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Simultaneous determination of fat and water content in caramel using time domain NMR

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Abstract

In the production process of caramel mass it would be desirable to determine the contents of water and fat simultaneously in one single analytical step.

In classic time domain NMR methods, the differentiation of water and fat is not sufficiently good as the differences in relaxation times of oils and fats are relatively small. As only signal amplitudes at given times are evaluated in classic time domain NMR, the contrast between the compounds is too small to allow a quantification. Therefore, samples are always pre-dried, allowing the determination of the fat content by time domain NMR.

Therefore a new TD-NMR method using a combined relaxation analysis was used, where the magnetisation at a certain time is determined by both T_1 and T_2. This combination leads to an increase of contrast and therefore opens up the possibility of quantification of water and fat by time domain NMR simultaneously. For data-processing, a chemometric method was employed to establish correlation with the results of the reference methods for fat and water content.

Keywords: Time domain NMR; Fat content; Water content; Simultaneous determination

1. Introduction

Some nuclei, like protons, possess a magnetic moment caused by their spin which of course reacts on magnetic fields. The spins can be polarised by means of an external magnetic field. At thermal equilibrium all spins contribute to a macroscopic magnetisation, which is proportional to the total number of nuclei – in the present case the total number of protons in the sample – and also proportional to the magnetic field.

The macroscopic magnetisation can be observed by irradiating the sample with radio frequency (rf) of an appropriate wave length and duration. This rf pulse, will lead to a rotation of the magnetisation from its equilibrium position aligned with the B_0 field by a defined angle, resulting in an oscillating magnetic field that induces an alternating voltage, the NMR signal. The NMR response is then measured as a function of time. After excitation, the sample will gradually return to its initial equilibrium state by various relaxation processes. The most important relaxion processes are the longitudinal relaxation T_1 and transverse relaxation T_2. Both reflect the molecular dynamics in the neighbourhood of the protons in a special frequency range and are therefore material specific. Also fat and water compartments can be differentiated by exploiting their relaxation properties. Different pulse sequences (e.g. Hahn-echo, Inversion Recovery, CPMG spin-echo) are known in NMR, which allow relaxation measurements for material investigations.

In food science and industry, classic TD-NMR is used to determine the concentration of fats or of water. The

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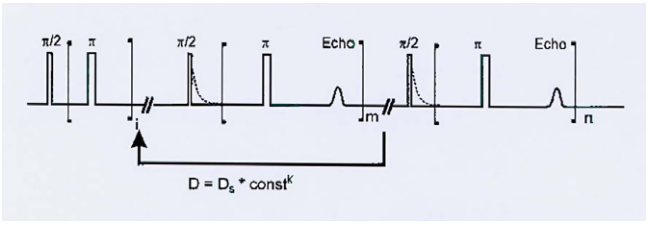

Fig. 1. The pulse sequence applied in combined relaxations experiment. First, the magnetization is saturated, followed by a recovery sequence which is extended by CPMG trains for simultaneous measurement of the transverse relaxation.

Table 1
Parameters of applied pulse sequence

Parameter	Value
Recycle delay	0.1 s
No. echos (=n)	500
Echo time	1.5 ms
Second No. echos (=m)	100
Second echo time	2.5 ms
First T_1 delay (=D_s)	20 ms
Last T_1 delay	1.500 s
T_1 relax points (=k)	29
Acquisition window	0.5 ms

products analysed with TD-NMR contain either a major amount of water or of fat. This restriction is due to the fact that the longitudinal and transverse NMR relaxation properties (characterised by T_1 and T_2) of fat and water molecules are relatively similar and therefore the contrast is generally not sufficient for quantification (for example Todt et al., 2006). In order to release the above mentioned restriction, a combined relaxation experiment is applied (see Fig. 1), where the magnetisation at a given time is determined by both relaxation processes, namely the longitudinal and transverse NMR relaxation (Guthausen, König, & Kamlowski, 2001). In this combined relaxation experiment, a number of radio frequency pulses is applied. In detail, the nuclear magnetization is first saturated by a conventional saturation sequence, followed by a T_1 recovery, which is extended by CPMG trains. The result is a magnetization spectrum which is given at each experiment time by longitudinal and transverse relaxation times. As an analytical approach of data-processing (i.e. conventional fit of relaxation functions) is rather time consuming and inaccurate in this case, a statistical point of view was chosen. The magnetization spectrum is regarded as a finger print of the samples components, which is analysed by chemometric methods. A similar approach is used in the NIR spectroscopy with good success. For further details we refer to for example http://www.chemometrics.net/2006. The task for the chemometric modelling is to correlate the magnetization spectrum with material properties like water and fat content.

In this paper, the applicability of the described method on caramel mass was examined to determine its water and fat content simultaneously.

2. Methods

A 20 MHz mq 20 minispec system was used for the measurements of caramel mass samples. Data were acquired using the minispec software and the measurement sequence shown in Fig. 1. The acquisition and timing parameters are shown in Table 1. The parameters were optimised on original samples and samples with varying water and fat contents via addition of water or drying, respectively. The data analysis and the calibration were performed in the Bruker OPUS software. The NMR based results for fat and water content were correlated with the established reference values.

Determination of the water content of the samples was carried out by Karl Fischer titration (KFT), using a Karl Fischer system of Metrohm AG, Herisau, Switzerland. Volumetric determinations were performed via two-component technique using Hydranal-Titrant 5 as titrating agent and Hydranal-Solvent as working medium. For better dissolution of the sample, Hydranal-Formamide dry was added to the working medium and the temperature of the working medium was elevated to 40 °C. The chemicals used for KF titrations were provided by Sigma–Aldrich Laborchemikalien, Seelze, Germany.

The fat content of the caramel masses was determined using the reference method of Weibull–Stoldt. After digestion with hydrochloric acid a Soxhlet-extraction was carried out with petroleum ether as solvent.

3. Samples

This study was carried out using nine sets of caramel masses (Table 2) obtained from Nestlé Research Center, Lausanne, Switzerland (see Section 5 for details). The caramel masses were produced in three pilot plants, by using different recipes for every set of samples, the samples from each set only differing in their percentages of water and fat content. During manufacturing the amount of water left in the caramel mass is a critical value as it affects the quality properties of the product. Correct and fast determination

Table 2
Overview of used caramel masses

Caramel mass	Calibration set (samples)	Measuring set (samples)
Caramel 1	19	6
Caramel 2	10	3
Caramel 3	7	4
Caramel 4	2	3
Caramel 5	4	2
Caramel 6	2	2
Caramel 7	2	2
Caramel 8	2	1
Caramel 9 (mixture of several masses)	2	2

of water and fat during the manufacturing is important for the quality control of caramel.

4. Procedures and parameters

The first step is the establishment of a correlation of NMR signals with material parameters. This procedure is often called calibration of the instrument. A caramel mass sample was measured in a glass tube without any sample pretreatment. For creating a calibration model the results of the reference methods were correlated with the relaxation time spectra of the TD-NMR measurements. In order to create predictive models for the water and fat content of the samples, PLS regression and cross validation were applied. The quality of the calibration model can be estimated by statistic quantities such as the correlation coefficient R^2 and the root mean square error of cross validation (RMSECV) (see Table 3). For an optimum calibration, R^2 is one and the prediction error is zero.

The first series of measurements were performed on caramel masses produced following the same recipe. The samples differed only in water and fat content. The NMR data of this set was measured at 20 and 40 °C (Caramel 20 A and Caramel 40 A). The calibration ranges for water and fat were from 4.5–28% and 11.5–16.5%, respectively.

To extend the measuring range for fat, caramel masses with different recipes were measured and calibrations were established (Caramel B). To identify the optimal measurement temperature, 20, 50 and 60 °C were tested. The water and fat content of the samples were measured by TD-NMR and the reference methods on the same day. Moreover a calibration was created, Caramel C, containing only two sets of caramel mass. These samples were measured at 20 °C. Samples with unknown water and fat contents were examined using the above described calibrations. The results were compared to the reference values. The reference values were supposed to be 100% correct. On basis of this comparison the recovery rate and standard deviation were calculated. The applicability of these calibrations was controlled by surveying of these standard values.

5. Results and discussion

It is well known that relaxation behaviour of protons and consequently their relaxation times (T_1, T_2) depend on temperature, therefore the NMR measurements were carried out at 20, 40, 50 and 60 °C in order to find the best contrast for quantification. In Table 3 the correlation coefficient R^2 and prediction error (RMSECV) of calibrations of Caramel A measured at 20 and 40 °C are shown. The statistical data are better for Caramel 20 A, which means that a sample temperature of 20 °C is the most appropriate for a quantitative analysis. The calibrations were then examined with samples differing in water and fat content from the calibration set but with the same composition (five samples, see Fig. 2). The obtained results were compared to the reference values. The Caramel 20 A delivers more precise results which is reflected in the recovery rate and the smaller standard deviation (see Fig. 2). According to Schlimme and Buchheim (1998) the milk fat that represents the main fat compound in the caramel mass exists almost completely as liquid phase at 40 °C, so the evaluation of signals of both components becomes indistinct.

Table 3
Statistics of calibrations

Calibration	Water/ R^2	Water/ RMSECV	Fat/ R^2	Fat/ RMSECV	Number of samples
Caramel 20 A	99.76	0.348	97.9	0.185	18
Caramel 40 A	98.62	0.829	97.18	0.214	18
Caramel 20 B	98.67	0.498	93.53	0.998	22
Caramel 50 B	97.96	0.747	98.47	0.715	19
Caramel 60 B	95.25	1.4	94.22	0.952	21
Caramel 20 C	99.76	0.348	97.9	0.185	15

In order to extent the measuring range for fat, further sets of caramel masses of larger compositional variations were used (Caramel 1–9). The evaluation of these calibrations is shown in Table 3. A better correlation for fat is given at 50 °C, for water at 20 °C. The high prediction error and low correlations coefficient at 60 °C can be explained with loss of water during the heating of the samples. Overall the results for fat and water content obtained from the calibrations (Caramel 20 B, Caramel 50 B and Caramel 60 B) using different sets of caramel masses during a examination are not significant, which means that the results deviate from the reference values. Fig. 3 shows that the recovery rates for all three calibrations differ strongly from the optimum and in addition show high standard deviations. The relaxation times not only depend on temperature but also on other parameters like varying compounds (emulgator, addition of oil or flavours) in caramel mass. The relaxation behaviour from varying sets of caramel mass seems to be too different and calibrations do not deliver acceptable results. It is difficult to identify a general optimal measuring temperature, as the differences in recipes play a major role.

Therefore a calibration, Caramel C (Table 3), was created, consisting of two sets of caramel masses (Caramel 2 and 3). Fig. 4 shows the results obtained from

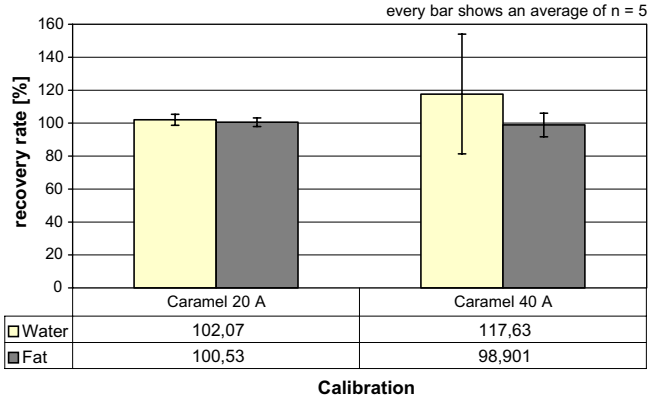

Fig. 2. The recovery rates of Caramel 20 A and 40 A using caramel masses with unknown water and fat content.

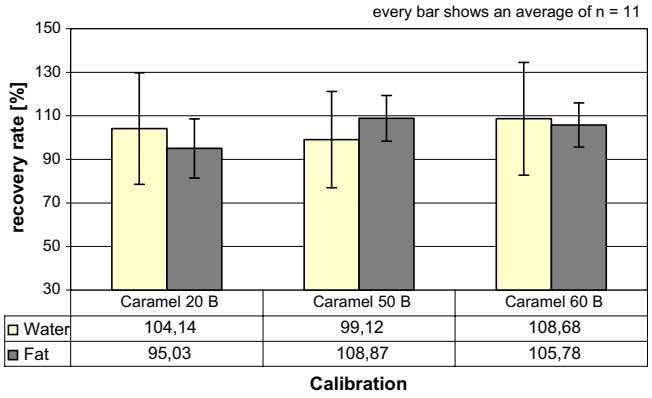

Fig. 3. The recovery rates of calibrations at different temperature using caramel masses from measuring set at the same temperature in each case.

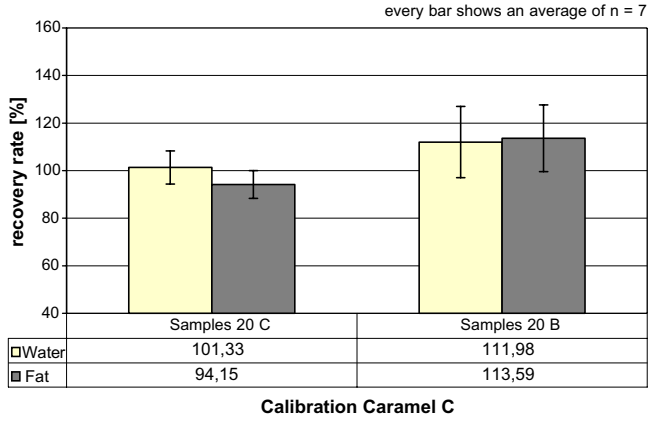

Fig. 4. Recovery rates contained by the examination of the Caramel 20 C with the samples of the same set (Samples 20 C) and with mixtured samples (Samples 20 B).

examinations of samples with the same recipes and of samples of the other caramel sets. Since the compositions of these caramel masses are more consistent than mixed samples, recovery rates are better and standard deviations smaller.

6. Summary

Caramel mass has a complex matrix, consisting of high amounts of ingredients. All of the ^{1}H-containing constituents contribute to the measurable NMR signal of caramel mass. Here, the water and the fat content were correlated with reference methods. The evaluation of the spectra of caramel samples is difficult, as several constituents apart from water and fat were varied in the samples used for one calibration. To obtain a reliable calibration model with satisfying recovery factors, the calibration sets should contain either only one type of caramel masses or a number of caramel masses with similar recipe. The measurements at lower sample temperature (20 °C) showed improved correlations. Further investigations have shown that more variation of parameters (pre-processing of the raw data before applying PLS, variation of factor (rank), widening of measuring range) gives room for improvement of the calibration statistics and correspondingly the applicability of the method.

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References

Guthausen, G., König, J., & Kamlowski, A. (2001). *Fat, water and protein determination by combined relaxation analysis using time domain NMR.* Comprehensive quality control of food: Silberstreifen, D-76287 Rheinstetten, Germany.

Schlimme, E., & Buchheim, W. (1998). *Milch und ihre Inhaltstoffe, Chemische und physikalische Eigenschaften* (Vol. 2). Auflage, Gelsenkirchen: Th. Mann.

Todt, H. et al. (2006). Water/moisture and fat analysis by time domain NMR. *Food Chemistry, 96*, 436–440.