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Summary

The growing complexity of global supply chains means that ensuring the safety and authenticity of food and non-food agricultural products is an ever-increasing challenge. This article focuses on fats, which, together with proteins and carbohydrates, make up the bulk of the human diet.

Over 86% of globally consumed edible fats are plant-derived (or vegetable) oils. In contrast, animal-derived fats account for about 14% of worldwide oil and fat consumption, mostly in developed countries with higher incomes and where meat and dairy agriculture is traditional.

With increasing affluence and a world population expected to reach almost ten billion by 2050, demand for fats and oils continues to accelerate. In some cases, this can lead to economically motivated malpractices such as adulteration, mislabeling or ‘unsustainable’ operations.

These activities create significant challenges for global supply chains that require more rigorous auditing and greater deployment of both existing and newly emerging analytical techniques to ensure the safety and reliability of traded agricultural products.

Keywords

Adulteration, analysis, certification, DNA markers, fats, provenance, spectroscopy, sustainability, traceability, vegetable oils

Abbreviations

AFLPs, Amplified Fragment Length Polymorphisms; CAD, Charged Aerosol Detection; CAPs, Cleaved Amplified Polymorphic sequences; CPO, Crude Palm Oil; DAF, Amplification Fingerprinting; DNA, Deoxyribonucleic Acid; EVOO, Extra-virgin Olive Oil; FA, Fatty Acids; FAMEs, Fatty Acid Methyl Esters; FFA, Free Fatty Acid; GC – FID, Gas Chromatography – Flame Ionisation Detection; GC – IMS, Gas Chromatography – Ion Mobility Spectrometry; GC – MS, Gas Chromatography – Mass Spectrometry; GIS, global imaging systems; HT – GC, High Temperature – Gas Chromatography; IR, Isotope Ratio; IV, Iodine Value; MS, Mass Spectrometry; OP, Oil palm; PCR, Polymerase Chain Reaction; PGI, Protected Geographical Indication; PDO, Protected Designation of Origin; PKO, Palm Kernel Oil; PLS – DA, Partial Least Squares – Discriminant Analysis; PO, Palm oil; PTR, Proton Transfer Reaction; RFLPs, Restriction Fragment Length Polymorphisms; RAPDs, Randomly Amplified Polymorphic DNAs; RP – HPLC, Reversed Phase – High Performance Liquid Chromatography; RSPO, Roundtable on Sustainable Palm Oil; SCARs, Sequence-Characterised Amplified Regions; SIMCA, Soft Independent Modelling by Class Analogy; SNPs, Single Nucleotide Polymorphisms; SSR, Simple Sequence Repeat; TAGs, Triacylglycerols; TSG, Traditional Speciality Guarantee; QA/QC, Quality Assurance/Quality Control; VOCs, Volatile Organic Compounds

Introduction

According to the OECD-FAO "*agricultural supply chains refer to the system encompassing all the activities, organisations, technology, information, resources and services involved in producing agricultural food products for consumer markets*".

Agricultural supply chains are at the centre of many national economies and are a major source of income for many communities.

A responsible agricultural supply chain is one that acknowledges the risk that negative impacts could have along supply chains and recognizes responsibility to respect human and labour rights, abide by health and safety regulations and to contribute to sustainable practices (particularly poverty reduction, food security and nutrition, animal welfare, sustainable use of natural resources and environmental protection) (1).

Within the coming decade the demand for food is expected to increase significantly as a result of growing populations, higher incomes and changing diets. Prices for most agricultural products are therefore projected to increase above those that occurred in the years before the 2007/08 global food price spike (1).

As has already been the case in isolated instances in several parts of the world, the potential for increased profitability can lead to malpractice and mislabeling of foodstuffs. It is therefore essential to continue working to ensure that all global agricultural supply chains operate responsibly.

With increasing media coverage of food incidents and malpractice in agricultural supply chains, consumer demands for improved traceability and sustainability within agricultural supply chains have grown and can now increasingly be met thanks to developments such as reliable technological methods for traceability monitoring (e.g. global imaging systems (GIS)) and more robust certification schemes (e.g. Roundtable on Sustainable Palm Oil (RSPO)).

There are several new analytical methods to verify the geographical provenance of vegetable oil samples (and particularly that they represent a ‘sustainable’ origin) and to rapidly and cheaply detect a wide range of contaminants.

In the case of palm oil (PO) these include undesirable constituents such as 3-MCPD (3-monochloropropanediol) and oil processing-related components, illicitly blended non-palm fats (including pig-derived lard) and illicit additives, such as potentially toxic colouring agents.

In line with these developments, major vegetable oil industry players, such as IOI-Loders Croklaan, have recently named sustainability & traceability as among the ‘mega trends’ that will drive future food innovation.

In February 2018 Unilever became the first consumer goods company to publicly disclose the suppliers and mills from which they source palm oil, which they state as marking a major achievement to creating a more traceable and sustainable industry (2).

Meanwhile the global spread of oil palm (OP) plantations is gathering pace, most notably in West Africa and South America. In some of these cases, the use of new high yield certified planting materials and niche products such as organic PO is creating additional markets in the US and EU. In summary, the sector is facing unprecedented new challenges and opportunities in order to fulfil its role as a true global ‘wonder crop’.

Safety and Reliability of Food Products

In general, international food agreements provide a fairly uniform level of protection in terms of public health and food standards; however, there are continual efforts to remove technical barriers to trade via the harmonisation of global standards.

One challenge is that legislation differs from country to country and so it is vital that food processors, manufacturers and traders use contemporary developments in legislation to ensure they are operating within the law.

A supply chain consists of a sequence of processes related to the production and distribution of a commodity. Supply chains are complex and dynamic networks of supply and demand, involving many parties (producers, manufacturers, wholesalers, retailers, consumers, exporters, etc.). Subsequently, there are often associated risks (endogenous and exogenous) which can lead to supply chains failing.

Quality, technical, ergonomic, logistic and management risks are all considered to be endogenous, whilst exogenous risks are usually caused by natural, market and policy environments (3). A prime example of an exogenous risk leading to supply chain failure was the 2017 'lettuce crisis' experienced in the UK, which was caused by flooding in Spanish agricultural regions, following a three-year drought.

This led to a supermarket ban on bulk purchasing by customers and increased prices (4). There are other instances where supply chain operations fail or are covertly abused by participants, leading to so-called 'food incidents' that can garner a great deal of adverse media attention.

Food incidents are defined as "*any event where, based on the information available, there are concerns about actual or suspected threats to the safety or quality of food and feed that could require intervention to protect consumers' interests. They are defined broadly into two categories:*

- *Accidental/deliberate contamination of food or animal feed throughout supply chains (including processing, distribution, retail and catering)*
- *Environmental pollution incidents such as fires, chemical/oil spills or radiation leaks* (5) Some particularly notable food incidents prior to 1995 are listed below:
- 1955 Morinaga Milk Arsenic Poisoning (Japan)– An industrial grade of Monosodium phosphate additive, which inadvertently contained 5-8% arsenic, was added to milk fed to infants. Over 600 infants died and over 6,000 people suffered health issues
- 1981 Spanish Toxic Oil syndrome (Spain) – Thousands of people were permanently damaged and over 600 killed, by consuming industrial colza oil denatured with aniline and sold as olive oil
- 1994 Adulteration of Ground Paprika by Lead Oxide (Hungary) – Ground paprika was found to be deliberately adulterated with lead oxide to improve colour and increase weight. It led to several deaths and dozens of illnesses

Following these incidents, the European Commission established a set of general principles and requirements regarding food and feed law at a Union level, resulting in the establishment of the General Food Law Regulation (EC) No 178/2002 (6).

This regulation states, *"European citizens need to have access to safe and wholesome food of the highest standard"* and covers all sectors of the food chain. However, even with tightened legislation and regulations worldwide, there are still recent instances whereby food products have become contaminated, both accidentally and deliberately:

- 2006 Pork and Clenbuterol (China) – Pork was found to contain the illegal steroid, Clenbuterol, after pigs were illegally fed the chemical to enhance fat oxidation and muscle growth. Over 300 people were affected after consumption;
- 2008 Melamine baby milk scandal (China) – over 51,000 hospitalisations and 6 infant deaths occurred when melamine was added to a baby milk formulation. More than $30M was paid out in compensation and trade restrictions were imposed by 68 countries;
- 2013 Horsemeat scandal (UK) (Fig. 1) – It was discovered that horsemeat contaminated beef burgers had been on sale in many stores. Of 27 beef burgers tested, ten tested positive for horse DNA, while 23 tested positive for pig DNA;
- 2017 Fipronil eggs contamination (Europe and Asia) – The spread of Fipronil insecticide into egg supply chains resulted in contamination of eggs and egg products destined for human consumption. Although nobody was harmed, it led to 180 farms to be shut down and dozens of products being removed from supermarkets.

Vegetable Oils: Contamination, Adulteration and Economic Fraud

Vegetable oils are a major global food source. In 2016, almost 24 million tonnes of vegetable oils were consumed in Europe alone.

Palm Oil (PO) accounted for about 60% of this, whilst soyabean accounted for around 54% (7). Although consumption of vegetable oils in Europe is expected to decrease slightly in the coming years, total worldwide consumption is projected to increase significantly, especially in India and China where markets are growing.

Consumption of speciality oils (high oleic sunflower oil, virgin coconut oil, organic cold-pressed oils) in Europe has grown steadily and is expected to continue increasing, despite the recent economic crisis (8).

With growing demand for speciality and premium products, the risk of economically motivated malpractice within supply chains is likely to increase owing to the potential for greater profit margins. It should be recalled, however, that fraudulent activity within vegetable oil (and other food) supply chains is not new, with well documented reports of adulteration dating back to the early 1900s.

Challenges within Olive Oil Supply Chains

Incidents within olive oil supply chains have perhaps garnered the most attention over the years. Olive oil is an edible vegetable oil which is obtained from the fruit of *Olea europaea*.

There are five grades based on specific processing and organoleptic criteria: extra virgin (EVOO), virgin, refined, olive pomace and lampante. EVOO is the highest grade which must come from the first pressing of fresh olives and be processed within 24 hours of harvesting. It must be extracted by mechanical means only and without the use of excessive heat.

The free fatty acid (FA) content must not exceed 0.8% and it must be defect free (having perfect taste and aroma). The production costs of EVOO are far greater than those of the lower grades and so it demands much higher retail prices.

In January 2018, Italian EVOO reached an average price of €422.50 per 100 kg, compared €325.50 per 100 kg for Italian lampante olive oil (9).

Premium olive oil markets are further enhanced by Protected Geographical Indication (PGI), Protected Designation of Origin (PDO) or Traditional Speciality Guarantee (TSG) schemes, which were developed under EU Regulation No 1151/2012 of the European Parliament to promote and protect names of quality agricultural products and foodstuffs and to ensure that only products genuinely originating in the specified region are allowed to be identified as such in commerce.

There are many documented instances of fraud within olive oil supply chains including:

- In 2015 it was reported that seven of Italy's best-known olive oil companies were being investigated for allegedly selling inferior virgin olive oil as EVOO. Investigations found that of the 20 brands that were tested, nine were found to be lower quality oil. Producers involved in the investigation included Berolli, Santa Sabina, Primadonna and Antica Badia (10);
- In February 2017, Italian police arrested 33 suspects in the Calabrian mafia's Piromalli clan, a criminal enterprise which authorities believe is a major player in 'agromafia', including an elaborate fake olive oil scheme. They were apparently importing olive pomace oil (oil extracted from already-pressed fruit pulp using chemical solvents) and mislabeling it as EVOO before exporting it to the USA. The products were then sold through retail chains in New York, Boston and Chicago (11)
- In November 2017, Greek police arrested seven gang members for adding dyes to sunflower seed oil to simulate the green colour and appearance of olive oil. The investigation found that from September to November, the organisation exported around 100,000 litres of artificially coloured sunflower oil, labelled as EVOO, to Germany, Belgium and the Netherlands (12)

In September 2017, Walter Zane, the managing director of Fillip Beria explained why instances of EVOO fraud may have increased, stating "import costs have gone up by over 40% – a combination of the devalued pound and the cost rising because last years' harvest was not particularly good.

There is also extreme resistance at retail levels to accept any increases caused by Brexit and currency devaluation. It puts tremendous pressure on the supply chain" (13).

Challenges within Palm Oil Supply Chains

Derived from *Elaeis guineensis*, the Oil Palm (OP) supply chain is another example that has many associated risks. PO came to the UK as a new ingredient which suppliers regarded as 'good for frying as well as in pastries, cakes and ice cream', shortly after 1954 when the then-Minister of Food in Britain ended rationing.

Its versatility as an ingredient is attributed to its high melting point and while animal fats are also characterised by high melting points, their production is far costlier. OP is the most productive oil crop worldwide and bears oil-rich fruits, from which PO can be extracted (Fig. 2).

Palm fruits consist of a fleshy mesocarp and a central nut or kernel, similar in structure to a peach (Fig. 3). PO is obtained from the mesocarp whilst Palm Kernel Oil (PKO) is extracted from the nut. The global popularity of OP as a commercial crop is attributed to the usefulness and versatility of both oils, and they are said to be present in up to 50% of supermarket products.

Having a crop that is as productive as OP, now yielding around 10 times more oil per hectare than its nearest oilseed competitors, is a significant benefit in a world challenged by an increasing global food shortage.

OP can also be harvested year-round, providing continuous work for many people and while it makes up 38% of the global supply chain, it only accounts for 5% of farmland used for that production (14–16). PO is not only used in food industries; its versatility means it is utilised by the cosmetic and oleochemical industries.

To achieve the correct consistency prior to use, its competitors (rapeseed, sunflower, soybean) would have to undergo additional processing (partial hydrogenation), resulting in the addition of controversial and possibly harmful *trans* fats (16).

Although it is viewed by many growers and in much of the food industry as a 'wonder crop', oil palm has an unfavourable reputation with some consumers and lobby groups owing to its associations with adverse environmental impact and alleged human and land rights violations. One particularly emotive issue is the alleged role of forest conversion to oil palm plantations in the reduction in Orangutan populations.

This has prompted strong consumer demand for sustainably sourced and/or organic palm oil. However, the demand for and production of such products can increase risks within the supply chain. In general, consumers more commonly associate PO from Malaysia and Indonesian with habitat destruction than PO from South America or Africa.

This is probably because the Indonesian and Malaysian industries account for up to 80% of global PO production and so reports of sustainability violations in these countries are more frequent than countries that produce less PO. Consumer demand for improved traceability of PO has led many companies to include the geographical origin on product labels, but like olive oil, PO with a known (and 'approved') geographical origin tends to command higher retail prices and is more desirable to consumers.

This can lead to mislabelling by some manufacturers, i.e. PO from Malaysia labelled as PO from South America, for economic gain. Whilst such an occurrence is not threatening to human health it is obviously misleading to consumers. Certification schemes are another result of consumer demand. Perhaps the most notable scheme established in 2004 is Roundtable on Sustainable Palm Oil (RSPO) that guarantees and certifies that a particular batch of PO comes from a sustainable source.

The RSPO logo on food products is easily recognizable and can reassure supply chain members and shoppers about the provenance of the oil (see Fig 4). Those involved in the supply chain can voluntarily sign up to a range of certification schemes and must abide by strict principles and criteria to become certified sustainable members. RSPO certified sustainable PO is defined by eight governing principles with associated criteria and indicators, negotiated by RSPO stakeholders:

1. Commitment to transparency

2. Compliance with applicable laws and regulations

3. Commitment to long-term economic and financial viability

4. Use of appropriate best practices by growers and millers

5. Environmental responsibility and conservation of natural resources and biodiversity

6. Responsible consideration of employees and individuals and communities affected by growers and mills

7. Responsible development of new plantings

8. Commitment to continuous improvement in key areas of activity

Certified sustainable PO products have a greater desirability to consumers, which again increases the risk of fraud within the PO supply chain. There is a danger that non-certified PO could be fraudulently sold as certified-sustainable PO due, both to the greater demand and for economic gain.

Nevertheless, consumers may check whether a particular producer or processor is really a certified member by accessing: https://www.rspo.org/members/all.

Adulteration is another major risk within PO supply chains. Perhaps the most commonly reported is the addition of colouring agents/dyes. The deep orange-red colour of freshly pressed PO is attributed mainly to its high β-carotene content and is usually an indication of quality.

There have been many reported incidents of the addition of Sudan IV dye to low quality PO to improve its colour so that it can be sold as high-quality PO. Sudan IV is an industrial dye, which is classified by the FSA as a carcinogenic compound.

The Swiss Federal Food Safety and Veterinary Office (FSVO) issued a warning to consumers in January 2018 after Sudan IV had been discovered in PO sample produced by the companies, ZOMI and POLIFUDS (17) (Fig. 5).

There have also been several reports of Sudan IV-adulterated PO being sold in the UK. The UK Food Standards Agency issued a warning in 2015 that several stores had been selling unlabeled Ghanaian PO which contained Sudan IV (18).

Methods for ensuring the safety and reliability of products in vegetable oil supply chains

Regulations and legislation in place for ensuring the safety and reliability of products in agricultural supply chains provide a reasonable level of protection for consumers and act as deterrents to those considering operating fraudulently.

However, supply chain traceability is another important factor that can further enhance consumer protection. Traceability is defined as *'the ability to trace a food product from the production until the distribution stage'* (19). Traceability is usually conducted by administrative controls and inspections.

In the case of PO supply chains, this allows traceability one step up and one step down, from any single point within the supply chain. However, administrative controls and inspections may be subject to misuse, with bribery reportedly being common within many agricultural supply chains (20–22).

It is therefore necessary to develop and utilise molecular (i.e. DNA-based techniques) and analytical methods (i.e. chemical or isotopic techniques) to verify product authenticity and/or provenance within supply chains, for traceability.

When vegetable oils are imported, samples must be taken and analysed to determine the quality and authenticity of the oil. Whilst there is a vast array of analytical and molecular techniques used within vegetable oil supply chains for QA/QC and authentication purposes, the conventional methods are often tedious, time-consuming and lack sensitivity.

As a result, there is an ever- increasing need to develop more rapid, cost-effective and sensitive methods, which can be operated by non-technical persons, to meet the demand of large sample numbers at import.

Malpractice within olive oil supply chains is long documented and frequently reported, so analytical methods for olive oil authentication are robust and well used. However, in the case of PO, far fewer approved methods exist because regulations and legislation are not yet as advanced as in the olive industry.

This has prompted researchers to develop novel, state-of-the-art techniques for PO analysis, predominantly focusing on verification of geographical origin and adulterant detection, the two major issues facing the industry.

Whilst the newer techniques have many advantages compared to conventional methods (higher separation efficiency/resolution, rapid analysis, non-destructive, less expensive), they are not usually adopted as official measurements of authenticity owing to the fact that most studies use a small, limited and ill-defined sample sets.

Analytical methods for vegetable oil authentication

Vegetable oil authentication is possible via the assessment of major compounds (Fatty Acids (FAs) and triacylglycerols (TAGs)) (commonly known as triglycerides), minor components (carotenes, tocopherols, tocotrienols and sterols), stable isotopes and smaller volatile organic compounds (VOCs).

This section will briefly discuss techniques used to successfully assess vegetable oil composition, with a particular focus on recent advances with regards to palm oil authentication.

Fatty Acid Composition

FA composition is an important indicator of oil composition for QA/QC and detection of contaminants. Iodine Value (IV), the saponification value or the slip point are classical physico- chemical tests used in the palm oil industry (23).

However, such measures only give an indication of oil composition, and additional chromatographic and/or spectroscopic techniques should therefore be used for more accurate determination of oil composition.

A prominent study by Tres *et al*. (2013), utilised Gas Chromatography-Flame Ionisation Detection (GC-FID) for analysis of crude palm oil (CPO) fatty acid methyl esters (FAMEs) to classify samples by geographical provenance.

The authors discovered that several FAs significantly varied between CPO from three continents (South East Asia, South America and Africa). Saturated FAs including lauric (C12:0), myristic (C14:0) and palmitic acid (C16:0) were higher in South East Asian CPO, whereas African CPO had a higher content of alpha-linolenic acid (C18:3 n-3).

South American CPO was richer in monounsaturated FAs such as oleic acid (C18:1 n-9) and eicosenoic acid (C20:1 n-9). They concluded that the verification of geographical origin, at least on a continental scale, by means of FA fingerprinting combined with chemometrics was feasible.

Triacylglycerol profile

The evaluation of TAG profiles has been successfully utilised to authenticate vegetable oils, given that biosynthetic pathways of lipids tend to be species specific.

However, TAG analysis can be problematic due to the high number of possible FA combinations of the glycerol backbone (25).

Interpretation of TAG profiles is also more complex than FA profiles (26). TAG analysis has been performed using several chromatographic techniques (thin-layer chromatography, Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) and High Temperature-GC (HT-GC)). Ruiz-Samblás *et al.* (2013) developed authentication models based on HPLC-Charged Aerosol Detection (CAD) and HTGC-Mass Spectrometry (MS) fingerprinting of TAGs from CPOs from South East Asia, Africa and South America.

A chemometric classification method, partial least square discriminant analysis (PLS-DA), was applied to discriminate the origin of the oils based on their TAG fingerprints. The rates of successful prediction, based on TAGs, of the geographical origin varied between 70% and 100%.

Unsaponfiable fraction

The analysis of minor components has also been used to assess the authenticity of vegetable oils to overcome issues presented by analysis of FA and TAG composition.

GC and HPLC techniques are most commonly utilised, however they tend to require complex sample preparation (26). Spectroscopic techniques have also proved successful (27). Pérez-Castaño *et al.* (2015) obtained sterolic chromatographic fingerprints using HPLC, to determine the geographical origin (South-East Asia, West Africa and South America) of edible CPO.

Two conventional chemometric classification methods (Soft Independent Modelling by Class Analogy (SIMCA) and PLS-DA) were applied. The sterolic chromatographic profiles from African samples showed no specific patterns and some were similar to the ones of American and Asian samples.

The authors concluded that technically, none of the classifiers showed enough assurance to be applied in order to discern the geographical origin of any sample of palm oil, given that 15% of samples would be erroneously classified.

Stable Isotopes

Stable isotope ratios of carbon ($^{13}C/C^{12}$), nitrogen ($^{15}N/^{15}N$), hydrogen ($^{2}H/^{2}H$), oxygen ($^{18}O/^{18}O$) and sulfur ($^{34}S/^{34}S$) are often used in food authentication studies and have been successfully employed for verification of olive oil provenance (26).

Isotope ratios are affected by a range of conditions such as climate, fertilisation regime and soil type. Faberi *et al.* (2014) therefore used FA composition and ^{13}C of bulk and individual FAs as markers for authenticating Italian PDO/ PGI EVOO, by means of Isotopic Ratio-MS (IR-MS). The work enabled FA ^{13}C characterization of all the Italian PDO/PGI olive oils related to the crop year 2010/2011, which allowed the creation of a database based on composition and isotopic parameters. The authors concluded that discrimination of olive oil samples by geographic and climatic parameters using ^{13}C results of bulk oils and individual FAs was possible and that ^{13}C isotopic values are a robust marker of origin.

Portarena *et al.* (2014) also explored the variation of isotopic compositions (^{13}C and ^{18}O) of EVOOs from different geographical locations in Italy. A plot of ^{13}C versus ^{18}O mean values highlighted a strong correlation resulting from variations in latitude, temperature and rain. No correlation was found by comparing isotope compositions with elevation, longitude and atmospheric relative humidity.

The authors concluded that environmental conditions could not be considered the only factors affecting variation in isotopic compositions and stated that further research would be required to determine the effects of other factors such as genotype.

Volatile Organic Compounds (VOC)

VOC profiles of vegetable oils vary according to geographical origin, climatic conditions, growing conditions, variety, species etc. Oils can therefore be discriminated accordingly.

VOC profiles are usually determined by GC-MS, Proton Transfer Reaction-Mass Spectrometry (PTR-MS) and sensor technology (known as electronic nose systems) (31–33).

Ruiz-Samblás *et al.* (2012) utilised PTR-MS for determining VOC fingerprints of EVOOs of five different olive fruit varieties (Arbequina, Cornicabra, Frantoio Hojiblanca, and Picual). PTR-MS requires no sample pre-treatment, it allows rapid analysis and is extremely sensitive (down to parts per trillion, ppt).

When PLS-DA was applied on the full spectral fingerprints of the measurements, 79.4% of samples were successfully classified according to their variety using a fitted model with six components. The authors concluded that VOC fingerprinting is useful for the assessment of the overall product quality and to differentiate oils based on quality characteristics.

Ion Mobility Spectrometry (IMS) is a relatively new technology, which is becoming a frequent choice for VOC analysis of vegetable oils due to its analytical potential, with regards to its operational speed, high sensitivity and selectivity.

Zhang *et al.* (2016) developed an IMS method with a runtime of just 20 s for detection of adulterants in sesame oil. Chemometric classification methods were employed and the results indicated that the discriminant models could identify adulterated sesame oil samples (10) with an accuracy value of 94.2%. IMS fingerprints also enabled detection of counterfeit sesame oils produced by adding sesame oil essence into cheaper edible oils.

The chemical composition of vegetable oil is the result of complex interactions among genes, environmental conditions, fruit ripening and oil extraction technology, thus the selection of appropriate chemical markers for verification of geographic origin is often complicated.

This has stimulated interest in the use of DNA-based markers for vegetable oil authentication as an alternative method (35). DNA-based markers, or molecular markers, represent differences in genomic DNA sequences of different individuals in a population.

The DNA composition of such molecular markers can vary within or between species as a result of genetic alterations (mutations, insertions and deletions) (36).

Several molecular marker (or 'DNA fingerprint') systems have been developed based on throughput, cost, reproducibility, abundance and ease of use. These systems detect three types of sequence polymorphisms: variation at single nucleotides, insertion/deletion of one or more bases, and variation in the number of tandem repeats of several nucleotides.

Examples of molecular marker systems include Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNAs (RAPDs), DNA Amplification Fingerprinting (DAF), Amplified Fragment Length Polymorphisms (AFLPs), Sequence-Characterised Amplified Regions (SCARs), Simple Sequence Repeat (SSR) polymorphisms, Cleaved Amplified Polymorphic sequences (CAPs) and Single Nucleotide Polymorphisms (SNPs) (36).

Molecular markers have proved particularly useful for DNA fingerprinting of vegetable oils. The cultivar and region of production directly affects the quality traits of olive oil, subsequently, EVOOs are considered premium products due to specific characteristics. Such oils are often protected by PDO and PGI certification labels so it is imperative that verification of the genetic identity of EVOO is possible.

Attempts to relate chemical composition of olive oil to cultivar have found that geographical and environmental effects strongly affect this approach. DNA-based methods should therefore be used alongside chemically based approaches for authentication and traceability as they are more reliable, specific, sensitive and absent of environmental influence. Note that molecular markers are also useful for detection of adulterants in vegetable oils (37–39).

Despite its huge potential, the use of molecular markers in processed foods such as vegetable oils present several challenges. The extraction of adequate quality and quantity of DNA is essential for the development and improvement of PCR-based DNA fingerprinting methods. The quantity of DNA extracted from highly refined, solvent extracted vegetable oils is tiny, but it can be sufficient for molecular marker analysis.

The presence of PCR inhibitors (fats and residual polysaccharides) and nuclease activity can inhibit PCR amplification. However, DNA purity is the most critical factor in DNA fingerprinting methods for ensuring validity and reproducibility (40). The ideal DNA extraction method for refined/unrefined vegetable oils should be simple, reproducible and cheap. It should also be capable of recovering stable DNA, which is free of PCR inhibitors.

Recently, Ramos-Gómez *et al.* (2014) designed a DNA isolation method for the application of DNA markers for food safety and traceability in commercial vegetable oils (including palm oils). The suggested DNA extraction protocol was deemed more suitable than commercial kits as it was less time consuming required a reduced volume of oil and was cheaper.

Several efforts to fingerprint olive oils have been documented, with varying degrees of success. An early effort to fingerprint four Italian olive oils using AFLPs was not successful due to high variation (30%) between the AFLP profiles of leaf and oil, attributed to differences in the level of DNA degradation between the two sample types (41).

Montemurro *et al.* (2008) later described AFLP profiles of 10 Italian cultivars by optimising DNA extraction protocols and restriction/ligation conditions. However, other molecular markers such as SSRs and SNPs combined with high- performance analytical platforms have been now developed, given the requirement of high-quality DNA and short storage time for reliable traceability.

The efficiency of several molecular markers (RAPDs, Inter-Simple Sequence Repeats (ISSRs) and SSRs) for varietal identification of 23 Portuguese olive oils was evaluated by Martins-Lopes *et al.* (2007) . An ISSR marker system was identified as the most informative compared to the inadequate efficiency of RAPD primers.

Conclusions

In the coming decades, the global vegetable oil industry will continue to expand owing to the increasing global population and changing diets.

It is therefore likely that the industry will experience an increase in accidental and deliberate food incidents within supply chains. Although worldwide quality and authenticity regulations and legislation have been implemented to protect products and consumers, there are still instances where these fail or are abused.

Whilst the importance of developing new state-of-the-art techniques for the assessment of quality, authenticity and geographic/botanical origin is quite clear, there are still several challenges which need to be overcome in order for such techniques to be adopted as official methods.

Many of the newer methods described in this review combine the use of advanced analytical techniques with the application of chemometrics, overcoming the limitations presented by conventional analytical methods.

However, despite the documented potential of such methods, there are two major limitations which are persistent in most studies: (i) the use of a small and limited sample set (too few samples and of restricted variation) which could limit application on a wider scale, and (ii) the upfront and operational costs of each technique.

Whilst a method could show great potential, cost is an extremely important limiting factor to consider when developing a new method. If the method is too costly then it will not be accessible to laboratories around the world and so is unlikely to ever be adopted as an official method.

The methods discussed in this review are extremely promising and have increased understanding of and helped to overcome some challenges faced by the vegetable oil industry and other key global food supply chains. However, there is still a long way to go to improving traceability and authenticating vegetable oils and their associated products, within what can be very long and complex supply chains.

Researchers should therefore continue to use recently developed methods with larger and more representative sample sets, while seeking to minimize their costs. The methods developed should be accessible to laboratories worldwide. By this means the methods are more likely to be adopted as standard official methods to tackle the challenges facing global vegetable oil supply chains.

By doing this they will play an important part in improving the safety and sustainability of food supply around the world.

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Figures

Figure 1. It is worth noting that news outlets tend to focus on the problems which are common knowledge and rarely harmful to human health, as in 2013 when the horse meat scandal was headline news in all major UK newspapers. It was widely documented and discussed for many weeks after the initial reports. However, items of greater significance are often ignored or under- reported (for example, the presence of aflatoxin B1 in the plasma of 96% of women surveyed in Nepal, likely resulting from consumption of contaminated staple grains) *(Source: PR Week [https://www.prweek.com/article/1171444/horsemeat-scandal-damaging-trust-say-majority-pr- chiefs](https://www.prweek.com/article/1171444/horsemeat-scandal-damaging-trust-say-majority-pr-chiefs))*

Figure 2. Left: How a typical oil palm plantation looks with rows of planted Elaeis guineensis trees (Oil palm) (Source: https://www.sciencenews.org/article/bad-karma-can-ruin-palm-oil-crops); Right: A plantation worker collecting harvested

fresh fruit bunches to take to the mill for processing into oils (Source: http://www.scmp.com/week-asia/geopolitics/article/2129698/time- bomb-our-stir-fry).

Figure 3. Left: crude palm oil being poured from the glass bottle (notice its bright orange-red colour); Right: several palm fruits (the external orange ring is the fleshy fruit from which PO is extracted, and the white central nut is from where PKO is extracted) (Source: https://financialtribune.com/articles/economy-business-and-markets/64622/iran-palm-oil-market- to-top-600m-by-2025)

Figure 4. RSPO certified-sustainable PO logo (Source: https://www.rspo.org/file/TM_Logo%20Usage%20&%20Guidelines.pdf)

Figure 5. Photos of the palm oil seized by SFVO that was found to contain the illegal Sudan IV (Source: https://lenews.ch/2018/01/31/swiss-government-warns-of-cancerous-palm-oil/)

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