Key challenges and developments in non-targeted methods or systems to identify food adulteration

Developing smart agri-food supply chains

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Key challenges and developments in non-targeted methods or systems to identify food adulteration

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1 Introduction
2 Terminology for non-targeted food authentication
3 Developments in non-targeted methods to identify unknown adulterations
4 Challenges in non-targeted methods to identify unknown adulterants
5 How the key challenges are being addressed
6 Future trends for non-targeted methods
7 Where to look for further information
8 References

1 Introduction

Technological advancement enables scientists to actively address food integrity challenges through the development of innovative analytical techniques, and assists in developing smart agri-food supply chains in the long run. The food industry is continually implementing these advances and techniques into their production systems to improve food integrity, which involves producing foods that are of high quality, authentic, safe, nutritious and sustainable. Furthermore, if a novel method could replace or reduce the traditional usage of laborious confirmatory analytical methods, it may offer many advantages, such as being more cost-effective, providing rapid results for decision makers, increasing the number of samples that are routinely tested or screened.

Food fraud is the intention to cause a mismatch between food product claims and the product's innate characteristics (CEN, 2019). When there is a match between a food product's characteristics and claims, it is deemed authentic. Therefore, for the authentication of food products, the authenticity of the product is verified by means of analytical approaches. This chapter explores

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the authenticity of foods, an important aspect under the food integrity umbrella, given the fast increase in research within this field as well as the application of non-targeted (also termed untargeted) techniques. Furthermore, depending on the adulterant that is added to a food product, there can be detrimental effects on the quality, safety, nutrition and sustainability of the product. For example, if a cheap, unregulated and toxic compound is added to a product, it decreases its quality and safety, impacts nutrition and, depending on how the adulterant was produced and sourced, jeopardises the sustainability of the product. Moreover, the need to combat food fraud is also a part of the new 'Farm to Fork Strategy' of The European Green Deal (https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_en; accessed February 20th, 2021) – highlighting its importance towards ensuring foods with integrity for the future.

Targeted methods detect only one compound at a time, and since numerous adulterants can be present in a product, the use of targeted analysis alone is regarded as an inefficient authentication strategy, unless only one specific adulterant is expected to be present (Ballin and Laursen, 2019; Jiménez-Carvelo et al., 2021). For the purpose of authenticating food products, the potential of rapid non-targeted methods has gained a considerable amount of attention since the year 2000 (Callao and Ruisánchez, 2018; Ballin and Laursen, 2019). Especially, a non-targeted testing system is more effective at addressing the large variations in food authenticity issues that can exist, such as geographic origin or production system, substitution with cheaper similar ingredients, extension of food etc., as it encapsulates the chemical analysis of the whole food matrix to generate a unique food fingerprint (McGrath et al., 2018). Non-targeted methods can also deliver a high throughput by enabling the screening of multiple food products within a short period of time.

However, when using non-targeted methods, there are also many challenges that need to be addressed before they can be implemented. The conventional targeted methods have been the official norm for food fraud testing, as they can be easily standardised, validated and harmonised. This also makes the results of studies comparable across various disciplines, which is often not the case for non-targeted methods. The multitude of challenges that exist for non-targeted methods will be further explored in this chapter. Furthermore, this chapter aims to explore the recent developments in rapid non-targeted (fingerprinting) methods or systems to identify unknown adulterations of food products. As it is impossible to discuss all the different types of non-targeted methods that exist, this chapter will focus on the two key types of techniques: spectroscopic- and spectrometric-based methods.

2 Terminology for non-targeted food authentication

The continuous technological developments and interdisciplinary nature of research nowadays mean that different approaches and nomenclatures are inconsistently used (Ballin and Laursen, 2019). The major issue with this inconsistency arises in legal disputes involving non-targeted approaches, as these analytical methods should be harmonised, validated and standardised, and should apply common procedures for data evaluation, interpretation and reporting. As a first step towards harmonisation, a mutual understanding of the analytical principles of targeted versus non-targeted food authentication is required. The commentary by Ballin and Laursen (2019) aimed to address this by proposing novel definitions and nomenclature of targeted and non-targeted authentication methods, as summarised in Table 1.

It is important to note that a specific analytical method is not necessarily always targeted or non-targeted. In fact, it depends on the general approach taken to authenticate the product through the targets used as well as the statistical methods employed. Targeted methods are aimed at measuring a single marker or only a few known markers (e.g. analysing the concentrations of single compounds) versus the profiles (of known compounds) or fingerprints (any feature) assessed in non-targeted analysis (Table 1). The measurement of unspecified targets or data points (often >100) and the application of advanced multivariate statistics are characteristic of non-targeted methods (Alewijn et al., 2016). For example, an analytical fingerprint or physico-chemical profile is combined with multivariate classification methods to establish a class discrimination based on product characteristics. Although this chapter is focussed on non-targeted methods, it is vital to highlight that the application of both targeted and non-targeted methods to address a specific authentication issue can vastly increase the reliability and robustness of the results (Ballin and Laursen, 2019). To select the most appropriate combination of methods, the following sources can be consulted:

- Galanakis, C. M. (2021), Food Authenticity and Traceability, Academic Press, Burlington. https://doi.org/10.1016/C2019-0-03808-3.
- Sun, D. W. (2018), Modern Techniques for Food Authentication, (2nd ed.), Academic Press, Burlington. https://doi.org/10.1016/C2017-0-01345-9.

3 Developments in non-targeted methods to identify unknown adulterations

The recent developments in non-targeted methods can be separated into those for analytical techniques and those for the multivariate approaches used. Both will be explored in this section.

Table 1 Targeted compared to non-targeted techniques as adapted from Ballin and Laursen (2019)

Method type	Targeted	Non-targeted
Description	• Single or dual targets that are primary marker(s) for direct authentication through legal limits. • Single or dual targets that are secondary marker(s), for indirect authentication through threshold value(s) or database comparison. • Profiling using secondary markers, for indirect authentication through threshold value(s) or database comparison.	• Fingerprinting using unspecified targets or data points for indirect authentication through a database or reference comparison.
Targets	• Primary markers: chemical compounds that often have specific legal limits. • Secondary markers: chemical elements, isotope ratios, metabolites, chemical breakdown products and derivatives or macromolecules (DNA, lipids, proteins, sugars) evaluated using established and internationally acknowledged threshold values or conversion factors.	• Unspecified targets or data points: provide a fingerprint that is a display of multiple non-targeted parameters comprising information from an analytical method.
Key features	• Quantitative and/or semi-quantitative. • Identification of known adulterants. • High selectivity and sensitivity. • Extensive sample preparation. • Validation often supported by matrix-matched certified reference materials (CRMs). • Information extracted through conventional/univariate statistical analyses or calibration. • Control limits are publicly available.	• Qualitative and/or semi-quantitative. • Detection of unknown adulterants. • High throughput. • Simple or no sample preparation. • Validation is more difficult to perform. • Information extracted through advanced multivariate statistics. • Control limits relate to specific reference databases.

3.1 Advances in analytical non-targeted methods

The analytical techniques typically used for non-targeted fingerprinting methods generate detailed instrumental fingerprints (which vary depending on the technique) of the authentic samples being tested (Jiménez-Carvelo et al., 2021). This fingerprint denotes the characteristic unspecific instrumental signal (i.e. spectrum, voltamperogram, thermogram, chromatogram, electropherogram or image) from the analysed sample, which can be related to its unique chemical composition. Ultimately, these authentic fingerprints form part of the library or reference database that is used to determine the authenticity of a sample in question. Multivariate statistics (chemometrics) is used to determine the difference between the new sample and the library of fingerprints gathered previously that represent historical material that is fit-for-purpose to establish its authenticity.

For an overview of the stages involved in non-targeted analysis, see McGrath et al. (2018). The various non-targeted methods used to identify unknown adulterants in foods are shown in Table 2. For technical details on the techniques, the reader is directed towards the numerous papers cited in this chapter. The two key types of techniques explored are spectroscopic analysis and spectrometric analysis. In short, spectroscopy is the science of studying the interaction between matter and radiated energy (matter subjected to electromagnetic radiation), whereas spectrometry is the measurement of the interactions between light and matter, and the reactions and measurements of radiation intensity and wavelength.

3.1.1 Spectroscopy-based non-targeted methods

Spectroscopic techniques allow rapid, objective, high-throughput and non-destructive analysis of foods which is required for the successful management of a complex and fast-paced global food supply network. These methods are also considered more user-friendly and can easily be used onsite or in-line, for example, at food production facilities. As seen in Table 2 they have been used on a wide range of products to detect adulteration. An important advancement for these technologies is the development of miniaturised portable devices (further discussed under Section 3.1.3). Some other advancements will be discussed in the following section.

Other advancements include the modification of the method, such as the integration of high-resolution NMR spectroscopy with chemometrics, which aids in the growth of food NMR metabolomics (Siddiqi and Nollet, 2018). Variations in metabolite fingerprints are caused by the effects of external factors (i.e. type, geographical origin, pedoclimatic conditions, animal or plant specialties, agricultural practices etc.) on the chemical composition of samples. These

Table 2 Examples of non-targeted methods used to identify adulterants or a food authenticity issue in foods

	Non-targeted method	Food commodity (adulterants/authenticity issue investigated)	Reference
Spectroscopy	UV–Visible spectroscopy	Pomegranate juice (filler juices and water)	(Boggia et al., 2013)
		Wine (wine from different varieties)	(Sen and Tokatli, 2016)
	FT-IR spectroscopy	Butter (mutton fat)	(Fadzlillah et al., 2013)
		Hazelnut paste (almond paste and chickpea flour)	(López et al., 2014)
		Coffee (coffee from different origins)	(Bona et al., 2017)
		Milk powder (melamine)	(Limm et al., 2018)
	MIR spectroscopy	Beef burgers (beef offal)	(Zhao et al., 2014)
		Milk (formaldehyde, hydrogen peroxide, sodium carbonate, sodium citrate, starch	(Gondim et al., 2017)
	NIR spectroscopy	Milk (melamine)	(Chen et al., 2017)
		Rice (rice from different varieties)	(Shannon et al., 2021)
		Chinese lotus root powder (cassava, sweet potato, potato and maize starches)	(Xu et al., 2013)
		Chinese kudzu starch (talcum powder, sweet potato, potato, maize and cassava starches)	(Xu et al., 2015)
	Raman spectroscopy	Extra virgin olive oil (hazelnut oil)	(Georgouli et al., 2017)
		Bakery products (margarine)	(Üçüncüoğlu et al., 2013)
		Beef burgers (beef offal)	(Zhao et al., 2015)
		Milk powder (melamine)	(Karunathilaka et al., 2018)
	HSI	Black pepper (papaya seeds)	(Orrillo et al., 2019)
		Herbal tea (different tea blends)	(Sandasi et al., 2018)
		Nutmeg (spent powder)	(Kiani et al., 2019)
		Minced pork meat (leaf lard)	(Jiang et al., 2020)
		Milk powder (melamine)	(Karunathilaka et al., 2018)
	NMR spectroscopy	Refined olive oil (refined hazelnut oil)	(Agiomyrgianaki et al., 2010)
		Honey (honey from different botanical sources)	(Boffo et al., 2012)
		Coffee (corn, coffee husks, barley and soybean)	(De Moura Ribeiro et al., 2017)
		Ceylon cinnamon (Chinese cinnamon)	(Farag et al., 2018)
	BARDS	Salts (salts of different origins)	(van Ruth et al., 2019)

Mass spectrometry (MS)	DI-MS	Rice (rice from different origins)	(Lim et al., 2017)
	UHPLC-QTOF-MS	Saffron (inferior saffron material)	(Senizza et al., 2019)
		Guarana seeds (guarana from different origins)	(Da Silva et al., 2017)
	MALDI-TOF-MS	Fish meat (different fish species)	(Stahl and Schröder, 2017)
		Feta and mozzarella (milk from different species)	(Rau et al., 2020)
		Buffalo ricotta (bovine milk)	(Russo et al., 2016)
	PTR-MS / PTR-TOF-MS	Lamb meat (lamb from different origins)	(Erasmus et al., 2017)
		Cocoa beans (cocoa beans from different origins)	(Acierno et al., 2019)
		Honey (different honey varieties)	(Schuhfried et al., 2016)
	SIFT-MS	Extra virgin Argan oils (oils from different origins)	(Kharbach et al., 2018)
		Extra virgin olive oil (corn oil, sunflower oil, high oleic sunflower oil and olive oil)	(Ozcan-Sinir, 2020)
Ambient mass spectrometry (AMS)	DART-MS	Chicken meat (chickens fed bone meal)	(Cajka et al., 2013)
		Milk (milk from different species; plant oils)	(Hrbek et al., 2014)
		Extra virgin olive oil (hazelnut oil)	(Vaclavik et al., 2009)
		Cinnamon (different *Cinnamomum* species)	(Avula et al., 2015)
		Chinese star anise (Japanese star anise)	(Shen et al., 2012)
	FI-ESI-MS	Cocoa (cocoa from different origins)	(Acierno et al., 2018)
	EASI-MS	Olive oil (olive oil from different origins)	(Riccio et al., 2011)
		Salted caviar (pasteurised caviar)	(Porcari et al., 2014)
	PS-MS	Coffee (coffee from different origins)	(Garrett et al., 2013)
	LESA-MS	Meat (meat from different species)	(Montowska et al., 2014)
	REIMS	Fish (fish from different species)	(Black et al., 2017)
		Minced chicken and pork meat (protein powders)	(Kosek et al., 2019)

(UV) ultraviolet; (FT-IR) Fourier transform infrared; (MIR) mid infrared; (NIR) near infrared; (HSI) hyperspectral imaging; (NMR) nuclear magnetic resonance; (BARDS) broadband acoustic resonance dissolution spectroscopy; (DI-MS) direct-infusion mass spectrometry; (UHPLC-QTOF-MS) ultra-high-performance liquid chromatography coupled to electrospray quadrupole-time-of-flight mass spectrometry; (MALDI-TOF-MS) matrix-assisted laser desorption ionisation time-of-flight mass spectrometry; (PTR-TOF-MS) proton transfer reaction time-of-flight mass spectrometry; (SIFT-MS) selected ion flow tube mass spectrometry; (DART-MS) direct analysis in real-time mass spectrometry; (FI-ESI-MS) flow infusion-electrospray ionisation-mass spectrometry; (EASI-MS) easy ambient sonic-spray ionisation-mass spectrometry; (PS-MS) paper spray-mass spectrometry; (LESA-MS) liquid extraction surface-mass spectrometry; (REIMS) rapid evaporative ionisation mass spectrometry.

compositional differences can be seen with NMR spectroscopy and exploited via chemometrics through the application of multivariate statistical models. The models analyse the vast collections of whole NMR spectra to identify the factors responsible for the variation and classify the samples accordingly.

Other recent NMR advances originate from the hardware point of view (Siddiqi and Nollet, 2018). Aiding the use of NMR approaches for people less familiar with the method, the latest NMR spectrometers run in a completely automated fashion and have pre-programmed routines for running advanced 2D NMR experiments with minimal setup. Furthermore, solid-state probes, particularly high-resolution magic-angle spinning (HR-MAS) probes that allow the direct NMR profiling of semi-solid and solid food samples, have become more widely available. Lastly, another beneficial advancement for NMR metabolomics is the availability of multi-samplers operating in automated mode in the NMR spectrometers. They usually depend on a large sample space to obtain the high statistical relevance needed for developing accurate classification authentication models (Siddiqi and Nollet, 2018).

A developing spectroscopy method (not included in Table 2) is far-infrared (THz) spectroscopy. The THz region is the gap between the microwave and IR regions of the electromagnetic spectrum. The application of the THz waves is still in its infancy when compared to the other established and widespread spectroscopy methods used for food authentication (Jiménez-Carvelo et al., 2021). Using THz spectrometry, one can study the vibrational activities of molecules. What makes it an enticing method is the ability of the THz wave to penetrate various commonly used nonpolar dielectric materials and indicate the vibrational modes of many biomolecules. The low-frequency THz region can also be used to observe the torsional and rotational modes of molecules. Another advantage is that the wavelength of the THz spectral band is longer and will not be easily affected by scattering, unlike the regularly used UV–Vis and IR spectroscopy.

A spectroscopy method not widely reported is molecular fluorescence spectrometry. It is considered a powerful analytical tool for food authentication, as it is rapid, non-destructive, relatively inexpensive, user-friendly, has good sensitivity and selectivity and is instrumentally versatile (Jiménez-Carvelo et al., 2021). When compared to other spectroscopic analytical techniques (i.e. UV–Vis and IR spectroscopy), it has a higher sensitivity and can be used to analyse materials including endogenous fluorescent compounds (even at low concentrations). Molecular fluorescent spectrometry can be used alone or coupled with separation techniques. Fluorescence excitation–emission matrix (EEM) spectroscopy generates a total fluorescence spectrum by methodically varying the excitation and emission wavelengths and collecting the resulting data matrix. The observed electronic transitions reflect the structure and properties of a material. The spectra’s 2D character implies a three-way nature (map of fluorescence characteristics) for a set of samples that need to be analysed by adequate chemometric methods. Currently, multiway methods coupled with EEM fluorescence spectrometry are mainly used for quantitative analysis, while limited studies have explored classification analysis.

Another advancement worth mentioning is the use of a product's acoustic properties to identify or authenticate it (van Ruth et al., 2019). The technique that has been successfully applied to measure the acoustic properties is broadband acoustic resonance dissolution spectroscopy (BARDS). Although currently, there are limited studies on its use for food authentication purposes, this method has been used to distinguish different salts (van Ruth et al., 2019). BARDS may be a very useful screening method in the future due to the minimal sample preparation that is required and its rapid nature. When a powdered sample is added to a liquid, small gas bubbles (adhered to or trapped within the particles) form in the liquid due to dissolution of the powder. The presence, production and consequent disappearance of the bubbles are indirectly detected in real time via the monitoring of the frequency change of acoustic resonances. The change in gas/bubble volume in the solution due to the addition of the powdered material is measured indirectly with BARDS.

3.1.2 Spectrometry-based non-targeted methods

Spectrometric techniques are effective tools used for the detection of adulteration. In Table 2, the methods have been separated as mass spectrometry (MS) (the conventional MS methods) and ambient mass spectrometry (AMS) (a relatively new field of MS). These MS methods have been used on a variety of foods for the detection of adulteration, with applications becoming increasingly widespread across food commodities.

An advance for conventional MALDI-MS is MALDI MS imaging (MALDI-MSI) – a valuable tool that can be used to non-destructively visualise food components as well as the geographical origin, among others (Morisasa et al., 2019). MALDI-MSI uses matrix-assisted laser desorption ionisation as an MS imaging method in which the sample (typically a thin tissue section) is moved in two dimensions while the mass spectrum is recorded. It is anticipated that MALDI-MSI will soon be widely applied in the food industry.

The application of AMS is a relatively new field of analytical chemistry which has the potential to overcome issues where techniques require long and complex sample preparation and assay times, whilst giving results that are comparable with other conventional techniques (Black et al., 2016). In fact, an important advantage is that AMS methods require no sample preparation and minimal sampling time; producing fast (obtained near-instantaneously within 2–3 seconds) and accurate results – making AMS appear like the 'holy grail' of rapid non-targeted methods. The quick growth of AMS has meant that there are currently over 30 different techniques available. Although not all of them will be capable of detecting adulteration, some (DART-MS, EASI-MS, LESA-MS and PS-MS) have already been proven to improve and assist in the system used for adulterant detection.

Under the AMS methods, REIMS is an innovative method that can achieve semi-quantitative results for solid samples without the need for any form of sample preparation within a liquid solution (Black et al., 2017). It appears to be a very promising screening tool for meat authentication. It has been used to detect protein-based adulterants (reliability at 2.5%) in minced pork and chicken (Kosek et al., 2019). Furthermore, REIMS has been proven to be efficient in assisting with fraud detection in fish, while it has the potential to be utilised by fisheries to conduct their own quality control checks for fast and accurate results – an accolade held by very few rapid methods (Black et al., 2017).

3.1.3 Portable instruments for onsite measurements

Portable (miniaturised) instruments, particularly spectral devices, are being increasingly used in the food industry and particularly for food fraud testing beyond the laboratory. They are also termed handhelds, and are generally promoted as rapid screening tools for onsite detection of adulteration.

A growing number of studies prove the high versatility of the applications of portable NIR spectroscopy and hyperspectral imaging (HSI) (Liu et al., 2018; Acierno et al., 2019; Kiani et al., 2019). For instance, using the latest Specim IQ portable HSI camera (https://www.specim.fi/iq/), it is possible to generate spectral fingerprints for authentic material and common adulterants.

The battery-operated MicroNIR OnSite-W (https://www.viavisolutions.com/en-uk/osp/products/micronir-onsite-w) is a handheld NIR analyser that connects wirelessly to a tablet or computer. Like most handhelds, it is compact/small and designed to be used for applications in the field or on the manufacturing floor. The SCiO Sensor is another handheld NIR device that operates through a mobile application (Consumer Physics, https://www.consumerphysics.com/) and was marketed as the world's first pocket-sized molecular sensor. Although it utilises only a short spectral range between 740 nm and 1070 nm, it has been proven to be an effective tool for food authentication (Shannon et al., 2021). It also recognises the need to have a powerful cloud that stores the material database along with chemometric models and algorithms that process the scan, which is essential for these non-targeted approaches (Müller-Maatsch et al., 2021). A portable attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectrometer, combined with soft independent modelling of class analogy (SIMCA), has also been used to successfully detect milk powder adulteration by using dry-blended and wet-blended samples with melamine as the test adulterant (Limm et al., 2018). Furthermore, portable handheld Raman spectroscopic devices and on-board chemometric analysis have been investigated for the detection of milk powder adulteration (Karunathilaka et al., 2018). The portable devices had similar levels of performance (concerning sensitivity and specificity) as the benchtop Raman spectrometer for classifying test samples as genuine milk powder or melamine blends.

There are also new advances for portable NMR in food analysis. The development of unilateral NMR sensors was a breakthrough for low-field NMR application (Capitani et al., 2017). Portable devices with unilateral NMR sensors allow for non-invasive assessment of the compositional and microstructural characteristics of foods. Their portability and easy sample access make them desirable for quality control in industrial environments and directly on sealed packaged foods. Unfortunately, a drawback is the decrease in sensitivity due to inhomogeneity of the magnetic field. Yet, developments in unilateral magnets design could help to improve their performance.

Although there is a notable rise in the portability of instruments, the same principles for analytical and multivariate method development still apply. In fact, the scientist operators should have increased awareness of this, especially when the goal is to develop standardised methods. For non-scientist operators who require an actionable answer, developers have put vast resources into reliable identification algorithms, databases and calibrations (Crocombe, 2018). Standardisation of operating conditions is a vital step, especially with spectroscopic techniques, as variations in environmental conditions are likely to have a greater effect than the variations in the samples being analysed (Müller-Maatsch et al., 2021).

To succeed in the application of these portable techniques to screen for fraud and adulteration, substantial effort must be invested for analysing a large set of (authentic) samples by standard methods, documenting sample metadata, collecting spectra with a complete understanding of sampling and the characteristics of each method, and using the data to build validated chemometric models. This cannot be done in a crowdsourced manner (asking consumers to scan samples), but it must be performed using good laboratory practice and requires familiarity with big data analytics. In parallel, dedicated spectral databases need to be constructed and maintained to enable the widespread application of portable optical screening devices (Müller-Maatsch et al., 2021).

For a detailed overview of portable spectroscopic devices, the following papers are advised:

- Crocombe, R. A. (2018), 'Portable spectroscopy', *Applied Spectroscopy*, 72(12), 1701-1751. https://doi.org/10.1177/0003702818809719.
- Müller-Maatsch, J. et al. (2021), 'The spectral treasure house of miniaturized instruments for food safety, quality and authenticity applications: A perspective', *Trends in Food Science & Technology*, 110, 841-848. https://doi.org/10.1016/j.tifs.2021.01.091.

3.2 Advances in multivariate qualitative methods

Non-targeted methods rely on multivariate data analysis (chemometrics) to solve a specific food fraud problem. These statistical approaches are fundamental for the success of a non-targeted application (Alewijn et al., 2016). Establishing a multivariate qualitative method involves the following steps: data collection, pre-treatment, exploration techniques (unsupervised analysis), classification techniques (supervised analysis using pattern recognition methods) and/or data fusion and method validation (model optimisation by internal validation, model testing by external validation and stability testing of the model by system challenges) (Riedl et al., 2015; Callao and Ruisánchez, 2018).

For a detailed overview of the whole process for multivariate qualitative method development and validation, readers are referred to the paper by Callao and Ruisánchez (2018). The review by Riedl et al. (2015) offers a generic scheme for multivariate model validation, while the paper by Alewijn et al. (2016) provides a concept for a full validation of multivariate classification methods in practice. A general overview of the different steps is provided in Table 3. It should be noted that for non-targeted methods, the qualitative approach is typically adopted (primarily considered in this chapter), although quantitative (linear and non-linear methods) also exist for multivariate analysis. These quantitative methods can, for example, be used to predict the level of adulteration.

As shown in Table 3, the unsupervised models (i.e. PCA, CA) are used first to provide an overview of the variance of a dataset. This is followed by supervised models that offer an increased discriminatory power that allows samples to be classified. The most common multivariate approaches use SIMCA and PLS-DA classification techniques, or some variation thereof. The SIMCA method can be used to build models for each class independently from all others in such a way that it enables them to be used for either one-class or multi-class classification. Details about the modelling power and discriminating power of variables can also be obtained. Conversely, PLS-DA is a discrimination technique based on the PLS regression technique, adapted to a supervised classification task. It requires that more than one class be defined (two-class or multi-class), and as an outcome, the samples are always assigned to one class. A new variation of the technique, one-class partial least squares (OCPLS), has been developed for the one-class approach, and seems to have become more popular amongst researchers (Callao and Ruisánchez, 2018).

Currently and in the future, the main challenge in chemometrics will be the generalised and standardised use of multivariate methods, which are still not completely accepted by analytical chemists. However, the usage of and the interest in these methods are growing, owing to requirements for more analytical and data processing skills (Siddiqi and Nollet, 2018). In the validation

Table 3 Overview of the steps for multivariate qualitative method development

Steps	Purpose	Examples
Data collection	Analyse collected samples that are representative of each category.	Fingerprints of authentic samples and/or adulterated samples are produced.
Pre-treatment	Remove any erratic information from the raw data or convert it to a suitable data format/matrix.	Baseline correction, variable reduction deconvolution, derivation, bucketing or binning, noise filtering (smoothing), normalisation, data transformation etc.
Exploration techniques	Provide information about the relationship between samples (natural groups or trends in sample distribution), between variables (which of them give complementary information and which give similar or redundant information) and/or between samples and variables (which variables are important/not for distinguishing groups of samples).	PCA, CA
Classification techniques	Individually assign an unknown sample characterised by the same variables to one (or none) of the predefined classes.	LDA, QDA, KNN, PLS-DA, SIMCA, UNEQ, SVM; OCPLS
Data fusion	Obtain a single result from more than one source.	Fusion of different spectroscopic data.
Method validation[1]	Establish the quantifiable attributes/performance parameters that define the method's quality (evaluate model to assure its proper performance).	Linearity, selectivity, LOD, LOQ, accuracy (precision and trueness/bias), robustness, measurement uncertainty, R^2X, R^2Y, Q^2, etc.

(PCA) principal components analysis; (CA) cluster analysis; (LDA) linear discriminant analysis; (QDA) quadratic discriminant analysis; (KNN) k-nearest neighbours; (PLS-DA) partial least squares discriminant analysis; (SIMCA) soft independent modelling of class analogy; (UNEQ) unequal dispersed classes; (SVM) support vector machines; (OCPLS) one-class partial least squares; (LOD) limit of detection; (LOQ) limit of quantification; (R^2X) goodness-of-fit parameter; (R^2Y) proportion of variance of the response variable that is explained by the model; (Q^2) predictive ability parameter; [1] For external validation of a classification model, the most common figures of merit are accuracy, precision, sensitivity (percentage of samples correctly classified) and specificity (percentage of samples correctly rejected) (Siddiqi and Nollet, 2018).

step of multivariate qualitative models, the dataset can be split into a test and training dataset, provided that there is a sufficient number of samples (Callao and Ruisánchez, 2018). The chemometric model is then optimised and validated via internal cross-validation and external validation using training datasets.

Performance parameters define the quality of the method and evaluate the model to assure its good performance (Table 3). If the model is for a quantifiable sample property, additional performance parameters such as decision limit, detection capability and unreliability region are determined. The multivariate classification strategy of Callao and Ruisánchez (2018) introduces data fusion techniques to find suitable parameters if one dataset does not deliver sufficient results. For a detailed discussion on the conventional validation process and a new approach for establishing the validation requirements of the qualitative screening methods, see Jiménez-Carvelo et al. (2021).

Cuadros-Rodríguez et al. (2020) have proposed the use of new applicability indicators. The tutorial shows a quick and easy-to-apply methodology to validate, from a broader perspective, an analytical screening method designed to analyse many samples and to verify those that meet certain characteristics or attributes. A new parameter, which has never been considered before in multivariate analysis, is named occurrence (OCURR), and is the occurrence value used to properly validate the analytical method in real conditions (Jiménez-Carvelo and Cuadros-Rodríguez, 2020). The knowledge of this parameter is crucial to verify that the screening method works correctly for the intended purpose. From the intuitive values of these indicators and by determining the confidence limit or error rate that the scientist or organisation is prepared to accept, the validation requirements of the screening methods can be quickly determined.

Data fusion is a new trend in the use of data, which involves combining the output data from multiple instrumental sources. It provides a larger set of data and thus increases the chances of obtaining a better and more accurate understanding about a specific food/sample. It has been used to authenticate the origin of the material and assess the quality of a wide range of foods (Di Rosa et al., 2017). Compared to the use of one single technique, findings show that it can significantly enhance the classification performance (lower error rates and less uncertainity in predictions) or quality evaluation (Borràs et al., 2015; Di Rosa et al., 2017), providing improved validity of authentication results (Siddiqi and Nollet, 2018).

Many of the studies to date combined the data from gas (E-nose) and liquid sensors (E-tongue) and UV–Vis spectroscopy or computer vision sensors (E-eye) (Di Rosa et al., 2017). For example, in food sensory analysis, the data from sensors can be used to model a food's sensory attributes that would conventionally be measured by a human panel. Furthermore, the synergistic effects of spectroscopic methods like IR, Raman spectroscopy and NMR spectroscopy have been studied by concatenating the data (fusing at low level) (Siddiqi and Nollet, 2018). Borràs et al. (2015) reviewed the fusion of multivariate instrumental techniques applied in the authentication and quality assessment of beverages and food commodities. The authors found that of the three levels of data fusion (low, mid and high), low- and mid-level fusion approaches were mostly used;

10% of the applications used high-level fusion, 30% mid-level fusion and 50% low-level fusion. Improved classification and/or prediction results were found following fusion, compared to those obtained using a single technique. When data are very different in size or scale, mid-level fusion can yield better results.

Further advances for data-fusion approaches will be driven by the growing availability and use of rapid non-destructive methods in industry and laboratories. It is anticipated that the advances will lead to improved prediction and understanding of complex intrinsic food properties that cannot otherwise be achieved with a single technique. This will in turn improve food authentication strategies (Borràs et al., 2015). New approaches are emerging to predict product quality, like hybrid applications that combine low- and mid-level data fusion, fusion of second- and higher-order data (i.e. hyperspectral images or data from hyphenated techniques) and the combination of first order-data from online monitored systems (Borràs et al., 2015). As for model performance, one can anticipate that data fusion will increase the global classification/prediction ability and decrease the uncertainty of each individual result, thereby enabling better outlier detection in prediction. This still needs to be studied more thoroughly. The following sources are recommended for more information on data fusion methodology and applications:

- Cocchi, M. (2019), *Data Fusion Methodology and Applications*, vol. 31, Elsevier Ltd., Amsterdam.
- Borràs, E. et al. (2015), 'Data fusion methodologies for food and beverage authentication and quality assessment – A review', *Analytica Chimica Acta*, 891, 1–14. https://doi.org/10.1016/j.aca.2015.04.042

4 Challenges in non-targeted methods to identify unknown adulterants

Various challenges exist for fingerprinting techniques, ranging from points of improvement in the method itself, to the holistic approach that needs to be adopted to ensure an effective transition from targeted to non-targeted methods for the authentication of food in routine analysis and official control.

4.1 Challenges for analytical non-targeted and multivariate qualitative methods

Figure 1 gives an overview of the workflow for setting up non-targeted methods and of the challenges that currently exist. The main challenges are shown at the different steps, while best-practice recommendations are also provided in the boxes.

Most studies using spectroscopic non-targeted methods focus on the detection of adulteration (presence of an undeclared substance), with fewer studies focusing on providing information on the geographic origin (McGrath et al., 2018). A reason for this could be the challenge (linked to logistics, access and costs) in collecting a representative authentic sample set of a defined origin. Instead, it is easier to adulterate a few batches of authentic samples with known adulterants at different concentrations. However, the other challenges that exist with adulterated sets are that while scientists often collect the 'authentic' sample from supermarkets or retail outlets, they do not have access to the adulterants used in practice, or the way in which the sample is adulterated does not reflect how it is done in practice. For example, inferior material such as black pepper spent (material obtained after the oil has been extracted) or papaya seeds are added to black pepper during the industrial grinding process, whereas studies (e.g. Orrillo et al., 2019) grind the samples using a knife mill and mix using a mortar. In effect, it is likely that the laboratory-validated method will not be easily transferable to industrial product solution, unless researchers work closely with industry and aim to match the industrial process as closely as possible.

Hyperspectral imaging is a promising non-targeted method, given the vast number of spectra and spatial data it provides. Yet, its non-targeted application for food authentication and method validation is mostly unexplored, with limited applications in practice (McGrath et al., 2018). A framework for developing and validating HSI as a standard protocol accepted by regulatory authorities has been developed (Fernández Pierna et al., 2010). This protocol was tested and validated in line with International Standard ISO 17025, which is essential for it to be transferred to other laboratories and to be introduced as official control at the laboratory level. Essentially, this paves the way towards standardising non-targeted methods.

To facilitate the extensive use of MALDI-MSI, some challenges of this technology need to be addressed, in particular the limited detection of molecules present at low concentrations or ionisation efficiency (Morisasa et al., 2019). It is also recommended that further studies are conducted to make additional improvements to the method and/or explore new developments in the equipment, to enable the sensitive detection of molecules.

A huge concern is that most studies do not go beyond the laboratory proof-of-concept to indicate whether the non-targeted method will work in the field (McGrath et al., 2018). This can be addressed by performing a real sample survey that entails the collection of samples from the relevant node in the supply chain network (e.g. samples received from the trader or commercial samples from the market), followed by non-targeted testing, and ultimately, the confirmation of anomalies that exist, using secondary testing. Another approach could be to determine the feasibility of conducting the measurements with variable conditions in the field after initial in-house method development.

When the authentication of food is required, fingerprinting methods are suitable and valid for working in-house. A challenge that exists is the transfer of these methods for acceptance by producers, authorities and consumers. For this, the use of food reference materials is essential. For example, a reference material will enable the normalisation of the fingerprint signal between equipment. As a second step, the compositional information on significant chemical markers could be used for confirmation and for transferability (Siddiqi and Nollet, 2018).

Data fusion (as discussed in Section 3.2) is becoming progressively more important in food authentication; however, it also requires appropriate pre-processing and model validation.

4.2 Moving from targeted to non-targeted methods for official and routine testing

A comprehensive review article by McGrath et al. (2018) extensively explored the scientific challenges that exist for moving from targeted to non-targeted spectroscopy-based methods for food fraud testing. They (and others) identified the challenges that are faced by research and routine-testing communities, as described in Table 4.

Method validation (to verify the relevance of modelling results) is a vital step towards the harmonisation and standardisation of food fingerprinting (Riedl et al., 2015; Alewijn et al., 2016; Cavanna et al., 2018). It is also still a huge challenge that impacts harmonisation and standardisation that are needed as prior conditions for the authentication of food in routine analysis and official control. Although method validation is essential for non-targeted approaches, it is often used insufficiently or inconsistently in studies (Cavanna et al., 2018). Yet, to become truly standardised, the issue of acceptance of non-targeted testing in legislation must be addressed before these methods can be widely applied in practice (McGrath et al., 2018).

The application and interpretation of chemometrics is overburdened by difficulties, as illustrated in a special issue by Pretsch and Wilkins (2006). Often, scientists do not have sufficient statistical expertise to perform chemometrics and resort to blindly following an approach that is generally accepted for publication. In fact, given the wide array of available multivariate analyses and non-targeted analytical approaches, it is not surprising that it is still a challenge to develop a standard guide for multivariate qualitative method development. Apart from the lack of programming and statistical skills, advanced chemometric modelling software is also generally expensive and often requires some computer programming skills and understanding.

A common problem that occurs is model overfitting, as fingerprints consist of far more variables than samples for building a model. Essentially, an overfitted

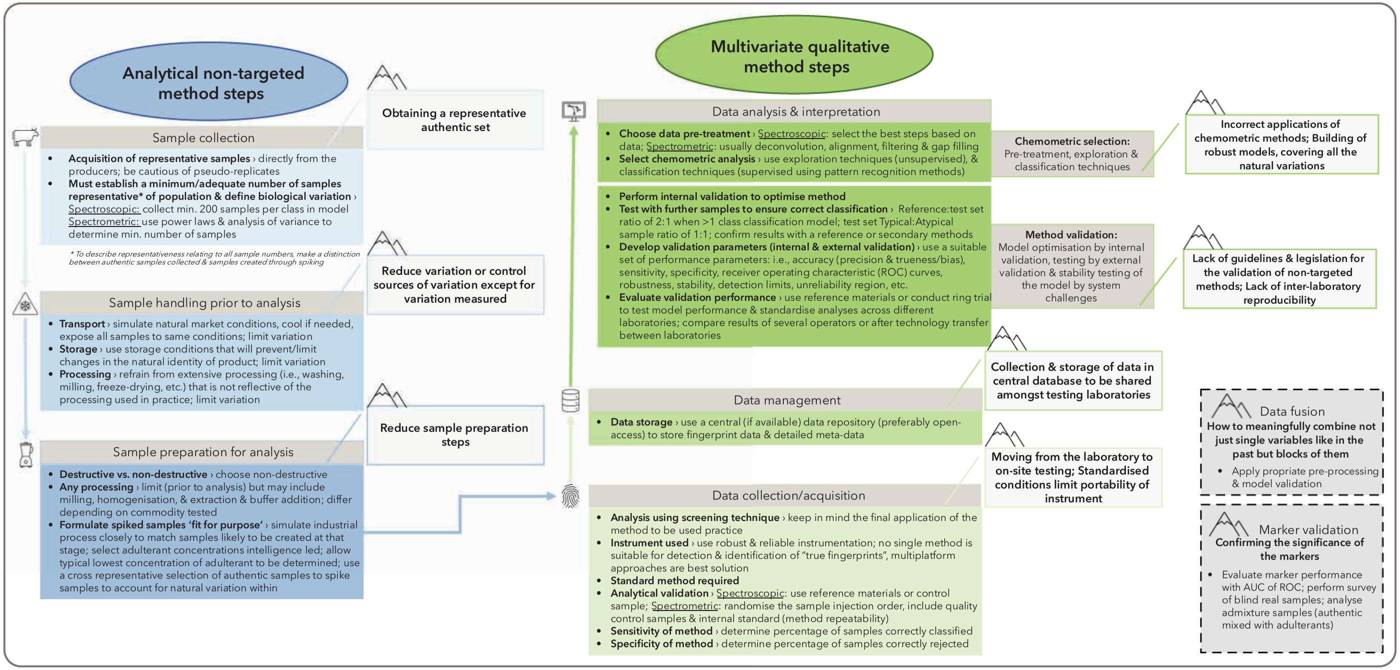

Figure 1 Overview of the workflow of setting up non-targeted methods and the challenges (⛰) that currently exist. The main challenges are shown at the different steps, while best practice recommendations are also provided.

model is unsuitable to receive valid results from new sample measurements. Characteristically, an overfitted model will have a high performance result that looks good for the paper published, but in reality, it is a weak result. This might explain why, despite the numerous studies on non-targeted methods, the uptake concerning routine surveillance has been limited to date.

Developing more robust models is an important challenge as well, as these models provide results that are independent of experiential influences (external variation) and in a best-case scenario address temporal changes of the biological material. Model robustness can be studied by testing the long-term stability of the model using a so-called system challenge.

After developing a validated model with sufficient performance, and before the method can be accepted as an official procedure, there is still another challenge to overcome – it requires a high degree of result reproducibility across different platforms (Cavanna et al., 2018). Hence, it should be able to deliver consistent results across different laboratories, instruments and analysts. There has been limited attention on inter-laboratory reproducibility, as most methods have been developed and validated in-house. The standardisation of methods is a huge but essential challenge to make data broadly comparable.

5 How the key challenges are being addressed

To address the key challenges and advance the widespread use of non-targeted methods for routine analysis, guidelines and legislation need to be developed for non-targeted methodologies. The publication of protocols from experts such as the EUROLAB Guide (Schönberger et al., 2015) is essential. This guide was the first published protocol to establish standard criteria and minimum requirements for NMR spectrometry, to ensure that the right validation of an analytical multivariate method (qualitative and quantitative) is applied.

Much of the expense (skilled operators, sample collection and preparation, analytical and chemometric analyses) to develop and maintain non-targeted testing is on the research and development side (McGrath et al., 2018). The entities developing the methods are not necessarily the same as the ones using them, and the research cost could be regained through the commercialisation of fully developed and validated methods. The end users then pay for access to the sample protocols and the model interface.

There are also a multitude of successful commercial applications that use non-targeted screening. Within the dairy industry, DairyGuard (PerkinElmer) is used for analysing milk powder (https://www.perkinelmer.com/product/dairyguard-milk-powder-analyzer-l1280080) and works by comparing unknown samples against a library containing spectra of authentic/unadulterated samples and known adulterant materials. FOSS Analytics created the MilkoScan™ FT2 (https://www.fossanalytics.com/en-gb/products/milkoscan-ft2) to analyse all liquid dairy products using targeted and untargeted adulteration screening.

Bruker Corporation has developed a database of several thousands of reference products that were sourced from production sites all over the world. The database is used with the FoodScreener™ (https://www.bruker.com/en/products-and-solutions/mr/nmr-food-solutions/food-screener.html), along with the Profiling™ technique, to compare non-targeted spectral ^{1}H NMR data, with the reference spectra using verification models. Samples are prepared and measured following a strict protocol in the laboratory, after which the data are evaluated on a single central Bruker server that runs the verification models and generates a report for the user. A problem with these proprietary databases is that concerns about the nature of the samples may arise (McGrath et al., 2018). Instead, the use of open access databases is suggested as a more robust solution.

A company that has successfully commercialised databases for the verification of origin is Oritain (https://oritain.com/). The verification signifies that the tested product can be scientifically verified as being from its claimed origin. Oritain will perform independent, scientific sampling and analysis for each brand. To begin, Oritain collects authentic samples of the product for analysis, and then product profiles are created – the 'origin fingerprint'. Finally, testing will be conducted against the origin fingerprint to scientifically verify whether the product is consistent with its claimed origin.

A good example of how huge amounts of non-targeted data are being made available to all interested investigators is MetaboLights (https://www.ebi.ac.uk/metabolights/). MetaboLights is an online data repository that allows the sharing of data by following a data protocol procedure (Kale et al., 2016). All details about each experiment are included. The database is cross-species and cross-technique and contains metabolite structures (with reference spectra), along with their biological roles, locations, concentrations and experimental data. If sharing of data in a standardised way can be promoted, raw data could be used as input for external validation. It is mentioned that the chemometric model should be able to correctly cluster these samples, even though the number of extracted features will likely not be exactly the same (Cavanna et al., 2018). Furthermore, in cases where the aim is to identify relevant markers, the shared results could assist in identifying robust compounds possibly already detected in different laboratories or, if not, to merge complementary results (Cavanna et al., 2018). A viable first step for this would be for scientific journals to make data-protocol-algorithm deposition a pre-requisite for publication.

The difficulties that investigators experience with the use of chemometrics are also being addressed on a commercial level. For example, HSI is becoming widely used in different fields, where scientists do not always know where to

Table 4 The challenges that exist in non-targeted methods used to identify adulteration

Challenge	Reference
• Lacking guidelines and legislation for: the development and validation (establishing a set of measurable attributes/performance parameters that define the method's quality) of non-targeted methodologies; describing how and at what concentrations spiked samples should be prepared to build robust models.	(Callao and Ruisánchez, 2018; Cavanna et al., 2018; McGrath et al., 2018; Jiménez-Carvelo et al., 2021)
• Determining if a method is fit for the purpose: defining the purpose, goal and scope of the method; outlining which samples to include in a database; establishing how many and which performance characteristics to use; deciding beforehand what is an acceptable performance.	(Alewijn et al., 2016)
• Developing a generally accepted and integrated validation approach.	(Alewijn et al., 2016)
• Basic performance parameters (i.e. sensitivity and specificity) are used, but others like robustness, stability, detection limits and the unreliability region are lacking.	(Callao and Ruisánchez, 2018; Cavanna et al., 2018)
• No common definition of terms, leading to difficultly in interpretation of data.	(McGrath et al., 2018; Ballin and Laursen, 2019)
• Lacking certified reference materials.	(Cavanna et al., 2018; McGrath et al., 2018; Ballin and Laursen, 2019)
• Difficulty in sourcing authentic samples with full traceability data for model building.	(McGrath et al., 2018)
• There is no single chemometric modelling software that offers all the algorithms required by developers.	(McGrath et al., 2018)
• Deciding which pre-processing approaches and classifiers to compare, given the endless choice. • Establishing criteria to choose the best approaches.	(Alewijn et al., 2016)
• Poorly understood (scientists lack the fundamental knowledge of the capabilities and the limits of the chosen method) and incorrect applications of chemometric methods.	(Pretsch and Wilkins, 2006)

start when it comes to processing the spectra. Consequently, perClass Mira (https://www.perclass.com/perclass-mira/product) has been developed to assist. Its logical and simple-to-use interface allows any field expert or research and development specialist to build solutions for automatic segmentation of spectral images. Once the image is uploaded, the user can define classes and build image interpretation solutions. In fact, no programming or machine learning expertise is required as its powerful automatic machine learning engine selects the best statistical model for the labelled samples. The developed models can be stored and used for automatic classification.

6 Future trends for non-targeted methods

Considering the vast technological advancements and the generation of large datasets and information, it is imperative to explore how these developments can be further advanced and applied towards the detection of food adulterants, as well as ways to benefit actors along the food supply chain networks.

Spectroscopic techniques have gained increasing attention, not only due to their non-destructive nature, but also due to their fast generation of information and their ability to increase automation. Various spectroscopic- and sensor-based methods have the potential to quantitatively predict chemical components and ensure food authenticity through product screening. Hence, it is envisioned that these methods will be more widely used in the food industry for product screening onsite or in-line at various nodes within the supply chain (i.e. port, food distribution centre, supermarket etc.) that are highly vulnerable to fraud.

Furthermore, the use of IT-based tools has the potential to benefit stakeholders (public, businesses, traders, government etc.) by providing an integrative platform for decision making in real time, based on the ability of screening methods to determine the presence of fraud. Hence, big data analytics and its integration with non-targeted techniques, as part of the IoT, are likely to gain increasing attention in the future. McGrath et al. (2018) suggested potential future advancements that entail the development of applications to provide and facilitate access to databases and information sharing in a cloud-based environment.

Future trends in analytical chemistry on food fraud will rely on the implementation of non-targeted methods obtained by highly informative techniques, such as MS and NMR fingerprints, which provide additional information rather than mere compliance or noncompliance (Jiménez-Carvelo et al., 2021). It is expected that there is still a lot of development to come for the analytical non-targeted methods in the coming years. Considering NMR spectroscopy as an example, it is envisioned that the field of food metabolomics will continue to grow (Siddiqi and Nollet, 2018). This expansion will be due to the development of hardware in the design of NMR probes and electronics to allow high throughput and cost-effective analysis of large food sample sets, as well as software improvements that will allow metabolite database integration for the semiautomatic assignment of NMR spectra to metabolites of interest.

In view of this, expansion of the Food Component Database (www.foodb.ca/) and other integrated food metabolite databases will be vital to the scientific community (Siddiqi and Nollet, 2018). Moreover, solid and semi-solid food authentication by NMR analysis has only recently been exploited; therefore, extensive development is expected in this field, especially given that most food materials are solid, and researchers are gaining increasing access to solid-state NMR spectrometers. This is particularly true for solid-state cross polarisation magic-angle spinning (CP-MAS) NMR, which has been unexploited in food fingerprinting. As low-cost permanent magnet-based liquid-state NMR spectrometers are becoming increasingly available in research, industry and government laboratories, the attention of the scientific community toward NMR applications in food authentication will likely increase.

Further advancements also focus on the best methodological approach. A two-tiered monitoring system comprising non-targeted screening run together with confirmatory techniques has been demonstrated (Shannon et al., 2021), and is suggested to be the system necessary to have broad-spectrum and cost-effective monitoring programmes ready to cover the complex and developing field of food fraud in the future.

Even though the steps for conducting a multivariate qualitative analysis are well established and documented, further research is still being done to seek improvements by experimenting with new data sources or developing new algorithms (Callao and Ruisánchez, 2018). In this regard, the advancement of data fusion (still to be studied more comprehensively) and the synergic information obtained from more than one technique will be beneficial for chemometrics.

As modern laboratories have a range of analytical instruments, any data fusion strategy is a feasible way of dealing with qualitative analysis. The promising new data fusion approaches include applications combining low- and mid-level data fusion, fusion of second- and higher-order data, such as data from hyphenated techniques, and the combination of first-order data from evolving systems to predict product quality (Siddiqi and Nollet, 2018). By combining the information from instrumental sources, improved results can be obtained, but one should carefully evaluate the improvement based on cost–benefit ratio, as using more than one method adds extra costs. Another trend to consider for data fusion is the permanent storage of data and the possibility of automation for online monitoring of industrial scale operations. For example, the combination of sensors and computer vision allows the combined sensing and visualisation of foods. See the review by Di Rosa et al. (2017) for an overview of fusing sensor data/hybrid systems using an electronic nose, electronic tongue and computer vision for animal-source food authentication.

It is without doubt that future advances in non-targeted techniques, combined with novel data analytics and computerized outputs, will contribute to building more resilient and integrated food supply chain networks. Technological advances in computing will continue to result in smaller and more powerful devices that can be used in a multitude of applications beyond the laboratory. The generation of vast amounts of data will be better utilised in the new era of digitisation and the building of a world through automation. In fact, it is anticipated that automation will offer improved food fraud monitoring and real-time decision making across the entire supply chain.

7 Where to look for further information

The following books provide a good overview of the non-targeted methods for food authentication:

- Sun, D.-W. (ed.) (2018). *Modern Techniques for Food Authentication*, Elsevier Science, United Kingdom.
- Siddiqi, K. S. and Nollet, L. M. L. (eds.) (2019). *Fingerprinting Techniques in Food Authentication and Traceability*, CRC Press, New York, U.S.
- González-Domínguez, R. (ed.) (2020). *Food Authentication: Techniques, Trends and Emerging Approaches*, MDPI AG.
- Galanakis, C. M. (ed.) (2020). *Food Authentication and Traceability*. Academic Press, Amsterdam. https://www.sciencedirect.com/science/book/9780128211045.
- Jiménez-Carvelo, A. M. et al. (2021). Nontargeted fingerprinting approaches, in *Food Authentication and Traceability*. Elsevier, pp. 163–193. https://doi.org/10.1016/b978-0-12-821104-5.00010-6.

The following articles provide a good overview of the multivariate data analysis strategies for non-targeted analysis of foods:

- Fisher, C. M. (2021). Data processing strategies for non-targeted analysis of foods using liquid chromatography/high-resolution mass spectrometry, *Trends in Analytical Chemistry*, 136, 116188.

Key research in this area can be found at the following organisations:

- The Food Authenticity Network (http://www.foodauthenticity.global/).
- The Institute for Global Food Security (IGFS) (https://www.qub.ac.uk/Research/GRI/TheInstituteforGlobalFoodSecurity/).
- The Centres of Expertise (CoEs) in food authenticity testing (http://www.foodauthenticity.global/uk-centres-of-expertise-coes-in-food-authenticity-testing).

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