Analytical methods

Crocin bleaching antioxidant assay revisited: Application to microplate to analyze antioxidant and pro-oxidant activities

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ABSTRACT

The crocin bleaching assay (CBA) is a common method for evaluating the antioxidant activity of hydrosoluble samples. It is criticized due to its low reproducibility, problematic quantification of results, differences in reagent preparation, doubtful need for a preheating phase and sensitivity to factors such as temperature, pH, solvents and metals. Here, the critical points of the method were extensively revised, and a highly reproducible procedure for microplate readers redeveloped. The problems of using quantification procedures, disregarding kinetic considerations, are discussed in detail and a model is proposed for quantifying simultaneously anti- and pro-oxidant activities as function of concentration and time. Thus, the combined use of a reproducible procedure and robust mathematical modeling produced consistent and meaningful criteria for comparative characterization of any oxidation modifier, taking into account the dose-time-dependent behavior. The method was verified by characterizing several commercial antioxidants and some