Enzymatic cross-linking at oil-water interfaces – a rheological study

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ABSTRACT

Potential food-compatible technologies for novel oil-in-water emulsions include interfacial cross-linking by enzymatic means. The objective of the present research is to elucidate the mechanisms, by which protein interfaces control transfer of low molecular weight compounds. As a first step, the impact of enzymatic cross-linking on mechanical properties of β-casein layer was studied. The dependence of interfacial properties on bulk phase concentration suggests, that the dynamics of cross-linking are influenced by the thickness and/or packing of the interfacial layer.

INTRODUCTION

Many food products are complex emulsions and their stability is greatly dependent on mass transfer phenomena. Oxidative stability and taste-masking are often major concerns especially in foods including unsaturated fatty acids or other health promoting components. The primary stage of oil oxidation involves transfer of oxygen across the interface from the aqueous phase to the dispersed oil phase. Mass transfer related instability is also caused by volatile secondary oxidation products, which can penetrate the interfacial layer and subsequently deteriorate the sensory quality of the product due to their low odour thresholds.

Therefore, the properties of the interfacial layer are the key in controlling oxidative stability of oil-in-water emulsions. Proteins play a central role in formation and stabilisation of food emulsions. Food proteins although very different in size and structure and hence, in adsorption dynamics, are still quite similar in terms of their interfacial rheology and charge. Technologies for engineering their interfacial behaviour are needed. Potential food-compatible technology for these novel oil-in-water emulsions is an interfacial cross-linking by enzymatic means. The objective of the present research is to elucidate the mechanisms, by which protein interfaces control transfer of low molecular weight compounds. As a first step, the impact of enzymatic cross-linking on the mechanical properties of the interfacial layer was studied.

MATERIALS AND METHODS

The protein studied was β-casein from Sigma. As oil phase, tetradecane (>99%, Aldrich) was used.

The interfacial visco-elasticity measurements were performed by a TA Instruments AR-G2 rheometer equipped with a Du Noüy ring. In prelimary experiments, significant contribution of ring position at the interface was found in G'. To standardize the position of the ring at the air-water surface prior to addition of tetradecane, protein solution was weighed and a standard gap value was used.

The protein concentration was varied between 0,001% and 0,01% in 10mM NaP buffer (pH 7). Into a cup, 45 g of solution was weighed, and the flamed ring was then immersed in the liquid to wet it. Then ring was lifted to a pre-determined gap and 10 ml of tetradecane was carefully added on the top. The measurement was immediately started and after equilibrating the surface for 1 h, 3 µl (50 000 nkat/g of protein in 0,001% solution) of transglutaminase enzyme was injected into the aqueous sub-phase with a Hamilton syringe (Figure 1). The enzyme dosage /g protein varied at different concentrations, as the surface area of the interfacial layer was constant. The evolution of surface shear moduli were followed for 8 h at 0,005 Hz and a strain of 0,02, after which a frequency sweep (strain 0,02) and a strain sweep (at 0,005 Hz) were performed.

From the linear visco-elastic region, the frequency of measurements (0,005 Hz) in time sweeps was chosen based on the raw phase degree, which was used as an indication for the contributions of sample and instrument inertia.

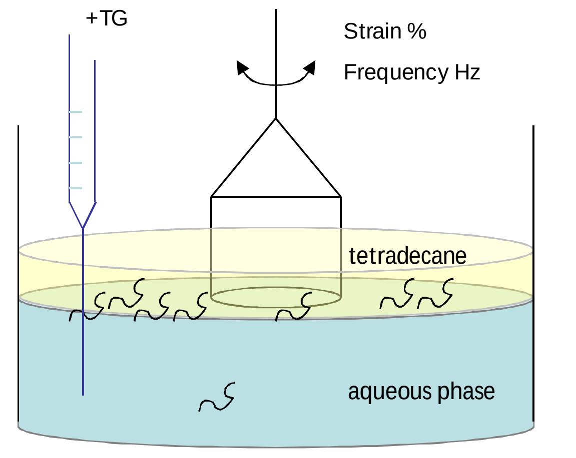

Figure 1. Experimental set-up for measuring visco-elastic properties of cross-linked β-casein layer.

RESULTS AND DISCUSSION

The interfacial layer, which was formed by adsorption of β-casein was very weak, independent of the protein concentration in the bulk. This surfactant-like behaviour of β-casein is well documented in the previous literature. After addition of TG, the elastic modulus starts to increase (Figure 2), which is in agreement with the findings of Faergemand et al. [1]. The slopes of the modulus increase are very dependent on the protein concentration in the bulk. This could be due to the equal amount of enzyme dosage in all samples, assuming that constant area of the layer would be equally linked by the enzyme.

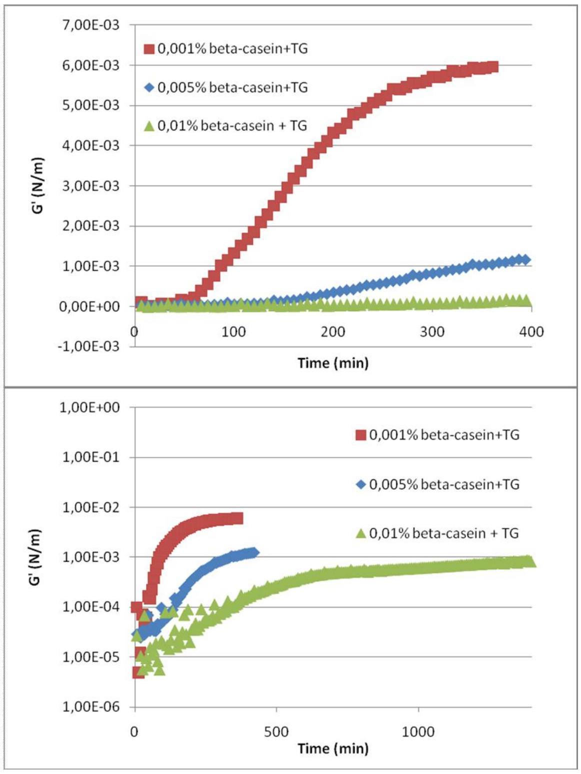

Figure 2. Evolution of surface shear elastic modulus at tetradecane-water interface by TG-induced cross-linking of β-casein layer.

Thus, the results imply that the amount of protein associated with the layer is strongly dependent on its concentration in the bulk even if there are no detectable differences in the mechanical properties of the uncross-linked layers. Based on earlier studies, it is known that casein forms multi-layers rather than monolayers at interfaces [2].

Due to higher amount of protein associated with the interface, it seems that it takes a longer time for the formed cross-links to affect the mechanical properties. This could be either due to slower diffusion of the enzyme to close proximity of the interface or due to formation of local clusters of cross-linked material in the deficiency of enzyme.

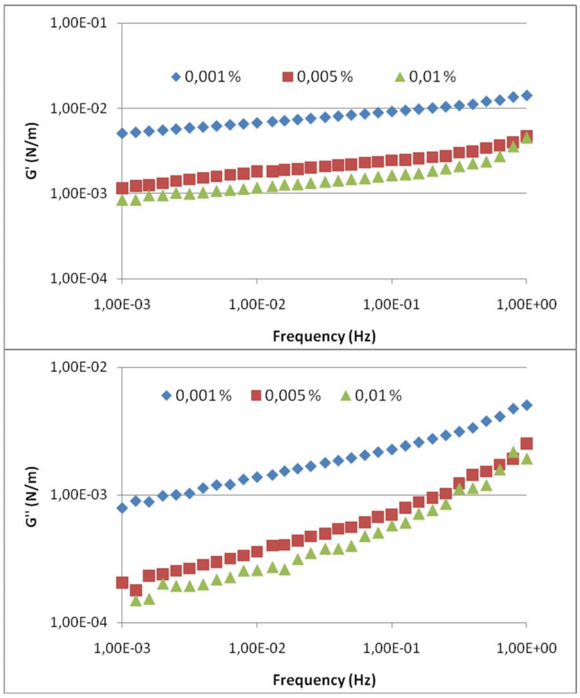

Figure 3. Frequency dependence of storage (G') and loss (G'') moduli of the cross-linked β-casein layer. Protein concentration is indicated in the legends.

In Figure 3, the frequency dependence of the storage modulus is shown for the cross-linked layer. In that respect, all the layers show very similar behaviour, the G' increasing with increasing frequency. However, at high frequencies, the raw phase (degrees) is dramatically increasing, suggesting that data quality criteria are no longer met. The loss modulus values are lower than the values for storage modulus in the whole frequency range. This result suggests, that the interfacial layer formed by TG-induced cross-linking is a continuous network rather than separate aggregates.

There is some difference in the strain sweeps of the cross-linked films. It should be noted that due to slow formation of the 0,01% film, the first sweep is made after 25 h incubation in time sweep, whereas for the lower concentrations, the subsequent sweeps were performed after only 7 h incubation. The strongest film at lowest bulk protein concentration collapsed at a strain of 0,2, whereas the 0,005 % film is only affected by the applied strain of 1. The modulus of the film with highest bulk concentration starts to decrease gradually with increasing strain.

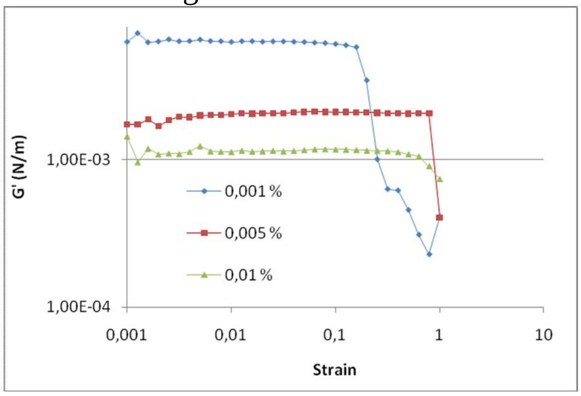

Figure 4. Strain sweep of the cross-linked β-casein layer. Protein concentration is indicated in the legends.

CONCLUSIONS

The dependence of interfacial properties on bulk phase concentration suggests that the dynamics of cross-linking are influenced by the thickness and/or packing of the interfacial layer.

ACKNOWLEDGMENTS

The research was supported by Academy of Finland in post-doctoral researcher's project *Novel protein-based emulsions by engineering interfacial mass transfer* (no: 133339), which is gratefully acknowledged by Riitta Partanen.

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