

**Title:**

Application of Electron Spin Resonance to Study Antioxidative Activity Of Retinyl Palmitate

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We have long been interested in studying the mechanisms by which diet constituents possess anti- and/or pro-oxidative activities, and employing electron spin resonance (ESR) spin trapping techniques to quantify free radical formation in biological systems. Vitamin A is an essential human nutrient that plays an important role in many physiological functions. Retinyl palmitate (RP) is the predominant storage form of vitamin A. In this study, we use ESR spin trapping technique to evidence that RP can scavenge free radicals. In a superoxide radical anion generating system, containing 1 mM xanthine and 0.05 U/mL xanthine oxidase in 20% acetonitrile in water, 25 mM the spin trap 5-*tert*-butoxycarbonyl 5-methyl-1-pyrroline *N*-oxide (BMPO), 0.1 mM DTPA, and RP (0, 1, 2, and 4 mg/mL, respectively) was incubated at ambient temperature for 10 min. The results indicate that RP scavenged superoxide radical in a dose response manner. In a separate experiment, hydroxyl radical (HO<sup>•</sup>) was generated by Fenton reaction involving the reaction of 0.2 mM FeSO<sub>4</sub> and 0.2 mM H<sub>2</sub>O<sub>2</sub>. The spin trap 5,5-dimethyl *N*-oxide pyrroline (DMPO, 50 mM) and RP (1, 2, and 4 mg/mL, respectively) were added to the reaction. Upon reaction, the resulting DMPO-OH ESR signal intensity was reduced by RP in a dose-response relationship. 2,2'-Bipyridyl, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH<sup>•</sup>) is a stable nitrogen-centered free radical. The ESR signal of DPPH<sup>•</sup> (0.5 mM) in ethanol was significantly reduced by 1.25 mg/mL RP and completely quenched by 5 mg/mL RP. Our results indicate that RP can effectively scavenge reactive oxygen species (ROS) superoxide radical anion and hydroxyl radical as well as the stable DPPH<sup>•</sup>; and that RP is a powerful antioxidative agent. (This article is not an official guidance or policy statement of U.S. Food and Drug Administration (FDA). No official support or endorsement by the U.S. FDA is intended or should be inferred.)