PCA and PLS-DA algorithms to see if it was possible to quickly distinguish adulterated samples from pure samples. The data was pre-processed using SNV, 2nd Derivative algorithm with Savitzky-Golay smoothing (11 and 2). **Figure 17** shows the PCA and PLS-DA models indicating that there clusters for each adulterated level from 50-100% olive oil demonstrating that it may be possible to use this approach to quickly screen for adulteration.

Figure 17: PCA and PLS-DA Calibration Models for Olive Oil Adulterated with Sunflower oil



For quantitative analysis the various chemometric parameters tested are highlighted in **Table 14**. The models were assessed and the best models will have a high value for the correlation coefficient (R^2) and low values for the RMSEC and RMSEP using the quantitative chemometric algorithms, PLS and PCR. **Figure 18** shows a typical quantitative calibration model obtained with actual concentration of olive oil along the x-axis and the NIRS predicted concentration along the y-axis.

Table 14: Quantitative calibration models generated using NIR spectral data and chemometric software

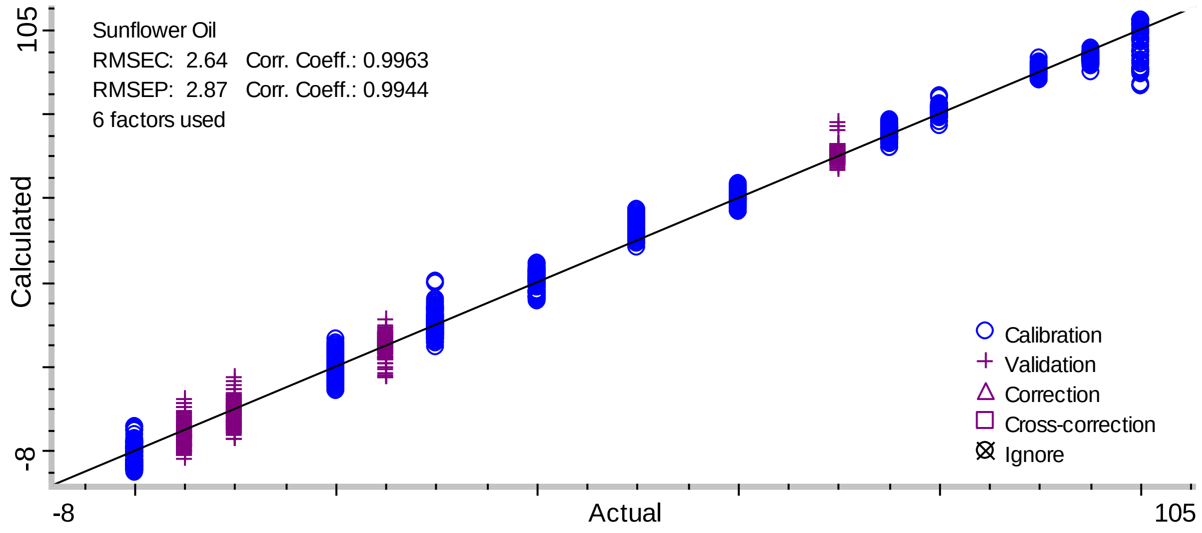


NIR - Olive Oils Spiked with Sunflower Oil

			Raw Data	1st Derivative	2nd Derivative					1st Derivative	2nd Derivative
PLS	SNV	R^2	0.9957	0.9963	0.9955		PLS	SNV	R^2	0.9950	0.9967
		RMSEC (%)	2.87	2.64	2.91				RMSEC (%)	3.09	2.49
		RMSEP (%)	3.18	2.88	3.24				RMSEP (%)	3.58	2.54
		Factors	4	6	5				Factors	4	10
Savitzky-Golay				7, 2	9, 3		Norris			5, 5	5, 5
PCR	SNV	R^2	0.9960	0.9959	0.9953		PCR	SNV	R^2	0.9956	0.9956
		RMSEC (%)	2.76	2.78	2.98				RMSEC (%)	2.89	2.9
		RMSEP (%)	3	3.030	3.38				RMSEP (%)	3.21	3.04
		PCs	10	10	10				PCs	10	10
Savitzky-Golay				7, 2	9, 3		Norris			5, 5	5, 5
PLS	MSC	R^2	0.9957	0.9958	0.9945		PLS	MSC	R^2	0.9972	0.9949
		RMSEC (%)	2.86	2.82	3.24				RMSEC (%)	2.32	3.12
		RMSEP (%)	3.19	3.14	3.7				RMSEP (%)	2.44	3.49
		Factors	5	5	3				Factors	10	4
Savitzky-Golay				7, 2	9, 3		Norris			5, 5	5, 5
PCR	MSC	R^2	0.9957	0.9953	0.9953		PCR	MSC	R^2	0.9958	0.9958
		RMSEC (%)	2.870	3.01	2.980				RMSEC (%)	2.82	2.83
		RMSEP (%)	3.27	3.390	3.4				RMSEP (%)	2.970	2.930
		PCs	10	10	10				PCs	10	10
Savitzky-Golay				7, 2	9, 3		Norris			5, 5	5, 5

Regions = 4500-9000cm^{-1}

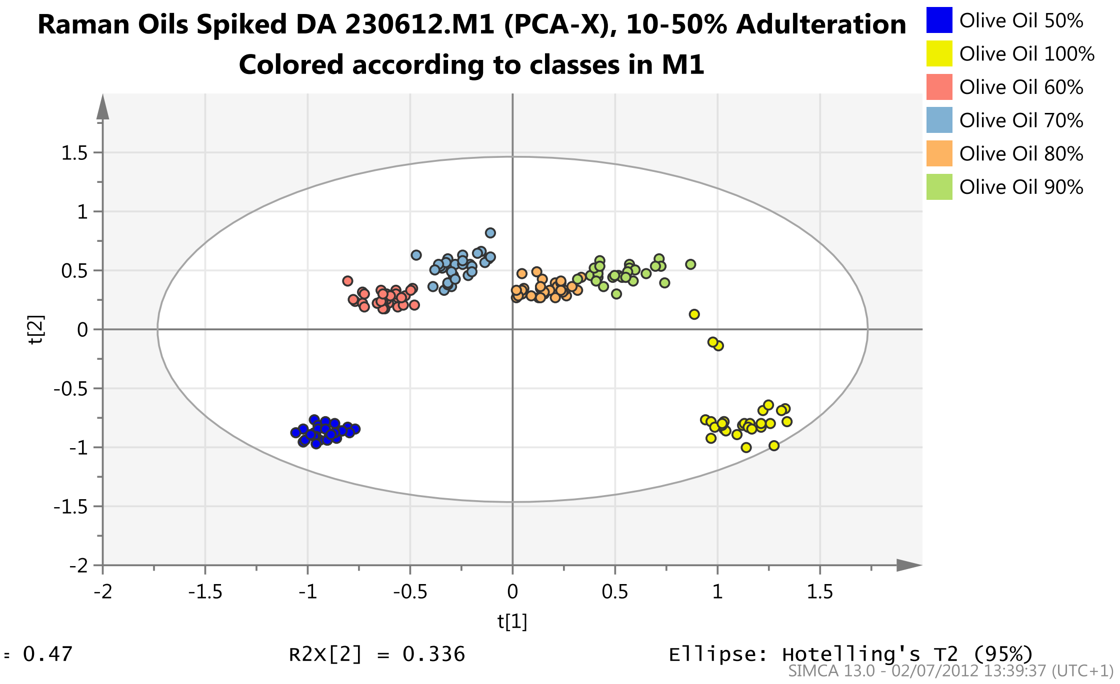
Figure 18: Typical Quantitative Calibration model constructed using NIR spectral data

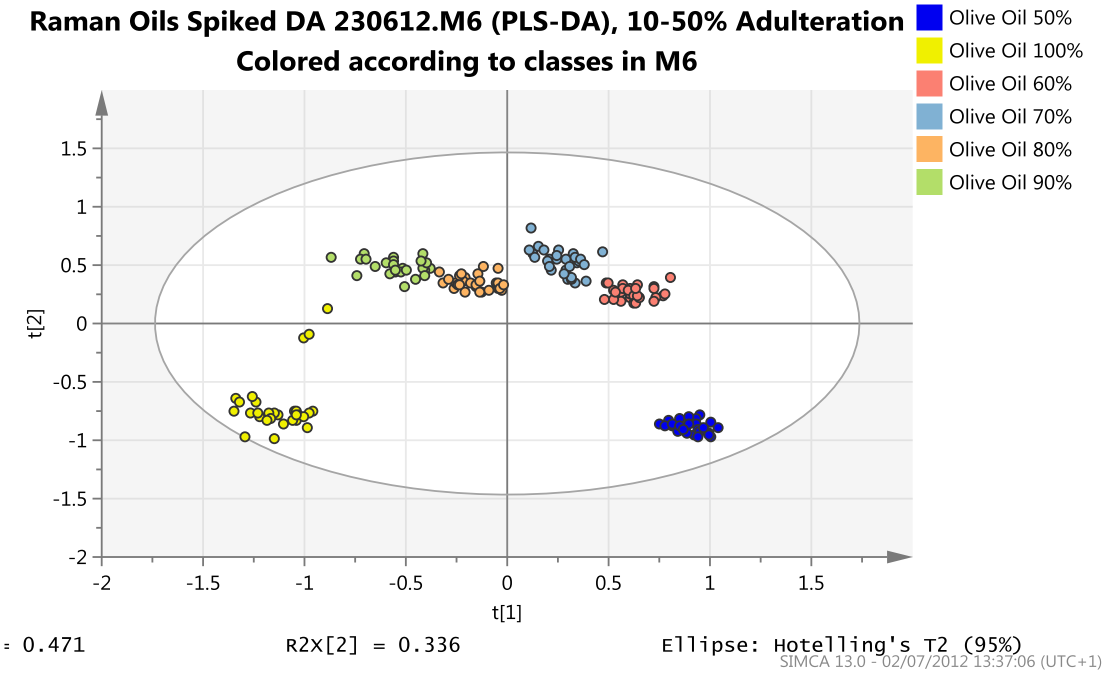


B2.2.2.2 Qualitative Analysis of Raman Spectral Data of Olive Oils adulterated with Sunflower Oil

The olive oils were adulterated at incremental levels from 0-100% with sunflower oil. These samples were run on the Raman instrument. The chemometric software was used to construct a qualitative model using the unsupervised PCA and the supervised PLS-DA algorithms to see if it was possible to quickly distinguish adulterated samples from pure samples. The data was pre-processed using SNV, 1st Derivative algorithm with Savitzky-Golay smoothing (9 and 2). **Figure 19** shows that there are clusters for each adulterated level from 50-100% olive oil indicating that it may be possible to use this approach to quickly screen for adulteration/gross contamination.

Figure 19: PCA and PLS-DA Calibration Models for Olive Oil Adulterated with Sunflower oil



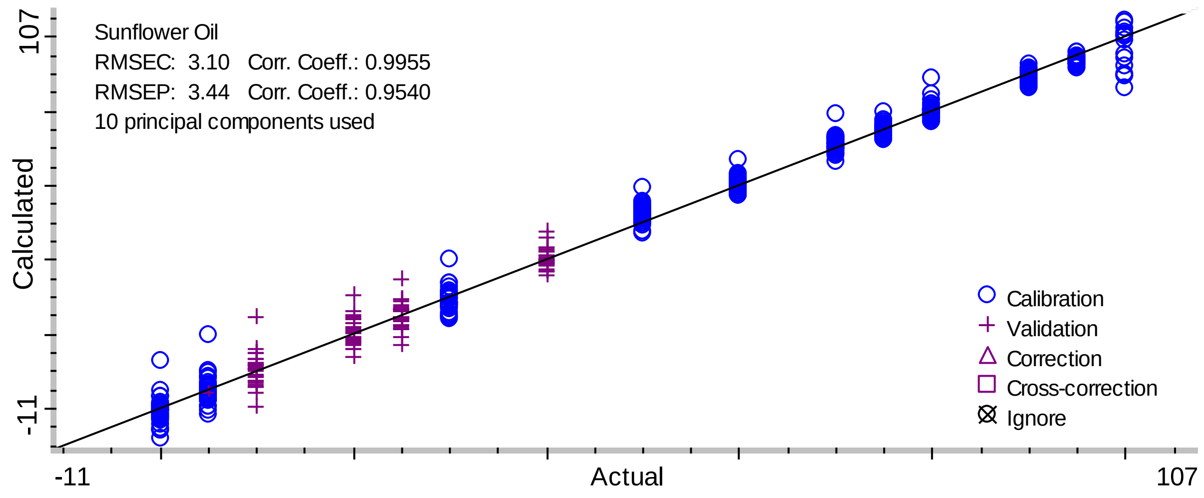


For quantitative analysis the various chemometric parameters tested are highlighted in **Table 15**. The models were assessed and the best models will have a high value for the correlation coefficient (R^2) and low values for the RMSEC and RMSEP using the quantitative chemometric algorithms, PLS and PCR. The error of prediction (RMSEP) indicates the typical sensitivity of the calibration model. **Figure 19** shows a typical quantitative calibration model obtained with actual concentration of olive oil along the x-axis and the Raman predicted concentration along the y-axis.

Table 15: Quantitative calibration models generated using Raman spectral data and chemometric software

			Raman - Olive Oil spiked with Sunflower Oil								
			Raw Data	1st Deriva	2nd Derivative					1st Derivative	2nd Derivative
PLS	SNV	R^2	0.99483	0.99593	0.99644		PLS	SNV	R^2	0.99602	0.99627
		RMSEC (%)	3.31	2.94	2.75				RMSEC (%)	2.91	2.81
		RMSEP (%)	3.63	3.6	3.61				RMSEP (%)	3.62	3.67
		Factors	2	4	10				Factors	6	9
Savitzky-Golay				7, 2	9, 3		Norris			5, 5	5, 5
PLS	MSC	R^2	0.99305	0.99553	0.99618	0.99621	PLS	MSC	R^2	0.99535	0.99618
		RMSEC (%)	3.84	3.08	2.85	2.84			RMSEC (%)	3.14	2.84
		RMSEP (%)	4.22	3.66	3.74	3.79			RMSEP (%)	3.58	3.69
		Factors	2	4	8	7			Factors	4	9
Savitzky-Golay				7, 2	9, 3	11, 4	Norris			5, 5	5, 5
PCR	SNV	R^2	0.9958	0.99539	0.99389		PCR	SNV	R^2	0.99545	0.99419
		RMSEC (%)	2.98	3.13	3.6				RMSEC (%)	3.11	3.51
		RMSEP (%)	3.63	3.51	4.38				RMSEP (%)	3.47	3.97
		PCs	10	10	10				PCs	10	10
Savitzky-Golay				7, 2	9, 3		Norris			5, 5	5, 5
PCR	MSC	R^2	0.99563	0.99519	0.99362		PCR	MSC	R^2	0.99546	0.99398
		RMSEC (%)	3.04	3.19	3.68				RMSEC (%)	3.1	3.57
		RMSEP (%)	3.82	3.56	4.5				RMSEP (%)	3.44	4.02
		PCs	10	10	10				PCs	10	10
Savitzky-Golay				7, 2	9, 3		Norris			5, 5	5, 5
	Regions =	1200-1290									
		1600-1700									

Figure 20: Typical Quantitative Calibration model constructed using Raman spectral data



B2.2.2.3 Conclusions to the oils study

It has been clearly demonstrated that both NIR and RAMAN were capable of detecting adulteration of olive oil with sunflower oil. Food fraud is a topic of growing concern in the UK Olive oil is recognised globally as one of the main types of foods subject to serious fraud. Over 13,000 bottles of substandard olive oil were seized in the EU by Europol in 2011. It is a high value product, noted for its health benefits and its consumption in the UK has risen substantially over the past decade. All of the olive oil we consume in the UK is imported and currently there is little or no checking to ensure any of this food has been adulterated with cheaper, lower quality oils. The methods develop should be taken forward and fully developed and validated to allow the rapid and inexpensive testing of olive oils to ensure what the UK public is purchasing is the bona fide product.

No such rapid, low cost method current exists in the world and the innovative approach demonstrated in this pilot study is a clear indication that such a procedure could be produced and used in routine monitoring programmes. The ease of use and relative low cost of the techniques make the NIR and RAMAN techniques a highly useful procedure for industry to adopt to undertake their own testing.

C1 General Conclusions and Contractor recommendations for further work

The development of NIR and Raman spectroscopy based methods to determine adulteration/gross contamination of spices and oils has highlighted the potential of these rapid, non-destructive techniques as the first line defence in food quality and safety. These tests were developed for adulteration/gross contamination scenarios where the health effects to the consumer would be higher and not to test for hazards at the legislative limits. The latter would take modification of the spectroscopies which would not be permitted in the time scale of this project. The prototype calibration models will need more data included to make these tests more robust and reduce the chances of over fitting of the models. It should be noted that there are no set validation protocols for spectroscopy techniques coupled to chemometrics.

The development of spectroscopic techniques over the last 20 years has seen this type of analysis becoming low cost, portable and easy to use. This coupled with the fact that the testing does not destroy the sample, i.e. non-destructive, should appeal to the authorities and the food industry in general as a means to prove conformity of their foodstuffs.

The contractors would like to recommend the following possibilities related to future work:

1. FoodAlert Register Database

- What has been presented in this report is a first attempt to provide an easy to use tool that would serve as an alert to guide surveillance and enforcement in the UK. A more comprehensive and robust system using a structured approach comprising an element that would build together knowledge about past alerts such as number of notifications for different food types, geographic origins of alerts etc coupled with another element that would seek to inform potential hazards by combining numerical factors (based on assessment of relevant knowledge of substances such as ‘genotoxic’ or regulatory criteria e.g. ‘not authorised’)’.

2. Fingerprinting Techniques

- To take the preliminary work performed on the oils and fully develop and validate a method capable of detecting adulteration of olive oil with cheaper vegetable oils types. As olive oil is believed to be ranked among the food types linked with adulteration there would appear to be an urgent need for such a method.

- NIR and Raman fingerprinting of other foodstuffs and hazards could lead to further development of calibration models based on these spectroscopy techniques. For example rice, wheat and other cereals, powdered foods such as eggs, milk, infant formula, sugars, fruit juices etc.

- The techniques selected in the present project were NIR and RAMAN. However there are a number of other techniques which are becoming lower cost, portable and can be potentially used in the field e.g. ports of entry. The choice of technique may depend on the commodity and the associated hazard but could include mid infrared (MIR), fluorimetry, x-ray fluorescence (XRF), biosensors and lateral flow devices (LFD).

- Development of validation protocols for spectroscopic techniques coupled with chemometrics would help bring consistency to this area of calibration model building.

Acknowledgements

The authors would like to thank the staff, students and friends of IGFS for participating in the collection of spice and oil samples from the UK, Ireland and around the world. The authors would also like to thank the FSA for funding this important project.

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