

G.B. PLENZLER, D.M. NAPIERAŁA, H.M. BARANOWSKA

HYDRATION OF STARCH AND PROTEIN SEEDS IN EARLY PHASE OF GERMINATION

Summary

Seed swelling in the first phase of imbibition involves mainly development of seed colloids. Chemical affinity of such colloids to water differs depending on the surface properties of the macromolecules. The biopolymer surface could perturb the dynamic and static state of water. For this reason the structure and composition of seeds, especially proteins, starch, and lipid content, can control the course of the swelling process.

The study presents the microscopic and macroscopic parameters describing the swelling pea seeds and triticale grains. Differences in corresponding parameters were observed. Measurements of water uptake rate in both species showed higher water uptake in triticale grains compared to that in pea seeds in the first step of the process but lower in the subsequent phase. The results of pulse $^1\text{H-NMR}$ measurements have revealed two groups of water protons, each in a different magnetic environment responsible for a different relaxation rate. These two groups correspond to water molecules differing in mobility, such as free and bound water, respectively.

The difference in results obtained for triticale and pea are related to size, different permeability of seed envelopes, different mobility of seed water and chemical content mainly determined by starch. Its structure and physicochemical properties are also very important.

Introduction

Starch is the major carbohydrate used extensively in food industry as a water binder, thickener, texturizer, emulsifier and gelling agent. It resides in plant tuber and seed endosperm where it occurs as granules [11], each typically containing several million amylopectin molecules accompanied by a higher number of smaller linear amylose molecules. The relative proportions of amylose to amylopectin depend on the natural source of starch [7]. Of the two components of starch, amylose is more useful as a hydrocolloid. Its expanded chain conformation [5] is responsible for the high vis-

cosity of water soluble starch and hydrophobic inner surface, which is not able to hold water strongly and the water molecules can be easily replaced by more hydrophobic ones such as lipids and aromatic compounds.

Seed swelling process in the first phase of imbibition mainly involves hydration of seed colloids. Chemical affinity of colloid forming macromolecular compounds to water depends on their surface properties [5, 6, 8]. The biopolymer surface could perturb the dynamic and static state of water near the surface. For this reason the structure and composition of seeds, especially the proteins, starch, and lipid content, can control the course of the process. The starch-water interactions are very important for the behaviour of the system. The interactions involve the molecular mobility of water which can be determined by $^1\text{H-NMR}$ spectroscopy [1, 3].

In the present study we have compared the macroscopic (the rate of water uptake) and microscopic parameters (transverse relaxation time of two fraction of water) describing the process of germination in triticale and pea seeds, belonging to starch and protein rich starch seeds, respectively.

Material and methods

Pea seeds, cv. Sześciotygodniowy (six-weeks old) and triticale grains, cv. Presto from and SH Choryń and CNOS Poznań, respectively, were used for all the experiments. Five groups of 20 seeds each were placed on Petri dishes and moistened with distilled water and then incubated in a germination chamber at stable temperature of 294 K. Relative mass change and relative water content ($\text{g H}_2\text{O/g dry mass}$) were measured with E50 S Gibertini balance (Italy), in standard conditions.

The state of water in seeds was studied in relation to moisture content by the laboratory made pulse NMR spectrometer, operating at 30 MHz using the CPMG pulse sequence ($90^\circ-\tau-180^\circ$). The results of the experiments were analyzed using a non-linear least-squares curve-fitting procedure. Each set of data was fitted to the one-, two- or three term exponential and the best fit was taken based on the chi-square and correlation coefficient value.

Results and discussion

Water sorption kinetics in pea seeds and triticale grains are presented in Figure 1. As can be seen, the water uptake rate in triticale is higher than in pea in the first phase of this process and then decreased. It may stem from a higher relative surface of individual seeds and different seed cover capacity to transport water in both types of seeds studied. Moreover, triticale belongs to starchy seeds of high starch content (68% carbohydrates and 11% proteins), whereas pea is characterised by high protein content (53% carbohydrates and 24% proteins) [4]. The effect observed in Fig. 1 is consistent

with general trend in which the oleic seeds absorb less water than seeds containing more starch and, first of all, protein seeds [10].

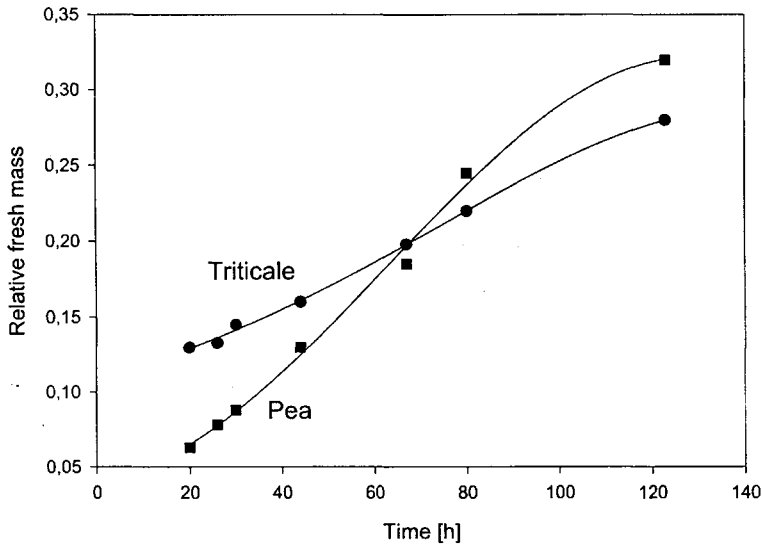


Fig. 1. Water uptake kinetic in pea and triticale seeds.

$^1\text{H-NMR}$ studies of the seed hydration have been most often performed on NMR-spectrometers designed to observe the signal from liquids [2, 3]. This method is useful for water entrapment (caging) in tissues or for in-situ water circulation investigations [1]. Two-dimensional high-resolution NMR experiments and molecular dynamics calculations [2] have indicated that in biopolymer systems and biological tissues it is usually sufficient to consider three states of water, this is, structural or bound water, surface water and bulk water. Structural water is that hydrogen bonded inside of grooves and cavities of globular proteins and polysaccharides, which plays an important role in determining the structure and dynamics of the biopolymer chains. The second fraction of water molecules sorbs at the biopolymer surface. In globular protein there are three types of water adsorbing sites, deriving from three types of amino acid residues: charged, polar and non-polar. The charged or dissociating groups chemisorb individual water molecules with high affinity [8]. The permanently available water molecules can form clusters around the polar groups, with decreasing affinity to water molecules. As more moisture was available, water aggregated over non-polar residues. This surface water extends for several molecular layers from the surface of macromolecules and was extremely mobile. The bulk of the cell water is normal liquid water and exists only when the water content in seeds exceed $0.8 \text{ g H}_2\text{O/g dry mass}$. Since the water content

in our samples is lower than that required for bulk water appearance. This water component is beyond of our current considerations.

The ^1H -NMR spin-spin relaxation time study (T_2) in triticale grains and pea seeds (Figs. 2 and 3) distinguished between two states of water. The relaxation time T_2 for two fractions of water took higher values for pea seeds than for triticale grains. For the two groups of seeds the humidity dependence of transverse relaxation time was not linear.

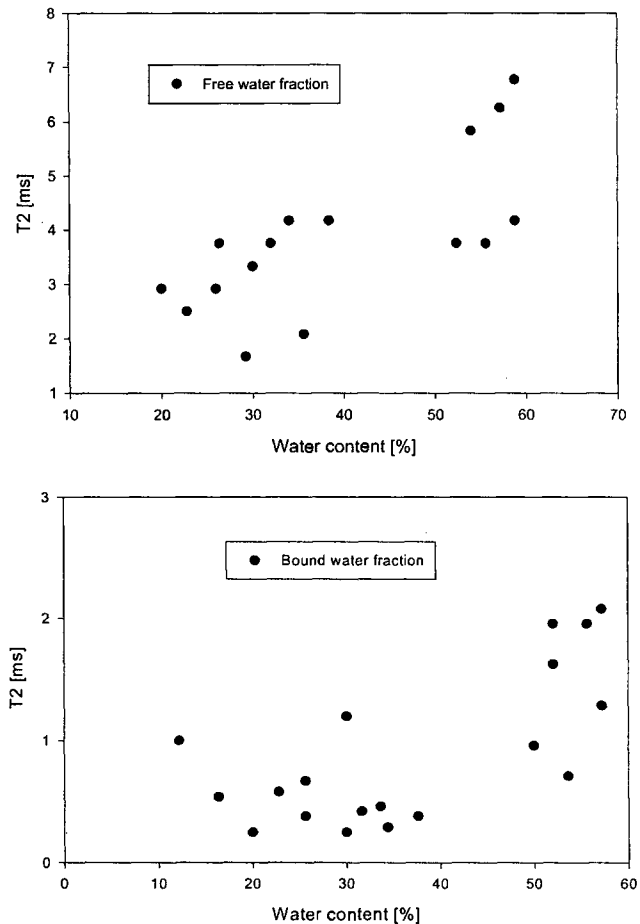


Fig. 2. Transverse relaxation times T_2 for bound and free water fraction in triticale grain.

The compact molecules possessed a reduced mobility as compared to less compact molecules. The reduced mobility provided a longer relaxation time, i.e. a time in which the molecules must return to equilibrium. Observed differences in transverse

relaxation time T_2 in pea and triticale could be explained as a result of diverse in starch structure in both investigated species.

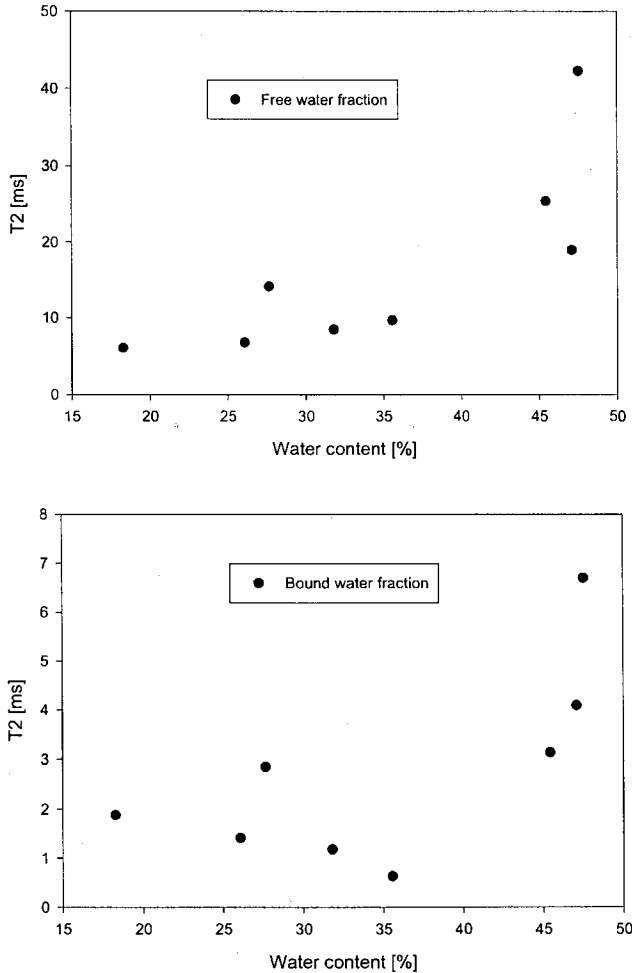


Fig. 3. Transverse relaxation times T_2 for bound and free water fraction in pea seeds.

Starch granules are complex structures consisting of crystalline and amorphous areas. The short chains in the amylopectin molecule are organised into double helices some of which are crystallized into lamellae. These lamellae alternate with the amorphous layers [11]. Amylopectin double-helical chains can either form the more open hydrated hexagonal crystallites (type B), the denser crystallites (type A) or, such as in pea, contain both polymorph forms: A and B [11]. Such structures determine the mobility of water in studied systems.

Conclusions

Presented investigations have shown that well documented differences in chemical content and structure of starch granules in pea and triticale correspond to differences in microscopic parameter – transverse relaxation time T_2 – in those seeds. The used NMR method is nondestructive and can be adapted to other noninvasive investigations of in vivo starch systems.

References

- [1] Belton P.S., Radcliffe R.G.: NMR and compartmentation in biological tissues. *Prog. NMR Spectr.*, **17**, 1985, 241-279.
- [2] Brunné R. M., Liepinsh E., Otting G., Wuthrich K., van Gunsteren W.F.: Hydration of proteins, a comparison of experimental residence times of water molecules solvating the bovine trypsin inhibitor with theoretical model calculation. *J. Mol. Biol.*, **231**, 1993, 1040-1048.
- [3] Di Nola A., Fabrizio G., Lamba D., Segre A.L.: Solution conformation of a pectic acid fragment by ^1H NMR and molecular dynamics. *Biopolymers*, **34**, 1994, 457-462.
- [4] Grzesiuk S., Kulka K.: *Fizjologia i biochemia nasion*. PWRiL, Warszawa, 1981
- [5] Hoover R.: Composition, molecular structure, and physicochemical properties of tuber and root starches; a review. *Carbohydr. Polym.*, **45**, 2001, 253-267.
- [6] Li I.Y., Yeh A.J.: Relationships between thermal, rheological characteristic and swelling power for various starches. *J. Food Eng.*, **50**, 2001, 141-148.
- [7] Parker R., Ring S.G.: Aspects of the physical chemistry of starch. *J. Cereal Sci.* **34**, 2001, 415-420.
- [8] Peeters D.: Hydrogen bonds in small water clusters: A theoretical point of view. *J. Mol. Liquids*, **67**, 1995, 49-61.
- [9] Tanaka H.: Cavity distribution in liquid water and hydrophobic hydration. *Chem. Phys. Lett.*, **282**, 1998, 133-138.
- [10] Walters Ch., Hill L.M.: Water sorption isotherms of seeds from ultradry experiments. *Seeds Sci. Res.*, **8** (Suppl.), 1998, 69-73.
- [11] Wang T. L., Bogracheva T. Y., Hedey C, L.: Starch: as simple as A, B, C? *J. Exp. Bot.*, **49**, 1998, 481-502.

HYDRATAcja NASION SKROBIOwYCH I BIAŁKOWYCH WE WCZESNEJ FAZIE KIELKOWANIA

Streszczenie

Pęcznienie nasion w pierwszej fazie polega głównie na hydratacji koloidów nasiennych. Powinowactwo chemiczne tych związków do wody różni się zależnie od właściwości powierzchniowych ich makromolekuł. Powierzchnie biopolimerów zakłócają dynamiczny i statyczny stan wody. Z tego względu struktura i skład nasion, w szczególności zawartość protein, skrobi oraz lipidów może warunkować przebieg procesu pęcznienia.

Proces pęcznienia nasion grochu i ziarniaków pszenżyta opisano w przedstawionych badaniach za pomocą parametrów makroskopowych (względny przyrost masy) oraz mikroskopowych (czasy relaksacji spin-spin).

Grawimetrycznie określony pobór wody przez nasiona pszenżyta, początkowo większy niż w grochu, w późniejszej fazie obniżał się. Metodą spektroskopii $^1\text{H-NMR}$ ujawniono obecność dwóch frakcji wody, które znajdując się w różnym otoczeniu różniły się ruchliwością, opisaną przez czasy relaksacji spinowo-spinowej. W przypadku grochu ten parametr mikroskopowy był zdecydowanie większy niż dla pszenżyta. Obserwowane różnice są dyskutowane przy uwzględnieniu różnic w wymiarach nasion i ziarniaków, różnej przepuszczalności okryw nasiennych oraz przede wszystkim różnic w składzie chemicznym, w którym dominujący udział ma skrobia. ❖