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Nuclear magnetic resonance for foodomics beyond food analysis ☆

Luca Laghi, Gianfranco Picone, Francesco Capozzi *

Department of Agri-Food Sciences & Technologies, Alma Mater Studiorum – University of Bologna, Piazza Goidanich 60, 47521 Cesena, FC, Italy

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ABSTRACT

Nuclear magnetic resonance (NMR) spectroscopy has a long tradition as a powerful platform in the hands of modern food scientists, with several applications related to food safety, traceability and authenticity. The continual advances in instrumental sensitivity and electronic stability, together with rapid growth in new, potent algorithms for multivariate data analysis, facilitate the use of NMR spectroscopy as a competitive, complementary analytical platform for observing the food metabolome. By adapting the holistic views of metabolomics research, foodomics emerges as a new discipline bringing food science and nutritional research closer together.

This review mostly focuses on recent efforts dedicated to extraction and interpretation of NMR data, rather than providing technical details about their acquisition. With this aim, we present new trends in the exploitation of the information gained by NMR of food matter. We critically describe and illustrate, via representative examples, the limitations and the counterbalancing advantages of the technique.

Contents

1. From food safety to food for health through foodomics

Nuclear magnetic resonance (NMR) spectroscopy is an investigation technique that, with a minimum sample preparation, offers the possibility to obtain quantitative and structural information of any molecule characterized by atoms with an intrinsic magnetic moment and angular momentum. The elements mainly present in foods, such as H, O, C, N, and P, have at least one detectable isotope, thus granting NMR spectroscopy the title "universal detector". The widespread application of this high-throughput technique, together with mass spectrometry (MS), is leading to a change in the goals of food analysis, as emerging from the systematic examination of the works published in the past 10 years [1]. The first goal of food analysis has traditionally been, and still is, to ensure food safety. Food

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* Corresponding author. Tel.: +39 0547 338105; Fax: +39 0547 382348.
E-mail address: francesco.capozzi@unibo.it (F. Capozzi).

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safety has been flanked by food traceability [2] and food authenticity [3], with the current frontier represented by studies on chemically or genetically fortified foods tailored to promote health [1].

The coverage of recent literature on NMR-based analyses is better in and extensively reported by other dedicated papers [4–8].

The success of these studies passes through evolution from a reductionist approach to food towards the observation of food "as a whole" [9]. For a holistic view of a food, ideally one should:

- know the structure and the concentration of all its molecules, by analogy to what has been done in the genetics field with the map of the entire genetic code [10];
- gain information about the metabolic networks and fluxes characterizing the food, where fluxes concern the kinetics description of all the transformations occurring within the same metabolic network [11]; and,
- understand how environmental factors or technological treatments modulate food composition.

In other words, the description of food composition must have the same high definition reserved to the human being (i.e., with details at the level of its genome, proteome and metabolome). Such a degree of definition, indeed, is required when the link between foods and their health effects must be demonstrated with a clear molecular mechanism. Meta-analysis of the information obtained from all omics, applied to both food and human, is the approach best addressing the mechanistic definition of nutrient activity [12]. In this context, foodomics collects the omics information about food products, which is necessary to define their safety, quality and nutritional value comprehensively.

2. Foodomics for a holistic approach to food

Foodomics has been defined as "the discipline that studies the food and nutrition domains through the application of advanced omics technologies in order to improve consumer well-being, health and confidence" [13]. Foodomics traditionally takes advantage of genomics, transcriptomics, proteomics and metabolomics data. Among them, metabolomics is the one geared towards providing an essentially unbiased, comprehensive qualitative and quantitative overview of the metabolites present in an organism [14].

The purpose of foodomics is to define food by applying the omics approaches, because the food is the result of, e.g., selection, production, processing, and storage, on the genome-transcriptome-proteome-metabolome of the originating organisms, or parts of them (Fig. 1). Whilst metabolomics pertains to the study of the metabolome of biological systems, foodomics studies the effect of different factors on the genome, the transcriptome, the proteome and the metabolome of the biological systems during their transformation in food. Then, foodomics continues its role by defining the evolution of food during digestion, absorption and interaction with humans, looking at nutrition and health from the food perspective.

Foodomics is ideally positioned for use in many areas of food science [4] for two main reasons:

- the metabolome can be considered downstream of genome, transcriptome and proteome, and is therefore the best representation of the food phenotype, so it can give a direct view of the substances that interact with our organisms upon eating; and,
- it is known from both metabolic control analysis [15] and experiments [16] that concentration changes of individual active enzymes might be expected to have little effect on the corresponding metabolic fluxes, but significant effects on the concentrations of other numerous individual metabolites due to cascade effects, feedback action or pleiotropy.

An example of the latter point is a recent study describing the perturbation caused by the insertion of one or three copies of the same exogenous gene into the metabolome of transgenic grapes [17]. The exogenous DefH9-iaaM construct encodes for tryptophan-2-monoxygenase, which is the enzyme regulating the synthesis of the auxin hormone indoleacetic acid. The experiment showed that, whatever the mechanism, the changes occurring in the grape composition were not directly predictable on the basis of type and copy number of the additional genes. For this reason, the metabolome (i.e., the molecular phenotype) could be considered as the monitor integrating the comprehensive perturbations at all omics levels.

3. NMR for food analysis and foodomics

The complete characterization and quantification of the molecules constituting the food metabolome can be thought as representing one dimension of the foodomics space, by analogy with and extending the metabolomics space described elsewhere [18]. An analytical technique ideally tailored for its exploration should be characterized by [19]:

- ease of quantification and identification;
- the high number of metabolites that can be measured through a single-pass, for which automation is important;
- short time and low costs needed for analysis, including sample preparation; and,
- the possibility to store the data into a database with extensive details and enriched by sufficient descriptors to allow the information to be retrieved by user-specific criteria.

The majority of the foodomics studies performed through NMR focus on hydrogen because it gives the highest sensitivity, compared to ^{31}P, ^{13}C, ^{17}O or ^{15}N. Nevertheless, until the potential of ultrasensitive applications, such as dynamic nuclear polarization [20] is expressed, ^{1}H-NMR spectroscopy still has to be considered a poorly sensitive technique compared to other spectrometries [e.g., MS, spectrophotometry, and electron-spin resonance (EPR)]. A second limitation of ^{1}H spectra in the exploration of the foodomics space has been traditionally identified as the relatively reduced resonance window of proton spectroscopy compared to ^{13}C or ^{31}P, so that many signals appear overlapped, especially when complex mixtures are analyzed. A reasonable number of molecules that can be unambiguously identified and simultaneously quantified in a food extract is in the range 50–100 [21].

These limitations are counterbalanced by the fact that the only variables modulating an NMR spectrum are the solvent, the magnetic field, and the pulse sequence employed to transfer magnetization to the observed nuclei, with little instrument drift [22]. The high reproducibility makes NMR spectroscopy the choice for obtaining data that can be directly organized into a database, since pattern recognition and multivariate analyses can be directly applied to raw spectra. To appreciate the importance of the possibility of analyzing unassigned sequences of numbers (such as spectra) or strings of letters (such as codons), it is sufficient to consider the impact that the publication of databases accessible to the entire scientific community had in the genomics field, even at their first appearance when most of the DNA or protein sequences were not yet annotated. The advantage of using raw spectra for the comparison of different metabolomes encouraged the exploitation of NMR foodomics for classification of food products.