Measurement of Fat Content of Food with Single-Sided NMR

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ABSTRACT: Fat content is one of the important parameters of quality control in many food products. Several techniques are used to determine the amount of fat in a given product. In this work, the applicability of a portable dedicated nuclear magnetic resonance (NMR) analyzer to measure the fat content in a packaged product without destruction of the material was examined. Two different low-field NMR methods, namely, a ratio method and a relaxation time method, were applied and discussed. The processed NMR signal was linearly correlated with the fat content obtained by reference methods. The linear correlation allowed the application of single-sided NMR for fat measurements.

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The rationale for commercial development of a bench-top pulsed NMR spectrometer was to apply the benefits of low-resolution (LR-NMR) or time-domain NMR (TD-NMR) to quality control in food products. The first application of LR-NMR was the determination of the solid fat content (SFC) in various fat compositions (1–3). For this application, the analyzer was designed to record the decay of the total NMR signal from protons in solid and liquid environments. The difference in transverse relaxation properties of solid and liquid fats was exploited to determine the relative solid and liquid contents. As a result of the success of this approach to SFC, TD-NMR was later applied to a number of applications in the food industry. For example, total fat or oil content was easily measured in seeds and other dry materials. TD-NMR methods were developed to measure parameters such as fat and water content in foods and droplet size distribution in emulsions. In these cases, commonly known NMR pulse sequences were implemented from research on high-resolution NMR systems. Current TD-NMR systems are simple and easy to use. For instance, predefined applications include routines for self-tuning and self-validation of the system. However, there are restrictions on the sample size and handling: The material to be investigated has to be filled into sample tubes with a given diameter and filling height. This restriction poses limitations for measurements on living subjects or in packaged goods.

The dimension restrictions of TD-NMR are relaxed when using a single-sided NMR device. One of them is the NMR-MOUSE® (Mobile Universal Surface Explorer, trademark of the RWTH Aachen). It is a portable NMR device used to measure NMR signals from near-surface volumes of arbitrarily shaped samples (4,5). It contains two permanent magnets mounted with antiparallel polarization on a yoke. In the gap between the magnets, a radio frequency (RF) tank circuit is positioned, which generates the RF field ($\boldsymbol{B}_1$) necessary for the observation of an NMR signal. Details of the construction and use of the NMR-MOUSE were described by Eidmann *et al.* (4) and Guthausen *et al.* (5).

Several publications showed promising experimental results on the use of single-sided NMR in quality control. The NMR-MOUSE® was first used in the rubber industry for measuring the cross-link density of the polymer chains (6). The cross-link density strongly influences the mechanical properties of the products, which are of prime importance. A variety of experiments revealed that the transverse relaxation rate is a sensitive and easily available NMR parameter that provides a means to compare samples. From NMR theory, it is well known that the fluctuation in dipolar interactions caused by molecular motion is one of the factors that determine the ^{1}H relaxation times.

All single-sided NMR devices are inherently equipped with a strong magnetic field gradient and are therefore inherently sensitive to diffusion processes. The field gradients are on the order of about 15 T/m and vary depending on the position and distance from the surface of the device. Therefore, not all sequences and methods known in conventional NMR are applicable in measurements by NMR devices using magnetic stray fields. As in the case of TD-NMR with traditional TD-NMR magnets, the inhomogeneity of the $\boldsymbol{B}_0$ field leads to line broadening such that chemical shift information or line splitting due to spin interactions is obscured. In single-sided NMR, the inhomogeneity is usually very significant and determines the decay time T_2^*. The free induction decay (FID) shows a decay time, T_2^*, of around 7 μs, which is on the same order of magnitude as the probe dead times available in TD-NMR. To overcome the fast decay time restriction of the FID, echoes are used for signal detection. Echoes are known to decay due to the generally longer transverse relaxation times T_2 or T_{2e} instead of T_2^*. Other relaxation times such as the longitudinal relaxation time T_1 as well as the longitudinal relaxation time in the rotating frame, $T_{1\rho}$, can be measured using a variety of sequences (5,6).

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In this study, the Bruker single-sided NMR device was used to show its capability in measuring the fat content of several oil/water emulsions. The relative amounts of fat and water are of major interest in the food industry. Several techniques are commonly used. Most wet chemical methods are time and labor consuming and often generate chemical waste. NIR methods often require extensive calibration, have a very limited penetration depth, and may require complex chemometric modeling to separate the sample signal from the container signal. TD-NMR, however, has been applied for years in this field (7,8).

In the case of single-sided NMR devices, the NMR sensor is brought to the sample to be measured. There are no special requirements on the properties of the surface, except it should not exhibit a high electrical conductivity because of RF penetration.

EXPERIMENTAL PROCEDURES

Margarine and mayonnaise samples were provided by Unilever (The Netherlands). The fat content was determined using the wet chemical reference method by Unilever. Briefly, the samples were freeze-dried and then extracted with dichloromethane. The result of the chemical analysis is called "reference fat content" in the following discussions. The coffee creams were from a supermarket and used as available to the final consumer. The fat content from the package was used as a reference value for the coffee creams.

The Bruker single-sided NMR device used for the NMR measurements consisted of (i) the minispec electronic unit, which was connected *via* Ethernet to (ii) a Windows PC, allowing the control of the experiment *via* front-end software; (iii) the NMR sensor, which was connected to (iv) a preamplifier unit where the signal routing and amplification took place.

RESULTS AND DISCUSSION

Single-sided NMR and its consequences on the measurements. Figure 1A is a photograph of the Bruker single-sided NMR device, which gives an impression of its relatively small size. It is equipped with an insert tuned to a certain frequency, which corresponds to a specific measurement depth (Fig. 1B): Taking account of the strong $\boldsymbol{B}_0$ gradient of the device due to the stray field of the magnets, a certain depth selectivity can be achieved *via* the NMR frequency ω_0 ($\omega_0 = \gamma \boldsymbol{B}_0$, γ being the gyromagnetic ratio). For example, inserts tuned for measurements on the surface, 3 mm, and 5 mm differ in frequency by about 800 kHz in the device used in this study. The signal-to-noise ratio (*S/N*) depends strongly on the measurement depth (Fig. 1B) and is low compared with NMR in homogeneous fields. It decreases rapidly at greater measurement depths, mainly owing to the $\boldsymbol{B}_1$ stray field. A strong radial dependence is observed for the stray field as well as for the NMR response (reciprocity principle).

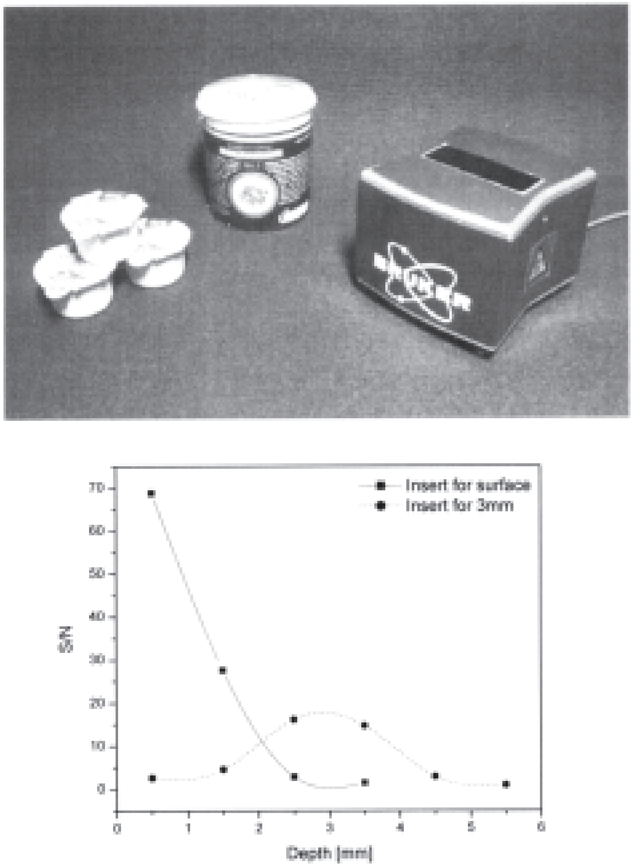

FIG. 1. (Top panel) Photograph of the Bruker sensor. For size comparison, dairy products are also shown. In the middle of the sensor, the insert allowing depth selective measurements can be seen (black rectangle). (Bottom panel) Signal-to-noise ratio (*S/N*) of two inserts tuned for surface near measurements and measurements in 3-mm depth. The *S/N* drops rapidly at greater depths and depends on the shape of the NMR coil.

Apart from the technical features discussed, the number of spins contributing to the NMR signal determines the *S/N*. Unlike NMR in homogeneous fields, where the sample is placed into the NMR coil and where all spins in the sample contribute equally to the NMR signal, only spins within the sensitive spot contribute in single-sided NMR. Essentially, this sensitive volume is determined by the geometry of the RF coil and the magnets as well as by the NMR frequency. Its shape and therefore the *S/N* are strongly dependent on measurement depth. This property of single-sided NMR devices can be used to measure only in a representative region of a given sample. In this study, the surface insert was used to measure packaged creams. The thickness of the package is quite small. The NMR signal of the package material as a hard polymer decays very fast (T_2 in the order of 60 µs), owing to the large dipolar couplings of solid polymers, and is hardly detected in the sequences used in this study. Even at the shortest possible echo times, the signal amplitude of the package material amounts to less than 12% of the liquids signal. In addition, NMR data were interpreted from the ratios of signal regions and magnetization decay time, which do not depend on the absolute signal intensity. Thus, variations in the package thickness are negligible. Only the *S/N* may change owing to the slightly different filling factor. As surface coils show the highest *S*/*N* near the surface and as the packing material is very thin for packed creams, the surface insert gave the best results. For the measurements on mayonnaises and margarines, where the packaging material is thicker, the 3-mm measurement depth was chosen to ensure that the signals from molecules near the packing as well as the bulk material were measured.

It is known that NMR relaxation times are usually temperature dependent. Therefore, environmental temperature stability and sample temperature equilibration are necessary to allow the methods to be applied reliably in quality control.

Single-sided NMR methods used for the measurement of fat content. In principle, two main physical properties can be used to distinguish between fat and water molecules in TD-NMR measurements. First, the ^{1}H transverse and longitudinal relaxation times depend on molecular mobility and therefore on the molecular structure. Second, fat and water differ in diffusion properties. These two material-related NMR properties are the basis for the methods applied in this study.

(i) Diffusion weighted method. As already stated, the diffusion coefficients D of fat and water molecules are different by more than an order of magnitude (oil $9.8 \cdot 10^{-12}$ m^2/s, water $2.3 \cdot 10^{-9}$ m^2/s). This difference can be exploited to create sufficient contrast between water and fat compartments. Several NMR methods are known: The conventional Hahn echo sequence exhibits a dependence on D proportional to exp $(-D\tau^3)$, where τ is the echo time. Consequently, at large echo times the influence of diffusion is more pronounced than at short times. In contrast to the Hahn echo sequence, the dependence on the decay time t is proportional to exp $(-Dt)$ in multi-echo sequences. However, the decay can be measured within one scan, leading to a measurement time advantage over the Hahn echo method. In addition to echoes exploiting mainly transverse magnetization, stimulated echo sequences are known, often being used in restricted diffusion experiments, where only the slower longitudinal relaxation time allows measurements at larger times.

Considering the specific prerequisites on experiments in single-sided NMR, the multi-echo approach seems to be the most promising for measurements in an industrial environment. The advantage is that the measurement is very fast compared with measurements using Hahn or stimulated echoes. A disadvantage is that an analytical understanding of the experiments is relatively difficult, as longitudinal and transverse magnetization components are mixed due to the inherent imperfections of $\boldsymbol{B}_0$ and $\boldsymbol{B}_1$. Figure 2 shows a pulse sequence developed for the special needs of the fat/water differentiation: In the first few echoes the total magnetization of the sample in the sensitive volume is observed except for magnetization contributions due to components with $T_2 < 100$ µs, which are essentially solid-like materials such as packing materials. The echo time τ_1 is small in order to avoid a large influence of relaxation and diffusion effects. Several echoes are averaged in order to improve the *S*/*N* ratio. In the second interval, the diffusion filter is applied such that only the signal of the fat component is left at the end of the filter period. The third part of the sequence is designed for the acquisition of the fat signal. Again, several echoes are averaged to improve precision. In principle, this sequence can be applied to any substances having two components with different diffusion coefficients. Only the number of echoes in any of the three parts of the sequences and the echo times have to be adapted to the specific requirements of the samples.

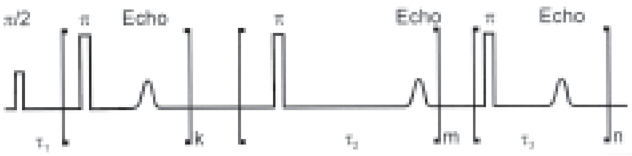

FIG. 2. Pulse sequence for measuring fat and water content *via* the transverse magnetization decay, which is influenced by relaxation processes and diffusion. For details see text.

Finally, for $k = n = 0$ (see Fig. 2), the case of the CPMG sequence [a multi-echo sequence named after the inventors Carr, Purcell, Meiboom, and Gill (9,10)], the complete echo decay is correlated with the fat content, rather than the calculated ratios of echoes at particular times. This method can also be applied in fat content measurements in fat/water mixtures but was found to be less reliable than the specially designed method exploiting diffusion properties and relaxation time differences in a specially designed way.

(ii) Relaxation time method. It is well known that relaxation times of fat and water molecules differ. Therefore, sequences can be constructed to maximize the contrast between them in order to get the maximum reliability. Several methods can be applied for the investigation of relaxation times in single-sided NMR. T_1 can be measured using saturation recovery, an inversion recovery sequence, or steady-state sequences (5,11). Or, in the case of T_2, single- and multi-echo sequences are possible in either thermal or dynamic magnetization equilibrium (11). Preliminary experiments on the skin and subcutaneous fat of humans and pigs showed the possibility of distinguishing both components *via* relaxation time measurements.

The sequence in this study used a combination of T_1 and T_2 relaxation measurements such that the magnetization was first destroyed by a train of pulses. The recovery was combined with transverse relaxation measurements in such a way that the relaxation time properties were reflected also in the T_2 curves. As hundreds of data points were acquired, each containing specific information about the fat/water composition, data processing was performed by a statistical approach, using principal component analysis.

Measurement results and interpretation. In Figures 3 and 4, correlation diagrams of NMR ratio and relaxation data with reference values are shown. For the measurements on coffee cream samples (Fig. 3), the surface frequency of the device was used, implying a reasonable *S*/*N* ratio. As already discussed, the packing material was relatively thin and the

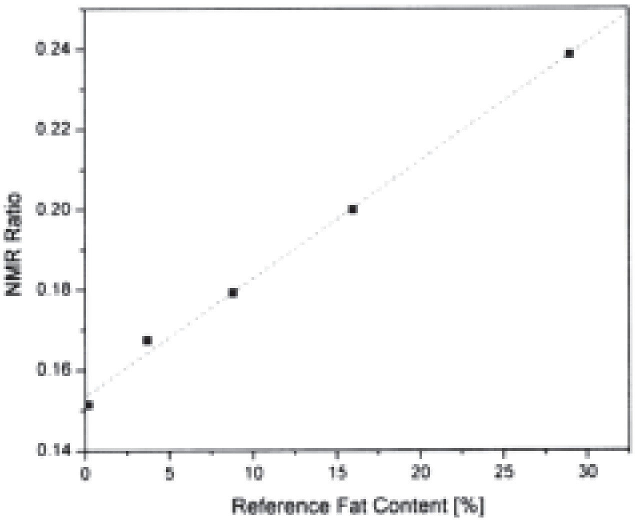

FIG. 3. Correlation of the NMR ratio with the reference fat content of some coffee creams. The correlation coefficient is 0.996. The sequence used for this experiment is the diffusion-weighted ratio method shown in Figure 2. The measurements were performed using the surface insert.

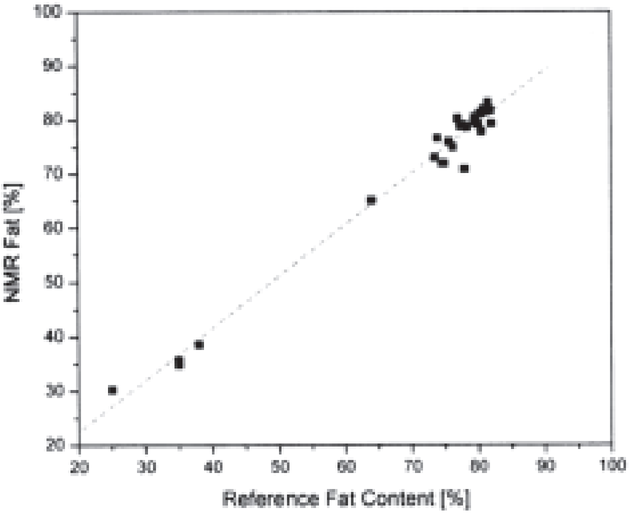

FIG. 4. Correlation diagram for measurements on mayonnaises and margarines, using the relaxation time method. The data were analyzed by a chemometric approach. The NMR measurement depth was 3 mm. The correlation coefficient is 0.991.

polymer material exhibited a short T_2 relaxation time. Thus, the surface insert was the most appropriate experimental setup. The diffusion-weighted sequence used in this study led to measurement times on the order of 1 min. As mentioned, the ratio of the final echoes and the first few echoes was taken as the relevant NMR parameter, named NMR ratio in Figure 3. Plotting this NMR ratio as a function of the reference fat content resulted in a linear dependence that could be addressed by linear regression. The correlation coefficient of 0.996 showed that a reasonable determination of the fat content in quality control was possible. Generally, the *S/N* of the measurement directly influences the quality of the calibration curve, i.e., the precision of the measurement. The *S/N* could be improved by increasing the number of repetitions of the experiment, which leads, however, to longer measurement times. The accuracy and precision of both calibration and measurement of samples with unknown fat content also depend strongly on the quality of the reference values. We were aware that the values on the packages might be rather inaccurate. However, for demonstrating the principle of this method, we chose these values as a good indicator.

In Figure 4 the calibration curve of mayonnaises and margarines obtained by the relaxation time method is shown. Although the composition, the fat content, and the texture of the samples were different, both sample types could be integrated into one calibration curve, indicating that the overall NMR relaxation properties did not depend so strongly on the details over a wide range of fat content. The data were processed using the chemometric approach (Bruker OPUS software) using a partial least squares algorithm, resulting in a prediction of the fat content measured by NMR (referred to as NMR Fat% in Fig. 4). These values were to be compared to the reference fat content. As shown in Figure 4, a linear correlation between NMR Fat% and reference values was observed. The correlation coefficient *R* was 0.991, slightly less than the *R* of the surface measurements using the diffusion-weighted ratio method (Fig. 3). The measurements were performed at a depth of 3 mm, leading to an inherent decrease of the *S/N* by a factor of about 3–4. This means that the measurement time should be increased by a factor of 9 to obtain the same *S/N*. Here, the measurement time was limited to 5 min, which corresponds to 64 scans, owing to the long T_1 of water and oil in most food products. Of course, an RF insert could be constructed with less pronounced depth selectivity for integration over a larger area, as the samples in this study were homogeneous.

Data were also obtained by homogeneous field measurements and compared with the reference values for a collection of samples including mayonnaises, margarines, and some sauces. The correlation coefficient for the relaxation time analysis in homogeneous fields was 0.997. The measurement time for one homogeneous field measurement was about 1 min. However, when the sample preparation time was included, differences in the total measurement times for single-sided and homogeneous field measurements were insignificant. For the diffusion-weighted ratio method, the measurement times were shorter, as T_1 relaxation times played a role only in the repetition time but not during the scan. For surface measurements, similar results were obtained as with gradient measurement in homogeneous fields. The diffusion-weighted method was developed for special experimental circumstances applicable to the single-sided NMR devices and cannot be transferred directly to homogeneous fields.

This study proved the applicability of single-sided NMR in quality control of a variety of food products. The major advantage of the single-sided NMR device is that no sample preparation is required. Packaged products can be examined without additional sample preparations. The sample can remain in the sealed package, provided a measurement can be made through a nonmetallic part of the package. However, it may be advantageous for the *S/N* issues if the package is thin so that the measurement can be performed at lower depths. Similar measurements can be performed *in vivo* in the surface near regions of biological tissues. The quality and accuracy of measurements depend strongly on the *S/N* available within a certain measurement time. Since the single-sided NMR devices use only stray fields, the relatively low *S/N* is an inherent feature. However, *S/N* can be improved by using stronger magnetic fields or larger measurement volumes.

The Bruker single-sided NMR device was shown to be capable of measuring the fat content in packaged food products. The two methods introduced in this study could also be used in other fields of application where the substances exhibit different relaxation or diffusion properties. The *S/N* determines the quality of the measurement and the correlation, as long as the temperature is stable. Improvements of the *S/N*, i.e., the better correlation or reduction of the measurement time, can be achieved by increasing the resonance frequency and the measurement volume.

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