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CONTEMPORARY METHODS OF TESTING HUMAN MILK QUALITY

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ABSTRACT

Infant nutrition is essential for their growth and development. This research aims to determine the total antioxidant capacity of the infant food for preterm infants and indicate adequate methods for testing the quality and biological value of milk and infant food. The total antioxidant capacity (TAC) was determined in human milk and an infant formula for premature infants. The determination of the total antioxidant capacity was made using cyclic and differential pulse voltammetry, potentiometry and electron paramagnetic resonance spectroscopy. The results of three comparative electrochemical methods indicate that human milk has a higher antioxidant potential compared to infant formula, which contributes to better physiological development of the child. Fenton-based electron paramagnetic resonance spectroscopy method provides additional insight into TAC analysis, whereby a carbon-centered radical and an ascorbyl radical are formed in infant food. All methods can be used to determine TAC, since the results obtained individually with each method follow the same trend.

INTRODUCTION

Adequate nutrition is essential for the growth and development of infants and young children. Human milk is the most complete source of nutrients, especially adapted to baby's needs. When breast milk is not available, infant formulas are an adequate source of macro and micronutrients for infant development. The nutrition of premature babies poses a specific challenge, because it requires a high-energy intake, adequate nutrients and antioxidants. In a complex system such as milk, several redox systems are active at the same time, and their effect on antioxidant potential depends on several factors, such as system reversibility, oxidant-reductant ratio and concentration of active system components[1].

In addition to the commonly used enzymatic and spectrophotometric methods, modern electrochemical techniques—differential pulse voltammetry (DPV), cyclic voltammetry (CV), and potentiometry (POT)—are used to determine the total antioxidant capacity (TAC) of biological samples [2]. These methods could find their application in the daily control of milk quality before thermal treatments. The addition of fortifier to human milk is very important for the nutrition of premature infants, so it is necessary to determine the antioxidant capacity for infants in neonatal units. Electron paramagnetic resonance spectroscopy (EPR) is a "fingerprint method" for determining the activity of biologically active compounds [3].

There is no simple method to determine the freshness of milk. The freshness and quality of milk reflect its redox state. The aim of this paper is to compare electrochemical methods that determine the redox potential of infant food, as carriers of protective activity and milk quality.

METHODS

Human milk samples (MM) were collected from mothers of preterm infants, delivered before 37 weeks of gestation, at the Institute of Neonatology, Belgrade, Serbia (E82501/4). Special infant formula (IF) for preterm and low birth weight infants was produced in Serbia in accordance with recommendations (Codex Alimentarius Commission FAO / WHO). IF was prepared by dissolving 16g of powder in 90 ml of water, for the final volume of 100ml of meal.

Potentiometric determination of the total antioxidant capacity of milk samples is based on the Noyhouzer method [4], which determines the redox state and redox capacity of milk. Total antioxidant activity of milk was determined potentiometrically using iodine / iodide redox reaction in a two-electrode cell. Fisher's Pt electrode was a working electrode, and saturated calomel electrode was the reference. Redox potential was measured in solution after adding constant volumes of redox mediator 0.1 M J^-/ J2. Portions (10 μL) were added to 50 ml of the titrant solution every 90 s. Cyclic and differential pulse voltammograms were recorded on a CHI760B instrument (CHInstruments, Austin, USA), in a three-electrode cell: a glass electrode (GC) was used as the working electrode (Model CHI 104) and an additional large Pt electrode (platinum wire) (Model CHI 221) and Ag / AgCl electrode (Model CHI 111) as auxiliary and reference electrodes. Electrochemical cells have a volume of 5 ml. The measurements were performed in an anaerobic atmosphere, with the introduction of nitrogen into the cell. Voltammogram CV was recorded in the range -400 to +1000 mV, with a recording scan rate of 100 mVs^{-1}, DP voltammogram from -100 to +700 mV with a scan rate of 100 mVs^{-1}. Before each recording, the working GC electrode was polished with aluminum powder (1 and 0.5 μm, Buehler, IL, USA), and then washed with distilled water [2]. The EPR spectrum was recorded on a Varian E104-A EPR spectrometer (Varian, Palo Alto, CA.USA) X-band (9.572 GHz). Spin-trap stimulation of 5-tert-butoxycarbonyl-5-methyl-1-pyrroline-N-oxide (BMPO) was used [3]. All data were expressed as means ± Standard Deviation (SD). Comparison between groups was done using NCSS-2001 (NCSS, Kaysville, UT) statistical software. Kruskal–Wallis ANOVA test was used for multiple comparisons.

RESULTS AND DISCUSSION

The total antioxidant capacity was examined in HM and IF for the nutrition of premature infants. Table 1 shows the results obtained by different methods used to determine TAC.

Table 1. Comparison of methods for determination of TAC of milk and IF

Method	MM	IF
POT (mV)	250±2	205±2
CV (μC)	0.398±0.263	0.204±0.011
DPV (μC)	0.737±0.298	0.532±0.016
EPR*	81±10	107±15

* The signal intensity of the BMPO adduct with carbon-centered radical

Mechanisms for determining the TAC by CV and DPV differ from potentiometry. CV and DPV reactive species are electrons from the electrode, while in potentiometry, reactive species are electron donors, while iodine solution as an oxidant is an acceptor, which is an indicator of the measured antioxidant potential. The values of antioxidant potential in CV and DPV were in good agreement (MM 100%, and IF 80% activity), while the results obtained by potentiometry show slightly higher values (IF 82% of total activity compared to MM). This can be explained by the longer measurement time, since in potentiometric analyzes the time between adding two aliquots of iodine is 90 s, which is enough time to use up all iodine—it oxidizes with antioxidants from milk—and to get a more realistic insight into antioxidant content. By comparing the three electrochemical

methods (CV, DPV and POT, Figure 1) to determine the total antioxidant potential of breast milk and infant formula, it is shown that human milk gives a significantly stronger TAC, i.e., it has a higher redox potential compared to infant formula.

Based on the above results, it can be concluded that all three methods can be used to determine TAC, since the results obtained individually with each method follow the same trend. The Kruskal-Wallis multiple Z-value comparison test (Table 2), which shows that the Z value for the confidence interval does not exceed the critical value (z-value 1.9600), confirmed the experimental results and showed that potentiometry, CV and DPV can be equally used to determine TAC of different milk samples.

Table 2. Kruskal-Wallis Multiple-Comparison Z-Value Test

Variable	POT	CV	DPV
POT	0.0000	0.0271	0.4611
CV	0.0271	0.0000	0.4339
DPV	0.4611	0.4339	0.0000

Regular Test: Medians significantly different if z-value > 1.9600
Bonferroni Test: Medians significantly different if z-value > 2.3940

The disadvantage of the potentiometric method is that the time required for analysis is longer—about half an hour per sample analysis—while the time required to obtain the TAC by cyclic and differential pulse voltammetry is much shorter, since the analyses only take a few seconds.

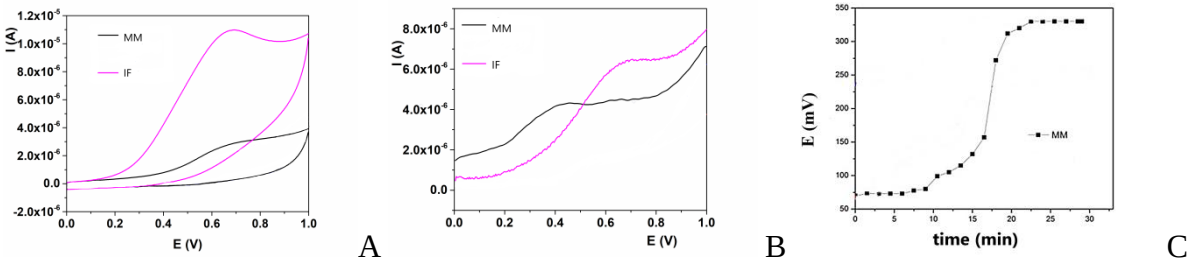

Figure 1. Application of different electrochemical methods for detection of human milk quality: A) CV; B) DPV; C) Potentiometry

Mother's milk and infant formula catch the hydroxyl radical to form carbon-centered and ascorbyl radical. The formation of these species is lower, with the equal production of ·OH radicals in IF compared to MM (Table 1). Both MM and IF scavenge the ·OH radical, whereby a carbon-centered radical and an ascorbyl radical are formed in MM, and a carbon-centered radical is formed in IF. The TAC of breast milk is obviously 3 times higher than IF, especially if we consider that the carbon-centered radical targets different biological molecules (Figure 2).

The applied electrochemical methods are very fast, cheap and reliable in determining the total antioxidant capacity of human milk because they are based on direct measurement of the electron-donating components of milk and enable quantitative determination of TAC of MM and IF. Electrochemical methods are very important for milk freshness control, especially in neonatal units. Spectroscopic examination using electron paramagnetic resonance with spin trapping, tests the ability of human milk and infant formula to reduce the production of free radicals in the Fenton system. Fenton-based EPR method completes the insights into TAC analysis of MM and IF. This is particularly important for the immature defense system of infants, which makes them more sensitive

to various environmental stressors and disorders within the system associated with the production of reactive oxygen species.

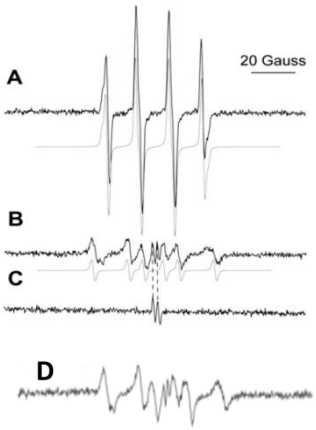

Figure 2. Application of EPR for detection of human milk (B and C) and IF (D) quality (A: Fenton system)

CONCLUSION

Our results represent valuable data guiding us into further research on electrochemical methods for rapid, routine and daily determination/monitoring of TAC in human milk and infant formulas. Electrochemical techniques can exceed the limits of spectrophotometric analysis, such as lower sensitivity and slower response, and can be successfully applied as a replacement for long-running spectrophotometric methods and in clinical trials.

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