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HYDRATION OF THE *CC400* PROTEIN PREPARATION

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Abstract. The paper presents concentration and temperature interrelationships of relaxation parameters in hydrated *CC400* protein preparation. Investigations were conducted within the range of concentrations from 0.08 g g^{-1} to 0.21 g g^{-1} and the range of temperatures from 20°C to 60°C. On the basis of concentration investigations, the hydration value of the preparation was determined and it was found to amount to 0.20 g per 1 g of non-hydrated preparation and did not depend on temperature. Temperature experiments allowed determining the temperature of the phase transition of the hydrated preparation. This temperature amounted to 39°C. The molecular dynamics was studied analysing the parameter proportional to the average reorientation correlation time of water molecules. It was found that, at the concentration of 0.12 g g^{-1}, the correlation time was the longest, which testifies to the constraint of the dynamics of water molecules in the system. Restriction of the molecule rotation at that concentration indicates that the developed gel was the strongest.

Keywords: hydration, NMR, protein preparation, water

INTRODUCTION

It is becoming increasingly common to manufacture food using functional additives acting as substitutes of a part of their basic constituents. In the case of the production of finely comminuted sausages, part of meat is often substituted by preparations containing animal proteins which, simultaneously, improve the quality of the product.

Investigations carried out on the rheological properties (Rezler *et al.* 2006) and the state of water binding (Baranowska *et al.* 2006) in finely comminuted sausage forcemeats and in sausages with the addition of the *CC400* protein preparation showed that the best results were achieved using initial hydration at the ratio of 1:7.5 (1 part of the preparation to 7.5 parts of water). This level of hydration was neither the highest nor the lowest. That is why investigations were

undertaken with the aim to determine concentration correlations of relaxation parameters in the water systems of the preparation suspensions within the range of concentrations including the hydrations of the preparation employed earlier.

MATERIAL AND METHODS

The experimental material was the *CC400* protein preparation obtained form pieces of pork skins, manufactured by the INTER JJP Company (INTER JJP 2004). This is an extract which contains 91% protein of which 70% is collagen and 9% – fat, Water suspensions were made, with concentrations ranging from 0.08 g g^{-1} to 0.21 g g^{-1}. The range of concentrations corresponded to hydrations from 1:11.25 to 1:3.75. Samples were prepared directly in measuring test tubes of 0.8 cm^3. Additionally, hydration of the preparation was conducted for 18 days from the gaseous phase, at the temperature of +20^{o}C. Samples of dry preparation, of 0.8 g, were placed in measuring test tubes and put into a desiccator over distilled water. In the system hydrated from the gaseous phase, the amount of water per 1 g of unhydrated preparation (hydration H) and the relaxation time spin-lattice T_1 were measured. Measurements of the spin-lattice T_1 and spin-spin T_2 relaxation times in the hydrated systems were conducted. The investigations were carried out with the assistance of the *NMR* impulse spectrometer operating at the frequency of 30 MHz. Times of the spin-lattice relaxation were investigated using the inversion-recovery pulse sequence (Fukushima *et al*. 1981), while the Carr, Purcell, Meiboom and Gill (Carr *et al.* 1954, Meiboom *et al.* 1958) impulse sequence was used to measure the spin-spin relaxation times. Relaxation studies of the hydrated system from the gaseous phase were carried out at controlled temperature of +20^{o}C. Measurements of the hydrated systems were carried out at temperatures ranging from +20^{o}C to +60^{o}C. Analysis of the results of direct measurements revealed a mono-exponential re-growth of the value of the longitudinal constituent of magnetisation and a mono-exponential disappearance of amplitudes of spin traits. This testifies to a rapid exchange of protons in the system.

RESULTS AND DISCUSSION

Concentration correlations of the spin-lattice R_1 and spin-spin R_2 relaxation rates in the systems of the hydrated protein preparation are shown in Figures 1 and 2.

Values of both relaxation rates change depending on the concentration and temperature. In the case of the lowest concentrations, the R_1 values change from about 0.3 s^{-1} for the temperature of +20^{o}C to about 0.8 s^{-1} for the temperature of +60^{o}C, respectively, while the R_2 values change from 1.5 s^{-1} (T = +20^{o}C) to about 2.1 s^{-1} (T = +60^{o}C). For the highest concentrations, the values of relaxation times

change as follows: R_1 – from about 0.7 s^{-1} (T = +20°C) to about 1.3 s^{-1} (T = +60°C), and R_2: respectively from 3.2 s^{-1} (T = +20°C) to about 5.0 s^{-1} (T = +60°C). It was found that in the examined systems, the spin-spin relaxation was three times faster than the spin-lattice relaxation.

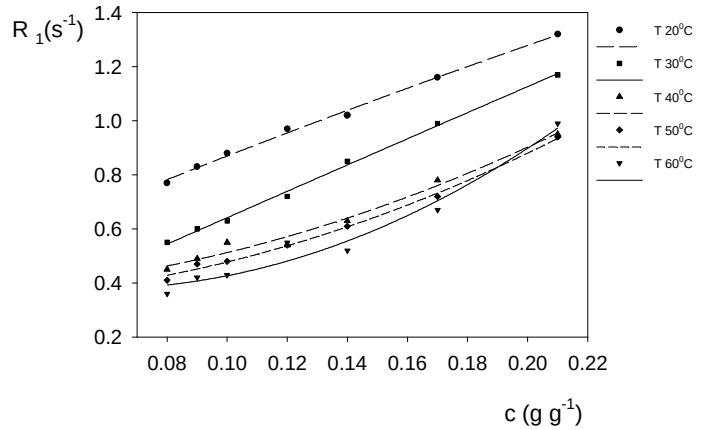

Fig. 1. Concentration correlations of the spin-lattice relaxation rates in the systems of the hydrated protein preparation *CC400*

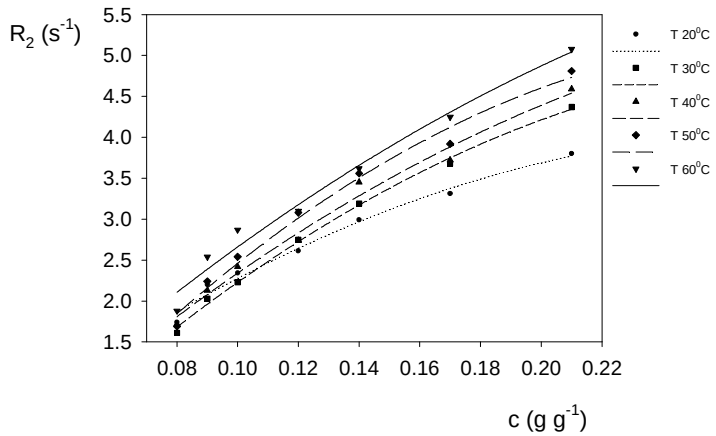

Fig. 2. Concentration correlations of the spin-spin relaxation rates in the systems of the hydrated protein preparation *CC400*

The R_1 relaxation rate is the opposite of the T_1 spin lattice relaxation time. In hydrated biopolymer systems, concentration relationships of the relaxation rate are described by the equation (Zimmerman *et al*. 1957, Daszkiewicz *et al*. 1963)

$$R_1 = \frac{H \cdot c}{1-c}\left[R_{1h} - R_{1w}\right] + R_{1w} \qquad (1)$$

where: c is the biopolymer concentration expressed in g g^{-1}, R_{1h} is the rate of water relaxation bound on the biopolymer surface, and R_{1w} is the relaxation rate of non-bound water.

Data obtained from the relaxation measurements allow ascertaining the hydration of the protein preparation H and determining the value of the relaxation rate of bound – R_{1h} and unbound – R_{1w} water.

Table 1 presents the hydration values obtained for different temperatures.

The hydration of the protein preparation does not depend on temperature and amounts to 0.20 g g^{-1}. The value of this parameter determined with the assistance of the desiccator method amounts to 0.18 g g^{-1}. The observed discrepancies in the hydration values of the examined preparation can, most probably, be attributed to the fact that the preparation was not properly dried prior to the hydration process.

Table 1. Hydration values *H* for the *CC400* protein preparation at different temperatures

T°C	H g g^{-1}
20	0,20
30	0,21
40	0,19
50	0,20
60	0,21

The relaxation rate of the spin-lattice R_1 of bound water, determined for the sample subjected to hydration from the gaseous phase, amounts to 16.80 s^{-1}. Using formula (1), the value of the relaxation rate of the free water fraction and the bound water fraction at various temperatures was determined. At the temperature of +20°C, the relaxation rate of the bound water fraction determined from the relaxation measurements amounts to 17.01 s^{-1}. The obtained results indicate that the *NMR* relaxation technique can be employed to determine the hydration values.

Figure 3 shows the concentration relationships of the relaxation rate for free water.

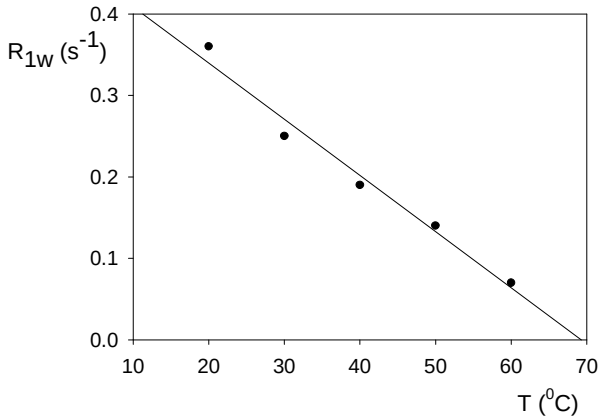

Fig. 3. Temperature correlations of the spin-lattice relaxation rate of free water in the hydrated *CC400* protein preparation

Together with temperature increase, the relaxation rate of this water fraction declines linearly. The value of the relaxation rate at the temperature of +20°C amounts to 0.36 s^{-1}, which is in agreement with literature data for the 30 MHz frequency. Value changes in the spin-lattice relaxation rate for bound water are shown in Figure 4.

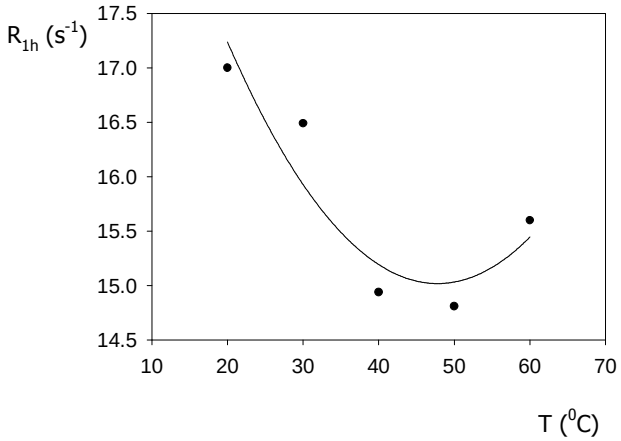

Fig. 4. Temperature correlations of the spin-lattice relaxation rate of bound water in the hydrated *CC400* protein preparation

In contrast to the unbound water, the spin-lattice relaxation rate of bound water is not a linear function of temperature. Minimum R_{1h} value is observed at the temperature at which the conformation change of the biopolymer probably takes place.

The relaxation rate is a function of the spin precession frequency and τ_c (sometimes called correlation time) which approximates the time required to change the molecule orientation by 1 radian. In accordance with the theory of Blombergen, Purcell and Pound (Bloembergen *et al.* 1948, Hennel *et al*. 1993), both relaxation rates are described by the formulas:

$$\frac{1}{T_1} = \frac{3}{10}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma^4\hbar^2}{r_0^6}\left[\frac{\tau_c}{1+(\omega\tau_c)^2} + \frac{4\tau_c}{1+(2\omega\tau_c)^2}\right] \tag{2}$$

$$\frac{1}{T_2} = \frac{3}{20}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma^4\hbar^2}{r_0^6}\left[3\tau_c + \frac{5\tau_c}{1+(\omega\tau_c)^2} + \frac{2\tau_c}{1+(2\omega\tau_c)^2}\right] \tag{3}$$

where: μ_0 – vacuum magnetic permeability, γ– gyromagnetic ratio, $\hbar = (h/2\pi)$ – h – Planck's constant, r_0 – radius of rotating molecule.

In systems characterised by low viscosity, a multiple change of the molecule orientation occurs within one period of spin precession. This is what happens, for example, in the case of clean water. In this case, the $\omega\tau_c$ factor << 1 and both relaxation rates are identical and their values are proportional to the value of the correlation time and do not depend on the precession frequency. In systems in which the correlation time is long, the $\omega\tau_c$ factor >> 1 and, in this situation, differences in values of both relaxation rates were observed. In this case, formulas (2) and (3) take the form:

$$R_1 = \frac{6}{10}\frac{\gamma^4\hbar^2}{r_0^6}\frac{1}{\varpi^2}\frac{1}{\tau_c} \tag{4}$$

$$R_2 = \frac{9}{20}\frac{\gamma^4\hbar^2}{r_0^6}\tau_c \tag{5}$$

The spin-lattice relaxation rate is inversely proportional to the correlation time value and depends on the precession frequency. The spin-spin relaxation rate does not depend on the precession frequency and is proportional to the correlation time value. Therefore, it can be said that:

$$\frac{R_2}{R_1} = \frac{3}{4}\varpi^2\tau_c^2 \tag{6}$$

The ratio of both relaxation rates is proportional to the value of the mean correlation time τ_c.

Employing formula (6), measurements of the relaxation rates allow estimating the value of the mean correlation time of water molecules in the system. The temperature dependence of the correlation time can be described with satisfactory approximation using the Arrhenius equation:

$$\tau_c = \tau_0 \exp\left(\frac{E_a}{RT}\right) \tag{7}$$

where: R – gas constant, T – temperature in absolute scale, τ_0 – constant, E_a – energy barrier for molecule reorientation.

Using the above dependence, the temperature of the phase system transition of the hydrated *CC400* protein preparation was determined (Fig. 5 and 6).

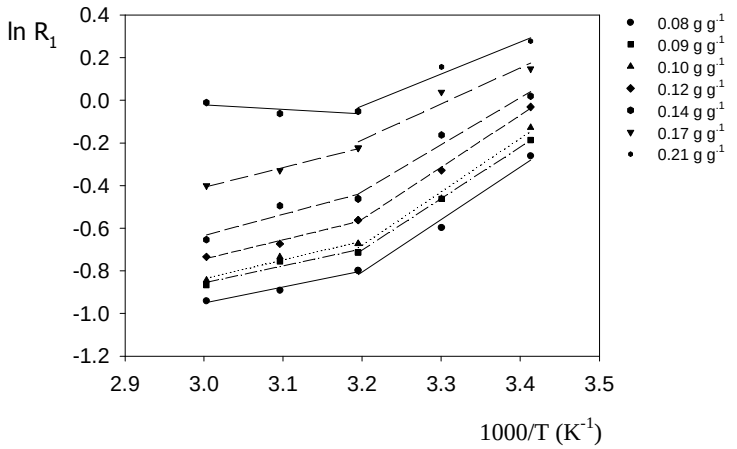

Fig. 5. Temperature correlations of the R_1 spin-lattice relaxation rate of the hydrated system of the *CC400* protein preparation

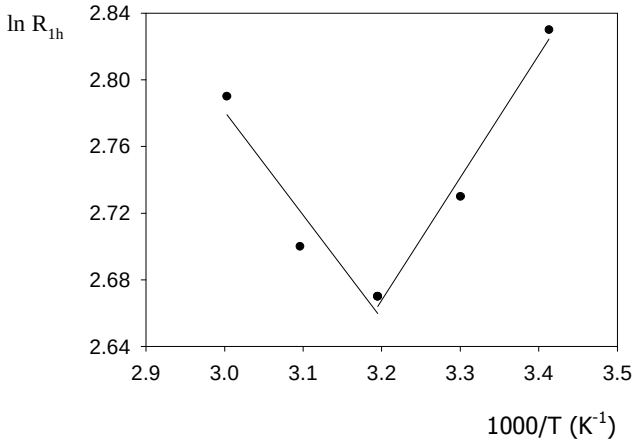

Fig. 6. Temperature correlations of the R_{1h} spin-lattice relaxation rate of bound water

Graphically determined temperature of the phase transition amounts to 39°C and does not depend on the preparation concentration. The same temperature of phase transition was obtained when analysing temperature value changes of relaxation rates of the bound water fraction.

Figure 7 presents concentration correlations of the spin-spin relaxation rate ratio to the spin-lattice relaxation rate which reflects the mean correlation time of water molecules in the system.

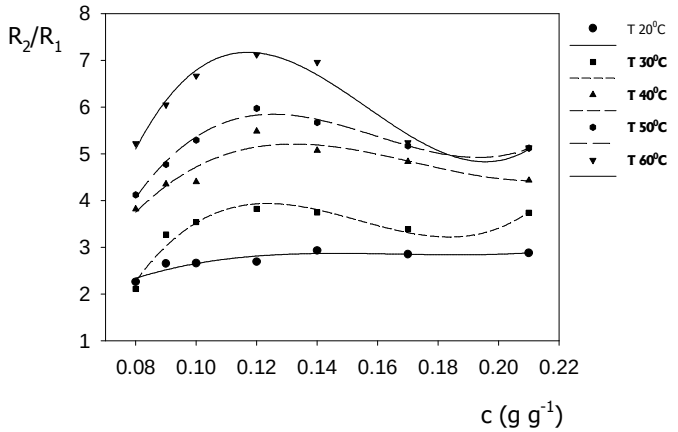

Fig. 7. Concentration correlations of the R_2 spin-spin relaxation rate ratio to the R_1 spin-lattice relaxation rate of the hydrated system of the *CC400* protein preparation

A maximum R_2/R_1 value is observed, and it occurs for the concentration of 0.12 g g^{-1}. Therefore, it can be concluded that, at this preparation concentration, the values of the mean correlation time of water molecule reorientation are the highest. The restriction of the molecular dynamics of water confirms its strong binding in the system and the developed gel is stiff. This explains why the addition of the *CC400* preparation, hydrated at the ratio of 1:7.5, to sausages exerted the most beneficial impact on the rheological properties and the dynamic state of water in those systems.

CONCLUSIONS

1. Hydration of the *CC400* protein preparation does not depend on temperature. The hydration value can be determined on the basis of the concentration correlations of the spin-lattice relaxation rate.

2. On the basis of relaxation investigations, the temperature of the phase transition of the *CC400* preparation was determined. It was found that the temperature of the phase transition does not depend on the concentration of the *CC400* preparation and amounts to 39°C.

3. A maximum of the mean correlation time τ_c was found for the system characterised by the concentration of c = 0.12 g g^{-1}. Water molecules exhibit the smallest dynamics at this concentration.

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HYDRATACJA PREPARATU BIAŁKOWEGO *CC400*

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S t r e s z c z e n i e . W pracy przedstawiono stężeniowe i temperaturowe zależności parametrów relaksacyjnych w hydratowanym preparacie białkowym *CC400*. Badania prowadzono w zakresie stężeń od 0,08 $g \cdot g^{-1}$ do 0,21 $g \cdot g^{-1}$ i w zakresie temperatur od 20^{o}C do 60^{o}C. Na podstawie wyników badań stężeniowych wyznaczono wartość hydratacji preparatu, która wynosi 0,20 g na 1 gram nieuwodnionego preparatu i nie zależy od temperatury. Badania temperaturowe pozwoliły na określenie temperatury przejścia fazowego hydratowanego preparatu. Temperatura ta wynosi 39^{o}C. Dynamikę molekularną prześledzono analizując parametr proporcjonalny do średniego czasu korelacji reorientacji molekuł wody. Stwierdzono, że przy stężeniu 0,12 $g \cdot g^{-1}$ czas korelacji jest najdłuższy co świadczy największym ograniczeniu dynamiki molekularnej wody w układzie. Ograniczenie rotacji molekuł przy tym stężeniu sugeruje, że utworzony żel jest najsilniejszy.

S ł o w a k l u c z o w e : hydratacja, MRJ, preparat białkowy, woda