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Evaluation of Vitamin A, E &C In Human Milk And Infant Formulae For Different Storage Conditions

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----------ABSTRACT----------
Storage of human milk for further use in infant feeding, now is more frequently, as a result of the social-economic activities of breastfeeding mothers. Expressed breast milk is usually stored frozen for consumption at a later time. Currently, human milk is stored in containers with headspace, where the removal of oxygen should minimize the oxidative effects in compounds of stored breast milk during shelf life, and improve nutritional quality.The purpose of this study was to analyze the quantity of some vitamins and compare any variation in vitamin A, E & C in milk frozen in two different containers; one with air in the headspace versus vacuum sealed storage containers. Vitamin A and E content were analyzed using high-performance liquid chromatography (HPLC). Fresh, mature, human milk donated by 4 women with healthy infants was used for analysis. Infant formula as powder and rehydrates liquid was also analyzed. According the results of analyses, the values of infant formula nutrient are generally higher than the human milk values.

Keywords *- Vitamins A, E &C high-performance liquid chromatography, human milk, infant formula*

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I. INTRODUCTION

Breastfeeding women working outside the home commonly utilize a breast pumping device to provide milk for their infants. Human milk storage for use later in infant feeding is on the increase as a result of the economic activities of breastfeeding mothers. One reason for providing expressed milk to an infant is the anti-oxidant properties of human milk in the form of vitamins. It is known that fresh human milk has a higher antioxidant capacity than infant formula. [1]. Expressed breast milk is usually stored frozen for consumption at a later time. It is known that changes occur in human milk stored either via refrigeration or frozen. Many nutrients, including vitamins, are susceptible to oxidation. Breast milk is subject to a strong peroxidation either at room temperature or at -20 degrees C. The oxidation of food products is well documented. Changes in product color, odor and taste are the most easily detected effects of oxidation in foods.[2]. Nutrient loss, rancidity, and microbial spoilage are all a part of milk degradation as well. This degradation, over time, is caused in part, by the presence of oxygen, and oxidation of the food components.[3]. Currently, human milk is stored in containers with headspace. Removal of headspace oxygen should minimize the oxidative effects in stored breastmilk, prolong shelf life, and improve nutritional quality. The purpose of this study was to analyze and compare any differences in vitamin A, E and C in milk frozen in containers with ambient air in the headspace versus vacuum sealed storage containers. [4]

II EXPERIMENTAL

Fresh, mature, human milk donated by four women with healthy infants was used for analysis. Infant formula from powder and liquid was also analyzed. Baseline analysis of each sample was completed within 24 hours of collection for Vitamin A and E. Each sample was divided into two sets, set S (standard) and V (vacuum) with aliquots of 1 (ounces) each: i.e., Day 10, Day 20, and Day 40. A 20 ml sample was removed from each aliquot and tested for the nutrients of interest. Half the samples were then vacuum sealed and placed in the freezer at -20 °C. The other half of the samples were sealed with ambient air and placed in freezer at -20 °C. After 10 days, one aliquot from each group was removed for analysis. At 20 days and 40 days the process was repeated. Vitamin A and E content were analyzed using high-performance liquid chromatography (HPLC) [5]. Prior to testing, the samples were briefly immersed in warm water (40°C) to thaw them, then mixed using a vortex to provide a homogenous sample. One ml of the sample was transferred to a centrifuge tube and 3 ml of ethanol were added. The samples were mechanically shaken and 1 ml of hexane was added, then shaken for another

minute. After resting the samples for 5 minutes, 3 ml of saturated NaCl was added to aid separation. The mixture was shaken by inversion. The samples were centrifuged for 5 minutes at 3000 rpm at room temperature. The hexane phase was recovered and directly filtered through a .22 µm nylon filter and collected in a 1 ml amber glass vial. Twenty µl was injected into the HPLC system.

Vitamin C content was analyzed using [6]. The samples were protected from light by wrapping tubes and flasks with aluminum foil and preparing the samples in a darkened room. Three hundred µl of milk mixed with 300ul of 0.56% meta-phosphoric acid solution were added to the same centrifuge and filtration tube, which was shaken for 30 seconds and centrifuged at 10°C (10 minutes, 3000 X g). Ascorbic acid was identified by comparing the retention time of the sample peak with that of the ascorbic standard at 254 nm. Quantification was carried out using external standardization.

III. RESULTS AND DISCUSSION

Descriptive statistics were calculated and nutrient mean values were compared using paired t-tests. Statistical significance was determined when $p < .01$.

The baseline values for each sample and nutrient are listed in TABLE 1. These values are highly variable, as would be expected in human milk. The infant formula nutrient values are generally higher than the human milk values. The range for vitamin A values was 196 mcg/L in human milk to a high of 1147 mcg/L in infant formula. For vitamin E, the lowest level was 1.633 mg/ml to a high level of 4.594mg/ml. The Vitamin C levels ranged from 32 mg/ml to a high of 89 mg/m.

	A (mcg/L)	E (mg/L)	C (mg/L)
HM1	453	2.142	51
HM2	694	3.247	87
HM3	196	1.633	32
HM4	302	4.365	89
IF1	712	3.238	61
IF2	1147	4.594	81
Average	584	3.203	66.8

Table 1. Baseline values by nutrient and sample

Due to the high variability of the values for human milk the mean values were used for comparison. The decrease in levels of vitamin A and E was higher in the S group than in the V group and was statistically significantly different.

The baseline values and the final values for each group are listed in TABLE 2.

The mean values for the human milk samples are depicted in TABLE 3.

Table 2.Values at baseline and 40 days.

	A(mcg/L)			E(mg/L)			C(mg/L)		
	Baseline	S40	V40	Baseline	S40	V40	Baseline	S40	V40
HM1	453	337	441	2.142	1.372	1.932	51	0	21
HM2	694	602	634	3.247	2.789	2.901	87	28	68
HM3	196	179	182	1.633	1.480	1.501	32	0	20
HM4	302	267	297	4.365	4.201	4.328	89	46	71
IF1	712	482	484	3.238	2.266	2.328	61	489	41
IF2	1147	1038	1038	4.594	3.001	3.581	81	0	54
Average	584	484	512	3.203	2.518	2.761	66.8	93.8	45.8

Table 3. Mean values of vitamins in human milk.

		10 days		20 days		40 days	
Vitamin	Baseline	S Group	V Group	S Group	V Group	S Group	V Group
A IU/ml	1.213	1.204*	1.210	1.114	1.196	1.137*	1.161
E mcg/ml	2.344	2.222*	2.243	2.159	2.187	2.040*	2.182
C mg/ml	0.058	0.041	0.052	0.034	0.042	0.018	0.041

*Statistically significantly below baseline at $p < 0.01$.

For the samples that were refrigerated, the nutrient levels were higher in the V group than in the S group. Each time-point measurement indicated consistent decreases in the level of all nutrients in the S group compared to slight decrease in the V group. While the differences were not statistically different, there was practical meaning of the differences. A larger sample size may have yielded significant differences.

IV. CONCLUSIONS

All of the nutrient levels dropped for the duration of the time in storage in both groups but consistently less in the V group. For example, at baseline, most subjects had adequate levels of vitamin A at baseline to meet the RDI for their infant. However, by the end of the 40 days storage time not all samples contained the RDI. by the end of the 40 days storage time not all samples contained the RDI for vitamin C of 50 mg/day for infants of less than 1 year of age. In particular, vitamin C content from the S group was reduced to non detectable levels in four of the human milk samples by the last measurement, while the V group all had Nutrients were better preserved by vacuum sealing, providing nutrient levels near the RDI for infants under the age of 1 year. Women around the world are storing their milk frozen for times of separation from their infants. [7] Providing a storage system that preserves the nutrients in frozen human milk is critical for the long-term health of those infants fed with frozen milk.

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