Critical Evaluation of DPPH Assay Applied on the Determination of the Antioxidant Activity of Rosemary Extracts

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DPPH assay is a method commonly used for the evaluation of the antioxidant activity based on the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by antioxidants present in the test sample. The reduction of $DPPH^{\bullet}$ during the reaction is followed by monitoring the decrease in absorbance (typically at 515 nm). High radical scavenging activity (RSA) is associated to high antioxidant activity. The assay is popular due to its simplicity, however, no standardized procedure has been published, which makes the comparison of various results extremely difficult. Several different protocols have been proposed for the evaluation of the RSA of antioxidants dissolved both in the solvent (usually methanol or ethanol) and, more recently, in complex systems such as edible oils.

In this study the applicability of the DPPH assay for the evaluation of the antioxidant activity of five oil-soluble rosemary extracts dissolved both in a pure solvent (methanol and toluene) and in sunflower oil has been investigated. For the latter one, only toluene has been used in order to ensure a complete dissolution of the samples.

A good correlation has been found between the two sets of samples (pure solvent and sunflower oil) despite the fact that the presence of the oil in the reaction mixture had a significant impact on the results. Further investigations showed that both the amount and the oxidative status of the oil affected the radical scavenging reaction. Finally, the RSA and the oxidative stability (evaluated by an accelerated oxidation test) of the oil spiked with rosemary extracts have been compared.