



Mobility of Lipids in Low Moisture Bread as Studied by NMR

G. Roudaut*, D. van Dusschoten†, H. Van As†, M. A. Hemminga† and M. Le Meste*

*ENSBA, Campus Universitaire- 1, Esplanade Erasme- F- 21000 Dijon, France and †Wageningen NMR Centre, Department of Molecular Physics, Wageningen Agricultural University, PO Box 8128, NL 6700 ET Wageningen, The Netherlands

Received 6 May 1997

ABSTRACT

The mobility of the lipids contained in glassy bread was studied with low resolution ¹H-NMR to measure their relaxation times (T_1 and T_2) and their translational diffusion coefficient (D), as a function of temperature and water content. The mobility of lipids detected with this method is independent of the water content of the samples. The behaviour of lipids in bread is observed to be comparable to that of lipids in bulk fat in the same temperature range. D measured for lipids is much higher than the values for water soluble solutes in glasses as provided by the literature. It was concluded that the lipids were distributed in globules which are dispersed in the glassy bread matrix and within which they diffuse.

© 1998 Academic Press

Keywords: mobility, glass, diffusion, lipids, bread.

INTRODUCTION

Most food products are rather complex systems which can be either homogeneous or composed of different phases, depending on their composition. The contribution of ingredients, such as lipids, to the material properties is dependent upon their repartition i.e. either homogeneously distributed and eventually interacting with the other ingredients, or organised in a dispersed phase. While in a dispersed phase, the contribution depends on the volume fraction¹ occupied by this phase and

on the difference between the respective properties (modulus, viscosity) of the continuous and dispersed phases. The overall stability of a food product is known to be controlled by the physical state of its constituent phases. From a chemical point of view, when the dispersed phase is entrapped within a glassy matrix, it may be protected from the outside environment. Indeed, stability is often associated with the glassy and crystalline states. The glassy state is a non-equilibrium state stabilised by the reduced molecular mobility; however, some local movements of small molecules such as water or oxygen may persist and affect the stability². Temperature and water activity are among the most important parameters responsible for changes in stability due to their effect on matrix mobility. Whereas the effects of these parameters are relatively well known for a continuous polymeric matrix³, in the case of multiphase systems the resulting behaviour of the dispersed phase is much less known.

This work is aimed at the study of the mobility

ABBREVIATIONS USED: NMR = Nuclear Magnetic Resonance; CPMG = Carr Purcell Meiboom Gill; FID = Free Induction Decay; T_2 = Transverse relaxation time; T_1 = Longitudinal relaxation time; DMTA = Dynamic Mechanical Thermal Analysis; D = translational diffusion coefficient; DSC = Differential Scanning Calorimetry; ESR = Electron Spin Resonance; CMC = Carboxy Methyl Cellulose

Corresponding author: C. Roudaut.

of lipid protons within a glassy bread matrix as a function of temperature and for three different moisture contents, using pulsed $^1\text{H-NMR}$. Relaxation time and diffusion coefficient measurements were carried out in order to measure the rotational and translational mobilities of lipids. Use of Nuclear Magnetic Resonance (NMR) has been extensively reported for the study of fat in food products—i.e. in imaging experiments^{4,5} to study the distribution of the fat in different products, pulsed NMR studies for determination of relaxation times of lipids^{6,7} or the solid/liquid fat ratio⁸. Furthermore, NMR diffusion measurements were also employed to measure diffusion of lipids in cheese⁹.

In previous studies, performed on low moisture bread using Dynamic Mechanical Thermal Analysis (DMTA) and Thermal Analysis^{10,11}, we demonstrated that the samples studied were in the glassy state with the exception of samples with a moisture content of 9% and at high temperatures ($T > 50^\circ\text{C}$). No event assignable to lipid behaviour was detected with these latter methods.

EXPERIMENTAL

$^1\text{H-NMR}$ methods

Theory

In NMR, there are two intrinsic parameters which correlate to the rotational diffusion, namely the relaxation times T_1 and T_2 . They each have a characteristic dependence upon the rotational correlation time τ_c ; T_1 shows a minimum when τ_c^{-1} is comparable to the measuring frequency ω_0 and increases linearly from this point with τ_c for $\tau_c < \omega_0^{-1}$ and with τ_c^{-1} for $\tau_c > \omega_0^{-1}$. The T_2 on the other hand is roughly proportional to τ_c^{-1} , but it reaches a minimum when the T_2 is comparable to τ_c itself. Furthermore, both relaxation times are inversely proportional to r^6 , where r is the proton–proton distance. This means that an identical rotational diffusion, the value of T_2 of fat is longer than that of water, since $r \sim 1.6 \text{ \AA}$ for water and $r \sim 1.8 \text{ \AA}$ for fat.

All NMR measurements were carried out on a 20 MHz spectrometer consisting of an electromagnet (Bruker, Karlsruhe, Germany), a SMIS imaging console (SMIS, Guildford, U.K.) and a 30 mm diameter probe (Doty Scientific Inc. Columbia, U.S.A.). 3 g (± 0.1 g) of sample were packed in a 15 mm external diameter tube, closed and placed in a dewar. The samples were cooled

down to -25°C , and then heated at 1°C per min. The NMR measurements were performed at 5°C intervals. A 5 min-waiting time was set at each 5°C step before the experiment was started, and measurements were repeated twice per temperature step. The sample temperature was measured with a thermocouple which was located above the sample in the NMR tube, and adjusted such that the signal to noise ratio was not deteriorated by the presence of the thermocouple.

Relaxation time studies

T_2 measurements were performed with the Carr Purcell Meiboom Gill (CPMG) sequence¹², which consists of a 90° pulse followed by a train of 180° pulses to refocus the NMR signal. At the beginning of the CPMG pulse train we also sampled the FID (Free Induction Decay) with 200 data points. The decay of the echo train (excluding the FID) was fitted with a multi-exponential NLLS (Non Linear Least Square) fitting routine by which mean values for the T_2 are obtained. The magnetisation decay of the FID was fitted to a two-exponential-model. By combination of the fits of the FID and the echo train, three different components could be discriminated, two of which were both present in the FID and the echo train.

The above mentioned sequence was also combined with a traditional¹³ inversion recovery experiment to measure the T_1 , using a 180° - τ_{IR} - 90° sequence with variable τ_{IR} .

In this way, a two dimensional data set was obtained. This set was analysed as a whole (2D data analysis), thereby obtaining much more accurate values for both the T_1 and the T_2 . This approach is identical to the one used for combined diffusion and T_2 measurements¹².

In our experiments, we used a 90° pulse of $8 \mu\text{s}$. A dead time of $20 \mu\text{s}$ was taken before data of the FID could be sampled. In the CPMG echo train, the echoes (in total 48) were sampled each 3.2 ms.

Diffusion coefficient measurements

Translational diffusion cannot be extracted directly from pulsed NMR experiments. However, NMR can be made extremely sensitive to motion by the use of magnetic field gradients. In a field gradient, the frequency at which spins resonate is dependent on their position. Spins resonating at different frequencies cause an extra dephasing of the NMR signal on top of T_2 relaxation, which results in an attenuated signal. The CPMG sequence is used to reduce the effect of this often

unwanted attenuation. Motions of spins in these gradient fields, however, still causes additional signal attenuation. The magnitude of this attenuation is related to the diffusion constant and the strength of the magnetic field gradient. By using controlled pulsed field gradients, this effect can be accurately exploited. The basis of this experiment¹⁴ is the use of two identical pulsed field gradients sandwiched around a 180° pulse.

The signal attenuation is given by:

$$A(G)/A(0) = \exp(-D\gamma^2 G^2 \delta^2 (\Delta - \delta/3)) \quad (1)$$

where $A(G)$ and $A(0)$ are the amplitudes respectively in the presence and absence of field gradient, G the magnitude of the gradient in the polarising field, D the self diffusion coefficient of the nuclear spins, γ the gyromagnetic ratio of the protons, the time interval Δ between the field gradient pulses and δ the duration of the gradient pulse. D is obtained by exponentially fitting the signal attenuation as a function of G^2 and represents the value along the direction of the gradient. In case of restricted or hindered diffusion, the root mean square displacement ($D = z^2/2t$) is obtained and an effective diffusion constant, dependent on the observation time Δ , can be calculated. In our case, we used a special sequence which combines diffusion and T_2 measurements in one experiment, in order to increase the accuracy and resolution of the diffusion measurement¹². In our experimental conditions, $\Delta = 10.3$ ms, $\delta = 9$ ms and $G_{\max} = 0.390$ T/m.

Standard deviation values of the different parameters were determined from four repetitions (two samples per water content and two repetitions per temperature and per sample). An average value was calculated for each sample per water content and for the temperature range studied.

Sample preparation

The white bread was purchased from a local supermarket. The composition as provided by the manufacturer was: wheat flour, sugar, honey, milk powder, milk fat, salt, yeast, lecithin, calcium propionate and water (37%). The chemical analysis of the bread gives, on a dry basis, the following values: 13.5% protein, 6% fat and 74.6% carbohydrates. The slices were cut into pieces (approximately $30 \times 15 \times 10$ mm) after removing the crust. The samples were dried for 90 mins in an

oven at 80 °C, then stored in desiccators over saturated solutions of $MgNO_3$, $MgCl$, and P_2O_5 at an ambient temperature of 25 °C. This corresponds to water activity levels of about 0.52, 0.33 and 0 respectively¹⁵. The samples were tested after at least 21 days; a time sufficient to reach a constant weight and hence practical equilibrium.

The moisture content of the samples was determined by weighing samples before and after drying for 5 h at 103 °C. The values were expressed as percent water on a wet basis (w/w). The final water contents of the bread samples were 2, 6 and 9%.

Defatting treatment

The defatting of the crushed bread was carried out at room temperature by two successive chloroform extractions for 2 h with an excess of chloroform. The bread sample was dried for several hours under a vacuum desiccator at room temperature until the residual solvent was evaporated.

RESULTS

Identification of the fractions

The amplitude of the decaying NMR signal reflects the amount of protons relaxing during the acquisition time. Protons with very low mobility ($\tau_c \approx 10$ μ s) normally relax within the dead time of the experiment (< 20 μ s) and do not take part into the observed signal.

For all the bread samples studied (normal bread with 2, 6 or 9% moisture, and defatted bread with 2% of water), the data could be described by three components: one with a T_2 lower than 0.2 ms (fraction 1), and two others with T_2 's between 5 to 300 ms (fraction 2 and 3). The signal amplitude corresponding to fraction 1 increased with moisture content over the range studied [Fig. 1(a)] due to an increase of the pool of protons with a T_2 higher than the dead time.

On the opposite, for all the water contents studied, the amplitude of fraction 2 increased from -25 to 15 °C and decreased up to 85 °C [Fig. 1(b)]; it is however noticeable that for the 9% water sample, the amplitude exhibits a second maximum between 50 and 75 °C. The amplitude of fraction 3 increased from -15 to about 40 °C, and then levelled off [Fig. 1(c)]. The amplitudes of these two latter fractions are insensitive to the water content.

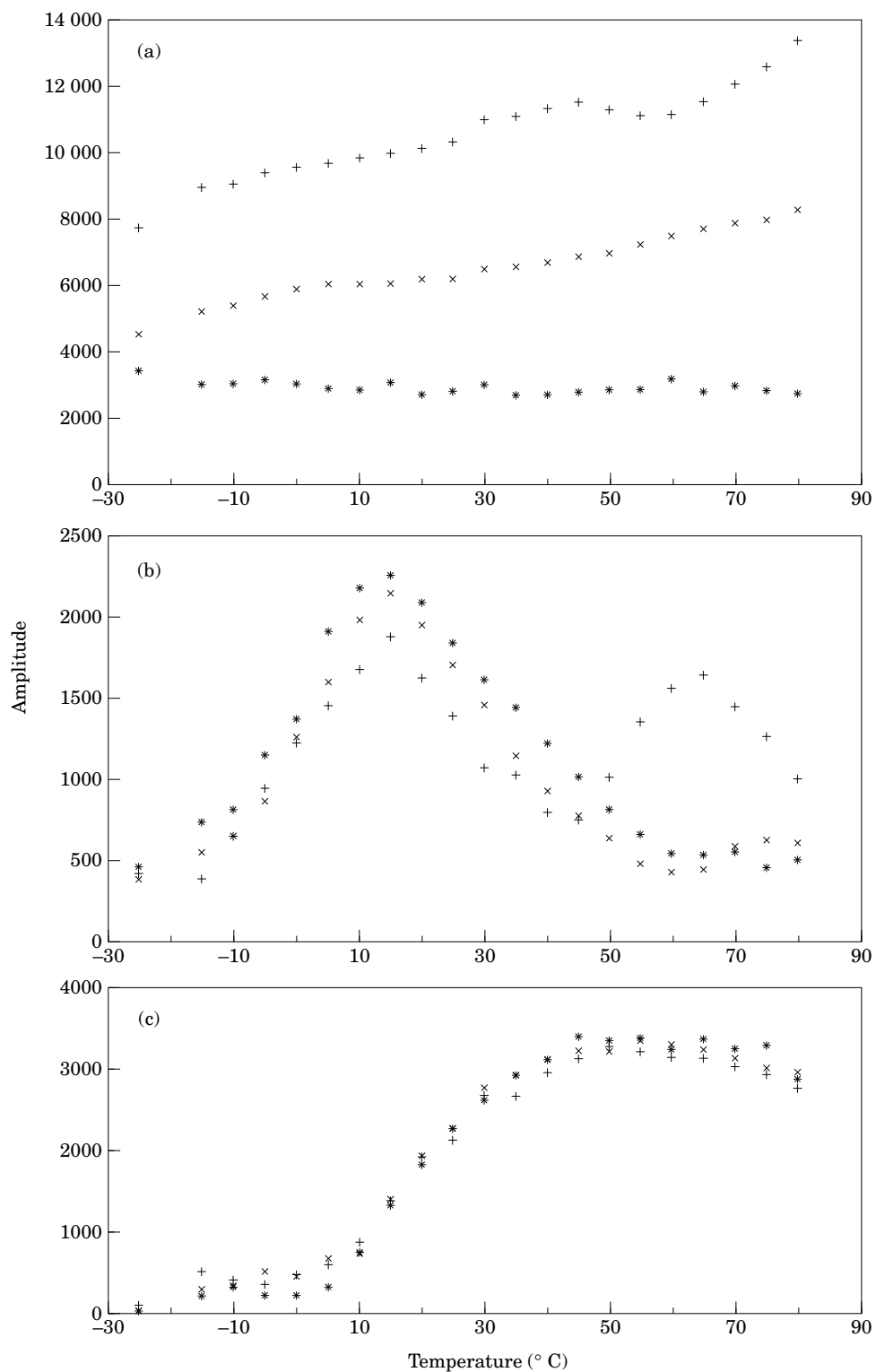


Figure 1 Temperature dependence of the signal amplitude of fraction 1(a), fraction 2(b) and fraction 3(c) for bread with (+) 9%, (x) 6% and (*) 2% of water. The standard deviations for the amplitude of fraction 1, 2 and 3 are 6.7%, 13.8% and 14% respectively.

The chloroform extraction modified the amplitudes of the three fractions, but the 2nd and 3rd fractions were most affected by the extraction. For example, at 35 °C, the summed signal amplitude of fractions 2 and 3 was reduced to 27% of the initial value (results not shown). On lipid removal, the overall amplitude loss is greater than that attributable to extracted lipid protons. Indeed total amplitude loss is 57% whereas the chemical analysis indicates that after the chloroform treatment the sample has lost 38% of its initial lipid content. Such a difference was also observed by Belton *et al.*⁶ upon gluten defatting, and could result from the difference in sensitivity of these methods. A small part of the less mobile fat might be undetected by NMR, but could be detected by the chemical analysis. On the basis of both their sensitivity to chloroform extraction and the insensitivity of their amplitude to moisture content, we assumed that the slower relaxing protons (2nd and 3rd fractions) are associated with the lipids contained in the bread. Furthermore, it seems that the fast relaxing fraction should be mainly associated with the water contained in the bread, perhaps with only a minor contribution of lipids. Due to very broad and overlapping signals, no chemical shift could be accurately measured for the different fractions, and thus could not help in the identification of the fractions. In the following work, we will focus mainly on the lipid fractions, that is the protons sampled as the 2nd and 3rd components of the decay. The behaviour of the water protons will be described in a separate article.

Mobility of the lipids

From -25 to 5 °C, the detectable T_2 's of fractions 2 and 3 were rather constant (~ 5 and 15 ms respectively). The difference in T_2 's between the two lipid fractions becomes more pronounced above 10 °C. Above 10 °C, the T_2 values of fraction 2 [Fig. 2(a)] increase and reach a maximum at 35, 45 and 50 °C with the 9%, 6% and 2% water samples respectively. The maximum value of T_2 for fraction 2 (especially in bread with 2% moisture) is comparable to the one obtained by Callaghan *et al.*⁸ for fat in pure milk fat at 30 °C (36×10^{-3} s). The T_2 's of fraction 3 exhibits a regular increase above 10 °C, characterised by an activation energy of 15 kJ/mol. As can be clearly seen, the T_2 values of fraction 2 and 3 were insensitive to the changes in moisture content, except above 35 °C for frac-

tion 2. The T_2 's of both fraction 2 and 3 were not affected by the defatting treatment (data not shown).

The inversion recovery data were analysed with a similar fitting routine as the T_2 's data, thus the amplitudes are the same for both T_1 and T_2 results. From -25 to 80 °C, the T_1 for both fractions 2 and 3 increased from 70 ms at -25 °C to 700 ms at 80 °C for bread with 2% of water, with an apparent activation energy of 19 kJ/mol. Similar results were obtained with bread containing 6 and 9% of water.

The variation of the translational diffusion coefficient D with temperature is presented in Figure 3 for bread samples with 6 and 9% of water. No diffusion could be precisely detected below 0 °C, because of insufficient signal amplitude. The temperature effect on the diffusion coefficient is characterised by an activation energy of ~ 20 kJ/mol.

DISCUSSION

The bread studied contains intrinsic lipids i.e. the flour lipids. Previous studies have been performed on flour lipids^{6,7}. Belton *et al.*⁶ have carried out pulsed $^1\text{H-NMR}$ measurements on dry gluten and defatted gluten. A fraction characterised with a T_2^* of 23 ms at 20 °C was attributed to the lipids. Thus, in the present case, the protons of the gluten lipids might be fitted in fraction 2. However, since gluten represents only 12% of the bread and contains only 4 to 7% lipids by weight (equivalent to 6–10% of the protons in gluten), these protons may only contribute to a small extent to the signal of fraction 2 of bread (the lipids protons represent approximately 6% of the total protons amount). Similarly, due to the very low lipid content of the starch (less than 1% per weight), the intrinsic lipids of the starch are expected to represent a very limited pool of protons. Moreover, they are expected to have a very low mobility⁷ and to contribute to the signal amplitude of fraction 1 or even to relax within the dead time.

According to the composition provided by the manufacturer, milk fat is the main fat added to the bread. The thermal behaviour of bulk milk fat studied by Differential Scanning Calorimetry⁸ exhibits an endothermic event associated with crystal melting spreading from -30 to 35 °C. Figure 4 illustrates the evolution of the summed amplitudes of fractions 2 and 3, as compared to the variation with temperature of the solid/liquid

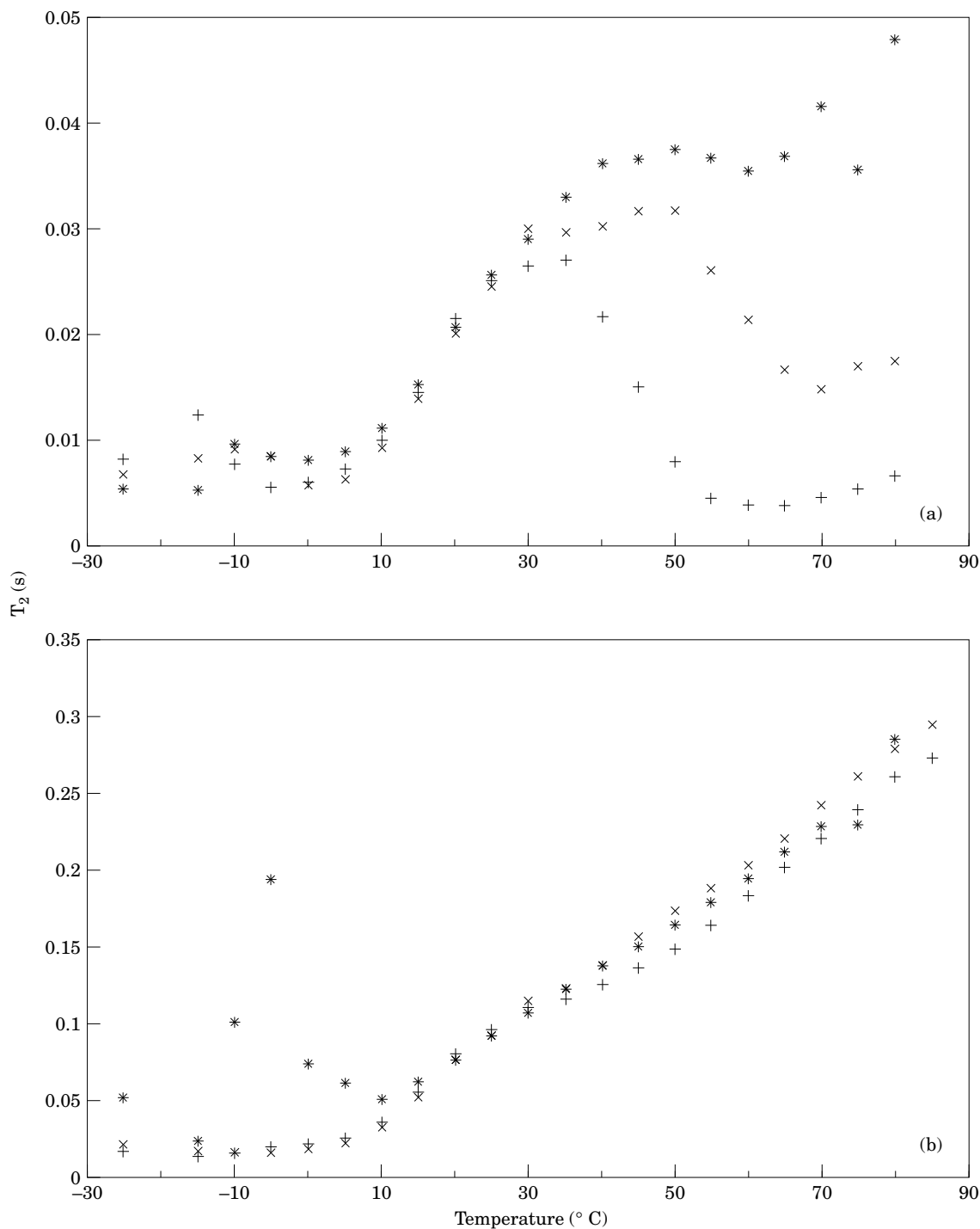


Figure 2 Temperature dependence of the T_2 of fraction 2(a) and fraction 3(b) for bread with (+) 9%, (x) 6% and (*) 2% of water. The standard deviations are 19.6% and 15.3% respectively for fraction 2 and 3.

fat ratio (measured by NMR) in milk fat⁸. It is noticeable that the signal amplitude of the sum of fractions 1 and 2 and the liquid fat index (Fig. 4) exhibits a similar temperature sensitivity. Thus it

can be concluded that both fractions 2 and 3 should represent mainly the signal of liquid lipids, and that their behaviour is very close to that of the liquid bulk fat.

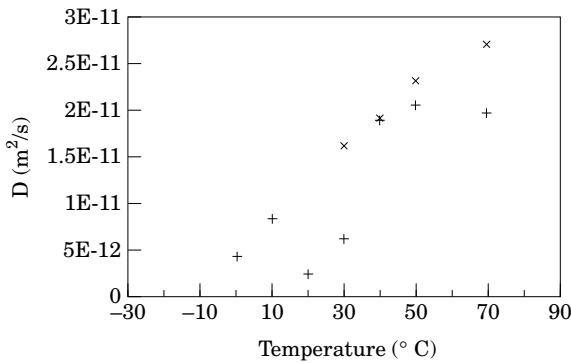


Figure 3 Temperature dependence of translational coefficient diffusion of fat in bread with (*) 6% and (+) 9% of water.

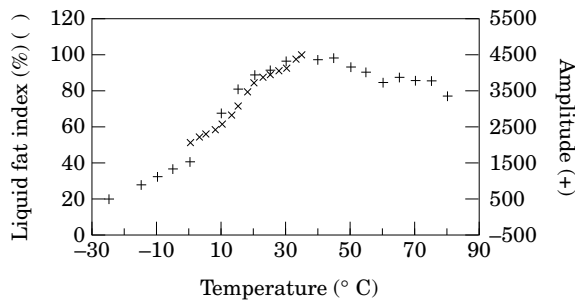


Figure 4 Temperature dependence of the amplitude of the added lipid fraction (+) in bread with 2% of water and of the liquid fat index (x) of milk fat determined by NMR (after reference 7).

In our study, from -25 to 35 °C, the bread is expected to contain both solid and liquid fat. Above 35 °C all the milk fat is expected to be liquid. Up to 35 °C, the total amplitude of fat fractions increases due to the melting of the fat, which becomes observable only when liquid. The measured parameters (D , T_2 , and T_1) reflect the effect of temperature on liquid fat. The changes in the T_2 [Fig. 2(a,b)] with increasing temperature are similar to the evolution of the rotational correlation time τ_c of a nitroxide probe homologous of a fatty acid, measured in lard¹⁶ with ESR.

Considering both the signal amplitude and the T_2 for fractions 2 and 3, it was observed that water content had no effect on their evolution with temperature below 35 °C [Fig. 1(b,c) Fig. 2(a, b)]. However, the T_2 of fraction 2 exhibited a behaviour that was dependent on hydration above 35 °C. From that point onwards, in bread samples with 6 and 9% water content, the T_2 decreased with increasing temperature. The reason for this decrease lies in the nature of the discrete fitting

procedure. Whether a certain fraction is detected as either belonging to a fast or a slow relaxing fraction depends on the relative distribution of T_2 's. This is caused by fitting a distribution of T_2 's with only two or three exponentials. It can therefore occur that, at a certain temperature, part of a fast relaxing fraction moves to the next, slower one. This may alter both the fractional amplitudes and the average T_2 's of both fractions, since these are in fact weighted averages. In our case, some water protons might skip from fraction 1 to fraction 2. Indeed, due to the higher temperatures, the T_2 of water increased such that it started to contribute to the CPMG echo train, which caused the amplitude of fraction 2 to increase and its T_2 to decrease. This hypothesis is supported by the fact that the drop of the T_2 occurs at a lower temperature for the bread with 9% of water than for the one with 6%, and that the T_2 associated with water is longer for the 9% bread than for the 6% at identical temperature.

Using Electron Spin Resonance, Bouanda¹⁷ measured the rotational correlation time of a nitroxide probe (homologous to a fatty acid) dissolved in linoleic acid in a Carboxy(methyl)cellulose (CMC) matrix. At 30 °C, this value of τ_c was comparable to the τ_c measured in bulk fat¹⁶. The author showed that the hydration (from 2 to 14%) did not modify the rotational mobility of the probe. The hydrophobicity of lipids induces a phase separation and this would explain the insensitivity of lipid mobility to changes in the water content. It should be noticed that the rotational mobility of the lipids is the same in partially defatted and in normal bread, suggesting that this property is not dependent on the amount of dispersed phase in the product.

The translational diffusion coefficients measured for lipids in low moisture bread are much higher than the coefficient measured for water molecules in sugar or biopolymer glasses at similar water contents^{18,19}. The value we obtained at 30 °C is of the same order as the one measured for the diffusion of lipids (mainly triglycerides) in bulk milk fat by Callaghan⁹ at the same temperature. Our values are only slightly affected by the hydration changes (from 6 to 9%). Using the profile concentration method, Naesens *et al.*²⁰ studied the translational diffusion of fatty acids in CMC matrix at low water contents. Due to the low water solubility of the fatty acids and the resulting phase separation, the authors suggested that the solutes might be transported as lipodic globules.

In previous work¹¹, we showed that in the hy-

dration range studied, the bread matrix was in the glassy state up to approximately 150, 90 and 50 °C for the bread at 2, 6 and 9% water content, respectively. Therefore, except for the sample with 9% water content above 50 °C, in all the samples the fat globules should be trapped in the glassy matrix and thus, translational motions of these particles should be hindered². However, we observed a relatively high translational diffusion coefficient of lipids with a low sensitivity to moisture content. We can thus assume, that this corresponds to the intrinsic motion, i.e. motion of lipids within lipid globules dispersed in the polymeric matrix. The distribution of lipids in globules entrapped in the matrix has already been suggested for gluten lipids by several authors^{7,22}. The diffusion properties of these globules may depend on the viscosity and physical state of the surrounding matrix. Thus, the travelled distance value during a time $\Delta = 19.3$ ms may provide a value for the minimal diameter of the globule. It can be thus calculated that at 40 °C, the minimum diameter would be of the order of 9×10^{-7} m, which is rather large and may reflect a relatively coarse emulsion.

Previous molecular studies of glassy bread using Dynamical Mechanical Thermal Analysis and DSC¹¹ did not give evidence of such a polyphasic character. Indeed, Ross and MacRitchie²³ described bread as a polymeric network (formed with gluten and starch) controlling the rheological properties, and in which the lipidic phase does not play a role. The amount of lipids present in the bread was probably not sufficient for the observation of the melting process with DSC or DMTA. However, even if the lipid phase (in limited proportion) is not expected to play a significant role on the mechanical properties of a glassy cereal product, it might contribute to its flavour either in a negative way, when oxidation reactions are involved, or in a positive way, as a solvent for aroma compounds.

In conclusion, we have demonstrated that NMR relaxation time and diffusion coefficient measurements appeared particularly suitable to study mobility and phase transition of species even at low concentration. The results obtained in this work confirm those obtained in model systems with different techniques. Our results suggest that both the rotational and translational mobilities of lipids are similar in bread and in bulk fat. The organisation of lipids in differentiated globules within a biopolymeric network does not prevent the lipid translational diffusion, which however

should remain limited to diffusion within fat globules when the surrounding matrix is a glass.

Acknowledgements

This work was supported by the European Community activity Large-Scale Facility Wageningen NMR Centre (ERBCHGECT 940061) and the European Commission Contract ERBF AIRCT 1085.

REFERENCES

1. Ferry, J.D. In 'Viscoelastic Properties of Polymers'. (3rd edn), John Wiley and Sons, Inc, New York (1980).
2. Le Meste, M. In 'Food Preservation by Moisture Control'. (V. Barbosa-Canovas and J. Welti-Chanes eds). Technomic. Pub. Co., Lancaster USA (1995) pp 209–225.
3. Roos, Y.H. Phase Transitions in Foods. Academic Press, San Diego (1995).
4. McCarthy, M.J. and Kauten R.J. *Trends in Food Science and Technology* December (1990) 134–139.
5. Desbois, P. and Le Botlan, D. *Journal of Food Science* **59** (1994) 1088–1090.
6. Belton, P.S., Duce, S.L. and Tatham, A.S. *Journal of Cereal Science* **7** (1988) 113–122.
7. Marion, D., Le Roux, C., Akoka, S., Tellier, C. and Gallant, D. *Journal Cereal Science* **5** (1987) 101–115.
8. Lambelet, P. *Lebensmittel Wissenschaft und Technologie* **16** (1983) 90–95.
9. Callaghan, P.T., Jolley, Y.K.W. and Humphrey, R.S. *Journal of Colloid and Interface Science* **93** (1983) 521–529.
10. Le Meste, M., Huang, V.T., Panama, J., Anderson, G. and Lentz, R. *Cereal Foods World* **37** (1992) 264.
11. Le Meste, M., Roudaut, G. and Davidou, S. *J. Thermal Analysis* **47** (1996) 1361–1376.
12. van Dusschoten, D., Moonen, C.T.W., De Jager, P.A. and Van As, H. *Magnetic Resonance Medicine* **36** (1996) 907–913.
13. Harris, R.K. In 'Nuclear Magnetic Resonance Spectroscopy': A Physicochemical View. Pitman, London (1976).
14. Tanner, J.E. and Stejskal, E.O. *Journal of Chemical Physics* **49** (1968) 1768–1777.
15. Greenspan, L. *Journal of Research of the National Bureau of Standards* **81A** (1977) 89–96.
16. Le Meste, M., Cornilly, Y.G. and Simatos, D. *Lipids* **20** (1985) 296–302.
17. Bounda, R. Thèse de 3^{ème} cycle, Thèse, Université de Bourgogne (1983).
18. Ablett, S., Darke, A.H., Izzard, M.J. and Lillford, P.J. In 'The Glassy State In Food', (J.M.V. Blanshard and P.J. Lillford, eds), Nottingham Press, Nottingham (1993) pp 189–206.
19. Parker, R. and Ring, S.G. *Carbohydrate Research* **273** (1995) 147–155.
20. Naesens, W., Bresseleers, G. and Tobback, P. *Journal of Food Science* **46** (1981) 1446–1449.

-
21. Naesens, W., Bresseleers, G. and Tobback, P. *Journal of Food Science* **47** (1982) 1245–1249.
 22. Hargreaves, J., Le Meste, M. and Popineau, Y. *Journal of Cereal Science* **19** (1994) 107–113.
 23. Ross, A.S. and MacRitchie, F. In ‘Ingredients Interactions: Effects on Food Quality’, (A.G. Gaonkar, ed.), Marcel Dekker, Inc., New York (1995) pp 321–356.