Food Chemistry 115 (2009) 1411–1416

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Changes in vitamin content of powder enteral formulas as a consequence of storage

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ARTICLE INFO

Article history:
Received 30 September 2008
Received in revised form 15 December 2008
Accepted 26 January 2009

Keywords:
Powder enteral formula
Thiamine
Vitamin E
Vitamin A
Storage

ABSTRACT

The thiamine, vitamin E (α-, γ- and δ-tocopherol) and vitamin A (all-*trans* and 13-*cis*-retinol) contents of four commercial powder enteral formulas (A, B, C and D) have been determined. The vitamin intake provided by the studied formulas was always above the US daily recommendations. Powder enteral formulas A and D were stored at 30 °C for up to 6 months with a water activity of 0.44 (A_w = 0.44), and formula A was also stored under atmospheric conditions for 3, 4 and 6 months. Formulas A and D kept at 30 °C and A_w = 0.44 suffered a gradual loss in vitamin content (from 3% to 4% after 1 month to 58–60% after 6 months). Formula A, stored at 30 °C under atmospheric conditions, underwent a slight reduction in vitamin content after 3 months, similar to that found after 1 month with A_w = 0.44, and from that time onward, this decreased steadily (to 30% after 6 months). The RDA of thiamine, vitamin E and vitamin A for women and men were met only when the powder enteral formulas were stored at 30 °C with A_w = 0.44 up to 1 month and without A_w up to 3 months. These results show that A_w and storage period have a marked effect on the stability of thiamine, vitamin E and A during the storage of powder enteral formulas and should be taken into consideration for the shelf-life of the product.

1. Introduction

Enteral formulas are designed to provide complete or supplemental nutritional support to individuals, who are unable to ingest adequate amounts of food in a conventional form, or to provide specialised nutritional support to patients with particular physiological and nutritional needs. Commercial formulas contain a specific combination of proteins (in the form of intact protein, peptides or free amino acids, giving rise to polymeric, oligomeric or elemental formulations, respectively), carbohydrates (in the form of glucose polymers or maltodextrins, disaccharides, and oligosaccharides), different amounts of fat (partially digested to mono-, di-, and triglycerides), and are fortified with micronutrients, such as minerals and vitamins. There is a range of commercial enteral formulas with varied components that provide all the nutrients required by patients with different clinical conditions when ingested at 2000 Kcal/day.

The general steps of powder enteral formula manufacture include sterilisation and spray-drying processes. In addition, these formulas are frequently stored after their manufacture in uncontrolled environmental conditions. All these treatments may affect the formula ingredients, leading to loss of bioavailability of necessary nutrients (García-Baños, Del Castillo, Sanz, Olano, & Corzo, 2005).

Vitamin fortification is one of the key operations by which formulations of enteral feeding products are tailored to meet the nutritional requirements for humans. However, formulas are often not consumed immediately after manufacture and can be degraded due to several factors, such as the type of packaging and the length and conditions of storage (e.g. light, oxygen exposure and high temperatures); (Frías & Vidal-Valverde, 2001; Sforzini et al., 2001). For this reason, the vitamin content should be evaluated after manufacture and storage in order to ensure the amount provided.

Vitamins have variable stability properties, due to their different chemical structures. Thiamine is stable below pH 5.5 but above pH 7.0 is destroyed rapidly, even at room temperature. Vitamin E (α-tocopherol, δ-tocopherol and γ-tocopherol) and vitamin A (all-*trans*-retinol and 13-*cis*-retinol) seem to be very stable in a nitrogen atmosphere in a dark cold place, but are readily oxidised with exposure to air and light (Machlin, 1984; Shils, Olson, Shine, & Ross, 1999). Taking these considerations into account, the storage conditions of powder enteral formulas could affect their vitamin content.

Different authors have reported the effect of different storage conditions on the thiamine, vitamin E and A contents in parenteral nutrition solutions (Baumgartner, Henderson, Fox, & Gondi, 1997; Montero, Vilchez, Cantarrana, & Atienza, 1990), in liquid enteral feeding formulas (Frias and Vidal-Valverde, 2001), and in liquid and powder infant milk (Albalá-Hurtado, Veciana-Nogués, Vidal-Carou, & Mariné-Font, 2000; Chávez-Servín, Castellote, & López-Sabater, 2008a; Chávez-Servín, Castellote, Rivero, & López-Sabater,

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0308-8146/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2009.01.070

2008b; Miquel, Alegría, Barberá, Farré, & Clemente, 2004). In general, the authors observed a reduction in the vitamin content of different products, and the magnitude of this reduction seemed to depend on the storage conditions. However, there is no available information on the vitamin content in powder enteral formulas and their stability during different storage conditions, including the effect of water activity (A_w). Manufacturers recommend a shelf-life of one year, although changes in these micronutrients seem to depend on the storage conditions and any variation in the vitamin content will be reflected in the daily intake consumed by patients, and RDAs may not be met.

The aim of this work was to study the thiamine, vitamin E (α-tocopherol, δ-tocopherol and γ-tocopherol) and vitamin A (all-*trans*-retinol and 13-*cis*-retinol) contents in four commercial powder enteral formulas and to evaluate, in two of them, the influence of time (up to 6 months) and A_w during storage at 30 °C on vitamin content.

2. Material and methods

2.1. Samples

Four commercial powder enteral formulas (samples A, B, C and D), sold in boxes of four bags, were purchased at a local pharmacy; the contents of protein, lipids and carbohydrates per 100 g indicated on the label are shown in Table 1. The bags were opened and freshly analysed.

2.2. Storage conditions

Storage assays were carried out with sample A (oligomeric) and sample D (elemental), just after the bags were opened. Samples were equilibrated to $A_w = 0.44$ in a desiccator over saturated K_2CO_3 solution, using the method of Labuza and Saltmarch (1981) and then stored at 30 °C for 1, 2, 3, 4 and 6 months. Samples of formula A were also stored at 30 °C at atmospheric conditions (without controlled A_w) for 3, 4 and 6 months.

2.3. Determination of vitamins

Two replicates of two different bags of each formula were analysed for thiamine, vitamin E and its vitamers (α-tocopherol, γ-tocopherol, and δ-tocopherol), and vitamin A and its vitamers (all-*trans*-retinol and 13-*cis*-retinol), according to Sierra, Prodanov, Calvo, Olano, and Vidal-Valverde (1996).

Thiamine was extracted with acid and enzymatic hydrolysis and quantified by HPLC, using a Waters μBondapak C_{18} column (300 × 3.9 mm i.d.) with postcolumn derivatisation and Waters 470 scanning fluorescence detector set at $\lambda_{exc} = 360$ nm and $\lambda_{em} = 435$ nm (Waters Corporation, Milford, MA). The mobile phase consisted of methanol/water/acetic acid (31/68.5/0.5), containing 5 mM sodium hexasulfonate (Sigma–Aldrich, St. Louis, MO), and was pumped at a flow rate of 1.5 ml/min. The column temperature was 35 °C and the injection volume was 50 μl.

Tocopherols and retinols were saponified and extracted with diethyl ether. Quantification was carried out by HPLC using a Lichrosorb (Merck, Darmstadt, Germany) Si 60 5 μm (250 × 4.6 mm i.d.) column and Waters 470 scanning fluorescence detector set at $\lambda_{exc} = 295$ nm and $\lambda_{em} = 330$ nm for tocopherols and at $\lambda_{exc} = 330$ nm and $\lambda_{em} = 470$ nm for retinols. The mobile phase consisted of *n*-hexane/diisopropyl ether (90/10) for tocopherols and (70/30) for retinols, and was pumped at a flow rate of 1.5 ml/min. The column temperature was 30 °C and the injection volume was 50 μl.

Vitamin E activity of the formulas was defined in terms of RRR-α-tocopherol calculated equivalents (α-*TEs*). One α-*TE* is the activity of 1 mg of *RRR*-α-tocopherol. The vitamin E activity was calculated using the factors for conversion of tocopherols to *RRR*-α-tocopherol equivalents (Eitenmiller & Landen, 1999):

$$\text{Vitamin E activity}(\alpha - TE/100\ \text{ml}) = \alpha - \text{tocopherol(mg)} \times 1.0 + \gamma - \text{tocopherol(mg)} \times 0.1 + \delta - \text{tocopherol(mg)} \times 0.03.$$

Vitamin A activity of the formulas was quantified by conversion of the vitamin A active components to retinol equivalents (*RE*), where 1 *RE* = 1 μg of all-*trans*-retinol (Ball, 1988):

$$\text{Vitamin A}(RE/100\ \text{g}) = \text{all} - trans - \text{retinol}(\mu\text{g}) \times 1.0 + 13 - cis - \text{retinol}(\mu\text{g}) \times 0.75.$$

Table 1
Proximate composition of powder enteral formulas.

Powder enteral formulas	Protein (%)	Lipid (%)	Carbohydrates (%)	Type
A	16.4	15.1	61.1	Oligomeric
B	14.2	3.9	73.9	Oligomeric
C	19.2	17.4	54.6	Elemental
D	12.6	7.4	75.9	Elemental

3. Results and discussion

The contents of thiamine (vitamin B_1), vitamin E and its vitamers (α-tocopherol, γ-tocopherol and δ-tocopherol), and vitamin A and its vitamers (all-*trans*-retinol and 13-*cis*-retinol) in commercial powder enteral formulas (samples A–D) are shown in Table 2. Powder enteral formulas A and C possessed a thiamine content of 0.36 and 0.39 mg/100 g, respectively, whilst the content of powder enteral formulas B and D was higher (0.58 and 0.61 mg/100 g, respectively). The vitamin E activity was similar ($p \leqslant 0.05$) in the powder enteral formulas A, B and C (~4 α-*TE*/100 mg), whilst powder enteral formula D possessed significantly higher vitamin E activity (4.2 α-*TE*/100 mg) ($p \leqslant 0.05$). α-Tocopherol was the main component, whilst γ-tocopherol and δ-tocopherol only contributed to vitamin E activity at 10% and 3%, respectively. Powder enteral formula A possessed the lowest vitamin A activity (267 *RE*/100 g), followed by formula D (280 *RE*/100 g), formula C (339 *RE*/100 g) and the highest vitamin A was found in sample B (370 *RE*/100 g), in which all-*trans*-retinol was the main contributor. In the studied formulas the RDA of thiamine, vitamin E and vitamin A were always over the US RDA for adults (Eitenmiller & Landen, 1999). Our contents were higher than those reported on the label of each commercial formula. Chávez-Servín et al. (2008b) suggested that most manufacturers add amounts of vitamins above the levels specified on the labels to compensate for the potential losses of these micronutrients during production and storage. This fact was also observed in liquid enteral formulas by Frias and Vidal-Valverde (2001).

Tables 3 and 4 compile the thiamine, vitamin E and A contents of powder enteral formulas A and D after storage at 30 °C for 1, 2, 3, 4 and 6 months at $A_w = 0.44$, and for powder enteral formulas A stored at 30 °C for 3, 4 and 6 months without controlled A_w. Fig. 1 shows the effect of storage on vitamin retention in powder enteral formulas A and D under these conditions. The storage at 30 °C produced changes in the content of vitamins of enteral powder formulas that depended on the storage conditions.

When formulas A and D were stored at 30 °C with $A_w = 0.44$ the thiamine content underwent a slight but significant decrease of 4–5% ($p \leqslant 0.05$) after 1 month. When the period of storage was extended to 2, 3, 4 and 6 months, gradual decreases in thiamine con-

Table 2
Vitamin content in powder enteral formulas*.

Powder enteral formulas	Thiamine (mg/100 g)	α-Tocopherol (mg/100 g)	γ-Tocopherol (mg/100 g)	δ-Tocopherol (μg/100 g)	Vitamin E activity (α-TE/100 g)	All-*trans*-retinol (mg/100 g)	13-*cis*-retinol (μg/100 ml)	Vitamin A activity (*RE*/100 g)
A	0.362 ± 0.003[a]	3.98 ± 0.077[ab]	0.722 ± 0.020[a]	12.6 ± 0.146[a]	4.05 ± 0.07[a]	0.264 ± 0.004[a]	2.64 ± 0.020[b]	267 ± 4.34[a]
B	0.577 ± 0.008[b]	3.86 ± 0.062[a]	0.797 ± 0.012[b]	17.4 ± 0.384[c]	3.94 ± 0.06[a]	0.366 ± 0.002[d]	2.55 ± 0.021[a]	369 ± 2.38[d]
C	0.387 ± 0.018[a]	3.92 ± 0.108[a]	0.707 ± 0.005[a]	16.1 ± 0.112[b]	3.99 ± 0.11[a]	0.335 ± 0.003[c]	2.87 ± 0.014[c]	339 ± 2.48[c]
D	0.611 ± 0.049[b]	4.10 ± 0.087[b]	1.18 ± 0.004[c]	26.7 ± 0.112[d]	4.22 ± 0.09[b]	0.275 ± 0.002[b]	4.03 ± 0.015[d]	280 ± 1.88[b]

Values are in dry matter and they are the mean of four determinations ± standard deviation. Values in the same column for each vitamin with the same superscript are not significantly different ($p \leqslant 0.05$).

Table 3
Changes in vitamin content of powder enteral formula A after different storage conditions*.

Storage conditions	Thiamine (mg/100 g)	α-Tocopherol (mg/100 g)	γ-Tocopherol (mg/100 g)	δ-Tocopherol (μg/100 mg)	Vitamin E activity (α-*TE*/100 g)	All-*trans*-retinol (mg/100 mg)	13-*cis*-retinol (μg/100 g)	Vitamin A activity (*RE*/100 g)
Control	0.362 ± 0.003[h]	3.98 ± 0.077[g]	0.722 ± 0.020[g]	12.6 ± 0.146[g]	4.05 ± 0.07[g]	0.264 ± 0.004[f]	2.64 ± 0.020[g]	267 ± 4.34[f]
Storage at 30 °C								
1 month								
A_w 0.44	0.344 ± 0.014[g]	3.84 ± 0.043[f]	0.688 ± 0.007[f]	12.0 ± 0.163[f]	3.91 ± 0.04[f]	0.254 ± 0.001[e]	2.46 ± 0.015[f]	257 ± 1.06[e]
2 months								
A_w 0.44	0.312 ± 0.004[f]	3.49 ± 0.032[e]	0.587 ± 0.006[d]	9.47 ± 0.113[d]	3.55 ± 0.03[e]	0.230 ± 0.001[d]	1.87 ± 0.003[d]	233 ± 1.09[d]
3 months								
A_w 0.44	0.243 ± 0.009[c]	2.70 ± 0.046[c]	0.492 ± 0.005[c]	8.58 ± 0.088[b]	2.75 ± 0.05[c]	0.186 ± 0.002[c]	1.45 ± 0.022[f]	188 ± 1.62[c]
No controlled A_w	0.342 ± 0.003[g]	3.82 ± 0.035[f]	0.693 ± 0.003[f]	11.9 ± 0.012[f]	3.89 ± 0.03[f]	0.249 ± 0.001[e]	2.47 ± 0.028[f]	252 ± 0.87[e]
4 months								
A_w 0.44	0.174 ± 0.04[b]	2.02 ± 0.044[b]	0.355 ± 0.007[b]	5.66 ± 0.056[b]	2.05 ± 0.04[b]	0.136 ± 0.005[b]	1.25 ± 0.036[b]	137 ± 4.43[b]
No controlled A_w	0.300 ± 0.002[e]	3.38 ± 0.052[d]	0.615 ± 0.005[e]	10.1 ± 0.129[e]	3.45 ± 0.05[d]	0.222 ± 0.001[d]	2.16 ± 0.017[e]	225 ± 1.42[d]
6 months								
A_w 0.44	0.149 ± 0.004[a]	1.61 ± 0.020[a]	0.308 ± 0.004[a]	4.49 ± 0.016[a]	1.64 ± 0.02[a]	0.109 ± 0.001[a]	0.906 ± 0.022[a]	111 ± 12.72[a]
No controlled A_w	0.254 ± 0.005[d]	2.77 ± 0.085[c]	0.503 ± 0.003[c]	8.65 ± 0.106[c]	2.82 ± 0.09[c]	0.187 ± 0.007[c]	1.87 ± 0.069[d]	190 ± 6.81[c]

Values are in dry matter and they are the mean of four determinations ± standard deviation. Values in the same column for each vitamin with the same superscript are not significantly different ($p \leqslant 0.05$).

Table 4
Changes in vitamin content of powder enteral formula D after different storage conditions*.

Storage conditions	Thiamine (mg/100 g)	α-Tocopherol (mg/100 g)	γ-Tocopherol (mg/100 ml)	δ-Tocopherol (μg/100 g)	Vitamin E activity (α-TE/100 g)	All-*trans*-retinol (mg/100 g)	13-*cis*-retinol (μg/100 g)	Vitamin A activity (*RE*/100 g)
Control	0.611 ± 0.049[g]	4.10 ± 0.087[g]	1.182 ± 0.004[g]	26.7 ± 0.143[g]	4.22 ± 0.09[g]	0.275 ± 0.002[g]	4.03 ± 0.015[g]	280 ± 1.88[g]
Storage at 30 °C								
1 months								
A_w 0.44	0.587 ± 0.006[e]	3.96 ± 0.032[e]	1.124 ± 0.011[e]	25.1 ± 0.299[e]	4.07 ± 0.03[e]	0.266 ± 0.001[e]	3.73 ± 0.013[e]	271 ± 0.40[e]
2 months								
A_w 0.44	0.529 ± 0.028[d]	3.59 ± 0.062[d]	0.929 ± 0.005[d]	19.4 ± 0.043[d]	3.68 ± 0.06[d]	0.238 ± 0.0002[d]	2.92 ± 0.018[d]	242 ± 1.75[d]
3 months								
A_w 0.44	0.407 ± 0.004[c]	2.78 ± 0.127[c]	0.804 ± 0.014[c]	18.2 ± 0.101[c]	2.86 ± 0.13[c]	0.194 ± 0.001[c]	2.09 ± 0.015[c]	197 ± 1.34[c]
4 months								
A_w 0.44	0.293 ± 0.008[b]	2.04 ± 0.032[b]	0.588 ± 0.004[b]	12. ± 0.160[b]	2.10 ± 0.03[b]	0.143 ± 0.001[b]	1.89 ± 0.011[b]	146 ± 1.19[b]
6 months								
A_w 0.44	0.255 ± 0.004[a]	1.64 ± 0.052[a]	0.499 ± 0.009[a]	9.38 ± 0.070[a]	1.69 ± 0.05[a]	0.116 ± 0.003[a]	1.38 ± 0.009[a]	117 ± 3.17[a]

Values are in dry matter and they are the mean of four determinations ± standard deviation. Values in the same column for each vitamin with the same superscript are not significantly different ($p \leqslant 0.05$).

tent were observed and reductions of 13–14%, 33%, 52% and 58–59%, respectively, were found (Tables 3 and 4, Fig. 1). Milder reductions in thiamine content were observed at the same periods of time when formula A was stored at 30 °C without A_w (5%, 17% and 30% after 3, 4 and 6 months, respectively).

The vitamin E content in powder enteral formulas A and D decreased after their storage at 30 °C with A_w = 0.44, and reductions of 3–5%, 12–14%, 32–33%, 49–52%, and 59–60% were obtained after 1, 2, 3, 4 and 6 months, respectively (Tables 3 and 4, Fig. 1). When formula A was stored at 30 °C without controlled A_w the vitamin E content decreased by only 4%, and reductions of 15% and 30% were obtained after 4 and 6 months, respectively (Table 3, Fig. 1).

The vitamin A content of formulas A and D also underwent a reduction during storage at 30 °C with A_w = 0.44. One month storage under these conditions led to a reduction of 3–4% in vitamin A, while longer periods caused decreases of 13–14%, 30%, 48–49% and 58–69% after 2, 3, 4 and 6 months, respectively (Tables 3 and 4, Fig. 1). The stability of vitamin A was higher when powder enteral

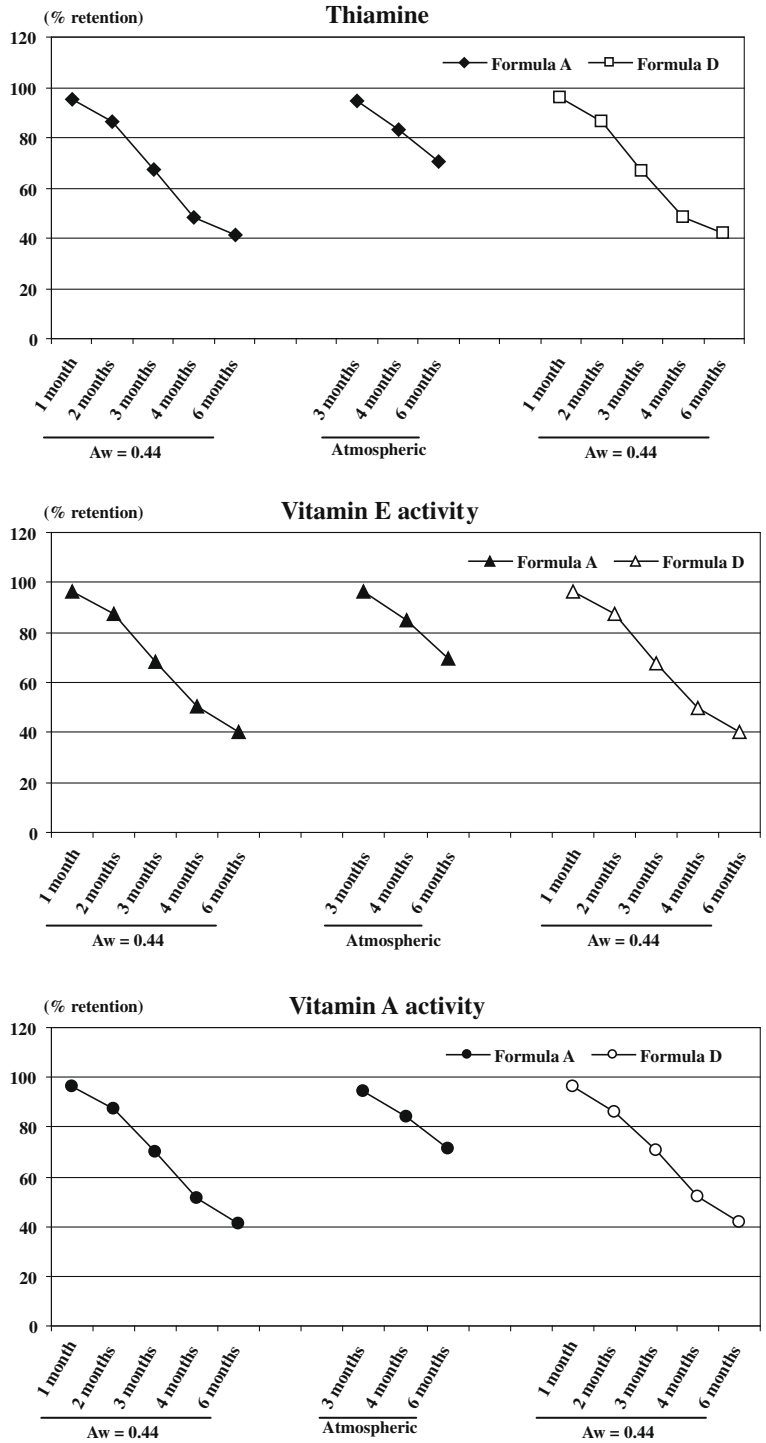

Fig. 1. Effect of storage at 30 °C on the vitamin retention of powder enteral formulas.

formula A was stored at 30 °C without controlled A_w, where reductions of 6%, 16% and 29% were obtained after 3, 4 and 6 months, respectively.

The powder enteral formulas are usually well packed in bags protected from light and oxygen, to prevent the alteration of their components. However, once opened, the formulas are exposed to external factors that contribute to the oxidation and degradation of vitamins. Furthermore, A_w can also affect the stability of vitamins, as shown in the present work.

No information has been found about the effect of storage conditions, time and water activity on the vitamin content of powder enteral formulas. For liquid enteral formulas stored at 4 °C, 20 °C and 30 °C for 3, 6 and 9 months, Frias and Vidal-Valverde (2001) reported thiamine losses of 7–9% after 3 months, which were even more pronounced after 6 and 9 months (20% and 52%, respectively), and similar results were reported for vitamin E. For vitamin A, however, they found greater losses in enteral liquid formula stored at 30 °C after 6 and 9 months (45% and 85%, respectively). In parenteral nutrition solutions, thiamine was found to be very unstable after 24 h at room temperature (Baumgartner et al., 1997) and under refrigeration at 4 °C (Montero et al., 1990). In powder and liquid infant milk formulas, thiamine content re-

mained unchanged for 12 months when kept at 37 °C (Albalá-Hurtado et al., 2000).

Different effects have been observed in vitamin E and A contents after storage of infant formulas under different conditions. Chávez-Servín et al. (2008b) reported that vitamin E was stable while vitamin A decreased (4.7–34.3%) when 20 commercial infant milk-based powdered formulas were stored at 25 °C for 70 days. Chávez-Servín et al., 2008a observed that the vitamin E content was reduced by 23–28%, and vitamin A dropped by 27–29% when two infant milk-based powdered formulas were stored at 40 °C for 18 months. Albalá-Hurtado et al. (2000) found that the storage of liquid and powder infant milk formulas for 12 months at 37 °C did not affect the vitamin E content but brought about reductions of 35% in vitamin A levels. On the other hand, Miquel et al. (2004) observed that four milk-based infant formulas kept at 37 °C for 17 months underwent vitamin E reductions of ~50%. Vidal-Valverde, Ruiz, and Medrano (1992), Vidal-Valverde, Ruiz, and Medrano (1993) reported losses in α-tocopherol (17%) and retinol (16%) in powder milk stored at 20 °C and A_w = 0.44 for 20 days.

It is not only vitamins which are altered during the storage of powder enteral formulas, but carbohydrates also seem to undergo isomerisation and degradation reactions, as shown by Garcia-Baños et al. (2005) in the powder enteral formulas A and D studied here. These authors reported a decrease in carbohydrates after 6 months of storage at 30 °C and A_w = 0.44, which could be due to their participation in the Maillard reaction, shown by the increase in furosine, 2-furoylmethyl-Ala, and α-2-furoylmethyl-Lys, after storage at 30 °C and A_w = 0.44. Furosine was the most abundant component after 6 months of storage in powder enteral formula A and D (579 mg/100 g and 221 mg/100 g), respectively.

Tables 5 and 6 show the daily intake ratio of thiamine, vitamin E and vitamin A of enteral powder formulas A and D *vs.* US RDA, assuming intakes of 2000 Kcal/day and values of US RDA for thiamine of 1.1 for women and 1.5 for men, of 12 mg α-*TE* for vitamin E for both women and men, and for vitamin A of 800 *RE* for women and 1000 *RE* for men (Eitenmiller & Landen, 1999). Formulas A and D satisfied the vitamin requirements in different ways. Formula D provided higher thiamine content than formula A (3.3–2.0-fold and 1.8–1.1-fold of the RDA, respectively). However, the contents for vitamins E and A of formula D were only slightly higher than those of formula A (Tables 5 and 6).

Formula A covered the RDA of thiamine for women when stored with A_w = 0.44 at 30 °C up to 3 months, but the RDA for men were only met when this formula was stored up to 1 month (Table 5). In the case of formula D stored at 30 °C with A_w = 0.44, the RDA of thiamine for women was reached even when the sample was stored for 6 months but the RDA for men was only satisfied up to 3 months (Table 6). Formulas A and D stored at 30 °C with A_w = 0.44 satisfied the women and men's RDAs of vitamin E when they were kept up to 3 and 2 months, respectively, and formulas A and D provided the RDA of vitamin A up to 2 and 1 month for women, and up to 3 and 2 months for men, respectively, (Tables 5 and 6). Therefore, only when samples A and D are stored up to 1 month at 30 °C with A_w = 0.44 can the RDA of the three vitamins (thiamine, vitamin E and vitamin A) be satisfied for both women and men.

Table 5
Daily vitamin intake ratio of powder enteral formula A* *vs.* US RDA for women and men.

Formula A storage conditions	Thiamine formula A/RDA women	Thiamine formula A/RDA men	Vitamin E formula A/RDA women and men	Vitamin A formula A/RDA women	Vitamin A formula A/RDA men
Control	1.80	1.09	1.53	1.21	1.51
Storage at 30 °C					
1 month					
A_w 0.44	1.71	1.04	1.25	1.16	1.45
2 months					
A_w 0.44	1.55	0.94	1.34	1.05	1.32
3 months					
A_w 0.44	1.21	0.73	1.04	0.85	1.06
No controlled A_w	1.70	1.03	1.47	1.14	1.42
4 months					
A_w 0.44	0.87	0.52	0.77	0.62	0.78
No controlled A_w	1.49	0.90	1.30	1.02	1.27
6 months					
A_w 0.44	0.74	0.45	0.62	0.50	0.62
No controlled A_w	1.26	0.77	1.06	0.86	1.07

Daily intake of enteral formula (2000 Kcal).

Table 6
Daily vitamin intake ratio of powder enteral formula D* *vs.* US RDA for women and men.

Formula D storage conditions	Thiamine formula D/RDA women	Thiamine formula D/RDA men	Vitamin E formula D/RDA women and men	Vitamin A formula D/RDA women	Vitamin A formula D/RDA men
Control	3.27	1.98	1.71	1.39	1.74
Storage at 30 °C					
1 month					
A_w 0.44	2.54	1.54	1.33	1.06	1.33
2 months					
A_w 0.44	2.29	1.39	1.20	0.95	1.19
3 months					
A_w 0.44	1.76	1.07	0.94	0.77	0.97
4 months					
A_w 0.44	1.27	0.77	0.69	0.57	0.72
6 months					
A_w 0.44	1.10	0.67	0.55	0.46	0.58

Daily intake of enteral formula (2000 Kcal).

Formula A stored at 30 °C without controlled A_w provided RDA of thiamine up to 6 months for women and up to 3 months for men. The women and men's RDAs of vitamin E were covered when this formula was stored up to 6 months, while the RDA of vitamin A were only satisfied when the process was carried out up to 4 months for women and 6 months for men (Table 5). Therefore, the RDA of the three vitamins (thiamine, vitamin E and A) for both women and men were satisfied when the powder enteral formula A was stored at 30 °C without controlled A_w up to 3 months.

In conclusion, A_w and storage period play an important role in the vitamin content of powder enteral formulas and, therefore, in their daily intakes. These results should be taken into consideration by manufacturers in order to recommend the storage conditions when establishing the shelf-life in which RDA of vitamins can be satisfied.

Acknowledgements

This work was supported by the Comisión Interministerial de Ciencia y Tecnología (CICYT), Project No. ALI 97-0606-CO2-01.

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