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# Antioxidant activities of nano-bubble hydrogen-dissolved water assessed by ESR and 2,2'-bipyridyl methods

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## Abstract

We prepared nano-bubble hydrogen-dissolved water (nano-H water) which contained hydrogen nano-bubbles of <717-nm diameter for 54% of total bubbles. In the DMPO-spin trap electron spin resonance (ESR) method, the DMPO-OH:MnO ratio, being attributed to amounts of hydroxyl radicals (OH), was 2.78 for pure water (dissolved hydrogen [DH]≤0.01 ppm, oxidation-reduction potential [ORP]=+324 mV), 2.73 for tap water (0.01 ppm, +286 mV), 2.93 for commercially available hydrogen water (0.075 ppm, +49 mV), and 2.66 for manufactured hydrogen water (0.788 ppm, -614 mV), whereas the nano-H water (0.678 ppm, -644 mV) exhibited 2.05, showing the superiority of nano-H water to other types of hydrogen water in terms of OH-scavenging activity. Then, the reduction activity of nano-H water was assessed spectrophotometrically by the 2,2'-bipyridyl method. Differential absorbance at 530 nm was in the order: 0.018 for pure water, 0.055 for tap water, 0.079 for nano-H water, 0.085 for commercially available hydrogen water, and 0.090 for manufactured hydrogen water, indicating a prominent reduction activity of hydrogen water and nano-H water against oxidation in ascorbate-coupled ferric ion-bipyridyl reaction. Thus, nano-H water has an improved antioxidant activity as compared to hydrogen water of similar DH-level, indicating the more marked importance of nano-bubbles rather than the concentration of hydrogen in terms of OH-scavenging.

**Keywords:** 2,2'-Bipyridyl method; Antioxidant; Electron spin resonance; Hydrogen water.

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