# **Stability of Thiamine and Vitamins E and A during Storage of Enteral Feeding Formula**

### Juana Frias and Concepción Vidal-Valverde\*

Instituto de Fermentaciones Industriales (Consejo Superior de Investigaciones Cientificas), Juan de la Cierva 3, E-28006 Madrid, Spain

The content of thiamine (vitamin  $B_1$ ), vitamin E ( $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherol) and vitamin A (all-*trans*retinol and 13-*cis*-retinol) in five commercial enteral feeding formulas was studied. These formulas provide a large amount of vitamins: 2.5–3, 3.1, and 1.6-fold above the U.S. daily recommendations (U.S. RDA) for thiamine, vitamin E, and vitamin A, respectively. The stability of thiamine and vitamins E and A of two of the enteral feeding formulas was followed throughout 9 months of storage in the dark at 4°, 20°, and 30 °C. According to our results in all of the storage conditions studied, the enteral formula covered the U.S. RDA levels for thiamine and vitamin E, but in the case of vitamin A the U.S. RDA requirements are met only when the enteral sample has been kept in storage less than 3 months. After 6 months of storage, the decrease in vitamin A was so drastic that the level of vitamin covered only 0.9–0.3-fold of the U.S. RDA and after 9 months it covered only 0.2–0.0-fold of U.S. RDA. The manufacturer shelf life of the formula studied was 1 year; and, because enteral feeding solutions are often placed in storage conditions of uncontrolled temperature and humidity, these results should be taken into consideration with regard to the shelf life.

**Keywords:** Enteral feeding formula; enteral nutrition; thiamin; vitamin E; tocopherol; vitamin A; retinol; vitamins; storage

## INTRODUCTION

Formula diets for enteral nutrition meet all of the nutritional requirements which are especially suited for nutritional support of those people who cannot ingest or digest sufficient amounts of food. The increasing popularity of enteral feeding in various clinical states can be attributed to the availability of a wide variety of commercial enteral feeding formulas with different nutrient components that allow for a choice of suitable formulas for patients with limitations in gastrointestinal function or for those who require special nutrition.

The compositions of commercial formulas are based on protein (in the form of amino acids, peptides, or intact protein), carbohydrates (in the form of glucose polymers or maltodextrins, disaccharides, and oligosaccharides), and different amounts of fat (partially digested to monoor diglycerides); and most commercially available liquid diets are fortified with minerals and vitamins. The enteral feeding formulas contain sufficient amounts of vitamins, including water-soluble and fat-soluble vitamins, so that intake of 1500 to 2000 calories per day provides adequate amounts of these micronutrients.

Enteral feeding formulas are not necessarily consumed immediately after manufacture; they are frequently stored without adequate control of temperature for periods of time. Vitamins may be affected by various factors such as the type of packaging and the length and condition of storage (e.g., exposure to oxygen, light, and high temperatures).

Thiamine (vitamin  $B_1$ ) is quite stable below pH 5.5 but above pH 7.0 can be destroyed very fast even at

room temperature. Vitamin E ( $\alpha$ -tocopherol,  $\delta$ - tocopherol, and  $\gamma$ -tocopherol) and vitamin A (all-*trans*retinol and 13-*cis*-retinol) seem to be very stable under nitrogen atmosphere in a dark cool place, but they are particularly sensitive to oxidation by air in the presence of light (*1*). Considering this information, the conditions of storage of enteral feeding formulas could affect, to some extent, their vitamin content.

Stability of thiamine in total parenteral nutrition admixtures (TPA) and infant formula after different storage conditions has been reported by different authors. Montero et al. (2) reported that thiamine in TPA was not stable even under refrigeration. Baumgartner et al. (3) also observed that thiamine undergoes a significant degradation at room temperature after 24 h. In liquid infant milks, however, thiamine seems to be more stable with regard to temperature (4).

Vitamin E seems to be stable under refrigeration and darkness in TPA for 20 days (5,  $\theta$ ) and in liquid infant formula stored at 20° and 30 °C for 12 months (4).

Exposure to light and presence of oxygen seem to be the major factors destroying vitamin A in TPA, and Kishi et al. (5) recommended protecting the formulas from daylight. McCarthy et al. (8) have correlated the degradation of vitamin A with the presence of oxygen during the storage of milk. Furthermore, it is very unstable in atmospheric conditions, and Shenai et al. (7) recommended increasing the vitamin A concentration in TPA severalfold because of the substantial loss during administration.

Although some information has been found on vitamin degradation in TPA and infant formulas during storage conditions, there is no information available on the stability of vitamins in enteral feeding admixtures, although the manufacturers suggest a shelf life of one

<sup>\*</sup>To whom correspondence should be addressed. Telephone: +34 91 5622900. Fax: +34 91 5644853. E-mail: ificv12@ifi.csic.es.

 Table 1. Protein, Lipid, and Carbohydrate Contents in

 Enteral Feeding Formulas (g/100 mL)

EFF	proteins	lipids	carbohydrates	
I	5.5	3.6	11.4	
II	3.7	3.9	12.5	
III	4	3.9	12.3	
IV	4	1.0	18.8	
V	1.2	5.1	12.3	

year. Any changes of these micronutrients in the enteral feeding formula could be crucial for patients; therefore, further efforts are required to establish the stability of vitamins in these admixtures.

The objectives of the present paper were, first, to survey the content of thiamine, vitamin E ( $\alpha$ -tocopherol,  $\delta$ -tocopherol, and  $\gamma$ -tocopherol), and vitamin A (all-*trans*-retinol and 13-*cis*-retinol) in five commercial enteral feeding formulas as sold to the consumer, and, second, to study the influence of different storage conditions (4°, 20°, and 30 °C for 3, 6, and 9 months) on those vitamins in two selected enteral feeding admixtures.

#### MATERIALS AND METHODS

**Samples.** Five commercial enteral feeding formulas (EFF) (samples I-V) were purchased at a local pharmacy. The content of protein, lipids, and carbohydrates per 100 mL (as indicated on the label) are presented in Table 1.

Two of these formulas containing different protein content (hyperproteic and normoproteic [EFF I] and [EFF II], respectively, Table 1) were selected for use to carry out storage trials at different temperatures and periods of time. Samples of these enteral formulas were freshly prepared by the industry and immediately submitted to storage studies (samples Ia and IIa).

**Industrial Elaboration for Storage Trials.** The elaboration of EFF Ia and EFF IIa consisted of reconstitution of principal components (proteins, malto dextrins, and vegetable fat) in water, pre-sterilization (indirect UHT, 136 °C for 3-4 s), homogenization, addition of vitamins, packaging in a light-inhibiting glass container, and sterilization at 118 °C for 9 min.

**Storage Conditions.** EFF Ia and EFF IIa were stored at  $4^{\circ}$ ,  $20^{\circ}$ , and  $30 \,^{\circ}$ C for 3, 6, and 9 months in darkness. Vitamin content in control samples was determined just after elaboration.

**Determination of Vitamins.** Two batches each of EFF Ia and EFF IIa were submitted to extraction of thiamine, vitamin E and its vitamers ( $\alpha$ -tocopherol,  $\gamma$ - tocopherol, and  $\delta$ -tocopherol), and vitamin A and its vitamers (all-*trans*-retinol and 13-*cis*-retinol), and subsequently quantified by HPLC according to a previous paper (9).

The vitamin E activity of the formula was defined in terms of RRR- $\alpha$ -tocopherol calculated equivalents ( $\alpha$ -*TEs*). One  $\alpha$ -*TE* is the activity of 1 mg of RRR- $\alpha$ -tocopherol. The vitamin E activity was calculated using the factors for conversion of

tocopherols to RRR- $\alpha$ -tocopherol equivalents (10):

Vitamin E activity ( $\alpha$ -*TE*/100 mL) = ( $\alpha$ -tocopherol (mg) × 1.0) + ( $\gamma$ -tocopherol (mg) × 0.1) + ( $\delta$ -tocopherol (mg) × 0.03)

The vitamin A activity of the formula was quantified by conversion of the vitamin A active components to retinol equivalents (*RE*). 1 RE = 1 mg of all-*trans* retinol (*11*):

Vitamin A (
$$RE/100$$
 mL) =  
(all-*trans* retinol (mg) × 1.0) +  
(13-*cis*-retinol (mg) × 0.75)

**Statistical Methods**. Data were subjected to multifactor ANOVA using the Statgraphics Program (Statistical Graphics System 5.0 Computer Software). The least-squares difference test was used.

#### **RESULTS AND DISCUSSION**

The contents of thiamine, vitamin E and its vitamers ( $\alpha$ -tocopherol,  $\gamma$ -tocopherol,  $\delta$ -tocopherol) and vitamin A and its vitamers (all-*trans*-retinol and 13-*cis*-retinol), of five commercial enteral feeding formulas (EFF I–V) are presented in Table 2. Our data were higher than the label information. In the case of vitamins A and E, the amount indicated on the label was in agreement with the results obtained for the all-trans retinol and  $\alpha$ -tocopherol vitamers, respectively; this could be because the manufacturer may have determined only the major vitamer. Furthermore, it is well-known that the most important vitamin A vitamer in foods is all-transretinol, wheras 13-cis-retinol has 75% of the vitamin A biological activity. The main component of vitamin E in foods is  $\alpha$ -tocopherol, but other vitamers such as  $\gamma$ and  $\delta$ -tocopherol have vitamin E activity (10 and 3%, respectively) (10).

In the enteral feeding formulas (EFF) thiamine content ranged from 0.14 to 0.17 mg/100 mL, the vitamin E activity was between 1.15 and 1.58  $\alpha$ -*TE*/100 mL, and the vitamin A activity was between 70.75 and 80.23 *RE*/100 mL (Table 2). These amounts were always above the U.S. daily recommendations, assuming an intake of two liters per day (2000 Kcal) (Table 5). For adults the U.S. RDAs are 1.1 to 1.2 mg for thiamine, 10 mg  $\alpha$ -*TE* for vitamin E in tocopherol equivalents. and 1000 *RE* for vitamin A in retinol equivalents (*12*). Our data showed that the freshly prepared enteral feeding formulas provided a large amount of vitamins, 2.5 to 3, 3, and 1.6-fold above the U.S. daily recommendations for thiamine, vitamin E, and vitamin A, respectively. Enteral feeding admixtures EFF I and EFF II (the

hyperproteic and one of the normoproteic) also showed

Table 2. Content of Vitamins in Different Enteral Feeding Formulas (EFFs)<sup>a</sup>

				0				
EFF	vitamin B <sub>1</sub> (mg/100 mL)	α-tocopherol (mg/100 mL)	γ-tocopherol (mg/100 mL)	$\delta$ -tocopherol ( $\mu$ g/100 mL)	vitamin E activity (α- <i>TE</i> /100 mL)	all- <i>trans</i> -retinol (µg/100 mL)	13- <i>cis</i> -retinol (µg/100 mL)	vitamin A activity ( <i>RE</i> /100 mL)
Ι	$\begin{array}{c} 0.165 \pm 0.002 \\ (0.11) \end{array}$	$1.547\pm0.028^{\scriptscriptstyle 1}$	$0.337\pm0.0101$	$5.663 \pm 0.148^{\rm 1}$	$\begin{array}{c} 1.581 \pm 0.025^{1} \\ (1.50) \end{array}$	$75.983 \pm 0.828^{\rm 1}$	$5.662\pm0.148^{\scriptscriptstyle 1}$	$\begin{array}{r} 80.230 \pm 0.846^{1} \\ (75.00) \end{array}$
II	$\begin{array}{c} 0.138 \pm 0.002 \\ (0.11) \end{array}$	$1.518 \pm 0.032^{\scriptscriptstyle 1}$	$0.339\pm0.0061$	$5.668 \pm 0.130^{\scriptscriptstyle 1}$	$egin{array}{c} 1.552 \pm 0.029^1 \ (1.50) \end{array}$	$75.333 \pm 0.715^{1}$	$5.668 \pm 0.130^{\rm 1}$	$\begin{array}{c} 79.585 \pm 0.779^{1} \\ (75.00) \end{array}$
III	$0.159 \pm 0.004$ (0.10)	$1.119 \pm 0.049^{\scriptscriptstyle 2}$	$0.279 \pm 0.011^{\scriptscriptstyle 2}$	$5.267 \pm 0.219^{\scriptscriptstyle 2}$	$\begin{array}{c} 1.147 \pm 0.044^{2} \\ (1.10) \end{array}$	$66.800 \pm 1.049^{\scriptscriptstyle 2}$	$5.267 \pm 0.219^{\scriptscriptstyle 2}$	$70.750 \pm 1.089^{2}$ (66.70)
IV	$\begin{array}{c} 0.154 \pm 0.003 \\ (0.10) \end{array}$	$1.115 \pm 0.041^{\scriptscriptstyle 2}$	$0.284 \pm 0.004^{\scriptscriptstyle 2}$	$5.346 \pm 0.099^{\scriptscriptstyle 2}$	$\begin{array}{c} 1.144 \pm 0.037^{2} \\ (1.10) \end{array}$	$68.283 \pm 0.768^{\scriptscriptstyle 3}$	$4.921\pm0.052$	$71.974 \pm 0.763^{3} \\ (66.70)$
V	$\begin{array}{c} 0.149 \pm 0.004 \\ (0.10) \end{array}$	$1.117 \pm 0.041^{\scriptscriptstyle 2}$	$0.281 \pm 0.012^{\scriptscriptstyle 2}$	$5.362 \pm 0.132^{\scriptscriptstyle 2}$	$\begin{array}{c} 1.145 \pm 0.038^2 \\ (1.10) \end{array}$	$67.350 \pm 0.532^{2,3}$	$5.362 \pm 0.132^{2}$	$71.371 \pm 0.607^{2,3} \\ (66.70)$

<sup>*a*</sup> Values are the mean of four determinations  $\pm$  standard deviation. Values in the same column for each vitamin with the same superscript are not significantly different ( $P \leq 0.05$ ). Values in parentheses are those claimed on the label by the manufacturer.

Table 3. Changes in Vitamin Content of EFF Ia after Different Storage Conditions<sup>a</sup>

EFF Ia	vitamin B <sub>1</sub> (mg/100 mL)	α-tocopherol (mg/100 mL)	γ-tocopherol (mg/100 mL)	$\delta$ -tocopherol ( $\mu$ g/100 mL)	vitamin E activity (α- <i>TE</i> /100 mL)	all- <i>trans</i> -retinol (µg/100 mL)	13- <i>cis</i> -retinol (µg/100 mL)	vitamin A activity ( <i>RE</i> /100 mL)	
control	$0.165\pm0.002^{1}$	$1.514\pm0.042^{1}$	$0.340 \pm 0.008^{1,2}$	$5.699 \pm 0.036$	$1.548 \pm 0.041_1$	$75.917 \pm 0.882^{1}$	$5.700\pm0.035$	$80.192 \pm 0.839^1$	
storage at	4 °C								
3 months	$0.165 \pm 0.002^{1}$	$1.489 \pm 0.027^{1}$	$0.345 \pm 0.011^{1}$	$5.577 \pm 0.078^{1}$	$1.523\pm0.027$	$75.650 \pm 0.387^{\rm 1}$	$5.606 \pm 0.041$	$79.855 \pm 0.298^{\rm 1}$	
6 months	$0.164 \pm 0.002^{1}$	$1.167\pm0.018$	$0.301\pm0.005$	$5.009 \pm 0.009^3$	$1.197\pm0.018$	$41.175 \pm 1.720$	$4.296\pm0.014$	$44.397\pm1.323$	
9 months	$0.105\pm0.006$	$0.957\pm0.011$	$0.211\pm0.013$	$3.454\pm0.073$	$0.978\pm0.012$	$11.450 \pm 0.580$	$2.081\pm0.025$	$13.011 \pm 0.337$	
storage at	20 °C								
	$0.164 \pm 0.001^{1}$	$1.442\pm0.019$	$0.335\pm0.001^{\scriptscriptstyle 2,3}$	$5.605 \pm 0.054^{1}$	$1.476\pm0.019$	$74.900 \pm 0.356^{1}$	$5.062 \pm 0.014$	$78.696 \pm 0.293$	
6 months	$0.164 \pm 0.002^{1}$	$1.076 \pm 0.031^2$	$0.290\pm0.004$	$4.968 \pm 0.048^{\scriptscriptstyle 3}$	$1.105\pm0.030$	$34.825\pm2.055$	$3.811 \pm 0.022$	$37.683 \pm 1.963$	
9 months	$0.088 \pm 0.015$	$0.837\pm0.015$	$0.164\pm0.009$	$3.008\pm0.046$	$0.853\pm0.014$	$7.800 \pm 1.180$	$1.869\pm0.014$	$9.202\pm0.432$	
storage at	storage at 30 °C								
3 months	$0.153 \pm 0.005$	$1.388 \pm 0.026$	$0.331 \pm 0.002^3$	$5.481 \pm 0.045$	$1.421 \pm 0.026_2$	$71.550 \pm 0.957$	$4.813\pm0.025$	$75.160 \pm 0.693$	
6 months	$0.127\pm0.003$	$1.063 \pm 0.047^2$	$0.246 \pm 0.003$	$4.216\pm0.006$	$1.088\pm0.047$	$17.650\pm0.412$	$2.619 \pm 0.022$	$19.614\pm0.204$	
9 months	$0.079 \pm 0.006$	$0.749 \pm 0.005$	$0.139 \pm 0.006$	$2.687 \pm 0.040$	$0.763 \pm 0.005$	ND	$1.322\pm0.019$	$0.991 \pm 0.011$	

<sup>*a*</sup> Values are the mean of four determinations  $\pm$  standard deviation. Values in the same column for each vitamin with the same superscript are not significantly different (*P* < 0.05). The same subscript in the same row between Tables 3 and 4 are not significantly different (*P* < 0.05).

Table 4. Changes in Vitamin Content of EFF IIa Formula after Different Storage Conditions<sup>a</sup>

EFF IIa	vitamin B <sub>1</sub> (mg/100 mL)	α-tocopherol (mg/100 mL)	γ-tocopherol (mg/100 mL)	δ-tocopherol (μg/100 mL)	vitamin E activity (α- <i>TE</i> /100 mL)	all- <i>trans</i> -retinol (µg/100 mL)	13- <i>cis</i> -retinol (μg/100 mL)	vitamin A activity ( <i>RE</i> /100 mL)
control	$0.135 \pm 0.003^{1}$	$1.521\pm0.061$	$0.343\pm0.005$	$5.651 \pm 0.091^{1}$	$1.556 \pm 0.061_1$	$75.617 \pm 0.852^{1}$	$5.650 \pm 0.090^{1}$	$79.854 \pm 0.617^1$
storage at	4 °C							
3 months	$0.135 \pm 0.002^{1}$	$1.444\pm0.017$	$0.335 \pm 0.003^{1}$	$5.584 \pm 0.030^{1,2}$	$1.478\pm0.018$	$75.550 \pm 0.645^{1}$	$5.618 \pm 0.039^{1}$	$79.763 \pm 0.588^{1}$
6 months	$0.128 \pm 0.002^3$	$1.142\pm0.012$	$0.301\pm0.004$	$4.259 \pm 0.007$	$1.173\pm0.012$	$39.775 \pm 0.350$	$4.237\pm0.059$	$42.953 \pm 0.215$
9 months	$\textbf{0.089} \pm \textbf{0.001}$	$\textbf{0.888} \pm \textbf{0.024}$	$\textbf{0.188} \pm \textbf{0.003}$	$3.072\pm0.023$	$0.907 \pm 0.024$	$10.075 \pm 0.411$	$2.105\pm0.040$	$11.654 \pm 0.355$
storage at	20 °C							
3 months	$0.135 \pm 0.002^{1}$	$1.405 \pm 0.013^{1}$	$0.330 \pm 0.003^{1,2}$	$5.590 \pm 0.031^2$	$1.439 \pm 0.012^{1}$	$71.975 \pm 0.299$	$4.985\pm0.020$	$75.714\pm0.198$
6 months	$0.126 \pm 0.001^{\scriptscriptstyle 2,3}$	$1.062\pm0.025$	$0.290\pm0.005$	$3.992 \pm 0.001$	$1.091\pm0.025$	$30.425\pm1.425$	$3.894 \pm 0.050$	$33.346 \pm 1.264$
9 months	$0.064 \pm 0.003^4$	$0.776 \pm 0.022$	$0.159 \pm 0.007$	$2.600\pm0.029$	$0.792 \pm 0.022$	ND	$1.819\pm0.006$	$1.364\pm0.003$
storage at	: 30 °C							
	$0.123 \pm 0.005^{\scriptscriptstyle 2}$	$1.389 \pm 0.016^{1}$	$0.326 \pm 0.002^2$	$5.402 \pm 0.028$	$1.422 \pm 0.016^{1}{}_{2}$	$70.075 \pm 0.602$	$4.624 \pm 0.019$	$73.544\pm0.524$
6 months	$0.108 \pm 0.006$	$0.938 \pm 0.034$	$0.238 \pm 0.002$	$3.439 \pm 0.006$	$0.962\pm0.064$	$14.125\pm0.650$	$2.394 \pm 0.034$	$15.920\pm0.670$
9 months	$0.063 \pm 0.001^4$	$0.692\pm0.011$	$0.131\pm0.003$	$2.257\pm0.020$	$0.705\pm0.012$	ND	$1.102\pm0.032$	$0.826\pm0.023$

<sup>*a*</sup> Values are the mean of four determinations  $\pm$  standard deviation. Values in the same column for each vitamin with the same superscript are not significantly different (*P* < 0.05). The same subscript in the same row between Tables 3 and 4 are not significantly different (*P* < 0.05).

Table 5.	Daily	Intake	<b>Ratio</b> for	EEF	' Ia and	EEF IIa	Vitamin	<b>Content</b> <sup>a</sup>	vs U.S. RDA

	vitamin B1 EFF Ia/ RDA	vitamin B1 EFF IIa/ RDA	vitamin E EFF Ia/ RDA	vitamin E EFF IIa/ RDA	vitamin A EFF Ia/ RDA	vitamin A EFF IIa/ RDA
control	3.0	2.5	3.1	3.1	1.6	1.6
storage at 4 °	°C					
3 months	3.0	2.5	3.1	3.0	1.6	1.6
6 months	3.0	2.3	2.4	2.4	0.9	0.9
9 months	1.9	1.6	2.0	1.8	0.3	0.2
storage at 20	°C					
3 months	3.0	2.5	3.0	2.9	1.6	1.5
6 months	3.0	2.3	2.2	2.2	0.8	0.7
9 months	1.6	1.2	1.7	1.6	0.2	0.0
storage at 30	0°C					
3 months	2.8	2.2	2.8	2.8	1.5	1.5
6 months	2.3	2.0	2.2	1.9	0.4	0.3
9 months	1.4	1.2	1.5	1.4	0.0	0.0

<sup>a</sup> Daily intake 2000 mL of enteral formula (2000 Kcal).

the highest and lowest thiamine content, and both presented the highest value for fat-soluble vitamin content. Samples of these formulas were industrially prepared and bottled, and they were immediately stored in dark conditions at different temperatures and for different periods of time (referred to as EFF Ia and EFF IIa). Tables 3 and 4 show the changes for thiamine, vitamin E ( $\alpha$ -tocopherol,  $\gamma$ -tocopherol, and  $\delta$ -tocopherol) and vitamin A (all-*trans*-retinol and 13-*cis*-retinol) in EFF Ia and EFF IIa after storage at 4°, 20°, and 30 °C for 3, 6, and 9 months. Figure 1 indicates the vitamin

retention after storage of EFF Ia and EFF IIa under those conditions.

Thiamine content in EFF Ia and EFF IIa (Tables 3 and 4, Figure 1), did not show any significant changes (P < 0.05) when they were stored at 4 °C for 3 months and only a slight, but significant (P < 0.05), decrease (5%) was observed in EFF IIa after 6 months. When the storage was prolonged to 9 months an important decrease (35%) was observed in both formulas. The same effects appeared when the formulas were kept at 20 °C but the decrease in thiamine content was more pro-

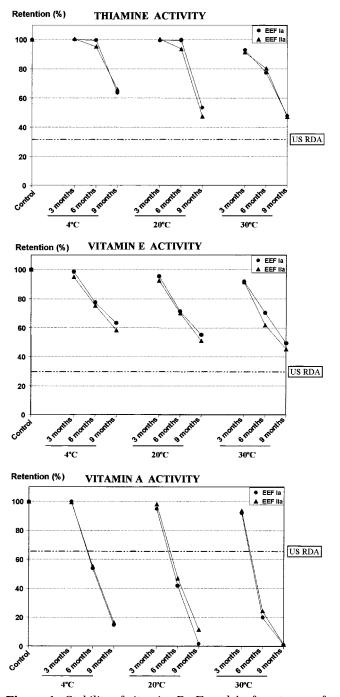


Figure 1. Stability of vitamins  $B_1$ , E, and A after storage of enteral feeding formula.

nounced (7% and 47–53% for 6 and 9 months, respectively). When both formulas were stored at 30 °C for 3 months, a small, but significant (P < 0.05), decrease (7–9%) in thiamine content was observed. The reduction was more noticeable after 6 and 9 months (20–23% and 52%, respectively).

Although the stability of thiamine has not been studied in enteral feeding formula, we can compare our results with those found for related admixtures. Thiamine is quite unstable even under refrigeration after 24 h in TPA (2), and a large degradation is shown at room temperature (3). In milk, a decrease of 6% is reported after storage at 4° to 7 °C for 7 days (9, 13), whereas in liquid infant milk thiamine levels remain constant during storage for 12 months at 20°, 30°, and 37 °C (4). The EFF sample showed a good stability for

thiamine after storage for 3 months, whatever the temperature studied, and for 6 months at 4° and 20 °C, but a drastic reduction (34-36%) was observed after 9 months of storage.

The vitamin E activity of EFF Ia and EFF IIa steadily but significantly decreased with storage (P < 0.05), and the decrease was less pronounced at lower temperature. The initial vitamin E content was similar in both formulas, but after storage this content decreased less in the hyperproteic formula (EFF Ia) than it did in the normoproteic formula (EFF IIa), except when the formula was stored at 30 °C for 3 months. This could be due to a protective effect of proteins on tocopherols, although this effect seems to be less effective at higher temperature. When the EFF Ia and EFF IIa were kept at 4 °C, after 3, 6, and 9 months, the vitamin E decreased between 2 and 5%, 23 and 25%, and 37 and 42% respectively. After storage at 20 °C for the same periods of time, a reduction of vitamin E content in EFF Ia and EFF IIa of 5–8%, 29– 30%, and 45–59% was observed, and this decrease was more pronounced when the formulas were kept at 30 °C (8-9%, 30–38%, and 51-55%, respectively) (Tables 3 and 4, Figure 1). A related formula, such as TPA, stored for 20 days at 4 °C or ambient temperature, has unchanged vitamin E levels even in daylight (5, 6). Liquid infant milks stored for 12 months at 20°, 30°, and 37 °C do not change their vitamin E content (4). Milk kept for 90 days at room temperature (15 to19 °C) does not show any changes in the vitamin E content, but losses of 52% in  $\alpha$ -tocopherol at 30 °C are observed (14). In EFF, a slight reduction in vitamin E took place when the samples were kept at 20 or 30 °C for 3 months. However, when the EFF was kept at 30 °C for 9 months a decrease of 51-55% was found, similar to what is reported for milk stored at this temperature for 3 months (15).

The vitamin A activity in EFF Ia and EFF IIa did not show appreciable changes after storage at 4 °C for 3 months (P < 0.05), but losses of 45–46% and 84–85% were observed after 6 and 9 months. When these formulas were kept for 3, 6, and 9 months at 20° or 30 °C a reduction in vitamin A was also observed, ranging between 2 and 5%, 53 and 58%, 89 and 98% for EFF Ia, and 6 to 8%, 76 to 80%, and 99% for EFF IIa. Exposure to light seems to be a major factor destroying vitamin A in TPA (5, 6), and temperature and storage also modify vitamin A stability in infant formula (4) and milk (15). Minor reductions in vitamin A content (6-8%) were observed for the EFF samples when these products were kept at the same conditions as milk (30 °C for 3 months), but vitamin losses were greater than those observed for milk when the period of storage time was extended up to 6 or 9 months (80-100%, respectively). As the EFF samples were always protected from daylight (bottled in dark containers) and stored in darkness, the observed losses in vitamin A content during storage should not be related to light effect and could possibly be linked to some other factor such as the presence of oxygen in the formulas (8, 16).

Table 5 shows the ratio for vitamin levels delivered from the enteral feeding formulas that were freshly prepared (control) and after storage versus their U.S. recommended daily intakes (assuming that the daily intake of enteral formula is 2 L = 2000 Kcal). When EFF Ia and EFF IIa were stored for 3, 6, or 9 months at 4°, 20°, or 30 °C, decreases in thiamine and vitamin E intakes were observed in most of the conditions studied. But, even taking into consideration the least favorable conditions (30 °C for 9 months), the enteral feeding formula still contained sufficient thiamine and vitamin E to meet the U.S. RDA. In the case of vitamin A, however, the recommendations are only covered after 3 months of storage in any of the temperatures studied. After 6 months the decrease was so drastic that at 4 °C the content of vitamin A in both formulas covered only 0.9-fold of the U.S. RDA, and at 20° and 30 °C only 0.7– 0.8-fold and 0.3–0.4-fold of the U.S. RDA, respectively. When the formulas were kept for 9 months a larger reduction of vitamin A content was observed: EFF could supply only 0.2–0.3-fold, 0–0.2-fold, and 0.0-fold of the U.S. RDA for vitamin A when stored at 4°, 20°, or 30 °C, respectively.

It has been shown that the freshly prepared enteral feeding formula studied here provided a large amount of vitamins, exceeding several times the U.S. RDA for thiamine, vitamin E, and vitamin A. It has been shown that conditions of storage affect to a great extent the stability of thiamine and vitamins E and A in EFF, which was quite acceptable during the first 3 months. However, after 6 or 9 months the vitamin levels suffered a decrease which was more acute for fat-soluble vitamins. These changes affected mostly vitamin A, which seems to be the most labile. Its content felt dramatically below the U.S. RDA after 3 months of storage.

#### LITERATURE CITED

- Machlin, J. L. Handbook of vitamins. Marcel Dekker: New York, 1984; pp 1–297.
- (2) Montero, C. G.; Vilchez, T.; Cantabrana, F.; Atienza, M. Thiamine stability in parenteral nutrition. *Nutr. Hosp.* **1990**, *5*, 32–35.
- (3) Baumgartner, T. G.; Henderson, G. N.; Fox, J.; Gondi, U. Stability of ranitidine and thiamine in parenteral nutrition solutions. *Nutrition* **1997**, *13*, 547–553.
- (4) Albalá-Hurtado, S.; Veciana-Nogués, M. T.; Riera-Valls, E.; Mariné-Font, A.; Vidal-Carou, C. Stability of vitamins during storage of liquid infant milks. *J. Dairy Res.* 2000, *67*, 225–231.
- (5) Kishi, H.; Yamaji, A.; Kataoka, K., Fujii, Y.; Nishikawa, K.; Ohnishi, N.; Hiraoka, E.; Okada, A.; Kim, C. Vitamin

A and E requirements during total parenteral nutrition. *JPEN, J. Parenter. Enteral Nutr.* **1981**, *5*, 420–423.

- (6) Billion-Rey, F.; Guillaumont, M.; Frederic, A.; Aulagner, G. Stability of fat-soluble vitamins (retinol Palmitate), E (tocopherol acetate), and K1 (Phylloquinone) in total parenteral nutrition at home. *JPEN*, *J. Parenter. Enteral Nutr.* **1993**, *17*, 56–60.
- (7) Shenai, J. P.; Stahlman, M. T.; Chytil, F. Vitamin A delivery from parenteral alimentation solution *J. Pediatr.* **1981**, *99*, 661–663.
- (8) McCarthy, D. A.; Kakuda, Y.; Arnott, D. R. Vitamin A stability in ultra-high-temperature processed milk. *J. Diary Sci.* **1986**, *69*, 2045–2050.
- (9) Sierra, I.; Prodanov, M.; Calvo, M.; Vidal-Valverde, C.; Olano, A. Vitamin stability and growth of psychotrophic bacteria in refrigerated raw milk acidified with carbon dioxide. J. Food Protect. **1996**, 59, 1305–1310.
- (10) Eitenmiller, R. R.; Landen, W. O. Vitamin Analysis for the Health and Food Sciences. CRC Press LLC: Boca Raton, FL, 1999.
- (11) Ball, G. F. M. Fat-soluble vitamin assays in food analysis. A comprehensive review. Elsevier Applied Science: London, 1988; pp 7–24
- (12) Shils, M. E.; Olson, J. A.; Shine, M.; Ross, A. C. Modern nutrition in health and disease. William & Wilkins: Baltimore, MD, 1999; pp 305–390.
- (13) Lavigne, C.; Zee, J. A.; Simard, R. E.; Beliveau, B. Effect of processing and storage conditions on the fate of B<sub>1</sub>, B<sub>2</sub> and C and on the shelf life of goat's milk. *J. Food Sci.* **1989**, *54*, 30–34.
- (14) Ford, J. E.; Porter, J. W. G.; Thompson, S. Y.; Toothill, J.; Edwards-Webb, J. Effects of ultra-high-temperature (UHT) processing and subsequent storage on the vitamin content of milk. *J. Dairy Res.* **1969**, *36*, 447–454.
- (15) Vidal-Valverde, C.; Ruiz, R.; Medrano, A. Stability of retinol in milk during frozen and other storage conditions. Z. Lebensm. Unters. Forsch. 1992, 195, 562–565.
- (16) Le Maguer, I.; Jackson, H. Stability of vitamin A in pasteurised and ultra-high-temperature processed milk. *J. Dairy Sci* **1983**, *66*, 2452–2456.

Received for review October 13, 2000. Revised manuscript received February 7, 2001. Accepted February 7, 2001. This work was supported by the Spanish Comision Interministerial de Ciencia y Tecnología ALI-97-0606-C02-01.

JF001243D