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FTIR and NDIR spectroscopies as valuable alternatives to IRMS spectrometry for the $\delta^{13}C$ analysis of food

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ABSTRACT

The $^{13}C/^{12}C$ carbon isotope ratio is a chemical parameter with many important applications in several scientific area and the technique of choice currently used for the $\delta^{13}C$ determination is the isotope ratio mass spectrometry (IRMS). This latter is highly accurate (0.1‰) and sensitive (up to 0.01‰), but at the same time expensive and complex. The objective of this work was to assess the reliability of FTIR and NDIRS techniques for the measurement of carbon stable isotope ratio of food sample, in comparison to IRMS. IRMS, NDIRS and FTIR were used to analyze samples of food, such as oil, durum, cocoa, pasta and sugar, in order to determine the natural abundance isotopic ratio of carbon in a parallel way. The results were comparable, showing a close relationship among the three techniques. The main advantage in using FTIR and NDIRS is related to their cheapness and easy-to-operate in comparison to IRMS.

1. Introduction

Stable isotope analysis has gained increasing interest in the study of the geographic origin of food products [1–4] giving the possibility for testing food authenticity [5–7], quality and typicality [8–15], particularly in the areas where conventional analytical methods cannot provide unambiguous results. Moreover, it has been recognized as an important technique for the traceability of animal-derived food products including cheese [16], milk [17] and butter [18]. In 2000 the EU mentioned traceability as one of the basic principles of consumer protection [19].

The highly sensitive (up to 0.01‰), but equally expensive system based on isotope ratio mass spectrometry (IRMS) is the method of choice for the ^{13}C measurements in terms of $\delta^{13}C$ [20]. However new and cheaper measuring equipment have been designed as alternatives to the IRMS for the ^{13}C analysis [21]. By the use of NDIRS an accuracy of ± 0.4‰ can be obtained [22–24]. Several comparative studies have shown the reliability of this technique in measuring ^{13}C enrichment, thus allowing it to be considered as a valid alternative to IRMS [25–28]. Moreover, some spectroscopic methods of $\delta^{13}C$ analysis have been proposed as alternatives to IRMS, based on the fact that isotopic substitution will affect the distribution of vibrational and rotational energy states of a molecule. Consequently, each distinct isotopomer of CO_2 has its own rotational – vibrational infrared spectrum. However, the main difficulty in measuring the contribution of each isotopomer is that the peaks are overlapped to an extent that does not allow an accurate determination of the relative contribute to the overall area. In the recent literature, a few attempts are reported for the measurement of $\delta^{13}C$ by using Fourier Transform Infrared Spectroscopy. Kidness and Marr, utilizing high-resolution spectrometers (0.25 cm^{-1}, and 1 cm^{-1} at elevated sample pressures) obtained an accuracy of ± 12‰ and ± 8‰ [29,30]. To execute accurate FTIR (Fourier Transform Infrared) analysis, we developed a method based on the absorption of CO_2 into polystyrene films to provide narrow, sharp, and well resolved IR absorption bands for the $\nu 3$ antisymmetric stretching mode of both $^{12}CO_2$ and $^{13}CO_2$ isotopomers [31]. This feature can be attributed to the reduction in the rotational mode of CO_2 as a result of the interaction between CO_2 molecule and the aromatic ring of polystyrene. By this way an accuracy of ± 2.5‰ can be obtained [31]. In a previous work we compared IRMS, NDIRS and FTIR for ^{13}C-urea breath test in the non-invasive diagnosis of *Helicobacter pylori* infection [32] showing that the newly developed FTIR methodology is a reliable and accurate analytical tool, low cost and easy-to-operate, which permits a highly specific measurement of ^{13}C

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enrichment in breath samples. As well as IRMS and NDIRS it allows to adequately discriminate between infected and non-infected subjects. FT-IR technique has been used for the quantification of adulterants in Mexican honeys [33] and, at the best of our knowledge, neither FT-IR nor NDIRS have ever been used for identification of isotopic composition of other kind of food samples.

In this paper we used these two techniques for the analysis of $\delta^{13}C$ in different kind of food in order to assess their reliability in comparison with IRMS.

2. Materials and methods

2.1. Materials

This study was conducted on 19 selected samples of pasta, oil, chocolate, cocoa, flour, sugar, typical components of the "Mediterranean Diet". These samples were collected within the framework of the Campus Project (Introduction and valorization of healthful foods and productive rationalization in the traditional industries of the Campania region) funded by the Italian Campania Region (POR CAMPANIA FESR 2007–2013). Samples were used as received without any preliminary treatment. More detailed description of the samples is reported in Table 1.

2.2. Stable carbon isotope ratio analysis

2.2.1. Isotopic analysis of bulk materials by Elemental Analysis/Isotope Ratio Mass Spectrometry (EA/IRMS)

A Delta Plus V Isotope Ratio Mass Spectrometer (ThermoFinnigan, Bremen, Germany) equipped with a Flash EA 1112 Elemental Analyzer (ThermoFinnigan) was used to measure $\delta^{13}C$. The $\delta^{13}C$ isotopic values were calculated using a homogenized in-house protein standard which was itself calibrated against international reference materials: L-glutamic acid USGS 40 (IAEA International Atomic Energy Agency, Vienna, Austria), fuel oil NBS-22 (IAEA) and sugar IAEA-CH-6 for $^{13}C/^{12}C$. The measurement uncertainty, expressed as 2 SR (standard deviation of reproducibility), is 0.3‰.

2.2.2. NDIRS

For NDIRS (Non Dispersive Infrared Spectroscopy) analysis the samples were reduced to carbon dioxide. The samples were combusted in pure oxygen at T = 1000 °C. Carbon dioxide gas was collected in an impringer filled with 100 mL of barium hydroxide, $Ba(OH)_2$, 0.3 M. This reaction started with a flash and carried on by itself. CO_2 reacted with $Ba(OH)_2$ and it was quantitatively converted in barium carbonate, $BaCO_3$.

$$Ba(OH)_2 + CO_2 \rightarrow BaCO_3 + H_2O$$

Before analysis, $BaCO_3$ was washed with boiled water and dried under vacuum at 120 °C for 1 h. 100 mg carbonate were introduced into a 10 mL glass flask, that was evacuated, and 2.5 mL of orthophosphoric acid was syringed to produce carbon dioxide. CO_2 gas produced was collected in a specific aluminized bag. NDIRS spectroscopy was conducted by means of a Heli-FANplus analyzer (Medimar Srl, Milan) equipped with a single beam non-dispersive infrared industrial photometer. The aluminized bags were directly connected with the inlet ports of the NDIR spectrometer for sequential measurements. The NDIRS device was interfaced to a computer system that enables the software-guided measurement and calculation of results.

All the chemicals were purchased from Sigma Aldrich.

NDIRS calibration was performed by using international standard purchased from the International Atomic Energy Agency (IAEA) (marble, $\delta^{13}C = +2.5 \pm 0.1$‰).

The measurement uncertainty is 0.6‰, expressed as 2 SR (standard deviation of reproducibility).

2.2.3. FTIR

For FTIR analysis, the sample was reduced to carbon dioxide gas

Table 1
The composition of samples used for the $\delta^{13}C$ analysis.

Identification	Sample	Description	Composition
1	Oil	Sunflower oil	Commercial sunflower oil
2	Oil	Home made extravirgin olive oil	Italian extravirgin olive oil 100%
3	Oil	Extravirgin olive oil	Commercial extravirgin Olive Oil "100% Italian"
4	Oil	Extravirgin olive oil with omega 3	Extravirgin olive oil 100% Italian with 2% wt of fish oil (with omega 3 and containing about 18% EPA[a] and 9% DHA[b]). Final oil should be about 0.4 g of EPA and 0.2 g DHA per 100 g. Extract of fish oil with about 18% EPA[a] and 9% DHA[b]
5	Oil	Fish oil extract	Extract of fish oil with about 18% EPA[a] and 9% DHA[b]
6	Wheatflour 0	Wheat flour 0	Italian wheat flour, a bit less refined about 70% of the grain, and a bit darker.
7	Wheatflour 00	Wheat flour 00	The softest, finest, Italian flour; very finely ground like a fine powder and very white.
8	Cane Sugar	Cane Sugar	Sucrose extracted from sugar cane
9	semolinaflour	Commercial semolinaflour	Semolina from Italian durum wheat
10	Semolinaflour	Commercial semolinaflour	Semolina from Italian durum wheat
11	Semolinaflour	Commercial semolinaflour	Semolina from Italian durum wheat
12	Pasta	Pasta with luteina	Italian durum wheat; lutein extracted from tomato skins local (20 mg/100 g)
13	Pasta	Commercial pasta	Italian durum wheat
14	Cornstarch	Cornstarch	Derived from the corn grain
15	Chocolate	Dark chocolate	Cocoa paste (70%) from Belgium; cocoabutter (20%); stevia + maltodextrin (produced by Nestevia); inulin (stardardprovided by Sigma Aldrich)
16	Cocoa	Commercial Cocoa	Cocoa 100%, the starting product obtained after extraction from plants
17	Cocoa	Commercial Cocoa	Cocoa 100%, the starting product obtained after extraction from plants
18	Chocolate	Chocolate with stevia	Cocoa paste (70%) from Belgium; cocoabutter (20%); stevia + maltodextrin (produced by Nestevia); inulin (stardardprovided by Sigma Aldrich)
19	Chocolate	Dark chocolate	Commercial dark chocolate, 50% cocoa. Ingredients: cocoa paste, sugar, butter, anhydrous vaccine, emulsifier lecithin, natural vanilla flavor

[a] EPA = Eicosapentaenoic acid.
[b] DHA = Docosahexaenoic acid.

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