# MODULATING PROTEIN INTERACTION ON A MOLECULAR AND MICROSTRUCTURAL LEVEL FOR TEXTURE CONTROL IN PROTEIN BASED GELS

A.H. Martin[1,2], D. Baigts Allende[1], C.D. Munialo[1,3], V. Urbonaite[1,3], L. Pouvreau[1,4] , H.H.J de Jongh[1,3]

[1]Top Institute Food & Nutrition (TIFN), PO Box 557, 6700 AN Wageningen, the Netherlands
[2]TNO, PO Box 360, 3700 AJ Zeist, The Netherlands
[3]Wageningen University and Research centre, Food Physics Group, PO Box 8129, 6700 EV Wageningen, The Netherlands
[4]NIZO food research, PO Box 20, 6710 BA Ede, The Netherlands

## 1 INTRODUCTION

The exploration of microstructures and textures of protein based systems is essential to understand (oral) breakdown properties and thereby textural aspects, or macroscopic functionalities such as water holding. Upon structure breakdown, the applied energy (W) is primarily directed towards fracture ($W_f$) for particulate gels. For stranded gels the applied energy is either elastically stored ($W_s$) or dissipated ($W_d$). The energy balance can then be denoted as $W= W_s+W_d+W_f$.[1] The fore mentioned mechanical properties have been shown to relate to texture perception that may go from 'spreadable' to 'crumbly' for particulate and stranded networks, respectively.[2,3] Understanding of how to control the energy balance with regard to microstructure and texture perception is of key importance for the food industry to modulate their products towards desired sensory properties, especially when new (alternative) protein sources are involved.

Texture is to a large extent determined by the properties of the structural elements and their mutual interactions. These structural aspects stem from the aggregation behaviour of the individual proteins, which in turn is determined by the molecular characteristics and their ability to interact during processing. Subsequently, an assembled microstructure may consist of molecules (nm) and protein molecules that are assembled into flexible fine stranded structure elements (0.1-5 μm), and coarse stranded or particle shaped structure elements (5-50 μm). At any length scale, protein (structure elements) can be subjected to (food grade) chemical or enzymatic modification to tune their function in a spatial network. Up to now, this was however only done for proteins at a molecular level. [4-6]

Functionalization of specific structure elements and their interactions is a tool in understanding which length scales are relevant for tuning texture and breakdown properties. The type, shape and dimensions of these structure elements determine the efficiency and gel strength of the established spatial network. Hence, this work sketches the potential of different types of structure elements made from gelatin, whey protein and soy protein to direct macroscopic behaviour. On a molecular scale, modulation of gelatine is performed to alter its assembly into fine stranded networks and the subsequent macroscopic breakdown behaviour. Modification of whey protein is performed on an aggregate level to show the efficiency of thiolation of different supramolecular structures (fibrillar and amorphous aggregates) with regard to gelation propensity. On a

microstructural level, particulate soy protein networks are tuned through the presence of calcium salts for their fracture behaviour. We show that control over texture and macroscopic properties can be obtained by modulation of protein functionality at different levels of protein organization.

## 2 METHODS AND RESULTS

### 2.1 Steric hindrance in the assembly in fine-stranded networks

For fine-stranded protein gels the structural building blocks are typically linear and have limited thickness. Gel strength is dominated by the flexibility (thickness) of the strands, the mesh size of the stranded network, and the interaction energy in the assembly points of the individual strand making up the spatial structure. The presence of sugar moieties cross-linked to gelatine molecules may impair with strand assembly, both kinetically and interaction energy. Recently we have shown that one can introduce significant numbers of such steric moieties along a gelatine molecule using the Maillard reaction[4] without losing the major functional property of adopting triple helix configurations, believed to be essential for efficient network formation. Also differences in charge appear to have a limited impact on the propensity and kinetics of gel formation[5]. From small deformation rheology (not shown), or when applying large deformation rheology (Figure 1A) it becomes evident that at a mechanical level the introduction of steric groups is very effective, as illustrated by the gradual lower Young's modulus with increasing degree of modification (DM). Combined with calorimetric and structural characterization we have been able to postulate the hypothesis that it is not the number of junctions that is affected by steric modulation, but the interaction energy of the junctions[7].

Figure 1B shows that despite the decreasing Young's modulus with increasing DM, the recoverable energy is not affected. Previously it has been shown that recoverable energy of semi-solid protein gels was directly related to the sensory attribute "crumbliness".[8] Evidently from this work it can be concluded that Maillardation of gelatines are likely not to lead to changes on this sensory property. The true fracture stress and strain (Figure 1C and D), do again follow the same dependence to DM as the Young's modulus. These rheological properties have been related to the number of particles formed during for example oral processing and thereby affecting indirectly the texture attribute "spreadability", an important pre-requisite for perceptions like creamy.[8] As for the melting behaviour of the Maillardated gelatines the enthalpic changes (results not shown) follow the reduced fracture stress, while the transition temperatures are not affected, it can be concluded that already limited degrees of modification will affect this latter texture attribute, while evaluation up to the fracture point might be preserved.

The example given above illustrates how food-grade modification of gelatines provides important consequences for the energy balance in gelatine gels. Maillardation has apparently no significant effect on the stored energy ($W_s$), as the recoverable energy is not affected by DM. The fracture energy ($W_f$) is gradually reduced with DM. The impact of Maillardation on the dissipated energy ($W_d$) is not clear at the moment and is difficult to assess. It could be speculated that ligated sugar moieties facing entrapped serum in the network would increase the viscous flow within the pores and thereby would increase the friction contribution. To substantiate this a more in-depth study would be required.

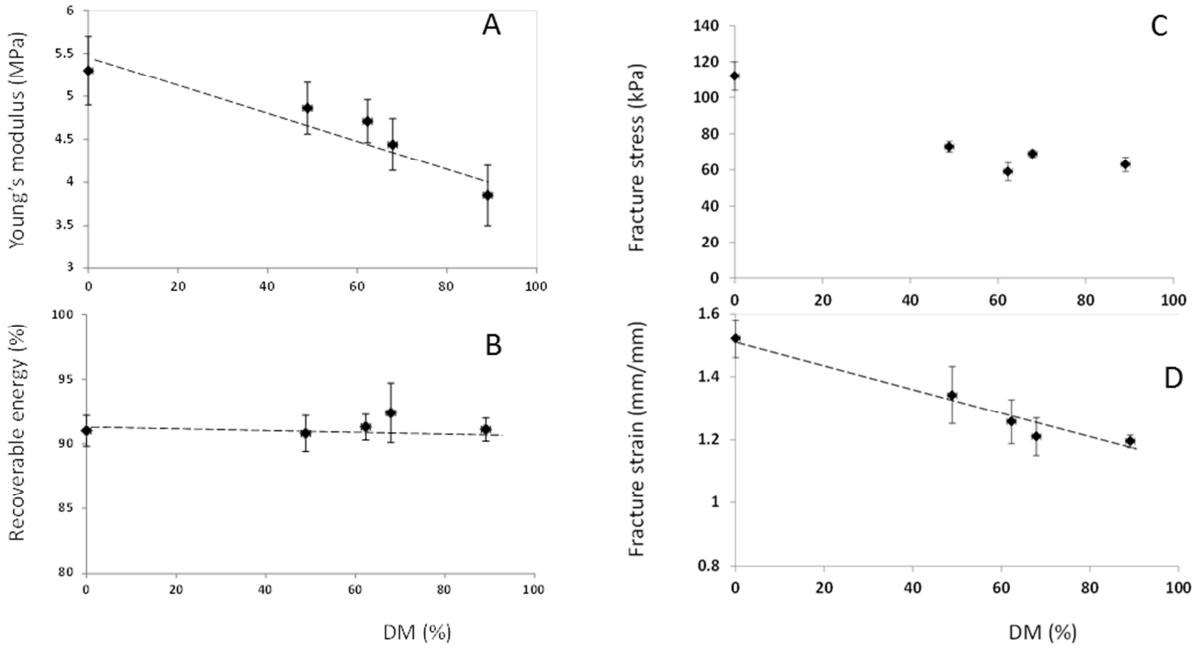


**Figure 1** *Young's modulus (A), recoverable energy (B) from uniaxial compression experiments using a deformation up to 90% of the fracture strain, true fracture stress (C) and fracture strain (D) of gelatine gels with different DM of available lysines that can undergo a Maillard reaction with glucose (5% w/v) at 20 °C (pH 6.5). Deformation speed was 1 mm/s.*

### 2.2 Chemical cross-linking of fibrils to further boost gel strength

It has been documented that the type and shape of aggregates formed in a gelling process determine the efficiency and gel strength of the established network. Typically, the critical (minimum) gelling concentration is lower for fibrillar aggregates compared to more amorphous ones. Amorphous aggregates (micrometer scale) and fibrillar aggregates (nanometer scale) can be assembled into a spatial network using cold-set gelation, e.g. using GDL (glucono-δ-lactone). Whereas for amorphous aggregates this has been widely studied[9,10], for fibrils this is not the case. Due to their differences in dimensions and hence the interactions between the structure elements, gelation propensity and network properties will vary significantly between amorphous and fibrillar aggregates.

Intermolecular crosslinking has appeared to be very relevant in determining gel strength. Typically, when additional functional groups were introduced that would enhance intermolecular chemical crosslinking higher gel strengths were observed. Alternatively, when chemical reactivity was suppressed weaker gels were obtained.[10] An effective way to increase the potential of chemical cross-linking between structure elements, is the introduction of chemical reactive thiol groups. Thiolation of whey protein on a molecular level was shown to affect the aggregation mechanism or propensity and might affect thereby the development and final functionality of the formed network.[5,6,11] Recently we have circumvented this latter issue by modifying whey protein on an aggregate level (either fibrillar or amorphous aggregates) introducing a (limited) number of thiol groups per structural building block using S-acetylmercaptosuccinic anhydride (S-AMSA) as previously described[12]. Thiol groups were successfully linked to both types of aggregates, as the number of thiol groups increased drastically (see Table 1). As a result, the chemical

reactivity was boosted significantly by the thiolation, as established from the SEI-index.[13] These two types of aggregates with and without modification were used as building blocks to form a spatial network. From Figure 2A it can be seen that the concentration dependence of G' is almost doubled by introducing additional chemical reactivity. Also for amorphous aggregates (see Figure 2B) G' increases as a result of thiolation although the effect for fibrillar aggregates is far more pronounced. Moreover, at similar protein concentration, fibrillar aggregates form much stronger gels than amorphous aggregates. From this work it can be suggested that, in addition to tuning the spatial dimensionality of the building block, the network forming properties can be further boosted by thiolation, especially when the building blocks have a more anisotropic structure. In this way, protein interaction on a microstructural level can be tuned very efficiently. In doing so, gel properties like firmness or fracture stress can be easily tuned bearing a direct impact on the afore discussed energy balance.

**Table 1** *Number of thiol groups (mM/mM protein) for native, unmodified (U) and modified (SX) fibrillar (F) and amorphous (A) aggregates of whey protein, as determined by OPA assay*

| | Number of thiol groups |
|---|---|
| Native WPI | 0.20±0.03 |
| UF | 0.60±0.07 |
| UA | 0.20±0.02 |
| SXF | 1.76±0.02 |
| SXA | 1.57±0.01 |

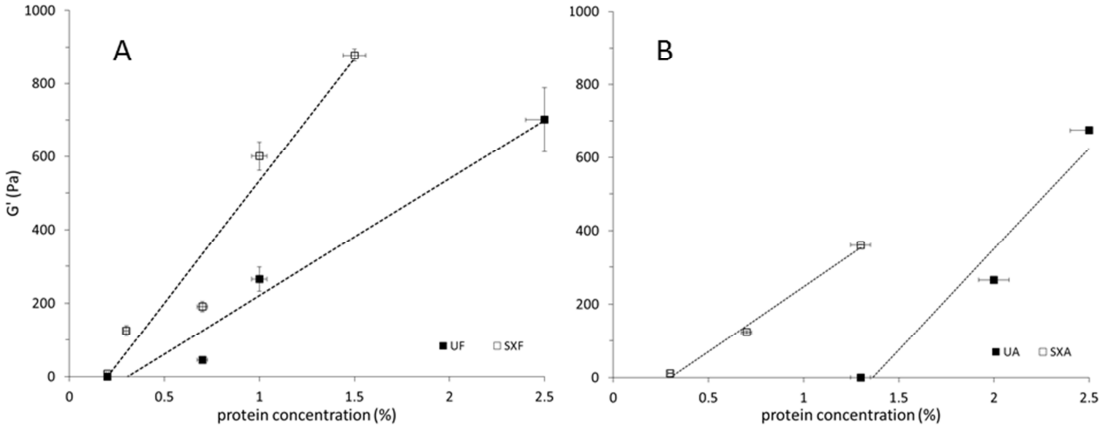


**Figure 2** *The storage modulus (G') as a function of the protein concentration for A) fibrillar (F) aggregates and B) amorphous (A) aggregates either unmodified (U) or thiolated (SX)*

### 2.3 Selecting the right ions to balance fracture properties and water holding capacity

Serum release or water expelled from the protein network during compression (e.g. during oral processing) contributes to dissipated energy in the energy balance equation. Opposite to serum release is the extent to retain water or water holding, which in literature is

generally accepted to be set by the network (micro)structure. Hence, following the energy balance, the microstructure determines both mechanical properties and release of water.[14] However, we postulate that the microstructure as such is not the determining factor for the water holding capacity of soy protein gels. To prove this, particulate soy protein networks are tuned on a microstructural level through the presence of different calcium salts and compared for their microstructure, mechanical properties and water holding capacity. Water holding capacity is measured using an adapted method of Kocher and Foegeding[15] in which the sample is placed in a micro-centrifuge and the speed of centrifugation is increasing stepwise (20-1000g for 10 min.). This approach allows us to not only to determine the amount of water remaining in the gel after centrifugation but also the kinetics of water expulsion as a function of centrifugational force applied.

Heat-set soy protein gels were prepared from native soy protein isolate (SPI) in the presence of two calcium salts ($CaCl_2$ and $CaSO_4$) as a trigger for protein aggregation. Whereas $CaCl_2$ is highly soluble in water, $CaSO_4$ only has a limited solubility at room temperature. Heating in the presence of the two calcium salts resulted in particulate soy protein networks (as confirmed by CLSM) with different microstructure (see Figure 3) in terms of pore size and strand thickness/density. These differences can be partially explained by the fact that the soluble calcium from $CaCl_2$ binds to the protein instantly while the poorly soluble calcium from $CaSO_4$ is released more slowly during heating creating a much more dense network.

Differences in the mechanical properties of the gels can be directly related to the microstructure. The more dense network formed with $CaSO_4$ gives a firmer gel with a higher Young's modulus and higher fracture stress than gels triggered by with $CaCl_2$ (see Table 2). With regard to the energy balance, for $CaSO_4$ the energy is more directed towards fracture instead of storage as is indicated by the lower recoverable energy and lower fracture strain (not shown). However, despite the differences in microstructure and mechanical properties, the water holding capacity (at maximum centrifugation speed) for the two protein networks is very comparable (see Table 2). A relevant factor appeared to be the kinetics at which water is released from the gel as $CaCl_2$ induced gels loose water faster upon applied g-force than $CaSO_4$ induced gels. This corresponds directly with the more dense structure of the
$CaSO_4$ gels.

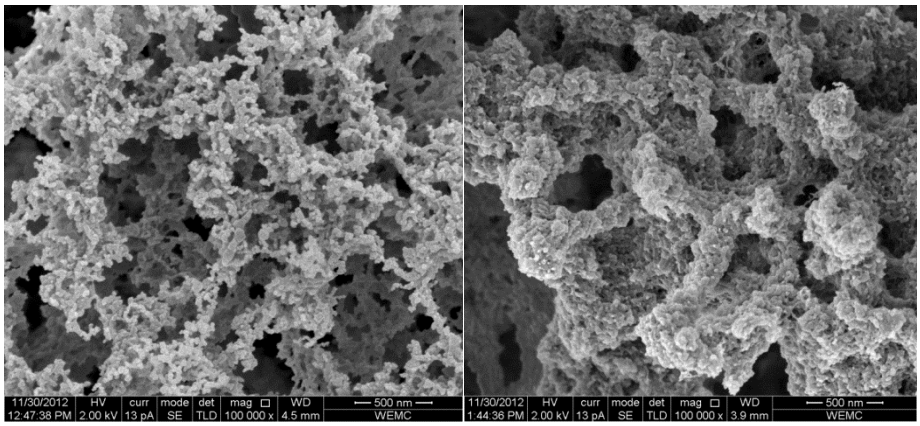


**Figure 3** *Microstructure (imaged by scanning electron microscopy (SEM)) of 100mM $CaCl_2$ (left) and $CaSO_4$ (right) induced 10% w/w soy protein gels heated for 30 minutes at 95°C*

**Table 2** *Rheological properties and water holding capacity (WHC) of calcium induced heat set soy protein gels (10%)*

| | $CaCl_2$ | $CaSO_4$ |
|---|---|---|
| Young's modulus (kPa) | 10.3±0.02 | 16.6±3.5 |
| Fracture stress (kPa) | 4.5±0.3 | 13.7±0.3 |
| Recoverable Energy (%) | 58.3±11.8 | 40.5±1.5 |
| WHC (%) | 43.8±1.1 | 41.1±1.5 |

Stepping down a length scale from microstructure to the formation of the structural elements, we have observed that besides the availability (solubility) of the calcium ion also the type of anion had an effect on the soy protein aggregation. Studying the aggregation mechanism at dilute protein concentration in the presence of calcium salts, sulphate resulted in smaller aggregates than the chloride anion. Subsequently, these smaller aggregates assemble in such a way that more dense and thicker strands and hence a denser network is formed for calcium sulphate.

In summary, for soy protein both the formation of structure elements and the subsequent assembly into a spatial network can effectively be tuned by the addition of different calcium salts. Modulation of protein interactions resulted in differences in aggregate formation, microstructure and hence rheological properties. Whereas water holding capacity cannot directly be related to microstructure as such, we have shown that the formation and type of aggregates influence the kinetics of the water expelled from the network.

## 3 CONCLUSION

Modification and hence functionalization of protein can be achieved at levels varying from molecular scale (nm) to structure element (μm) to tune texture and breakdown properties of protein networks. Examples were given on the modification of gelatin, whey protein aggregates and soy protein microstructure and this shows that modulation of protein interaction can be achieved resulting in shifts in the energy balance equation and hence oral breakdown properties.

## References


1 T. van Vliet and P. Walstra, in Food Colloids and Polymer, Stability and Mechanical Properties. (Royal Society of Chemistry, Cambridge, 1993), 175-190

2 L. v.d. Berg, T. van Vliet, E. van der Linden, M.A.J.S van Boekel and F. v.d. Velde Food Biophysics 2008 **3**, 198.

3 L. v.d. Berg, A.L. Carolas, T. van Vliet, E. van der Linden, M.A.J.S van Boekel and F. v.d. Velde Food Hydrocoll 2008 **22**, 1404.

4 K. Broersen, A.G.J. Voragen, R.J. Hamer and H.H.J. de Jongh, *Biotechnology and Bioengineering* 2004, **86**, 78.

5 E.D. Strange, V.H. Holsinger and D.H. Kleyn, *J. Agric.Food Chem.* 1996, **44**, 54

6 S.C. Kim, N.F. Olson and T. Richardson, *Milchwissenschaft* 1990, **45**, 627.

7 D. Baigts Allende and H.H.J de Jongh, submitted to *Food Biophysics* (2013)
8 H.H.J de Jongh, *Food Biophysics* 2011, **6**, 303.
9 A.C. Alting, R.J. Hamer, C.G. de Kruif, M. Paques and R.W. Visschers, *J Agric Food Chem*, 2003, **51**, 3150.
10 A.C. Alting, M. Weijers, M.A. Cohen Stuart. R.J. Hamer, K.C.G. de Kruijf, and R.W. Visschers, *J. Agric.Food Chem*. 2004, **52**, 623.
11 K. Broersen, A.M.M. Van Teeffelen, A. Vries, A.G.J. Voragen, R.J. Hamer, and H.H.J. de Jongh, *J. Agric. Food Chem*. 2006, **54**, 5166.
12 I.M. Klotz and V.H. Stryker, *Biochem. Biophys. Res. Comm*. 1959, **1**, 119.
13 R.K. Owusu-Apenten, C. Chee and O.P. Hwee, *Food Chem.* 2003, **83**, 541.
14 A.-M. Hermansson and J. M. Aguilera (2008). Structuring Water by Gelation. Food Materials Science, Springer New York: 255-280.
15 P.N. Kocher and E. A. Foegeding, *J of Food Science* 1993, **58**(5), 1040.