

# Osmotic Properties of Gluten

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## ABSTRACT

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A model for dough is proposed in which the distribution of water between hydrated gluten and starch paste explains a number of practical observations such as 1) the extreme sensitivity of the consistency of dough to the amount of water in the recipe, and 2) the fact that working of the material results in an increase in consistency. The model assumes dough to be a composite material consisting of a starch paste and gluten filaments. During kneading, the starch granule paste in dough dries to become a phase with a yield stress as a result of the uptake of water by the stretching gluten filaments. This study focused on one particular aspect of this model: the osmotic properties of gluten during stretching.

The results suggest that gluten can be hydrated more efficiently in the stretched state than in an unstretched conformation. Gluten hydration tends to change slowly over a period of weeks, which is accompanied by water expulsion or uptake, depending on the osmotic properties of the solvent. The rate of change does not seem to depend very much on pH and osmotic pressure for the current experimental conditions. The level of hydration of relaxed gluten depends strongly on pH, as expected. The experiments allow the construction of an osmotic pressure versus gluten concentration diagram over the range  $4.6 < \text{pH} < 5.8$ . The level of hydration of the gluten is consistent with the proposed model for dough.

An important property of a dough is its capacity to hold the gas produced during proofing. The stability of the dough films between growing gas cells, necessary for the gas-holding capacity, requires that dough exhibits an adequate degree of strain hardening during the characteristic deformation conditions for proofing (van Vliet et al 1992; Dobraszczyk and Roberts 1994; Kokelaar et al 1996). Strain hardening implies that regions in the dough that have been stretched more than their surroundings, tend to stiffen to a degree that opposes further stretching. We will formulate a hypothesis that relates strain hardening of the dough to the physicochemical processes taking place in and between starch granules and gluten filaments. Such an explanation of a continuum property like strain hardening in terms of particle properties is justified because the length scale of the proofing process (i.e., the size of the gas bubble) is much greater than that of the starch granules or gluten filaments.

Kneading of a dough (essentially a mixture of gluten, starch, and water) is often described as unfolding of the gluten and their subsequent cross-linking, thus focusing almost exclusively on processes taking place on a molecular scale. However, it is unlikely that this can provide the full explanation of dough rheology. On the one hand, it is hard to imagine that the large deformations imposed during kneading would allow the development of a real network (e.g., the type that can be found in polymer gels) as it is intuitive that networks are broken up rather than formed in a kneading process. On the other hand, it is difficult to understand why the amount of water added to the flour is so critical to the performance of the dough if kneading would involve merely the unfolding and cross-linking of the gluten.

Any alternative model for kneading of a dough should complement rather than contradict the current molecular model on the phenomena taking place during kneading. Furthermore, the model should explain two intriguing observations: 1) dough becomes stiffer as a result of extensive mechanical working during kneading (instead of dramatically losing consistency) before the optimum; 2) the degree of stiffening is extremely sensitive to the amount of water present in the formulation compared with a typical biopolymer network.

In addition, the model should explain why starch is so vital for the performance of a dough, while it can be washed out easily. The easy separation suggests the absence of a pronounced interaction between starch and gluten.

These observations can be explained tentatively by a mechanism involving the microstructure of the dough on a mesoscopic scale, featuring the following elements (Fig. 1). 1) Dough can be considered a biphasic, composite system of gluten and starch phases that have the solvent (water) in common. 2) Water may be transferred from one phase to another during kneading of a dough. 3) Gluten is able to take up water when stretched. 4) The starch paste goes through a transition from a fluid slurry to a solid on drying. The yield stress of such a solid is very sensitive to the amount of water. (The inverse situation, reduction of yield stress with increasing water content, plays a role in landslides after heavy rainfall.)

The model describes the kneading process as follows. When water is added to the flour, gluten hydrates and swells, while the starch granules remain basically unaffected. As a result, dough before kneading is a composite containing elastic gluten-rich regions and starch-rich regions. The starch-rich regions will have a yield stress that depends on their moisture content. During the macroscopic deformation process of kneading, the gluten will become stretched and broken-up to a degree at which its internal stresses become commensurate with the yield stress of the plastic starchy regions. This implies that stretching of the gluten is actually controlled by the yield stress of the starch paste.

Strain hardening can now be understood if further stretching of gluten allows it to accommodate more water (as a consequence of the unfolding of the gluten proteins). The amount of water that is taken up by the gluten is controlled by the osmotic pressure  $\Pi$  of the gluten. The term osmotic pressure deserves some explanation in this context. Equilibrium in the dough two-phase system requires that external pressure equals pressure on solute plus pressure on solvent (in gluten phase) equals pressure on solvent plus pressure on the starch granules (in starch paste). Therefore, the pressure on the solute in the gluten phase (osmotic pressure) should equal the pressure on the starch granules because the pressure on the solvent is equal in both phases. Thus, it can be concluded that the starch granules are pressed together, which generates a frictional force between the granules, which gives the starch paste a yield stress. The water taken up by the gluten is withdrawn from the interstitial water in the starch paste which, as a consequence, dries during kneading. Water withdrawn from the starch phase increases the pressure on the starch granules and thus the yield stress of the paste which, in turn, enables further stretching of the gluten. Note that the model proposes that kneading is required to stretch the gluten into filaments and not (necessarily) to form a network.

Essential for the model is that the gluten and starch phases remain separate and do not mix on a molecular scale. This requirement will be fulfilled up to the point at which the gluten molecules or agglomerates become comparable in size with the voids between the starch granules. The starch granule dispersion will only exclude

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gluten agglomerates that are big enough. Overworking could tentatively be explained as a state where the breakdown of the gluten has progressed to a stage that the starch no longer excludes the gluten. The separation of starch and gluten phase does explain that washing out of starch from a dough is fairly easy. Note that this type of separation is different from the phenomenon usually referred to as thermodynamic incompatibility, as discussed by Tolstoguzov (1997).

In a system with too much water, the starch paste will be too thin and will act as a kind of a lubricant for the gluten particles, which cannot be stretched at kneading and will fail to absorb water. As a result, the starch paste will not dry and not gain in yield stress, and the dough will not develop any strain hardening.

In a system with too little water, the gluten will be able to take up the required amount of water. However, the starch will not be soaked in water. Also, air will enter the voids between the granules. The starch will behave as (relatively) dry sand and will no longer be very sensitive to the amount of water.

In the narrow range of flour+water compositions, where an optimally developed dough can be prepared, the starch paste is dry enough to develop the yield stress required to keep the gluten stretched. At higher and lower water content, the starch phase is expected to behave as a slurry or a dry powder, respectively. The interdependence of the yield stress in the starch phase and the stretching of the gluten ensures the proofing stability of the dough by means of strain hardening during biaxial extension. Furthermore, the decreasing deformation rate during proofing might be the mechanism causing the loss of strain hardening of the dough.

The Mohr-Coulomb equation (de Bruijne and Bot 1999) is adequate for estimating the value of the osmotic pressures in a proofing dough. This equation states that the yield stress of a dispersion like the starch paste is proportional to the pressure on that dispersion. This pressure can be identified as the underpressure in the interstitial water in the pores of the starch paste, which is equal to the osmotic pressure on the gluten. The proportionality constant is not known exactly but it is usually  $\approx 0.3$ . The implication is that the osmotic pressure will be approximately three times the rheological stresses in the dough. Because rheological stresses are  $\approx 5$  kPa, one expects osmotic pressures in the range of 10–20 kPa. The overpressure in the gas bubbles in a proofing dough depends on the gas fraction in the dough and the average rheological stress. In a well-proofed dough, where most of the dough material resides in the films between the gas cells, one may set up a force balance between the biaxial stress in the films and the gas pressure in the cells. One might use here the analogy with the Laplace pressure, relating the gas pressure inside a bubble with the surface tension and the bubble radius. Analogously, for every cell, the film force (the product of the biaxial stress and half the film thickness) has to equal half the product of the excess pressure in the cell and the cell radius. Applying this to all cells in a proofing dough, one may conclude that the ratio between the biaxial dough stresses and the excess gas pressure in the cells has to equal the ratio of the gas fraction and the dough fraction in the system. The ratio of these fractions is about unity at the end of proofing, which means that one expects also  $\approx 5$  kPa for the gas pressure. This was indeed the order of magnitude found by Bailey (1955).

The model described above was set up to guide us in finding new routes for improvement of the baking performance of doughs. This is of particular importance in the case of preproofed frozen doughs, which suffer great losses in baking performance after long frozen storage. Such performance losses appear to be associated with small changes in gluten hydration of  $\approx 1\%$  over three weeks (Bot 2003). This article does not aim at providing a complete proof of the model, but aims at exploring whether osmotic effects are of the expected order of magnitude.

The model has two critical ingredients: the starch paste rheology, and the osmotic properties of the gluten. In a qualitative sense, it is easy to demonstrate that a starch granule dispersion can have

either a yield stress or be quite fluid over a relatively small range of water contents near close packing of the granules. The order of magnitude of the osmotic pressure of the gluten in the relevant hydration range, however, is not known, and only limited data on the properties of gliadin can be found in the literature (Burk 1938). The determination of these properties is not straightforward, unfortunately, because osmotic pressure measurements on materials like hydrated gluten are very uncommon. Because more advanced standard techniques do not seem appropriate for this type of material, a very basic approach was chosen to estimate the osmotic pressure of gluten for different gluten concentrations and handling procedures. Although the current approach has certain flaws, it serves its current purpose to assess whether the osmotic pressure has the correct order of magnitude, not to invalidate the physical model introduced above, and as a basis for later, more advanced studies.

## MATERIALS AND METHODS

Dialysis bags were prepared from dialysis tubes (width 3.2 cm when flat) that were cut in pieces  $\approx 10$  cm (Spectra/Por 4 molecular-porogen regenerated cellulose dialysis membrane, Spectrum Chromatography, Houston, TX). The molecular weight cut-off (MWCO) of the membrane is 12–14 kDa. This will keep the gluten inside the bag, but some lower molecular weight protein may diffuse out. The tubes were sealed at the bottom by using UHU Plus two-component adhesive, and heating for 15 min at 100°C in an oven. Subsequently, the mass of the bags was determined both in a dry state and in a hydrated state.

The bags were filled with 3–6 g of commercial wheat gluten (Sigma, lot 127H0169). The dry powder contains 80.7% protein, 6.4 wt% water and 7 wt% fat. The material was either added in a dry form, or as a gluten dough. Stresses in the dry state originating from the prehydration process (kneading) are avoided. Initially, prehydration was done manually. Later kneading was done in a more reproducible way using a farinograph (Brabender).

During hydration in a constant-atmosphere cabinet at 20°C, the bags were suspended in 550 mL of dextran solutions of different concentrations (Dextran T500, Pharmacia Biotech, lot 243862, molecular weight 464 kDa as determined by light scattering,  $M_w/M_n = 2.9$ ). Sodium azide was added at a 0.01 wt% level as a germicide. The composition of dextran solutions will not change significantly during the exchange of water with gluten, due to the large amount of dextran solution relative to the amount of wet gluten. The osmotic pressure  $\Pi$  of dextran as a function of concentration is known from the literature:  $\Pi = 730 \cdot (c/2.5)^{9/4}$  (Pa), where  $c$  is the dextran concentration in wt% (Koning et al 1993). To achieve osmotic pressures of 10–20 kPa in the dialysis bath, solutions containing 7–11 wt% dextran should be used. This is well above the overlap concentration (Smit et al 1992) and molecular weight effects of the dextran play no role in the present study.

By determining the hydration level of equilibrated gluten in a solution of known osmotic pressure, it is possible to determine the osmotic pressure in gluten as a function of concentration. Hydration of the bags was monitored for typically two months by measuring the weight of the bags. In general, initially dry samples hydrate over time, and prehydrated samples dehydrate over time, except when submerged in the least concentrated dextran baths. The pH of the dextran bath was measured at regular intervals, because the solubility (and thus the osmotic pressure) is rather dependent on pH (MacRitchie 1979).

After completion of the hydration experiment, samples were dried by heating them for two days in a vacuum oven at 100°C (some small holes were punched in the bags to allow air to escape from the bags). The dried samples were weighed.

For various specific experiments the above standard procedure was modified. For pH-dependent experiments, appropriate amounts of NaOH and HCl were added to the dextran solutions. The osmotic pressure of the dextran is not expected to be affected

because dextran is a neutral polymer. For the kneading experiments, a small systematic error was introduced in the determination of the dry matter content by using a normal oven instead of a vacuum oven. The correct dry weight was reconstructed by matching the level of hydration for relaxed gluten at the relevant pH and dextran concentration. This procedure did not affect any trends in the data because all samples were treated in the same way.

## RESULTS

### Effect of pH and Osmotic Pressure on Relaxed Gluten

Figure 2 shows a typical example of the change of the hydration level as a function of time for samples dialyzed against solutions containing different concentrations of dextran. The sample takes up water at the lowest dextran concentration, whereas all other samples lose water in time. The change in the hydration level occurs very gradually and the process takes weeks to be completed.

A series of preliminary experiments showed that two processes contribute to these slow changes. The first process dominates during the first two to three weeks and was related to exchange of water between the gluten and the dextran bath. The second process is related to a gradual change in pH during storage. Possible causes were slow hydrolysis of the gluten (by enzymatic degradation) or contamination of the gluten samples by microorganisms. Note, however, that if the pH reduction were caused by hydrolysis, it is not very likely that this would affect the osmotic pressure of the gluten for the same reason that dextran molecular weight does not affect osmotic pressure in the current experiments. At a gluten weight fraction of 0.3, the gluten is expected to be considerably above the overlap concentration. Possible microbiological degradation was nevertheless thwarted by adding sodium azide as a germicide, but the dose may have been insufficient to completely prevent

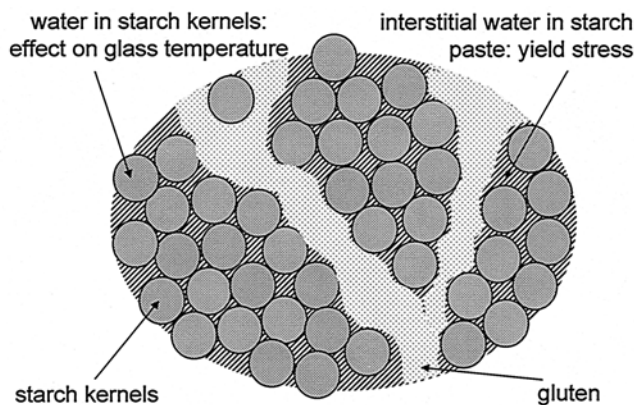


Fig. 1. Proposed water exchange process in dough. Interstitial water in the starch paste is exchanged with hydration water in the gluten.

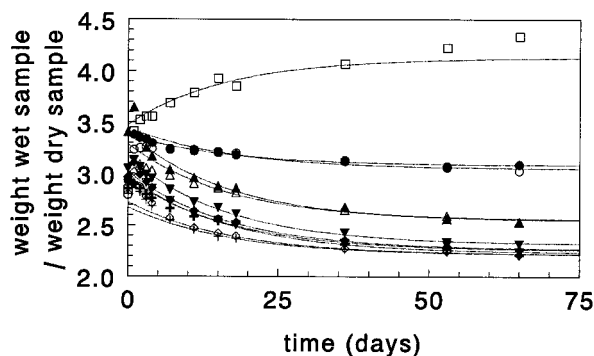


Fig. 2. Hydration of gluten as a function of time. Samples in a dextran bath of different dextran concentrations (wt%): 2 (□), 4 (●), 6 (▲), 8 (▼), 9 (◇), and 10 (+). Duplicated experiments are indicated by open and filled symbols.

microbiological activity over such long periods. From these preliminary experiments, it was concluded that, besides an increase of the sodium azide concentration, it is of major importance to monitor the pH of the dextran bath during the experiments.

For this reason, the first experiment consisted of constructing a diagram for the osmotic hydration level of gluten as a function of both the osmotic pressure of the dialysis bath (i.e., the dextran concentration) and the pH of the solution. To this end, the ratio of the weight of the hydrated gluten and the dry gluten was determined. To avoid interference of the water exchange processes during the first weeks of storage, the values were taken after a storage period of three weeks. The result is shown in Fig. 3, which features experimental data taken for samples that were prepared at pH 4.8, 5.2, and 5.6. The osmotic pressure is lower at higher pH, but increases more steeply with gluten concentration. Also plotted in Fig. 3 are profiles for osmotic pressure  $\Pi$  versus gluten weight fraction  $x$  for various pH values based on an empirical equation which was obtained on basis of experimental data in this section and some further experimental data that will be introduced later.

$$z = 6.80 \cdot \exp(-(x - 7.67)/6.25) \cdot \exp(-(y - 4.26)/0.83) + 1.86 \cdot 10^{-5} \cdot x^{0.53} \cdot y^{5.79} \quad (1)$$

where  $x$  is the dextran concentration in the dialysis bath in wt%,  $y$  is the pH of the dialysis bath, and  $z$  is the weight ratio of wet and dry gluten (not corrected for the presence of 7 wt% fat and 14 wt% water in the gluten powder). Equation 1 does not have any physical basis.

The cusp in the fit at  $\approx$  pH 5.5 at higher gluten weight fractions in Fig. 3 is tentatively attributed to the minimum in solubility observed for gluten proteins as a function of pH (MacRitchie 1979). It is likely that this minimum coincides with the isoelectric point of gluten, although higher values have been reported in the literature as well (Wu and Dimler 1963a,b).

Note that the accuracy of the original experimental data leading to Fig. 3 is limited, and that the fitting procedure induces some skewing of the  $\Pi$ - $x$  profile. Systematic errors in gluten weight fraction may be as large as 15%. As the exact value for the osmotic pressure at a given condition will depend on the chemical details of the gluten (i.e., wheat cultivar) under study (MacRitchie 1979), this inaccuracy is taken for granted. Thus, Fig. 3 should be treated with care.

Nevertheless, an interesting confirmation of the model proposed earlier can be derived from this plot. Osmotic pressures of 10–20 kPa are typically attained for gluten weight fractions of 0.4. Indeed, similar concentrations are thought to be present in a dough (Bushuk 1966; Bloksma and Bushuk 1988). It should be kept in mind, however, that this confirmation is semiquantitative also.

### Effect of Kneading on Osmotic Properties of Gluten

The second experiment focused on the processes taking place during the two to three weeks before equilibrium was achieved. First, it should be realized that this very long time scale is rather peculiar. The diffusion coefficient of water is  $2.26 \cdot 10^{-9}$  m<sup>2</sup>/sec (Atkins 1989), a day lasts  $0.864 \cdot 10^5$  sec, so the average distance over which a water molecule can be expected to move in the course of one day is  $\sqrt{(2.26 \cdot 10^{-9} \text{ m}^2/\text{sec} \cdot 0.864 \cdot 10^5 \text{ sec})} = 0.0139$  m, or just over a centimeter. This is well in excess of the thickness of the dialysis bags. Thus, equilibration is expected to take place within one day, rather than within several weeks.

The most obvious alternative explanation is that the slow process is related to the transport of low molecular weight gluten out of the dialysis bags and not to the transport of water. There are two objections to this interpretation. First, gluten itself does not come in units with molecular weights <40 kDa, which is well above the MWCO of the membrane (but of course, other ingredients could be involved). Second, this interpretation would suggest that the hydration level would always decrease for long equilibration times. However, Fig. 2 shows that this is not always the

case. For this reason, the explanation of the slow equilibration process, in terms of low molecular weight compounds from the gluten that diffuse out of the dialysis bag, is rejected.

Alternatively, one could think of very slow processes taking place in the gluten itself. This could involve the refolding processes of secondary structures in the glutenin proteins that were unfolded during kneading (Khatkar and Schofield 1997; van Velzen et al 2003). If these processes are at the origin of the slow water exchange phenomenon, it is expected that the degree of kneading of the gluten determines the extent to which the exchange process will take place. To investigate this hypothesis, the hydration behavior was investigated of kneaded gluten samples that had been subjected to different degrees of kneading.

The results are plotted in Fig. 4. Three gluten samples were kneaded for different periods in a farinograph (7.5, 17, and 60 min in a 300-g mixer at 300 BU). The sample that was kneaded for the longest period contains the largest amount of water. For longer periods, all three hydration curves superimpose, and the variation is relatively limited. This clearly suggests that the differences in dehydration behavior during the first  $\approx 25$  days are due to the preparation procedure, most probably involving stretching of the gluten sample. These differences diminish during storage, and the curves superimpose after  $\approx 25$  days. Similar results have been observed for different samples and different osmotic pressures of the dextran bath.

The results in Figs. 2 and 4 suggest that gluten can hydrate to a larger extent in the stretched state than in an unstretched conformation. The level of hydration of the gluten slowly changes over two to three weeks of storage. A reduction in the hydration of the gluten might be understood in terms of refolding of secondary protein structures of the gluten (van Velzen et al 2003), which is likely to be accompanied by water expulsion. The rate of the process does not seem to depend very much on pH and osmotic pressure in the present range of experimental conditions. The process might relate to the observation that the water activity of a dough increases slightly during relaxation (Czuchajowska et al 1989).

## DISCUSSION AND CONCLUSIONS

The present results should be considered as a first attempt to determine the osmotic properties of gluten. It should be realized that the measurement of osmotic properties of this type of material is quite uncommon, and there are no well-established methods available to do these experiments. As such, the present experiments leave much room for further improvement.

The osmotic properties were investigated over relatively long periods in the current experiments, which caused some problems with a drift in pH in specific experiments. This drift is not considered to be a major problem in the current experiments, as the characteristic times relevant for proofing and baking are actually quite short and, therefore, short-term behavior of gluten is considered far more important. Experiments on frozen storage of dough suggest that rather minor changes  $<1\%$  in water distribution may result already in considerable differences in dough performance (Bot 2003). Such changes take place over much shorter time periods, and the present experiments suggest that these changes can occur within less than one day, over which changes in pH in the current experiments can be neglected. The long time periods over which the present systems were followed were merely intended to show that the different gluten systems that were prepared using different kneading regimes relax to the same state of hydration over time when subjected to identical storage conditions and because changes in state of hydration are much more difficult to demonstrate over shorter periods. Furthermore, the hydration behavior of relaxed samples with slightly drifted pH was consistent with the hydration behavior in Fig. 3, which shows samples that were prepared at a specific pH.

A second issue deserving closer attention is the effect of electrolytes. Gluten is a polyelectrolyte, giving rise to a Donnan equilibrium for the ions in the dialysis system. In such a system, the true osmotic pressure of the gluten (pressure of the polymeric chain and not that of the counter ions) is only obtained at high salt con-

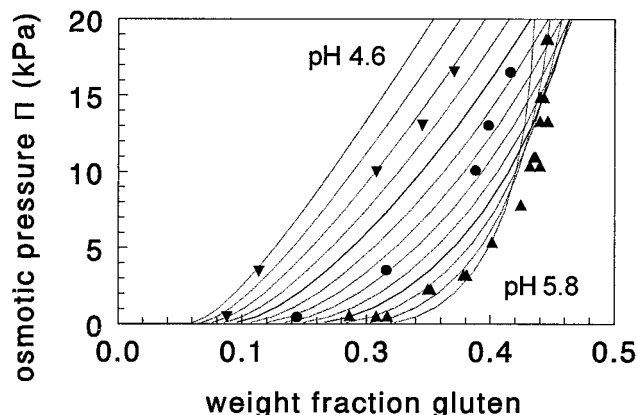


Fig. 3. Lines represent the osmotic pressure  $\Pi$  as a function of gluten weight fraction for pH values of the solution at 4.6–5.8 at 0.1 intervals. Solid curves = fits for pH 5.0, 5.5. Lines cusp back near pH 5.5. Also shown are experimental data at various pH values: 4.8 ( $\nabla$ ), 5.2 ( $\bullet$ ), 5.6 ( $\blacktriangle$ ). Gluten concentration corrected for fat and water present in commercial gluten powder.

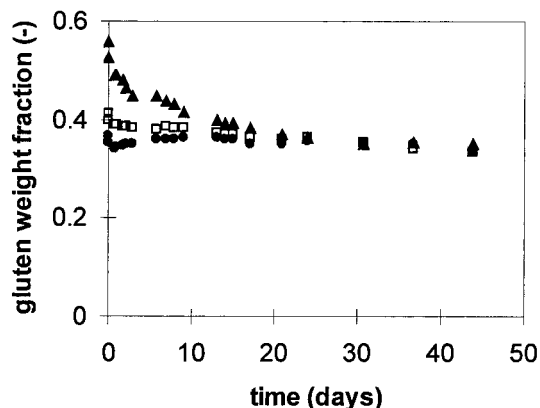


Fig. 4. Effect of various degrees of kneading on the (de)hydration of gluten at  $\Pi$  10.0 kPa and pH 5.5. A small systematic correction (equal to all three samples) according to Equation 1 is applied to account for a shift in the pH of the dextran bath during storage: short kneading ( $\blacktriangledown$ ), intermediate kneading ( $\square$ ), long kneading ( $\bullet$ ).

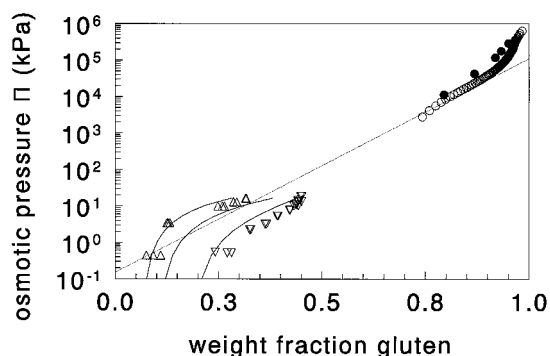


Fig. 5. Osmotic pressure of gluten as a function of gluten concentration as derived from various techniques. Osmotic pressure data from present study at pH 4.8 and 5.6, respectively ( $\Delta, \nabla$ ). Vapor pressure data taken from Bushuk and Winkler (1957) ( $\bullet$ ). NMR data taken from Cheria and Chinachoti (1996) ( $\circ$ ). Fits to osmotic pressure data Fig. 3, from left to right pH 4.8, 5.2, 5.6 (—).

centration (Atkins 1989). (Note that addition of salt would not lead to an unrealistic condition because a typical dough formulation contains 2 g of salt in 60 g of water.) Future experiments should aim at separating these two contributions to osmotic pressure.

The results suggest that gluten hydrates more efficiently in the stretched state than in an unstretched conformation. One might identify stretching with the breakdown of secondary protein structures and unstretching with the formation of these structures. The latter process is apparently very slow and takes typically about three weeks. The rate of the process does not seem to depend very much on pH and osmotic pressure in the present range of experimental conditions. The present experiments show that the osmotic pressure of dough decreases with time, and that water activities go up. But it should be noted that the effects are too small to be detected by water vapor measurements. Thus, it is not possible to use the present data as direct support for earlier observations that the water activity of a dough seems to increase slightly during relaxation (Czuchajowska et al 1989). Nevertheless, Fig. 5 shows the present osmotic pressure data for relaxed gluten are qualitatively in line with water activity data derived from vapor pressure data (Bushuk and Winkler 1957) and NMR (Cherian and Chinachoti 1996), although a more quantitative comparison should explicitly take into account effects of pH, etc.

Summarizing, the osmotic properties of gluten are consistent with a model in which the dough is described as a composite system where the exchange of water between the hydrated gluten and the starch paste determines the rheology of the dough.

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