

NUCLEAR MAGNETIC RESONANCE

A possible answer to actual and future analytical problems

Summary: Within a context of production and internationalised marketing, the development of analysis methods must respond to the needs of more and more numerous, specific and precise analyses. The spectroscopic methods, which are non-destructive and permit simultaneous multiple determinations, are privileged tools for these needs. In Particular, high-resolution NMR spectroscopy presents characteristics and a potential capable of answering the dairy industry's needs. This method is not yet very widespread in control laboratories, but, to date, the results obtained seem promising.

New analytical objectives :

The needs in analytical methods for measuring the components of milk and dairy products, are now covered as much by the reference methods as by the fast routine methods.

However, research and development in matters of analysis methods has to face up to new needs relating to product quality. The dairy sector cannot escape from this trend.

Indeed, with increasing international exchanges and consumer protection, a more and more complex international regulation is being set up (CE, Codex) concerning :

- nutritional allegations and health,
- the absence of chemical, biochemical and microbiological contamination,
- product conformity (falsification, adulteration),
- AOP product authenticity.

Moreover, the needs for control concern essentially consumption products, but they can also concern raw materials, milk in this case, whose quality is regularly verified by the dairy inter-profession through its milk payment laboratories network. For example, the increased knowledge of fat could lead to a differential classification of milk on the basis of its health and nutritional quality.

Considering the diversity and frequency of the imposed controls due to increasing commercial exchanges, a regular control of product quality and conformity is hardly feasible with reference methods, which are often long, expensive and sometimes require a high technicality. To be precise, the identification and the quantification of particular molecules in a complex mixture (i.e. food matrix) require a previous separation of the analytes before analysis. The reference methods are established on this principle. The control of the product composition in relation to contract conditions multiplies the analyses by the number of analytes concerned, generating a very important cost of the collected information.

The development of fast analysis methods proves to be necessary.

Spectroscopic methods

Spectroscopic methods became the privileged tools to analyse the composition of dairy products thanks to their rapidity, their non-destructive and non-polluting character, and their aptitude to measure simultaneously several analytes or components of the matrix.

In the dairy sector, the actual fast routine analysis methods are principally based on mid and near infrared spectrometry. As they are not very specific, they are limited to a restrictive number of components or component families (e.g. fat, protein, sugar) and they generally present a low precision for the minor components when they can be measured. Low-resolution nuclear magnetic resonance is less used because of its limited number of specific applications (water and fat).

Considering these limits, it seems necessary to identify other fast spectroscopic analytical methods to permit the specific measures of a large diversity of molecules in food products, particularly in dairy products, and to develop applications.

High-resolution nuclear magnetic resonance spectroscopy (HR-NMR)

A fast method with potential

NMR spectroscopy addresses the atomic nucleus, permitting obtention of a characteristic spectral signature of the atomic and electronic environment of each atom.

Principle: NMR measures the difference between the resonance frequency and that of a reference standard. This is called the chemical shift. Each molecule can therefore be characterised, in theory, by all the chemical shifts of each nucleus considered, and so constituting a characteristic spectrum of the molecule. In a molecular mixture, the spectrum obtained reflects the sum of the individual spectra.

Born in the United States in 1945, the NMR principle obtained the Nobel Prize for physics in 1952 for its two principal inventors, Edward M. Purcell and Felix Bloch. Since then, this method has been stated at many levels according to the applications sought

after. Principally, two types of NMR can be distinguished : low-resolution and high-resolution.

Not all the elements are visible in NMR, but the principal elements that constitute living matter (C, H, O, N, P) are, either directly, indirectly according to their environment or via one of their isotopes .

Low-resolution NMR : this method uses low intensity fields and the resonance of the hydrogen nucleus, for which the maximum intensity of the signal is obtained. The cost of this method is not very high and its instrumentation not yet very bulky. It is applied to the direct quantitative analysis of certain elements (water and fat) and is used in routine analyses for product control. It also permits to distinguish the different forms of water and to study the repartition of water in the organic matrix by nuclear imaging.

High- resolution NMR : this method uses high intensity fields and has been for a long time reserved to research laboratories, within the context of structural and dynamic study of organic components and the reactional mechanisms. It is potentially applicable to all the "sensitive" nuclei, that is to say nuclei with a magnetic moment. Apart from the expensive material, this method requires a high technicity facing the various techniques used to exploit the spectral information.

The state of mobility of the elements in the matrices allows distinction of NMR with solids, for which a high-resolution is less easy to obtain than with liquids and for which particular techniques have had to be developed. It is more particularly via sample presentation modalities, that a very significant gain in resolution can be obtained, with the introduction of magic angle rotation (MAR). A rotation of the sample according to an angle of $54^{\circ}44''$ cancels out many effects, especially interactions between nuclei (direct coupling effects) and sample heterogeneity . In the favourable case of products with partial molecular mobility (semi-solids), it is possible to reach a resolution near to that obtained with liquids. This technique is called high-resolution NMR magic angle rotation (HR-MAR). It is particularly efficient for polyphasic heterogeneous matrices (e.g. paste and gel). This technique permits to envisage a fine analysis of food and foodstuffs.

A constantly improved tool :

Improvements in high-resolution NMR have been developed thanks to the progress in supra-conductor electromagnet technology, in electronics and in the treatment of the signal and the spectral information. Thanks to scientific, technical and technological developments, the sensitivity and the resolution of the method have seen considerable improvements.

The method is, therefore, more and more adapted to direct quantitative and qualitative analysis of complex products :

- improvement in magnet technology (more regular, powerful and protected fields),
- taller sample racks (better signal),
- low temperature probe (better signal/noise ratio)
- multiple probes permitting simultaneous scrutiny of several nuclei (increase in specificity),
- improved electronics (better signal/noise ratio),
- quicker signal treatment (deconvolution, Fourier transformation),
- treatment of the spectral information thanks to advances in data processing and software.

To be more accessible to a larger number of users, user-friendly automated systems with micro-computing and software dedicated to the treatment have been perfected (e.g. ADVANCE INCA™ and Metabolic Profiler, by Bruker; Varian NMR System, by Varian Inc.).

More particularly, integrated systems have been developed to associate chromatographic separations before the NMR measures, for example, LC-NMR (Liquid chromatography NMR, Spraul and al., 2005).

Now, applications dedicated to routine analysis laboratories in the food industry are being developed through collaborations with research and development laboratories.

To date, work in the area of food analysis is still rare and oriented to a more qualitative rather than quantitative aspect. It is often associated with product characterisation. Examples of work on quantitative analyses are rare.

Development for specific quantitative analysis :

The work by De Angelis Curtis and al. (2000) gives a possible example of application in the dairy industry. Indeed, other than proposing a method for following the ripening of Grana Padano cheese they have shown that amino acid profiles during ripening can be established by ^1H (proton) NMR at 500 MHz.

The amino acid composition is a major element of food nutritional value and, in the case of foodstuffs dedicated to sensitive populations (e.g. infant foods), its control is, to date, limited by the lack of quick or economic analytical techniques. NMR could be an option to establish an aminogram by direct scrutiny of a sample in the context of product control.

Development for qualitative analysis and characterisation :

In the dairy industry, NMR had its first success in the 80s, with the isotopic hydrogen ratio measurement technique for the detection of chaptalization during vinification (SNIF-NMR®). The method, that has since been used for other applications, is based on the

preliminary identification of a tracer (for example, ethanol), for which the spectral fingerprint is determined, and that varies according to the factor to be identified (e.g. nature and origin of the fermented sugars).

More global approaches, using multidimensional statistical treatments to exploit the NMR spectra, have appeared. They permit recognition and classification according to various combinations of spectral zones that can be linked to various biochemical tracers, which together, characterise the products.

NMR is efficient in the characterisation of various products in the food industry. In the dairy industry, recent work by the TRACES laboratory (Universities of Provence and Paul Cezanne in Marseille) has shown the possibility to characterise different steps during ripening of Parmigino Reggiano cheese by the application of HR-MAR using ^1H NMR at 400 MHz. In parallel, it is possible to assign the spectral lines to the principal tracer element during ripening (Shintu et al., 2004). Another study, led by the same team, on 20 Emmental cheeses from 5 European countries and using the same technique, permits to establish a distinct discrimination of the cheeses according to the origin of country. It also allows cheeses made from heat treated milk to be identified (Shintu et al., 2006).

For the protection of Registered Designation of Origin (RDO) products, this method is an alternative to the necessary traditional methods for establishing the authenticity of a product's origin and its conformity to the RDO type contract. The need for rapid controls has increased with the development in the commercialisation of portions, for which the reference of the cheese round is no longer possible for the consumer.

Hypotheses and conjectures in relation to emerging needs

With competition for market shares, nutritional and health allegations are more and more important. Those relating to a particular composition must be verifiable by the manufacturer and the regulation control services.

The incidence of food fat quality on human health is part of this problem, to regulate the maximal trans-fatty acid content and to guarantee minimal unsaturated and polyunsaturated fatty acid content. In the future, it will be possible to have the definition of new quality criteria of fat.

The fast specific quantitative analysis of these fatty acids could become necessary to establish characteristic fatty acid profiles.

To date, for many fatty acids, tables of chemical shifts for ^1H and ^{13}C nuclei in various positions of the molecules have been set up. It is notably possible to appreciate the spectral differences between fatty acids.

At the development of analytical methods level, various authors summarise the state of quantitative analyses on unsaturated fatty acids (AGM, AGPI, ω -3) in different fat matrices (oil and fish fat) by ^1H and ^{13}C NMR (Knothe, 2005).

However, the work published to date only concerns a limited number of particular fatty acids in relatively homogenous and simple matrices.

The technical developments realised with NMR, associated with possible couplings with separative techniques, permit to expect increased possibilities relating to molecule recognition and quantification.

Therefore, it is not excluded that NMR brings fast alternative solutions applicable to dairy fat, to partially substitute the long and costly methods actually required for the establishment of fatty acid profiles.

Conclusion

NMR is still ill-known and little used in the dairy sector. However, since 2000, various studies have opened up applications to dairy products. The method presents an attractive character by its versatility so much as for analysis of dairy matrices as for analysis of measurable elements in these products. The qualitative approach in relation with the characterisation of product quality seems to be more advanced than the developments in quantitative analysis.

Lines for development and priorities will therefore have to be defined.

Bibliographic references:

De Angelis Curtis S., Curini R., Delfini M., Brosio E., D'Ascenzo F., Bocca B., (2000) : Amino acid profil in the ripening of Grana Padano cheese : A NMR study. *Food Chemistry*, Vol. 71, 4, 495-502.

Knothe G., (2005) : ^1H NMR Spectroscopy of Fatty Acids and their derivatives – Quantitation by ^1H NMR. The Lipid Library, www.lipidlibrary.co.uk.

Knothe G., Kenar J.A., (2004) : Determination of the fatty acid profil by ^1H NMR spectroscopy. *Eur. J. Lipid Sci. Technol.*, 106, 88–96 (2004).

Shintu L. , Caldarelli S., (2006) : Towards the determination of the geographical origin of Emmental

cheese via High Resolution MAS NMR : A preliminary investigation. (envoyé pour publication).

Shintu L. , Caldarelli S., (2004) : High-Resolution MAS NMR and chemometrics : Characterization of the ripening of Parmigiano Reggiano cheese. Journal of Agriculture and Food Chemistry.

Spraul M., Humpfer E., Schäfer H., (2005) : Application of hyphenated NMR to the study of food.

From « Magnetic Resonance in Food Science : The Multivariate Challenge. The Royal Society of Chemistry 2005. ISBN 0-85404-648-8.

Royer A., Naulet N., Lees M., Martin G.H., (1997) : Isotopic analysis for the authentication of extra-virgin oils. Food Authenticity 4th European Symposium, La Baule, 4-6 juin.