## Antioxidative properties of infant formulas against hydroxyl radical production in the Fenton reaction

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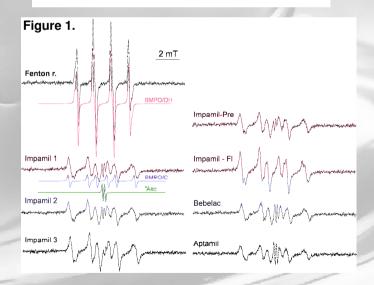
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Electron paramagnetic resonance (EPR) spin-trapping spectroscopy was used to compare the antioxidative capacities of commercial infant formulas against hydroxyl radical production in the Fenton reaction. Infant formula used in our experiments was Impamil-Serbia: Impamil 1, Impamil 2, Impamil 3, Ipamil PRE, Impamil FL, and Danone-Holland: Bebelac and Milupa-Germany: Aptamil marked as samples 1-7 Table 1). All formulas were prepared by dissolving 15 g of dry powder in 90 ml water (36 g/L). EPR spectra were recorded using a Varian E104-A EPR spectrometer operating at the X-band (9.572 GHz) with the following settings: modulation amplitude: 0.2 mT: modulation frequency: 100 kHz; microwave power: 20 mW; time constant: 32 ms; scanning time: 4 min. The temperature in the cavity was controlled at 293°K. Spin-trap BMPO (5-tert-butoxycarbonyl-5-methyl-1-pyrroline-Noxide) was purchased from Enzo Life Sciences International (Plymouth Meeting, PA, USA). Recordings were performed using EW software (Scientific Software, Bloomington, IL, US). The Fenton reaction was performed by adding Fe<sup>2+</sup> (FeSO<sub>4</sub> Sigma-Aldrich) and H<sub>2</sub>O<sub>2</sub> (Renal. Budapest, Hungary) at final concentrations of 0.6 mM and 3 mM, respectively, followed after the addition of BMPO (10 mM). The samples were drawn into 10 cm long gas-permeable Teflon tubes (wall thickness: 0.025 mm; internal diameter: 0.6 mm; Zeus industries, Raritan, NJ, USA) in order to maintain constant pO2 levels. Measurements were performed using quartz capillaries in which the Teflon tubes were placed. Recordings were conducted 2 min after the start of the reaction. Spectral simulations of each spectrum were performed using the WINEPR SimFonia computer program (Bruker Analytische Messtechnik GmbH, Darmstadt, Germany). In all samples the generation of hydroxyl radical led to the formation of carboncentred and ascorbyl radicals. In samples 1-5, the level of carboncentred radical was significantly higher than in samples 6 and 7 but those samples have higher signal of ascorbyl radical in comparation with other samples. In addition to hydroxyl radical scavenging, Fenton reaction may be hampered by the removal of redox active (inappropriately chelated) iron. That fact limit amount of ascorbate which may be added in Formulas and other possible mechanisms for increased antioxidative capacity are discussed (Figure 1).

## Table 1

sample 1	Impamil 1-Serbia five samples: Impamil 1-infant formula for 0-6 month old babies
sample 2	Impamil 2 - infant formula for 6-12 month old babies
sample 3	Impamil 3 - infant formula for babies from 12 month
sample 4	Impamil PRE- infant formula for premature babies
sample 5	Impamil FL - infant formula lactose free
sample 6	Bebelac 1(Danone, Holland) - infant formula for 0-6 month old babies
sample 7	Aptamil (Milupa, Germany) - infant formula for 0-6 month old babies



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