DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY OF MILK USING A MODIFIED CUPRAC ANTIOXIDANT ASSAY

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Milk and dairy products contain valuable antioxidant constituents that help to protect consumers from oxidative stress-originated diseases (coronary disease, cancer, ageing, etc.). These antioxidant compounds may also prevent lipid peroxidation and contribute to milk nutritional quality. An important gap in literature is the unknown antioxidative contribution to total antioxidant capacity (TAC) of milk proteins, because due to inadequacies of analytical methodology in most TAC assays, proteins are initially separated by precipitation from the main matrix. Recently the CUPRAC method has been modified for measuring the antioxidant capacities of thiol-containing proteins [1]. In the modified CUPRAC method, the classical pH 7 ammonium acetate (1 M) buffer has been replaced with 8 M urea buffer of which the pH was adjusted with the addition of 6 M HCl to pH 7. The function of urea is to make the embedded thiol groups of proteins open to oxidative attack so that they would be more easily oxidized by the antioxidant assay reagent. The aim of this work is to measure TAC of milk with standard reference methods and the modified CUPRAC method, and to identify the contribution of milk proteins, especially thiol-containing proteins, to the measured TAC. Additivity of TAC is a prerequisite for comparing the TAC values of complex samples. For investigating this, various antioxidants and selected thiol compounds were added to real complex matrices, namely egg white, gelatin and whey [1]. The results showed that the modified CUPRAC method demonstrated the absorbance additivity required by Beer’s law for complex mixtures. Because milk fat caused turbidity, preliminary experiments were carried out with skimmed milk purchased from local markets. To determine TAC, the modified CUPRAC method was applied to whole milk after dilution with pH 8 standard buffer of the Ellman method with the exception that the masking agent in the buffer was Na-citrate instead of Na₂-EDTA [2]. The same method was applied to separated and redissolved milk proteins and remaining liquid phase after necessary operations. In whole milk experiments, effect of surfactants on removal of turbidity was also investigated. Sodium dodecyl sulfate (SDS) and Triton X-100 were successful in removing turbidity whereas cetyl trimethylammonium bromide was not effective. It was also noteworthy that SDS, being independently non-responsive to CUPRAC, caused increases in CUPRAC absorbance of milk proteins, probably as a result of increased exposition of thiols and certain amino acid moieties on proteins. As opposed to the Ellman method responsive to only thiols but not other common antioxidants, CUPRAC is most advantageous in regard to its strong response to both thiols and antioxidants. Ellman method may be used to reveal the thiol-only contribution to TAC of milk essentially measured by the modified CUPRAC method.

References