Isotopomic ^{13}C-NMR for Characterization of complex mixtures

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As a general rule, the quality of the manufactured products (but also the raw materials and intermediates) is controlled by analyses that are developed and validated for several domains as food, nutrition, health, pharmaceuticals. The purpose of these analyses is to protect the health of consumers, guarantee the origin and traceability of products and fight against fraud and especially against the presence on the market of EMA (Economically Motivated Adulteration). In this context, characterization of complex mixtures by Nuclear Magnetic Resonance (NMR) can be obtained by the determination of the metabolic profile but also by the determination of the isotopic fingerprint of major components. Isotopomics is the combination of these two strategies in a global approach where the metabolomic and isotopic profiles can be obtained from the same NMR acquisition.

In order to validate this approach, we chose the authenticity control and the characterization of edible oils which has gained increasing attention in the past few years. The search for origin markers of olive oils has been the object of several studies [1]. In this respect, the fatty acid compositional data, obtained by means of the gas chromatography (GC) method, have been used as analytical variables in order to classify oils and to ensure their botanical and geographical traceability [2]. An alternative can be found with NMR spectroscopy, which delivers direct information about the molecular composition of fatty acids in triacylglycerol matrices without any previous chemical preparations. For instance, ^{1}H-NMR method provides percentages of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), linoleic and linolenic acids (the only fatty acids that can be individually quantified by this method), and allows identifying minor components in oils. In a previous work, we investigated the potential of ^{13}C-NMR to afford additional information regarding the individual fatty acids amounts [3, 4], based on its broad range of resonance frequencies. In this respect, the use of an adiabatic ^{13}C-INEPT (Insensitive Nuclei Enhanced by Polarization Transfer) pulse sequence was introduced [5] to overcome the low sensitivity and the long experiment time of regular ^{13}C-NMR, *i.e.*, the one-pulse sequence.

In this study, a set of olive oil samples with known composition (*i.e.*, their fatty acid profile determined by GC) was analyzed by ^{13}C-INEPT and their individual fatty acids amounts were predicted by means of the previous multivariate models. The values obtained were compared to those determined by GC. Moreover, aiming to evaluate the potential of ^{13}C-INEPT, relative to GC and ^{1}H-NMR, in the discrimination of olive oils according to their origin, thirty-six authentic olive oil samples were considered. Corresponding analytical data were used as inputs in the canonical discriminant analysis (CDA) and the linear discriminant analysis (LDA) to classify the oil samples according to the altitude of the olive field and to the color of olives. In addition, the long term repeatability of the three aforementioned analytical techniques were determined. Comparable accuracies were obtained by GC and ^{13}C-INEPT techniques in the quantification of fatty acids. However, ^{13}C-INEPT presents the following advantages over GC: no need to prior chemical manipulations, which permits to eliminate a source of error; once implemented, it is calibration-free; it affords the positional distribution of major fatty acids on the glycerol backbone; and permits to determine the relative position-specific isotopic ^{13}C content at different sites of the triacylglycerol molecules. Furthermore, all these information are obtained from the same spectrum in only 8 minutes. ^{13}C-INEPT has therefore a higher potential in the classification of olive oils according to morphological and geographical factors than ^{1}H-NMR and GC. Its higher efficiency is explained by a significant contribution of variables only obtained by this technique: the isotopic and compositional variables, as the positional distribution of oleic and linolenic acids on the glycerol backbone.

This analytical methodology, validated for olive oils, could be used for the characterization of other triacylglycerol matrices. To implement it, a set of samples should be first analyzed by GC then by ^{13}C-INEPT in order to construct the fatty acid quantification models. Moreover, since triacylglycerols are quasi-universal food constituents and present in animal tissues and blood, our methodology constitutes a powerful isotopomic tool to find out biomarkers of food origin, of dietary intake, and of diseases such as coronary artery disease and lipid metabolism disorders. Finally, such strategy could be extended to numerous other fields in health, pharmaceutic or forensic sciences.

References:

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