Effects of Thermal Processing and Storage on Available Lysine and Furfural Compounds Contents of Infant Formulas

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The Maillard reaction-related effects that thermal treatments during the manufacturing process and storage (at 20 and 37 °C) have on powdered adapted and follow-up milk-based infant formulas were estimated by measuring the available lysine and furfural compounds contents of raw cow milk used in manufacturing, intermediate products and formulas. A fluorimetric method was used to measure the available lysine contents, and free and total furfural compounds were determined by HPLC. Statistically significant losses in available lysine (about 20%) in the infant formulas with respect to raw milk were found. The storage period did not affect the available lysine contents of adapted formulas but reduced (16%) the contents of the follow-up ones (from 6.61 to 5.33 g/100 g of protein). No furfural compounds were detected in raw milk, and free and total furyl methyl ketone (FMC) and methylfurfural (MF) were not observed in the analyzed samples. After 6 months of storage, an increase in free hydroxymethylfurfural (HMF) (from 0.34 to 0.77 mg/100 g of protein) and furfural (F) (from nondetectable to 0.1 mg/100 g of protein) in adapted formulas and free HMF (from 1.84 to 2.62 mg/100 g of protein) in follow-up formulas was observed.

Keywords: *Thermal treatment; storage; furfural compounds; available lysine; infant formulas*

INTRODUCTION

Infant formulas are sometimes the only food given to infants, and therefore, they must fulfill all their nutritional needs. However, the components of infant formulas can be modified and interact (Maillard reactions) during thermal processing treatments and also during storage of the product. This leads to changes that depend on the raw materials, formula composition, manufacturing process applied, and packaging and storage conditions (Van Mil and Jans, 1991).

In the initial stages of the Maillard reaction the amino acid lysine and the carbohydrate lactose are the main compounds involved, and as a consequence lysine can be present in milk-based formulas as available lysine or as a component of a Schiff base or lactulosyl•lysine. At advanced stages, undesirable compounds such as furfurals can be found. To evaluate the intensity of the thermal treatment applied and the effects of storage, it can be useful to measure the losses of available lysine and determine furfural derivates (Ferrer et al., 1999).

Several studies have been carried out on the effect of thermal treatment on the available lysine contents using either cow milk (Arteaga et al., 1994; Hewedy et al., 1994; Morales et al., 1995) or model systems (Baisier and Labuza, 1992; Morales et al., 1995) and ratios between temperature and time that do not reflect the usual conditions in the manufacturing process. However, studies on the loss of lysine and furfural formation as a consequence of thermal treatments in liquid (Rossi and Pompei, 1991a) or powdered (Anantharaman and Finot, 1993) infant formulas are scarce.

In addition, the available reports on lysine and furfural evolution during infant formula storage give a wide range of values ascribable to differences in the quality of the raw material (Hewedy et al., 1994; Morales et al., 1995), the heating treatments applied during processing, and the storage time and temperature (Pompei et al., 1987; Albalá-Hurtado et al., 1997a,b). The high temperatures often used (60−70 °C) enhance the Maillard reaction (Hurrell et al., 1983; Ford et al., 1983).

In Spain, infant formulas are mainly used in the powdered form. The adapted ones (infant milks) are used to feed infants up to 4−6 months of life, after which follow-up formulas are used and constitute the main liquid food in a progressively diversified diet. They combine a set of factors that makes them highly sensitive to Maillard reactions, that is, their high lactose and lysine contents, relatively high temperatures applied during the manufacturing process, and storage for long periods of time (Palombo et al., 1984). Moreover, due to the enrichment of infant milks with various compounds including vitamin A, iron, and lactose, these types of milk can be more susceptible to the Maillard reaction than cow's milk (Caric et al., 1984). However, data on the effect of thermal treatments and storage on the available lysine and furfural of powdered infant formulas are scarce. We therefore consider it of interest to study the effect of thermal treatment and of subsequent storage of powdered infant formulas marketed in Spain.

Our purpose was to contribute to the knowledge of Maillard reaction consequences by studying the global effect of the thermal treatments applied during the manufacturing process on two types (adapted and follow-up) of powdered infant formulas by monitoring the available lysine and furfural contents at different

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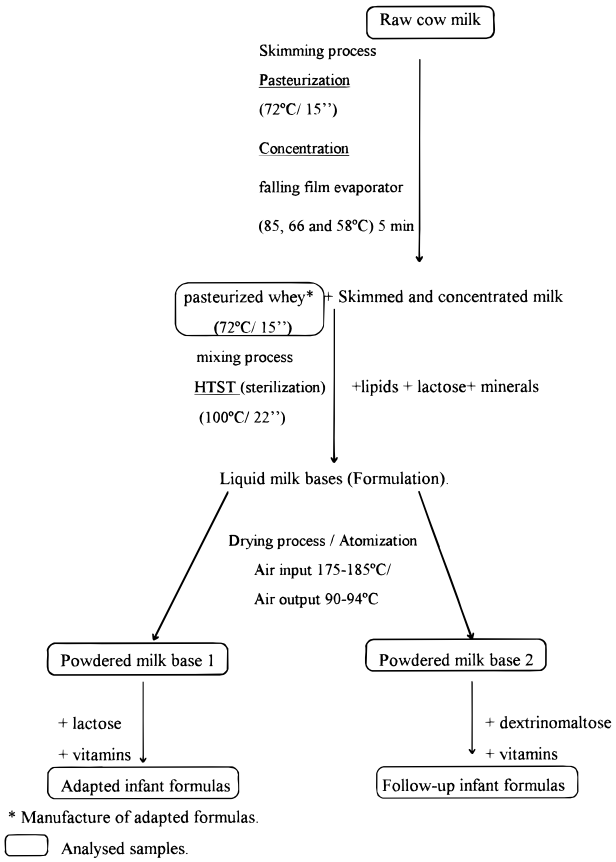

Figure 1. Outline of the infant formulas manufacturing process.

stages of the manufacturing process. We also study the evolution of these compounds in both types of formula, during a 6-month storage at 20 and 37 °C.

MATERIALS AND METHODS

Samples. Samples of adapted and follow-up milk-based formulas were collected at different points of the manufacturing process (Hero España S.A), see Figure 1.

The following products were collected and analyzed: (1) Raw cow milk (class A, means <400 000 somatic cells/mL and <100 000 cfu/mL); (2) Concentrated and pasteurized whey (from three different batches used in three adapted formula manufacturing processes); (3) Powdered milk bases 1 and 2, used in the manufacture of adapted (raw cow milk: whey 50/50, w/w) (from three manufacturing batches) and follow-up infant formulas (from five manufacturing batches), respectively. All these products were stored in polyethylene containers at 0−4 °C until analyzed.

Samples of three and five manufacturing batches of adapted and follow-up infant formulas, respectively, were collected. The composition of the adapted infant formulas as given on the label was proteins 11.6%, carbohydrates (lactose) 55%, lipids 28%. The composition of the follow-up infant formulas as stated on the label was proteins 16.%, carbohydrates 54% (lactose 32.4% + maltodextrine 21.6%), lipids 24%. The adapted and follow-up formulas were vacuum packed in commercial airtight 1-kg containers in a N_2/CO_2 (<3% O_2) modified atmosphere. For each type of formula, enough packages from the same batch to carry out the storage study were sampled.

The adapted and follow-up formulas were stored at 20 and 37 °C in a storage chamber (with <10% relative humidity and temperature controlled by a BJC heater with a Omron E5EW thermostat) for 6 months and analyzed just after manufacturing (at zero time) and after 3 and 6 months of storage. Samples were maintained in their airtight containers until analysis.

Taking into account the ratio of casein to whey proteins in raw cow milk and in the final products together with the available lysine and furfural compound contents of these products and whey, we estimated the contents of intermediate products (which we called formulation).

The following thermal treatments were applied during processing: (1) Pasteurization (72 °C/15 in.) of raw cow milk and milk whey, the latter obtained by ultracentrifugation; (2) Concentration of milk in long-tube vertical, falling film evaporator combining three thermal effects (85, 66, and 58 °C) over a 5 min period; (3) Sterilization (HTST 100 °C/22 in.); and (4) Spray-drying (air input 175−185 °C/air output 90−94 °C) of the mixture of concentrated milk, pasteurized whey, and lipids, lactose, and minerals.

Analytical Methods. *Available Lysine.* To measure available lysine, a fluorimetric method adapted in the laboratory that takes the method proposed by Goodno et al. (1981) as the starting point was applied.

Reagents. Hydrochloric acid 37.5% (Merck) (d = 1.19 g/mL), deionized water (Millipore-Milli Q), β-mercaptoethanol minimum 99% (Merck), casein from bovine milk purified powder (Sigma), anhydrous ethanol 99.5% (Panreac), sodium hydroxide 97% (w/w) (Panreac), *o*-phthaldialdehyde (OPA) 99% (Merck), sodium dodecylsulfate (SDS) >99% (Merck), sodium tetraborate (Borax) 99.5−102.5% (Panreac); tetraborate buffer (pH = 9) was used to prepare the casein solutions; tetraborate buffer (pH = 9.7−10.0) was used for the OPA reagent, which was prepared daily according to Goodno et al. (1981): 80 mg of *o*-phthaldialdehyde in 2 mL of ethanol (95%), 50 mL of sodium tetraborate (0.1 M), 5 mL of SDS (20%, w/w), and 0.2 mL of β-mercaptoethanol.

(1) Determination of available lysine in milk samples: 1 mL of SDS 12% was added to 950 μL of water and 50 μL of liquid sample or whey reconstituted at 2%, milk bases at 5%, and powdered infant milk at 13% (65−130 μg of proteins). It was allowed to cool at 4 °C for 12 h and then sonicated for 15 min at 25 °C. A 100 μL aliquot was taken, and 3 mL of OPA was added. The mix was incubated at 25 °C in a shaking bath for 2 min, and the fluorescence (fluorimeter Shimadzu RF-5000) $\lambda_{excitation}$ = 340 nm and $\lambda_{emission}$ = 455 nm was measured.

(2) Determination of possible interferences originated by small peptides (non-proteic components), free amino acids, and amines (analysis of the supernatant obtained after 10% TCA precipitation): TCA was added to 2 mL of liquid sample or reconstituted samples (65−130 μg of proteins), 2 mL of 10% TCA, and then centrifuged at 3000 rpm for 15 min. A 900 μL amount of water and 1 mL of 12% SDS were added to 100 μL of supernatant, and the above-described procedure was applied.

A standard of casein bovine milk was used to prepare a calibration curve. A set of casein standards with lysine contents ranging from 7.65 to 76.5 mg of lysine/mL was prepared using tetraborate buffer (pH = 9) as the solvent. The conversion factor of casein to lysine was calculated considering that the lysine content of κ-casein was negligible and that the α-casein to β-casein ratio was 1:1, with 7% and 5.2% residues of lysine, respectively (Swaisgood, 1982).

Analytical Parameters of the Method. Infant formulas: linearity in the range from 0.1 to 2 mg casein/mL (r = 0.995; A = 1.73; B = 16.4). Detection limit: 4.4 μg/mL and 17.6 mg/100 g in the assay and infant formula, respectively. Precision (relative standard deviation percent): intraday 3.4%, interday 4.7%. Accuracy (recovery assays): (104 ± 4.1)%. Determinations were carried out in quadruplicate.

Free and Total Furfural Compounds. The HPLC method proposed by Albala-Hurtado et al. (1997b) to measure free and total furfural compounds was applied. The furfural group includes four different compounds: hydroxymethylfurfural (HMF), furfural (F), furyl methyl ketone (FMC), and methylfurfural (MF). The analytical parameters of the method are reported in Table 1.

Total Protein Determination. The Kjeldahl method (Kjeltec system 1026 distilling unit) was used to measure total nitrogen (AOAC, 1980). The analyses were carried out in triplicate.

Table 1. Analytical Parameters of Furfural Compounds [Hydroxymethylfurfural (HMF), Furfural (F), Furyl Methyl Ketone (FMC), and Methylfurfural (MF)] Determination

	HMF	F	FMC	MF
interday precision				
μg/100 mL of sample	13.1 ± 1.4	4.4 ± 0.6	12.8 ± 1.2	14.3 ± 2.1
RSD%[a]	11.2	14.5	9.4	14.4
recovery (%)	98.5 ± 3.5	96.3 ± 3.6	96.6 ± 3.7	86.7 ± 3.2
detection limit				
μg/100 mL of sample	1.0	2.0	2.0	2.0
μg/mL of assay	0.005	0.01	0.01	0.01
linearity (μg/mL of assay)		0.01−0.5		

[a] RSD: relative standard deviation.

Statistical Analysis. (*1) Thermal Processing*. A paired data test was applied to evaluate the global effect of thermal treatments on available lysine contents.

(2) Storage. A three-factor ANOVA test was applied to lysine, free and total HMF, and total F contents in order to detect differences between the two types of formulas (adapted and follow up), the storage conditions (20 and 37 °C), and the studied storage period (0, 3, and 6 months). A two-factor (temperature/time) ANOVA test was applied to free furfural (F) contents only in follow-up formulas, because this compound was not detected in adapted formulas.

RESULTS AND DISCUSSION

The total protein contents (%, mean value ± standard deviation of three measurements) of the analyzed samples are raw cow milk, 3.0 ± 0.1; whey, 75.3 ± 3.2; powdered milk base 1, 13.9 ± 0.5; powdered milk base 2, 19.9 ± 0.6; adapted formulas, 12.7 ± 0.2; and follow-up formulas, 18.3 ± 0.7.

The available lysine contents (expressed as g per 100 g of product and 100 g of protein), together with the percentages of available lysine (expressed as g per 100 g of protein), in the infant formulas with respect to the available lysine content of the raw cow milk (100%) used in the manufacturing process are reported in Table 2.

Free and total furfural contents (HMF and F) (expressed as μmol per kg of product and g per 100 g of protein) are reported in Tables 3 and 4, respectively.

The values reported for whey, bases 1 and 2, and adapted and follow-up formulas are the mean values of the analyzed batches.

Decreases in the available lysine contents of the infant formulas with respect to the raw cow milk ranged from 15.7% to 24.8% and from 15.2% to 26.7% for the adapted and the follow-up formulas, respectively. Decreases in the available lysine contents of formulation with respect to raw cow milk ranged from 6.3% to 6.6%. Decreases in the available lysine contents of the infant formulas with respect to the formulation ranged from 8.8% for adapted formulas to 18.6%. This indicates that spray-drying atomization decreased the lysine contents more than pasteurization and sterilization.

To evaluate whether the thermal processing applied during the manufacturing process affects the available lysine content, a paired data test was applied to the following pairs: raw cow milk/adapted formula; raw cow milk/formulation; formulation/adapted formula; and raw cow milk/follow-up formula. The results obtained are reported in Table 5. Statistically significant differences ($p < 0.05$) were found between the available lysine content of adapted and follow-up formulas with respect to raw cow milk, while there was no statistically significant difference ($p < 0.05$) between raw cow milk and the formulation or between the latter and the infant-adapted formula. These findings explain why no differences were found when the effect of each of the treatments was evaluated but did appear in the global evaluation of the thermal treatments.

The application of the three-controlled factor ANOVA test showed statistically significant differences ($p < 0.05$) between the available lysine contents of adapted and follow-up formulas that must be ascribed to the differences in protein (nature and contents) between the two types of formulas. The storage temperatures, 20 and 37 °C, were also responsible for statistically significant differences ($p < 0.05$) during the storage period, while the storage time did not affect the available lysine content. It should be noted that the importance from a nutritional point of view of the differences induced by the storage temperature (mean values of 6.5 and 6.8 g of lysine/100 g of protein for 20 and 37 °C, respectively) is scarce. The significant interaction detected between two of the factors, type of sample and time of storage, means that during the considered period of time, lysine has a different behavior in adapted and follow-up formulas.

As was expected, no furfural compounds were detected in raw cow milk and free and total FMC and MF were not observed in any of the analyzed samples.

HMF and F were detected in samples that have been subjected to the thermal process, since their contents are higher in follow-up formulas than in adapted ones. As expected, total contents were higher than free contents.

After 6 months of storage, an increase of free HMF and F in adapted formulas and of free HMF in follow-up formulas was observed. These furfurals were probably formed from the precursors in the initial sample. It should be noted that in consonance with this, the total furfural contents suffered a small increase during the storage period as compared to the free furfural contents.

Application of the three-way ANOVA test to the results showed that the storage temperature (20 and 37 °C) did not affect HMF (free and total) and F (total) contents. However, statistically significant differences ($p < 0.05$) were found between the HMF (total and free) contents of adapted (0.49 and 1.68 mg/100 g of proteins) and follow-up formulas (2.24 and 4.16 mg/100 g of proteins). The fluctuations found in free and total HMF contents and in total F contents are probably responsible for the statistically significant differences detected. This can be ascribed to the variability in the method at the low furfural levels of the analyzed samples (see Tables 3 and 4).

Application of two-way ANOVA test to free furfural contents of follow-up formulas showed no statistically significant differences between the two storage temperatures (20 and 37 °C), and as previously mentioned, the obtained differences could be ascribed to the variability in the method, given the low contents of free F (0.08−0.11 mg of F/100 g of proteins).

The contents of available lysine of the analyzed infant formulas agree with those reported by other authors for the same type of samples (Angelini et al., 1984; Albalá-Hurtado et al., 1997a, 1998) and are slightly higher than those mentioned by Pompei et al. (1987), even though these authors analyzed samples stored for a longer period of time (between 10 and 12 months).

Table 2. Available Lysine Contents in Ingredients and Infant Formulas (Fresh and Stored)

samples	mean[a]	confidence interval[a]	mean[b]	confidence interval[b]	raw milk[c]	formulation[c]
raw milk	2.03	1.97−2.09	8.36	8.01−8.72	100	
whey	5.36	5.01−5.85	7.11	6.94−7.62	86.6−87.4	
formulation[d]	3.69	3.49−3.97	7.73	7.48−8.17	93.4−93.7	100
base 1[e]	1.38	1.21−1.55	9.93	8.71−11.15		
base 2[f]	1.89	1.83−1.89	9.36	9.07−9.65		

T[g]	month	mean[a]	confidence interval[a]	mean[b]	confidence interval[b]	raw milk[c]	formulation[c]
			Adapted Formulas−Storage				
20	0	0.84	0.79−0.89	6.67	6.29−7.05	75.2−84.3	81.4−91.2
20	3	0.86	0.80−0.92	6.71	6.28−7.14	75.1−85.4	81.2−92.4
20	6	0.9	0.84−0.96	7.07	6.55−7.59	78.3−90.8	84.7−98.2
37	0	0.89	0.84−0.94	6.96	6.56−7.36	78.5−88.0	84.8−95.2
37	3	0.86	0.80−0.92	6.75	6.31−7.19	75.5−86.0	81.6−93.0
37	6	1.02	0.97−1.07	8.02	7.62−8.42	91.1−100.0	98.6−100.0
			Follow-Up Formulas−Storage				
20	0	1.21	1.12−1.30	6.61	6.13−7.09	73.3−84.8	
20	3	1.17	1.12−1.22	6.37	6.10−6.64	73.0−79.4	
20	6	0.98	0.92−1.04	5.33	5.00−5.66	59.8−67.7	
37	0	1.19	1.13−1.25	6.47	6.15−6.79	73.6−81.2	
37	3	1.15	1.11−1.19	6.29	6.08−6.5	72.7−77.8	
37	6	1.04	0.97−1.11	5.69	5.32−6.06	63.6−72.5	

[a] g/100 g of sample. [b] g/100 g of proteins. [c] % available lysine. [d] Formulation: mixture of raw milk and whey at the ratio that gaves a 50/50 casein to whey protein relationship. [e] Base 1: powdered milk base 1 (adapted formulas). [f] Base 2: powdered milk base 2 (follow-up formulas). [g] °C.

Table 3. Free Furfural Contents in Ingredients and Infant Formulas (Fresh and Stored)[a]

	HMF				F			
samples	mean[a]	confidence interval[a]	mean[b]	confidence interval[b]	mean[a]	confidence interval[a]	mean[b]	confidence interval[b]
whey	0.03	0.02−0.04	0.06	0.04−0.07	nd	nd	nd	nd
base 1[c]	0.02	0.02−0.03	0.14	0.12−0.15	nd	nd	nd	nd
base 2[d]					nd	nd	nd	nd

		HMF				F			
T[e]	month	mean[a]	confidence interval[a]	mean[b]	confidence interval[b]	mean[a]	confidence interval[a]	mean[b]	confidence interval[b]
				Adapted Formulas−Storage					
20	0	0.03	0.03−0.04	0.34	0.26−0.42	nd[f]	nd[f]	nd[f]	nd[f]
20	3	0.04	0.03−0.05	0.37	0.29−0.45	nd[f]	nd[f]	nd[f]	nd[f]
20	6	0.05	0.04−0.06	0.51	0.50−0.60	0.008	0.007−0.009	0.06	0.05−0.07
37	0	0.05	0.03−0.07	0.51	0.29−0.73	nd[f]	nd[f]	nd[f]	nd[f]
37	3	0.05	0.04−0.06	0.51	0.40−0.62	nd[f]	nd[f]	nd[f]	nd[f]
37	6	0.08	0.06−0.09	0.77	0.64−0.90	0.01	0.01−0.02	0.1	0.08−0.12
				Follow−up Formulas−Storage					
20	0	0.26	0.21−0.33	1.84	1.44−2.24	0.01	0.007−0.02	0.08	0.04−0.12
20	3	0.30	0.18−0.42	2.04	1.22−2.86	0.01	0.01−0.02	0.08	0.07−0.09
20	6	0.36	0.27−0.45	2.50	1.88−3.12	0.02	0.008−0.03	0.09	0.04−0.14
37	0	0.30	0.21−0.38	2.05	1.47−2.63	0.02	0.02−0.03	0.11	0.08−0.14
37	3	0.34	0.22−0.45	2.31	1.53−3.09	0.01	0.01−0.02	0.08	0.07−0.09
37	6	0.38	0.29−0.47	2.62	2.02−3.22	0.01	0.01−0.02	0.06	0.04−0.08

[a] μmol/kg of sample. [b] mg/100 g of proteins. [c] Base 1: powdered milk base 1 (adapted formulas). [d] Base 2: powdered milk base 2 (follow-up formulas). [e] °C. [f] nd: not detectable.

It should be pointed out that in most of the studies the effect of thermal treatments on the available lysine content is examined in different model systems−sugar/protein (casein or whey) or sugar/amino acid−and using different temperature/time conditions (Labuza and Massaro, 1990; Baisier and Labuza, 1992; Arteaga et al., 1994; Morales et al., 1995; Naranjo et al., 1998). This makes it difficult to compare the results, but in all cases, the decrease in the lysine content follows a first-order kinetics equation and it can be either followed or not followed by a nondecreasing period. The possible deviations from the first-order kinetics responded to differences in the composition of the sample and in the thermal treatment applied.

In our study, statistically significant losses of available lysine (about 20%) in the studied infant formulas with respect to raw cow milk as a consequence of the thermal treatments (pasteurization 72 °C, 15 in./sterilization 100 °C, 22 in./atomization: air input 175−185 °C, air output 90−94 °C) were found. Finot et al. (1981) studied the effect of thermal milk treatment at 115 °C for 10 and 20 min and reported lysine losses of 10% and 12%, respectively. Rossi and Pompei (1991a) indicated decreases in available lysine contents ranging from 18.4% to 26.3% in liquid infant formulas after different heating treatments (pasteurization, UHT, and sterilization). During the storage at 4, 20, and 38 °C for 20 months, irregular changes in the available lysine contents were observed (Rossi and Pompei, 1991b). Anantharaman and Finot (1993) found a decrease in available lysine of about 5−10% in spray-dried infant formulas and of 20−50% in the rolled drying, while Morales et al. (1995) reported similar losses of available lysine after treatment of raw cow milk at 140 °C for 15

Table 4. Total Furfural Contents in Ingredients and Infant Formulas (Fresh and Stored)

	HMF				F			
samples	mean[a]	confidence interval[a]	mean[b]	confidence interval[b]	mean[a]	confidence interval[a]	mean[b]	confidence interval[b]
whey	0.05	0.04−0.06	0.08	0.06−0.09	nd[c]	nd[c]	nd[c]	nd[c]
base 1[d]	0.05	0.04−0.06	0.47	0.39−0.55	0.02	0.01−0.03	0.16	0.08−0.24
base 2[e]					0.02	0.01−0.03	0.11	0.03−0.19
		HMF			F			
T[f]	month	mean[a]	confidence interval[a]	mean[b]	confidence interval[b]	mean[a]	confidence interval[a]	mean[b]	confidence interval[b]
					Adapted Formulas−Storage				
20	0	0.23	0.13−0.32	2.23	1.33−3.13	0.04	0.03−0.04	0.27	0.22−0.32
20	3	0.14	0.11−0.18	1.41	1.08−1.74	0.02	0.02−0.03	0.17	0.13−0.20
20	6	0.14	0.10−0.18	1.37	1.01−1.73	0.02	0.02−0.02	0.12	0.11−0.13
37	0	0.24	0.16−0.32	2.36	1.57−3.15	0.04	0.03−0.04	0.29	0.25−0.34
37	3	0.14	0.11−0.17	1.36	1.07−1.65	0.02	0.02−0.03	0.17	0.14−0.20
37	6	0.17	0.15−0.18	1.64	1.47−1.81	0.03	0.02−0.03	0.19	0.18−0.20
					Follow-Up Formulas−Storage				
20	0	0.60	0.43−0.77	4.12	2.97−5.27	0.05	0.04−0.06	0.25	0.21−0.29
20	3	0.51	0.36−0.66	3.52	2.47−4.57	0.04	0.02−0.05	0.21	0.15−0.31
20	6	0.61	0.45−0.78	4.21	3.08−5.34	0.03	0.03−0.04	0.16	0.13−0.19
37	0	0.60	0.46−0.74	4.11	3.13−5.09	0.05	0.03−0.06	0.25	0.18−0.32
37	3	0.58	0.38−0.78	3.99	2.59−5.39	0.04	0.02−0.05	0.20	0.12−0.28
37	6	0.72	0.57−0.88	4.98	3.93−6.03	0.04	0.03−0.05	0.21	0.18−0.24

[a] μmol/kg of sample. [b] mg/100 g of proteins. [c] nd: not detectable. [d] Base1: powdered milk base 1 (adapted formulas). [e] Base 2: powdered milk base 2 (follow-up formulas). [f] °C.

Table 5. Paired Data of Available Lysine Contents

sample	$\bar{d}$[a]	S_d	n	t_{n-1}	$d/(S_d/\sqrt{n})$	$d/(S_d/\sqrt{n}) \leq t_{n-1}$
raw cow milk−adapted formula	1.81	0.61	3	4.303	$1.81/0.61\sqrt{3}$	5.14 > 4.303
raw cow milk−formulation	0.63	0.51	3	4.303	$0.63/0.51\sqrt{3}$	2.25 < 4.303
formulation−adapted formula	0.92	0.72	3	4.303	$0.92/0.72\sqrt{3}$	2.21 < 4.303
raw cow milk−follow-up formula	1.79	0.41	5	2.776	$1.79/0.41\sqrt{5}$	9.97 > 2.776

[a] d = differences of available lysine of samples. S_d = standard deviation. n = number of batches. t_{n-1} = t student ($p < 0.05$).

min. These authors found that when the same temperature/time conditions were used, in model systems (lactose/casein and lactose/whey proteins) as in cow milk, the losses were greater. These differences could be ascribed to the lack of interactions between whey proteins and casein and to the absence of fat in the model system.

In our study the available lysine content of adapted formulas did not decrease during the storage period while a statistically significant decrease (16%) from the first to the sixth month of storage was found in follow-up formulas, regardless of the storage temperature. Greater losses (40−80%) of available lysine were observed in samples stored at higher temperatures (60−70 °C) for shorter storage times (2−2.5 months) (Hurrell et al., 1983; Ford et al., 1983). However, Albalá-Hurtado et al. (1998) did not report significant decreases in the available lysine contents of follow-up formulas stored at 20/30 and 37 °C. It should be noted that their initial available lysine contents were lower (5.6 ± 0.2 g of lysine/100 g of protein) than ours.

Our adapted and follow-up formulas differ in the type of carbohydrates, lactose, and lactose + maltodextrine, respectively, and in the casein:whey protein ratio, 40:60 and 80:20, respectively. It should be pointed out that the reactivities of maltose and lactose with available lysine were similar in a model system (casein:sugar) heated at 37, 50, and 60 °C (Naranjo et al., 1998). In agreement with these results, we found similar available lysine losses in adapted and follow-up formulas (~20%). Furthermore, the percentages of blocked lysine ranged from 10% to 25% and from 17% to 24.6% in follow-up formulas with lactose or lactose−maltodextrine, respectively (Evangelisti et al., 1993, 1994).

The analyzed follow-up formulas had lower free and total HMF and F contents than those reported by Albalá-Hurtado et al. (1998) for infant formulas of the same type and with the same temperature and storage time. The origin of these differences could be the quality of raw materials and the thermal treatment applied (temperature and time of heating) during the manufacturing process (Hewedy et al., 1994; Morales et al., 1995). Similarly, we found lower total HMF contents than those reported by Pompei et al. (1987) and the free and total HMF contents mentioned by Angelini et al. (1984), even though Pompei et al. (1987) carried out the analysis after 10−12 months of storage and Angelini et al. (1984) used a method (complex formation with TBA) that overestimates the furfural contents. Rossi and Pompei (1991a) reported lower free and total HMF contents than ours in liquid infant formulas subjected to pasteurization, UHT, and sterilization treatments that were stored for 0−5 month periods. This could be ascribed to differences in the formula composition and in the treatments to which the liquid formulas are subjected. Rossi and Pompei (1991b) reported, as we do, irregular changes in the total HMF contents during storage periods of 100−120 days.

CONCLUSIONS

The thermal treatments involved in the manufacturing of infant formulas reduced their available lysine contents, while storage affected only the available lysine contents of follow-up formulas. The effect of the atomization drying process on available lysine content is greater than that of pasteurization and sterilization.

In the analyzed infant formulas, only HMF and F were detected. Their free contents increased at a higher

rate than the total ones, probably at the expense of the bound forms.

Although as a consequence of the manufacturing process Maillard compounds are formed, their contents in the product are very low and do not involve any risk for the consumer. No maximum content limits have been established for these compounds. However, the European Society of Pediatry Gastroenterology and Nutrition (ESPGAN, 1987) recommends for preterm infants that the amount of blocked lysine should be as low as possible.

The adapted and follow-up formulas did not behave in the same way with regard to the Maillard reaction. Although the raw materials and thermal treatments are the same for both types of formulas, they differ in the carbohydrates fraction (lactose in adapted and lactose + maltodextrine in follow-up formulas); the presence or lack of whey serum as a component; and the protein content (higher in follow-up formulas than in adapted ones). Even when lysine losses originated by thermal treatments are similar in both types of formulas, the Maillard reaction continues in follow-up formulas during storage and there is a decrease in the available lysine content and an increase in total HMF. These changes are not observed in adapted formulas. Therefore, the type and contents of proteins and carbohydrates play an important role in the course of the Maillard reaction, regardless of the quality of the raw material, the thermal treatments applied, and the storage temperature (20 and 37 °C).

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