DETERMINATION OF HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF PHENOLICS AND FLAVONOIDS WITH A MODIFIED CUPRAC METHOD

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Hydrogen peroxide (H$_2$O$_2$) is a biologically relevant, non-radical, oxidizing species, and may be formed in tissues through oxidative processes, but there has been limited information regarding its scavenging by polyphenolic antioxidants. H$_2$O$_2$ scavenging activity is one of the methods for the estimation of reactive oxygen species (ROS) scavenging ability in biological and plant materials. It cannot be evaluated as a 'total antioxidant activity' assay due to the fact that antioxidants can act directly by scavenging ROS (O$_2^-$, H$_2$O$_2$, OH) or by inhibiting their generation, or indirectly by regulating endogenous antioxidant defenses. The present study was undertaken to investigate the hydrogen peroxide scavenging (HPS) activity of several polyphenolic compounds, including a series of flavonoids, simple phenolic and hydroxycinnamic acids and other antioxidants (e.g., ascorbic acid). HPS activity has usually been determined by following the rate of H$_2$O$_2$ consumption in an incubation system (H$_2$O$_2$ + scavenger) using the classical UV-method at 230 nm. Since some polyphenolics have strong absorption in the UV-to-visible region, HPS activity of polyphenolics was alternatively determined without interference by directly measuring the concentration of H$_2$O$_2$ using the modified CUPRAC (cupric reducing antioxidant capacity) spectrophotometric method at 450 nm in the presence of a Cu(II) salt (since H$_2$O$_2$ is a relatively stable compound, not scavenged unless transition metal compounds are present as catalysts). The CUPRAC [1] absorbance of the incubation solution due to the reduction of Cu(II)–neocuproine reagent by the products of the incubation system decreased in the presence of polyphenolics, the difference being proportional to the HPS scavenging ability of the tested compound. Among benzoic acid derivatives, gallic acid (GA) was found to be the most efficient H$_2$O$_2$ scavenger with its HPS activity being 62.8±0.9 %. Rosmarinic acid (RA) was the strongest antioxidant amongst cinnamate derivatives with a HPS of 26.0±0.5 %, classifying it as a poor hydrogen peroxide scavenger. Comparison between the two groups revealed that benzoate derivatives are much stronger hydrogen peroxide quenchers relative to cinnamic acids. The findings of the developed method for polyphenolics were statistically alike with those of reference GSH-Px method (DTNB method) [2]. In addition to polyphenolics, some fruit juices and green tea extract were evaluated for their HPS activity using the developed method. The proposed spectrophotometric method was practical, low-cost, rapid, and could reliably assay H$_2$O$_2$ in the presence of polyphenols (flavonoids, simple phenolic acids and hydroxycinnamic acids), and less open to interferences caused by UV-absorbing substances.

References