

EXECUTIVE SUMMARY

1. PROTOCOL FOR THE MEASUREMENT OF WATER ACTIVITY (a_w) BY LOW RESOLUTION NMR

Purpose. The purpose of our investigations has been to find an optimum NMR procedure (transverse relaxation times, T_2) to characterise a_w in foodstuffs. We propose two different protocols for the measurement of T_2 , which differ by the type of mathematical processing (i.e. mono- and multi-exponential fitting) and by the information, which is obtained (i.e. water state in the *bound* or *free* compartment of the food system).

Materials and Methods. Samples of potato starch with different a_w were analysed by two distinct Carr-Purcell-Meiboom-Gill (CPMG) pulse sequences, which characterised water or oil decays. The cumulated relaxation curve of water and oil allowed a tri-exponential fitting for the T_2 calculation. A mono-exponential fitting procedure has been tested in order to find a more convenient, fast and bust protocol for routine characterisation of a_w in food industry. For this purpose, the T_2 has been measured with a pulse sequence that covered the first 10 ms of water decay.

Results. A distinction between water and fat protons during NMR measurement is essential, and it is the only possibility to find a relationship between the time constants of water and a_w , in complex systems. The multi -exponential fitting procedure reveals in the same time a complete information about the transverse relaxation of proton populations in the system, i.e. *bound* water, *free* water, oil. However, this method can be used with a high prudence when the food products contain other proton rich compounds, e.g. oil. The mono-exponential processing of the first 10 ms of water decay provides accurate results, which reflect with confidence the a_w of a food system. An automation of this technique should allow its implementation as a routine procedure for the assay of a_w in food industry.

Conclusion. Although the multi -exponential fitting procedure requires a large amount of data and is more time-consuming, it could be useful when a detailed information on the substrate state of hydration is required during food processing, i.e. the hydration of flour substrates during dough preparation. The mono-exponential protocol is faster and more practical, and provides a reliable characterisation of a_w even in the presence of oil. We have to emphasise that such a protocol is reproducible only for a certain type of product and for the same time domain parameters used in the acquisition.

2. THE QUANTIFICATION OF SOLID, WATER AND OIL IN FOOD SYSTEMS BY NMR METHODS

Purpose. Several low-resolution NMR methods are known in food industry to quantify the solid, water and oil in food products, but obvious problems still remain when their moisture content is greater than 5%. In the present study, some NMR protocols were proposed, which describe simultaneously the ratios solid/liquid or water/oil and the T_2 relaxation times in foodstuffs when they are required for the measurement of a_w . The novelty of the method developed in the University of Mons-Hainaut relies on the fact that it tries to surpass the actual constraints related to the quantity of water or oil in the food products, and imposed by the current techniques used in industry.

Materials and Methods. For the measurement of the solid/liquid ratio in samples containing only starch (or mousline) and water we used the free induction decay (FID) method. The FID+one spin echo (SE) and FID+CPMG sequences were used to quantify the percentages of solid, water and oil in the samples, which contained also oil (or margarine) in their composition. We replaced the "f-factor" (currently used to determine the amplitude of the FID signal at time 0) by an extrapolation function, which takes into account the T_2 of solid and the type of signal decay. In all experimental situations, the results were calculated both by including or not the specific hydrogen proportion in the starch, water and oil molecules, and the data were compared.

Results. An accurate determination of the solid/liquid ratio has been possible in starch containing samples, excepting the ones with lower water content (16%), where a deviation from the real value was been observed. For complex model systems (starch, water, oil), the specific proton proportion improve the data at inferior water levels (< of = 20%). Higher water contents in these food models can cause errors in oil quantification.

Mousline powder contains in its grains about 16% "liquid-like" material, which can interfere with water signal and result in its overestimation. Furthermore, margarine contains about 9% solid at 25°C, which can contribute to the solid signal that belongs to the mousline powder and finally can result in its overestimation. A deviation from the real value has been obvious for the samples with lower water (63-70%) and margarine content (3%), while the percentage composition of each element has been generally better estimated by taking into account the correction factor for specific proton abundance.

Our results demonstrated that both NMR techniques (FID+SE and FID+CPMG) used in this study are adequate for the determination of the percentage composition in complex food systems, and their approach may depend on the type of information which is required. For example, the FID+CPMG procedure gives simultaneously the following information:

the percentage content of solid, water (including bound and free water) and oil; the T₂ of different water populations (e.g. solid-like, bound water, free water) and the one of oil. The automation of this technique will allow an on-line protocol for industry, and will be able to assay concomitantly the a w and the percentage content of different components in food systems.

Conclusion. The low-resolution NMR methods proposed here to quantify the solid, water and oil in food systems give accurate results even when moisture content is higher than 6%. If we add the complex information furnished by them, we can assert that our procedures have an obvious original contribution to the ones known up-to-date. The fact that NMR methodology is non-destructive, fast and reproducible represents another argument for their advantages above other conventional methods.

3. THE MEASUREMENT OF STARCH RETROGRADATION BY NMR METHODS

Purpose. The present study is supported by the importance of retrogradation for the quality of starch containing products, and by the theoretical and practical advantages afforded by NMR techniques. Due to the dynamic interaction between starch polymer and water molecules, the process of starch retrogradation can be monitored in time by ¹H-NMR techniques. For this reason, the kinetics of potato starch retrogradation has been studied in the present work by low and high-resolution ¹H NMR spectroscopy.

Materials and Methods. The transverse relaxation times (T₂, mono- or multi -exponential) and solid/liquid ratio (FID technique), the proton exchange times (measured by Goldman-Shen sequence), and texture analysis on MR images were analysed as useful parameters that describe starch retrogradation in starch gels and potato.

Results. The evolution of the transverse relaxation times (T₂₁ and T₂₂; T₂₃ can be also obtained by a tri-exponential processing) suggests that polymers' re-crystallisation is associated with a dynamic process of water binding - release, the dominating one being the water binding. Beside the time constants (T₂₁, T₂₂), A₁ OR A₂ (the percentage amplitude of *bound* and *free* water) can add a supplementary information about the gel's potential to hold water in its structure. This is an important attribute for the food quality and preservation.

The mono-exponential T₂ decreased concomitantly with the progress of retrogradation, which suggests that water molecules have a lower mobility due to their holding in the re-crystallised polymer network. This procedure is accurate and fast, and can be implemented in industry for the assay of starch retrogradation. The NMR method does not need any special preparation of the sample, it is not destructive, and can give the result in a short time.

The solid content increased both in starch gels and potato concomitantly with starch retrogradation. The solid T₂ exhibited the tendency to decrease, which could confirm the water holding and entrapping in re-crystallised molecules.

The Goldman-Shen experiment showed a constant increase of the bound exchangeable phase of water protons, which confirms our former investigations on transverse relaxation and solid/liquid ratio. A general tendency of the exchange times to increase in potato samples has been observed. This is a prove of the strong interaction between water protons and polymer molecule, which consequently facilitates the phenomenon of cross-relaxation.

Texture analysis reflects potato as a homogeneous paste interrupted by a dispersed distribution of some points of starch nucleation. Furthennore, the evolution of texture parameters seems to be a result of a decreased T₂ and proton density. The method of texture analysis is promising and can find its applicability in a multitude of branches in food industry.

Conclusion. Different NMR methods may provide a particular information about starch retrogradation, and this depends on our requirements when we intend to analyse a food product, i.e. the state of water in the starch gel (e.g. its mobility), the percentage of bound water in its composition, the solid content which corresponds to the level of re-crystallized polymers.

4 NMR METHODS FOR LIPID ANALYSIS

Purpose. Low- and high-resolution ¹H and ¹³C NMR were used to analyse edible fats with different degrees of unsaturation, i.e. cocoa butter, animal fat, fish fat (hydrogenated fat), peanut oil, corn oil, sunflower oil. The purpose of our investigations has been to identify the more suitable NMR methods to analyse the physical and chemical properties of edible fats. Finally, we have analysed their usefulness as tests of authenticity for cocoa butters.

Materials and Methods. The samples were analysed by ¹H and ¹³C NMR spectroscopy, and by low resolution NMR (T₁ as an index of fats' unsaturation, and the solid fat content, SFC).

Results / Conclusions

¹H NMR spectroscopy can be used to characterise the general structure of triacylglycerols. The ¹H NMR spectra can furnish quantitative information, such as the molecular weight, the percentage hydrogen content of the fat molecule, the degree of unsaturation (iodine number), the average number of double bonds per fat molecule.

The **¹³C NMR spectroscopy** is a useful tool for fat analysis. This method can provide a complex and accurate qualitative (chemical structure) and quantitative information (e.g., the relative proportion of unsaturated and saturated fatty acids, the average molecular mass, the degree of unsaturation). ¹³C NMR spectroscopy can be used in fat industry both for the quality control analysis and as a test of authenticity.

The analysis of T1 as a parameter that describes the degree of unsaturation revealed its usefulness, excepting some fats such as the fish fat (hydrogenated fat). Therefore, the method can be used in fat industry for the fast measurement of the degree of unsaturation.

The **SFC** has been determined according to the methods described in literature, but some changes were made in the University of Mons-Hainaut in order to improve the actual methodology. These improvements refer to the NMR signal processing which results in an accurate measurement of SFC.

NMR methods for the test of cocoa butter authenticity. The new regulation adopted by the European Commission stipulates that 5% of chocolate can be replaced by other tropical vegetable fats known as *cocoa butter equivalents* (CBE). Consequently, there is an urgent need at European level for methods capable to detect and quantify the CBE.

Our studies evidenced the following differences between cocoa butters and their equivalents: (i) the higher content of palmitic acid in CBE and in their mixtures with authentic cocoa butter; (ii) a lower stearic acid / palmitic acid ratio for CBE and their mixtures with cocoa butter; (iii) the ¹³C spectra of some CBE (e.g. industrial cocoa butter substitute, illipé butter, and in the mixture of cocoa butter with palm oil) present supplementary signals assigned to diacylglycerols; (iv) the higher level of solids in some CBE (e.g. industrial cocoa butter substitute, illipé butter). However, our studies identified lower levels of solids in mixtures if we compare with their origin samples. This property is difficult to be used as a test of authenticity if we take into account the great variability of cocoa butters.