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ORIGINAL PAPER

Bean cotyledons microporosity under hydration conditions

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Abstract Positron Annihilation Spectroscopy (PAS), proton Nuclear Magnetic Relaxation (NMR) and hydration kinetics measurements were applied to study the free volume formation process in the initial stages of bean cotyledons imbibition. These methods allowed us to analyse the behaviour of bound water in the bean cotyledon molecular structure, and to observe free volume regions formed there. They also made it possible to describe their dimensions and concentration quantitatively. Good correlation was found between the increase of the free volume radius and the amount of water bound to the bean cotyledon solid matrix.

Key words bound water • free volume • PAS • proton NMR

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Introduction

Seeds of grain legumes used for human food require hydration to prepare for cooking and eating. The imbibition of water by bean seeds leads to the softening of the seed coat and cotyledon. Water in the seeds plays also an important role in chemical transformations such as protein denaturation and starch gelatinization important in cooking process. Swanson and co-workers [18] reported that the stage of water imbibition is reflected by grain legume seeds coat and cotyledon microstructure. Water absorption characteristics was shown to influence the textural characteristics of soaked legume seeds. Sefa-Dedeh and Stanley [16] suggested that microstructure of the seeds is important to water imbibition and movement throught its tissues. Hosfield's, Uebersax's [11], and Moh's [13] examination of beans seeds "San Fernando" and "Nep-2", which are almost identical genetically, shows that observed differences of water absorption capacity, several physicochemical characteristics and many of their culinary properties are related with their different microstructure. The investigations of hydration of seeds are very important not only for food technology but for agriculture and botany too. There is a number of scientific papers reporting relationship between the microstructure of seeds and water absorption rate [1, 5, 9, 10] but quantitative descriptions of such processes are continually deficient.

This study was undertaken to determine if the imbibition of bean cotyledons could produce measurable changes of their microstructure. The aim of the study was to answer the question whether free volume regions are formed there and to correlate their numbers and dimensions with the concentration of bound water. To solve this problem, we used proton NMR relaxation and the PAS method.

The proton NMR relaxation [2, 6] is sensitive to molecule mobility. The Spin Grouping Technique [15] allows one to distinguish signals coming from free and bound water, and to

find their relative amounts. The PAS is useful to observe behaviour of free volumes in molecular systems [3, 4, 8, 17].

Material and methods

Investigations of the initial stages of seed imbibition were performed on bean cotyledons (Atlas variety). The dry mass (m_0) of the bean cotyledon was estimated after 48 h of drying in an oven at a temperature of 72°C. All measurements were performed at room temperature. The separated bean cotyledons were immersed in distilled water, dried using a filter paper and weighed.

The proton NMR relaxation is an efficient method to observe the molecular dynamics of water in the condensed matter. In particular, on the basis of different molecular mobility, proton magnetic relaxation can distinguish fractions of bound and free water. Free Induction Decay (FID) signal of bean cotyledon consists of two components. The first, gaussian shaped, has a very short decay time (μs). That component comes from the protons of the solid matrix. The other component has a much longer decay time (ms) and is attributed to the protons of water [15]. Such behaviour is typical for the water in microheterogeneous systems. It was observed in paper pulp [2], wheat grains [10] or lichen thallus [9]. As far as the FID is concerned, there is a fast exchange between free and bound water resulting in an average relaxation time of water component. The "Inversion Recovery" pulse train was applied and after each time space between pulses, full FID is stored in the computer memory. Therefore, 2D data matrix was recorded. The CracSpin program [20] applying a two--dimensional time-domain Spin-Grouping procedure [15] was used to analyse relaxation data. CracSpin gives an opportunity to analyse data starting either from T_1 or from T_2^*. For example, values of T_2 are calculated for every FID and are averaged for all fitting windows. Next, those fitted values are used to fit amplitudes of FID components. The values of reconstructed amplitudes create a curve of the perpendicular magnetisation decay, and T_1 relaxation times may be calculated. Use of Spin Grouping procedure improves ability to detect different spin groups.

The sample for NMR measurement was put into a 5-mm sample tube. Water was added to the sample tube prior to the experiment. Then, the sample tube was sealed immediately and put into an NMR spectrometer. Further stages of the absorption process were observed without removing the sample from the magnetic field. The NMR measurements were performed using the computer-controlled WNS HB65 spectrometer (Waterloo NMR Spectrometers Inc.). The spectrometer operates at proton resonance frequency of 30 MHz, with a dead time of about 10 μs and a length of π/2 pulse of 1.2 μs. Data were digitised by the oscilloscope card Compuscope CS220 installed in the computer.

Positron annihilation measurements were used to observe the intermolecular free volumes of the sample structure at the first stage of their imbibition. Application of PAS to determine the size and distribution of free volumes is based on examination of the lifetime of positronium atom (Ps) [12, 14]. It was well established experimentally that Ps, which is a bound state of an electron and a positron, could be formed only in empty space of molecular structure [3, 4]. Free-volume studies base on the fact that decay of the ortho-Ps in the pick-off process depends on its size. The correlation between the o-Ps lifetime (τ_3) and the size of the free volume hole was found by Tao [19]:

$$\tau_3 = 0.5 \Bigg/ \left[\frac{\Delta R}{R_0} + \frac{1}{2\pi} \cdot \sin\left(2\pi \frac{R_0 - \Delta R}{R_0} \right) \right] \tag{1}$$

where ΔR=0.1656 nm [18]. In the literature one can find several other, much more complex models [4], which generally show an increase of the o-Ps lifetime when the free volume radius increases. The aim of our paper was to analyse the general trends of positron behaviour in the bean cotyledon molecular structure, and we did not want to test the o-Ps decay model. Therefore, we took the simplest, which seems to be appropriate, as it was used e.g. for description of the experimental data for cotton cellulose [7].

The PAS measurements were performed on two bean cotyledons which were sandwiched with a positron source ^{22}Na, enveloped in a polyethylene foil to prevent drying and located in a positron lifetime spectrometer. Source activity was 0.2 μCi, time resolution (FWHM) of the fast-fast lifetime spectrometer with NE 111 scyntilators was equal to 300 ps. One positron lifetime spectra contained 10^6 counts.

Results

Hydration kinetic measurements

Increase of the relative mass of water absorbed by the bean cotyledon ($\Delta m/m_0$) as a function of hydration time is presented in Fig. 1. At the beginning of the water absorption process, ($\Delta m/m_0$) strongly increased with the hydration time and then saturated. This time dependency was well described by the following formula:

$$\Delta m/m_0 = A_1[1 - B_1 \exp(-t/C_1)] \tag{2}$$

The solid line in Fig. 1 represents the best fit to this relation with the following fitted parameters: A_1=1.17±0.01, B_1=0.91±0.01, C_1=78.01±2.92 min.

An exponential behaviour indicates that the water absorption is caused by the gradient of the matrix potential between the dry cotyledon structure and the water environment. Relation

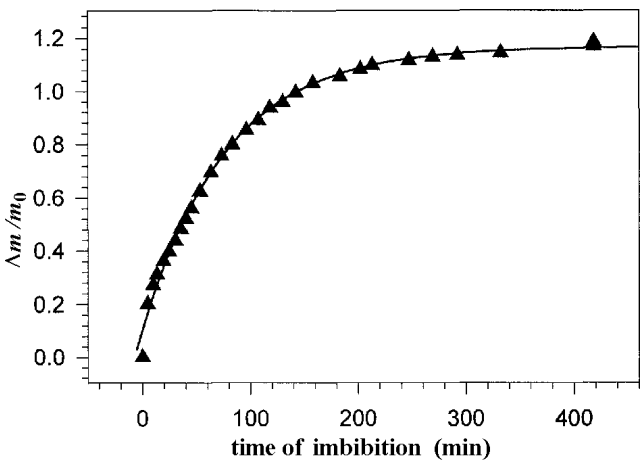

Fig. 1. Relative absorbed water mass increase ($\Delta m/m_0$) as a function of bean cotyledon imbibition time.

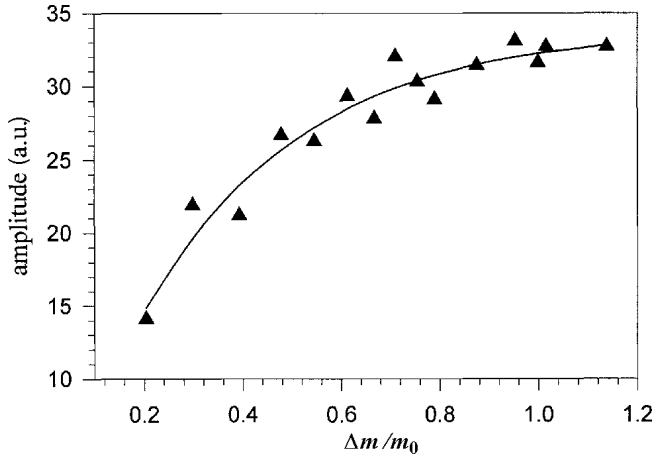

Fig. 2. Amplitude of bound water, in bean cotyledonary cell structure, as a function of relative absorbed water mass increase ($\Delta m/m_0$).

(2) was used to calculate the relative mass increase of water absorbed by the bean cotyledon during the NMR experiment. For positron annihilation lifetime experiments, the mass of the sample was controlled both before and after the measurement.

NMR measurement

In proton NMR relaxation data three components with different relaxation properties were observed. The first one, described by T_2^*=(12±1) μs and T_1=(143±20) ms, was attributed to the solid matrix. Two other components had the same values of T_2^*=(3.23±0.21) ms and different values of T_1. The component with T_1=(112±20) ms was attributed to the bound water, while the component described by T_1=(461±131) ms was attributed to the free water. The bound water has restricted mobility and, therefore, the value of its T_1 relaxation time is similar to the value of T_1 of the solid matrix. The free water is more mobile and the T_1 relaxation time is much longer. The amplitude of the bound water component, which is proportional to the amount of bound water in the bean cotyledon molecular structure, is presented in Fig. 2. This amplitude increases with increasing relative mass of the water absorbed by the bean cotyledon, and may be described by the following empirical formula:

$$I_{BW} = A_2\left[1 - B_2 \exp\left(-\frac{\Delta m / m_0}{C_2}\right)\right] \quad (3)$$

The solid line in Fig. 2 presents the best fit, where the values of the fitted parameters are as follows: A_2=(3.39±1.37) a.u., B_2=1.05±0.16, C_2=0.32±0.07. Such behaviour suggests that during the first stage of hydration, water was absorbed only by the solid matrix of the cotyledon tissue and could not be stored as free water. From Fig. 2 we can conclude that for ($\Delta m/m_0$) greater than 0.6, the water absorbed in bean cotyledonary cells hardly changes its mobility. This is only possible when the absorbed water is placed far away from the surface of solid matrix.

Positron annihilation spectroscopy measurements

In the positron lifetime spectra measured for the bean cotyledon, three lifetime components were detected. The shortest lifetime component (τ_1) was related to the para-Ps. The lifetime component (τ_2) was due to free positron annihilation in a singlet configuration with respect to orbital electrons. The longest one (τ_3) was attributed to the o-Ps decay in the pick-off process in the free volume regions. Fig. 3a presents a value of the longest component as a function of ($\Delta m/m_0$) One can see that τ_3 increases for ($\Delta m/m_0$) from 0 to 0.3, and next saturates. Basing on equation (1) we can conclude that the free volume radius increased from (0.246±0.004) nm to (0.278±0.004) nm during imbibition. As in the case of NMR studies, the exponential function was applied to describe the relative increase of the o-Ps lifetime:

$$\tau_3 = A_3\left[1 - B_3 \exp\left(-\frac{\Delta m/m_0}{C_3}\right)\right] \quad (4)$$

The best fit is presented in Fig. 3 as a solid line. The values of the fitted parameters were as follows: A_3=(1923.20±2.20) ps, B_3=0.16±0.02, C_3=0.17±0.05.

The dependence of the free volume radius R evaluated from equation (1), on ($\Delta m/m_0$) is presented in Fig. 4. The free volume size strongly increased for ($\Delta m/m_0$) in the range of (0–0.3) and next saturated.

On the basis of the similar type of relations (3) and (4), we conclude that the amount of bound water in the bean cotyledon molecular structure is responsible for the increase of the free volume radius. The value of parameter C_2 in equation (3) is about twice as large as the value of parameter C_3 in equa-

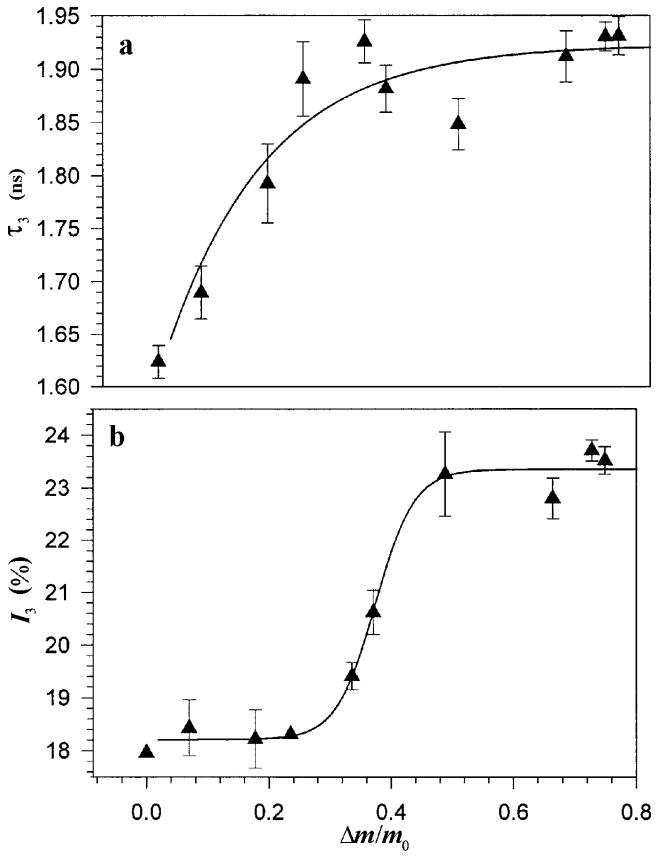

Fig. 3. The o-Ps lifetime in bean cotyledon molecular structure: a – as a function of intensity of o-Ps lifetime component, b – as a function of relative absorbed water mass increase ($\Delta m/m_0$).

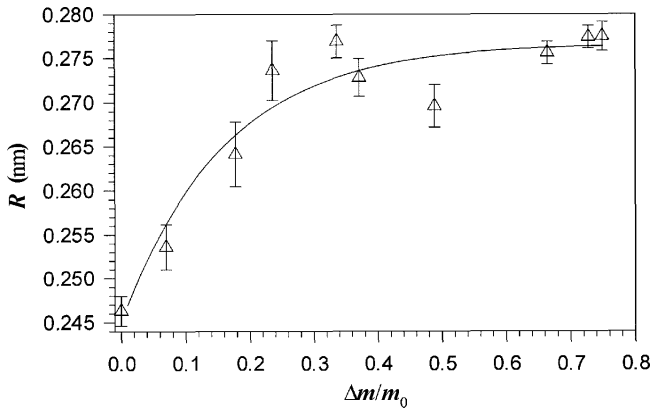

Fig. 4. Free volume radius in bean cotyledon molecular structure, *vs.* relative absorbed water mass increase ($\Delta m/m_0$).

tion (4). This may be explained by the fact that only a half of the amount of bound water is related to the increase of the free volume radius. The rest of the amount of bound water participates in forming the new free volume regions, but this is possible when the value of ($\Delta m/m_0$) is higher than 0.3. This could be seen in Fig. 3b, where intensity of τ_3 is presented. Intensity of this component was attributed to the concentration of free volumes [13, 16] and can be described by the following function:

$$I_3 = \frac{\left[A_4 + B_4 \exp\left(\frac{\Delta m / m_0}{C_4}\right)\right]}{\left[1 + D_4 \exp\left(\frac{\Delta m / m_0}{C_4}\right)\right]} \tag{5}$$

where: A_4=18.21±0.16, B_4=0.0002±0.0007, C_4=0.032±0.011, D_4=(8±3)×10^{-6}

This behaviour suggested that for ($\Delta m/m_0$) in the range of (0.25–0.45) violent fluctuation in concentration of free volumes took place and that a new stage of the imbibition was observed.

Conclusion

At the initial stages of bean cotyledons imbibition water is very fast absorbed by the sample and bound to its solid matrix (see Figs. 1, 2). The amount of bound water is mainly determined by hydration of storage substances that are abundant in cotyledons. The hydration of the cotyledon structure evokes reduction of the surface and interior tension and loosening structure of the sample. There are good conditions to increase the dimensions of the empty spaces in the sample microstructure, which is consistent with the results obtained from positron lifetime spectra. They indicate that for ($\Delta m/m_0$) lower than 0.3 the absorbed water causes increase of the free volume dimensions, but does not change their number (see Figs. 3a, 3b). For ($\Delta m/m_0$) in the range 0.3, 0.5 the absorption of the water causes strong increase of free volumes number (see Fig. 3b). It could be explained by the fact that under high hydration condition reconstruction of the cotyledons microstructure takes place, a lot of free volume regions are formed, some of existing ones are divided into parts by hydration products. For ($\Delta m/m_0$) higher than 0.5 the absorption of water in the bean cotyledon molecular structure does not change, as well as the number and dimensions of the free volume regions.

The comparison of the results obtained from NMR and PAL methods suggested that only a half of the amount of bound water induced increase in the existing free volume hole dimensions. The rest affected the formation of new free volume regions.

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