Determination of Antioxidant Properties of Thiol–Containing Proteins with Modified Cupric Antioxidant Assay

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Proteins are not considered as true antioxidants but are known to protect antioxidants from oxidation in various antioxidant activity assays. This study aims to investigate the contribution of proteins, especially thiol-containing proteins, to the observed overall antioxidant capacity measured by known methods. To determine the antioxidant properties of thiol-containing proteins, the CUPRAC method of antioxidant assay [1] using the oxidizing reagent Cu(II)-neocuproine previously used for simultaneous analysis of cystine and cysteine [2] was adopted. While the CUPRAC method is capable of determining all compounds comprising antioxidants and thiols in the sampled matrices, the Ellman method of thiol quantitation [3] either does not react or reacts to a small extent with antioxidants. The antioxidant quantities in the selected samples were assayed with the ABTS and FRAP methods as well as the CUPRAC method. In all applied methods, the dilutions were made with a standard pH 8 buffer used in the Ellman method by substituting the Na₂EDTA component of the buffer with sodium citrate. On the other hand, the standard CUPRAC protocol was modified by substituting the pH 7 buffer component ammonium acetate (at 1 M concentration) with 8 M urea buffer adjusted to pH 7 by neutralizing with 6 N HCl. All methods used in the estimation of antioxidant properties of proteins (i.e., CUPRAC, Ellman, ABTS, and FRAP) were first standardized with a simple thiol compound, cysteine, by constructing the calibration curves. The molar absorption coefficients of these methods for cysteine were: \( \varepsilon_{\text{CUPRAC}} = 7710 \), \( \varepsilon_{\text{Ellman}} = 13652 \), \( \varepsilon_{\text{ABTS}} = 20612 \), and \( \varepsilon_{\text{FRAP}} = 2982 \). Then these methods were applied to various mixtures containing thiols, such as glutathione (reduced form: GSH), egg white, whey proteins, and gelatin. Additionally, known quantities of selected antioxidants were added to these mixtures to investigate the additivity of responses.

References