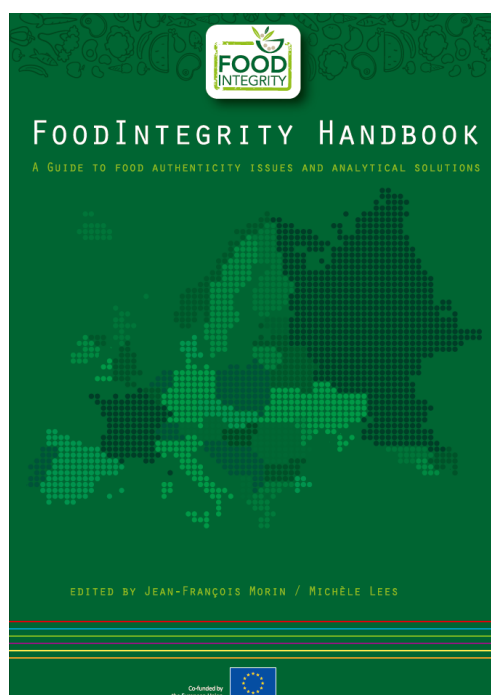# FOODINTEGRITY HANDBOOK

A GUIDE TO FOOD AUTHENTICITY ISSUES AND ANALYTICAL SOLUTIONS

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Spices

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General overview of the product

Spices, seasonings, herbs are all products that are generally added to both fresh and processed food to enhance flavour. With documents showing trade in spices as early as 3000 BC, the spice trade itself can be considered as one of the earliest drivers of globalisation. More recently, the global spices and seasoning market has been valued at around USD 12.7 billion in 2012 and is expected to grow to about USD 16.6 billion by the end of 2019 (source: www.statista.com). Among the forces pushing up consumption in spices are the perceived health benefits. Spices contain plant-derived chemical compounds that have been shown to help prevent certain diseases. With concern among the general population about side effects of commonly used drugs, interest is shifting towards other forms of medicine such as Ayurvedic and Traditional Chinese medicines, which focus on the use of herbs and spices.

This chapter provides a detailed review of current legislation defining spices, the problems of fraud that the spice industry faces, and potential analytical methods to deal with authenticity issues. Because of its importance as one of the most expensive spices, saffron is dealt both in the general chapter on spices and in its own chapter, where more details on analytical methods for saffron authentication are given.

1. Product Identity

1.1. Definition of the product and manufacturing process

1.1.1. FAO

According to the FAO, spices can be defined as "vegetable products used for flavouring, seasoning and imparting aroma in foods". Herbs, considered a subset of spices, are leafy spices, and some, like dill and coriander, can provide both spice seeds and leafy herbs [1].

1.1.2. Codex Alimentarius

Spices and Dried Aromatic Herbs are defined as dried components or mixtures of dried plants used in foods for flavouring, colouring, and imparting aroma [2]. This term equally applies to whole, broken, ground and blended forms.

1.1.3. FDA (Code of Federal Regulations, 21CFR101.22)

The term "spice" is defined in the U.S. Code of Federal Regulations for specific labelling requirements (21 CFR Sec. 101.22 (2)) [3]. The term spice means any aromatic vegetable substance in the whole, broken, or ground form, except for those substances which have been traditionally regarded as foods, such as onions, garlic and celery; whose significant function in food is seasoning rather than nutritional; that is true to name; and from which no portion of any volatile oil or other flavouring principle has been removed. Spices include the spices listed in 182.10 and part 184 of this chapter, such as the following: allspice, anise, basil, bay leaves, caraway seed, cardamom, celery seed, chervil, cinnamon, cloves, coriander, cumin seed, dill seed, fennel seed, fenugreek, ginger, horseradish, mace, marjoram, mustard flour, nutmeg, oregano, paprika, parsley, pepper, black pepper, white pepper, red rosemary, saffron, sage, savory, star aniseed, tarragon, thyme, turmeric and saffron.

1.2. Current standards of identity or related legislation

In the General Food Law Regulation EC 178/2002 [4], the general principles and requirements of food law and procedures of food safety are outlined. With regard to the consumer's interest, the General Food law aims to prevent, "fraudulent or deceptive practices, the adulteration of food, and any other practices which may mislead the consumer".

The European Food Safety Authority (EFSA) was established legally in 2002 under the General Food Law, following a number of food crises in the late 1990s. EFSA provides scientific advice and communicates risks within the food chain.

In the United States, the FDA and the US Department of Agriculture (USDA) are the principle federal agencies working on food safety. Border protection and import authorities, as well as food safety, food defence and food quality authorities broadly look after food fraud across a number of federal agencies [5]. The primary food safety law administered by the FDA is the Federal Food, Drug and Cosmetic Act (FFDCA) [6]. This act tightened control over food, drugs, and consumer protection, and gave the government enforcement ability. The Food Safety Modernization Act [7] was then passed by US congress. This Act amended Section 415 of the FFDCA with the aim to prevent rather than respond to contamination and outbreaks.

Specific organisations have become involved in the protection of the herb and spice industry. The European Spice Association (ESA) is a non-profit organisation made up of national federations of the spice industry from the EU, Turkey and Switzerland. It has an aim to protect the industry and its members with regard to processing, packaging, quality assurance, food safety and marketing in the herb and spice industry. The American Spice Trade Association (ASTA) works similarly in the US, to ensure clean and safe spices, and enhance the industry and the business interests of its members. The ESA has a set maximum level of 2 % w/w extraneous matter in herbs and 1 % w/w maximum level in spices in the Quality Minima Document [8] whereas ASTA [9] has set a level of extraneous matter at 0.5-1 % w/w. One of the difficulties in keeping the herb and spice industry free from fraud is the issue of long industry supply chains that can exist over many countries.

2. Authenticity issues

2.1. Identification of current authenticity issues

2.1.1. Complex Supply Chains

Supply chains in the herb and spice industry tend to be long, complex and can pass through many countries. Such complexities present many opportunities for criminals to carry out EMA. 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 [10].

"Fraud control measures" can be implemented in companies to detect fraud opportunities or motivations that may occur either internally, or externally of the company [11]. The processing and manufacturing need to be carefully monitored to ensure food protection. Cleanliness and protection of the product from contamination and adulteration is vital. The cost of maintaining these standards can be high. The blending and packaging stage provides an early opportunity for adulteration and needs to be carefully monitored. In more modern processing plants, the product is often enclosed during this process. In addition, careful monitoring is required for the preparation of ready meals i.e. precooked meals, and other food products that have herbs and spices added to them towards the end of the supply chain.

The ESA Adulteration Awareness Document [12] advises companies on ways to prevent adulteration: 1. "Evaluation of the supply chain" (knowing the history of the supply chain, adherence to legal requirements, traceability, adherence to HACCP (Hazard Analysis and Critical Control Points) and adherence to accreditation standards), 2. "The nature of the material" (whole or ground, botanical species and commercial grade), 3. "Product testing" (there is a range of methods being developed for the rapid and accurate detection of fraud). It is important to have these precautions in place for both industry and the consumer, however, cases of adulteration continue to occur, and there may be useful lessons in reviewing old examples of adulteration.

2.1.2. Economically Motivated Adulteration

A large global industry such as the herb and spice sector is under constant threat from fraudsters. With valuable condiments such as saffron, oregano, vanilla, turmeric and paprika, substantial amounts of money can be made by carrying out adulteration of these products at the expense of the consumer and potentially the reputation of food businesses. The long, complex supply chains and the increase in crushed and ground herbs and spices provide excellent opportunities for EMA. However, other vulnerabilities that may affect the chances of adulteration include seasonality and availability of the crop, weather events, cultural and geo-political events, economic indicators, food safety laws, prevalence of corruption and advances in technology to mask fraud [10]. The 2016 garlic crop had potential to become vulnerable to adulteration following severe weather events of heavy rain and snow in late 2015, causing a surge in the price of garlic [13]. This surge in the price caused stockpiling of garlic. Circumstances such as these can all provide motivation for adulteration. Preventative measures can include; knowing product specification, supplier assurance, product type (ground and crushed and where did this process take place), knowing the supply market and being aware of vulnerabilities in the supply chain. Verification and testing can be carried out to confirm the preventative measures are effective. This can involve devising representative sampling and inspection programmes for products, a suitable testing strategy that

meets objectives, a test method in an accredited laboratory, and supply chain verification measures which may include pre-delivery of samples prior to purchase for approval, or evidence of authenticity from an accredited laboratory [10]. The prevention of fraud is not in detecting each individual fraud and controlling one type, but reducing the vulnerabilities, as the fraudsters are always evolving and looking for their next crime [14]. The herb and spice industry has been a victim of EMA on numerous occasions. Table 1 focuses on examples where substitution adulteration occurred with various herbs and spices.

Table 1: Examples of Substitution Adulteration in the Herb and Spice Industry

Ingredient	Adulterant	Reference
Chilli	Oil, rice flour, bran	[15]
	Ziziphus nummularia fruits	[16]
	Plant husks, rice powder, sawdust, stone powder	[17]
Oregano	Sumac, olive leaves	[18]
	Olive leaves, myrtle leaves	[19]
	Satureja montana L. and *Origanum majorana* L.	[20]
	Cistus incanus L., *Rubus caesius L.* and *Rhus coriaria* L	[21]
Cumin	Almond, peanut, tree nuts, peach and cherry	[22]
	Fennel seeds	[23]
	Peanut shell	[24]
Black pepper	Chilli	[25]
	Buckwheat or millet	[26]
	Papaya	[27]
Cinnamon	Coffee husk	[26]
Chinese star anise	Japanese star anise	[28]
Nutmeg	Coffee husks	[26]
Paprika	Almond	[29]
	White pepper, curcuma, barium sulphate, brick powder	[30]
	Defatted paprika	[26]
	Paprika of inferior quality substituting paprika from the Protected Designation of Origin (PDO) 'La Vera' region.	[31]
	Falsely declared Szegedi paprika substituted for *Szegedi Füszerpaprika* PDO	[32]
Saffron	Saffron of unknown origin labelled as being cultivated in the PDO region in Spain can be used for substitution.	[33]
	Beet, pomegranate fibres, dyed corn stigmas, red dyed silk fibres, safflower, marigold to red stigma	[34]
	Safflower, gardenia, meat fibres, gelatine fibres, curcuma, sandalwood, campeche wood powder, stigmas of other saffron types, flowers, starch, glucose	[35]
Turmeric	*Curcuma zedoaria, Curcuma malabarica*	[36]
	Chalk powder	[37]

The addition of colour to spices to improve their value is a common occurrence. Colour can influence the perception of food and stimulate appetite, therefore, increase the value of a product [38]. The addition of colourants to foodstuffs dates back to at least 1500 BCE, and up until the middle of the 19th century, ingredients such as the spice saffron was added for a decorative effect in certain foodstuffs [38]. Natural dyes were commonly used in food around this time, however, as the 1900s began, the use of synthetic dyes became the colouring of choice with ease of production, less expense and superior colouring ability [38].

As with other types of food adulteration, there is a likelihood that certain synthetic dyes may be a threat to public health, and historical records show that injuries and even death occurred following ingestion of toxic colourants [38]. Allergic and asthmatic reactions as well as DNA damage have also been reported [39]. Therefore, the use of most synthetic dyes is forbidden in Europe. The two main types of dyes that may be illegally added to food include azo dyes and triphenylmethanes [40]. Examples of these illegal azo dyes include Sudan I, II, III, IV, para red, orange II, methyl yellow and rhodamine B. Malachite green and its metabolite leucomalachite green are examples of triphenylmethane dyes considered genotoxic and/or carcinogenic.

In May 2003, Sudan 1 was found to be illegally present in chilli powder and foods containing chilli powder in the EU [40]. Following this event, in 2005 and 2006, numerous tests were carried out for the presence of illegal dyes by the UK Food Standards Agency (FSA) [41]. Regulatory legislation was put in place following the scandal, and member states were required to monitor high risk products and provide analytical reports for the presence or absence of Sudan dyes as an emergency measure in the European Commission Decision 2005/402/EC [42]. This legislation was later repealed in the European Commission Regulation (EC) No. 669/2009 [43] to a less intensive testing regime due to a reduction in the presence of Sudan dyes.

Legislation varies in different countries, which can cause problems for importers and exporters [41]. In the EU, Regulation (EC) No. 1333/2008 [44] on food additives was developed "…with a view to… ensuring a high level of protection of human health and a high level of consumer protection…." With regard to food colours, there are currently 25 natural, and 15 synthetic dyes on Annex II of this regulation that can be allowed in food [41]. The US FDA regulates food additives in the US. To indicate the variation between countries, three synthetic dyes approved in the US are not approved in the EU, and nine synthetic food colours in the EU are not approved in the US [41]. There is still a continued risk of adulteration with dyes in spices.

Table 2: Adulteration with dyes [45,46]

Spice	Adulteration
Red Pepper Chili powder	Sudan 1, Sudan 4, Metanil Yellow, Sudan 3, Oil Orange SS, Rhodamine B, Auramine O, Orange II, Dimethyl Yellow, Fast Garnet GBC, Malachite Green, Allura Red
Paprika powder	Sudan 1, Sudan 4, Acid Black 1, Orange II, Annatto
Turmeric powder	Sudan 1, Mentanil Yellow, Orange II, Lead Chromate
Sumac	Amaranth Red, Basic Red 46
Curry powder	Auramine O, Chrysoidin (Basic Orange II)
Saffron flower	Acid Orange II, Mentanil Yellow, Sudan I, Ponceau 4R, Ponceau 6R
Cayenne pepper	Crystal Violet
Five spice powder	Auramine O

The results in Table 2 summarises reported cases of adulteration of spices with dyes from 2013 to 2017 in the US. In this work the most common dyes reported were Sudan 1 and Sudan 4. These results indicate that adulteration with dyes is ongoing. Continued surveillance of spices to detect and prevent adulteration with dyes is vital to the herb and spice industry as well as the safety of consumers. Health risks can occur alongside both substitution and addition adulteration. They can cause more than an economic threat to the consumer.

2.2. Potential threat to public health

The main motivation for the addition to, or substitution of the authentic product is for economic reasons, however, with the cases outlined in Table 3, a number of health risks were a detrimental result of this criminal behaviour. There is an increasing concern over the introduction of hazards from food fraud. It is a constant and growing concern in the food industry, with greater actions needed to be put in place to detect it.

There are three types of food fraud risks that pose a threat to the public: 1. Direct: The consumer is put at immediate risk from a short-term exposure leading to acute toxicity or lethality, 2. Indirect: The consumer is put at risk over long-term exposure with potential chronic effects, 3. Technical: Food documentation may not be representative of the food content [47]. A serious example of a technical fraud risk could be an allergic reaction to an unknown product that has not been outlined in the label.

The detection of undeclared nut protein in cumin and paprika in 2015 was one case where adulteration did not result in just economic losses [22]. This crime had serious consequences for public health and strengthened the demand for food protection. With food allergies affecting approximately 3-4 % of the adult population, an estimated 0.6 % are allergic to peanut and 0.5 % allergic to tree nut [48]. All products that come into contact with nut protein need to be labelled accurately as the risk of an unsuspecting sensitive individual coming into contact with this can be fatal. In a study by Bock, Muñoz-Furlong, and Sampson [49], it was found that out of 32 fatal cases of anaphylaxis from 1994-1999, 94 % of the cases were caused by peanut or tree nuts, indicating that the vast majority of food induced anaphylaxis is caused by these foodstuffs. The adulteration of spices with nuts is a serious public health risk for susceptible individuals.

Table 3: Examples of Economically Motivated Adulteration with Possible Health Impact

Herb/Spice	Adulterant	Possible Health Impact	Reference	Food Fraud Risk
Cumin, Paprika	Nut protein	Anaphylaxis	[22,48]	Direct
Chinese star anise	Japanese star anise	Neurological and gastrointestinal problems	[28]	Direct
Black pepper	Papaya seeds	Liver and stomach problems	[27]	Direct
Turmeric	Yellow chalk powder	Face swelling, loss of appetite, nausea, and vomiting	[37]	Direct
	Curcuma zedoaria	Toxicity in rats and chickens	[51]	Direct
	Lead chromate	Delayed mental and physical development	[52]	Indirect
Cumin	Fennel seeds coated with marble dust and dye	Possible health risk from the use of dye and marble dust	[23]	Indirect
Oregano	Olive leaves	Presence of pesticides-Toxicity, carcinogenicity, mutagenicity	[53]	Indirect

Chinese star anise (*Illicium verum*) is infused in teas to relieve the symptoms of colic in children. The adulteration of Chinese star anise with Japanese star anise (*Illicium anisatum*) has in previous years resulted in the intoxication of children. Japanese star anise looks similar to Chinese star anise, and they are often even more difficult to distinguish as they can be sold in broken or ground form. Therefore, chemical analysis is required to distinguish them. Japanese star anise contains neurotoxins and can result in a child having neurological and gastrointestinal problems [28].

Papaya seeds have been used to adulterate and bulk black pepper. However, these papaya seeds can cause liver and stomach problems, and therefore pose a health risk to the unsuspecting consumer [27].

Turmeric can contain various adulterants that threaten public health. Yellow chalk powder has been used to add bulk to turmeric as it is a cheap material [37,50]. This adulterated product however can cause swelling of the face, loss of appetite, nausea and vomiting. *Curcuma zedoaria* can be used to adulterate turmeric [36], and was found to have toxic effects in rats and chickens by Latif et al. if not processed properly [51]. Lead chromate added to turmeric was used as a dye as well as a bulking powder. Over exposure to lead can cause delayed mental and physical development [52].

In a case reported in the Times of India [23], poor grade fennel seeds were coated with waste marble dust and dye, and mixed in with the cumin product. In this case, it was the treatment of the fraudulent product that caused the public health risk rather, than the fennel seeds themselves.

The use of other plant cuttings such as olive leaves in the adulteration of oregano [19] can also pose a health risk to the consumer. As these leaves are not produced for consumption, it is unknown how these cuttings may be treated. In the case of olive leaves in particular, evidence of pesticides can be found (Elliott, C- personal communication). Pesticide residues pose a health risk, and hazards such as toxicity, carcinogenicity and mutagenicity are associated with them [53].

There are many possible risks with food adulteration. Therefore, it is vital that there is adequate policing of the supply chains and the food industry to deter and try to prevent any fraud before it is too late. Illegal dyes are a constant threat to the international food industry and are found intermittently, as indicated by the alerts in Rapid Alert System for Food and Feed (RASFF) [54]. Examples from RASFF and the possible health impacts can be seen in Table 4.

Table 4: The Possible Health Impacts of Common Illegal Dyes

Common Illegal Dyes	Possible Health Impact	Examples of Spices
Sudan 1	Genotoxic and carcinogenic in rats	Cayenne pepper, Turmeric, Chilli, Paprika, Curry
Sudan 4	Potentially genotoxic and possibly carcinogenic	Curry, Turmeric, Chilli, Paprika, Sumac
Para Red	Potentially genotoxic and possibly carcinogenic	Chilli, Cayenne pepper, Paprika
Orange II	Potentially genotoxic, insufficient data on carcinogenicity	Chilli, Safflower, Sumac, Paprika
Methyl Yellow	Possibly carcinogenic to humans	Curry
Rhodamine B	Potentially genotoxic and potentially carcinogenic	Sumac, Chilli, Paprika, Turmeric, Curry

It is vital that authentication testing is carried out to detect cases of economic fraud and to verify that preventative measures are effectively in place [10]. This prevention not only maintains quality and consumer trust, but also helps to prevent the possibility of public health risk [55].

3. Analytical methods used to test for authenticity

Fast, reliable and competent analytical techniques are required to confirm the authenticity of food with this increasing trend of food adulteration [55]. According to the database records collected by Moore, Spink and Lipp [56], from 1980 to 2010, the top two methods used for detecting food adulteration were liquid-chromatography and infrared spectroscopy. Visual inspection and microscopy are common methods used to detect adulteration in herbs and spices as reported by the British Retail Consortium, the Food and Drink Federation, and the Seasoning and Spice Association in 'Guidance on Authenticity of Herbs and Spices' [10]. However, it requires highly trained analysts and analysis can take a long time, therefore research is continuously being carried out to develop new methods for the detection of adulteration in herbs and spices. Fraudsters tend to be one-step ahead of the food safety agencies but also, techniques for food adulteration are becoming more and more advanced [27]. Recent analytical methods for the detection of adulterants are listed in section 4.

3.1. DNA analysis

DNA analysis is increasingly being used in the fight against food fraud as advances in methods provide cheaper, more efficient and accurate means of detection of fraud. It can be seen from section 4 that DNA analysis plays an important role in the detection of substitution adulteration in herbs and spices. In recent years, Sequence Characterised Amplified Region – Polymerase Chain Reaction (SCAR-PCR) and DNA barcoding are becoming desirable methods for the detection of food adulteration.

SCAR-PCR is an advancement on the use of Random Amplified Polymorphic DNA (RAPD) markers in DNA analysis. RAPD analysis is considered a useful starting point as it has low operating cost and can distinguish between botanical varieties [57,58]. Although RAPD markers are a fast and cheap method, their downfall is that repeatability is low and exchanging results between laboratories creates difficulties [59]. This problem with RAPD markers was corrected with the development of SCAR primers and this increased specificity and reliability [60]. The use of SCAR-PCR was observed for the detection of bulking agents in saffron, where, the method screened large batches with a fast, reliable sensitive and low cost screening method [57]. The detection of adulteration of oregano with *Cistus incanus L.*, *Rubus caesius L.*, and *Rhus coriaria L.*, was carried out by Marieschi et al. using RAPD [58] and subsequently with SCAR-PCR [21] to improve the robustness of the method.

Other SCAR-PCR methods include the detection of olive leaves, *Satureja montana L.*, and *Origanum majoranan L.* in oregano [20,60], the presence of *Curcuma zeodoaria / Curcuma malabarica* in turmeric [36] and the presence of plant based materials in chilli [16]. The development of a SCAR and Internal Transcriber Spacer (ITS) region multiplex PCR method allowed the detection of both the adulterant safflower and the spice saffron in the one analysis [59]. It is evident that the use of SCAR-PCR has potential for EMA adulteration detection in a number of herbs and spices. SCAR-PCR is a sensitive method with detection limits at 1 % for the adulteration

of oregano with *Cistus incanus L.*, *Rubus caesius L.*, and *Rhus coriaria L.* [21], 1 % for the detection of olive leaves in oregano [61] and a limit of detection (LOD) of 10 g/kg for the presence of *Curcuma zeodoaria / Curcuma malabarica* in turmeric [36] indicate this. However, a limitation of SCAR-PCR is the need for sequence data for the PCR primers design [61].

DNA barcoding is a relatively new method that was first developed in 2003 and is based on the variability within a standard region of the genome, the 'DNA barcode' [62]. It has become increasingly used since its development, and there is successful evidence of this method in the detection of adulterants in herbs and spices. This method has been used for the detection of adulterants in saffron [63], and chilli adulteration in black pepper [25]. DNA barcoding is a fast, reliable sensitive method for a wide range of food commodities, and even strongly processed foods and there is also the possibility of building reference databases to improve the chances of it becoming a routine test for food quality, and traceability [64].

DNA purity and integrity are concerning with regard to DNA barcodes, which, can be a limitation of the test. Poor quality DNA may reduce amplification success of DNA barcodes [65]. DNA barcoding also relies on the availability of sequence libraries to reference against [66].

Whole genome sequencing is becoming a possibility and it has potential for the detection of food adulteration with Next Generation Sequencing (NGS). However, so far, little work in this area has been carried out with the complex work flow and high costs associated with this method [67].

The methods for the detection of adulteration in herbs and spices using DNA analysis described are qualitative. Quantitative methods often result in high measurement uncertainty, although advancements in PCR technologies are improving in this way [67]. Overall, the limitations with DNA analysis may include poor integrity and purity of the DNA, poor efficiency of the extraction, and the risk of contamination is a concern with these methods. Also, low level accidental contamination can be misinterpreted as intentional substitution.

3.2. Mass spectrometry

Mass Spectrometry (MS) is a powerful tool in the fight against food fraud, and in many industries, it is considered the gold standard technique. Methods include Gas Chromatography (GC-MS), Liquid Chromatography (LC-MS), Isotope Ratio (IR-MS) and Inductively Coupled Plasma (ICP-MS). Once a targeted method is developed, mass spectrometry can provide a highly specific and sensitive technique that can quantify known analytes to sub-μg concentrations [68]. Although an expensive technique that requires significant expertise and laboratory surroundings, it is highly regarded as a confirmatory technique.

In the study by Black et al., Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC-HRMS) was used as part of a two-tier approach to detect the presence of adulterants in oregano with LC-HRMS used as a confirmatory technique [19]. The analysis was untargeted, and with the use of Principal Component Analysis (PCA) and Orthogonal Partial Least Squares – Discriminant Analysis (OPLS-DA) chemometrics, biomarkers specific to the classes (oregano and various adulterants) were identified. The identification of such biomarkers allowed further developments in the detection of adulteration with targeted mass spectrometry [69]. Wielogorska et al. [69] used targeted FTIR (Fourier Transform Infrared) and LC-MS/MS to quantitatively detect adulteration in oregano. These studies [19,69] were an improvement on the work of Bononi and Tateo [70] as they identified biomarkers for a number of adulterants, as well the development of a quantitative method. In the work by Bononi and Tateo [70], a targeted method was developed for

the detection of a characteristic marker of olive leaves, the phenolic compound oleuropein, in both oregano and sage with the use of Liquid Chromatography-Electrospray Ionization Mass Spectrometry (LC-ESI-MS/MS). This compound oleuropein was later found to be also present in myrtle leaves by Wielogorska et al. [69]. Similarly, the use of untargeted Ultra High Performance Liquid Chromatography coupled to High Resolution Mass Spectrometry (UHPLC-HRMS) merged with chemometrics, OPLS-DA proved to be a successful powerful tool in determining products from the PDO of saffron [33]. Falsely declared saffron from a PDO can be used in substitution of the authentic product.

GC-MS is another method that has been used to detect possible adulterants such as with the study carried out in 2015 investigating detection methods for known fruit adulterants in fennel seed [71]. Essential oils of fennel seed and two adulterants were profiled, and distinct differences between fennel seed and two of its adulterants were observed. Bononi, Fiordalise and Tateo were able to use GC-MS to detect olive leaves in oregano and sage by using GC-MS with a detection limit of 1 % [72]. The benefits of this method included the ease of use and reproducibility of the results. However, with regard to the detection of adulteration in herbs and spices, an issue that may occur with the use of GC-MS is that, only the volatile oils are investigated. Therefore, the addition of volatile oils to a product may cheat the GC-MS adulteration detection method.

ICP-MS along with PCA and Canonical Discriminant Analysis (CDA) was a method developed to detect falsely declared Szegdi paprika (PDO) [32]. The Sr isotopic composition and the multi-elemental analysis are indicative of paprika from the region.

Upgrades in mass spectrometry involve the use of real time analysis of samples by directly introducing the samples to the mass spectrometer. Ambient mass spectrometry is a relatively new analytical technique that gives comparable results to conventional techniques without complex sample preparation [73]. Examples of its use include the detection of the adulterant Japanese star anise in Chinese star anise using Direct Analysis Real Time-High Resolution Mass Spectrometry (DART-HRMS) by detecting the presence of anisatin [74]. Advances on this method involves the use of direct plant spray combined with orbitrap-HRMS [75]. This method can detect between the neurotoxic Japanese star anise and the Chinese star anise in seconds, and without sample pre-treatment. DART ionisation has slightly higher selectivity, no solvents added and the absence of high voltages when compared to direct plant spray. The benefits of direct plant spray over DART ionisation include the low cost, lower standard deviations and simplicity. Direct plant spray and DART ionisation techniques are more successful qualitative methods than quantitative methods.

Currently the disadvantages of mass spectrometry in comparison to spectroscopy are the cost and the requirement of a laboratory setting and highly trained analysts. However, advances to overcome this are ongoing with aims to miniaturize the instrumentation, and for the data to be presented so that it is easily interpreted. However, these developments require further optimization and are not readily available [68]. Similarly to spectroscopy, the validation procedure for non-targeted methods in mass spectrometry has not been standardised. This can reduce consistency between laboratories.

3.3. Spectroscopy

Vibrational spectroscopies, along with chemometrics, have become well known as rapid, non-destructive, fingerprinting techniques and are valuable screening tools in the detection of adulteration / authentication in the food industry. A range of spectroscopic analytical techniques

used in the food industry include FTIR, Fourier Transform Near infrared (FT-NIR), Raman, Hyperspectral Imaging (HSI) [76] and Nuclear Magnetic Resonance (NMR) [77].

In the detection of adulteration of herbs and spices for economic gain, a number of spectroscopic methods continue to be developed. Work has been carried out to develop competent models to detect cornstarch in garlic powder by FTIR [76] and onion powder by FTIR and NIR [78]. Raman has also been used to detect cornstarch in onion powder and garlic or ginger powder [79,80]. Starch may be added to white powders such as garlic and onion powder to add bulk to the product. In these studies, a quantitative model was built using the algorithm Partial Least Squares Regression (PLSR) in chemometrics. The Raman, FTIR and NIR spectral data based models described here are capable of detecting adulteration in onion powder, garlic and ginger with starch up to 35 %.

In a study by Black et al. on the detection of adulteration in oregano, FTIR was used alongside the confirmatory technique LC-HRMS [19]. Following the identification of biomarkers for both oregano and its adulterants, and the development of spectroscopic classification models using the unsupervised PCA and supervised OPLS-DA chemometric algorithms, a rapid screening method and confirmatory method was developed. The benefit of this method was that a number of different adulterants could be added to the database that was used to build the model. The developed screening technique therefore was robust and could identify numerous adulterants at each screening in the survey that was subsequently carried out. The results of the survey indicated that adulteration was ongoing, but also, it displayed the use of a rapid screening technique to help the fight against food fraud. Further development on these analytical techniques was carried out with the development of targeted quantitative methods using FTIR with PLSR and LC-MS/MS for the detection of adulteration in oregano [69].

Raman and FTIR methods analyse the sample in the mid infrared region of the electromagnetic spectrum. The spectral data consist of sharp bands representing inelastic scattering, or information on the fundamental vibrations of the sample respectively. This is in comparison to the vibrational overtones and combination peaks of the NIR, which does not provide as much information [68]. However, in the detection of starch in onion powder, NIR with PLSR chemometric algorithm was determined the most suitable method [78]. NIR has the ability to penetrate deeper into the sample and therefore is more suitable for bulk samples that have little or no sample preparation. Raman has advantages over NIR and FTIR as it is not affected by water, and inorganic materials can be analysed more easily. Analysis through packaging or glass is also a possibility [79]. Recent improvements to Raman also include the use of Surface Enhanced Raman Scattering (SERS) and Spatially Offset Raman Spectroscopy (SORS) which has shown its ability to detect counterfeit products through packaging [68].

The use of Proton Nuclear Magnetic Resonance (^{1}H-NMR) combined with chemometrics (PCA, OPLS-DA, O2PLS-DA) was investigated and was proven successful at determining the quality and authenticity of saffron [81], allowing the detection of common adulterants such as Sudan dyes [82], other dyers mixed with stabilizing agents or bio-adulterants such as gardenia, safflower or curcuma [77] whose specific markers have been identified. Additionally to these targeted studies (use of markers), some untargeted approaches coupled to chemometrics were also developed to assess saffron authenticity and detect the presence of unexpected adulterants [83]. That sort of approach can be transferrable to other spices, given the availability of consequent authentic database. Quantitative metabolomics analysis were also performed to distinguish cinnamon varieties and showed encouraging results [84]. Others spices such as safflower [85] were also studied by NMR. ^{1}H-NMR was shown to give reproducible results rapidly, however, this technique requires solvent extraction and is then limited to extracted metabolites. Additionally only organic compounds are visible with this technique. Further work carried out using DRIFTS on FTIR

minimized the process of sample preparation and proved to be successful along with PLS-DA classification and quantitative PLSR models at detecting six known saffron adulterants [86].

Although these spectroscopy methods are often successful on their own, further developments are being made to improve the methods by:

1) Combining data: Wang et al. [87] carried out a study that improved FTIR and NIR results for the detection of the adulterant *Iuicium lanceolatum A.C. Smith* (ILACS) in Chinese star anise. This method involved combining the NIR and FTIR spectral data and the use of PCA and Linear Discriminant Analysis (LDA) chemometric techniques. Although the FTIR performed better than NIR in this study when analysed separately, the classification results from the combined approach proved to be even more successful.

2) Increasing sensitivity: Vermaak et al. [88] used hyperspectral imaging with PCA and PLS-DA to distinguish between the neurotoxic Japanese star anise and Chinese star anise. This emerging method incorporates spectroscopy and imaging to produce both spatial and spectral data from a sample [89] (Gowen, O'Donnell, Cullen, Downey and Frias, 2007). This method is also non-destructive and rapid with the added advantage that with the acquisition of several predictions on the sample, the statistics are better [88]. The quantification of adulterants, buckwheat or millet, in ground black pepper was carried out using FTIR and NIR with hyperspectral imaging with PLSR chemometrics. NIR with hyperspectral imaging was seen to produce the best calibrations which, in this case was largely to do with the larger sample area used with NIR, and the spatial information from the imaging system used with it [90]. Galaxy Scientific's Classical Least Squares (CLS)-based Advanced-ID algorithm has been developed to detect screening samples to a level as low as 0.01 % [91]. When it was used to detect paprika adulterants, it detected Sudan 1 dye at 0.1 %, tomato skin at 0.5 % and brick dust at 5 %.

3) Analysis through packaging: Terahertz spectroscopy was used to overcome the barrier of common packaging materials such as plastics and papers [37]. This method is a promising non-intrusive technique that was used for the detection of yellow chalk powder in turmeric.

It is apparent that further improvements and developments are ongoing with the use of spectroscopy. Developments seen in benchtop spectroscopic instruments are also being transferred to handheld devices. An added benefit as discussed by Ellis et al. [68] would be to use the advantages of the NIR and FTIR combined, and developed into a handheld device. Overall, the ability to transfer this technology to portable and handheld devices allows the user to determine authenticity in the field, and can focus on vulnerable points of the supply chain. This not only allows improvements in traceability and detection of fraud, but at a basic level, it can also act as a deterrent. If food fraud criminals are aware of this possibility, they may be less likely to take the risks of committing a crime in the first place.

Limitations of spectroscopy must not be overlooked. Spectroscopy is used as a rapid screening technique and therefore, further investigations may need to be carried out by confirmatory techniques that require more expertise, time and cost more, such as mass spectrometry. This is also true when building models using chemometrics, the purity of samples needs to be assured in order to build accurate models. Another limitation of spectroscopy, as a non-targeted method, is the lack of a standardised validation procedure for all laboratories.

Following a review of more than sixty scientific publications, it was found that spectroscopic techniques are the major analytical techniques used to determine adulteration of herbs and spices in high concentrations [92]. Overall, these techniques provide a good first point of control in the

fight against food fraud. Although the use of other confirmatory techniques such as mass spectrometry may be required in some circumstances, the bulk of screening herbs and spices for EMA are possible with spectroscopy.

Although not a spectroscopic technique, an analytical screening technique called the 'electronic nose', capable of detecting aroma fingerprints, was used alongside PCA and Artificial Neural Networks (ANN) to detect adulteration in saffron. This technique was found to be promising, as detection was possible at higher than 10 % adulteration, enough to detect EMA [34].

3.4. Combination of detection methods

In some circumstances, there is a need to use more than one technique to verify results. Along with the combination of methods already described by Black et al. [19], the combination of microscopy and GC-MS was also carried out for the detection of adulteration of fennel seeds [71]. Screening tests are often carried out with rapid techniques, but they have their limitations. In 2014, the USA recalled over 675 products due to the presence of undeclared nut protein in cumin. In a study carried out by Garber et al. [22], it reported failings in the antibody-assay based technologies involved in screening products for allergens. Although these methods are robust, and can detect as little as 1µg of allergen, they are not always specific to the allergen they are developed to detect. Therefore, with this analytical weakness, DNA and mass spectrometry-based tests are often used for further investigations. With the use of DNA and mass spectrometry analysis, additional allergens were detected; however, further work on the development of biomarkers for accurate analysis of a range of possible allergens may improve detection. This case indicates the limitations of screening methods with single analyte testing in some cases, and the need for multiple testing methods to understand the adulteration further.

3.5. Chemometrics

Chemometrics is used to improve the chemical data obtained from analytical instruments and to correlate the properties of samples with the use of mathematics and statistical methods [76]. Chemometrics has been used in the calibration analysis of spectroscopic and spectrometric data. It has been used with both targeted and untargeted methods to detect the presence of fraud in food or to determine authenticity [92]. The use of pre-processing is carried out in chemometrics to amplify desirable information from raw data and reduce the effects of undesirable information in the spectra. There are three key stages in the use of chemometrics, data pre-processing, development of a robust model, and the validation of a model and the analysis of results. Two commonly used pre-processing techniques include scatter correction methods, and spectral derivatives. Scatter corrective techniques can include Multiplicative Scatter Correction (MSC), Standard Normal Variate (SNV) and, normalisation to reduce the effects of physical variability caused by scattering [93]. The two commonly used spectral derivatives are Norris-Williams (N-W) and Savitzky-Golay (S-G). The spectral derivatives aim to smooth the spectra without reducing the signal to noise ratio in the spectra too much.

The analysis of adulteration using spectroscopy and in some cases mass spectrometry requires further investigation with chemometrics. The most common algorithms used for the determination of authenticity or the detection of fraud are the classification/discrimination algorithms such as the unsupervised PCA, and the supervised LDA, PLS-DA or OPLS-DA. For the quantification of adulterant in a sample, PLSR analysis is used frequently.

3.6. Detection methods for the addition of illegal dyes

An extensive review of detection methods for illegal dyes has been carried out by Oplatowska-Stachowiak and Elliott [41]. Liquid Chromatography is the most common method of detection of illegal dyes. Other chromatography techniques were used with various detection methods including voltammetric, spectrophotometric and capillary electrophoresis. The use of Enzyme-Linked Immunosorbent Assay (ELISA) is also a common method of detection in this field.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Ingredient	Adulterant	Reference	Detection Methods	Chemo-metrics
Saffron	*Carthamus tinctorius, Chrysanthemum x morifolium, Zea mays, Nelumba nucifera*	[65]	DNA barcoding	
Black pepper	Chilli	[25]	DNA barcoding	
Saffron	Safflower	[59]	SCAR and ITS Multiplex PCR	
Saffron	Saffron	[94]	Barcoding Melting Curve	
Chilli	Dried red beet pulp and powdered *Ziziphus nummularia* fruits	[16]	PCR-SCAR markers	
Oregano	*Satureja montana* L. and *Origanum majorana* L.	[20]	SCAR-PCR	
Oregano	Olive leaves	[61]	SCAR-PCR	
Oregano	*Cistus incanus* L., *Rubus caesius* L. and *Rhus coriaria* L	[21]	SCAR-PCR	
Saffron	*Arnica montana* L., *Bixa orellana* L., *Calendula officinalis* L., *Carthamus tinctorius* L., *Crocus vernus* L., *Curcuma longa* L., and *Hemerocallis* sp.	[57]	SCAR-PCR	
Turmeric	*Curcuma zedoaria/Curcuma malabarica*	[36]	SCAR-PCR	
Cumin	Almond, peanut, tree nuts, peach and cherry	[22]	DNA analysis, Antibody based technology, Microscopy, Mass spectrometry	
Saffron	Saffron of unknown origin labelled as being cultivated in the PDO region in Spain can be used for substitution.	[33]	LC HRMS	PCA, OPLS-DA
Fennel seed	*Anethum graveolens* fruit (AGF) and *Cuminum cyminum* fruit (CCF)	[71]	Light microscopy, fluorescence microscopy, GC-MS	
Chinese star anise	Japanese anise	[75]	Plant spray DART-HRMS	
Chinese star anise	Japanese anise	[74]	DART-HRMS	

Ingredient	Adulterant	Reference	Detection Methods	Chemo-metrics
Oregano	Olive leaves, myrtle leaves, hazelnut leaves, sumac	[69]	LC-MS/MS, FTIR	PLSR
Oregano	Olive leaves	[70]	LC-ESI-MS/MS	
Sage	Olive leaves	[70]	LC-ESI-MS/MS	
Oregano	Olive leaves	[72]	GC/MS	
Paprika	Falsely declared Szegedi paprika substituted for *Szegedi Füszerpaprika* PDO	[32]	ICP-MS	PCA, CDA
Oregano	Olive leaves, myrtle leaves, cistus, hazelnut leaves, sumac	[19]	FTIR , LC-HRMS	PCA, OPLS-DA
Garlic	Cornstarch	[76,78]	Raman, FTIR	PLSR
Ginger	Cornstarch	[78]	Raman	PLSR
Onion Powder	Cornstarch	[78,79]	Raman, FT-NIR, FTIR	PLSR
Saffron	*Crocus sativus* stamens, turmeric, safflower, gardenia	[77]	^{1}H-NMR	PCA, OPLS-DA, O2PLS-DA
Saffron	*Crocus sativus* stamens, calendula, safflower, turmeric, buddleja, and gardenia	[86]	DRIFTS-FTIR	PLS-DA, PLSR
Chinese star anise	ILACS	[87]	NIR/MIR	LDA, PCA
Chinese star anise	Japanese star anise	[88]	SWIR-HIS	PCA, PLS-DA
Black pepper	Buckwheat or millet	[90]	NIR hyperspectral imaging, FTIR	PLSR
Paprika	Tomato skins, brick dust	[91]	FT-NIR & Advanced-ID algorithm	
Turmeric	Yellow chalk powder	[37]	Terahertz spectroscopy	
Saffron	Safflower dyed corn stigma	[34]	Electronic Nose	PCA, ANN

5. Conclusion

It is evident that EMA is a constant threat in the growing herb and spice industry. Cases of fraud have an economic impact on the industry as well as reducing consumer confidence. Potential public health risks following adulteration, such as the case of nut protein in cumin and paprika, are a major concern in the industry. Advances in DNA analysis include the use of SCAR-PCR and DNA barcoding provide faster and cheaper methods of analysis. Further advancement may include the use of NGS as it moves into the area of food fraud. Mass spectrometry, commonly used for the detection of food fraud is also improving by becoming faster and cheaper with the introduction of ambient techniques. Spectroscopic methods along with chemometric techniques are increasingly being used in the fight against food fraud and offer a rapid, robust screening technique that is cost effective and requires little expertise. There is an increasing need for screening techniques that can detect EMA over a range of products in the growing herb and spice industry.

Acknowledgements

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