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ELECTROCHEMICAL DETERMINATION OF REDOX POTENTIAL IN INFANT FORMULA AND HUMAN BREAST MILK

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Introduction

Antioxidant molecules are known to have specific antioxidant roles against lipid peroxidation, scavenging reactive oxygen species (ROS) and quenching chain reactions, as well as for having effects on cellular functions by binding themselves to specific receptors in order to initiate signal transduction cascades, inhibiting or activating enzymes and regulating gene expression. However, when mother is not able to breastfed, infant formula is a good replacement, so a baby can grow and thrive normally. Cow's milk is not used routinely in the nutrition of infants, but it is modified in the form of infant formulas that are more like human milk. These formulas for infants and premature babies are enriched with small molecules of vitamins E, A, C and minerals, in order to have excessive chain-breaking antioxidants as compared to mother's milk. Although the composition of individual milk antioxidants is known, there is a necessity to develop methods for the detection of the total antioxidant activity of milk. Several methods have already been used to test the total antioxidant activity of mother's milk, among which are spectrophotometric methods, and the enzymatic method for the determination of the enzyme activity component of mother's milk. Human breast milk is a best dietary choice for newborn baby, and as it consider as a gold standard all the manufacturers of infant formula aim to produce these products with composition very similar to that of human breast milk. The objects of research of this study is to investigate the significance of breast milk and infant formula in the prevention of oxidative stress, by electrochemical determination of the total antioxidant potential and commonly used DPPH method, demonstrating the relationship between the antioxidant capacity of milk and postnatal age.

Materials and methods

Two different infant formulas supplemented with prebiotics (IF) (produced by IMPAMIL d.o.o., Serbia) were used as sample food for infants of different age: MIL 1® the milk formula for the nourishment of younger age infants and MIL PRE® special formula for preterm and low weight infants. Human breast milk - mother milk (MM) samples were collected from 10 healthy women in the 9th week of lactation, after uncomplicated delivery. Mother's milk from woman's after preterm delivery (PMM) samples were collected from 10 healthy women in the 3rd week of lactation. Commercial UHT milk with 3.2% content of milk fat (CM) was used as a control sample. The vitamin C was used as a reference material in the concentration range 0,5 – 1,5 mmol/L as compared to vitamin C. Cyclic voltammograms and differential pulse voltammograms were recorded using a CHI760B instrument (CHInstruments, Austin, USA). A three electrode cell was employed, including glassy carbon (GC) electrode as the working electrode (Model CHI104), an auxiliary platinum electrode of large area (Model CHI 221) and an Ag/AgCl reference electrode (Model CHI 111). The electrochemical cell volume was 5 ml. CV scans were made from -400 to +1000 mV at a scan rate of 100 mV s^{-1}, and DP scans from -100 to +700 mV at a scan rate of 100 mV s^{-1}. The DPPH assay measures the reducing ability of antioxidants in milk samples towards the DPPH radical using a UV-vis spectrophotometer.

Results

Electrochemical measurements indicates that human breast milk has highest redox potential (250 mV), while skimmed UHT milk has very low (100 mV). Infant formulas have also high potential of 180mV. DPPH method confirmed results obtained by electrochemical methods. The free radical scavenging activity is highest for human breast milk (92%) and lowest for UHT milk sample (39%). Infant formulas have also high free radical scavenging activity (90-91%).

The electrochemical behaviour of milk and semi-quantitative determination of vitamin C were detected by cyclic voltammetry. The figure 1 displayed CV voltammograms obtained for the PMM and MIL PRE milk samples as well as for MM, MIL 1 and CM. The figure 2 displayed DPV voltammograms for milk samples.

Cyclic and differential pulse voltammograms were also recorded for the vitamin C in the concentration range (0.5 -1.5 mmol/L). A calibration curve obtained for this standard was used to calculate the total redox potential of the studied milk samples. All three methods can be used to determine the redox potential and the total antioxidant activity, and that the results obtained by these three techniques are consistent and follow the same trend. The values of the antioxidant activity obtained by differential pulse voltammetry and cyclic voltammetry are consistent, while the results obtained by DPPH method are slightly different, expressing somewhat higher values.

Conclusions

The higher antioxidative capacity of mother's milk could be attributed to direct scavenging of radicals. This suggests that breast milk possesses much stronger antioxidative potential as compared with the examined infant formulas. This is of particular importance for the immature defence systems in infants, which renders them more susceptible to different environmental stressors and to in-system fluctuations that are accompanied by an increased production of reactive oxygen species. From the results of all the three comparative electrochemical methods, it may be concluded that the IF for infants (MIL 1), and for prematurely born children (MIL PRE) has a very high AOA capacity (80-70%) as compared to breast milk (100%) and mother's milk from woman's after preterm delivery , which contributes to the physiological development of the child. The main advantage of electrochemical methods used to assess the total antioxidant activity of milk was that they directly monitored the electron-donating ability of the compounds and could be used for the quantitative analysis of the total antioxidants of different types of milk. The electrochemical procedure could be highly relevant for the quick and routine measurement of the total antioxidant capacity of milk and infant formula, and of the freshness of milk, as well as for the quantitative determination of the total antioxidant capacity of milk.

Acknowledgements

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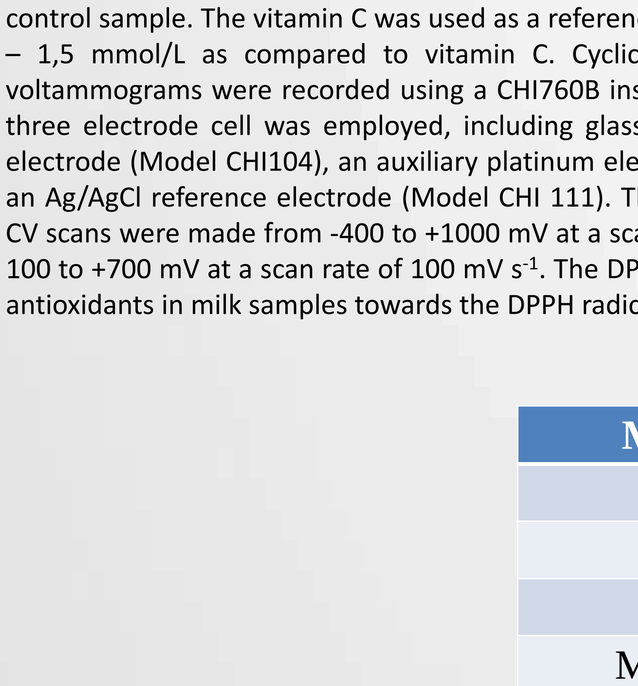
Method	CV	DPV	DPPH
MM	100	100	92
MIL1	70	66	91
PMM	72	72	80
MILPRE	80	80	90
CM	38	43	39

Table: Comparison of all methods for determining the antioxidant activity of the milk samples, in percentages.



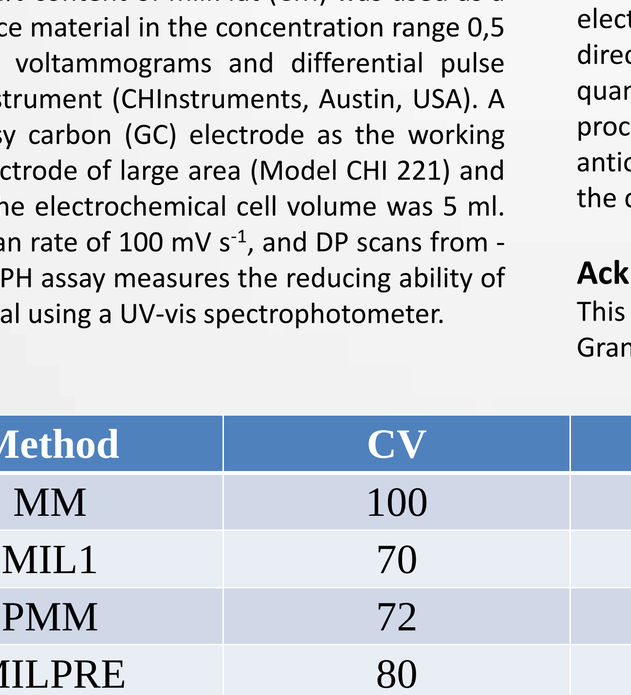


Figure 1: CVs recorded at a GC electrode at the scan rate 100mVs^{-1} in the potential range of -400 to 1000 mV.

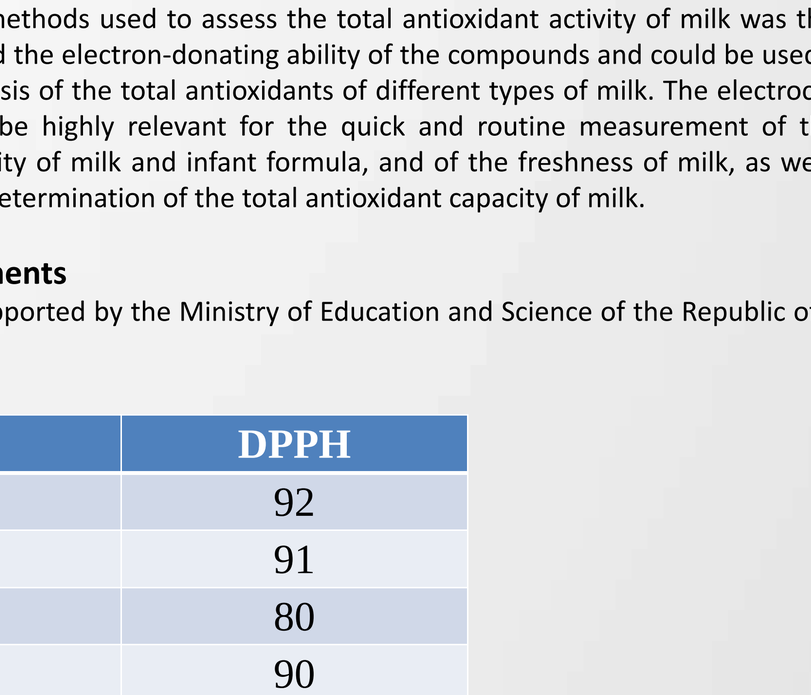


Figure 2: DP voltammograms of recorded milk samples at the scan rate of 100 mVs^{-1}, pulse amplitude 100 mV, initial potential -400 mV and final potential +1000 mV.

References

1. Zarban, A., Taheri, F., Chahkandi, T., Sharifzadeh, G., Khorashadizadeh, M. (2009). Antioxidant and radical scavenging activity of human colostrum, transitional and mature milk. Journal of Clinical Biochemistry and Nutrition, 45, 150-154.
2. Nikoleta M. Lugonja, Dalibor M. Stanković, Snežana D. Spasić, Goran M. Roglić, Dragan D. Manojlović, Miroslav M. Vrvić, „Comparative Electrochemical Determination of Total Antioxidant Activity in Infant Formula with Breast Milk", Food Anal. Methods. 7(2) (2014) 337-344