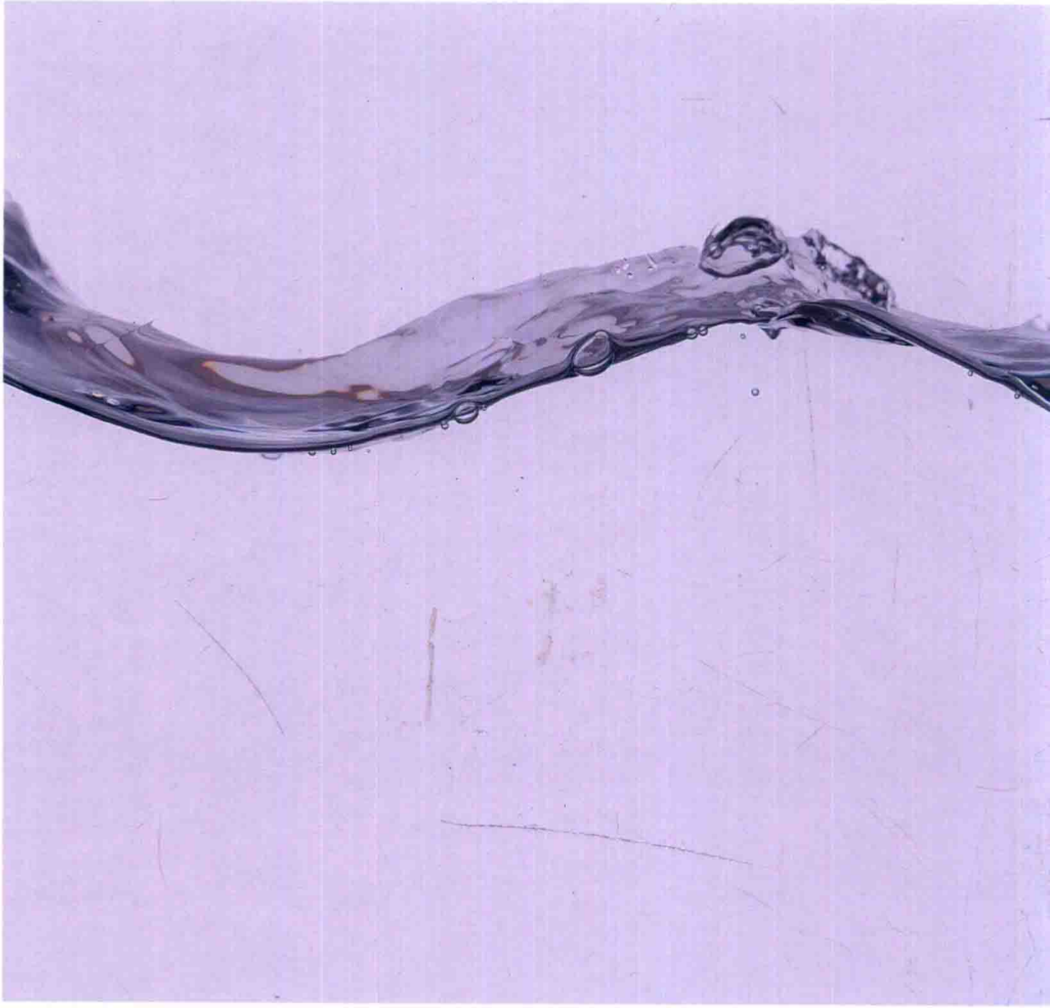
I.A. Farhat, P.S. Belton and G.A. Webb

Magnetic Resonance in Food Science

From Molecules to Man



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Preface

The modern challenges of food science and technology require a better understanding of the utilisation of food by the human body and the determinants of food quality. We need to know how raw materials, processing, and changes during storage impact upon the sensory and nutritional attributes of foods. Magnetic resonance is well positioned to help address these challenges. This is being achieved through a combination of the following developments:

- Advances in hardware, in particular in terms of technically and economically achievable field strength.
- Advances in data analysis e.g. through the use of complex multivariate protocols.
- Affordability and robustness of instruments, widening the access to magnetic resonance.

The 8th *International Conference on the Applications of Magnetic Resonance in Food Science* was held at The University of Nottingham on the 16th - 19th July 2006. As the principal conference in the field of magnetic resonance in food sciences, the event attracts contributions from internationally acknowledged experts from academia and industry and an audience from all over the world. This edition was inaugurated by a lecture by Sir Peter Mansfield, Medicine Nobel prize laureate for his discoveries in magnetic resonance imaging.
The 8th edition of the proceedings is entitled *From Molecules to Man* in order to truly reflect the breath of the applications of nuclear magnetic resonance in all aspects of food sciences: from the studies of subtle molecular motion modes to macroscopic scale mass transfer during processing and from structure elucidation to authentication, quality and metabonomics.

This book is based on contributions to the conference technical programme. It is structured into four major sections:

- Food in the human body which includes MRI and metabonomics studies.
- Food quality which includes papers on animal metabonomics, structure of food systems, food stability and authentication.
- Food processing with particular emphasis on dynamic processes such as water migration and phase transformations.
- New techniques, novel data analysis and exploitation covering innovations in NMR methodologies, hardware and data analysis, for example using multivariate approaches.

We would like to thank the contributors to the book and the conference, the staff in the Division of Food Sciences and the Sir Peter Mansfield Magnetic Resonance Centre at the University of Nottingham who supported the event, in particular Mrs Val Street, and last but not least, The Royal Society of Chemistry for the realisation of the book.

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1 INTRODUCTION

1.1 Role of NMR in the Foods Industry

Current State of the Industry

A main challenge for the foods industry for the next decades is to address the growing concerns of society with respect to public health. In the whole western world obesity, cardiovascular disease, arthrosis, and diabetes are on the rise (1,2) and the link with eating patterns is scientifically well established. The industry is now addressing the growing demand of consumers and legislators for products that promote sustainable wellbeing and health. In one route, the industry is responding by reducing high levels of fat, sugar and salt, since these are clearly linked to adverse health effects. Since, these ingredient critically determine taste and texture of the current generation of food products, the industry is now posing the challenge of re-designing their microstructures (3). In a second route, the industry is searching for food ingredients that actively promote health. Both routes require significant scientific and technological investments, in particular since new legislation is requiring that claims should be sustained by sound scientific evidence.

What to Measure

In order to be successful, the industry recognizes the key role of science and technology. Since 'all good science is measurement' (Helmholtz), this offers ample opportunities for NMR. Within the realm of measurement technologies, NMR takes a unique position due to its wide range of application areas. NMR can be deployed in diverse areas as the measurement of product meso- and microstructures, product compositions as well as the physiological and psychological impact of products on consumers.

How to Measure

Within science we are witnessing a shift from hypothesis testing towards hypothesis generation. This is particularly apparent within the emerging 'omics' life sciences (4,5). Such approaches can only be successful when measurement technologies are available that are not particularly biased by prior expectations. NMR has established itself a position in this field to its comprehensiveness and unbiased nature. In this review examples will be

presented from the nutritional metabonomics, and we will also demonstrate how NMR can be applied in an analogous manner in the microstructural area.
Although NMR is still considered as a technique that requires expensive instrumentation, trained operators and data interpreters, non-NMR experts can already obtain useful data from benchtop NMR instruments. Developments in this area are speeding up, and we can now even envisage NMR (self)-measurements by consumers.

1.2 Define, Discover, Design, Deploy, Deliver

The competitive position of foods industries strongly relies on the efficiency with which they can bring new and healthy product innovations to the market. Within the innovation process one can roughly discern 5 phases: Define, Discover, Design, Deploy and Deliver (Figure 1A). In the Define phase, understanding of consumer needs is translated into outlines for novel products and services that eventually can be brought to market. In the Discovery phase, the food scientist acquires new insights that allow the design of novel food products. In the Design phase, ideas that were conceived in the Discovery phase are transformed into product prototypes. Subsequently, in the Deployment phase, food engineers scale up processes up to mass production. Another critical success factor is the efficiency of the supply chain, which Delivers food products to the consumer. This involves safe manufacturing and efficient distribution through a range of logistic channels (retail, food services etc.).

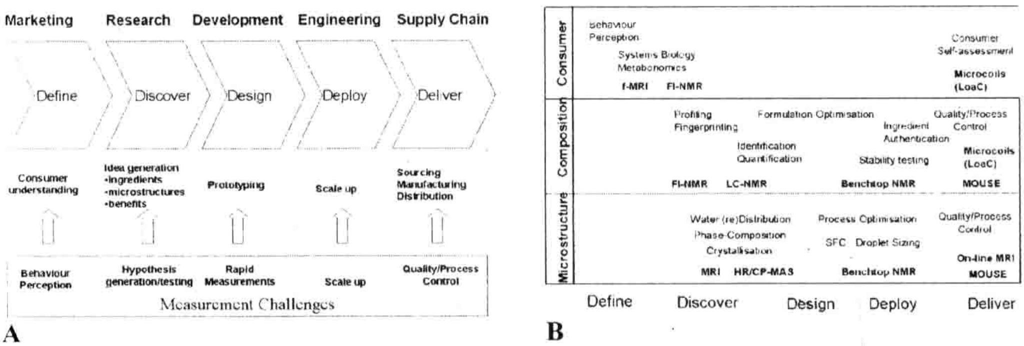


Figure 1 *(A) Schematic depiction of the industrial innovation process. (B) Overview of applications of NMR throughout the 5 innovation phases (horizontal) and main NMR/MRI application areas (vertical). Methods are indicated in bold, applications in italics (see text).*

Within the foods industry, NMR plays a unique role since it can make important contributions in all DDDDD phases. It can do so by providing insight in product composition and microstructure, and their interactions with the consumer. An overview of the vast and broad amount of NMR applications in the foods industry is given in Figure 1B. In this review, the industrial opportunities of NMR will be illustrated by two examples from the author's own practice. Two product concepts will be tracked through different innovation phases, involving measurements on both product and consumer level. Trends in the industrial field will be illustrated by recent work and reviews, but no attempt has been made to give an exhaustive overview.

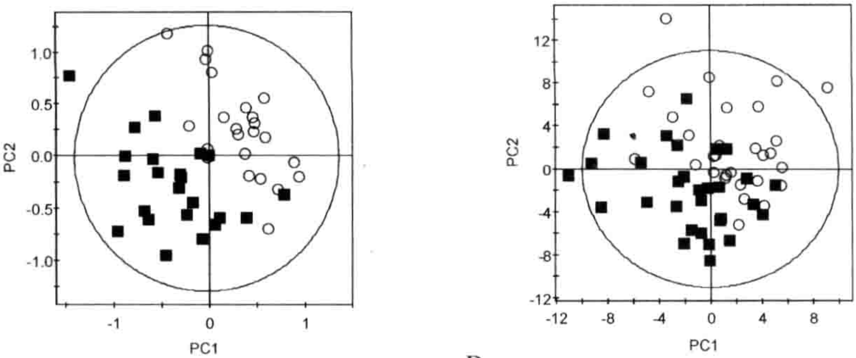
2 HEALTH IMPACT OF FLAVONOIDS

2.1 Flavonoids

In their quest for 'natural' functional ingredients with beneficial health effects, the foods industries have developed a strong interest in polyphenols. These compounds have been associated with prevention of diseases, in particular cardiovascular disorders (6,7). Among the polyphenols, beneficial effects are best articulated for the flavonoids. Most evidence, however, is based on a limited number of biomarkers and clinical end-points. Flavonoids do have an impact on oxidative stress markers, but there is a growing awareness that the 'antioxidant theory' is a naïve simplification (6). Hence there is a need to pursue further research in this area.

2.2 Discovery: health benefits of flavonoids

Until recently, nutritional research was hypothesis-driven and departed from pre-identified markers and benefits. The relatively slow progress made in the last decades is driving a movement towards more exploratory, hypothesis generating 'omics' approaches, which assess living organisms in a holistic manner (8). Sofar most nutritional applications of metabonomics relied on NMR for unbiased profiling of body fluids (9,10), and used multivariate data analysis techniques (11) to recognise patterns and establish relations with accepted biomarkers. Metabonomics has taken a firm position within the foods industry since can provide direct feedback on health status and metabolic effects of nutritional interventions (12-15). Figure 2 shows examples of the type of information that can be obtained from nutritional intervention studies. In a (double blind) cross-over trial, volunteers were taking grape/wine extract and a placebo. The metabolic impact was assessed by measuring ^{1}H NMR spectra of body fluids collected from the volunteers. Figure 2A and 2B show that a grape/wine extract had a significant impact on the metabolite composition of these urine and plasma, respectively. Several metabolites that are responsible for the clustering in Figure 2 have been identified and could be attributed to both exogenous (xenobiotic) and endogenous effects. The exogenous impact mainly involved low molecular weight flavonoid degradation products. This points towards bioconversion by gut microflora, and is in line with metabonomic studies on the impact of other flavonoid sources (16-19). It has been recognised that bioavailability of intact dietary flavonoids is limited, and that the explanation of their beneficial effects may lie in secondary metabolites produced by gut microflora (6). The role of gut microflora in human health is gaining considerable interest, and metabonomics will be critical for gaining further insights (15,20,21). In the next years, when the hypotheses in this area will become more focussed, more sensitive and targeted metabolic profiling techniques based on mass spectrometry will be used ((22). Meanwhile, NMR will remain the preferred technique to obtain comprehensive and unbiased metabolic profiles, with minimal sample pre-treatment (23).



A B

Figure 2 *PLSDA scores plot of (A) urine and (B) plasma ^1H NMR spectra obtained from volunteers on grape/wine extracts (squares) and a placebo (circles).*

2.3 Design/Deploy: rational product formulation

Flavonoids are typically sourced as natural product extracts (NPE's), which have a considerable compositional complexity (24). This poses a challenge for the product developer, since this complicates sourcing of raw materials, reproducible product formulation and also raises regulatory issues with respect to product safety (25). At present, suppliers typically provide results of crude analytical or functional tests, but such data mostly do not relate to product performance, and can also easily be manipulated (26). This is circumvented by recording compositional profiles, where many different compounds are assessed simultaneously, and in an unbiased manner (27). NMR meets these requirements (28), as is illustrated in Figure 3A. Here, a range of commercially available grape/wine extracts were profiled by NMR and represented in a PCA scores plot. One can observe that many extracts cluster in different groups indicating compositional similarity. Such information can be obtained rapidly, and can be used to aid in the selection of raw materials and suppliers in a rational manner.

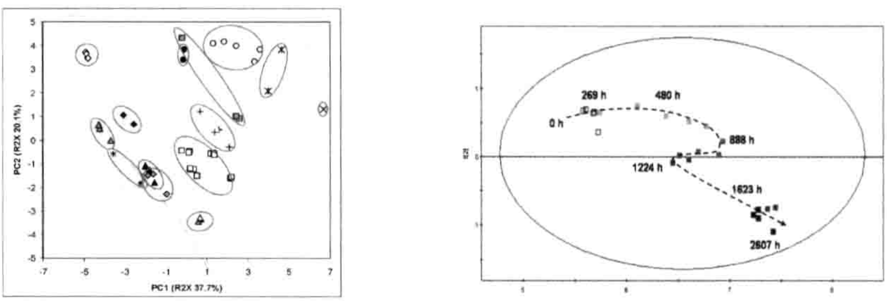


Figure 3 *(A) ^1H NMR based PCA compositional map of commercially available grape extracts. (B) Result of a storage test where the flavonoid composition within a product format was monitored in time by means of ^1H NMR profiles.*

The compositional complexity of NPE's also makes it difficult to make predictions of their stability within formulated products. Also this issue can be addressed by acquiring comprehensive compositional NMR profiles. An example is presented in Figure 3B, which shows the trajectory of the compositional flavonoid profile within a product formulation during a storage test. The model loadings (not shown) indicate the disappearance of narrow signals, and the appearance of broad ones. These effects are most pronounced for the aromatic region of the 1H NMR spectrum and suggest flavonoid aggregation. This is valuable information for the food technologist, and can be used to make rational formulation adjustments.

3 NOVEL FOOD MICROSTRUCTURES

3.1 Food Microstructures-Property relations

Food quality is generally considered to be related to composition, but often the dominating factor is the microstructure of the product (29). Product innovations are often hampered by the lack of understanding of the relation between sensory/physical parameters and the underlying microstructures. The current 'deductive' research strategy in this area is depicted in Figure 4. In time-consuming first step, high-end measurement techniques (imaging, spectroscopy) are deployed, in order to derive quantitative structure descriptors. Next, these descriptors need to be related to sensory parameters. Both steps are projects in themselves, and work on time-scales, which often do not match with the required pace of innovation. Similar to the approach adopted in the Discovery of innovative health ingredients (*vide supra*), we now also witness deployment of explorative modelling in the microstructural area (30,31). As a profiling tool mostly NMR relaxometry is used, due to its reputation in probing food microstructures (32,33) .

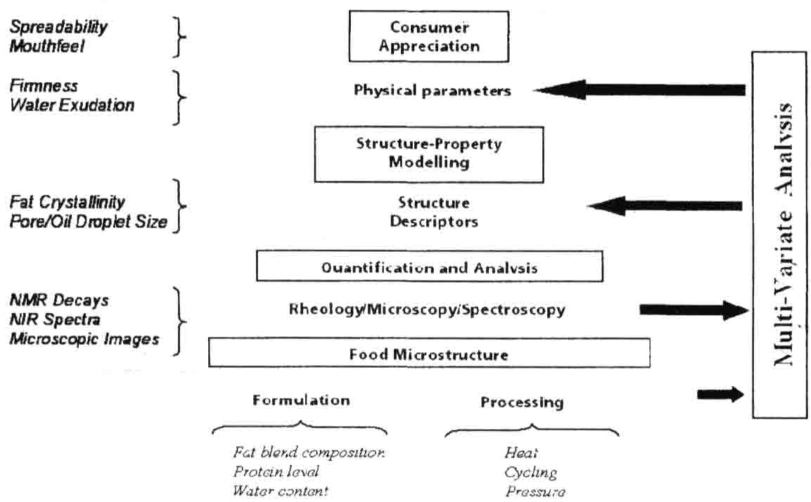


Figure 4 *Structure-property relations established by the 'deductive' rational strategy (pyramid), and by the 'inductive' multi-variate analysis route (right). At the left side examples are given of measurements, structural parameters and, consumer related parameters (bottom to top).*

3.2 Discovery: model emulsion microstructure-property relations

Protein-stabilised oil-in-water emulsions consist of oil droplets stabilised in a protein aggregate network (34) in which water is dispersed in pores with a range of sizes (Figure

5A). An important quality parameter for these food materials is Water Exudation during shelf-life. A range of these emulsions was prepared with varying levels of fat, protein and water, and NMR relaxation decays were recorded to probe their microstructure. These decays contain comprehensive information on microstructure of the food emulsions, which is illustrated in a condensed form in the PCA scores plot in Figure 5B. One can clearly observe clustering, which could be attributed to the presence/absence of a biopolymer.

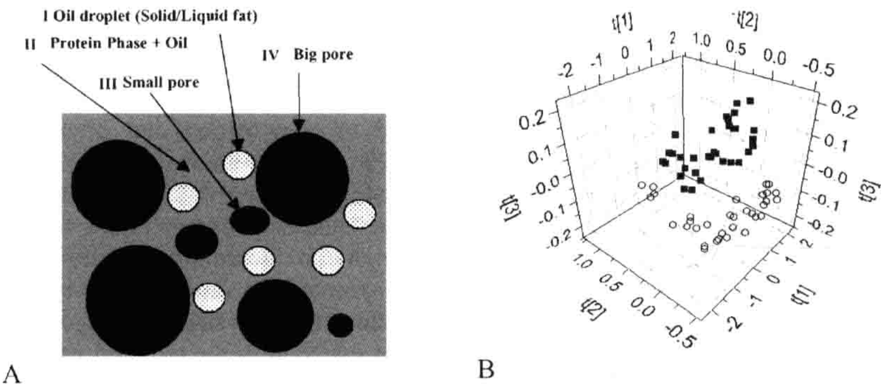


Figure 5 *(A) Schematic representation of microstructures of protein stabilised O/W emulsions. (B) PCA scores plot derived from transversal relaxation decays (^1H NMR, 20 MHz) recorded for a series of these emulsions.*

Figure 6A shows the performance of a multivariate model that predicts Water Exudation from NMR decays recorded for emulsions prepared with biopolymer. (35,36). The model performs poorly for samples without biopolymer, indicating a difference in microstructure. This is visualized in the loadings for models built for samples with/without biopolymer (Figure 6B). The samples without biopolymer have a contribution of small pores, which is absent in samples with biopolymer. This is indicating that the biopolymer had a water retaining effect on the emulsions.

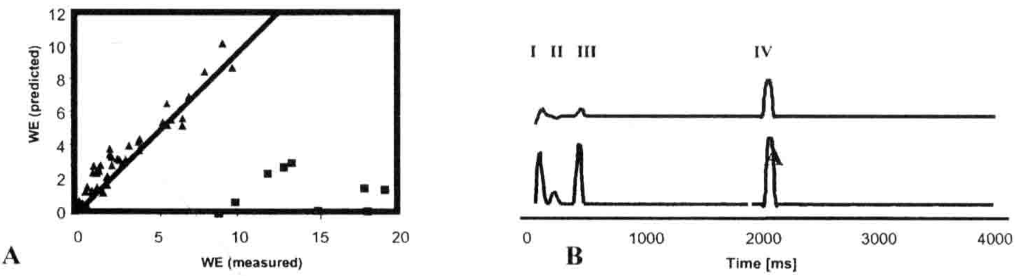


Figure 6 *Goodness of fit for multivariate model based on samples with biopolymer (triangles). In the plot also samples without biopolymer (squares) have been presented (prediction values based on model for samples with biopolymer. (B) T_2 components that explain WE for model based on samples with (top) and without (bottom) biopolymer.*

3.3 Design: rapid and cheap assessment of microstructures

In the Design phase, the pressure to bring a product to the market is increasing, but meanwhile the prototypes under investigation are still complex, both on microstructural and on compositional level. In this stage, food product Designers prefer methods that provide rapid feedback on meso- and microstructure of food product prototypes. Understanding and/or control of food structures at the laboratory bench/kitchen table or manufacturing pilot plant requires relatively cheap and easy-to-use measurement technologies that can be operated by non-(NMR) expert users. Already in the 70's, such systems became available for the routine measurement of Solid Fat Content (37,38) in fat blends, and this rapidly abolished the cumbersome classical method (39). Later, benchtop NMR equipment was extended with the capability to assess water (40,41) and oil (42,43) droplet sizes in food emulsions. Recently, also benchtop NMR methods were presented for assessment of fat /water content (44,45), phase composition and microstructure (46,47) of complex food products.

3.4 Deploy/Deliver: through-package assessment of microstructural quality

In the Deploy and Deliver phases, we are witnessing a transition from the 'classical' process and quality control taking place in an 'off-line' laboratory, towards non-invasive, on-line and real-time measurement of product quality parameters. Most of these measurement tools only provide *chemical compositional* information of the food system of interest, however. Benchtop NMR is well suited for rapid microstructural assessment, but still requires sampling in NMR tubes (48). Recently, NMR has also been presented in a truly non-invasive mode, by deploying one-sided magnets with built-in (49) measurement coils, also denoted as the MObile Universal Surface Explorer (MOUSE). The first applications of the NMR MOUSE were in non-invasive assessment of polymer quality (50), but also the first applications in food technology have appeared. These food applications of the MOUSE focussed on the non-invasive assessment of *compositional* parameters (51,52). However, the aforementioned sensitivity of NMR to distribution and dynamic state of water implies that the MOUSE should also be a versatile sensor for the *microstructural* quality of foods (53). We have explored this in a recent study where we investigated whether the microstructural quality of the aforementioned model emulsions could be assessed by the NMR MOUSE. As a measure for microstructural quality, Water Exudation (WE) was taken, which is commonly assessed by a cumbersome and destructive gravimetric procedure.

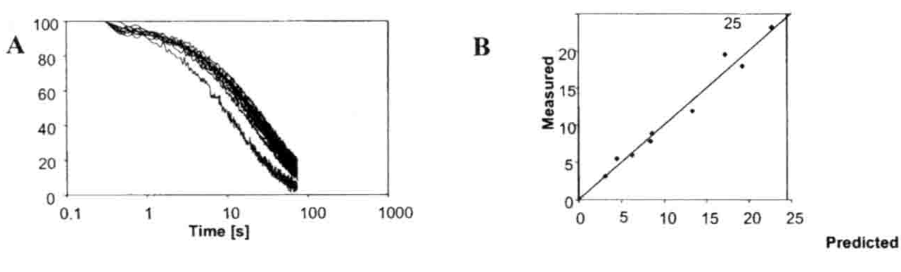


Figure 7 *(A) MOUSE decays of food emulsions that were used for non-invasive (though-package) predictions of water-exudation (B).*

In Figure 7A the NMR decays are presented of a series of emulsions with different WE values. By means of multi-variate methods, models can be built that correlate these decays to WE values (Figure 7B). Validation of these models indicated that the MOUSE yielded reliable 'through-package' measurements of WE (54). This opens opportunities for non-invasive and on-line testing of the microstructural quality of food products in manufacturing environments and in the supply chain.

4 CONCLUSIONS AND PERSPECTIVES

In the Discovery phase, NMR is playing an important role in holistic assessment of complex materials and organisms like humans. The most impressive example is metabonomics, which has profited significantly from the stability and unbiased and comprehensive nature of NMR. These characteristics can also be exploited in the explorative building of understanding of the microstructural origin of the properties of food products and materials. We expect that NMR will keep playing an essential role in the systematic and unbiased building of both physical and biological understanding. For the sake for conciseness we have not been able to address the developments in Discovery of the underlying neurophysiological factors of consumer behavioural/perception where functional MRI (55) has rapidly acquired an essential position.

We have also illustrated how NMR is being applied in the Design/Deploy phases. Implementation of relaxometric and diffusometric NMR methods on benchtop instrumentation, has brought NMR close to the workbench of the food technologists and product developer. We envisage that further miniaturisation of NMR instrumentation will take place in the next years, thus moving NMR technology further into the Deploy and Deliver phase. The MOUSE sensor may only present a first example of a revolution that may profit from developments in magnet design (56) and microcoils (57). These hardware developments may enable truly online sensing (58,59) of meso/micro/macro-structural product quality. Ultimately, we may even envisage the use of hand-held sensors for consumer (health) self measurement (60).

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