**Exploring the impact of Supercritical CO2 on protein stability in crowded environment – a model system**

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Amid global challenges in sustainability and food security, the exploration of innovative processes and functional ingredients has become a priority for the food industry. Supercritical CO2 (ScCO2) has emerged as a promising solvent due to its tunable properties (temperature T > 31°C, pressure P > 74 bar) and potential for eco-friendly applications in food engineering1. Despite its growing interest, the impact of ScCO2 on the structural stability of proteins and their interactions within complex matrices, such as those encountered in food systems, remains poorly understood.

This study investigates the effects of ScCO2 on a simplified model composed of C-Phycocyanin (CPC), a globular protein obtained from cyanobacteria (*Arthrospira platensis*), and polyethylene glycol (PEG), a neutral polymer that mimics the macromolecular crowding typically found in food matrices. Macromolecular crowding can stabilize protein structures by mimicking dense environments2,3,4; however, the behavior of such systems under ScCO2 conditions is largely unexplored.

Preliminary results in fluorescence spectroscopy and differential scanning calorimetry revealed alterations in the protein's microenvironment leading to dissociation and aggregation, while suggesting a potential protective effect of PEG on the thermal stability of CPC. Other investigations including small-angle X-ray scattering (SAXS) and molecular dynamics (MD) simulations are planned to provide molecular-level insights into CPC-PEG interactions.

This work offers new perspectives on the use of ScCO2 in food systems, particularly for stabilizing protein-based ingredients in crowded matrices. By enhancing the understanding of protein-polymer interactions in ScCO2, this research contributes to the development of sustainable and functional food formulations.

*References*:

1Reverchon, E. and De Marco, I. (2006). Supercritical fluid extraction and fractionation of natural matter. *The Journal of Supercritical Fluids*, 38, 146–166.

2Wu, J., Zhao, C., Lin, W., Hu, R., Wang, Q., Chen, H., Li, L., Chen, S. and Zheng, J. (2014). Binding characteristics between polyethylene glycol (PEG) and proteins in aqueous solution. *Journal of Materials Chemistry B*, 2, 2983

3 Fonin, A. V., Silonov, S.A., Sitdikova, A.K., Kuznetsova, I.M., Uversky V.N. and Turoverov, K.K. (2017). Structure and conformational properties of D-Glucose/D-Galactose binding protein in crowded milieu. *Molecules*, 22, 244

4 Somkuti, J., Török, Z., Pfalzgraf, F., Smeller, L. (2017) Low crowding agent concentration destabilizes against pressure unfolding. *Biophysical Chemistry*, 231, 125-134