MRI mapping of structural anisotropy in plant-based protein extrudates.

S.A. Kuijpers[1], E.D. Garina[2], M.I. Gobes[1], F.J. Vergeldt[1], J.P.M. van Duynhoven[1,3], C. Terenzi[1]
[1]Laboratory of Biophysics, Wageningen University and Research, Wageningen, The Netherlands, [2]Department of Radiation Science and Technology, Delft University of Technology, Delft, The Netherlands, [3]Unilever Global Foods Innovation Centre, Wageningen, The Netherlands.

Introduction: High-moisture extrusion (HME) of plant-based proteins is the dominant industrial processing method for producing fibrous meat alternatives. Yet, the mechanisms by which fibrous anisotropic structures are formed in protein extrudates are not fully understood [1]. Conventional measurement techniques adopted for structural characterisation [2] typically do not provide spatially-resolved and/or molecular-level information. In our work, we used complementary and multi-modal techniques based on Time Domain (TD-)NMR, Chemical Exchange Saturation Transfer (CEST), Diffusion Tensor Imaging (DTI) MRI, Diffuse Reflectance (DR) [3] and Small Angle Neutron Scattering (SANS) [4], to spatially describe the development of structural anisotropy in extrudates, based on soy protein concentrate (SPC), from the molecular to macroscopic scale.

Methods: Samples were extruded using an XtruTech XTS19 at lab-scale and a Bühler PolyTwin at pilot-scale. MRI images were acquired using a 25-mm bore 600 MHz (14T) Bruker MRI spectrometer. T_1- and T_2-weighted RARE and DTI images were acquired on frozen-thawed and swollen extrudates at a resolution of 39x39x500 μm^3. RARE images were analysed using Rotation Fourier Transform, resulting in Weighted Order Parameter (WOP) maps [5], and DTI data was processed according to Kingsley (2006) [6]. CEST images were acquired using a 5-mm bore 1200 MHz (28T) Bruker MRI spectrometer at a resolution of 50x50x100 μm^3 and analysed according to Mayar *et al.* (2023) [7].

Results and Discussion: Low-field TD-NMR and high-resolution MRI revealed the anisotropic phase-separation of extrudates into protein-rich (PR) and water-rich (WR) lamellae. Ultra-high resolution CEST MRI data confirmed the presence of protein inside the WR lamellae. Freeze-thawing enhanced delamination of the WR and PR domains. MRI images and WOP maps revealed that lamellar phase separation is mostly visible close to the cooling die wall, and is dominantly oriented along the extrusion direction. Similar findings were obtained by SANS, at the nm-scale, and by DR, at the μm-scale. During passage of the cooling die, a clear increase in the volume fraction of the lamellar phase was seen by MRI. By resorting to DTI, information about structural anisotropy beyond the MRI spatial resolution limit could be obtained at length scales down to tens of μm. Inside the PR domains, and along the extrusion flow direction, diffusion appears predominantly anisotropic, in contrast to the nearly isotropic water diffusion within the WR domains.

Conclusion: In this work, we have shown how MRI could be used to quantify structural anisotropy of plant-based protein extrudates at length scales from tens of μm to sub-mm. For deadstop extrudate samples, it was observed that, upon entering the cooling die, the hot protein melt cools and solidifies, into anisotropic lamellae, dominantly in the regions close to the walls of the die. Complementary use of DR and SANS methods have shown a similar anisotropic structure development, respectively, at the μm- and nm-scale. Therefore, MRI is a powerful technique to underpin current hypotheses on shear- and temperature-induced phase separation of plant-proteins, and to provide the experimental data required to validate current *in silico* modelling efforts. Ongoing work is targeting the application of flow-MRI to study HME *in situ* using a benchtop-scale extruder.

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