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Introduction

Wheat gluten proteins form a complex protein network, not soluble in water, which displays a unique viscoelastic behavior. Gluten is composed of a blend of gliadins (gli) and glutenins (glu) and the respective roles of the two classes of proteins are not well understood. Recently, we have developed an extraction method to obtain protein extracts with different glu/gli mass ratio R in order to study the interaction between these two classes of proteins. In an ethanol/water mixture, these protein blends display an upper critical solution temperature. The aim of this experiment was to study the structure of protein blends in an ethanolo/water solvent and their dependence with temperature, concentration and composition (R) .

Experimental and Results

Firstly an Ultra Small Angle X-ray Scattering (USAXS) configuration was used to investigate samples during their liquid-liquid phase separation. A Linkam heating stage (THMS600/TMS94) was used to quench samples to different final temperatures with a cooling rate of 80 K min^{-1}. Measurements were carried out as a function of time for 45 sec for samples with different R (R = 0.04, R = 0.8 and R = 1.9) at a total protein concentration C = 237 $g.l^{-1}$. For R=0.04 and R=1.9, once samples are phase- eparated, the USAXS spectra display a broad peak at q* and a q^{-4} slope at high q (fig. 1). We characterized the spectra by the position of the peak that defines a characteristic distance ($\xi=2\pi/q^*$) between the two phases formed, and the prefactor of the

q^{-4} evolution (Porod regime: $I(q) = 2\pi\Delta\rho^2 S/Vq^{-4}$) that defines the contrast between the two phases ($\Delta\rho^2$) times the specific area (S/V).

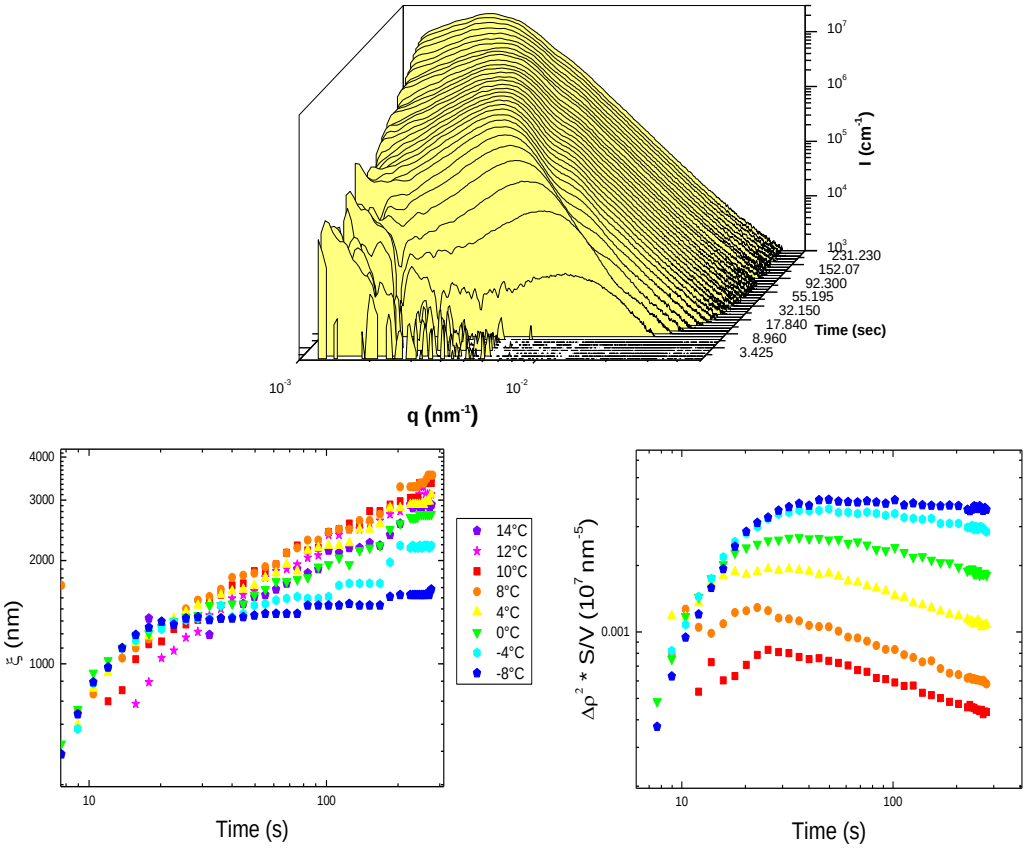

Fig.1: 3D plot of the intensity as a function of *q* and time during a temperature quench at 4°C for the sample with R=0.8 (top). Characteristic length ξ (bottom left) and contrast times specific area ($\Delta\rho^2$ S/V) (bottom right) versus time for different quenching temperatures.

Before 20s, ξ and $\Delta\rho^2 S/V$ increase rapidly wathever the quenching temperature. Then the kinetics is slowed down. For temperatures quenches above 0°C the evolutions follows a growth process model ($\xi \propto t^{1/3}$) whereas an arrested state is observed below 0°C. We observed that both the liquid-liquid phase separation temperature and the arrested state temperature increase with the glu/gli ratio and is associated to the increase of molar mass. For R=1.9, the spectral signature of the liquid-liquid phase separation is different. No peak is observed, but the scattering profile display a bump that can be fitted by a Debye Buëche model ($I(q) = C/(1+(\Xi q)^2)^2$) defining thus a correlation length Ξ. The time evolution of this length does not follow the growth model after the rapid increase and has to be further investigated.

In a second time, samples with different ratio R were investigated at room temperature in a wide range concentration from C=10 $g.l^{-1}$ to C=400 $g.l^{-1}$ using either a flow through cell (C=10$g.l^{-1}$) or capillaries (other concentrations). The SAXS measurements were carried out with three different distance sample-detector (D) configurations, D = 1.5 m, 7 m, and 30 m, covering a *q*-range from 2.10^{-3} to 7 nm^{-1}. Fig.2 (left) displays spectra measured in the dilute regime (C=10 $g.l^{-1}$). For all ratios, a q^{-2} slope is measured at high q, indicating a local polymer statistic in a theta solvent. The mean radius of gyration, well defined for the lowest ratio R=0.04, is 4 nm. The low q slope increases with R and is associated to the presence of glutenin polymers.

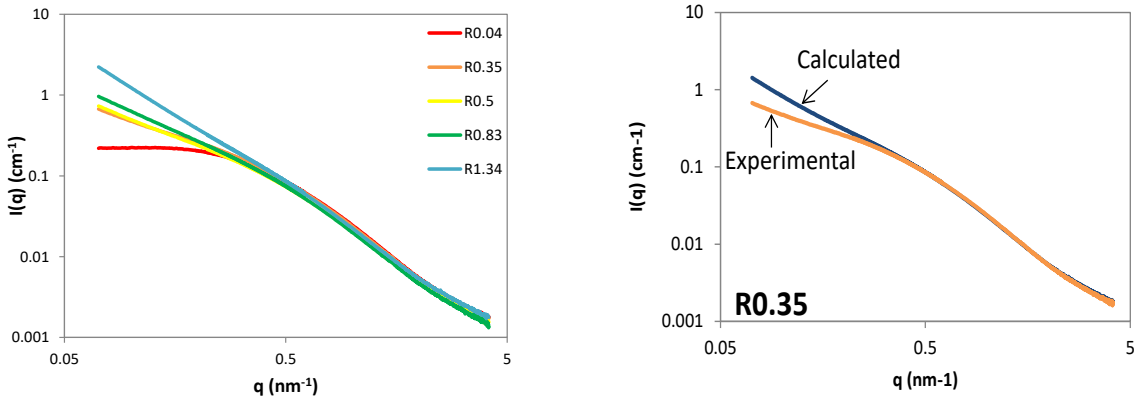

Fig.2: (Left) SAXS spectra of dilute samples (C=10g.l-1) at different ratio R, measured at room temperature (Right) Comparison of experimental and calculated spectra from mixture of two samples.

Finally, to investigate the interaction between gliadin and glutenin, the structure of dilute (C=10 $g.l^{-1}$) mixtures of gliadin-rich (R0.04) and glutenin-rich (R1.3) samples were investigated. It was shown that systematically the experimental spectra of the mixtures display lower intensity at low q than expected by a linear combination of spectra measured for R0.04 and R1.3 (Fig.2 right). The non-additivity of the signals indicates an interaction between gliadin and glutenin that would result in smaller scattering objects.