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TEMPERATURE CHARACTERISATION OF STARCH AND STARCH-PROTEIN DISPERSIONS

Summary

The aim of the study was to analyse changes in the parameters describing molecular dynamics of water in starch and starch-protein dispersions taking place in the process of gelation. The study was performed by the nuclear magnetic resonance (NMR) method for starch dispersion samples of the concentration of 0.10g/cm^3 (*Triticum durum* wheat starch) and starch-protein dispersion samples (gluten obtained from wheat) of the concentration c = 0.1g/cm^3. NMR measurements were performed in the range of 20°–70°C. The parameters describing molecular dynamics of water in retrograded gels obtained at 70°C and 100°C were also determined.

The process of starch gelation was found to occur already at temperatures lower than 70°C. The spin-lattice relaxation times were observed to decrease despite of increase in temperature. It suggested a decreased mobility of water molecules in the system studied. It would result from the formation of spatial lattice formation already in the process of gelation. In the starch-protein samples, the relaxation time, T_2, slightly increased with increase in temperature over the whole range studied. In this system water molecules had unlimited mobility, which suggested a lack of gelation. These results were confirmed by analysis of the relaxation times for both systems of retrograded gels obtained at different temperatures. For the starch gel, the relaxation times were identical at both temperatures of gelation. For starch-protein gel the relaxation times are longer for the gel obtained at 70°C than at 100°C. This observation confirmed temperature of 70°C was insufficient for lattice formation.

Introduction

Gelatinization is a transformation occurring on heating of starch-water dispersions. Above certain temperature, starch granules swell and alter their structure [1, 2, 6]. A gradual loss of birefringence occurs, and the low molecular weight components leach into water. During this process, the secondary bonds that maintain the granule structure are broken and the micelar network is pulled apart.

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Several methods can be used to follow the gelatinization process: loss of birefringence, increase in viscosity, and susceptibility to enzymatic degradation, decrease in enthalpy, and loss of X-ray diffraction pattern. Among them only susceptibility to enzymes and variation of enthalpy are used in quantification of the extent of gelatinization. These methods, however, are either time-consuming (enzyme susceptibility) or show poor reproducibility (calorimetry).

Lelievre and Mitchell [7] showed that heating of starch-water dispersions above 60°C, resulted in an increase in the relaxation time measured by pulsed-proton nuclear magnetic resonance (NMR). This behaviour was attributed to an increase in the mobility and hydration of starch polymers, suggesting an increase in the number of protons linked to the liquid phase. Although the relaxation time due to the protons in the solid phase was not measured, it would be lower than those in the liquid phase. Thus, in a starch-protein sample with different degrees of gelatinization and different content of proteins, there would be different relations between the protons in the liquid phase and those in the solid phase. Thus, a study of gelatinization of starch water-protein systems with involvement of the ratio of the protons in the liquid and those in the solid phase as determined by pulsed-proton NMR.

In this study possibility of using pulsed-proton NMR to measure the degree of gelatinization of starch-protein systems was recognised. Simultaneously, gelatinization kinetics of wheat starch-gluten-water systems was investigated.

Material and methods

Materials: Wheat starch (*Triticum durum), (*Sigma, MC = 9%) and starch-protein (gluten from wheat containing 80% protein and 7% fat), (Sigma, MC = 8%) dispersions.

Sample preparation: The total concentration of the polymers was 0.10g/cm3. The starch-proteins mixtures were prepared following the starch to protein ratio of 9:1, 8:2, 7:3, 6:4, 5:5. Directly after preparation the samples were placed in NMR tubes and closed.

The NMR experiment was performed on a pulse NMR spectrometer ELLAB Poznań operating at 15 MHz. The spin-lattice relaxation times T_1 were measured applying the inversion-recovery pulse sequence (π–τ–$\pi/2$).The pulse distance τ was changing from 10 to 5300 ms. The repetition time TR was 10 s. The spin-spin relaxation times T_2 were measured by the CPMG pulse train. The distance between τ pulses was established for 3 ms, the number of spin-echos was 50. The CracSpin calculated program [11] was used to obtain the vales T_1, while T_2 values were calculated by the fit to the formula:

$$M_{x,y} = M_0 \left\{ \exp \frac{-\tau}{T_2} \right\}$$

where: M_0 is the equilibrium value of magnetisation, T_2 the spin-spin relaxation time. Temperature was controlled with precision of ±0.5°C.

Results and discussion

Recognition of molecular mechanisms of changes in the starch structure which took place at different levels of its organisation under hydrothermal treatment is of great importance. Gelation is the most important processes occurring in starch systems at moderate temperatures and in the presence of water, temperature of gelation is one of the most important parameters characterising the starch system.

The temperature dependencies of the spin-lattice relaxation times are shown in Fig. 1.

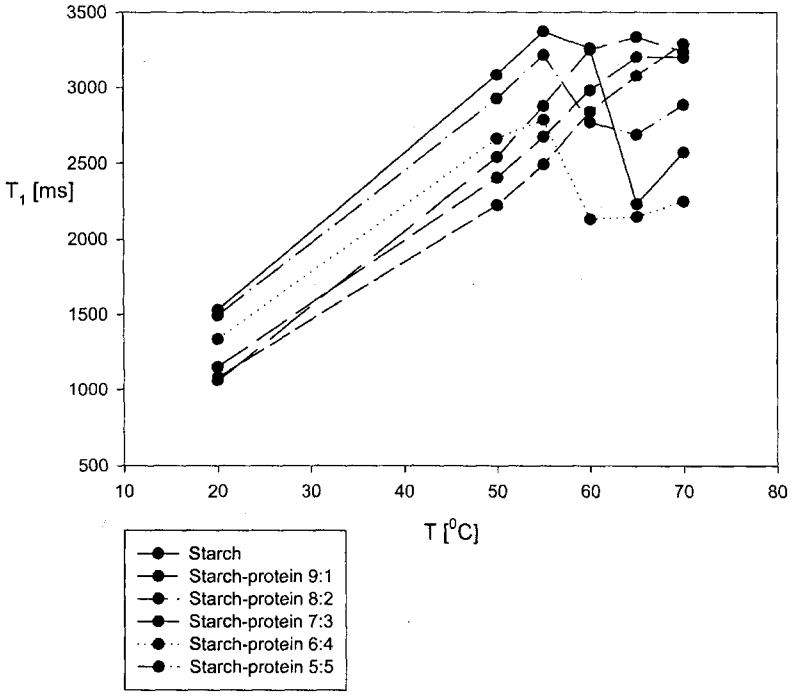

Fig. 1. The temperature dependencies of the spin-lattice relaxation times.

For starch dispersions, above +60°C the T_1 relaxation time decreases in spite of increase in temperature.

In aqueous dispersions of starch heated above the gelation temperature the irreversible process of swelling takes place, accompanied by disappearance of the crystalline ordering and release of amylose to the solution [3, 4, 8, 10]. The degree of the crystal structure of the system is determined by the contribution of bihelical forms among the high-molecular starch components. The temperature dependence of this

contribution reveals a transition from the ordered helical fragments of the polymer chains to the state of spatially disordered coil. Some of the water molecules penetrating into the swelling starch grains become immobile and bound to the starch hydroxyl groups unshielded in the process of gelation. Simultaneously, the crystal phase melts and the concentration of the active segments of the amorphous lattice decreases with increase in temperature, causing a deshielding of more hydrophilic group. They bound more water molecules limiting their dynamics. This influences the temperature dependence of T_1 relaxation time (Fig. 1).

The spatial lattice of macromolecular gel starts forming under conditions when stable bonds can form among fragments of different macromolecules. This process occurs on cooling of hot starch gels of sufficient both concentration and degree of homogeneity. The intermolecular bonds form as a result of coiling of neighbouring fragments of the macromolecule chains taking place on decreasing temperature, and their association to bihelical forms, characteristic of native and retrograded starch. In order to determine the effect of the temperature of gelation on the final structure of the macromolecular gel, the relaxation time was measured for starch systems gelled at +70°C and +100°C. The measurements were performed at +20°C at 24 hours after the completion of gelation. The T_1 relaxation time values obtained were within the error limit (±5%) for both systems (Table 1). This result leads to a conclusion that in spite of different temperatures of gelation, the spatial lattices formed as a result of the retrogradation processes are similar and temperature of +70°C is sufficient for starch gelation within the concentration range studied.

For the starch-protein systems two different courses of temperature dependencies of T_1 relaxation time were observed. For the systems of starch-to-protein concentrations ratio of 9:1, 8:2 and 7:3, the value of T_1 relaxation time increased with increase in temperature. This dependence was interpreted as follows. The water molecules initially bound with the protein are released as a result of the protein denaturation process above +50°C [5, 9]. At the same time, because of the uncoiling of the starch molecules, the water molecules take part in the formation of the lattice. In general, these two processes evoked no macroscopic change in the water molecules dynamics. The situation is different for the systems containing starch and protein at the concentration ratio of 6:4 and 5:5, for which the temperature dependencies of T_1 relaxation time revealed maxima and minima in the temperature range studied. At the beginning, the T_1 value increases with temperature increasing up to +55°C, which suggested the release of water molecules as a result of protein denaturation and an increase in their mobility. A significant decrease in T_1 observed at +60°C for the system of the ratio of 6:4 and at +65°C for the system of the ratio of 5:5, was probably related to the uncoiling of starch chains as well as participation of water molecules in the lattice formation and bonding of some of the water molecules at the hydrophilous sites of the peptide chains de-

shielded as a result of protein denaturation. Further slow increase in T_1 is explained as a consequence of an increase in the mobility of those water molecules which are not involved in the lattice formation.

Table 1

The spin-lattice and spin-spin relaxation times for starch and starch-protein dispersions before and after gelation.

	T_1 [ms]			T_2 [ms]		
	Before gelation	After gelation at 70°C	After gelation at 100°C	Before gelation	After gelation at 70°C	After gelation at 100°C
Starch	1527	669	680	718	191	176
Starch-protein 9:1	1060	1040	796	894	792	484
Starch-protein 8:2	1154	1142	886	689	621	447
Starch-protein 7:3	1083	1059	960	788	978	334
Starch-protein 6:4	1337	1020	928	927	531	462
Starch-protein 5:5	1492	1161	1108	894	1213	519

The temperature dependencies of spin-lattice relaxation times are shown in Fig. 2.

The T_2 relaxation time slightly increases with increasing temperature for all the systems studied. The most significant change in T_2 was noted in the range 20–50°C. It is interpreted as being related to increase in mobility of the water molecules not involved in the network formation.

Conclusions

1. The transition from the ordered spiral forms of the fragments of the polymer chains to the state of spatially disordered coil, taking place in the starch systems above 60°C facilitates the process of bonding of the water molecules to the hydroxyl groups of starch (unavailable at lower temperatures). It leads to a decrease in the water molecule dynamics.
2. In spite of different temperatures of gelation, the spatial lattices formed as a result of the processes of retrogradation are similar and the temperature of +70°C is sufficient for gelation of starch in the systems without proteins in the concentration range studied.
3. For starch-protein mixtures of the same total concentration, temperature of +70°C is insufficient for gelation of the starch in the system.

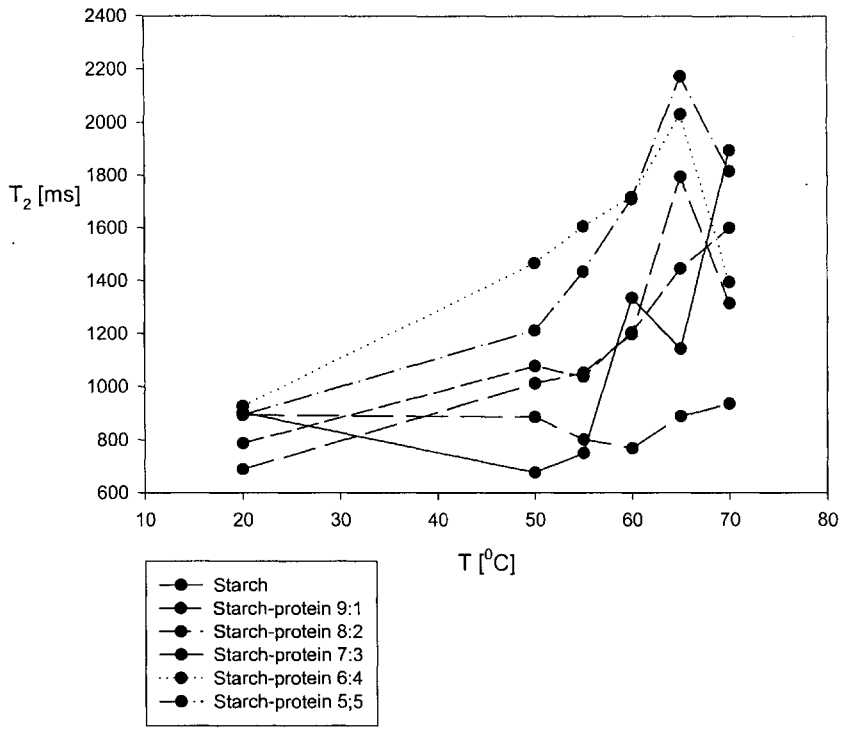

Fig. 2. The temperature dependencies of the spin-spin relaxation times.

4. There are two different ranges of starch-protein concentrations under which the dynamics of the water molecules depends on the concentration of protein in the system.
5. The relaxation times determined for starch-protein gels at 70°C are longer than these at 100°C. It confirms that for such systems the temperature of 70°C is insufficient for the lattice formation.

References

[1] Cherian G., Chinachoti P.: ^{2}H and ^{17}O nuclear magnetic resonance study water in gluten in the glassy and rubbery state. Cereal Chem., **73**, 1996, 618-624.

[2] Collison R., Chilton, W.G.: Starch gelation as a function of water content. J. Food Technol., **9**, 1974, 309-315.

[3] Erdogdu G., Czuchajowska Z., Pomeranz Y.: Wheat flour and defeated milk fractions characterised by differential scanning calorimetry. I. DSC of flour and milk fractions. Cereal Chem., **72**, 1995, 70.

[4] Erdogdu G., Czuchajowska Z., Pomeranz Y.: Wheat flour and defeated milk fractions characterised by differential scanning calorimetry. II. DSC of interaction products. Cereal Chem., **72**, 1995, 76.

[5] Jacoba M., Renkema S., van Vliet T.: Heat-induced gel formation by soy proteins at neutral pH. J. Agric. Food Chem., **50**, 2002, 1569-1573.

[6] Leach H.W.: Starch Chemistry and Technology, Vol. 1, R.L. Whistler and E.F. Paschall. Eds. Academic Press. New York, 1965, 297.

[7] Lelievre J., Mitchell J.: A pulsed NMR of some aspects of starch gelatinization. Starch, **4**, 1975, 113-115.

[8] Renkema S., Knabben H.M., van Vliet T.: Gel formation by β-conglycinin and glycinin and their mixtures. Food Hydrocoll., **15**, 2001, 407-404.

[9] Rezler R., Baranowska H.M.: Molecular dynamics of water and polymer chains in starch protein gels during structure formation. Properties of Water in Foods, Agricultural Univ. Press. Warsaw, 2000, 7-16.

[10] Vodovotz Y., Hallberg L., Chinachoti P.: Effect of aging and drying on thermomechanical properties of white bread as characterised by dynamic mechanical analysis (DMA) and differential scanning calorimetry (DSC). Cereal Chem., **73**, 1996, 264-270.

[11] Węglarz W., Harańczyk H.: Two-dimensional analysis of the nuclear relaxation function in the time domain: the program Crac Spin. J. Phys. D. Appl. Phys., **33**, 2000, 1909-1920.

TEMPERATUROWA CHARAKTERYSTYKA DYSPERSJI SKROBIOWYCH I SKROBIOWO-BIAŁKOWYCH

Streszczenie

Celem podjętych badań była analiza zmian parametrów określających dynamikę molekularną wody w dyspersji skrobiowej i dyspersjach skrobiowo-białkowych w trakcie procesu kleikowania. Badania przeprowadzono na próbkach dyspersji skrobiowej o stężeniu 0,10 g/cm^3 (skrobia pszenicy *Triticum durum*) i skrobiowo-białkowych (gluten uzyskany z pszenicy) o stałym stężeniu mieszaniny c = 0,1 g/cm^3. Pomiary prowadzono w zakresie temperatur 20°–70°C. Dodatkowo porównano parametry określające dynamikę molekularną wody w zretrogradowanych żelach kleikowanych w temperaturze 70°C i 100°C. Do badań wykorzystano technikę magnetycznego rezonansu jądrowego. Stwierdzono, że skrobia kleikuje w temperaturze niższej niż 70°C. Zaobserwowano spadek wartości czasu relaksacji spin-sieć mimo wzrostu temperatury. Sugeruje to, że w układzie obniżona została mobilność molekuł wody. Jest to efektem formowania się struktur przestrzennych sieci już w trakcie kleikowania. W układzie żeli skrobiowo-białkowych w całym analizowanym zakresie temperatury wartości czasów relaksacji T_2 nieznacznie rosną ze wzrostem temperatury. Molekuły wody w tym układzie nie mają ograniczonej mobilności co sugeruje brak efektu kleikowania. Powyższe wyniki potwierdza analiza wartości czasów relaksacji obu układów zretrogradowanych żeli kleikowanych w różnej temperaturze. Wartości czasów relaksacji żelu skrobiowego są takie same w obu zakresach temperatury kleikowania. Czasy relaksacji żelu skrobiowo-białkowego są dłuższe w przypadku kleikowania w temperaturze 70°C niż w przypadku kleikowania w temperaturze 100°C. Potwierdza to, że temperatura 70°C jest niewystarczająca do uformowania sieci takiego układu.