

DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY IN HUMAN MILK AND INFANT FORMULA

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ABSTRACT

Implementation of new methods to assess the total antioxidant capacity of human milk is very important with regard to the role of antioxidants in preventing diseases associated with oxidative stress. The objects of research were to investigate the total antioxidant capacity of human milk, human milk supplemented with fortifier and infant formula, by electrochemical and DPPH methods. Electrochemical measurements indicated that human milk supplemented with fortifier has higher antioxidant potential than human milk and infant formula. Similarly, the free radical scavenging activity measured by DPPH method was the highest for human milk supplemented with fortifier and infant formula. The electrochemical techniques studied directly monitored the electron-donating ability of the compounds in milk substrate and could be used for the quantitative analysis of antioxidant potential.

INTRODUCTION

Adequate nutrition during infancy and early childhood is essential to ensure the growth, health, and development of children to their full potential [1]. Human milk is a gold standard in infant nutrition, and its composition is highly variable with energy and nutrients and provides strong protection against the potentially harmful effects of oxidative stress in newborns [2]. Human milk from mothers delivering preterm infants is different from that of mothers delivering at term in some of the biological properties, and it requires additional care in fortification to fulfill nutrient and energy requirements [3]. Human milk has antioxidant properties and provides antioxidant protection. Synergism of action of several enzymatic and non-enzymatic antioxidants of human milk helps to eliminate free radicals in newborns [4]. The measurement of total antioxidant capacity of human milk, besides qualitative and quantitative analysis, could be a useful tool for examination of this complex food and relevant indicator of oxidative stress and health status of the subject [5].

Implementation of new fast, accurate and sensitive method to assess the total antioxidant capacity of human milk is important with regard to the role of antioxidants in preventing diseases associated with oxidative stress. The aim of the research was to investigate total antioxidant capacity (AOC) of human milk, human milk supplemented with fortifier and infant formula by electrochemical determination of the total antioxidant potential and commonly used DPPH method.

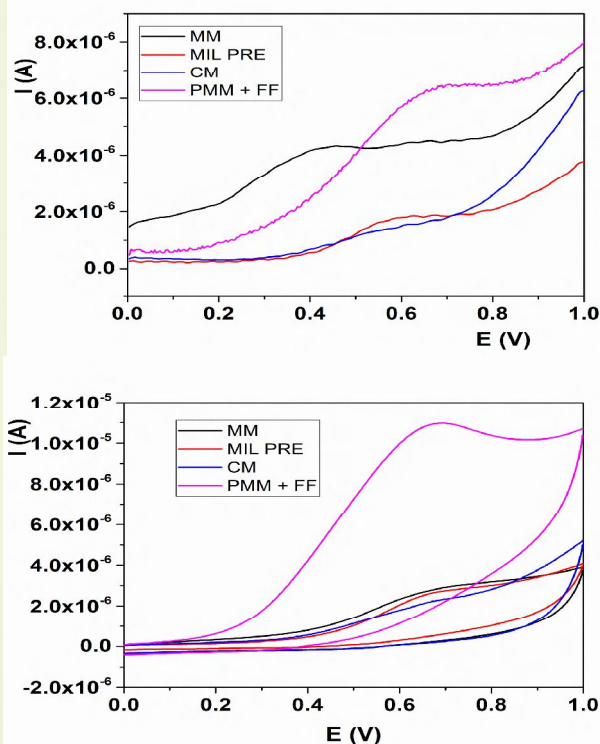


Figure 1. Differential pulse and cyclic voltammograms obtained for the tested samples.

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Experimental

We collected pool sample of 10 human breast milks from mothers of preterm infants (PMM). Human milk fortifier designed for preterm neonates (FF - Mil Fortifier, produced in Serbia) was used to supplement PMM milk samples (PMM+FF samples). Fortifier (5 g) was added to 100 mL of milk. Infant formula for preterm infants (MIL PRE, produced in Serbia) was prepared by dissolving 16 g MIL PRE in 90 mL of water. Commercial cow milk (CM) was used as reference milk (2.8% MM, produced in Serbia). The study protocol was approved by the Research Ethics Board of the Institute for Neonatology. Cyclic voltammograms (CV) and differential pulse voltammograms (DPV) of milk samples were recorded with the boron doped diamond electrode as the working electrode, an accessory platinum electrode, and an Ag/AgCl reference electrode, from 0 to +1000 mV at a scan rate of 100 mVs⁻¹. Samples were prepared adding known amount of KCl (0.1 M, Sigma Aldrich, USA) as supporting electrolyte. Prior to each measurement the working electrode active surface was polished with alumina. Vitamin C was used as a reference standard material to produce calibration curves for both the CV and DPV methods, in the concentration range of 0.5–1.5 mmol/L. The calibration curves were used to calculate the AOC of the studied breast milks samples, and the results represent the total reducing capacity in terms of vitamin C equivalents (vit C mM). The determination of radical scavenging activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed according to Zarban et al. [6], with slight modifications. The absorbance of the incubated sample supernatant was measured at 517 nm. The percentage of radical scavenging activity was expressed as scavenging rate %: $\text{Radical scavenging rate \%} = [1 - (\text{Abs sample}/\text{Abs blank})] \times 100$. Data are presented as means with standard deviations.

Results and Discussion

Comparison of all three methods for determining the antioxidant capacity of the milk samples are shown in the Table 1. PMM+FF has the highest AOC (92.87 % by DPPH and 0.209 mM vit C by CV and 0.194 mM vit C DPV method). Similarly, high AOCs are obtained for infant formula MIL PRE and PMM. As it is expected CM has the lowest AOC compared to PMM and MIL PRE. These results are shown in Figure 1, with representative differential pulse and cyclic voltammograms obtained for these samples. The tested samples indicated dominant oxidation peak at potential of around 0.6–0.75 V. Areas below oxidation peaks are proportional to the quantity of antioxidant compounds in milk samples. The AOC can be illustrated by the oxidation half potential, which reflects the particular reducing power of a substance, and the intensity of the oxidation peak current, which is proportional to the total concentration of the antioxidant compounds. The DPPH method appeared more limited by high concentrations of antioxidants, and at higher concentrations of antioxidants would be an unreliable indicator of breast milk antioxidant levels [7].

Table 1. Total antioxidant capacity in human milk of mothers of preterm infants and infant formula.

Method	CV (vit C mM)	DPV (vit C mM)	DPPH (% radical scavenging)
PMM	0.044±0.002	0.045±0.002	81.27±0.01
PMM + FF	0.209±0.001	0.194±0.002	92.87±0.01
MIL PRE	0.020±0.001	0.053±0.001	91.08±0.01
CM	0.006±0.001	0.012±0.001	40.53±0.01

CONCLUSION

In this work, it was shown that tested electrochemical method offer fast and accurate procedures for estimation of AOC. Results obtained with these methods are comparable with standard spectrophotometric method and could be suitable replacement for other, time consuming and expensive techniques.

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