

Spectral Imaging for Spatially-Resolved Structural and Chemical Analysis of Starch–Protein Regions in Chymosin-Induced Protein Gels

By Dionysios D. Neofytos

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Interactions and structuring processes at the mesoscale are key for the structure–function relationship in food matrices (Aguilera, 2024; Aguilera & Lillford, 2008). In multi-domain systems containing different proteins (e.g. whey and micellar casein) and structures, or mixed components such as starch and proteins, conventional microscopy lacks the ability to provide both spatial resolution and detailed chemical information. High-resolution spectral imaging techniques, such as FTIR and Raman imaging, address this limitation by enabling simultaneous localization of structural domains and characterization of their molecular composition (Neofytos et al., 2024; Neofytos et al., 2025). This study exemplifies the transformative nature of new approaches using these complementary techniques. Mixed gels containing casein micelles and whey proteins in combination with low- and high-amylose starches were evaluated under three processing conditions: native, heat-treated, and mixed. Raman imaging differentiated the serum phase from the protein network, offering detailed insights into protein structure, distribution, and density along network branches. Distinct regions of denatured proteins were localized within the gel matrix, separated from the serum phase, along with starch granules and interfacial protein layers between starch and the surrounding protein/serum phases. Spectral analysis revealed treatment-dependent differences in starch granule hydration, strongly influenced by protein composition. FTIR imaging complemented these results by providing additional information on protein composition and mapping high-starch and high-sugar regions. This enabled visualization of sugar distribution patterns around starch-rich domains, further elucidating processing effects on microstructural organization. Complementary confocal laser scanning microscopy (CLSM) confirmed starch granule distribution within the protein network, while rheological measurements quantified differences in gel strength and viscoelasticity arising from starch type and processing. Overall, the results demonstrate that starch type and processing history significantly influence microstructure formation, starch–protein interactions, and starch hydration state. This work highlights the advantages of combining FTIR and Raman spectral imaging with microscopy and rheology to investigate complex food systems. The findings not only advance understanding of mesoscale structural organization and its functional consequences but also showcase the

potential of spectral imaging for designing and optimizing multi-component food formulations.