# Water/moisture and fat analysis by time-domain NMR

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## Abstract

Time-domain nuclear magnetic resonance (TD-NMR) is widely used within food industries for QC/QA applications. Precise determination of moisture and water is of fundamental interest. To determine moisture and water in the presence of lipids, the mini-spec makes use of differences in their respective NMR relaxation properties. The minispec applications analysing bound water are well-known and have become International standard methods. Different methods to analyse food samples with high water content or free water, like some dairy products, are addressed in this paper. Explicitly, the well-known pre-drying methods are compared to NMR diffusion experiments and a combined relaxation time analysis. Finally the single-sided NMR approach is discussed to analyse packages food.
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## 1. Introduction

Although NMR is ubiquitously applied for chemical analysis and structural research as well as in its clinical adaptation of magnetic resonance imaging (MRI), time-domain NMR is often not very well known. This field – also known as low-resolution NMR – has started about 35 years ago in a cooperation of Unilever Research (The Netherlands) and Bruker Physik AG. The idea was raised to build a small tabletop time-domain (TD)-NMR analyzer for the solid-to-liquid ratio analysis on fat compositions. Nowadays, this method is widely adapted and well known as the so-called solid fat content (SFC) determination (Bruker minispec: Food Industry Application, 1970). It is recognized as an International Standard Method (American Oil Chemist Society, 1993; IUPAC Norm Version 2.150 (ex 2.323), 1987; ISO 8292 International Standard, 1991).

Moreover the classic use of TD-NMR in food science and industry is devoted to products containing either a dominating amount of water or fat with the aim to determine the concentration or the amount of a specific component, respectively. The restriction to either low water or low fat products is due to the fact that in TD-NMR almost no spectral resolution is available and that the transverse and longitudinal NMR relaxation properties of fat and water molecules are not substantially different. Moreover, the selectivity in commonly used TD-NMR pulse sequences is too small to guarantee a clear separation of signals. The classic approach was to pre-dry the samples either for example by an oven, chemical reagents like $CuSO_4$ or via infrared or microwave drying processes, rendering the TD-NMR application a two-step approach. Due to the technical development of TD-NMR spectrometers, especially of

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the electronic part, in the last years, also more sophisticated experiments can be implemented and run for quality control in food science and industry, allowing the method to be a simple one step procedure.

In this paper the physical background and selected examples of these new applications will be discussed as well as the standard approaches. In the first section however, TD-NMR in regards to water/moisture signals in food will be briefly addressed.

## 2. Time-domain NMR and water/moisture analysis

### 2.1. Experimental

As TD-NMR experiments are either based on relaxation or diffusion measurements, i.e. on differences of the samples in their NMR-related properties, there is no need for highly homogenous magnetic fields or high field strengths as commonly required in FT-NMR. Thus, permanent magnet technology is well suited. This significantly reduces overall system costs as well as running costs (no cryogens are required like in case of superconducting magnets). Most common TD-NMR applications are based on very simple NMR pulse sequences, like the Free Induction Decay (FID) or the Hahn-echo acquisition (Hahn, 1950). TD-NMR systems can, however, also be used to measure relaxation time constants, like the relaxation times $T_1$, $T_2$ and $T_{1\rho}$ or to perform pulsed field gradient (PFG)-NMR experiments.

### 2.2. Applications

The NMR properties such as relaxation times of water molecules are strongly dependent on the neighbouring surrounding. Whereas crystal water exhibits relaxation times in the order of some tens of microseconds, bound water has an intermediate relaxation behaviour, the NMR signal decays within a few hundreds of microseconds. Free water may show NMR signal up to seconds. Therefore, TD-NMR is suitable for a qualitative and quantitative examination of food samples with respect to the water binding.

Making use of the different relaxation properties of various water types, TD-NMR is appropriate for detection of glass transitions or ice melting behaviours, like for example in dough. Water freezing in food can be followed up quantitatively using low temperature equipment.

Usually total signal amplitudes can be used to determine moisture in a quantitative way. As food samples usually contain also lipids exhibiting relaxation times of a few hundred milliseconds, the minispec methods have to differentiate these components, using typically relaxation properties as contrast parameter.

## 3. Simultaneous oil and moisture analysis in food (moisture below 15%)

Only few years after the introduction of the SFC method with TD-NMR, the application area of TD-NMR has been widened by applying the Hahn-echo NMR pulse sequence (Fig. 1) to determine the total oil and moisture content in food samples. Almost all types of food samples could be analyzed provided the water content is minor (typically below 15%) (Bruker minispec: Food Industry Application, 1975), see Table 1. Again, the oil and moisture analyses for seeds and seed residues have become International Standards Methods (American Oil Chemist Society, 1995; ISO/CD 10632 for Oilseed Residues, 1993; ISO 10565 for Oilseeds, 1995). According to these methods, both the FID signal amplitude, $S_1$, and the echo amplitude, $S_2$, are measured (Fig. 1). The amplitudes $S_1$ (at about 50 µs following the 90° pulse) and $S_2$ (at $\tau = 7$ ms) represent the sum of the oil and moisture signals and the oil content, respectively. Thus, the amplitude difference, $S_1 - S_2$, is related to the moisture content (see also Todt, Guthausen, Burk, Schmalbein, & Kamlowski (in press)).

The oil and moisture contents can be measured simultaneously after previous calibration. The calibration is performed only once and will be automatically stored. Advantages of NMR, compared to other standard methods like chemical oil extraction, are a minimum sample preparation and a fast analysis time which is in the order of seconds. Moreover, chemical solvents are not required releasing the problem of waste disposal.
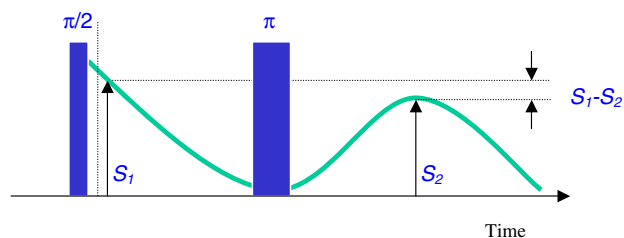


Fig. 1. Hahn-echo NMR pulse sequence with amplitudes and amplitude difference for the determination of both the moisture and fat content of low water products.

Table 1
Summary of food and feed samples that can be analysed with respect to the moisture and the fat/oil content by standard TD-NMR methods

- Moisture and fat/oil content in (Dry) food systems:
- Cocoa powder, cocoa beans and chocolate (mass and liquor)
- Snacks, cereals, chips, brezel, fischli, bread
- All types of nuts and almonds, cookies, beans, lentils, linseed
- Milk powders, bakery powders, baby food, low moisture cheese, soup powders
- Flavors, flour, semolina, starch, sugar beet

- Moisture and oil in all types of seeds and residues.
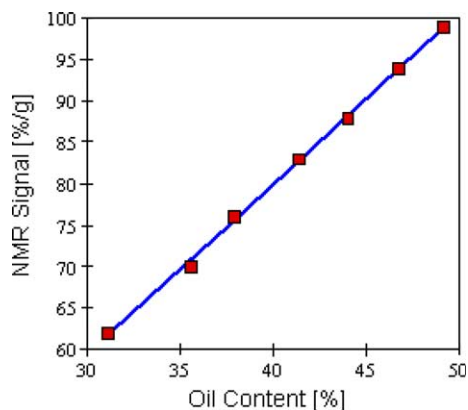
- Moisture and fat content in animal feed.

Fig. 2. Typical calibration curve of a standard oil content analysis. The regression model is simply linear, as the NMR signal amplitudes are directly proportional to the number of hydrogen nuclei in the oil phase. Here the oil content of seeds has been analyzed.

Naturally, the TD-NMR systems are turnkey systems which do not require skilled personnel.

Obviously, different products (e.g., rape seeds/sunflower seeds) require different calibration curves. In Fig. 2 a typical calibration curve is depicted. Generally, only a few reference samples – typically 3–5 – are required. The values from reference methods, like Soxhlet extraction and Karl-Fischer water determination, are utilized to calibrate the TD-NMR equipment. In seed breeding applications the oil calibration is performed differently: Pure oil samples are utilized for system calibration. In addition, the oil sample mass is taken into account by weighing. This way, systematic errors and uncertainties from the reference methods are excluded. For inhomogenous samples like big seeds or nuts, TD-NMR systems with measurement volumes up to 100 cm$^3$ are available.

Another standard method to be mentioned is the determination of fat content in chocolate, more precisely in chocolate mass and paste as well as in chocolate liquor (Bruker minispec: Food Industry Application, 1980). This method requires that the fat has completely melted. Calibration of the system can be done conveniently with pure cocoa butter. Alternatively, well-characterized chocolate samples can be used for the calibration.

## 4. Oil content determination in pre-dried samples

Oil and moisture determination on food samples containing only bound water can be performed easily, as de-

scribed before. However, samples with high amounts of water/free water cannot be treated this way. The NMR signal from water will interfere with the oil signal. This is because the relaxation times of free water and oil are rather similar and chemical shift dispersion is obscured in TD-NMR by the magnets inhomogeneity. Under these circumstances, samples (like olives, sausages, fish, meet) need to be pre-dried to remove free water. Following this preparation step, a Hahn-echo sequence is adapted to determine the oil content precisely. This method has found more than a hundred of users only in Spain for the precise oil content determination in olive paste (Bruker minispec: Food Industry Information, 1995).

## 5. Gradient experiments for fat and water content determination

In parallel to the already discussed relaxation parameters, diffusion properties can be exploited by PFG experiments in order to separate signal contributions of different molecules. For example, diffusion coefficients of water and oil differ by approximately a factor of 100. A variety of PFG sequences is available, allowing dedicated approaches to the individual problem. As an example, the conventional Hahn Echo equipped with pulsed gradients and the PFG stimulated echo sequence are shown in Fig. 3.

The key application – as discussed – is the determination of the fat/oil content in fresh food in the presence of a high water content. By adjusting the timing of the sequence properly, the contrast of the components to be differentiated can be optimized. In Fig. 4 the echo attenuation of a fresh salmon sample is depicted as a function of the square of the gradient strength. The fast and slowly decaying components are due to water and oil, respectively. Extrapolation of the slowly decaying phase (solid line) to zero gradient strength provides a measure of the oil content. Calibration of the signal against known standards allows the determination of the relative oil or fat concentration. The calibration can be done very quickly and requires only few well-known reference samples for each type of product.

Clearly, the PFG-NMR approach implies that the sample can be readily investigated without the need of pre-drying. The method was examined for different products such as sauces, mayonnaises, margarines, and
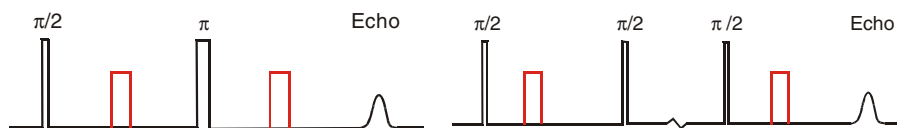


Fig. 3. PFG Hahn-Echo (left) and PFG stimulated echo PFG-STE (right) used in TD-NMR for discriminating components exhibiting different diffusion properties. The application of Hahn or stimulated echo depends on the relaxation and diffusion properties of the product to be investigated.
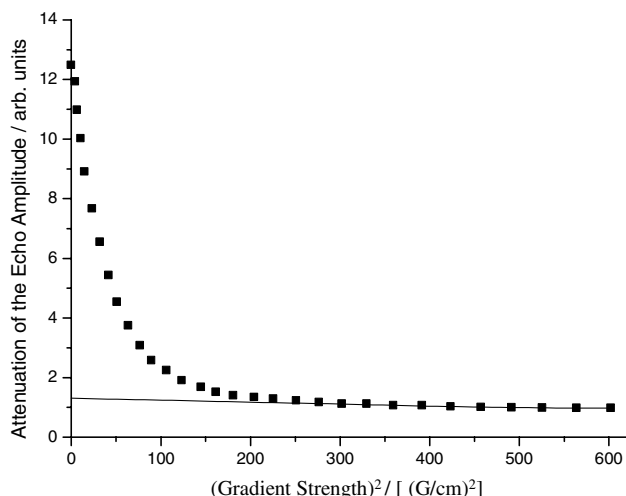
Fig. 4. Echo attenuation as function of the squared gradient strength measured on a fresh salmon sample. The diffusion coefficients of water and oil differ strongly so that, at larger gradients, only the oil signal is left (Todt et al., 2001).

dairy products with fat content in the range of 4% to about 80%.

## 6. Combined relaxation analysis in foods with high water content

It is well known that the transverse and also the longitudinal relaxation times of water and fat differ. The same is valid for several other ingredients, for example proteins. The relaxation properties of these components are exploited in the application discussed in this section. Clearly, as the differences in relaxation times are relatively small and as the magnetization relaxation depends also on other parameters like temperature, salt concentration, i.e., the local environment of the molecules, a common measurement of well known relaxation times like $T_1$ and $T_2$ is not sufficient for establishing a good correlation to the sample properties. Therefore, a combined relaxation experiment is to be applied, where the magnetization at a given time is determined by both, the longitudinal and the transverse relaxation (Fig. 5). There are several approaches to realize this aim. One of them uses a steady state dynamic equilibrium (Guthausen, Zimmer, Blümler, & Blümich, 1998), others (Tinsley, Taicher, & Heiman, 2004) make use of the longitudinal recovery of magnetization and measure the transverse magnetization at each point of the $T_1$ curve by CPMG. Using a modified sequence which also acquires the FID in addition to the echoes, 'hard' components can also be measured and analyzed. Thus, the relaxation properties $T_1(M(t, T_2), M(t, T_2^*))$, $T_2(M(t, T_1))$ and $T_2^*(M(t, T_1))$ can be measured within one experiment (Guthausen, Todt, Burk, Schmalbein, & Kamlowski, in press-a), whereby $M$ is
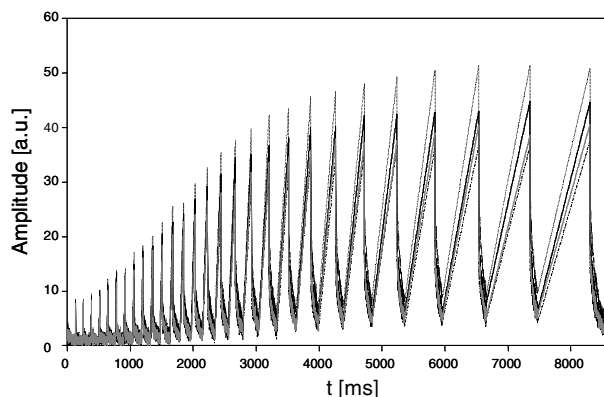


Fig. 5. Time dependence of the NMR signal measured by a combined relaxation time experiment. Four different samples each with a specific composition are shown. The differences in the time dependence due to the specific relaxation times are obvious.

the nuclear magnetization and $T_2^*$ is the inhomogeneous transverse relaxation time.

The information obtained by this experiment is huge and not easily analyzed quantitatively in terms of classic relaxation time analysis. Therefore, another approach was chosen for data processing, using statistical, i.e. chemometric methods (http://www.chemometrics.net/). These methods are commonly applied for example in near infrared studies. One of the advantages is, that a correlation with several sample properties can easily be established. As an example in Fig. 6 the results of fat analysis on mayonnaises, margarines, and sauces being characterized by wet chemical methods are shown (samples from Unilever Bestfoods, the Netherlands). Please note that the three types of products fall onto the same
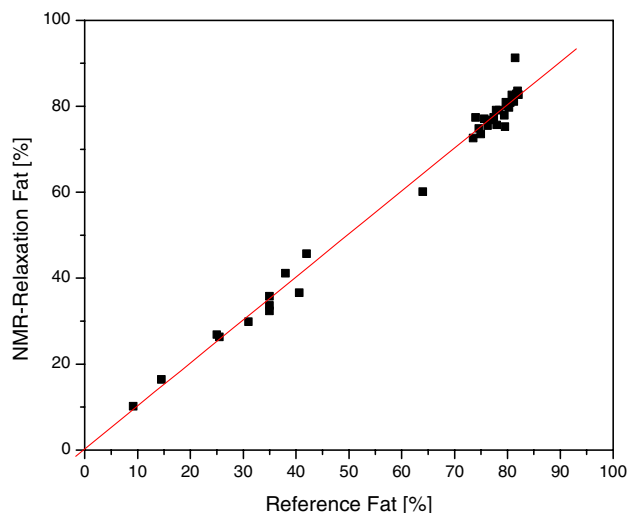


Fig. 6. Fat content as determined by the combined relaxation analysis for margarines, mayonnaises, and sauces. A clear correlation with the reference fat content could be established. The correlation coefficient amounts to 0.994, the statistic F-factor to 2814. The correlation considered 35 different samples with a fat content between 9.2% and 82.1%.

Fig. 7. The Bruker single-sided NMR device called minispec mq-ProFiler, specially designed for industrial needs. Creme samples are shown for comparison of size.

calibration curve. This implies the possibility to establish a common calibration model which will be valid for several products thus reducing the effort which is usually necessary for chemometric data processing.

## 7. The Bruker single-sided NMR approach

Similar to the above described relaxation time and diffusion approaches (see Sections 3 and 4), single-sided NMR can be applied to analyze water and fat content with correlations of the same order of magnitude. The big advantage of this approach is related to the fact that the sample can be directly placed on the surface of this device without any tube filling actions. The analysis can be even performed through packaging material (Guthausen, Todt, Burk, Schmalbein, & Kamlowski, in press-b; Guthausen et al., 2004).

Therefore, Bruker built a single-sided NMR device called minispec mq-ProFiler (Fig. 7), especially for the needs in industrial environments. The device is small and equipped with exchangeable rf parts for allowing fast and easy change of the measurement depth, i.e. tailoring to the application problem. Currently, three measurement depths are available, which are at the surface or 3 or 5 mm in the interior of the sample. Further developments are in progress.

## 8. Conclusion

Due to the numerous advantages of the NMR methods presented in this article the popularity of TD-NMR is steadily increasing. For the water and oil/fat content determination in food especially the 'one step' NMR methods are designated to gain more and more importance. Depending on the special requirements of the users, a tailored method can be chosen from the variety of available solutions.

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