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Water, ice and sucrose behavior in frozen sucrose–protein solutions as studied by ^{1}H NMR

Tiphaine Lucas*, François Mariette, Sandra Dominiawsyk, Dominique Le Ray

Cemagref, Food Process Engineering Research Unit, CS 64426, 17 avenue de Cucillé, 35044 Rennes Cedex, France

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Abstract

This work aimed at characterizing both the spin–spin (T_2) and spin–lattice (T_1) relaxations of water in frozen samples. Pure water and aqueous solutions (sucrose and/or casein) were studied, temperatures ranging from −13 to 20 °C. Three relaxation components could be distinguished after signal fitting. For example, the shorter spin–spin relaxation time was only observed at the frozen state and attributed to the ice crystals protons and the longer relaxation time was attributed to the liquid water and some of the sucrose protons. The exploitation of the 'solid' relaxation parameters gave information on ice content (T_2) and on ice structure (T_1). The method developed for ice content calculation was coherent with calorimetric data. The changes in the spin–spin relaxation of sucrose protons with temperature showed that at low temperature (−13 °C) it could be fully distinguished from the relaxation of water protons and at positive temperature this discrimination was not possible anymore.
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Keywords: Relaxation; Water; Protein; Temperature; Spin–spin; Spin–lattice; Ice content; Unfrozen water

1. Introduction

In freezing/thawing processes assessing the amount and the state of unfrozen water is of a crucial importance to predict the stability of the frozen product. This liquid water is indeed available for diffusion (sublimation of water at the air/food interface, contribution to the ice recrystallization) as for biochemical or enzymatic reactions. Although these processes are limited by low temperatures and by the reduced amount of available liquid water, they definitely govern the slow organoleptic deteriorations in frozen foods.

Unfortunately the behavior of unfrozen water in frozen food products is not available by conventional techniques. Water activity cannot be assessed experimentally, but calculated by extrapolation. The glass transition temperature T_g' gives information on the mobility of molecules at its vicinity, but its use remains limited as in many food products, the glass transition temperature is often much lower than the storage temperatures commonly used. At last, the presence of both solid and liquid phases makes difficult, if not impossible, the interpretation of measurements from indirect techniques (e.g. viscosity). Measuring the amount of frozen water and then deducing the amount of unfrozen water, often remains the only information available.

The amount of frozen water is usually determined by a calorimetric technique [differential scanning calorimetry (DSC), differential thermal analysis (DTA)]. Nuclear magnetic resonance (NMR) has also been shown as a powerful technique to calculate the amount of unfrozen water into a food sample. The current method consists of measuring the variation of the amount of liquid proton including the liquid water and the non water proton, and after temperature correction according to the Curie's law, to express it as a function of the total water content (Hays & Fennema, 1982; Katayama & Fujiwara, 1980; Weisser & Harz, 1984). This method only assumes that the solid phase is pure ice water alone. As calorimetric techniques, NMR does not require any calibration with a reference technique but uses a reference measurement on pure water samples. Two main advantages of the NMR method

* Corresponding author. Tel.: +33-2-2348-2121; fax: +33-2-2348-2115.

E-mail addresses: tiphaine.lucas@cemagref.fr (T. Lucas).

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Nomenclature

PD	proton density (g mol g^{-1} mol^{-1})
I	relative population associated to the spin–spin relaxation component, expressed in intensity unit (V)
m	weight of the sample or of an ingredient in the sample (g)
MI	mass intensity (V g^{-1})
ω	mass fraction (g g^{-1})
T	relaxation time (s)
t	time (s)
θ	temperature (°C)
θ_m	initial freezing temperature (°C)
X_{s-w}	fraction of sucrose protons involved in the water relaxing component, relative to the total sucrose protons

Superscripts

ℓ	attached to a quantity which is expressed per gram of remaining liquid solution (in the case of partially frozen samples)
S	attached to a quantity which is expressed per gram of sample
tw	attached to a quantity which is expressed per gram of total water

Subscripts

$1CP_j$	identifies the spin–lattice relaxation parameters (time and relative population) as well as the component number
$2CP_j$	identifies the spin–spin relaxation parameters (time and relative population) as well as the component number
C	identifies the caseins
i	identifies the ice crystals
s	identifies the sucrose
S	identifies the sample
tw	identifies the total water (liquid water and ice)
w	identifies the liquid water

towards the DSC method, could be outlined. First, the NMR technique does not involve any thermal processes to assess the amount of ice and thus can be performed at stable temperature. Next the relaxation parameters of water could be used to provide information on the water–non water molecule interactions. For assessing both the amount of the unfrozen water and the water–non water molecules interactions, the NMR relaxation parameter most commonly measured is the spin–spin relaxation time and its corresponding population. But if many works have been focused on the unfrozen water behavior, the ice behavior has surprisingly attracted little attention compared to the issues of recrystallization during storage and the consecutive modifications of food texture. Moreover, as far as frozen food products are concerned, very few results have been published based on spin–lattice relaxation time of water. One of its interests compared to the spin–spin relaxation is (1) that experimentally the spin–lattice determination of the solid phase is less sensitive to the fitting method as the spin–spin relaxation suffers from inaccuracy because of the dead time of the probe which in turn impedes the sampling of the first points, (2) because of the strong dipole–dipole interaction the spin–spin relaxation is a less sensitive parameter (maximum range of variation from 10^{-2} to 40 10^{-2} ms) compared to spin–lattice relaxation (maximum range of variation 1000–10 000 ms).

In the case of water molecules, ^1H spin–lattice relaxation is mainly governed by the dipole–dipole interactions (Abragam, 1961; Bloembergen, Purcell, & Pound, 1948). In previous papers it was found that the non-Arrhenius temperature dependence of the spin–lattice relaxation time T_1, for the numerous nuclei ^{17}O, ^2H as well as ^1H in water could be explained on the basis that the relaxation involves two processes, one dominant at positive temperature and a second important at negative temperature (Hindman, 1974). At positive temperatures the rotational diffusion of non or weakly bonded water molecules is involved, and the activation energy for this process was found to be very low: 13.8 kJ mol^{-1}, value which corresponds to the activation energy of a single hydrogen bond. However, at negative temperatures, the activation energy in ice is close to 59 kJ mol^{-1}: 59 kJ mol^{-1} from T_1 (Barnaal & Lowe, 1968), 60.6 kJ mol^{-1} from diffusion coefficients (Dengel, Jacobs, & Riehl, 1966). Various relaxation processes giving similar values indicate that the relaxing molecules are in a well-defined hydrogen-bonded structure. It is known that the internal motions in ice are strongly influenced by minute amounts of impurities (Hobbs, 1974). Changes in the activation energy are directly related to the nature of defects in the ice structure (Bilgram, Roos, & Gränicher, 1976; Gran, Hansen, & Pedersen, 1997; Kopp, Barnaal, & Lowe, 1965). For example, the activation energy deduced from the variation of the T_1 of ice versus temperature decreases with increase in the number of defects induced by the HF molecules in doped ice (Barnaal, Kopp, & Lowe, 1977).

The present paper reports the behavior of both spin–lattice and spin–spin relaxations of the sucrose protons, the unfrozen water and the ice water protons in aqueous sucrose solutions. Sucrose has been chosen because numerous data are available from NMR and non NMR techniques. Moreover, sucrose solutions are often used as a model to reproduce the freezing behavior in foods or in at least sucrose-containing foods. The effects of the

temperature (from −13 to 20 °C), of the sucrose concentration (42.86 and 61.29% w/w of water) and of the addition of casein (3% w/w of water) on these parameters will be studied. The behavior of pure water as a function of temperature will also be investigated with the aim of getting a reference (absence of solutes) for further comparison.

2. Materials and methods

2.1. Sample preparation

The day before, solutions were prepared by completely dissolving commercial-grade sucrose (99.3% dry matter) and powder of native phosphocaseinate, called 'casein' (INRA, Rennes, France) in demineralized water at room temperature and under agitation. The concentrations of sucrose, casein and water in the aqueous solution are expressed in grams per 100 g of total water. Two concentrations of sucrose were studied: 42.86 and 61.29%. The initial freezing temperature of the sucrose–water (SW) solutions is denoted θ_m and equals, respectively −2.7 and −4.0 °C (Young & Jones, 1949). The casein–sucrose–water (CSW) solution contained 42.85% of sucrose and 3.06% of casein.

Then the NMR tubes were filled with samples (approximately 0.5 g), hermetically closed, weighed and then placed in a domestic freezer (−30 °C) for freezing and storage over night. The freezing rate, i.e. time needed to lower the core temperature from θ_m to −10 °C (International Institute of Refrigeration, 1986) was close to 0.5 °C/min (Fig. 1).

2.2. NMR measurement

Before the NMR measurement, the tube was placed in the NMR probe for thermal equilibration. The temperature of the probe could be controlled in the range (−18 to 80 °C) with a standard error of 0.15 °C with a cryothermostat (Ministat, Huber, Biobloc Scientific, F-67403 Illkirch, France). The time needed to reach thermal equilibrium at the different measurement temperatures was previously determined with a series of reference samples with a thermocouple (type T, Ø 1 mm) placed at the center of the sample.

Once the temperature was stabilized within the sample, NMR measurements were performed with a low field NMR spectrometer (0.47 Tesla) operating at 20 MHz for the ^{1}H (Minispec PC120, Bruker SA, F-67166 Wissembourg, France). The spin–spin relaxation (T_2) was measured from the free induction decay (FID) and the Carr Purcell Meiboom Gill (CPMG) curve. The sampling rate for the acquisition of the FID was 1 point per 1 μs, and the delay between the 90° and 180° pulses of the CPMG sequence varied from 0.2 to 3 ms as a function of the temperature. The spin–lattice relaxation (T_1) was measured using a saturation recovery (SR) sequence. One hundred points were acquired from 30 ms to the recovery delay values, RD. The RD varied from 35 s at −14 °C to 10 s at 20 °C. For all acquisitions the receiver gain was the same.

Each sample is used only once, i.e. it does not serve for any another measurement at a higher temperature. Most experimental conditions (defined by one type of solution and one level of temperature) were repeated at

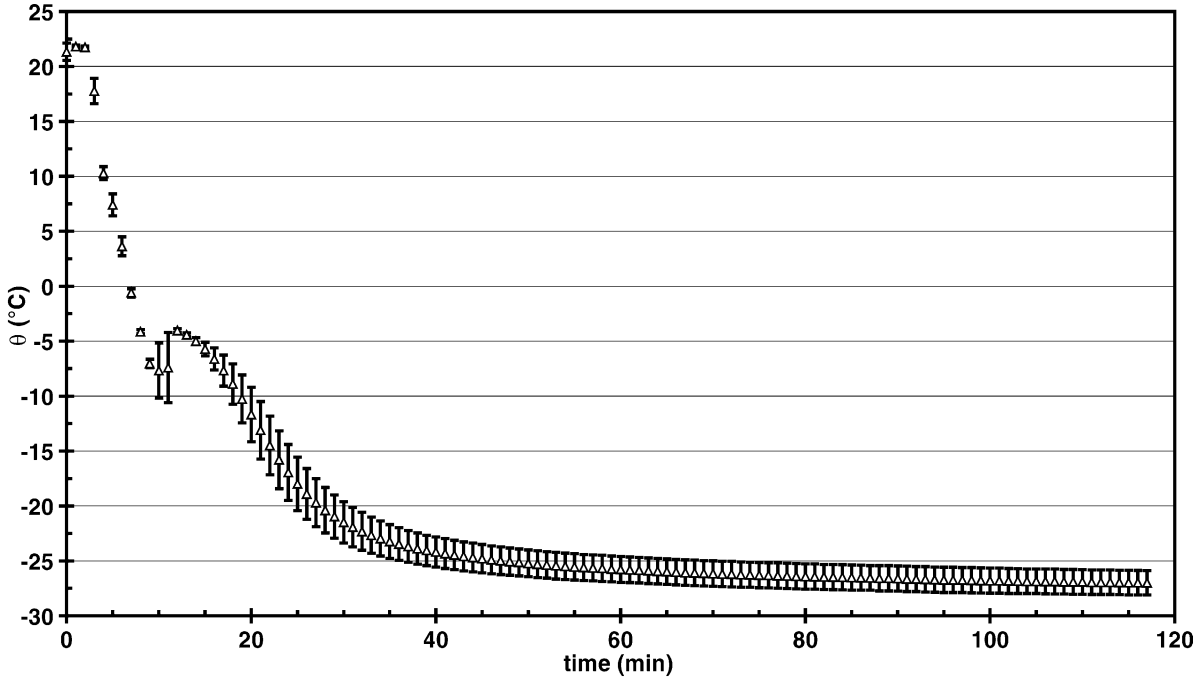

Fig. 1. Core temperature evolution with time in the NMR sample (42.8% sucrose–water solution) during freezing.

least twice (three runs). Final values were expressed as a mean value with associated standard error (2σ).

2.3. Data expression

2.3.1. NMR relaxation times and intensities

Determination of the relaxation time and its relative population is known to be sensitive to the fitting methods selected. In order to be exempt of mis-adjustement, we compared the results obtained with two different methods: the discrete method (Marquardt, 1963) and the continuous maximum entropy method (MEM; Mariette, Guillement, Tellier, & Marchal, 1996). This strategy was applied both on CPMG and SR sequences (it was not applied to the FID sequence as the number of points was too small to be fitted by the MEM). Table 1 compared the spin–spin relaxation times (T_2) and the associated populations expressed in relative intensities, obtained by the discrete method and the MEM, for a 42.86% sucrose–water solution at two temperatures, one in the liquid state ($\theta = -2\ ^\circ\mathrm{C} > \theta_m$) and the other partially frozen ($\theta = -6\ ^\circ\mathrm{C}$). The results given by both methods were consistent. Consequently, only the results from the Marquardt method were presented.

Spin–spin relaxation parameters (relaxation time and relative population) have been identified from the reconstituted relaxation curve (FID + CPMG). The FID decay curve from 11 to 70 μs was adjusted with a gaussian function. The electronic noise of the transmitter was taken into account by a measurement of the signal from an empty tube after the application of a single 90° pulse.

With respect to the spin–spin relaxation components attributed to protons from the liquid phase, the values of their relaxation time and relative intensity identified from the reconstituted relaxation curve could slightly differ from the ones identified when using only the data from the CMPG sequence. Both the presence of data from the FID sequence and the lack of data in the range (70 μs–200 ms) contributed to changes in the adjustment of the very first component representing the liquid-like behaving protons. Such change modified in turn the parameter values of the longer components. At the exception of Table 1, all data presented in Section 3 have been identified applying the Marquardt algorithm to the reconstituted FID + CPMG curve, which make them all comparable.

2.3.2. Calculation of the ice fraction

The ice fraction is denoted ω_i^{tw} and expressed in gram per g of total water (or 'initial' water). Two modes of calculation were applied in the present work, one based on the data from Young and Jones (1949), the other one based on our NMR data.

Data from calorimetric measurements (Young & Jones, 1949) were available in the forms of the initial freezing temperature of sucrose–water solutions as a function of the initial sucrose concentration. These data were re-expressed as the sucrose concentration in the remaining liquid phase as a function of the operating temperature and were fitted by a ratio of quadratic equations (*T*able Curve, Jandel, USA):

$$\omega_s^{\ell}\{\theta\} = \frac{2.4110^{-3} - 1.7710^{-1}\theta - 4.9310^{-3}\theta^2}{1 - 1.8810^{-1}\theta - 6.3810^{-3}\theta^2},\ r^2 = 0.99 \quad (1)$$

where $\omega_s^{\ell}\{\theta\}$ is the concentration of sucrose relative to the remaining liquid water in the frozen solution. In thermodynamic conditions, the ice fraction ω_i^{tw} can be expressed from the sucrose concentration as follows:

$$\omega_i^{tw}\{\theta\} = 1 - \frac{\omega_s^{tw}}{\omega_s^{\ell}\{\theta\}} \quad (2)$$

where ω_s^{tw} is the concentration of sucrose when the sucrose–water solution does not contain ice crystals ('initial' sucrose content). Combining Eqs (1) and (2) gives the ice fraction as a function of temperature for a given sucrose–water solution (fixed sucrose concentration).

The NMR method is based on the fact that solids, in contrast to liquid, have very short T_2 relaxation times in

Table 1
Comparison between the MEM method and the Marquardt method for the adjustment of the CPMG signals obtained at two temperatures: −2 °C (liquid state) and −6 °C (partially frozen), for a 42.86% sucrose–water solution

θ (°C)	T_{2CP2} (ms)		T_{2CP3} (ms)		I_{2CP2} (%)		I_{2CP3} (%)	
	MEM	Marquartdt	MEM	Marquartdt	MEM	Marquartdt	MEM	Marquartdt
−2	50	56	376	382	8	9	92	91
	50	61	363	360	11	12	89	88
	50	63	376	372	10	11	90	89
−6	25	25	137	137	25	26	75	74
	22	23	137	136	22	23	78	77
	25	24	137	137	25	25	75	75

the range of microseconds. Then, the signal intensity I (V) of relaxation component with $12 < T_2 < 20$ μs deduced from the FID decay fitting could be directly related to the amount of ice:

$$I_i = I_{2CP1} \tag{3}$$

The ice fraction was then calculated according to:

$$\omega_i^{tw}\{\theta\} = \frac{I_i\{\theta\}}{I_{tw}\{\theta\}} \tag{4}$$

I_{tw} is the intensity of total water present in the sample. It could be obtained at each temperature by applying Curie's Law which gives a linear relationship between the mass intensity of pure water MI_{tw} (V/g of water) and the inverse of temperature (in K). The linear relationship was validated on pure water at positive temperatures (Fig. 2). Then, intensities for negative temperatures were deduced by extrapolation. Because the NMR intensity is proportional to the total amount of hydrogen detected by the coil, the total water intensity expected in the sample, I_{tw}, was deduced from the mass intensity, MI_{tw}, corrected from the total water fraction in the sample:

$$I_{tw} = m_{tw} M_{tw} \tag{5}$$

where

$$m_{tw} = \omega_{tw}^{S} m_S.$$

The attribution of the relaxing component to the different proton fractions was deduced from the mass intensities of sucrose and of water, respectively. The mass intensity of the sucrose was calculated from the mass intensity of water and corrected by the proton density ratio as follows:

$$MI_s = \frac{PD_s MI_{tw}}{PD_{tw}} \tag{6}$$

Two proton fractions could be separated if they relax at a different rate and if they are not involved in a chemical exchange mechanism. At ambient temperature most of the sucrose protons relax faster than the water protons, but it has already been demonstrated that the chemical exchange proceeds between sucrose and water protons (Guillou-Charpin, Le Botlan, Tellier, & Mechin, 1990). Then the fraction of sucrose protons involved in the liquid water relaxing component relative to the total sucrose protons was calculated according to:

$$X_{s\text{-}w} = \frac{m_s MI_s - I_{2CP2}}{m_s MI_s} \tag{7}$$

where $m_s MI_s$ is the intensity of total sucrose protons in the sample.

3. Results and discussion

3.1. Attribution of the relaxation components

Table 2 presents the effect of solution composition on the spin–spin relaxation times and their relative intensity at −6 °C. The relaxation signal was reconstituted from the FID and CPMG sequences. In the case of water

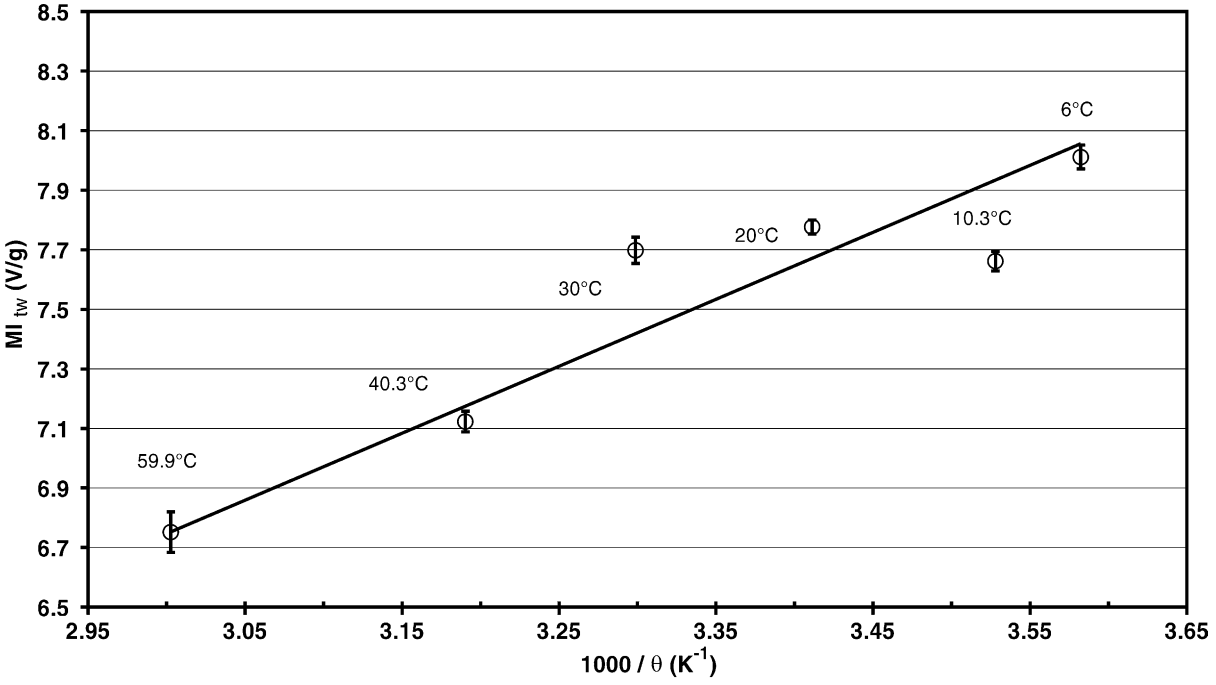

Fig. 2. Mass intensity (V/g) of pure water (liquid state) versus the reciprocal of temperature (K^{-1}).

Table 2
Effect of solution composition on the spin–spin relaxation times and their relative population at −6 °C

	T_{2CP1} (μs)	2σ	T_{2CP2} (ms)	2σ	T_{2CP3} (ms)	2σ	I_{2CP1} (%)	2σ	I_{2CP2} (%)	2σ	I_{2CP3} (%)	2σ
Water	**16.2**	0.2	–	–	–	–	**100**	–	–	–	–	–
SW 42.86%	**15.7**	0.2	**17.57**	2.49	**103.35**	4.87	**43.1**	0.2	**14.6**	1.2	**42.3**	1.3
SW 61.29%	**15.6**	2.5	**15.19**	0.89	**110.40**	5.57	**24.0**	2.5	**19.6**	0.4	**56.4**	2.8
CSW 3.06–42.85%	**15.9**	0.6	**8.33**	3.63	**31.85**	1.12	**41.6**	1.6	**11.1**	2.7	**47.3**	4.2

alone, the relaxation curve could be fitted by a single gaussian function. The value of the identified relaxation time was very short (16.2 μs) and in accordance with the relaxation time of protons involved in the crystal network. In the case of sucrose–water or casein–sucrose–water solutions, the relaxation curve could be resolved into one very fast component (fitted by a gaussian function) and two longer components (fitted by a bi exponential function). The shorter relaxation time was not significantly different from the relaxation time of ice crystals (data on water alone). Moreover the crystallization temperature of sucrose was never reached and the amount of casein protons was too low to significantly participate to the solid component. Consequently this component could be attributed to the protons involved in the ice crystal network. In the view of their order of magnitude, the two other relaxation times from the CPMG decay could be attributed to the liquid phase. The shorter one could be related to the non exchangeable sucrose protons (Guillou-Charpin et al., 1990). The longer relaxation time could then be attributed to the water protons belonging to the cryo-concentrated phase in exchange with sucrose protons. The attribution of the three relaxation times to ice crystals, sucrose and liquid water will be further validated and discussed later.

Fig. 3 presents the changes in ice fraction with temperature at thermodynamic equilibrium. The method used for the calculation of ice fraction from NMR data was based on the intensity of the shorter spin–spin relaxation time (I_{2CP1}) relative to the mass of water contained in the sample. It has been further detailed in the Section 2. The standard error observed on NMR data was equal to 2.5% for sucrose solutions and 3% for casein–sucrose–water solutions. The mean values calculated from NMR data were very close to the calorimetric data calculated from Young and Jones (1949) (deviation less than 2–5%). These results confirmed the preview attribution of the shorter spin–spin relaxation time to ice crystals.

Table 3 compares for the 42.86% sucrose–water solution the sum of the relative intensities I_{2CP1} and

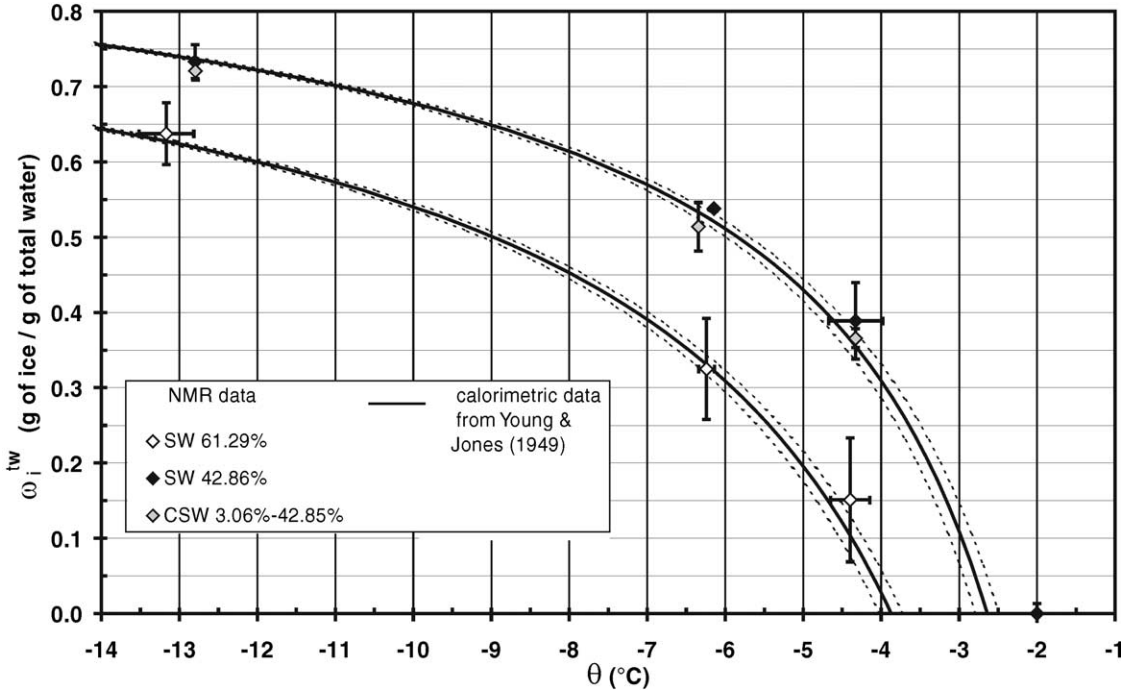

Fig. 3. Ice fraction (g/g total water) calculated from spin–spin relaxation signals and calorimetric measurements (Young & Jones, 1949) versus temperature (°C). Effect of sucrose concentration and protein addition. Data from Young and Jones (1949) are presented with the standard error the authors reported for the calculated parameter θ_m (±0.1 °C).

Table 3
Comparison for the 42.8% sucrose–water solution between the sum of the relative intensities I_{2CP1} and I_{2CP3} (V/g total water) with the expected total water intensity (deduced from the mass intensity of pure water, see Section 2)

θ (°C)	I_{2CP1} (V)	I_{2CP2} (V)	I_{2CP3} (V)	$I_{w\ expected}$ (V)	$I_{w\ expected} - (I_{2CP1} + I_{2CP3})$ (V)
	2.56	0.82	0.98	3.49	**−0.05**
−12.8	2.55	0.85	1.00	3.53	**−0.02**
	2.55	0.77	0.93	3.41	**−0.07**
	1.86	0.63	1.81	3.44	**−0.23**
−6.1	1.81	0.64	1.76	3.38	**−0.20**
	1.87	0.61	1.87	3.48	**−0.26**
	1.29	0.54	2.41	3.40	**−0.30**
−4.2	1.25	0.55	2.44	3.40	**−0.29**
	1.41	0.54	2.26	3.38	**−0.29**
	–	0.29	3.84	3.30	**−0.54**
3.6	–	0.29	3.60	3.18	**−0.42**
	–	0.30	3.60	3.19	**−0.41**
	–	0.33	3.70	3.23	**−0.47**
20.3	–	0.39	3.48	3.11	**−0.37**
	–	0.29	3.51	3.05	**−0.46**

I_{2CP2} was reported for full data availability but was not used for the calculus.

I_{2CP3} (V/g total water) with the expected total water intensity (deduced from the mass intensity of water, see Section 2). At −12.8 °C the sum of the relative intensities I_{2CP1} and I_{2CP3} was not significantly different from the expected total water intensity. This tends to confirm the attribution of the component CP_3 to liquid water as previously assumed. However, when temperature increased, the relative intensity of the total water was significantly overestimated when adding the intensities of components CP_1 and CP_3. This can be explained by the contribution of the sucrose protons to the component CP_3. In carbohydrate solutions, two mechanisms should be considered to interpret the relaxation time of water (Hills, 1991). Firstly, the chemical exchange of protons between water and carbohydrate hydroxyl groups is the major mechanism affecting the water proton transverse relaxation. Secondly, the sucrose–water interaction can additionally induce a slight anisotropy in the reorientational motion of the water.

Fig. 4 presents, as a function of temperature, the sucrose protons ratio which contributes to the longer relaxation component CP_3. Assuming that these protons were the sucrose hydroxyl protons (so called exchangeable protons) and that the exchange rates of all the hydroxyl protons were comparable, the maximum exchangeable proton ratio could be deduced from the sucrose formula and was equal to 8/22 = 36.36%. This value was obtained at −4 °C and decreased as the temperature decreased. This effect was in accordance with

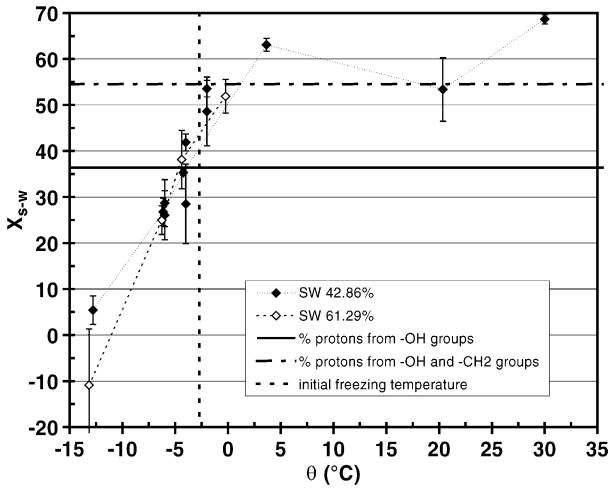

Fig. 4. Sucrose protons ratio contributing to the longer relaxation component CP_3 expressed as a function of temperature (°C). Calculated from Eq. (7).

the exchange mechanism which is temperature dependant (Harvey & Symons, 1978); it was shown that the exchange rate becomes sufficiently slow at or below 0 °C so that the resonance for sucrose hydroxyl protons can be resolved. Moreover, it could be noticed that the same ratio was obtained whatever the initial sucrose concentration. This last observation simply illustrates that in the frozen state and in thermodynamic equilibrium conditions, the sucrose concentration in the remaining liquid phase is independent of the initial sucrose concentration. Our data at −12.8 °C suggests that the fast chemical exchange condition might no longer be valid below such a temperature, the rate of exchange was slower than the water relaxation rate and the two populations could be distinguished. However, those values suffer from large standard deviations resulting from the limits of signal acquisition (small signal to noise ratio) and of the extrapolation method to calculate the expected signal for sucrose protons (see Section 2).

For temperatures above 0 °C the exchangeable protons ratio reaches a plateau, and the value obtained was different from the maximum exchangeable proton ratio. This could be explained by the high sucrose concentration studied. Indeed, in that case the contribution of the non-exchangeable sucrose protons might not be neglected (Guillou-Charpin et al., 1990). A value of 55% is reached if the exchangeable hydroxyl sucrose protons and a part of the non exchangeable sucrose protons (CH_2 groups) are now considered. This ratio is close to the data measured above 0 °C. The slight variation of the ratio between 0 and 20 °C could be explained by a slight variation of the correlation time of the sucrose molecules. These results underline that at positive temperatures the sucrose protons contribute up to 12% to the liquid water signal.

The attribution of the spin–lattice relaxation times has been carried out relying on the preceding attribution of the spin–spin relaxation times. In the case of pure water, and whatever the phase (liquid or crystallized), the spin–lattice relaxation curve could be fitted by a single exponential function. Below 0 °C, the component was attributed to the ice crystal protons. In the case of solutions, the spin–lattice relaxation curve could be resolved into three exponential components that were attributed to ice crystal protons, less mobile non-exchangeable sucrose protons, water protons with more mobile non-exchangeable and exchangeable sucrose protons.

3.2. Temperature dependence of the relaxation time

3.2.1. Ice phase

Our results presented in Fig. 5 were in complete agreement with previous works (Barnaal & Lowe, 1968; Hindman, 1974). First, we observed a linear dependency of the logarithm of the spin–lattice relaxation time versus the reciprocal of temperature (K^{-1}). Secondly, the activation energy deduced (64±0.5 kJ/mol) was consistent with the earlier results. In fact, the motional process causing the spin–lattice relaxation can be accurately described by a single activation energy of 60.6±0.4 kJ/mol over a wide range of temperatures (from 0 °C to −60 °C; Barnaal and Lowe, 1968). Nevertheless, an offset between the two series of data could be observed which is explained by two different NMR frequencies used for the experiments: 20 MHz for the present paper and 10 MHz for Barnaal and Lowe (1968). Moreover, we showed that the activation energy was insensitive to the sucrose amount or to the addition of protein (3%) (Fig. 6). These results demonstrated that the water proton mobility in ice is unaffected by the presence of large molecules such as sucrose or protein. Since the activation energy is very sensitive to the ice structure defaults and decreases notably when small amounts of dopant molecules (HCl, HBr, HI, NaCl) are added (Gran et al., 1997), our results illustrate that the ice water phase in frozen water–sucrose solutions was made of pure water.

Despite the fact that the relaxation mechanism involved in the spin–spin relaxation time is different from that of the spin–lattice relaxation mechanism, a linear relationship between the logarithm of the spin–spin relaxation time and the reciprocal of temperature (K^{-1}) was also observed (Fig. 7). The value found for the activation energy was 16.1±0.5 kJ/mol.

3.2.2. Liquid phase

The relaxation times T_1 and T_2 decreased with decreasing temperature (Figs. 8 and 9). A single process could not be used to describe the behavior of the relaxation over all the range of studied temperatures. Above the initial freezing temperature of water, the relaxation T_1 was governed (1) by the intra and inter dipole–dipole interactions between water molecules and (2) by the dipole–dipole interaction between the water molecules and the effective bonding sites of the sucrose molecules. These interactions are modulated by the temperature dependence of the

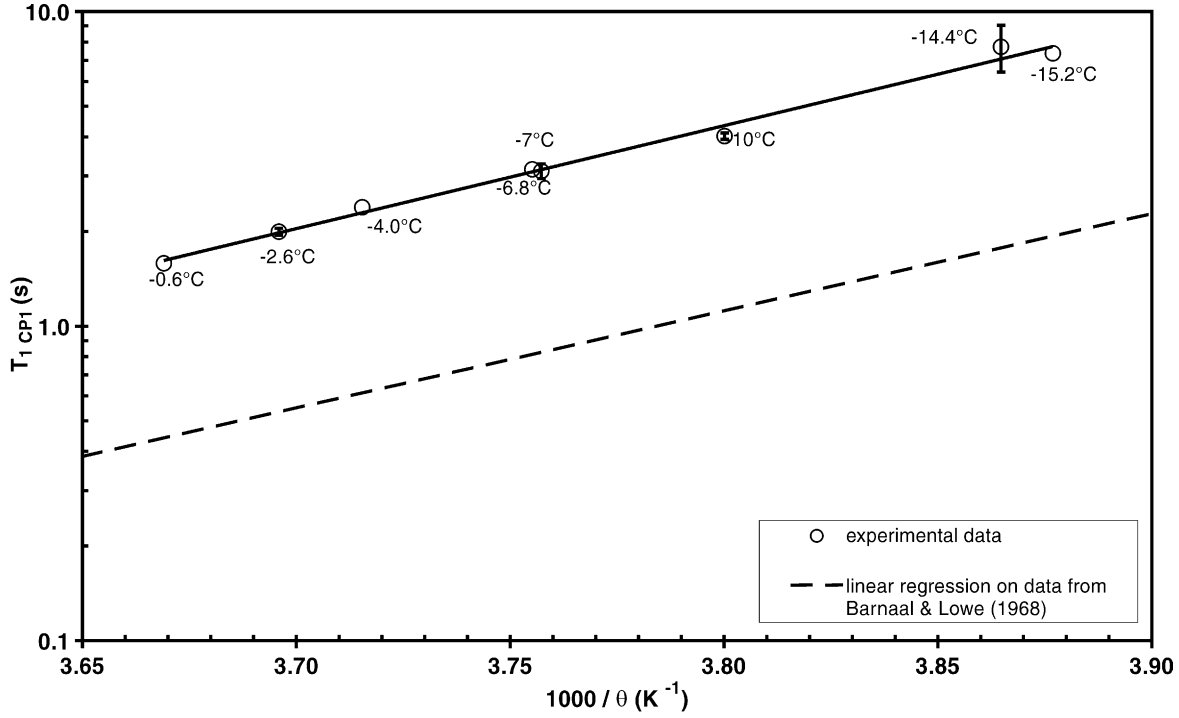

Fig. 5. Ice crystal spin–lattice relaxation time in pure water.

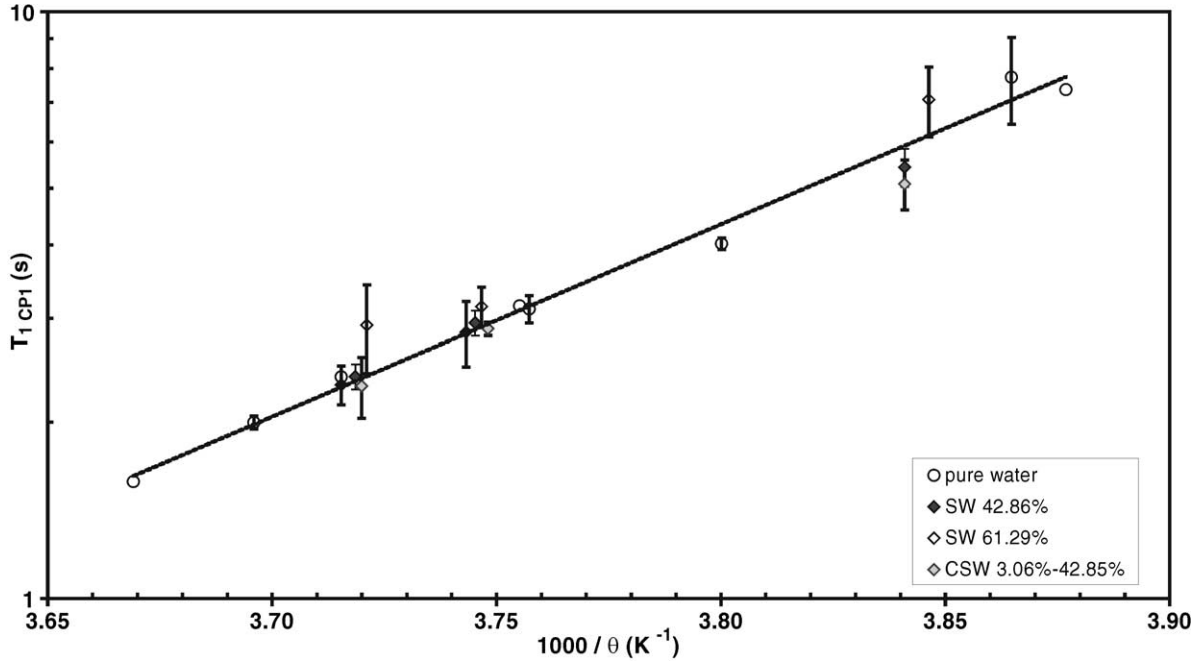

Fig. 6. Ice crystal spin–lattice relaxation time versus the reciprocal of temperature (K^{-1}). Effect of solution composition.

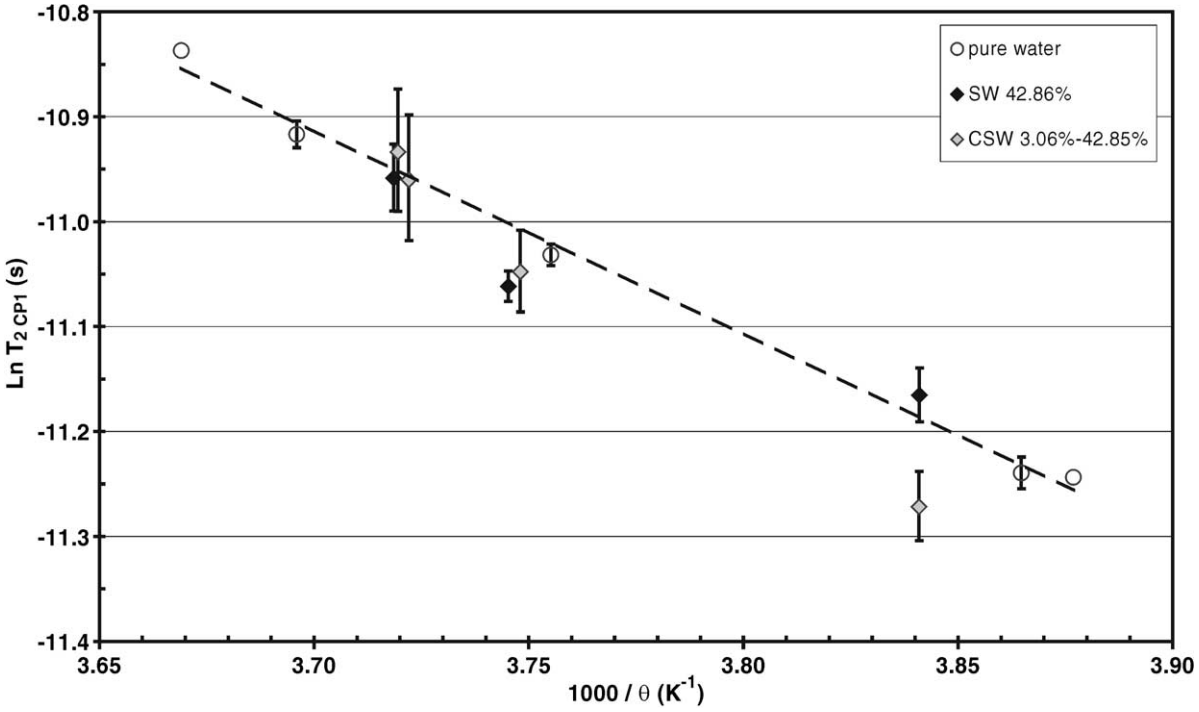

Fig. 7. Logarithm of ice crystal spin–spin relaxation time versus the reciprocal of temperature (K^{-1}). Effect of solution composition.

correlation time. For T_2, chemical exchanges between water protons and exchangeable hydroxyl sucrose protons should also be considered. If we suppose that the water–sucrose interactions involved hydrogen bonding and since the sucrose did not undergo any major conformational change as a function of the temperature (Cornell, Dudley, Joubran, & Parris, 1994), the activation energy deduced at positive temperature should be consistent with the hydrogen bond energy (16 kJ/mol). The activation energy obtained from the linear regression of our T_1 values at positive temperatures is 21.5 ± 0.5 kJ/mol. The difference could be explained by the contribution of the non-exchangeable sucrose protons to the water relaxation as already mentioned.

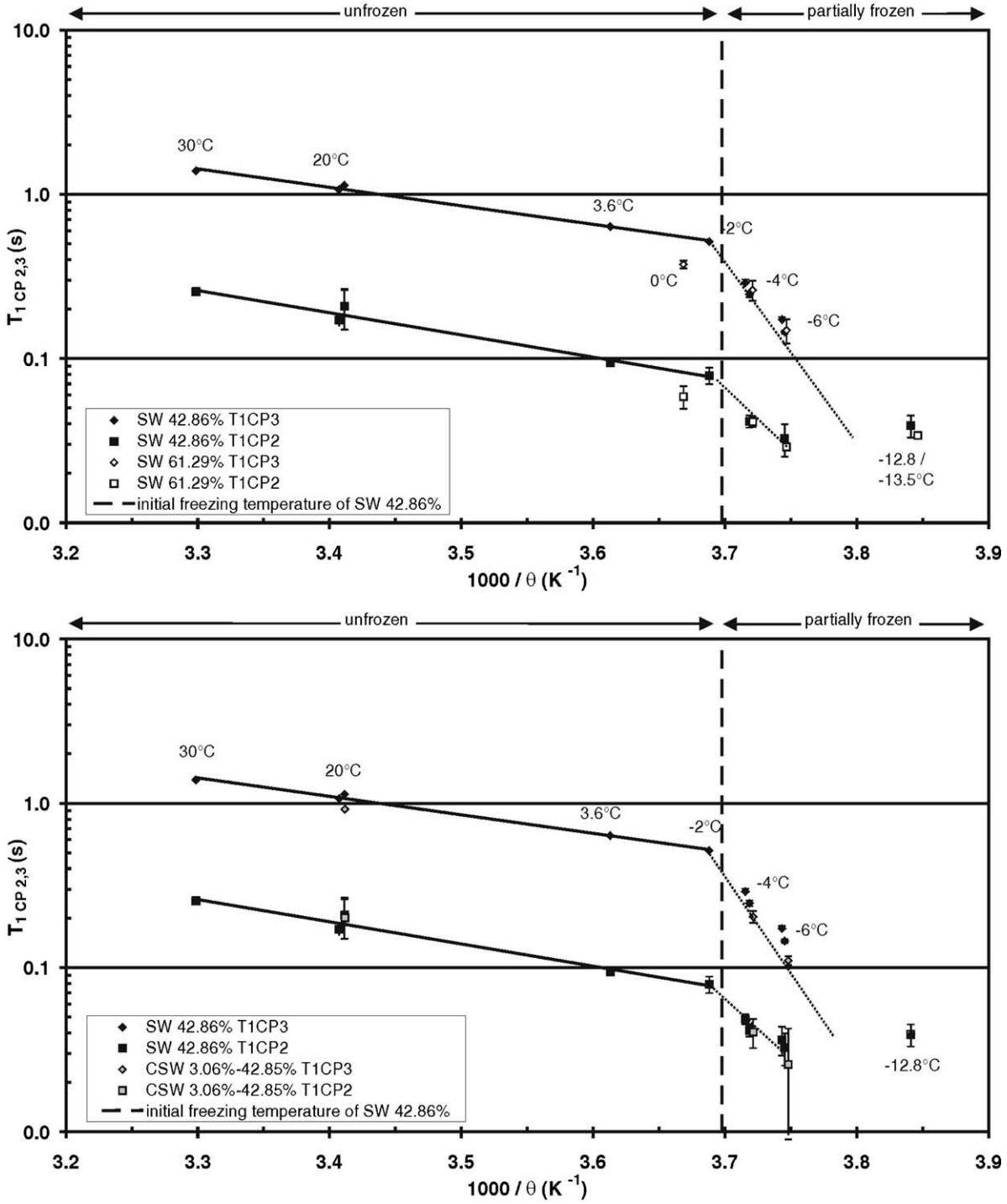

Fig. 8. Spin–lattice relaxation time attributed to the liquid phase versus the reciprocal of temperature (K^{-1}) in a sucrose–water solution. Effect of sucrose concentration and of the addition of protein.

When the water starts to crystallize, then the relaxation times T_2 and T_1 of water present a sharp decrease as a function of temperature. This behavior could be attributed to the effect of the cryo-concentration. Indeed, as the temperature decreases and the ice fraction increases, the amount of unfrozen water remaining decreases and this results in an increased sucrose/water ratio in the remaining liquid phase. The cumulative effect of the intrinsic effect of temperature and of the decreasing concentration in liquid water on the water relaxation explained the sharp decrease in the relaxation time for temperatures below the initial freezing temperature of water. The calculation of the activation energy is of no sense in this case. Another consequence

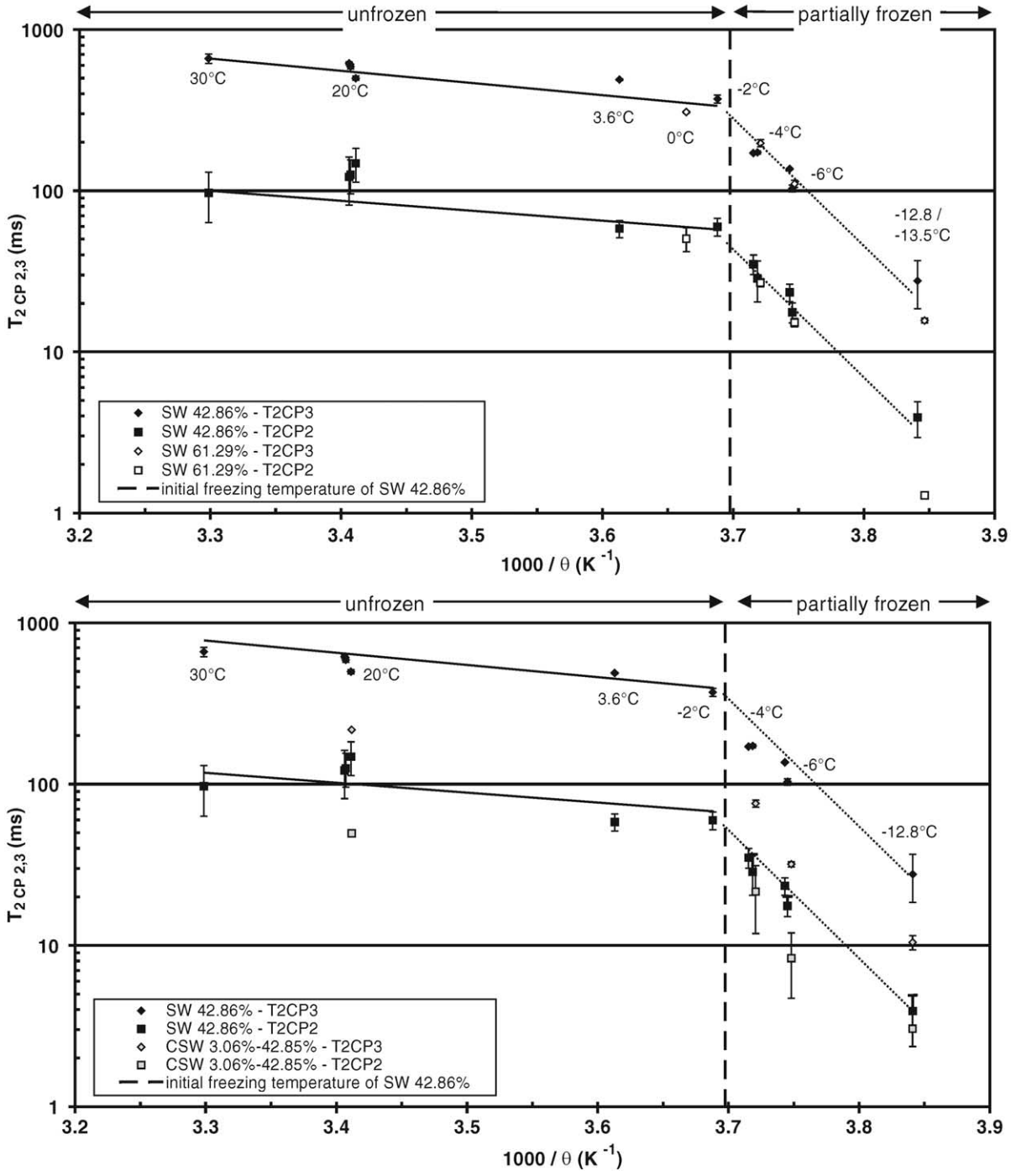

Fig. 9. Spin–spin relaxation time attributed to the liquid phase versus the reciprocal of temperature (K^{-1}) in a sucrose–water solution. Effect of sucrose concentration and of the addition of protein.

is that at low temperatures (e.g. −13.5 °C) the two spin–spin relaxation times attributed to the sucrose protons and to the water protons were hardly distinguished ($T_2 = 10.6$ and 3.3 ms, respectively) and only one spin–lattice relaxation time was observed. Moreover, the relaxation time of water appeared insensitive to the initial sucrose concentration of the solution. At temperatures below the initial freezing temperature, the amount of ice, and then the amount of non-freezing water differ with temperature from one initial sucrose concentration to another, but the non-freezing water/sucrose ratio remains the same once freezing is in process. Consequently the water relaxation times in frozen samples appeared independent of the initial sucrose concentration.

Above the initial freezing temperature of water, the plot of the Logarithm of the relaxation time T_1 and T_2 for non-exchangeable sucrose protons versus the reciprocal

of temperature (Figs. 8 and 9) were linear. This behavior was coherent with the molecular dynamic of the sucrose molecule in solution which showed that the conformation of sucrose in solution is independent of concentration (Cornell et al., 1994; McCain & Markley, 1986, 1987). Below the initial freezing temperature, the effect of the temperature (and of the cryo-concentration) and the relaxation time of sucrose can not be interpreted as explained above.

4. Conclusion

The NMR technique was able to distinguish the liquid from the solid water in aqueous sucrose solutions. This permitted the calculation of the ice fraction in thermodynamic equilibrium conditions. The originality of the NMR method developed in this work compared to previous works relies on the exploitation of the solid part of the signal. Since the solid phase is composed of only water, it was not necessary to take into account the non aqueous protons to determine the ice content. The exploitation of the liquid part of the signal implies taking into account the contribution of non aqueous protons and a correction should be used. Our calculated data showed excellent agreement with calorimetric data from previous works. As with calorimetric techniques, the NMR method developed in the present work does not need any reference (except the intensity expected for water). The main advantage of the NMR measurement compared to the conventional calorimetric measurements is that it does not require any cooling or warming process, i.e. measurement can be performed at constant temperature.

Previously published results from NMR studies on frozen solutions were mainly focused on spin–spin relaxation measurements, and spin–lattice ones have been little considered. However the main advantages of using spin–lattice relaxation measurements could be outlined. First, spin–lattice relaxation provides information about the ice molecular structure (defaults). Results presented in this work confirmed that the ice phase in casein or sucrose solutions is composed of pure water. Future works should investigate the effect of smaller molecules such as ions to determine if such molecules can be imbedded in the ice crystal network. Next, the spin–lattice relaxation could be useful to determine the ice content in the case of samples exhibiting multiple solid phases (e.g. water–lactose solutions). The behavior of liquid water could be assessed even at low temperatures with both T_1 and T_2 relaxation times. The effect of the freezing (temperature decrease plus cryoconcentration of the remaining liquid phase) could be detected, as, below the initial freezing temperature, the behavior of the liquid water deviated from the Arrhenius behavior exhibited at positive temperatures. However the sensitivity of these parameters greatly decreased with decreasing temperature. At temperatures as low as -13.5 °C, the sucrose and water protons T_1 relaxations could not be distinguished anymore. Finally, the evolution of the sucrose protons involved in the water proton relaxation during freezing could be assessed from a comparison of the measured intensities with the intensities expected for sucrose and total water. Our results suggested that at temperatures as low as $-12/-13$ °C no more sucrose protons would exchange with water and that above the initial freezing temperature (-2.7 to 20 °C), the percentage of sucrose protons involved in the water protons relaxation would include exchangeable (–OH protons) and non-exchangeable protons ($-CH_2$ protons).

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