In vitro gastric digestion study of casein gel structure in H_2O and D_2O

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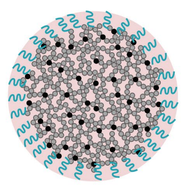
BACKGROUND

Understanding of the role of food microstructure to control nutrient release during digestion offers a unique opportunity to design food structures targeted towards a specific health function.

Casein micelle



Different modes of casein gelation leads to the formation of unique nano- and microstructures.

Heavy water (D_2O) is a widely used solvent for neutron scattering and it has been assumed that replacing hydrogen with deuterium would not influence gel formation and structural elements within the gel protein network.

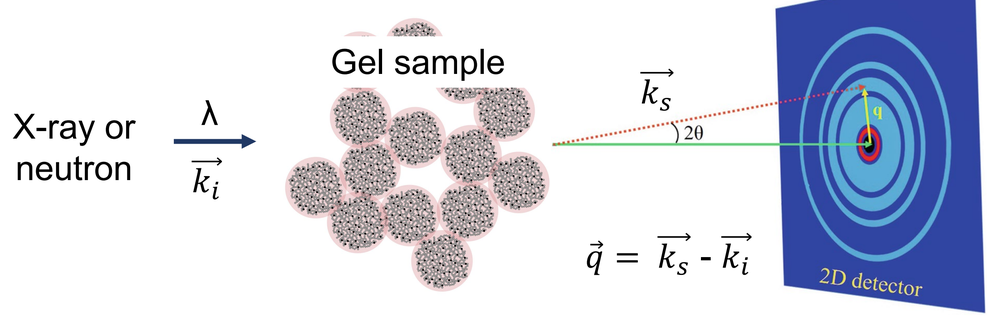
The aim of this study was to examine the influence of solvent environment (D_2O vs H_2O) on the structural formation of a rennet-induced (RG) protein gel, and subsequent structural devolution under simulated *in vitro* gastric digestion conditions.

METHODOLOGY

Structural changes of a gel were prepared and analysed both in H_2O and D_2O during simulated *in vitro* digestion. The digestion protocol was performed with and without pepsin to elucidate the respective contribution of both the acidic simulated gastric fluid and enzymatic actions to structural deformation.

Rheological characteristics were followed by monitoring the storage (G′, Pa) and the loss modulus (G″, Pa) at a frequency of 1 Hz. A frequency sweep was performed where G′ and G″ were measured as a function of frequency from 0.1 to 10 Hz at a constant strain of 0.1%, where the ratio between G′ and frequency is related to the nature and strength of a gel.

Ultra-small (USANS) and small-angle neutron scattering (SANS) enabled structural changes of casein at nano (CCP), micro (micelle) and macro (micellar gel) scales to be followed as a function of digestion time (0, 15, 120 minutes).



Transmission electron microscopy (TEM) were used to visualise the casein gel and digesta samples collected.

RESULTS

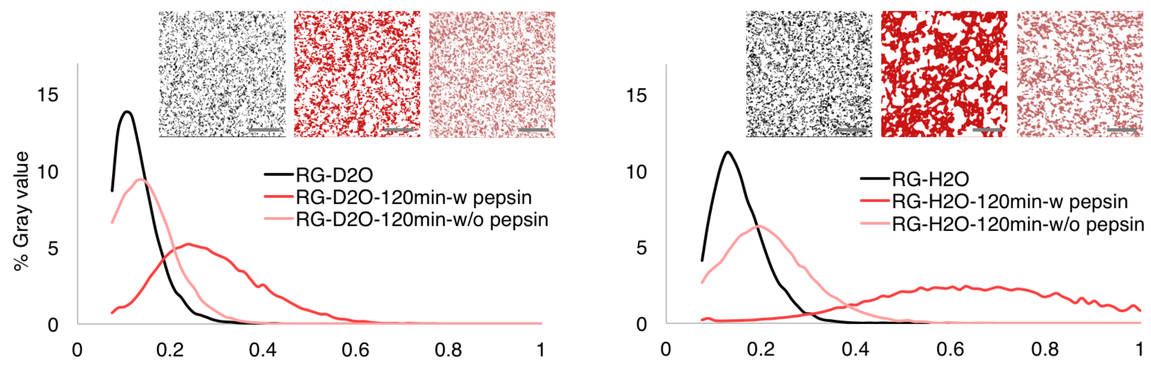
Rheology and particle size distribution upon mastication

- Gels prepared in D_2O exhibit earlier onset of gelation, are firmer yet more brittle compared to H_2O.
- The lower tan δ (the loss tangent, G″/G′) for RG-H_2O indicates higher gel elasticity compared to RG-D_2O

Characterisation of initial and digesta gel structure

- **Protein micelle aggregates of the gel structure**

TEM images suggests a narrower particle size distribution in D_2O where gels have a finer and more homogeneous network structure. The change in microstructure was more pronounced in the presence of pepsin.



D_2O gels indicated a densely packed aggregate network structure, whereas gels formulated with H_2O had a looser and less dense network on a micro-scale.

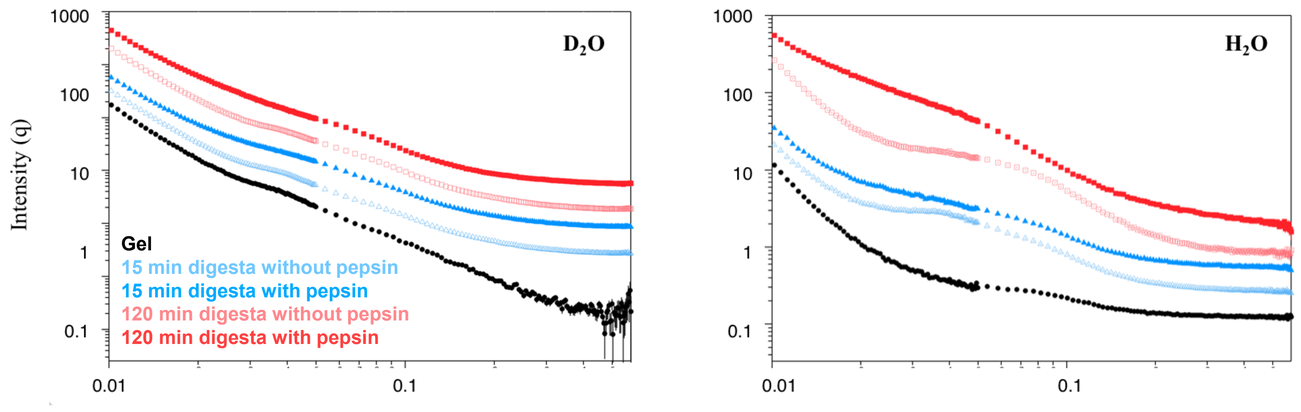
- **The size of micelle aggregates fused together to form the network strands**

The apparent aggregate size increased from 153 nm to 501 nm with pepsin in RG-H_2O; while decreased significantly between the initial gel and 15 min digesta for RG-D_2O.

		With pepsin		Without pepsin	
Gel type	**Initial Gel**	**15 min**	**120 min**	**15 min**	**120 min**
RG-D2O	150.1 ± 4.3^{cd}	114.0 ± 3.6^{ab}	115.5 ± 3.5^{ab}	100.2 ± 2.8^{a}	158.5 ± 4.3^{d}
RG-H_2O	152.8 ± 6.1^{d}	501.2 ± 3.5^{g}	314.0 ± 1.5^{f}	293.0 ± 32.9^{f}	212.0 ± 5.4^{e}

- **Colloidal calcium phosphate (CCP) nanoclusters located within the interior of the micelle**

A more pronounced neutron signal was observed for samples in D_2O, indicating a more dense internal structure and stronger hydrogen bonding. This high-q regions allows for the estimation of partial CCP dissolution where a wider inflection indicates less dense CCP nanoclusters, and shift to a smaller q suggests an increase in CCP spacing. RG in H_2O became broader and less intense due to higher solubilisation of CCP.



CONCLUSIONS

- This is the first study to monitor gastric devolution of casein gels using USANS and SANS.
- The first study examining the structural changes of a gel both in H_2O and D_2O during *in vitro* digestion.
- The strength of the hydrophobic interactions between the proteins of a gel formed in D_2O are greater than in the H_2O environment leading to a higher number of aggregates and/or cross-linking junction points. As a result, D_2O gels have a **finer** and more **homogeneous** network structure with decreased porosity and **smaller pores.**
- The firmer structured gels formed in D_2O have a **brittle** structure that facilitated gel disintegration and consequently digestibility.