

Changes in Furfural Compounds during Storage of Infant Milks

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The progress of the Maillard reaction in two infant milks (liquid and powdered forms of the same commercial brand) was monitored for 9 months of storage at 20, 30, and 37 °C. An increase in furfural compounds [hydroxymethylfurfural (HMF) and furfural (F)] was observed while no changes in available lysine occurred during storage at any temperature. In reconstituted powdered infant milk, at the three storage temperatures, the contents of free and total HMF ranged from 10.3 to 23.5 $\mu\text{mol/L}$ and from 15.0 to 34.7 $\mu\text{mol/L}$, respectively. The content of free and total F ranged from 1.1 to 5.3 $\mu\text{mol/L}$ and from 1.8 to 6.8 $\mu\text{mol/L}$, respectively. Free and total HMF content in liquid infant milk ranged from 0.6 to 1.6 $\mu\text{mol/L}$ and from 9.0 to 12.2 $\mu\text{mol/L}$, respectively. The content of free and total F ranged from 0.3 to 0.6 $\mu\text{mol/L}$ and from 1.4 to 2.3 $\mu\text{mol/L}$, respectively. Although free and total HMF and F were always higher in powdered milk than in liquid infant milk, in the latter the ratio total HMF/free HMF was more than 10 times that in powdered infant milk. On the basis of the increase in furfural compounds and by using the sum of free HMF and free F, an equation has been derived by which the shelf life of such products can be predicted.

Keywords: HMF; F; furfural compounds; available lysine; infant milks

INTRODUCTION

Spanish infant milks, in line with other European milks, follow the nutritional recommendations established by the Committee of Nutrition of the ESPGAN (European Society for Pediatric Gastroenterology and Nutrition). Three types of infant milks are available in Spain: "starting infant milks", milk for infants up to 4 or 6 months old; "follow-up infant milks", which constitute the main liquid feed as the infant's diet progressively diversifies; and "junior milks" or "third age milks" used in feeding infants after the first year of life.

Infant milks are heat-treated to guarantee their safety and to prolong their storage life. These objectives can be achieved by the application of sterilization processes (liquid infant milks) or by the reduction of the water content (powdered infant milks). However, in both cases chemical reactions of nonenzymatic origin can still occur during storage, which can change both nutritional and sensorial properties (Renner, 1988) and, therefore, reduce their shelf life.

The Maillard reaction can occur during the manufacture and storage of infant milks and involves the carbonyl groups from reducing sugars and the amino compounds mainly from amino acids. The intensity of the changes produced by the Maillard reaction depends on the severity of heat treatment, although it also seems to be correlated with the concentration of lactose (Evangelisti et al., 1994), casein, vitamin A, and iron (Caric et al., 1984; Park and Hong, 1991). The time the product is kept in storage and the temperature during storage are also critical to the progress of the Maillard reaction (Hurrell and Finot, 1983; Van Mil and Jans,

1991), while water activity (a_w) would also seem to play an important role in its promotion.

Browning reactions greatly modify food properties and are responsible for various changes in flavor, aroma, color, nutritional value, stability, and shelf life (Cuzzoni et al., 1988). Certain low molecular weight products of the Maillard reaction such as aldehydes and ketones give pleasant odors, but at high levels these can become undesirable. The final products of the Maillard reaction are high molecular weight compounds, such as polymers and complex brown pigments (premelanoidins and melanoidins), which produce undesirable color changes in milks (Renner, 1988).

Both mutagenic and antimutagenic properties have been described for compounds formed during the Maillard reaction, though always in model systems. Cuzzoni et al. (1988) reported the genotoxic activity of various intermediates, whereas Kong et al. (1989) and Yen et al. (1992) studied the antimutagenic properties of certain products of the Maillard reaction, mainly attributed to their antioxidative activity (Kim et al., 1986; Hayase et al., 1989; Yen et al., 1992).

Milk is relatively rich in the essential amino acid lysine (Mittal et al., 1989), and the ϵ -amino group of this amino acid is the most sensitive group to be involved in the Maillard reaction leading to lactulose-lysine, which is the first relatively stable intermediate of the browning reaction in milk products (Möller, 1977). This reaction, which yields nutritionally unavailable lysine, has been employed by some authors to monitor the Maillard reaction in powdered infant milks by means of the furosine method (Calcagno et al., 1996). Therefore, the Maillard reaction is undesirable in infant milks as these products might be the only source of lysine for babies (Erbersdobler and Hupe, 1991). In addition, the Maillard reaction, along with the denaturation of protein and the crystallization of lactose, has a pronounced

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Table 1. Initial Contents of Furfural Compounds, Available Lysine, and pH Values in Powdered and Liquid Infant Milk Formulas

| samples | free furfural | | total furfural | | available lysine (g of Lys/100 g of prot) | pH values |
|----------|-----------------------------|-------------------------|---------------------------|-------------------------|--|----------------|
| | HMF ($\mu\text{mol/L}$) | F ($\mu\text{mol/L}$) | HMF ($\mu\text{mol/L}$) | F ($\mu\text{mol/L}$) | | |
| powdered | 10.3 \pm 0.4 ^a | 1.1 \pm 0.1 | 15.0 \pm 0.3 | 1.8 \pm 0.1 | 5.6 \pm 0.2 | 6.65 \pm 0.1 |
| liquid | 0.6 \pm 0.1 | 0.3 \pm 0.1 | 9.0 \pm 0.4 | 1.4 \pm 0.1 | 5.5 \pm 0.3 | 6.63 \pm 0.1 |

^a Mean \pm standard deviation ($n = 2$).

effect on the losses of solubility in powdered milk (Van Mil and Jans, 1991).

Hydroxymethylfurfural (HMF) is formed during the Maillard reaction and has been used in various foods as an indicator of the severity of heat treatment and also of the length and conditions of storage (Guerra-Hernández et al., 1992; Villalón-Mir et al., 1992; García-Villanova et al., 1993; Lo Coco et al., 1994).

In contrast with other foods, no more data are available about the usual contents of furfural compounds in infant milks, with the exception of the total HMF values reported by Park and Hong (1991) in Korean commercial infant milks. 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.

Infant milks are usually consumed immediately after manufacture, but no previous data are available about the progress of browning reactions during infant milk storage. Therefore, the aim of this work is to study the influence of the length and temperature of storage on the extent of the Maillard reaction in infant milks by monitoring changes in HMF and F (furfural) content and in the availability of lysine. Special emphasis will be given to comparisons between the changes in solid and liquid infant milks. Kinetic equations are established to predict the influence of storage conditions on these parameters. Moreover, the water activity (a_w) in powdered infant milk is measured in order to determine if there is any relationship between the Maillard reaction and a_w values.

MATERIALS AND METHODS

Sampling. Two Spanish commercial milks of the same commercial brand were studied. All samples were follow-up infant milks. Powdered samples were obtained by spray-drying, and liquid samples were sterilized. The composition of powdered samples was (g/100 g) as follows: proteins (15.5), fat (21.0), lactose (31.3), dextrinmaltose (20.0), starch (6.0), and minerals (3.2). The composition of liquid samples was (g/100 mL) as follows: proteins (2.3), fat (3.1), lactose (4.7), dextrinmaltose (3.0), starch (0.9), and minerals (0.5).

Thirty-two samples of each kind of milk belonging to the same production lot were split into three batches and stored in their original package at 20 ± 1 °C in an oven, FR30 Frimatic (Afora, Spain); at 30 ± 1 °C in a climatic test chamber, HC0020 Heraeus Vötschs (Frommern, Germany); and at 37 ± 1 °C in an oven, CC/500 Radiber (Afora, Spain). Samples for analysis were taken in duplicate at zero time (just after manufacture) and after 1, 3, 5, 7, and 9 months. In addition, samples corresponding to 10 and 12 months of storage were also analyzed to check if the predicted values from the kinetic equation are close to the experimental data.

Analytical Methods. *Free and Total Furfural Compounds.* Determinations were carried out using an HPLC technique according to the procedures previously described by us (Albalá-Hurtado et al., 1997a), which allow the determination of free and total HMF and F compounds in a single run. Each furfural was determined at its optimal absorption wavelength. For total furfurals, samples were treated with oxalic acid

(prepared fresh daily) and heated in a water bath at 100 °C for exactly 25 min. A TCA (trichloroacetic acid) solution was added to clarify the samples. For free furfurals, sample preparation was the same as for total furfurals, but the heating at 100 °C was omitted. Powdered samples were reconstituted at 15% (w/v).

Available lysine was determined by using an HPLC procedure with UV detection (Albalá-Hurtado et al., 1997b). Samples were derivatized with FDNB (1-fluoro-2,4-dinitrobenzene) followed by acid hydrolysis. The time of hydrolysis was drastically reduced to 2 h and 30 min by use of 8 M HCl in a Tekator digestion flask connected to a vacuum system and immersed in an oil bath, which allowed a constant temperature of 160 °C.

Water content (%) was measured in powdered infant milks according to Method 26 of the International Dairy Federation (1964).

Water activity (a_w) was determined in powdered infant milks at 25 °C in a Novasina RTD-33 TH-2 (Defensorage, Switzerland).

pH. The pH was measured at 25 °C with a micropH 2001 pHmeter (Crison, Italy). In liquid samples, the pH was measured directly, whereas in powdered samples a reconstitution (15%) was needed.

Visible Color Change. The visible color change was evaluated by a panel of 10 food science postgraduate students. The criterion was dichotomous: change or no change in comparison with a control sample from sample zero that had been stored at -20 °C in the dark. The criterion used to assign an actual change in color samples was the positive response in more than one-half of the total number of panel members.

Statistical Analysis. Nonparametric tests were applied when data were not normally distributed. Pearson's regression, Friedman, and *t*-Student's tests were performed using the Statistical Software Package for Windows 6.0.1 (SPSS Inc., Chicago, IL, 1994).

RESULTS AND DISCUSSION

Numerous furfural compounds can be formed in processed food during thermal processing or storage at inappropriate temperatures (Lo Coco et al., 1994). Although the analytical method used allows the determination of HMF, F, FMC (furyl methyl ketone), and MF (methylfurfural), we did not detect FMC or MF in any of the analyzed samples.

Table 1 shows the initial contents of furfural compounds, available lysine, and pH values in powdered and in liquid infant milks just after manufacturing (time = 0). Available lysine contents and pH values were very similar in both milks. Obviously, in both milks, free furfural levels were lower than total furfural levels (free furfural compounds plus the potential furfural compounds derived from other browning intermediates). Free and total F were always lower than 2 $\mu\text{mol/L}$ in both samples, but they were lower in liquid than in reconstituted powdered infant milks. Free and total HMF levels were higher than those of F, and in addition, they were much higher in powdered than in liquid infant milks. Caric et al. (1984) also observed higher levels of furfural compounds in powdered milks than in liquid milks. In general, the differences in furfural

Table 2. Contents of Free and Total Furfural Compounds in Reconstituted (15%) Powdered Infant Milk

| time (months) | free furfurals | | | | | | total furfurals | | | | | |
|------------------|------------------------------|----------------------------|------------------------------|----------------------------|------------------------------|----------------------------|------------------------------|----------------------------|------------------------------|----------------------------|------------------------------|----------------------------|
| | 20 °C | | 30 °C | | 37 °C | | 20 °C | | 30 °C | | 37 °C | |
| | HMF ($\mu\text{mol/L}$) | F ($\mu\text{mol/l}$) | HMF ($\mu\text{mol/L}$) | F ($\mu\text{mol/L}$) | HMF ($\mu\text{mol/L}$) | F ($\mu\text{mol/L}$) | HMF ($\mu\text{mol/L}$) | F ($\mu\text{mol/L}$) | HMF ($\mu\text{mol/L}$) | F ($\mu\text{mol/L}$) | HMF ($\mu\text{mol/L}$) | F ($\mu\text{mol/L}$) |
| 0 | 10.3 \pm 0.4 ^a | 1.1 \pm 0.1 | 10.3 \pm 0.4 | 1.1 \pm 0.1 | 10.3 \pm 0.4 | 1.1 \pm 0.1 | 15.0 \pm 0.3 | 1.8 \pm 0.1 | 15.0 \pm 0.3 | 1.8 \pm 0.1 | 15.0 \pm 0.3 | 1.8 \pm 0.1 |
| 1 | 11.4 \pm 0.5 | 1.0 \pm 0.1 | 12.6 \pm 0.5 | 1.8 \pm 0.1 | 13.5 \pm 0.5 | 2.3 \pm 0.1 | 19.2 \pm 1.3 | 1.5 \pm 0.1 | 20.0 \pm 1.0 | 2.9 \pm 0.1 | 22.5 \pm 0.5 | 3.8 \pm 0.2 |
| 3 | 12.4 \pm 0.5 | 1.4 \pm 0.1 | 15.1 \pm 0.6 | 2.7 \pm 0.1 | 15.3 \pm 0.5 | 2.9 \pm 0.1 | 20.1 \pm 1.0 | 2.1 \pm 0.1 | 25.1 \pm 0.9 | 3.5 \pm 0.1 | 29.7 \pm 0.7 | 3.9 \pm 0.1 |
| 5 | 13.0 \pm 0.3 | 1.7 \pm 0.1 | 16.3 \pm 0.6 | 3.0 \pm 0.1 | 17.2 \pm 0.6 | 3.5 \pm 0.1 | 22.0 \pm 1.1 | 2.3 \pm 0.1 | 27.2 \pm 0.8 | 3.5 \pm 0.1 | 30.4 \pm 0.6 | 4.6 \pm 0.2 |
| 7 | 14.3 \pm 0.4 | 2.2 \pm 0.1 | 19.3 \pm 0.4 | 4.7 \pm 0.2 | 22.9 \pm 0.8 | 4.9 \pm 0.1 | 27.3 \pm 1.0 | 3.9 \pm 0.1 | 31.8 \pm 0.9 | 4.0 \pm 0.1 | 34.4 \pm 0.5 | 6.2 \pm 0.2 |
| 9 | 16.8 \pm 0.6 | 2.5 \pm 0.1 | 19.8 \pm 0.6 | 4.5 \pm 0.2 | 23.5 \pm 0.6 | 5.3 \pm 0.2 | 29.8 \pm 1.2 | 4.0 \pm 0.1 | 33.9 \pm 1.2 | 5.3 \pm 0.3 | 34.7 \pm 1.0 | 6.8 \pm 0.3 |

^a Mean \pm standard deviation ($n = 2$).

Table 3. Contents of Free and Total Furfurals in Liquid Infant Milk

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

^a Mean \pm standard deviation ($n = 2$).

compounds between powdered and liquid infant milks could be related to a more severe temperature/time treatment that powdered milk undergoes in its manufacture, which includes a drying process that leads to an increase in the Maillard reaction products.

In the initial samples, the low level of free HMF found in the liquid milk (0.6 $\mu\text{mol/L}$) in comparison with that in powdered (10.3 $\mu\text{mol/L}$) would be indicative of minor developments in the Maillard reaction during the manufacture of liquid infant milks. However, the ratio total HMF/free HMF was about 10 times higher in liquid milks than in powdered infant milks. This finding might indicate a greater presence of the Amadori products in liquid infant milks than in solid infant milks. Thus, by considering only the total HMF values, we cannot predict if the greater presence of Amadori products in liquid than in solid infant milks might yield a higher intensity in the Maillard reaction during storage.

A significant increase was observed in all furfural compounds during the storage of the powdered milk at the three temperatures (Table 2). All the correlations between time and furfural compounds were significant ($p < 0.01$) with r values that ranged from 0.9122 to 0.9743. By using a Friedman test, we verified the influence of the temperature of storage on the formation of furfural compounds. Thus, the production of furfural compounds, after the same elapsed time, was statistically greater ($p < 0.01$) when samples were stored at 37 °C than when they were stored at 20 or 30 °C.

The evolution of the contents of furfural compounds in the liquid infant milk is shown in Table 3. As in the powdered infant milk, free and total F and free and total HMF values increased during the storage, and except for total HMF values at 20 °C, the increases were correlated with the time of storage ($p < 0.01$). Nevertheless, a correlation with temperature of storage was found only for total HMF and free F.

No previous data are available about the changes of furfural compounds, about the usual contents of free and total furfural compounds in infant milk products, or about the factors that might favor their accumulation

during processing with the exception of those of Park and Hong (1991), who reported high contents (21.0–43.9 $\mu\text{mol/L}$) of total HMF in Korean commercial infant milks. These authors also reported that Korean milks enriched with iron and vitamin A showed higher contents of HMF than nonenriched infant milks. Similar results have been reported by Caric et al. (1984) in enriched cow's milk. Likewise, we also reported that furfural compounds were higher in infant milks than in cow's milk (Albalá-Hurtado et al., 1997a). Infant milks are usually enriched with lactose, vitamin A, and iron; therefore, they are susceptible to contain high levels of furfural compounds. The increase observed in HMF during storage is in agreement with that reported by Caric et al. (1984) in liquid and dried cow milk during 8 months of storage at room temperature. In addition, these authors also found higher levels of HMF in powdered milk than in liquid cow's milk.

No changes in available lysine occurred during storage at any of the three temperatures. Previous reports on the degree of loss of available lysine during the manufacture and storage of cow's milk differ considerably. This is probably due to differences in treatments and storage conditions (Renner and Dorguth, 1980; Finot et al., 1981; Hurrell and Finot, 1983; Caric et al., 1984; Kessler and Fink, 1986; King et al., 1991; Evangelisti et al., 1994). Our results agree with those of Van Mil and Jans (1991) in powdered cow's milk stored at 20 °C for 3 years and with those of King et al. (1991), who found no changes in available lysine stored for 1 year at 20 °C in an infant milk which they themselves designed. However, Van Mil and Jans (1991) found a 10% decrease in lysine in samples stored at 35 °C, and King et al. (1991) reported a 21% loss after 1 year of storage at 37 °C.

A relationship has been reported between the loss of lysine and the increase in moisture content during the storage of powdered cow's milk. (Erbersdobler and Dehn-Müller, 1989; Dworschäck and Hegedüs, 1974). Despite this, here the water content, water activity, and available lysine were constant throughout storage at the three temperatures described.

The increase in furfural compounds accompanied by no changes in available lysine was unexpected since the Maillard reaction in milk occurs between lactose and lysine, leading to a negative correlation between furfural compounds and available lysine. Renner (1988) concluded that the losses of available lysine could be estimated from the HMF values, but according to our results, this estimation would not always be possible. A hypothetical explanation for the absence of a relationship between lysine and furfural compounds might be that furfural compounds came only from the precursors (compounds resulted from the initial steps of the Maillard reaction) formed during the thermal treatments applied in manufacture. Therefore, the blocking of lysine should be done mainly during thermal treatment. In addition, no changes in available lysine may be explained because other amino acids different from lysine could be also involved in this reaction. Thus, Ashoor and Zent (1984) reported the reaction between lactose and a wide variety of natural amino acids in model systems. In addition to the Maillard reaction, the potential contribution of other chemical reactions should also be considered to explain the increase in furfural compounds.

Some authors reported that browning reactions only occur during the storage of powdered dairy products at room temperature, if their moisture content is $\geq 5\%$ (Renner, 1988). However, Maillard reactions occurred in powdered milk stored under the conditions described above, even though the sample moisture was always lower than 5%. The mean values for moisture and water activity were $3.34 \pm 0.1\%$ and 0.22 ± 0.1 , respectively, and no statistical differences were found between samples and temperatures of storage ($p > 0.05$).

The pH value decreased slightly during storage, with the greatest reduction being recorded in samples stored at 37 °C. These data are in accordance with those reported by Kieseker and Clarke (1984) and McKenna (1988), who found a decrease in pH values during the storage of reconstituted powdered cow's milks.

Organoleptical Changes. The organoleptical changes caused by the Maillard reaction are undesirable in dairy products. Samples stored at 20 °C did not undergo an appreciable organoleptical change after 12 months of storage. However, both liquid and reconstituted powdered samples at 30 and 37 °C showed a yellow-brown color after 5 months of storage, which was more intense in samples at 37 °C than those at 30 °C. After 7 months of storage, a yellow sediment was observed in liquid samples, and a decrease in the solubility was observed after 10 months of storage at each of the three temperatures in the powdered infant milk. Both the sediments in the liquid milk and the decreased solubility in the powdered milk could be attributed to changes in the protein structure and/or to the formation of insoluble products in the Maillard reaction (Saito and Taguchi, 1980).

Furfural Compounds Kinetic in Infant Milks. To check if the accumulation of furfural compounds could be predicted during the storage of infant milks, the corresponding kinetic equations were calculated. Table 4 shows the coefficients of determination (r^2) and the slopes from each relation for zero- and first-order reactions for all parameters studied in both infant milk samples. In addition, the sum of free HMF and free F contents and the sum of total HMF and total F contents

Table 4. Coefficients of Determination (r^2) and Slopes for Zero- and First-Order Reactions in Powdered and Liquid Infant Milks

| | order 0 | | | order 1 | | |
|---------------|--|------------------|------------------|-------------------|------------------|------------------|
| | 20 °C | 30 °C | 37 °C | 20 °C | 30 °C | 37 °C |
| | Powdered | | | | | |
| free HMF | 0.9284 ^a 0.6423 ^b | 0.9485 1.0443 | 0.9493 1.4676 | 0.9413 0.0485 | 0.9157 0.0696 | 0.9338 0.0881 |
| total HMF | 0.9331 1.5280 | 0.9469 2.0218 | 0.8320 2.0080 | 0.9193 0.0692 | 0.8936 0.0838 | 0.7568 0.0808 |
| free F | 0.9568 0.1701 | 0.9258 0.3966 | 0.9606 0.4462 | 0.9471 0.1040 | 0.8864 0.1506 | 0.8569 0.1534 |
| total F | 0.8684 0.2893 | 0.8807 0.3147 | 0.9106 0.4965 | 0.8812 0.1096 | 0.8266 0.0948 | 0.8045 0.1225 |
| free HMF + F | 0.9589 0.8122 | 0.9522 1.4409 | 0.9565 1.9140 | 0.9734 0.0545 | 0.9191 0.0813 | 0.9296 0.0973 |
| total HMF + F | 0.9409 1.8173 | 0.9497 2.3364 | 0.8650 2.5043 | 0.9387 0.0732 | 0.8923 0.0852 | 0.7781 0.0864 |
| | Liquid | | | | | |
| free HMF | 0.7369 0.0375 | 0.7106 0.0693 | 0.9199 0.1108 | 0.7207 0.0479 | 0.6245 0.0704 | 0.9239 0.1053 |
| total HMF | 0.0692 -0.0469 | 0.5680 0.0796 | 0.7401 0.3062 | 0.0577 -0.0051 | 0.5536 0.0084 | 0.7134 0.0284 |
| free F | 0.6617 0.0280 | 0.7535 0.0218 | 0.8500 0.0342 | 0.5888 0.0845 | 0.7569 0.0581 | 0.8476 0.0830 |
| total F | 0.8358 0.0915 | 0.8996 0.0921 | 0.8367 0.0901 | 0.8307 0.0509 | 0.9009 0.0511 | 0.8197 0.0544 |
| free HMF + F | 0.8779 0.0655 | 0.7896 0.0911 | 0.9194 1.1449 | 0.8782 0.0573 | 0.7238 0.0661 | 0.9316 0.0987 |
| total HMF + F | 0.0597 0.0445 | 0.8697 0.1613 | 0.8340 0.3963 | 0.0662 0.0048 | 0.8303 0.0573 | 0.8114 0.0317 |

^a Coefficients of determination (r^2). ^b Slopes obtained from each relation.

are included. Close examination of the coefficients of determination (r^2) showed that, in general, they were higher in the zero-order rate than in the first order. Thus, the zero order was found to be the most suitable, implying that the rate of increase of the furfural compounds was constant throughout the storage period.

To describe the influence of temperature on each parameter studied, the linear regression method was employed taking the Arrhenius equation as the mathematical model. Table 5 shows the collision factor ($\ln K_0$), the activation energy (E_a), and the coefficient of determination (r^2) obtained from the relation between the reciprocals of temperature and the natural logarithms (\ln) of kinetic constants at each temperature for each of the parameters studied. To choose an indicator that is temperature-dependent and that allows stability predictions in infant milk, the r^2 coefficients need to be compared. In both powdered and liquid infant milk, the highest r^2 values were found in free HMF ($r^2 = 0.9999$ and 0.9996, respectively) and in the sum of free HMF and free F ($r^2 = 0.9907$ and 0.9631, respectively). Although in both cases the r^2 were slightly higher in free HMF than in the sum of free HMF and free F, it is generally recognized that the measurement of a combination of several compounds rather than that of a single analyte is much less prone to intersample variability. In addition, most methods used to evaluate the extent of the Maillard reaction are based on the determination of total furfural compounds by colorimetric methods, using the reaction with thiobarbituric acid (TBA), which reacts with any carbonyl compound produced in the Maillard reaction. Thus, both HMF and F would react with TBA. Therefore, we concluded that the sum of free HMF and free F would be more appropriate than only free HMF for developing stability studies in infant milks.

According to the Arrhenius model, the relationship between \ln of rate constant and the inverse of the

Table 5. Arrhenius Parameters for Powdered and Liquid Infant Milks

| | powdered infant milk | | | liquid infant milk | | |
|---------------|----------------------|---------------|---------|--------------------|---------------|---------|
| | ln K_0 | E_a (J/mol) | r^2 | ln K_0 | E_a (J/mol) | r^2 |
| free HMF | 14.25 | 35800.83 | 0.99997 | 15.93 | 46827.61 | 0.99964 |
| total HMF | 5.54 | 12404.48 | 0.81150 | 53.04 | 140010.25 | 1.00000 |
| free F | 16.00 | 43113.49 | 0.90376 | -0.84 | 6911.89 | 0.12628 |
| total F | 7.84 | 22287.47 | 0.79147 | -2.63 | 593.62 | 0.37315 |
| free HMF + F | 15.18 | 37467.87 | 0.99071 | 11.10 | 33746.27 | 0.96314 |
| total HMF + F | 6.44 | 14191.17 | 0.95216 | 28.10 | 75092.55 | 0.99978 |

absolute temperature range of 20–37 °C can be expressed by

$$\ln K = \ln K_0 - E_a/R(1/T) \quad (1)$$

which corresponds to a straight line, and where K ($\mu\text{mol L}^{-1} \text{month}^{-1}$) is the rate constant at temperature T , K_0 is the collision factor, T is the absolute temperature (K), E_a (J/mol) is the activation energy, and R is the universal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$). This equation enables us to obtain the rate constant at 25 °C (or at any temperature between 20 and 37 °C) for each infant milk. For powdered infant milk, $K_{25} = 1.0051$; for liquid infant milk, $K_{25} = 0.0772$. Thus, at the same temperature the formation of the sum of free HMF and F is approximately 30 times faster in powdered than in liquid infant milk.

The general order zero equation is

$$C_f = Kt + C_0 \quad (2)$$

where C_f and C_0 are the final and the initial concentrations of the sum of free HMF and free F, respectively ($\mu\text{mol/L}$); K is the slope of the straight line defined by concentration–time; and t is the duration of storage (months). The final concentration of furfural compounds in both infant milks produced by different duration/temperature of storage combinations can be calculated from eq 3, which is obtained by inserting eq 1 into eq 2. The parameters in eq 3 are the same as those calculated in the two-step approach previously reported. No attempt was made to obtain a multiple regression equation to model the system:

$$\text{powdered } C_f (\mu\text{mol/L}) = 12.1 + 3.9 \cdot 10^6 e^{-4506.6/T} t \quad (3a)$$

$$\text{liquid } C_f (\mu\text{mol/L}) = 0.94 + 6.6 \cdot 10^4 e^{-4058.9/T} t \quad (3b)$$

By using these equations, it is possible to estimate the content of furfural compounds after a period of storage (t) at temperature (T) without previously having to calculate the rate constant at a specific temperature.

Table 6 shows that the predictive values (obtained with the above equations) and the real values (experimental values) for the 10 and 12 months are very close with no statistical differences being found between them ($p > 0.05$). In addition, the contents of available lysine in both powdered and liquid infant milks were the same as that in the initial samples ($p > 0.01$), which confirms that the available lysine remains constant throughout storage.

In summary, storage at 20, 30, and 37 °C gave rise to the development of the Maillard reaction, since there was an increase in furfural compounds. In contrast, the available lysine content remained constant during storage of powdered and liquid infant milks. Both F and HMF were found throughout the storage of liquid and

Table 6. Predictive and Real Values of Furfural Compounds in the Sum of Free HMF and Free F in Powdered and Liquid Infant Milks at Three Temperatures at 10 and 12 Months of Storage

| | powdered infant milk | | liquid infant milk | |
|-------|----------------------|----------------------|--------------------|---------|
| | pred/obsd | % error ^a | pred/obsd | % error |
| | 10 Months | | | |
| 20 °C | 20.3/19.4 | 4.4 | 1.6/1.5 | 4.6 |
| 30 °C | 25.7/25.1 | 2.2 | 1.9/2.0 | 3.0 |
| 37 °C | 31.1/29.9 | 4.0 | 2.3/2.3 | 0.0 |
| | 12 Months | | | |
| 20 °C | 21.9/20.6 | 6.3 | 1.7/1.6 | 6.2 |
| 30 °C | 28.4/28.1 | 1.0 | 2.1/2.2 | 2.3 |
| 37 °C | 34.9/31.5 | 10.8 | 2.6/2.5 | 2.8 |

^a |observed – predicted|/observed \times 100.

powdered infant milks. Free and total F and HMF were higher in solid samples than in liquid milks because of the more severe heat treatment in the manufacture of powdered infant milks. A kinetic study of furfural compounds showed that the combination of the two free furfurals (HMF + F) can be used to predict the increase of these compounds in both liquid and powdered infant milks since no statistical differences were found between experimental and predictive values.

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LITERATURE CITED

- Albalá-Hurtado, S.; Veciana-Nogués, M. T.; Izquierdo-Pulido, M.; Vidal-Carou, M. C. Determination of free and total furfural compounds in infant milks by high performance liquid chromatography. *J. Agric. Food Chem.* **1997a**, *45*, 2128–2133.
- Albalá-Hurtado, S.; Bover-Cid, S.; Izquierdo-Pulido, M.; Veciana-Nogués, M. T.; Vidal-Carou, M. C. Determination of available lysine in infant milks by high performance liquid chromatography. *J. Chromatogr. A* **1997b**, *778*, 235–241.
- Ashoor, S. H.; Zent, J. B. Maillard browning of common amino acids and sugars. *J. Food Sci.* **1984**, *49*, 1206–1207.
- Calcagno, C.; Evangelisti, F.; Zunin, P. Relationship between storage and protein quality in powdered infant formulas. *Riv. Sci. Aliment.* **1996**, *25*, 381–386.
- Caric, M.; Gavarić, D.; Milanović, S. Determination of intensity of Maillard reactions in dairy products. *Spec. Publ.—R. Soc. Chem.* **1984**, *49*, 303–311.
- Cuzzoni, M. T.; Stoppini, G.; Gazzani, G. Influence of water activity and reaction temperature of ribose-lysine and glucose-lysine Maillard systems on mutagenicity, absorbance and content of furfurals. *Food Chem. Toxicol.* **1988**, *26*, 815–822.
- Dworschák, E.; Hegedüs, M. Effect of heat treatment on the nutritive value of proteins in milk powder. *Acta Aliment.* **1974**, *3*, 337–347.

- Erbersdobler, H. F.; Dehn-Müller, B. Formation of early Maillard products during UHT treatment of milk. *Bull. Int. Dairy Fed.* **1989**, *238*, 63–67.
- Erbersdobler, H. F.; Hupe, A. Determination of lysine damage and calculation of lysine bio-availability in several processed foods. *Z. Ernährungs-wiss.* **1991**, *30*, 46–49.
- Evangelisti, F.; Calcagno, C.; Zunin, P. Relationship between blocked lysine and carbohydrate composition of infant formulas. *J. Food Sci.* **1994**, *59*, 335–337.
- Finot, P. A.; Deutsch, R.; Bujard, E. The extent of the Maillard reaction during the processing of milk. *Program Food Nutr. Sci.* **1981**, *5*, 345–355.
- García-Vilanova, B.; Guerra-Hernández, E.; Martínez Gómez, E.; Montilla, J. Liquid chromatography for the determination of 5-(hydroxymethyl)-2-furaldehyde in breakfast cereals. *J. Agric. Food Chem.* **1993**, *41*, 1254–1255.
- García-Hernández, E.; García-Villanova, B.; Montilla-Gómez, J. Determination of hydroxymethylfurfural in baby cereals by high performance liquid chromatography. *J. Liq. Chromatogr.* **1992**, *15*, 2551–2559.
- Hayase, F.; Hirashima, S.; Okamoto, G.; Kato H. Scavenging of active oxygens by melanoidins. *Agric. Biol. Chem.* **1989**, *53*, 3383–3385.
- Hurrell, R. F.; Finot, P. A. Storage of milk powders under adverse conditions. *Br. J. Nutr.* **1983**, *49*, 343–354.
- International Dairy Federation. Determination of the water content of dried milk. Standard method 26. IDF: Brussels, 1964 (confirmed 1982).
- Kessler, H. G.; Fink, R. Changes in heated and stored milk with an interpretation by reactions kinetics. *J. Food Sci.* **1986**, *51*, 1105–1155.
- Kieseker, F. G.; Clarke, P. T. The effect of storage on the properties of nonfat milk powders. *Aust. J. Dairy Technol.* **1984**, *39*, 74–77.
- Kim, S. B.; Hayase, F.; Kato, H. Desmutagenic effect of melanoidins against amino acid and protein pyrolysates. *Dev. Food Sci.* **1986**, *13*, 383–392.
- King, J.; De Pablo, S.; Montes de Oca, F.; Uauy, R. Formulación, fabricación y evaluación de una fórmula lactea para el Programa Nacional de Alimentación Complementaria en Chile. *Rev. Chil. Pediatr.* **1991**, *62*, 1–7.
- Kong, Z. L.; Shinohara, K.; Mitsui, M.; Murakami, H.; Omura, H. Desmutagenicity of furan compounds towards some mutagens. *Agric. Biol. Chem.* **1989**, *53*, 2073–2079.
- Lo Coco, F.; Valentini, C.; Novelli, V. High-performance liquid chromatographic determination of 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde in processed citrus juices. *J. Liq. Chromatogr.* **1994**, *17*, 603–617.
- McKenna, A. B. Influence of heating on the formation of caramel flavour, brown colour and hydroxymethylfurfural in reconstituted concentrated skim milk. *N. Z. J. Dairy Sci. Technol.* **1988**, *23*, 363–372.
- Mittal, S. B.; Hourigan, J. A.; Zadow, J. G. Effect of UHT treatment, storage time and temperature on the available lysine of UHT recombined and UHT recombined lactose hydrolyzed milk. *Aust. J. Dairy Technol.* **1989**, November, 88–90.
- Möller, A. B.; Andrews, A. T.; Cheeseman, G. C. Chemical changes in ultra-heat-treated milk during storage. *J. Dairy Res.* **1977**, *44*, 267–275.
- Park, Y. H.; Hong, Y. H. Comparison of the heat treatment intensity in infant formulae. *Kor. J. Food Sci. Technol.* **1991**, *23*, 627–632.
- Renner, E. Storage stability and some nutritional aspects of milk powders and ultrahigh-temperature products at high ambient temperatures. *J. Dairy Res.* **1988**, *55*, 125–142.
- Renner, E.; Dorguth, H. Untersuchungen zur eiweißqualität (Investigations on the protein quality of UHT milk in the Federal Republic of Germany). *Dtsch. Milchwirtsch.* **1980**, *31*, 505–506, 508.
- Saito, Z.; Taguchi, A. Crystallization of lactose in stored skim milk and whey. *Jpn. J. Dairy Food Sci.* **1980**, *29*, 133–140.
- Van Mil, P. J. J. M.; Jans, J. A. Storage stability of whole milk powder: effects of process and storage conditions on products properties. *Neth. Milk Dairy J.* **1991**, *45*, 145–167.
- Villalón-Mir, M.; Quesada Granados, J.; López García de la Serrana, H.; López Martínez, M. C. High performance liquid chromatography determination of furanic compounds in commercial brandies and caramels. *J. Liq. Chromatogr.* **1992**, *15*, 513–524.
- Yen, G. C.; Tsai, L. C.; Lii, J. D. Antimutagenic effect of Maillard browning products obtained from amino acids and sugars. *Food Chem. Toxicol.* **1992**, *30*, 127–132.

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