COMPOUND-SPECIFIC ANALYSIS OF $\delta^{13}C$ AND $\delta^{2}H$ OF OLIVE OIL FATTY ACIDS

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Abstract– An analytical method was developed to measure $\delta^{13}C$, and for the first time, $\delta^{2}H$ of fatty acid methyl esters (FAMEs) extracted from olive oil triglycerides, after base-catalysed transesterification with methanol. FAMEs are analysed using Gas Chromatography Combustion/Pyrolysis Isotope Ratio Mass Spectrometry (GCC/Py-IRMS). Accuracy, precision and uncertainty of the whole method (from preparation to measurement) were determined as well as the impact of methylation on $\delta^{13}C$ and $\delta^{2}H$. The obtained performance of the method is satisfying and allow the determination of reliable data.

Keywords: *olive oil; fatty acids; GC-C\Py-IRMS; $\delta^{13}C$; $\delta^{2}H$*

1. INTRODUCTION

In the last few years, due to increasing numbers of fraud cases, extravirgin olive oil (EVOO) has suffered a dramatic loss of consumer confidence. Currently, stable isotope ratio analysis (SIRA) offers one of the most promising approaches for tracing the geographical origin of food [1]. Indeed, stable isotope content of bioelements reflects local agricultural practices and the geo-climatic characteristics of the area of production [2]. Traditionally, stable isotope analysis has relied on measurement of bulk EVOOs, in particular, $\delta^{13}C$ and $\delta^{2}H$ have been allowed on some extent the differentiation of products from different geographical origin [3–5]. However, analysis of individual chemical compounds can provide more information on this topic. In particular, the isotopic content of specific sub-components adds further detailed information, for example on local conditions (e.g. micro-climate, soil and water availability) and can therefore improve geographical discrimination [2,6]. Olive oil is composed mainly of triglycerides and small quantities of free fatty acids, glycerol, pigments, flavour compounds and sterols. The isotopic composition of fatty acids is influenced by environmental as well as biological factors and can thus exhibit spatial differentiation in terms of $\delta^{13}C$ and $\delta^{2}H$ values [7,8]. Up to now, there are some studies on the $\delta^{13}C$ of fatty acids [7,9,10] in olive oil in the literature, whereas no studies concerning their $\delta^{2}H$ composition have been published. Recently, the $\delta^{2}H$ of fatty acids has been measured in other matrices, such as milk [8,11], phytoplankton [12,13] and pelagic microorganisms [14].

In this study, the performance of a 'partially' new fast method to measure the $\delta^{13}C$ and $\delta^{2}H$ values of individual fatty acids extracted from olive oil using base-catalysed transesterification with methanol by Gas Chromatography Combustion/Pyrolysis Isotope Ratio Mass Spectrometry (GC-C/Py-IRMS) is presented. In particular, the analytical protocol for hydrogen isotope ratio analysis of the fatty acids contained in olive oil was developed. Accuracy, precision and uncertainty were evaluated in determining both $\delta^{13}C$, and for the first time $\delta^{2}H$ of EVOOs fatty acids.

2. EXPERIMENTAL

Triglycerides were subjected to base-catalysed transesterification with methanol to obtain the corresponding fatty acid methyl esters (FAMEs) in a one-step reaction. For the preparation FAMEs the olive oil sample was mixed with hexane and, then 2 M methanolic sodium hydroxide solution according to the EU official method (annex XA of EC Reg. 702/2007). 1 μL of the hexane solution was injected into the GC-C-IRMS for the analysis of $\delta^{13}C$ and GC-Py-IRMS for the determination of $\delta^{2}H$. More information can be found in Paolini et al. [15].

3. RESULTS AND DISCUSSION

The accuracy, precision and uncertainty of the whole method (from preparation to measurement) were determined. Furthermore, the impact of methylation reaction on $\delta^{13}C$ and $\delta^{2}H$ values of the obtained FAMEs was investigated.

Determination of accuracy of GC-C\Py-IRMS: a mixture of FAMEs was prepared miming the composition of olive oil. The $\delta^{13}C$ and $\delta^{2}H$ values measured using GC-C\Py-IRMS were compared with the isotopic values determined by EA-IRMS and TC/EA-IRMS. The found differences were no more than ± 0.2‰ and ± 1.9‰ respectively for $\delta^{13}C$ and $\delta^{2}H$.

Determination of precision of GC-C/Py-IRMS: a reference FAME mixture was analysed 10 times with GC-C/Py-IRMS. The precision (1 standard deviation) of GC-C/Py-IRMS determination was on average ± 0.2‰ for δ13C and ± 2‰ for δ2H.

Determination of repeatability of the method: an olive oil was treated 10 times (from preparation to analysis) and each of the obtained derivatives was measured using GC-C/Py-IRMS. The standard deviations obtained were on average ± 0.3‰ and ± 3‰ respectively for $\delta^{13}C$ and $\delta^{2}H$.

Determination of measurement uncertainty (estimated according to Dunn et al. [16]): it was ± 0.3‰ and ± 3‰ for the $\delta^{13}C$ and $\delta^{2}H$ of methyl palmitate, methyl stearate and methyl linoleate. The uncertainty obtained for methyl oleate, the main olive oil fatty acid, was ± 0.2‰ for $\delta^{13}C$ and ± 2‰ for $\delta^{2}H$.

Effects on $\delta^{13}C$ and $\delta^{2}H$ values due to the addition of extra carbon and hydrogen due to methylation: an empirical correction was applied to determine the actual $\delta^{13}C$ and $\delta^{2}H$ values of fatty acids after methylation considering the contribution of methanol to the obtained FAMEs (Paolini et al., 2017). The isotopic values of the fatty acids and the standard deviations remained almost unchanged after correction of the added carbon and hydrogen atoms. The reason for this is that the majority of carbon and hydrogen isotopes measured in FAMEs derive from the fatty acids themselves, and less than 10% come from the derivatising reagent. Moreover, the influence of the methyl group on the isotopic value of the final FAME depends on the difference between their isotopic values. In this study the effect of methanol on the real isotopic value of fatty acids was almost negligible, being the isotopic values of methanol not far from the corresponding values of the fatty acids.

4. CONCLUSIONS

From the validation of the whole method (from preparation to analysis) it can be asserted that the analytical methodology developed to measure the $\delta^{13}C$ and $\delta^{2}H$ values of fatty acids from triglycerides in olive oil using GC-C/Py-IRMS proved to be able to provide reliable $\delta^{13}C$ and $\delta^{2}H$ data. As $\delta^{13}C$ and $\delta^{2}H$ in bulk EVOOs have already demonstrated to be useful in tracing their geographical origin, the isotopic analysis of fatty acids can be proposed as a tool for improving this capability.

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