DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY OF MILK USING POTENTIOMETRIC TITRATION

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INTRODUCTION: Human milk is the best food during infancy as it is the best source of nutrients and it also supplies a number of defense factors for the growing infant. It has a number of enzymatic and nonenzymatic antioxidant constituents, such as superoxide dismutase, glutathione peroxidase, catalase, vitamin E, vitamin C, ß-carotene, which can protect newborns against reactive oxygen species at the early stage of life. Mother's milk is a synergistic mixture of multiple interacting factors and it is affected by the maternal antioxidant status, which in turn can influence the antioxidant status of breast-fed infants.

Although human breast milk is the best dietary choice for a newborn, infant formula is a good enough replacement, so that babies not only survive but thrive on it. There is a constant search for an improved way of monitoring the quality and freshness of infant formula. The freshness and quality of milk is undoubtedly affected and reflected by its redox state. In a complex fluid such as milk, several oxidation-reduction systems are active simultaneously and their effect on the oxidation-reduction potential depends on several factors, such as the reversibility of the system, its E^0 value, the ratio of oxidant to reductant, and the concentration of the active compounds of the system.

The aim of this research was to determine the similarity and difference in total antioxidant capacity between formula and breast milk for the purposes of preventing oxidative stress and modifying infant formula.

METHODS: Human breast milk, collected from 10 mothers in the 9th week of lactation, 3% skimmed milk UHT, and an infant formula supplemented with prebiotics were used as food for infants under 6 months of age. Milks were diluted (1:1 v/v) in a phosphate buffer solution, (pH 6.7) in order to maintain a constant pH during the measurements. The total antioxidant activity was potentiometrically measured using iodine/iodide redox couple with a two-electrode cell. The Pt wire was used as a working electrode and a saturated calomel electrode was used as a reference electrode. The total antioxidant activity (redox potential) was measured by a Pt electrode in a solution consisting of 1: 1 v/v milk and phosphate buffer (0.1 M pH 6.7) upon adding constant volumes of redox mediator 0.1 M J^-/J2. Portions of 10 µL of the titrant were added into 50 mL of solution every 300 s.

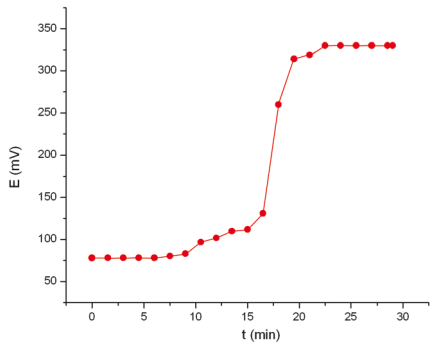
Cyclic voltammograms were recorded on a CHI760B instrument (CHInstruments, the USA). The cell was equipped with a GC electrode, an accessory platinum electrode of larger area (Model CHI221, cell top including a Pt wire counter electrode) and an Ag/AgCl reference electrode (Model CHI111). All measurements were taken at ambient temperature.

RESULTS: Potentiometric data of all milks indicate that total antioxidant capacity was high in both human breast milk and infant formula. Figure 1 shows determination of total antioxidant activity of human breast milk (a), infant formula (b) and 3% skimmed milk UHT (c), using potentiometric titration with 0.1 M J2 .The human breast milk has redox potential of 260 mV, infant formula 180 mV, while 3% skimmed milk (UHT) has redox potential of 70 mV. In the case of skimmed milk UHT, it was also shown that there are lipid and water phases which have different redox state. 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 (d).

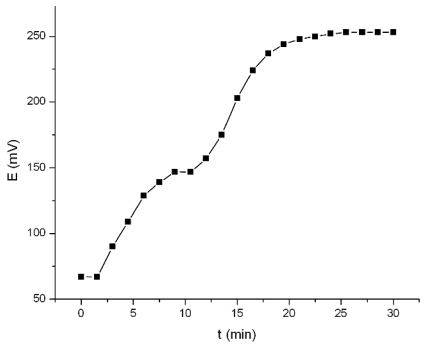
Electrochemical behavior of milk and semiquantitative determination of vitamin C were detected by cyclic voltammetry (figure2). Figure 3 shows calibration curve of Vitamin C, with concentrations 0.5, 1, and 2 mM.

CONCLUSION: Based on our results, it can be concluded that milk is not a well-poised system due to the presence of at least two different oxidizable species, one of which is hydrophobic while the other is hydrophilic. It was observed that total antioxidant capacity of infant formula is similar to the one of breast milk.

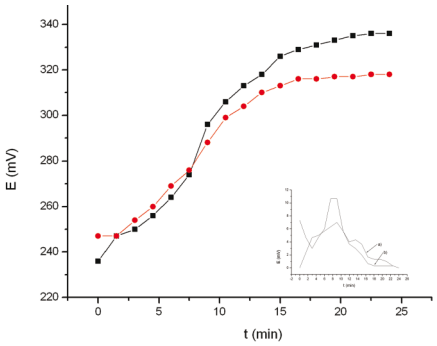
Figure 1 Determination of total antioxidant activity of human breast milk (a), infant formula (b) and 3% skimmed milk UHT (c) using potentiometric titration with 0,1 M J2.



(a) Human breast milk



(b) Infant formula



c) Skimmed milk UHT, lipid and water phase (black), and water phase (red).

d)Two major oxidizable components of milk (UHT) are present and sequentially oxidized by iodine

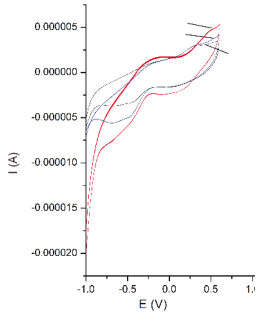


Figure 2 Electrochemical behavior of milk detected by cyclic voltammetry

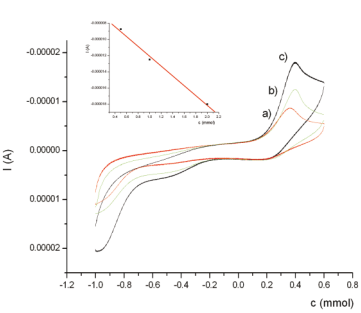


Figure 3 Calibration curve of Vitamin C with concentrations 0.5, 1, and 2 mM