LSI SUPPORT FOR PhD-STUDENTS IN APPLIED AREAS

Synchrotron X-ray Microtomography for in-situ studies of the freeze-drying process in probiotics

THE PURPOSE OF THE PHD PROJECT

Freeze-drying is one of the most common ways to increase the shelf-life of probiotics and pharmaceutics. The impact of freeze-drying process on structure poses a significant challenge for probiotics, as inadequate cell encapsulation due to too thin material can harm the stability. The main purpose of this study is to follow the freeze-drying process in-situ to understand how the freezing, annealing and drying steps affect the final material structure. The study has affirmed the strength of using µCT imaging techniques to study freeze-dried probiotic products, which is of value for BioGaia AB.

USING A LARGE-SCALE INFRASTRUCTURE

Tomographic imaging techniques, both lab-based X-ray microtomography (µCT) and Synchrotron µCT (SRµCT) offer the potential to evaluate the 3D-structure of a freeze-dried product and to follow the evolution of the matrix during freeze-drying. Capturing the dynamic events of freeze-drying in 4D requires the of use synchrotron radiation in combination with an in-situ sample environment, offering a higher photon flux, thus decreasing the acquisition time. This study utilised a novel in-house designed freeze-drying sample environment designed for µCT at ForMAX beamline at MAX IV Synchrotron (Fig. 1) to investigate the structure during freeze-drying of a 20% maltodextrin solution and pre-frozen pellets.

***Figure 1**. The sample environment under installa-tion at ForMAX*

RESULTS AND IMPACT

To evaluate the efficacy of µCT as a characterization method for quantifying the material of freeze-dried samples, we performed tomography experiments using a lab-based µCT at the 4D Imaging Lab (Lund University, Lund, Sweden) and at the ForMAX beamline. The µCT experiments gave valuable insight into the structure of dry pellets, with a total scan time of 6 hours per sample, which is too slow to study kinetics in 4D.

A critical challenge was to achieve sufficient contrast between ice and freeze-concentrated solution. Using SRµCT at the ForMAX beamline a high spatial resolution and high contrast were achieved at a total scan time of only 45 s per sample. This enabled real-time capture of the structural changes during freezing and drying. Fig. 2 shows reconstructed 2D slices of various steps of freeze-drying. We were able to study both pre-frozen pellets and samples frozen directly in the freeze-dryer. The sample size was 3-4 mm in diameter. The sample holder temperature was varied from -25°C to 0°C during drying and the pressure varied between 40-65 Pa.

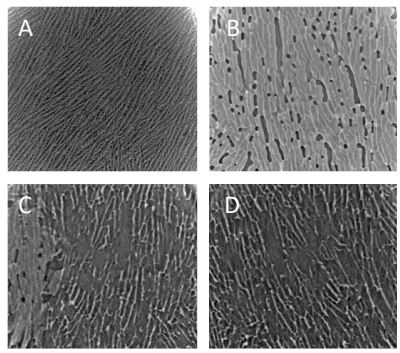

***Figure 2**. Reconstructed 2D slices of the same sample, captured at the ForMAX beamline, MAX IV Synchrotron. Sample directly frozen (A), annealed at -9 °C (B), partly dried with ice, (C), dried sample (D).*

The results provided novel insights into the annealing process, revealing the transformation from a very fine frozen structure to a structure with larger ice crystals. Formation of cracks in the ice occurred due to the annealing process. The material changes only to a minor degree during drying, which also occurs faster in some parts of the samples. It was confirmed that the final ice crystal structure is the key factor for the structure of the dried product.

This project has given the PhD student good insight into planning and executing tomographic imaging experiments, and carrying out image reconstruction, processing, analysis and 3D-rendering. The method developped forms a large part of the thesis work.

Contacts: Shuai Bai Palmkron – PhD-student at Lund University, shuai.bai@food.lth.se
Marie Wahlgren – Supervisor at Lund University marie.wahlgren@food.lth.se
Björn Bergenståhl – Supervisor at Lund University bjorn.bergenstahl@foood.lth.se
Anna Millqvist Fureby – Supervisor at RISE, anna.fureby@ri.se
Emanuel Larsson – X-ray Tomography Expert at Lund University, emanuel.larsson@med.lu.se
Sebastian Håkansson – probiotics expert at BioGaia, sh@biogaia.se
Vinnova's project No: 2020-00824 **Duration:** May 2020—September 2023
Funded by Sweden's Innovation Agency, Vinnova, in order to increase knowledge and regarding utlization of large-scale research infrastructures such as MAX IV and ESS for PhD students in applied