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Effects of Light on Riboflavin and Ascorbic Acid in Freshly Expressed Human Milk

Jimi Francis
The University of Texas at Tyler, jfrancis@uttyler.edu

Darby Dickton

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Abstract

Millions of dollars each year is spent funding dairy research to better understand every aspect of milk processing, storage, handling, and shelf life. The dairy industry has shown that in animal milks vitamin C is photo-oxidized when exposed to light, which can cause a cascade of other nutrients that may be affected. Expressed human breast milk has had limited research published, mainly recommendations for storage duration secondary to bacterial growth, with scant research on nutrient quality during handling compared to the animal models. In this study, freshly expressed human milk was placed in containers of varying color/UV sensitivity and exposed to light over 6 hours. The laboratory analysis showed riboflavin and ascorbic acid concentrations rapidly decreased in clear containers. The containers wrapped in foil and those of amber color appear to have prevented the photo-oxidation of riboflavin and ascorbic acid. The concentrations of riboflavin and ascorbic acid consistently decreased over a relatively short space of time when stored in translucent containers. The control of photo-oxidation is an important component of maintaining nutrient quality, particularly in foods intended for infants. Minimizing light exposure would provide protection to the nutrients that are susceptible to oxidation. More research is needed to update recommendation for handling expressed human milk to ensure integrity of fragile nutrients in expressed human milk. The authors concluded that amber and other darkened containers that can prevent photo-oxidation of the breast milk could prevent degradation of certain nutrients in stored expressed human milk and possibly the shelf-life. While more research is needed to further identify harmful and helpful aspects of breast milk storage, these findings can establish a foundational understanding and new perspective on human breast milk handling.

Keywords: Breast milk; Ascorbic acid; Riboflavin; Storage; Photo-oxidation; Dairy

Research Article

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Jimi Francis[1]* and Darby Dickton[2]

[1]*Department of Health & Kinesiology, University of Texas, USA*
[2]*Western University of Health Sciences, USA*

***Corresponding author:** Jimi Francis, University of Texas at Tyler, 3900 University Blvd Tyler, TX 75799, USA, Tel: 903-565-5522, Email: jfrancis@uttyler.edu

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Abbreviations: WPS: Well Plate Sampler; HPLC: High Performance Liquid Chromatography

Introduction

While human mothers are more often expressing their milk to use later with little thought to storage conditions, the dairy industry is acutely aware of how storage conditions such as light, heat, and oxygen affect the flavor and nutritional value of other mammals' milk [1]. One nutrient abundant in all milk that is highly light-sensitive is riboflavin (vitamin B2) [2], which, in cows' milk is destroyed when exposed to light [3] and produces byproducts responsible for a rancid (off-flavor) of milk [4,5]. This degradation leads to the formation of the free radicals superoxide (O2-) and singlet molecular oxygen (1O2) [6], which degrade other nutrients such as vitamin C [7].

Research reports from the dairy industry indicate that vitamin C is reduced during milk processing [2], supporting the premise that exposure to light and oxygen causes a loss of nutrients other than vitamin C. According to Cakmakci et al., milk should be delivered in light-proof packaging materials and stored in refrigerated conditions immediately following production to prevent destruction of vitamin C [7]. Other dairy studies have shown that milk loses 30% of riboflavin after 30 minutes exposure to sunlight [8]. It is unknown if these reactions also occur in human milk.

According to the International Dairy Federation, the light transmittance through packaging into the food must not exceed 2% at 400 nm and 8% at 500 nm to protect the contents sufficiently from light oxidation [7-9]. Controlling photo-oxidation is an important component of maintaining nutrient quality in all milks, which requires more human milk studies to confirm the findings of the dairy industry. The objective of this study is to evaluate ascorbic acid and riboflavin in freshly expressed human milk when stored in containers of varied colors, materials, and UV resistance.

Materials and Methods

Breastfeeding mothers were recruited from various lactation support sources to participate in this research project. Written informed consent was obtained from the women that agreed to participate in the study. Each participant was brought into a room which had dim lighting and expressed milk using a standardized breast pump. The expressed milk sample was immediately wrapped in foil and placed in a small cooler to be transported into the laboratory. Each sample was removed from the cooler in the lab under red light working conditions within 10 minutes of being

expressed and a baseline sample was immediately analyzed. Aliquots were split between the variable micro-centrifuge 1mL tubes. Into each of the tubes, clear, opaque, blue, green, amber and foil. 1mL of milk was pipetted gently while placing the tip against the side of the tube in order to avoid oxidation. The tubes were then exposed to light and samples were removed from each tube at the respective times of 30 minutes, 1 hour, 2 hours, 4 hours, and 6 hours. Twenty-seven samples were analyzed in triplicate. Every sample taken for testing was placed in amber HPLC vials, wrapped in foil under red light working conditions, and taken immediately for analysis under red-light working conditions. Riboflavin and ascorbic acid concentrations were identified and quantified on the basis of standard solutions used as external standards (R=0.9827). Isocratic chromatographic separation was carried out using a mobile phase of Milli-Q water with acetic acid (0.1%, v/v) and methanol in a relative proportion of 95:5 (v/v) using all HPLC grade reagents. One microliter of filtrate was injected by a WPS-3000 auto-sampler (Dionex) onto a Polar Advantage II (C18 3 um, 4.6 × 150 mm, Dionex) equipped with a guard column (PAII C18, 5 um, 4.3 × 10 mm, Dionex) and chromatographed on the Ultimate 3000 HPLC (Dionex) using the LPG-3000 loading pump in conventional HPLC mode. Ascorbic acid was identified by comparing the retention time of the sample peak to the peak from the ascorbic acid standard at wave length 254 nm. Riboflavin was identified by comparing retention times of the sample peak to the standard peak at wave length 450 nm.

All values were entered into a Microsoft Excel spreadsheet. The mean of each triplicate sample set was calculated. The mean values were used to calculate the amount of ascorbic acid in milligrams per liter of milk. Standard deviation and percentage of baseline was calculated for each mean value. Analysis of variance was used to determine significant differences in ascorbic acid levels for the time periods being studied. Alpha error was set at $p < 0.01$ for all tests.

Results

Both ascorbic acid and riboflavin concentrations declined quite rapidly in the clear containers. Samples in the Control and Amber containers maintained levels above 90% of the original ascorbic acid and riboflavin concentrations while in the remaining containers the concentrations decreased to less than 65% of the baseline level. The concentrations of riboflavin and ascorbic acid consistently decreased over a relatively short space of time when stored in translucent containers.

The mean riboflavin values were stable in the control tubes covered in aluminum foil and the amber tubes at above 95% of baseline values. In the other colored tubes, the mean level of riboflavin dropped below 75% by 30 minutes and continue to decrease over the 6 hours of testing to <35% of baseline (Figure 1).

The mean level of ascorbic acid in the control tubes covered in aluminum foil and the amber tubes at around 98%. In all other tubes the level decreased by the 30 minute sampling to < 68% of the Baseline level (Figure 2).

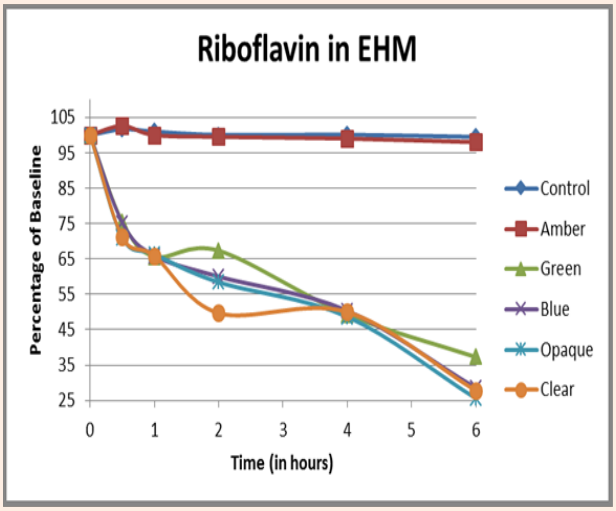

Figure 1: Mean level of riboflavin in freshly expressed human milk in various colored containers.

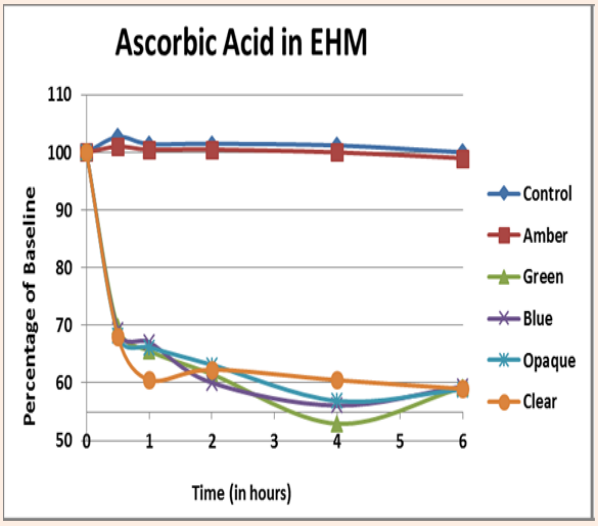

Figure 2: Mean level of ascorbic acid in freshly expressed human milk in various colored containers.

Conclusion

The control of photo-oxidation is an important component of maintaining nutrient integrity in foods intended for consumption, particularly in foods intended for infants. As infants fed expressed human milk, below the age of 6 months, rely solely on one source of nutrition, the importance of maintaining the integrity of all the nutrients contained in that milk is critically important for the infant's health and growth. The changes in nutrient integrity may impact other aspects of the milk. Notably, decreases of the nutrients evaluated in this study may also lead to light-induced flavor changes in the milk as are seen in the dairy industry. The aspect of flavor of human milk requires further study. Minimizing

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light exposure would provide protection to the nutrients that are susceptible to oxidation, and also potentially protect the flavor of the milk, though at this time there has been limited research into the effects of light on the taste of human milk. More research is needed to update recommendations for storage and handling of expressed human milk to ensure integrity of fragile nutrients in expressed human milk.

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