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Review of methods for the analysis of culinary herbs and spices for authenticity

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Final Report

FSA FS900293

DOI: 10.46756/sci.fsa.fod541

February 2024

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Report no. LGC/R/2023/858

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1. Executive Summary

Herbs and spices are a commodity group that consistently appear in the [top ten commodities](#) most reported as being adulterated. Due to the large variety of products that fall within the category of herbs and spices, complex global supply chains and commercial production processes, methods for verifying their authenticity / detecting fraud are not straightforward. The focus of this report is on methods for the analysis of culinary herbs and spices, in their dehydrated or dried form, for authenticity with a focus on detecting deliberate adulteration rather than adventitious contamination.

Stakeholder engagement revealed that with respect to authenticity, herbs and spices of most concern are oregano followed by black pepper. Chilli powder, saffron, paprika and turmeric, were next, with all of them rated of equal concern by stakeholders. The main authenticity issues encountered are substitution, adulteration, and concealment. Unified strategies for the sampling of herbs and spices were not identified, with the type of sampling employed i.e., targeted, general screening, intelligence led, spot check, etc. dependent on the stakeholder. There was unanimous support for provision of guidance in relation to the authenticity testing of herbs and spices.

There is a lack of commercially representative reference materials for five of the six herbs and spices of most concern to stakeholders i.e. black pepper, chilli powder, saffron, paprika and turmeric. Four Proficiency Testing (PT) providers for authenticity testing of herbs and spices were identified, who currently offer 17 different relevant PT rounds, which cover authenticity, quality and safety. There are currently no PT rounds available for saffron or black pepper. Most Public Analyst (PA) Official Laboratories (OLs) stated they had difficulty in accessing appropriate reference materials and PT rounds for herbs and spices for authenticity.

There are several excellent examples of open access food authenticity databases, which are supported / originated from large publicly funding programmes. Private contractors also have set-up commercial food authenticity databases and due to the expense involved in their development, they are protected by IP. In addition, the larger herb and spice producing companies, have their own within-company reference libraries, which have been modelled to reflect 'what good looks like' for their organisations. Whilst there is evidence of information sharing within confidential supply chains, sharing of this type of

data does not occur between companies, mainly because product specifications are bespoke and confidential. PA OLs recognise the demands of setting up reference databases and would therefore be willing to work with other PA OLs to reduce setup costs or would welcome access to 3rd party data.

Authenticity testing of herbs and spices is a complex matter, and the analytical approaches used for the authenticity testing of herbs and spices are very much dependent on the stakeholder category and the purpose of testing. The priorities for authenticity testing vary between different stakeholders, as do the herbs and spices of primary concern, the sampling strategies adopted, and the analytical methods employed. Spectroscopy-based, mass spectrometry-based and DNA-based techniques feature most in the scientific literature. Within these broad technique categories, NIR and PCR are the techniques that are most commonly used followed by FT-IR, GC-MS, LC-MS, microscopy, NGS, Ash, Acid-ash, ICP-MS and HPLC, which all featured equally in publications.

Although, there is a wide portfolio of methods used for verifying the authenticity of herbs and spices, the evidence shows a preference for the use of initial rapid screening approaches, within a tiered system of testing, where a non-compliant result can be followed up with appropriate confirmatory analysis of the same sample using an alternative technique. NIR and FT-IR appear to be gaining favour for use as initial screening tools. Both NIR and FT-IR are rapid to apply, non-destructive, non-targeted screening methods, with the potential to generate results in a comparatively short time span (~ 20 minutes) which is preferable for screening-based approaches.

Currently there is a lack of laboratories, including UK OLs, that can provide accredited authenticity tests for NIR and FT-IR in herbs and spices. Within the UK, Queens University Belfast (QUB) Institute for Global Food Security (IGFS) and Bia Analytical (BA), a commercial subsidiary of IGFS, are recognised as leading experts in the field of NIR and FT-IR for food authenticity testing. BA currently provide FT-IR authenticity testing services for nineteen herbs and spices, two of which have been fully ISO 17025 UKAS accredited (oregano and sage) at QUB, with the remaining seventeen pending

final authorisation¹. Once accreditation has been granted, BA will be the only UK laboratory with extensive UKAS accredited FT-IR based methods for verifying the authenticity of herbs and spices.

There is agreement among stakeholders that the threshold for economically motivated adulteration is typically at 5% w/w or greater so there is no requirement to detect very low concentrations of the adulterant. Stakeholders recognised that validated qualitative results could be used to take prosecution action but most agreed that ideally, a quantitative result would be preferable.

Currently the FT-IR method has been validated for the detection of economically motivated adulteration (EMA), but there is potential for it to be validated so that it can be used as both a quality and authenticity screening tool. However, this would require further work to validate the method at the 1-2% level used by the food industry.

Stakeholders expressed an interest in the adoption of portable platforms (point of contact devices (POC)), which are becoming more readily available with enhanced performance characteristics. This increased interest is fuelling the development of non-targeted multianalyte platforms for food authenticity testing (e.g. multispectral, FT-IR and NGS).

All methods and technologies used for the authenticity testing of herbs and spices suffer from the same issues of the lack of reliable reference samples, difficulty of accessing proprietary validated datasets, on which to validate the methods to demonstrate their fitness for purpose, and relevant chemometric models for use in interpreting the outputs. This is especially concerning as 73% of papers in the academic literature review exploited chemometric and machine learning algorithms as methods of data analysis, for example POC methods of analysis (Defra project [FA0178](#)). Although, evidence from another country, demonstrates that the judiciary is willing to accept this type of data, which is encouraging.

¹BA are also expected to receive UKAS accreditation status for FT-IR analysis of herbs and spices following UKAS audit

As there is no “one size fits all” analytical approach for herbs and spices, it is likely that a number of different techniques / methods may need to be used to verify their authenticity and a weight of evidence (WoE) approach used to determine whether samples have been adulterated. Given the trend in methods towards greater use of chemometrics and artificial intelligence (AI) as methods of data analysis, it is likely that data fusion methods may need to be employed to enable a judgement to be made on the authenticity of a particular herb or spice that has been analysed by a number of different methods.

There is no statutory requirement to analyse herbs and spices for authenticity at ports, so samples are not currently being done at UK ports. PA OLs stated that herb and spice authenticity is not a priority for local authorities so consequently they receive very few samples for analysis. This has a knock-on effect on PA OLs as it is difficult to maintain UKAS accreditation for a wide analytical scope if very few or no samples are analysed annually. In the 2022 survey of PA OLs in England conducted by the Government Chemist, methods for herb and spice authenticity were identified as a gap.

This project has found that PA OLs currently have capability for undertaking the authenticity testing of herbs and spices using optical microscopy, but this is a resource intensive approach (typically 2 hours/sample, but can be longer depending on the nature of the sample). The majority of PA OLs have, or have access to, equipment suitable for instrumental analysis of herbs and spices for authenticity. PA OLs currently employ multiple established traditional analytical techniques to gain an “overall impression” of the composition of a sample (WoE approach), rather than relying on the results generated by a single approach. Microscopy is often the first tool used to identify gross adulteration / contamination issues. PA OLs would welcome methods / technology that could increase the robustness of testing or reduce resource requirements provided it is economically viable to do so. All PA OLs would also welcome further training in the authenticity testing of herbs and spices.

Based on current available methodology and techniques, skills in PA OLs and ease of transfer, FT-IR and qPCR based methods represent those best suited to being transferred to PA OLs. Of the two approaches, FT-IR employs short sample preparation times, is a multitargeted approach, and returns a result in a short period of time (~ 20 minutes). Quantitative real-time PCR (qPCR) requires longer sample preparation times, is a targeted approach, and takes longer to generate a result than with FT-IR (~ 2 hours).

PA OLs expressed a preference for rapid screening approaches, therefore FT-IR methods would be the preferred technique to adopt. It is a rapid, non-destructive, non-targeted, multianalyte approach compared to qPCR, which is slower, destructive to the sample, and focused on the quantification of a single or small number of analyte targets. Another important factor is that QUB / BA have achieved UKAS accreditation for FT-IR based methods for two herbs and spices, with accreditation pending for the remaining suite of herbs and spices.

Outside the PA OLs, there is sufficient on-land (UK) capability and capacity with seven private organisations offering commercial herb and spice authenticity testing. In many cases, these organisations operate independently curated reference databases, across different techniques, which allows cross checking / cross validation in cases of non-compliance. This is already happening on a commercial basis and could also be utilised by PA OLs to gather additional evidence, using an alternative technique, if required. In addition, three international organisations, specialising in herb and spice authenticity testing, also offer commercial services.

1.1 Recommendations for further work

- Based on the wealth of evidence gathered for this project, the following recommendations are proposed.

1.1.1 Reference materials and Proficiency testing

- Ensure the availability of commercially representative reference samples for the herbs and spices identified as of highest concern with respect to authenticity; this would allow greater comparability between methods, techniques, and laboratories, enabling greater confidence in the results produced.
- Review future provision of authenticity PTs to ensure inclusion of those herbs & spices that have been highlighted of significant concern, including saffron and black pepper, for which there are currently no rounds. If this is not possible, FSA should consider funding closed PT rounds for PA OLs to enable them to demonstrate ongoing competence. This will assist PA OLs in maintaining UKAS accreditation in core areas supporting the resilience of the UK official food and feed control system.

1.1.2 Support PA OLs by:

- Considering how PA OLs can access the specialist sample preparation equipment, namely ball mills, required to sufficiently homogenise samples prior to undertaking authenticity testing of herbs and spices by FT-IR.
- Providing training on food authenticity testing methods (targeted and non-targeted) with a focus on those that use chemometric and/or machine learning algorithms for data analysis.
- Providing PA OLs with an inventory of laboratories offering food authenticity testing for herbs and spices by technique and herb / spice.
- Assessing the costs of implementation of new methods so that PA OLs can be supported to ensure maximum uptake. An important part of this is to ensure sustained demand for herb and spice authenticity testing in PA OLs.
- Organising a visit to a herb and spice producer to help PA OLs gain greater appreciation of the complex supply chains and the commercial production processes involved for herbs and spices.
- Providing updated training on the use of external DNA databases such as the BOLD and NCBI (GenBank) databases. It is proposed that both the factory visit, and this training could be put forward as ideas for delivery via the Joint Knowledge Transfer Framework for Food Standards and Safety (if a 2023 – 2026 programme is approved).

1.1.3 Roll-out of FT-IR method to PA OLs

PA OLs have expressed a preference for rapid screening approaches. Spectroscopy-based approaches address this requirement and the FT-IR-based methods developed at QUB / BA have been validated for nineteen herbs and spices, with UKAS accreditation already granted to QUB for two (oregano and sage) and decisions are pending on the remaining seventeen at the time of writing this report.

Roll-out the QUB / BA FT-IR method², coupled with chemometrics, for the economically motivated adulteration of oregano and black pepper; the first step would be to establish how many PA OLs have FT-IR instruments that satisfy the minimum requirements (as specified in the QUB / BA method). For PA OLs who do not have FT-IR instruments that satisfy the minimum requirements and want to implement the method in their laboratories, FSA should investigate options for them to acquire / have access to appropriate FT-IR instruments. Resource sharing is already recognised and used by two of the PA OLs interviewed in Phase 2 of the project.

The roll-out would include training on the FT-IR method including information on how the data library was constructed, and how it is used to interpret the data to provide a test result.

1.1.4 Guidance

Consider development of guidance / regulator-industry code of practice for authenticity testing of herbs and spices as it would provide greater clarity on the required standards for industry, commercial and control laboratories, and local and port health authorities.

1.1.5 Future research

- Data fusion methods should be understood as they may need to be employed to enable a judgement to be made on the authenticity of a particular herb or spice that has been analysed by a number of different methods.
- Challenge the QUB / BA method with mixtures lower than 10% w/w of adulterants to assess its potential to be used as a screening tool for quality and as well as for detection of EMA.
- Further work is required to assess the suitability of using NGS for the purposes of species identification / verifying the authenticity of herbs and spices.

² UKAS accreditation awarded to QUB for sage and oregano, award pending for remaining 17 herbs and spices. UKAS accreditation to BA for all 19 herbs and spices pending at the time of compiling the report.

- Further work should be undertaken to identify relevant PCR based methods for determination of herbs and spices. Depending on the validation status associated with the relevant methods, method verification should be undertaken, in line with [best measurement](#) practice guidance and a laboratory's internal Quality Management System, to ensure that the methods are fit for purpose. This could be conducted by each PA OL, or by a central laboratory prior to transfer to a PA OL in order to minimise the resource / cost impact on the PA OLs as was requested by them.
- Provision of support and guidance to SMEs should be considered as it will enhance the resilience and integrity of the herb and spice supply chain.

2. Introduction

Spices, and herbs are products that are generally added to both fresh and processed food to enhance flavour. The trade in spices can be traced back to as early as 3000 BC and can be considered as one of the earliest drivers of globalisation. More recently, the global spices and herbs market for 2022 was estimated at USD 79.1 billion and is expected to grow to USD 126 billion by the end of [2023](#).

Herbs and spices are a commodity group that consistently appear in the top ten commodities most commonly reported as being [adulterated](#). Reasons for this include that supply chains for herbs and spices can be long, global and complex, and can pass through many countries. The stages of the supply chain can include grower, collector, primary processor, local traders, secondary processor, exporter, importer, trader, processor / packager, food manufacturer / retailer / wholesaler, and finally the consumer; at any stage of this supply chain, a number of fraud opportunities can occur including misrepresentation, adulteration and substitution. The main motivation for the addition to, or [substitution](#) of herbs and spices is economic gain.

In addition, herbs and spices are often blended and processed to produce retail products, which can influence the analytical test methods used to verify authenticity. Unlike analytical test methods for food safety components, where testing is performed to check compliance against a legislative limit or some other recommendation such as maximum residue limits, food authenticity methods compare unknown samples against a reference set of 'authentic' samples. The challenge of obtaining traceable, internationally accepted reference samples for comparison remains one of the biggest challenges for food authenticity testing methods and is a barrier to the universal application / standardisation of methods.

Due to the above-mentioned complexities, methods for verifying the authenticity of / detecting fraud in herbs and spices are not straight forward.

3. Phase 1

3.1 Objective

The objective of the first Phase of the project was to undertake a review of current and emerging methods for the analysis of herbs and spices, comparing and contrasting methods by:

1. Establishing needs via five stakeholder category based virtual focus groups.
2. Conducting a thorough review of the literature (both academic and grey) to identify current and emerging targeted and non-targeted analytical methods for the detection, identification and quantification of herbs and spices and their potential adulterants.
3. Collecting broader stakeholder views on methods (available, emerging and currently being used) for identification and quantification of herbs and spices via an e-survey on the Food Authenticity Network.
4. Using the output of the literature review and the stakeholder engagement focus groups to produce a summary table that compares the relevant key characteristics of the identified methods that are representative of the technologies most commonly used.
5. Assessing the practicability of testing herbs and spices in laboratories where the method of analysis requires the use of a reference database and how different targeted and non-targeted methods might be used as part of a screening process to verify evidence of adulteration.
6. Conducting a search to establish the availability of reference materials (RMs) and proficiency testing schemes (PTs) for herb and spice authenticity.

3.2 Approach

The following activities were undertaken.

3.2.1 Stakeholder engagement

Five stakeholder category-based focus groups were held in January 2023 with a total of 28 participants. The groups and participating organisations are presented in Table 1.

Table 1: Stakeholder focus groups and participating organisations

Group	Category	Participating organisations
1	The Food Industry	• McCormick and Company • The Bart Ingredients Company Ltd • The British Retail Consortium (BRC)
2	UK Government, Local Authorities, Port Health Authority & Non-Departmental Public Body	• Bristol Trading Standards (former regulatory authority for Barts) • Buckinghamshire and Surrey Trading standards (the primary authority for McCormick) • Suffolk Coastal Port Health Authority • Association of Public Analysts • Defra • FSA (England, Wales and Northern Ireland) • FSS • The National Food Crime Unit • Royal Botanic Gardens (RBG) Kew
3	Instrument manufacturers	• Thermo-Fisher Scientific • Waters • Videometer Multispectral Imaging • Oxford Nanopore Technologies
4	Testing laboratories	• Eurofins • Intertek • SGS • Food Forensics • Bia Analytical • Campden BRI
5	Official Control Laboratories	• Aberdeen Scientific Services • Edinburgh Scientific Services • Glasgow Scientific Services • Lancashire Scientific Services • Kent Scientific Services¹ • Public Analyst Scientific Services (Eurofins)

¹In partnership with Hampshire Scientific Services

The questions used as a basis for discussion in focus groups are provided in Annex 1.

3.2.2 e-Survey

An e-survey was sent to the following membership categories of the Food Authenticity Network:

- ‘Food Authenticity Analysts’ (954 members*), including the Food Authenticity [Centres of Expertise](#) excluding LGC
 - ‘Regulator’ (502 members*)
 - ‘Food Industry’ (1,013 members*).
- * membership as of 31 January 2023.

Questions were tailored to each member category and sent to members via the Food Authenticity Network. The questions used for the e-surveys are provided in Annex 2.

3.2.3 Literature review

A review of academic and grey literature was conducted by Queens University Belfast (QUB). The approaches used are presented in Annex 5 (academic review) and Annex 6 (grey literature review).

3.2.4 Reference materials and Proficiency tests

A review was conducted to identify available Reference Materials (RMs) and PTs for the authenticity of herbs and spices. In addition to reviewing websites and current catalogues for relevant products, searches were carried out via the [COMAR](#) database for reference materials, and the [EPTIS](#) database for proficiency testing schemes.

3.3 Results

The results of the stakeholder engagement exercises conducted are presented below.

3.3.1 Stakeholder category-based focus groups

The responses from all five focus groups were collated and a subjective appraisal was undertaken to produce a summary response against the main questions. Detailed summarised responses are presented in Annex 3.

The herbs and spices that were mentioned most frequently in response to the question ‘What are the main herbs and spices of concern to you when it comes to authenticity?’ were oregano and saffron, closely followed by black pepper, turmeric and chilli pepper /

chilli powder / cayenne powder / capsicums. Other herbs and spices that were mentioned, in descending order, were cinnamon, sage, white pepper, cumin, nutmeg, coriander, garlic, ginger and paprika. i.e.

Oregano and saffron > black pepper, turmeric and chilli pepper / chilli powder / cayenne powder / capsicums >>>> cinnamon, sage, white pepper, cumin, nutmeg, coriander, garlic, ginger and paprika.

Substitution was cited as the main authenticity issue encountered, followed by adulteration, concealment and dilution.

A range of techniques were reported as being used or recommended to verify authenticity. The techniques, in order of frequency of use were:

1. FT-IR / IR / NIR
2. GC-MS / LC-MS / LC-HRMS
3. Microscopy
4. NGS / PCR
5. Ash and acid insoluble ash
6. ICP-MS
7. Multispectral
8. NMR
9. UV Spectroscopy
10. Volatile oils.

Apart from Government bodies and Port Health who said that validated qualitative results could be used to take prosecution action, the other stakeholder groups responded that they either supplied or required both quantitative and qualitative results. Stakeholders agreed that ideally, a quantitative result would be preferable.

The Public Analysts generally advocated a two-tier approach i.e. using classical techniques such as light microscopy and FT-IR as screening techniques to obtain an initial indication of the authenticity of samples, followed, if necessary, by more focussed techniques such as GC or LC-MS in order to validate any noncompliant findings.

However, Public Analysts indicated that optimally they require a rapid screening method to serve as a triage approach for the initial analysis of herbs and spices. GC and LC-MS were highlighted to have long sample preparation and analysis times, were more expensive to conduct and needed a greater skill level of analysts undertaking such analysis in comparison to FT-IR-based methods.

Databases such as National Center for Bioinformatics (NCBI) and Barcode of Life Data System (BOLD), together with in-house databases, are used for some analyses. One participant commented that public databases could be corrupted by uploading profiles of fraudulent samples as 'authentic', which highlights the importance of verified reference samples and databases.

Issues or gaps that stakeholders identified included the need for a WoE approach, for example when assessing the data from multiple techniques or results from multiple labs, and development of databases.

The need for further guidance in the area of herbs and spices authenticity was universally supported. The need for the development of a regulator-industry code of practice in particular was highlighted, as was the provision of additional guidance on importing for suppliers. In addition to the development of new and additional guidance, food industry stakeholders drew attention to the fact that a number of the existing standards are no longer fit for purpose and need revising, e.g., the ISO standard for water activity method for herbs and spices. The relevant ISO standard ([ISO18787](#)) has been in review for some time, but the herb and spice industry no longer work to water activity, but to moisture in terms of specifications.

3.3.2 e-Survey

Responses to the survey were submitted by 64 participants; 15 'Regulators', 24 'Analysts' and 25 'Industry'. The respondents comprised of the following job roles:

- Food Safety Manager (15)
- Food Authority, Trading Standards (8)
- Analyst – Contract (7)
- Analyst – Enforcement (4)

- Management (4)
- Enforcement Officer, e.g., Port Health (3)
- Food Safety Manager (3)
- Quality Manager (3)
- Supply Chain Assurance (2)
- Laboratory Manager (1)
- Other (14).

58 % of the respondents' employers had headquarters based in the UK and 42% were outside the UK.

Summaries of the responses received by Food Authenticity Network member category are presented in Annex 4.

In descending order, the herbs and spices of most concern were:

- Paprika
- Oregano
- Chilli Powder
- Saffron
- Black Pepper
- Garlic
- Cinnamon and Cassia
- Cumin
- Turmeric
- Cayenne Pepper
- White Pepper
- Coriander
- Sage.

Other items of concern were onion powder, all spice/pimento, ginger, mixed herbs, curry powder and medicinal herbal pills/tablets.

The top five herbs and spices of concern differed slightly by member category, as shown in Table 2.

Table 2: Herbs and spices of concern by member category

Order of concern	Regulators	Analysts	Industry
1	Saffron	Oregano	Paprika
2	Paprika	Saffron	Garlic
3	Turmeric	Black Pepper	Chilli Powder
4	Chilli Powder	Paprika	Black Pepper
5	Cinnamon and Cassia	Chilli Powder	Oregano

The main authenticity issue in relation to herbs and spices was substitution, followed by mislabelling. Concealment was also considered to be an issue but more so by the food industry members than regulators and analysts.

The techniques most reported as being used by analysts were nucleic acid testing, including PCR and sequencing (NGS) and microscopy, whereas the techniques being most used by industry were chromatographic, e.g., HPLC and chemical, e.g., colorimetric.

92 % of respondents answered yes to the question 'Would the availability of additional guidance and best practice for the analysis of herbs and spices be of value to your organisation?'

Suggested forms for guidance were ranked in the following descending order:

- Guidance (35)
- Specific methods and their application (28)
- Reference databases (23)
- Legislation (22)
- Reference materials (22)
- Specialised equipment (5).

3.3.3 Literature review

The results of the literature review are presented in Annex 5 (academic review) and Annex 6 (grey literature review).

3.4 Reference materials and Proficiency tests

3.4.1 Reference materials

No relevant certificated reference materials (CRMs) were identified.

A summary of currently available quality control (QC) materials relevant to authenticity of herbs and spices testing is presented in Table 3.

Table 3: Quality control materials for authenticity testing of herbs or spices

Supplier	Code	Matrix	Analyte
FAPAS	T29104ABCbundleQC	Herb (Salvia spp.) (Sage)	Deliberate adulterant: Olea (Olive), Myrtus (Myrtle). Inadvertent adulterant: Origanum (Oregano).
FAPAS	T2980ABCbundleQC	Herb (Origanum spp.)	Deliberate adulterant: Olea (Olive), Cistus (Rockrose). Inadvertent adulterant: Satureja (Savoury)
FAPAS	T2985ABCbundleQC	Herb (Origanum spp.)	Deliberate adulterant: Myrtus (Myrtle), Satureja (Savoury), Rhus (Sumac). Inadvertent adulterant: Olea (Olive)
FAPAS	T2990ABCbundleQC	Herb (Origanum spp.)	Deliberate adulterant: Cistus (Rockrose). Inadvertent adulterant: Olea (Olive)
FAPAS	T2996ABCbundleQC	Herb (Origanum spp.)	Deliberate adulterant: Myrtus (Myrtle), Olea (Olive), Corylus (Hazel), Satureja (Savoury).
FAPAS	T27297AQC	Garlic Powder	Peanut / Peanut protein
FAPAS	T27317BQC	Spice – Cumin	Gluten (Qualitative), Sesame (Qualitative)
FAPAS	T27325AQC	Garlic Powder	Peanut/Peanut Protein (Qualitative), R-Biopharm RIDASCREEN Peanut (R6811)
FAPAS	T29102ABCDbundleQC	Garlic Powder	Deliberate adulterants: Rice powder, clay

In addition to the above quality control materials, the [American Herbal Pharmacopoeia](#) supply botanical reference materials for use in the determination of identity. Their

catalogue contains various botanical reference materials, including cinnamon, black pepper, and sage, for use in routine quality control testing.

[ChromaDex](#) and [LGC Standards](#) also supply a number of botanical reference materials including pepper and turmeric.

The Royal Botanic Gardens, Kew, maintain and curate collections of plant specimens that are available for research purposes on request. The three relevant collections are:

- i. [The Spirit Collection](#)
- ii. [The DNA and tissue bank](#) and
- iii. [The Millennium Seedbank](#).

3.4.2 Proficiency tests

The following suppliers produce PTs involving the analysis of herbs or spices:

- [FAPAS, Fera Science Ltd, UK](#)
- [LGC AXIO Proficiency Testing, LGC Ltd, UK](#)
- [BIPEA, France](#)
- [DLA Proficiency Tests GmbH, Germany](#)

A summary of PTs relevant to the authenticity of herbs and spices testing is presented in Table 4.

Table 4: Proficiency tests for authenticity testing of herbs or spices

	Code	Matrix	Analyte
FAPAS	29114	Herb (Petroselinum spp.) (Parsley)	Adulteration (Three Petroselinum spp. test materials that may or may not be adulterated with other herb or non-herb leaves. Suitable for both microscopy and FT-IR methods.)
FAPAS	2996	Herb (Origanum spp.)	Adulteration (test materials that may or may not be adulterated with other plant powders, spent material, non-spice fillers and dyes).
FAPAS	29104	Herb (Salvia spp.) (Sage)	Adulteration (test materials that may or may not be adulterated with other plant powders, spent material, non-spice fillers and dyes).
FAPAS	27351	Spice - Cumin	Gluten, sesame
FAPAS	27358	Garlic Powder	Peanut / Peanut protein
FAPAS	27387	Spice - Cumin	Gluten, sesame
FAPAS	29111	Paprika	Adulteration (Three spice test materials that may or may not be adulterated with other plant powders, spent material, non-spice fillers and dyes.)
FAPAS	29115	Vanilla Extract	4-Hydroxybenzoic Acid, Coumarin, Ethyl Vanillin, Vanillin
LGC AXIO proficiency testing	PT-FC- 886	Spices (e.g. chilli or turmeric)	Illegal dyes (Contains a minimum of 5 dyes from a list of 21.)
LGC AXIO proficiency testing	PT-FC- 825 FC326	Oregano	Confirmation of authenticity (Would you consider this sample to satisfy your specification for authenticity? If you consider this sample as not authentic, what adulterant have you detected? What is the specification assessment based on?)

	Code	Matrix	Analyte
LGC AXIO proficiency testing	PT-FC-825 FC332	Cumin	Confirmation of authenticity (Would you consider this sample to satisfy your specification for authenticity? If you consider this sample as not authentic, what adulterant have you detected? What is the specification assessment based on?)
LGC AXIO proficiency testing	PT-FC-850	Vanilla extract	Vanillin, Ethyl alcohol, Density, Refractive index at 20°C
LGC AXIO proficiency testing	PT-FC-870	Spices	Bulk index, Bulk Density, Colour strength, Mercury
LGC AXIO proficiency testing	PT-FC-879	Herbs	Moisture, Acid Insoluble Ash, Ash, Volatile Oil, Water activity
BIPEA	395	Turmeric	Sudan Orange G, Sudan I, Sudan IV, Rhodamine B
BIPEA	195	Paprika	Sudan Orange G, Sudan I, Sudan IV, Rhodamine B
DLA Proficiency Tests	ptAL09	Barbecue Spice Mix (with onion, garlic and paprika powder)	Peanut, Almond and Molluscs

PTs and RMs from the below listed suppliers were also reviewed but no products relating to herb or spice authenticity were identified:

- [NIST, USA](#),
- [JRC, Belgium](#),
- [BAM, Germany](#),
- [TestQual, Spain](#).

3.5 Discussion

A review of information collected from the stakeholder focus groups, and responses from the Food Authenticity Network eSurvey and the literature review demonstrates that the authenticity testing of herbs and spices is a complex matter.

The priorities for authenticity testing vary between different stakeholders (e.g., food industry, regulatory, testing laboratories and instrument manufactures), as do the herbs and spices of primary concern, the sampling strategies adopted, and the analytical methods employed.

3.5.1 Herbs and spices of most concern

Data from the stakeholder focus groups and responses to the Food Authenticity Network eSurvey were compared and this information is presented in Table 5.

Table 5: Top five herbs & spices of concern by activity and stakeholder category

Priority	Stakeholder focus group Industry	Stakeholder focus group Testing Labs	Stakeholder focus group PA Labs	Stakeholder focus group Instrument manufacturers	Stakeholder focus group Gov ¹	FAN e-survey Regulators ²	FAN e-survey Analysts	FAN e-survey Industry
1	Black Pepper	Black Pepper	Oregano	Oregano	Chilli Powder	Saffron	Oregano	Paprika
2	White Pepper	Oregano	Sage	Sage	Cumin	Paprika	Saffron	Garlic

Priority	Stakeholder focus group Industry	Stakeholder focus group Testing Labs	Stakeholder focus group PA Labs	Stakeholder focus group Instrument manufacturers	Stakeholder focus group Gov ¹	FAN e-survey Regulators ²	FAN e-survey Analysts	FAN e-survey Industry
3	Turmeric	Turmeric	Cinnamon	Black Pepper	Coriander	Turmeric	Black Pepper	Chilli Powder
4	Oregano	Paprika	Nutmeg	White Pepper	Turmeric	Chilli Powder	Paprika	Black Pepper
5	Sage	Garlic	Saffron	Chilli Pepper	Saffron	Cinnamon and Cassia	Chilli Powder	Oregano

¹ Gov relates to the stakeholder focus group that was comprised of Trading Standards, Port Health and Government

² Regulators relates to a generic member category of the Food Authenticity Network

The results show that whilst there was general consensus for the top five herbs and spices of concern named by stakeholders, there are differences in the relative priorities by activity and for different stakeholder types. This is not altogether surprising given the differing perspectives involved, for example:

- Some commercial laboratories actively undertake horizon scanning to identify potential emerging risks, using intelligence led as well as general sample screening to better understand the basis of current and future threats to the marketplace.
- UK port authorities priority lists reflect their primary role in UK statutory screening programs for monitoring aflatoxins and pesticide residues. There is no legal requirement to undertake testing for authenticity / adulteration for herbs and spices.
- PA OLs indicated that they had inputted into recent proposed national sampling initiatives on the basis of their local market intelligence. However, sampling priorities remain the responsibility of local authorities with some direction from central government. Local authorities do not consider herb & spice authenticity to be high priority and consequently not many samples are taken.
- The priority list for instrument manufactures closely matches that of the commercial testing laboratories. Priorities tended to be governed by customers rather than regulatory bodies, although workflow compliance with recognised and established codes of practice and Global Food Safety Initiative (GFSI) certified manufacturing [schemes](#) was also acknowledged.

- The priority lists specified by relevant UK regulatory bodies (FSA, FSS, Defra, NFCU, SFCIU) diverged the most from other stakeholders, with several inclusions justified on the basis of historical known fraud incidents. Whilst the availability of limited funding may necessitate this approach, it is unlikely to identify emerging issues. Current statutory testing is undertaken for aflatoxins and pesticide residues, and adulteration is usually only indicated where an aberrant result is returned.

The data was combined by giving all stakeholders and activities equal weighting to identify the top five herbs & spices of concern when it comes to authenticity, which is presented in Figure 1.



Figure 1: Word cloud graphical representation of the top five herbs & spices of concern

Figure 1 demonstrates that with respect to authenticity, there is most concern about oregano followed by black pepper. Chilli powder, saffron, paprika and turmeric, were named next, with all of them rated of equal concern when assessing the pooled data.

3.5.2 Authenticity issue most encountered

The main authenticity issues encountered are substitution, adulteration, and concealment. To a lesser extent, counterfeiting of herbs and spices is also encountered but as this relates to the falsification of packaging / documentation, it does not usually involve analytical testing.

3.5.3 Sampling strategies

From the information gathered during Phase 1, it is clear that there is no unified approach to the sampling of herbs and spices. The two large commercial manufacturers interviewed indicated that they screen incoming batches, with mid-infrared (MIR) and near infrared (NIR) technology using within company reference databases that have been constructed to model 'what good looks like' from their organisations perspective following GMP. One of the organisations reportedly does not currently experience cases of non-compliance as they have migrated the majority of their supplier base to using the same screening approach. Whereas the other organisation has access to a large portfolio of techniques / methods, including their own NGS instrument(s), to investigate non-compliant results. Both organisations said that, where possible, the industry is moving to a more global screening approach in which 'proof (of authenticity) before export' is becoming a requirement of the larger commercial manufacturers.

With minor exceptions, the commercial laboratories expressed that they had little insight into the sampling strategies used by their customers and typically undertook analysis of the samples submitted by their customers. However, the commercial laboratories emphasised the need to use multiple techniques / methods in the case of non-compliance, and some even deploying multiple methods routinely, to provide assurance that samples are not adulterated.

PA OLs stated that they were only able to undertake sample analysis when requested by their local authority as PAs have no statutory powers to procure samples although they often work in collaboration to collect samples. As such, regional sampling is informed by a combination of local authority and central government priorities and dictated by the available funding and resourcing. Sampling strategies employed appear to be primarily

established on risk-based assessments which have an established historical basis. As well as targeted, risk-based sampling, it would be beneficial to undertake some market surveillance type sampling and analysis to assess the authenticity of herbs and spices on sale in the UK.

3.5.4 Analytical techniques

The literature review identified a total of 132 analytical techniques used for herb and spice authenticity between 2015-2022 and 52%, 23%, 14% and 12% of all analytical techniques reported were spectroscopy-based, mass spectrometry-based, DNA-based and a combination of other techniques, respectively as shown in Figure 2 of Annex 5 (reproduced below).

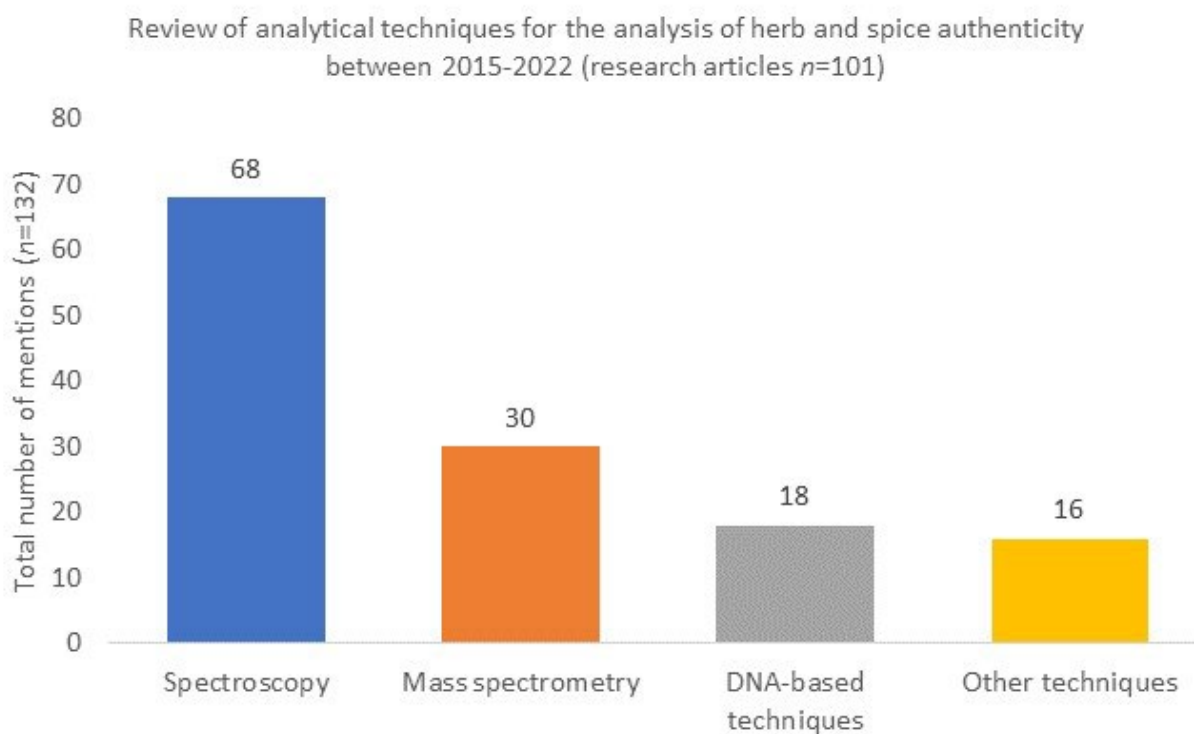


Figure 2 of Annex 5

Data from the stakeholder focus groups, responses to the Food Authenticity Network eSurvey and the literature review were compared (equally weighted) and assessed to identify the techniques most commonly used for the analysis of herbs & spices for authenticity and is presented in Figure 2.

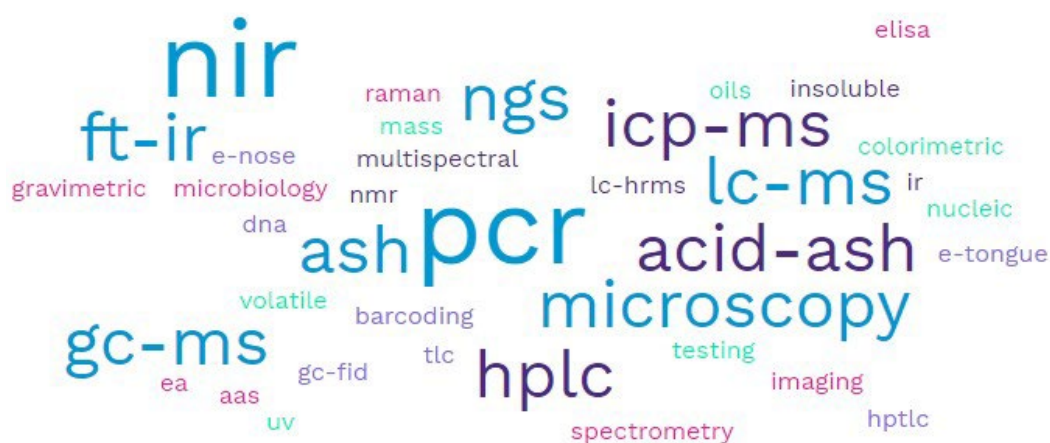


Figure 2: Word cloud graphical representation of the most commonly used techniques

Figure 2 shows that NIR and PCR are the most commonly used techniques followed by FT-IR, GC-MS, LC-MS, microscopy, NGS, Ash, Acid-ash, ICP-MS and HPLC, which were all equally used by stakeholders.

73% of papers used in the academic literature review exploited chemometric and machine learning algorithms as methods of data analysis. This result was higher than expected, considering most analytical techniques which did not use chemometrics or data analytics were DNA-based technologies. Overall, this highlights the popularity and importance of using advanced data analytics in combination with both targeted and untargeted methodologies to assist in the analysis/detection of herb and spice authenticity/adulteration.

3.5.5 Key characteristics of commonly used techniques

The data from the literature review was assessed and the key characteristics of the most commonly used techniques were identified and are presented in Table 6.

Table 6: Key characteristics of commonly used techniques

Characteristic	NIR	FT-IR	LC-MS	GC-MS	HPLC / TLC / HPLCTLC	GC-FID / E-nose / E-tongue	DNA barcoding (Sanger sequencing)	Targeted qPCR	General NGS applications
Targeted / non-targeted / both?	Non-targeted	Non-targeted	Both	Both	Targeted	Both	Both	Targeted	Both
Qualitative / Quantitative / both?	Qualitative/semi-quantitative	Qualitative/semi-quantitative	Both	Both	Quantitative	Both	Qualitative	Fully quantitative	Currently qualitative
Chemometrics required?	Yes (e.g., PCA, PLS-DA, OPLS-DA, ANN, KNN, SVM, PLSR, LDA etc.)	Yes	Depends on application	Not always but typically used for adulteration	Depends on application	Depends on application but are commonly used	No	No	No
IP protected chemometric software required?	Depends on the algorithms needed (e.g., SIMCA, TQ Analyst, MATLAB, Python, Unscrambler)	Depends on the algorithms needed	Depends on the algorithms needed	Depends on the algorithms needed (e.g., SIMCA, R Studio, Python)	Depends on the algorithms needed	Depends on the algorithms needed	No	No	Depends on the algorithms needed
IP protected Reference database required?	Yes (library development software sometimes provided by manufacturer)	Yes	Depends on application	Depends on application (e.g., NIST library, Wiley database, AroChemBase database)	Depends on application	Yes, but depends on application	No – can use publicly available DNA databases	No	Depends on application but can also access publicly available DNA databases
Typical limit of detection	Usually limits based around levels related to EMA (e.g., 0.25-1% adulteration or 90-100% for model predictions)	Usually limits based around levels related to EMA	Depends on application	Depends on application	Depends on application	Depends on application	Typically around 1% (m/m)	Can be fully quantitative down to <0.1% (m/m) on an ingredient by ingredient basis	Typically around 1% (m/m)

Characteristic	NIR	FT-IR	LC-MS	GC-MS	HPLC / TLC / HPLCTLC	GC-FID / E-nose / E-tongue	DNA barcoding (Sanger sequencing)	Targeted qPCR	General NGS applications
Validation status	Single lab and collaborative trials	Single lab and collaborative trials	Full validation	Full validation	Depends on application	Depends on application	Full validation and SOPs	Full validation and SOPs	Published scientific papers and SOPs
Accreditation status	Can be accredited	Can be accredited, evidence of method accreditation from grey literature review.	Can be accredited	Can be accredited	Can be accredited	Not commonly accredited	Can be accredited	Easily accredited	Can be accredited
Expense of instrument	Low to medium (depending on portable/bench top and manufacturer)	low to medium	High	High	High	High	Low	Low	Low – High
Ease of method transfer	Easy-medium	Potentially transferable	Difficult	Difficult	Difficult	Difficult	Easy	Easy	Potentially difficult
Ease of use	Easy	Easy	Difficult	Difficult	Difficult	Difficult	Medium	Easy	Difficult
Portability / potential for point of use	Some handheld instruments available (e.g., Neospectra, Scio, Flame-NIR)	Some portable instruments available	No	No	No	No	No	Some portable instruments available	Good – poor, depending on instrument
Time to result* (mins)	Depends on instrument/collection length typically seconds / minutes	Depends on instrument/minutes	Hours, depends on application	Depends on application, can range from 30 min to several hours	Depends on application, usually hours	Can be seconds, minutes or several hours	Instrument dependent: 30 mins to a few hours	Typically 30 mins to 2.5 hours	Instrument dependent: 15 mins to 2 days

Characteristic	NIR	FT-IR	LC-MS	GC-MS	HPLC / TLC / HPLCTLC	GC-FID / E-nose / E-tongue	DNA barcoding (Sanger sequencing)	Targeted qPCR	General NGS applications
Advantages	Rapid, non-destructive, requires little or no sample preparation, straightforward	Modern techniques using ATR are rapid, non-destructive, requires little or no sample preparation	Confirmatory methodology, simplified sample preparation for some samples (e.g., dilute-and-shoot), high sensitivity	High sensitivity, faster than HPLC, provides mass spectral information (compared to GC retention time only)	Precise, accurate	Automated and requires a short analysis time. Does not require the identification of the volatile compounds to create a model.	Accepted practice to use DNA barcoding for speciation Supported by a number of international consortia Freely accessible databases which are actively curated Requires no a priori information	Many labs have qPCR instruments Easy to use Many SOPs available Can be fully quantitative Cheap Supported by a number of SOPs	Sequencing for unknown or unauthorised herbs and spices Good for looking at mixed samples Requires no a priori information Some instruments provide results in real-time
Disadvantages	The technique is not very sensitive, so usually it can be applied only to major components. Building a database of spectral information needed.	May require standardisation, rigorous data collection, and expertise in the chemometric analysis of spectra. Building a database of spectral information needed.	Typically a very large, expensive instrument needed. User needs a high degree of training and expertise.	Only a relatively small range of volatile, thermally stable compounds can be analysed. Typically used as a means of identifying geographical origin, which may not necessarily confirm the authenticity of a sample. Database required.	Analysis time can be long, complex to perform, sophisticated equipment, skilled personnel required to conduct.	Creation of database required. Sensitivity/ selectivity/ validation parameters poor or not reported.	Not easily applicable to mixed samples	Requires <i>a priori</i> information on the target of interest	Requires investment or access to bioinformatics infrastructure Costly

* NB: Time to result subject to some interpretation as for DNA would need to extract nucleic acids from sample first and NGS needs additional processing of the DNA.

Table 6 demonstrates that there are a large number of techniques available for authenticity and adulteration of herbs and spices, boasting a range of different characteristics. Within each technique, a range of further application specific approaches are available, for example, Next Generation Sequencing (NGS) can provide targeted and non-targeted analysis, metabarcoding, metagenomic and whole genomes sequencing applications.

This plethora of techniques/methods and associated performance characteristics, coupled with the diversity of plants species and matrices classified as “herbs and spices” means that it is unrealistic to expect just one technique to be suitable for all analyses and all herbs and spices. Each technique needs to be examined on a case-by-case basis, and full method validation should be applied for a specific application / matrix, in order to generate objective evidence that the technique is fit for purpose in line with the defined scope of the method. To further qualify the fitness for purpose of a method, the exact application of that method needs to be defined (e.g., analysis of powders, leaf materials, compound samples, etc.) as well as defining the exact analytical question (e.g., presence or absence of one species, presence of a specific or any type of adulterant, a quantitative estimate relative to a threshold, etc.,).

The relative merits of each of technique, captured in Table 6 based on typical performance characteristics, can be used as a very general guide for informing on the potential utility of that technique for a particular application. For example, should portability and field operation be a priority, then NIR, FT-IR and some qPCR and NGS instrumentation would be applicable. However, if aspects such as sensitive quantitative determination or open access databases are required, then groups of techniques inclusive of NIR/qPCR and sequencing (Sanger and NGS) respectively, may be more appropriate.

As there is no “one size fits all” analytical approach for herbs and spices, it is likely that a number of different techniques/methods need to be used to verify their authenticity and a WoE approach used to determine whether samples have been adulterated. Given the trend towards methods that use chemometrics and AI as methods of data analysis, it is also likely that data fusion methods may need to be employed to enable a judgement to be made on the authenticity of a particular herb or spice that has been analysed by a number of different methods.

The output of the stakeholder focus groups and eSurvey were not as specific in terms of specifying analytical techniques used but were generally consistent with the findings represented in Table 6.

3.5.6 Reference Databases

It is acknowledged that it is difficult to set-up, curate and maintain food authenticity databases because of the need for them to be representative of the ‘unknown’ samples that they are used to test, which will most likely, in the case of herbs and spices, have been blended, processed and be subject to seasonal, climate and other environmental conditions that can affect their chemical or biological characteristics. The [Food Integrity Project Scientific Opinion](#) details what the expert authors consider to be the key considerations in building and curating food authenticity databases; there are extensive considerations related to the set-up and on-going curation of food authenticity databases.

Private contractors have set-up their own commercial food authenticity databases and due to the expense involved in their development, they are usually protected by IP. Hence, access to the data is restricted, which acts as a potential barrier for the uptake of the method. There are excellent examples of open access databases, but these are supported/originated from large publicly funding programmes; these include:

- The [Barcode of Life Data Systems](#) (BOLD) is an initiative of the [Consortium for the Barcode of Life](#) (CBOL), which is fostering development of international research alliances to build a barcode library for all eukaryotic life. BOLD is a web platform specifically devoted to DNA barcoding and currently contains sequences for ~296,000 formally described species (~7 million specimens).
- [GenBank](#) is a nucleic acid sequence database and is hosted by the [National Center for Biotechnology Information](#) (NCBI) in the US, as part of the [International Nucleotide Sequence Database Collaboration](#).
- [EU Wine Databank](#) contains the isotopic composition of wines collected from across the EU so that competent authorities in Member States can request that information from the EC when disputes or court cases arise.

Both BOLD and GenBank are open access with a good level of curation associated with them, largely being regarded as the two main public databases of DNA barcode data for

animals, plants and fungi. As such, they provide an excellent corner-stone underpinning comparability and confidence in DNA based sequencing results.

QUB recently designed and implemented a multi-laboratory study using cheap handheld NIR instruments globally to determine the authenticity of oregano samples. Although the study achieved a high level of success, one of the drawbacks was that the results determined by using the cloud-based analysis associated with the instruments were not as good as those determined using the spectra with external chemometric algorithms to generate results. This indicates shortcomings that might be encountered using off-the-shelf cloud-based solutions.

These challenges are not unique to herbs and spices testing, as the efficacy of any analytical approach is dependent upon the availability of appropriate reference materials and/or curated databases.

Availability of appropriate reference materials and validated data sets were flagged by stakeholders as a significant obstacle to authenticity testing capability. Section 3.4 provides the results of searches conducted to identify reference materials for herbs and spices and shows that certified reference materials are not available for herbs and spices, which is a limitation for authenticity analysis. Although botanical reference samples are available, it is likely that they will not be representative of commercially produced retail samples and available PT QC materials may not be of the required quality for inclusion in reference libraries. If commercially representative reference samples were available for the herbs and spices identified as of high concern with respect to authenticity, it would allow greater comparability between methods, techniques and laboratories, enabling greater confidence in the results produced.

In the absence of such materials, manufacturers indicated a preference for the use of in-house resources, of which specific examples included databases of IR scans for a range of ingredients, and from numerous trusted suppliers, growing seasons, ambient conditions, and geographical locations. If a mechanism could be developed and brokered for the sharing of proprietary food authenticity datasets akin to the [Food Industry Intelligence Network](#), then it would represent a game-changer for food authenticity testing. [The Food Authenticity Network](#) has just launched a call for the sharing of food authenticity data,

which seeks to invite stakeholders to share their food authenticity data, via links, on its website.

There is a need for independently curated, reliable authenticity databases but as they require significant investment to set-up, it is not anticipated that governments currently have funds to conduct such activities for the large number of herbs and spices that are of commercial importance to the UK.

Apart from comparative approaches for data analysis, commercial testing labs indicated that they address the issue by undertaking intensive internal data analysis (e.g., Principal Component Analysis) to look for potential outliers, cross validating aberrant results with open access databases such as GenBank and Bold and other laboratories that use alternative techniques.

A further option that was mentioned by stakeholders was the utilisation of established biobank resources (e.g., Alliance Biodiversity & CIAT), which utilise a voucher-based system using archived samples. These resources were identified as extremely versatile and valuable resources by instrument manufacturers as they can provide both test materials and relevant data.

3.5.7 Targeted Vs non-targeted methods

The development and validation of non-targeted analytical techniques is an area that has been subject to much discussion in recent times due to the lack of guidelines or standardisation covering these types of tests. There have been a couple of publications which have tried to address the lack of guidelines governing both the development and validation of non-targeted methodologies in relation to [spectroscopy](#) and [mass spectrometry](#) (QUB co-authors on both publications). These studies reviewed and assessed the current state of the art with regards to the non-targeted analysis of food for the purposes of fraud detection and proposed harmonised workflows for these types of applications.

Since the publication of these papers, the European Committee for Standardization, CEN, has set up a technical committee on food authenticity [CEN TC460](#) and a number of working groups to standardise methods in relation to food authenticity; WG 5 is producing

a harmonized standard on “Validation of non-targeted testing methods in food and feed authenticity — General considerations and definitions”, with work well progressed.

The feedback received, shows that the analytical approach used is very much dependent on the stakeholder category and the purpose of testing. NIR and FT-IR appear to be gaining favour for use as initial screening tools, although concern was expressed about the difficulty of accessing proprietary validated spectral libraries and relevant chemometric models for use in interpreting the outputs of this technology. Although the availability of multiple, independent libraries could be beneficial, as they potentially offer an opportunity to independently verify the authenticity of a sample on multiple analytical platforms.

Potential problems associated with the impact of differing ingredient processing on reference libraries and subsequent interpretation was also highlighted as being a significant limitation, in that in some cases, even inter batch changes in a product may result in it being flagged as non-compliant by a particular method. Thus, it is important that reference materials reflect commercial processing conditions.

Preference was expressed for forms of rapid, non-destructive screening approaches within a tiered system where a noncompliance could be followed up with appropriate confirmatory analysis, using an alternative technique, of the same sample. There were good examples of commercial laboratories using each other’s ‘unique’ services to provide additional independent evidence in cases on non-compliance. PA OLs can access such services through commercial offerings.

The concept of a tiered testing system has been highlighted previously, where industry, commercial and PA OLs all indicated that they routinely employ conventional light microscopy as an initial screening tool before progressing with more advanced approaches, particularly as validated primary reference samples are generally available for microscopical examination.

PA OLs expressed a preference for employing multiple but established analytical techniques in order to gain an “overall impression” of the composition of a sample (WoE approach), rather than relying on the results generated by a single approach as this was considered preferential in providing evidence for assessing contraventions against the

Food Safety Act 1990 / Food Safety Order 1991 and thus, could provide the basis for enforcement action.

Although, in laboratories, the most commonly employed technologies are currently mass spectrometry, elemental analysis, and conventional DNA-based analyses (PCR-based), the literature review showed that 31% of the reviewed research articles performed more than one analytical technique, for example, using a combination of both screening and confirmatory analysis. It also showed a move towards non-targeted analysis and the use of chemometric models and AI as methods of data analysis and interpretation.

Stakeholders appreciated that a tool-kit of methods can be applied depending on what the purpose of the testing is. For routine purposes, a two-tier approach i.e. using classical techniques such as light microscopy and FT-IR as screening techniques to obtain an initial indication of the authenticity of samples, followed, if necessary, by more focussed techniques such as GC or LC-MS was advocated.

Also, Stakeholders expressed an interest in the adoption of portable platforms (e.g., adaptation of LC-MS to handheld NIR), which are becoming more readily available with enhanced performance characteristics. However, these portable technologies still suffer from the same issues of the lack of reliable reference samples and reference libraries on which to [validate](#) the methods to demonstrate their fitness for purpose.

These findings correspond with discussions with the instrument manufacturer stakeholder focus group, the majority of whom are developing non-targeted multianalyte platforms (e.g. multispectral, FT-IR and NGS).

3.5.8 Qualitative or quantitative

In terms of reporting or requiring qualitative or quantitative results, the unanimous response was that where possible, both should be reported, but with some caveats. Regulators said that enforcement action could be taken on the basis of a validated qualitative method.

Stakeholders agreed that fraudsters who commit food fraud for economic gain would adulterate samples at concentrations of typically at least 30% w/w for it to be worth their while financially, with evidence from a 2017 survey of Turkish producers indicating levels of at least 40% w/w. Thus, in general terms, there isn't a requirement to detect very low

concentrations of the adulterant. It is more important to have a qualitative method with a validated LOD that is capable of enforcing agreed actions levels.

3.5.9 Legislation / Standards

There are no legislative limits governing the authenticity for herbs and spices, and standards relating to herbs and spices vary globally. However, the Codex Alimentarius Commission (CAC), a global food standards setting body funded by WHO/FAO, sets standards to prevent, and assist in the resolution of, trade disputes before WTO. Codex texts are voluntary and need to be translated into national legislation or regulations to be enforceable. The CAC is comprised of 189 Members, including 188 Member Countries and 1 Member Organisation (EU).

The Codex Committee on Spices and Culinary Herbs (CCSCH) aims to elaborate worldwide standards for spices and culinary herbs in their dried and dehydrated state in whole, ground, and cracked or crushed form. CCSCH has developed 8 standards based on a priority assessment on the need for world trade, which are shown in Table 7.

Table 7: CCSCH quality standards for herbs and spices based on a priority assessment on the need for world trade.

Reference	Title	Committee	Last modified
CXS 326-2017	Standard for Black, White, Green Peppers	CCSCH	2021
CXS 327-2017	Standard for cumin	CCSCH	2021
CXS 328-2017	Standard for dried thyme	CCSCH	2021
CXS 342-2021	Standard for dried oregano	CCSCH	2021
CXS 343-2021	Standard for dried roots, rhizomes and bulbs: dried or dehydrated ginger	CCSCH	2021
CXS 344-2021	Standard for dried floral parts: cloves	CCSCH	2021
CXS 345-2021	Standard for dried basil	CCSCH	2021
CXS 347-2019	Standard for dried or dehydrated garlic	CCSCH	2019

With over a hundred known herbs and spices, CCSCH has a large work programme that is moving towards a grouping system, where relevant, based on origin e.g., rhizomes, floral parts. CCSCH's focus is on defining the herb or spice and setting essential composition and quality standards. The standards do not address adulteration but there is an expectation that the product should not be adulterated. The standards specify limits for physical characteristics including for extraneous vegetable matter (EVM) ("Vegetative matter associated with the plant from which the product originates but not accepted as part of the final product"). Codex EVM limits are presented in Table 8.

Table 8: % Extraneous Vegetable Matter (EVM) specified in Codex Standards

Codex Standard	Herb / Spice	Extra	Class I / Grade I	Class II / Grade II	Class III / Grade III	Requirement
CXS-326-2017	Black Pepper	-	1	2	2	-
CXS-326-2017	White Pepper	-	1	1.5	2	-
CXS-326-2017	Green Pepper	-	0.5	1	2	-
CXS-327-2017	Cumin	-	1	2	3	-
CXS-328-2017	Dried Thyme	-	-	-	-	0.5
CXS-342-2017	Dried Oregano - Whole or crushed/rubbed oregano	0.5	2	2	-	-
CXS-343-2017	Dried or dehydrated ginger - whole or pieces	-	-	-	-	1
CXS-344-2017	Dried Cloves - whole & ground	-	-	-	-	1
CXS-345-2017	Dried Basil - Whole/Intact	-	-	-	-	0.5
CXS-345-2017	Dried Basil - Crushed/ Rubbed/ Flaked	-	-	-	-	1
CXS-347-2017	Dried or dehydrated garlic	-	-	-	-	0.5

Food Industry representatives at the stakeholder focus group said that the global industry is essentially “self-policing”. Food Industry works to trade association [guidance](#) produced by the British Retail Consortium, the Food and Drink Federation and the Seasoning and Spice Association based on good manufacturing practice. This guidance references the European Spice Association Quality Minima [Document](#) (Rev. 5), which is used by the vast majority of the UK industry. Both industry guides specify generic limits for EVM) of 2% w/w in herbs, and 1% w/w in spices.

Comparing the %EVM limits specified in the individual Codex standards agreed to date with the %EVM limits specified in [Annex 1](#) of the industry guide reveals there is consistency for most herbs and spices.

The exceptions are shown in Table 9.

Table 9: Divergence of % EVM in Codex standards compared to the industry guide

Herb / Spice	Description	Codex	Industry guide
Herb	Dried Thyme	0.5	Herbs max. 2%, Spices max. 1% by weight
Herb	Dried Basil - Whole/Intact	0.5	Herbs max. 2%, Spices max. 1% by weight
Spice	Dried or dehydrated garlic	0.5	Herbs max. 2%, Spices max. 1% by weight

It should be noted that the industry guide uses a generic approach for %EVM across all herbs and spices whereas, Codex has developed minimum standards appropriate to individual herbs or spices or groups, where relevant, based on practices in worldwide trade. In addition, Annex 1 of the industry guide gives ‘Types and Methods of Adulteration, including Recommended Controls’. The recommended controls for checking for adulteration of herbs or spices with EVM from the same plant are “Visual inspection, microscopy and/or analysis to meet the standards defined in the European Spice Association Quality Minima Document (1% for spices and 2% for herbs); Volatile Oil Content”.

Food Industry representatives at the stakeholder focus group said that having lower %EVM limits in the Codex Standards compared to those specified in the ESA quality minima document could present problems for the food industry. However it is noted that in

many cases, industry's own private quality specifications, which are agreed between buyers and sellers, will be superior to the generic minimum quality criteria set by ESA of 1% for spices and 2% for herbs.

3.5.10 Laboratory Capacity

The number of PA OLs has declined over the years to the current number of nine in the UK. Restrictions in local authority budgets and resources have resulted in a declining number of official food control samples. This has a knock-on effect on PA OLs as it is difficult to maintain UKAS accreditation for a wide analytical scope if very few or no samples are analysed annually. In the 2022 survey of PA OLs in England conducted by the Government Chemist, PA OLs identified methods for herb and spice authenticity as a training need.

PA OLs stated that herb and spice authenticity is not a priority for local authorities so consequently they receive very few samples for analysis. A port health representative told us that port health authorities only analyse herbs and spices where they have a statutory requirement to do so (typically aflatoxins, pesticides, veterinary residues). In view of this, regulators need to ensure that adequate sampling is conducted to enable maintenance of core capability in PA OLs.

However, the grey literature review did show that there is sufficient on-land (UK) capability and capacity with seven private organisations offering commercial herb and spice authenticity testing. In many cases, these organisations operate independently curated reference databases, across different techniques, which allows cross checking / cross validation in cases of non-compliance. This is already happening on a commercial basis. This also offers PA OLs the opportunity to make use of these commercial services to build up their WoE for a particular herb or spice if it is required, provided that appropriate quality control is used to generate the result, and the contracting lab are willing to work 'under the direction' of a public analyst.

Three international organisations, specialising in herb and spice authenticity testing, also offer commercial services. In addition, five large instrument manufacturers (Perkin Elmer, Thermofisher Scientific, Waters Corporation, Bruker & Agilent), actively market food authenticity applications for their instruments.

3.5.11 International case studies

The grey literature review revealed that there has been sustained global interest in the authenticity of herbs and spices. Interestingly, a court ruling in Australia was based on the verification of authenticity of oregano based on a spectroscopic screening technology with interpretation made using chemometric modelling against an IP protected reference data library. This shows that the judiciary, albeit in another country, is willing to accept this type of data.

3.5.12 Gaps

The findings from Phase 1 suggest, that the larger food businesses have good systems in place to ensure that they have done everything reasonably within their power to comply with regulatory requirements / international standards. However, it was recognised that this is more challenging for SMEs to do, making them more vulnerable to food fraud. Provision of support and guidance to SMEs, will enhance the resilience and integrity of the herb and spice supply chain.

A further review of the information indicated a number of gaps over and above those already discussed, which require addressing. Food manufacturers highlighted the need to revise and repeal numerous existing standards. For example, [ISO 939:2021](#) the standard for moisture determination in herbs and spices, was reported as being superseded by advances in technology.

Stakeholders suggested that centrally funded databases are required for official control purposes, and they recognised the need for adopting cross validation policies between laboratories and databases. In addition, they highlighted the need for reliable relevant reference materials.

In terms of maximising the impact of existing technology, the development of a practical WoE approach was considered a promising option for further consideration. Defra is currently funding a WG, chaired by the Government Chemist, that is producing guidance on applying a WoE approach to food authenticity. It is anticipated the output of this WG will address this gap.

The development of new methodologies particularly those that would enable the undertaking of non-targeted multianalyte analyses was generally supported as this is seen as the way forward. The ability to generate technology driven analytical fingerprints of food ingredients in general would enable adulteration to be detected at an earlier stage in the supply chain than currently possible. Recent advances in methods for data analysis, including chemometric modelling, machine learning and AI are accelerating the development of these newer methods. Given the trend towards methods that use advanced data analysis methods, further work may be required on data fusion methods to support a WoE approach for herb and spice authenticity.

4. Phase 2

Building on Phase 1 of the project, the aim of Phase 2 was to understand the needs of PA OLs to enable them to implement new technologies which would improve, support or increase efficiency in herbs and spices authenticity testing.

4.1 Objective and Approach

The objective of the second Phase of the project was to understand the current capabilities of the PA OLs with regards to authenticity testing for herbs and spices, and how this capability could be built on to increase appetite and ability for testing. Specifically:

1. Establish current instrument portfolios.
2. Understand current skills.
3. Ascertain appetite for adoption of new technologies, including non-targeted methods.
4. Identify skills gap to implement point 3.
5. Identify current barriers which may impede adoption of new techniques, including accessibility of databases, and feasibility of creating them.
6. Understand current gaps in quality procedures, such as access to PT schemes.
7. Identify any further barriers to herbs and spices authenticity testing.

All nine PA OLs in England, Wales and Scotland along with the Government Analyst for the Isle of Man (IoM³), were invited to participate in one-to-one interviews. Interviews (via MS Teams) were conducted with seven laboratories (participants are listed in Annex 7) using a proforma questionnaire (presented in Annex 8) to guide discussions. Two labs declined to participate due to a lack of activity in testing herbs and spices. The questionnaire was designed to obtain direct answers to specific questions and included open questions allowing the interviewee to provide sufficient detail to establish needs.

³ IoM is not a UK PA OL but has been referred to as such for ease of reference in this report.

4.2 Results and discussion

The current capabilities of each laboratory was established.

4.2.1 Current practices

All PA OLs currently conduct herb and spice authenticity testing using optical microscopy. All considered themselves skilled in this approach with the majority having UKAS accreditation; 7 PA OLs confirmed UKAS accreditation for identification of herbs and spices by optical microscopy as presented in Table 10. This is not surprising, considering microscopical examination and identification of unknowns are a core part of the Mastership in Chemical Analysis examination syllabus; it is not possible to obtain the qualification without demonstrating sufficient skill.

Table 10. UKAS accreditation for identification of herbs and spices by optical microscopy

Laboratory	Accreditation held for identification of herbs and spices
PASS Ltd.	Yes
Glasgow SS	Yes
Edinburgh SS	Yes
Kent SS	Yes
Lancashire SS	Yes
Minton Treharne and Davies Ltd.	Yes
Government Analyst, Isle of Man.	No

4.2.2 Equipment

Turning to the equipment that is currently available in PA OLs, all PA OLs have some or all the equipment, based on the outputs of Phase 1, that could be utilised to introduce

instrumental methods for the analysis of herbs and spices. The equipment available in each participating PA OL is presented in Table 11.

Table 11 (a): OL instrument capability as March 2023 - First action or screening methods

Laboratory:	Glasgow	MTD	Kent ¹	Edinburgh	Lancashire	PASS	IoM
Microscope	YES	YES (inc. SEM)	YES	YES	YES	YES	YES (inc. SEM)
FT-IR	YES – but old	YES	YES	YES	NO	YES	YES
NIR	NO	NO	NO	NO	NO	NO	NO
Raman	NO	NO	NO	NO	NO	NO	NO
End point PCR	NO	YES	YES	YES	YES	YES	NO
qPCR	YES	YES	YES	YES	YES	YES	YES
Other	Oil profiling	TGA / Pyrolysis GCMS	-	UV for saffron	-	NGS	-

Table 11 (b): OL instrument capability as March 2023 - Confirmatory Methods

LC-MS	YES	YES	YES	YES	NO	YES	YES
GC-MS	YES	YES	YES	YES	YES	YES	YES
qPCR	YES	YES	YES	YES	YES	YES	YES

¹Kent and Hants partnership, equipment split between the two PA OLs.

Table 11 shows the current range of equipment available in each PA OL. Other than microscopy, the equipment is not currently used for herb and spice authenticity testing. The age and frequency of use of the equipment varied significantly between labs and different instruments.

All PA OLs also reported a wide range of sample preparation equipment available, comprising grinders, blenders and similar types of cutters. Only two of the seven PA OLs

also reported having ball type mills, which are used to produce fine powders and are required to sufficiently homogenise samples of herbs and spices prior to analysis by FT-IR. Only one of the seven PA OLs has cryo-capability for sample preparation.

None of the PA OLs reported having any automation for herbs and spices authenticity analysis, however, other capability exists that could be used e.g., automated DNA extraction equipment.

All PA OLs felt that they were competent in the use of the equipment in their lab. As previously noted, most PA OLs hold UKAS accreditation for the identification of herbs and spices using optical microscopy, however, this was seen by all PA OLs as being a time-consuming test (typically taking ~2hrs per sample) and required a significant investment in training to maintain proficiency. All would welcome new approaches that would reduce this analysis time – it was evident that PA OLs did not ‘promote’ the (microscopy) test due to the resource implications for analysis of large numbers of samples.

4.2.3 Training

When considering the adoption of new first action or confirmatory methods, training requirements fell broadly into the following areas:

- **Sample preparation:** it was recognised that sample preparation was key to robust analysis when using screening techniques, such as FT-IR. Good procedures executed by well trained staff were considered paramount to enable data generated to be used in enforcement action, should this be required.
- **Implementation of specific methods:** all PA OLs identified lack of method development time (both in terms of researching new methods, and implementation once methodology identified) as a factor in adopting new methodology, assistance to implement methods with the minimum impact is required.
- **Limitations of the technique:** this was identified by all respondents, a detailed knowledge of what the method was capable of and more so what its limitations are, were key to understanding how to interpret data generated and allow decisions on next steps as required.
- **Metadata and libraries:** The concerns regarding the use of metadata and libraries fell broadly into two areas: i) resources required to create such information in-house (both

in terms of time / cost and ensuring materials used to create the data were genuine) and ii) when using third party data, the robustness of the data (mutatis mutandis self-generated data) and the (on-going) costs to access the data.

-

4.2.4 Quality

As noted above, the reliability of reference standards is a concern for PA OLs. All PA OLs hold their own set of herb and spice reference materials for comparative purposes, however, none of these are certified materials, and most have been built up over many (>50) years. These reference banks are supported by standard texts such as Winton's microscopy of foods (Winton, A.L. and Winton, K.B., 1945. The analysis of foods.). It is unlikely that the materials held by the PA OLs would be suitable for generating comparative data using instrumental techniques, for a number of reasons, but mainly due to the significant age of the materials causing, inter alia, loss of volatiles. None of the PA OLs currently hold any libraries of data relating to the authenticity of herbs and spices other than aforementioned microscopical references.

All PA OLs reported difficulties in identifying suitable PT schemes. To address this, small groups of PA OLs were undertaking blind 'round robin' exercises. The lack of suitable PT schemes was identified as an area of significant concern.

Utilisation of external databases drew mixed responses. For example, for DNA based techniques, most PA OLs were competent to use open access resources such as the [Barcode of Life Database](#) (BOLD). However, some reported concerns with the reliability of the data, and suggested that further training in this area would be beneficial.

Most PA OLs noted the existence of 3rd party restricted access databases, however, had not used them due to cost or lack of relevant methodology in their laboratory. All respondents suggested that engagement with these databases would be beneficial providing that the quality of the metadata could be established, and any financial costs could be recovered.

4.2.5 Other considerations

Participants were asked to identify other barriers or aids that could be addressed in order to implement new techniques for the authenticity testing of herbs and spices:

- All PA OLs noted the significant resource required to set up each methodology, particularly where non-targeted techniques are used, and all would be willing to collaborate with other PA OLs to reduce the burden on each individual PA OL.
- The majority of PA OLs felt their understanding of the herbs and spice supply chain could be improved, and would welcome training in this area, including, if possible, a visit to a producer.
- All PA OLs would welcome the implementation of new technologies to improve their herb and spice authenticity testing, however, this would need to be economically viable, either via support during implementation of methods and maintenance of accreditation or through a targeted sampling program.
-

4.3 Phase 2 Conclusions

- All PA OLs have the capability to conduct herb and spice authenticity testing, however, it is currently using time consuming microscopy methods.
- The current frequency of testing is low because herb and spice authenticity is not a priority for most local authorities and consequently little or no sampling is currently being undertaken.
- All PA OLs would be willing to introduce new technology to improve their capability, however, this would need to be properly supported. All PA OLs have or have access to suitable equipment to effect this.
- None of the PA OLs currently holds any libraries of data relating to the authenticity of herbs and spices other than microscopical references, most of which are historical. The lack of commercial reference materials and relevant PT schemes is of concern.
- Additional training in interpretation of data using non-targeted and metadata-based techniques is essential.

5. Project Conclusions

The conclusions from this project are:

- **Herbs and spices of most concern:** with respect to authenticity, stakeholders are most concerned about oregano followed by black pepper. Chilli powder, saffron, paprika and turmeric, were next, with all of them rated of equal concern.
- **Authenticity issue commonly encountered:** the main authenticity issues encountered by stakeholders are substitution, adulteration, and concealment.
- **Sampling strategies:** Unified strategies for the sampling of herbs and spices were not identified. The type of sampling employed i.e. targeted, general screening, intelligence led, spot check, etc. is very much dependent on the stakeholder and their reasons for undertaking sampling and analysis, for example, manufacturers have different requirements to regulators.
- **Reference materials:** the lack of appropriate reference materials and validated data sets was flagged by stakeholders as a significant obstacle to authenticity testing capability. No CRMs are available for herb and spice authenticity. However, four sources of botanical reference samples were identified, which could prove useful in the identification of species of herbs and spices but they are unlikely to be representative of commercially produced retail samples. QC materials are currently available for sage, oregano (4 different types), cumin and garlic powder (3 different types) relating to authenticity testing. The lack of reference materials is of concern to PA OLs.
- **Proficiency tests** Four PT providers for authenticity testing of herbs and spices were identified, who currently offer 17 different relevant PT rounds. There are currently no PT rounds available for saffron and black pepper. PA OLs stated they found difficulty in accessing appropriate rounds for herb and spices for authenticity.
- **Reference Datasets:** There are several excellent examples of open access databases, which are supported / originated from large publicly funding programmes. Private contractors have set-up commercial food authenticity databases and due to the expense involved in their development, they are usually protected by IP. Hence, access to the data is restricted, which acts as a barrier for widespread adoption of the associated method. In addition, the larger herb and spice producing companies, have their own within-company reference libraries, these are not shared outside of the organisation / its supply chain. Some PA OLs wanted further training on the use of external DNA databases. Given the eSeminar on DNA sequencing ([DNA sequencing to support food labelling enforcement e-seminar](#)), which included DNA databases, is based on material over 12 years old, and was case specific, an update / further training on the BOLD and NCBI (GenBank) databases would be useful to PA OLs.

- **Analytical testing:** The priorities for authenticity testing vary between different stakeholders, as do the herbs and spices of primary concern, the sampling strategies adopted, and the analytical methods employed.
 - The literature review identified a total of 132 analytical techniques used for herb and spice authenticity between 2015-2022, with spectroscopy-based, mass spectrometry-based, DNA-based and a combination of other techniques being reported.
 - NIR and PCR are the techniques that are most commonly used followed by FT-IR, GC-MS, LC-MS, microscopy, NGS, Ash, Acid-ash, ICP-MS and HPLC.
 - NIR and FT-IR appear to be gaining favour for use as an initial screening tool, although stakeholders expressed concern on the difficulty of accessing proprietary validated spectral libraries and relevant chemometric models for use in interpreting the outputs of these technologies.
 - The PA OLs expressed a preference for employing multiple but established analytical techniques in order to gain an overall impression of the composition of a sample (WoE approach), rather than relying on the results generated by a single approach. Microscopy is often the first tool to identify gross adulteration / contamination issues.
 - Stakeholders expressed an interest in the adoption of portable platforms (e.g., adaptation of LC-MS to handheld NIR), which are becoming more readily available with enhanced performance characteristics. These findings correspond with discussions with the instrument manufacturer stakeholder focus group, the majority of whom are developing non-targeted multianalyte platforms (e.g. multispectral, FT-IR and NGS).
 - There is a wide portfolio of methods used for verifying the authenticity of herbs and spices, the evidence shows a preference for the use of initial rapid screening approaches, within a tiered system of testing, where a non-compliant result can be followed up with appropriate confirmatory analysis of the same sample using an alternative technique.
 - It is likely that a number of different techniques/methods may need to be used to verify their authenticity and a WoE approach used to determine whether samples have been adulterated. Given the trend towards methods that use chemometrics and AI as methods of data analysis, it is also likely that data fusion methods may need to be employed to enable a judgement to be made on the authenticity of a particular herb or spice that has been analysed by a number of different methods.

- There is agreement that thresholds for economically motivated adulteration is typically at concentrations of 5% w/w or greater so there is no requirement to detect very low concentrations of the adulterant. It is more important to have a qualitative method with a validated LOD that is capable of enforcing agreed actions levels.
- **Demand:** There is no statutory requirement to analyse herbs and spices for authenticity at ports so it is not done in the UK. PA OLs stated that herb and spice authenticity is not a priority for local authorities so consequently they receive very few samples for analysis, which negatively impacts maintenance of UKAS accreditation. In the 2022 survey of PA OLs in England conducted by the Government Chemist, methods for herb and spice authenticity were identified as a gap.
- **Commercial services:** There is sufficient on-land (UK) capability and capacity with seven private organisations offering commercial herb and spice authenticity testing. In addition, three international organisations, specialising in herb and spice authenticity testing, also offer commercial services.
- **International case study:** A court ruling in Australia was based on the verification of authenticity of oregano using a spectroscopic screening method that uses chemometric modelling for data analysis and interpretation against an IP protected reference data library. This shows that the judiciary, albeit in another country, is willing to accept this type of data.
- **Guidance:** There was unanimous support for provision of guidance in relation to the authenticity testing of herbs and spices.
- **PA OL Capability:**
All PA OLs have the capability to conduct herb and spice authenticity testing, however, it is currently using time consuming microscopy methods. The current frequency of testing is low because herb and spice authenticity is not a priority for most local authorities and consequently little or no sampling is currently being undertaken.

All PA OLs would be willing to introduce new technology to improve their capability, however, this would need to be properly supported to embed the method into PA OLs with minimum resource / financial impact, and to ensure there is sufficient demand to maintain UKAS accreditation.

The most-suitable methods for testing the authenticity of herbs and spices for enforcement purposes are:

- Microscopy

- Traditional methods to support product quality e.g. ash content or solubility.
 - Liquid chromatography techniques for illegal dyes
 - FT-IR
 - qPCR.
-
- Given that the first three approaches are well established in PA OLs, and based on current available methodology, skills in PA OLs and ease of transfer, FT-IR and qPCR based methods are best suited as complementary techniques to being transferred to PA OLs. The rationale for this recommendation is presented in Table 12.

Table 12: Techniques available in PA OLs and their ease of transfer

Technique	Widely available	Ease of transfer to PA OLs	Comment
Microscopy	YES	In place	Too laborious
FT-IR	YES – but varying capability	High	Good option
NIR	NO	Low	-
Raman	NO	Low	-
End point PCR	NO	Low	-
qPCR	YES	High	Good option
NGS	NO	Medium	Outsource
LC-MS	YES	High	Authenticity applications evolving
GC-MS	YES	High	Authenticity applications evolving

All PA OLs have or have access to suitable equipment to implement this recommendation, although, additional training for PA OLs in interpretation of data using non-targeted and metadata-based techniques is essential.

Although, there is a wide portfolio of methods used for verifying the authenticity of herbs and spices, the evidence shows a preference for the use of initial rapid screening approaches, within a tiered system of testing, where a non-compliant result can be followed up with appropriate confirmatory analysis of the same sample using an alternative technique. NIR and FT-IR appear to be gaining favour for use as initial screening tools. Both NIR and FT-IR are rapid to apply, non-destructive, non-targeted

screening methods, with the potential to generate results in a comparatively short time span (~ 20 minutes) which is preferable for screening-based approaches. Within the UK, Queens University Belfast (QUB) IGFS and Bia Analytical (BA), a commercial subsidiary of IGFS, are recognised as leading experts in the field of NIR and FT-IR for food authenticity testing. BA currently provide FT-IR authenticity testing services for nineteen herbs and spices, two of which have been fully ISO 17025 UKAS accredited (oregano and sage) at QUB, with the remaining seventeen pending final authorisation⁴. As such, BA will be the only UK laboratory with extensive UKAS accredited FT-IR based methods for herbs and spices.

PA OLs were asked to provide the specification of their FT-IR instruments, and whether it would meet the requirements of the QUB / BA method. At the time of compiling this report, three had responded confirming that they have FT-IR instruments that meet the required specification. Although a definitive minimum specification has yet to be established, QUB / BA advocate the use of an FT IR system that employs an Attenuated Total Reflection (ATR) accessory, ideally containing a solid diamond, for full spectral range (although there is also some evidence that an ATR with a zinc selenide (Zn-Se) crystal may also work), utilising a wavenumber range of 549-4000cm⁻¹ (potentially use wavenumbers within this range too), and with the resolution set to 4.

6. Recommendations

Based on the wealth of evidence gathered for this project, the following recommendations are proposed.

6.1 Reference materials and Proficiency testing

- Ensure the availability of commercially representative reference samples for the herbs and spices identified as of highest concern with respect to authenticity. This could be achieved by discussion with leading PT providers and/or funding the production of QC materials for black pepper, chilli powder, saffron, paprika and turmeric. The availability

⁴ BA are also expected to receive UKAS accreditation shortly, following a recent audit.

of appropriate reference materials will allow greater comparability between methods, techniques, and laboratories, enabling greater confidence in the results produced.

- Share the results of this project with leading PT providers and seek to influence their provision of future rounds for authenticity to include spices of concern to stakeholders, especially for saffron and black pepper as there are currently no rounds for them. If this is not possible, FSA to consider funding closed PT rounds for PA OLs to enable them to demonstrate ongoing competence in core areas, which will assist in maintenance of their UKAS accreditation.

6.2 Supporting PA OLs

- Provide PA OLs training on food authenticity testing methods (targeted and non-targeted) that use chemometric and/or machine learning algorithms for data analysis. Aspects of this will be covered for the PA OLs to whom the FT-IR method is transferred (refer to 6.3). However, for the remaining PA OLs, a closed workshop could be organised at QUB / BA to achieve this. BA are the only UK laboratory with extensive, validated FT-IR based methods for herbs and spices (currently pending UKAS accreditation).
- Provide PA OLs a list of laboratories offering food authenticity testing for herbs and spices by technique and herb / spice so they have ready access in cases where their data requires corroboration.
- The costs of implementation of new methods into PA OLs, validation and accreditation need to be assessed and how the PA OLs can be supported to ensure maximum uptake. An important part of this is to ensure sustained demand for herb and spice authenticity testing in PA OLs.
- Organise a visit to an herb and spice producer to help PA OLs gain greater appreciation of the complex supply chains and the commercial production processes involved.
- Provide updated training on the use of external DNA databases such as the BOLD and NCBI (GenBank) databases. Both the factory visit and this training could be delivered via the Joint Knowledge Transfer Framework for Food Standards and Safety.
- It is essential that all PA OLs undertaking authenticity testing of herbs and spices by FT-IR have, or have access, to ball mills in order to sufficiently homogenise samples.

6.3 Roll-out of FT-IR method to PA OLs

The IGFS at QUB developed authenticity tests, based on FT-IR coupled with chemometrics, for detection of the economically motivated adulteration of oregano and sage, which were [ISO/IEC 17025](#) accredited by UKAS in 2019.

To help to commercialise these tests, QUB spun out BA to support the food sector in detecting food fraud by providing ISO/IEC 17025 accredited authenticity testing services. The oregano and sage accredited methods were exclusively licensed to BA with a right to purchase. Since then, BA has developed authenticity tests for seventeen other herbs and spices (includes all those identified by stakeholders to be of concern) including:

1. Black Pepper
2. Turmeric
3. Paprika
4. Ginger
5. Cumin
6. Garlic
7. White Pepper
8. Coriander
9. Thyme
10. Parsley
11. Basil
12. Fennel
13. Cinnamon & Cassia
14. Chilli Powder
15. Rosemary
16. Mint
17. Saffron Powder.

These tests have recently undergone an audit by UKAS and are now ISO/IEC 17025 accreditation pending.

QUB, BA and one of the PA OLs are currently in discussion regarding whether databases and methods developed at QUB / BA can be transferred to PA OLs. It is proposed that this work is taken forward in collaboration with UK government.

Task 1: Ascertain how many PA OLs have FT-IR instruments of the required specification.

- **Task 2:** FSA to explore options for PA OLs without FT-IR instruments or that don't meet the required specification to acquire / have access to suitable FT-IR instruments.
- **Task 3:** Transfer QUB FT-IR method to all PA OLs that have / have access to suitable FT-IR instruments as follows:
 - Provide SOP including instructions for instrument set-up to give optimal performance, critical evaluation of meta data and how data interpretation is performed to obtain the final result.
 - Provide pre-characterised (by QUB) samples for analysis by PA OL for one herb (oregano) and one spice (black pepper) at varying levels of adulteration from 10% up to 100%.
 - PA OLs to undertake analysis and provide data to BA in CSV format for data analysis.
 - BA to undertake data analysis and report on accuracy of PA OL analysis (test validated at an LOD of 10% (aimed at detecting EMA) with 95% confidence).
 - Follow-up discussion with PA OL on outcome.

The data generated in this inter-laboratory study should be assessed and the outcome reported to PA OLs and FSA.

6.4 Guidance

Development of guidance / regulator-industry code of practice for authenticity testing of herbs and spices would provide greater clarity on the required standards for industry, commercial and control laboratories, and local and port health authorities. Such guidance could then be used as a basis for the development of a practical system of screening herbs and spices for authenticity for the purposes of official controls that uses industry best practice, international standards, existing expertise in PA OLs,

supplemented as required, by commercially available methods that use independently curated reference datasets for interpretation across a number of different technologies. In addition, manufacturers highlighted the need to revise and repeal numerous existing standards. For example, ISO 939:1980, the standard for moisture determination in herbs and spices, was reported as being superseded by advances in technology.

7. Future research

- Given the trend towards methods that use chemometrics and AI as methods of data analysis, it is proposed that data fusion methods are understood as they may need to be employed to enable a judgement to be made on the authenticity of a particular herb or spice that has been analysed by a number of different methods.
- Challenge the QUB/BA method with mixtures lower than 10% w/w of adulterants to assess its potential to be used a screening tool for quality and as well as for detection of EMA.
- 56% of all DNA based techniques identified in the Phase 1 literature review used DNA barcoding methods. [NGS](#) is a powerful tool for rapidly and cost-effectively identifying and characterising plant, animal and microbial species present in mixed food samples. Further work is required to assess the suitability of using NGS for the purposes of species identification / verifying the authenticity of herbs and spices. LGC has just (March 2023) been awarded an 18 month Defra research contract to assess NGS for application to food authenticity that is expected to provide valuable evidence.
- As all PA OLs have capability to undertake PCR based methods, further work should be undertaken to identify relevant PCR based methods for determination of herbs and spices. Depending on the validation status associated with the relevant methods, method verification should be undertaken, in line with best measurement practice guidance and a laboratory's internal Quality Management System, to ensure that the method is fit for purpose. This could be conducted by each PA OL, or by a central laboratory prior to transfer to a PA OL in order to minimise the resource / cost impact on PA OLs as was requested by them.
- In recognition that SMEs do not have the same resources available to them to help prevent food fraud, consider whether the resources / training developed for PA OLs can be made available more widely so all stakeholders benefit from best practice

information. Provision of support and guidance to SMEs, will enhance the resilience and integrity of the herb and spice supply chain.

8. Annex 1: Stakeholder focus groups – example questions

8.1 Questions used for Group 1 (Food Industry) and Group 2 (UK Government, Local Authorities, Port Health Authority & Non-Departmental Public Body)

1. What are the main herbs and spices of concern to you when it comes to authenticity (e.g., cayenne pepper, coriander, oregano, saffron, etc)?
2. What are the main food authenticity issue/s you encounter (concealment, counterfeiting, substitution, etc)?
3. What sampling strategy does your organisation recommend (e.g., country specific targeted approach, general screening, intelligence led, spot check, etc)?
4. At what stage in the supply process does your organisation employ / recommend authenticity testing (e.g., at source, at the port of entry, upon receipt, etc)?
5. What are the current practices / methods do you employ / recommend to verify authenticity (e.g., chromatographic, chemical/elemental, nucleic acid testing, mass spectrometry etc)?
6. Do you report / require qualitative or quantitative measurements, or both?
7. Action limits required / LOD?
8. What current standards and regulations in relation to herbs and spices composition and labelling do you adhere to?
9. Would the availability of additional guidance and best practice for the analysis of herbs and spices be of value to your organisation?
10. If the answer to the previous question was yes, what form of guidance would be beneficial (e.g., legislation and regulations, methods and their application, reference materials etc)?
11. Are there any issues / gaps that you consider need to be addressed?

8.2 Questions used for Group 3 (Instrument Manufacturers)

1. Do you have any current application notes in the area of herbs and spices authenticity?

2. In developing new authenticity testing techniques /methodologies which herbs / spices would you prioritise (e.g., cayenne pepper, coriander, oregano, saffron, etc)?
3. What are the main food authenticity issue/s you addressing ~~encounter~~ (concealment, counterfeiting, substitution, etc)?
4. What are the current practices / methods do you offer / recommend to verify authenticity (e.g., chromatographic, chemical/elemental, nucleic acid testing, mass spectrometry etc)?
5. In the test you offer, how many components / targets can target / detect (e.g., 1, 2, 3 and above)?
6. Do you report / require qualitative or quantitative measurements, or both?
7. LOD?
8. What current standards and regulations in relation to herbs and spices composition and labelling do you adhere to?
9. Would the availability of additional guidance and best practice for the analysis of herbs and spices be of value to your organisation?
10. If the answer to the previous question was yes, what form of guidance would be beneficial (e.g., legislation and regulations, methods and their application, reference materials etc)?
11. Are there any emerging technologies that could be important to verify herbs and spice authenticity?
12. Are there any issues/gaps you consider need to be addressed?

8.3 Questions used for Group 4 (Testing Laboratories and Public Analyst Official Control Laboratories)

1. What are the main herbs and spices of concern with respect to authenticity (e.g., cayenne pepper, coriander, oregano, saffron, etc)?
2. What within laboratory sampling strategy does your organisation generally adopt for authenticity testing?
3. What are the main food authenticity issue/s you encounter (concealment, counterfeiting, substitution, etc)?
4. What are the current practices / methods do you employ / recommend to verify authenticity (e.g., chromatographic, chemical/elemental, nucleic acid testing, mass spectrometry etc), are you accredited for any of these?
5. Do you report / require qualitative or quantitative measurements, or both?

6. What is the limit of detection that you currently work to?
7. In the absence of a reference value, what do you define as a positive finding?
8. What current standards and regulations in relation to herbs and spices composition and labelling do you adhere to?
9. Would the availability of additional guidance and best practice for the analysis of herbs and spices be of value to your organisation?
10. If the answer to the previous question was yes, what form of guidance would be beneficial (e.g., legislation and regulations, methods and their application, reference materials etc)?
11. Are there any issues / gaps that you consider need to be addressed?

9. Annex 2: e-Survey questions

9.1 Sent to the 'Analyst' member category of the Food Authenticity Network

1. Which sector(s) do you work in? (Please select which suggestions apply)
 - Enforcement
 - Contract
 - Other
2. Is your organisation's headquarters based in the UK?
 - Yes
 - No
3. Approximately how many authenticity testing requests have you received in the last 12 months?
 - 0
 - Less than 10
 - 10 to 20
 - 20 and above
4. What was the nature of the food authenticity request? (please select any that apply)
 - Concealment, e.g., masking lower quality material
 - Counterfeiting, e.g., replacing a material with a 'look-a-like'
 - Mislabelling, e.g., inaccurate ingredient statement, country of origin
 - Substitution, e.g., replacing some or all with a cheaper material
 - Unapproved additives, e.g., used to trick analytical tests
 - Other
 - Not Applicable (0 requests)
5. Was the authenticity related analysis requested possible?
 - Yes
 - No
 - Not Applicable
6. If the answer to question 5 was no, what was the reason?
 - No suitable method available

- Equipment not available
- Reference materials not available
- Lack of resources
- Other

7. Which methods do you generally employ?

- Chromatographic, e.g., HPLC
- Chemical, e.g., colorimetric
- Elemental, e.g., EA, AAS, ICP-MS
- Gravimetric, e.g., mass
- Immunological, e.g., ELISA
- Imaging
- Nucleic acid testing, including PCR and sequencing (NGS)
- Mass spectrometry
- Microbiology
- Microscopy
- Spectroscopic, including NIR and Raman
- Other (please specify)

8. How long is generally required to perform a single test, but not perform an analysis of the result?

- Less than 30 minutes
- Between 30 minutes and sixty minutes
- More than 60 minutes

9. How long is generally required to perform an analysis of the test output?

- Less than 30 minutes
- Between 30 minutes and sixty minutes
- More than 60 minutes

10. Does the analysis of the test result require access to a reference database?

- Yes
- No

11. If the previous answer was yes, is the database an open, or restricted access resource?

- Open access (Public Domain)
- Restricted access (Commercial IP Restricted Access Resource)

12. What is the limit of detection that you currently work to?

13. With respect to each authenticity method used / offered, are you accredited?
- Yes
 - No
14. How much training/expertise is generally required to conduct a test? (Please select which suggestions apply)
- Minimal, e.g., no background technical qualifications and only basic operator training required
 - Moderate, e.g. A-Level/BTEC with some training
 - Extensive, e.g., degree/post-graduate degree with training
 - Professional, e.g., degree/post-graduate degree with comprehensive training
 - Other
15. How much training/expertise is generally required to interpret a test? (Please select which suggestions apply)
- Minimal, e.g., no background technical qualifications and only basic operator training required
 - Moderate, e.g. A-Level/BTEC with some training
 - Extensive, e.g., degree/post-graduate degree with training
 - Professional, e.g., degree/post-graduate degree with comprehensive training
 - Other
16. Do you report require qualitative or quantitative measurements, or both?
- Qualitative
 - Quantitative
 - Both
17. What are the top five herbs and spices that are of most commercial concern to your organisation?
- Cayenne Pepper
 - Chilli Powder
 - Black Pepper
 - White Pepper
 - Cinnamon and Cassia
 - Coriander
 - Cumin
 - Garlic
 - Oregano

- Paprika
- Saffron
- Sage
- Turmeric
- Other (please specify)

18. Which authenticity questions do you most frequently encounter? (Please select which suggestions apply)

- Is the product what was ordered?
- Is the product of the correct grade/quality?
- Is the product of the correct functionality, e.g., nutritional content.
- Is the product of the correct composition?
- Does the product originate from the specified country of origin?
- Other (please specify)

19. What standards and regulations in relation to herb and spice authenticity does your organisation work to?

- National legislation
- ISO
- CODEX
- Other (please specify)

20. Are you aware of any emerging technologies that could be important to verify herb and spice authenticity?

- Yes
- No

21. What would be an acceptable level of expenditure in adopting a new technology / method for authenticity?

- Less than £1000
- Less than £10,000
- Less than £50,000
- More than £50,000
- Not applicable

22. Would the availability of additional guidance and best practice for the analysis of herbs and spices be of value to your organisation?

- Yes
- No

23. If the answer to question 26 was yes, what form of guidance would be beneficial?

(please select which suggestions apply)

- Legislation
- Guidance
- Specific methods and their application
- Specialised equipment
- Reference materials
- Reference databases
- Other (please specify)

9.2 Sent to the 'Regulator' member category of the Food Authenticity Network

1. Which of the following best describes your occupation?

- Enforcement Officer, e.g., Port Health
- Authority, Trading Standards
- Food Safety Manager
- Laboratory Manager
- Management
- Supply Chain Assurance
- Quality Manager
- Other

2. Approximate number of employees in your organisation?

- Less than 10
- Between 11 and 50
- Between 51 and 250
- More than 250

3. Is your organisation's headquarters based in the UK?

- Yes
- No

4. What sampling strategy does your organisation generally recommend for authenticity testing of herbs and spices? (please select any that apply)

- Country specific targeted approach

- General screening approach
 - Intelligence led approach
 - Spot check approach
 - Not applicable
5. Please select the food authenticity issue/s being tested? (please select any that apply)
- Concealment, e.g., masking lower quality material
 - Counterfeiting, e.g., replacing a material with a 'look-a-like'
 - Mislabelling, e.g., inaccurate ingredient statement, country of origin
 - Substitution, e.g., replacing some or all with a cheaper material
 - Unapproved additives, e.g., used to trick analytical tests
 - Other
6. Do you require the reporting qualitative or quantitative measurements, or both?
- Qualitative
 - Quantitative
 - Both
 - Not applicable
7. What are the top five herbs and spices that are of most concern to your organisation?
- Cayenne Pepper
 - Chilli Powder
 - Black Pepper
 - White Pepper
 - Cinnamon and Cassia
 - Coriander
 - Cumin
 - Garlic
 - Oregano
 - Paprika
 - Saffron
 - Sage
 - Turmeric
 - Other (please specify)
8. In terms of authenticity, which of the following are of significant concern to your organisation? (Please select which suggestions apply)
- Is the product what was ordered?

- Is the product of the correct grade/quality?
- Is the product of the correct functionality, e.g., nutritional content.
- Is the product of the correct composition?
- Does the product originate from the specified country of origin?
- All of the above
- Other (please specify)

9. Where does your organisation normally survey herbs and spices authenticity? (Please select which suggestions apply)

- At source
- At the port of entry
- Wholesale
- Retail
- Other

10. In relation to herbs and spices authenticity, what regulatory control does your organisation work to?

- National legislations
- ISO
- CODEX
- Other (please specify)

11. What level of annual expenditure does your organisation allocate to addressing food authenticity issues?

- Less than £1000
- Less than £10,000
- Less than £50,000
- More than £50,000
- Not applicable

12. Would the availability of additional guidance and best practice for the analysis of herbs and spices be of value to your organisation?

- Yes
- No

13. If the answer to question 12 was Yes, then what form of guidance would be beneficial? (please select which suggestions apply)

- Legislation
- Guidance

- Specific methods and their application
- Specialised equipment
- Reference materials
- Reference databases
- Other.

9.3 Sent to the 'Food Industry' member category of the Food Authenticity Network

1. Which of the following best describes your occupation?

- Analyst
- Food Safety Manager
- Laboratory Manager
- Management
- Sales/Marketing
- Supply Chain Assurance
- Technician
- Quality Manager
- Other

2. Approximate number of employees in your organisation?

- Less than 10
- Between 11 and 50
- Between 51 and 250
- More than 250

3. Which sector do you work in?

- Consultancy/training
- Distribution
- Equipment/instrumentation
- Farming/primary production
- Manufacturing
- Primary processing
- Research
- Retail

- Testing
- Other

4. Are your organisation's headquarters based in the UK?

- Yes
- No

5. Please select the food authenticity issue/s being tested? (please select any that apply)

- Concealment, e.g., masking lower quality material
- Counterfeiting, e.g., replacing a material with a 'look-a-like'
- Mislabelling, e.g., inaccurate ingredient statement, country of origin
- Substitution, e.g., replacing some or all with a cheaper material
- Unapproved additives, e.g., used to trick analytical tests
- Other

6. Which methods do you generally employ for authenticity testing of herbs and spices?

(Please select any that apply)

- Chromatographic, e.g., HPLC
- Chemical, e.g., colorimetric
- Elemental, e.g., EA, AAS, ICP-MS
- Gravimetric, e.g., mass
- Immunological, e.g., ELISA
- Imaging
- Nucleic acid testing, including PCR and sequencing (NGS)
- Mass spectrometry
- Microbiology
- Microscopy
- Spectroscopic, including NIR and Raman
- Other
- Not applicable

7. For any authenticity testing you request, how many components / targets are evaluated?

- 1
- 2
- 3 and above
- Not sure

8. Do you require the reporting of authenticity test results to be qualitative or quantitative measurements, or both?

- Qualitative
- Quantitative
- Both
- Not applicable

9. What are the top five herbs and spices that are of most commercial concern to your organisation?

- Cayenne Pepper
- Chilli Powder
- Black Pepper
- White Pepper
- Cinnamon and Cassia
- Coriander
- Cumin
- Garlic
- Oregano
- Paprika
- Saffron
- Sage
- Turmeric
- Other (please specify)

10. Which herbs and spices do you have the most problem with regarding authenticity?

- Cayenne Pepper
- Chilli Powder
- Black Pepper
- White
- Cinnamon and Cassia
- Coriander
- Cumin
- Garlic
- Oregano
- Paprika
- Saffron

- Sage
- Turmeric
- Other (please specify)

11. Which authenticity questions do you most frequently encounter? (Please select which suggestions apply)

- Is the product what was ordered?
- Is the product of the correct grade/quality?
- Is the product of the correct functionality, e.g., nutritional content.
- Is the product of the correct composition?
- Does the product originate from the specified country of origin?
- Other (please specify)

12. At what stage in the supply process does your organisation generally employ authenticity testing?

- At source
- At the port of entry
- Upon receipt
- Not applicable

13. What standards and regulations in relation to herb and spice composition does your organisation work to?

14. Are you aware of any emerging technologies that could be important to verify herb and spice authenticity?

- Yes
- No

15. What would be an acceptable level of expenditure in adopting a new technology / method for authenticity testing by your organisation?

- Less than £1000
- Less than £10,000
- Less than £50,000
- More than £50,000
- Not applicable

16. Would the availability of additional guidance and best practice for the analysis of herbs and spices be of value to your organisation?

- Yes
- No

17. If the answer to question 16 was yes, what form of guidance would be beneficial?

(please select which suggestions apply)

- Legislation
- Guidance
- Specific methods and their application
- Specialised equipment
- Reference materials
- Reference databases
- Other

10. Annex 3: Summary of responses from stakeholder focus groups

1. What are the main herbs and spices of concern to you when it comes to authenticity (e.g., cayenne pepper, coriander, oregano, saffron, etc)?

Volume wise

[Industry ¹]	[Testing Labs ^{7,8,9,10}]	[PA Labs ^{2,3}]	[Instruments]	[Gov]
Black Pepper	Black Pepper	Oregano	Oregano	Chilli Powder ^{11,12}
White Pepper	Oregano	Sage ⁴	Sage	Cumin
Turmeric	Turmeric	Cinnamon ⁵	Black Pepper	Coriander
Oregano	Paprika	Nutmeg	White Pepper	Turmeric ^{13,14}
Sage	Garlic	Saffron ⁶	Chilli Pepper	Saffron ^{13,14}
Saffron	Saffron	-	-	Cayenne Powder
[Cinnamon]	Cumin	-	-	Capsicums
[Ginger]	Cinnamon	-	-	-
-	Nutmeg	-	-	-

¹ Individual or as mixed herb.

² Local authorities choosing not to test (survey at random).

³ Recent FSA survey work.

⁴ Herb on its own, but also in foods such as sausages.

⁵ Substitute for Cassia.

⁶ Whole saffron not powdered.

⁷ Opinion is doesn't matter what test some supplies less pure than others.

⁸ Anything you look at will start to see differences.

⁹ Challenge is to look at blends (higher risk category).

¹⁰ See in all spices regardless.

¹¹ Chilli powder, cumin and coriander from India on the basis of aflatoxin screening

¹² Port Health, no funding for screening, only undertake statutory requirement which covers aflatoxin and pesticide residues.

¹³ FSS driven in Scotland, high failure rate due to presence of lead chromate.

¹⁴ FSA driven in England and Wales but primarily through testing for mycotoxins and metals.

Authenticity wise

[Industry]	[Testing Labs]	[PA Labs]	[Instruments]	[Gov]
Oregano ^{1,2}	In all if look ^{3,4,5,6}	N/A	Turmeric ⁷	-
Black Pepper	-	Saffron ⁷	-	-
White Pepper	-	-	-	-

¹ Of most concern historically.

² Each manufacturer has different supply chains, hence different risk for each ingredient.

³ Opinion is doesn't matter what test some supplies less pure than others.

⁴ Anything you look at will start to see differences.

⁵ Challenge is to look at blends (higher risk category)

⁶ See in all spices regardless.

⁷ FSS driven through high failure rates due to lead chromate.

2. What are the main food authenticity issue/s you encounter (concealment, counterfeiting, substitution, etc)?

Risk perspective

[Industry]	[Testing Labs]	[PA Labs]	[Instruments]	[Gov]
Concealment ¹	Not Counterfeit	Not Counterfeit	Substitution	All apart from counterfeit
Substitution ^{2,3}	Substitution	Range issues	Adulteration	-
-	Adulteration	-	-	-
-	Dilution	-	-	-

¹ Notably through use of dyes with saffron, both ground and unground ingredient.

² Turmeric with use of dyes and spent product, also capsicums using spent product which has no oils.

³ RASFF doubling for use of illegal dyes 2018 – 2022, use to conceal for substitution, particularly fillers, 2018 - 8 cases, 2019 – 9 cases, 2020 - 6 cases, 2021 - 9 cases, 2022 - 21 cases.

3. What sampling strategy does your organisation recommend (e.g., country specific targeted approach, general screening, intelligence led, spot check, etc)?

[Industry]	[Testing Labs]	[PA Labs]	[Instruments]	[Gov]
General screen	Test what sent, no insight required	FSA driven ^{4,5}	Two tier strategy ⁹	Country targeted ¹⁰
Strategy where undertaken ^{1,2}	Limited surveillance activities ⁸	FSS driven ^{6,7}	-	Global Screen Proposed ^{11,12}
Deep dive if non-compliant	If available use surveillance to target	-	-	Proof before export ¹³
FOOD DEFENSE ³	-	-	-	-

¹ Manufacturer dependent.

² One supplier only finished product primarily purchased.

³ FOOD DEFENSE, different commodities have different risk, different location have different risk (political unrest, Russian/Ukraine war).

⁴ Some PA's asked to input into list based on own intel, but primarily FSA driven by their current interests/priorities.

⁵ Local authority directed, but PAs have restricted powers of entry and seizure. Can request TS but depends on their priorities and resources.

⁶ Scotland do have the "Sampling Surveillance Group" into which PA's input, but primarily FSS driven.

⁷ FSS driven, also depends on priorities in area of lab. Some local authority samples, some PA sampling but restricted powers of entry and seizure.

⁸ Limited general surveillance, specifics do in depth sweep broad range products (FF).

⁹ Instrument development very much driven by perceived need for a 2-tier scheme of general screen followed by deep dive.

¹⁰ FSA directive based on risk assessment (link to pesticides in rice from India and Pakistan), also FSA Regulation 669 strategy samples of high risk (pesticides and aflatoxins). Country targeted focus also based on historical evidence and therefore knowing where to look which achieves maximum from limited government funding resources.

¹¹ In reality recommended to look more broadly and will find hence FSS Global Screening proposal.

¹² Few non-targeted surveys currently run on even an annual basis, FSS led intelligence operation “A basket of Food” look at authenticity on an annual basis.

¹³ Relates to container at point of entry, but not currently a statutory requirement. Many larger retailers do undertake this but primarily for microbial contamination not adulteration. On the whole no “digital-chain” of evidence, reliance on paper audit trail. But most retailers require GFSI approved supply chain integrity, if not accredited require different approach.

4. What are the current practices / methods do you employ / recommend to verify authenticity (e.g., chromatographic, chemical/elemental, nucleic acid testing, mass spectrometry etc)?

[Industry]	[Testing Labs]	[PA Labs ³]	[Instruments]	[Gov]
IR Spec each batch ¹	FT-IR	Microscopy ⁴	FT-IR	GC-MS & LC-MS ¹²
Microscopy	GC & LC-MS ⁹	UV Spectroscopy ⁵	NGS	NIR ^{13,14,15,16,17}
MS & LC-HRMS	NGS & PCR	Ash and acid insoluble ash ⁶	Multispectral	-
NMR	Microscopy	FT-IR ^{7,8}	GC-MS	-
NGS & PCR ²	Volatile oils	-	LC-MS	-
-	ICP-MS	-	-	-

¹ IR profile match database, with 6.5K profile database, models for turmeric and pepper

² NGS for general screen, don't currently generally recommend, PCR preferable as quantitative

³ PAs advocated "Broad Brush Approach" of classical techniques. These provide a general inference that sample not consistent with that generally reported by PAs

⁴ Many PA labs accredited for microscopy, applications depends largely on nature of sample (botanical or powder, although the addition of brick dust (saffron) and sand (in ginger) has been reported)

⁵ With saffron, single wavelength, British Standard

⁶ Used with ginger (95% w/w) case where sand (5% w/w) added to ginger

⁷ Restricted use as limited access to instrumentation. Good for initial screen, depending on models, though reported not to be suitable for use with saffron. Critical to have good standards for developing models.

⁸ FT-IR Chemometric models sensitive to processing leading to false non-compliant findings

⁹ GC-MS/LC-MS for biomarkers of adulterant rather than product

¹⁰ Two tier system for analysis

¹¹ Advocate be unpredictable to fraudsters test wise

¹² Advocate GC and LC-MS as provides for qualitative as well as semi quantitative results

¹³ Move to portable point of contact NIR devices. Indian market widely use IR to provide a yes / no type analysis. Non-compliance require further analysis. Employ IR with chemometric models for oregano authentication, with over 1000 reference samples

¹⁴ Recent FAPAS Oregano PT screen highlighted use of multiple different methods to determine compliance but highlighted the need for the development of reference databases of size and variability

¹⁵ Look to transfer LC-MS and GC-MS to NIR

¹⁶ Look to validate result through cross comparison of LC-MS with NIR, or with DNA, so multi technique confirmatory approach.

¹⁷ Recent proficiency trial of multiple commercial databases against public offerings (FAPAS?).

4a References Material and Authentication

[Industry]	[Testing Labs]	[PA Labs]	[Instruments]	[Gov]
In-House ⁷	PCA for outliers ¹	-	Biobanks ⁶	EU-JRC Wine database ⁸
-	Cross validate between labs and databases ^{2,3}	-	-	-
-	Ref samples from trusted source	-	-	-
-	Verified reference databases ^{4,5}	-	-	-

- ¹ Chemometric analysis problematic as processing can mimic non-compliance
- ² Combinatorial databases, NCBI, BOLD and in-house
- ³ Where no ID agreement take up a level, “genus”
- ⁴ Public databases corrupt as criminal accessible and false annotation
- ⁵ NGS more specific at NCBI, Refseq
- ⁶ Representative sample voucher-based resources (initial form of BOLD)
- ⁷ Particularly for IR and FT-IR batch scans, some NGS but mainly reliant on GenBank and BOLD
- ⁸ Only example of government owned/funded database.

5. Do you report / require qualitative or quantitative measurements, or both?

[Industry]	[Testing Labs]	[PA Labs]	[Instruments]	[Gov]
Both	Both	Both, depends ¹	Both, depends	Both
Deep dives lend to quant	NGS quality ^{2,3,4}	-	Majority semi quant ⁶	Qualitative (Good Enough) ^{6,7,8,9}

¹ Depends on sample and requirements. On a legal perspective as a PA, a semi-quant range is easier to defend in court than an absolute value.

² However, number of reads taken as indicative of relative abundances, provides useful information, but some overreporting of species.

³ NGS not an accredited method

⁴ FT-IR Bia Analytical UKAS accreditation pending for Oregano and Sage, plus a further 17 herbs and spices following successful audit

⁵ NGS multianalyte, single screen, semi quantitative based on numbers of reads

⁶ Port Health consider qualitative good enough as non-compliant substance should not be there. Highlighted Almond in Cumin as Almond should not be in same factory so unlikely to be there by accident

⁷ Likelihood of establishing a prosecution on basis of qualitative measurement is slim and holding 20,000 kg at border difficult as cost implication

⁸ Need for additional guidance highlighted, e.g., FSA of presence of EM. Codex currently stands at 2% w/w herbs and 1% w/w spices although about to enforce 1% for herbs and 0.5% for spices without industrial consultation

⁹ Question of Codex integrity has been raised as country driven, but conflict of interest if industry residing within the lead country is exploiting political influence to control the market.

6. Action limits required LOD

[Industry]	[Testing Labs]	[PA Labs]	[Instruments]	[Gov]
NIR 2% ¹ & French 5%	Nominal EM 2% & 1% ⁶	Case by Case ⁶	Technology and case-based ⁸	Codex
One supplier zero tolerance ²	Non-compliant if detected ⁷	Codex	-	-
EM 2% herbs 1% spice ^{3,4}	-	Contravention Food Safety Act	-	-

¹ Adulteration not expected at such low levels

² No agreed industrial limits apart from EM

³ Codex to specify 1%herbs 0.5% spices

⁴ Initiatives European CCP Project, AFNOR (France) Accredita (Italy)

⁵ In line with current Codex, above threshold, i.e., doesn't fit with database, relates to "Non-Compliance"

⁶ Too many variables for simple PA answer. Broadly based on information to hand, e.g., "My colleagues and I have analysed more than 400 samples and the range of this component generally falls between x and y and this sample has a value of w". Hence opinion and cross checking between labs on findings (commercial and PA labs report this)

⁷ American Spice Trading Association monographs indicate likely adulterants, utilise gravimetric determinations

⁸ Low limit of detection not advocated with adulteration as not economic to consider below ~10% w/w.

7. What current standards and regulations in relation to herbs and spices composition and labelling do you adhere to?

[Industry]	[Testing Labs]	[PA Labs]	[Instruments]	[Gov] ¹
Authenticity Industry led	Nature of request	Codex	GFSI Guidance	BRC Guidance
Comply national regs	Codex and ISO	-	FDF	FSA Guidance
GFSI	-	-	SSA	-
BRC vs. 9	-	-	CCP	-
ESA	-	-	AOC	-
SSA	-	-	SMPR ²	-

¹ Possible future implementation of a trusted trader scheme

² Standard Minimum Performance Requirements (AOAC), new one for new method or new commodity.

8. Would the availability of additional guidance and best practice for the analysis of herbs and spices be of value to your organisation?

[Industry]	[Testing Labs]	[PA Labs]	[Instruments]	[Gov]
Enable to self govern ^{1,2,3}	-	Code of practice	SMPR ⁶	Code of practice
Update existing standards ⁴	-	-	-	Trade guidance ⁵

¹ Industry already produce own standards.

² Not commercially beneficial to work to lower standards by enforcing Codex.

³ Codex potential to be political due to lead country being influenced by industry.

⁴ Many existing standards are no longer fit for purpose and need revising, e.g., moisture method for herbs and spices. The ISO 9391980 has spent a number of years in review and is now 9392020 or 9392021? But in the industry, the herb and spice industry don't tend to work to water activity, but to moisture in terms of specifications.

⁵ GFSI, BRC etc produce guidance but good to have guidance on how it fits with legislation for bodies such as Port Health and also in general for food safety requirements for importers.

⁶ Standard Minimum Performance Requirements (AOAC).

9. Are there any issues / gaps that you consider need to be addressed?

[Industry]	[Testing Labs]	[PA Labs]	[Instruments]	[Gov]
New methodologies	Need WoE approach ⁴	Local authority surveillance ^{1,2}	-	Databases ⁷
Need to evolve risk management ³	Database development ⁵	-	-	Regulatory ⁸
-	Cross validation validity ⁶	-	-	-

¹ PA state none or limited local authority surveillance, enforcement sampling negligible, if not for recent FSA sampling would be unlikely any undertaken.

² Allergens appear current priority.

³ Recent changes in worldwide trading requires the adaptation of risk management. Move away from a sole testing strategy to supply line management.

⁴ Use of multiple methods advocates the development and acceptance of a more WoE style approach.

⁵ Funded development of reference databases for labs and industry.

⁶ Cross checking and results consideration. Where labs get inconsistent results doesn't mean one wrong, points to a "non-compliance" with available specification. Points back to need to WoE approach and use of panel of methods.

⁷ Cross government department agreement on funding core database development

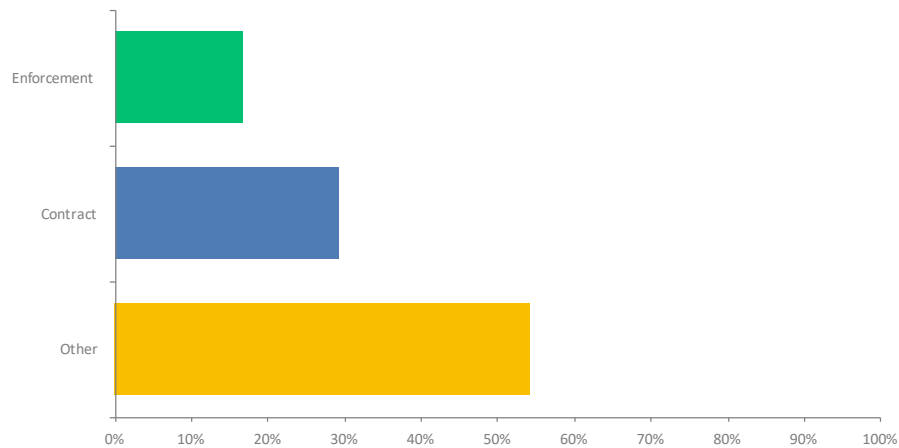
⁸ Port Health would like to have input as a port health authority on the bio security and the public health side of things.

11. Annex 4: Summary of responses from e-survey

11.1 E-survey results - Analysts

Q1: Which sector(s) do you work in? (Please select which suggestions apply)

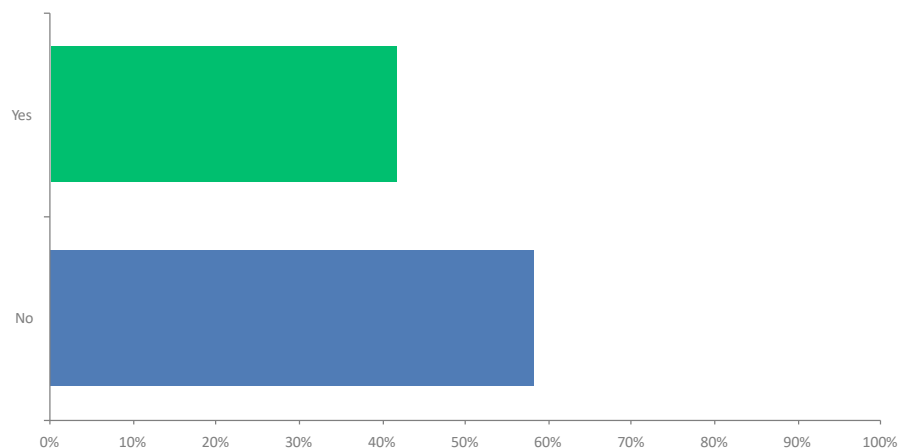
Answered: 24 Skipped: 0



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Q2: Is your organisation's headquarters based in the UK?

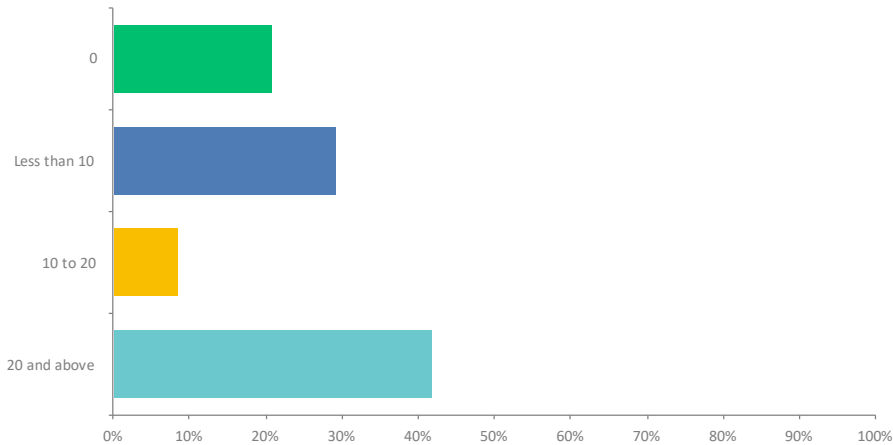
Answered: 24 Skipped: 0



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Q3: Approximately how many authenticity testing requests have you received in the last 12 months?

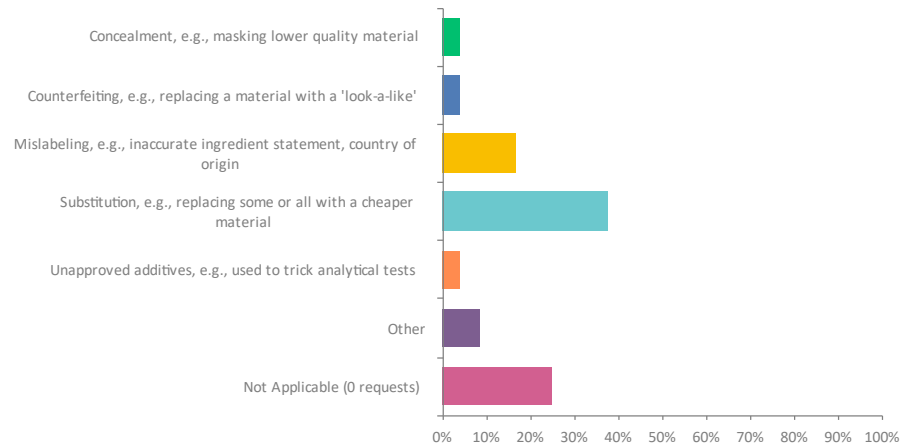
Answered: 24 Skipped: 0



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Q4: What was the nature of the food authenticity request (please select any that apply)

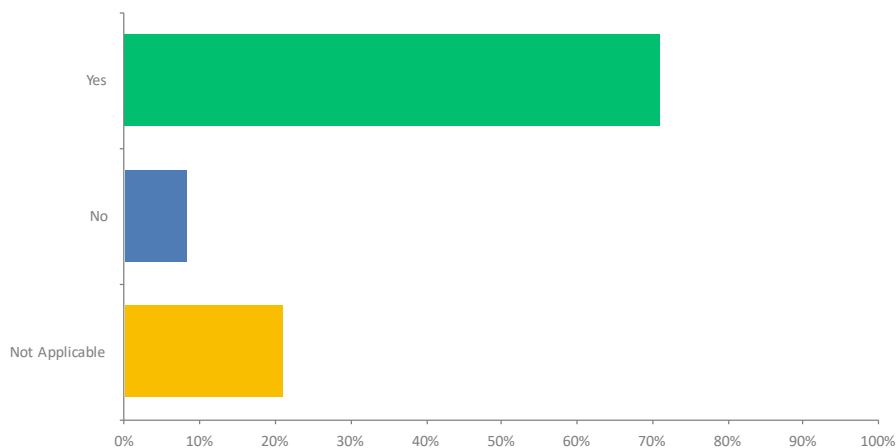
Answered: 24 Skipped: 0



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Q5: Was the authenticity related analysis requested possible?

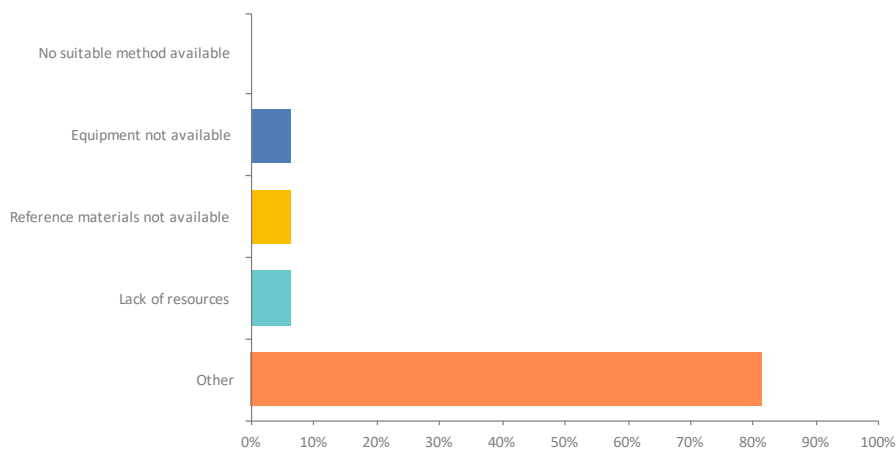
Answered: 24 Skipped: 0



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Q6: If the answer to question 5 was no, what was the reason

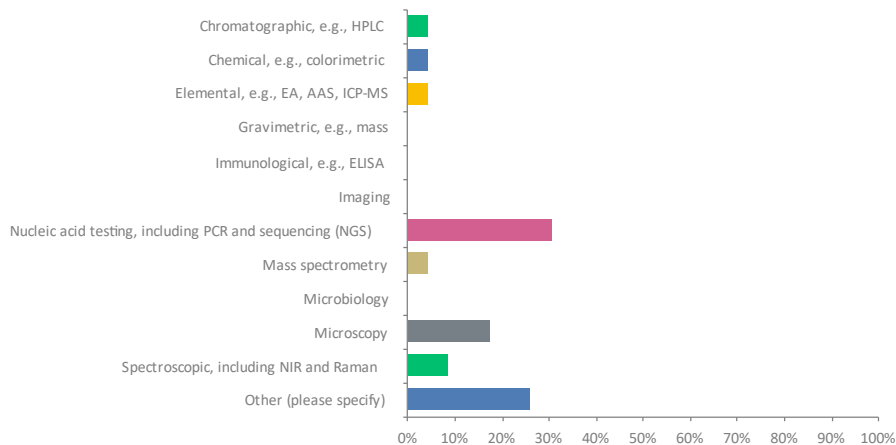
Answered: 16 Skipped: 8



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Q7: Which methods do you generally employ?

Answered: 23 Skipped: 1

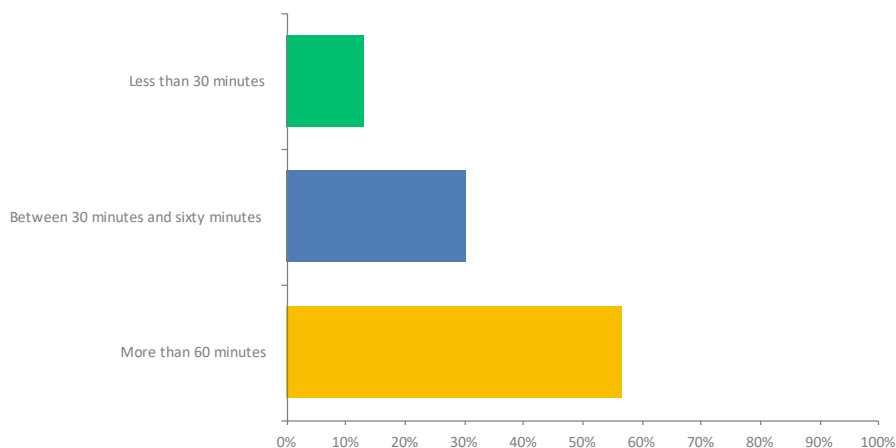


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#	OTHER (PLEASE SPECIFY)
1	SIRA, DNA, micro, mass spectrometry, spectroscopic
2	Stable isotope ratio MS
3	Nuclear Magnetic Resonance NMR
4	IRMS
5	as applicable from the above
6	Low Field NMR

Q8: How long is generally required to perform a single test, but not perform an analysis of the result?

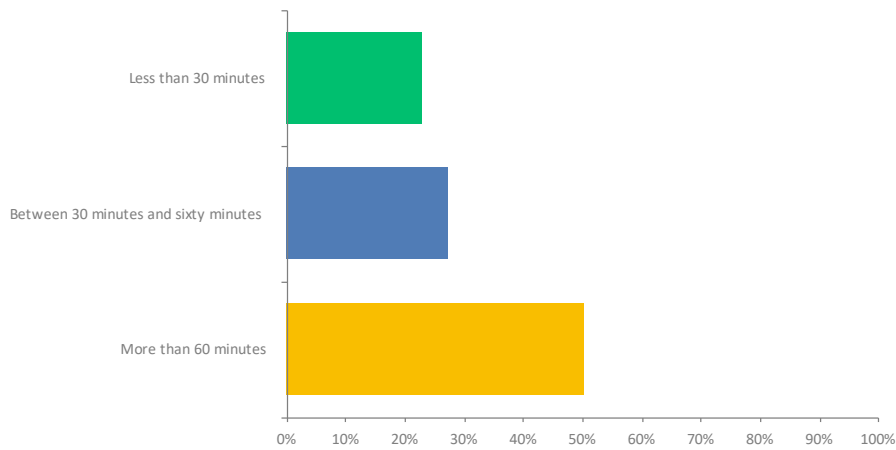
Answered: 23 Skipped: 1



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Q9: How long is generally required to perform an analysis of the test output?

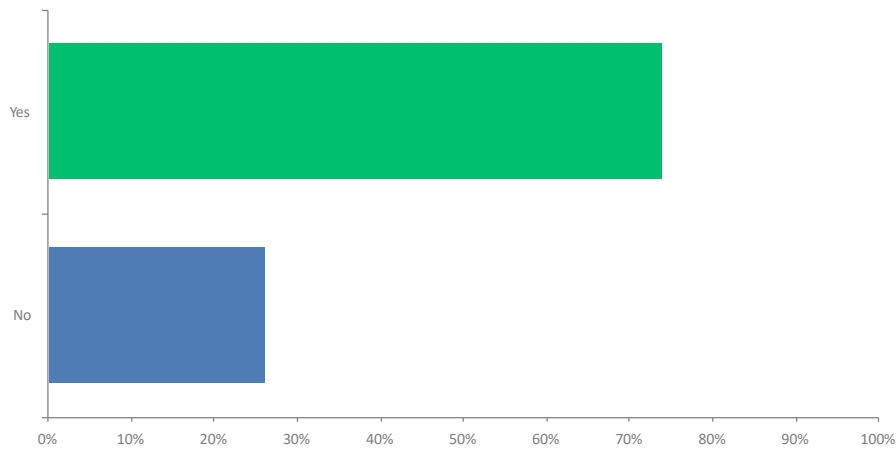
Answered: 22 Skipped: 2



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Q10: Does the analysis of the test result require access to a reference database?

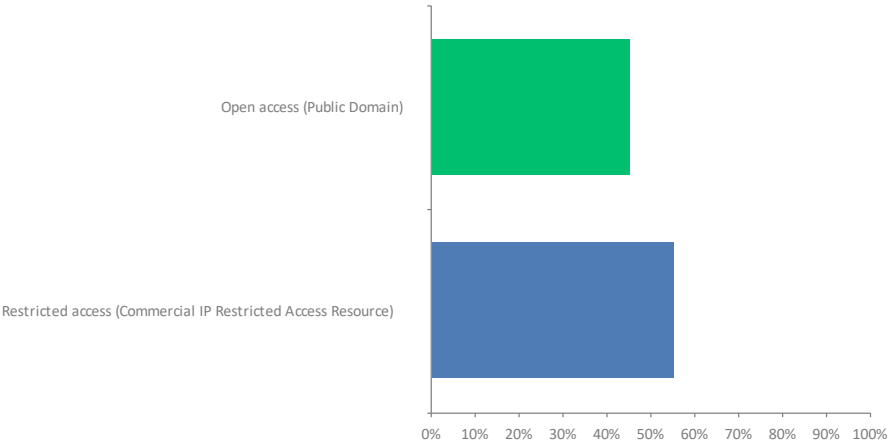
Answered: 23 Skipped: 1



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Q11: If the previous answer was yes, is the database an open, or restricted access resource?

Answered: 20 Skipped: 4



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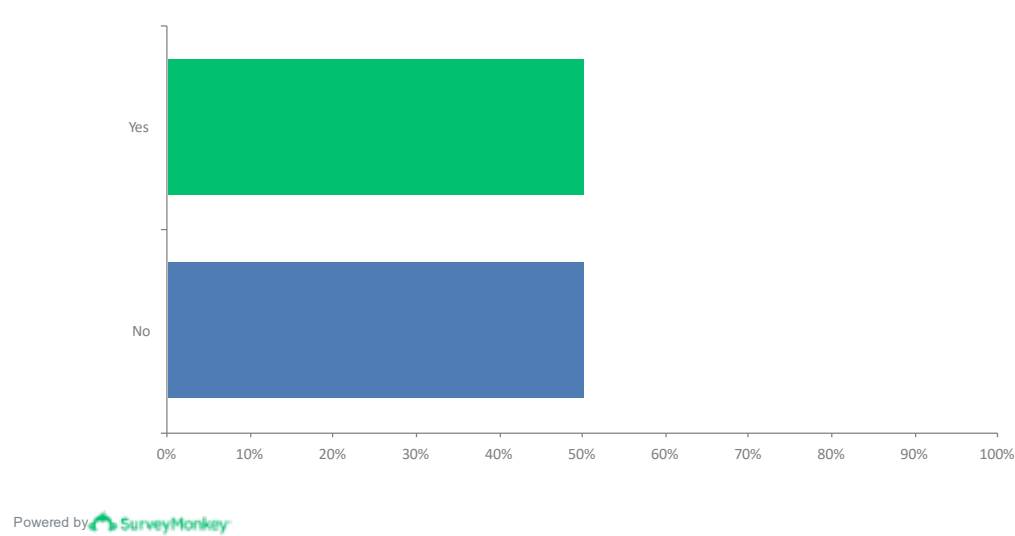
Q12 What is the limit of detection that you currently work to?

Answered: 18 Skipped: 6

#	RESPONSES	DATE
1	various	2/7/2023 5:05 PM
2	2%	2/7/2023 9:37 AM
3	Difficult to say as it is microscopy, approx. 5%	2/6/2023 8:16 AM
4	0	2/3/2023 4:23 PM
5	30%	2/3/2023 10:31 AM
6	Zero	2/3/2023 9:45 AM
7	5%	2/3/2023 8:43 AM
8	0.5%	2/3/2023 8:38 AM
9	variable	2/3/2023 8:26 AM
10	The limit of detection is species-dependent (generally in the range 0,1-1%)	2/3/2023 8:05 AM
11	0.01 mg/kg	2/3/2023 5:41 AM
12	0.01%	2/3/2023 4:30 AM
13	0.01mg/kg	2/3/2023 4:10 AM
14	0.0001%	2/3/2023 1:24 AM
15	0.1% (w/w)	2/2/2023 10:47 PM
16	0.001	2/2/2023 9:21 PM
17	ug/kg	2/2/2023 8:00 PM
18	n/a	2/2/2023 5:21 PM

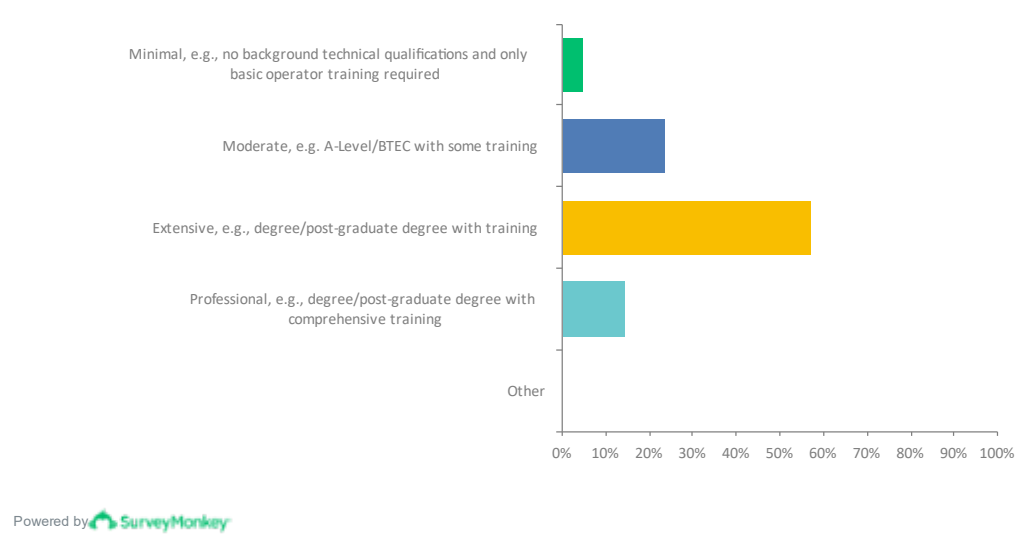
Q13: With respect to each authenticity method used / offered, are you accredited?

Answered: 22 Skipped: 2



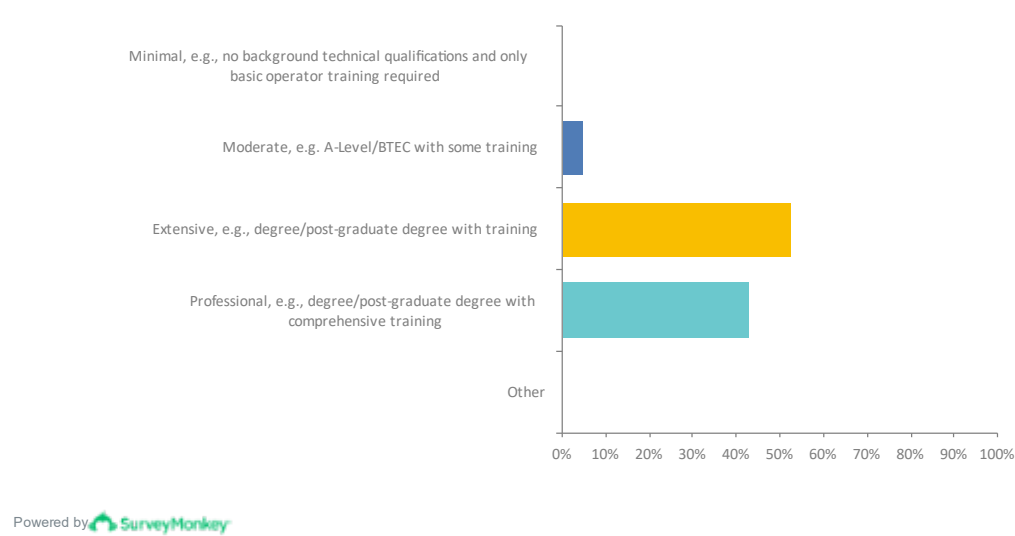
**Q14: How much training/expertise is generally required to conduct a test?
(Please select which suggestions apply)**

Answered: 21 Skipped: 3



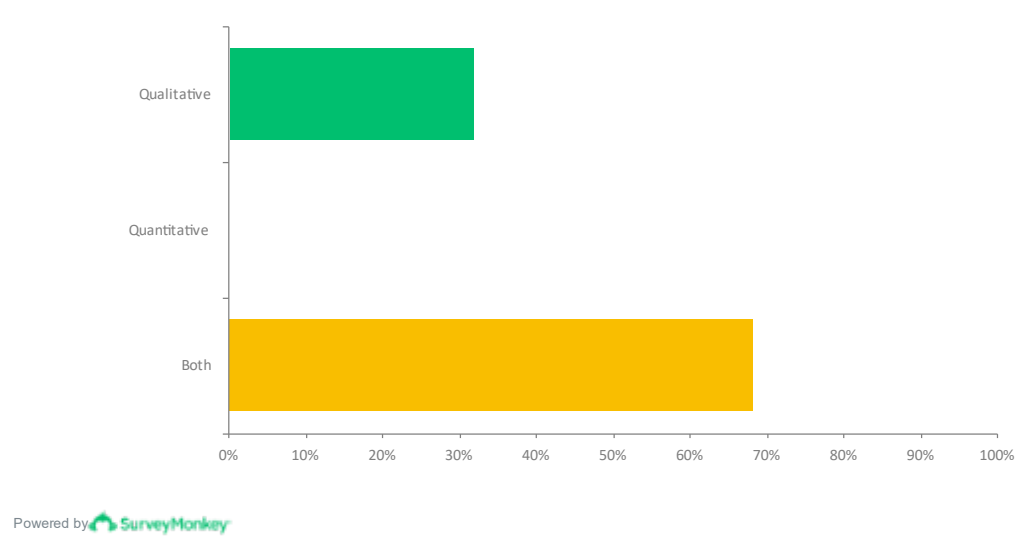
Q15: How much training/expertise is generally required to interpret a test? (Please select which suggestions apply)

Answered: 21 Skipped: 3



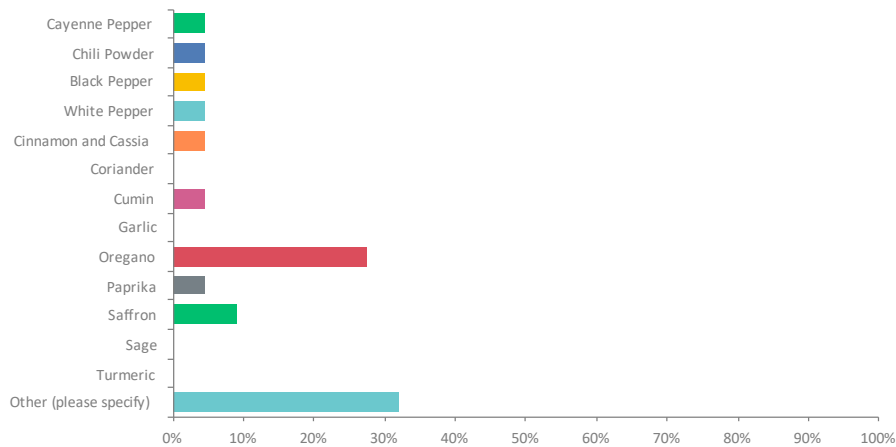
Q16: Do you report require qualitative or quantitative measurements, or both?

Answered: 22 Skipped: 2



Q17: What are the top five herbs and spices that are of most commercial concern to your organisation?

Answered: 22 Skipped: 2

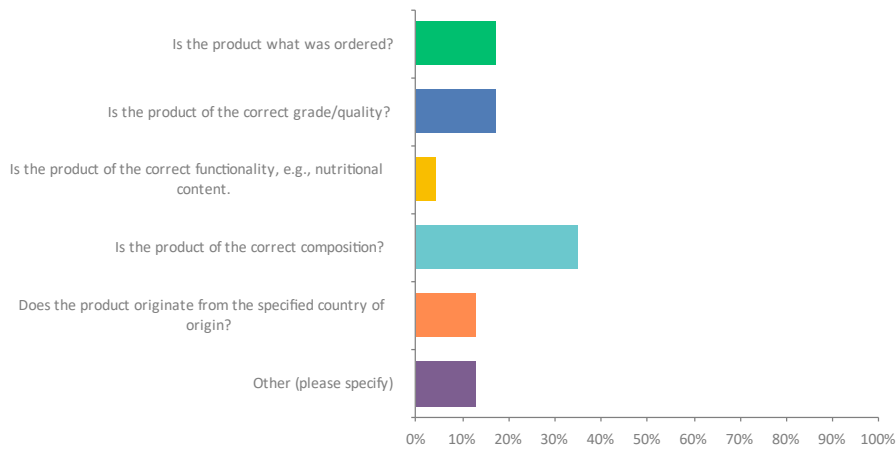


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#	OTHER (PLEASE SPECIFY)
1	system only allows 1 but questions asks for 5? black pepper, saffron, garlic, oregano, sage
2	Paprika, Cumin, Turmeric, Saffron, Pepper, Oregano
3	saffron, paprika, black pepper, cinnamon, garlic
4	Onion powder
5	mixed herbs, curry powder, medicinal herbal pills/tablets
6	—
7	saffron, Chilli, Oregano

Q18: Which authenticity questions do you most frequently encounter?
(Please select which suggestions apply)

Answered: 23 Skipped: 1

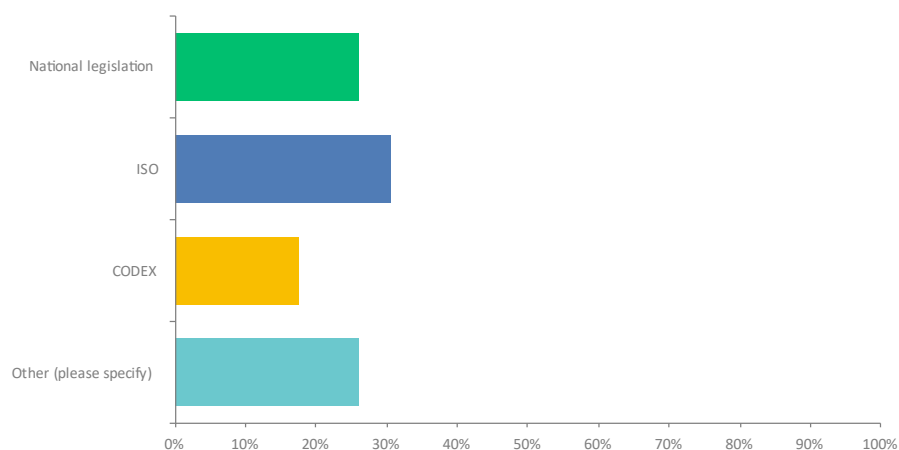


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#	OTHER (PLEASE SPECIFY)
1	product that was ordered, grade, COO,
2	is there any contamination
3	-

Q19: What standards and regulations in relation to herb and spice authenticity does your organisation work to?

Answered: 23 Skipped: 1

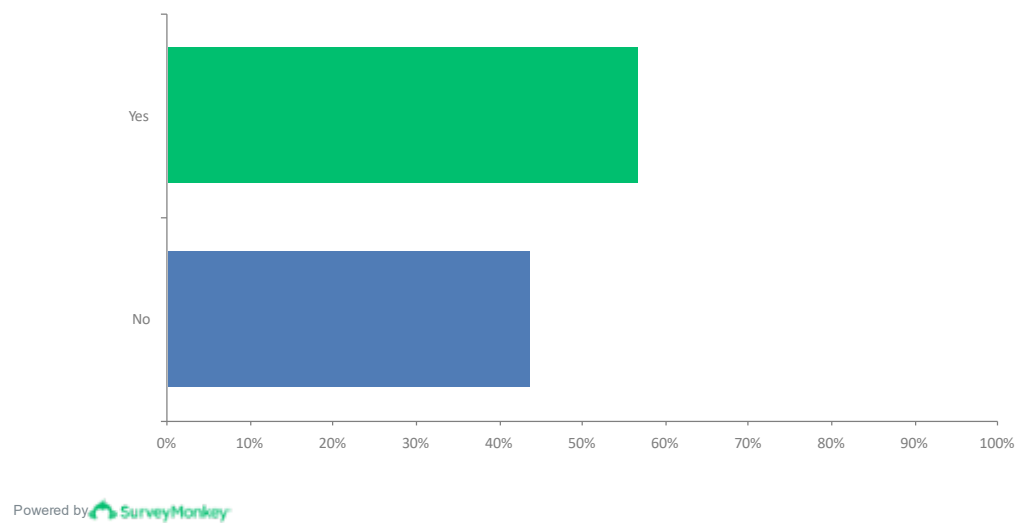


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#	OTHER (PLEASE SPECIFY)
1	codex and NL
2	Reference books and control samples
3	multiple (depending on the area of operations)
4	No standards available
5	Food Industry
6	-

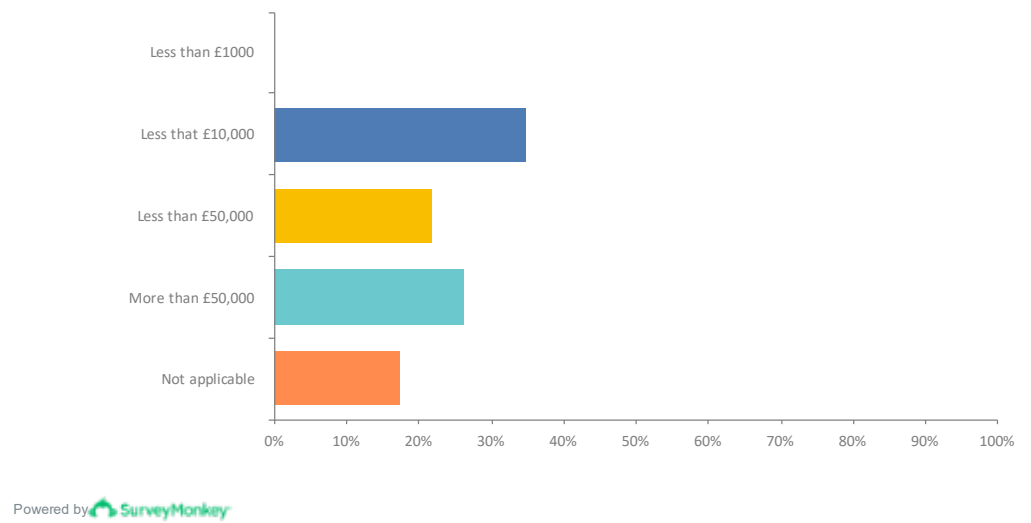
Q20: Are you aware of any emerging technologies that could be important to verify herb and spice authenticity?

Answered: 23 Skipped: 1



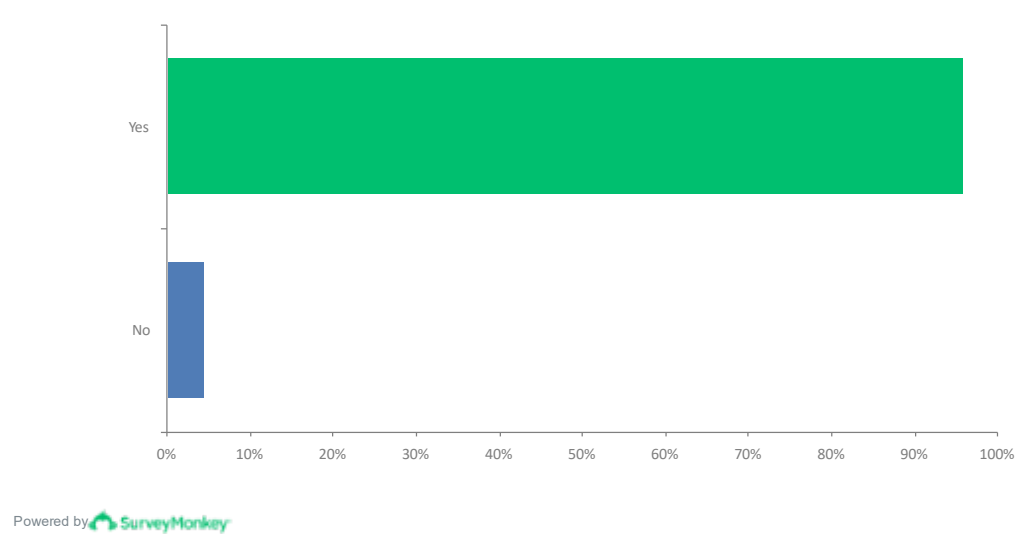
Q21: What would be an acceptable level of expenditure in adopting a new technology / method for authenticity?

Answered: 23 Skipped: 1



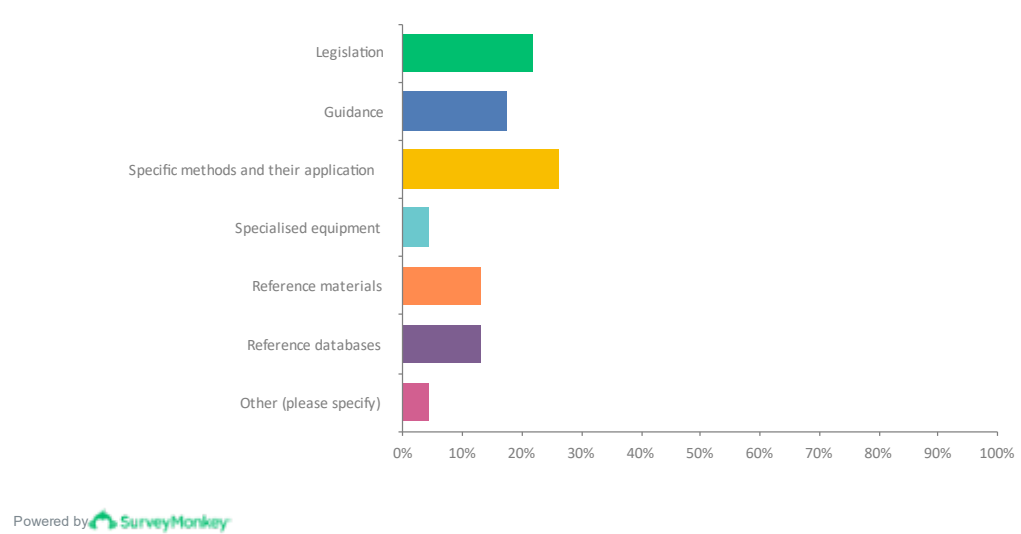
Q22: Would the availability of additional guidance and best practice for the analysis of herbs and spices be of value to your organisation?

Answered: 23 Skipped: 1



Q23: If the answer to question 26 was yes, what form of guidance would be beneficial (please select which suggestions apply)

Answered: 23 Skipped: 1

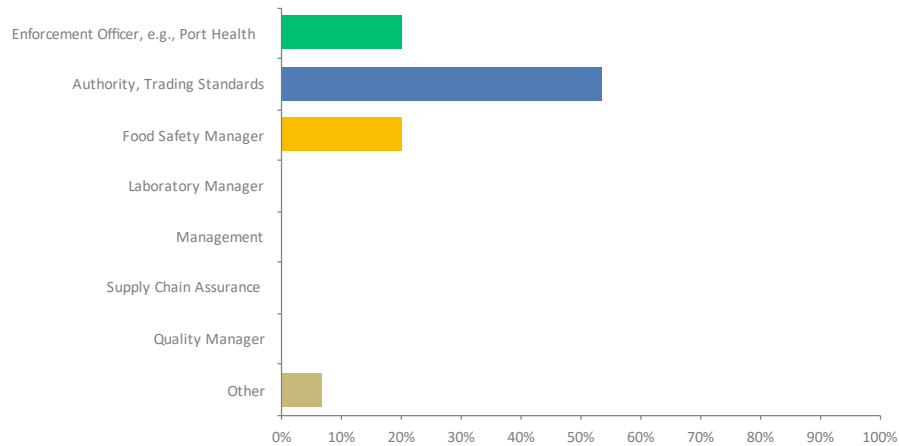


#	OTHER (PLEASE SPECIFY)
1	guidance, specific methods and their application, reference material and databases

11.2 E-survey results - Regulators

Q1: Which of the following best describes your occupation?

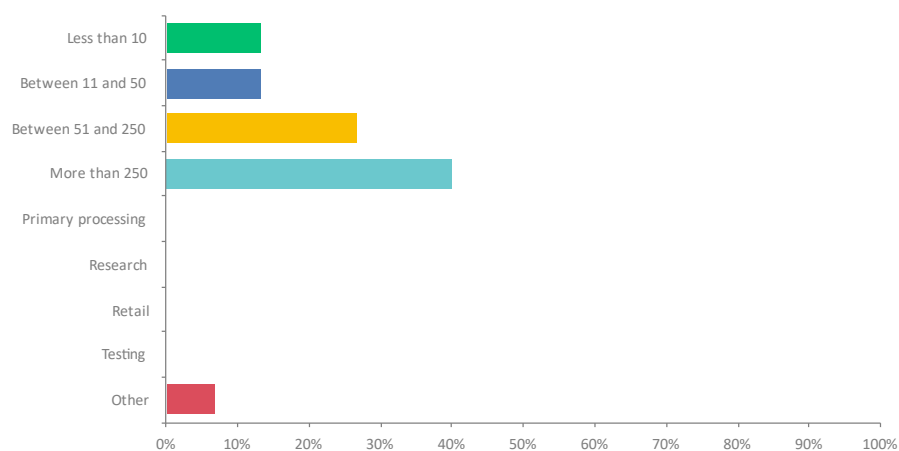
Answered: 15 Skipped: 0



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Q2: Approximate number of employees in your organisation?

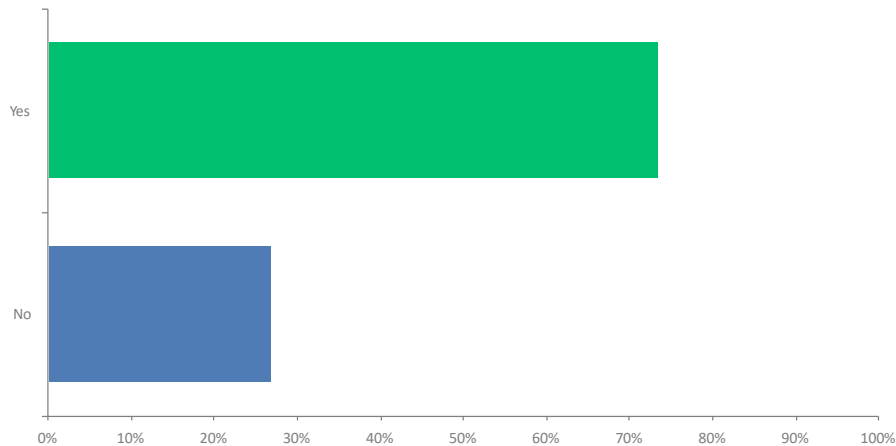
Answered: 15 Skipped: 0



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Q3: Is your organisation's headquarters based in the UK?

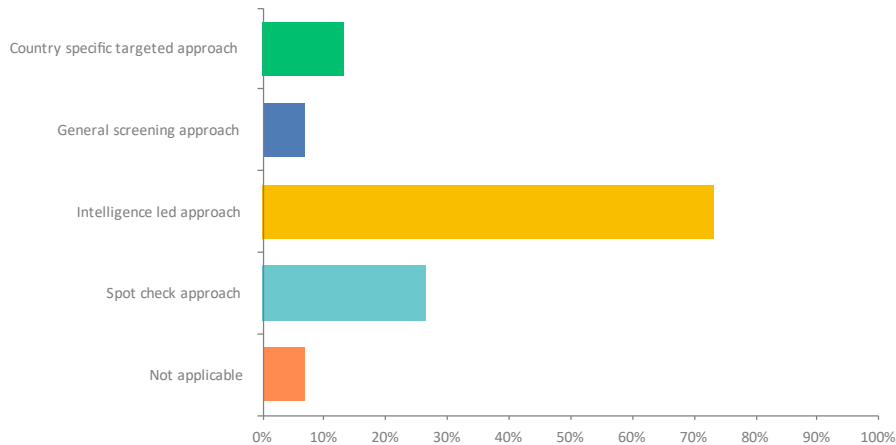
Answered: 15 Skipped: 0



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Q4: What sampling strategy does your organisation generally recommend for authenticity testing of herbs and spices (please select any that apply)

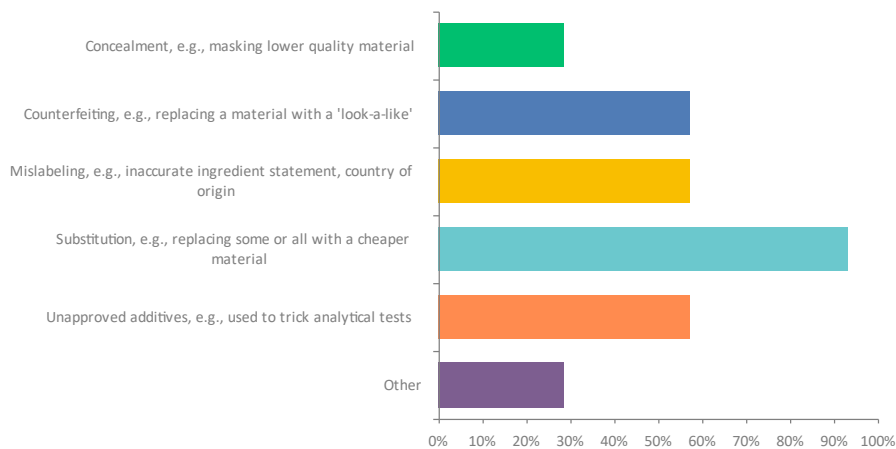
Answered: 15 Skipped: 0



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Q5: Please select the food authenticity issue/s being tested (please select any that apply)

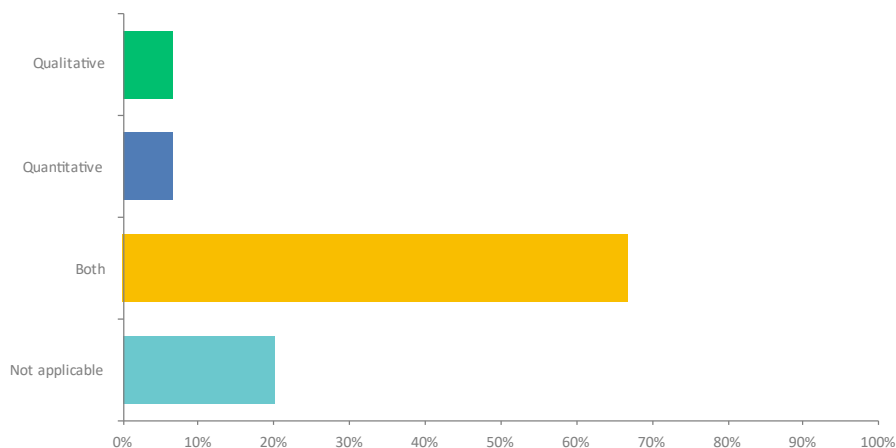
Answered: 14 Skipped: 1



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Q6: Do you require the reporting qualitative or quantitative measurements, or both?

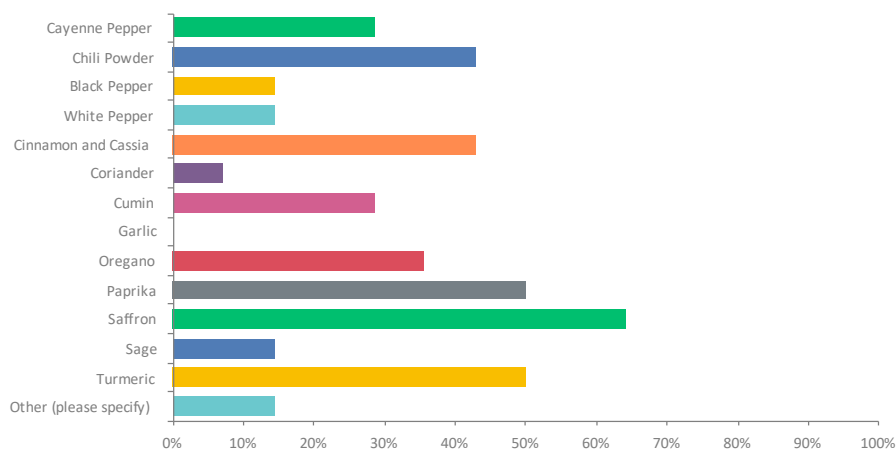
Answered: 15 Skipped: 0



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Q7: What are the top five herbs and spices that are of most concern to your organisation?

Answered: 14 Skipped: 1

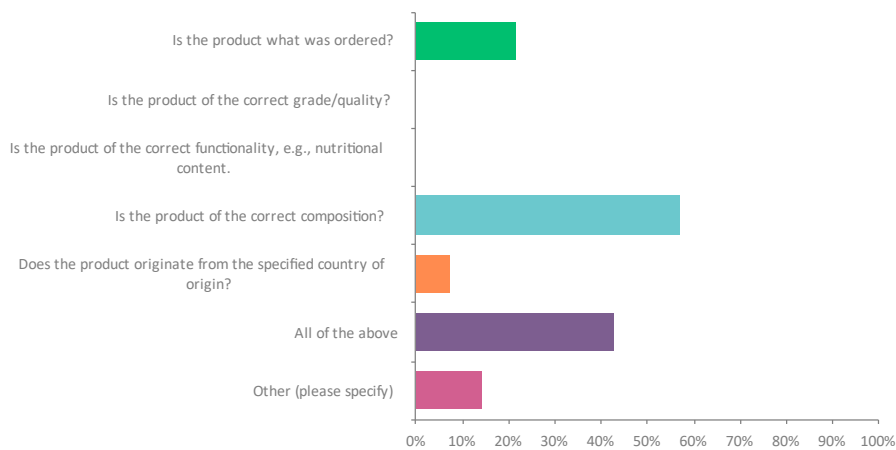


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#	OTHER (PLEASE SPECIFY)
1	We currently are not looking at authenticity
2	no sampling has taken place over the last 12 months

Q8: In terms of authenticity, which of the following are of significant concern to your organisation? (Please select which suggestions apply)

Answered: 14 Skipped: 1

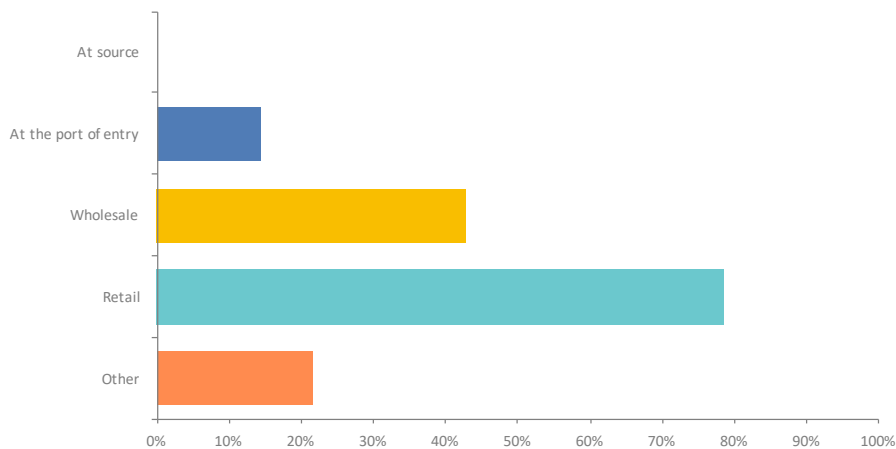


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#	OTHER (PLEASE SPECIFY)
1	Has the product undergone undeclared irradiation treatment
2	Food safety

Q9: Where does your organisation normally survey herbs and spices authenticity? (Please select which suggestions apply)

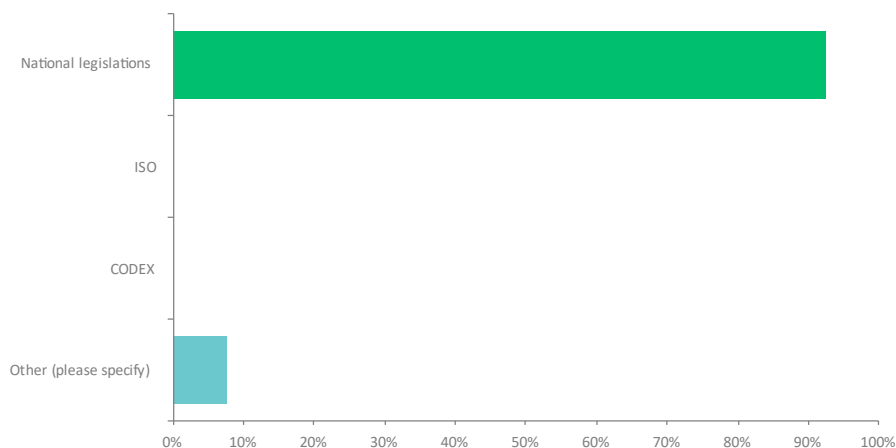
Answered: 14 Skipped: 1



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Q10: In relation to herbs and spices authenticity, what regulatory control does your organisation work to?

Answered: 13 Skipped: 2

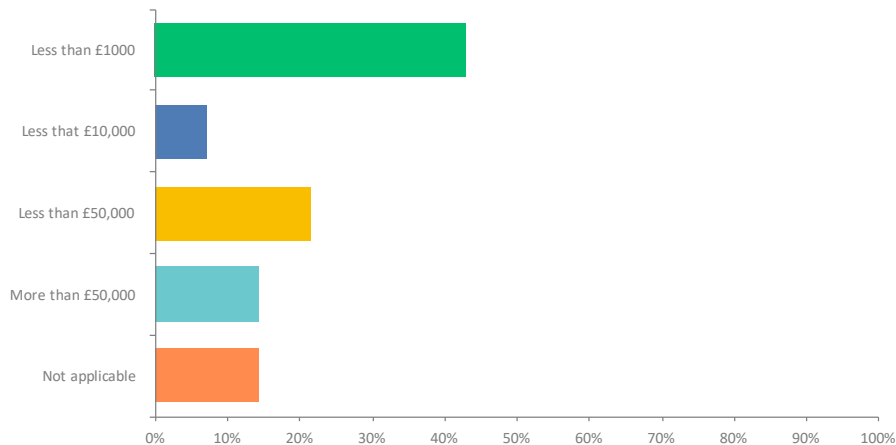


Powered by SurveyMonkey

#	OTHER (PLEASE SPECIFY)
1	EU legislation

Q11: What level of annual expenditure does your organisation allocate ton addressing food authenticity issues?

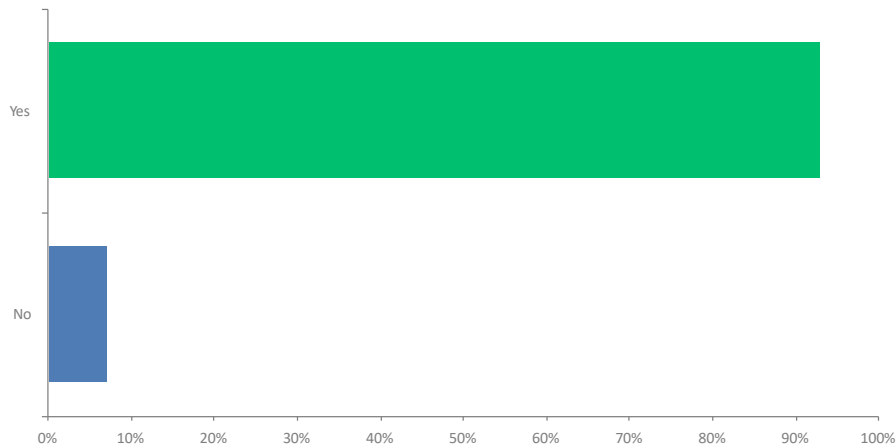
Answered: 14 Skipped: 1



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Q12: Would the availability of additional guidance and best practice for the analysis of herbs and spices be of value to your organisation?

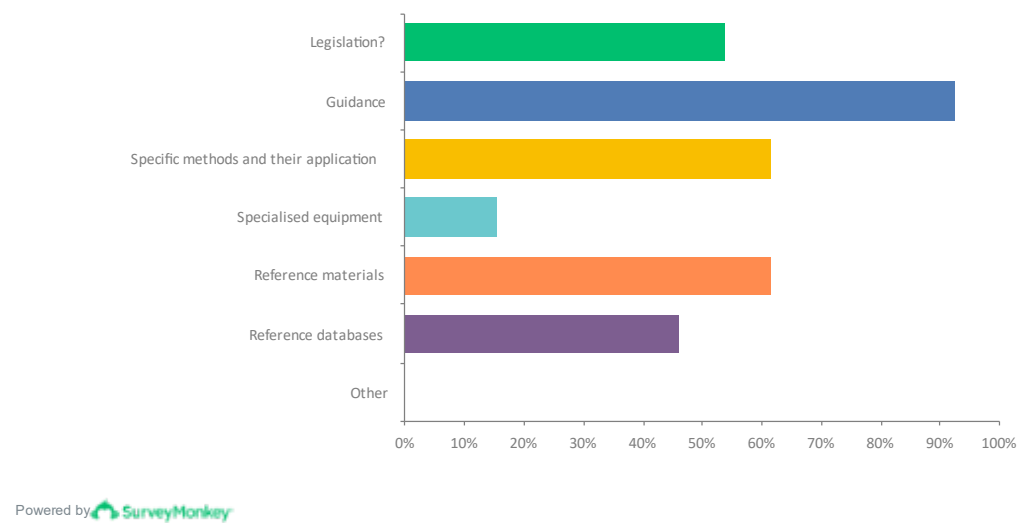
Answered: 14 Skipped: 1



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Q13: If the answer to question 12 was Yes, then what form of guidance would be beneficial (please select which suggestions apply)

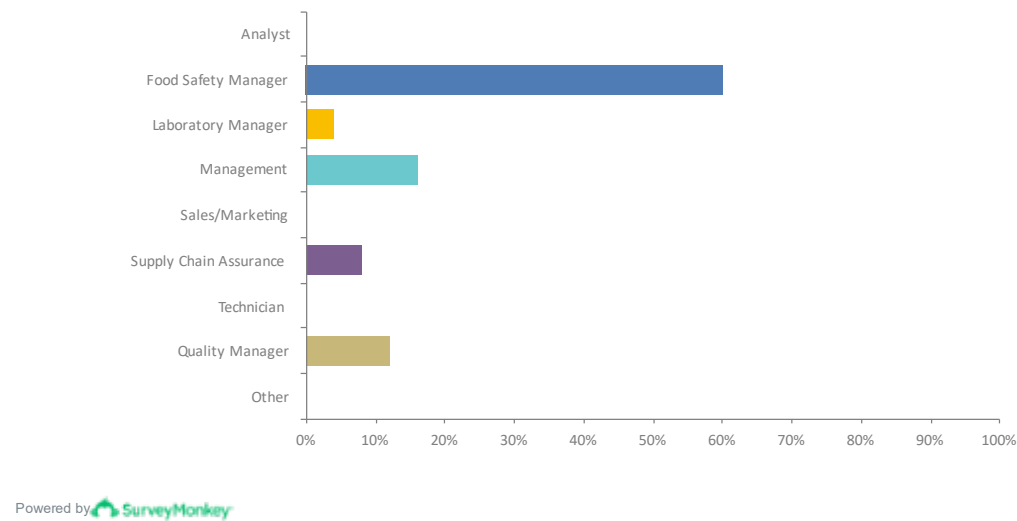
Answered: 13 Skipped: 2



11.3 E-survey results – Industry

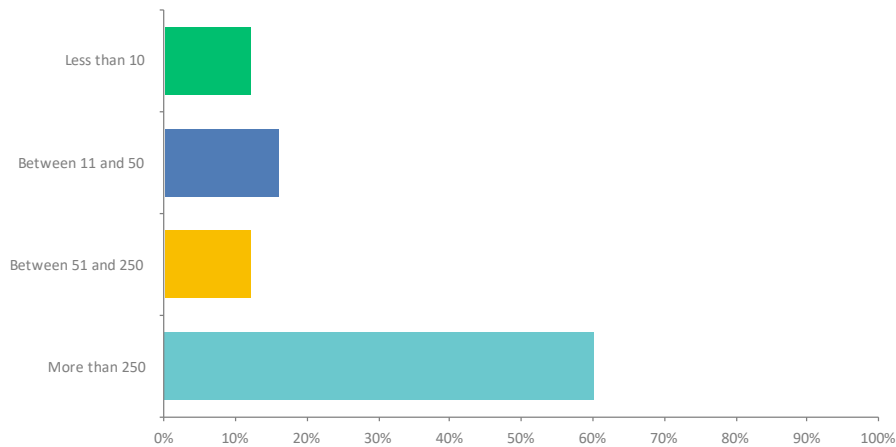
Q1: Which of the following best describes your occupation?

Answered: 25 Skipped: 0



Q2: Approximate number of employees in your organisation?

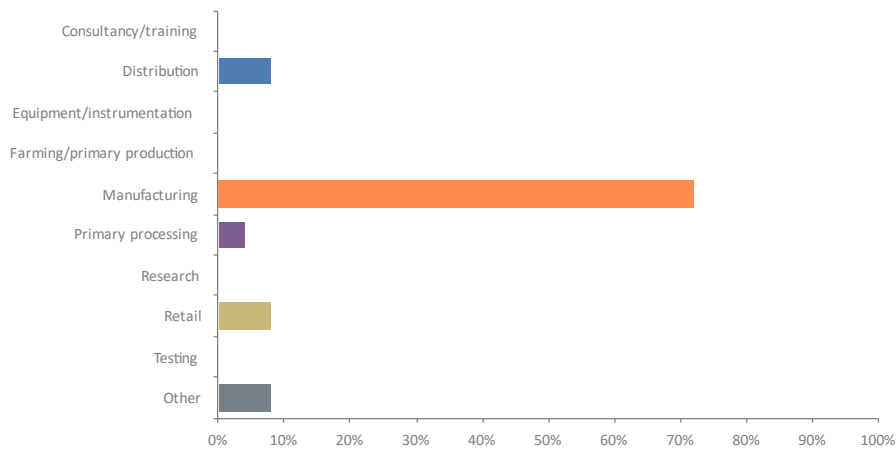
Answered: 25 Skipped: 0



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Q3: Which sector do you work in?

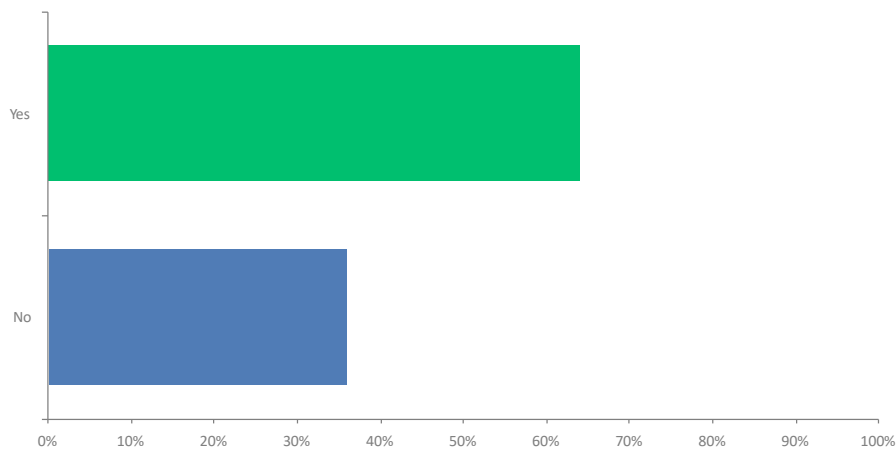
Answered: 25 Skipped: 0



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Q4: Are your organisation's headquarters based in the UK?

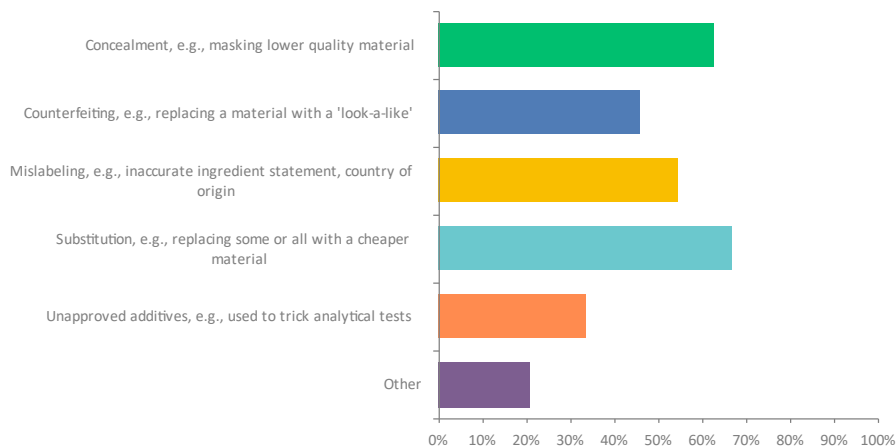
Answered: 25 Skipped: 0



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Q5: Please select the food authenticity issue/s being tested (please select any that apply)

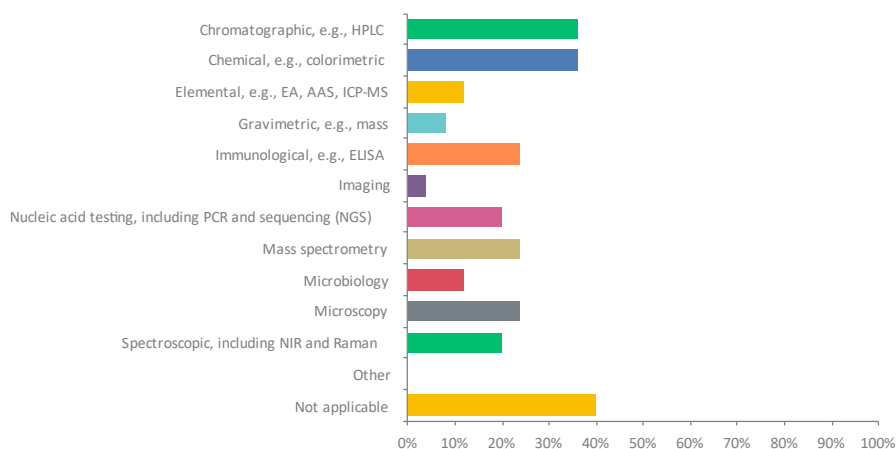
Answered: 24 Skipped: 1



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Q6: Which methods do you generally employ for authenticity testing of herbs and spices? (Please select any that apply)

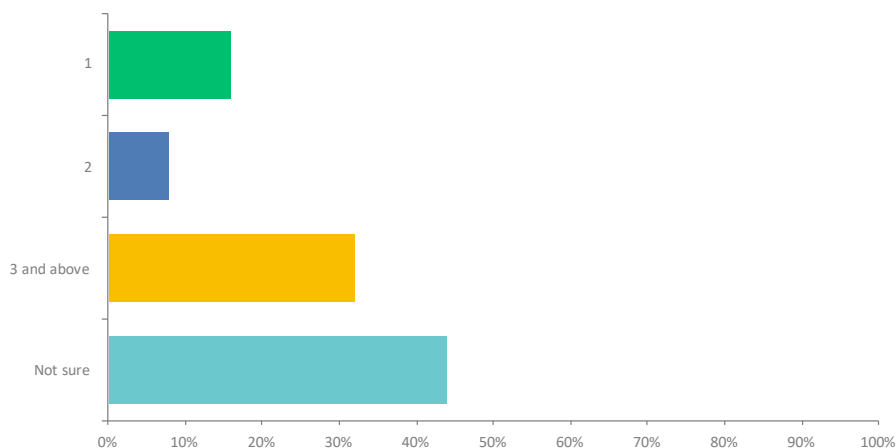
Answered: 25 Skipped: 0



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Q7: For any authenticity testing you request, how many components / targets are evaluated?

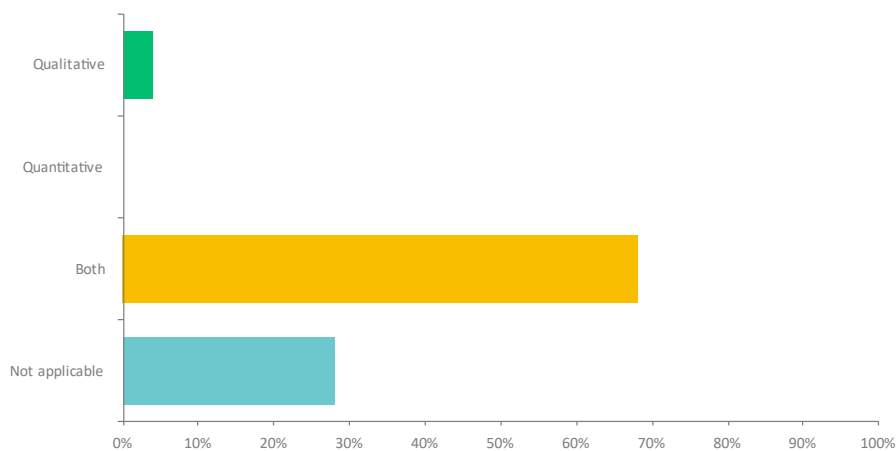
Answered: 25 Skipped: 0



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Q8: Do you require the reporting of authenticity test results to be qualitative or quantitative measurements, or both?

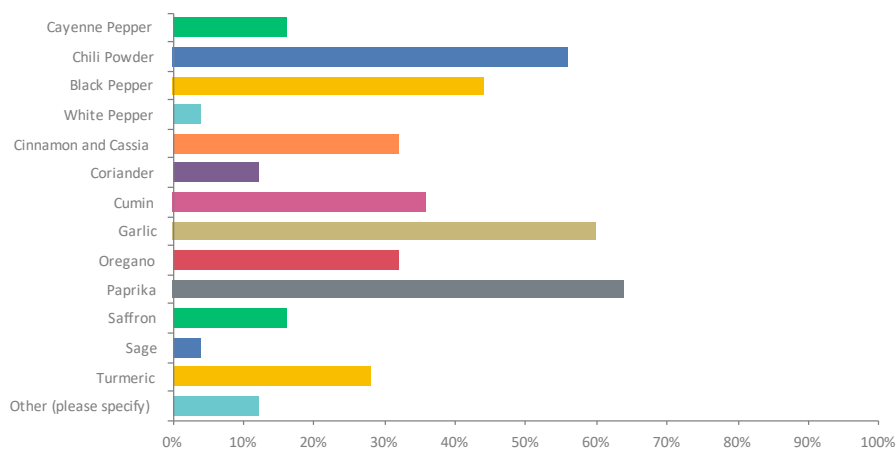
Answered: 25 Skipped: 0



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Q9: What are the top five herbs and spices that are of most commercial concern to your organisation?

Answered: 25 Skipped: 0

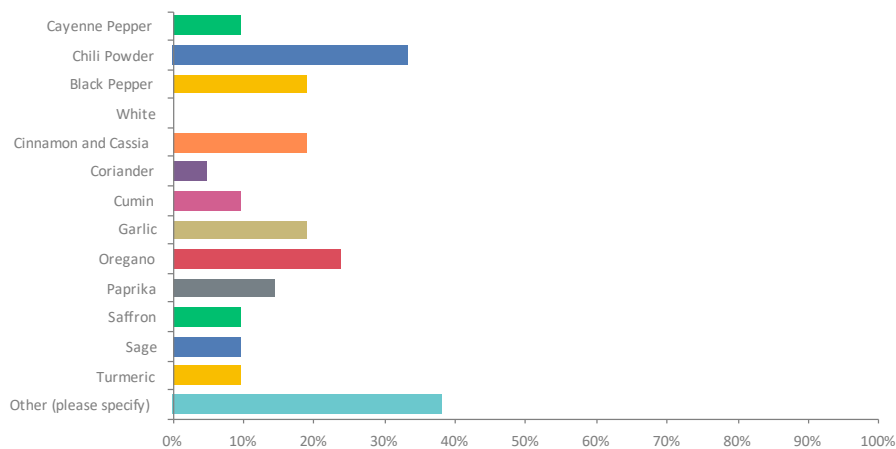


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#	OTHER (PLEASE SPECIFY)
1	Oregano
2	Allspice/Pimento
3	Ginger

Q10: Which herbs and spices do you have the most problem with regarding authenticity?

Answered: 21 Skipped: 4

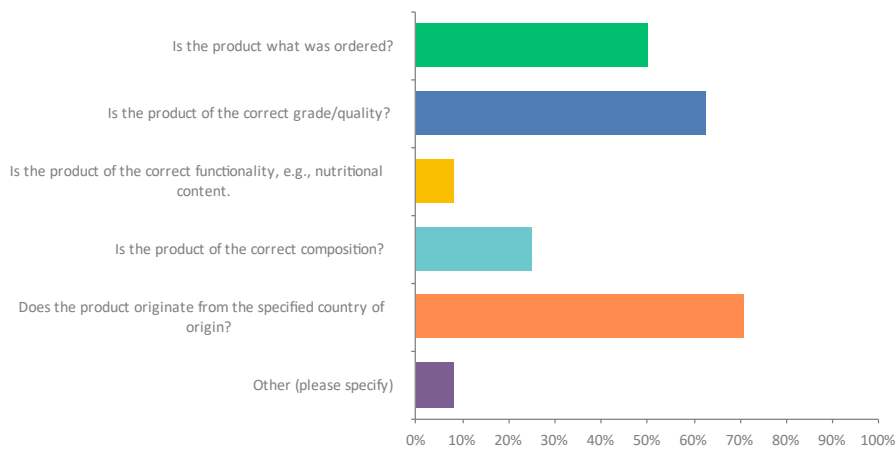


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#	OTHER (PLEASE SPECIFY)
1	oregano
2	So far no issues
3	dried /powdered onion, dried whole chiles
4	we haven't had any issues identified
5	Ginger
6	None. We find that our suppliers are good at protecting us from this
7	Not applicable
8	We have found any issues as of yet

Q11: Which authenticity questions do you most frequently encounter? (Please select which suggestions apply)

Answered: 24 Skipped: 1

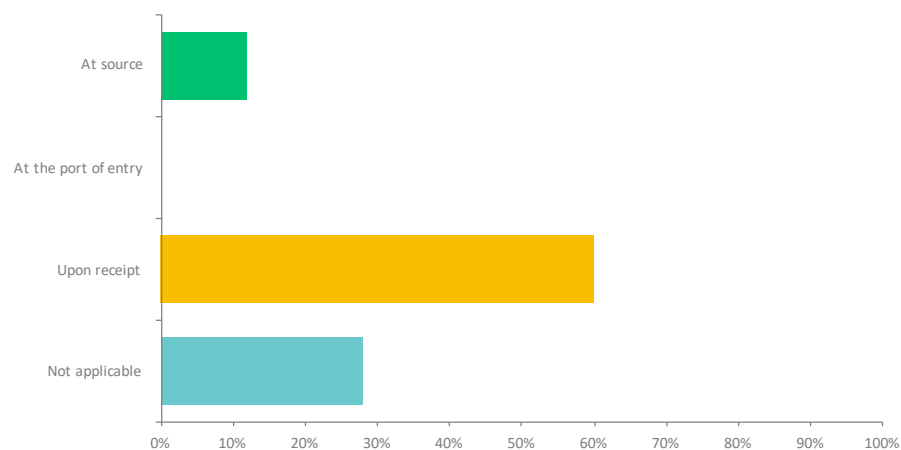


Powered by SurveyMonkey

#	OTHER (PLEASE SPECIFY)
1	bacterial load
2	Not applicable

Q12: At what stage in the supply process does your organisation generally employ authenticity testing?

Answered: 25 Skipped: 0



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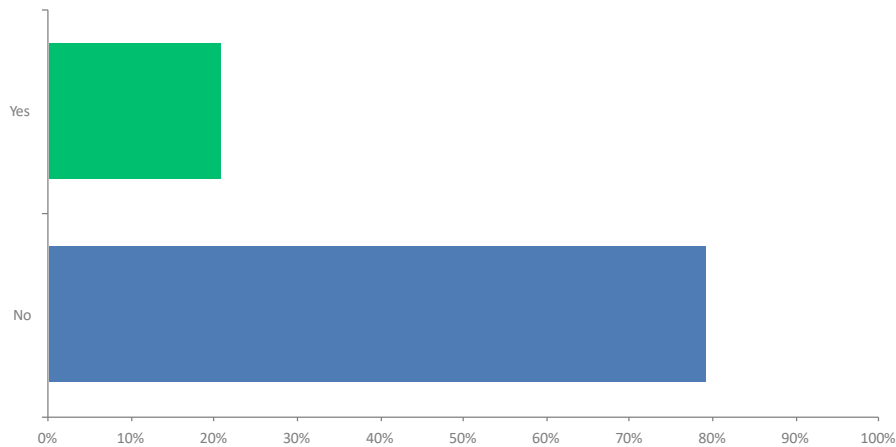
Q13 What standards and regulations in relation to herb and spice composition does your organisation work to?

Answered: 22 Skipped: 3

#	RESPONSES	DATE
1	General UK and export country legislation.	2/10/2023 2:03 PM
2	ISO standards where available and in the absence ASTA standard or internal guideline	2/8/2023 11:13 AM
3	Agreed specifications, customer requirements and legal requirements	2/8/2023 11:08 AM
4	Internal standards and UK/EU legislation	2/8/2023 11:05 AM
5	BRCGS Food Issue 9	2/7/2023 2:28 PM
6	EU & UK Law, BRCGS Food	2/7/2023 11:30 AM
7	EU regulations	2/7/2023 10:58 AM
8	Legislation and customer requirements	2/6/2023 2:14 PM
9	FSA guidance	2/6/2023 12:23 PM
10	FDF & customer specific requirements	2/6/2023 12:06 PM
11	ESA standard/EU and UK Legislation	2/6/2023 9:27 AM
12	FDA	2/3/2023 4:22 PM
13	garanteeing the authenticity (bases BRC/IFS)	2/3/2023 12:25 PM
14	These usually come as part of a compound ingredient so we are relying on supplier assurance	2/3/2023 11:05 AM
15	UK	2/3/2023 8:39 AM
16	We rely on our supplier testing mechanism	2/3/2023 8:18 AM
17	EU / national legislation	2/3/2023 7:32 AM
18	not yet	2/3/2023 1:52 AM
19	American Spice Trade Association	2/2/2023 10:57 PM
20	Not applicable	2/2/2023 8:08 PM
21	The material must meet the agree specification	2/2/2023 6:55 PM
22	USP	2/2/2023 6:45 PM

Q14: Are you aware of any emerging technologies that could be important to verify herb and spice authenticity?

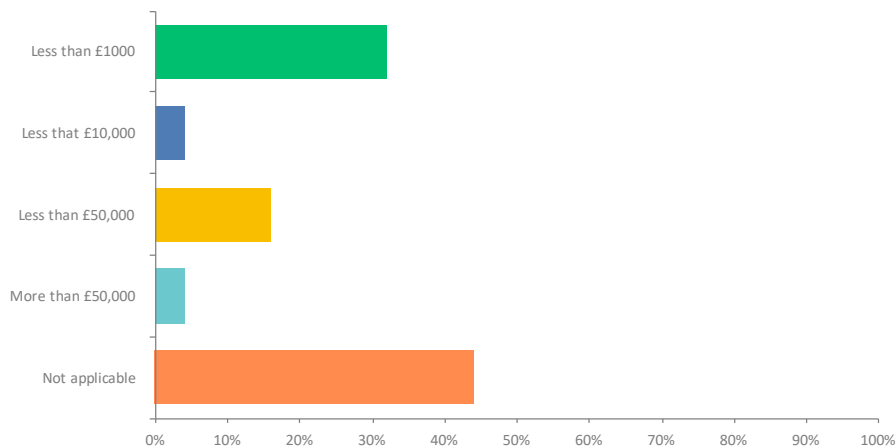
Answered: 24 Skipped: 1



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Q15: What would be an acceptable level of expenditure in adopting a new technology / method for authenticity testing by your organisation?

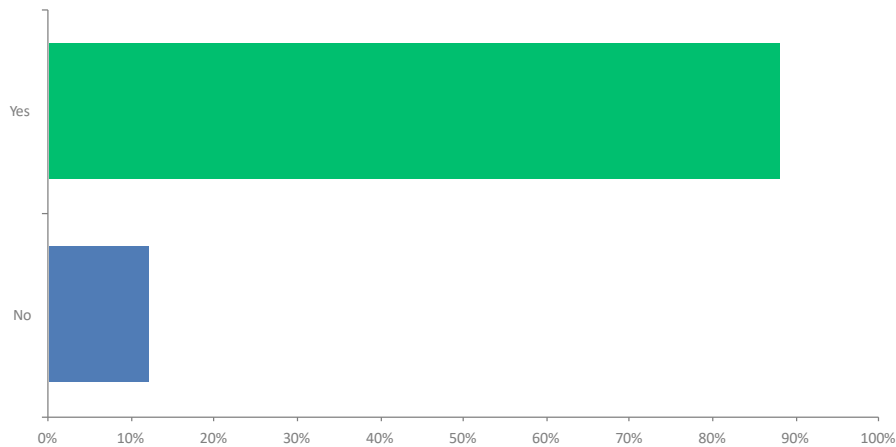
Answered: 25 Skipped: 0



Powered by 

Q16: Would the availability of additional guidance and best practice for the analysis of herbs and spices be of value to your organisation?

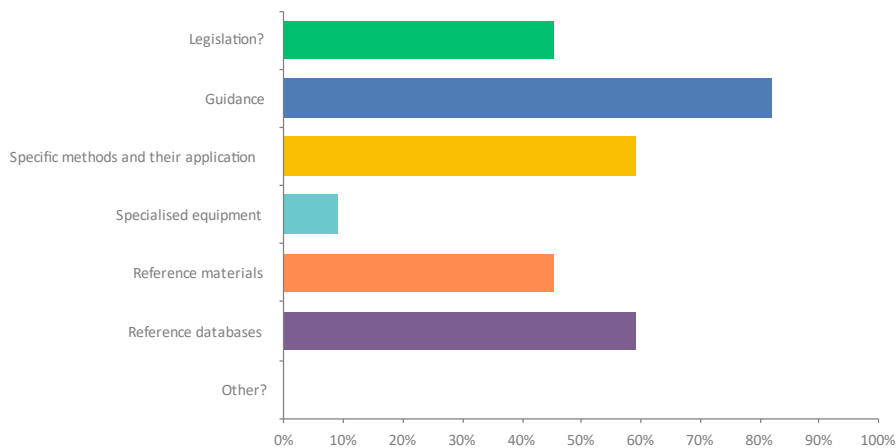
Answered: 25 Skipped: 0



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Q17: If the answer to question 16 was yes, what form of guidance would be beneficial (please select which suggestions apply)

Answered: 22 Skipped: 3



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12. Annex 5: Academic literature review

12.1 Introduction

This is a report of the literature review conducted at Queens University Belfast (QUB) and provides evidence-based recommendations on the most-suitable methods for the verification of the authenticity of herbs and spices for enforcement purposes. It also provides further recommendations for the research and development of current or emerging methods considered suitable for the testing of herbs and spices.

12.2 Methodology

12.2 1 Research aims and objectives

As part of work package 1, task 1b is to undertake a review of current and emerging methods for the analysis of herbs and spices. This will include conducting a thorough review of the literature (both academic and grey) to identify current and emerging targeted and non-targeted analytical methods for the detection, identification and quantification of herbs and spices and their potential adulterants.

The objectives of the literature review are to:

- Produce a summary table comparing the relevant key characteristics/parameters of the identified methods representative of the technologies most used.
- Assess the practicability of testing herbs and spices in laboratories where method of analysis requires the use of a reference database.
- Determine how different targeted and non-targeted methods might be used as part of a screening process to verify evidence of adulteration.
- Document the availability of reference materials and proficiency testing schemes for herb and spice authenticity.

The [Campbell Methods Guide](#) was followed for the retrieval of information for the systematic review. Relevant articles were initially identified using title and abstract

screening and secondly by full text. The records were double screened by two researchers and discrepancies settled by both researchers. All citations were exported to EndNote and duplicates removed prior to full text screening.

12.2.2 Search strategy

A review of peer-reviewed and grey literature was conducted to understand and obtain empirical evidence of what works in practice rather than the conceptual models or frameworks that have yet to be evaluated. Electronic searches of the academic literature were performed using the following databases: Scopus, Web of Science and PubMed. The following keywords and search strings were applied to the electronic literature databases outlined previously.

(Herb*) AND (spice*) AND (authenticity OR fraud* OR adulteration* OR analysis* OR methods* OR validation* OR targeted* OR non-targeted* OR untargeted* OR commercial rapid test*).

12.2.3 Inclusion criteria

Studies were identified by searching literature published in English between 2015 and December 2022. These dates are particularly relevant as a follow on to the review published by LGC in 2013, the FSA and LGC's influence in the UK's response to the 2015 allergens in spice issue and the rapid method based on FTIR and chemometrics developed by QUB in 2015 to determine oregano authenticity. Whilst these methods and recommendations remain valid the work conducted in this review provides a vital update in line with current technologies, methods and best measurement practice guidance advice that have evolved since 2015. No restrictions were placed on geographical regions and no study design was specified. The search strategy was piloted to test the suitability of the electronic databases and the selected keywords. Research articles were cross-referenced with the literature included in the published review articles to ensure that all methodologies from 2015 were included.

12.2 4 Exclusion criteria

Any publications not relevant to herb and spice authenticity were excluded including, those articles which only mention that the developed methods could be useful to confirm the

authenticity or adulteration of herb and spices. If the method was not tested, validated, or originally designed for the purpose of determining authenticity/adulteration in herbs and spices and shows no evidence of this the articles were removed. Conference proceedings were excluded as access was restricted and the results obtainable from abstracts is limited. Review articles were excluded (but used to cross-reference with the research articles) and references within the reviews outside of the 2015 to 2022 scope were excluded. All duplicates across the three academic databases were removed.

12.2 5 Critical Appraisal

Critical appraisal of the publications ensured that only relevant high-quality studies were included in the review and low-quality studies were excluded. To be included in the review, papers had to adequately answer citation, title, and abstract screening questions such as:

- Does the citation indicate that the publication is within the time period specified?
- Is the title and abstract in English?
- Is the aim of the study highlighted clearly?
- Does the abstract mention determination or detection of herb and spice authenticity/adulteration?

If the answers to the above questions were unclear after first title and abstract screening, the articles must answer the following questions during the second full text screen to be included in the review. These questions included:

- What were the main findings of the paper?
- Was the technologies' main purpose to detect/determine herb & spice authenticity?
- What was the strengths and limitations of the methodology?
- Did the author acknowledge any limitations?

12.3 Literature review results

Using the described search strategy, 461 references were retrieved and subjected to screening and critical appraisal. Both qualitative and quantitative studies were included in the review. Overall, 101 papers passed the critical appraisal process and were included. A summary of the critical appraisal process has been included in Figure 1.

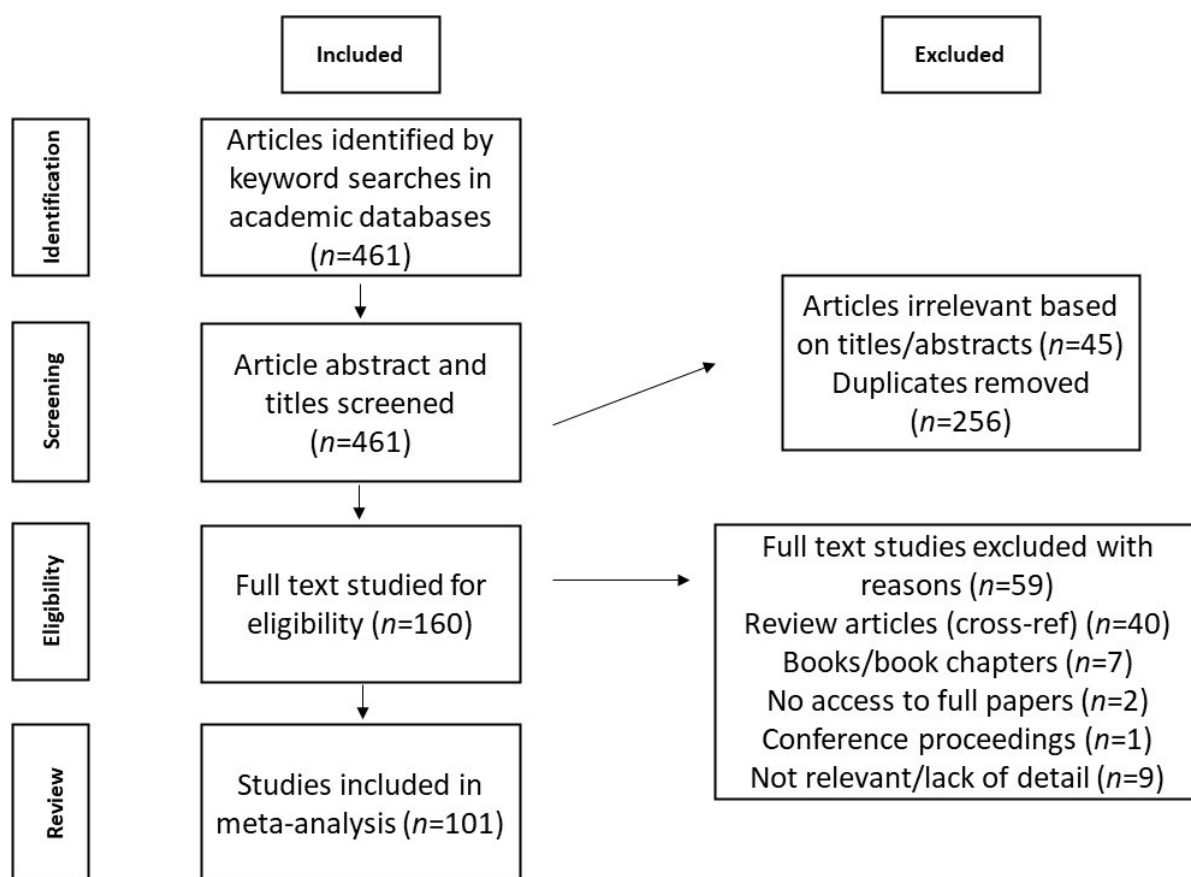


Figure 1: Summary of screening and critical appraisal process performed to obtain research articles suitable for review (n=101).

Results of the grey literature search is presented in Annex 6. Sources of information retrieved included international and government agency websites, providing legislative documents, reports, guidelines, and standards.

The findings of the literature review are stored in four Excel databases, organised by technique (LGC/R/2023/858_Annex5_Research Article Databases 1 - 4), which give details, key findings/conclusions and methods used for each research article reviewed (n=101). The four Excel databases have been provided by email to the FSA.

The databases provide information on:

- sample matrix
- targeted or non-targeted
- qualitative or quantitative
- analytical technique
- data analysis

- sensitivity
- specificity
- sample collection and validation parameters
- reference databases and software required
- expense
- transferability
- availability of instrumentation
- difficulty level
- portability
- time to result
- sample preparation
- advantages of results/methods
- limitations of results/methods
- author
- date
- URL/DOI link.

If the parameter was not applicable 'n/a' was recorded. If the information was not provided by the author 'not specified or not reported' was recorded in the databases.

A full list of the academic research papers reviewed in the Excel databases are detailed below (Di Anibal, Rodriguez et al. 2015, Haughey, Galvin-King et al. 2015, Parvathy, Swetha et al. 2015, Rajabi, Sabzalian et al. 2015, Rani, Medhe et al. 2015, Black, Haughey et al. 2016, Chen, Yu et al. 2016, Genualdi, MacMahon et al. 2016, Kim and Baik 2016, Rubert, Lacina et al. 2016, Tahri, Tiebe et al. 2016, Zhao, Shi et al. 2016, Hu, Wang et al. 2017, Lohumi, Joshi et al. 2017, Osathanunkul, Ounjai et al. 2017, Petrakis, Cagliani et al. 2017, Petrakis and Polissiou 2017, Swetha, Parvathy et al. 2017, Villa, Costa et al. 2017, Horn, Esslinger et al. 2018, Kar, Tudu et al. 2018, Matsushita, Zhao et al. 2018, Schwack, Pellissier et al. 2018, Vannozzi, Lucchin et al. 2018, Vera, Ruisanchez et al. 2018, Wielogorska, Chevallier et al. 2018, Barbosa, Nogueira et al. 2019, Bessaire, Savoy et al. 2019, Dhakal, Schmidt et al. 2019, Drabova, Alvarez-Rivera et al. 2019, Galvin-King, Haughey et al. 2019, Hansen, Almonacid et al. 2019, Hwang, Moon et al. 2019, Kar, Tudu et al. 2019, Khilare, Tiknaik et al. 2019, Kiani, van Ruth et al. 2019, Kong, An et al. 2019, Marquez, Ruisanchez et al. 2019, Morozzi, Zappi et al. 2019, Orrillo, Cruz-Tirado et al. 2019, Parveen, Tehen et al. 2019, Rohaeti, Muzayanah et al. 2019, Silvis, Luning et al.

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A review of the research articles listed above ($n=101$) revealed a total of 132 analytical techniques used for herb and spice authenticity between 2015-2022, as 31% of the reviewed research articles performed more than one analytical technique. For example, using a combination of both screening and confirmatory analysis. Therefore, all targeted and untargeted technologies were counted individually across all 101 papers. The results confirmed that 52% (66/132), 23% (30/132), 14% (18/132) and 12% (16/132) of all analytical techniques reported were spectroscopy-based, mass spectrometry-based, DNA-based and a combination of other techniques, respectively (**Figure 2**).

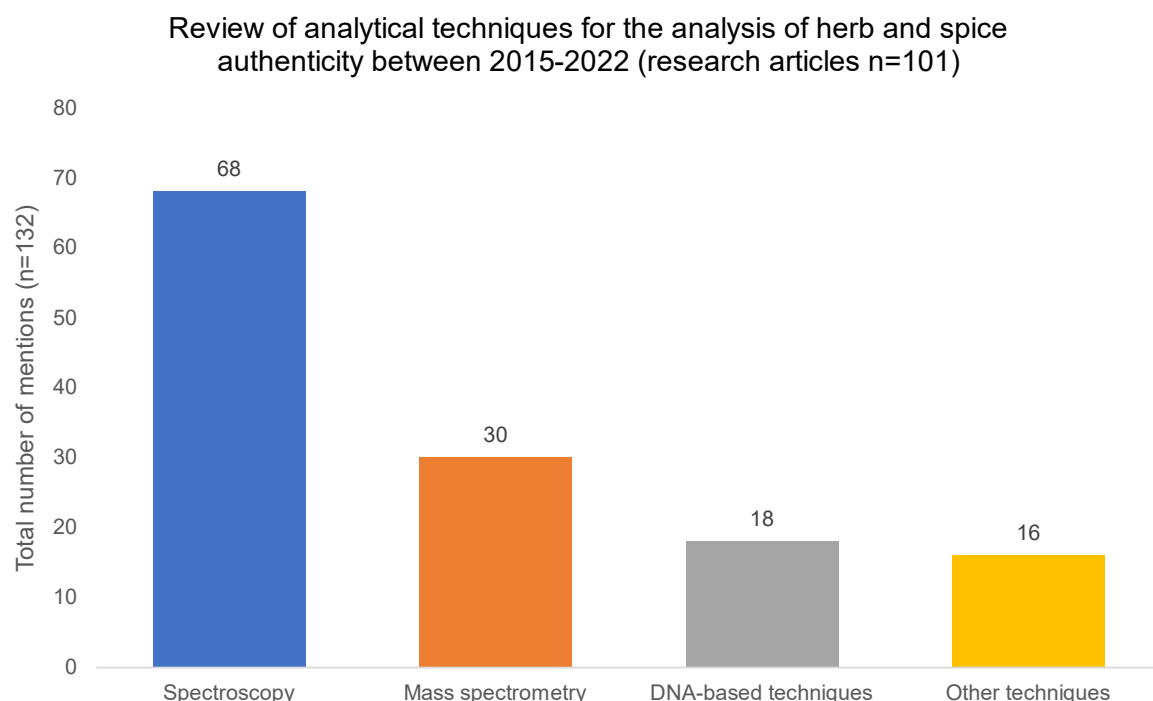


Figure 2: Review of analytical techniques (n=132) for the analysis of herb and spice authenticity published between the years 2015-2022.

Additionally, the number of reviewed articles exploiting chemometric and machine learning algorithms was highlighted. The results confirmed that 74 out of 101 (73%) papers exploited these methods of data analysis, whilst 27 out of 101 (27%) papers did not (**Figure 3**). This result was higher than expected, considering most analytical techniques which did not use chemometrics or data analytics were DNA-based technologies.

Overall this highlights the popularity and importance of using advanced data analytics in combination with both targeted and untargeted methodologies to assist in the analysis/detection of herb and spice authenticity/adulteration.

% of analytical techniques performing chemometrics (n=101)

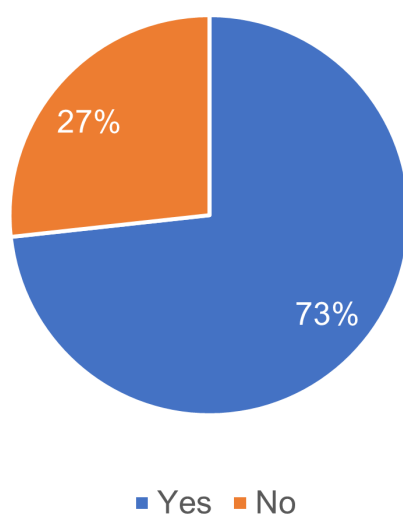


Figure 3: Number of reviewed research articles reporting chemometric and machine learning algorithms for data analysis.

Finally, a thorough review of the analytical techniques performed for herb and spice authenticity analysis revealed that NIR spectroscopy (22) and FT-IR spectroscopy (20), LC-MS (11) and GC-MS (10), DNA barcoding (10) and PCR (4) and HPLC (inc. TLC and HPTLC) (6) and GC (inc. E-nose and E-tongue) (5) were the most frequently reported analytical techniques across the four categories of spectroscopy (**Figure 4**), mass spectrometry (**Figure 5**), DNA-based technologies (**Figure 6**) and other reviewed technologies (**Figure 7**), respectively.

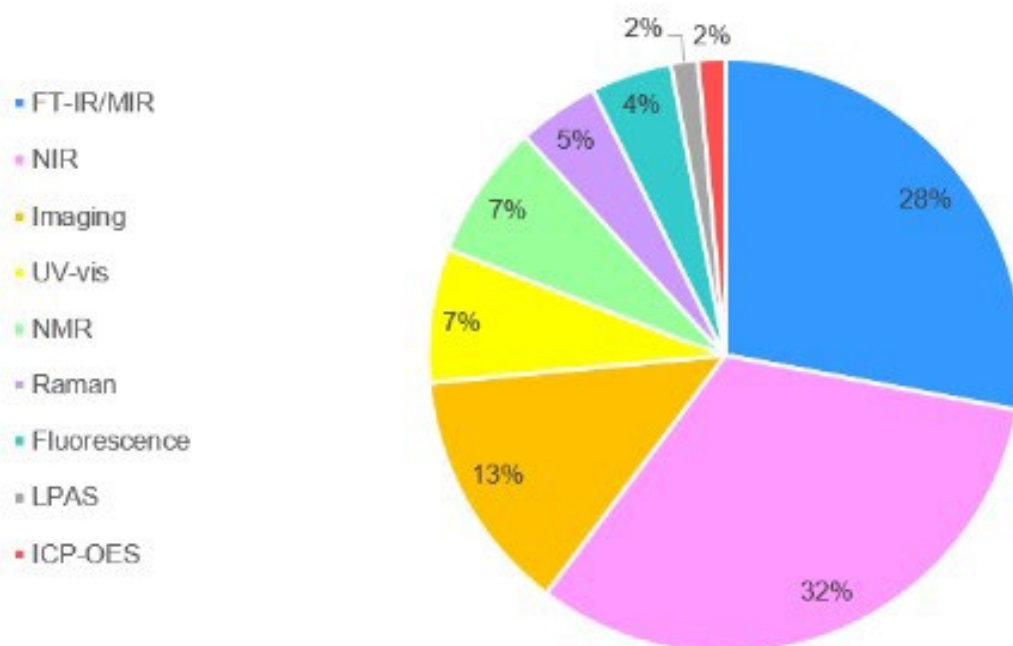


Figure 4: Spectroscopy techniques for herb & spice authenticity (n=68)

Figure 4: Spectroscopy techniques for herbs and spice authenticity (N=68)

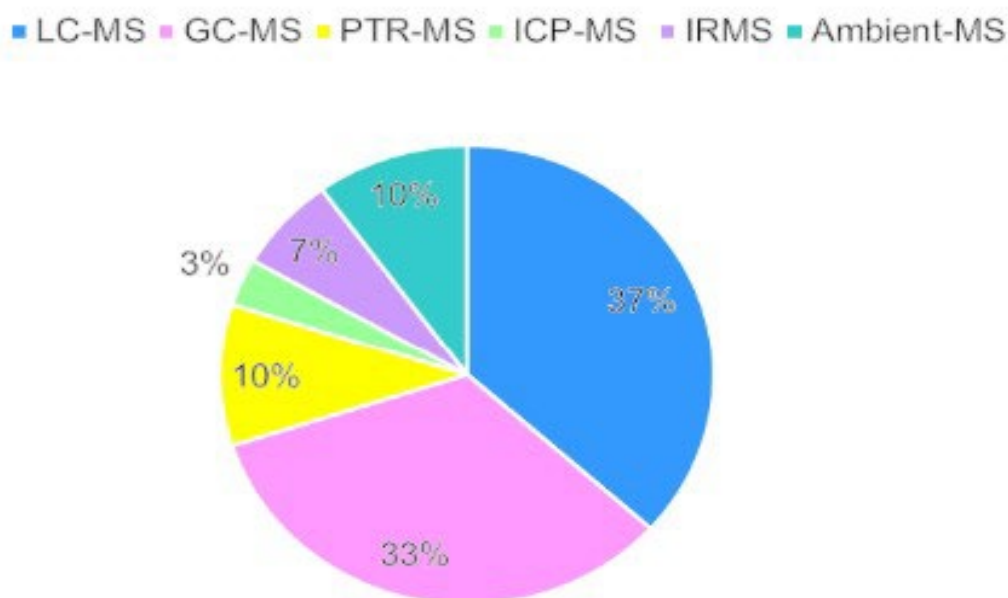


Figure 5: Mass spectrometry techniques for herb & spice authenticity (n=30)

Figure 5: Mass spectrometry techniques for herbs and spice authenticity (n=30)

■ DNA barcoding ■ PCR

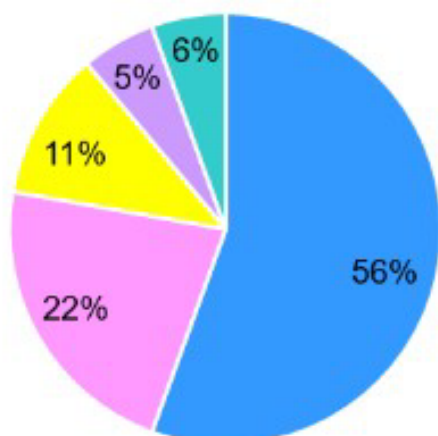


Figure 6: DNA-based techniques for herb & spice authenticity (n=18)

Figure 6: DNA-based techniques for herbs and spices authenticity (n=18)

■ HPLC/TLC/HPTLC
 ■ GC-FID/enose/nose
 ■ Microscopic analysis
 ■ FIAC
 ■ QCM
 ■ LFD

Legend

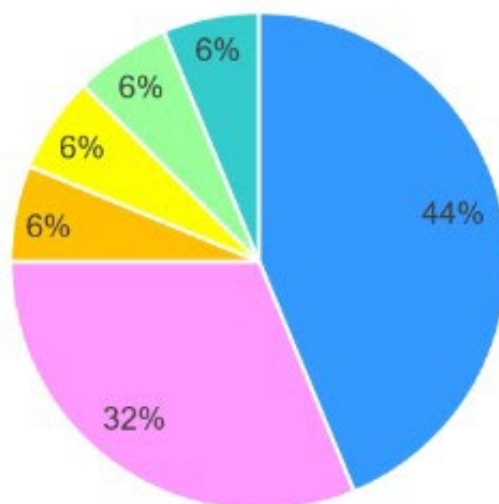


Figure 7: Other techniques for herb & spice authenticity (n=16)

Figure 7: Other techniques for herbs and spice authenticity (n=16)

Overall, these results confirm the wide range of techniques that have been developed by researchers to determine/detect herb and spice authenticity / adulteration between 2015-2022. However, many of the methodologies still rely on spectroscopy and mass spectrometry-based techniques. Also, the number of spectroscopy-based technologies reviewed outnumbered the mass spectrometry, DNA-based and other technologies combined, which could be an important result going forward.

13. Annex 6: Grey literature review

The Google search engine was interrogated to identify any relevant international, government and commercial entities associated with authenticity testing for herbs and spices and identify any other relevant grey literature associated with the topic. Due to the nature of this search, many research articles were found as Google Scholar is part of the Google search engine but are not reported here as academic databases were interrogated separately. Many of the hits were also associated with contamination events related to food safety, e.g. mycotoxins, pesticides, and are not included as this review as the remit was specifically for methods related to herb and spice authenticity analysis.

However, information to the testing of herbs and spices for authenticity was identified as presented below:

13.1 International Standards/UK and Global Trade Bodies

13.1.1 ISO

There are a series of [ISO International Standards](http://www.iso.org/standard) for Herbs and Spices that can be purchased from www.iso.org/standard. An example is the international standard for Oregano (ISO 7925) and it specifies extraneous matter as “all that does not belong to the leaves of oregano (*Origanum* genus, species and sub-species) and all other extraneous matter of animal, vegetable and mineral origin shall be considered extraneous matter”. It goes on to say that extraneous matter should be >1% for processed oregano and 3% for semi-processed oregano. The Standard then says that the extraneous matter should be determined using ISO 927, which states the following:

“ISO 927:2009 specifies a general procedure for visual examination, or with magnification not exceeding 10 times, of whole spices for the determination of macro filth. ISO 927:2009 is applicable to dehydrated herbs and spices.”

So according to these ISO Standards **visual inspections and magnification** are the recommended methods. No mention of modern analytical methods or issues related to EMA.

13.1.2 [Codex](#)

The Codex Alimentarius is a collection of internationally adopted food standards and related texts presented in a uniform manner. These food standards and related texts aim at protecting consumers' health and ensuring fair practices in the food trade.

The publication of the Codex Alimentarius is intended to guide and promote the elaboration and establishment of definitions and requirements for foods to assist in their harmonization and in doing so to facilitate international trade.

Codex has produced commodity standards for nearly 200 traded products, from apples and wheat to frozen fish and bottled water, which define the physical and chemical characteristics of the foods.

Refer to section 3.5 for Codex information relating specifically to herbs and spices.

13.1.3 [AOAC](#)

AOAC INTERNATIONAL brings together government, industry, and academia to establish standard methods of analysis that ensure the safety and integrity of foods and other products that impact public health around the world.

AOAC's Food Authenticity Methods (FAM) program focuses on identifying analytical tools to better locate and characterize the intentional and economically motivated adulteration of foods.

The FAM launched with two working groups (Targeted and Non-Targeted Testing) with the following goals:

- Survey the Targeted Testing landscape to identify most commonly tested ingredients and their associated targeted adulterants
- Identify existing methodologies for food fraud Targeted Testing and their (AOAC) status and identify reference materials and Proficiency Testing programs.
- Identify Targeted Testing analytical gaps and set priorities for developing AOAC standards
- Map an accelerated process for AOAC Targeted Testing standards development and review for use in the event of a major international food fraud incident

- Develop standards for Non-Targeted Testing (NTT) of foods including a generic SMPR to evaluate NTT methodologies for their reliability and usefulness
- Develop acceptance criteria for NTT methodologies for use as ingredient screening tools in domestic and international trade.

To date, six Standard Method Performance Requirements (SMPRs®) have been produced for vanilla, saffron, and turmeric, honey, milk products, and extra virgin olive oil.

13.1.4 Seasoning and Spice Association (SSA)

The mission of the SSA is to be the leading voice of the UK Seasoning and Spice industry in the interests of members, food manufacturers and consumers alike. Its principal objective is to promote the interests of its members in all aspects of the importation, processing and distribution of seasonings, herbs, spices and related products. This organisation in partnership with the Food and Drink Federation and the British Retail Consortium published “*Guidance on Authenticity of Herbs and Spices: Industry Best Practice on Assessing and Protecting Culinary Dried Herbs and Spices*”. This document was developed to provide Industry Best Practice Guidance on vulnerability assessment for culinary dried herbs and spices (including blends), in order to mitigate against potential adulteration and substitution. It also recommends using the following methods to determine adulteration depending on the issue to be addressed: **visual inspection, microscopy, HPLC, LC-MS.**

13.1.5 European Spice Association (ESA)

ESA a non-profit association representing the interests of its members in all matters pertaining to the processing, packing, quality assurance and food safety and/or marketing of herbs, spices and spice products. The objectives of this organisation are as follows:

- “-represent the interests of its members vis-à-vis the competent bodies and departments of the European Union, as well as international institutions and organisations;
- promote the interests of members in respect of the products concerned and to protect the image of the products and the sector;
- study subjects of common interest to the members in the scientific, legislative, technological and economic fields.”

ESA have produced a publication called "[European Spice Association Quality Minima Document](#)" that describes the quality minima for dried herbs and spices, which should be demanded by buyers when these products are purchased for further processing within the EU. The document specifically mentions "adulteration" and says that commodities "should be free from" and also refers to another publication called "*ESA Adulteration Awareness Paper*".

In terms of methodologies to determine extraneous matter (Herbs max. 2%, Spices max. 1% by weight), the quality minima document refers to the ISO Standards. However, their adulteration awareness document gives other examples of the tests that should be used to determine adulteration e.g. (i) **Targeted analysis**, such as dye testing; (ii) Confirmation of purity using **non-targeted fingerprinting techniques** and **chemometrics**; (iii) Organoleptic assessment; (iv) **Microscopic** Identification. ESA have also published a white paper on the use of **Next Generation Sequencing (NGS)** analysis applied to herbs and spices. The main recommendations were as follows:

- NGS is not able to detect any adulterations with economically motivated additions of endogenous material.
- NGS can be a secondary tool to confirm and identify only additions of exogenous plant matters containing DNA when detected by reference methods.
- NGS is not able to detect any adulterations with economically motivated additions of materials not containing DNA.
- NGS should not be used as a direct tool to authenticate herbs and spices nor to detect or quantify them as ingredients in mixtures.
- Reference methods like classical microscopy or validated non-targeted chemical/physical methods (using **NMR**, **NIR/MIR spectroscopy**, **mass spectrometry**) or a combination of them should be used as primary analyses to prove herb and spice authenticity.
- NGS methods using short DNA fragments should be chosen to mitigate the effect on analysis results of processes applied to herbs and spices (milling, steam-treatment, etc.).
- Any internal herb and spice databases should be stated, and the use of reliable reference materials should be demonstrated.

13.1.6 [American Spice Trade Association \(ASTA\)](#)

The American Spice Trade Association represents the interests of the U.S. spice industry (approximately 200 members) including companies that grow, dehydrate, and process spices. ASTA's members include U.S.-based agents, brokers and importers, and companies based outside of the U.S. that grow spices and ship them to the U.S. and other companies associated with the U.S. spice industry. ASTA members manufacture and market the majority of spices sold in the U.S. for industrial, food service and consumer use. ASTA works to ensure the supply of clean, safe spice, shape public policy on behalf of the global industry, and advance business interests of its members. The Official Analytical Methods of the American Spice Trade Association provide the industry standard for analytical testing of spices and includes all previous existing ASTA methods, revised and updated, as well new methods. ASTA members can download individual methods for free or purchase hard copies at the following rates: \$50.00 for Members, \$125.00 for US Non-Members, \$150.00 for International Non-Members.

Adulteration is one of the major concerns of the spice industry and ASTA has produced a guidance document ([Identification and Prevention of Adulteration](#)), which provides a tool for companies to identify circumstances when adulteration may occur and then take steps to prevent it. The ASTA guidance is based on a publication developed by the British Retail Consortium, the Food and Drink Federation and the Seasoning and Spice Association³. The recommended methods for analysis include macroscopic, microscopic, TLC, HPLC, LC-MS depending on the issue to be addressed and it mostly recommends methods in their Official Analytical Methods publication.

13.2 Articles and Blogs

Since the [WHICH?](#) Magazine study from 2015, which suggested that up to 25% of oregano sold in the UK and Ireland was adulterated, there has been a number of articles written on this issue and more surveys have been undertaken.

In 2016, CHOICE consumer magazine in Australia tested 12 different brands of oregano using **FT-IR and chemometrics** method of analysis. This represented ~83% of brands of oregano sold by retail outlets. The survey found that only five of twelve samples tested were 100% authentic oregano. The tests indicated that food fraud is occurring in the

supply chain of oregano, with seven of the twelve dried oregano samples containing other ingredients, including olive leaves and sumac leaves.

In November 2016, on the back of these survey results, the [Australian Competition and Consumer Commission \(ACCC\)](#) acted against a number of brands sold in Australia. Aldi and Menora had to regularly commission tests to prove that products labelled as 'oregano' are authentic. The brands have also agreed to test other herb and spice products for possible adulteration. The ACCC took a somewhat lighter touch with smaller brands G Fresh Oregano Leaves, Mediterranean, Master of Spices Oregano Leaves and Spice & Co. Oregano Leaves, who have agreed through an "administrative resolution" to stop supplying adulterated oregano products and make sure their oregano products remain unadulterated going forward.

This was followed by another ruling in December 2016 where ACCC accepted a court enforceable undertaking from Anchor Foods Pty Ltd trading as Spencers Gourmet Trading, following an investigation into alleged misrepresentations made as to the composition of Spenders' 'oregano' product. In an enforceable undertaking given to the ACCC, Spencers had undertaken to obtain annual testing of its oregano product by an internationally accredited testing laboratory, as well as testing of random samples of its other herbs and spices products for a period of three years.

Furthermore, in 2017, Hoyt Food Manufacturing Industries Pty Limited (Hoyt's Food) paid a penalty of \$10,800AUD following the issue of an [infringement](#) notice by the ACCC. The ACCC issued the infringement notice because it had reasonable grounds to believe that Hoyt's Food had made false or misleading representations in contravention of the Australian Consumer Law, after it tested the composition of a sample from a batch "Hoyt's Oregano Leaves rubbed". Upon being notified by the ACCC of its concerns, Hoyt's Food took steps to change its supply arrangements and indicated it would test its oregano products in the future. The oregano adulteration story made headlines across many media outlets in Australia and Asia (1 – 4).

These articles demonstrate that the adulteration of herbs and spices is a global problem. In light of these findings, in 2019, the European Commission set up a coordinated control plan inviting the EU Member States, Norway and Switzerland to sample certain herbs and spices and send them for analysis in its Joint Research Centre (JRC). It is noteworthy that the UK did not take part due to leaving the EU.

The results for this wide-ranging [survey](#) were published in November 2021. In summary, nearly 10,000 analyses were carried out by the JRC on 1885 samples, using a range of state-of-the-art analytical techniques to assess the authenticity of six different herbs and spices. The analytical techniques used included:

- High performance liquid chromatography coupled to high resolution mass spectrometry (HPLCHRMS)
- Energy dispersive X-ray fluorescence spectroscopy (ED-XRF)
- Thermogravimetric analysis (TGA)
- Digital droplet polymerase chain reaction (ddPCR)
- Next Generation Sequencing (NGS)
- Real-time PCR (qPCR)
- Fourier transform infrared spectroscopy.

The percentage of samples which were deemed at risk of adulteration were 17% for pepper, 14% for cumin, 11% for curcuma, 11% for saffron and 6% for paprika/chilli. Oregano was identified as the most vulnerable with 48% of samples at risk of contamination, with olive leaves in most cases. Authenticity and purity of herbs and spices was assessed against relevant ISO standards. In case a sample did not comply with these provisions for extraneous matter and total ash, it was considered to be suspicious of adulteration.

In addition, the outcome of additional tests targeting certain biomarkers of herbs and spices was used as supporting evidence. Based on these results, the Commission has already called on the operators for an immediate action plan to remedy the situation that is detrimental to consumers' interests and health, but also to the herbs and spices sector itself and its fair operators. The Commission also invited national authorities to increase official controls in the sector with a view to continuing deterring fraudulent practices and sanctioning fraud perpetrators.

The release of this report in 2021 led to many global media outlets running this story (5 – 8). This emphasized that the adulteration of herbs and spices was truly a global issue.

13.3 Commercial Tests for Herb and Spice Authenticity

The following organisations offer commercial tests for authenticity testing of herbs and spices with services available in the UK.

13.3.1 [Bia Analytical \(BA\)](#) offer a test, FT-IR spectroscopy coupled with chemometrics, to authenticate a wide range of herbs and spices (Oregano, Sage, Black pepper, Turmeric, Paprika, Ginger, Cumin, Garlic, White Pepper, Coriander, Thyme, Parsley, Basil, Fennel, Cinnamon & Cassia, Chilli Powder, Rosemary, Mint & Saffron). The major advantage of this methodology is a 3-day turnaround time from receipt of sample to issuing of Certificate of Analysis. The test is specifically for EMA with limits of detection typically 10% of adulterants. A number of the tests are ISO 17025 accredited with the others already run under this quality management system with full accreditation expected in 2023.

13.3.2 [Food Forensics](#) offer an ISO 17025 accredited Next Generation Sequencing test for authenticity of herbs and spices. In addition, due to global supply chains there are often price differentials for different origins or production methods, they also offer Stable Isotope Ratio Analysis that provide a solution for these challenges.

13.3.3 [Eurofins](#) claim to be a specialist in authenticity analyses in the herbs and spices segment. They recently developed two methods that can be applied to herbs and spices: the 'NGS' (Next Generation Sequencing) and the 'NMR profiling'. These two so-called 'non-targeted' analyses are unique and by applying these techniques can increase the levels of herb and spice authenticity. In addition, they offer the possibility to determine the origin of raw materials by means of their patented SNIF-NMR technology.

13.3.4 [ALS Global](#) ALS has access to a wide range of chemical and DNA based methods to determine the nature of food products and ingredients, these include:

- **PCR** and **NGS** for species identification
- **IRMS (SIRA)** and **ICP-MS** techniques which are useful for identifying the origin of products

- **NMR** for detailed breakdown of components
- **FT-IR** and **NIR** for rapid spectroscopic screening
- **HPLC**, **GC**, and **MS** for specific targeted compounds

With these techniques, ALS can investigate foods using a combination of targeted and non-targeted analysis. The tests are accredited to ISO 17025.

13.3.5 [SGS](#) claim that they help defend against food fraud by verifying the authenticity of the ingredients. Their isotope ratio mass spectrometry (IRMS) testing can be used to verify geographic origin, test the authenticity of ingredients labelled as natural, identify undeclared additives and detect the use of synthetic fertilizers in organic products. Their next-generation sequencing (NGS) testing methods are a powerful tool for food analysis, food authenticity testing, and the identification of ingredients, pathogens, allergens and potential adulteration. NGS of DNA samples offers an untargeted test that answers the questions traditional DNA testing (PCR) cannot.

13.3.6 [Campden BRI](#) provide a comprehensive range of analytical services and consultancy services to help ensure the quality and safety of herbs and spices. Available analysis and testing services for herbs and spices include (but are not limited to): Aflatoxins, Allergens, Artificial colours, Ethylene oxide, Heavy metals, Illegal dyes, Irradiation, Microscopy small scale screening, Ochratoxin A, Pesticide screening, Polycyclic aromatic hydrocarbons (PAHs), Scoville units, Volatile oils, Volatile profiling.

13.3.7 [Premier Analytical Services \(PAS\)](#) has over 30 years' experience in validating the authenticity of foods and raw materials, continuously developing unique and highly sophisticated techniques to prove the authenticity of foods and ingredients. PAS offer a range of testing in support of this, including the latest innovation in analysis – Next Generation Sequencing, under ISO 17025 accreditation. Commodities analysed: Meat, Plants (including Herbs & Spices), Fish and Crustacea.

13.4 Other testing laboratories globally

The following are international companies that use multiple analytical techniques to determine Herb and Spice authenticity which suggests they follow a WoE approach to determine if a commodity has been adulterated:

13.4.1 [Lifeasible](#) and [CD-Biosciences Food Test](#) appear to be the same company, based in the USA, offering herb and spice authenticity testing using a suite of analytical methods.

Lifeasible has many years of experience in the study of herbs and spices. In order to ensure the quality of herb and spice products, they offer a complete package of techniques, which include, but not limited to:

- **Physical methods** - The physical methods for the authentication of herbs and spices are **macroscopic and microscopic analysis**. Macroscopic analysis involves the morphological description of the plant part(s) such as shape, size, colour, texture, odour, surface characteristics, etc. Microscopy techniques ranging from light to electron microscopy techniques are commonly employed for powdered samples, basing on the observations of structural, cellular, and internal tissue features to differentiate among plant species.

Analytical techniques - The analytical techniques allow identification and authentication of herbs and spices by evaluating the chemical compositions or organic components present in the plants. Depending on the basic principles, the techniques can be grouped into chromatographic techniques, electrophoretic methods, spectroscopic analysis, and chemometric techniques. Chromatographic techniques include high-performance liquid chromatography (HPLC) with different types of separation methods (normal Phase, reverse, and ion exchange) and detection methods (absorption in ultraviolet and visible region-UV/Vis, fluorescence, pulsed amperometric detector-PAD, and refractive index-RI), gas chromatography (GC) with a capillary column and flame ionization detector (FID), thermionic detector (TID), or mass spectrometry (MS), and so on. The commonly used electrophoretic method is capillary electrophoresis. The spectroscopic analysis allows non-destructive testing using small portion of samples for

compound identifications. The spectroscopy techniques include UV, visible, mid- or near infrared (MIR, NIR), Raman, fluorescence, and nuclear magnetic resonance (NMR) spectroscopy. Chemometric approaches are multivariate statistical analysis methods used along with other analytical techniques to provide sample classification and discrimination. The commonly used chemometric approaches involve principal component analysis (PCA), linear discriminant analysis, soft independent modelling of class analogy (SIMCA), artificial neural networks (ANNs), k nearest neighbour analysis (KNN), etc..

Biotechnological methods - Biotechnological methods are DNA based methods employed to identify plant species adulterations. They are classified into three main types: PCR-based, hybridization-based, and sequencing-based. Chloroplastic region-ribulose biphosphate carboxylase large chain (rbcL) and the nuclear ribosomal region- internal transcribed spacer 2 (ITS2) have been reported to be effective DNA barcodes for detection of plant-based adulterants in herb and spice products.

Immunological methods - Immunological assays utilize reaction of an antibody with specific plant protein to identify adulteration of other plant materials in herbs and spices.

Biosensors - Biosensors, such as electronic nose, are a robust indicators for the detection of adulteration in herbs and spices using nanotechnology.

Stable isotopic analysis - Stable isotope analysis is based on the isotope ratio of C, H, O, N, which are commonly used for verification of geographical origin of herb and spice products.

CD-Bioscience Food Test

Herb and Spice Adulteration Testing Platform

Ultra-high performance liquid chromatography-High resolution mass spectrometry (UHPLC-HRMS) merged with chemometrics: Quantitative and qualitative detection of herbs and spices, analysis of mixed substances.

Proton Nuclear Magnetic Resonance (1H-NMR) combined with chemometrics (PCA, OPLS-DA, O2PLS-DA): Quality and authenticity testing of herbs and spices. **Inductively coupled plasma mass spectrometry (ICP-MS)** along with principal components analysis (PCA) and canonical discriminant analysis (CDA): The isotope composition and multi-element analysis can identify the geographic origin of herbs and spices.

Sequence feature amplified region polymerase chain reaction (SCAR-PCR) and DNA barcoding detect adulteration of herbal and spice substitutes.

Near-infrared (NIR) spectroscopy analysis: Qualitative and quantitative analysis of organic matter in herb and spice. Turnaround Time: 7-10 working days.

13.4.2 [Cultivator Phyto Lab](#)

Based in Jodhpur in India and offer multiple analytical techniques to determine authenticity of Herbs and Spices. They claim that adulteration of these commodities has quite an adverse effect as it leads to the erosion of their medical and nutritional values as well as posing a threat to consumer health and safety. Cultivator Phyto Lab claims to offer a complete package of techniques to ensure quality and authenticity:

The Physical Method of Evaluation

When it comes to the physical methods for authentication of herbs and spices, microscopic analysis is the most widely used method. It involves the morphological description of spices that includes shape, size, colour, texture, odour, and surface characteristics. **Microscopy techniques** typically include light and electron microscopy and are employed for powdered samples. These tests are based on the observations of structural, cellular, and internal tissue features.

Analytical Methods of Evaluation

Through analytical techniques, the identification and authentication of herbs and spices are conducted by evaluating the organic components and chemical compositions present in the spices. The techniques can be categorised **into mass spectrometric methods, chromatographic methods, chemometric**

techniques, spectroscopic analysis, IR methods, Physio-Chemical methods, and others. The spectroscopic analysis allows non-destructive testing using a small portion of samples for compound identification. The techniques generally include **UV, visible, mid, or infrared, fluorescence**, and others. For chromatographic analysis, **high-performance liquid chromatography** with different types of separation methods is used. Chemometric approaches involve principal component analysis, soft independent modelling of class analogy, linear discriminant analysis, artificial neural networks, and K-nearest neighbour analysis. Other methodologies are mentioned on their website but they have not implemented them yet.

13.5 Instrument Manufacturers and Authenticity Testing

Several instrument manufacturers refer to methods for food authenticity testing using their instruments/devices:

13.5.1 [Perkin Elmer](#) recommend their NIR instrument coupled with specialised software to build your own in-house herb and spice authenticity database. They do not supply a commercial database.

13.5.2 [ThermoFisher Scientific](#) mention food authenticity testing and various approaches to tackle food fraud:

- (i) **Genomic** approaches utilize nuclear or mitochondrial DNA to generate genetic profiles;
- (ii) **Proteomics** characterizes food and food products through the analysis of proteins and peptides;
- (iii) **Metabolomics** provides deep insights into the overall composition of food products;
- (iv) **Isotopologomics** can provide the analysis of isotopic ratios and rare earth elements. In their [brochure](#) on food adulteration and testing, many different instruments are referred to in relation to the approaches mentioned above. With regards to herbs and spices, it appears to suggest **spectroscopy and NGS based techniques** for authenticity analysis.

13.5.3 [Waters Corporation](#) has a dedicated webpage dealing with Food and Beverage Authenticity. Within this website there is an application note on “[RADIAN ASAP LiveID](#) as a Routine Screening Solution for Substitution Fraud in Dried Herbs”. This is a direct ambient mass spectrometry technique, coupled with chemometrics, that can give real time results to indicate whether a sample is adulterated or not. The prediction accuracy was shown to 100% based on an independent validation sample set.

13.5.4 [Bruker](#) also has a dedicated webpage to food analysis but there appears to be no applications available for herb and spice authenticity testing.

13.5.5 [Agilent](#) have dedicated pages on their website specifically for authenticity of many foods but not herbs and spices.

13.6 Grey Literature Review Cited References

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14. Annex 7: List of participant laboratories for Phase 2 interviews.

OL	Website
Glasgow SS	http://www.glasgow.gov.uk/scientificservices
Minton Treharne & Davies	http://www.minton.co.uk
Kent SS*	http://www.kentscientific.co.uk/
Edinburgh SS	http://www.edinburgh.gov.uk/scientificservices
Lancashire SS	https://www.lancashire.gov.uk/business/business-services/scientific-services/
PASS Ltd.	http://www.publicanalystservices.co.uk
Government Analyst of the Isle of Man	http://www.gov.im/govlabs

*Hampshire SS also contributed by email as part of the Hampshire & Kent partnership.

15. Annex 8: Questionnaire

The questionnaire is to establish what would be needed to support the setting up of methodology in your lab to undertake herbs and spices authenticity testing. The questionnaire will look at equipment, training needs and any other barriers to establishing capability.

1. In the context of herbs and spices authenticity testing capabilities:
 - a) In terms of potential herbs and spices sample adulteration, what types of instruments do you currently possess that could be applied for *initial (first action)* analytical purposes, for example (but may not be currently used for such testing):
 - I. Microscope
 - II. Infrared spectrometer (IR) -specify range
 - III. Near Infrared spectrometer (NIR) – specify range
 - IV. Fourier Transform Infrared spectrometer (FT-IR) – specify range
 - V. Fourier Transform Near Infrared spectrometer (FT-NIR) specify range
 - VI. Raman spectrometer
 - VII. End Point PCR (e.g., PCR-RFLP)
 - VIII. Real-time PCR (e.g., species specific assays)
 - IX. Other
2. Do you associate the proficiency of any of these instruments with any recognised limitations? For example, high levels of background fluorescence.
3. Existing confirmatory herbs and spices authenticity testing capabilities
 - a) In terms of potential herbs and spices sample adulteration, what types of instruments do you currently possess that could be applied for *confirmatory* analytical purposes in the event of a sample being judged non-compliant, for example:
 - I. LC-MS
 - II. GC-MS
 - III. End Point PCR (e.g., PCR-RFLP)
 - IV. Real-time PCR (e.g., species specific assays)
 - V. Other

4. In terms of any instruments employed for confirmatory analysis, what is the effective working range they are generally employed with?
5. Do you associate the proficiency of any of these instruments with any recognised limitations? For example, high levels of background fluorescence.
6. With respect to the instruments listed, are you competent in their use to generate data. Would you benefit from any further specific training in their use for determining the authenticity of herbs and spices? If so which specific instrument(s)?
7. With respect to the instruments listed, are you competent in the interpretation of the data they generate. Would you benefit from any further specific training in the interpretation of generated data for determining the authenticity of herbs and spices? If so what specific area?
8. With respect to the instruments listed, are you currently accredited for any methods that involve their utilisation to generate a result?
9. In general, are you competent with interpreting meta data generated from targeted and non-targeted analytical methods. Would you benefit from any specific training in this area? If so, what specifically?
10. What is your current capacity for processing samples?
11. Do you have access to relevant auto sampling technologies, and if so in what form?
12. Would you be willing to share resources and work with other laboratories as part of an officially recognised network/consortium?
13. Do you recognise any of the following issues as being resource limiting:
 - a. Access to relevant databases
 - b. Access to reference materials
 - c. Access to relevant PT schemes
14. Do you currently maintain any in-house generated reference resources, for example reference materials, datasets, databases?

15. Is there anything further that you need / would like to assist with testing herbs and spices for authenticity? E.g., a visit to an herb and spice processor to understand how they operate?

16. Annex 9: Glossary

AAS: Atomic Absorption Spectroscopy

ACCC: Australian Competition and Consumer Commission

AI: Artificial Intelligence

ANN: Artificial Neural Network

AOAC INTERNATIONAL: Association of Official Analytical Collaboration International

ASTA: American Spice Trade Association

BA: Bia Analytical Ltd

BOLD: Barcode of Life Data System

BRC: British Retail Consortium

CAC: Codex Alimentarius Commission

CBOL: Consortium for Barcode of Life

CCSCH: Codex Committee on Spices and Culinary Herbs

CEN: European Committee for Standardization

CIAT: International Center for Tropical Agriculture

CRM: Certified Reference Materials

Defra: Department for Environment, Food & Rural Affairs

DNA: Deoxyribonucleic Acid

EA: Elemental Analysis

ELISA: Enzyme-linked Immunosorbent Assay

EMA: Economically Motivated Adulteration

enose: electronic nose

ESA: European Spice Association

etongue: electronic tongue

EC: European Communities

EU: European Union

EVM: Extraneous Vegetable Matter

FAM: Food Authenticity Methods

FSA: Food Standards Agency

FSS: Food Standards Scotland

FT-IR: Fourier-Transform Infrared

GC: Gas Chromatography

GC-FID: Gas Chromatography-Flame Ionization Detection

GC-MS: Gas Chromatography Mass Spectrometry
GMP: Good Manufacturing Practice
HPLC: High Performance Liquid Chromatography
HPLCTLC: High Performance Liquid Chromatography-Thin Layer Chromatography
ICP-MS: Inductively Coupled Plasma Mass Spectrometry
IGFS: Institute for Global Food Safety
IP: Intellectual Property
IRMS: Isotope Ratio Mass Spectrometry
ISO: International Organization for Standardization
JRC: Joint Research Centre of the European Commission
KNN: K-Nearest Neighbours
LC-HRMS: Liquid Chromatography High Resolution Mass Spectrometry
LC-MS: Liquid Chromatography Mass Spectrometry
LDA: Linear Discriminant Analysis
LOD: Limit of Detection
MIR: Mid-Infrared
NCBI: National Center for Biotechnology Information
NFCU: National Food Crime Unit
NGS: Next Generation Sequencing
NIR: Near Infrared
NIST: National Institute of Standards and Technology
NMR: Nuclear Magnetic Resonance
NTT: Non-Targeted Testing
OPLS-DA: Orthogonal Projections to Latent Structures Discriminant Analysis
PA OL: Public Analyst Official Laboratory
PASS: Public Analyst Scientific Services
PCA: Principal component analysis
PCR: Polymerase Chain Reaction
PLS-DA: Partial Least Squares-Discriminant Analysis
PLSR: Partial Least Squares Regression
PT: Proficiency Test
QC: Quality Control
qPCR: Real-time quantitative Polymerase Chain Reaction
RASFF: Rapid Alerts System for Feed and Food
RBG: Royal Botanical Gardens (Kew)

SFCIU: Scottish Food Crime and Incidents Unit
SIRA: Stable Isotope Ratio Analysis
SME: Small and medium-sized enterprises
SMPR: Standard Method Performance Requirements
SNIF-NMR: Site-Specific Natural Isotope Fractionation studied by Nuclear Magnetic Resonance
SOP: Standard Operating Procedure
SSA: Seasoning and Spice Association
SVM: Support Vector Machines
TLC: Thin Layer Chromatography
UK: United Kingdom
UKAS: United Kingdom Accreditation Service
USD: United States Dollar
UV Spectroscopy: Ultra-Violet Spectroscopy
WG: Working Group
WHO/FAO: World Health Organization/Food and Agriculture Organisation
WoE: Weight of Evidence
WTO: World Trade Organisation.