Supplementary Information for

The origin of water loss from soy protein gels

Introduction

To investigate whether capillary suction measurements would give deeper insight in water holding (WH) of protein based gels, as alternative to the centrifugal measurements described in the main paper, studies were performed using equilibration of the gel in contact to filter paper. The capillary pressure difference of a spherical cavity in comparison to its surrounding is given by the Young-Laplace equation: $\Delta P = 2\gamma/R$, where γ is the interfacial tension between the cavity and its surrounding, and R is the radius of the cavity. Accordingly, water should be more tightly held in gels with smaller pore size, more hydrophilic network surface (small contact angle), and higher solvent surface tension.[1] Our paper describes a number of gel morphologies based on soy protein, formed in the presence of 0-100 mM magnesium salts. From the results it was concluded that gel microstructure (gel coarseness) was not the unique determinant to WH. This supplementary material treats the capillary effect on WH in these results.

Materials and Methods

The capillary suction method was adapted from Labuza and Lewicki (1978).[2] A custom designed plate containing twelve 22.5 mm in diameter and 4.5 mm in height cylinder wells was made. Gels were cut in 2 mm height and 14 mm diameter cylinders using a cork borer and carefully placed in the middle of each well and covered with 7 layers filter paper 20 mm in diameter (Whatman grade nr.1). Dimensionality of the porosity was determined by image analysis of the filter paper and varied from 70 µm to 20 nm (**Figure 1**). The ratio of total water present in the sample and maximum amount of water that could be absorbed by the filter paper (1 : 2.5) was determined by dipping filter papers in demi water and weighing the maximum amount of water adsorbed. Using a lower or higher ratio permits faster or slower equilibration, but does not affect the equilibration amount. A filled plate was covered with silicone rubber, an aluminum plate and a 2 kg weight load was placed on top to reduce pressure build-up in the well while assembling the stack and to prevent evaporation of water during equilibration. After storage for 24 h at 4°C, the filter papers were carefully removed and weighted. Absorbed water by capillary suction was recalculated to remaining water in the gel named WH_{CS} (%) (**Equation 1**), where W_T is total amount of water in the sample (g), and W_r is removed water from the sample. Measurements were performed in triplicates.

$$WH_{CS} = \frac{W_T - W_r}{W_T} \cdot 100 \quad [\%] \qquad (1)$$

Results

In **Figure 2** a positive linear correlation of WH and RE, shown in the main paper (**Figure 10a**), is presented in a 3D graph to illustrate if the salt concentration affects WH and RE. Careful analysis of the ion concentration on the gel structure formation and WH (**Figure 2**) does not provide an indication that WH is proportional to ionic strength. Though, it could be argued that the osmotic pressure in the gels is different by employing different salts and concentrations. To evaluate whether the determination of WH by capillary forces would show such an ionic strength dependence, studies were performed using equilibration of the gel in contact to a filter paper as described in the method section.

A steady state capillary suction method was used to investigate WH of a gel. Results of WH_{CS} are shown in **Figure 3**. It can be observed that neither salt concentration (ionic strength), nor the anion type influence the WH as measured by static capillary suction. No obvious pattern of increasing osmotic pressure with increasing ionic strength on WH was observed. WH varied between gels (45 - 60%), but did not depend on salt concentration.

As discussed in the main paper, WH measured by centrifugal forces shows a clear correlation with Young's modulus and RE. Exudation of water from a gel changes its spatial structure (density) and thereby its osmotic potential.[2] Consequently both morphology (stiffness and elasticity) and ionic strength of the gel were expected to have an effect on WH. In **Figure 3a** and **b** correlation plots of WH_{CS} and Young's modulus, and WH_{CS}-RE are shown for all soy protein gels studied in the main paper. No obvious correlations were found between these properties. The obtained results suggest that neither gel morphology nor ionic strength have an effect on WH measured by capillary suction. From this study can be concluded that not the ionic strength or osmotic pressure, but apparent gel morphology sets the WH measured by centrifugal forces.

The capillary suction pressure has an order of magnitude of $0.1/R \sim 10^5$ Pa (when using a surface tension ~0.1 N/m and $R \sim 10^{-6}$ m). The centrifugal pressure equals $h*\Delta\rho*g_c$, where h is the height of the sample, $\Delta\rho$ the density difference between aqueous phase and protein, and g_c the centrifugal force. With the sample height of 0.2 cm that was used, and approximating $\Delta\rho=100$ kg/m^3, the centrifugal pressure has an order of magnitude of $200*g_c$.

The capillary pressure becomes negligible when $0.1/R < 200*g_c$ or when $R>5*10^{-4}/g_c$.

Taking g_c in units of g (gravitational acceleration), and R in microns, we find that the capillary pressure is negligible when $R/\text{micron}>50/(g_c/g)$. For a typical $10\ g< g_c <100\ g$, we find 0.5 micron $<R<5$ micron.

Summarizing, the ionic strength contribution to capillary forces that might be present in determination of WH using gravitational pressure is not apparent. Moreover, evaluation of the pore sizes suggests that a contribution of capillary pressures on WH may be negligible in these systems.

References

(1) Stevenson, C. D.; Dykstra, M. J.; Lanier, T. C., Capillary Pressure as Related to Water Holding in Polyacrylamide and Chicken Protein Gels. *J. Food Sci.* **2013,** *78*, C145-C151.

(2) Labuza, T. P.; Lewicki, P. P., Measurement of gel water-binding capacity by capillary suction potential. *J. Food Sci.* **1978,** *43*, 1264-1269.

Figure Captions

Figure 1: SEM images of filter paper (a) 300 μm and (b) 1 μm length scale.

Figure 2: Salt concentration dependency on WH-RE relation of 10% (w/w) SP gels prepared in the presence of 0-100 mM $MgSO_4$.

Figure 3: WH measurements by static capillary suction method of 10% (w/w) SP gels without salt (open bars), and mixed with 5-100 mM $MgSO_4$ (filled bars) or with 5-100 mM $MgCl_2$ (striped bars).

Figure 4: WH_{CS} as a function of (a) Young's moduli and (b) RE, where (●) $MgSO_4$ and (▲) $MgCl_2$. Horizontal error bars stand for WH, vertical for Young's moduli and RE.

Figures

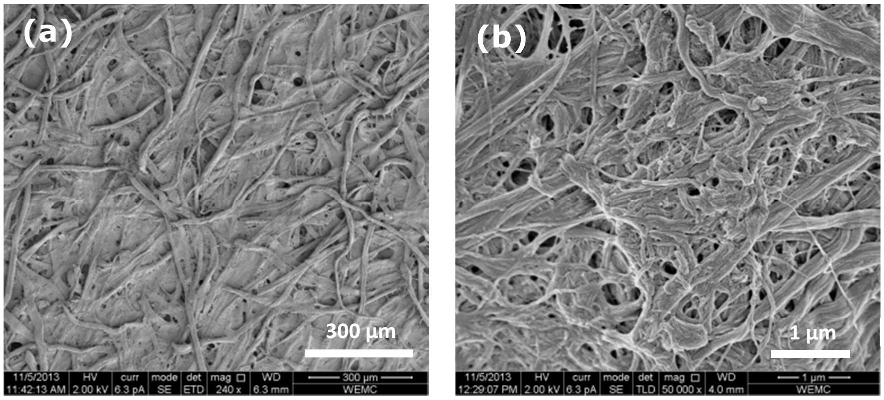
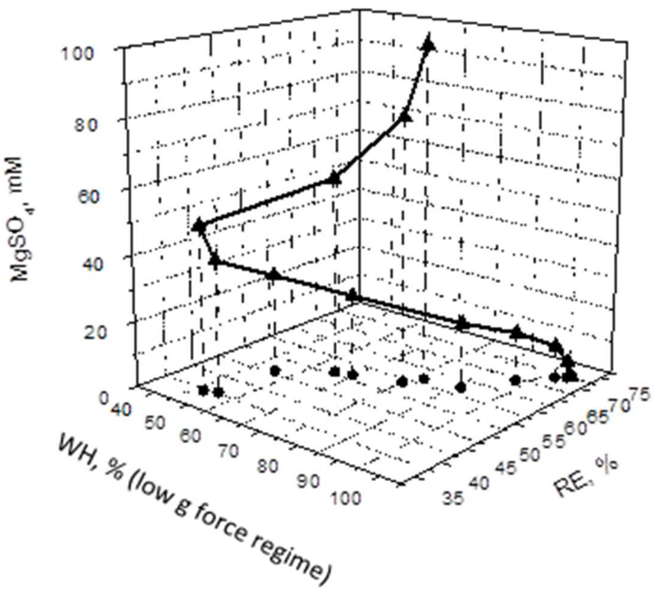
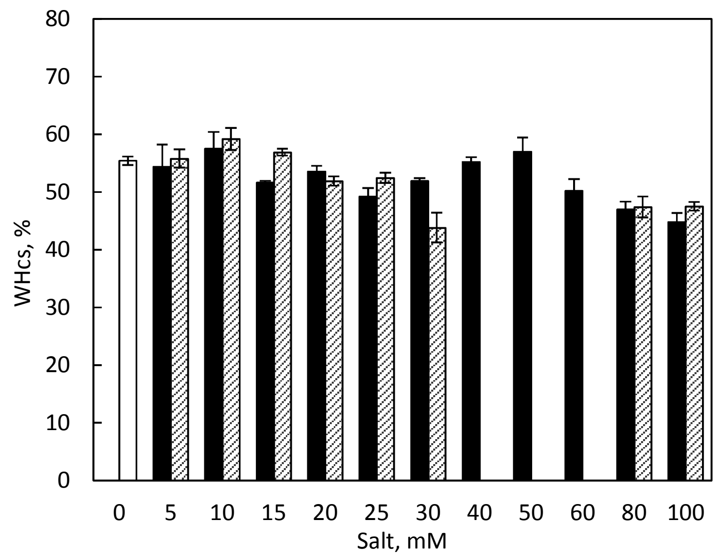


Fig. 1



100
80
60
40
20
0
MgSO4, mM
40
50
60
70
80
90
100
WH, % (low g force regime)
35
40
45
50
55
60
65
70
75
RE, %

Fig. 2



80
70
60
50
40
30
20
10
0
WHcs, %
0
5
10
15
20
25
30
40
50
60
80
100
Salt, mM

Fig. 3

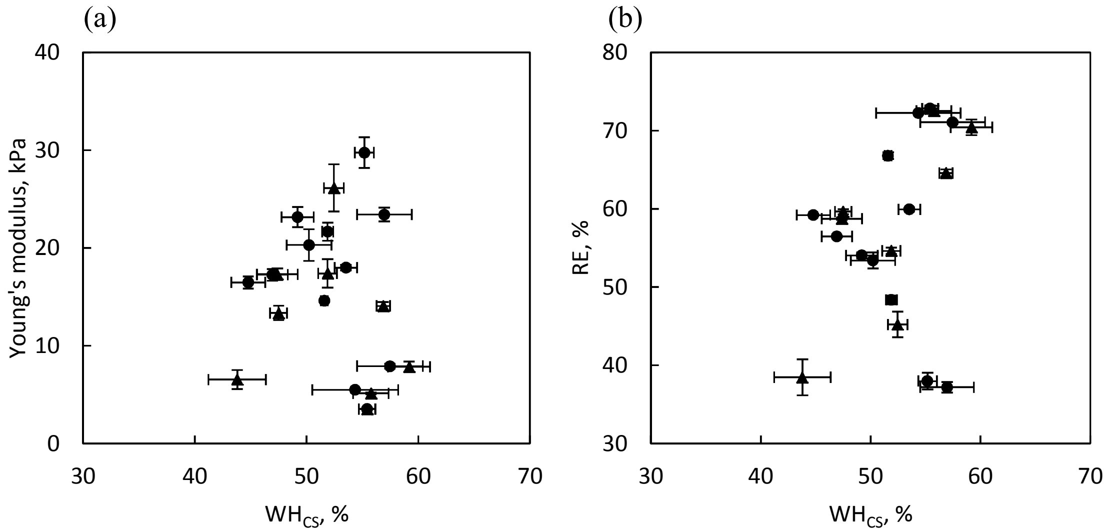

(a)
(b)
40
30
20
10
0
Young's modulus, kPa
30
40
50
60
70
WHCS, %
80
70
60
50
40
30
RE, %
30
40
50
60
70
WHCS, %

Fig. 4