A review of Fourier Transform Infrared (FTIR) spectroscopy used in food adulteration and authenticity investigations

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Abstract

The increasing demand for food and the globalisation of the supply chain, have resulted in a rise in food fraud, and recent high profile cases, such as the Chinese milk scandal in 2008 and the EU horsemeat scandal in 2013 have emphasised the vulnerability of the food supply system to adulteration and authenticity frauds. Fourier Transform Infrared (FTIR) spectroscopy is routinely used in cases of suspected food fraud as it offers a rapid, easy and reliable detection method for these investigations. In this review we first present a brief summary of the concepts of food adulteration and authenticity as well as a discussion of the current legislation regarding these crimes. Thereafter, we give an extensive overview of FTIR as an analytical technique and the different foods where FTIR analysis has been employed for food fraud investigations as well as the subsequent multivariate data analysis have been applied successfully to investigate case of adulteration or authenticity. Finally, we give a critical discussion of the applications and limitations of FTIR, either as a standalone technique or incorporated in a test battery, in the fight against food fraud.

Key words: FTIR, food fraud, food adulteration, food authenticity, spectroscopy
Introduction

With the increasing globalisation of the food supply chain, with several stakeholders in between ‘farm to fork’, and the intrinsic vulnerability of such a system, food quality and safety have become of increased concern for consumers, food producers and governments (US Food and Drug Administration, 2009; European Commission, 2018). Scandals such as the addition of melamine in baby formula in China in 2008 (Pei et al., 2011), the European horse-meat scandal in 2013 (Premanandh, 2013), and the issue of peanuts and almonds found in ground cumin and paprika in Europe and the US in 2015 (Agres, 2015), all illustrate the negative impact of food fraud, including both adulteration and authenticity.

Although there is no harmonised definition of food fraud in the EU, it is generally accepted by the member states that food fraud are committed ‘intentionally for financial gain through consumer deception’ (European Commission, 2018). The criminal activity of intentionally adulterating food has gradually become more prevalent due to the associated incomes (Galvin-King, Haughey and Elliott, 2018). Food adulteration can be defined as the process in which the quality of food is purposefully degraded either by the addition of low-grade quality material or by extraction of valuable ingredients (Spink and Moyer, 2011). Nowadays, the most common food categories susceptible to any type of food fraud are in descending order; olive oil, fish, organic foods, milk, grains, honey and maple syrup, coffee and tea, spices, wine and certain fruit juices (Moore, Spink and Lipp, 2012; Hoffman, 2013a). The so-called premium food products, such as meat (Rohman et al., 2011; Nunes et al., 2016a), spices and herbs (Lohumi et al., 2017a; Wielogorska et al., 2018), honey (Amiry, Esmaili and Alizadeh, 2017), olive oil (Rohman and Man, 2010; Georgouli, Martinez Del Rincon and Koidis, 2017a), and coffee (Reis et al., 2017) are particularly susceptible to adulteration, especially when they are produced and supplied through complex supply chains (Black et al., 2016). Expensive commodities are vulnerable to economically motivated adulteration (EMA) due to the large incomes generated from these products (Galvin-King, Haughey and Elliott, 2018).

Conversely, cheap foods, such as milk and grains, are also susceptible to adulteration, mainly due to the low margins of profit (Smith, Manning and McElwee, 2017). For instance, the increased consumption of milk, due to its high nutritional value, has made it prone to fraudulent activity.
Additionally, 21st century agriculture faces multiple challenges due to increasing global population, such as an increasing demand on food production for humans and livestock as well as increased demands on feedstock for the bioenergy market (FAO, 2009). The agricultural sector also faces demands due to the developments in the agriculture-dependent developing countries and the adaptation to more efficient and sustainable production methods and climate change (FAO, 2009). All of these factors are important when understanding the reasons for food fraud. Although it is not currently known how common food fraud is, it is estimated that the cost for the global food industry could be as high as US$40 billion dollars annually (PwC, 2016).

Depending on the adulterant used, food fraud could have severe adverse health effects, such as the development of cramps, nausea, diarrhoea, vomiting, nerve damage, allergic reactions and paralysis (Sicherer, Burks and Sampson, 1998). For instance, in 2015 there were reports of the addition of peanuts and almonds to cumin and paprika powder in the USA and Europe (Agres, 2015). The unintentional ingestion of these two allergens could potentially lead to severe or even lethal allergic reactions (Sicherer, Burks and Sampson, 1998). Other common allergens found in contaminated food are fruits, soybeans, fish, milk, egg, tree nuts, wheat and shell fish (Añíbarro, Seoane and Múgica, 2007) all of which can cause severe health problems.

The scandals concerning fraudulent foods have increased the pressure on food laboratories to develop fast and reliable screening methods for the detection of food fraud. One of the most commonly used screening techniques for food fraud today currently is Fourier Transform IR (FTIR) based on mid infrared (MIR) vibrational spectroscopy, as it offers a rapid and reliable detection method and in this review we describe the use of FTIR as a tool to investigate food adulteration and food authenticity. The first part of this review offers an overview of food adulteration and authenticity from a historical and current global perspective. The second part introduces FTIR as a screening technique and discusses the theory behind this technique. Finally, the third part of this review offers a critical discussion of the use of FTIR and multivariate analytical tools, on their own or as part of a battery of techniques as a method to investigate different food matrices.
This is to our knowledge the first critical review of FTIR as a tool to investigate food adulteration and authenticity since Rodriguez-Saona and Allendorf published their review in 2011 (Rodriguez-Saona and Allendorf, 2012) and considering the recent advances in the analytical techniques as well as the recent high profile food scandals, it is therefore a timely addition to the discussion of food authenticity and adulteration.

**Food adulteration and authenticity**

*Legislation and industry standards*

Food fraud is a collective term that encompasses the deliberate substitution, addition, tampering or misrepresentation of food, food ingredients or food packaging, or false or misleading statements made about a product for economic gain (Spink and Moyer, 2011; Lakshmi and Pradesh, 2012). Within the EU there is currently no harmonised definition of food fraud, however, there are four operational criteria for establishing food fraud (European Commission, 2018). These include the violation of the EU Food Law, intention, economic gain and deception of customers (European Commission, 2018). In the General Food Law Regulation EC 178/2002 (European Commission, 2002), the broad principles of food safety and regulation are outlined. For instance, under Article 8 – ‘Protection of consumers’ interests’, the General Food Law states its aim to prevent ‘fraudulent or deceptive practices’ and ‘the adulteration of food’ (European Commission, 2002). Furthermore, the EC 178/2002 regulation also established the European Food Safety Authority (EFSA) to provide scientific advice (European Commission, 2002) following some high profile European food scandals during the previous two decades.

In the United States (US), the two primary agencies for food safety, and thus for food fraud and authenticity, are the US Department of Agriculture (USDA) and the US Food and Drug Administration (Johnson, 2014). The primary legislation concerning food safety; governing food, drug and consumer protection, is the Federal Food, Drug and Cosmetic Act (FFDCA) from 1938 (United States Congress, 1938). In 2011, the Food Safety Modernization Act (FSMA) was signed into law,
awarding the FDA further authorities such as a mandatory recall authority (United States Congress, 2011), deemed necessary due to reported incidents of foodborne illnesses during the early 2000’s.

It is important to emphasise that not all levels of contamination are considered intentional adulteration. Most national and international legislations as well as industrial organisations today have a threshold for extraneous matter to allow distinction between accidental and intentional contamination. For instance, the threshold for gross adulteration for horsemeat in the UK is when food items contain 1% weight by weight (w/w) or above of extraneous material (Food Standards Agency, 2015). In the US, the FDA have established the Food Defect Action Levels in order to distinguish between ‘natural or unavoidable’ defects, where adulteration is one of many causes, for a range of commodities (US FDA, 2018). An example of an industry organ with its own thresholds is the European Spice Association (ESA), with a maximum of 2% w/w for extraneous material for herbs and 1% w/w for spices (ESA, 2015). However, with recent developments in increasing sensitivity of the methods employed, such as DNA tests and analytical chemical methods, to detect food fraud, very low concentrations of a contaminant could be detected (Galvin-King, Haughey and Elliott, 2018). These thresholds are therefore in place to differentiate between contamination and intentional adulteration, and are set based on the limit of detection for the particular analytical technique used to detect the adulteration (Downey, 2016).

Testing for food authenticity is important to ensure that food offered for sale or sold is of the nature, substance and quality expected by the purchaser (Defra et al., 2014). If manufactures fail to correctly and honestly state what ingredients are present within the food item, it becomes deception and is then classified as food crime (Defra et al., 2014). There are therefore harmonised regulations in the EU on food labelling, presentation and advertising aim to protect consumers and facilitate trade inside and outside Europe (European Commission, 2000). In 2006 the European Council Regulation introduced three different types of quality logos for ensuring authenticity of agricultural products and foodstuff (The Council of the European Union, 2006). Two of these logos - the Protected Designation of Origin (PDO) and the Protected Geographical Indication (PGI) - have a specific link to the region where the product comes from, while the third one - the Traditional Speciality Guaranteed (TSG) logo
highlights a traditional production process (The Council of the European Union, 2006). In practicality, this directive regulates food or drinks that are produced must adhere to a precise set of specifications to be labelled with any of the three logos (The Council of the European Union, 2006). As an example, as wine is a premier agricultural product of the EU and exported worldwide, the EU has taken serious action to maintain the reputation of the wine producing companies and other food manufacturers. Since 2011, the EU also has specific regulations for wine authenticity since 2011. Wine adhering to the specifications set by the framework is now categorised according to PDO and PGI (The European Commission, 2014b, 2014a).

**Food adulteration throughout history**

One subcategory of food fraud is Economically Motivated Adulteration (EMA), which can be defined as the intentional contamination of food for increased profit (Spink and Moyer, 2011). Not only is EMA a fraudulent activity that might harm consumers trust in a specific food category or company (GMA and Kearney, 2010; Kendall et al., 2018), it might also directly or indirectly have adverse effects on consumers health by the addition of potentially harmful substances to the food (Spink and Moyer, 2011). However, EMA is by no means a contemporary phenomenon and is likely to be as old as the food processing and production systems themselves (Ellis et al., 2012). Historical examples of EMA are the addition of lead to soured wine in ancient Rome, or adding the drug *cocculus indicus* in order mask the smaller amounts of hops and malt used during brewing or mixing alum with flour in bread to make it whiter (Evershed and Temple, 2016).

The first researcher to apply scientific methods to detect food adulteration was Frederick Accum, who made the first serious attempt to expose both the extent and dangers of food adulteration in 1820 (Accum, 1820). However, more than half a century after Accum first published his book, food adulteration was still so rife that a journalist for the *New York Times* wrote that most people were not aware of ‘what abominable messes they are constantly putting down their throats under the most innocent disguises’ (Evershed and Temple, 2016).
Even though modern food adulteration is usually more sophisticated than these historical examples, it is no less prevalent, and only in the last decade, there have been several high profile cases linked to food adulteration and authenticity. In 2008, elevated levels of melamine was found in the products of 22 Chinese dairy companies (The Associated Press, 2008), and this contamination led to the death of six infants and the hospitalisation of over 52,000 small children (Pei et al., 2011). Melamine is an organic base which is used to make plastics, fertilisers and concrete, and can cause kidney stone formation that in certain risk groups can trigger acute renal failure (Skinner, Thomas and Osterloh, 2010). When melamine is added to food products, it mimics a higher protein content of a diluted food (Pei et al., 2011), and this unapproved enhancement of the milk is a case of the devastating consequences EMA can have (Lakshmi and Pradesh, 2012). The melamine scandal in China also illustrates the vulnerability of food safety regulation if official controls do not efficiently assure the implementation of existing legislation (Pei et al., 2011).

Another highly publicised food scandal is the so-called ‘2013 horsemeat scandal’ in Europe in which food products marketed as beef were found to contain high levels, sometimes up to 100%, of undeclared or improperly declared horse meat (BBC News, 2013). This case, where cheaper horsemeat was mislabelled as expensive beef, highlighted several vulnerabilities within the European food supply chain (Department of Agriculture Food and Marine, 2013; Elliott, 2013). For instance a complex network of traders and grocers, which may contain farmers, primary processor, local traders, secondary processor, exporter, importer, trader, processor/packager, retailer and finally consumer, cause a lack of transparency in this part of the supply chain (Department of Agriculture Food and Marine, 2013; Elliott, 2013; Galvin-King, Haughey and Elliott, 2018). In the aftermath of the horsemeat scandal, it was concluded that the control of food safety also need to involve checks on food authenticity (Department of Agriculture Food and Marine, 2013).

One of the more recent cases of EMA with potentially severe adverse health effects for the public is the adulteration of ground cumin and paprika sold in Europe and the US by the addition of peanuts and almonds (Agres, 2015). The unintentional ingestion of peanuts or almonds could potentially lead to severe or even lethal allergic reactions (Sicherer, Burks and Sampson, 1998) and detection of
such adulterations is of high interest. This is also an example whereby adulteration associated with
EMA is driven by crop failures (Bawden, 2015). Whenever there is a crop failure of a certain
commodity, that commodity shows an increased susceptible to food fraud, similar to any food with a
sudden increase in price or demand (Bawden, 2015; Black et al., 2016).

Yet another example of adulteration of a food product in high demand is the dilution of
expensive extra virgin olive oil (EVOO) with cheaper vegetable and seed oils (Dalmia, 2015). This also
results in a lower the nutritional value further adding to the fraudulent nature of the adulteration
(Dalmia, 2015). The adulteration of olive oil using unhealthy substitutes is therefore considered a threat
for public health (Ok, 2017). The increased global demand for olive oil coupled with the recent years
of drought in the Mediterranean regions, has seen a steep rise in the prices of EVOO (Schrieberg, 2017),
making adulteration particular profitable. Adulteration of EVOO has therefore recently been recognised
as a common problem for regulatory agencies, oil suppliers and consumers (Dalmia, 2015). For
instance, in 2012, two Spanish businessmen were sentenced to prison after they were found trading
hundreds of thousands of litres of EVOO adulterated with sunflower oil (70-80%) (Henley, 2012).

Food authenticity as an emerging issue

The authenticity of food can be understood as how honestly and accurately the food item has been
marketed and trading of inauthentic foods is currently a large economic problem (Hansen, 2015). In
order to protect consumers and implement food laws, it is important to verify the labelling of food in
relation to composition, processing or origin (Primrose, Woolfe and Rollinson, 2010). The
determination of authenticity is important so that consumers can make informed choices in relation to
health concerns, religious beliefs or lifestyle choices (Primrose, Woolfe and Rollinson, 2010). Food
products that are marketed claiming to contain natural ingredients or originating from a special location
produced using local and traditional methods become difficult to verify unless thoroughly checked
(Hansen, 2015). Trading of inauthentic foods is currently a large economic problem (Hansen, 2015).

Food authenticity is a growing concern due the consequences deceptive marketing can
have on consumer health and trust. Moreover, the country or region of origin of certain commodities or
products that meet the standards of organic farming, such as saffron (Anastasaki \textit{et al.}, 2010), olive oil (Gouvinhas \textit{et al.}, 2015), honey (Gok \textit{et al.}, 2015) as well as wine (Cozzolino \textit{et al.}, 2009a) and wine derived products (Ríos-Reina \textit{et al.}, 2017) will greatly impact the retail price. This increased price makes these products vulnerable to food fraud due to the economic gains associated with the substitution with a cheaper ingredient. For instance, the price of saffron is very much dependent on the country of origin (Anastasaki \textit{et al.}, 2010), and there have been reports showing that more than 50\% of saffron labelled as the prestigious and Protected Designation of Origin (PDO) Spanish saffron could be grown and/or processed in other countries, such as Iran (Govan, 2011; Rubert \textit{et al.}, 2016).

Similarly, olive oils from certain PDO regions or countries, such as Sabina (Lazio), will also fetch a higher price due to their unique qualities in flavour and/or production technique (Bevilacqua \textit{et al.}, 2012a). The verification of authenticity of a food product is however a complex issue, where the entire food product is usually analysed in a non-targeted analysis (Bevilacqua \textit{et al.}, 2012a). In these cases, no foreign material has been added to the food product and only small chemical differences in several variables between the authentic and the fraudulent product are analysed, which requires multivariate statistical techniques, such as partial least squares (PLS), principal component regression (PCR), principal component analysis (PCA) or Soft Independent Modeling Class Analogy (SIMCA) to distinguish between foods with very similar chemical composition (Granato \textit{et al.}, 2018).

From the cases presented above it is clear that food fraud is neither a new phenomenon, nor something that is expected to decrease within the foreseeable future. Even if the food safety legislation has become more stringent in modern times, there is need for official controls using scientific methods to ensure that these regulations are implemented. It is clear that food fraud has been and will be posing a threat not only to the food industry in terms of loss of consumer trust and ultimately profits, but also, and more importantly, to consumers in the shape of potentially adverse health effects. These examples of food fraud also illustrate the need for a rapid, easy and reliable detection technique in order to analyse a larger subset of food for adulteration and authenticity, and thus discourage fraudulent activities.
FTIR vibrational spectroscopy as a detection technique

Introduction to infrared vibrational spectroscopy

Mid-IR (MIR) spectroscopy as an analytical technique measures the absorbance of IR radiation of dipole bonds within functional groups of molecules (Chalmers and Griffiths, 2002a; Gauglitz and Vo-Dihn, 2003). The absorbance of IR radiation excites the electrons in the bonds to a higher state of vibration (Gauglitz and Vo-Dihn, 2003), causing bending or stretching motions of the molecular bonds (Chalmers and Griffiths, 2002b). A molecule will absorb IR radiation only if the absorption of the particular wavelength causes a change in the at-rest and excited vibrational states (Amand and Tullin, 1999). Most chemical compounds, except for instance elemental diatomic gases such as N₂, H₂ and O₂, have a dipole moment and can be analysed by their characteristic infrared absorption (Amand and Tullin, 1999).

For organic compounds, characteristic stretching vibrations usually occurs between 4000-1500 cm⁻¹, in the so-called MIR region (Gauglitz and Vo-Dihn, 2003). However, below 1500 cm⁻¹, vibrations, generally bending, result in a characteristic fingerprint of the molecule or a large part of the molecule and can therefore give structural information useful for identification (Stuart, 2005). Moreover, the intensity of the absorbance is directly proportional to the concentration of a pure compounds or that particular feature when analysing mixtures (Gauglitz and Vo-Dihn, 2003), further making IR spectroscopy suitable for quantitative purposes.
Development of FTIR spectroscopy

FTIR instruments utilise the interference between two IR beams to yield a signal, called an interferogram, which is a function of the change in path length between the two beams (Stuart, 2005). This is usually achieved using a Michelson interferometer, see Figure 1. The generated signal from a Michelson interferometer can then be decomposed into the frequencies that form a signal, using Fourier Transform algorithms (Stuart, 2005). The Michelson interferometer, first described in 1881 (Michelson, 1881, 1927), consists of a light source, such as a mercury arc, tungsten or a globar lamp, a semi-reflecting beamsplitter and two perpendicular mirrors, one stationary and one moving (Stuart, 2005; Albert, Albert and Quack, 2011a). In the first part of the interferometer a collimated IR beam is split with equal intensity by a beamsplitter (Gauglitz and Vo-Dihn, 2003). These divided beams are then reflected by both the stationary and moving mirrors back to the beamsplitter, where they recombine and interfere, see Figure 1 (Gauglitz and Vo-Dihn, 2003). This interference, either constructive or destructive in the recombined beam, are caused by the moving mirror producing a difference in path length (Stuart, 2005).

Figure 1. Schematics of a Michelson interferometer used in modern FTIR instruments. Adapted from Albert, Albert and Quack (2011)
Although the Michelson interferometer was developed in the late 1800’s, it was not until the late 1970’s the methodology revolutionised high resolution IR, when the bottleneck of computational power to perform an Fourier transformation on high resolution IR data was overcome (Albert, Albert and Quack, 2011b). The Fourier transformation convert the obtained interferograms, presenting intensity versus time, to an IR spectrum, showing intensity versus frequency (Gauglitz and Vo-Dihn, 2003). Prior to the computer revolution in the late 1970’s, the resolution of most FTIR instruments were comparable to the dispersive instruments (Albert, Albert and Quack, 2011b). Currently, the resolution of an FTIR instrument is limited by the maximum path difference between the two beams reflected by the mirrors (Stuart, 2005), see Figure 1, which is proportional to the pathlength difference between the Michelson interferometer arms, so that a longer distance would increase the resolution (Gauglitz and Vo-Dihn, 2003). More precisely, the limiting factor to achieve even higher resolution with modern FTIR instruments is the precision of the optic and movement mechanisms for the moving mirrors in the interferometer (Stuart, 2005).

**Analysis of foods adulteration and authenticity using FTIR**

**Foods analysed by FTIR**

Traditionally, FTIR has been used to detect food adulteration in virgin oil (Rohman and Man, 2010; Georgouli, Martinez Del Rincon and Koidis, 2017b), honey (Rios-Corripio et al., 2011; Gok et al., 2015) and beef (Alamprese et al., 2013; Nunes et al., 2016b). However, recently it has also been used to analyse adulteration and authenticity in spices and herbs, such as saffron and oregano (Black et al., 2016; Lohumi et al., 2017b; Petrakis and Polissiou, 2017; Wielogorska et al., 2018), authenticity of wine and wine based products (Cozzolino et al., 2009b; Ríos-Reina et al., 2017) and nectars and jams (Miaw et al., 2018). All in all, IR spectroscopy has been used to detect fraud of many of the food products listed as most at risk (Hoffman, 2013b), emphasising the effectiveness of FTIR analysis in the battle against EMA and authenticity frauds.
Analysis of solid foods in FTIR can be carried out using alkali halide discs made from potassium bromide (KBr), as this simple inorganic salt do not generate any vibrations in the MIR region (Nyquist and Kagel, 2012). Solid materials can therefore be analysed by mixing the analyte with KBr and subsequently pressing the mix to a fused disc which can be introduced into the light beam of a spectrophotometer (Simmons, 1960; Gauglitz and Vo-Dihn, 2003). The use of KBr discs is seemingly the preferred method in some studies, such as for the detection of fraud in saffron samples (Anastasaki et al., 2010; Ordoudi et al., 2017; Petrakis and Polissiou, 2017), see Table 1. One of the main disadvantages by using KBr discs is the issue of reproducibility, as conditions such as matrix ratios and homogeneousness needs to be exactly the same for each sample. Even with the addition of internal standards, the discs and mulls must be prepared under precisely the same circumstances to avoid any changes in path length and thus response (Stuart, 2005). Moreover, KBr pellet method is susceptible to interfering moisture effects, that may cause water absorption peaks in the infrared spectrum or fluctuations in the baseline due to scattering caused by the clouding of KBr pellets (Stuart, 2005). Furthermore, due to the mixing of analyte and KBr this sample preparation technique is destructive.

As seen in Table 1, using Attenuated Total Reflectance (ATR) FTIR seem to be favoured nowadays when investigating food authenticity or adulteration, most likely due to its minimal, sometimes non-existent, sample preparation. In ATR FTIR the sample is pressed against a crystal, usually made from germanium, diamond or zinc selenide (Gauglitz and Vo-Dihn, 2003). The technique operates by measuring the changes that occur in a internally reflected IR beam when the it comes into contact with a sample surface (see Figure 2) (Kazarian and Chan, 2013). When the IR beam travels inside the crystal a standing wave of radiation, called the evanescent wave, is created (Gauglitz and Vo-Dihn, 2003). ATR has in recent years revolutionised solid and liquid sample analyses by FTIR as it combats the most challenging aspects of infrared analyses, namely sample preparation and spectral reproducibility (Kazarian and Chan, 2013), thus increasing the reproducibility of the analyses. This method does not only minimise issues with reproducibility it also allows for a rapid analysis. As with any screening technique, ATR also has its limitations, caused by the optical contact between the sample
and the ATR crystal (Lindenberg et al., 2012). This is of particular concern when solid samples are analysed, and in order to overcome this issue the accessories have devices that clamp the sample to the crystal surface and apply pressure (Lindenberg et al., 2012).

**Figure 2.** Schematics of an Attenuated total reflectance (ATR) cell.

**Multivariate analysis of FTIR results**

Two of the most common multivariate analyses employed for the quantification of adulteration is partial least squares (PLS) and principal component regression (PCR). These statistical techniques are normally used for calibration purposes and uses the full spectra region rather than smaller region of pre-specified wave numbers (Tasumi, 2015). Both these methods also use statistically independent covariates from lower dimensions (Granato and Ares, 2014). The main difference between PLS and PCR is that while PCR seeks a high variance distance between the space of the covariates, PLS seeks the space between covariates that will predict the best outcome, using the concentration information (Tasumi, 2015). To establish calibration curves, PLS is therefore more suitable for analysing noisy spectra when the noise is random, whereas PCR is more appropriate for spectra where a systematic noise is observed (Tasumi, 2015).

Another statistical approach, particularly employed for screening purposes, is principal component analysis (PCA) which is an exploratory method (Ríos-Corripio et al., 2011; Jaiswal et al., 2015a; Ríos-Reina et al., 2017). The aim of a PCA is to depict as much variability in a data set, such as an IR spectra, using as few variables as possible (Currell, 2015) and a linear combination of the original variables is used to generate new variables (Callao and Ruisánchez, 2018). The output of a PCA can then be visualised in a score plot, where the position of each sample is positioned according to its values.
of the principal components (PCs), centralised around zero (Currell, 2015). A PCA approach is typically used to visualise the difference between suspected adulterated foods and pure foods, with the expectation that the two should for separate clusters in the resulting scatter plot. However, a major drawback of PCA is that this method generates PCs in an unsupervised manner (Vidal, Ma and Sastry, 2016). This unsupervised learning means that there is not a specifically predicted target variable, which is commonly used for finding general patterns in the data set (Cady, 2017).

There is however, supervised classification techniques which aims to individually assign unknown samples to a predefined class based on sample characteristics (Callao and Ruisánchez, 2018). Examples of classification techniques commonly used for food fraud investigations are linear discriminant analysis (LDA), k nearest neighbours (kNN) and partial least squares discriminant analysis (PLS-DA), see Table 1. These techniques all require at least two defined classes, and will classify unknown samples to the class it most closely resembles based on a set of characteristics (Callao and Ruisánchez, 2018). However, a major drawback of these statistical methods is the need to add known adulterants to authentic foods when building the model, which means that they are not suitable for non-target analyses. Moreover, the detection of outliers within a dataset is more difficult using these methods, as all samples are assigned to a class (Currell, 2015; Callao and Ruisánchez, 2018).

Due to the drawback with both unsupervised and classification methods soft supervised learning models, such as Soft Independent Modeling Class Analogy (SIMCA), has in recent years gained attention for analysing suspected adulteration of food, see Table 1. In a SIMCA, the classification is made based on a set of PCs from training data, such as a calibration set (Salzer and Siesler, 2014). These classes are then applied on test data, where a class is independently assigned to the test data based on the closeness of that data to the classes in a multidimensional space (Salzer and Siesler, 2014; Callao and Ruisánchez, 2018). SIMCA has especially been shown to be useful when comparing known adulterated samples with unknown samples (Santos, Pereira-Filho and Rodriguez-Saona, 2013; Li et al., 2015; Black et al., 2016). As SIMCA only classifies the sample in one class, it is more suitable for cases where the adulterant is not known, and only the adulterated class needs to be
defined (Callao and Ruisánchez, 2018). However, due to this one class assignment the results can sometimes be ambiguous or inconclusive (Callao and Ruisánchez, 2018).

Moreover, some studies using FTIR to detect food adulteration have presented novel statistical tools, such as Continuous Locality Preserving Projections (CLPP) in recent years (Georgouli, Martinez Del Rincon and Koidis, 2017b) and hierarchical models (HM) (Gok et al., 2015; Reis et al., 2017) in recent years, to detect food adulteration based on FTIR data. CLPP can model mixtures of food and adulterant as data series rather than discrete data points, thus enabling a better visualisation of the classification compared to a PCA (Georgouli, Martinez Del Rincon and Koidis, 2017b). Just as PLS or PCR, HM is a type of linear regression model. However, in HM the variables fall into strictly hierarchical levels, also known as nested levels (Kery and Royle, 2016). Although HM has traditionally been used in biology to describe biodiversity (Kery and Royle, 2016), it can be used to classify non-adulterated versus adulterated food and even identify which particular adulterant has been used (Reis et al., 2017). However, to be able to identify the specific adulterant it must be used when building the model, and thus HM is not suitable for non-target analysis.
Table 1. Summary of studies analysing different foods for adulteration and authenticity using FTIR as an analysis technique.
<table>
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<tr>
<th>Food</th>
<th>Adulterants</th>
<th>Determining adulteration</th>
<th>Sampling technique</th>
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<th>Comments</th>
<th>Study</th>
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</thead>
<tbody>
<tr>
<td>Honey</td>
<td>Different Anatolian honey samples</td>
<td>N/A</td>
<td>ATR</td>
<td>Mid-IR (1800–750 cm⁻¹)</td>
<td>Hierarchical clustering and PCA</td>
<td>Developed method able to differentiate between honey floral source</td>
<td>(Gok et al., 2015)</td>
</tr>
<tr>
<td></td>
<td>Inverted beet sugar adulteration</td>
<td>Scanned multiple samples of honey with different concentrations of beet invert sugar (0.5 to 25% w/w)</td>
<td>ATR</td>
<td>Mid-IR (950–1500 cm⁻¹)</td>
<td>PLS and PCR</td>
<td>88-94% of the validation set were classified correctly</td>
<td>(Sivakesava and Irudayaraj, 2001)</td>
</tr>
<tr>
<td></td>
<td>Corn syrup, cane sugar syrup</td>
<td>Honey samples from central Mexico were adulterated (0-90%)</td>
<td>ATR</td>
<td>Mid-IR (900–1140 cm⁻¹)</td>
<td>PCA</td>
<td>Method able to discriminate between honey and cheaper syrup.</td>
<td>(Rios-Corripio et al., 2011)</td>
</tr>
<tr>
<td>Olive oil</td>
<td>Sunflower oil, corn oil, and canola oil</td>
<td>Extra virgin olive oil was adulterated with varying concentrations of palm oil (1.0–50.0% wt./wt)</td>
<td>ATR</td>
<td>Mid-IR (1500–1000 cm⁻¹)</td>
<td>PLS and PCR</td>
<td>Developed method able to discriminate between extra virgin olive oil and the adulterants tested</td>
<td>(Rohman and Man, 2010)</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>47 samples of extra virgin olive oil from both PDOs and other origins were analysed</td>
<td>ATR</td>
<td>Mid-IR (4000–630 cm⁻¹) NIR (10,000–4000 cm⁻¹)</td>
<td>PLS-DA and SIMCA</td>
<td>NIR was found to provide better predictions than MIR, year of harvest were found to impact spectra</td>
<td>(Bevilacqua et al., 2012b)</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>Monovarietal extra virgin olive oils from three cultivars</td>
<td>ATR</td>
<td>Mid-IR (700–740, 950–1050, 1100–1250, 1350–1500, 1700–1800 and 2750–3000 cm⁻¹)</td>
<td>PLS-DA and PCA</td>
<td>PLS-DA able to discriminate between samples from the three different cultivars</td>
<td>(Gouvinhas et al., 2015)</td>
</tr>
<tr>
<td></td>
<td>Sunflower, corn, soybean and hazelnut</td>
<td>Extra virgin olive oil samples were adulterated (0%, 5%, 10%, 30%, 50%, 75% and 100%)</td>
<td>KBr discs</td>
<td>Mid-IR (4000–500 cm⁻¹)</td>
<td>LDA</td>
<td>Adulterant levels down to 5% could be detected using the method developed</td>
<td>(Lerma-García et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>Hazelnut oil</td>
<td>Four extra virgin olive oil samples with different origins were adulterated with hazelnut oil (1–90%)</td>
<td>ATR</td>
<td>Mid-IR (4000–550 cm⁻¹)</td>
<td>CLPP</td>
<td>Method developed can be used for screening purposes on even low levels of adulteration (1%). Raman spectroscopy also investigated.</td>
<td>(Georgouli, Martinez Del Rincon and Koidis, 2017b)</td>
</tr>
<tr>
<td></td>
<td>Canola, hazelnut, pomace and high linoleic/oleic sunflower</td>
<td>Extra virgin olive oil was adulterated with varying concentrations of adulterating oil (5–40.0% wt./wt)</td>
<td>ATR</td>
<td>Mid-IR (4000–700 cm⁻¹)</td>
<td>PLS</td>
<td>Method developed were able to discriminate between olive oil and adulterants and determine level of adulteration</td>
<td>(Maggio et al., 2010)</td>
</tr>
<tr>
<td>Meat Type</td>
<td>Adulterants</td>
<td>Analysis Methodology</td>
<td>Identification and Quantification Methodology</td>
<td>References</td>
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<tr>
<td>Beef</td>
<td>Injecting solutions of non-meat ingredients (NaCl, phosphates, carrageenan, malodextrin)</td>
<td>Seized meat products from five slaughterhouses were analysed</td>
<td>The addition of addition NaCl to bovine meat causes specific β-sheets vibrations of proteins</td>
<td>(Nunes et al., 2016b)</td>
<td></td>
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<tr>
<td>Pork</td>
<td>Analysis of fats from pork and beef mince meat</td>
<td>ATR Mid-IR (1200-1000 cm⁻¹) PLS</td>
<td>Method able to quantify pork traces in beef meatballs</td>
<td>(Rohman et al., 2011)</td>
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<tr>
<td>Turkey mince</td>
<td>Minced beef adulterated with turkey meat (5-50% w/w)</td>
<td>ATR Mid-IR (4000-400 cm⁻¹) PCA, LDA, and PLS</td>
<td>Combining information from UV/vis, NIR and MIR spectroscopy improved the confidence in the results</td>
<td>(Alamprese et al., 2013)</td>
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<tr>
<td>Horse meat, fat</td>
<td>Mixtures of beef minced meat and the adulterants were prepared (2–90% (w/w))</td>
<td>ATR Mid-IR (4000–650 cm⁻¹) PCR and SIMCA</td>
<td>Method able to identify and quantify adulterant</td>
<td>(Meza-Márquez, Gallardo-Velázquez and Osorio-Revilla, 2010)</td>
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<tr>
<td>Saffron</td>
<td>Six characteristic adulterants of plant origin, i.e. C. sativus, calendula, safflower, turmeric, buddleia, and gardenia</td>
<td>Saffron was adulterated with adulterants (5-20% w/w) KBr discs Mid-IR (4000-600 cm⁻¹) PCA and PLS-DA</td>
<td>Proposed method involved a three-step process for the detection of adulteration as well as for the identification and quantification of adulterants</td>
<td>(Petrakis and Polissiou, 2017)</td>
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<tr>
<td>Saffron</td>
<td>Tartrazine, sunset yellow along with propane-1,2-diol, propan-2-ol and acylglycerols</td>
<td>Synthetic commercial sample tested that mimicked the appearance and specific UV-Vis absorbance values of trade standard test KBr Mid-IR (4000-400 cm⁻¹) Linear regression</td>
<td>Multistep workflow using data acquired from HPLC-UV/Vis, UV-Vis, mid-infrared (FTIR) and nuclear magnetic resonance (NMR)</td>
<td>(Ordoudi et al., 2017)</td>
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<tr>
<td>N/A</td>
<td>Saffron from four countries, including from the region of Castilla-La Mancha</td>
<td>KBr discs Mid-IR (4000-400 cm⁻¹) PCA and DA</td>
<td>Method able to correctly classify saffron from different countries in 90-98% of the cases depending on origin</td>
<td>(Anastasaki et al., 2010)</td>
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<tr>
<td><strong>Calendula flowers or ground turmeric</strong></td>
<td>Quantification of crocin in the samples</td>
<td>ATR</td>
<td>Mid-IR (1700–900 cm(^{-1}))</td>
<td>Target analysis, PLS</td>
<td>Validated against UV-Vis standard (ISO/TS3632)</td>
<td>(Lee, Htar and Akowuah, 2015)</td>
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<tr>
<td><strong>Milk, whey, hydrogen peroxide, synthetic urine, urea and synthetic milk</strong></td>
<td>Milk was spiked with each of the adulterant at five different levels</td>
<td>ATR</td>
<td>Mid-IR (1400–1800 cm(^{-1}))</td>
<td>SMCA and PLS</td>
<td>Method able to discriminate samples based on levels of adulteration, and also type of adulterant</td>
<td>(Santos, Pereira-Filho and Rodriguez-Saona, 2013)</td>
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<tr>
<td><strong>Soy milk</strong></td>
<td>Milk samples were adulterated with soymilk (2-40%)</td>
<td>ATR</td>
<td>Mid-IR (4000–500 cm(^{-1}) and 1680–1058 cm(^{-1}))</td>
<td>PCA</td>
<td>Method able to identify and quantify adulterant in ranges tested</td>
<td>(Jaiswal et al., 2015b)</td>
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<tr>
<td><strong>Melamine</strong></td>
<td>Milk samples were adulterated with melamine (0.0625–25% w/w)</td>
<td>ATR</td>
<td>Mid-IR (4000–650 cm(^{-1}))</td>
<td>PLS</td>
<td>Developed method able to distinguish between unadulterated milk samples and that containing 2.5 ppm MEL (threshold)</td>
<td>(Jawaid et al., 2013)</td>
<td></td>
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<tr>
<td><strong>Margarine</strong></td>
<td>Butter was mixed with margarine (0-100% w/w)</td>
<td>ATR</td>
<td>Mid-IR (900–1800 and 2800–3040 cm(^{-1}))</td>
<td>PLS</td>
<td>Method able to predict adulteration with high confidence in selected adulteration ranges (0–5%, 0–25% and 20–60%)</td>
<td>(Koca et al., 2010)</td>
<td></td>
</tr>
<tr>
<td><strong>Cheap animal fats; lard</strong></td>
<td>Butter adulterated with lard (0.5–80%)</td>
<td>ATR</td>
<td>Mid-IR (4000–650 cm(^{-1}))</td>
<td>PLS</td>
<td>Method determined for rapid identification of adulteration of butter with lard</td>
<td>(Nurrulhidayah et al., 2015)</td>
<td></td>
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<tr>
<td><strong>N/A</strong></td>
<td>In total, 54 unsalted butter samples from three different regions of Morocco were tested</td>
<td>ATR</td>
<td>Mid-IR (4000–600 cm(^{-1}))</td>
<td>PCA, PLS and PLS-DA</td>
<td>PLS-DA were able to discriminate accurately between butter samples from the three different regions</td>
<td>(Bassbasi et al., 2014)</td>
<td></td>
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<tr>
<td><strong>Cheese, formaldehyde</strong></td>
<td>Soft white cheese were spiked with formaldehyde (1-100 mg/100 g)</td>
<td>ATR</td>
<td>Mid-IR (1600–850 cm(^{-1}))</td>
<td>PLS</td>
<td>Method developed suitable for determination of formaldehyde/formalin in cheese samples</td>
<td>(Alkhalf and Mirghani, 2017)</td>
<td></td>
</tr>
<tr>
<td><strong>Oregano</strong></td>
<td>Mixtures of oregano and each adulterant were prepared (0–100% w/w)</td>
<td>ATR</td>
<td>Mid-IR (4000–550 cm(^{-1}))</td>
<td>PLS and SIMCA</td>
<td>Liquid Chromatography High Resolution Mass spectrometry (LC-HRMS) to confirm oregano adulteration using biomarkers</td>
<td>(Black et al., 2016; Wielogorska et al., 2018)</td>
<td></td>
</tr>
<tr>
<td>Material</td>
<td>Adulterant</td>
<td>Technique 1</td>
<td>Technique 2</td>
<td>Technique 3</td>
<td>Reference</td>
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<tr>
<td>Paprika and chili powder</td>
<td>Sudan dye</td>
<td>ATR</td>
<td>Mid-IR (1400-1800 cm(^{-1}))</td>
<td>HLA/GO (hybrid linear analysis)</td>
<td>Technique determined to be cost-effective in comparison to main alternative technique, LC. (Lohumi et al., 2017b)</td>
<td></td>
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<tr>
<td>Onion powder</td>
<td>Starch</td>
<td>ATR</td>
<td>Mid-IR (4000-650 cm(^{-1})), Near-IR (10000-4000 cm(^{-1}))</td>
<td>PLS</td>
<td>FT-NIR data was determined to have a higher predictive value than FTIR. (Lohumi et al., 2014)</td>
<td></td>
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<tr>
<td>Coffee</td>
<td>Spent coffee grounds, roasted coffee husks, roasted corn, and roasted barley</td>
<td>ATR and diffuse reflectance, DR</td>
<td>Mid-IR (4000-550 cm(^{-1}))</td>
<td>HM and DF</td>
<td>Method able to identifying adulterants in coffee, even in complex mixtures. (Reis et al., 2017)</td>
<td></td>
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<tr>
<td>Java tea (Orthosiphon stamineus)</td>
<td>N/A</td>
<td>KBr discs</td>
<td>Mid-IR (4000-550 cm(^{-1}))</td>
<td>PLS and SIMCA</td>
<td>Sample origin seems to have more dominant effect to the chemical constituent of the plant compared to plant varieties (Sim et al., 2004)</td>
<td></td>
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</tr>
<tr>
<td>Organic wine (Australian)</td>
<td>Non-organic wine (Australian)</td>
<td>Bacchus flow cell</td>
<td>Mid-IR (4000-400 cm(^{-1}))</td>
<td>PCA, discriminant PLS and LDA</td>
<td>DPLS was able to correctly assign 85% of all samples (Cozzolino et al., 2009a)</td>
<td></td>
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</tr>
<tr>
<td>Wine vinegars (PDO)</td>
<td>N/A</td>
<td>ATR</td>
<td>Mid-IR (1800-900 cm(^{-1}))</td>
<td>PLS and PCA</td>
<td>Method able to distinguish between different wine vinegars aged different times (Ríos-Reina et al., 2017)</td>
<td></td>
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<tr>
<td>Walnut oil</td>
<td>Soybean oil</td>
<td>ATR</td>
<td>Mid-IR (4000-650 cm(^{-1}))</td>
<td>PLS and SIMCA</td>
<td>Method could determine adulteration by soybean oil down to 10% (Li et al., 2015)</td>
<td></td>
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<tr>
<td>Avocado oil</td>
<td>Sunflower, canola and soybean oils</td>
<td>ATR</td>
<td>Mid-IR (4000-550 cm(^{-1}))</td>
<td>PLS and SIMCA</td>
<td>Developed method gave standard errors of prediction between 0.09 and 2.81 for the adulteration (Quiñones-Islas et al., 2013)</td>
<td></td>
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<tr>
<td>Nectars (Grape, orange, peach and passion fruit)</td>
<td>Syrup, apple and cashew juices</td>
<td>ATR</td>
<td>Mid-IR (4000-650 cm(^{-1}))</td>
<td>PLS</td>
<td>The mean relative errors of prediction using developed model varied from 3.0 to 6.7% (Miaw et al., 2018)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jams and juices</td>
<td>Glucose syrup, synthetic flavour</td>
<td>KBr discs</td>
<td>Mid-IR (4000-400 cm(^{-1}))</td>
<td>Analysis of variance</td>
<td>Developed method able to distinguish adulterated jams (Mohamed et al., 2011)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(strawberry and apricot jam, orange, apple and strawberry juices) and pigment (allura red and sunset yellow) and 50% glucose syrup and 100% glucose syrup. Juices were prepared from fruits</td>
<td>Tea</td>
<td>Talcum powder</td>
<td>210 samples of tea powder with 13 dose levels of talcum powder (0.15-1.40 mg g⁻¹)</td>
<td>KBr discs</td>
<td>Mid–IR (990–1055 cm⁻¹)</td>
<td>PCA</td>
<td>Method developed able to detect talcum powder in tea</td>
</tr>
</tbody>
</table>
Several studies have combined FTIR analysis with other analytical chemical techniques such as UV-Vis, near-IR (NIR) or Raman spectroscopy (Bevilacqua et al., 2012b; Alamprese et al., 2013; Lee, Htar and Akowuah, 2015; Georgouli, Martinez Del Rincon and Koidis, 2017a; Ordoudi et al., 2017), liquid chromatography coupled to either UV/Vis (Ordoudi et al., 2017) or high resolution mass spectrometry (LC-HRMS) detectors (Black et al., 2016; Wielogorska et al., 2018) and DNA barcoding (Wielogorska et al., 2018) in order to either compare a newly developed FTIR method with validated or standardised methods or to complement the FTIR analysis. For instance some studies have used FTIR analysis as a part of a spectroscopy test battery (Bevilacqua et al., 2012b; Alamprese et al., 2013; Georgouli, Martinez Del Rincon and Koidis, 2017a). Some of these studies have shown that combining FTIR with other spectroscopy techniques, such as UV-Vis and NIR, can improve the models for predicting adulteration of for example turkey mince in in beef (Alamprese et al., 2013). This was recently showcased in a case of saffron adulteration where the syntethic components used as adulterants were tailored to surpass the standard ISO 3632 testing solely relying on UV-Vis detection (Ordoudi et al., 2017). This further emphasises the need to use a combination of techniques in order to detect the increasing and more sophisticated cases of food fraud.

### FTIR in combination with other vibrational spectroscopy techniques

Although there are similarities between NIR and MIR there are also important differences which affect the ability to detect some adulterants. In general, the intensity of NIR (NIR, 780-2500 nm) absorbance is much lower than MIR making it more difficult to detect (Sun, 2009). Additionally, specific chemical groups are difficult to associate with a specific IR frequency due to the weak, broad overtone and combination bands associated with NIR absorbance (Sun, 2009). NIR is also not as sensitive as MIR spectroscopy because its radiation will penetrate the sample to a greater extent compared to MIR (Sun, 2009). However, in some studies where onion powder and olive oil were analysed, NIR was found to give better predictions of adulteration than FTIR (Bevilacqua et al., 2012a; Lohumi et al., 2014). This can most likely be explained by the fact that NIR is better suited to analyse highly absorbing bulk samples, such as powders and liquids, due to the lower intensity of the NIR absorbance compared to
MIR. FTIR has also been compared to Raman, specifically when the spectra were analysed using CLPP. For example Georgouli, Martinez Del Rincon and Koidis (2017) found that the prediction accuracy of adulteration of olive oil were similar between FTIR and Raman. One of the advantages of Raman compared to NIR and FTIR is that this type of spectroscopy does not exhibit interference from water, something that otherwise might be an issue when analysing foods without prior sample preparation.

**FTIR in combination with hyphenated mass spectrometry (MS) techniques**

Although FTIR analysis is a cheap and rapid screening technique that has been applied on many different food matrices, one drawback is that information regarding individual compounds or components in a complex mixture cannot be extracted. Therefore, it often needs to be complemented with other techniques such as hyphenated chromatography, for example LC coupled to high resolution MS (Black et al., 2016; Wielogorska et al., 2018) for confirmatory analyses in order to identify and quantify specific biomarkers, see Table 1. One advantage of combining FTIR with chromatography is that different compounds in a complex biological matrix such as foods, is first separated according to their physicochemical properties (Hird et al., 2014). Hyphenated to mass spectrometry this becomes very a powerful as the mass-to-charge ratio of ions is detected making it possible to provide both quantitative and structural information about the compounds in the sample (de Hoffman and Stroobant, 2007). However, one of the disadvantages of these HRMS methods are that the data analysis is often complex and laborious.

Using LC-HRMS for screening or non-target analyses is also an important technique in other areas of food safety, for instance for the screening of pesticides (García-Reyes et al., 2007; Malato et al., 2011; Gómez-Ramos et al., 2013), detection of contaminants in food packaging materials (Bengtström et al., 2016; Pérez-Ortega et al., 2016; Pieke, Smedsgaard and Granby, 2017) and identification of biomarkers for allergens (Korte, Lepski and Brockmeyer, 2016). With recent advances in non-target analysis of biomarkers in complex biological matrices using HRMS, it is expected that the two-tier approach using FTIR screening and LC-HRMS confirmation suggested by Black et al. (2016) and Wielogorska et al.(2018) for analyses of adulteration of oregano, will be implemented on a broader scale for analyses of food fraud.
One of the major drawbacks with all analytical chemical techniques is that contamination by any other biological species, such as other species of ground plant material in spices or substituted minced meat, is not easily identifiable by chemical profiles alone. Novel interdisciplinary strategies combining analytical chemistry such as FTIR screening and HRMS identification of biomarkers, with molecular biology, such as that suggested for detecting adulterating oregano with olive and/or myrtle leaves suggested by Wielogorska et al. (2018). DNA techniques such as DNA barcoding, real-time polymerase chain reaction (PCR) and Sequence Characterised Amplified Region (SCAR) PCR have been extensively used to detect food adulteration in spices such as oregano (Marieschi et al., 2011b, 2011a), turmeric (Dhanya et al., 2011) and saffron (Marieschi, Torelli and Bruni, 2012; Villa et al., 2016) as well as meat products (Ali et al., 2015; Hou et al., 2015; Amaral et al., 2017).

However, these methods all require access to species specific sequence data in order to design suitable primers to be used in the amplification process, ensuring the amplification of the correct DNA molecule. One approach to remove this limitation is to use next generation sequencing (NGS). However, NGS is an expensive technique that often involves complex workflows, limiting its use in routine analysis (Burns et al., 2016). Although, these molecular methods are generally considered specific and sensitive they are still considered to be more qualitative than quantitative due to their high uncertainty in measurements (Burns et al., 2016). The sensitivity of these techniques means that even low level of accidental contamination can be analysed. However, due to the high level of quantitative uncertainty, even these low-level contaminations can be interpreted as intentional, and thus fraudulent (Galvin-King, Haughey and Elliott, 2018).
Conclusions

The food production chain has become increasingly globalised, making food vulnerable to adulteration. There is therefore a growing demand on both authorities and food producers to develop fast and efficient methods for the comprehensive monitoring of food. Over the last decades, FTIR has proven itself to be a powerful tool for screening of foods for adulteration and authenticity. It is a fast, easy and generally cost effective method to detect food adulteration and in some cases it can also provide information about the geographical origin of certain foods which can be useful for investigating of authenticity. In many cases, FTIR analysis in combination with multivariate data analysis is sufficient to predict the level of adulteration. However, for some purposes, the use of FTIR as part of an extended test battery, such as other spectroscopy techniques, hyphenated chromatographic methods or DNA analysis, is needed to confirm authenticity or quantitative adulteration. Developing test batteries employing several different analysis methods is highly desirable as there are an increasing number of reports where adulterating agents have been designed to comply with certain standardised analyses. Thus the latest development in the fight against food fraud emphasise the need to use at minimum of two techniques, based on different chemical principles, as is already standard in other areas of forensic science.

Ideally, confirmatory analysis using hyphenated MS techniques or nuclear magnetic resonance (NMR) should be used to identify any biomarkers that could potentially be quantified. For instance, recent developments in tentative identification, i.e. identification without certified reference materials, by HRMS are promising for these purposes and recent studies have shown the large potential of this two-tier approach for screening and confirmation of cases of food fraud. This two-tier strategy should therefore be used to detect fraud of more groups of food, especially for groups where the fraud is suggested to become increasingly sophisticated. Furthermore, recent advances in DNA sequencing, such as technical advances in NGS and more powerful bioinformatics tools for downstream analyses, have the potential to identify adulteration down to the species level without prior sequence knowledge. This is particularly useful in the case of suspected adulteration of mincemeat and ground spices and herbs, as the potential to add virtually any meat or plant material to these foods without chemical detection is large. Information gained from the combined use of analytical chemistry and DNA analyses
may even in certain cases be able used to trace the origin of any adulteration within the food supply chain.

Food fraud by adulteration or authenticity is by no means a new phenomenon, but recent scandals have illustrated the vulnerability of the modern and increasingly global food supply chain. Developing rapid and easy screening techniques, using for instance FTIR, are therefore imperative to ensure future food safety. With the increasing complexity of food fraud, future methods need to be able combine rapid screening of large quantise of food as well as confirmatory analyses using for instance a combination of detection techniques based on different chemical principles.
1. References


BBC News (2013) *Findus beef lasagne contained up to 100% horsemeat, FSA says*. Available at:


