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A Spectrophotometric Microtiter-Based Assay of the Ability of UHT-Treated Milk to Reduce XTT

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A spectrophotometric microtiter-based assay was established for quantification of the ability of UHT-treated milk to reduce 3'-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}-*bis* (4-methoxy-6-nitro)benzenesulfonic acid hydrate (XTT). Under the optimized conditions, 40 μ l of a milk sample was mixed with 60 μ l of 0.5 mM XTT solution (pH 7.0) saturated with menadione in each well of a 96-well microtiter plate. The formed XTT formazan in the reaction mixture was determined by an increase in the absorbance difference between that at 492 nm and at 600 nm during the incubation for 20 min at ambient temperature. The use of the absorbance at 600 nm as a reference wavelength permitted the determination of XTT formazan without any interference from the turbidity caused by the addition of the milk sample. The present microtiter plate assay could differentiate two kinds of UHT-treated milk with a different extent of thermal treatment. In addition, the reducibility of the UHT-treated milk gradually decreased during storage dependent on the period or temperature. The method was very convenient and economical; it is promising as a method of estimating the extent of the heat treatment of milk and the storage conditions.

Keywords: milk, Maillard reaction, tetrazolium salt, microplate, heat treatment





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