ISOTOPE METHODS FOR THE CONTROL OF FOOD PRODUCTS AND BEVERAGES

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Abstract. The measurement of the stable isotope contents provides useful information for the detection of many frauds in food products. Nuclear magnetic resonance (NMR) and isotopic ratio mass spectrometry (IRMS) are the two main analytical techniques used for the determination of stable isotope contents in food products. These analytical techniques have been considerably improved in the last years offering wider possibilities of applications for food analysis. A review of the applications for the control of food products and beverages is presented. The need for new reference materials is discussed.

1. INTRODUCTION

For several decades the measurement of the natural abundance of stable isotopes has been mainly used in geochemistry and environmental research. Recently, isotope techniques have seen a growing interest in many other fields of research. Due to the improvement of the techniques numerous applications have been published in biomedical science, ecology, pharmacy and also within the field of consumer protection for detection of frauds in food and beverages. The measurement of the various isotope ratios $^{2}H/^{1}H$ (D/H), $^{18}O/^{16}O$, $^{13}C/^{12}C$, $^{15}N/^{14}N$ in different fractions of a product often provides information about the origin of starting materials. Isotope methods have therefore shown to be a major tool for checking the compliance of food products with national and international regulations. The technological progress of the last years has led to an increased use of hyphenated techniques for measuring isotope ratios using isotope ratio mass spectrometry (IRMS) coupled to a continuous flow elemental analyser (EA-IRMS), a pyrolysis unit (Py-IRMS) or a gas chromatograph (GC-IRMS). Nuclear magnetic resonance of deuterium (SNIF-NMR) has also demonstrated to be useful for site-specific measurement of intramolecular $^{2}H/^{1}H$ ratios of some organic molecules. These applications and techniques require establishment of new international standards to ensure comparability of isotope analyses of food products at an international level. This paper does not try to exhaustively review the application of isotope techniques in food analysis. It aims at presenting some basic aspects of isotope fractionation in plants and introduce analytical techniques used for the determination of isotope ratios as a tool for authenticity control of food products. The various possibilities offered by isotope techniques for food analysis are illustrated by selected applications for some of the major products of interest for consumers. Furthermore, implications for choosing new reference are also discussed.

2. STABLE ISOTOPES AND ISOTOPE FRACTIONATION

In nature, all of the major organic bio-elements (C, H, N, and O) are mixtures of two or more stable isotopes. The mean isotopic abundances observed for C, H, O and N are presented in Table I. It has been observed that the isotope ratios of a given molecule vary depending on its origin. This variability is linked to the isotope abundance of the starting pools and to the isotope fractionation associated with the various physical processes, chemical reactions and/or biochemical pathways involved during the formation of the molecule. In the water cycle, a well-known isotope fractionation takes place during the evaporation of the water from the oceans where depletion in heavy isotopes is observed in the vapour with respect to that of the liquid state. A similar isotope fractionation occurs in the transpiration of water from plants. The isotope ratios observed in plant water are positive relative to those of the corresponding ground water. Plants can be classified in three categories according to their photosynthetic pathways [1–3]. Plants belonging to the first category fix the atmospheric CO_2 by carboxylation of ribulose 1,5-diphosphate leading to two molecules of phosphoglycerate (chain of three carbon atoms, hence the name C_3 plants). This RuBisCo reaction is accompanied by a strong ^{13}C isotope effect causing a large depletion in the carbon-13 content of the plant (carbohydrate $\delta^{13}C$

TABLE I. ISOTOPE RATIOS OF STANDARDS AND TECHNIQUES USED FOR ISOTOPE DETERMINATION IN FOOD PRODUCTS

	Hydrogen	Carbon	Oxygen	Nitrogen
Isotope Ratio	$^{2}H/^{1}H$	$^{13}C/^{12}C$	$^{18}O/^{16}O$	$^{15}N/^{14}N$
R x 10^{6}	155.76	11237.2	2005.2	3676.5
Standard	V-SMOW	V-PDB	V-SMOW	Air
(molecule)	H_2O	$CaCO_3$ *	H_2O	N_2
Technique	IRMS, NMR	IRMS	IRMS	IRMS

* PDB (Pee Dee Belemnite carbonate) is exhausted. It has been replaced with NBS 19 (V-PDB scale). Other international standards available from IAEA include: IAEA-CH-7 (polyethylene, ex PEF1), NBS22 (oil), IAEA-CH-6 (sucrose).

values of these plants are ranging from –28‰ to –23‰). Most plants belong to this group (e.g. grape, rice, barley, wheat, soybean, potato, rye, sugar-beet). The second category are the so-called C_4 plants. They fix CO_2 by carboxylation of phosphoenolpyruvate (PEP-Carboxylase reaction) leading to four-carbon product, oxaloacetic acid. The PEP-Carboxylase reaction shows almost no isotope fractionation with respect to the carbon-13 content of atmospheric CO_2. Only the diffusion of CO_2 into the intercellular space exhibits a small fractionation of about 4‰. Products derived from C_4 plants show higher carbon-13 contents than analogous products from C_3 plants ($\delta^{13}C$ values of carbohydrates from C_4 plants are generally around –10‰). Cane, sorghum, millet and maize are the most important representatives of this group from the agro-economical point of view. The third category are CAM (Crassulacean Acid Metabolism) plants which have an intermediate metabolism. As a result intermediate carbon-13 contents are found for the products derived from these plants (carbohydrate $\delta^{13}C$ values range from -18‰ to -12‰). Pineapple, vanilla and agave are the more important plants from this group from an economic point of view.

Following photosynthesis secondary metabolisms which transform carbohydrates into proteins and lipids are accompanied by a further ^{13}C depletion leading to a large variety of $\delta^{13}C$ values in a given organism.

3. THE TECHNIQUES

Two analytical techniques are mainly used for the measurement of stable isotope ratios in food and beverages. These are the Isotope Ratio Mass Spectrometry (IRMS) for $^{2}H/^{1}H$, $^{18}O/^{16}O$, $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ ratios and the deuterium Nuclear Magnetic Resonance (^{2}H-NMR) for the intramolecular distribution of deuterium.

$$\delta D(‰) = \frac{R_{Sample} - R_{VSMOW}}{R_{VSMOW}} \times 1000$$

IRMS has a constant magnetic field which separates the different isotope species of the measuring gas (generally CO_2, H_2 or N_2) introduced into the ion source where they are ionised. The determination of the deuterium abundance in an organic compound requires conversion of the hydrogen from the original chemical form to molecular H_2. The H_2^+ ($m/z = 2$) and HD^+ ($m/z = 3$) species are then separated by the magnetic field of the IRMS and their corresponding ion currents (i) are measured on two different collectors leading to the ratio $R_{sample} = i_3/i_2$ (i_3 has to be corrected for the contribution of H_3^+ species formed from H_2 and H_2^+ in the source) [4]. The ratio obtained for the sample is compared to that of the International Standard V-SMOW (Vienna-Standard Mean Ocean Water) and the content in deuterium can therefore be expressed in ‰ on the δD scale [5].

Similarily, the carbon-13 content is determined on carbon dioxide gas resulting from the combustion of the sample. The various possible combinations of the ^{18}O, ^{17}O, ^{16}O and ^{13}C, ^{12}C, isotopes are found at mass 44 ($^{12}C^{16}O_2$), mass 45 ($^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$) and mass 46 ($^{12}C^{16}O^{18}O$). The mixed isotopomer species $^{13}C^{17}O^{16}O$ and $^{12}C^{17}O_2$ can often be neglected due to their low abundance. The corresponding ion currents are determined on three different collectors. The ion current measured for mass 45 is corrected for the contribution of $^{12}C^{17}O^{16}O$ which is computed from the intensity current measured on the detector for mass 46 by considering the relative abundance of ^{18}O and ^{17}O (Craig correction) [4]. The comparison with a reference calibrated on the international V-PDB scale allows the precise calculation of the carbon-13 content in $\delta^{13}C$ units. The nitrogen-15 content is determined against that of N_2 in air. The results are expressed in $\delta^{15}N$ units.

One method for the determination of oxygen-18 in water fractions has been originally published by Epstein and Mayeda. It is widely used for the control of wines and fruit juices [6]. Its principle is based on the isotope equilibration of the liquid water sample with CO_2 gas. Through equilibration, the ^{18}O information of the water is transferred to the gas phase. The ^{18}O abundance, expressed in $\delta^{18}O$ units, is determined by IRMS against that of the reference water V-SMOW which defines the international scale.

During the past decade, the analytical capabilities of IRMS have been considerably enhanced owing to the development of on-line techniques which for instance couple elemental analysers or gas-chromatographs with isotope ratio mass spectrometers. These techniques have increased the productivity of isotope laboratories considerably. They allow to carry out a large number of analyses per day or to analyse the isotopic profile of several organic compounds extracted from the same initial matrix in a single chromatographic run.

Oxygen isotope ratio measurements in organic matter are usually carried out using time-consuming off-line pyrolysis techniques not suitable for analysis of a larger number of samples. More recently, several groups have introduced on-line techniques based on high-temperature pyrolysis of organic samples. Here, carbon monoxide needs to be produced in a quantitative manner from the original sample. CO is directly used as the analyte gas [7–11].

The NMR technique is used for the determination of the site specific deuterium content of an organic molecule [12–15]. In spite of a considerable lack in sensitivity which implies the use of relatively large sample sizes (minimum of 1 mmol), deuterium NMR presents interesting features for the characterisation of organic compounds. It provides a " fingerprint" of the deuterium content within the molecule which is difficult to mimic while maintaining a sizeable profit from adulteration on an industrial scale. The deuterium content measured by NMR is generally expressed in absolute ratio D/H in ppm units. Here, 1 ppm corresponds to 6.4‰ on the V-SMOW scale.

4. APPLICATION OF ISOTOPE TECHNIQUES FOR FOOD AND BEVERAGE ANALYSIS

4.1. Wine

Wine has always been one of the products steadily analysed either for improvement of quality or for detection of possible frauds. Wine is obtained by fermentation of grape must and its alcohol grade is proportional to the initial sugar concentration of the must. An increase of the alcohol grade of wine can be obtained by addition of foreign sugars before or during the fermentation. In the European Union this practice, called chaptalisation, must be in compliance with the European regulations that stipulate maximum levels of enrichment for the various European vine growing areas [16]. The main botanical sources of sugar being used are cane (C_4) and beet-sugar (C_3). As it was shown by Bricout, the chaptalisation with cane sugar is easily detectable by IRMS because of the significant increase of the carbon-13 content of the ethanol resulting from the fermentation of the mixture of C_4 cane and C_3 grape sugar [17]. On the other hand, because the C_3 metabolism of grape and beet being the same, the chaptalisation with beet sugar can not be detected by the same carbon-13 IRMS method. By using

quantitative deuterium NMR, Martin showed that the internal distribution of deuterium in ethanol, measured by the ratio R which represents the D/H ratio of the methylene site against that of the methyl site, is very different for grape and beet [12]. It can be used to quantify mixtures of ethanols from these two botanical origins and therefore to detect chaptalisation with beet sugar [13]. It was also shown that this technique enables the detection of chaptalisation with cane sugar. Further development of the methodology improved the precision considerably [14, 18]. The method was adopted by the European Community as the official method for the detection of chaptalisation of wine (and grape must) which was followed by the decision to establish a E.U. wine database [19, 20]. More examples of using isotope techniques for the analysis of wine and other beverages include:

- $^{18}O/^{16}O$ IRMS for the detection of addition of water and for the characterisation of the origin of wines [21]. Further to O.I.V.[1], the European Community has adopted the determination of oxygen-18 as an official method for analysis of wines and has included this parameter in the E.U. Wine Database [22, 23].
- ^{2}H-NMR for detection of edulcoration.
- Carbon-13 IRMS for the characterisation of natural gaseification of sparkling wines [24].

Isotope ratios have also been used for the characterisation of the geographical origin of wines [25–28]. Finally on-going research using isotope ratios is made in order to trace possible addition of glycerol [29].

4.2. Sugar

Since the two major economical sources of sucrose belong to two different groups of plants (C_3 for beet and C_4 for cane) it is relatively easy to discriminate the sugars from these two botanical origins by measuring their carbon-13 content by IRMS [17]. The same distinction is possible by using the deuterium NMR on the ethanol obtained by fermentation of sugar: ethanol derived from sugar of C_4 origin has a higher deuterium content in the methyl group.

However, additional information is also given by the deuterium content which concerns the physiology of the plant from which the sugar originates [30, 31]. It has been found that C_3 aerial plants like grape or apple tree and C_3 cereals like wheat, rye or barley exhibit a higher deuterium content than beet which grows underground. The high deuterium content of carbohydrates from C_4 plants, such as cane or maize sugars, allows an easy recognition of these botanical origins. Adulteration of grape sugar products (e.g. concentrated rectified must) with exogenous sugars, in particular beet sucrose, can be evidenced by isotope methods used together with compositional analysis of polyalcohols [32].

4.3. Alcohol and spirits

Spirits and more generally alcoholic drinks are derived from fermentation of sugars. Thus, isotope methods used for ethanol also allow to control fraud in such beverages. It has been shown that the deuterium content $(D/H)_I$ of the methyl site of ethanol depends mainly on the deuterium content of the fermented sugar while that of the methylene site $(D/H)_{II}$ is governed mainly by the deuterium content of the fermentation water [33, 34]. In other words, $(D/H)_I$ bears the information about the botanical origin while $(D/H)_{II}$ holds information upon the fermentation process. It is therefore possible to check the botanical origin of a variety of alcoholic products. High $(D/H)_I$ ratios as well as high carbon-13 contents are found for rum and tequila which should originate from cane and agave respectively. More negative isotope ratios are encountered for spirits derived from a C_3 origin (plum, cherry, apple, grape barley or potato) [35]. An example of rums adulterated with beet alcohol (C_3 plant) is shown in Fig. 1.

[1] Office International de la Vigne et du Vin — 18 rue d'Aguesseau 75008 Paris (France).

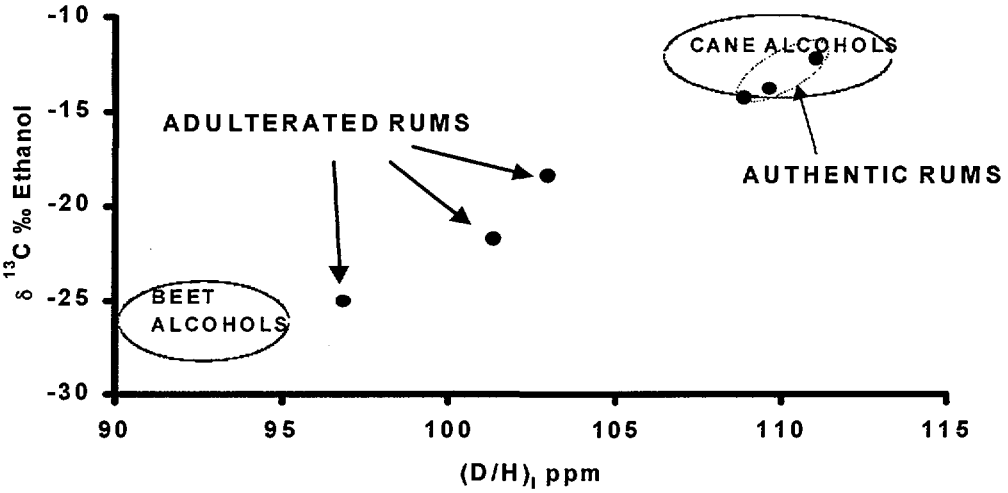

Fig. 1. Control of authenticity of spirits: rum. The absolute deuterium ratio of methyl site of ethanol $(D/H)_I$ is measured by ^{2}H-NMR.

Maize is widely used in blending with malt (barley) for the elaboration of some commercial whiskies. Since maize is a C_4 plant it can be easily distinguished from the C_3 barley on the basis of the isotope ratios which have therefore been proposed for the determination of the percentage of maize in commercial whiskies [36].

The isotope techniques are particularly interesting for the control of extra-rectified neutral alcohol that does no more contain the characteristic "impurities"(esters and higher alcohols) usually analysed by gas-chromatography for checking the origin (grape or cereal) of alcohol. In this case, only the combined use of NMR and IRMS allow for the determination of the botanical origin of the raw material (e.g. beet, cane, maize, potato). Synthetic alcohol is easily identified by its very high deuterium contents on both methyl and methylene sites [12].

4.4. Vinegars

Vinegar is often used as ingredient in many food products. Methods for identification of its botanical origin have been proposed based both on IRMS and on NMR techniques [37, 38]. Similarily to what is observed in the isotope filiation sugar-alcohol the isotope information is also kept after oxidation of ethanol to acetic acid (vinegar). This provides a convenient tool for control of the authenticity of the "expensive" vinegars derived from fruits. It could also be useful for the characterisation of some particular origins which produce "special" vinegars according to particular traditional processes. It may be emphasized that many canned products contain vinegars that should be of natural origin and sometimes claimed to be from one single botanical origin (generally wine). In 1993 AFNOR[2] adopted a method for the control of the vinegar used for canned macquerels [39].

4.5. Fruit juices

Many studies using isotope techniques have been carried out on fruit juices since about 20 years. One of the most widely known application is the distinction between the direct juices and the juices made from concentrates by redilution with "tap" water on the basis of the IRMS determination of deuterium and oxygen-18 content of the water of the juice [40]. An example of recognition between these two categories of fruit juice is shown in Fig. 2. The fruit juice industry has taken into account the possibilities of control offered by the isotope techniques and has published indicative ranges of values for isotope contents of genuine fruit juices [41].

[2] Association Française de Normalisation-Tour Europe , 92049 Paris, La Défense (France).

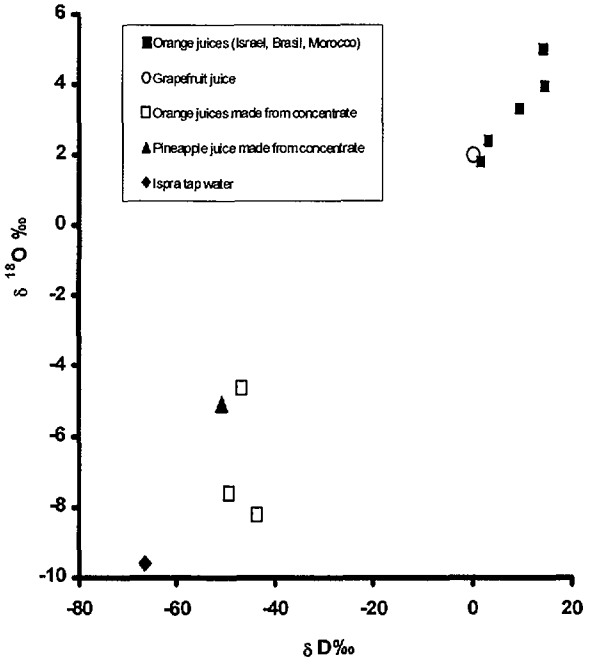

Fig. 2. Discrimination between direct fruit juices and juices made from concentrates based upon $\delta^{18}O$ and δD values of the juice. Isotope values of our laboratory tap water (Ispra tap water) are shown for comparison.

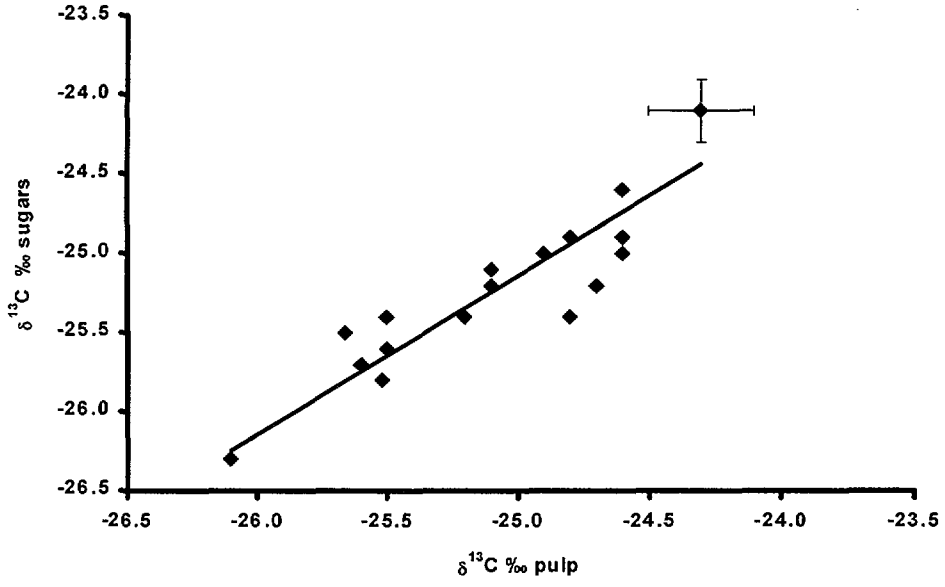

Fig. 3. Comparison of $\delta^{13}C$ ratio of the pulp and sugar fraction in orange juices from Sicily. A representative uncertainty of measurement (single standard deviation) is indicated for one sample.

Different isotope techniques have also been proposed for the detection of adulteration of fruit juices by addition of foreign sugars. For the detection of addition of beet sugar, Bricout and Doner have proposed the derivation of sugars as nitrate esters prior to their determination of their (D/H) ratio by IRMS [42], [43]. The detection of addition of cane or maize sugar is carried out by measuring ^{13}C abundance of the sugar fraction. For orange juices, a better sensitivity is obtained when an internal standardisation against the pulp fraction is carried out [42]. In Fig. 3 we present values for $\delta^{13}C$ of pulp and sugar fractions of genuine orange juices from Sicily (I) measured in our laboratory.

An indirect method has been studied by Brause et al. in the case of fruit juice concentrates adulterated by syrups. The principle is determining the ^{18}O abundance of the residual water from the concentrate: typically a pure fruit juice concentrate should show more positive $\delta^{18}O$ values (> +12 ‰) because of the concentration process which enriches the residual water in heavy isotopes [44].

The simultaneous use of deuterium NMR and carbon-13 IRMS has been shown to be very powerful for the control of authenticity of fruit juices [45, 46]. Due to the natural variation of isotope ratios, the development of databases containing the isotope ratios of authentic products is needed and a better precision would be achieved for the determination of adulteration when the precise geographical origin of the product is known.

More recent studies have shown that the $\delta^{15}N$ value of pulp could be a parameter indicative of the geographical origins of fruit juices [47].

4.6. Edible oils and lipids

The low $^{13}C/^{12}C$ ratio of lipids is shown to result from isotope fractionation during the oxidation of pyruvate to acetyl coenzyme A [48, 49]. It has been demonstrated that a large difference exists in the isotope content between the methyl and carbonyl carbon atoms of acetyl coenzyme A and in the carbon atoms of deriving lipids. This was confirmed by a positional carbon-13 isotope analysis of pyruvate and acetate by stepwise quantitative degradation [49].

Carbon isotope ratios of edible seed oils have been reported in literature, regarding in particular C_3 plants oils (sunflower about -27 ‰, soybean -28 ‰, palm -27 ‰, coconut -25 ‰, peanut -28 ‰) and C_4 plant oils (maize -12 ‰) [50, 51]. Due to its C_4 botanical origin, maize oil is easily recognised by measurement of the $^{13}C/^{12}C$ isotope ratio. GC-IRMS determination of $\delta^{13}C$ values of individual fatty acids have been proposed as a method to detect adulteration of maize oil with other vegetable oil [52]. Although not sufficient for an unambiguous recognition of C_3 oils this technique provides information that can be used in conjunction with other oil analyses to detect adulterations [53].

Concerning olive oil a study was carried out on the ^{13}C abundance of oil and some of its classes of compounds [54]. The isotope values for the bulk oil, aliphatic alcohols, sterols and glycerol were those expected given their biosynthetic origin, but distinctly different for each class of compounds. Based on those differences a further study was carried out to detect the adulteration of olive oil with pomace oil [55].

The characterisation of the geographical origin of virgin olive oil from various producing countries of the Mediterranean basin has been studied by measuring the $^{18}O/^{16}O$ ratio of bulk oil by pyrolysis-IRMS technique [11, 56]. Another study for determination of the geographical origin and of the purity of extra virgin olive oil has been performed by measuring the $^{13}C/^{12}C$ ratios of fatty acids by GC-IRMS [57].

A few studies on edible oils and fatty acids have been carried out using deuterium NMR, providing new information on isotope fractionation caused by biochemical, physiological and natural environmental effects. In particular, the site specific deuterium distribution in the fatty acids has been found to be related to the mechanism of fatty acids biosynthesis [58, 59].

4.7. Honey

Adulteration of honey by addition of syrups seems to be a frequent and widespread practice. An official method of analysis using the carbon-13 IRMS technique has been early adopted by the A.O.A.C. for the detection of the addition of High Fructose Corn Syrup (HFCS) [60].

However due to the variety of botanical origins, a large range of $\delta^{13}C$ variation may be expected in some cases (e.g. catsclaw, citrus). It results a lack of sensitivity of the method and a large "gray area" where the adulteration of a commercial product cannot be clearly established. In order to reduce this "gray area" the method was further improved by considering an internal standardisation of the $\delta^{13}C$ of honey against that of its protein fraction [61, 62]. Fig. 4 presents the application of this method using data published by White and Winters [61].

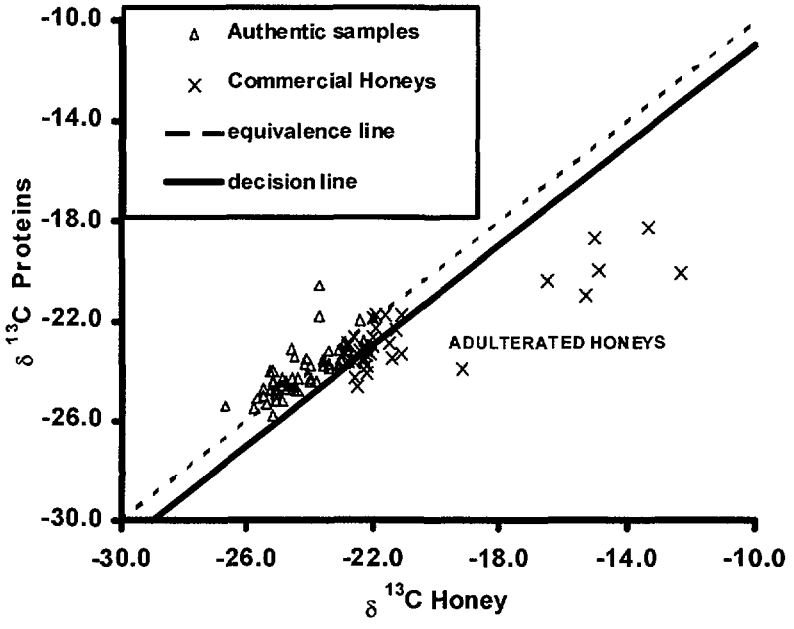

Fig. 4. Internal standardisation for the detection of adulteration in honey products with C_4 sugar (data from White and Winters [61]).

4.8. Flavours

Isotope ratios of flavour compounds have been studied in great detail since many years [63]. The isotope techniques have demonstrated their ability to distinguish between natural products extracted from plants or obtained by biogenesis from their cheaper synthetic or hemisynthetic homologues. The modern hyphenated IRMS techniques, in particular GC-IRMS, allowing the isotope analysis of several components of an essential oil, permit to obtain valuable information regarding the authenticity and the origin of the product. The number of applications of GC-IRMS will certainly increase in the next years. We will not further extend our discussion on the possibilities offered by this technique but present some typical examples of characterisation of flavour compounds by stable isotope ratio analysis.

Vanillin is the most widely used flavour compound in the food industry. A strong incitement for frauding vanillin exists because of very big price differences between natural vanillin extracted from vanilla beans, vanillin from synthetic origin from eugenol or guaiacol and vanillin from hemisynthetic origin derived from lignin or curcumin. The isotope ratios of vanillin have been extensively studied in order to detect the possible adulteration of this flavour. Vanilla is a CAM plant and consequently the first method proposed for detection of adulteration of vanillin was the determination of its $\delta^{13}C$ value. The values for $\delta^{13}C$ of vanillin extracted from vanilla-beans should be in the range -17 ‰ to -21 ‰ while other sources show much lower values (generally < -26 ‰) [64-66]. This method was successful until vanillin appeared on the market which was slightly enriched in ^{13}C on either methoxy or carbonyl sites in order to mimic the global carbon-13 content of natural vanillin extracted from vanilla-beans.

In order to detect this sophisticated fraud it has been proposed to chemically degrade vanillin prior to the determinating the isotope ratio of the carbon corresponding to these positions [66-68]. Information about the origin of vanillin is also obtained from the site specific deuterium content measured by NMR [69-70]. Now the development of the GC-IRMS technique allows the determination of the carbon isotope ratios of a series of components in a 'vanilla extract'. This 'isotope profile' can be used to assess the authenticity of the extract [71].

Anethole is another molecule that has been studied extensively. This compound enters in the composition of some popular aniseed spirits like Pastis and Ouzo. According to the European regulation about spirits, the anethole should originate from star anise or green anise [72]. Like in the case of vanillin, the site specific deuterium contents of anethole determined by NMR allow a clear distinction of the various possible synthetic or botanical origins [15].

The major compound responsible for the flavour of raspberry is 4-(4-hydroxyphenyl) butan-2-one and is called raspberry ketone. In nature, it occurs only at very low concentrations in plants so that its extraction is not economically feasible for its use by food industries. Alternatively it can be obtained by bioconversion of natural precursors, or by chemical reactions (chemical catalysts) using natural precursors. In the first case it can be labelled 'natural'. On the other hand, the use of chemical synthesis and/or precursors from petrochemical origin permit to produce this flavour at lower cost but in this case it cannot be labelled 'natural'. The site specific deuterium contents of raspberry ketone and its precursors have been studied by ^{2}H-NMR in order to identify the origin of these molecules and the processes that have been used to produce them. It has been found that the deuterium distribution on the H-atoms of the aromatic ring gives information about the natural or synthetic origin of one precursor (para-hydroxybenzaldehyde). The deuterium content of the methylenic positions could inform about the process (catalytic hydrogenation or fermentation) used to reduce the double bond of the precursor [73]. Further determinations of the D/H, $^{13}C/^{12}C$ and $^{18}O/^{16}O$ isotope ratios of raspberry ketone extracted from *Taxus baccata* and obtained by oxidation of plant extrative betuligenol has been carried out to study the isotope pattern of extracted or biogenerated natural raspberry ketone [74].

4.9. Coffee and tea

The isotope contents of caffeine from various geographical origins have been studied by Dunbar in 1982 [75]. Interestingly, the first aim of this work was not the characterisation of the caffeine as a component of a food product but rather the establishment of a new methodology for the determination of the geographical source of illegal drugs such as morphine and cocaine. Indeed, because of the difficulty to legally obtain samples of morphine or cocaine the authors chose to take caffeine as a model alkaloid for testing their C, H, O isotope fingerprinting method by IRMS. Their results showed that both organic oxygen-18 and deuterium abundance in caffeine provide information about the geographical origin of tea or coffee. Further experiments using ^{2}H-NMR confirmed that the geographical origin of coffee can be checked by the determination of its isotopic contents [76].

5. CONSIDERATIONS ABOUT REFERENCE MATERIALS

Most of the calibration standards used for isotope analysis were first established taking into consideration the needs of biogeochemistry. Few of the International Standards available by IAEA are relevant for $\delta^{13}C$ analysis of food products with an elemental analyser-IRMS. However, none of these materials is really suitable for GC-IRMS techniques now used for control of flavours in food products. Moreover, these international standards are generally covering the $\delta^{13}C$ determinations but leave gaps for $\delta^{15}N$ and in particular for $\delta^{18}O$ determinations in organic compounds [77]. It is necessary for the scientific community that these gaps are filled in the near future. Analysts need international standards suitable for on-line stable isotope analysis in order to ensure the best traceability of isotope determinations against the primary international standards. For GC-IRMS as many applications as those already established for gas chromatographic separations may be envisaged. It is certainly not realistic to establish international standards for all possible applications. It seems more reasonable to concentrate the efforts on a selection of few candidate compounds. These selected compounds should cover most of the chromatographic conditions taking into account the polarity of columns and volatility of analytes. Moreover they should preferably be calibrated for several isotope ratios $^{13}C/^{12}C$, $^{15}N/^{14}N$, D/H, $^{18}O/^{16}O$ and possibly $^{34}S/^{32}S$. The establishment of these new international standards could be carried out by the scientific community in collaboration with IAEA and other institutions experienced in reference materials like NIST, USGS and the Institute of Reference Materials and Methods (IRMM) of the JRC-Geel.

TABLE II. REFERENCE MATERIALS PREPARED IN THE FRAME OF THE PROJECT REFMAT (SMT 4 CT96-2086)

	REFERENCE MATERIAL				
Parameters to be certified	Ethanol E (96%, vol)	Sugar S	Synthetic Wine W1 7% alc vol	Synthetic Wine W2 12% alc vol	E + water Mixture W3 12% alc vol.
	CRM 656	CRM 657	CRM 658	CRM 659	CRM 660
$\delta^{13}C$ of ethanol (IRMS)	X				X
$(D/H)_i$ of ethanol (^{2}H-NMR)	X				X
$\delta^{13}C$ of sugar (IRMS)		X			
$\delta^{18}O$ of water (IRMS)			X	X	
$(D/H)_w$ of water (IRMS)					X
Alcoholic grade	X				X

Regarding NMR some certified reference materials have been available for a few years and are routinely used by laboratories as working standards (e.g. tetramethylurea) for calculating D/H values and for quality control monitoring of NMR determinations (NMR sealed tubes) [78, 79].

It is worth mentioning that five reference materials, dedicated to the authentication of wines and sugars, are currently in preparation within the European project REFMAT [80]. These reference materials presented in Table II have been chosen to cover the main applications of isotope techniques used to analyse these products and will be applied to control the isotope ratio determinations by IRMS and NMR and also preparation steps such as the distillation. These materials have been subjected to stability and homogeneity testing. They will be proposed soon for certification and should then be available as Certified Reference Materials by IRMM.

6. CONCLUSION

Isotope methods have proven their ability to characterise the authenticity of a variety of food products as well as of alcoholic or non-alcoholic drinks since a long time. Thanks to technical improvement in instrumentation it is likely that many other applications will be developed in the coming years. The continuous flow techniques now widely used in IRMS allows for high sample throughput with a high reliability of results. This is convenient for constituting isotope ratio databases of authentic samples as well as for the routine control of many marketed products. Moreover the GC-IRMS technique developed in the decade has brought a very powerful tool to the food analyst. It is also probable that multi-isotope fingerprinting will lead to a better characterisation of the (geographical) origin of a substance in question. Internal isotope ratio standardisation as proposed for honey and some fruit juices may be extended to other food products and should improve the sensitivity for the detection of fraudulent practices.

Finally authenticity control of food and beverages is only one of many possible uses for isotopic techniques: many studies have shown that nutrition problems as well as efficiency tests of pharmaceutical products can be tackled with success. Applications for recognising the origin of illegal drugs or detecting doping in sports have also been developed.

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