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Determination of fat, lactose and water content in milk

In this application note we study how the Spinsolve benchtop NMR spectrometer can be used to quantify water, fat, and lactose content in milk. Milk contains around 87% of water, therefore its signal dominates the spectrum and overlaps with other peaks in the sample.

Figure 1 shows the NMR spectra of a cow's milk sample measured on a Spinsolve 60 ULTRA spectrometer with a standard pulse sequence (top) and a PRESAT solvent suppression sequence (bottom).

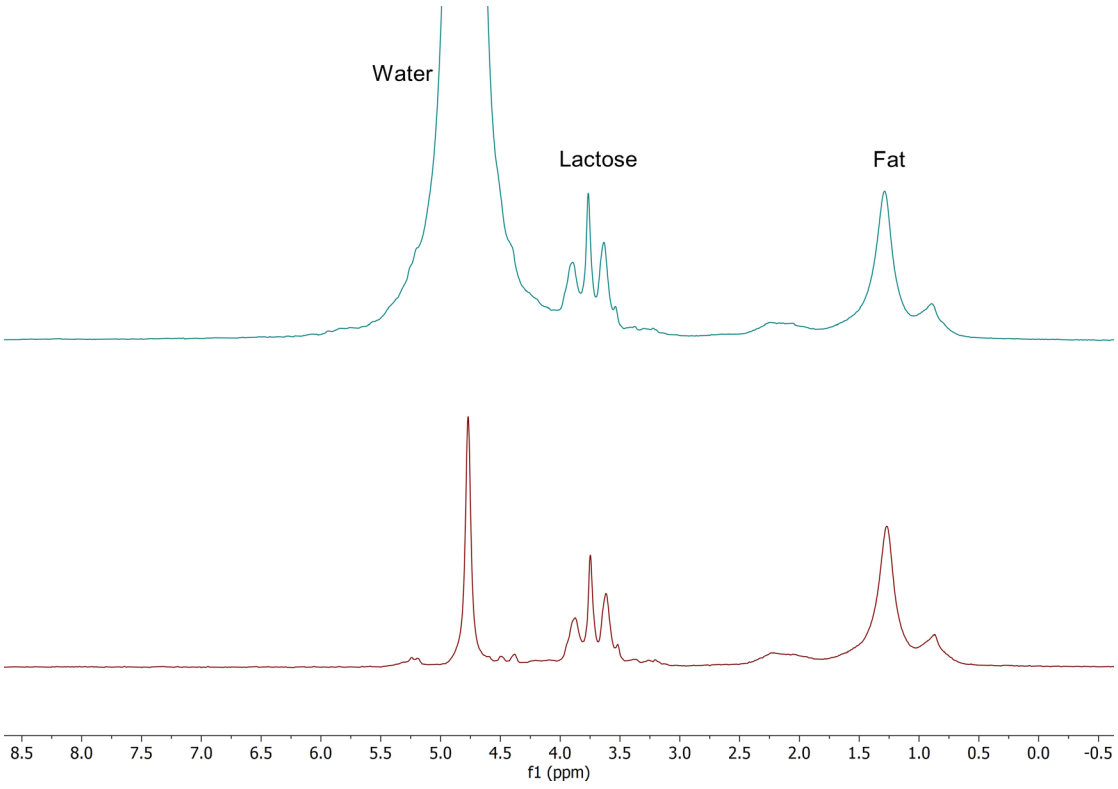

Figure 1: 60 MHz ^{1}H NMR spectra of a cow's milk sample recorded on Spinsolve 60 Ultra with (bottom) and without (top) solvent suppression. The spectra were acquired using 4 scans, a repetition time of 60 seconds, and an acquisition time of 6.4 seconds.

The spectra show 3 main regions that are of interest. Region 1 (between 0.5 and 3 ppm) shows the aliphatic signals of the fat, region 2 (between 3 and 4 ppm) shows predominantly peaks from lactose, and region 3 (centred at 4.7 ppm) contains mainly the water signal, but also some peaks from fat and lactose that lay under the water signal. Integral values of these 3 regions are shown below in Table 1:

Spectrum	Integral region, ppm	Integral [a.u.]	Comment
Milk	0.20 - 2.90	568.33	Fat
Milk	2.80 - 7.00	19254.96	Water + others
Milk (PRESAT)	2.90 – 3.86	191.93	Lactose

Table 1: Integral values of fat, lactose, and water regions in milk spectrum

Determination of fat content

Fat in milk is a complex mixture of triglycerides, which are comprised of a glycerol backbone and three fatty acid chains. There are more than 400 different fatty acids in cow's milk ranging in carbon chain length and degree of unsaturation. The fat in cow's milk consists of around 65% saturated fatty acids (no double bonds), 30% monounsaturated fatty acids, (one double bond), and 5% of polyunsaturated fatty acids (more than one double bond). Fat content can be determined from the NMR spectrum by means of a simple calibration curve obtained by measuring milk samples with known amount of fat. A set of eight samples were measured with the same experimental parameters as before (4 scans, 60 s repetition time and 6.4 s acquisition time). The spectral region between 0.20 and 2.90 ppm was integrated for all eight samples and these values were used to build the calibration curve shown in Figure 2.

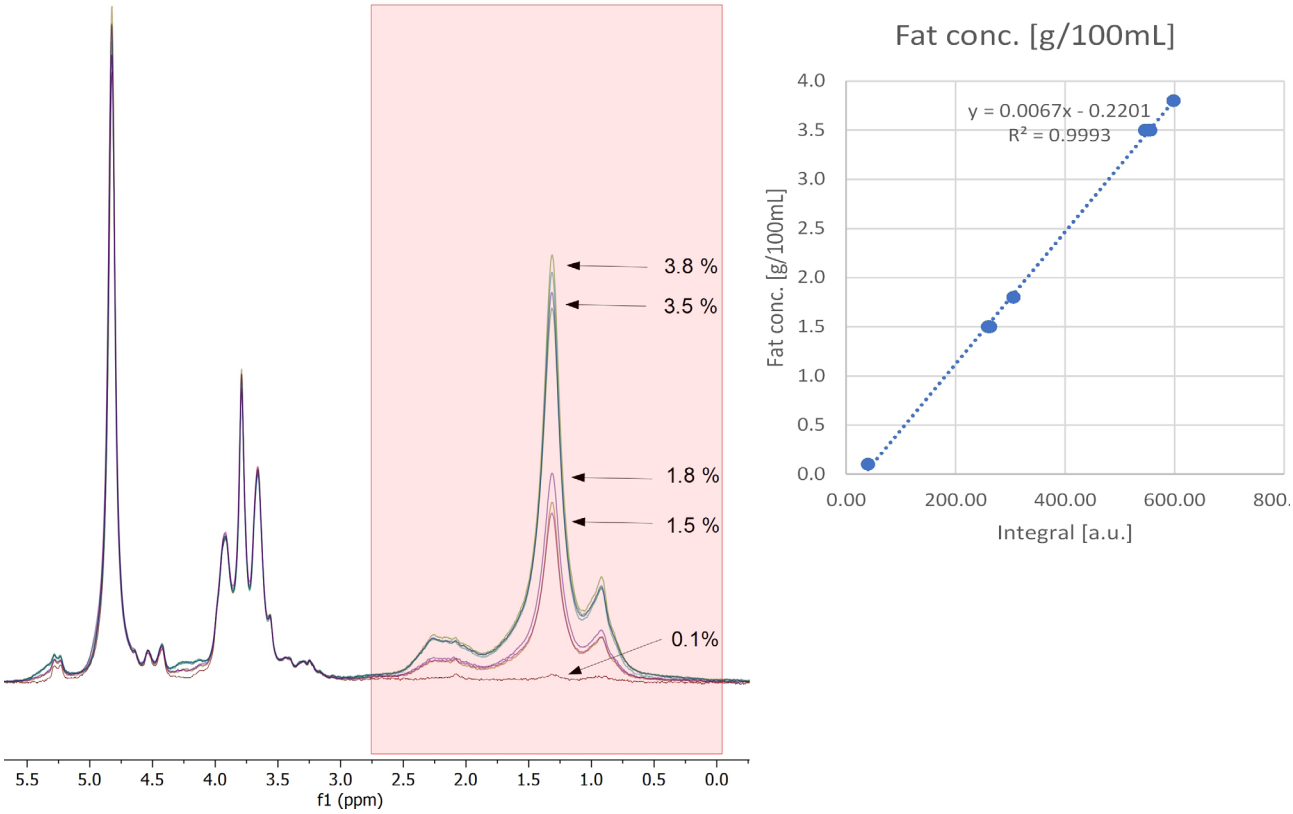

Figure 2: 60 MHz ^{1}H NMR spectra of 8 milk samples recorded on Spinsolve 60 Ultra used to build a calibration curve for fat content determination.

The calibration equation that can then be used to calculate the fat content in the milk samples in [g/100mL] is

$$C_{Fat} = 0.0067x - 0.2201, \qquad (1)$$

where x is simply the integral value of the region between 0.20 and 2.90 ppm.

Determination of lactose content

Lactose is a disaccharide made up of glucose and galactose. Its peaks are spread between 3.4 ppm and 5.5 ppm region of the milk spectrum. These peaks overlap with the water signal at 4.7 ppm. However, the largest lactose resonances at around 3.79 ppm can be fully separated from the water signal by running a PRESAT experiment to suppress the water signal (see Fig. 1 bottom spectrum), and therefore its integral can be used to quantify lactose content.
To determine the concentration of lactose in milk, a calibration curve was built by measuring four samples with known lactose concentrations in water. The PRESAT spectra of these four samples were measured on a Spinsolve 60 Ultra spectrometer with the same acquisition parameters as before (4 scans, 60 s repetition time and 6.4 s acquisition time). The spectral region between 2.90 – 3.86 ppm was integrated for all four samples and these values were used to build the calibration curve for lactose shown in Fig. 3.

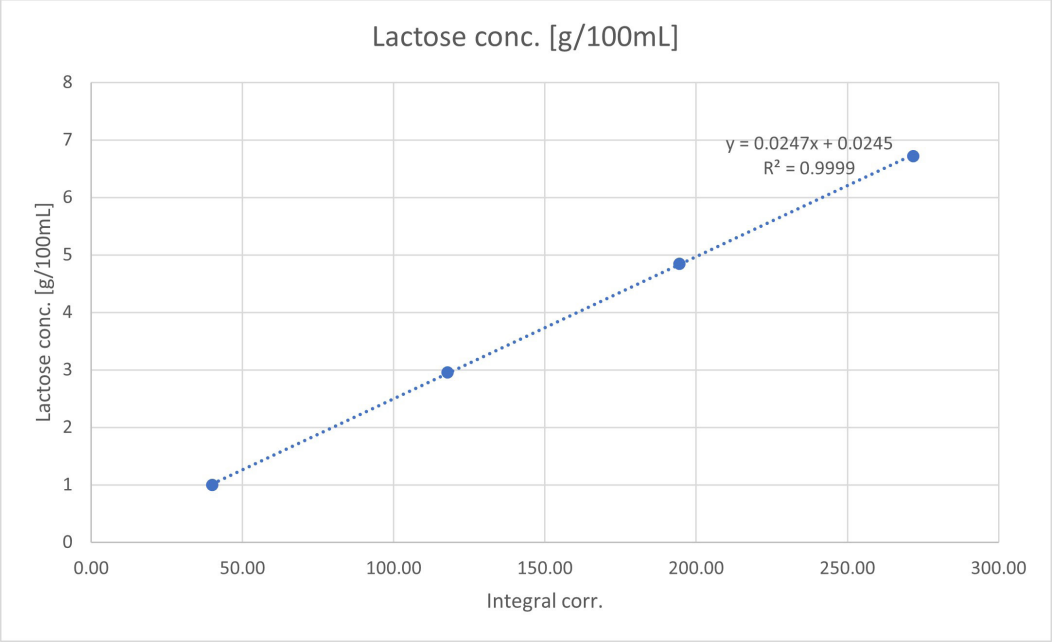

Figure 3: Calibration curve for determining the lactose concentration.

By simply applying linear regression to these for points the following calibration curve was obtained

$$C_{Lac} = 0.0247x + 0.0245. \qquad (2)$$

The equation provides the lactose content in [g/100mL] and x is an absolute value of the integral region 2.90 - 3.86 ppm. Typical lactose and fat contents from different brands of milk can be seen in the table below (Table 2). The results of calculations show very similar concentrations to the ones stated on the labels of the products.

Milk	Lactose, label [g/100mL]	Fat, label [g/100mL]	Lactose, NMR [g/100mL]	Fat, NMR [g/100mL]
Brand 1	4.9	1.8	5.01	1.83
Brand 2	4.8	3.5	4.96	3.51
Brand 3	4.9	3.5	4.81	3.45
Brand 4	4.9	1.5	4.88	1.52
Brand 5	4.8	3.8	4.94	3.80
Brand 6	4.8	1.5	5.01	1.55
Brand 7	4.8	3.5	4.94	3.49
Brand 8	5.0	0.1	4.88	0.05

Table 2: Fat and lactose content calculated for different brands of milk and compared to the values stated on the labels.

Determination of water content

As mentioned previously milk's major component is water. It gives rise to a broad signal at 4.7 ppm which dominates the overall milk spectrum and overlaps with lactose and some of the fat peaks. Because of these overlaps it is not possible to get the accurate water content directly from the integral value of its peak. First, we need to calculate contributions from lactose and fat and subtract them from the total integral of region 3. Note that to quantify water we need to use milk spectra recorded without solvent suppression.
To calculate the signal generated by lactose in the water region we first need to quantify the amount of lactose in the sample by means of the PRESAT sequence. Once the lactose content has been determined, we can predict the amount of signal that needs to be subtracted in the standard 1D proton spectrum by means of the following simple calibration

$$I_{Lac} = \frac{C_{Lac}}{C_{Lac,ref}} \times I_{Lac,ref} \, . \qquad (3)$$

In this equation C_{Lac} is the concentration of lactose in the milk sample, $C_{Lac,ref}$ is the concentration of lactose in the reference sample, and $I_{Lac,ref}$ is the signal generated by the calibration sample in the region integrated for the water quantification.
To estimate what is the contribution of fat to the integral value of the water signal it is important to look at the spectrum of cow's milk fat stripped of water. Figure 4 shows a 60 MHz NMR spectrum of clarified butter dissolved in CDCl3 (50:50). Clarified butter is milk fat rendered from butter to separate the milk solids and water from the butterfat.

The calibration equation that can then be used to calculate the fat content in the milk samples in [g/100mL] is

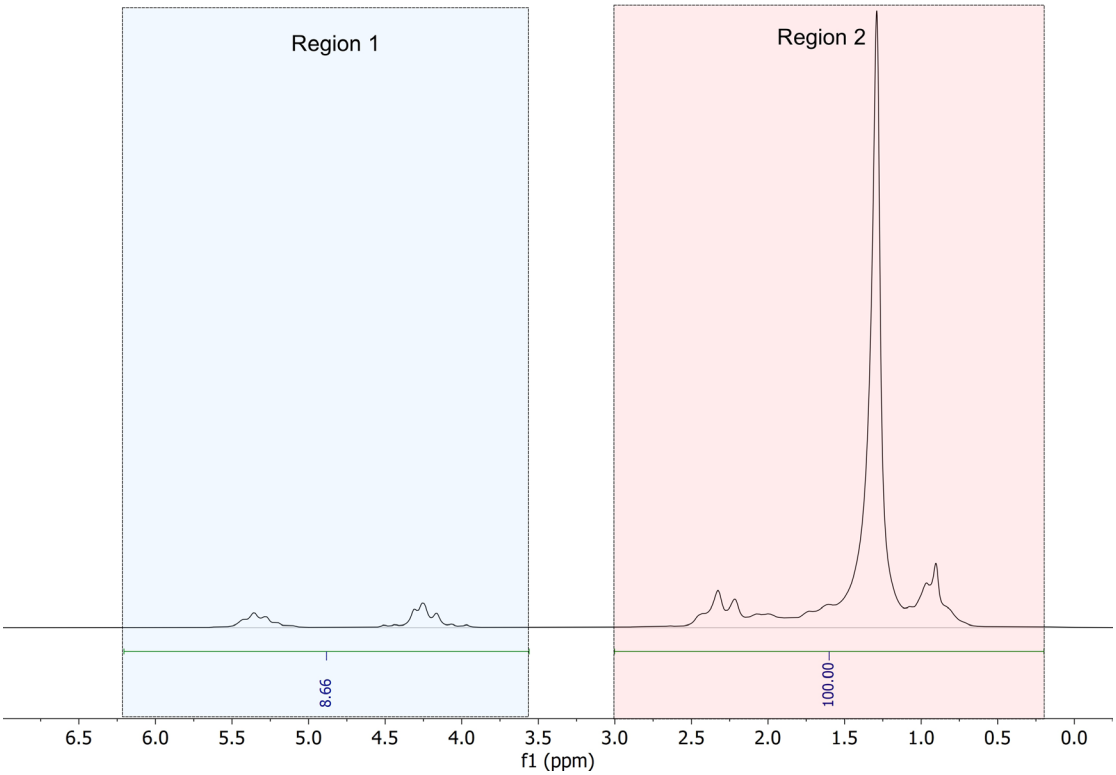

Figure 4: 60MHz ^{1}H NMR spectrum of clarified butter.

In the spectral region between 3.50-6.20 ppm there are peaks from the glycerol and double bond protons, which will be covered by the water peak in the actual milk sample. Therefore, knowing the ratio between the two signal regions, Region 1 (3.50 -6.20 ppm) and Region 2 (0.20 -2.90 ppm), allows us to correct the water signal once the amount of fat in the milk sample is determined from the PRESAT experiment. Based on Figure 4 the ration of the two signal regions of fat Region1/ Region2 is 8.66/100. The experimental observation is that this ration is quite stable for milk samples of different brands.

Once we know the contributions from lactose and fat to the integral value obtained for the water region, the integral can be corrected to obtain:

$$I_{water,corr} = I_{water,total} - I_{Lac} - I_{fat\ region1} \qquad (4)$$

Finally, the water content in the milk sample can be calculated in [mol/L] as:

$$C_{water} = \frac{I_{water,corr}}{I_{water,ref}} \times C_{water,ref} \qquad (5)$$

or in [g/100mL] as:

$$C_{water} = 48.76\frac{mol}{L} \times 18\frac{g}{mol} = 87.76\frac{g}{100mL} \qquad (6)$$

The measurement of milk was repeated five times and the results are shown in the Table 3 below:

	Lactose [g/100mL]	Fat [g/100mL]	H2O [g/100mL]
1	4.77	3.59	87.76
2	4.72	3.60	87.35
3	4.72	3.71	87.36
4	4.73	3.55	87.37
5	4.77	3.51	87.69
Mean	4.74	3.59	87.50

Table 3: Lactose, fat and water content calculated from 5 repeat measurements of milk sample.

Conclusion

In this note we have demonstrated that the Spinsolve Ultra benchtop NMR spectrometer can be successfully used for the determination of water, lactose, and fat content in milk. The results show a good reproducibility and agree with good precision with literature values.