Anal Bioanal Chem (2003) 376 : 424–430
DOI 10.1007/s00216-003-1804-6

REVIEW

N. Ogrinc · I. J. Košir · J. E. Spangenberg · J. Kidrič

The application of NMR and MS methods for detection of adulteration of wine, fruit juices, and olive oil. A review

Received: 13 December 2002 / Accepted: 15 January 2003 / Published online: 27 March 2003

Abstract This review covers two important techniques, high resolution nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), used to characterize food products and detect possible adulteration of wine, fruit juices, and olive oil, all important products of the Mediterranean Basin. Emphasis is placed on the complementary use of SNIF-NMR (site-specific natural isotopic fractionation nuclear magnetic resonance) and IRMS (isotope-ratio mass spectrometry) in association with chemometric methods for detecting the adulteration.

Keywords Nuclear magnetic resonance · Mass spectrometry · Stable isotopes · Adulteration · Wine · Fruit juices · Olive oils

Introduction

Making fraudulent profit from misrepresentation of food has been a feature of society from historical times. Nowadays frauds in various consumer sectors are commonly practised. The addition of beet or cane sugar or concentrated rectified must to grape must or wine before or during fermentation is used to increase the natural content of ethanol and therefore the value of wine, which commands higher prices on the market. Consumers are thus deceived since added sugar is not declared on the product. Another type of economic fraud is mixing high quality wines with low quality ones that often originate from other geographical regions or countries. A memorable example is the adulteration of Austrian wine and also some Italian and German wines with the poisonous antifreeze ethylene glycol with intention to give the impression of a wine with a greater body.

Identifying fraudulence related to fruit juices is also of great economic importance because of the large quantities of juice consumed. The types of adulteration include diluting with water, the addition of sugar solution, citric and tartaric acid, and colorants to the pure juice and the addition of cheaper juices originating from other fruits, mainly from grapefruit.

The consumption of virgin olive oil, which is defined as oil obtained only by mechanical means is increasing due to its nutritional properties arising from the high content of unsaturated acids (oleic and linoleic acids). Natural phenolic compounds present only in virgin olive oil are responsible for its oxidation stability and for its characteristic sensory attributes. The high sensory and nutritional quality and consequently higher commercial value of virgin olive oil has lead to its adulteration with low-grade foreign oils (seed oils), esterified oils or refined olive oils and olive-pomace oils, which due to the refining process and solvent extraction have lost phenolic compounds.

The result of the antifreeze fraud was the establishment of quality-control schemes such as Appellation Controle that are being applied to other food areas. Authenticity control is regulated in EU and in the USA and is also spreading to other countries with the important principle that if adulteration with potentially safe materials is not properly policed the danger exists that ever more toxic materials will be used in search for quick profits.

To undertake necessary controls and to detect the adulteration of food products many analytical techniques are used: HPLC, GC, GC–MS, GC–FTIR, UV, AAS/AES, ICP–AES, ICP–MS, IRMS, DSC, IR, and NMR [1]. In this review we focus on use of NMR and MS for detecting the adulteration of wine, fruit juices, and olive oil that

N. Ogrinc
"J. Stefan" Institute, Department of Environmental Sciences,
Jamova 39, 1000 Ljubljana, Slovenia

I. J. Košir · J. Kidrič (✉)
National Institute of Chemistry,
Hajdrihova 19, 1000 Ljubljana, Slovenia
e-mail: jurka.kidric@ki.si

J. E. Spangenberg
Institut de Minéralogie et Géochimie, Université de Lausanne,
BFSH-2, 1015 Lausanne, Switzerland

Present address:
I. J. Košir
Institute of Hop Research and Brewing of Slovenia,
Žalskega tabora 2, 3310 Žalec, Slovenia

are important products of the Mediterranean Basin. To day both NMR and MS have an outstanding role in the chemical analysis of food products. Though less sensitive than HPLC, GC, and capillary electrophoresis NMR has many advantages. It is nondestructive, selective and capable of simultaneous detection of a great number of low molecular mass components in complex mixtures and sample preparation is simpler and less time consuming. MS is capable of yielding analytical data on picomole, femtomole or even attomole amounts of target compounds. Food constituents such as polysaccharides, proteins and lipids are frequently analyzed by MS. In the analysis of beverages MS is usually coupled to gas chromatography (GC) or liquid chromatography (LC). Sample preparation techniques vary from rudimentary to very elaborate depending on the analytical problem to be solved. Especially powerful are SNIF-NMR and IRMS methods applied for determination of the authenticity of wine, fruit juices and olive oil. These methods are based on the measurements of the stable isotope content of a product or of a specific component of the product.

The aim of this article is to review the use of SNIF-NMR, IRMS and other NMR and MS techniques in studies of adulteration. We illustrate the application of these methods on our research on fruit juices, wine and olive oil.

IRMS and SNIF-NMR methods

Each plant has its own unique pattern of naturally occurring stable isotopes of carbon (^{12}C, ^{13}C), nitrogen (^{14}N, ^{15}N), hydrogen (^{1}H, ^{2}H) and oxygen (^{16}O, ^{18}O), whose distribution has been influenced by a number of physical and/or biochemical properties and geoclimatic conditions. The isotope content of natural products depends on their botanic and geographical origin.

The procedure of IRMS consists in measuring the isotope ratio ($^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$) of an analyte converted into a simple gas, isotopically representative of the original sample, before entering the ion source of an IRMS. The stable isotopic data are expressed in the delta (δ) notation as the per mil (‰) deviation of the isotope ratio of a sample relative to that of a standard. V-PDB (Vienna-Peedee Belemnite Limestone) is used as international standard for carbon ($\delta^{13}C$), AIR for nitrogen ($\delta^{15}N$) and V-SMOW (Vienna-Standard Mean Ocean Water) for oxygen ($\delta^{18}O$) and deuterium (δD). The carbon and hydrogen isotopic ratios play key roles in determining the plant origin of sugars. The discrimination offered by carbon depends largely on plant variety. The ^{13}C content is particularly good for distinguishing organic products originating from C_3 and C_4 plants [2, 3]. In the early days of IRMS, detection of added sugar in fruit juice and wine (C_3 plants) was simple as mainly cheap corn syrup (maize: C_4 plant) was predominately used to boost sugar and/or ethanol content. In this case, $\delta^{13}C$ values of glucose or bulk carbon was sufficient to assess the adulteration. However, the addition of sugars from other C_3 plants (sugar beet; concentrated and deflavored grape juice) could not be detected by these measurements. The deuterium content of sugars isolated from fruit juice could be used as a useful tracer to detect the addition of exogenous sugar [4, 5, 6].

The most sophisticated and most specific method for detecting such adulteration is SNIF-NMR. This method is based on the measurement of deuterium/hydrogen (D/H) ratios at the specific sites of the ethanol molecule [7, 8]. To detect the addition of sugar or modified sugar syrups with this method the sample must be fermented under controlled conditions and the resulting alcohol distilled off. The (D/H) ratios are determined at the methyl $(D/H)_I$ and methylene $(D/H)_{II}$ sites of the ethanol molecule. One of the first applications of SNIF-NMR was the detection of the adulteration of wine [9]. Results obtained since then from a survey of all EU wine-growing regions have begun to be checked to ascertain whether they can additionally provide a means for identifying the provenance of European wines [10, 11, 12, 13, 14]. ^{2}H NMR-measurements are backed-up by determining the $\delta^{13}C$ in ethanol [13, 15, 16] and $\delta^{18}O$ values of water in wine, which add relevant information regarding wine origin. The purpose of $\delta^{18}O$ measurements is to detect the possible addition of water and in particular, to support the geographical correspondence [17, 18, 19]. All these methods are well established in the EU (EC Regulations [20, 21], European Committee for Normalization, CEN, [22]) and internationally (Office International de la Vigne, O.I.V. [23] and the Association of Official Analytical Chemists, AOAC [24]).

The natural variation of isotopic values within the same fruit type makes it difficult to detect the addition of small amounts of sugars (≤10%) by measuring only one constituent of the juice. The potential of stable isotope techniques to detect economic adulteration is considerably improved by analyzing several components of the same product and investigating their intermolecular correlations [25]. This is achieved by coupling a GC system to an IRMS to enable precise compound-specific isotope analysis (CSIA) at natural isotopic abundance level. Several studies show that specific correlations exist between $\delta^{13}C$ values of sugars, L -ascorbic acid [26, 27] L -tartaric acids [27] L -malic acids [28], and citric acids [29, 30, 31, 32] by taking one of these metabolites as internal reference. Any deviation from the expected correlation is indicative of the addition of at least one of these compounds from an exogenous source. These methods based on high-precision CSIA were used to prove fraudulent addition of sugars and even vitamin C [33].

Further, ^{13}C depletion position specific for the C-1 position of glycerol of authentic origin was found. This unique feature might be used to test for illegal addition of synthetic glycerol to wine [34].

High-quality vegetable oils are another target for adulteration by partial or total substitution of minor quality, hence cheaper oils for the high quality products. The overlap in fatty acids composition in different single seeds oils makes difficult to detect adulteration and authenticity frauds. Furthermore, natural variations in fatty acid composition may mask the adulteration of premium or gourmet

oils by adding small amount (up to 10%) of cheaper oils. Consequently, comparison of the fatty acid composition is not a reliable indicator of mixing of vegetable oils. The isotopic composition of individual fatty acids from different vegetable oils can be used as a tool for assessing their origin [35, 36, 37, 38, 39, 40]. It helps to distinguish between the natural variations of $\delta^{13}C$ of genuine C_3 and C_4 oils and admixtures of oils of different varieties of C_3 plant. The studies also show that the saturated 16:0 fatty acids are more depleted in ^{13}C that the corresponding unsaturated fatty acids 18:1 and 18:2 [35]. Therefore, substantial separation of the oils from the 1:1 line in the $\delta^{13}C_{16:0}$ versus $\delta^{13}C_{18:1}$ graph suggests an impurity or adulteration. Further, $\delta^{13}C$ of the aliphatic alcoholic oil fractions can be used to detect adulteration of olive oils [41]. In the same study it was found that higher grade olive oil has more positive $\delta^{13}C$ values for isoprenoids and methylsterols isolated from each grade of olive oil.

Fruit juices

In our research studies the IRMS method was first introduced to detect adulteration of commercially available juices in Slovenia. The research focused on ^{13}C and the deuterium content of sugars [42]. In natural (orange, lemon, and grapefruit juice made in the laboratory) and some commercially available juices (orange juices, and concentrates, apple juices, grapefruit and juices made from mixed fruits) sugars were isolated according to the procedure proposed by Koziet et al. [43]. Sugars were then directly analyzed for $\delta^{13}C$ and nitrated for δD determination. The $\delta^{13}C$ values of the samples were between –23‰ and –27‰ covering the interval of the $\delta^{13}C$ values of beet sugar, the most often used sugar in Slovenia. It is evident that it is not possible to detect this kind of adulteration using only $\delta^{13}C$ measurements. Therefore, the isotopic composition of hydrogen was also determined in these samples. The averaged δD value for Slovenian nitrated beet sugar was –118‰. It was estimated that orange juice adulterated with beet sugar should have the δD value between –100‰ and –60‰. The deuterium content determined in analyzed samples ranged between –53‰ and –15‰ suggesting that no exogenous sugars were added to the juices. The main disadvantage of this complementary method is time, since several steps are involved in deuterium determination: isolation, nitration of sugars and their combustion in a special unit. A more promising approach is the SNIF-NMR method. Martin et al. applied SNIF-NMR alone [8] or SNIF-NMR and IRMS analysis to detect added sugar and to assess authentication of fruit juices [44]. This interpretation could be illustrated on an adulteration triangle constructed from the plane of $\delta^{13}C$ values vs. $(D/H)_I$ values in ethanol. Isotopic parameters for authentic reference alcohol from beet, cane and authentic sample are represented as a triangle. Possible adulteration can be detected from the position in the triangle. This technique could be applied to any food product, if there is a large knowledge base or database for comparison purposes. This approach has also been tested in our research on Slovenian wines.

Wines

For widespread application of isotopic methods for wine authentication it is necessary to know both the reference mean values and the sources of variations of the different bioelements' stable isotopes within a given wine-producing area. Thus, a special database on the stable isotope parameters of Slovenian wines has been collected since 1996 according to EU Regulations [20, 21] and O.I.V. protocol [23].

Our data bank consists of $(D/H)_I$, $(D/H)_{II}$, R, and $\delta^{13}C$ isotope ratios of wine ethanol for 1996, 1997 and 1998 vintages in three different wine-growing regions in Slovenia obtained by SNIF-NMR and IRMS. Additionally, our data bank also contains the values of $\delta^{18}O$ isotope ratios of wine water for 1997 and 1998 vintages and δD isotope ratios of wine water for 1997 vintage obtained by IRMS. In such studies it is convenient to use multivariate analysis to interpret the results. By applying the appropriate chemometrics tools, such as Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) we were able to determine the geographical origin of a specific wine and under certain circumstances even the year of production [19].

To test the detection power of the isotopic measurements in wine ethanol, samples with added beet or cane sugar ($40\,g\,L^{-1}$) were prepared. Isotopic parameters for authentic reference alcohol from beet, cane and wine are represented as a triangle on the graph where $\delta^{13}C$ values are plotted against $(D/H)_I$ values (Fig. 1). Cut-off points

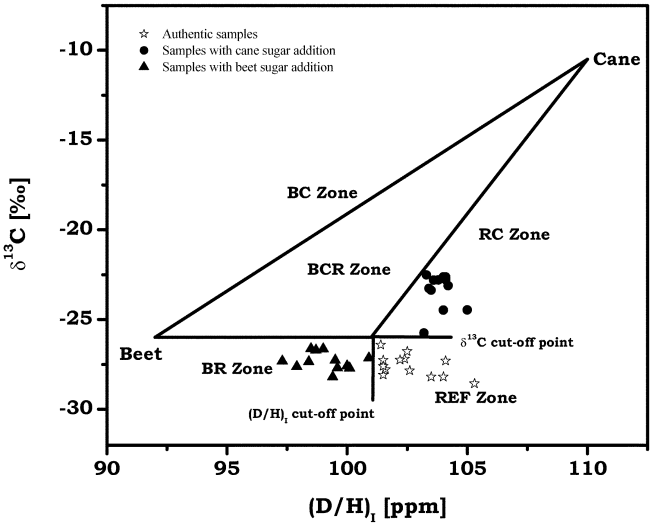

Fig. 1 Adulteration triangle. Samples are represented in a plane of $\delta^{13}C$ content and of the normalized hydrogen isotope ratio $(D/H)_I$ of wine ethanol. The reference zone (REF) is determined from authentic samples of Posavje region for 1997 vintage. If beet, cane or a mixture of these sugars is added, then the sample will fall in zones BR, RC, BCR. A sample in zone BC is not a mixture of sugar wine. This may indicate a high level of adulteration

for the wine reference group were established from authentic samples. It is seen from Fig. 1 how both types of adulteration can be detected. For complex mixtures of cane and beet sugar, the sample will fall in the middle of the triangle. Considering all the aspects outlined above, proving the authenticity of a certain wine is only possible when the results are compared with a reference (not adulterated) sample from the data bank of the same vintage from the same region.

The use of chemometric methods is necessary for evaluating the results obtained by NMR and MS methods used to detect the adulteration or authenticity of the food. We compared PCA, KANN (Kohonen Artificial Neural Networks) and cluster analysis to find the optimal method for discriminating between natural and enriched wines. PCA and KANN give equal information regarding the separation of natural wines and wines enriched by cane sugar, but PCA is faster. The best separation between natural wines and wines enriched by beet sugar is obtained by KANN. In all cases cluster analysis shows poorer efficiency than PCA and KANN [45].

Olive oils

The cold-pressed olive oil samples from the main producing regions in the Mediterranean and Adriatic were characterized by GC and GC–C-IRMS of individual fatty acids, and the results were evaluated by PCA. The combined chemical and isotopic data were used to distinguish the geographical origin of the samples [38, 46]. The bulk olive oil samples have isotopic composition between –26.5‰ and –30.6‰ typical of C_3 plant. The $\delta^{13}C$ values of the virgin olive oil fatty acids vary between –28.5‰ and –36.5‰ (Fig. 2). The isotopic shift can be partially explained by geographical origin, year of harvest and chemical changes (transmerization and oxidation) during refining. Blending of olive oil with edible oils with slightly different fatty acid composition (olive pomace, sunflower, hazelnut) might be detected by using $\delta^{13}C_{16:0}$ versus $\delta^{13}C_{18:1}$ covariations combined with molecular information and carbon isotopic composition of the bulk oil. Furthermore, some of the variations of the isotopic composition of the individual lipids of the oil samples may be due to climatic and plant growing condition including atmospheric carbon dioxide and cultivation practices. These factors may affect the isotopic composition of the main fatty acids in a similar way and, consequently, the oil-samples would move along the 1:1 line in the plot of $\delta^{13}C_{16:0}$ versus $\delta^{13}C_{18:1}$.Therefore, it is important to establish a data base that provides isotopic information for the authenticity of olive oil.

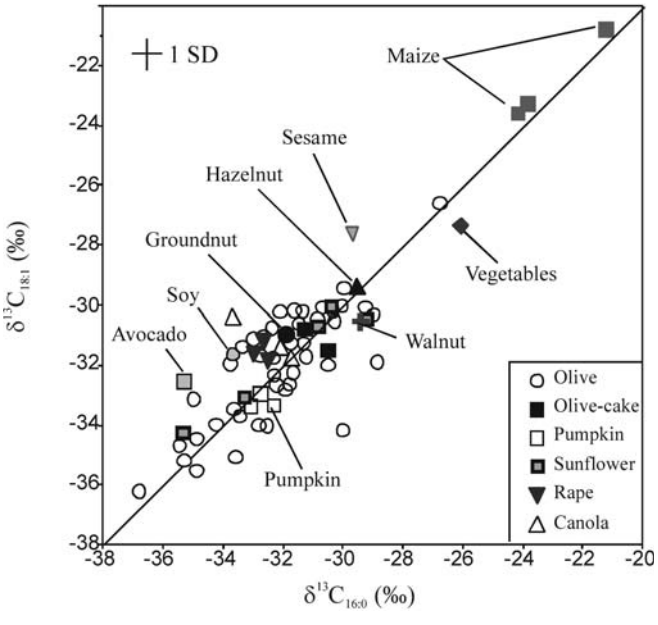

Fig. 2 Carbon isotope composition of oleic acid ($\delta^{13}C_{18:1}$) versus that for palmitic acid ($\delta^{13}C_{16:0}$) of the vegetable oils from the major oil-producing regions in Mediterranean

Other NMR and MS methods

Development of high-field NMR spectrometers, the possibility of recording two- and multi- dimensional NMR spectra, detecting NMR of different nuclei, and using "nano" probes for microliter quantities of sample make possible the analysis of complex mixtures at the molecular level. In the last several years new and advanced MS methods have been developed: matrix-assisted laser desorption/ionization MS (MALDI–MS), MALDI-time-of-flight MS (MALDI–TOF–MS), tandem MS (MS–MS), electrospray ionization MS (EI–MS), chemical ionization MS (CI–MS), pyrolysis MS (Py–MS). Recent developments of both NMR and MS methods offer new possibilities for detecting adulteration of foods.

Fruit juices

Juices differ by amino acid pattern and this can be used to detect the adulteration of juices with inexpensive amino acids. Chromatographic methods were used for determining the amino acid pattern in fruit juices [47]. Belton et al. [48, 49] have assigned the ^1H NMR signals of different fruit juices to characterize several classes of compounds, among which are also minor compounds such as amino acids. The full capacity of the NMR approach for determining amino acid pattern in fruit juices has yet to be exploited. The potential of NMR and multivariate analysis methods to detect the adulteration of orange juice with pulp wash has been examined [50]. The chemical composition of pulp wash is similar to that of orange juice but it is paler, more bitter and regarded as a lower quality product. The addition of pulp wash to orange juices is at present forbidden in the EU. A fast method for detection of cheap sweeteners (sucrose from cane or beet, starch hydrolyzates) based on combination of MALDI–TOF-MS with capillary zone electrophoresis is an efficient tool for

determining the adulteration of orange juice [51]. Py–MS has a considerable potential as a rapid method for the detection of adulteration. In combination with multivariate analysis it has been used for detecting adulteration of juices by sucrose [52]. Recently, the mass-spectrometer based electronic nose has been utilized to differentiate between grapefruit juices that differ only in the concentration of a single component [53].

Wine

^{1}H and ^{13}C NMR spectroscopy in combination with chemometric methods is a suitable approach for studying wine adulteration in terms of varieties, regions of origin and vintage and also for detecting the addition of undesirable or toxic substances. ^{13}C NMR was introduced in wine analysis by Rapp et al. [54]. It has been shown that ^{13}C NMR can successfully be used for the detection of sugars, alcohols, organic acids and amino acids [55, 56]. Amino acids can be used as fingerprints for monitoring of European wines [57]. ^{1}H and ^{13}C NMR spectra of wines of different variety and geographical area differ in the intensity of particular signals and also in the appearance of some signals which offers the possibility to follow the variability in their chemical composition [58]. By using the ^{1}H signal intensities of amino acids in chemometric analysis a good separation of Slovenian wines according to the vine variety has been obtained, while by adding also the signal intensities of glycerol, butylene glycol and succinic acid, the Slovenian wines can be separated according to their geographical origin [59, 60]. Recent development in MS is the use of ESI Fourier transform ion cyclotron resonance MS (ESI FT-ICR–MS) for identifying the presence and relative abundance of compounds in wine (carbohydrates and phenolics) without any prior separation or purification steps. An important MS study concerns the determination of ochratoxin A (OTA) in wine [61]. OTA is a mycotoxin produced by several Aspergillus and Penicillium species in different agricultural commodities. It is a nephrotoxin and a hepatotoxin with teratogenic, mutagenic and immunosupressive effects. It was also classified as a potential carcinogen for humans. The consumption of OTA-contaminated food is linked to the occurrence of Balkan endemic nephropathy, a disease characterized by severe kidney damage. The most important sources of OTA are cereals, coffee, beer and wine. In their study Leitner et al. [61] have shown that MS sensitivity is sufficient to measure OTA concentrations in contaminated samples that could pose a treat to human health, taking WHO guidelines and proposed OTA tolerance levels within the EU into account.

Olive oils

The composition of olive and of any vegetable oil is generally defined in terms of the nature and distribution of the fatty acids present in the triacylglycerols and also of the positions at which these fatty acids are attached to the glycerol backbone. In terms of triacylglycerol composition, olive oil contains mainly oleyl and linoleyl unsaturated groups in addition to palmitic and stearic saturated groups. Acyl and acyl positional distribution vary between different oil varieties. In the case of olive oil it may depend on the area of production and the technique of production itself. The detection of acyl and acyl positional distribution is therefore important for determining adulteration. Currently chromatographic methods are used to detect adulteration of virgin olive oil by other oils. However, they suffer from several disadvantages. They are not specific, they are destructive, time-consuming, and qualitative.

This prompted the development of applications of high-resolution ^{1}H and ^{13}C NMR spectroscopy for the analysis of virgin olive oil. The major results have been reviewed by Vlahov [62] and Sacchi [63]. Adulterations with seed oils (soybean, peanut, maize, etc., characterized by high content of n-3 linolenic acid can be detected [64]. Particularly useful is the application of ^{13}C NMR to determining the fatty acid composition. Olefinic, methylenic and carbonyl resonance are suitable for the direct and structure-specific analysis of the relative amounts of different fatty acids present in olive oil. The total content of diacylglycerols and the ratio sn-1,3-diacylglycerols/total diacylglycerols (sn-1,2 + sn-1,3-diacylglycerols) determined by NMR can provide a good discrimination between virgin olive oils and refined oils ("olive oils" and "olive pomace oils") [65].

The detection of *trans* fatty acids in virgin olive oil is another determination for which NMR can be used as an alternative to official gas-chromatographic methods [66]. The absence of *trans* fatty acids is considered as a purity index for virgin olive oil: refined olive and olive pomace oils contain detectable levels of oleyl, linoleyl and linolenyl *trans* isomers. Capillary GC standard methods are used in the EU at present. Using ^{13}C NMR and GC samples of virgin olive and refined olive oils have been studied [63]. No detectable *trans* isomers were found in virgin olive oil while refined ones showed a variable level of *trans* fatty acids (0.3–1%).

Further information about virgin oil purity can be obtained by analysis of the unsaponifiable matter of virgin olive oil constituted mainly of squalene, β-sitosterol and aliphatic alcohols. ^{13}C NMR of the unsaponifiable matter has been used in combination with multivariate statistical analysis for the discrimination of virgin olive oil from olive-pomace and refined olive oils [67]. The analysis of olive oils mixed with esterified oils, which are considered non-edible in EU, is presently carried out using standard methods recognized by EU [68, 69]. Not only is this procedure complicated involving several steps, the information on the positional distribution of the individual fatty acids moieties on the glycerol backbone is lost. ^{13}C NMR on the other hand yields immediate results without any chemical manipulation and it seems to represent the only direct instrumental method by which the positional distribution of fatty acids on glycerols can be specifically iden-

tified [70, 71]. Recently ^{1}H NMR has been used for the characterization of Italian olive oils which contribute about one-third to world production [72, 73]. In their study Brescia et al. have shown that ^{13}C NMR can successfully be used for olive oil cultivar classification purposes [74]. In comparison to NMR MS methods have been much less used for the detection of olive oil adulteration. MS has been applied mostly in combination with GC. GC is frequently used in association with a large set of analyses and with chemometric data treatment [1]. Py-MS has been used for rapid assessment of olive oil adulteration [75]. Recent research shows that GC combined with time-of-flight mass spectrometry (TOF-MS) reliably detects adulteration of virgin olive oil with hazelnut oil down to a concentration of about 5% [76].

Conclusions

Advances of NMR and MS techniques have led to new applications for detecting adulteration of foods. One- and multidimensional ^{1}H and ^{13}C NMR methods in combination with chemometric methods have been especially successful in detecting adulteration in terms of varieties, geographical origin and vintage of wines and for detecting the addition of low quality material or toxic compounds to wine, fruit juices, and olive oil. All this information should be obtained on a defined number of authentic samples. Stable isotopes have a wide range of application in food and drink quality and adulteration studies and they are used routinely and in combination with other chemical criteria. Despite recent technical advances many fundamental challenges still remain. Further development is required leading to:

- extension of this approach to the measurement of other isotopes (^{15}N, ^{18}O, ^{2}H) in the minor trace components (proteins, phenolic compounds, trace oligosaccharides;
- routine application to detect intermolecular isotope pattern approach; and
- routine CSIR of hydrogen isotopes after GC separation.

Only an integrated approach based on compositional and isotopic fingerprints constructed from as many components as possible of the food product and a statistical model for data evaluation and interpretation would provide an efficient means for combating fraud in adulteration. Additionally, the main emphasis of food authenticity testing is to provide a means of enforcing product labeling to guide consumer choice.

Acknowledgements We are grateful to Professor D. Hadži for critical comments. This work was supported by the Ministry of Education, Science and Sport and Ministry of Agriculture and Forestry of the Republic of Slovenia.

References

1. Cordela C, Moussa I, Martel A-C, Sbirrazzuoli N, Lizzani-Cuvelier L (2002) J Agric Food Chem 50:1751
2. O'Leary MH (1988) Bioscience 38:328
3. O'Leary MH (1995) In Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant water-relations. Academic Press, San Diego, CA, USA
4. Dunbar J, Schmidt H-L, Woller R (1983) Vitis 22:375
5. Dunbar J, Schmidt H-L (1984) Fresenius Z Anal Chem 317: 853
6. Bréas O, Guillou C, Reniero F, Sada E, Tanet G (1996) Rapid Commun Mass Spectrom 10:246
7. Martin GJ, Martin ML (1981) Tetrahedron Lett 22:525
8. Martin GG, Wood R, Martin GJ (1996) JAOAC Int 79:917
9. Martin GJ, Martin ML (1988) Mod Methods Plant Anal (Wine Anal) 6:258
10. Martin GJ, Guillou C, Martin ML, Cabanis M-T, Tep Y, Aerny J (1988) J Agric Food Chem 36:316
11. Monetti A, Reniero F, Versini GZ (1994) Z Lebensm Unters Forsch 199:311
12. Day MP, Zhang B-L, Martin GJ (1994) Am J Enol Vitic 45:79
13. Monetti A, Versini G, Dalpiaz G, Reniero F (1995) J Agric Food Chem 44:2194
14. Day MP, Zhang B-L, Martin GJ (1995) J Sci Food Agric 67: 113
15. Rossmann A, Schmidt H-L, Reniero F, Versini G, Moussa I, Merle MH (1996) Z Lebensm Unters Forsch 203:293
16. Gimenez-Miralles JE, Salazar DM, Solana I (1999) J Agric Food Chem 47:2645
17. Bréas O, Reniero F, Serrini G (1994) Rapid Commun Mass Spectrom 8:967
18. Rossmann A, Reniero F, Moussa I, Schmidt HL, Versini G, Merle MH (1999) Z Lebensm Unters Forsch A 208:400
19. Ogrinc N, Košir IJ, Kocjančič M, Kidrič J (2001) J Agric Food Chem 49:1432
20. EC Regulation No. 2676/90, Sept 17, 1990, Off J Eur Communities L 272:64
21. EC Regulation No. 822/97, 1997, Off J Eur Communities L 117:10
22. CEN/TC 174 No 108–110, 1996
23. O.I.V. Office International de la Vigne et du Vin—Resolution OENO/SCMA/00/177 Détermination du rapport isotopique de l'ethanol, 2001
24. Cunniff P (1995) (ed) Fruit and fruit products, Official Method 995.17: Beet sugar in fruit juice. AOAC International, Gaithersburg, MD
25. Meier-Augenstein W (1999) J Chromatogr A 842:351
26. Gensler M, Rossmann A, Schmidt HL (1995) J Agric Food Chem 43:2662
27. Weber D, Gensler M, Schmidt HL (1997) Isotopes Environ Health Stud 33:151
28. Rossmann A, Schmidt HL (1997) Anal Chim Acta 347:359
29. Jamin E, Gonzalez J, Remaud G, Naulet N, Martin GG, Weber D, Rossmann A, Schmidt, HL (1997) Anal Chim Acta 347:359
30. Jamin E, Gonzalez J, Remaud G, Naulet N, Martin GJ (1997) J Agric Food Chem 45:3961
31. Jamin E, Gonzalez J, Bengoechea I, Kerneur G, Remaud G, Naulet N, Martin GG (1998) JAOAC Int 81:604
32. Weber D, Rossmann A, Schwartz S, Schmidt HL (1997) Z Lebensm Unters Forsch 205:158
33. Schmidt HL, Butzenlechner M, Rossmann A, Schwarz S, Kexel H, Kempe K (1993) Z Lebensm Unters Forsch 196:105
34. Weber D, Kexel H, Schmidt HL (1997) J Agric Food Chem 45:2042
35. Woodbury SE, Evershed RP, Rossell JB, Griffith RE, Farnell P (1995) Anal Chem 67:2685
36. Kelly S, Parker I, Sharman M, Dennis J, Goodall I (1997) Food Chem 59:181
37. Woodbury SE, Evershed RP, Rossell JB (1998) J Am Oil Chem Soc 75:371
38. Spangenberg JE, Macko SA, Hunziker J (1998) J Agric Food Chem 46:4179
39. Royer A, Gerard C, Naulet N, Less M, Martin GJ (1999) JAOCS 76:357

40. Royer A, Gerard C, Naulet N, Less M, Martin GJ (1999) JAOAC 76:365
41. Angerosa F, Camera L, Cumitini S, Gleixner G, Reniero F (1997) J Agric Food Chem 45:3044
42. Gucek M, Marsel J, Ogrinc N, Lojen S (1998) Acta Chim Slov 45:217
43. Koziet J, Rossmann A, Martin GJ, Ashurst PR (1993) Anal Chim Acta 271:31
44. Martin GG, Hanote V, Lees M, Martin YL (1996) JAOAC Int 79:62
45. Kosir IJ, Kocjancic M, Ogrinc N, Kidric J (2001) Anal Chim Acta 429:197
46. Spangenberg JE, Ogrinc N (2001) J Agric Food Chem 49:1534
47. Fang TT (1988) Modern methods of plant analyses, vol 8, Analysis of non-alcoholic beverages. Springer, Berlin, p 51
48. Belton PS, Delgadillo I, Holmes E, Nicholls A, Nicholson JK, Spraul M (1996) J Agric Food Chem 44:1483
49. Belton PS, Delgadillo I, Gil AM, Roma P, Casuscelli F, Colquhoun IJ, Dennis MJ, Spraul M (1997) Magn Reson Chem 35:S52
50. Le Gall G, Puaud M, Colquhoun IJ (2001) J Agric Food Chem 49:580
51. Židkova J, Chmelik J (2001) J Mass Spectrom 36:417
52. Garcia-Wass F, Hammond D, Mottram DS, Gutteridge CS (2000) Food Chem 69:215
53. Goodner KL, Rouseff RL (2001) J Agric Food Chem 49:250
54. Rapp A, Markowetz A, Spraul M, Humpfer E (1988) GIT Fachz Lab 9:375
55. Rapp A, Markowetz A, Niebergall H (1991) Z Lebensm Unters Forsch 192:1
56. Rapp A, Spraul M, Humpfer E (1997) In Vino Analytica Scientia, Bordeaux, 359
57. Holland MV, Bernreuther A, Reniero F (1995) In: Belton PS, Delgadillo I, Gil MA, Webb G (eds) Magnetic resonance in food science. Royal Society of Chemistry, Cambridge, p 136
58. Kosir IJ, Kocjancic M, Kidric J (1998) Analusis 26:97
59. Kosir IJ, Kidric J (2002) Anal Chim Acta 458:77
60. Brescia MA, Kosir IJ, Caldarola V, Kidric J, Sacco A (2003) J Agric Food Chem 51:21
61. Leitner A, Zöllner P, Paolillo A, Stroke J, Papadopoulou-Bouraoui A, Jaborek S, Anklam E, Lindner W (2002) Anal Chim Acta 453:33
62. Vlahov G (1999) Progr Nucl Magn Reson Spectrosc 35:341
63. Sacci R (2001) In: Webb GA, Belton PS, Gil MA, Delgadillo I (eds) Magnetic resonance in food science. Royal Society of Chemistry, Cambridge, pp 213
64. Sacchi R, Patumi M, Fontanazza G, Barone P, Fiordiponti P, Mannina L, Rossi E, Serge AL (1996) J Am Oil Chem Soc 73: 747
65. Sacchi R, Addeo F, Paolillo L (1997) Magn Reson Chem 35: S133–S145
66. Sacchi R, Addeo F, Guidicianni I, Paolillo (1989) Riv Ital Sost Grasse 56:171
67. Zamora R, Navarro JL, Hidalgo FJ (1994) J Am Oil Chem Soc 71:361
68. Regulation no. 2568/91, EC Official J. L248, September 5th, 1991
69. Regulation no. 656/95, EC Official J. L69), March 29th, 1995
70. Sacchi R, Addeo F, Giudicianni I, Paolillo (1992) Ital J Food Sci 4:117
71. Mavromoustakos T, Zervou M, Bonas G, Kolocouris A, Petrakis P (2000) J Am Oil Chem Soc 77:405
72. Sacchi R, Mannina L, Fiordiponti P, Barone P, Paolillo L, Patumi M, Segre A (1998) J Agric Food Chem 46:3947
73. Sacco A, Brescia MA, Liuzzi V, Reniero F, Guillou C, Ghelli S, van der Meer P (2000) JAOCS 77:619
74. Brescia MA, Alviti G, Liuzzi V, Sacco A (2003) J Am Oil Chem Soc, in press
75. Goodcare R, Kell DB, Bianchi G (1993) J Sci Food Agric 63: 297
76. Sies A, Loescher R, Tablack P, Guth H (2002) AOCS Archives, 93rd AOCS Annual Meeting and Expo Abstracts, Montreal, Canada, May, 2002