The aim of presented work was determination of macromolecular crowding influence on structural stability of protein (lysozyme) in water environment with use of calorimetric, densitometric and spectroscopic methods. As macromolecular crowding agent polymers – poly(ethylene) glycols, PEG, of molecular masses: 6000, 10000, 20000 were used.

It was found that values of apparent molar volumes of lysozyme, $V_\Phi^{298}(298K)$, and values of excess molar heat capacities, $\Delta C_p(298K)$ depend on molecular mass and concentration of added polymer. For PEG 6000 these dependences were observed in the slightest degree, whereas in the case of PEG 10000 and 20000 both, $V_\Phi^{298}(298K)$ and $\Delta C_p(298K)$ dependences are clear, especially when polymer concentration reached $8 - 10 \text{ g·dm}^{-3}$.

In order to explain observed changes of $V_\Phi^{298}(298K)$ and $\Delta C_p(298K)$ as a functions of PEG concentration, the fluorescence spectroscopy method was applied. Received results were interpreted with an assumption of tryptophane residues division into two groups: a) on the surface, and b) buried inside of lysozyme molecule. It was shown that increasing PEG 20000 concentration causes a decrease of maximum of fluorescence intensity of lysozyme, $I_{0,max}$. The characteristic point of trend-change of $I_{0,max}=f(\%\text{PEG})$ dependence agreed with those observed for $V_\Phi^{298}(298K)$ and $\Delta C_p(298K)$ under the same conditions of temperature, pressure and pH.

The results indicate that there is a change in solvation shell of lysozyme in the presence of increase of macromolecular crowding.

The investigations of lysozyme unfolding process, caused by increasing temperature under macromolecular conditions were conducted with use of adiabiatic, differential, scanning calorimetry. It was shown that lysozyme unfolding process is two-state process regarding received agreement between calorimetrically determined values of $\Delta H_{\text{U}}^{\text{c}}$ and calculated van’t Hoff enthalpies, $\Delta H_{\text{vH}}$.

Determined values of temperatures of conformational change of lysozyme, $T_{\text{m}}$, as a function of both, molecular mass and concentration of PEG indicate on structural stability changes of investigated protein. The observation that unfolding enthalpies, $\Delta H_{\text{U}}^{\text{c}}$ do not depend on molecular mass and concentration of polymers indicates for entropic character of investigated process.