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A flavour of omics approaches for the detection of food fraud
David I Ellis¹, Howbeer Muhamadali¹,², David P Allen¹, Christopher T Elliott² and Royston Goodacre¹

Food fraud has been identified as an increasing problem on a global scale with wide-ranging economic, social, health and environmental impacts. Omics and their related techniques, approaches, and bioanalytical platforms incorporate a significant number of scientific areas which have the potential to be applied to and significantly reduce food fraud and its negative impacts. In this overview we consider a selected number of very recent studies where omics techniques were applied to detect food authenticity and could be implemented to ensure food integrity. We postulate that significant reductions in food fraud, with the assistance of omics technologies and other approaches, will result in less food waste, decreases in energy use as well as greenhouse gas emissions, and as a direct consequence of this, increases in quality, productivity, yields, and the ability of food systems to be more resilient and able to withstand future food shocks.

Addresses
¹ Manchester Institute of Biotechnology, School of Chemistry, University of Manchester, 131 Princess Street, Manchester M1 7ND, UK
² Queen’s University Belfast, School of Biological Sciences, Institute for Global Food Security, Belfast BT9 5AG, County Antrim, Northern Ireland, UK

Corresponding author: Ellis, David I (D.Ellis@manchester.ac.uk)

Introduction
In the spring of 2016 the latest report from Operation Opson (Opson V) was released detailing the largest ever seizures of hazardous fake food and drink ever recorded [1]. This joint international Interpol/Europol operation originally began in 2011 and initially included 10 European countries, with it now expanding to include 57 countries across the world. Perhaps not surprisingly the release of the report led to a slew of media headlines involving terms such as monkey meat, copper sulfate painted olives, fertiliser contaminated sugar, and tonnes of locusts and caterpillars seized, to name but a few; alarmist headlines perhaps, but all of them true (see Table 1 for a summary of Opson V seizures). It should also be pointed out that these large-scale and record seizures of fake and counterfeit foods and beverages, carried out at shops, markets, industrial estates, air- and seaports, all occurred during a relatively short period from November 2015 to February 2016, and are only a snapshot of the severity of the problem. The news of Opson V appeared to coincide with the release of the first report [2] from the UK’s newly formed National Food Crime Unit (NFCU) which covered the period November 2014 to July 2015. Whilst informative, the only seizures mentioned within the NFCU report involved counterfeit and adulterated alcoholic beverages, including 35 000 bottles of counterfeit vodka originating from Ukraine, and 8 000 litres of vodka from Lithuania with forged duty stamps. More worryingly, these seizures also included 20 000 counterfeit branded vodka bottles and material suggesting adulteration with anti-freeze, as well as 130 000 litres of potentially toxic spirits alongside bottling and labelling materials from another raid [2]. More recently, reports of organised international food fraud involved seven countries and thousands of tonnes of wheat, corn, soybeans, rapeseed and sunflower seed imported from multiple non-EU countries, mislabelled as organic and shipped to EU countries via Malta or Italy [3]. These reports readily illustrate and reinforce the fact that food fraud can be international in scope, with no country being immune from its reach and impacts, and that this transboundary criminal activity can be both opportunistic, as well as highly organised. Here though, as analytical scientists, we are primarily concerned with the single greatest, or what could be termed grand challenge of food adulteration; its unequivocal detection. Therefore, we have selected a very small number of recently reported omics and related technologies that are being developed to enable the detection of food authenticity and integrity.

Omics technologies
During the past two decades molecular-based technologies have rightly proven themselves as an invaluable option for the detection of food authenticity and integrity [4]. Such DNA-based methodologies generally rely on specific DNA sequences (markers) that can be used for detection of food adulterants and/or approving the authenticity (i.e. quality and origin) of raw ingredients [5].
DNA-barcoding, named due to this technique using a specific region of the genome described as the DNA-barcode, is considered as one the most common identification systems for taxonomic discrimination [5]. However, the successful application of this approach for the separation of food and foodstuffs relies on the availability of comprehensive reference sequence libraries, such as the barcode of life database (www.barcodeoflife.org).

DNA-barcoding is of particular interest when it comes to authentication of seafood products [6]. This interest is mainly due to the presence of a wide-range of species, morphological similarities between species, as well as a loss of the structural and visible characteristics of the raw material during different food processing procedures (i.e. heat treatment, or cooking). Several studies have successfully applied this approach for seafood authenticity testing, such as those by Cutarelli and co-workers, who reported the application of mitochondrial cytochrome b (Cytb) and cytochrome oxidase subunit I (COI) genes as DNA markers for the identification of 58 Mediterranean marine fish samples sold on the Italian market. Whilst Pereira et al. demonstrated the efficacy of barcode methodology (COI gene), for the highly accurate (99.2%) identification of 254 species of freshwater fish samples, Kim and co-workers picked up the metaphorical baton, taking this approach a step further by employing a combination of DNA-barcoding and stable isotope analysis for the identification and verification of the origin of Hairtail fish and shrimp. This strategy also allowed these authors to differentiate between natural and farmed shrimp [7], an important and significant area for fish authenticity and traceability, which was only possible as the stable isotope analyses allowed the phenotype of the organism to be measured.

As might be expected, DNA-based methodologies have been applied to the authentication and traceability of a wide-range of food products, including the detection of the mislabelling or cross-contamination of halal meat [8] and the detection of species such as horse in ground meats [9]. With one very recent article of significant interest involving the development of a real-time PCR approach for the relative quantitation of horse DNA in raw beef mixtures [10**]. Other studies have involved labelled milk and milk products, such as yogurt and cheese, which were traced through a production chain via DNA tags, in this case silica particles with encapsulated DNA (SPED) [11], with the applicability of synthetic and naturally occurring DNA sequences demonstrated. Identifying species specific differences in herbal medicines [12], chilli adulteration of traded black pepper powder down to 0.5% adulteration [13], and tracing/tagging of edible oils (e.g. olive oil) using encapsulated DNA in heat-resistant and inert magnetic particles [14] have also been reported. However, the application of DNA-barcoding

| Country | Summary of seizures |
|---------|---------------------|
| Australia | 450 kg of honey found to be blended or adulterated. Peanuts repackaged and relabeled as pine nuts. |
| Belgium | Several kilos of monkey meat found at Zaventem airport. |
| Bolivia | Warehouse containing thousands of cans of sardines, and fake labels from a famous Peruvian brand of sardines. |
| Burundi | 36,000 liters of illicit alcohol seized during the operation, as well as nine Kalashnikov rifles, ammunition, and three grenades |
| France | 11 kg of locusts and 20 kg of caterpillars seized and destroyed. |
| Greece | Three illicit factories producing counterfeit alcohol. Equipment, fake labels, caps, empty bottles and 7,400 bottles of fake alcohol seized. |
| Hungary | Counterfeit non-alcoholic sparkling wine, chocolates, sweets. |
| India | See Thailand below. |
| Indonesia | 70 tonnes of chicken intestines preserved in formalin seized. |
| Italy | 310,000 illegal food products found behind piles of tiles in a warehouse and believed to be smuggled from Malaysia. |
| Lithuania | Counterfeit non-alcoholic sparkling wine, chocolates, sweets. |
| Malaysia | See Indonesia above. |
| Romania | Counterfeit non-alcoholic sparkling wine, chocolates, sweets. |
| South Korea | Arrest made associated with the online sale of fake dietary supplements/weight loss products estimated to have generated US$170,000 in a 10 month period. |
| Sudan | 9 tonnes of counterfeit sugar contaminated with fertilizer. |
| Thailand | Four tonnes of meat smuggled by one individual from India. Further investigation led to discovery of illicit network operating across 10 provinces. Recovery and destruction of more than 30 tonnes of illegal beef and buffalo meat, unfit for human consumption and destined for sale in supermarkets. |
| Togo | 24 tonnes of imported tilapia. |
| United Kingdom | 10,000 litres of fake or adulterated alcohol, including wine, whisky and vodka. |
| Zambia | 1,300 bottles of fake whisky in original packaging. Over 3,200 cartons of diet powder drinks with modified expiration dates. |

* Aimed at children and destined for export to West Africa.
using the more traditional sequencing methods, such as Sanger sequencing, can suffer from some drawbacks including their low-throughput nature and the necessity for food samples with high DNA purity and concentration [15]. That said, such inherent limitations have been largely overcome using more recent technologies such as next generation sequencing (NGS), which present themselves as high-throughput and low cost approaches in comparison to earlier genomics methods, and allow for the sequencing of millions of DNA molecules in parallel [16]. These include microfluidic and nanofluidic devices such as the nanopore [17]. Readers with a particular interest in genomic methods for detecting food authenticity and integrity are directed to far more comprehensive reviews in this area [4,5,12,16].

The applications of analytical platforms common within other omics approaches have also been proven valuable in providing critical information regarding the biochemical composition of various food products. Proteomics, whilst a well-established and continually developing field in many areas of research, has been said to be emerging as a complementary methodology [18] to the DNA-based approaches (as well as antibody approaches [19,20]), for food authenticity detection, as the amino acid sequence, just like the DNA sequence, is species specific. Given the recent resurgence in public interest in food adulteration following the horsemeat scandal in the UK, it is perhaps to be expected that several of the recent proteomics approaches have involved adulteration of meat products. These have included the identification of novel heat stable peptides for horsemeat speciation in highly processed foods such as corned beef and baby food. After in-depth analyses of these two well-known products, muscle food mixtures of various meat species such as horse, cow, pig, and chicken were used to emulate these complex processed food matrices. This semi-targeted approach then utilised nanoflow liquid chromatography mass spectrometry (nLC–MS/MS) to produce a database of markers for a total of nine animal species [18], and was able to detect levels of processed and raw horse meats in meat mixtures as low as 0.5% (w/w).

Other analysts have used high-performance liquid chromatography–mass spectrometry (HPLC–MS) analysis of tryptic digests of protein extracts from a number of species, to detect horsemeat and pork in a range of these muscle foods, including halal beef. They developed multiple reaction monitoring (MRM) methods which allow for signal enhancement and the detection of 0.55% adulteration/contamination of horse or pork in a beef matrix, with still further enhancements in sensitivity (referred to as MRM³) allowing for the detection of 0.13% pork in halal beef [21].

More recently Obana and co-workers used LC–MS/MS shotgun spectral matching for the speciation as well as quantification of a wide-range (16 mammalian and 10 avian species) of raw, cooked, and mixed meat types [22]. While others have recently trod a similar path of proteomic meat speciation and quantification but with less success in terms of the limit of detection (LOD) than the studies already discussed above, with targeted levels of meat speciation down to 1% (w/w) [23]. Of course proteomic detection methods involve many food products in addition to meats. Milk has been one of these products for example, where matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS) methods have been used for the combined peptidomic and proteomic profiling of raw and thermally treated cow, goat, buffalo and sheep milk and combinations of these with skimmed milks and water at various concentrations. Results were said to permit rapid and accurate estimates of the extent of fraud at either the protein or peptide level, and were also said to extend previous MALDI-MS approaches in this area via the analysis of heat treatment and complementary peptide profiling, thereby potentially broadening the applicability of these methods [24].

There have also been a number of recent reviews in the field of proteomics for food fraud and related areas, including topics such as: biomarker discovery and applications for foods and beverages [25]; proteomics as a tool to understand the complexity of beers [26]; fruits and beverages [27]; and yet again, meat and muscle for animal origin authentication [28], as well as MS-based omics strategies concerning the analysis of the complex links between food science and nutrition, referred to as foodomics [29].

The more recent omics disciplines are of course metabolomics and lipidomics, the latter so relatively recent that very few standalone lipidomic studies of food fraud or authenticity have been reported to date (with the odd exception [30]). These do however include Fourier transform infrared (FT-IR) spectroscopy as a tool to authenticate the origin of wild and farmed European sea bass, as well as the additional benefit of providing useful information on the composition of nutritionally beneficial lipids [31]. That being said, there are additional lipidomics food authenticity methods that have been integrated within metabolomics studies, and we would expect this, as the lipidome is a subset of the more chemically diverse metabolome. Metabolomics is a rapidly expanding field within the omics finding useful and growing applications across a very broad range of disciplines. Metabolomics is downstream of genomics and proteomics (see Table 2 for a description of the omics approaches) and said to be the link between genotype and phenotype. Thus, it is not surprising that there are significantly more metabolomics studies related to food authenticity and integrity to be found during the last few years, than those from the other omics technologies.
Table 2

| Omics approaches | Description | Approaches and technologies |
|------------------|-------------|-----------------------------|
| Genomics         | The study and assessment of variability and function of DNA sequences | Whole-genome sequencing (WGS), Next generation sequencing (NGS), Amplified fragment length polymorphism (AFLP), Single nucleotide polymorphism (SNP), Simple sequence repeat (SSR) |
| Proteomics       | The study of structure, function and abundance of different proteins and peptides (or complexes), post-translational modifications (PTM) in a system | Two-dimensional gel electrophoresis, MALDI-TOF-MS, LC–MS and high-resolution tandem mass spectrometry |
| Metabolomics     | The comprehensive and systematic study of low molecular weight compounds (metabolites) involved in metabolism in a system | Metabolic fingerprinting (FT-IR and Raman spectroscopies), Metabolic profiling (GC/LC–MS, NMR), Targeted metabolomics analysis |
| Lipidomics       | The study of pathways and networks of different lipid species in a system | Lipid profiling (GC/LC–MS, NMR), shot-gun MS, lipid fingerprinting (MALDI-TOF-MS), ultra-performance LC–MS (UPLC-MS) |

mentioned here, especially as metabolites are often essential for human nutrition.

Indeed, these recent metabolomics studies appear to encompass not only a wider range of technological methods but also a more diverse range of applications within this area. These include nuclear magnetic resonance (NMR) spectroscopy for the determination of the country of origin of coffee by Arana and co-workers [32], using a fingerprinting approach [33] and showing high classification rates of large numbers of spectra of coffee extracts. Whilst others have used metabolomics to elucidate discriminant markers for the authentication of the world’s most expensive coffee, Kopi Luwak, an exotic Indonesian blend made from coffee beans that have been eaten by the Asian palm civet (*Paradoxurus hermaphroditus*). Here gas chromatography–mass spectrometry (GC–MS) based multimarker profiling was used to elucidate significant metabolites as markers to determine original, fake Kopi Luwak, regular coffee, and blended samples containing only 50% Kopi Luwak [34]. In another study of a well-known high value food product the evaluation of the adulteration of saffron (*Crocus sativus* L.) by a variety of other cheaper plant bulking agents (i.e., *Crocus sativus* stamens, safflower, turmeric, and gardenia) was undertaken using NMR metabolite fingerprinting [35]. Herbs and spices can be an expensive commodity and have, along with other sectors, long and complicated supply chains making them vulnerable to adulteration. One very recent study [36] elegantly demonstrated a two-tier LC–MS-based metabolic profiling and FT-IR metabolic fingerprinting approach, which alarming showed that 24% of the 78 samples of commercially available oregano purchased from retail outlets in the UK and online had some form of adulterant present. These included myrtle, olive, sumac, cistus, and olive leaves, and ranged from 30 to 70% adulteration.

High resolution magic angle spinning nuclear magnetic resonance (HR-MAS-NMR) spectroscopy has also been used to determine the metabolic profile of a highly prized and designated Sicilian lemon variety (Interdonato Lem- on of Messina PGI) in order to determine commercial fraud [37]. With fruit juice authenticity also being the subject of a study by Jandric and co-workers [38]. Where 21 metabolites were selected and found to contribute to the separation of pineapple, orange, grapefruit, apple, clementine, and pomelo juices and their admixtures (pineapple juice adulterated with orange, apple, grapefruit and clementine at 1%, 5%, 10%, 15% adulteration, and orange juice adulterated with apple, grapefruit and clementine at the same levels of adulteration). Additional metabolomics studies published very recently of note also include: a salient reminder of the China melamine [39] crisis and ongoing issues (in some countries) with infant formula, via the classification and evaluation of the degradation and contamination (with melamine) of multiple infant formulas [40]; fully automated NMR analysis of wine authenticity [41] and honeys [42]; and direct analysis in real time mass spectrometry (DART–MS) of poultry meat with metabolomics for the retrospective control of feed fraud via contamination by banned bone meal [43] (yet another reminder of recent history, this time of the BSE crisis). A very recent integrated metabolic profiling and lipidomics approach led to a panel of metabolites for the identification of pork adulteration of minced beef, with additional insights from the analysis of lipids due to the integrated nature of this study [44]. As with the other omics mentioned all too briefly above, those readers with an interest in metabolomics applied to the detection of food authenticity and integrity [45] are directed to a number of more comprehensive reviews [33, 46, 47, 48, 49–51].

**Discussion**

In this very short overview, it has not been our intention to become entangled in discussions regarding the nature of food fraud or criminality, and we will leave the continually evolving and at times unhelpful terminology and quibbling semantics used to describe these various
practices to others. To those in the small, decreasing, and
mumpsimus minority and new to this field of study (post
Horsegate in 2013), whose comments regarding food
fraud/crime include the refrain ‘where is the evidence?’,
we kindly remind them that absence of evidence is not
evidence of absence, and point them to the international
reports, record seizures and other practices mentioned in
the brief introduction above and accompanying Table 1. Evidence of these practices will not be found,
or detected, if it is not being actively sought out.

Whilst we have only been able to show a ‘flavour’ of some
of the recent applications of omics technologies for the
detection of food authenticity and integrity in this article,
we hope it is apparent that the omics have much to offer
in this area. It must also be pointed out that there can be
issues and caveats that are specific to each of the omics
fields, such as the measurement issues associated with
molecular and mass spectrometry analysis of food mat-
rices, with a very recent and timely reassessment of their
quantitative potential [4]. As food and beverage products
already present themselves as complex physical and
(bio)chemical matrices, the composition of which can
be strongly dependant on a variety of environmental
factors such as climate, seasonality, and storage condi-
tions, prior to any other analytical considerations specifi-
cally related to the detection of food fraud. Furthermore,
one must think more laterally as food fraud involves
more complex issues for analytical scientists (and regulators)
than the simple adulteration of one high value food
product with a less expensive one. Multiple factors can
be involved and for example can include issues related to
adulterated feed for livestock, and therefore the impor-
tance of the detection of chemical residues [52–54] in
foods, resulting from either hormonal or antibiotic
treatment of livestock, aquatic products [55], or herbicide/

Figure 1

Venn diagram which has been adapted from a graphical model of Routine Activity Theory [84,68] and more recently repurposed for its potential
relevance to food fraud by Ellis et al. [61**]. This approach is originally based on the three necessary conditions for crime (such as food crime) to
occur. The three conditions require: (1) a likely offender (i.e. potential food adulterer/fraudster); (2) a suitable target (i.e. food supply system/
network); and finally (3) the absence of a capable guardian (i.e. omics and other disruptive detection technologies). The opportunities/vulnerable
areas for crime to occur exist where the so-called capable guardian is absent. We have forwarded a technology-based capable guardian system.
Whereby a wide-range of current and future sensor/detection platforms and technologies, along with future predictive computational methods
could together take on the capable guardian role, and assist in significantly reducing the areas of vulnerability to fraud within food supply chains.
Note the area vulnerable to food fraud is dynamic and the detection technologies not static.
pesticide [56] treatment of crops. One recent example from this area is a metabolomics screening method for the adulterated hormonal status of cattle [57]. Other food fraud issues can of course be related directly to consumer safety and health such as the inclusion, whether deliberately or inadvertently, of unwanted contaminants. These can have severe health impacts and include peptides, proteins or a variety of other compounds acting as food allergens [58]. With a recent review discussing the serious issues relating to the ability to measure food allergens reproducibly, their traceability, and the identification of substantial gaps within the international analytical community [59**].

The latter review by Michael Walker of the Government Chemist programme and colleagues from Manchester and Belfast [59**] takes an integrated approach, and whilst each of the main areas of omics briefly discussed here have their own pros and cons, the integration of several omics methods can be very effective indeed, and lead to more practicable knowledge and insights which can then have the potential for implementation within food supply chains. The study by Trivedi and co-workers [44**] mentioned above provides a very recent example of this integrated omics approach, as have others involving topical food pathogens such as Campylobacter [60]. New findings from the omics technologies such as the elucidation of omics markers of authenticity or adulteration can be used in knowledge transfer, and have the potential to be incorporated into a range of commercially available or future technologies for the rapid detection of food fraud. Whether these are to be used in laboratory based detection technologies, wi-fi connected and highly mobile point-and-shoot handheld devices out in supply chains [61**], or at/on/in-line sensors [62,63]. This of course would necessitate co-operation with other disciplines, with the hope that such disruptive detection technologies could act as so-called capable guardians [64] (Figure 1) within food supply chains, with the potential to reduce the areas vulnerable to food fraud. At some point in the future this may also include predictive analysis of points of vulnerability within food networks via one or more omics related techniques from the computational and informatics sciences [65], such as scale-free networks [66] for example. These forms of analyses may have the potential in future to be developed and assist in identifying/predicting nodes which are especially vulnerable to food fraud within complex food supply chains. Allowing for the rapid intervention of disruptive technologies, and/or be aided in this ‘identify/predict’ function via data automatically collected from omics and related technologies and relayed across networks (Figure 2). Data from these, as well as the other interdisciplinary approaches discussed here, will of course require large-scale and open-access data repositories, and significantly more data sharing than is practised to date.

Such interdisciplinary co-operation, across multiple and at times unrelated disciplines, including engineering, informatics, as well as the social sciences, would require all those involved to see well beyond the boundaries of their own respective fields of research and truly collaborate for the common good. As significant reductions in food fraud will have multiple benefits across international food supply chains. These benefits include reductions in: food waste, energy use, greenhouse gas (GHG) emissions, as well as negative health impacts. The integration of multiple disciplines we believe will as a direct consequence lead to increases in: food security via the maximisation of product yields, and improvements in food quality, as well as sustainability, with the result that food supply chains would have the potential to be far more resilient to withstand future food shocks [67].

In conclusion, the individual omics discussed here and their related approaches hold a great deal of promise for the detection of food authenticity and integrity, and especially so when using an integrated omics approach (in tandem with future technological and computational advances). With knowledge and expertise from a wide-range of sources leading to valuable new insights and applications; themselves inducing further technological leaps, and reaping beneficial outcomes for an equally wide-range of areas with complex intrinsic and extrinsic links to global food systems.
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