

Apostolos Spyros and Photis Dais

NMR Spectroscopy in Food Analysis

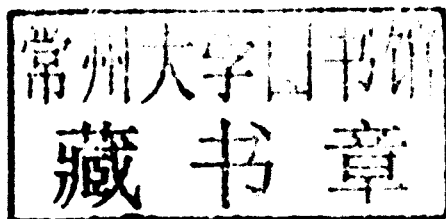


NMR Spectroscopy in Food Analysis

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Preface

During the last few decades, NMR spectroscopy has found increased use in the analysis of food materials at an academic, industrial and government regulation level. The first NMR experiments aimed at studying food were probably the product of the curiosity of NMR spectroscopists (it was easier to find food in close proximity to NMR spectroscopists than NMR spectrometers close to food scientists!). Presently, the full exploitation of NMR spectroscopy in food analysis requires the involvement of food scientists with a deep understanding of food materials, in designing and implementing NMR experiments directed to specific analytical problems. The need for a book that could serve as an introductory tool in NMR spectroscopy for food scientists is thus clear and present. We hope we will succeed, at least partially, in covering this void with our present contribution.

The organization of the material is such that in the first few chapters, theoretical aspects and instrumentation are discussed, followed by sample preparation and various experimental aspects. After briefly describing chemometrics, several chapters are devoted to NMR applications in specific food categories. In each chapter, relatively simple NMR methodologies are discussed first, followed by more advanced techniques. The presentation of the chapter material is organized so that the reader can obtain easily an “NMR overview” of a specific food category. Ample use of figures (especially NMR spectra/images) has been made, in an effort to make the material more appealing and easier to digest. The coverage of NMR applications in different food categories is quite extensive, although not complete, as this would extend beyond the scope of any introductory book.

It is our hope that after reading this book food scientists, especially those not very familiar with NMR spectroscopy, will possess the ability to comprehend

NMR related applications in the ever growing “NMR in food” literature, to acquire the basic skills necessary to design an NMR experimental approach, and to select the NMR methodology suitable for addressing the food-related problem at hand.

A. Spyros, Ph. Dais

Contents

Chapter 1	Introduction	1
Chapter 2	Theoretical Aspects	5
2.1	Nuclear Spins and Energy States	5
2.2	A Collection of Spins—the Vector Model	10
2.3	NMR Parameters	12
2.3.1	Chemical Shift	12
2.3.2	Spin-Multiplicity and Coupling Constant	13
2.3.3	Signal Intensity	16
2.4	Nuclear Relaxation	17
2.5	NOE	18
2.6	Pulsed Fourier Transform NMR	20
2.6.1	Rotational Frame of Reference (RFR)	20
2.6.2	The Single-Pulse Experiment	22
2.7	Pulses	25
2.7.1	Non-Selective Pulses	26
2.7.2	Selective Pulses	26
2.7.3	Composite Pulses	27
2.7.4	Shaped Pulses	29
2.7.5	Field Gradient Pulses (PFG)	30
2.8	Useful Pulse Sequences	31
2.8.1	Spin-echo	31
2.8.2	Spin-Lock	31
2.8.3	Polarisation Transfer	32
2.8.4	Spectral Editing	33

RSC Food Analysis Monographs No. 10

NMR Spectroscopy in Food Analysis

By Apostolos Spyros and Photis Dais

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2.9	Two-Dimensional (2D) NMR Spectroscopy	35
2.9.1	The Principle	35
2.9.2	The Appearance of 2D NMR Spectra	38
2.9.3	Description of a Simple 2D NMR Experiment	39
2.9.4	Types of 2D NMR Experiments	41
2.10	Solid-State NMR	45
2.10.1	Line-Broadening Factors	45
2.10.2	Removal of Dipolar Coupling and CSA	47
2.11	MRI	50
2.11.1	Spatial Encoding and Decoding	50
2.11.2	Basic Imaging Techniques	55
2.11.3	Basic MRI Scans	57
2.12	Time Domain (TD) NMR	57
2.12.1	TD NMR Methods	58
	Further Reading	64
	Liquid-State NMR	64
	Solid-State NMR	65
	Magnetic Resonance Imaging	65
	References	65

Chapter 3 Instrumentation 67

3.1	The NMR Spectrometer	67
3.1.1	The Magnet	67
3.1.2	The Probe	70
3.1.3	The Console	73
3.2	Sensitivity of the NMR Experiment	75
3.2.1	Technological Advances to Improve NMR Sensitivity	76
3.3	High-Throughput NMR	77
3.3.1	Technological Advances	77
3.3.2	Hyphenated NMR (LC-NMR)	78
3.4	TD NMR	81
	References	82

Chapter 4 Sample Preparation 84

4.1	Sampling	84
4.2	Foodstuff Pre-treatment	85
4.2.1	Conservation	85
4.2.2	Removal of Extraneous Matter	85
4.2.3	Reduction	86
4.2.4	Drying	86
4.2.5	Sample Extraction	86

4.3	Extraction	86
4.3.1	LLE	87
4.3.2	Microwave Assisted Extraction (MAE)	88
4.3.3	SPE	89
4.3.4	LLE <i>versus</i> SPE	91
4.4	NMR Samples Preparation	91
4.4.1	Liquid-State NMR	92
4.4.2	NMR Solvent Selection	93
4.4.3	Solid-State NMR	93
4.4.4	MRI	94
	References	94
Chapter 5	Experimental Conditions and Processing	96
5.1	Qualitative Liquid NMR	96
5.1.1	Setting Up the High-Resolution 1D NMR Experiment	97
5.1.2	Setting Up the 2D NMR Experiment	106
5.1.3	Data Processing	108
5.2	Quantitative NMR	116
5.2.1	1D NMR	116
5.2.2	2D NMR	122
	References	124
Chapter 6	Chemometrics in Food Analysis	126
6.1	Why Chemometrics?	126
6.2	Before the Application of Chemometrics	127
6.2.1	Sample Selection	127
6.2.2	Sample Preparation	128
6.2.3	Data Pre-Processing	128
6.3	Targeted or Metabolic Profiling	130
6.4	The Chemometric Approach or Metabolic Fingerprinting	131
6.5	Chemometric Techniques	132
6.5.1	Unsupervised Pattern Recognition Methods	133
6.5.2	Supervised Pattern Recognition Methods	135
6.6	Validation of Chemometric Methods	144
6.7	Which Statistical Method to Choose for Food Analysis?	146
	References	147
Chapter 7	Fats and Oils	149
7.1	Vegetable Fats and Oils	149
7.1.1	Triacylglycerols	149
7.1.2	Minor Compounds	156

7.1.3	Quality Assessment	164
7.1.4	Authentication	171
7.1.5	Geographical Origin and Cultivar	173
7.2	Animal Oils and Fats	175
7.2.1	Milk Fat	175
7.2.2	Margarines and Hydrogenated Fats	181
7.2.3	Fish Oils	184
	References	195
Chapter 8	Wine and Beverages	202
8.1	Alcoholic Beverages	202
8.1.1	Wine	202
8.1.2	Vinegar	210
8.1.3	Beer	213
8.1.4	Spirits	217
8.2	Non-Alcoholic Beverages	219
8.2.1	Coffee	219
8.2.2	Cocoa	223
8.2.3	Tea	225
8.2.4	Juices	228
	References	233
Chapter 9	Fruits and Vegetables	241
9.1	Fruits	241
9.1.1	Composition and Metabolite Profiling	241
9.1.2	Growth and Ripening	244
9.1.3	Quality Evaluation	249
9.1.4	Processing	254
9.2	Vegetables	260
9.2.1	Compositional Analysis and Metabolite Profiling	260
9.2.2	Quality Evaluation	264
9.2.3	Processing	267
	References	271
Chapter 10	Milk and Dairy Products	280
10.1	Milk	280
10.1.1	Composition and Metabolite Profiling	280
10.1.2	Quality and Processing	283
10.2	Cheese	285
10.2.1	Composition and Metabolite Profiling	285
10.2.2	Quality and Processing	287

10.3	Yogurt	290
10.4	Ice Cream	290
	References	291

Chapter 11 Meat 295

11.1	Composition and Metabolite Profiling	295
11.2	Quality	298
	11.2.1 Post Mortem Metabolism	298
	11.2.2 Water Holding Capacity	301
11.3	Processing	302
	11.3.1 Freezing	302
	11.3.2 Cooking	303
	11.3.3 Curing	304
	References	306

Subject Index 310

CHAPTER 1

Introduction

Nuclear magnetic resonance (NMR) spectroscopy is an effective analytical technique, which has been used systematically in food analysis and authentication in recent years. Its origin is traced back to 1946 when two groups of scientists at Harvard University (Purcell, Torrey and Pound) and at Stanford University (Bloch, Hansen, and Packard), working independently, observed proton resonance signals from paraffin wax and water, respectively. For their discovery, Purcell and Bloch were jointly awarded the Nobel Prize in Physics in 1952.

The first application of NMR in food science dates back to 1957 when low-resolution NMR measured moisture in foods. Consistent and widespread application of NMR in food science started in the 1980's mainly due to deficiencies in instrumentation and the complexity of food matrices. Since then, an explosive publication of research and review articles dealing with NMR applications in food science has appeared in scientific journals and several books. Figure 1.1 shows diagrammatically the explosion of publications in food science after 1988.

Also, numerous oral and written communications have been presented in domestic and international conferences. In particular, an *International Conference on Application of Magnetic Resonance in Food Science* is held in Europe every two years. This conference started in 1992 and gave the opportunity for scientists worldwide to present new applications of NMR in food science and technology. It is worth mentioning that NMR methods have been approved as official methods by the European Union (*e.g.* detection of wine fraud). There are several reasons for this development: (a) the increasing sophistication and the user-friendly NMR instrumentation; (b) the increasing need of the food industry to understand and innovate its products and processes; and (c) the necessity for the development of new and more effective analytical techniques for the quality control and authentication of foods and thereby the reinforcement of pertinent legislation.

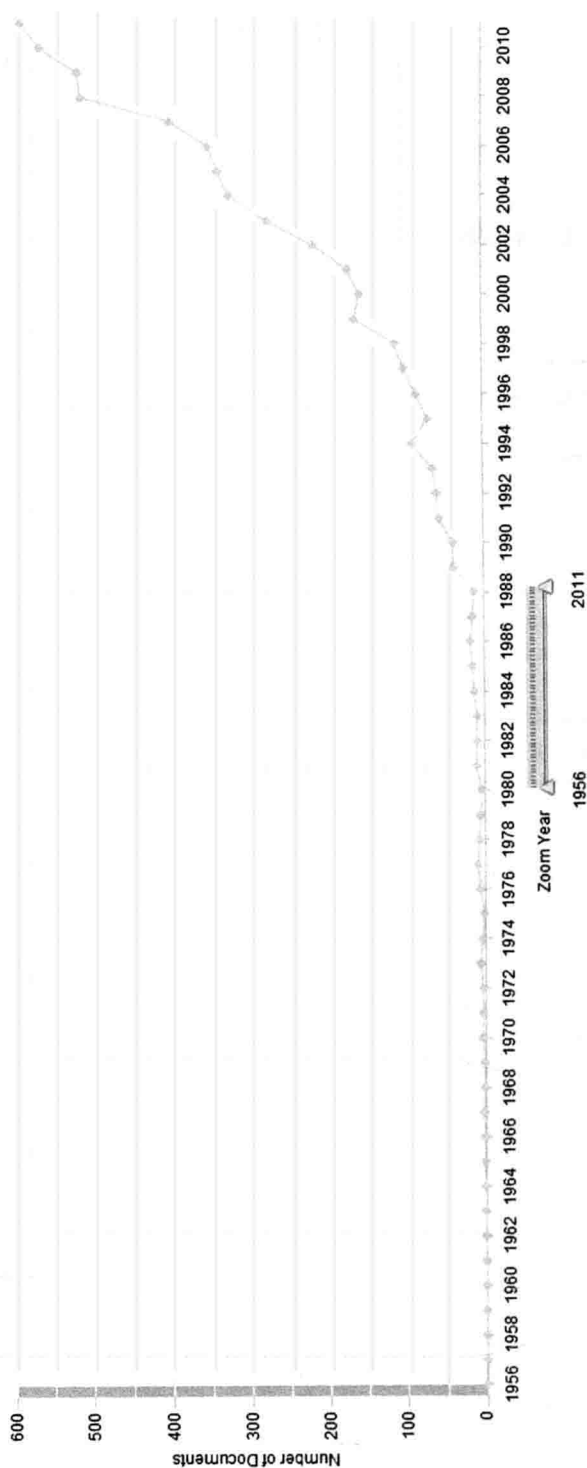


Figure 1.1

The graph presents the number of publications per year that include the words 'food' and 'NMR' or 'MRI' (either as acronyms or in full text) in their title, keywords or abstract, as indexed in Scopus. (Retrieved on May 25, 2012 from www.scopus.com.)

Foods are very complex and highly heterogeneous systems comprising a large number of chemical compounds, the composition of which varies considerably under certain circumstances (*e.g.* agronomical or slaughter practices, industrial processes, storage, maturation, *etc.*). To this direction, one-dimensional (1D) liquid or solid-state high-resolution NMR spectroscopy can provide in a single experiment a wealth of structural and quantitative information in the form of the NMR parameters, namely chemical shifts, coupling constants and signal intensities. For the same sample the researcher can choose different nuclei, such as ^1H , ^{13}C , ^{31}P , ^{19}F —to mention the most popular nuclei—that allow the study of food samples under different perspectives and to extract the maximum information about its natural or industrial condition. These experiments need no separation of the various food components and no serious sample pre-treatment. Moreover, NMR spectroscopy is sensitive to dynamics, which allows differentiation between molecules or groups of molecules with different mobility through spin–lattice and/or spin–spin relaxation measurements.

In cases where the complexity of the food sample is so severe, causing extensive signal overlap in 1D spectra, the arsenal of NMR spectroscopy provides a large number of analytical techniques starting from the homonuclear and heteronuclear multi-dimensional NMR to its hyphenation with effective separation techniques, such as liquid chromatography (LC-NMR). In particular, two-dimensional (2D) NMR techniques, such as COSY, TOCSY, NOESY, HSQC, *etc.*, based on the inherent ‘communication’ of nuclei with each other (through spin–spin and dipolar coupling), spread out the spectroscopic information in two dimensions unravelling hidden nuclear connectivities and facilitating the structural characterisation of the molecules in the food sample. Although NMR spectroscopy is not a destructive analytical technique and recovery of the analyte can be easily achieved after experimentation, industrial needs may require the examination of food products under different processing conditions by non-invasive means. Magnetic resonance imaging (MRI), used extensively in medicine, has been exploited in recent years in food analysis. The ability of MRI to show spatial resolution within the food product and the judicial application of MRI techniques allows the monitoring of the fate of certain molecules (*e.g.* water) and reveals various molecular interactions and changes in tissue structure that occur during food processing or storage (*e.g.* food freezing and thawing).

The combination of NMR spectroscopy with multivariate statistical methods provided an alternative possibility of analysing and maximising the information recovery from complex NMR spectral data of foods. This methodology, usually called metabonomics, does not necessarily require the identification of the individual signals in the spectrum as in quantitative NMR, but seeks to find subtle spectral features that can identify unequivocally the presence of metabolites or useful biomarkers. Pattern recognition techniques (supervised or unsupervised) can be used to map the NMR spectra of a large number of samples, and locate spectral fingerprints that reflect either metabolic changes or used to distinguish sample classes.

The disadvantage of the early days of NMR spectroscopy related to the low sensitivity and high cost of the analysis does not hold true for the NMR instrumentation of the present day. These drawbacks have been largely compensated by the development of modern hardware comprising strong magnetic fields up to 23.5 T and cryogenic probes that allow easy detection of food components at the level of μg and even ng. Moreover, the progress in sophisticated software and innovations in automation allow the screening of a large number of samples (overnight run), reducing the experimental time to a few minutes even for the less sensitive nuclei (e.g. ^{13}C , ^{15}N).

In concluding this introductory chapter, we could add that it is not only the unique information that NMR provides, but also the versatility of methods, instruments and probes that make it an important tool for qualitative and quantitative analysis.

This book has been organised as follows: *Chapter 1* is the book's introduction. *Chapter 2* gives an account of the theory underlying the physical phenomenon of NMR and grouping the most useful NMR techniques to better understand the core principles, which appear in subsequent chapters. Since NMR is a well-documented spectroscopic technique and it is well described in several introductory and advanced books, this chapter will be kept to a minimum. *Chapter 3* describes the NMR instrumentation in an attempt to familiarise the reader with the hardware and software components of modern high-resolution and solid-state NMR spectrometers, and their functions and automation. Relevant information about NMR spectrometers and the implementation of its components may help the reader to choose the right spectrometer and accessories for their needs. Also, this chapter includes useful information about the hardware systems and experimental designs to perform sophisticated experiments, such as (HP)LC-NMR, time-domain NMR, and high-throughput and on line NMR. Appropriate guidance for obtaining pure samples from various food matrices that are suitable for NMR experiments will be presented in *Chapter 4*, whereas the experimental conditions described in *Chapter 5* may help the NMR user to choose the right input values for the critical parameters in the experimental setup in order to obtain the maximum possible information from the NMR experiment, and to perform quantitative analyses with high accuracy and precision. *Chapter 6* presents a few aspects of the supervised and unsupervised pattern recognition statistical methods employed for data exploration, classification of food samples, and the build-up of calibration–prediction models giving special attention to NMR metabolomics. The applications of NMR spectroscopy and its specialties to different food systems are discussed in *Chapters 7–11*. A detailed presentation of the available NMR methodologies and techniques for each food category is provided, whereas practical guidance and tips for performing concrete experiments is afforded. Every chapter starts with a short abstract and ends with relevant bibliographic coverage.

CHAPTER 2

Theoretical Aspects

2.1 Nuclear Spins and Energy States

An atomic nucleus is a collection of protons and neutrons (nucleons) that possess a quantum mechanical property called spin, which is characterised by spin angular momentum. Spin angular momentum is an intrinsically quantum mechanical property that does not have a classical analog. All subatomic particles are spin $\frac{1}{2}$ particles. The nucleus itself has a total spin angular momentum formed by the coupling of the individual spin angular momenta of its constituent protons and neutrons. The total nuclear spin angular momentum quantum number I may therefore take values: 0, $1/2$, 1, $3/2$, 3, $5/2$, etc. A nucleus with non-zero quantum numbers I behaves as a small magnet or magnetic dipole with a magnetic moment μ . The magnetic moment is an intrinsic property of the nucleus, and it is associated with the angular momentum of the nucleus. As a vector, nuclear magnetic moment has two properties: magnitude and direction. The magnitude of μ is quantised and given by eqn (2.1):

$$\mu = \gamma \hbar \sqrt{I(I+1)} \quad (2.1)$$

where $\hbar = h/2\pi$ is Planck's constant and γ is the magnetogyric ratio, an inherent property of the nucleus. This parameter is unique for each nucleus. When $I=0$, then μ is zero, and the nucleus does not have magnetic properties. Nuclei with $\mu=0$, such as ^{12}C , ^{16}O , ^{32}S cannot be studied by NMR. The smallest magnetic moments belong to protons and is called the nuclear magneton, μ_{N} ; its value is calculated from $\mu_{\text{N}} = e\hbar/2m_{\text{p}}c$ (e is the electric charge, m_{p} is the mass of proton, and c is the velocity of light) to $5.0505 \times 10^{-27} \text{ J T}^{-1}$. Under the influence of an external magnetic field of intensity (strength) B_0 fixed along the z -axis of a static Cartesian coordinate system, the magnetic moment assumes discrete orientations (the nuclear Zeeman effect) governed by the magnetic quantum number m_I . The allowed values of m_I are $-I, -I+1, -I+2, \dots, I-1, I$, giving rise to

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