

# Progress of Browning Reactions during Storage of Liquid Infant Milks

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Changes in furfural compounds and reactive lysine were monitored in three commercial liquid infant milks during 9 months of storage at 20, 30, and 37 °C. Samples consisted of two ultrahigh temperature (UHT)-treated milks and one conventionally sterilized milk. Reactive lysine remained constant throughout storage at the three temperatures, whereas, in general an increase in furfural compounds was observed. The heat treatment used in the manufacture of milk is an important factor that influences the levels of furfural compounds although the composition of the milk is also a critical factor. Finally, a study was conducted to find the kinetic equations describing furfural compounds changes and allowing for the prediction of the influence of time and temperature of storage on those changes.

**Keywords:** HMF; F; furfural compounds; reactive lysine; infant milks

## INTRODUCTION

Liquid infant milks are processed by ultrahigh temperature (UHT) treatment or by conventional sterilization process to improve their keeping quality and safety. Nonenzymatic browning reactions are often responsible for quality changes that occur during manufacturing and storage of foods and limit their shelf life. The Maillard reaction leads to changes in flavor, color, and nutritional value, and also, as recently reported in model systems, might yield new products having mutagenic and antimutagenic properties (Cuzzoni et al., 1988; Kong et al., 1989; Yen et al., 1992), antioxidant compounds, and compounds with antimicrobial and anti-allergen activities (Friedman, 1996). The relatively high concentrations of lactose and lysine-rich proteins in infant milk make it especially sensitive to thermally induced Maillard reaction (Hurrell et al., 1990), which is the main way of browning in infant milks. In addition, the presence of casein, vitamin A, and iron also seems to have a strong influence on this reaction (Caric et al., 1984; Park and Hong, 1991). The time and temperature that the product is kept in storage are also critical to the progress of the Maillard reaction (Renner, 1988; Van Mil and Jans, 1991).

Several methods have been used to determine the extent to which the Maillard reaction has progressed. Some of these are related to an early stage of the Maillard reaction, such as Amadori products (by furfural determination) (Evangelisti et al., 1994; Calcagno et al., 1996; Pizzoferrato et al., 1998), or losses of essential amino acids such as lysine (Fink and Kessler, 1988; Resmini et al., 1990; Morales et al., 1995; Albalá-Hurtado et al., 1998). Other parameters are related to advanced Maillard reaction steps, such as hydroxymethylfurfural (HMF) (Caric et al., 1984; Kessler and

Fink, 1986; Morales et al., 1992, 1995; Albalá-Hurtado et al., 1998).

For the same sterilizing effect, the conventional sterilization process of bottled milk causes greater chemical changes in milk than the ultrahigh temperature process (Olano et al., 1989). The level of total HMF has been used as criteria for distinguishing between them (Samuelson and Nielsen, 1970; Fink and Kessler, 1988). In addition, the determination of furfural compounds might be used as an indicator of the length and conditions of storage, as well as the illegal use of reconstituted powdered milk for the production of liquid milks, since powdered milks have higher levels of furfural compounds than liquid milks (Jiménez-Pérez et al., 1992; Baskaran et al., 1994; Albalá-Hurtado et al., 1998). Classical procedures to determine furfural compounds are spectrophotometric thiobarbituric acid (TBA) methods, although it should be pointed out their lack of specificity, due to interferences with furfurals other than HMF and to other carbonyl compounds.

The conversion of the reactive lysine into nutritionally unavailable lysine as a consequence of the Maillard reaction does not have nutritional significance in a healthy adult mixed diet; however, it should be taken into consideration in specific cases, for instance in infant milks (Hurrell et al., 1990). In addition, sensorial changes and potential unhealthy implications from Maillard reaction should also be taken into consideration. Thus, it is necessary to check this kind of products in order to ensure that an adequate intake of nutrients occurs and also to ensure that storage does not significantly modify their quality.

The work reported here aimed to study changes in reactive lysine and furfural compounds during liquid infant milks storage. Particular emphasis was given to comparisons between UHT and conventionally sterilized infant milks, to check if the different heat treatment applied influences in the extent of browning reactions. On the basis of the changes that occurred at three

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**Table 1. Initial Contents of Furfural Compounds and Reactive Lysine in Liquid Infant Milks**

samples	free furfurals ( $\mu\text{mol/L}$ )		total furfurals ( $\mu\text{mol/L}$ )		reactive lysine (g of Lys/100 g of protein)
	HMF	F	HMF	F	
A (conventionally sterilized)	$0.6 \pm 0.1^a$	$0.3 \pm 0.1$	$9.0 \pm 0.4$	$1.4 \pm 0.1$	$5.5 \pm 0.3$
B1 (UHT)	n.d. <sup>b</sup>	n.d.	$3.0 \pm 0.1$	$1.2 \pm 0.1$	$5.4 \pm 0.2$
B2 (UHT)	$1.0 \pm 0.1$	$0.3 \pm 0.1$	$13.2 \pm 0.2$	$2.5 \pm 0.1$	$5.5 \pm 0.2$

<sup>a</sup> Mean  $\pm$  standard deviation ( $n = 2$ ). <sup>b</sup> Not detected.

different temperatures, kinetic calculations would be applied to know the influence of time and temperature of storage on those changes.

## MATERIALS AND METHODS

**Infant Milk Samples.** Three Spanish commercial liquid milks from two different firms (A and B) were studied. Samples A and B1 were follow-on infant milks (from 4–6 to 12 months), and the B2 samples were junior milks (from 1 to 3 years). A samples were conventionally sterilized milks (10–15 min, 115 °C), canned in crown-capped brown glass with a total volume of 0.25–0.50 L. The composition of these samples was, according to the labels, as follows (g/100 mL): proteins (2.3), fat (3.1), lactose (4.7), dextrinmaltose (3.0), starch (0.9), and minerals (0.5). The content of iron was 0.90 mg/100 mL and of vitamin A and vitamin C were 67.5  $\mu\text{g}/100$  mL and 7.5 mg/100 mL, respectively; the energy content was of 72 kcal/100 mL. B1 and B2 samples were UHT treated (150 °C, 3 s) by steam injection and canned in Tetra-Brik containers with a total volume of 0.5 L. The composition of the B1 samples was as follows (g/100 mL): proteins (2.2), fat (2.9), lactose (7.9), and minerals (0.5). The content of iron was 1.2 mg/100 mL, and those of vitamin A and vitamin C were 60.0  $\mu\text{g}/100$  mL and 6.7 mg/100 mL, respectively; the energy content was 67 kcal/100 mL. The composition of the B2 samples was (g/100 mL): proteins (2.2), fat (2.9), lactose (3.2), dextrinmaltose (3.2), sucrose (1.0), honey (0.6), and minerals (0.5). The content of iron was 1.2 mg/100 mL; those of vitamin A and vitamin C were 80.0  $\mu\text{g}/100$  mL and 8.0 mg/100 mL, respectively; the energy content was 67 kcal/100 mL. All samples studied were ready to feed and had an assigned shelf life of 6 months.

Thirty-two samples of each milk belonging to the same production lot were split into three batches and stored in their original packaging at  $20 \pm 1$  °C in an oven, FR30 Frimatic (Afora, Spain); at  $30 \pm 1$  °C in a climatic test chamber, HC0020 Heraeus Vötschs (Frommern, Germany); and at  $37 \pm 1$  °C in an oven, CC/500 Radiber (Afora, Spain). Samples were taken in duplicate at zero time (just after manufacturing) and after 1, 3, 5, 7, and 9 months of storage. In addition, to check the suitability of kinetic models, additional samples ( $n = 36$ ) were taken after 10 and 12 months of storage.

**Analytical Methods.** Furfural compounds were determined by using a high-performance liquid chromatography (HPLC) technique as previously described Albalá-Hurtado et al. (1997a). The method allows the determination of free and total furfural compounds (free furfurals plus the potential furfurals derived from other browning intermediates) by using the optimal absorption wavelength for each furfural compound. Total and free hydroxymethylfurfural (HMF) and furfural (F) and also furfurylmethyl ketone (FMC) and methylfurfural (MF) can be quantified by using this method.

Reactive lysine was determined by HPLC, after derivatization of samples with FDNB (1-fluoro-2,4-dinitrobenzene), followed by acid hydrolysis for 2 h and 30 min with 8 M HCl in a Tekator digestion flask connected to a vacuum system according to the method of Albalá-Hurtado et al. (1997b).

**Visible Color Change.** A panel of 10 food science postgraduate students was trained to evaluate the visible color change. A dichotomous criterion was chosen: change or no change in comparison with a control sample from the zero time point, stored at  $-20$  °C in the dark. A positive response in more than half of the 10 members of the panel was considered as a change in color.

**Statistical Analysis.** All statistical tests were performed by the Statistical Software Package SPSS (SPSS, Inc., Chicago, IL). Pearson's regression, *t*-Student's test, and the nonparametric Friedman test were applied.

## RESULTS AND DISCUSSION

Initial contents of furfural compounds and reactive lysine, just after manufacturing (time = 0) are shown in Table 1. The reactive lysine content was similar in the three liquid infant milks studied. However, there were clear differences in the furfural compounds. Whereas free HMF and free F were found in the A and B2 samples, these compounds were not detected in the B1 samples. Likewise, levels of total HMF and total F were higher in the A and B2 samples than in B1 samples.

The ratio of total HMF/free HMF was very similar in the A (15.0) and B2 (13.2) samples and was higher than in B1 (3.0). In contrast, the ratio of total F/free F in the B2 samples was almost two times (8.3) that obtained in the A samples (4.6). Thus, taking into account only the HMF values, there seems to be a higher presence of browning precursors in the A and B2 samples than in the B1 samples. However, taking the F values, the presence of precursors would be higher in the B2 than in the A samples. This lack of coincidence indicates that there is not correlation between the formation of HMF and F from their precursors. This finding was unexpected because, in general, it is considered that the formation of both furfural compounds (HMF and F) is affected by the same factors.

The temperature/time treatment is more severe in conventional sterilization than in UHT processes (Fink and Kessler, 1988; Olano et al., 1989). The higher levels of furfural compounds found in the A samples (conventionally sterilized) than in the B1 samples (UHT) are consistent with previous reported data (Samuelson and Nielsen, 1970; Kessler and Fink, 1986; Fink and Kessler, 1988). However, the levels of furfural compounds were higher in the UHT steam heated B2 samples than in the A samples, showing that the extent of heat treatment is not the only factor involved in furfural compounds formation. Milk composition is also known as a factor that influences the Maillard reaction. B2 samples, which had the highest furfural levels, contained the highest values of both iron and vitamin A. Our results agree with those of Park and Hong (1991), who reported that the enrichment of infant milks with iron and vitamin A resulted in higher furfural compounds than nonenriched infant milks. Similar results have been reported by Caric et al. (1984) for enriched cow's milk. The increase in furfural compounds in samples enriched with iron cannot be related here with interferences from lipid oxidation products because any TBA colorimetric method was used.

In addition, in B2 samples, lactose was partially substituted by honey, which is known that already has

**Table 2. Changes of Free and Total Furfural Compounds during Storage of a Conventionally Sterilized Liquid Infant Milk (A Samples)**

time (months)	free furfurals ( $\mu\text{mol/L}$ )						total furfurals ( $\mu\text{mol/L}$ )					
	20 °C		30 °C		37 °C		20 °C		30 °C		37 °C	
	HMF	F	HMF	F	HMF	F	HMF	F	HMF	F	HMF	F
0	0.6 ± 0.1 <sup>a</sup>	0.3 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	9.0 ± 0.4	1.4 ± 0.1	9.0 ± 0.4	1.4 ± 0.1	9.0 ± 0.4	1.4 ± 0.1
1	0.7 ± 0.1	0.2 ± 0.1	1.1 ± 0.1	0.3 ± 0.1	0.8 ± 0.1	0.3 ± 0.1	7.5 ± 0.3	1.6 ± 0.1	9.6 ± 0.1	1.5 ± 0.1	10.8 ± 0.3	1.3 ± 0.1
3	0.9 ± 0.1	0.2 ± 0.1	1.1 ± 0.1	0.3 ± 0.1	0.9 ± 0.1	0.3 ± 0.1	7.6 ± 0.3	1.6 ± 0.1	9.6 ± 0.1	1.8 ± 0.1	10.9 ± 0.2	1.3 ± 0.1
5	0.8 ± 0.1	0.3 ± 0.1	1.2 ± 0.1	0.4 ± 0.1	1.0 ± 0.1	0.4 ± 0.1	7.7 ± 0.3	2.1 ± 0.1	9.8 ± 0.1	1.8 ± 0.1	12.0 ± 0.2	1.7 ± 0.1
7	0.9 ± 0.1	0.4 ± 0.1	1.3 ± 0.1	0.4 ± 0.1	1.5 ± 0.1	0.5 ± 0.1	7.9 ± 0.3	2.1 ± 0.1	9.9 ± 0.1	2.0 ± 0.1	12.1 ± 0.4	1.9 ± 0.1
9	1.0 ± 0.1	0.5 ± 0.1	1.4 ± 0.1	0.5 ± 0.1	1.6 ± 0.1	0.6 ± 0.1	8.0 ± 0.3	2.2 ± 0.1	9.9 ± 0.2	2.3 ± 0.1	12.2 ± 0.4	2.1 ± 0.1

<sup>a</sup> Mean ± standard deviation ( $n = 2$ ).

**Table 3. Changes of Free and Total Furfural Compounds during Storage of a UHT Liquid Infant Milk (B1 Samples)**

time (months)	free furfurals ( $\mu\text{mol/L}$ )						total furfurals ( $\mu\text{mol/L}$ )					
	20 °C		30 °C		37 °C		20 °C		30 °C		37 °C	
	HMF	F	HMF	F	HMF	F	HMF	F	HMF	F	HMF	F
0	n. d. <sup>a</sup>	n. d.	n. d.	n. d.	n. d.	n. d.	3.0 ± 0.1 <sup>b</sup>	1.2 ± 0.1	3.0 ± 0.1	1.2 ± 0.1	3.0 ± 0.1	1.2 ± 0.1
1	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	3.0 ± 0.1	0.6 ± 0.1	3.3 ± 0.1	1.2 ± 0.1	3.7 ± 0.1	1.2 ± 0.1
3	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	3.2 ± 0.1	0.8 ± 0.1	3.4 ± 0.1	1.5 ± 0.1	4.3 ± 0.1	1.4 ± 0.1
5	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	3.4 ± 0.1	0.9 ± 0.1	4.0 ± 0.1	1.7 ± 0.1	5.9 ± 0.1	1.8 ± 0.1
7	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	3.5 ± 0.1	0.9 ± 0.1	4.3 ± 0.1	2.3 ± 0.1	8.2 ± 0.2	2.9 ± 0.1
9	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	3.6 ± 0.1	0.9 ± 0.1	4.7 ± 0.1	2.4 ± 0.1	8.8 ± 0.2	3.2 ± 0.1

<sup>a</sup> Not detected. <sup>b</sup> Mean ± standard deviation ( $n = 2$ ).

**Table 4. Changes of Free and Total Furfural Compounds during Storage of a UHT Liquid Infant Milk (B2 Samples)**

time (months)	free furfurals ( $\mu\text{mol/L}$ )						total furfurals ( $\mu\text{mol/L}$ )					
	20 °C		30 °C		37 °C		20 °C		30 °C		37 °C	
	HMF	F	HMF	F	HMF	F	HMF	F	HMF	F	HMF	F
0	1.0 ± 0.1 <sup>a</sup>	0.3 ± 0.1	1.0 ± 0.1	0.3 ± 0.1	1.0 ± 0.1	0.3 ± 0.1	13.2 ± 0.2	2.5 ± 0.1	13.2 ± 0.2	2.5 ± 0.1	13.2 ± 0.2	2.5 ± 0.1
1	1.1 ± 0.1	0.3 ± 0.1	1.3 ± 0.1	0.5 ± 0.1	1.5 ± 0.1	0.7 ± 0.1	12.5 ± 0.1	2.3 ± 0.1	14.1 ± 0.2	2.6 ± 0.1	15.8 ± 0.3	2.9 ± 0.1
3	1.2 ± 0.1	0.3 ± 0.1	1.5 ± 0.1	0.6 ± 0.1	1.7 ± 0.1	0.8 ± 0.1	13.2 ± 0.2	2.3 ± 0.1	14.7 ± 0.2	2.7 ± 0.1	16.0 ± 0.3	3.0 ± 0.1
5	1.1 ± 0.1	0.4 ± 0.1	1.4 ± 0.1	0.7 ± 0.1	1.9 ± 0.1	0.9 ± 0.1	13.3 ± 0.3	2.5 ± 0.1	15.0 ± 0.2	2.8 ± 0.1	18.7 ± 0.2	3.1 ± 0.1
7	1.1 ± 0.1	0.5 ± 0.1	1.5 ± 0.1	0.8 ± 0.1	2.1 ± 0.1	1.1 ± 0.1	15.2 ± 0.2	2.5 ± 0.1	15.0 ± 0.2	2.9 ± 0.1	20.1 ± 0.2	3.3 ± 0.1
9	1.2 ± 0.1	0.5 ± 0.1	1.5 ± 0.1	0.9 ± 0.1	2.2 ± 0.1	1.2 ± 0.1	15.3 ± 0.2	2.6 ± 0.1	15.3 ± 0.3	3.0 ± 0.1	20.5 ± 0.2	3.4 ± 0.1

<sup>a</sup> Mean ± standard deviation ( $n = 2$ ).

furfural compounds. Moreover, the adding of honey (glucose + fructose) seems to increase the Maillard reaction rate more than the addition of lactose. This agrees with the results of Evangelisti et al. (1994), who found higher increase in the Maillard reaction progress after adding glucose than lactose. No data were found about the influence of fructose in the progress of Maillard reaction in infant milks. Thus, differences in furfural compounds between B1 and B2 samples seem to be more related to milk composition than thermal treatment. Despite higher casein/whey ratio has been related to higher furfurals compounds (Caric et al., 1984), here no correlation was found between them. Thus, B1 and B2 samples with the same casein/whey ratio (82:18) showed much different furfural contents. In addition, A samples with lower casein/whey ratio (40:60) had more furfurals than B1 samples.

Tables 2–4 show changes in free and total HMF and F in samples during storage. The levels of furfural compounds and the length of storage were statistically correlated ( $p < 0.05$ ), except free F in B1 samples, which were not detected at any time and temperature (Table 3), total F in B1 and B2 samples at 20 °C, and total HMF in A samples also at 20 °C. Likewise, excluding the total F and the free HMF in the A samples, a statistically significant correlation between temperature of storage and furfural compounds was found ( $p < 0.05$ ). Thus, furfural compound production was greater when samples were stored at 37 °C than at 30 and 20 °C.

Increases in furfural compounds were previously reported in powdered infant milks under different conditions of time/temperature storage (Baskaran et al., 1994; Nagendra et al., 1995). Likewise, Caric et al. (1984), Fink and Kessler (1986), and Jiménez-Pérez et al. (1992) also found HMF increases in UHT cow milks. With the exception of our previous work (Albalá-Hurtado et al., 1998), no data were found about changes of furfural compounds during liquid infant milks storage.

Many authors reported a reduction in reactive lysine in milk proteins during storage of milk under various time/temperature conditions in both liquid and cow milks and powdered infant milks (Kessler and Fink, 1986; King et al., 1991; Van Mil and Jans, 1991; Baskaran et al., 1994; Evangelisti et al., 1994; Nagendra et al., 1995; Calcagno et al., 1996). However, the reactive lysine content remained constant during storage of liquid infant milks at the three temperatures studied here. No previous data have been found about reactive lysine behavior during storage of liquid infant milks.

In this study, the increase in furfural compounds was not accompanied by changes in reactive lysine. The lack of a relationship between reactive lysine and furfural compounds during storage come only from the precursors formed by the thermal treatments in manufacturing. Therefore, the blocking of lysine would be done only during thermal treatment and not during storage. In



**Table 5. Arrhenius Parameters for Liquid Infant Milks (A and B2 Samples)**

	A samples				B2 samples			
	$\ln k_0^a$	$E_a$ (kJ/mol) <sup>b</sup>	95% confidence interval of slope	$r^2$ <sup>c</sup>	$\ln k_0$	$E_a$ (kJ/mol)	95% confidence interval of slope	$r^2$
free HMF	15.93	46.83	-6985.5 to -4279.3	0.9996	33.38	91.72	-15592.2 to -6471.3	0.9989
total HMF					13.31	36.02	-75927.9 to -67262.6	0.3715
free F	-0.84	6.91	-28616.7 to 26953.9	0.1263	17.68	51.84	-16112.8 to 3640.3	0.9847
total F	-2.63	0.59	-1104.5 to 1247.3	0.3731	20.63	59.50	-13685.9 to -627.9	0.9948
free (HMF + F)	11.10	33.75	-14148.4 to 6030.4	0.9631	25.60	70.15	-9639.4 to -7235.1	0.9999
total (HMF + F)	28.10	75.09	-12320.6 to -10471.3	0.9998	14.34	38.26	-65289.2 to -56083.8	0.4815

<sup>a</sup> Collision factor. <sup>b</sup> Activation energy. <sup>c</sup> Coefficient of determination from the relationship between the reciprocal of absolute temperature and the natural logarithms at each temperature for each parameter.

**Table 6. Comparison between Predictive and Experimental Values ( $\mu\text{mol/L}$ ) after 10 and 12 Months of Storage**

	kinetic equations <sup>a</sup>							
	A samples		free HMF		B2 samples		total F	
	$C_f = 0.69 + 8.27 \cdot 10^6 e^{-5632.39/T} t$		$C_f = 1.15 + 3.13 \cdot 10^{14} e^{-11031.76/T} t$		$C_f = 1.53 + 1.30 \cdot 10^{11} e^{-8437.23/T} t$		$C_f = 2.52 + 9.11 \cdot 10^8 e^{-7156.56/T} t$	
	pred/obsd	% error <sup>b</sup>	pred/obsd	% error	pred/obsd	% error	pred/obsd	% error
	10 Months							
20 °C	1.00/0.99	1.00	1.29/1.23	4.87	1.93/1.74	10.92	2.74/2.46	11.38
30 °C	1.39/1.50	7.30	1.63/1.70	4.11	2.58/2.46	4.88	3.02/3.09	2.26
37 °C	1.75/1.72	1.74	2.25/2.30	2.17	3.50/3.60	2.78	3.37/3.32	1.50
	12 Months							
20 °C	1.13/1.11	1.80	1.32/1.30	1.54	2.02/2.03	0.49	2.79/2.54	9.84
30 °C	1.53/1.60	4.37	1.73/1.80	3.88	2.78/2.73	1.83	3.12/3.20	2.50
37 °C	1.96/1.83	7.10	2.46/2.34	5.13	3.89/3.67	5.99	3.54/3.43	3.21

<sup>a</sup> Kinetic equations were obtained according to Fink and Kessler (1988).  $T$  is expressed in Kelvin and  $t$  in months. <sup>b</sup>  $| \text{predicted} - \text{observed} | / \text{observed} \times 100$ .

addition, the fact that amino acids other than lysine could also be involved in Maillard reaction might contribute to the increase in furfural compounds without a decreasing in reactive lysine. In this way, Ashoor and Zent (1984) reported the reaction between lactose and a wide variety of natural amino acids in model systems. Finally, chemical reactions other than the Maillard reaction, such as the heat degradation of lactose and lactulose, might also explain the formation of furfural compounds.

Organoleptical changes as a consequence of the browning reactions are undesirable in dairy products. At the start of this study (time = 0), the A samples (conventionally sterilized) had a yellowish color in comparison with the B1 and B2 samples (UHT), which had the typical white color. Throughout storage, no appreciable color changes were found in any of the samples stored at 20 °C, and neither in the B1 and B2 samples stored at 30 °C and 37 °C. However, the A samples had a more intense yellow-brown color after 5 months at 30 and 37 °C, being more intense at 37 °C. As mentioned above, a higher increase in furfural compounds was observed in the B2 samples than in the A samples, which underwent a visible color change. Thus, the visible color change is not enough to follow the extent of the Maillard reaction during storage.

The changes in furfural compounds in the A and B2 samples during storage followed an overall zero-order reaction kinetic. The B1 samples were not included in the kinetic study, since free HMF and free F were not found at any time and temperature of storage. The Arrhenius parameters for A and B2 liquid infant milks are in Table 5. Taking into account the 95% confidence interval of slopes, thermodynamic data from free HMF, free (HMF + F), and total F were statistically significant in B2 samples. However, in A samples only the relationship between temperature and free HMF was signifi-

cant. Therefore, to compare A and B2 samples, only the kinetic parameters obtained from free HMF were considered.

By using the data from Table 5, the rate constants at 25 °C ( $k_{25}$ ) were calculated, being 0.051 and 0.026  $\mu\text{mol L}^{-1} \text{month}^{-1}$  for A and B2 samples, respectively. Accordingly the speed of formation of the free HMF was two times higher in the A (conventionally sterilized) than in the B2 (UHT) samples. Concerning the activation energy ( $E_a$ ), differences were also found between A and B2 infant milks studied. Thus, for free HMF, the  $E_a$  values were 46.83 and 91.72 kJ/mol, respectively. In other words, the  $E_a$  values were two times more affected by a change in temperature in the B2 than in the A samples. Therefore, Arrhenius parameter values explain the differences observed in the increase of furfural compounds between A and B2 samples.

By combining of the general zero-order and the Arrhenius equations, we can obtain different equations (Table 6), which allow to estimate the content of furfural compounds after a certain period of storage ( $t$ , months) at one set temperature ( $T$ , K). The predictive furfural values calculated with those equations for samples after 10 and 12 months of storage were very close to the real ones. In all cases the percentage of error was less than 12. However, more data are needed to check if the free HMF is the more suitable furfural parameter to make this kind of predictions. In addition, the suitability of these calculations should be checked in other infant milks due to the influence of milk composition in the browning reactions rate.

As a conclusion, it should be pointed out that furfural compounds were better indicators of the extent of the browning reaction than reactive lysine or changes in color. In general, the storage of infant milks increases the furfural compounds but no changes were observed in reactive lysine. Differences in furfural compounds

accumulation during storage seem to be related not only to the heat treatment applied in samples manufacturing but also to the particular composition of some of these products. In addition, changes in furfural compounds seems to be adjusted to a kinetic model, which would allow to predict the influence of time and temperature on their levels during storage.

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#### LITERATURE CITED

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