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IRMS and NMR spectroscopy in food field

Potential applications and perspectives

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We want to dedicate this work to the memory of dear Professor Antonio Sacco, unfortunately passed away in January 2018. If the circumstances had been different, he would have had the pleasure of sharing with us the success of the publication of this book. In particular, we want to thank him for his continuous encouragement; he has taught us many things about his scientific activity and we like to think that part of our success is thanks to him.

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NUCLEAR MAGNETIC RESONANCE (NMR) AND ISOTOPE RATIO MASS SPECTROMETRY (IRMS)

IN THE ASSESSMENT OF THE GEOGRAPHICAL ORIGIN OF FOOD PRODUCTS AND FURTHER APPLICATIONS

Chapter I

Introduction

With the globalization of food market, and the increase of transactions, the number of cases of foodstuff frauds, by means of addition of external cheap products, or mislabelling practices, have also enormously increased causing economic detriment to both honest producers and unaware consumers. Thus, in recent years, food quality and authentication are becoming of primary importance for both consumers and industries, at all levels of the production process, from raw materials (farm) to finished products (fork). Moreover, consumers all around the world have shown an increasing interest for typical food products with reliable indicators of geographical origin. Typical food products have an important economical role both on National and International level as is confirmed by the several certifications and trademarks of quality (e.g. Protected Denomination of Origin, PDO, and Protected Geographical Identification, PGI) assigned to guarantee their typicity and quality standards. Italy has the greatest number of typical food products (113 PDO and 61 PGI) in the European Union (456 PDO and 361 PGI), and has more than 4000 traditional food products. The reasons for this can be traced to patriotism, media attention, health benefits or specific organoleptic qualities associated with regional products. In addition, recent food scandals have seriously undermined consumer confidence, increasing attention on traceability in the food chain, both among authorities and consumers. Traceability is by definition "the ability to trace and follow a food, feed, food–producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution" [EC regulation 178/2002]. Recently EU directives require that food products should be labelled with information such as:

a) species;
b) geographical origin;

c) production method (i.e. wild/farmed) [EC regulation 2065/2001].

Although, from the legislative point of view, quality standards have been established through the requirement of quality labels that specify the chemical composition of each product, appropriate controls and analysis are quite often not available to enforce these approaches. Just one example is the requirement for geographical origin of several foods, which consists of a paper documentation rather than an instrumental determination. Thus, a significant discrepancy exists between the higher standards of quality and the inappropriate analytical approaches used to address the problem. In the last decades, efforts have been done in order to find innovative and competitive methods to certificate the geographical origin, i.e. for the assessment of food quality and for establish authenticity and traceability of food products.

In this respect, this research work has focused on applying different analytical techniques to the determination of the geographical origin of foods, as well as authenticity and quality investigations.

1.1. Instrumental analyses for food quality evaluation

During the last decades, food characterization was obtained by using "classical methods" making use of spectroscopic, chromatographic, and enzymatic techniques. The following summarizes the most important analytical techniques used in food chemistry.

High–performance liquid chromatography (HPLC) is one of the most widely used techniques as a quality control tool, since it is able to separate several chemical compounds in mixtures. Numerous chemical compounds have been extensively analyzed by HPLC either for food products characterization or to detect adulteration [1–9]. In addition, GC is generally used to discriminate among different varieties of the same product, adulteration detection, and organic compound authentication and identification [10–12]. Nitrogenous content in food products such as cheese, milk, or honey is usually performed with numerous techniques based on the chemical properties of protein, peptides, and/or amino acids by means of spectrophotometric or fluorometric techniques [13–16], also employed to characterize other classes of substances, such as fatty acids, sugars, vitamins, or min-

eral elements, entering in the composition of a wide variety of food products [17–20].

Atomic Absorption/Atomic Emission (AAS/AES) and Inductively Coupled Plasma–Atomic Emission (ICP–AES) are very useful analytical techniques, that found their principal application in multi–element analysis of wines [21], sugar [22], fruit [23–24], cheeses [25], and honeys [26–27].

In recent years, even though most of the classic analytical methods are still used because they are part of certified methodologies, new analytical techniques are emerging and progressing more and more. These "Innovative" techniques are used and are usable to carry out analytical control with the final goal of food characterization or adulteration detection.

In this context, Nuclear Magnetic Resonance (NMR) spectroscopy has shown to be particularly well suited in the food analysis [28–29]. NMR is a non–specific analytical method in that the target molecules or structures do not need to be isolated for analysis. Thus, NMR spectra may be used as "fingerprints" to compare or classify food samples [30]. Indeed, NMR spectroscopy has been applied to a wide range of foodstuffs, including fruit juices [31], olive oil [32–33], wines [34], and honey [35–36].

In a recent review [37], different NMR technologies have been presented showing their advantages and drawbacks in food science. From these studies, it appeared that NMR spectroscopy combined with chemometric techniques can be a useful and rapid method to assess food authenticity. The use of chemometric methods is necessary because the comparisons and the evaluation of differences or similarities present within large data sets, such as obtained by NMR techniques, is possible only by using statistical analysis.

Another technique that has been widely applied for the determination of food authenticity and also for origin control of food products is the Isotope Ratio Mass Spectrometry (IRMS) [38]. The isotopic signatures in biological products, food included, depend upon geographical parameters, and seasonal effects [39]. Stable isotope analysis enables the differentiation of chemically identical substances, but with different origins, through their specific *isotopic fingerprints*. For example, the measurements of stable isotope ratio of light elements ($^{2}H/^{1}H$,$^{15}N/^{14}N$,$^{13}C/^{12}C$,$^{18}O/^{16}O$,$^{34}S/^{32}S$) have been used to detect re-

gional provenance and supply authenticity control information for products like fruit juices, wine, milk, butter and cheese [40–43].

1.2. Objectives of the thesis

The main objective of this PhD work is the assessment of the geographical origin of food products by using innovative techniques such as the Nuclear Magnetic Resonance and the Isotope Ratio Mass Spectrometry.

In particular, NMR and IRMS combined with appropriate statistical techniques were discussed in terms of advantages against classical techniques in the assessment of geographical origin of typical food products.

Finally, these innovative techniques, with reduced handling of samples and rapid acquisition of information, have been used aiming to propose them as methods officially which could be adopted by the European Community.

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