



HYDROXIL RADICAL SCAVENGING ACTIVITY OF PRETERM MOTHERS MILKS IN THE FENTON SYSTEM

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Object of Research

Mothers milk have antioxidative effects against Fenton system via HO• radical scavenging. Mother's milk from woman's after preterm delivery antioxidative properties are not examined in details. We examined hydroxyl radical scavenging activity of preterm mothers milks in the Fenton system .

Materials and Methods

We utilize electron paramagnetic resonance (EPR) spin-trapping spectroscopy to determine and compare activity of premature mother milk, skim milk and whey against Fenton system. The spin-trapping technique is based on the reaction of 'EPR silent' spin-trap with free radical which yields a more persistent EPR active nitroxide spin-adduct. DEPMPO, a sophisticated EPR spin-trap reagent, is applied in order to analyze reactive products of milk with HO• produced in Fenton reaction.

Results and Conclusion

It can be observed that full breast milk as well as fractions scavenge hydroxyl radical, which results in the production of urate and ascorbyl radicals. The intensities of DEPMPO signals in all milk-containing systems was drastically lower compared to control (Fenton) system indicating that milk samples scavenge HO•. There was no significant difference between the intensities of signals of urate radical adduct or ascorbyl radical between fractions – full milk, skim milk and whey. Main antioxidants (urate and ascorbate) in mature mother's milk from woman's after preterm delivery are in whey.

Premature mother milk was collected 6 weeks after premature baby delivery, from five exclusively breast-feeding mothers. The milk was then mixed, aliquoted, stored at -80°C. Skim milk was prepared by centrifugation (10000 g, 5 min at 4 °C).Whey was prepared from skim milk by acidification to pH 4.6 with lactic acid, incubation for 30 min at room temperature, centrifugation (as above), and readjustment of pH of the supernatant with NaOH to 6.7. Fenton reaction was performed by combining 1 mM H₂O₂, and 0.2 mM FeSO₄.

Table 1. The intensity (AU) of EPR signals of DEPMPO adduct with urate radical, and ascorbyl radical in systems with native breast milk and fractions.

	Milk	Skim milk	Whey
DEPMPO/C	676 ± 150	499 ± 147	606 ± 172
Ascorbyl radical	11.7 ± 3.1	15.1 ± 4.1	13.5 ± 4.3

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References

- Lugonja N, Spasic SD, Laugier O, Nikolic-Kokic A., Spasojevic I., Orešcanin-Dušić Z., Vrvic M.M. Differences in direct pharmacological effects and antioxidative properties of mature breast milk and infant formulas. *Nutrition* 2012; doi.org/10.1016/j.nut.2012.07.018
- Friel JK, Martin SM, Langdon M, Herzberg GR, Buettner GR. Milk from mothers of both premature and full-term infants provides better antioxidant protection than does infant formula. *Pediatr Res* 2002; 51(5): 612-618.

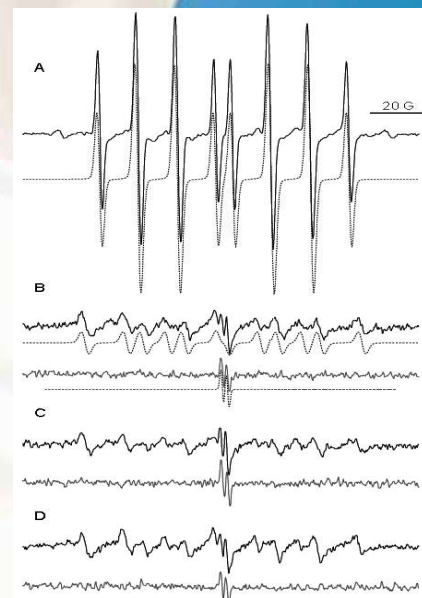


Figure 1: EPR signals of Fenton reaction (A), full breast milk (B), fresh skim milk (C) and fresh milk whey (D)