Impact of $scCO_2$ on C-Phycocyanin stability in presence of polyethylene glycol

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Amid worldwide economic and environmental challenges, the urge to find sustainable processes and new sources of proteins has positioned supercritical CO_2 ($scCO_2$) as an interesting alternative solvent in chemical engineering and food science [1]. Its solvation properties may indeed be tuned by changes in thermodynamic parameters such as temperature (T > 31°C) and pressure (P > 74 bar) and/or by the addition of co-solvents such as water or ethanol. However, the use of $scCO_2$ as an extraction solvent in complex matrices remains essentially empirical, given the poor knowledge on its solvation properties at molecular scale in crowded environments. Molecular interactions and excluded volume effects in biological matrices induce macromolecular crowding, which may stabilize and protect the native structure of proteins under high-temperature and/or high-pressure conditions [2], [4].

However, much less is known on how such interactions evolve in $scCO_2$ solvent. For this purpose, we considered a simplified model composed of a globular protein, C-Phycocyanin (CPC) and a polymer, polyethylene glycol (PEG) that mimics the macromolecular crowding found in complex food matrices. Two PEG with different molecular weight (PEG 4000 Da and PEG 35 000 Da) have been chosen to modulate the hydration of the protein. Moreover, two conditions have been applied (T=50°C; p=400 bars) and (T=50°C; p=800 bars) at which the density of scCO2 increases from 0.92 g/cm^3 to 1.04 g/cm^3.

The viscosity and density of PEG solutions did not significantly change after exposure to scCO2 thereby suggesting no irreversible influence of $scCO_2$ on the specific volume or on the conformation of the polymer. Spectroscopic and differential scanning calorimetry (DSC) measurements showed strong structural and environmental alterations of CPC in presence of PEG. Even though no protein aggregation was observed, fluorescence spectroscopy showed favorable interactions between PEG and protein hydrophobic residues, increasing their exposure to solvent, meanwhile DSC highlighted alteration of the oligomeric equilibrium and denaturation temperatures. Exposure to $scCO_2$, regardless of PEG size or concentration, resulted in notable protein aggregation and structural destabilization.

References:

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