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Determination of total fat and moisture content in meat using low field NMR

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Abstract

The use of low field Nuclear Magnetic Resonance (LF-NMR) is shown to be a fast and accurate alternative to the use of drying and solvent extraction, to determine the content of raw or total fat and moisture in a biological system. The proposed NMR method for fat determination in minced meat proves to be a robust method that does not require sophisticated post handling of the experimental data. The calibration procedure is very easy, as a calibration value from a sample of known weight containing 100% oil is the only calibration needed for the proposed experimental set-up. On three sets, each containing 42 samples of minced beef where the fat content varies from less than 1 to 14%, the fat content has been measured either by NMR on fresh tissue, NMR on dried tissue, or by the use of solvent extraction determining the content of raw fat [Foss-let fat analyser (AOAC Official Method 976.21]. Comparison of the three methods for determination of the fat content shows satisfactory agreement between the different methods. On six samples of minced pork meat, the fat and moisture content have been determined. The total fat content was determined by NMR both on fresh and dried tissue. The moisture content was determined by NMR of fresh tissue and by drying of the tissue. The different methods for determining fat and moisture content agreed for the minced pork samples.

Keywords: Quantification; Fat; Moisture; Meat; Nuclear magnetic resonance

1. Introduction

In spite of the innovative use of NIR (Near Infrared Reflectance) and NIT (Near infrared transmission) in food analysis, the food industry will benefit from developing fast methods that do not require time consuming and expensive calibration. Low field NMR (LF-NMR) is a fast and accurate alternative to these methods to determine the content of water and fat simultaneously. LF-NMR may be used to analyse most food (i.e. fish, meat, dairy) products along the production process from the raw material until the finished product.

The reference methods are still based upon the use of acid hydrolysis and solvent extraction to obtain the total amount of fat. Examples are the modified Soxhlet method (AOAC Official Method 991.36) and the SBR method (NMKL, 1989). These methods are examples of slow and laborious methods, which depend on experienced and properly trained personnel. Many of the routine methods used in food laboratories today still use hazardous solvents. An example of this is the Foss-let fat analyser (AOAC Official Method 976.21), introduced a few decades ago. The method uses a chlorinated solvent that can be absorbed through the skin and therefore requires skilled personnel to make an accurate and safe analysis of fat in meat.

One of the latest improvements was made by Büchi, the Büchi B-820, (BÜCHI, 1997) which introduced a semi automated dedicated GC-system to analyse for the total amount of triglycerides—however the system still uses some chemicals during the wet preparation of the samples before the analysis.

One important aspect is to know what is quantified; raw fat or total fat? The accuracy of the result depends

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on the level of extractability by the specific solvent or combination of solvents used. With the LF-NMR a specific proton signal is related to the absolute amount of hydrogen atoms which are proportional to the fat (lipids) in the sample. It is assumed that the phospholipid component that is decomposed by acid hydrolysis can be determined by use of LF-NMR. However this means the sample has to be warmed to 70 °C before analysis.

LF-NMR methods, do not use chemicals, and the low magnetic field means no safety risk for personnel operating the instrument. Training personnel to operate a LF-NMR and calibrating the instrument is a low cost task. In this work we apply two NMR methods for determination of the fat content in a series of minced beef and pork samples. The results are compared with the Foss-let method. The first method requires that the water within the samples is evaporated off to remove the water NMR signal from the total NMR signal. The second method makes use of pulsed magnetic field gradients in order to separate the fat NMR signal from that of water.

Although the second method is much faster it requires pulsed magnetic field gradients, and thus needs a NMR spectrometer equipped with a gradient probe and a stable gradient power supply.

2. Theory

The following theory is based on a PCT application (Patent Co-operation Treaty NO 01/00220, 2001) for the proposed method, and a more elaborate description of the method is found there.

When placing hydrogen in an external magnetic field, the nuclear magnetic moment will either align towards or against the direction of the external magnetic field. In thermal equilibrium a difference in population between upper and lower level is given by the Boltzmann factor

$$\frac{n_{\text{upper}}}{n_{\text{lower}}} = e^{-\frac{\Delta E}{kT}} \tag{L1}$$

where T is absolute temperature, k = Boltzmann's constant, and ΔE is the difference in energy between the levels. The difference in population will generate a net nuclear magnetic moment that depends on the content of hydrogen/proton, and in thermal equilibrium the magnetic moment will be aligned with the external magnetic field. When imposing an oscillating magnetic field, RF field, transverse to the external magnetic field Ho, transitions between the energy levels occur when the frequency corresponds to the resonance frequency (Price, 1997). The direction of the net nuclear magnetic moment will then move away from thermal equilibrium with the external field. When the RF field is switched off, the system will start to approach thermal equilibrium with the external field, the direction of Ho. The time it takes to get back to thermal equilibrium is given by the characteristic relaxation times T_1 (longitudinal relaxation) and T_2 (transverse relaxation) (Slichter, 1989).

The path back to thermal equilibrium in combination with an oscillating net nuclear magnetic moment transverse to Ho, will cause changes in the magnetic flux that can be recorded with the same RF coil as is used to excite the system. The current induced in the coil will then be proportional to the number of hydrogen atoms in the system, and from the intensity of the signal it is possible quantify their content in the system.

One may record the mobility of the hydrogen by making use of pulsed magnetic field gradient. The magnetic field gradient, g, imposes a position dependent frequency on the system, and with which the nuclear magnetic moment of the proton is oscillating in a plane transverse to Ho. A combination of RF pulses and pulsed magnetic field gradients in a NMR diffusion experiment (Price, 1997), dephases the net magnetic moment by

$$\varphi = \gamma g(z_2 - z_1) \tag{L2}$$

where (Z_2–Z_1) is the distance the protons have moved during the NMR diffusion experiment and where γ is the gyromagnetic ratio. For molecules moving faster during the NMR diffusion experiment, the induced current in the RF coil (the NMR signal) will decrease more from these molecules (as water) than from the slow diffusing molecules (as fat).

When assuming a Gaussian distribution of diffusivities and mono exponential attenuation of the NMR signal due to relaxation processes, the attenuation of the NMR signal is written

$$I = I_0 e^{-\frac{t_1}{T_2}} e^{-\frac{t_2}{T_1}} e^{-\gamma^2 g^2 D \int_0^t \left(\int_0^{t'} g(t'')dt'' \right)^2 dt'} \tag{L3}$$

where t_1 = duration the NMR-signal is influenced by transverse relaxation processes, t_2 = duration the NMR-signal is influenced by longitudinal relaxation processes $g(t'')$ = total magnetic field gradient, external and internal, D = diffusion coefficient, T_1 = characteristic longitudinal relaxation time, T_2 = characteristic transverse relaxation time, I_0 = initial intensity of the NMR signal.

To resolve the fat signal from the water signal, we apply a multi-pulsed magnetic field gradient spin echo experiment (m-PFGSE) (see Fig. 1). Using this experiment it becomes unnecessary to perform an extra correction for

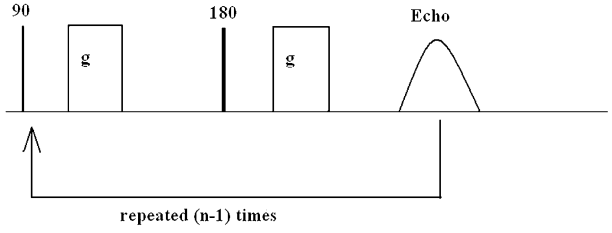

Fig. 1. A multi-pulsed field gradient spin echo experiment (m-PFGSE).

longitudinal relaxation processes, transverse relaxation processes, and the NMR signal will be refocused with respect to internal magnetic field gradients. In addition, the uncertainty due to eddy current field is minimised as one is using the same gradient strength throughout the experiment. An eddy current field is a magnetic field transient that follows the onset and offset of the magnetic field gradient pulses, and may cause unwanted dephasing of the NMR signal (Price, 1998).

The echo attenuation for the m-PFGSE-sequence in Fig. 1 is written

$$I = I_0 e^{-n\bullet\left[\frac{2\tau}{T_2}-\frac{2\tau^3}{3}\gamma^2 G_i^2 D\right]} e^{-n\bullet\left[\gamma^2 g^2 D\delta^2\left(\tau-\frac{\delta}{3}\right)\right]} \quad \text{(L4)}$$

where Gi is the internal magnetic field gradient caused by changes in magnetic susceptibilities throughout the sample, g is the externally applied magnetic field gradient, δ is the gradient pulse length, τ is the time interval between 90 degree RF pulse and 180 degree RF pulse, and n is the echo number ($n = 1,2,3$,etc.). By defining the unknown parameter.

$$K = \frac{2\tau}{T_2} + \frac{2\tau^3}{3}\gamma^2 G_i^2 D + \gamma^2 g^2 D\delta^2\left(\tau - \frac{\delta}{3}\right) \quad \text{(L5)}$$

The attenuation is then simplified to

$$I = I_0 e^{-n\bullet K} \quad \text{(L6)}$$

Terms including relaxation, diffusion due to internal magnetic field gradients and diffusion terms due to the applied magnetic field gradients, are thus collected as one unknown, K.

To separate NMR signal from fat/oil from the other components, one makes use of the difference in mobility and transverse relaxation time. Fat/oil has significant different mobility from water and sugar dissolved in water. By fitting the applied field gradient pulse such that the water signal and possible signal from sugar dissolved in water is suppressed at the first echo, the m-PFGSE experiment can be used to quantify the fat (oil) directly. Due to the very short transverse relaxation times ($\leqslant 1$ ms) of protein and solid sugar, their NMR signal will not contribute when the first measuring point ($n = 1$) in the m-PFGSE experiment is at approximately 5 ms or more. The attenuation is then written

$$I = I_{\text{fat}} e^{-n\bullet K_{\text{fat}}} \quad \text{(L7)}$$

A weighted linear fit (Press, Teukolsky, Vetterling, & Flannery, 1992) of the logarithm of L7 to the function

$$y = -ax + c \quad \text{(L8)}$$

yields a value for c where

$$I_{\text{fat}} = e^c \quad \text{(L9)}$$

By weighting the fit one takes into account that the model in (L7) is not valid at all times, as the fat may exhibit a distribution of T_2 relaxation times. However, one may assume a mono exponential decay at a short observation time, which is defined as an observation time that is shorter than the shortest T_2 value in the T_2 distribution. As the observation time approaches the first measuring point at time equal to 2τ ($n \rightarrow 1$), the validity of this assumption increases. The first measuring points are therefore given more weight than the last ones in the decay. With this fit one has corrected for diffusion and relaxation effects in the NMR signal, and the signal is thus a measure of the content of fat or oil in the sample.

To measure the moisture content as well, one may use the same experiment without pulsed field gradients, a Carr Purcell Meiboom Gill (CPMG)-sequence (Meiboom & Gill, 1958). Fitting the experimental data from the CPMG yields an initial NMR signal that contains signal from fat and moisture only, and a subtraction of the fat signal found in L9 results in the moisture signal from the sample. This signal is a measure of the moisture content within the sample. However, one should note that this simple procedure for quantification of moisture does not apply when sugar has been added to the system. Sugar dissolved in water will interfere with the moisture signal, and the initial NMR signals then contains signals from fat, moisture and sugar dissolved in water.

3. Experimental

The NMR 'dried' method requires drying of the sample prior to analysis. Minced meat (5 g) was dried for 16 h at 103 °C (NMKL, 1991). The dried sample was placed in 18 mm NMR-tubes and heated to 70 °C to ensure that the fat phase is liquid in order to acquire the total fat signal. The instrument used in this method is a Bruker Minispec pc 120, 20 MHz. The pulse sequence used is based upon a single spin echo sequence where the inter echo spacing, τ, is set to 310 μs. The instrument was calibrated by making a standard curve of known amounts of refined lard (high proton density).

To measure fat content in systems containing water as well, we made use of the proposed m-PFGSE experiment. The instrument used is a Maran23 MHz equipped with 18 mm gradient probe and access to 350 Gauss/cm. A typical result from the m-PFGSE experiment is shown in Fig. 2, where the gradient strength and duration of the gradient pulses were adjusted such that the water signal was suppressed to an insignificant level at the time of appearance of the first echo. When extracting the intensities of the echo peaks, and fitting these as a function of echo number, the fitted initial intensity at echo number 0, corresponds to the total fat signal. Effects from relaxation, diffusion, and eddy current field

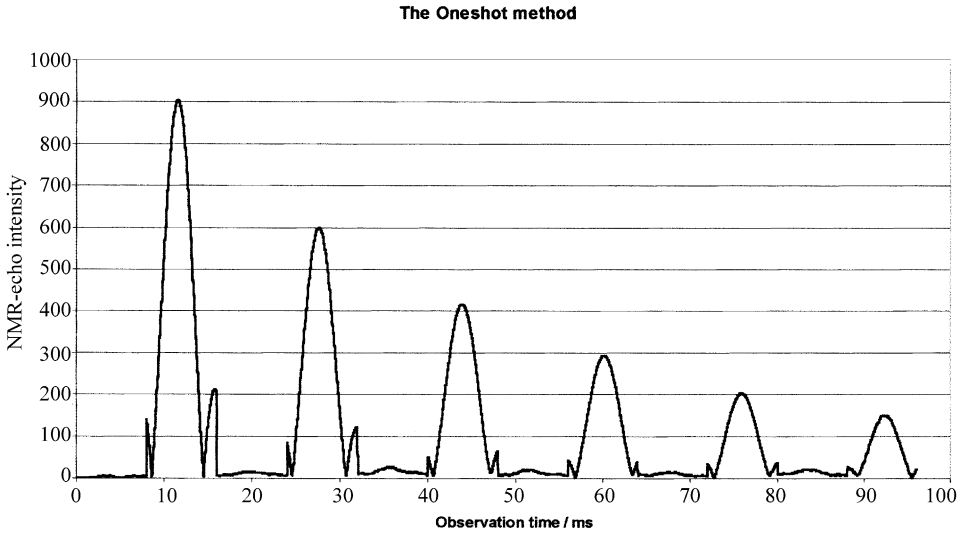

Fig. 2. A multi-PFGSE performed on a sample containing fat.

transients, are corrected for using this approach. This method is therefore just as simple to calibrate as the method where the moisture within the sample was evaporated. What is required is only a sample of 100% oil and of known weight.

The 42 samples of minced beef meat used in this experiment consisted of deep-frozen packed samples that were measured by NMR dried, NMR fresh, and Foss-let methods. The six samples of minced pork meat were measured by NMR dried and NMR fresh only. To avoid any signal contribution from protein in the protein-water interface, we recorded the CPMG attenuation and performed a multi-exponential fit of the data. The resulting T_2 distribution is shown in Fig. 3, where we find a small peak arising at approximately 1.5 ms. This signal cannot arise from water as free water is very mobile, with T_2 values much higher than 1 ms as the exchange between free and bound water is much faster than the duration of the τ-value in the CPMG-experiment (Bottomley, Hardy, Argersinger, & Allen-Moore, 1987). As long as the fat is in a liquid state, the peak at 1.5 ms cannot arise from fat, leaving the protein or other macromolecules as the only cause. Thus, to quantify the moisture content without any interference from other molecules such as protein or sugars, one should start the recording of the CPMG experiment at an observation time of at least 5 ms. Then the signal arising from the macromolecules is reduced to an insignificant amount.

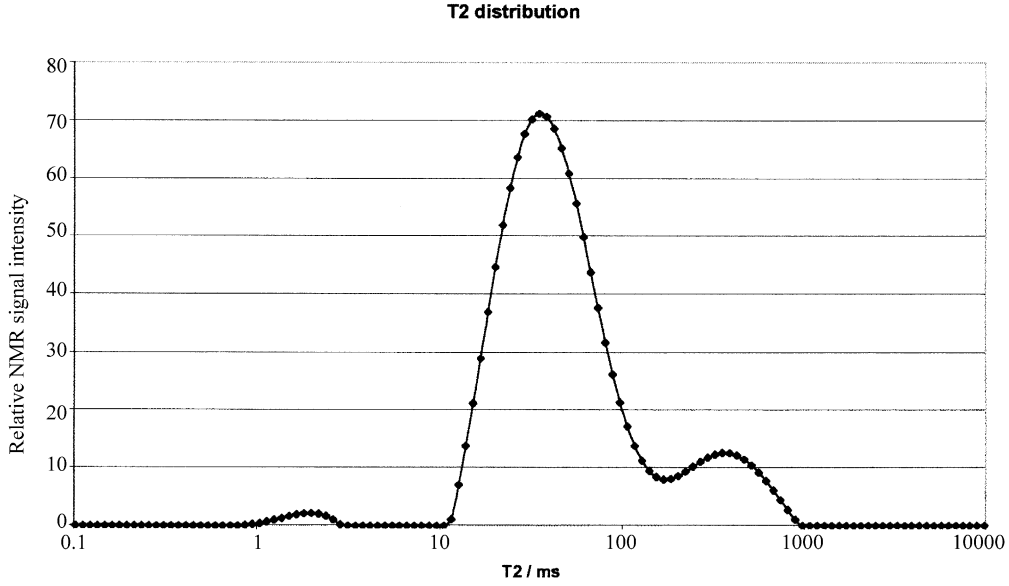

Fig. 3. T2 distribution of a sample containing minced pork meat.

4. Results and discussion

4.1. Accuracy

As the NMR method is non-invasive, the samples can be measured several times. It is therefore possible to produce an experimental set-up with the required accuracy. Fig. 4 shows a recording of 100 measurements on a 100% oil sample, where the standard deviation is 0.25, during a period of 4 months, this calibration value did not change significantly. The standard deviation for 100 measurements was measured every second week, and all values of the standard deviation were within 0.35. Within this period of time, the experimental parameters did not change, and the gradient probe was detached and attached to the Maran23 NMR spectrometer several times. As the standard deviation does not increase over a period of months, it reflects the stability of the m-PFGSE experiment. As long as the proton density of the calibration sample remains constant, the calibration value will also be constant.

A calibration as is shown in Fig. 4 is all that is needed when measuring the fat content of an unknown sample of fat content 0.1–100.0%. The basic assumption is that the proton density does not change significantly from sample to sample. We have measured the proton density of different types of fish oil extracted from wild salmon, bred salmon, herring, and mackerel. For the different types of fish oil the proton density remained constant within a relative error of ±1.0%. The uncertainty in a sample of fat content of 10.0% will thus be ±0.1% due to a possible variation in proton density. As the proton density of fat from pork is found to be 8% higher than of the fish oil, one would introduce a systematic error if fish oil was used as a reference oil when measuring fat in pork. It is therefore crucial to calibrate the NMR system with the same type of oil as is found in the samples that are to be investigated.

As the NMR fresh method corrects for systematic errors such as loss of signal due to relaxation, convection, and/or eddy current transients, we have found that the stability of the mains (power) is the most critical factor. Any instability of the mains may introduce extra and unwanted electrical currents looping through the gradient coils. This will generate an unwanted and random dephasing of the NMR echo signal that increases as the duration of the NMR-experiment is increased. Through a sophisticated, yet simple, experimental set-up we have reduced the duration of the NMR experiment such that the degree of random dephasing of the NMR echo signal does not vary the measured fat content significantly. This is verified in Fig. 4, where 100 repeated measurements of 100% fat yielded a standard deviation of ±0.26.

One should also note that the decays from the NMR fresh method were examined with and without weighting on the linear fit to obtain the NMR signal arising from fat. Within the noise in the experiment there was no difference between the two methods for fitting the experimental data. This confirms the validity of the assumptions of a mono exponential decay at short observation times.

4.2. Comparison with the reference material SMRD2000

In order to verify that the NMR method for fat determination in water containing systems truly measures the fat content, it was tested on a finely minced reference material made by Swedish Meats R&D

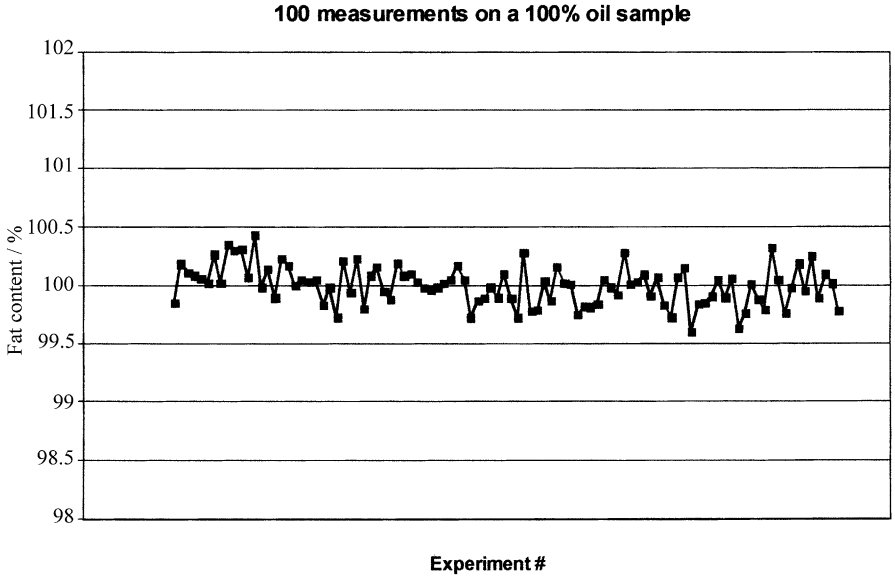

Fig. 4. One hundred measurements on a 100% oil sample using the NMR fresh method. The standard deviation is 0.26.

(SMRD, 2000). The reference sample contains lean pork, water, potato flour, and nitrite, and the reference oil value is 14.3% with ±0.4% as expanded uncertainty. Reference methods used were NMR on dried tissue, Soxhlet, Weibullt Stoldt, and SBR 70–100 °C. The reference value for moisture is 68.8%±0.1%, using drying at 100–110 °C as the reference method.

Four samples containing 3.5 g of the meat paste were measured. The fat content was found to be 14.3, 14.4, 14.6 and 14.7%. The water content for one sample was found to be 68.7. This sample was dried for a period of time at 104 °C and the moisture content on the remaining sample were measured again. This procedure was repeated several times, and the result of measured moisture content as a function of loss of weight is shown in Fig. 5. It is evident that there is a linear relationship between the measured moisture content and the loss of weight.

From the results achieved with the reference system SMRD2000, we conclude that the method for fat and water determination do separate the two components satisfactorily. When repeating the measurement 50 times as shown in Fig. 6, the standard deviation was found to be less than 0.1%.

4.3. Fat content in a variety of meat samples

Fig. 7 shows the fat content for the 42 samples of minced beef meat using the three different approaches, NMR fresh (Anvendt Teknologi), NMR dried (Matforsk), and Foss-let (NMA). For the NMR fresh method, the recording of the fat signal starts when the water signal is suppressed to an insignificant amount using the m-PFGSE sequence, and when the protein signal has decayed due to a short T_2 relaxation time. When drying the sample prior to analysis, the T_2 relaxation time of the protein is less than 100 us, and

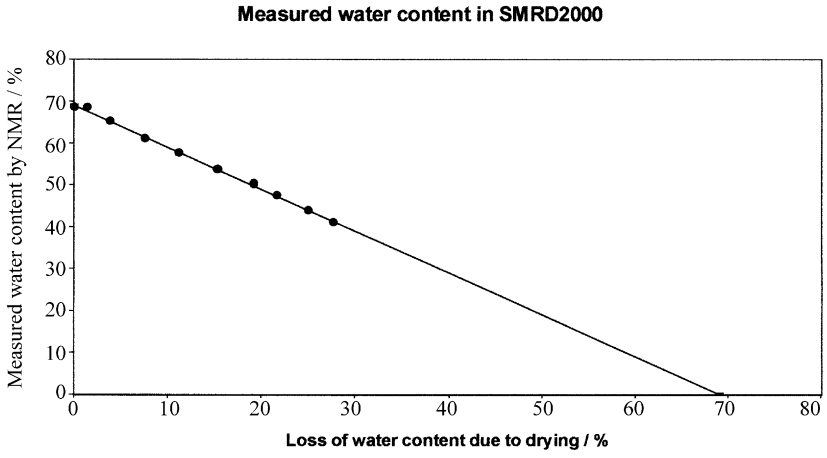

Fig. 5. Measurement of water in SMRD2000 as a function of loss of mass due to drying.

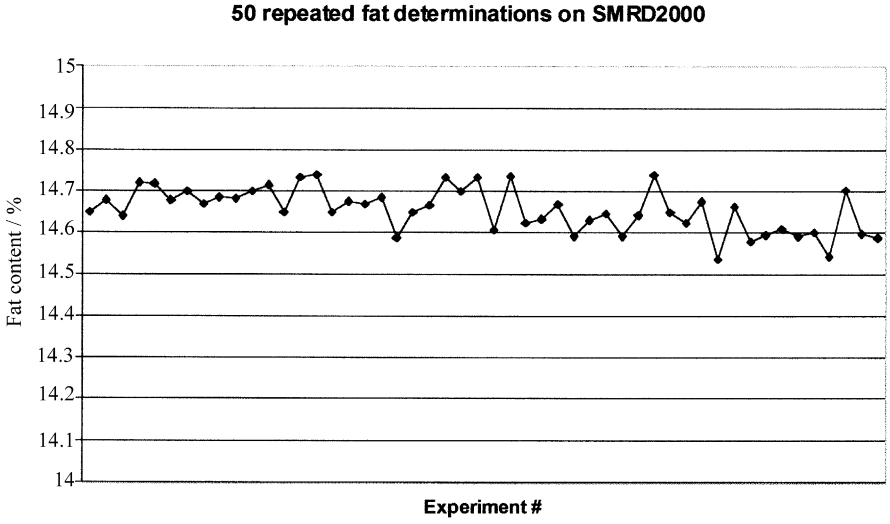

Fig. 6. Fifty repeated fat determinations in SMRD2000. The standard deviation is less than 0.1.

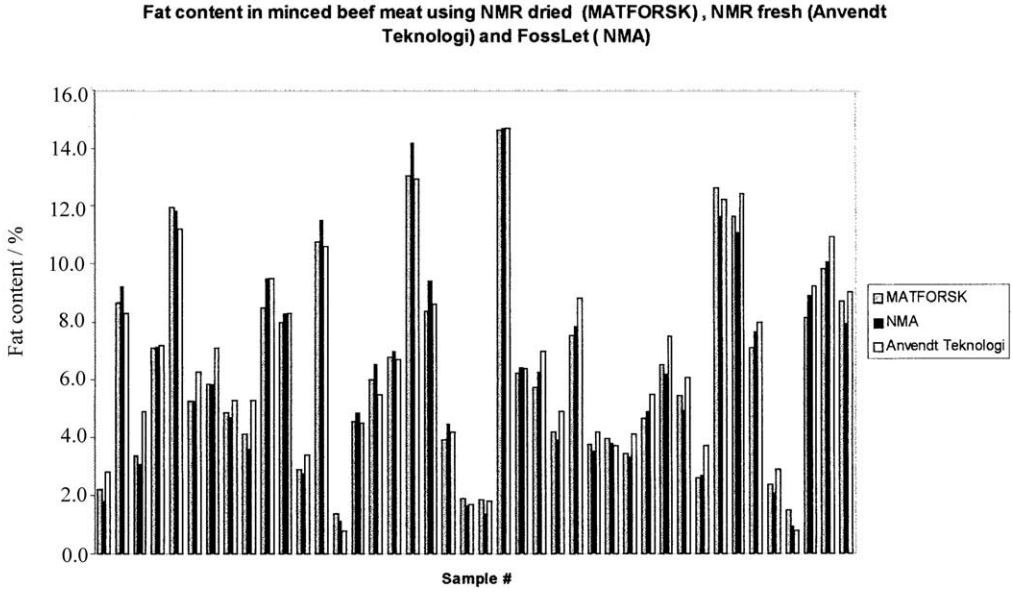

Fig. 7. Measured fat content in minced beef meat by NMR fresh, by NMR dried, and by Foss-let extraction.

one may record the fat signal at an observation time of approximately 500 us. At this observation time it is not necessary to correct for loss of NMR signal, as the T_2 relaxation of the liquid fat is more than 100 ms. Comparing the NMR fresh method against the NMR dried method and/or the Foss-let method we find satisfactorily correlation in all cases. The correlation is 0.975 when comparing the NMR fresh method against the Foss-let using Partial Least Squares Regression (PLS1) (Camo ASA, 2000), and against both the Foss-let and the NMR dried methods using Partial Least Squares Regression (PLS2) (Camo ASA (2000)). A slightly improved correlation of 0.981 is found when comparing the two NMR method using PLS1.

Table 1 shows the measured fat content and water content in six samples of minced pork. The water content is found by running an ordinary CPMG, where the attenuation is recorded from 10 ms and upwards. As shown in Fig. 3, the total area above 10 ms corresponds to the total fat and moisture signal. The water signal is then found by subtraction of the fat signal that is found from the m-PFGSE experiment. The proposed method is designed to measure the fat content directly using the NMR technique without the need for compensation of loss of NMR-signal due to a finite duration of the NMR-experiment. Thus the calibration procedure involves just measuring the proton density of a sample of known weight and containing 100% oil or a known amount of fat.

Table 1
Measured fat and moisture content in minced pork meat, either by NMR fresh, NMR dried, or by drying

Sample No.	Moisture by drying at 105 °C	Moisture by NMR-fresh	Fat by NMR-dried	Fat by NMR-fresh
1	64.3	65.5	14.6	15.0
2	55.8	55.9	26.2	25.5
3	60.0	59.9	20.8	21.5
4	62.5	62.2	17.8	17.8
5	62.2	61.7	17.7	18.2
6	72.1	70.1	5.1	4.7

5. Conclusion

The proposed NMR method for quantification of fat in the presence of water is a safe, fast and accurate alternative to other methods. In addition, water content may be measured as well in non-sugar containing systems, using a total experimental time of approximately 1 minute (Resonance Instruments Ltd., 2002).

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