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Analysis of vitamins A, E and C, iron and selenium contents in infant milk-based powdered formula during full shelf-life

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Abstract

The stability of vitamins A, E and C, and the iron and selenium content were determined in two types of long chain-polyunsaturated fatty acid (LC-PUFA) supplemented milk-based powdered infant formulas (IF), during an 18-month storage period at 25 and 40 °C. The first type (IF-A) was supplemented with vitamin A as retinol acetate. The second type (IF-B) was supplemented with vitamin A as retinol palmitate. Both types were also supplemented with vitamin E as α-tocopherol acetate and with vitamin C as ascorbic acid. The two formulas studied had higher vitamin A (140% and 139%), vitamin E (109% and 198%) and vitamin C (167% and 118%), but lower iron (65.0% and 65.3%) and selenium (72.9% and 79.4%) than the amounts declared on the label. As expected, all the studied vitamins showed decreases during storage, and these decreases were higher in formulas stored at 40 °C. The losses of vitamin A at 40 °C after 18 months of storage were 27.5% in IF-A and 29% in IF-B, while vitamin E losses under the same conditions were 23.1% and 28.1%, and vitamin C losses under the same conditions were 28.4% and 48.6%. All these losses justify the over-fortification of the aforementioned vitamins in these LC-PUFA supplemented IFs. Iron and selenium content remained unchanged throughout storage.

Keywords: Infant formula powder; Retinols; Tocopherols; Ascorbic acid; Iron; Selenium; Storage

1. Introduction

Human milk is the ideal food for newborns. It provides all nutrient needs, such as protein, carbohydrates and lipids. Human milk contains micronutrients, namely vitamins and minerals, which are essential during the first month of a baby's life. In general, vitamin A refers to all-*trans*-retinol, which is the most active form of this vitamin, while vitamin E is a collective term for tocopherols (α, β, γ and δ) and tocotrienols (Blake, 2004, 2005), and vitamin C (L-ascorbic acid) is a water soluble vitamin. Vitamin A is essential for the maintenance of healthy vision, healthy skeletal and tooth development, cellular differentiation, proliferation and reproduction, and integrity of the immune system (Olson, 1987, 1994; Spannaus-Martin, Cook, Tanumihardjo, Duitsman, and Olson, 1998; Tanumihardjo et al., 1990). The predominant physiological function of vitamin E is its antioxidant activity. Vitamin E protects the fatty acids by interfering with the free radical reactions that can result in cellular damage. Finally, vitamin C functions in the body as a cofactor for critical enzyme systems and as a reducing agent (Levine et al., 1996).

Iron-containing compounds serve many essential biological functions, including oxygen transport and storage (haemoglobin and myoglobin) and the generation of cellular energy as ATP, *via* oxidative metabolism, involving the

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iron-containing cytochrome enzymes (Yip, 1994). Finally, the biochemical importance and the essential nature of selenium are related to its presence in a number of functionally active proteins. It is an essential component of glutathione peroxidase, which functions to reduce lipid peroxidation, and it has a prooxidant role in products with low water content (Driskell, Giraud, Drewel, and Davy, 2006; Kim, Lora, Giraud, and Driskell, 2006).

Currently, it is suggested that milk-based powder infant formulas (IF) should contain long chain-polyunsaturated fatty acids (LC-PUFA), similar to those of human milk. Therefore, some IF manufacturers include LC-PUFA in formula composition. Fatty acid unsaturation increases the rate of oxidation and, although the amount of LC-PUFA in supplemented formulas is low, it needs substantial antioxidant protection (Gonzalez-Corbella, Tortras-Biosca, Castellote-Bargallo, and Lopez-Sabater, 1999). Tocopherols are naturally-occurring lipid antioxidants, which specifically inhibit the oxidation of polyunsaturated fatty acids (PUFA), such as linoleic (LA, C18:2 $n-6$), α-linolenic (ALA, C18:3 $n-3$), arachidonic (AA, C20:4 $n-6$) and docosahexaenoic (DHA, C22:6 $n-3$) acids. Tocopherols, retinol and ascorbic acid are added to IFs, both to improve vitamin content and to prevent lipid oxidation during manufacture and storage, thereby helping to extend product shelf-life. Fortification of IFs with the most stable vitamin esters, such as α-tocopherol acetate, retinol acetate, or retinol palmitate, is required (Blake, 2005; Brigelius-FlohE and Traber, 1999; Parrish, 1980). These molecules are more stable than their analogues from vegetable oils and less susceptible to oxidation.

The European Communities Commission (2006/141/CE) established the limits of vitamins A, E and C, which can be added to meet nutritional requirements and to guarantee the stability of the product. It is usual for manufacturers to add a higher quantity of vitamins (A, E and C) to the IFs than that indicated on the formulas' label, to compensate for losses during manufacture and storage. However, the real content of those vitamins after manufacture and storage at different temperatures needs to be checked, to ensure correct intake and the accuracy of the label statements. There exists a lack of information about the stability of the isomers of vitamin A and E, vitamin C, iron and selenium in LC-PUFA supplemented IF powder.

The main aims of this work were: first, to survey the content of vitamin A (as retinol palmitate or retinol acetate), vitamin E (as α-tocopherol, α-tocopherol acetate, γ-tocopherol and δ-tocopherol), vitamin C (as ascorbic acid), and the iron and selenium content in two types of LC-PUFA supplemented infant milk-based powdered formulas, in relation to the label statements; second, to evaluate the stability of the aforementioned micronutrients during the full shelf-life of the product under different storage conditions (25 and 40 °C); and, third, to study the compliance of those vitamin and mineral contents with the recently published European legislation (2006/141/CE).

2. Experimental

2.1. Reagents and standards

The chemicals used for sample preparation were of analytical reagent grade. Hexane, methanol and ethyl acetate, all of HPLC grade, were obtained from SDS (Peypin, France); absolute ethanol and HPLC-grade acetic acid from Panreac (Barcelona, Spain); *meta*-phosphoric acid, and α-tocopherol acetate standard from Fluka (Buchs, Switzerland); standards of α-, γ- and δ-tocopherol, all-*trans*-retinol palmitate, and retinol acetate from Sigma (St. Louis, MO). The ascorbic acid standard was obtained from Merck (Darmstadt, Germany).

2.2. Instruments

For vitamin A and E determination, we used a Hewlett-Packard (HP) liquid chromatographic system (Waldbronn, Germany) with an HP 1050 pump, an HP-1040 M photodiode-array detector, and a Waters 717 Plus autosampler injector (Milford, MA). A Pinnacle II silica short-narrow-bore column (50 × 2.1 mm i.d.) of 3-μm particle size, with a silica pre-column guard cartridge (10 × 2 mm) from Restek (Bellefonte, PA) was used.

For vitamin C determination, an HP liquid chromatographic system with HP 1050 pump, Waters 717 Plus autosampler injector and a UV–vis detector, SPD-10 AV VP (Shimadzu, Kyoto, Japan) was used. The analytical column used was a Tracer Excel 120 ODSB (250 × 4.0 mm I.D., 5 μm particle size) protected with a guard cartridge (Tracer, C_{18}, 5 μm), both from Tracer (Tecknokroma, Barcelona, Spain).

2.3. Samples

The two types of LC-PUFA supplemented IF powder samples were obtained from a pilot scale food plant. The first type (IF-A) was supplemented with vitamin A (640 μg of retinol equivalents [RE]/100 g), in the form of retinol acetate, and the second type (IF-B) was supplemented with vitamin A (606 μg RE/100 g), in the form of retinol palmitate. Both formulas were also supplemented with vitamin E as α-tocopherol acetate, IF-A containing 25 mg α-tocopherol equivalents [TE]/100 g and IF-B containing 6.1 mg α-TE/100 g, and vitamin C (IF-A: 60 mg/100 g, IF-B: 68 mg/100 g) as ascorbic acid. The formulas were packed in airtight containers flushed with a nitrogen-modified atmosphere N_2/CO_2 (<2% O_2). Formula composition is reported in Table 1.

2.4. Storage

To evaluate the evolution of selected vitamins and minerals during the shelf-life of IFs, the product was kept at 25 or 40 °C from production until 0, 1, 3, 6, 9, 12, 15 and

Table 1
Composition of the studied formulas, according to the information on the label

	Mean values per 100 g	
	IF-A	IF-B
Energetic value (kJ/kcal)	2038/487	2132/509
Protein (g)	10	9.5
Carbohydrate (g)	53.3	58
Lactose (g)	46.1	41
Fat (g)	26	26
Arachidonic acid (mg)	47	91
Docosahexaenoic acid (mg)	25	53
Sodium (mg)	175	129
Potassium (mg)	540	401
Chloride (mg)	330	341
Calcium (mg)	420	348
Phosphorus (mg)	230	174
Magnesium (mg)	42	45
Iron (mg)	6	6.1
Zinc (mg)	6	4.5
Copper (μg)	300	273
Iodine (μg)	100	53
Selenium (μg)	10.7	9
Vitamin A (μg RE))	640	606
Vitamin D (μg)	10.3	8.3
Vitamin E (mg α-TE)	25	6.1
Vitamin K (μg)	42	38
Vitamin B_1 (μg)	520	568
Vitamin B_2 (μg)	620	908
Vitamin B_6 (μg)	825	379
Vitamin B_{12} (μg)	2	1.3
Vitamin C (mg)	60	68
Niacin (mg)	6	8.3
Folic acid (μg)	42	68
Pantothenic acid (mg)	3.2	3
Biotin (μg)	16	15

18 months; 25 °C constitutes the usual ambient temperature in markets and food stores, while 40 °C is a temperature which can be reached under extreme conditions in stores without air conditioning in the summer. Once storage was completed, analytical determinations were subsequently conducted.

2.5. Vitamin A, E and C determinations

Tocopherols (α-tocopherol, α-tocopherol acetate, γ-tocopherol and δ-tocopherol) and retinol acetate or palmitate compounds in the formulas were measured according to a previously reported HPLC method (Chávez-Servín, Castellote, and López-Sabater, 2006). Vitamin C content in the formulas was measured according to the HPLC method reported by Romeu-Nadal, Morera-Pons, Castellote, and Lopez-Sabater (2006). All analyses were carried out in quadruplicate.

2.6. Iron and selenium determinations

Iron and selenium analyses were determined by graphite furnace atomic absorption spectrometry, as reported in Aleixo and Nobrega (2003).

2.7. Statistical analysis

For statistical analysis, we used one-way analysis of variance, as well as multiple comparisons, using the Tukey HSD procedure for each IF and storage time. We conducted statistical analysis using the SPSS package for Windows version 12 (SPSS, Chicago, IL). The level of statistical significance was set at 5% for all analyses.

3. Results and discussion

3.1. Vitamin A content

Vitamin A activity is expressed as retinol equivalents (RE), with 1 RE equal to 1 μg all-*trans*-retinol, 6 μg all-*trans*-β-carotene and 12 μg of other provitamin A carotenoids, (Anonymous, 1998; Olson, 1989). Table 2 reports the values for retinol acetate or palmitate and the respective values expressed as RE, following the equivalence:

$$1\ \mu g\ \text{retinol} = 1.146\ \mu g\ \text{retinol acetate} = 1.832\ \mu g\ \text{retinol palmitate}.$$

IFs are most commonly fortified with vitamin A in the form of retinol acetate (as IF-A) or retinol palmitate (as IF-B) because these molecules are more stable and less susceptible to oxidation than their respective analogues from vegetable oils. At the beginning of the study, in IF-A (time 0), a value of 0.90 mg RE/100 g was found, while the content according to the label was 0.64 mg RE/100 g; therefore, this represents an adequacy value of 140%. Also, in IF-B (time 0) a value of 0.85 mg RE/100 g was found, which means an adequacy value of 139% according to the label statements (Table 2). However, a constant decrease of vitamin A (as retinol acetate and retinol palmitate) during storage was observed in both formulas. At 25 °C, after 18 months of storage, IF-A recorded a value of 0.73 mg RE/100 g, which represents losses of 18% with respect to the initial content, and an adequacy of 114.5% according to the label. IF-B under the same conditions recorded a value of 0.67 mg RE/100 g, which represents losses of about 21%, with respect to the initial content. When the formulas were stored at 40 °C, losses of vitamin A were higher than expected. IF-A showed values of 0.65 mg RE/100 g, which represents losses of about 27.5% and a label adequacy of 102%, while IF-B showed values of 0.60 mg RE/100 g, showing losses of 29% and a label adequacy of 99%. In both formulas, vitamin A losses in the form of retinol acetate (IF-A) and retinol palmitate (IF-B) were observed in a similar way.

Over-fortification of vitamin A is regulated due to possible toxicity from excessive intake, because vitamin A is stored in the body. European legislation (2006/141/CE) establishes the limits for vitamin A in IF, the minimum being 60 μg RE/100 kcal and the maximum 180 μg RE/100 kcal. The studied formulas are between these limits

Table 2
Analysis of vitamin A content in infant formulas during storage

Sample	Storage (months)	Retinyl acetate[I] or palmitate[II] (mg/100 g)	RE Found (mg/100 g)	Label (mg/100 g)	Losses (%)	Adequacy (%)	μg RE/ 100 kcal
IF-A[I]	0	1.03 ± 0.03^{a}	0.90 ± 0.03^{a}	0.64	–	140.2	174 ± 5.10
25 °C	1	1.00 ± 0.01^{a}	0.87 ± 0.01^{a}		2.8	136.3	169 ± 2.04
	3	0.99 ± 0.03^{a}	0.86 ± 0.02^{a}		3.9	134.8	168 ± 4.65
	6	0.94 ± 0.01^{b}	0.82 ± 0.01^{b}		8.5	128.2	159 ± 2.41
	9	0.85 ± 0.04^{c}	0.74 ± 0.04^{c}		17.7	115.4	143 ± 7.42
	12	0.85 ± 0.04^{c}	0.74 ± 0.03^{c}		17.5	115.7	144 ± 6.78
	15	0.85 ± 0.01^{c}	0.74 ± 0.00^{c}		17.8	115.2	143 ± 0.97
	18	0.84 ± 0.00^{c}	0.73 ± 0.00^{c}		18.3	114.5	142 ± 0.37
IF-A[I]	0	1.03 ± 0.03^{a}	0.90 ± 0.03^{a}	0.64	–	140.2	174 ± 5.10
40 °C	1	0.99 ± 0.04^{a}	0.86 ± 0.04^{a}		3.8	134.9	168 ± 7.59
	3	0.97 ± 0.03^{a}	0.85 ± 0.02^{a}		5.8	132.1	164 ± 4.44
	6	0.82 ± 0.02^{b}	0.71 ± 0.02^{b}		20.7	111.3	138 ± 3.68
	9	0.78 ± 0.02^{c}	0.68 ± 0.02^{c}		24.2	106.3	132 ± 3.72
	12	0.77 ± 0.01^{c}	0.67 ± 0.01^{c}		24.9	105.3	131 ± 2.06
	15	0.77 ± 0.02^{c}	0.67 ± 0.02^{c}		25.5	104.5	130 ± 3.26
	18	0.75 ± 0.02^{c}	0.65 ± 0.01^{d}		27.5	101.7	126 ± 2.63
IF-B[II]	0	1.55 ± 0.04^{a}	0.85 ± 0.02^{a}	0.61	–	139.0	167 ± 4.30
25 °C	1	1.37 ± 0.03^{b}	0.75 ± 0.02^{b}		11.9	122.4	147 ± 3.30
	3	1.36 ± 0.05^{b}	0.74 ± 0.02^{b}		12.7	121.3	145 ± 4.87
	6	1.35 ± 0.01^{b}	0.74 ± 0.01^{b}		13.3	120.5	144 ± 1.33
	9	1.25 ± 0.09^{c}	0.68 ± 0.05^{c}		19.3	112.2	134 ± 9.18
	12	1.25 ± 0.08^{c}	0.68 ± 0.05^{c}		19.8	115.5	134 ± 8.85
	15	1.24 ± 0.03^{c}	0.68 ± 0.02^{c}		20.1	111.0	133 ± 3.24
	18	1.23 ± 0.03^{c}	0.67 ± 0.01^{c}		21.0	109.8	132 ± 2.94
IF-B[II]	0	1.55 ± 0.04^{a}	0.85 ± 0.02^{a}	0.61	–	139.0	167 ± 4.30
40 °C	1	1.37 ± 0.02^{b}	0.75 ± 0.01^{b}		12.0	122.3	147 ± 1.95
	3	1.35 ± 0.06^{b}	0.74 ± 0.03^{b}		13.3	120.5	144 ± 5.90
	6	1.28 ± 0.07^{b}	0.70 ± 0.04^{b}		17.9	114.1	137 ± 7.09
	9	1.24 ± 0.07^{c}	0.68 ± 0.04^{c}		20.1	111.0	133 ± 7.83
	12	1.22 ± 0.05^{c}	0.66 ± 0.03^{c}		21.7	108.8	130 ± 5.73
	15	1.18 ± 0.03^{c}	0.64 ± 0.01^{c}		24.0	105.6	127 ± 2.85
	18	1.10 ± 0.03^{d}	0.60 ± 0.02^{d}		29.0	98.7	118 ± 3.37

Values are expressed as means ± standard deviation of four determinations. Repetitions in superscripted letters within the same column and in formula indicate no significant differences ($p > .05$).

even after 18 month of storage at 40 °C (Table 2). At the beginning of the study, IF-A and IF-B contained higher amounts (2.90 times and 2.77 times, respectively) than this minimum level. Levels in IF-A were 174 μg RE/100 kcal and IF-B 166 μg RE/100 kcal, below the upper limit.

Delgado-Zamarreno, Bustamante-Rangel, Garcia-Jimenez, Sanchez-Perez, and Carabias-Martinez (2006) reported values of vitamin A (as retinol acetate) in the range of 0.59–0.74 mg RE/100 g in four types of IFs. These values were between 113% and 120% of adequacy respect to the labels' information. Similarly, Albalá-Hurtado, Veciana-Nogues, Vidal-Carou, and Font (2001) reported values of vitamin A of 0.64–1.06 mg RE/100 g in starting liquid, and 0.43–1.09 mg RE/100 g in starting powdered IF, so the reported values on the label were close to the values found (mean 134% ± 17) in samples. Other studies have reported values more than twice those declared on the labels in several IFs (Albalá-Hurtado, Veciana-Nogues, Vidal-Carou, and Marine-Font, 2000; Landen et al., 1985).

3.2. Vitamin E content

The standard for comparison is dl-α-tocopherol, which is defined to be 1.49 IU/mg or 1 tocopherol equivalent (TE). The activities of other isomers relative to the α-isomer (α-T) are about 70% (0.7 TE/mg) for dl-α-tocopherol acetate (α-TAc), 40% or 0.4 TE/mg for β-tocopherol acetate (β-T), 10–30% or 0.1–0.3 TE/mg for δ-tocopherol acetate (δ-T), and about 10% or 0.1 TE/mg for δ-tocopherol acetate (δ-T). The vitamin E content is expressed as α-TE, using the formula:

$$\alpha\text{-TE} = [\delta\text{-T}] + (0.2 \times [\delta\text{-T}]) + (0.1) \times ([\delta\text{-T}]) + (0.7) \times ([\alpha\text{-TAc}]).$$

The studied formulas were supplemented with α-tocopherol acetate at two levels, IF-A (25 mg α-TE/100 g) and IF-B (6.1 mg α-TE/100 g). However, IFs also contain tocopherols derived from the vegetable oils used for formula manufacturing. In both formulas, at the beginning of the

Table 3
Analysis of vitamin E content in different infant formulas during storage by NP-HPLC-DAD

Sample	Storage (days)	α-T (mg/100 g)	γ-T (mg/100 g)	δ-T (mg/100 g)	α-TAc (mg/100 g)	α-TE Found (mg/100 g)	α-TE Label (mg/100 g)	Losses (%)	Adequacy (%)	α-TE mg/100 kcal
IF-A	0	6.18 ± 0.29^{a}	1.62 ± 0.11^{a}	–	29.8 ± 1.28^{a}	27.3 ± 0.99^{a}	25	–	109	5.31 ± 0.19
25 °C	1	5.88 ± 0.08^{a}	1.48 ± 0.02^{b}	–	29.1 ± 0.92^{a}	26.5 ± 0.72^{a}		2.9	106	5.15 ± 0.14
	3	6.02 ± 0.15^{a}	1.48 ± 0.03^{b}	–	29.1 ± 0.88^{a}	26.7 ± 0.77^{a}		2.3	107	5.18 ± 0.15
	6	5.89 ± 0.09^{a}	1.42 ± 0.03^{b}	–	28.4 ± 0.50^{a}	26.0 ± 0.42^{a}		4.8	104	5.05 ± 0.08
	9	5.34 ± 0.27^{b}	1.29 ± 0.07^{c}	–	25.9 ± 0.87^{b}	27.3 ± 0.85^{a}		13.2	94.9	4.61 ± 0.16
	12	5.35 ± 0.25^{b}	1.30 ± 0.07^{c}	–	26.0 ± 0.85^{b}	23.8 ± 0.80^{b}		13.0	95.1	4.62 ± 0.16
	15	5.27 ± 0.13^{b}	0.82 ± 0.00^{d}	–	25.7 ± 0.14^{b}	23.4 ± 0.11^{b}		14.3	93.7	4.55 ± 0.02
	18	5.35 ± 0.48^{b}	0.74 ± 0.02^{e}	–	24.2 ± 0.61^{b}	22.4 ± 0.56^{b}		17.9	89.7	4.35 ± 0.11
IF-A	0	6.18 ± 0.29^{a}	1.62 ± 0.11^{a}	–	29.8 ± 1.28^{a}	27.3 ± 0.99^{a}	25	–	109	5.31 ± 0.19
40 °C	1	5.95 ± 0.17^{a}	1.59 ± 0.03^{a}	–	29.1 ± 1.77^{a}	26.6 ± 1.47^{a}		2.6	107	5.17 ± 0.29
	3	6.02 ± 0.15^{a}	1.58 ± 0.06^{a}	–	28.5 ± 1.43^{a}	26.3 ± 0.78^{a}		3.9	105	5.10 ± 0.15
	6	5.83 ± 0.11^{b}	1.40 ± 0.08^{b}	–	27.8 ± 1.40^{b}	25.5 ± 1.43^{a}		6.5	102	4.96 ± 0.28
	9	5.09 ± 0.14^{c}	1.29 ± 0.02^{c}	–	24.1 ± 1.76^{c}	22.2 ± 1.14^{b}		18.8	88.8	4.31 ± 0.22
	12	5.04 ± 0.20^{c}	1.23 ± 0.03^{d}	–	23.9 ± 1.74^{c}	22.0 ± 1.16^{b}		19.5	88.0	4.27 ± 0.23
	15	5.20 ± 0.09^{c}	0.81 ± 0.04^{e}	–	24.0 ± 1.61^{c}	22.2 ± 1.06^{b}		18.8	88.7	4.31 ± 0.21
	18	4.78 ± 0.06^{d}	0.71 ± 0.02^{f}	–	19.3 ± 1.04^{d}	21.0 ± 0.69^{b}		23.1	84.0	4.08 ± 0.13
IF-B	0	6.47 ± 0.21^{a}	4.90 ± 0.06^{a}	1.21 ± 0.03^{a}	6.46 ± 0.53^{a}	12.1 ± 0.57^{a}	6.1	–	198	2.38 ± 0.11
25 °C	1	6.06 ± 0.05^{b}	4.56 ± 0.06^{b}	1.15 ± 0.04^{b}	6.20 ± 0.53^{a}	11.4 ± 0.40^{a}		5.4	188	2.25 ± 0.08
	3	5.96 ± 0.05^{b}	4.65 ± 0.08^{b}	1.08 ± 0.05^{c}	5.89 ± 0.08^{a}	11.1 ± 0.10^{b}		8.1	182	2.18 ± 0.02
	6	5.93 ± 0.10^{b}	4.64 ± 0.09^{b}	0.71 ± 0.16^{d}	5.96 ± 0.15^{a}	11.1 ± 0.21^{b}		8.2	182	2.18 ± 0.04
	9	5.61 ± 0.14^{c}	4.48 ± 0.03^{c}	–	5.84 ± 0.21^{a}	10.6 ± 0.29^{b}		12.4	174	2.08 ± 0.06
	12	5.58 ± 0.10^{c}	4.45 ± 0.03^{c}	–	5.80 ± 0.15^{a}	10.5 ± 0.22^{b}		13.0	173	2.07 ± 0.04
	15	5.60 ± 0.11^{c}	4.57 ± 0.07^{c}	–	5.92 ± 0.26^{a}	10.7 ± 0.52^{b}		11.8	175	2.09 ± 0.10
	18	5.55 ± 0.11^{c}	4.50 ± 0.13^{c}	–	5.86 ± 0.23^{a}	10.6 ± 0.56^{b}		12.8	173	2.07 ± 0.11
IF-B	0	6.47 ± 0.21^{a}	4.90 ± 0.06^{a}	1.21 ± 0.09^{a}	6.46 ± 0.53^{a}	12.1 ± 0.57^{a}	6.1	–	198	2.38 ± 0.11
40 °C	1	5.97 ± 0.09^{b}	4.51 ± 0.12^{b}	0.99 ± 0.07^{b}	6.22 ± 0.30^{a}	11.3 ± 0.29^{a}		6.3	186	2.23 ± 0.06
	3	5.93 ± 0.12^{b}	4.56 ± 0.19^{b}	–	5.97 ± 0.36^{a}	11.0 ± 0.26^{b}		8.8	181	2.17 ± 0.09
	6	5.60 ± 0.18^{c}	4.54 ± 0.17^{b}	–	5.85 ± 0.45^{a}	10.6 ± 0.18^{b}		12.3	174	2.08 ± 0.04
	9	5.03 ± 0.10^{d}	4.51 ± 0.11^{b}	–	5.57 ± 0.42^{a}	9.83 ± 0.78^{b}		18.7	161	1.93 ± 0.15
	12	4.92 ± 0.12^{d}	4.29 ± 0.07^{c}	–	5.46 ± 0.33^{b}	9.60 ± 0.33^{c}		20.6	157	1.89 ± 0.08
	15	4.63 ± 0.03^{e}	4.06 ± 0.09^{d}	–	5.39 ± 0.49^{b}	9.22 ± 0.12^{c}		23.7	151	1.81 ± 0.06
	18	4.42 ± 0.10^{f}	3.49 ± 0.07^{e}	–	5.12 ± 0.36^{b}	8.70 ± 0.21^{d}		28.1	143	1.71 ± 0.04

Values are expressed as means ± standard deviation of four determinations. Repetitions in superscripted letters within the same column and formula indicate no significant differences ($p > .05$).

study (time 0), similar quantities of α-tocopherol were observed (IF-A: 6.18 mg/100 g and IF-B: 6.47 mg/100 g), as well as γ-tocopherol (IF-A: 1.62 mg/100 g and IF-B: 4.90 mg/100 g); δ-tocopherol was only detected in IF-B (1.21 mg/100 g) (Table 3). The initial value of α-TAc in IF-A was 29.75 mg/100 g, and at the end of the study, it was 24.19 mg/100 g (18.7% loss) at 25 °C and 19 mg/100 g (35.3% loss) at 40 °C. In IF-B, a value of 6.46 mg α-TAc/100 g was recorded at the beginning of the study (time 0). After 18 months of storage at 25 °C, 5.86 mg α-TAc/100 g remained (9.3% loss) and at 40 °C 5.12 mg α-TAc/100 g remained (20.7% loss) (Table 3).

According to the label, IF-A contains 25 mg α-TE/100 g, the measured value was 27.3 mg α-TE/100 g, which represents an adequacy value of 109.3%. However, after 18 months of storage the recorded values were 22.4 mg α-TE/100 g at 25 °C (which represents 89.7% of the stated label) and 21 mg α-TE/100 g at 40 °C (an adequacy of 84%). According to the label, the IF-B content is 6.1 mg α-TE/100 g, and the initial recorded value (time 0) was 12.1 mg α-TE/100 g, which represents an adequacy value of 198%. After 18 months of storage at 25 and 40 °C, recorded values were 10.5 and 8.7 mg α-TE/100 g, which represents adequacy values of 173% and 142%, with respect to label information.

In formula IF-A, over-fortification to ensure the content of the label information, was not sufficient; however, the average content of α-TE, according to the labels, in most commercial IFs is about 5.9 α-TE/100 g (in the range 3–13 α-TE). IF-A has a higher vitamin E content (25 mg α-TE/100 g), in comparison with commercial IFs. IF-B contained amounts closer to twice the declared values (Table 3), which is consistent with other reports (Albalá-Hurtado et al., 2001; Landen et al., 1985). Albalá-Hurtado et al. (2001) reported ranges of vitamin E in starting liquid milk from 8.9 to 24.44 mg α-TE/100 g and in starting powdered milk from 6.66 to 22.22 mg α-TE/100 g, after saponificating the samples and analyzing by RP-HPLC. Delgado-Zamarreno et al. (2006) studied tocopherols in several IFs, using pressurised liquid extraction and liquid chromatography with amperometric detection, obtaining values of vitamin E of 6.63 mg α-TE/100 g in a starter hypoallergenic

IF and 14.5 mg α-TE/100 g in a starter adapted protein IF. Miquel, Alegria, Barbera, Farre, and Clemente (2004) reported values of vitamin E by normal-phase HPLC of 2.20, 1.90, 1.61 and 1.78 mg α-TE/100 kcal in four IFs; the first two supplemented with α-T and the last two with α-TAc, immediately after opening packets, the mean adequacy value being 185% with respect to the composition stated on the labels. Human milk has been reported to contain between 3.0 and 5.6 mg/l (0.45–0.8 mg/100 kcal) of vitamin E (Barbas and Herrera, 1998; Bohm et al., 1997; Chappell, Francis, and Clandinin, 1985). European legislation (2006/141/CE) establishes a minimum vitamin E content in IF of 0.5 mg α-TE/g of PUFA, and never less than 0.5 mg α-TE/100 kcal. IF-A and IF-B have 10.62 times and 4.76 times the minimum recommended level of vitamin E (Table 3). Previously no maximum limit was specified for vitamin E (96/4/CE). Nowadays (2006/141/CE, 2006) a maximum level of 5 mg α-TE/100 kcal is currently specified for vitamin E. IF-A contained slightly higher levels (1.06 times) than this maximum limit, and it must be adapted to the new regulation.

3.3. Vitamin C content

At the beginning of the study, in IF-A (time 0), a value of 100 mg/100 g of vitamin C was found, while the label stated the value to be 60 mg/100 g, which represents an adequacy of 167%. In IF-B (time 0), a value of 80.5 mg/100 g of vitamin C was found, which means an adequacy value of 118%, according to the composition expressed in the label information (Table 4). Following storage, constant decreases were observed under the conditions tested. For formulas stored at 25 °C after 18 months of storage, IF-A contained 79.9 mg/100 g, which represents a loss of 20.4% with respect to the initial content (adequacy to label of 133%). Under the same conditions, IF-B recorded a value of 52.5 mg/100 g of vitamin C, which means a loss of 34.8%. In this case the value is below the label statement (adequacy of only 77.2%). IF-B showed values lower than the referred label value of 68 mg/100 g after only 9 months at 25 °C.

When formulas were stored at 40 °C, greater losses were observed. In IF-A after 18 months of storage, a value of

Table 4
Analysis of vitamin Ccontent in infant formulas during storage

Sample	Storage (months)	Vitamin C[x] (mg/100 g)	Label (mg/100 g)	Losses (%)	Adequacy (%)	mg/100 kcal
IF-A	0	100 ± 3.51^{a}	60	–	167	19.5 ± 0.53
25 °C	1	99.1 ± 2.19^{a}		1.30	165	19.2 ± 0.21
	3	98.0 ± 3.51^{a}		2.39	163	19.0 ± 0.52
	6	95.3 ± 2.01^{b}		5.13	159	18.5 ± 0.21
	9	88.3 ± 2.57^{c}		12.1	147	17.1 ± 0.25
	12	85.3 ± 3.52^{c}		15.0	142	16.6 ± 0.51
	15	80.1 ± 2.31^{d}		20.3	133	15.6 ± 0.23
	18	79.9 ± 3.47^{d}		20.4	133	15.5 ± 0.35
IF-A	0	100 ± 3.51^{a}	60	–	167	19.5 ± 0.53
40 °C	1	93.8 ± 5.54^{b}		6.57	156	18.2 ± 0.22
	3	91.7 ± 3.17^{b}		8.65	153	17.8 ± 0.31
	6	87.9 ± 3.71^{c}		12.5	146	17.1 ± 0.33
	9	83.0 ± 4.01^{c}		17.3	138	16.1 ± 0.41
	12	80.0 ± 2.21^{c}		20.3	133	15.5 ± 0.33
	15	75.1 ± 2.71^{d}		25.3	125	14.6 ± 0.32
	18	71.9 ± 2.22^{e}		28.4	120	14.0 ± 0.20
IF-B	0	80.5 ± 2.52^{a}	68	–	118	15.8 ± 0.25
25 °C	1	78.8 ± 2.01^{a}		2.11	116	15.5 ± 0.21
	3	76.5 ± 3.11^{a}		4.97	112	15.0 ± 0.34
	6	75.1 ± 2.19^{b}		6.67	110	14.8 ± 0.22
	9	63.3 ± 1.99^{c}		21.3	93.1	12.4 ± 0.21
	12	60.5 ± 2.56^{c}		24.8	89.0	11.9 ± 0.25
	15	56.2 ± 1.57^{d}		30.1	82.7	11.1 ± 0.19
	18	52.5 ± 2.02^{e}		34.8	77.2	10.3 ± 0.20
IF-B	0	80.5 ± 2.52^{a}	68	–	118	15.8 ± 0.25
40 °C	1	75.3 ± 2.03^{b}		6.49	111	14.8 ± 0.22
	3	73.1 ± 2.15^{b}		9.16	108	14.4 ± 0.22
	6	70.5 ± 1.81^{c}		12.4	104	13.9 ± 0.26
	9	60.9 ± 2.75^{d}		24.3	89.6	12.0 ± 2.27
	12	53.5 ± 1.97^{e}		33.5	78.7	10.5 ± 0.18
	15	45.8 ± 2.05^{f}		43.1	67.4	9.00 ± 0.11
	18	41.3 ± 2.37^{g}		48.7	60.8	8.12 ± 0.25

Vitamin C is referred to as ascorbic acid. Repetitions in superscripted letters within the same column and formula indicate no significant differences ($p > .05$).

[x] Values are expressed as means ± standard deviation of four determinations.

71.9 mg/100 g of vitamin C was recorded, a loss of 28.4%. However, this value complies with the label statements (adequacy of 119.80%). In IF-B, a value of 41.3 mg/100 g of vitamin C was recorded after 18 months of storage, which represents losses of 48.7% of the vitamin C, with respect to the initial content (adequacy 60.8%). After 9 months of storage at 40 °C, IF-B showed levels lower than the referred value on the product's label (Table 4). In IF-B, over-fortification of vitamin C was not enough to ensure the correctness of the label statement after 9 months of storage at both 25 and 40 °C.

The values found in the two formulas are in agreement with those reported by Behrens and Madere (1989) in powdered IF (46.4–86.3 mg/100 g) and by Martin et al. (1987) in IF manufactured in the United States (15.3 ± 3.2 mg/100 kcal in milk-based IF). Our values are slightly higher than the values reported by Esteve et al. (1995) on milk-based and soy-based infant formula (49.28 mg/100 g and 45.27 mg/100 g, respectively), and those reported by Fontannaz, Kilinc, and Heudi (2006), who found values of ascorbic acid in IF in the range of 39.5–62.2 mg/100 g.

The vitamin C content of mature milk varies widely from 30 to 100 mg/l (4.5 to 15 mg/100 kcal) (Bank, Kirksey, West, and Giacoia, 1985; Bates, Prentice, Prentice, and Whitehead, 1982; Byerley and Kirksey, 1985; Salmenpera, 1984; Sneed, Zane, and Thomas, 1981), and decreases during the course of lactation. The current European legislation (2006/141/CE, 2006) has established a minimum of 10 mg/100 kcal and a maximum of 30 mg/100 kcal. In all tested conditions the studied formulas complied with this new regulation, with the exception of IF-B at 40 °C after 15 months of storage (Table 4).

3.4. Iron contents

The iron levels in IF-A under all studied conditions were between 3.51 and 3.95 mg/100 g (0.68–0.77 mg/100 kcal), which represents an adequacy from 58.5% to 65.8% (Table 5), with respect to label information which specified a content of 6 mg/100 g. Values of iron in IF-B were between 3.98 and 4.59 mg/100 g (0.78–0.90 mg/100 kcal), which represents adequacy values from 65.3% to 75.3%, with respect to label information. In both studied formulas val-

Table 5
Analysis of iron content in infant formulas during storage

Sample	Storage (months)	Iron (mg/100 g)	Iron (mg/l)	Iron (mg/100 kcal)	Adequacy (%)
IF-A	0	3.90 ± 0.26^{a}	5.27	0.76	65.0
25 °C	1	3.82 ± 0.26^{a}	5.16	0.74	63.7
	3	3.75 ± 0.27^{a}	5.06	0.73	62.5
	6	3.54 ± 0.11^{a}	4.78	0.69	59.0
	9	3.52 ± 0.25^{a}	4.75	0.68	58.7
	12	3.79 ± 0.13^{a}	5.12	0.74	63.2
	15	3.72 ± 0.16^{a}	5.02	0.72	62.0
	18	3.80 ± 0.32^{a}	5.13	0.74	63.3
IF-A	0	3.90 ± 0.26^{a}	5.27	0.76	65.0
40 °C	1	3.84 ± 0.05^{a}	5.18	0.75	64.0
	3	3.61 ± 0.13^{a}	4.87	0.70	60.2
	6	3.58 ± 0.21^{a}	4.83	0.70	59.7
	9	3.65 ± 0.22^{a}	4.93	0.71	60.8
	12	3.95 ± 0.36^{a}	5.33	0.77	65.8
	15	3.51 ± 0.42^{a}	4.74	0.68	58.5
	18	3.89 ± 0.23^{a}	5.25	0.76	64.8
IF-B	0	3.98 ± 0.14^{a}	5.25	0.78	65.3
25 °C	1	4.00 ± 0.35^{a}	5.28	0.79	65.6
	3	4.24 ± 0.33^{a}	5.60	0.83	69.5
	6	4.22 ± 0.19^{a}	5.57	0.83	69.2
	9	4.47 ± 0.57^{a}	5.90	0.88	73.3
	12	4.37 ± 0.41^{a}	5.77	0.86	71.6
	15	4.35 ± 0.41^{a}	5.74	0.85	71.3
	18	4.21 ± 0.28^{a}	5.56	0.83	69.0
IF-B	0	3.98 ± 0.14^{a}	5.25	0.78	65.3
40 °C	1	4.24 ± 0.16^{a}	5.60	0.83	69.5
	3	4.56 ± 0.12^{a}	6.02	0.90	74.8
	6	4.50 ± 0.31^{a}	5.94	0.88	73.8
	9	4.42 ± 0.12^{a}	5.83	0.87	72.5
	12	4.48 ± 0.22^{a}	5.91	0.88	73.4
	15	4.59 ± 0.59^{a}	6.06	0.90	75.3
	18	4.55 ± 0.32^{a}	6.01	0.89	74.6

Values are expressed as means ± standard deviation of three determinations. Repetitions in superscripted letters within the same column and formula indicate no significant differences ($p > .05$).

ues of iron were lower than the values reported on the labels. The measured values are slightly lower than values reported by other authors in IF milk-based powder from Nigeria (8.49 ± 1.21 mg/l), United Kingdom milk-based liquid first and follow-on formulas (11.3 ± 2.26 mg/l), United States of America milk-based powder formulas (9.30 ± 0.46 mg/l) and soy-based powder formula (9.14 ± 0.29 mg/l) (Ikem, Nwankwoala, Odueyungbo, Nyavor, and Egiebor, 2002).

Human milk iron content is highest in early lactation, diminishes over the course of about 5 months, and gradually rises thereafter (Casey, Smith, and Zhang, 1995). The iron content of human milk does not appear to be affected by maternal iron status (Lonnerdal, 1986). The reported mean values for mature human milk range from 0.2 to 0.8 mg/l (0.03 to 0.12 mg/100 kcal) (Anderson, 1992; Feeley, Eitenmiller, Jones, and Barnhart, 1983; Hirai et al., 1990; Lonnerdal and Hernell, 1994). However, the ability to make recommendations about the appropriate iron content of infant formulas is limited by several factors including, among others:

(a) the lack of an ideal standard. Unlike other nutrients, human milk cannot be used as the standard for establishing minimum iron levels for infant formulas, primarily because of evidence to indicate that exclusively breast-fed infants are at risk of iron deficiency anaemia after the age of 6 months,
(b) the bioavailability of iron from various sources, including breast milk and various formulas, is not known,
(c) concerns about the impact of iron on gastrointestinal function and health of the infant,
(d) the potential nutrient interactions make estimation of appropriate iron content of infant formulas difficult,
(e) the safety of "low-iron" formulas, and
(f) the risks associated with excessive iron intake.

The difficulty in making recommendations about iron content in infant formulas is due in part to the incomplete understanding of iron status in infants, particularly breast-fed infants. An incongruity exists between the concentrations of iron in breast milk and the infant's ability to maintain adequate iron status, at least during the first 6 months of life. Some commercially produced "iron-fortified" formulas contain approximately 12 mg/l of iron (1.8 mg/100 kcal). Formula products labelled as "low-iron" contain between 1.3 and 4.7 mg/l (0.2 and 0.7 mg/100 kcal) of iron. The justification for low-iron products is that their use will alleviate gastrointestinal problems (mainly constipation), presumed to be associated with the iron content of conventional formulas. These perceptions have been tested empirically in a number of studies (Bradley, Hillman, Sherman, Leedy, and Cordano, 1993; Hyams et al., 1995; Nelson, Ziegler, Copeland, Edwards, and Fomon, 1988; Oski, 1980). Also, iron has been reputed to interact with the absorption of several other minerals, including copper, zinc and manganese (Solomons, 1986). In addition, a number of deleterious effects have been attributed to excessive iron intake. For example, iron acts as a catalyst for free radical generating reactions; consequently, an excess of free iron can enhance of cellular oxidative reactions, such as lipid peroxidation, which can lead to oxidative damage (Halliwell, 1994). The previous Commission of the European Communities (96/4/CE, 1996) specified a minimum iron level of 0.5 mg/100 kcal and a maximum iron level of 1.5 mg/100 kcal, a value which was recently modified (2006/141/CE, 2006) to limits of 0.3 mg/100 kcal and 1.3 mg/100 kcal, respectively. Analysis showed that iron levels remain unchanged during storage, and these levels complied with the current European legislation. It would be interesting to analyse Fe(II) and Fe(III) during storage, due to the oxidant activity of iron, in order to increase understanding of oxidation reactions during storage of powdered IF.

3.5. Selenium contents

Table 6 shows the selenium found in the analysed samples. Analysis showed that selenium levels remain unchanged throughout storage. The selenium levels in IF-A in all tested conditions were between 6.90 and 7.90 μg/100 g (1.34–1.53 μg/100 kcal), which represents adequacy values from 64.5% to 73.8% with respect to the label, where a content of 10.7 μg/100 g is specified. Values of IF-B were between 6.80 and 7.55 μg/100 g (1.34–1.48 μg/100 kcal), and these are numbers which represent adequacy values of 75.6–83.9% with respect to label statements. Both types of formulas contained lower levels of selenium than the labels stated. IF-A average content was 10.00 ± 0.48 μg/l and IF-B was 9.67 ± 0.27 μg/l. Using liquid chromatography hydride generation atomic fluorescence spectrometry, Viñas, Lopez-Garcia, Merino-Merono, Campillo, and Hernandez-Cordoba (2005) reported selenium in starting milk, follow-on milk, and soy-based IF in the range of 4.5–11.2 μg/100 g. Lower values, also using flow injection hydride atomic absorption spectrometry, were reported in Spanish milk-based IF in the range 1.8–5.0 μg/l. Using inductively coupled plasma atomic emission spectrometry with hydride generator, Navarro-Blasco and Avarez-Galindo (2004) found values in Spanish IF from 2.9 to 17.3 μg/l. L'Abbe, Trick, and Koshy (1996) used a diaminonaphthalene fluorimetric method and reported selenium in Canadian IF, finding values from 3 to 31 μg/l in unsupplemented IF and 16 to 35 μg/l in supplemented IF. Foster and Sumar (1996) determined selenium in IF available in the UK by hydride generation atomic absorption spectrometry and reported figures that ranged from 3.4 to 9.3 μg/100 g for bovine casein and whey-based powdered IF, 2.3–9.3 μg/100 g for preterm powdered formulas and 2.7–4.9 μg/100 g for hospital administered low birth weight formulas. Unfortified commercially available infant formulas marketed in the United States have been reported to contain 2.2–9.5 μg/l or 0.33–1.4 μg of selenium/100 kcal

Table 6
Analysis of selenium content in infant formulas during storage

Sample	Storage (months)	Selenium (μg/100 g)	Selenium (μg/l)	Selenium (μg/100 kcal)	Adequacy (%)
IF-A	0	7.80 ± 0.46^{a}	10.53	1.51	72.9
25 °C	1	7.64 ± 0.32^{a}	10.31	1.48	71.4
	3	6.90 ± 0.33^{a}	9.32	1.34	64.5
	6	7.08 ± 0.42^{a}	9.56	1.37	66.2
	9	7.04 ± 0.33^{a}	9.50	1.37	65.8
	12	7.58 ± 0.45^{a}	10.23	1.47	70.8
	15	7.44 ± 0.35^{a}	10.04	1.44	69.5
	18	7.60 ± 0.37^{a}	10.26	1.48	71.0
IF-A	0	7.80 ± 0.46^{a}	10.53	1.51	72.9
40 °C	1	7.68 ± 0.32^{a}	10.37	1.49	71.8
	3	7.22 ± 0.34^{a}	9.75	1.40	67.5
	6	7.16 ± 0.45^{a}	9.67	1.39	66.9
	9	6.90 ± 0.33^{a}	9.32	1.34	64.5
	12	7.90 ± 0.28^{a}	10.67	1.53	73.8
	15	7.02 ± 0.38^{a}	9.48	1.36	65.6
	18	7.78 ± 0.42^{a}	10.50	1.51	72.7
IF-B	0	7.15 ± 0.27^{a}	9.44	1.40	79.4
25 °C	1	6.80 ± 0.17^{a}	8.98	1.34	75.6
	3	7.38 ± 0.35^{a}	9.74	1.45	82.0
	6	7.17 ± 0.28^{a}	9.47	1.41	79.7
	9	7.52 ± 0.41^{a}	9.93	1.48	83.6
	12	7.43 ± 0.37^{a}	9.81	1.46	82.5
	15	7.40 ± 0.32^{a}	9.76	1.45	82.2
	18	7.16 ± 0.35^{a}	9.45	1.41	79.5
IF-B	0	7.15 ± 0.27^{a}	9.44	1.40	79.4
40 °C	1	7.21 ± 0.18^{a}	9.51	1.42	80.1
	3	7.35 ± 0.31^{a}	9.70	1.44	81.7
	6	7.52 ± 0.25^{a}	9.93	1.48	83.6
	9	7.45 ± 0.32^{a}	9.83	1.46	82.8
	12	7.55 ± 0.41^{a}	9.97	1.48	83.9
	15	7.45 ± 0.35^{a}	9.83	1.46	82.8
	18	7.52 ± 0.25^{a}	9.93	1.48	83.6

Values are expressed as means ± standard deviation of three determinations. Repetitions in superscripted letters within the same column and formula indicate no significant differences ($p > .05$).

(Daniels, Gibson, and Simmer, 1997; Johnson, Smith, Chan, and Moyermileur, 1993; Smith, Chen, and Thomas, 1995; Smith, Picciano, and Milner, 1982). Van Dael and Barclay (2006) reported on selenium levels in IF by using continuous flow hydride generation atomic spectrometry in several samples of infant and follow-on formulas from France, Germany, Spain, Netherlands, Brazil, Mexico, USA, China, India, Australia and Africa. The endogenous selenium levels found varied between 3.4 and 13.6 μg/l.

Although the data on the bioavailability of selenium from various milks fed to healthy term infants are limited, there are sufficient data to deduce that differences do exist and that they are related to the source and form of selenium in the diet (Daniels, 1996; Lonnerdal, 1985). An additional, consideration is the potential for interactions between selenium and other nutrients, including protein, iron, magnesium, vitamin B_6, and ascorbic acid (Jimenez, Planells, Aranda, SanchezVinas, and Llopis, 1997; Lonnerdal and Hernell, 1994; Yin et al., 1991). The practical implications of these potential interactions with selenium for the establishment of recommendations for the selenium content of infant formulas are unclear at this time. However, it appears that fortification of infant formula at selenium concentrations comparable to those found in human milk will result in a selenium status equivalent to that of breast-fed infants nursed by selenium-adequate mothers.

The Commission of the European Communities (2006/141/CE, 2006) has recently specified a minimum and maximum concentration of 1 and 9 μg/100 kcal for selenium in infant formula. The values found in the samples we studied are in agreement with this legislation.

Losses of vitamins A, E and C during storage were observed in both LC-PUFA supplemented infant formulations. In spite of the stability of α-TAc, in comparison with the tocopherol isomers (namely, α-T, γ-T and δ-T), losses of α-TAc were observed in IF-A, during storage. Vitamin C showed the greatest losses during storage of the studied vitamins. The iron and selenium values found remained unchanged during storage and were lower than the label statements in both formula types. The results justify the finding that over-fortification in these IFs with vitamin A, E and C is necessary to ensure that those vitamins meet the content stated on the labels. Further studies are required in other supplemented IFs.

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References

2006/141/CE. (2006). Directiva 2006/141/CE de la Comisión de 22 de diciembre de 2006 relativa a los preparados para lactantes y preparados de continuación y por la que se modifica la Directiva 1999/21/CE.L 401, pp. 1–33.

96/4/CE. (1996). Directiva 96/4/CE de la Comisión, de 16 de febrero de 1996, por la que se modifica la directiva 91/321/CEE relativa a los preparados para lactantes y preparados de continuación. DOUE n. 49 de 28/02/1996, pp. 12–16.

Albalá-Hurtado, S., Veciana-Nogues, M. T., Vidal-Carou, M. C., & Font, A. M. (2001). Concentration of selected vitamins in infant milks available in Spain. *Journal of Food Science, 66*(8), 1191–1194.

Albalá-Hurtado, S., Veciana-Nogues, M. T., Vidal-Carou, M. C., & Marine-Font, A. (2000). Stability of vitamins A, E, and B complex in infant milks claimed to have equal final composition in liquid and powdered form. *Journal of Food Science, 65*(6), 1052–1055.

Aleixo, P. C., & Nobrega, J. A. (2003). Direct determination of iron and selenium in bovine milk by graphite furnace atomic absorption spectrometry. *Food Chemistry, 83*(3), 457–462.

Anderson, R. R. (1992). Comparison of trace elements in milk of 4 species. *Journal of Dairy Science, 75*(11), 3050–3055.

Anonymous. (1998). Vitamins. *The Journal of Nutrition, 128*(11S), S2182.

Bank, M. R., Kirksey, A., West, K., & Giacoia, G. (1985). Effect of storage time and temperature on folacin and vitamin-C levels in term and preterm human-milk. *American Journal of Clinical Nutrition, 41*(2), 235–242.

Barbas, C., & Herrera, E. (1998). Lipid composition and vitamin E content in human colostrum and mature milk. *Journal of Physiology and Biochemistry, 54*(3), 167–173.

Bates, C. J., Prentice, A. M., Prentice, A., & Whitehead, R. G. (1982). Vitamin-C supplementation of lactating women in Keneba – A west-african rural-community. *Proceedings of the Nutrition Society, 41*(3), A124.

Behrens, W. A., & Madere, R. (1989). Ascorbic and dehydroascorbic acid content of infant formula. *Journal of Food Composition and Analysis, 2*(1), 48–52.

Blake, C. J. (2004). Committee on food nutrition. *Journal of AOAC International, 87*(1), 285–286.

Blake, C. J. (2005). Committee on food nutrition – Fat-soluble vitamins. *Journal of AOAC International, 88*(1), 325–329.

Bohm, V., Peiker, G., Starker, A., Weske, E., Schaarmann, G., Schubert, R., et al. (1997). The contents of vitamin B-1, B-2, A, and E as well as beta-carotene in transitional human milk and comparative examinations with maternal blood and cord blood. *Zeitschrift fur Ernahrungswissenschaft, 36*(3), 214–219.

Bradley, C. K., Hillman, L., Sherman, A. R., Leedy, D., & Cordano, A. (1993). Evaluation of 2 iron-fortified, milk-based formulas during infancy. *Pediatrics, 91*(5), 908–914.

Brigelius-FlohE, R., & Traber, M. G. (1999). Vitamin E: Function and metabolism. *FASEB Journal, 13*(10), 1145–1155.

Byerley, L. O., & Kirksey, A. (1985). Effects of different levels of vitamin-C intake on the vitamin-C concentration in human-milk and the vitamin-C intakes of breast-fed infants. *American Journal of Clinical Nutrition, 41*(4), 665–671.

Casey, C. E., Smith, A., Zhang, P. (1995). Microminerals in human and animal milks. In R. G. Jensen (Ed.), *Handbook of milk composition* (pp. 622-674). New York, NY.

Chappell, J. E., Francis, T., & Clandinin, M. T. (1985). Vitamin-A and vitamin-E content of human-milk at early stages of lactation. *Early Human Development, 11*(2), 157–167.

Chávez-Servín, J. L., Castellote, A. I., & López-Sabater, M. C. (2006). Simultaneous analysis of Vitamins A and E in infant milk-based formulae by normal-phase high-performance liquid chromatography-diode array detection using a short narrow-bore column. *Journal of Chromatography A, 1122*, 138–143.

Daniels, L., Gibson, R., & Simmer, K. (1997). Selenium status of preterm infants: The effect of postnatal age and method of feeding. *Acta Paediatrica, 86*(3), 281–288.

Daniels, L. A. (1996). Selenium metabolism and bioavailability. *Biological Trace Element Research, 54*(3), 185–199.

Delgado-Zamarreno, M. M., Bustamante-Rangel, M., Garcia-Jimenez, M., Sanchez-Perez, A., & Carabias-Martinez, R. (2006). Off-line coupling of pressurized liquid extraction and LC/ED for the determination of retinyl acetate and tocopherols in infant formulas. *Talanta, 70*(5), 1094–1099.

Driskell, J. A., Giraud, D. W., Drewel, B. T., & Davy, S. R. (2006). Dietary intakes and plasma concentrations of vitamin C, selenium, and carotenoids of a group of preschool children. *Nutrition Research, 26*(1), 23–26.

Esteve, M. J., Farre, R., Frigola, A., Lopez, J. C., Romera, J. M., Ramirez, M., et al. (1995). Comparison of voltammetric and high performance liquid chromatographic methods for ascorbic acid determination in infant formulas. *Food Chemistry, 52*(1), 99–102.

Feeley, R. M., Eitenmiller, R. R., Jones, J. B., & Barnhart, H. (1983). Copper, iron, and zinc contents of human milk at early stages of lactation. *American Journal of Clinical Nutrition, 37*(3), 443–448.

Fontannaz, P., Kilinc, T., & Heudi, O. (2006). HPLC-UV determination of total vitamin C in a wide range of fortified food products. *Food Chemistry, 94*(4), 626–631.

Foster, L. H., & Sumar, S. (1996). Selenium concentrations in soya based milks and infant formulae available in the United Kingdom. *Food Chemistry, 56*(1), 93–98.

Gonzalez-Corbella, M. J., Tortras-Biosca, M., Castellote-Bargallo, A. I., & Lopez-Sabater, M. C. (1999). Retinol and alpha-tocopherol in infant formulas produced in the EEC. *Food Chemistry, 66*(2), 221–226.

Halliwell, B. (1994). Free-radicals and antioxidants. A personal view. *Nutrition Reviews, 52*(8), 253–265.

Hirai, Y., Kawakata, N., Satoh, K., Ikeda, Y., Hisayasu, S., Orimo, H., et al. (1990). Concentrations of lactoferrin and iron in human milk at different stages of lactation. *Journal of Nutritional Science and Vitaminology, 36*(6), 531–544.

Hyams, J. S., Treem, W. R., Etienne, N. L., Weinerman, H., Macgilpin, D., Hine, P., et al. (1995). Effect of infant formula on stool characteristics of young infants. *Pediatrics, 95*(1), 50–54.

Ikem, A., Nwankwoala, A., Odueyungbo, S., Nyavor, K., & Egiebor, N. (2002). Levels of 26 elements in infant formula from USA, UK, and Nigeria by microwave digestion and ICP-OES. *Food Chemistry, 77*(4), 439–447.

Jimenez, A., Planells, E., Aranda, P., SanchezVinas, M., & Llopis, J. (1997). Changes in bioavailability and tissue distribution of selenium caused by magnesium deficiency in rats. *Journal of the American College of Nutrition, 16*(2), 175–180.

Johnson, C. E., Smith, A. M., Chan, G. M., & Moyermileur, L. J. (1993). Seleniumstatus of term infants fed human-milk or selenite-supplemented soy formula. *Journal of Pediatrics, 122*(5), 739–741.

Kim, Y. N., Lora, K. R., Giraud, D. W., & Driskell, J. A. (2006). Nonsupplemented children of latino immigrants have low vitamin E intakes and plasma concentrations and normal vitamin C, selenium,

and carotenoid intakes and plasma concentrations. *Journal of the American Dietetic Association, 106*(3), 385–391.

L'Abbe, M. R., Trick, K. D., & Koshy, A. (1996). The selenium content of Canadian infant formulas and breast milk. *Journal of Food Composition and Analysis, 9*(2), 119–126.

Landen, W. O., Hines, D. M., Hamill, T. W., Martin, J. I., Young, E. R., Eitenmiller, R. R., et al. (1985). Vitamin-A and vitamin-E content of infant formulas produced in the United States. *Journal of the Association of Official Analytical Chemists, 68*(3), 509–511.

Levine, M., ConryCantilena, C., Wang, Y. H., Welch, R. W., Washko, P. W., Dhariwal, K. R., et al. (1996). Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. *Proceedings of the National Academy of Sciences of the United States of America, 93*(8), 3704–3709.

Lonnerdal, B. (1986). Effects of maternal dietary intake on human milk composition. *Journal of Nutrition, 116*(4), 499–513.

Lonnerdal, B. (1985). Dietary factors affecting trace-element bioavailability from human-milk, cows milk and infant formulas. *Progress in Food and Nutrition Science, 9*(1–2), 35–62.

Lonnerdal, B., & Hernell, O. (1994). Iron, zinc, copper and selenium status of breast-fed infants and infants fed trace-element fortified milk-based infant formula. *Acta Paediatrica, 83*(4), 367–373.

Martin, J. I., Phifer, E. C., Hines, D. M., Hamill, T. W., Young, E. R., Eitenmiller, R. R., et al. (1987). Thiamine, riboflavin, and ascorbic acid levels in infant formulas manufactured in the United States. *Journal of Food Composition and Analysis, 1*(1), 76–80.

Miquel, E., Alegria, A., Barbera, R., Farre, R., & Clemente, G. (2004). Stability of tocopherols in adapted milk-based infant formulas during storage. *International Dairy Journal, 14*(11), 1003–1011.

Navarro-Blasco, I., & Avarez-Galindo, J. I. (2004). Selenium content of Spanish infant formulae and human milk: Influence of protein matrix, interactions with other trace elements and estimation of dietary intake by infants. *Journal of Trace Elements in Medicine and Biology, 17*(4), 277–289.

Nelson, S. E., Ziegler, E. E., Copeland, A. M., Edwards, B. B., & Fomon, S. J. (1988). Lack of adverse reactions to iron-fortified formula. *Pediatrics, 81*(3), 360–364.

Olson, J. A. (1987). Recommended dietary intakes (RDI) of Vitamin-A in humans. *American Journal of Clinical Nutrition, 45*(4), 704–716.

Olson, J. A. (1989). Upper limits of vitamin-A in infant formulas, with some comments on vitamin-K. *Journal of Nutrition, 119*(12), 1820–1824.

Olson, J. A. (1994). Vitamins – The tortuous path from needs to fantasies. *Journal of Nutrition, 124*(9), S1771–S1776.

Oski, F. A. (1980). Iron-fortified formulas and gastrointestinal symptoms in infants. A controlled study. *Pediatrics, 66*(2), 168–170.

Parrish, D. B. (1980). Determination of vitamin-E in foods – A review. *Crc Critical Reviews in Food Science and Nutrition, 13*(2), 161–187.

Romeu-Nadal, M., Morera-Pons, S., Castellote, A. I., & Lopez-Sabater, M. C. (2006). Rapid high-performance liquid chromatographic method for vitamin C determination in human milk versus an enzymatic method. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences, 830*(1), 41–46.

Salmenpera, L. (1984). Vitamin-C nutrition during prolonged lactation. Optimal in infants while marginal in some mothers. *American Journal of Clinical Nutrition, 40*(5), 1050–1056.

Smith, A. M., Chen, L. W., & Thomas, M. R. (1995). Selenate fortification improves selenium status of term infants fed soy formula. *American Journal of Clinical Nutrition, 61*(1), 44–47.

Smith, A. M., Picciano, M. F., & Milner, J. A. (1982). Selenium intakes and status of human-milk and formula fed infants. *American Journal of Clinical Nutrition, 35*(3), 521–526.

Sneed, S. M., Zane, C., & Thomas, M. R. (1981). The effects of ascorbic-acid, vitamin-B6, vitamin-B12, and folic-acid supplementation on the breast-milk and maternal nutritional-status of low socioeconomic lactating women. *American Journal of Clinical Nutrition, 34*(7), 1338–1346.

Solomons, N. W. (1986). Competitive interaction of iron and zinc in the diet: Consequences for human nutrition. *Journal of Nutrition, 116*(6), 927–935.

Spannaus-Martin, D. J., Cook, L. R., Tanumihardjo, S. A., Duitsman, P. K., & Olson, J. A. (1998). Vitamin A and vitamin E statuses of preschool children of socioeconomically disadvantaged families living in the midwestern United States (Vol. 51, p. 864, 1997). *European Journal of Clinical Nutrition, 52*(4), 310.

Tanumihardjo, S. A., Muhilal, Yuniar, Y., Permaesih, D., Sulaiman, Z., Karyadi, D., et al. (1990). Vitamin-A status in preschool-age Indonesian children as assessed by the modified relative-dose-response assay. *American Journal of Clinical Nutrition, 52*(6), 1068–1072.

Van Dael, P., & Barclay, D. V. (2006). Geographical, seasonal and formula-specific variations in the selenium levels of infant formulae. *Food Chemistry, 96*(4), 512–518.

Viñas, P., Lopez-Garcia, I., Merino-Merono, B., Campillo, N., & Hernandez-Cordoba, M. (2005). Determination of selenium species in infant formulas and dietetic supplements using liquid chromatography-hydride generation atomic fluorescence spectrometry. *Analytica Chimica Acta, 535*(1–2), 49–56.

Yin, S., Sato, I., Hosokawa, Y., Niizeki, S., Tojo, H., & Yamaguchi, K. (1991). The necessity of dietary vitamin-B6 to selenium biopotency for tissue selenium and glutathione peroxidase in rats. *Journal of Nutritional Science and Vitaminology, 37*(5), 509–516.

Yip, R. (1994). Iron-deficiency contemporary scientific issues and international programmatic approaches. *Journal of Nutrition, 124*(8), S1479–S1490.