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Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry for Traceability and Authenticity in Foods and Beverages

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ABSTRACT

As consumers demand more certainty over where their food and beverages originate from and the genuineness of ingredients, there is a need for analytical techniques that are able to provide data on issues such as traceability, authenticity, and origin of foods and beverages. One such technique that shows enormous promise in this area is gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). As will be demonstrated in this review, GC-C-IRMS is able to be applied to a wide array of foods and beverages generating data on key food components such as aroma compounds, sugars, amino acids, and carbon dioxide (in carbonated beverages). Such data can be used to determine synthetic and natural ingredients; substitution of one ingredient for another (such as apple for pear); the use of synthetic or organic fertilizers; and origin of foods and food ingredients, including carbon dioxide. Therefore, GC-C-IRMS is one of the most powerful techniques available to detect fraudulent, illegal, or unsafe practices in the food and beverages industries and its increasing use will ensure consumers may have confidence in buying authentic products of known origin.
Introduction

The global food industry is estimated to be worth US$4 trillion in retail sales annually (Services 2012). At the same time, consumer awareness of food issues is increasing as evidenced by more stringent labelling requirements, growing awareness of genetically modified organisms (GMOs) and diseases linked to food (‘mad cow disease’ and ‘bird flu’), as well as the increase in the market for premium products, such as organic food, Protected Denomination of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Speciality Guaranteed (TSG). In view of the value of the global food industry and greater consumer demands on food, there is a necessity for regulations that protect consumers from dangerous or fraudulent practices and analytical methods to assist in enforcing regulations and detecting untoward activities.

In the food and beverage industries, 2 keywords associated with consumer protection are “authenticity” and “traceability”. The word ‘authentic’ is defined (Dictionary.com 2013) as ‘the sense of actuality and lack of falsehood or misrepresentation. Authentic carries a connotation of authoritative certification that an object is what it is claimed to be.’ Non authentic food products arise through adulteration and fraud through practices such as mislabelling/misrepresentation, addition of flavors/aromas to enhance a product at a cheaper price, addition of non disclosed additives to increase bulk, among others. For the food and beverage industry the necessity for authentic products is in the best interest of the consumer, as non authentic products could pose health risks and/or consumer confidence may be dampened through an inferior product sold as the genuine item.
The word ‘trace’ is defined (Dictionary.com 2013) as ‘to follow, discover, or ascertain the course of development of (something),’ and ‘to follow or be followed to source; date back.’ For the consumer, traceability of a food provides a level of confidence in the product that is being sold as there is a level of transparency, there is nothing to conceal in the production of the product. Traceability is also beneficial to trace back the provenance of the ingredients and the source of any contamination.

For example in Europe, with the establishment of the European Economic Community (EEC), rules such as the ‘Common Agricultural Policy’ (CAP) in 1957 (Zobbe 2001), were introduced to control agricultural practices, supply and expenses. Such Euro-wide rules eventually led, towards the end of 1978, to regulations to address authenticity and traceability practices such as labelling of foodstuffs (Council Directive, 79/112/EEC). One of the main outcomes of these rules is the elimination of all unfair competition and the trading of fake products. The milestone of the European Union food safety and traceability regulation is the Regulation (EC) No 178/2002, which defines ‘traceability’ as ‘the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution’.

Methods for testing authenticity and providing data on traceability require robust analytical techniques that can be utilized by the various regulatory authorities around the world. The analytical methods currently used to determine the origin of a foodstuff or beverage and whether it has been adulterated are:

- separation techniques such as gas chromatography (GC) and high-performance liquid chromatography (HPLC);
structural analysis such as mass spectrometry (MS), high resolution-nuclear magnetic resonance (HR-NMR) spectroscopy, and infrared spectroscopy (IR);

- stable isotope analysis such as site-specific isotopic-fractionation nuclear magnetic resonance (SNIF-NMR) spectroscopy and isotopic ratio mass spectrometry (IRMS);

- trace element analysis such as inductively coupled plasmaatomic emission spectroscopy (ICP-AES) and atomic absorption spectroscopy (AA);

- bio-analytical techniques such as DNA profiling,

together with compositional analysis, data handling, and interpretation implementing multivariate statistics such as chemometrics (Lees 1998).

Of the many techniques available to aid in authenticity and traceability testing, isotope ratio mass spectrometry (IRMS) has been widely used due to the high precision of the method, the requirement for small samples, and the fact that the same technique can be used for almost any type of food or beverage. Since the first report in 1977 of IRMS to detect adulteration of honey, by the addition of high-fructose corn syrup (Doner and White 1977), other applications have included authenticity testing and traceability studies of olive oil (Camin and others 2010), orange fruit (Rapisarda and others 2010), lamb (Piasentier and others 2003), and beef (Boner and Forstel 2004). Even though IRMS has been successfully applied to the detection of adulteration across a wide range of foods and beverages, a major drawback is that it is a bulk technique, that is, the data are for the whole sample, which may contain many hundreds or thousands of different chemical compounds. In order to provide more information about specific compounds in foods and beverages, IRMS may be coupled to a GC unit so that separation of compounds occurs prior to isotope ratio monitoring. Coupling of the GC to the IRMS may be via either a combustion or pyrolysis chamber giving information about C/N or H/O isotopes, respectively. Indeed the
development of GC-combustion(C)/pyrolysis(P)-IRMS is a powerful technique for adulteration and authenticity testing (Greule and others 2010), as it allows specific compounds to be targeted that are important to the overall make-up of the particular foodstuff or beverage.

The aim of this review is to provide a broad overview of the GC-C-IRMS technique covering the historical development of the method, a discussion on the natural variability of carbon (C) and nitrogen (N) stable isotope ratios, and an overview of the applications of the technique to a variety of foods and beverages for authentication and traceability purposes. The emphasis of the review will be on C-IRMS for several reasons: historically GC-C-IRMS was developed before GC-P-IRMS; the principles of operation of the instruments are similar in most respects (Sessions 2006); and most importantly, in applications to food and beverages, results from combustion studies have been reported as stand-alone articles, whereas pyrolysis studies have almost always been reported in combination with combustion studies. In presenting this review, the power of the GC-C-IRMS technique is highlighted as a valuable tool in ensuring consumer confidence and safety in the global food industry.

**Historical Development of GC-C-IRMS**

The development of GC-C-IRMS instrumentation is underpinned by some of the most fundamental discoveries in science. As such, we briefly review these developments, which highlight the way in which this very applied technique has evolved due to fundamental developments in physics and chemistry.
In the middle to late 1800s (Svec 1985) chemists and physicists were exploring the nature of cathode rays, which led in 1895 to the discovery of particles with a negative charge (Griffiths 1997; Perrin 1986; Svec 1985; Thomson 2010). Joseph John Thomson replicated this experiment, improving the experimental design, and also utilized a Faraday cup to determine that cathode rays are definitely negatively charged particles (termed ‘electrons’) and their velocity and mass-to-charge ratio (Thomson 2010). These initial stages of measuring particles in cathode rays mark the beginning of isotope ratio mass spectrometry (IRMS) in the early 1900s with the design of the mass spectrograph by Thomson and others (Classen 1908; Dempster 1918). The various parties were experimenting with different methods of detection of elements and their isotopes.

During the decade 1910-1920 significant developments in mass spectrometry yielded the mass spectrum of CO₂ (Budzikiewicz and Grigsby 2006; Thomson 1912) and the discovery of 2 isotopes of neon (Budzikiewicz and Grigsby 2006; Thomson 1913). Initially it was thought that neon was composed of 2 gases giving a main ray at \( m/z \) 20 and a weaker ray at \( m/z \) 22. The assignment of the two rays to neon arose from the development of a new ‘velocity focusing’ mass spectrograph.

In 1918, another type of mass spectrograph was reported, termed ‘directional focusing’ (Budzikiewicz and Grigsby 2006; Dempster 1918; Dempster 1948; Squires 1998; Svec 1985) and by 1948 the isotopic composition of 83 elements had been determined (Dempster 1948). A ‘double focusing’ instrument was reported in 1934, which combined a radial electric field and a uniform magnetic field, creating velocity and direction focusing, thereby enhancing the measurement accuracy of the mass spectrograph due to sharpened images and intensity (Mattauch and Herzog 1934). Various versions of the ‘double focusing’ instrument appeared between 1936 and 1941 (Bainbridge and Jordan 1936; Nier 1940; Straus 1941; Svec 1985). During this
time natural variation in isotopic abundances was discovered and the variance present in specific isotope ratios may carry significant information (Nier 1937; Nier and Gulbransen 1939).

The ability to measure variations in the abundances of isotopes in gases such as CO₂ was enhanced by the design of a mass spectrometer with dual inlet system in 1947. The incorporation of a ‘changeover valve’ allowed 2 gas mixtures (sample and standard) to be alternatively interchanged, in seconds, and subsequently introduced to the mass spectrometer to be detected by 3 Faraday cups (Murphey 1947). Murphey employed this instrument to determine thermal diffusion factors of gas mixtures such as H₂ and D₂, H₂ and He, and D₂ and He (Murphey 1947). Improvements to this system, and the incorporation of a reference standard allowed variations in the isotope abundance ratios of carbon and oxygen in CO₂ and O₂ to be measured with great accuracy (Brand 2004; McKinney and others 1950).

Over the next quarter of a century there were continual improvements to mass spectrometer instruments and the first commercial (quadrupole) mass spectrometer coupled to a gas chromatograph was released in 1968 (Brock 2011). But it wasn’t until 1976 (Brand 1996; Sano and others 1976) that a combustion system was incorporated between the GC outlet and the mass detector to convert the eluent to simple gases whose isotope ratios can be measured. In 1976, Sano developed this instrument to implement an isotope tracer technique for complex matrices such as metabolites in urine samples as an aid in the research of drug metabolites. The system included a CuO combustion reactor positioned after the GC and a multiple ion detection scheme (detecting ions of \textit{m/z} 44 and 45) (Brand 1996; Sano and others 1976). This design was the prototype for the GC-C-IRMS instrument defined as ‘isotope-ratio-monitoring gas chromatography-mass spectrometry (IRM-GCMS)’. The first commercial GC-C-IRMS was released in 1988,
building on Sano’s design with various features such as: a water trap (magnesium perchlorate); a trap for CO₂ (when analyzing for N₂) and the removal of nitrogen oxides through a reduction oven over copper metal. The instrument measured isotope ratios at natural abundance levels (Matthews and Hayes 1978) for C and N in any organic molecule that could be analyzed by GC.

A further enhancement to the overall instrumental design of the GC-C-IRMS was the incorporation of a flame ionization detector (FID). This allowed the “normal” gas chromatogram to be monitored from the main eluent, while the remainder passed through to the combustion and isotope monitoring system. Interestingly, the application chosen to highlight the advantages of this advancement was the determination of the natural \(^{13}\)C abundance of flavorings found in different foods to develop an authenticity test for foodstuffs. Hence, the ability for isotope ratio monitoring to be utilized in food authenticity/traceability has been recognized from the earliest stages of the development of the technique.

One of the requirements for precise measurement of isotope ratios in GC-C-IRMS is the necessity for strict baseline separation of components of interest, which may be difficult in complex sample matrices. Hence there are ongoing efforts to improve separation of the peaks in the gas chromatograph so that the eluent travelling to the mass spectrometer is composed of a single component. One strategy for achieving this is to “heart-cut” the retention time interval of interest and divert the flow to a second column, where further separation can take place prior to introduction to the IRMS. This multi-dimensional (MD) GC approach does allow better resolution of overlapping peaks, but it suffers from the drawback that 2 runs are required – the first to determine which region is to be heart-cut, and the second where MDGC is performed. Alternatively in comprehensive two-dimensional GC (GC × GC) the whole sample is passed through a second column and therefore only one run is required. While the advantages of
comprehensive GC × GC-C-IRMS have been demonstrated in sports drug testing (Tobias and others 2008), so far no food or beverage applications have been published (to the best of the authors’ knowledge). Thus a potential area of fruitful research may be to utilize multi-dimensional and comprehensive GC-C-IRMS in authenticity and traceability studies.

Principles of Operation of GC-C-IRMS Instrumentation

As the name suggests, the basic components of a GC-C-IRMS are a gas chromatograph, which feeds eluent to a combustion system, and thence to an isotope ratio mass spectrometer. Details of these 3 instrumental components are documented in a review by Sessions (Sessions 2006) hence only a brief description is included in this review. Whilst the gas chromatograph needs modification to accommodate the combustion/reduction tubes and so on for GC-C-IRMS, the principles of gas chromatography are no different from any other GC instrument, such as GC-FID or GC-MS. Sample introduction to the GC can occur in a variety of ways depending on the sample, including split/splitless injection, on column injection, solid phase microextraction (SPME)and so on. The most important aspect is to guarantee the injection of analytes without fractionation (see below). After separation on the column the eluent is split with a small part diverted to a “standard” detector such as FID or MS, while the other portion of the flow continues to the combustion system.
Combustion of the eluent occurs in 3 stages: an oxidation step, a reduction step, and the removal of water. Oxidation is facilitated by high temperature (800-1100 °C) and the use of oxidation catalysts such as CuO/Pt or CuO/NiO/Pt (Meier-Augenstein 1999). The principal elements of organic compounds, C, H, and N, are oxidized to the gases CO₂, H₂O, and NOₓ, which are then passed into a reduction tube, where in the presence of elemental Cu at 500-700 °C, NOₓ is reduced to N₂ and any remaining O₂ is removed (note that oxygen originating from the sample cannot be analyzed by IRMS in the system described here, since atmospheric oxygen would change the isotope ratios of oxygen in the combustion gases).

After the reduction step, water is removed from the analyte gas stream. This is a necessary step as the water can react with the gases inside the ion source (in the IRMS) creating ions of differing compositions with conflicting masses to the ions to be measured (known as “isobaric interference”). For example, water could protonate the ¹²CO₂ to H¹²CO₂⁺ which has the same m/z as the ¹³C¹⁶O₂⁺ peak of 45. Removal of water is typically accomplished via a countercurrent drier based on a Nafion™ membrane (Leckrone and Hayes 1998). For nitrogen analyses only, CO₂ must be removed from the gas stream, generally by cryogenic trapping (Merrit and others 1994), to prevent possible isobaric interferences with N₂ in the ion source (from production of ¹²C¹⁶O).

An important component of the combustion assembly is a system for solvent diversion. During a run the solvent peak elutes first; this peak must be removed because the solvent reduces the performance of the oxidation column by overloading the column with organic material. The most common system, referred to as backflushing, acts to reverse the He flow through the oxidation reactor such that the solvent is vented out by an exhaust valve, before it can reach the oxidation column. Once the solvent is vented, eluent from the sample is allowed through the combustion system. The backflush
system can also play a role in regeneration of the oxidation reactor by introducing O\textsubscript{2} into the reversing He stream. A new system has recently been proposed which is based upon the use of a cooled injection system and auxiliary vacuum for solvent removal (Flenker and others 2007).

Dried gases from the combustion system travel to the IRMS detector and are subjected to electron-impact ionization and a succeeding magnetic-sector or electromagnetic analyzer, deflecting the generated ions by molecular weight to be subsequently detected by precisely positioned (in the MS focal plane) Faraday cups. For carbon there are 3 detecting Faraday cups that monitor the various isotopologues of CO\textsubscript{2} namely \textit{m/z} 44 \((^{12}\text{C}^{16}\text{O}_{2})\), \textit{m/z} 45 \((^{13}\text{C}^{16}\text{O}_{2} \text{ and } ^{12}\text{C}^{17}\text{O}_{2})\), and \textit{m/z} 46 \((^{12}\text{C}^{18}\text{O}_{2})\). For nitrogen these three Faraday cups are arranged to capture isotopologues of N\textsubscript{2} -- \textit{m/z} 28 \((^{14}\text{N}^{14}\text{N})\), \textit{m/z} 29 \((^{14}\text{N}^{15}\text{N})\), and \textit{m/z} 30 \((^{15}\text{N}^{15}\text{N})\). During the chromatographic run the Faraday cups continuously record ion currents and these data are processed to give relative isotopic abundances as described below.

**Processing of GC-C-IRMS Data**

The output from a GC-C-IRMS consists of partially overlayed chromatograms that correspond to the ions detected by the Faraday cups. For carbon isotope monitoring there will be 3 traces corresponding to \textit{m/z} 44, 45, and 46 (Figure 1a shows 2 traces; \textit{m/z} 44 \((^{12}\text{C}^{16}\text{O}_{2})\) and \textit{m/z} 45 \((^{13}\text{C}^{16}\text{O}_{2})\)), while for nitrogen, 2 traces are common \textit{m/z} 28 and 29, with a third trace optional (\textit{m/z} 30). The peaks of isotopologues are shifted in time, because the heavier isotopologue elutes milliseconds before the lighter isotopologue as a result of chromatographic isotope effects (Matucha and others 1991) (see Figure 1).
The first step in data processing is that of peak detection whereby peak start and stop parameters are defined (Zhang and others 2012). From there ion current ratios can be calculated for the detected peaks via summation, curve fitting or linear regression. The summation approach is most commonly used (Zhang and others 2012) and is explained in detail in Ricci and others (1994). Briefly, once peak start and stop times have been defined, major and minor isotope traces are integrated from which isotope ratios can be calculated; background correction on the ion currents is mandatory and can be performed either before or after peak detection and integration (Zhang and others 2012). It should be noted that algorithms have been developed (Bluck and Coward 2004; Goodman and Brenna 1994) to accommodate some co-elution of peaks in GC-C-IRMS, however to the best of our knowledge these algorithms have not been used in any real-life applications and we strongly emphasize the need for baseline separation of peaks.

Having calculated the isotope ratios (such as $^{13}\text{C}/^{12}\text{C}$) for each peak in the sample chromatogram, further data processing is required to report these ratios relative to ratios derived from standard reference substances (shown in Fig 1b). This relative deviation is expressed using the isotope delta ($\delta$) notation, which was first described for C by Urey (Urey 1948) and adapted as follows (Brand and Coplen 2012):

$$\delta_i^E = \left( \frac{R_{SA}}{R_{REF}} \right)^i - 1$$

where $^i$ is the mass number of the heavier isotope of element E (for example $^{13}\text{C}$ or $^{15}\text{N}$);

$R_{SA}$ is the respective isotope number ratio of a sample (such as for C: number of $^{13}\text{C}$ atoms / number of $^{12}\text{C}$ atoms or as approximation $^{13}\text{C}/^{12}\text{C}$);

$R_{REF}$ is that of international recognised reference materials, namely Vienna Pee Dee Belemnite (VPDB) for CO$_2$ and Air for N$_2$;
The delta values are multiplied by 1000 and are expressed in units of ‘per mil’ (‰).

In GC-IRMS correct calibration of the instrument is essential. This involves introducing the reference gas (either CO\textsubscript{2} or N\textsubscript{2}) during the run via the reference gas inlet when there is no interference with other eluting peaks and/or using organic standards which can be added to the sample if they elute in analyte free regions or analysed in the run in the proximity of the samples (Sessions 2006). The isotope ratios of both the reference gases and the standards are calibrated against secondary isotopic reference materials with certified values (Brand and others 2014), for example V-PDB for CO\textsubscript{2} and air for N\textsubscript{2}.

The precision of the GC-C-IRMS instrument has to be excellent, namely 10\textsuperscript{-5} atom % deviation to determine the $^{13}$C/$^{12}$C isotopic ratio (Brand 1996; Meier-Augenstein 1997). However, in order to achieve the necessary precision and accuracy the analytical workflow must be carefully considered. It is essential for reproducibility that the following are considered: maintaining a clean working environment; extensive method development aimed at producing the optimal running conditions of the instrument (columns, injector systems/methods, splits, flow rates, isotopic calibration and so on); appropriately selected internal standards (Caimi and others 1994; Mosandl 1995; Mosandl and others 1995); avoidance of analysis-induced fractionation, as a result of derivatisation, improper injecting methods, incomplete sample combustion and so on) and suitable sampling and storage techniques (Blessing and others 2008). Limits of detection and linearity should be determined, and runs should be examined for carryover effects as these could cause significant differences for the isotope ratio values (Mottram and Evershed 2003).
Isotopes – Natural Abundances and Ratios

Some discussion of isotopes and their natural abundances is essential in order to understand the principles of the GC-C-IRMS instrument and its applications. The term ‘isotope’ was first introduced by Soddy in 1913 as a condensed version of ‘They are chemically identical, and save only as regards the relatively few physical properties which depend on atomic mass directly, physically identical also.’ (Soddy 1913). The word ‘isotope’ is derived from the Greek, ‘isos’ meaning ‘equal’ and ‘topos’ meaning ‘place’, that is the chemical element, (same atomic number) with different atomic weights (different number of neutrons), resides in the same position on the periodic table. Isotopes exist in 2 forms, stable and unstable. The bio-elements H, C, N, O, and S exist in nature as 2 or more stable isotopes (Table 1).

Isotopes of an element have the same electronic structure and, therefore, very similar chemical reactivity. Nonetheless, isotopes show different natural abundance, through processes which may be collectively termed “fractionation”. Fractionation results in either enrichment or depletion of an isotope away from its mean natural abundance (Table 1). For example, since H$_2^{18}$O is heavier than H$_2^{16}$O, evaporation of water from the earth’s oceans will result in more H$_2^{16}$O in the atmosphere and consequently the atmosphere is depleted in H$_2^{18}$O relative to the ocean (Craig and Gordon 1965).

Fractionation processes are governed by kinetic and thermodynamic effects and are due to the isotope’s physical and chemical properties that often are proportional to their mass differences and which can affect either the rate of a process (kinetic isotopic effect) or the energy state of a system (thermodynamic isotopic effect) (Galimov 1985). As will be described in more detail below, fractionation processes that occur during the biosynthesis
of the components of foods or beverages can be used in authenticity and traceability studies. Small changes in the ratios of isotopes such as $^{13}\text{C}/^{12}\text{C}$ and/or $^{15}\text{N}/^{14}\text{N}$ can provide a fingerprint that provides a unique identifier for the origin of particular organic compounds within a food or beverage. By determining these ratios with GC-C-IRMS and, therefore, in specific food components, much information can be derived about foods and beverages, which can be used to ensure they meet consumer and regulatory requirements.

Carbon

Variations in the $^{13}\text{C}/^{12}\text{C}$ ratio in natural systems were discovered in 1939 (Nier and Gulbransen 1939) and are associated with the manner of their formation, beginning from the primary C sources; atmospheric CO$_2$. The $\delta^{13}\text{C}$ of atmospheric CO$_2$ is decreasing progressively, albeit very slowly with time, from -7.92 ‰ at the end of 1998 to -8.22 ‰ at the end of 2003 over the central Indian ocean (Longinelli and others 2005). Some major processes that incur isotopic fractionation in the inorganic and organic carbon cycles are the exchanges between the atmosphere and the hydrosphere that generate sedimentary carbonate deposits enriched in $^{13}\text{C}$ (+5 ‰ to -5 ‰) and biological processes cause biospheric carbon to be enriched in $^{12}\text{C}$ (typical ratios from -15 ‰ to -35 ‰) (Galimov 1985) (see Figure 2).

The fractionation of carbon isotopes within a biological vegetal system occurs through photosynthetic pathways, Calvin (C3), Hatch-Slack (C4), and the Crassulacean acid metabolism (CAM). The C3 plants (tomato, potato, wheat, rice, grape) that utilize the Calvin cycle to fix CO$_2$ have a $^{13}\text{C}/^{12}\text{C}$ ratio more depleted in $^{13}\text{C}$ than the C4 plants (such as corn and sugarcane (Winkler 1984)) that utilize the Hatch-Slack cycle (Smith and Epstein 1971).
The reason for this is that the Calvin cycle fixes atmospheric CO₂ in the first step with a carboxylation reaction utilizing the enzyme ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO) (Galimov 1985; Ting 1985) and this step is rate-determining, irreversible, and discriminates against ¹³C causing fractionation (O'Leary 1988). This carboxylation step leads to 2 molecules of phosphoglycerate containing 3 carbons and hence the term ‘C3’ given to plants implementing the Calvin cycle (Galimov 1985; Krueger and Reesman 1982; O'Leary 1988). The Hatch-Slack cycle, however, fixes atmospheric CO₂ via carboxylation, aided by the enzyme phosphoenolpyruvate (PEP) carboxylase into oxaloacetic acid which is then converted into molecules of either malate or aspartate, both of which contain 4 carbons, and hence the term ‘C4’ used for these plants (Galimov 1985; Krueger and Reesman 1982; O'Leary 1988). The molecule is then relocated to the bundle sheath cells where it will become a molecule of pyruvate (Galimov 1985) and a molecule of CO₂ is released (Galimov 1985; Krueger and Reesman 1982; O'Leary 1988). The CO₂ molecule is then incorporated into the Calvin cycle. As the limiting step in this photosynthetic cycle is the diffusion through the stomata (O'Leary 1988), and the carboxylation reaction via PEP carboxylase is efficient and irreversible, the molecules generated do not vary greatly from the atmospheric CO₂ (Krueger and Reesman 1982; O'Leary 1988), hence fractionation is not as great as for the Calvin cycle. As a consequence, the δ¹³C values for 80-90% of C3 plants are between -24 ‰ to -34 ‰ (Krueger and Reesman 1982), whereas those for C4 plants are between -10 ‰ and -16 ‰ (Winkler 1984). CAM plants (vanilla, pineapple, and cacti) tend to have ratios -12 ‰ to -30 ‰, (Winkler 1984), similar to C3 and C4 plants as they are able to utilize both forms of photosynthetic pathways depending on the conditions they are subjected to (Osmond and others 1973).

Secondary carbon metabolism induces kinetic isotope effects due to metabolic and branching reactions, including the extent of reversibility of these reactions (Schmidt and Kexel 1997; Schmidt 1999). These metabolism reactions tend to cause further fractionation and, therefore, their products are
generally more depleted in $\delta^{13}C$, for example the reaction of carbohydrates to proteins (2‰ depletion of $\delta^{13}C$) and to lipids (5‰ depletion of $\delta^{13}C$) (DeNiro and Epstein 1977; Winkler 1984).

Factors such as temperature, water availability, relative humidity, CO$_2$ concentration, fertilization, salinity, light intensity, and photorespiration (O'Leary 1981) also impact the $\delta^{13}C$ value of plants and their products because they have an effect on stomatal aperture and CO$_2$ diffusion into the leaf (Farquhar and others 1982; O'Leary 1995; Scheidegger and others 2000).

The fractionation processes described above mean that $\delta^{13}C$ can be used to detect authenticity of food when adulterated with compounds derived from plants with a different photosynthetic cycle (such as unpermitted addition of cane sugar to wine). When the adulteration has been made with compounds derived by plants with the same photosynthetic cycle, adulteration is more difficult to detect. One approach is to use the isotope ratio of a compound of known origin, which can be used as standard reference. Further challenges arise from the use of synthetic compounds as adulterants. Such compounds, derived from coal and petroleum originating from reservoirs of carbon formed from ancient C3 plants, have $\delta^{13}C$ generally ranging from -25‰ to -30‰ very similar to those of C3 plants today (Krueger and Reesman 1982). These synthetic compounds may be used as food additives to enhance or replace naturally derived compounds (Krueger and Reesman 1982). Detection of this substitution by isotope ratio monitoring may be difficult and may require the use of complementary analytical techniques.

Nitrogen
The natural environmental cycle of nitrogen is slightly more complicated than for carbon as it progresses through the atmosphere, plant and microbes and can occur in reduced and oxidized forms and there are more factors causing isotope fractionation. Atmospheric nitrogen is utilized as an international standard for $\delta^{15}$N measurements, since isotope $^{15}$N in the air remains constant with a natural abundance of 0.366 % (Ehleringer and Rundel 1989). Nitrogen fixation by biological or abiotic processes incurs a slight fractionation when atmospheric N$_2$ is converted to ammonia. This process occurs through bacteria, physical processes that produce high temperatures such as lightning and fire, and also human activities including the use of fertilizers and the production of energy (Fogel and Cifuentes 1993)(Figure 3).

The $\delta^{15}$N of soil can vary significantly on the basis of the processes occurring in the soil such as mineralization, nitrification, volatization, and nitrate reduction or denitrification, each incurring an isotopic fractionation (Choi and others 2006; Mariotti and others 1981; Mariotti and others 1982; Tang and Maggi 2012). The extent of these processes depends on factors such as soil depth, vegetation type, and climate and, therefore, the $^{15}$N natural isotope abundance ranges vary (Malchair and others 2010; Persson and Wiren 1995). For cropped soils, the main factor affecting $\delta^{15}$N is fertilizing practices. Synthetic fertilizers produced by the Haber process have $^{15}$N values in the range of -6 ‰ to +6 ‰ while organic fertilizers (enriched in $^{15}$N) have $^{15}$N values in the range of +0.6 ‰ to 36.7 ‰ (manure between +10 ‰ and +25 ‰)(Bateman and Kelly 2007a).

Plants display $\delta^{15}$N values that are linked to ammonia and nitrates in the soils and also associated with isotope effects due to the assimilation of these compounds into the plant (Werner and Schmidt 2002). Factors such as proximity to sea and water stress can induce enrichment in $^{15}$N (Heaton 1987).
Leguminous and nitrogen-fixing plants are an individual case as they can fix nitrogen directly from the air, and therefore have $\delta^{15}$N values around 0‰ (Martinelli and others 1992). Organic fertilizers such as manure also affect $\delta^{15}$N values as the nitrogen in the manure has been through various trophic levels (that is where an organism occurs in the food chain, for example plant, herbivore, microbe, and so on).

The $\delta^{15}$N analysis can be used for food authenticity, for example to verify the use of organic instead of synthetic fertilizers, as required by law for organic food production. $\delta^{15}$N analysis of bulk samples has proven to be useful in the differentiation of organic and conventional produce such as potato tubers (Camin and others 2007), wheat (Schmidt and others 2005; Senbayram and others 2008), carrots (Bateman and others 2005), tomatoes (Bateman and others 2007; Schmidt and others 2005), oranges, (Camin and others 2011; Rapisarda and others 2010), peaches and strawberries (Camin and others 2011), though most of these techniques require, for absolute authentication, other complementary techniques. This application of $\delta^{15}$N could be improved by analyzing, using GC-C-IRMS, single components, or an array of components, such as amino acids.

Isotopic fractionation within natural systems occurs via a multitude of processes and it provides an isotopic fingerprint for each biological system. Analytical tools are vital in investigating such systems and certifying these fingerprints. The GC-C-IRMS is one such tool that has the potential to be implemented in fingerprinting

**Application of GC-C-IRMS in foods and beverages**
GC-C-IRMS is increasingly being used within the food and beverage industries to assure product authenticity and curb product adulteration (Takeoka and Ebeler 2011). GC-C-IRMS can measure separately the ratio of stable isotopes of C and N of different compounds within a single matrix; this technique is known as compound-specific isotope analysis (CSIA). CSIA may be advantageous in detecting adulteration compared to elemental analyzer-isotope ratio mass spectrometry (EA-IRMS), which is utilized in bulk sample analysis. In EA-IRMS, the entire sample is combusted, not just a single component as in CSIA, and hence compound-specific data are lost in EA-IRMS.

CSIA can focus on a single compound within a matrix (such as a fatty acid in olive oil) which provides more detailed information on the final composition of the sample. In addition, the CSIA approach can be extended to monitor classes of molecules which may be of interest, for example, terpenes. As mentioned above, there are many ways in which the authenticity of foods or beverages may be compromised by substitution or addition of specific compounds and CSIA is a powerful technique in addressing these.

As well as detecting single compounds that may indicate food/beverage fraud, it is also useful to consider the isotopic authenticity range of a particular product. This range is determined by measuring a large number of authentic samples for compounds found specifically within that product, providing minimum, maximum, and/or 95% of variability (a range) per compound. Compilation of the compound-specific data facilitates the production of a profile for the specific product. To determine whether a sample is authentic, the sample must be analyzed and its values compared to the isotopic authenticity range previously established. If the sample has one or more compounds outside the “range” then that sample may be adulterated. Often an internal isotopic standard (i-IST) is used as part of the isotopic fingerprint to remove the underlying and predominant fractionation caused by CO₂
fixation in plants, allowing for the fractionation due to secondary metabolic synthesis to be revealed (Braunsdorf and others 1993b). The compound chosen to be an i-IST requires the following: be in sufficient quantity for analysis; be genuine; no isotopic fractionation should occur during sample preparation or analysis; should be biogenetically related to the other compounds in question; should not be sensorially important; and also be stable during storage (Braunsdorf and others 1993b). Thus GC-C-IRMS in conjunction with an i-IST can be used to study plant metabolic processes in their own right, as well as being applied to the detection of food/beverage fraud.

Another consideration for the implementation of GC-C-IRMS is the potential for fractionation to occur during the sample preparation stage of the analysis. Schumacher and others (1995) investigated fractionation during different extraction procedures (dynamic headspace, simultaneous distillation, and solvent extraction) for the analysis of volatile components from strawberries. Also studied were the effects of the additives ammonium sulfate and calcium chloride (typically added to the strawberry pulp during crushing to avoid enzyme activity) on the volatile component, with ammonium sulfate showing slight fractionation for one of the internal standards. Schumacher and others (1995) were able to provide $\delta^{13}$C data for selected strawberry volatiles such as butanoic acid, 2-methylbutanoic acid, hexanoic acid, $\gamma$-decalactone, and $\gamma$-dodecalactone. Further examples of the application of GC-C-IRMS to traceability and authenticity of foodstuffs, beverages, and related plant metabolic processes are discussed below and are summarised in Table 2. In the following discussion we report some data for $\delta^2$H and $\delta^{18}$O compound specific analysis using GC-P-IRMS (Sessions, 2006), considering only the studies when this technique was combined with GC-C-IRMS. The $\delta^2$H and $\delta^{18}$O values are expressed in per mil relative to the international reference material VSMOW (Vienna Standard Mean Ocean Water) (Coplen 1996).
**Aroma compounds in fruit**

Seasonal variation in rainfall, amount of sunlight, and so on can cause the quality and/or yield of food products to vary greatly (Siebert 2002). The addition of flavor and aroma compounds is widely implemented to achieve the desired food and beverage products that will sell on the market. Natural flavors are often expensive to produce in comparison to flavors produced synthetically and could lead to possible fraudulent activity to increase profits to the producer. To guard and inform consumers, labelling requirements were adopted for food products (as stated previously) to include more specific labelling to assign the use of flavors in foodstuffs as ‘natural’ products, as opposed to synthetic, artificial, or nature-identical (the latter term can mean compounds derived from microorganisms or enzyme treatments of natural products). For example, vanillin can be derived from a plant, made synthetically, or even biotechnologically derived (Greule and others 2010). It would appear from the literature that, as yet, there is no standard terminology for the different ways flavor/aroma compounds can be derived and as such, we will use the terminology as reported in the original literature.

**Discrimination between natural and synthetic aromas**

Due to the requirement for baseline separation in GC-C-IRMS, generally the compounds selected for study are characteristic or key aroma compounds of the fruit or fruit product which can be isolated chromatographically. In the case of pear, 7 compounds were investigated by gas chromatography-combustion/pyrolysis-isotope ratio mass spectrometry (GC-C/P-IRMS) to evaluate $\delta^{13}$C (combustion) and $\delta^2$H (pyrolysis), namely 1-butanol, hexyl
acetate, 1-hexanol, butyl acetate, methyl \(E,Z\)-2,4-decadienoate, ethyl \(E,Z\)-2,4-decadienoate, and ethyl \(E,E\)-2,4-decadienoate (Kahle and others 2005). These compounds were determined in a variety of matrices such as juices, brandies, and baby food, and additionally synthetic and “natural” (biotechnologically acquired) compounds were also analyzed. The synthetic samples (\(\delta^{13}C\): -25.0 \(\%\)o to -31.3 \(\%\)o; \(\delta^{2}H\) -63 \(\%\)o to -159 \(\%\)o) could be distinguished from “natural samples” (\(\delta^{13}C\): -12.4 \(\%\)o to -42.4 \(\%\)o; \(\delta^{2}H\) -140 \(\%\)o to -346 \(\%\)o), fruit (\(\delta^{13}C\): -29.7 \(\%\)o to -42.7 \(\%\)o; \(\delta^{2}H\): -48 \(\%\)o to -255 \(\%\)o), and fruit products (\(\delta^{13}C\): -27.2 \(\%\)o to -41.9 \(\%\)o; \(\delta^{2}H\) -46 \(\%\)o to -262 \(\%\)o) thus enabling this technique to be used as an authenticity tool.

Similar considerations and methodology have been applied to the study of typical aroma compounds of pineapple fruits including methyl hexanoate, ethyl hexanoate, methyl 2-methylbutanoate, ethyl 2-methylbutanoate, and 2,5-dimethyl-4-methoxy-3[2H]-furanone (Preston and others 2003). The C and H isotopic ratios of these compounds were measured in fruits (CAM plant; \(\delta^{13}C\): -12.6 \(\%\)o to -28.6 \(\%\)o; \(\delta^{2}H\): -118 \(\%\)o to -263 \(\%\)o), recovery aromas/water phases (\(\delta^{13}C\): -15 \(\%\)o to -29.4 \(\%\)o; \(\delta^{2}H\): -150 \(\%\)o to -265 \(\%\)o) and “natural” (\(\delta^{13}C\): -11.8 \(\%\)o to -32.2 \(\%\)o; \(\delta^{2}H\): -242 \(\%\)o to -323 \(\%\)o) and synthetic (\(\delta^{13}C\): -22.8 \(\%\)o to -35.9 \(\%\)o; \(\delta^{2}H\): -49 \(\%\)o to -163 \(\%\)o) versions of the compounds. The authors concluded that GC-C/P-IRMS may be a useful tool for recognizing authenticity of pineapple products, but would require a larger data bank of samples, including those from more regions such as the Philippines, Indonesia, and Brazil, to be used definitively.

\textit{Prunus} fruits such as peaches, apricots and nectarines were analyzed by Tamura and others (2005) for the isotopic ratios of \(\gamma\)-decalactone and \(\delta\)-decalactone (\(\delta^{13}C\) ranges of \(\gamma\)-decalactone and \(\delta\)-decalactone for fruits: -34.0 \(\%\)o to -38.4 \(\%\)o; “natural”: -27.7 \(\%\)o to -30.1 \(\%\)o; and synthetic: -27.4 \(\%\)o to -28.3 \(\%\)o and \(\delta^{2}H\) ranges of both compounds for fruits: -160 \(\%\)o to -228 \(\%\)o; “natural”: -185 \(\%\)o to -286 \(\%\)o; and synthetic: -151 \(\%\)o to -184 \(\%\)o). Berries
also have been subjected to isotope analysis, for example del Mar Caja and others (2007) analyzed raspberry for α-ionone, β-ionone, and α-ionol ($\delta^{13}C$ ranges of α-ionone, β-ionone, and α-ionol for fruits: -30.3‰ to -36.6‰; “natural”: -9.1‰ to -28.0‰; and synthetic: -24.5‰ to -29.0‰ and $\delta^2H$ ranges of both compounds for fruits: -176‰ to -225‰; “natural”: -43‰ to -257‰; and synthetic: -26‰ to -184‰). Both studies were able to differentiate between fruit, “natural”, and synthetic-derived compounds proving that the GC-C/P-IRMS is useful for authenticity assessments.

In gas chromatograms where the compound of interest cannot be baseline-resolved, it may be helpful to employ multi-dimensional chromatography to achieve better separation of the target compound. This was the approach of Sewenig and others (2005), who used a constant-flow multidimensional gas chromatography-combustion/pyrolysis-isotope ratio mass spectrometry (MDGC-C/P-IRMS) method to authenticate (E)-α(β)-ionone in raspberries and raspberry products. This technique allowed the sample to be passed through a pre-column and the target compounds to be “cut” from the rest of the chromatogram and introduced to the main column for further separation and purification (Figure 4). The method was able to distinguish between fruit ($\delta^{13}C$: -28.3‰ to -35.1‰; $\delta^2H$: -190‰ to -214‰), “natural” ($\delta^{13}C$: -14.9‰ to -15.6‰; $\delta^2H$: -155‰ to -204‰), and synthetic ($\delta^{13}C$: -21.6‰ to -25.8‰; $\delta^2H$: -28‰ to -213‰) (E)-α(β)-ionones, and the authenticity of the raspberry flavor was validated in a number of different products (Sewenig and others 2005).

Even more information about the origin of compounds can be found by combining results from enantioselective GC, multidimensional GC, and GC-IRMS. For example, Bernreuther and others (1990) were able to differentiate the origin of γ-decalactone from various natural sources including fruits (-28.2‰ to -40.9‰) and synthetic products (-24.4‰ to -26.9‰), while Nitz and others (1991) analyzed γ-lactones in strawberry, raspberry,
pineapple, passion fruit, plum, and coconut. More recently, Greule and others (2008) investigated blackberry flavors by the combined techniques for authentication purposes.

**Other applications of GC-C-IRMS for the study of aroma compounds**

Apart from authenticity (natural or synthetic) testing, GC-C-IRMS has been used to determine the origin of bananas and derived products, and the ripening stage of bananas used in those products by studying volatile compounds in banana aroma (Salmon and others 1996). Bananas from 3 different areas (Ivory Coast, Martinique, and Central America) could be differentiated based on the concentrations of selected aroma compounds and their $^{13}$C isotope ratios. Similarly, bananas at 2 ripening stages – early and late – could also be differentiated, confirming that banana nectar is made from very ripe fruit. The study also discovered that natural banana flavor could be distinguished from synthetic flavor.

Schipilliti and co-workers (2011b) implemented SPME combined with GC-C-IRMS to determine $^{13}$C isotope ratios in selected volatile compounds from strawberry. The compounds studied were chosen as contributors to the overall aroma of strawberries and their carbon isotope ratios were determined from fresh organic strawberries that established an authenticity range which included the use of an i-IST. The results showed that this technique can be used to determine food authenticity, and several products tested (including strawberry yogurts, strawberry flavored lolly ice) were shown to contain aroma compounds that were not derived from strawberries. Moreover, it was possible to distinguish between different fruits (pineapple, peach) containing volatile compounds common to strawberries, even when the volatile compounds were derived from plants sharing a
common photosynthetic pathway (both strawberry and peach are C3 plants). The authors noted that “This result appears useful to evaluate mixed-fruits flavor with common volatiles among their aroma active ones. However, more detailed studies should be carried out in this direction.”

As previously discussed, a combination of GC-C-IRMS with chiral chromatography can provide useful information on substitution of one natural product for another to achieve a similar aroma/flavor. Thus Weckerle and others (2001) provided isotopic and chiral data for the cactus pear (*Opuntia ficus indica*) flavor for future work in authenticity determinations, as products seeking a melon-like aroma could be enhanced with this extract.

Technological processing techniques such as heating, distillation, and vaporizing can cause isotopic fractionation of aroma compounds. Elss and others (2006) studied the effects of processing on the isotopic values and the quantitative profile of apple aromas. The aroma profiles for authentic apple juice, apple concentrate, and apple fruit were different and could be attributed to cultivar differences, storage, bottling, pasteurization and packaging, but the profiles during the industrial processing were determined to be sustained (Elss and others 2006). The isotopic ratios of C and H were measured for 3 aroma compounds of apple (*E*-2-hexenal, 1-hexanol, and *E*-2-hexenol) to determine if isotopic fractionation occurred during processing. The results showed that for $\delta^{13}$C, with all the compounds, there was no fractionation, though for *E*-2-hexenal and 1-hexanol the $\delta^{2}$H ratios were slightly depleted indicating that fractionation did occur. Authenticity assessment was not affected by this fractionation as it was too small.

**Aroma compounds in essential oils**
Discrimination between natural and synthetic aromas

Essential oils can be used as flavorings in foodstuffs, but their production is very expensive in comparison with the synthetic counterparts and, for this reason, fraudulent activity, such as stating the product is natural when it is synthetic, often occurs. A characteristic flavor compound of fennel and anise oils is trans-anethole and it is extracted as a flavoring additive for beverages and foodstuffs (Bilke and Mosandl 2002; Newberne and others 1999). trans-Anethole is also produced synthetically and could be potentially used as a substitute for the natural extract. Bilke and others (2002) developed an authenticity range for trans-anethole based on self-extracted fennel and anise oils implementing a combination of GC-C-IRMS and GC-P-IRMS, as separately these methods could not definitively determine authenticity. Bilke and others (2002) were able to show that “natural” fennel oil and anise oils that were commercially available ($\delta^{13}C$: -24.2‰ to -29.6‰; $\delta^2H$: -71‰ to -99‰) fell in the authenticity range ($\delta^{13}C$: -25.3‰ to -28.3‰; $\delta^2H$: -46‰ to -84‰) and that most of the synthetic compounds ($\delta^{13}C$: -29.6‰ to -32.1‰; $\delta^2H$: -20‰ to -79‰) and one commercial fennel oil sample were outside this range, therefore enabling this method to be used for authentication.

Fink and others (2004) investigated (E)-methyl cinnamate from basil and other sample extracts, several commercial natural aromas, and a synthetic compound by a combination of $\delta^{13}C$ and $\delta^2H$ ratios. They were able to measure a difference between synthetic (E)-methyl cinnamate ($\delta^{13}C$: -29.5‰ to -31.4‰; $\delta^2H$: +328‰ to +360‰) and that derived from basil ($\delta^{13}C$: -28.9‰ to -29.0‰; $\delta^2H$: -126‰ to -133‰), as well as showing that some of the commercial natural aromas ($\delta^{13}C$: -25.7‰ to -28.5‰; $\delta^2H$: -85‰ to -191‰) were incorrectly labelled.
Frank and others (1995) used the combination of enantioselective analysis, δ^{13}C and an i-IST, for the measurement of essential oils in coriander samples. The use of the i-IST allowed for the development of fingerprints using the terpenes found in the oils. Fingerprint matching between authentic and commercial oils identified one commercial sample as natural. Another commercial sample had a different fingerprint with respect to γ-terpinene and geraniol, which suggests that these compounds were not natural in this commercial sample.

Citrus fruits and their essential oils have been studied at great length by GC-C-IRMS owing to the fact that these aromas/essential oils are used in many food and beverage products. Examples include studies on lemons (Braunsdorf and others 1993b; Nhu-Trang and others 2006; Schipilliti and others 2012), oranges (Braunsdorf and others 1992, 1993a), mandarins (Faulhaber and others 1997a; Schipilliti and others 2010), and also lemon balms (and while not a citrus, its leaves have a lemon aroma and it is a C3 plant) (Nhu-Trang and others 2006; Wagner and others 2008). In the work of Nhu-Trang and others (2006), the focus was the terpenes citral (a mixture of neral and geranial, E/Z isomers of 3,7-dimethyl-2,6-octadien-1-al, respectively) and citronellal (3,7-dimethyl-6-octenal, which occurs as 2 enantiomers). Thus they employed chiral GC with GC-C-IRMS to determine enantiomer and ^13C and ^2H isotopic ratios for these compounds genuinely derived from different plants having the “lemon” aroma. The natural citral had δ^{13}C ratio of ca. −26 ‰ and synthetic citral had a δ^{13}C range from −30 ‰ to −28 ‰, whereas the δ^2H composition of natural citral (ca. −244 ‰) was very different from that of synthetic citral (−6 ‰ to 63 ‰). In addition to identifying synthetic and natural differences, other plant types (C3/C4) were also identified by isotope ratios of citral.
Vanillin extracted from the bean of the CAM plant vanilla is another flavoring agent of enormous economic significance. Early studies on vanillin by GC-C-IRMS were undertaken in 1994 by Breas and others (1994). Then a GC-C-IRMS method was used by Fayet and others (1995) to determine if the $\delta^{13}$C of extracted vanillin flavouring from ice cream was the same as the $\delta^{13}$C of the vanillin flavoring from the supplier or from a different source. The results showed that the $\delta^{13}$C of the vanillin flavoring from the supplier was similar to the $\delta^{13}$C of the vanillin found in the ice cream. Then in 1998, Hener and others (1998) measured various sources of vanillin using GC-C-IRMS for $\delta^{13}$C in comparison with GC-P-IRMS for $\delta^{13}$C and $\delta^{18}$O. The values for $\delta^{13}$C, when comparing methods, were very similar except for 2 samples, one of biotechnological origin and the other from Mexico. The $\delta^{13}$C values easily separated vanillin samples from different origins; biotechnologically derived (-37 ‰), synthetic (-27.4 ‰) and natural (-20.2 ‰ to -18.7 ‰).

In order to develop a more comprehensive analysis of compounds derived from vanilla, Kaunzinger and others (1997) implemented an isotopic fingerprint based on 4-hydroxy benzyl alcohol, vanillic acid, 4-hydroxybenzaldehyde, and vanillin. This enabled them to show that the $\delta^{13}$C values are stable from year to year and that for different varieties of vanilla the $\delta^{13}$C values for vanillin are quite different ($V. tahitensis$: -15.5 ‰; $V. planifolia$: -19 ‰), though for 4-hydroxybenzaldehyde the $\delta^{13}$C are similar (approx.: -16.5 ‰). Further studies of vanillin undertaken by Greule and co-workers (2010) in 2010 employed GC-C-IRMS for the authentication of vanillin in conjunction with an analysis for $\delta^2$H using the pyrolysis technique. Correlation plots of the isotopic bulk analysis data (synthetic - $\delta^{13}$C: -28.17 ‰ to -29.74 ‰; $\delta^2$H: 54.6 ‰ to 79.7 ‰; lignin - $\delta^{13}$C: -32.46 ‰; $\delta^2$H: -61.7 ‰; vanilla pods - $\delta^{13}$C: -14.58 ‰ to -18.52 ‰; $\delta^2$H: -47.4 ‰ to -58.5 ‰), and the isotopic values of the methoxyl group of vanillin for $\delta^{13}$C and $\delta^2$H were able to differentiate between vanillin produced by various sources such as guaiacol (synthetic; methoxyl group - $\delta^{13}$C: -29.73 ‰ to -52.24
‰; δ²H: -84.5 ‰ to -169.4 ‰) and lignin (semi-synthetic; methoxyl group - δ¹³C: -37.15 ‰; δ²H: -235.6 ‰), as opposed to vanillin produced naturally from tropical orchid vanilla pods (methoxyl group - δ¹³C: -7.08 ‰ to -24.08 ‰; δ²H: -149.0 ‰ to -181.6 ‰). Gruele and others (2010) conclude that the method could also be used to differentiate between the various varieties of vanilla (in line with Kaunzinger and others above), though this would require a much larger database of vanillin samples, and also that with this method adulterated vanillin samples could be detected.

However, not all studies on essential oils have been able to detect a difference between natural and synthetic aroma compounds. For the case of estragole and methyl eugenol, the differentiation between natural and synthetic products was not discernible due to the synthetic version being produced from natural compounds (Ruff and others 2002) and, therefore, this technique cannot be used for authentication purposes in this instance, although application of other complementary techniques may have assisted.

Aroma compounds in essential oils – adulteration

Another type of fraud commonly used for essential oils is blending the authentic high-value oil with another low-cost oil (or oils). Development of an isotope ratio authenticity range for the major compounds that are found in the essential oil is a reliable method to detect fraud (Greule and others 2010; Hrastar and others 2009; Sewenig and others 2003). For example, mandarin oil was shown to be adulterated by less expensive oils such as orange peel oil (Faulhaber and others 1997b), and cassia cinnamon oil is often used in the adulteration of Ceylon cinnamon oil (Sewenig and others 2003). A component typical of mandarin oil, methyl N-methylantranilate (MNM), is not found in any other citrus peel oil and for this reason can be
implemented in the detection of adulterants. More sophisticated adulterations have added synthetic MNM, which means that the detection method requires additional complementary methods to appropriately identify adulteration.

Such complementary methods can include HPLC, GC-FID, and enantioselective GC (Es-GC) and Schipilliti and others (2010) employed these and GC-C-IRMS to investigate 5 commercial mandarin essential oil samples. While all techniques provided useful information, GC-C-IRMS enabled the detection of synthetic and natural compounds that were added to 4 of the samples to enhance the overall aroma of the oils (Schipilliti and others 2010).

Bergamot (Dugo and others 2012; Mosandl and Juchelka 1997; Schipilliti and others 2011a), neroli (Bonaccorsi and others 2011), and lime oils (Bonaccorsi and others 2012) are further examples of citrus oils with which fraud by blending occurs. To assess these oils for authenticity several techniques have been employed such as MDGC, GC-FID, GC/MS, Es-GC, HPLC, and GC-C-IRMS. It has been found that GC-C-IRMS is the most sensitive to the slightest adulteration when compared to the other techniques, although it is recommended to use more than one technique for reliability, efficiency, comparison, sensitivity, and confirmation of the GC-C-IRMS results. For example, Schipilliti and others (2011a) used GC-FID and Es-GC in comparison with GC-C-IRMS and were able to determine that a number of the commercial bergamot oil samples analyzed were adulterated either due to the addition of adulterants or blending with a different oil into bergamot oil.

For lemon essential oils Schipilliti and others (2012) used a number of techniques, such as Es-GC, GC-FID, and spectrophotometry (CD), together with GC-C-IRMS to prove the viability and reliability of GC-C-IRMS for authenticity assessments. An isotopic fingerprint was developed using 30
genuine lemon essential oils and an i-IST for the analysis of commercial lemon essential oils and distilled oils. The authors showed that a sample from Brazil and a sample from Argentina had been adulterated as the values for certain targeted compounds were out of the authenticity range. The enantiomeric distributions of compounds within the samples were also outside of the normal range for those from genuine samples. The authors (Schipilliti and others 2012) concluded that the Brazilian sample was made up of a combination of sweet orange oil terpenes and distilled lemon oil, by combining results from the different techniques used.

The use of 3 isotopic ratios, $\delta^{13}C$, $\delta^2H$, and $\delta^{18}O$, together with enantioselective analysis has also been employed in the analysis of essential oils. For example, Jung and others (2005) analyzed lavender oils and key aroma compounds, linalool and linalyl acetate, and were able to determine that 2 commercial lavender oil samples did not originate from lavender. They concluded that “Owing to the variety of adulterations of lavender oils multielement/multicomponent IRMS and the consideration of $\Delta \delta$ values as well as enantioselective analysis are necessary for comprehensive authenticity assessment”. The comprehensive nature of the analyses required illustrates the degree of challenge in reliably detecting adulteration for some compounds and essential oils.

_Aroma compounds in essential oils – biochemical pathways_

Analysis of essential oils by GC-C-IRMS can be used to identify biochemical pathways within plants. Faber and others (1997) found that the composition of the essential oil is very different during the various stages of plant development in dill (*Anethum graveolens* L.). Of interest were the
biochemical pathways of monoterpenes (enantiomers of limonene, carvone, dill ether, α-/β-phellandrene) in the plant as their biological purpose is uncertain. Faber and others (1997) were able to postulate a pathway for the above-mentioned monoterpenes using the results gained by GC-C-IRMS and enantioselective analysis. By comparing δ¹³C in different plant parts during different stages of development, the authors were able to discover that different enzymes were responsible for the biosynthesis of limonene in dill herb to those in the buds, flowers and seeds. Monoterpenes generally have a specific aroma and are vital for the food and beverage industries to enhance flavors. Therefore, fundamental knowledge of their biosynthesis could be of benefit for developing new and more sophisticated adulteration detection methods.

**Edible fats and oils**

*Assessment of authenticity*

Edible oils such as olive oil and vegetable oils are often adulterated with cheaper oils (Angerosa and others 1997; Lees 1998; Mottram and others 2003) and IRMS on bulk oil (the ‘whole’ oil) was proposed as a possible means to detect this type of fraud over 30 years ago (Gaffney and others 1979). As well as bulk oil, various fractions of oil, such as aliphatic alcohols (Angerosa and others 1997), can be submitted to isotope ratio analysis to determine whether adulteration has occurred. The first use of GC-C-IRMS for the detection of fraudulent oils was published by Woodbury and others (1995) who looked at isotope ratios of individual fatty acids in maize oil (a C4 plant) and compared them to isotope ratios for fatty acids in
rapeseed oil (a C3 plant). This group went on to publish a comprehensive database of δ^{13}C values of the major fatty acids of more than 150 vegetable oils (Woodbury and others 1998a) and each particular oil was shown to have its own unique isotopic fingerprint. Arising from this work, the authors noted that variability in δ^{13}C values was related to the geographical origin of the oil, year of harvest, and the particular variety of oil (Woodbury and others 1998a). This suggests that both environmental and genetic factors contribute to the observed isotope ratios. One particular interest was the bimodal distribution of δ^{13}C values depending on whether the oils originated in the northern or southern hemispheres.

GC-C-IRMS has also been applied to the analysis of fatty acids in false flax oil (*Camelina sativa* oil) (Hrastar and others 2009), olive oil (Baum and others 2010; Royer and others 1999; Spangenberg and others 1998), vegetable oils (Kelly and others 1997; Mottram and others 2003; Spangenberg and Ogrinc 2001; Woodbury and others 1998b), and cooking oils (Liu and others 2012) for the purposes of authentication and determination of origin. A method using GC-C-IRMS in combination with bulk isotope ratio analysis has been developed by Richter and others (2010) to combat against possible frauds and adulterations of rapeseed oil. The bulk measurements determined the isotope ratios of C, H, and O giving details on geographical origin, whereas GC-C-IRMS measured the fatty acid components in the rapeseed oil. The results were that individual fatty acids can be used to differentiate between different species of C3 plants such as flax, poppy, and rapeseed, but this by itself would not be enough to determine fraud; bulk IRMS analysis would also be required.

Cocoa butter is a lipid-rich food ingredient that is prone to fraud due to its high value. Bulk IRMS and GC-C-IRMS were used to investigate cocoa butter of different origins, suppliers, and varieties and equivalents (Illexao 30-61, Illexao 30-71, Illexao 30-96, Choclin, Coberine, Chocosine-Illipé,
Chocosine-Shea, Shokao, Akomax, Akonord, and Ertina (Spangenberg and Dionisi 2001). The aim of this study was to determine if fatty acids (bulk fat, palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2)) could be differentiated by bulk and molecular isotopic ratios. Principal component analysis (PCA) showed that the addition of vegetable fats or cocoa butter equivalents of 15% were able to be detected, the exception being illipé fat, which was not possible to be detected in the cocoa butter. Spangenberg and Dionisi (2001) suggested that an improvement in precision of the method would be to use other qualitative and quantitative methods in combination with isotopic ratios.

Jung and others (2007) developed a method to detect glycerol and fatty alcohols of olive oil and other edible fats and oils by means of reductive ester cleavage, with the GC-C/P-IRMS for $^{13}$C and $^{18}$O. The correlation of $\delta^{13}$C and $\delta^{18}$O ratios for glycerol provided an edible fats and oils authenticity assessment and was able to prove that ratios from synthetic glycerol and glycerol from other sources were different from the glycerol derived from edible fats and oils. For fatty alcohols the analysis of $\delta^{18}$O was not possible with GC-P-IRMS as the concentration of oxygen in the fatty alcohols was not enough to be measured. Therefore, only the $\delta^{13}$C ratios for fatty alcohols was measured with GC-C-IRMS and were determined to be useful for the authenticity assessment of edible fats and oils.

**Beverages**

GC-C-IRMS has been implemented to characterize flavor and aroma compounds and to detect fraud and adulterations through the analysis of $\delta^{13}$C in a range of beverages such as teas (del Mar Caja and others 2009; Weinert and others 1999), spirits (Schumacher and others 1999), apple juice (Kelly
and carbonated drinks (Calderone and others 2007). Weinert and others (1999) have evaluated the carbon isotope ratios of the characteristic flavor compounds (\textit{trans}-2-hexenal, \textit{cis}-3-hexenol, \textit{trans/cis}-linalool oxides (furanoids), linalool, methyl salicylate, and geraniol) found in specific black teas (Ceylon, Assam, and Darjeeling) to differentiate between authentic teas and adulterated teas. Methyl salicylate is a major component of black teas and it is often added as an adulterant to enhance the overall flavor/aroma thereby increasing consumer sales (Abraham and others 1976; Weinert and others 1999). The authors (Weinert and others 1999) were able to produce isotopic fingerprints for each of the specific black teas studied and were able to prove that adulteration by methyl salicylate could be detected through the use of an i-IST. Although this method worked well, it was not perfect, with about 6\% of all Ceylon black teas investigated unable to be classified. Weinert and others (1999) stated that the use of another isotopic measurement (namely $\delta^{18}$O ratios by GC-P-IRMS) could help overcome this problem.

Alkylpyrazines, derived from roasting, are very important for the typical aroma of roasted coffee. Richling and others (2005) analyzed alkylpyrazines for $\delta^{15}$N and $\delta^{2}$H implementing GC-C/P-IRMS. Synthetic compounds and compounds claimed as “natural” were also analyzed for comparison to determine the origin of commercial samples as a check to deter possible adulterations. The results for some of the alkylpyrazines showed differentiation between synthetic and/or natural compounds and the alkylpyrazines from coffee. For example, 2-methylpyrazine synthetic references had a $\delta^{15}$N range of 0.3 \textperthousand{} to -1.2 \textperthousand{} and $\delta^{2}$H range of 15 \textperthousand{} to 40 \textperthousand{}, “natural” references had a $\delta^{15}$N range of -0.9 \textperthousand{} to -2.4 \textperthousand{} and $\delta^{2}$H range of -102 \textperthousand{} to -117 \textperthousand{}, whereas the coffee samples studied had a $\delta^{15}$N range of 2.5 \textperthousand{} to 2.8 \textperthousand{} and $\delta^{2}$H range of -13 \textperthousand{} to -77 \textperthousand{} clearly showing differentiation between the different references and the coffee samples. This study provided new isotopic data of N and H for alkylpyrazines and it has proven that isotopic
methods could be used for authenticity assessment of products containing coffee, though more data will be required before the method is used in commercial situations.

Schumacher and others (1999) studied the fusel alcohols 2-methylbutanol and 3-methylbutanol in alcoholic apple beverages. These alcohols are formed during fermentation from metabolism of isoleucine and leucine, respectively. The authors wanted to determine whether these alcohols had different $\delta^{13}C$ values depending on the source of carbohydrate used in fermentation (of C3 or C4 plants,) and whether there was a difference in $\delta^{13}C$ values between apple juice and processed alcoholic beverages. The $\delta^{13}C$ isotopic values for 2-methylbutanol in distillates from C3 plants (-17.0 ‰ to -26.1 ‰) were clearly separated from those of C4 plants (-7.7 ‰ to -13.4 ‰). Similarly, apple-derived alcoholic beverages had $\delta^{13}C$ isotopic values for 2-methylbutanol (-24.5 ‰ to -27.0 ‰, with one exception of -19.0 ‰) different from apple juices (-38.1 ‰ to -39.3 ‰). Further differentiation was evident for $\delta^{13}C$ isotopic values for 2-methylbutanol among C4 plants as maize starch and glucose syrup had $\delta^{13}C$ values of -7.7 ‰, which were much higher than those for cane sugar -13.4 ‰ to -13.9 ‰. Similar trends were observed for 3-methylbutanol. Additionally, $\delta^{13}C$ values were 4 - 5 ‰ lower than for 2-methylbutanol from the same product, typical for fermented products (Schumacher and others 1999).

In the EU, properties of CO$_2$ (origin, concentration, and purity) for addition to beverages are regulated and, hence, methodologies to ensure adherence to these regulations are required. The origin of CO$_2$ is either from industrial, botanical or geological sources, which generate a wide range of $\delta^{13}C$ values (Calderone and others 2007) that can be used for authentication purposes. Calderone and others (2007) have utilized GC-C-IRMS for the analysis of CO$_2$ in the headspace of sparkling and carbonated drinks and found that naturally carbonated waters had a $\delta^{13}C$ range of -4.5 to 1.05 ‰.
which matched the natural CO2 vent sources from which the products were derived. On the other hand, industrial carbonation of water produced more depleted carbon isotope values (-29.24 ‰ to -54.15 ‰). A study by Bencie and Vreca (2007) showed that bottled waters from different origins (bottling and processing procedures) could be differentiated based on their $^{13}$C isotopic ratios of dissolved inorganic carbon. The average $\delta^{13}$C value for sparkling water produced naturally was -3.3 ‰, whereas sparkling water produced artificially had a mean value $\delta^{13}$C of -36.5 ‰. The average $\delta^{13}$C ratio for still (-10.0 ‰) and flavoured (-11.0 ‰) waters were similar. Beers could be distinguished in light of their manufacturing, whereby those produced with C4 plant ingredients had a $\delta^{13}$C range for CO2 of -17.5 ‰ to -21.8 ‰ and double malt beers as well as a beer that was industrially carbonated had $\delta^{13}$C values of less than -30 ‰ (Calderone and others 2007). Similarly, sparkling wines could be differentiated based on CO2 stable isotope ratios due to the type of sugar (C3 or C4) added during the second fermentation. The CO2 $\delta^{13}$C range for sparkling wine with C3 sugar added was -19.23 ‰ to -21.12 ‰ and for the sparkling wine with C4 sugar added the result was -9.7 ‰ (Calderone and others 2007).

Still wines and distillates are also subjected to strict rules and regulations, especially in countries such as Italy and France and where certain alcoholic beverages (such as grappa, cognac, vodka, tequila, and so on) are of great economic importance. Protected Designation of Origin (PDO) assignments of these products are fundamental to protect and regulate wines and distillates from distinct regions. Fraudulent production of PDO beverages includes mislabelling/misdescription and adulteration such as addition of sugar (sugar cane and sugar beet) and addition of artificial/synthetic flavors and dilution with water (Dordevic and others 2013). Spitzke and others (2010) developed a GC-C-IRMS method that could be used to determine the isotopic ratios of wine ethanol and other alcohols, for example 2-methylpropan-1-ol, 2- and 3-methylbutan-1-ol; butan-2,3-diol, 2-phenyl-1-ethanol and glycerol. They were able to make correlations of the $\delta^{13}$C of the higher-alcohol compounds (such as 2-/3-methylbutan-1-ol) with $\delta^{13}$C wine
ethanol ($R^2 = 0.829$), for authenticity analysis of wines and distillates. Other groups (Calderone and others 2004a; Jung and others 2006) have also investigated wine ethanol and glycerol by GC-C-IRMS for alternative techniques to determine adulteration of wines.

Wines and distillates are generally evaluated by their perceived aroma by consumers, and this fact could lead producers to adulterate their products with added flavor compounds to increase profits. Previous studies (Bauer-Christoph and others 1997, 2003; Engel and others 2006; Winterova and others 2008) have measured the concentrations of flavor compounds found in distillates in combination with isotopic data of bulk ethanol to provide an authenticity profile of the distillate type (fruit spirits, wines, whiskeys, and so on), but the isotopic values of the flavor compounds were not investigated. A recent study undertaken by Schipilliti and others (2013a) employed a headspace solid phase microextraction (HS-SPME) coupled to a GC-C-IRMS to determine the authenticity of Italian liqueurs. They were able to measure the $\delta^{13}C$ of typical flavour compounds found in 3 types of Italian liqueurs, “limoncello”, “mandarinetto”, and “bergamino” and then apply an authenticity range using an i-IST. This technique was successful in determining adulterations such as the addition of essential oil fractions, blending with artificial flavors and the addition of other citrus oils.

**Miscellaneous applications of GC-C-IRMS**

Hattori and others (Hattori and others 2010, 2011) proposed 2 methods to analyze acetic acid in vinegar. One method analyzed the ferments of raw materials such as rice, tomatoes, and apple juice for C and H by HS-SPME-GC-IRMS (Hattori and others 2010). The method was able to differentiate the origins of the vinegars, especially between those derived from C3 and C4 plants. For example, a sample labelled as apple vinegar had $\delta^{13}C$ values
in the range of a C4 plant, yet apple is a C3 plant. The second method of Hattori and others (2011) analyzed the $\delta^{13}C$ of the methyl carbon and carboxyl carbon of acetic acid to determine whether these values could provide more information on the origin of samples. The results of this work showed that in all cases but one (determined to be not 100 % pure acetic acid derived from *Acetobacter*) the methyl C was more depleted than the carbonyl C and that the samples did show some discrimination of the isotopic differences ($\delta^{13}C_{\text{carboxyl}} - \delta^{13}C_{\text{methyl}}$) between origin, C3 (2.1 ‰ to 6.7 ‰), CAM (18.2 ‰) and C4 (11.6 ‰) plants. Hattori and others (2011) proposed that the data generated from this method were not sufficient for origin differentiation and that more samples from the different botanical origins were required to improve the database.

To this point in the review, the main isotope of interest has been $^{13}C$ with various applications also drawing on $^2H$ and $^{18}O$. There is however, growing utilization of $^{15}N$ in food authentication, especially with respect to organic produce. For example, organic fertilizers must be used and mineral or synthetic fertilizers are prohibited, which has led to investigations of N uptake by plants. Understanding the variation in $\delta^{15}N$ values in plants as a result of synthetic or organic fertilizer use requires knowledge of $\delta^{15}N$ values in soil, how they change as the plants assimilate nitrogen, and how metabolic processes within a plant subsequently affect $\delta^{15}N$ values; as well as isotopic ratios in the applied fertilizer. Mineral or synthetic fertilizers have $\delta^{15}N$ values around -6 to +6 ‰ as they originate from atmospheric $\delta^{15}N$ and the subsequent industrial processes induce slight, if any, fractionation (Bateman and Kelly 2007b). On the other hand, in the production of organic fertilizers such as manure and composts, there are more processes occurring that incur trophic shifts, and therefore isotopic fractionation is greater. Thus, organic fertilizers have much higher $\delta^{15}N$ values, from +1 ‰ to +37 ‰ (Bateman and Kelly 2007b) than mineral fertilizers.
Soil $\delta^{15}N$ values primarily reflect the added fertilizer, though other factors such as the type of soil, altitude, moisture, and processes like denitrification, mineralization, nitrogen assimilation, and leaching are also involved. The $\delta^{15}N$ values in the plants derive mainly from the uptake of nitrogen in the soil and can be influenced by external factors such as the timing of fertilizer application to the soil (Choi and others 2002), soil water availability (Choi and others 2003), nature of the fertilizer, such as liquid or pellet form (Evans and others 1996), and internal factors such as plant traits and metabolic processes (Senbayram and others 2008).

The main assimilation forms of inorganic nitrogen by plant roots are NO$_3^-$ and NH$_4^+$. Nitrate is transported to the chloroplasts, where it is reduced to NH$_4^+$, rapidly distributed to various organelles, and converted into organic forms by the glutamine synthetase reaction. According to Winkler (1984), plants assimilating nitrate to form NH$_4^+$ have an increase in $\delta^{15}N$ of about 10 ‰ therefore when the NH$_4^+$ is converted to proteins and amino acids, enrichments in $^{15}N$ (Winkler 1984) again occur. Isotopic differences for amino acid components of organic and conventional produce, as determined by GC-C-IRMS, could therefore be used to assess authenticity.

The metabolism of plant nitrogen was studied by Styring and others (2014) in barley, wheat, broad beans, and peas through an investigation of the $^{15}N/^{14}N$ ratios of amino acids. The results showed that different anabolic and catabolic processes in the plants cause isotopic fractionation and, therefore, the amino acids have different $\delta^{15}N$ values. The amino acids involved in the metabolism of nitrogen in barley and wheat had similar $\delta^{15}N$ values suggesting that the pathways were similar, whereas for the broad beans and pea seeds the $\delta^{15}N$ values were different, indicating different
pathways for development. It was shown by utilizing $\delta^{15}N$ values that amino acids follow known metabolic pathways. This information could be useful for more in-depth studies on N cycling within a plant and to determine the effect of external factors on plant metabolism.

Additional evidence that GC-C-IRMS could be implemented to study amino acids in plants comes from Bol and others (2002) who studied amino acid isotopic composition in relation to the available sources of N and uptake systems in temperate grasslands. Their results illustrated that the amino acids histidine and phenylalanine could be utilized to differentiate between plant species. Also, it was noted that the different isotopic N ratios were due to the diverse uptake mechanisms of N by the plants.

Thus, while $\delta^{15}N$ values of amino acids show promise for utilization in food authenticity and traceability studies, to date no such studies utilizing GC-C-IRMS have appeared (to the best of the authors’ knowledge). This represents a potentially fruitful area of research to complement the well-recognized applications of $\delta^{13}C$, $\delta^2H$, and $\delta^{18}O$ to detect fraudulent activity in the food industry.

**Conclusion**

Consumers around the world want to understand and be informed as to what they are eating, where it comes from, and how it was made. There is increasing information easily obtainable through electronic sources on healthy eating, types of produce, organic or conventional farming, and food processing protocols. Products that contain labels “organically grown” or are Protected Denomination of Origin, command premium prices and hence
there is a great need to be able to test foods and beverages to verify that all the contents are genuine (in terms of ingredients, origin, and so on) As demonstrated in this review, GC-C-IRMS is an analytical technique that has the potential to be applied across a wide range of foods, beverages, and ingredients to detect fraudulent activity. While the foundation of this technique can be traced to the very beginnings of mass spectrometry, modern-day applications require sophisticated, precisely calibrated instrumentation, and careful attention to detail in terms of sample preparation. Nevertheless, the tremendous versatility of GC-C-IRMS is evident from the numerous applications documented in this review and we anticipate that it will become a central technique in the detection of fraud either alone or in combination with other analytical methods.

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Author Contributions
van Leeuwen was responsible for sourcing, reading, analyzing and collating the literature as part of her PhD program. Prenzler, Ryan, and Camin were responsible for editing, synthesizing and structuring the review in their role as PhD supervisors for van Leeuwen.
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Table 1. Mean natural abundance of the stable isotopes of the bio-elements

<table>
<thead>
<tr>
<th>Element</th>
<th>Stable isotope</th>
<th>Mean natural Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hydrogen</strong></td>
<td>$^1$H</td>
<td>99.99</td>
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<tr>
<td></td>
<td>$^2$H (D)</td>
<td>0.01</td>
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<tr>
<td><strong>Carbon</strong></td>
<td>$^{12}$C</td>
<td>98.89</td>
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<tr>
<td></td>
<td>$^{13}$C</td>
<td>1.11</td>
</tr>
<tr>
<td><strong>Nitrogen</strong></td>
<td>$^{14}$N</td>
<td>99.63</td>
</tr>
<tr>
<td></td>
<td>$^{15}$N</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Oxygen</strong></td>
<td>$^{16}$O</td>
<td>99.76</td>
</tr>
<tr>
<td></td>
<td>$^{17}$O</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>$^{18}$O</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Sulfur</strong></td>
<td>$^{32}$S</td>
<td>95.00</td>
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<tr>
<td></td>
<td>$^{33}$S</td>
<td>0.76</td>
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<tr>
<td></td>
<td>$^{34}$S</td>
<td>4.22</td>
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<td></td>
<td>$^{36}$S</td>
<td>0.02</td>
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Table 2. Food and Beverage Analysis by GC-C-IRMS

<table>
<thead>
<tr>
<th>Food and Beverage</th>
<th>Purpose of study</th>
<th>Compounds</th>
<th>Outcome</th>
<th>Isotopic ratio</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Pear</td>
<td>Natural and synthetic aromas</td>
<td>1-Butanol, hexyl acetate, 1-hexanol, butyl acetate, methyl $E,Z$-2,4-decadienoate, ethyl $E,Z$-2,4-decadienoate, ethyl $E,E$-2,4-decadienoate</td>
<td>Naturally derived and synthetic compounds could be distinguished; authenticity range for various products (fruit, juice, brandy, baby food)</td>
<td>$\delta^{13}$C, $\delta^2$H</td>
<td>Kahle and others (2005)</td>
</tr>
<tr>
<td>Pineapple</td>
<td>Natural and synthetic aromas</td>
<td>Methyl hexanoate, ethyl hexanoate, methy 2-methylbutanoate, ethyl 2-methylbutanoate, 2,5-dimethyl-4-methoxy-3[2H]-furanone</td>
<td>GC-C/P-IRMS could distinguish natural and synthetic aromas</td>
<td>$\delta^{13}$C, $\delta^2$H</td>
<td>Preston and others (2003)</td>
</tr>
<tr>
<td>Fruit(s)</td>
<td>Aroma(s)</td>
<td>Compound</td>
<td>Analysis</td>
<td>Isotopes</td>
<td>Reference</td>
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<td>----------------------------------------------</td>
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<tr>
<td>Raspberry</td>
<td>Natural and synthetic aromas</td>
<td>$E$-α(β)-Ionone</td>
<td>MDGC-C/P-IRMS could distinguish the naturally derived compound from the synthetic one</td>
<td>$\delta^{13}$C, $\delta^2$H</td>
<td>Sewenig and others (2005)</td>
</tr>
<tr>
<td>Strawberry, raspberry, pineapple, passionfruit, plum, coconut</td>
<td>Natural and synthetic aromas</td>
<td>$\gamma$-Decalactone</td>
<td>Enantiomeric distribution and isotopic ratios in isolation were not capable of proving the genuineness of a flavour but when combined and with more isotopic data this could be achieved</td>
<td>$\delta^{13}$C, bulk</td>
<td>Nitz and others (1991)</td>
</tr>
<tr>
<td>Apricot, blueberry, guava, mango, passionfruit, peach, pineapple, plum, strawberry</td>
<td>Natural and synthetic aromas</td>
<td>$\gamma$-Decalactone</td>
<td>MDGC and HRGC-IRMS were used for analysis. Synthetic compounds, microbial compounds, stone fruits and strawberry could be differentiated based on the</td>
<td>$\delta^{13}$C, bulk</td>
<td>Bernreuther and others (1990)</td>
</tr>
<tr>
<td>Plant</td>
<td>Authentication Method</td>
<td>Compounds</td>
<td>Description</td>
<td>Isotopes/IRMS Analysis</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------</td>
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<tr>
<td>Cactus pear (Opuntia ficus indica)</td>
<td>Authenticity assessment</td>
<td>1-Hexanol, E-2-hexenol, E-2-nonenol, E,Z-2,6-nondienol</td>
<td>The isotopic results clearly showed that these compounds originated from CAM plants, which could be useful for flavor authenticity</td>
<td>$\delta^{13}C$</td>
<td>Weckerle and others (2001)</td>
</tr>
<tr>
<td>Blackberry</td>
<td>Authenticity assessment</td>
<td>Heptan-2-ol, trans-linalool oxide (furanoids)</td>
<td>Enantioselctive analysis and MDGC-C/P-IRMS in combination could be used for authenticity assessment. The jams, jelly, juices, and yogurts tested were not in the limit of quantitation for IRMS analysis due to insufficient quantities of the blackberry constituents</td>
<td>$\delta^{13}C$, $\delta^2H$, $\delta^{18}O$</td>
<td>Greule and others (2008)</td>
</tr>
<tr>
<td>Fruit</td>
<td>Study Title</td>
<td>Volatiles</td>
<td>Isotopic Ratios</td>
<td>References</td>
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<tr>
<td>Raspberry, <em>Litsea cubeba</em>, lemongrass</td>
<td>Authenticity assessment</td>
<td>α-Ionone, β-ionone, α-ionol</td>
<td>Synthetic compounds were differentiated from the natural compounds in raspberry. The compounds synthesised from C4 plants had a more enriched isotopic ratio</td>
<td>δ(^{13})C, δ(^2)H, bulk</td>
<td>del Mar Caja and others (2007)</td>
</tr>
<tr>
<td>Strawberry</td>
<td>Origin of aromas – from strawberry or other fruit</td>
<td>Methyl butanoate, ethyl butanoate, hex-(2E)-enal, methyl hexanoate, butyl butanoate, ethyl hexanoate, hexyl acetate, linalool, hexyl butanoate, octyl isovalerate, δ-decalactone and octyl hexanoate</td>
<td>Volatiles from different sources could be distinguished as natural, synthetic (nature-identical) or artificial strawberry</td>
<td>δ(^{13})C</td>
<td>Schipilliti and others (2011b)</td>
</tr>
<tr>
<td>Banana</td>
<td>Fruit origin and ripening stage</td>
<td>Pentan-2-one, isobutyl acetate, 2-methyl propan-1-ol, isoamyl acetate, isobutyl</td>
<td>GC, GC-MS, GC-C-IRMS used to determined that there are different aroma</td>
<td>δ(^{13})C</td>
<td>Salmon and others (1996)</td>
</tr>
<tr>
<td>Fruit</td>
<td>Natural, synthetic, and biotechnological aromas</td>
<td>Aromas</td>
<td>Method</td>
<td>Isotopes</td>
<td></td>
</tr>
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<td>---------------</td>
<td>------------------------------------------------</td>
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</tr>
<tr>
<td>Peach (Prunus persica var. persica), apricot (Prunus armeniaca), and nectarine (Prunus persica var. nectarina)</td>
<td>Natural, synthetic, and biotechnological aromas</td>
<td>γ-Decalactone and δ-decalactone</td>
<td>GC-C/P-IRMS enabled differentiation of fruit derived compounds from the synthetic and biotechnological derived compounds</td>
<td>δ13C, δ2H</td>
<td></td>
</tr>
<tr>
<td>Apple</td>
<td>To determine if processing can modify the isotopic ratios of apple juice aroma; to determine if processing can modify the isotopic ratios of apple juice aroma; to</td>
<td>E-2-Hexenal, 1-hexanol, E-2-hexenol</td>
<td>The C isotopic ratios for these aroma compounds remained constant though for H there were slight isotopic changes</td>
<td>δ13C, δ2H</td>
<td></td>
</tr>
</tbody>
</table>

Tamura and others (2005)
Elss and others (2006)
<table>
<thead>
<tr>
<th>Product</th>
<th>Method</th>
<th>Substance</th>
<th>Determination of Origin</th>
<th>δ13C, bulk</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acerola berries, camu-camu fruits (tropical fruits)</td>
<td>Determination of origin</td>
<td>Ascorbic acid</td>
<td>A combination of 13C SNIF-NMR, EA-IRMS, GC-C-IRMS and multivariate analysis was used to determine the origin of ascorbic acid, either natural or industrial</td>
<td>δ13C, bulk</td>
<td>Albertino and others (2009)</td>
</tr>
<tr>
<td>Strawberry</td>
<td>Method development</td>
<td>Butanoic acid, 2-methylbutanoic acid, hexanoic acid, γ-decalactone, γ-dodecalactone</td>
<td>A method was developed for strawberry volatiles without fractionation</td>
<td>δ13C</td>
<td>Schumacher and others (1995)</td>
</tr>
</tbody>
</table>

**Essential Oils**
<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Type</th>
<th>Chemical Components</th>
<th>Methodology</th>
<th>Stable Isotopes</th>
<th>Authors</th>
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</thead>
<tbody>
<tr>
<td>Fennel and anise oils</td>
<td>Natural and synthetic</td>
<td>trans-Anethole</td>
<td>Combination of GC-C- and GC-P-IRMS established an authenticity range for naturally derived trans-anethole</td>
<td>$\delta^{13}C$, $\delta^2H$</td>
<td>Bilke and others (2002)</td>
</tr>
<tr>
<td>Lemon essential oil, <em>Citrus limon</em> (L.) Burm.</td>
<td>Natural and synthetic</td>
<td>$\alpha$-Pinene, $\beta$-pinene, limonene, $\alpha$-terpineol, neral, geranial, neryl acetate, geranyl acetate, $\beta$-caryophyllene, <em>trans</em>-*$\alpha$-bergamotene, $\beta$-bisabolene, norbornanol, campherenol, $\alpha$-bisabolol</td>
<td>GC-C-IRMS together with Es-GC, GC-FID and CD can be used in combination for the control of authenticity</td>
<td>$\delta^{13}C$</td>
<td>Schipilliti and others (2012)</td>
</tr>
<tr>
<td>Mandarin essential oil (Citrus reticulata Blanco)</td>
<td>Natural and synthetic, blending of citrus</td>
<td>Methyl N-methylanthranilate, methyl anthranilate</td>
<td>GC-C-IRMS established that essential oils from Italy have</td>
<td>$\delta^{13}C$, $\delta^{15}N$</td>
<td>Faulhaber and others (1997a)</td>
</tr>
<tr>
<td>Products, Origin Assignment</td>
<td>Different Isotopic Ratios To</td>
<td>Differentiation Of Synthetic Compounds From Natural Was Possible</td>
<td>δ13C</td>
<td>Braunsdorf And Others (1992)</td>
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<tr>
<td>Orange Oil</td>
<td>Natural And Synthetic</td>
<td>Octanal, Nonanal, Decanal, Dodecanal, Neral, Geranial</td>
<td>Lemon Oils Could Be Authenticated Using GC-C-IRMS With The Use Of An Internal Standard Against Neral And Geranial</td>
<td>δ13C</td>
<td>Braunsdorf And Others (1993b)</td>
</tr>
<tr>
<td>Melissa, Lemon (Citrus limon), Lemongrass, Citronella, Litsea cubeba, Lippia citriodora, Lemon Myrtle (Backhousia)</td>
<td>Natural And Synthetic</td>
<td>Citral (Geranial + Neral), Citronellal</td>
<td>Combination Of GC-FID For Enantioselective Analysis And GC-C/P-IRMS Were Essential For The Determination Of The Adulteration Of Essential Oils With Cheaper Oils Or Synthetic</td>
<td>δ13C, δ2H</td>
<td>Nhu-Trang And Others (2006)</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Description</td>
<td>Prerequisites</td>
<td>Notes</td>
<td></td>
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<tr>
<td><em>citriodora</em>, and lemon gum (<em>Eucalyptus citriodora</em>)</td>
<td>Natural and synthetic</td>
<td>Citral (geranial and neral), citronellal, β-caryophyllene, germacrene D, caryophyllene oxide</td>
<td>Not all differences between natural and synthetic compounds established, but 4 of the lemon balms were shown to be citronella oils</td>
<td></td>
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</tr>
<tr>
<td>Lemon balm (<em>Melissa officinalis</em> L.)</td>
<td>Natural and synthetic</td>
<td></td>
<td>δ¹³C, bulk</td>
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<tr>
<td>Coriander (<em>Coriandrum sativum</em> L.)</td>
<td>Natural and synthetic</td>
<td>Limonene, γ-terpinene, p-cymene, linalool, geraniol, myrcene, geranyl acetate, β-pinene, camphene, terpinolene, sabinene</td>
<td>An isotopic fingerprint was determined with use of an i-IST. Also enantio-GC-IRMS was used for the isomers of linalool as an additional tool that could be used for authenticity assessment</td>
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</table>

Wagner and others (2008) 
Frank and others (1995)
<table>
<thead>
<tr>
<th>Source</th>
<th>Type</th>
<th>Compound</th>
<th>Methodology</th>
<th>Isotopic Data</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamon oil, <em>C. ceylanicum</em> (ceylon), <em>C. cassia</em> (cassia) and <em>C. burmanii</em> (cassia vera)</td>
<td>Natural and synthetic</td>
<td>Cinnamaldehyde</td>
<td>The combination of GC-C/P-IRMS enabled the establishment of an authenticity technique to differentiate cinnamaldehyde of different origin</td>
<td>$\delta^{13}C$, $\delta^2H$</td>
<td>Sewenig and others (2003)</td>
</tr>
<tr>
<td>Basil extracts, <em>(E)</em>-methyl cinnamate (various sources), commercial strawberry and blueberry aromas</td>
<td>Natural and synthetic</td>
<td><em>(E)</em>-Methyl cinnamate, Methanol and cinnamic acid</td>
<td>The differences between the isotopic data were great enough to allow for differentiation between synthetic and natural alcohol (methanol)</td>
<td>$\delta^{13}C$, $\delta^2H$</td>
<td>Fink and others (2004)</td>
</tr>
<tr>
<td>Estragole; methyl eugenol; tarragon oil; sweet basil oil; pimento oil; laurel</td>
<td>Natural and synthetic</td>
<td>Estragole, methyl eugenol</td>
<td>The methods developed were not sufficient enough to determine isotopic differences between natural and synthetic samples due to the synthetic</td>
<td>$\delta^{13}C$, $\delta^2H$, $\delta^{18}O$</td>
<td>Ruff and others (2002)</td>
</tr>
<tr>
<td>Substance</td>
<td>Type of Sample</td>
<td>Constituents</td>
<td>Techniques Description</td>
<td>Isotopes</td>
<td>Reference</td>
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</tr>
<tr>
<td>leaf oil; <em>Tagetes lucida</em> oil</td>
<td>Natural and synthetic</td>
<td>Linalool, linalyl acetate</td>
<td>A combination of techniques was necessary to determine authenticity of lavender oils; multielement/multicomponent IRMS and (GC) enantioselective analysis</td>
<td>δ¹³C, δ²H, δ¹⁸O</td>
<td>Jung and others (2005)</td>
</tr>
<tr>
<td>Lavender oil</td>
<td>Natural and synthetic</td>
<td>Linalool, linalyl acetate</td>
<td>A combination of techniques was necessary to determine authenticity of lavender oils; multielement/multicomponent IRMS and (GC) enantioselective analysis</td>
<td>δ¹³C, δ¹⁸O</td>
<td>Kaunzinger and others (1997)</td>
</tr>
<tr>
<td><em>Vanilla planifolia, Vanilla tahitensis</em></td>
<td>Natural and synthetic</td>
<td>Vanillin, 4-hydroxybenzyl alcohol, vanillic acid, 4-hydroxybenzaldehyde, anisic alcohol, anisic acid, and 4-hydroxybenzoic acid</td>
<td>GC-C IRMS profiles could distinguish different vanilla species and the origin of synthetic vanillin</td>
<td>δ¹³C</td>
<td>Hener and others (1998)</td>
</tr>
<tr>
<td>Vanillin</td>
<td>Natural and synthetic, origin determination</td>
<td>Vanillin</td>
<td>Simultaneous on-line analysis of vanillin using GC-C/P-IRMS. Synthetic and biotechnologically derived</td>
<td>δ¹³C, δ¹⁸O</td>
<td>Hener and others (1998)</td>
</tr>
</tbody>
</table>
vanillin and natural vanillin could be differentiated.

<table>
<thead>
<tr>
<th></th>
<th>Natural and synthetic aromas</th>
<th>Vanillin</th>
<th>There was no isotopic difference between the vanillin in the ice cream and the vanillin flavoring used</th>
<th>δ^{13}C</th>
<th>Fayet and others (1995)</th>
</tr>
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<tbody>
<tr>
<td>Vanilla ice cream</td>
<td>Natural and synthetic</td>
<td>Vanillin</td>
<td>A reliable and rapid method for authentication of vanillin</td>
<td>δ^{13}C, δ^2H</td>
<td>Greule and others (2010)</td>
</tr>
<tr>
<td>Vanilla, V. Planifolia, V.tahitensis, V. pompona</td>
<td>Natural and synthetic</td>
<td>Vanillin, cis-3-hexanol</td>
<td>The method developed allowed for small quantities to be measured without purification and therefore no fractionation. The method was able to determine natural and synthetic aromas</td>
<td>δ^{13}C</td>
<td>Breas and others (1994)</td>
</tr>
<tr>
<td>Essential Oil</td>
<td>Method</td>
<td>Chemicals</td>
<td>Description</td>
<td>δ¹³C</td>
<td>Reference</td>
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<tr>
<td>Mandarin essential oil (Citrus reticulata Blanco)</td>
<td>Develop an authenticity profile</td>
<td>α-Sinensal, limonene, γ-terpinene, α-thujene, β-pinene/sabinene, myrcene, terpinolene, MNM, linalool and octanal</td>
<td>An authenticity profile was developed which could be used to evaluate the authenticity of commercial mandarin oils</td>
<td>δ¹³C</td>
<td>Faulhaber and others (1997b)</td>
</tr>
<tr>
<td>Mandarin essential oil</td>
<td>Assessment of authenticity</td>
<td>α-Thujene, α-pinene, β-pinene, myrcene, limonene, γ-terpinene, terpinolene, terpinen-4-ol, α-terpineol, decanal, thymol, MNM, α-farnesene and α-sinensal</td>
<td>GC-C-IRMS together with Es-GC, GC-FID and HPLC are a useful combination for the control of adulteration; GC-C-IRMS especially for the detection of the addition of thymol, MNM and α-sinensal to commercial products</td>
<td>δ¹³C</td>
<td>Schipilliti and others (2010)</td>
</tr>
<tr>
<td>Neroli oil, petitgrain oil, bergamot oil</td>
<td>Assessment of authenticity</td>
<td>α-Pinene, β-pinene/sabinene, limonene, myrcene, linalool, linalyl</td>
<td>The combination of data from enantio-MDGC and GC-C-IRMS is useful in the</td>
<td>δ¹³C</td>
<td>Mosandl and others (1997)</td>
</tr>
<tr>
<td>Oil Type</td>
<td>Analysis Details</td>
<td>Detected Components</td>
<td>Isotopic Ratios</td>
<td>Reference</td>
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</tr>
<tr>
<td>Lemon petitgrain oil, mandarin petitgrain oil</td>
<td>Composition, authenticity assessment</td>
<td>acetate, neryl acetate, caryophyllene, γ-terpinene</td>
<td>δ¹³C</td>
<td>Schipilliti and others (2013b)</td>
<td></td>
</tr>
<tr>
<td>Bergamot oil, <em>Citrus bergamia</em> Risso</td>
<td>Authenticity assessment</td>
<td>α-Thujene, α-pinene, β-pinene, myrcene, α-terpineol, neral, geranial, neryl acetate, geranyl acetate, β-caryophyllene, MNM</td>
<td>δ¹³C</td>
<td>Schipilliti and others (2011a)</td>
<td></td>
</tr>
<tr>
<td>Bergamot oil</td>
<td>Authenticity assessment and characterization of cold-pressed and processed oils</td>
<td>α-Thujene, α-pinene, β-pinene, myrcene, limonene, γ-terpinene, linalool, linalyl</td>
<td>δ¹³C</td>
<td>Dugo and others (2012)</td>
<td></td>
</tr>
</tbody>
</table>
| Egyptian Neroli oil,  
(Citrus aurantium L.) | Authenticity assessment,  
geographic origin | was done using a variety of  
techniques, GC-FID, GC-MS,  
GC-C-IRMS, Es-GC, MDGC,  
HPLC and HPLC-MS-ion trap-TOF |  
|----------------------|------------------|-------------------------------------------------|---|
|                      | β-Pinene, myrcene,  
limonene, linalol, terpinen-4-ol, α-terpineol, nerol,  
neryl acetate, geranyl acetate, (E)-caryophyllene, (E)-nerolidol, (2E,6E)-farnesol | Along with data obtained from GC-FID, GC-MS, Es-GC and HPLC, GC-IRMS  
was used to develop an isotopic fingerprint with small variation and therefore could be used to determine authenticity and geographic origin |  
<p>|                      |                  | δ¹³C | Bonaccorsi and others (2011) |</p>
<table>
<thead>
<tr>
<th>Peppermint oil, (<em>M. piperita</em>)</th>
<th>Authenticity assessment</th>
<th>Isomenthone, methyl acetate, menthone, menthol, 1,8-Cineole</th>
<th>i-IST enabled an isotopic fingerprint of authentic peppermint oil. Addition of methyl acetate was able to be detected in adulterated samples. The use of a chiral column enabled isotopic ratio determination of the isomers of methyl acetate and also enabled determination of the amount of true methyl acetate in the adulterated sample</th>
<th>δ¹³C</th>
<th>Faber and others (1995)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lime essential oils, (<em>C. aurantifolia Swingle and C. latifolia Tanaka</em>)</td>
<td>Authenticity assessment</td>
<td>α-Pinene, β-pinene, limonene, α-terpineol, neral, geranial, β-</td>
<td>MD-GC and GC-C-IRMS provided a more reliable approach to detect adulteration. Use of an i-IST</td>
<td>δ¹³C</td>
<td>Bonaccorsi and others (2012)</td>
</tr>
<tr>
<td></td>
<td>Biochemical pathways in monoterpen synthesis</td>
<td>Limonene, carvone, dill ether, α-β-phellandrene</td>
<td>Pathways for monoterpenes were identified via GC-C-IRMS</td>
<td>δ\textsuperscript{13}C</td>
<td>Faber and others (1997)</td>
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<tr>
<td>Dill, <em>Anethum graveolens</em> L.</td>
<td>Biochemical pathways in monoterpenene synthesis</td>
<td>Logonene, carvone, dill ether, α-β-phellandrene</td>
<td>Pathways for monoterpenes were identified via GC-C-IRMS</td>
<td>δ\textsuperscript{13}C</td>
<td>Faber and others (1997)</td>
</tr>
<tr>
<td>Orange oil</td>
<td>Effect of technological processing</td>
<td>Octanal, nonanal, decanal, dodecanal</td>
<td>Isotopic ratio of C was not affected/influenced by sample clean-up during technological processing</td>
<td>δ\textsuperscript{13}C</td>
<td>Braunsdorf and others (1993a)</td>
</tr>
</tbody>
</table>

**Edible Oils and Fats**

<p>| Olive oils, Food fats | Authenticity assessment, origin assessment | Glycerol, fatty alcohols | The developed method serves as a tool for assessment of origin for food fats and oils., An authenticity range was developed for samples of C3 origin and glycerol from fats | δ\textsuperscript{13}C, δ\textsuperscript{18}O | Jung and others (2007) |</p>
<table>
<thead>
<tr>
<th>Oil Type</th>
<th>Authenticity Assessment</th>
<th>Fatty Acid Markers</th>
<th>Methodology</th>
<th>Isotopic Data</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive oil</td>
<td>Authenticity assessment</td>
<td>Palmitic acid, oleic acid</td>
<td>Bulk and fatty acids can be used to identify oil source. Blending may also be detected in combination with GC-MS data</td>
<td>$\delta^{13}$C, bulk</td>
<td>Spangenberg and others (1998)</td>
</tr>
<tr>
<td>Maize oil</td>
<td>Authenticity assessment</td>
<td>Palmitic acid, oleic acid, linoleic acid, sterols, tocopherols</td>
<td>The combination of isotopic ratios for fatty acids and minor components (sterols and tocopherols) allowed adulteration of maize oil to be detected at 5% (mass/mass)</td>
<td>$\delta^{13}$C</td>
<td>Mottram and others (2003)</td>
</tr>
<tr>
<td>Maize oil, rapeseed oil, groundnut oils</td>
<td>Authenticity assessment</td>
<td>Palmitic acid, oleic acid, linoleic acid</td>
<td>Detection of adulterant of above 5% in maize oil was possible</td>
<td>$\delta^{13}$C</td>
<td>Woodbury and others (1995)</td>
</tr>
<tr>
<td>Vegetable Oils</td>
<td>Authenticity Assessment</td>
<td>Fatty Acids</td>
<td>Notes</td>
<td>Stable Isotopes</td>
<td>Reference</td>
</tr>
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</tr>
<tr>
<td>Maize oil and other vegetable oils</td>
<td>Authenticity assessment</td>
<td>Palmitic acid, oleic acid, linoleic acid</td>
<td>The data generated in this study can help to identify adulterations to maize oil using the major fatty acids found in this oil</td>
<td>$\delta^{13}$C</td>
<td>Woodbury and others (1998a)</td>
</tr>
<tr>
<td>Groundnut oil, rapeseed oil, palm oil, sunflower oil</td>
<td>Authenticity assessment</td>
<td>Palmitic acid, stearic acid, oleic acid, linoleic acid</td>
<td>Sole use of fatty acids to determine adulterations in single seed oils is not possible but could be used in conjunction with other techniques</td>
<td>$\delta^{13}$C</td>
<td>Kelly and others (1997)</td>
</tr>
<tr>
<td>Olive oil, pumpkin seed oil, sunflower oil, soybean oil, sesame oil, maize oil, and rapeseed oil</td>
<td>Authenticity assessment</td>
<td>Palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid</td>
<td>Vegetable oils can be classified using the isotopic ratios of the bulk oil, the fatty acids and also the composition of the fatty acids</td>
<td>$\delta^{13}$C, bulk</td>
<td>Spangenberg and Ogrinc (2001)</td>
</tr>
<tr>
<td>Cooking oils, commercial vegetation oils, animal oils, illegal swill-cooked oils</td>
<td>Authenticity assessment</td>
<td>Myristic acid, palmitic acid, stearic acid</td>
<td>Isotopic ratios can determine the origin of the oil, though the method is not 100% reliable as there is some overlap of the isotopic ranges</td>
<td>$\delta^{13}C$, bulk</td>
<td>Liu and others (2012)</td>
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<tr>
<td>Cocoa butter, Cocoa butter counterparts</td>
<td>Authenticity assessment</td>
<td>Bulk fat, stearic acid, palmitic acid, oleic acid</td>
<td>Cocoa butter and its counterparts can be differentiated based on their isotopic ratios (bulk and fatty acids) and blending of more than 15% of cocoa butter counterparts to cocoa butter is able to be detected</td>
<td>$\delta^{13}C$, bulk</td>
<td>Spangenberg and others (2001)</td>
</tr>
<tr>
<td>Olive oil, hazelnut oil, sunflower oil, soybean oil, maize oil</td>
<td>Authenticity assessment</td>
<td>Palmitic acid, oleic acid, linoleic acid</td>
<td>Information on geographical, botanical and temporal characteristics of the oils can</td>
<td>$\delta^{13}C$</td>
<td>Royer and others (1999)</td>
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<tr>
<td>Olive oil</td>
<td>Authenticity assessment</td>
<td>Phytol, geranyl geraniol, citrostadienol, docosanol, tetracosanol, hexacosanol</td>
<td>The analysis is able to detect a 3 % adulteration of olive oil with pomace oil based on the isotopic ratio of the aliphatic alcohols</td>
<td>$\delta^{13}C$</td>
<td></td>
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<td>Angerosa and others (1997)</td>
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<tr>
<td>Rapeseed oil</td>
<td>Adulteration with refined or less expensive oils, geographical origin</td>
<td>Palmitic acid, oleic acid, $\alpha$-linoleic acid, linoleic acid</td>
<td>The fatty acid carbon ratios were different for the rape, flax and poppy oils, and differences in ratios were also observed within each species</td>
<td>$\delta^{13}C$, bulk</td>
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<td>Richter and others (2010)</td>
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<tr>
<td>Camelina sativa Oil</td>
<td>Adulteration with refined or less expensive oils, geographical origin</td>
<td>Fatty acids; C_{16:0}, C_{18:0}, C_{18:1n9}, C_{18:1n7}, C_{18:2n6}, C_{18:3n3}, C_{20:0}, C_{20:1n9}, C_{20:3n3}, C_{22:1n9}</td>
<td>PCA was able to separate the oil samples from different continents; the correlation between $\delta^{13}C_{18:2n6}$ and</td>
<td>$\delta^{13}C$, bulk</td>
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<td>Hrastar and others (2009)</td>
<td></td>
</tr>
<tr>
<td>Olive oil</td>
<td>Geographical origin</td>
<td>Methyl palmitoleate, methyl palmitate, methyl oleate</td>
<td>Use of 3 FAME peaks enabled greater differentiation between samples of different geographic origin compared to using the isotopic ratios of the bulk oils</td>
<td>$\delta^{13}C$</td>
<td>Baum and others (2010)</td>
</tr>
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</tbody>
</table>

**Beverages**

<p>| Coffee beans (Coffea arabica L. and Coffea canephora var. robusta) | Natural and synthetic | Alkylpyrazines such as 2-ethyl-3-methylpyrazine, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine and 2,3,5-trimethylpyrazine | GC-C/P-IRMS. For some of the alkylpyrazines the isotopic values of the natural compounds were differentiated from the synthetic ones | $\delta^{15}N$, $\delta^2H$ | Richling and others (2005) |</p>
<table>
<thead>
<tr>
<th>Product</th>
<th>Component Type</th>
<th>Component</th>
<th>Description</th>
<th>Isotopes</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Black and green tea, Rooibos tea</td>
<td>Natural and synthetic</td>
<td>(±)-Dihydroactinidiolide</td>
<td>The synthetic and natural educts reflected their origin in their isotopic ratios. The Rooibos tea was shown to be slightly more enriched than the black and green teas allowing for differentiation</td>
<td>δ(^{13})C, δ(^2)H</td>
<td>del Mar Caja and others (2009)</td>
</tr>
<tr>
<td>Wine</td>
<td>Natural and synthetic</td>
<td>Ethanol, 2-methylpropan-1-ol, 2- and 3-methylbutan-1-ol, butan-2,3-diol and 2-phenyl-1-ethanol</td>
<td>The developed method could be used to determine the addition of exogenous alcohols</td>
<td>δ(^{13})C</td>
<td>Spitzke and Fauhl-Hassek (2010)</td>
</tr>
<tr>
<td>Apple juice, sugar syrups</td>
<td>Authenticity assessment</td>
<td>Hexamethylenetetramine</td>
<td>The combination of δ(^{13})C and δ(^2)H data was proven to be reliable in detecting the addition of commercial beet and cane sugar syrups to the apple juice</td>
<td>δ(^{13})C, δ(^2)H</td>
<td>Kelly and others (2003)</td>
</tr>
<tr>
<td>Wine</td>
<td>Authenticity assessment</td>
<td>Glycerol</td>
<td>GC-C/P-IRMS was able to distinguish wine grape glycerol from synthetic glycerol and glycerol produced from cane sugar. Also depending on amount and origin, addition of foreign glycerol detection is possible. Origin assessment is possible too.</td>
<td>$\delta^{13}C$, $\delta^{18}O$</td>
<td>Jung and others (2006)</td>
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<tr>
<td>Citrus liquers; limoncello, bargamino, mandarinetto</td>
<td>Authenticity assessment</td>
<td>$\alpha$-Pinene, $\beta$-pinene, myrcene, limonene, $\gamma$-terpinene, linalool, terpinene-4-ol, $\alpha$-terpineol, decanal, neral, geranial, linalyl acetate, neryl acetate, MNM, $\beta$-caryophyllene,</td>
<td>Isotopic ratios enantioselective GC was able to prove genuineness of the citrus liquers and could be used to prove adulteration</td>
<td>$\delta^{13}C$</td>
<td>Schipilliti and others (2013a)</td>
</tr>
<tr>
<td>Category</td>
<td>Objective</td>
<td>Constituents</td>
<td>Bottled waters from various origins, bottling and processing procedures could be differentiated</td>
<td>δ$^{13}$C</td>
<td>Authors</td>
</tr>
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<td>--------------------------------</td>
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<td>------------------------------------------------------------------------------</td>
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<tr>
<td>Bottled waters; sparkling, still, flavored</td>
<td>Authenticity assessment, origin determination</td>
<td>Dissolved inorganic carbon (dissolved CO$_2$, carbonic acid, bicarbonate, carbonate)</td>
<td></td>
<td>δ$^{13}$C</td>
<td>Brencic and Vreca (2007)</td>
</tr>
<tr>
<td>Black Ceylon, Assam, and Darjeeling teas</td>
<td>Adulteration detection implementing isotopic fingerprinting</td>
<td>Linalool, trans-2-hexenal, cis-3-hexenol, cis-linalool oxide (fur.), trans-linalool oxide (fur.), methyl salicylate, geraniol</td>
<td>Adulterations of teas with methyl salicylate was detected by use of an internal standard and isotopic fingerprinting</td>
<td>δ$^{13}$C</td>
<td>Weinert and others (1999)</td>
</tr>
<tr>
<td>Wine</td>
<td>Endogenous and exogenous glycerol and characterization</td>
<td>Glycerol, ethanol</td>
<td>GC-C-IRMS enabled correlations between ethanol and glycerol but the parameters may not be enough to determine</td>
<td>δ$^{13}$C, bulk</td>
<td>Calderone and others (2004b)</td>
</tr>
<tr>
<td>Product Type</td>
<td>Origin of CO₂, authenticity assessment</td>
<td>CO₂, ethanol</td>
<td>Discrimination between natural C₃- and C₄-derived CO₂ and technological CO₂ was possible, but may not be enough for authenticity assessment</td>
<td>δ¹³C, δ²H</td>
<td>Authors and Year</td>
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<tr>
<td>Sparkling wine, beer, carbonated water, carbonated drinks</td>
<td>Origin of CO₂, authenticity assessment</td>
<td>CO₂, ethanol</td>
<td>Discrimination between natural C₃- and C₄-derived CO₂ and technological CO₂ was possible, but may not be enough for authenticity assessment</td>
<td>δ¹³C, δ²H</td>
<td>Caledrone and others (2007)</td>
</tr>
<tr>
<td>Distillates from fermented carbohydrates, distillates of fermented apple and grape juice, apple products</td>
<td>Biosynthetic pathways</td>
<td>2-Methylbutanol, 3-methylbutanol</td>
<td>Determined that C₃ and C₄ products were able to be differentiated</td>
<td>δ¹³C</td>
<td>Schumacher and others (1999)</td>
</tr>
<tr>
<td>Vinegars</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Notes:**
- CO₂ refers to carbon dioxide.
- δ¹³C and δ²H are isotopic ratios used for authenticity assessment.
- Caledrone and others (2007) refers to the reference source for the first row.
- Schumacher and others (1999) refers to the reference source for the second row.
<table>
<thead>
<tr>
<th>Japanese vinegars</th>
<th>Origin determination, control of quality</th>
<th>Acetic acid</th>
<th>Intramolecular carbon isotope distribution could be used to determine origin of acetic acid in food.</th>
<th>δ&lt;sup&gt;13&lt;/sup&gt;C</th>
<th>Hattori and others (2011)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice vinegar, apple vinegar, grape vinegar, tomato vinegar, pineapple vinegar, wheat vinegar, lychee vinegar</td>
<td>Botanical origin</td>
<td>Acetic acid</td>
<td>HS-SPME combined with GC-high temperature conversion-C-IRMS enabled the acetic acid to be discriminated by botanical origin</td>
<td>δ&lt;sup&gt;13&lt;/sup&gt;C, δ&lt;sup&gt;2&lt;/sup&gt;H</td>
<td>Hattori and others (2010)</td>
</tr>
</tbody>
</table>
Figure 1. Chromatographic traces generated by GC-C-IRMS. a) Time shift due to isotopic effect on retention time of CO$_2$. b) The resulting S-shaped peak due to the 45/44 ratio (Meier-Augenstein 1999).
Figure 2. Carbon isotope ratio natural abundance ranges (ratio ranges are based on various literature data presented in this review).
Figure 3. Nitrogen isotope ratio natural abundance ranges (ratio ranges are based on various literature data presented in this review).
Figure 4. Precolumn (A, FID) and main column (B, SIM detection) chromatogram of a raspberry extract (Sewenig and others 2005).