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A measuring system for the fast simultaneous isotope ratio and elemental analysis of carbon, hydrogen, nitrogen and sulfur in food commodities and other biological material

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The isotope ratio of each of the light elements preserves individual information on the origin and history of organic natural compounds. Therefore, a multi-element isotope ratio analysis is the most efficient means for the origin and authenticity assignment of food, and also for the solution of various problems in ecology, archaeology and criminology. Due to the extraordinary relative abundances of the elements hydrogen, carbon, nitrogen and sulfur in some biological material and to the need for individual sample preparations for H and S, their isotope ratio determination currently requires at least three independent procedures and approximately 1 h of work. We present here a system for the integrated elemental and isotope ratio analysis of all four elements in one sample within 20 min. The system consists of an elemental analyser coupled to an isotope ratio mass spectrometer with an inlet system for four reference gases (N_2, CO_2, H_2 and SO_2). The combustion gases are separated by reversible adsorption and determined by a thermoconductivity detector; H_2O is reduced to H_2. The analyser is able to combust samples with up to 100 mg of organic material, sufficient to analyse samples with even unusual elemental ratios, in one run. A comparison of the isotope ratios of samples of water, fruit juices, cheese and ethanol from wine, analysed by the four-element analyser and by classical methods and systems, respectively, yielded excellent agreements. The sensitivity of the device for the isotope ratio measurement of C and N corresponds to that of other systems. It is less by a factor of four for H and by a factor of two for S, and the error ranges are identical to those of other systems.

Isotope ratio measurements have become the most important and most frequently applied method for origin and authenticity determinations of food commodities,[1–3] fragrances or flavourings,[4] and other natural products.[5] They have also been indispensable for the treatment and understanding of environmental and ecological questions,[6] in food web and nutrition studies,[7,8] in archaeology,[9] and in forensic sciences.[10] All of these investigations demonstrate that it is advantageous, perhaps even obligatory, to perform the analyses, whenever possible, as a multi-element or multi-compound/multi-element isotope ratio determination. As the isotope ratio of each of the bioelements is individually correlated to the conditions of biosynthesis and secondary treatments of natural compounds, a multi-element isotope analysis will provide the greatest amount of independent information for the characterisation and assignment of origin of the material in question.

The source of adsorbed water and of bound oxygen and hydrogen in the biological material under investigation is always water; therefore, the corresponding isotope ratios reflect the isotopic signature of the local precipitation of the production area and so may be correlated to its latitude or its distance from the ocean. In some cases, the isotope ratio data of the tissue water of an unknown biological sample may be indicative of its geographic origin but often the isotope characteristics of this water can be modulated by secondary external influences. Therefore, it would be better and more reliable to measure the isotope ratios of the organically bound hydrogen or oxygen, which preserve the original information, and integrate it over a longer period. The carbon isotope ratio plant material is determined primarily by the photosynthesis type of the plant in question (C_3-, C_4-metabolism and crassulacean acid metabolism (CAM)), but also by local and

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temporal climate conditions, under which it has grown;[11] via food chains the isotope signature is correspondingly transferred to animal material. Nitrogen isotope ratios can give information on the primary nitrogen sources of plants such as the fertilisers used or, in case of animal material, on its primary protein source. Finally, sulfur isotopes and isotopes of heavier elements (e.g. Sr) are indicators for geological characteristics or for anthropogenic influences on the environment of the origin of the sample (e.g. Pb).

The isotope ratio analysis of an organic sample implies its conversion into simple gases by the Dumas combustion procedure using an elemental analyser and the subsequent mass spectrometric determination on the isotopologues of the product gas molecules. Normally, after the combustion of the sample, the product gas molecules are separated by gas chromatographic or cryogenic procedures. Water and SO_2 are (chemically) removed and only N_2 and CO_2 are used for the isotope ratio measurements of nitrogen and carbon isotopes, respectively. The isotope ratio analysis of sulfur as a minor element in biological material is normally carried out as a single measurement, using larger samples. Hydrogen and oxygen isotope analyses are performed on H_2 and CO, respectively, and a separate pyrolytic sample preparation must be applied for each element. Thus, the conventional multi-element isotope analysis of a compound including oxygen demands up to four independent weighing, conversion and measurement processes – a time- and labour-consuming procedure.

The Vario EL III elemental analyser from Elementar Analysensysteme GmbH (Hanau, Germany) has proved its suitability for the simultaneous isotope ratio determination of nitrogen, carbon and sulfur isotopes in a single run.[12,13] One of the major advantages of this instrument is the fact that it is capable of combusting up to 100 mg of organic samples corresponding to up to 30 mg of carbon. This size of such samples is large enough for the provision of sufficient amounts of SO_2 for the isotope analysis even from material with low sulfur content. A second characteristic of the elemental analyser is that the separation of the combustion gases is performed by reversible adsorption on a series of (molecular sieve) traps, each of which is allocated to a specific gas species. The aim of the present investigation is to extend the capability of the system by the further addition of a hydrogen isotope ratio measurement. This will be achieved by the integration of a reduction furnace with a suitable packing that will reduce the water of combustion directly to hydrogen gas, from which the hydrogen isotope ratio analysis will be made. This supplementation demands in addition the adaptation of a suitable mass spectrometer to the elemental analyser by the development of corresponding hardware and software in order to obtain an integrated automatic multi-element isotope ratio analysis system.

DISPLAY AND FUNCTIONAL DIAGRAM OF THE SYSTEM

The device consists of an Vario EL III elemental analyser from Elementar Analysensysteme GmbH with an automatic carousel for up to 80 samples, supplemented with a water reduction unit and an IsoPrime (GV Instruments, Manchester, UK) stable isotope ratio mass spectrometer (IRMS) with H/D collector and a reference gas injector for four gases, monitor, printer and IonVantage software control of the system (Figs. 1(A) and 1(B)).

Figure 2 shows a functional diagram of the elemental analyser and the reference gas injector; the system is additionally equipped with a thermoconductivity detector (TCD), providing the opportunity for the determination of the amounts of the combustion gases as a prerequisite for the quantitative elemental analysis.

Each sample analysis including elemental composition and the isotope ratio determination of N, C, H and S takes 20 min. The sequence of the inlets of the gases and their references is indicated in Fig. 3. The display for each

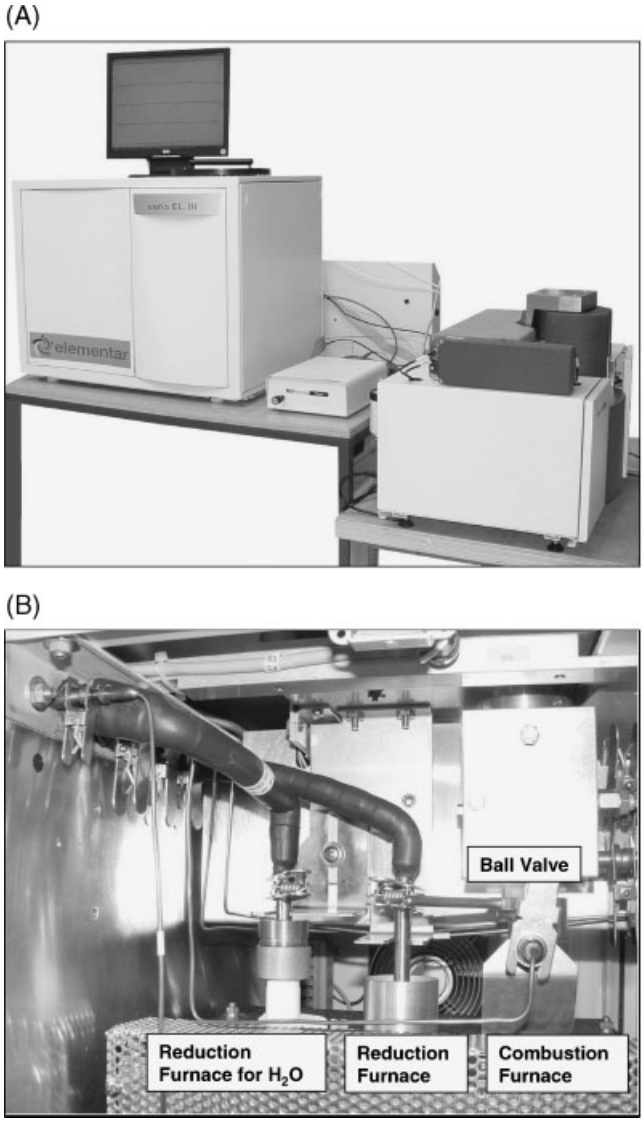

Figure 1. (A) The multi-element CHNS isotope ratio analyser. From left to right: monitor and supplemented Vario EL III elemental analyser; gas dilution system (front) with part of the quadruple reference gas inlet system (behind); GV Instruments IsoPrime mass spectrometer. (B) Interior of the elemental analyser, showing the combustion furnace, the NO_x and SO_3 reduction furnace and the additional furnace for the water reduction. In the upper right part the ball valve for the sample introduction can be seen.

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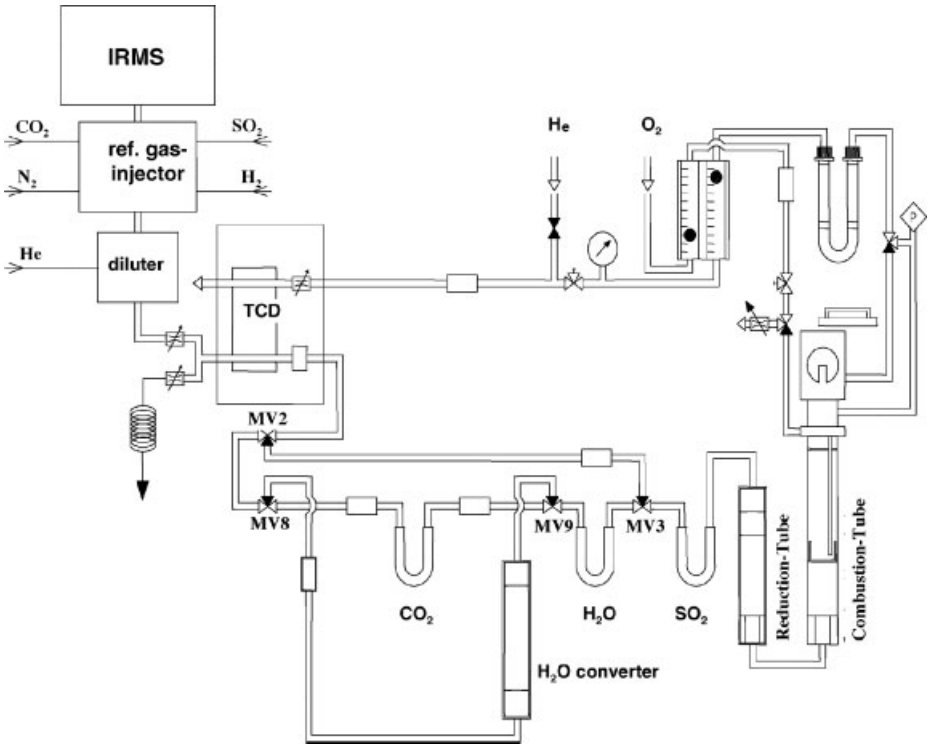

Figure 2. Functional diagram and running mode of the supplemented Vario EL III elemental analyser for the simultaneous elemental analysis and isotope ratio mass spectrometry of hydrogen, nitrogen, carbon and sulfur in a single sample (sample size up to 100 mg). MV = magnetic valve, TCD = thermoconductivity detector, IRMS = isotope ratio mass spectrometer (GV Instruments IsoPrime). By means of a ball valve the sample (in a tin capsule) is dropped into the combustion tube (WO_3 at 1150°C). The gases pass through a reduction tube (Cu at 850°C) for the reduction of NO_x and SO_3, then through three traps with specific adsorbers for the condensation of SO_2, H_2O and CO_2, respectively. N_2 is immediately transferred to the IRMS followed, after heating of the corresponding trap, by CO_2. The water trap is then heated and the water passed (by-pass via MV9/MV8) through the H_2O converter (Mg at 600°C). Finally, SO_2 is liberated and transported (short-cut MV3/MV2) to the IRMS.

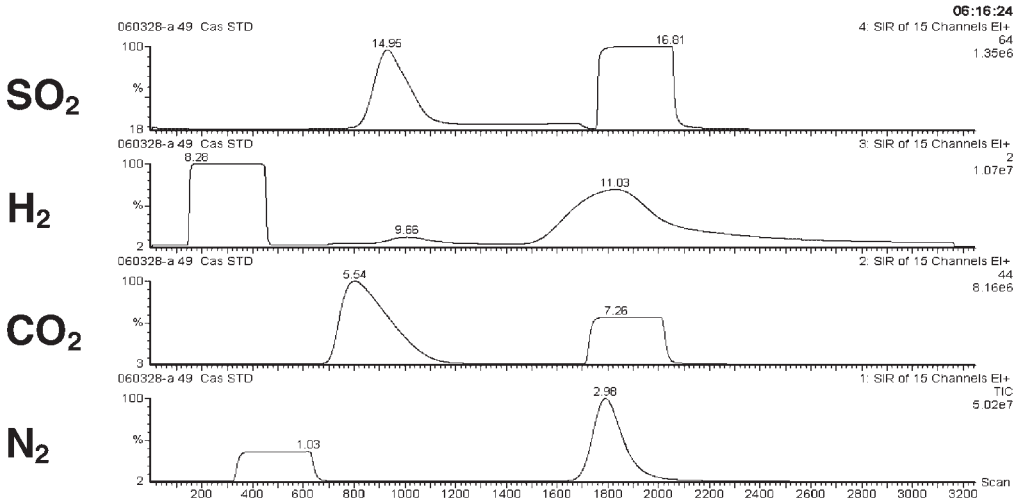

Figure 3. Display of the mass traces (from bottom to top) of the four gases for the isotope ratio determination of a sample of cheese protein (casein) with N, C, H and S. The measurement sequence is for N_2: reference – sample, for CO_2: sample – reference, for H_2: reference – sample and for SO_2: sample – reference. The scales for each measuring gas are relative to the corresponding maximum = 100%, the numbers on the tops of the peaks indicate the time after start of the run. The total time for one run, yielding the information of elemental content and isotope ratio for four elements, is 20 min. The printout provides elemental content in % and isotope ratios in ‰ relative to international standards.

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Table 1. Water reduction: memory effects and reproducibility (sample size 6 μL)

Sample	δ^2H [‰]$_{V\text{-}SMOW}$
Drinking water, Hanau	−73.7
	−58.6
	−56.9
	−57.7
SLAP (theoretical −428.0‰)	−352.8
	−414.9
	−418.6
	−420.4
	−419.4
Drinking water, Hanau	−127.2
	−57.7
	−57.3
	−57.4

SLAP: Standard Light Antarctic Precipitation.

individual gas is relative to the individual maximum concentration = 100%.

The equipment and the catalysts for the sample oxidation (WO_3-granulate) and for the reduction of NO_x and SO_3 (Cu at 850°C) are well established (Application Sheet from Elementar Analysensysteme) and need no further comments. The reduction of water for the hydrogen isotope analysis has formerly been performed with U,[14] a quite good but dangerous reductant. Although Ni, Zn and more recently Cr are most commonly used for reduction,[15] we checked the suitability of other metals. W, Mn, Fe and Mo were not satisfactory, as these metals are rapidly inactivated by forming surface layers of their oxides. The reduction of the combustion water with Mg at 600°C was quantitative and allowed a large sample throughput due to the 100% availability and conversion of the metal. As with other metals, memory effects were observed (Table 1). These were obviously due not to an intermediate binding of water to the reductant, but rather to the glass surfaces. As only the runs after a change between samples with quite large deuterium differences were affected, we decided to use Mg in our device.

Table 2. Comparison of results from conventional (conv.) method (HT pyrolysis for δ^2H) and simultaneous CHNS analysis of Ca-citrate (for detection of citric acid addition to fruit juices)

Sample		δ^2H [‰]$_{V\text{-}SMOW}$	δ^{13}C [‰]$_{V\text{-}PDB}$
Synthetic mixture of cane and beet sugars	conv.	−68.7	−16.86
	CHNS	−64.2 ± 3.8	−17.06 ± 0.04
■synthetic from beet sugar	conv.	−84.3	−24.92
	CHNS	−79.9	−25.22 ± 0.15
■synthetic from beet sugar	conv.	−79.5	−24.86
	CHNS	−78.3 ± 2.5	−24.91 ± 0.08
● synthetic/ natural mixture	conv.	−46.8	−24.47
		−41.5 ± 3.9	−24.38 ± 0.04
● synthetic/ natural mixture	conv.	−50.3	−24.42
	CHNS	−45.6 ± 3.5	−24.33 ± 0.09
natural from orange	conv.	−17.0	−23.99
	CHNS	−15.2 ± 3.6	−23.87 ± 0.06

■/● are blind duplicates.

COMPARISON OF CHNS ANALYSER RESULTS WITH THOSE FROM CONVENTIONAL ANALYSES

In order to check the trueness and reliability of the data obtained with the new system, samples were measured which had independently been analysed before using conventional methods and in other instruments. Hydrogen samples had been measured by a standard high-temperature (HT) pyrolysis technique[16] by Dr C. Schlicht, Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim, Germany, on a Thermo-Finnigan XP plus IRMS. These data are given in Tables 2 and 3.

Citric acid for the adulteration of fruit juices is prepared by fermentations of sugars with microorganisms. The use of inexpensive sugars from cane or high fructose corn syrup (C_4-plant origin) can easily be detected by the δ^{13}C-value, whereas C_3-sugar as source can only be found via the measurement of the deuterium content of the non-exchanging methylene groups. This measurement was originally performed by the esterification of the acid and the ^{2}H-NMR analysis of the ester, a very laborious procedure

Table 3. Comparison of data from conventional (conv.) method (HT pyrolysis for δ^2H) and simultaneous CHNS analysis. Casein from cheese of different Alpine valleys in Italy. Conventional values by Dr. C. Schlicht, Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Oberschleißheim, Germany

Sample		δ^{15}N [‰]$_{AIR}$	δ^{13}C [‰]$_{V\text{-}PDB}$	δ^2H [‰]$_{V\text{-}SMOW}$	δ^{34}S [‰]$_{V\text{-}CDT}$
Asiago	conv.	4.77	−19.25	−105.9	5.51
	CHNS	4.91 ± 0.03	−19.43 ± 0.02	−106.4 ± 1.5	5.35 ± 0.04
Fontina	conv.	4.68	−22.89	−121.6	4.51
	CHNS	4.93 ± 0.06	−23.20 ± 0.04	−123.4 ± 0.5	4.23 ± 0.17
Montasio	conv.	4.81	−18.55	−104.7	4.53
	CHNS	4.89 ± 0.15	−18.59 ± 0.16	−105.2 ± 1.1	3.15 ± 0.15
Nostr. Castf.	conv.	4.43	−22.10	−122.2	7.04
	CHNS	4.75 ± 0.14	−22.13 ± 0.05	−113.3 ± 0.8	6.63 ± 0.18
Spressa	conv.	4.48	−21.78	−119.6	5.47
	CHNS	4.66 ± 0.05	−21.83 ± 0.06	−116.4 ± 0.7	5.65 ± 0.11
Toma	conv.	5.27	−21.07	−119.6	3.80
	CHNS	5.64 ± 0.31	−21.05 ± 0.04	−111.6 ± 1.3	4.27 ± 0.15
Vezzena	conv.	4.21	−22.13	−110.9	5.21
	CHNS	4.55 ± 0.17	−22.01 ± 011	−113.8 ± 0.9	5.16 ± 0.05

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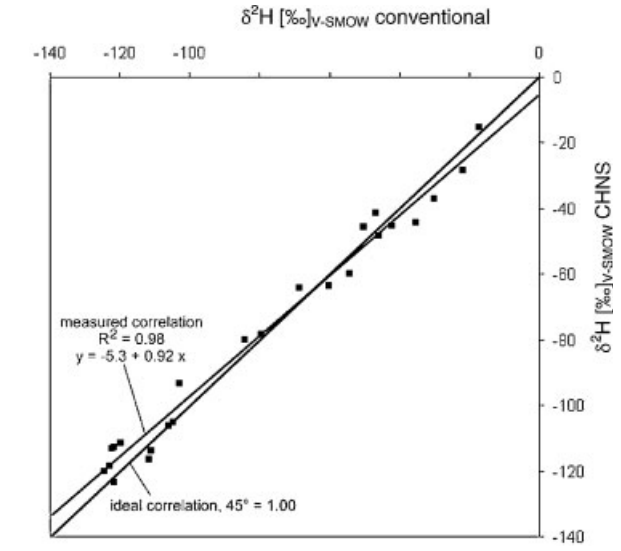

Figure 4. Comparison of δ^2H-values of water and organic samples obtained by conventional analysis (high-temperature pyrolysis) and with the CHNS isotope analyser.

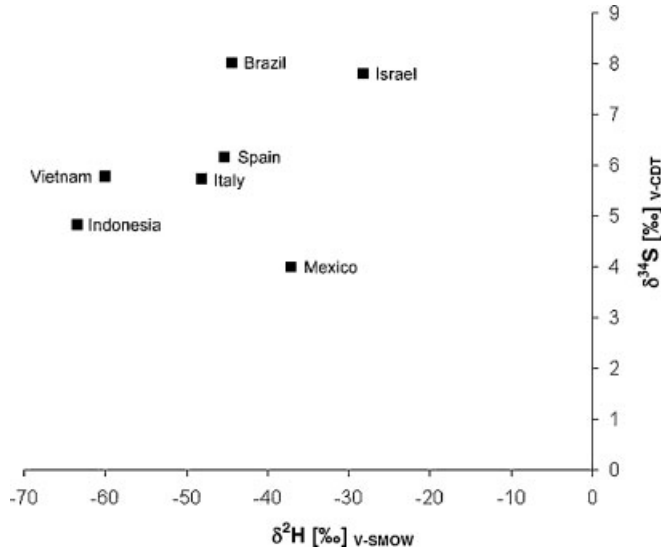

Figure 5. δ^2H- and δ^{34}S-values of fruit juice pulps obtained by simultaneous isotope analysis with the CHNS analyser. Values are from Table 4.

requiring very large samples.[17] The same result can be more easily obtained by the conventional HT pyrolytic deuterium analysis of Ca-citrate.[18] We have compared data of Ca-citrate samples from classical analyses (δ^{13}C by combustion, δ^2H by HT pyrolysis) with those simultaneously produced by our multi-element CHNS analyser and found excellent agreement (Table 2). Quite good agreement between results by the analyser and by conventional measurements was also obtained for all four elements in casein as the basis for the origin assignment of cheese from different Alpine valleys in Italy (Table 3). For samples with relatively high S content like human hair, an individual ^{2}H-determination by HT pyrolysis was more advantageous than that by the integrated water reduction. A comparison of all the conventionally measured ^{2}H-abundances of water and organic compounds and that acquired to date using the CHNS analyser is shown in Fig. 4. The correlation between the results from the two methods is quite convincing, and the differences between the data from the two modes are within the normal error range for deuterium analyses.

APPLICATIONS OF THE CHNS ANALYSER FOR THE ANALYSIS OF UNKNOWN SAMPLES

The advantage of the automated multi-element isotope analysis is that data from all elements are simultaneously available, providing the opportunity to select the most efficient combination for the solution of a given question even in cases where the other data are of minor value for an origin assignment. The δ^{13}C-values of the fruit juice pulp samples shown in Table 4 are in line with the origin of these samples from C_3- or CAM-plants excluding the addition of sugars from a source of the other group but not sufficient for excluding adulteration with sugars from the same group. Similarly, the δ^{15}N-values of the samples cannot be used for an origin assignment because there are insufficient references available in this case. However, a combination of the δ^2H- and δ^{34}S-values as indicators for local climate and soil characteristics (Fig. 5) is indicative of such an origin assignment because the values are in good agreement with results from a data bank established within a European research project ('PURE JUICE') on origin and authenticity investigations on fruit juices.[19] Similarly, satisfactory results have been obtained with the origin identification of Emmenthaler and other PDO (protected denomination of origin) cheese samples from different European countries by comparison with data from Pillonel *et al*.[20,21]

A promising result has also been obtained in the origin investigation of wine and fruit (juices) via the fermentation product ethanol. Traditionally, this investigation is performed by a combination of a δ^{13}C-value determination by conventional combustion-IRMS analysis with a positional

Table 4. CHNS simultaneous stable isotope analysis of fruit juice pulp from different countries

Sample	δ^{15}N [‰]$_{AIR}$	δ^{13}C [‰]$_{V\text{-}PDB}$	δ^2H [‰]$_{V\text{-}SMOW}$	δ^{34}S [‰]$_{V\text{-}CDT}$
Pineapple/Indonesia	1.17 ± 0.17	−13.69 ± 0.25	−63.4 ± 1.8	4.84 ± 0.10
Grapefruit/Israel	6.11 ± 0.02	−25.69 ± 0.13	−28.3 ± 0.9	7.82 ± 0.25
Pineapple/Vietnam	4.89 ± 0.03	−13.27 ± 0.07	−60.0 ± 1.8	5.79 ± 0.25
Orange/Mexico	5.77 ± 0.03	−25.96 ± 0.11	−37.2 ± 1.1	3.99 ± 0.11
Orange/Spain	5.57 ± 0.03	−25.38 ± 0.11	−45.3 ± 0.8	6.16 ± 0.25
Orange/Italy	5.54 ± 0.11	−24.80 ± 0.12	−48.2 ± 1.2	5.73 ± 0.08
Orange/Brazil	6.09 ± 0.09	−25.75 ± 0.25	−44.5 ± 1.3	8.02 ± 0.35

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deuterium analysis by nuclear magnetic resonance (NMR) spectroscopy. The latter demands large amounts of sample, long measuring times and very expensive instrumentation. We have combined the ^{13}C-analysis in our CHNS analyser with the simultaneous (average) ^{2}H-determination on the raw ethanol (distillate, >92% alcohol as used for the NMR analyses). Figure 6(A) shows that the average $\delta^{2}H$-values are in satisfactory agreement with the NMR data and Fig. 6(B) that the isotope data of the ethanol from samples of different European areas form clusters permitting the origin assignment of most of them. This indicates that NMR analysis is not absolutely necessary for wine origin analysis but that in many cases the average $\delta^{2}H$-values, simultaneously and gratuitously obtained together with the $\delta^{13}C$-values by IRMS, will be sufficient or useful for a preliminary check.

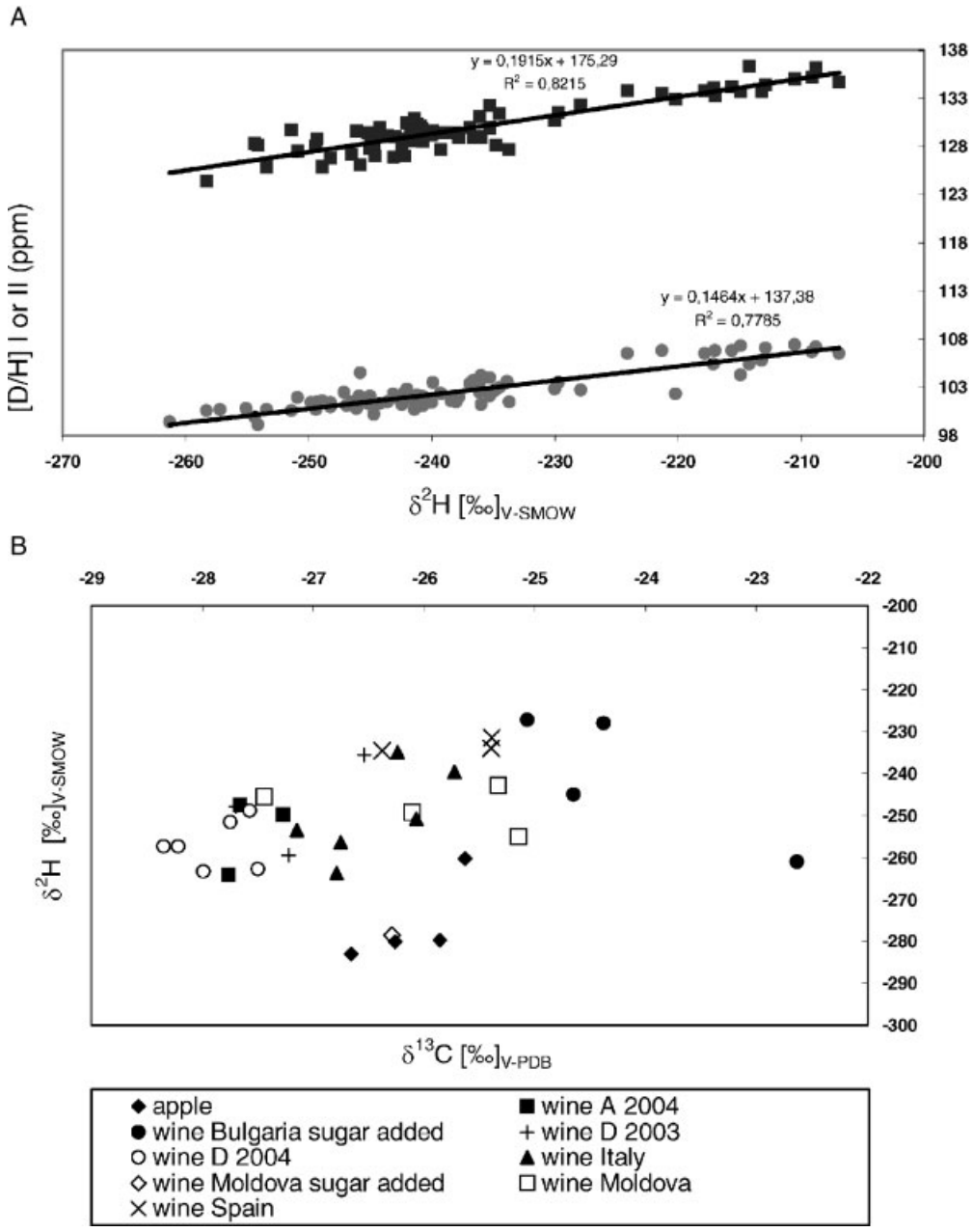

Figure 6. (A) Comparison of the average $\delta^{2}H$-values of ethanol from wine and fermented apple juice samples from different European countries with their positional NMR data (ppm). $(D/H)_{I}$-values (CH_3-group) lower curve, $(D/H)_{II}$-values ($-CH_2$-group) upper curve. Samples and NMR data are by courtesy of Dr. N. Christoph, Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Würzburg, Germany. As the average of the ppm-values (for corresponding calculation see Schmidt *et al.*[4]) would not imply the abundance of the hydrogen of the OH-group, it cannot be absolutely compared with the $\delta^{2}H$-values. The latter are, however, a valuable origin indicator. (B) Geographical origin clusters of ethanol from fermented apple juice and wine from different European countries by simultaneous $\delta^{2}H$ and $\delta^{13}C$ measurement with the CHNS analyser.

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Table 5. Sensitivity (minimum element amount needed) and recommended sample sizes in routine multi-element isotope ratio analysis. Values in parentheses are for HT pyrolytic analysis of hydrogen and for special sulfur isotope ratio analysis, respectively

Parameters	N	C	H	S
Minimum amount [μg]	100	100	200 (50)	20 (10)
Recommended working amount [μg]	300–500	1500–8000	300–800 (80–120)	30–100
Error range (δ-units)	0.2	0.1	3.0	0.3
Minimum rel. ratio to C	0.05		0.1	0.005

CONCLUSIONS

Multi-element isotope ratio mass spectrometry is the most suitable analytical method for the authenticity check and origin assignment of food commodities and many other organic products, and for the investigation of food chain, nutrition and environmental questions. The combination of a multifunctional elemental analyser, supplemented with a unit for water reduction, and a suitable mass spectrometer, provided a potent device for the simultaneous elemental analysis and isotope ratio determination of H, C, N and S. A complete determination of the elemental content and the isotope ratio of the four elements can be performed on a sole sample within 20 min. The capacity of the elemental analyser permits the combustion of organic samples up to 100 mg, providing the possibility to analyse material with very low elemental concentrations and extraordinary elemental ratios.

The sensitivity (minimum amounts needed) of the system for the isotope ratio measurement of C and N is identical to that of any conventional system. Compared with the direct high-temperature pyrolysis determination of δ^2H-values, the sensitivity of the system is less by a factor of 4, and the minimum amount of sulfur needed for an isotope analysis is about twice that reported for classical systems.[22] The characterisation and recommendations for the samples size needed in the practical routine analysis with the multi-element isotope ratio analyser are given in Table 5.

These results indicate that routine simultaneous isotope ratio analyses of a maximum of four elements will be possible with 10–15 mg crude plant dry matter (very low sulfur content), 2–6 mg of a protein and 4–5 mg organic acids or carbohydrates. The amounts of sample needed for the analysis of material with extreme element ratios will be checked in the future. The values given in the tables are means of three individual measurements. The standard deviations for the measurements of δ^2H-values is $\leq$3.0‰, for δ^{13}C-values 0.1‰, for δ^{15}N-values 0.2‰, and for δ^{34}S-values 0.3‰; these are identical to those obtained with other systems. A detailed discussion of the obtained results in regard to the origin assignment of the investigated samples is inappropriate in the present context but will be the topic of special reports on the application of the system, not only for food origin and authenticity determination, but also for investigations on questions in archaeology and forensic chemistry.

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