Belgrade Food International Conference

Food, health and well being

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Belgrade Food International Conference

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P 2.18. Comparative electrochemical determination of total antioxidant activity in breast milk with infant formula

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Human breast milk contains all of essential nutrients and is commonly known as best kind of nutrition for neonates. However, when mother is not able to breastfeed, infant formula is a good enough replacement, so that babies not only survive but thrive on it. The study aimed to consider the significance of breast milk and infant formula in preventing oxidative stress by electrochemical determination of total antioxidant potential, demonstrating the relationship between antioxidant capacity in milk and postnatal age. Human breast milk, UHT milk, and infant formulas supplemented with prebiotics were used. Milks were diluted in phosphate buffer solution and total antioxidant activity was potentiometrically measured using iodine/iodide redox couple with the Pt Fisher electrode as a working electrode and saturated calomel as reference electrode. Cyclic voltammograms and differential pulse voltammetry were recorded with GC electrode as working, an accessory platinum electrode and an Ag/AgCl reference electrode. Cyclic voltammograms were recorded for milk using oxidation potentials between -400 and +1000 mV versus Ag/AgCl electrode. Only one anodic peak was found in each milk sample, and no reduction wave was observed. The anodic peak potentials were located between 480 and 580 mV, suggesting that +200 mV should be a sufficiently high potential for a stationary electrode to oxidize the antioxidants in the samples. DP voltammograms were recorded between -100 and +700 mV, with anodic peak potential at +500 mV. Potentiometric 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. Plotting the derivative of the oxidant concentration with potential as a function of potential showed that all samples had a double-peak curve due to the presence of two major oxidizable components that are sequentially oxidized by iodine. A main advantage of the electrochemical methods used to assess total antioxidant activity in milk was that they directly monitored the electron donating ability of the compounds, and can be used for quantitative analysis of the total antioxidants in different types of milk.

COMPARATIVE ELECTROCHEMICAL DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY IN BREAST MILK WITH INFANT FORMULA

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INTRODUCTION

Human breast milk contains all of essential nutrients and is commonly known as a best kind of nutrition for neonates. However, when mother is not able to breastfeed, infant formula is a good enough replacement, so that babies not only survive but thrive on it. The study aimed to consider the significance of breast milk and infant formula in preventing oxidative stress by electrochemical determination of total antioxidant potential, demonstrating the relationship between antioxidant capacity in milk and postnatal age. In the present work, it has been investigated total antioxidant activity of milk samples prepared as a meal for infants with three different electrochemical methods.

MATERIAL AND METHODS

Human breast milk (MM), commercial cow milk with 3.2% content of fat (CM), and an infant formula (IF - MIL 1) for infants under 6 months of age, supplemented with prebiotics were used. Samples were diluted (1:1 v/v) with a phosphate buffer solution (pH 6.7) in order to maintain a constant pH during the measurements, and total antioxidant activity was potentiometrically measured using iodine/iodide redox couple with the Pt Fisher electrode as a working electrode and saturated calomel as reference electrode. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were recorded using a CHI760B instrument (CHInstruments, Austin, USA), with GC electrode as working, an accessory platinum electrode and an Ag/AgCl reference electrode. 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}.

RESULTS AND DISCUSSION

The potentiometric titration of milk exhibited two clear and separated redox processes associated with at least two oxidizable species (Figure 1). From separating the phases of milk it was concluded that at least one of these species is hydrophobic and tends to stay in the fatty phase, and one is highly hydrophilic and could be titrated in the aqueous phase after phase separation by centrifugation. Potentiometric measurement indicates that human breast milk has highest antioxidative activity (100%), while commercial cow milk has very low (40%). Infant formulas have also high antioxidative activity (70%). Plotting the derivative of the oxidant concentration with potential as a function of potential showed that all samples had a double-peak curve due to the presence of two major oxidizable components that are sequentially oxidized by iodine. It was investigated the use of the differential pulse votammetry with the same electrode system as for the determination of cyclic voltammetry, and the results obtained for different milk samples and their water phase are presented as % of total antioxidative activity, based on measured area under the curve, showed in the figure 2 and 3. Values obtained from the figures showed that the results obtained by DPV method as area under the curve at 500 mV (MM – 100%, Mil 1- 66 % and CM- 43%) are in good agreement with those obtained from potentiometric and CV determination as area under the curve at 200 mV (MM – 100%, Mil 1 - 70 % and CM- 38%) for the total antioxidant activity of milk meals.

CONCLUSION

Comparing the three methods with each other, it can be concluded from the above results that all three methods can be used to determine the total antioxidant activity and the results obtained with all three techniques agree well and follow the same trend.To assess the total antioxidant capacity of infant formula, human breast milk and commercial cow milk, three electrochemical methods including potentiometry, cyclic voltammetry and differential pulse voltammetry were applied. A main advantage of the electrochemical methods used to assess total antioxidant activity in milk was that they directly monitored the electron donating ability of the compounds, and can be used for quantitative analysis of the total antioxidants in different types of milk. According to the results all three methods, it can be concluded that IF for infants under 6 months of age has very high antioxidant capacity (70 %) compared to human breast milk (100%), as a gold standard in infant nutrition. It is very important for normal physiological development of infants and children. We believe that all three electrochemical procedures could be highly relevant to the quick and routine measurement of total antioxidant capacity of milk and infant formula, and of the freshness of milk, as well as for quantitative determination of total antioxidant capacity of milk.

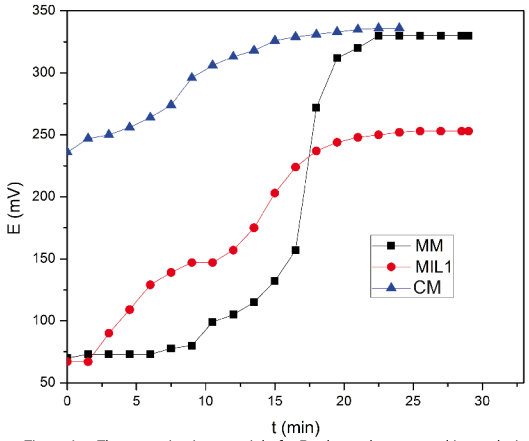

Figure 1: The open circuit potential of a Pt electrode measured in a solution of different milk samples in phosphate buffer pH 6.7 that were titrated with 1 mM of I^-/I_2. Human breast milk –meal and water phase (MM), Infant formula–meal and water phase (MIL 1), C. Commercial cow milk–meal and water phase (CM)

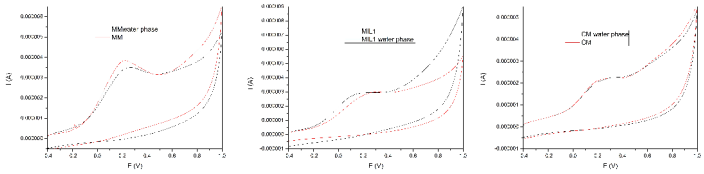

Figure 2: CVs recorded at a GC electrode in 0.1M phosphate buffer pH 6.7; scan rate 100mVs^{-1} in the potential range of -400 to 1000 mV. A: Human breast milk –meal and water phase (MM), B. Infant formula–meal and water phase (MIL 1), C. Commercial cow milk –meal and water phase (CM)

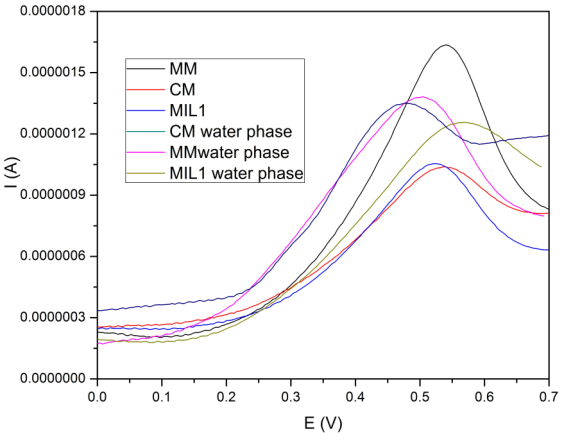

Figure 3: Shows DP voltammogram of recorded milk samples at scan rate 100 mVs^{-1}, pulse amplitude 100 mV, initial potential -400 mV and final potential +1000 mV. Human breast milk –meal and water phase (MM), Infant formula–meal and water phase (MIL 1), C. Commercial cow milk–meal and water phase (CM)