INDUSTRIAL PILOT PROJECT

Synchrotron SAXS-analysis of micellar recombinant casein properties for vegan cheese production

THE INDUSTRIAL CHALLENGE

For successful production of vegan cheese, Cassius needs to gain a better understanding of recombinant casein micelles, which have proven to behave differently compared with natural casein micelles. For example, they do not aggregate in the same way in response to presence of calcium. To develop a cheese production method, we must first characterize the behaviour of recombinant casein with addition of calcium. A conventional way to induce gel formation and renneting coagulation is to reduce the pH, but it is unknown how this affects the recombinant casein micelles.

WHY USING A LARGE SCALE FACILITY

To understand the size and microstructure difference between Cassius recombinant micelles and natural micelles from cows, small angle X-ray scattering (SAXS) is an excellent method of choice. The large size of the micelles (R_g >300Å) requires a sample to detector distance (>8m) that is longer than those available at lab-SAXS sources.

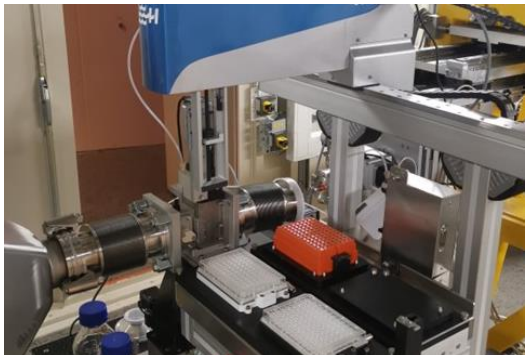

Figure 1: High-throughput SAXS setup at CoSAXS, with autosampler and flow cell.

HOW THE WORK WAS DONE

The purified recombinant casein micelles were measured at the CoSAXS beamline of MAX IV Laboratory that provides a sample to detector distance of 8m. We varied both environment conditions (0-200 mM NaCl and pH of 7 or 8) and the casein components, using between 0% and 40% Kappa casein alongside Alpha casein in the solution. For fitting our data, we used a spherical model for the low angles (corresponding to the overall shape of the micelle) and a Debye gaussian coil model for the larger angles (corresponding to the calcium-casein nanoparticle building up the micelle).

THE RESULTS AND EXPECTED IMPACT

We found that the size of the micelles decreased with increasing NaCl concentration, while pH had little or no effect. Increased ratio of Kappa to Alpha casein led to increased micelle size, but also increased sensitivity to the ion strength on the solution. We also measured the gels formed from recombinant casein micelles, as well as micelles from cows.

We found that recombinant micelles produced in this project formed much more monodisperse and well-ordered casein-calcium nanoparticles, which also appeared smaller than those from natural cow micelles. Overall, the structure found in recombinant micelle gel appeared smaller than that found in natural micelles. We also found that calcium chloride treatment of the casein solution prior to the gel formation appear to be the variable with the largest impact of final product. We therefore plan to investigate this further using among other techniques, small angle X-ray scattering.

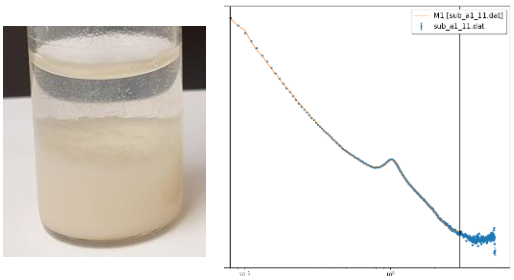

Figure 2. Left panel, recombinant casein solution after renneting and lowered pH. Right panel, SAXS data from recombinant casein after renneting. The pronounced peak in the curve announces high order at a length scale of approximately 6 nm.

"SAXS-measurements are vital for us to better understand our macroscopic results and for adapting our production processes" /Johan Krakau, Cassius AB

Contacts: Name Johan Krakau – Cassius AB, johan.krakau@cassiusway.se
Name Christopher Söderberg – RISE, christopher.soderberg@ri.se

Vinnova's project No: 2021-03823 **Duration:** November 2021 -- September 2022

Funded by Sweden's Innovation Agency, Vinnova, in order to build competence and capacity regarding industrial utilisation of large-scale research infrastructures such as MAX IV and ESS.