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Physical investigations of surface membrane–water relationship of intact and gelatinized wheat–starch systems

Albert Linton Charles,[a] Hsien-Ming Kao,[b] Tzou-Chi Huang[c,*]

[a] *Institute of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung, Taiwan*
[b] *Department of Chemistry, National Central University, Chung-Li, Taiwan*
[c] *Department of Food Science, National Pingtung University of Science and Technology, Pingtung, Taiwan*

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Abstract

Water mobility in intact and dried gelatinized starch was investigated by gravimetric water sorption, scanning electron microscopy (SEM), and solid-state nuclear magnetic resonance (NMR). A multi-component exponential model quantitatively measured different spin–spin relaxation times of two water components, namely bound water ($T\mathrm{s}_i$) at 3.16 ms and mobile or free water ($T\mathrm{s}_{ii}$) at 3.23 ms, as a function of water activity (a_w). The starch samples were moistened to 30% moisture content. SEM confirmed the disrupted, absorbent microstructure in dried, gelatinized starch powder and revealed starch granules in an incomplete gelatinized state, as compared to the complete membrane surface of the intact starch granule. Starch granules sorbed significantly differently at low a_w, but after $a_\mathrm{w} = 0.44$, sorption leveled similarly with increasing a_w. The presence and role of a surface membrane was concluded, in support of the hypothetical "water sink" properties of intact granules, and was considered to influence in part the sorption behavior of incompletely gelatinized starch granules.

Keywords: Starch; Surface membrane; NMR spectroscopy; SEM; Water activity

1. Introduction

Many food starches have been developed for particular applications, and have proved successful as ingredients in processed foods for many years. They are used principally to take up water and to produce viscous fluids, pastes, and gels to impart desired textural qualities. In certain cookies that are high in fat and low in water, about 90% of the wheat starch granules remain ungelatinized.[1] These ungelatinized, intact starch granules were postulated as early as 1968[2] to function as water sinks and as structure setters, in thermal setting processes during baking[3] and for immobilizing water molecules during firming in stored starch-based food systems.[4]

Water in food that is not bound to food molecules can support the growth of microorganisms, and the term water activity (a_w) is used to refer to this unbound water. The term a_w is the ratio of the water-vapor pressure in any kind of food system to the water-vapor pressure of pure water, and is depicted by a moisture sorption isotherm. The sorption isotherm is used to study the equilibrium moisture content of a sample at a given temperature.[5] The Brunauer–Emmet–Teller (BET) theory, using low a_w levels 0 to 0.5, defines the isotherm into three layers. The first layer, referred to as the BET monolayer, defines the most strongly sorbed and least mobile or bound water, and provides maximum stability of a dry product.[6] The second and third layers represent the continued adsorption of additional layers, referred to as free or mobile water. These water components have been studied using nuclear magnetic resonance (NMR); although the information obtained is still debatable.

Solid-state NMR has proved particularly successful for nondestructive determination of the content, structure, and dynamics of water.[5] Interpretation of NMR relaxation measurements in heterogeneous systems are

* Corresponding author. Fax: +886-8-774-0213.
E-mail address: tchuang@mail.npust.edu.tw (T.-C. Huang).

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still model-dependent, but because of a lack of understanding of factors that cause spin–lattice and spin–spin behaviors, these models may not be satisfactorily productive. Hence, the present research utilized physical and instrumental analysis to investigate the water characteristics in intact and gelatinized starch systems and to confirm the results expected from the hypothesis that the intact starch granule related to moisture content control, separates them as a novel ingredient in food processing.

The reason for studying the intact starch granule was motivated by the concept of the presence of a hypothetical surface membrane, and a theory that the intact starch granules have the ability to act as a "water sink". These features possibly make the intact starch granule a very useful functional ingredient in regulating moisture, texture development, and storage ability in bakery foods, improving and increasing the matrix crumbness, crispiness and firmness of snacks.[2] Although the functions of the intact starch granule are seemingly important, there are not many reports on the role of the intact starch granules in starch-based foods is being compared with the gelatinized starch granule based on their functional surface membrane. Hypothetically, the intact granular surface is expected to contain more moisture or exert some influence on water molecular properties. The inclusion of native, ungelatinized granules, in processing of low moisture starch-based foods as compared to the more popular use of pregelatinized starches in starch food processing, would introduce new methods of studying and enhancing food functional properties, and elucidate the function and control of water molecule in these foods.

2. Results and discussion

2.1. Gravimetric moisture sorption determination

Fig. 1 shows the moisture adsorption isotherm for the intact and gelatinized starch granules at 25 °C. At low water activity, the intact starch granules adsorbed little or no water until, at a_w level of 0.44, the granules begin to adsorb water. In contrast, the gelatinized starch gradually adsorbed water at all levels of a_w. Moisture adsorption isotherms (Fig. 1) were developed to illustrate the behavior of the starch samples to water sorption in different a_w environments. The isotherm shows that the intact samples adsorbed less water as compared to the increasingly higher water content of the gelatinized starch granules, possibly due to the larger surface area in the inner porous structure of gelatinized starch. This distinctive water-adsorbing behavior between the two starch forms seems to support the view that the surface membrane apparently has permeability properties, allowing for the free movement of free water in and out of the starch system.

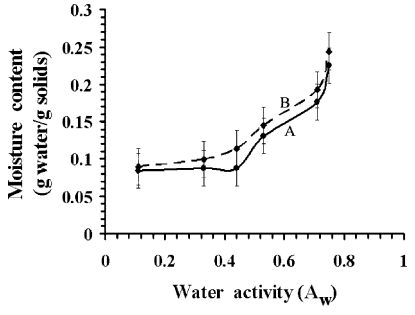
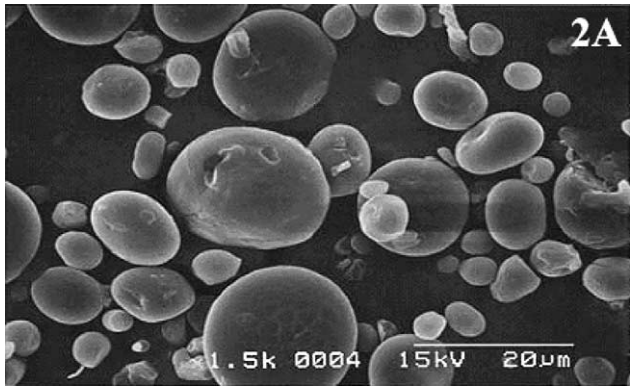


Fig. 1. Moisture adsorption isotherm for the intact and gelatinized starch cookies at 25 °C.

2.2. Scanning electron microscopy of starch granules

The scanning electron micrographs show the different structures of the intact and gelatinized starch (Fig. 2(a, b)). The intact starch granules are smooth, free from pores, cracks, or fissures, have round to oval shapes, and are relatively thick, whereas the gelatinized starch granules are either swollen or ruptured with cleaved surfaces. Fig. 2 indicates significant structure remaining after gelatinization of the starch granules. It is plausible,



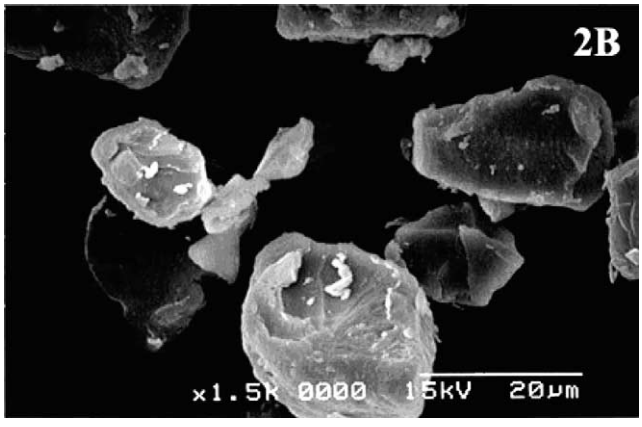


Fig. 2. Scanning electron micrographs of (A) intact and (B) gelatinized granule.

therefore, to assume that this controlled disruption could be attributable to the limiting presence of a membrane that is able to undergo partial destruction, resulting in the incomplete gelatinization of some granules. These morphological differences, similarly reported,[7] are useful for effectively controlling water activity, shelf life and to design the storage conditions of these products. Some granules reportedly[8] remain intact, embedded in the gelatinized matrix system, whereas others are completely and/or incompletely gelatinized (Fig. 2). From this structural spectrum, we can only speculate about the structure morphology, such as the proposed surface membrane, that would influence the water–starch relationship.

2.3. NMR measurements

Fig. 3 shows the ^{1}H MAS NMR spectra of intact and gelatinized starches of 30% moisture content. Only one isotropic resonance was observed, at 4.70 and 4.66 ppm, for intact and gelatinized samples, respectively. The line width of the intact sample is slightly larger than that of gelatinized sample (152.6 vs. 136.5 Hz). This small difference in the chemical shift and number of peaks was reportedly[9] significant in that a larger number of peaks or broader peaks were expected for heterogeneous samples than for homogenous samples. Fig. 3 indicates gelatinized starch granules exhibiting broader peak resonance and higher peak numbers. Where the significance of broad peaks is vague, the number of peaks on the gelatinized starch spectrum correlates with Fig. 1, depicting the granules in different stages of gelatinization. The broader NMR lines of gelatinized starch were also correlated to water molecules exhibiting restricted mobility in solid-like components. This reinforces the earlier observation that the different water behavior patterns in gelatinized starch may be attributed to the presence of a surface membrane.

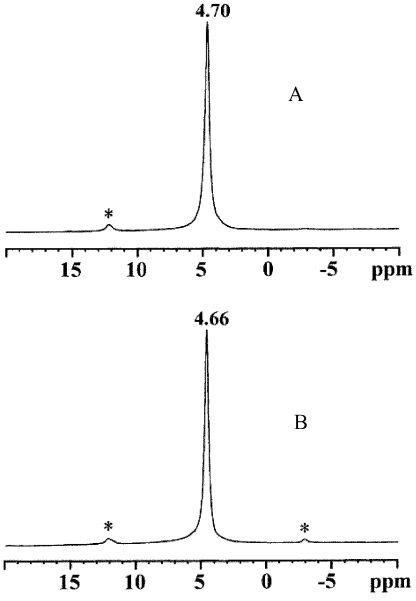


Fig. 3. ^{1}H NMR spectra of wheat (A) intact and (B) gelatinized starch containing 30% moisture content. * represents sidebands.

Spinning sidebands having small intensities were observed in both starch samples, suggesting high water mobility in both samples. Although the ^{1}H NMR spectra of both intact and gelatinized starches are to be reported similarly,[4] the earlier results showed no presence of the sidebands as reported in this study. These sidebands arise from the inhomogeneous characteristic of the ^{1}H–^{1}H dipolar interactions, largely decreased by the motion of water molecules. The appearance of spinning sidebands suggests that bound or less-mobile water is present in both samples. It is also noted that the spinning sideband manifold of the intact starch-granule system is more asymmetric as compared with that of the gelatinized starch system. This asymmetry in Fig. 3 may be explained by the different surface morphologies in Fig. 2, indicating varying starch water behavior. These observations imply that the water motion in intact samples exhibits more anisotropic character as compared to that in gelatinized sample. This may be interpreted as the water being rigidly bound to the membrane of the intact starch granule.

The ^{1}H NMR relaxation data, fitted with the discrete model (Eq. (1)), were resolved into two exponential components having distinct spin–spin relaxation times, labeled as T_{2i} and T_{2ii} respectively (Table 1). It is generally considered that bound water experiences faster relaxation than free water. Therefore, the component having the longer T_2 is assigned to free water and the shorter T_2 component to bound water. The bound water molecules of gelatinized starch relax faster ($T_2 = 3.16$ ms) as compared to that of the intact starch ($T_2 = 3.23$ ms). A shorter T_2 is generally interpreted as indicating less mobility of water molecules. Therefore, the bound water in intact starch exhibits higher mobility than that in gelatinized starch.

2.4. Water activity

The changes in distribution of water mobility in both starch forms, designated T_{2i} (less mobile) and T_{2ii} (more mobile), followed a different pattern and are compared in Table 1. It is observed that, although the moisture content of the intact starch granule was lower, it had higher initial T_{2ii} value (in the ms range) and the T_{2ii} values increased with a_w. At $a_w = 0.18$ (Fig. 4A), the distribution of mobility was uniform, but as a_w increased, the frequency distribution of water mobility became broader, indicating that water mobility became less uniform. There is (Fig. 4B) a similar moisture distribution in the gelatinized starch; however, the

Table 1
^{1}H NMR spin–spin relaxation data for intact and gelatinized starch as a function of a_w

Water activity (a_w)	Intact starch				Gelatinized starch			
	Bound water		Free water		Bound water		Free water	
	T_{2i} (ms)	%	T_{2ii} (ms)	%	T_{2i} (ms)	%	T_{2ii} (ms)	%
0.11	0.42	69	0.84	31	0.2	23	0.55	77
0.23	0.34	40	0.75	60	0.21	29	1.45	71
0.33	0.35	34	0.78	66	0.55	42	2.3	58
0.44	0.31	26	0.83	74	0.45	41	2.2	58

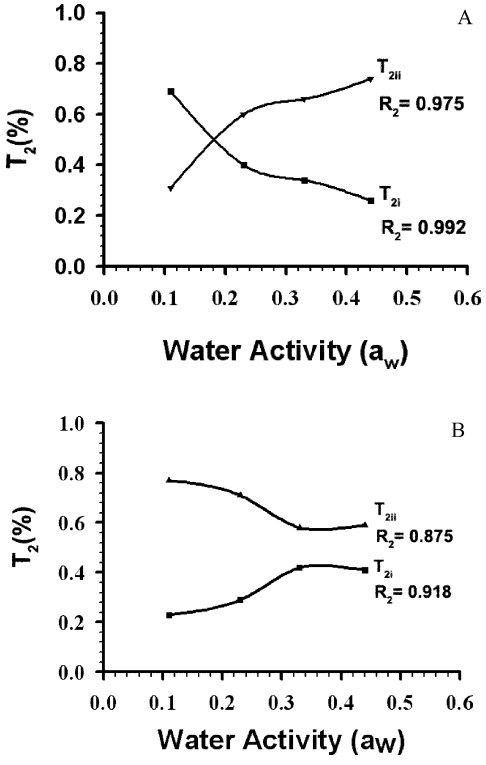


Fig. 4. The effects of water activity on (A) intact and (B) gelatinized starch granules, prepared by adsorption and desorption, on the NMR resonance signals at different water activity.

behavior of water mobility to the water activity environment is different. Free water (T_{2ii}) is observed to decrease with a corresponding increase in water activity, whereas the bound water (T_{2i}) slowly increases. The decrease of free water from the gelatinized starch powder is expected (Fig. 2B), since the membrane and crystalline arrays of the gelatinized starch are completely disrupted and the granules are ruptured. In contrast, for the intact starch granule, an increase in water mobility may be attributed to the limiting presence of the surface membrane surrounding the intact starch granule.

2.5. Water adsorption

It is evident (Fig. 1) that, as the water activity increased linearly with relative humidity, the water content of the intact granules also increases or the granules are resorbing. From the BET monolayer formula,[10] the monolayer water on the surface of the intact starch granule was determined to be 0.053 g water/g solid and for gelatinized starch, 0.0535 g water/g solids. This gives some indication of the presence of bound water within the starch granule and hence different types of water that may exist within the granule. It is assumed (Fig. 1) that the water distribution among the components in gelatinized starch is increased in the gelatinized matrix; hence the observed higher moisture content readings. The observed low or negligible adsorption readings of the intact starch at lower water activity may be accounted for by the limiting presence or permeability of the surface membrane and/or structural changes that occurred during sample preparation. The starch granule is chemically heterogeneous, containing both amylose and amylopectin and physically so, because it has both crystalline and amorphous phases. As a result, reactions with water are heterogeneous within the granule.

During drying, the pores originating from the surface membrane lead to collapse of the granule interior by hydrogen bonding of adjacent molecular chains during water removal. As a result, water molecules are bound to the exposed hydroxyl sites of the polysaccharide chains by hydrogen bonding, thus influencing the rate of water adsorption. Hence this change affects the movement of water through the membrane of the intact granules. This observation explains the higher water mobility in the intact starch granule as compared to the disrupted unstable system of the gelatinized starch with lower water mobility.

2.6. The effect of water activity on water mobility

Longer T_{2ii} (Fig. 4) indicates that, as water activity increased linearly with moisture adsorption, the amount of mobile or free water also increased. A similar

behavior of the free water in a sucrose–starch system at higher a_w and slow increase in tightly bound water was observed.[9] T_{2i} showed a decrease in relaxation time, indicating less mobile or free water molecules as the a_w increased. The shortened relaxation times observed, supported elsewhere,[5] could be accounted for by a small fraction of water molecules hydrogen bonded to macromolecules.

As shown in Fig. 4B, water molecules demonstrate different relaxation times and hence different environments of water. The trend (Fig. 4) in the gelatinized starch system shows that, as water activity increases, molecules experiencing longer relaxation times (T_{2ii}), decrease, whereas molecules with shorter relaxation times (T_{2i}) seem to increase. Within the system, some granules are adsorbing while some granules are simultaneously desorbing. The reduction in T_{2ii} may be the result of water evaporating out of the gelatinized system, or the molecules are hydrogen-bonding to free hydroxyl sites. The model we present in this discussion is that the reduction of T_{2ii} may be further explained by the incorporation of water molecules into the pore spaces of the surface membrane and as well as hydrogen-binding on its amylopectin–amylose complex structures. As a result of this, water molecules, limited by the permeability properties of the surface membrane, would then experience shorter relaxation times as they enter the starch system.

3. Conclusions

Resonance signals from intact and gelatinized starch samples revealed water molecules having different spin–spin relaxation times. These relaxation times, as a function of water activity, were correlated to the presence of a membrane on the starch-granule surface. The heterogeneous gelatinized-starch system exhibits different adsorption behavior and surface structure, whereas the homogenous intact-granule system, exhibits similar adsorption and structural properties. Further structural analysis studies need to be carried out on these starch systems to elucidate the physical and chemical properties of this surface membrane and its influence on starch properties.

4. Experimental

4.1. Sample preparation

The intact and the gelatinized starch were prepared by the Martin process,[8] which is the standard process used for isolating starch from gluten in flour in the food industry. A 2000 g sample of high protein content wheat flour was used. Immediately after centrifugation, the starch flour was dried in an oven at 50 °C for 48 h, and then ground and filtered to obtain uniform granule size and it was stored in a dessicator until further use. The reconstituted intact and gelatinized starch forms are expected to have similar chemical composition and identical chemical structures.

4.2. Gravimetric moisture sorption determination

Adsorption isotherms were determined through equilibration of predried samples over saturated salt solutions of known relative humidities at constant temperature (25 ± 2 °C).[11] Saturated solutions were prepared corresponding to a range of water activity levels from 0.07 to 0.97 (LiCl, KOAc, K_2CO_3, $MgCl_2$, $MgNO_3$, NaBr, $SrCl_2$, NaCl, and KCl).

Under the foregoing conditions the required equilibrium time was about 2 weeks. At least triplicate determinations were made for each duplicate sample (six replicates). When the difference between the weights of the sample was less than 1 mg/g solids within 2 consecutive weeks, the sample was considered to have reached equilibrium. Moisture content was determined by vacuum drying at 70 °C for 24 h and under the pressure of 50 Torr. Since the objective here is to investigate the behavioral tendencies of the starch systems in low moisture content foods, the moisture contents and related a_w data relevant to the research were selected in the low moisture range > 0.2–0.5. Significance of differences was defined at $P < 0.05$. The BET theory and model was used in the monolayer analysis for $a_w < 0.56$ in the starch systems.

4.3. Scanning electron microscopy

SEM was used to examine the intact and gelatinized starch samples. A small amount was used in the test. The starch powder was sprinkled on double-sided adhesive allowance tapes mounted on aluminum stubs. They were then coated with a gold ion-sputtering device (Ion-coater IB-2) and examined with a Hitachi S-2300 Scanning Electron Microscope (Hitachi Ltd., Tokyo, Japan) working at a 7 keV accelerating voltage.

4.4. NMR experiments

For NMR studies, two separate tests were carried out using two different techniques to study the water behavior in the starch samples. First, starch samples were similarly moistened to 30% water content. Secondly, starch samples were equilibrated over salt solutions by absorption or desorption in four a_w-controlled glass containers [0.11, 0.23, 0.33 and 0.44 a_w at 25 °C], using saturated solutions of LiCl, KOAc, $MgCl_2$, and K_2CO_3, respectively. The starch samples were left in the

a_w chambers for 1 week at 25 °C; after that weighed samples were packed into NMR rotors.

All NMR experiments were performed in triplicate on a Bruker Avance-400 spectrometer, equipped with a Bruker 7 mm probe head. The ^{1}H MAS (magic angle spinning) NMR spectra of starch samples containing 30% moisture content were recorded at a ^{1}H resonance frequency of 400.13 MHz. Typically, a 90° pulse length of 4 μs was used to acquire the free induction decay (FID) with a recycle delay of 2 s and at a spinning speed of 3 kHz. In general, for heterogeneous systems the acquired NMR signal intensities can be fitted with a multi-component exponential model:[12]

$$A(t) = \sum A_{0i} \exp\left(-\frac{t}{T_{2ii}}\right) \quad (1)$$

where $A(t)$ is the measured NMR signal intensity, T_2 is the spin–spin relaxation time of protons in the sample, A_0 is the signal intensity at equilibrium, i represents the sample with shorter relaxation times interpreted as tightly bound water to the macromolecule and ii (T_{2ii}) represents the sample with longer relaxation times or free water. NMR T_2 spectra were derived by a spin-echo pulse sequence with various evolution periods (Eq. (1)). All of the results shown in Fig. 3 were obtained using the Bruker Avance 400 XWin-NMR 1.3 on Iris2. A spectral width of 66 000 Hz 15 s pulse and short acquisition time (0.06 s) to obtain maximum sensitivity were used to obtain the single Lorentzian spectral lines.

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