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Analysis of potential and free furfural compounds in milk-based formulae by high-performance liquid chromatography☆ Evolution during storage

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Abstract

A simple and reproducible HPLC-diode array detection method for the qualitative and quantitative analysis of potential and free furfural compounds (5-hydroxymethyl-2-furaldehyde, HMF; 2-furaldehyde, F; 2-furyl methyl ketone, FMC; and 5-methyl-2-furaldehyde, MF) in milk-based formulae was developed and validated. The method showed good linearity with determination coefficients over 0.999. The limits of detection and quantification were acceptable for all furfurals. The relative standard deviations (RSDs) for repeatability and reproducibility were <4.28. Recoveries in all furfurals were between 94.5 and 98.7%. In addition, we report the evolution over shelf life of furfural compound levels in an experimental powder formula for pregnant women stored at 25 and 37 °C from production until 15 months.

Keywords: Furfural compounds; Milk-based formula powders; Maillard reaction; Storage

1. Introduction

The food industry has made several attempts to improve the quality and the nutrient content of milk-base products, and to develop products for specific stages of live (i.e. intrauterine, newborn, pregnancy and lactation [1]. Two of these products are the infant formulae (IFs) and the formulae for pregnant women (FPW), of which milk powder is one of the major constituents. One of the challenges of industry is to control the stability of these two kinds of product. Instability can occur because many factors make these powders susceptible to the Maillard reaction (MR), such as the reductor sugar content, lysine-rich proteins, high temperature applied during production and long storage times [2–6].

The reductor sugars and lysine are the main compounds involved in the initial states of the MR, and consequently a lactulosyl-lysine compound is produced [4,7–14]. In advanced states of MR undesirable compounds such as furfurals can be found [12,15,16]. These compounds can be useful indicators of food damage and can also be used to evaluate the extent of the MR [17,18].

Furfurals can be produced in two ways: via Amadori compounds (mostly ∈-*N*-deoxylactulosyl-L-lysine) from MR by enolization in acidic conditions, or through lactose isomerization [19,20], known as the Lobry De Bruyn-Alberda van Ekenstein transformation (L-A) and the subsequent degradation reactions [12,21]. Fig. 1 shows the schematic formation of furfural compounds from lactose and lysine.

To date, studies have focused on four furfural compounds in processed foods: HMF, 2-furaldehyde (F), 2-furyl methyl ketone (FMC) and 5-methyl-2-furfural (MF) [19,22–29].

Since the development of the Keeney and Basette method [28], a differentiation was made between free HMF and potential HMF. In this method, to determine the latter, the

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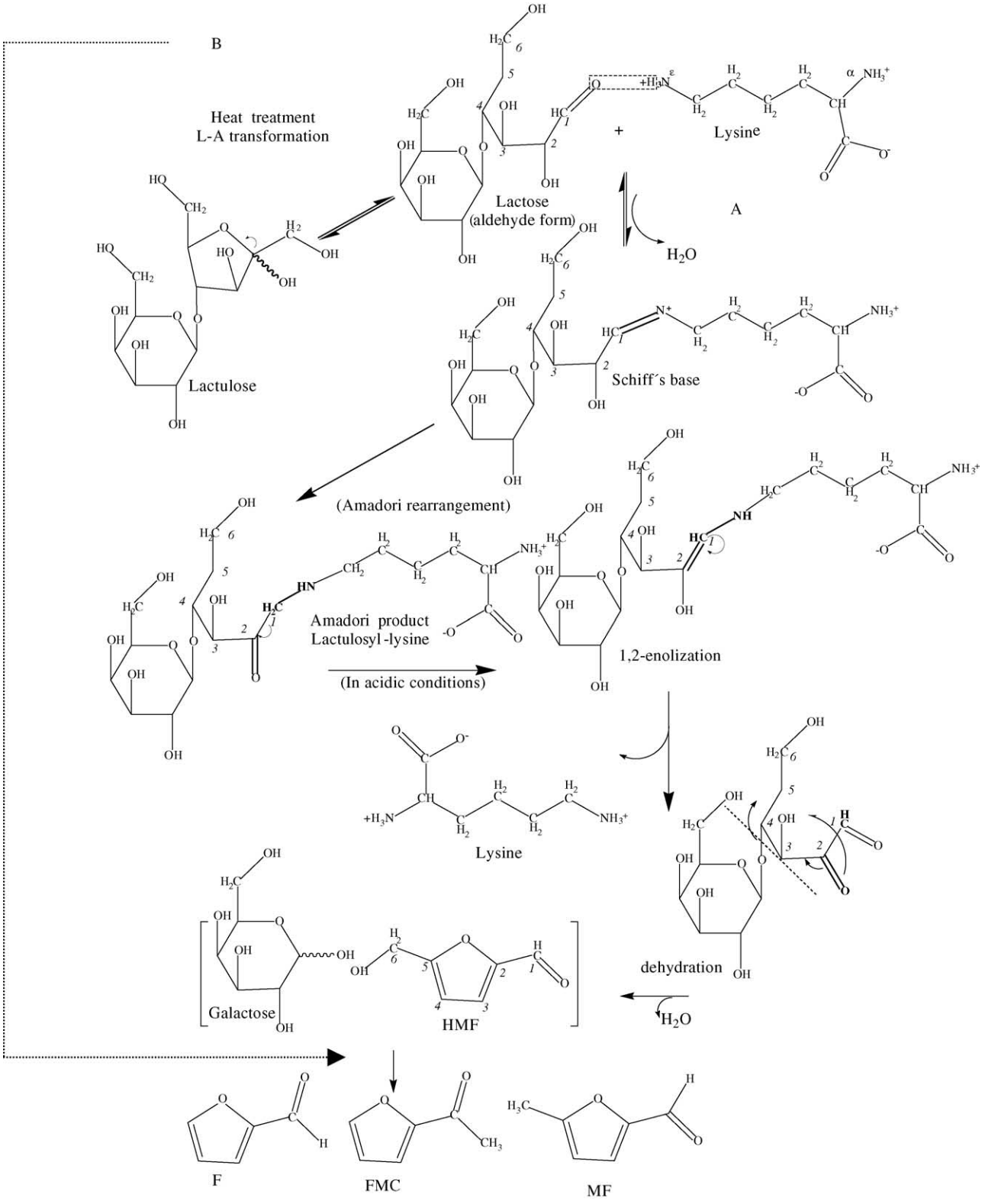

Fig. 1. Schematic presentation of furfural formation from amino ε-group of lysine and lactose in the Maillard reaction: (A) via Amadori compounds and (B) lactose isomerization (L-A). *Note*: Lactose isomerization to lactulose is not a MR process, but this reaction is important in the study of milk-based formulae.

heated-treated milk sample is reheated in 0.3 N oxalic acid at 100 °C to release HMF, because the formation of this furfural from the Amadori compound is induced under acidic conditions. Thus, potential HMF is the sum of the precursors of HMF (i.e. HMF bounded to protein; as Amadori products, HMF from reductor sugars, or novo HMF) and free HMF. Free HMF is determined by omitting the hydrolysis step.

Furfural compounds such as HMF can be measured by spectrophotometry with thiobarbituric acid (TBA). However, one disadvantage of this colorimetric method is that it is not specific for HMF detection because TBA lacks specificity for this compound. In addition, a strict control of time and temperature reaction is required because the reaction product measured colorimetrically is unstable [24]. This instability leads to highly variable results. At present, HPLC techniques can be used for accurate and reliable measurement of furfural compounds in several food products [16,21,26,29–33]. These techniques can determine furfural compounds specifically, and the formation of a colored derivative is not required because of the strong UV absorption of furfurals at approximately 280 nm.

Given that the formation of furfurals can be caused by many factors (temperature of heat treatment/time and composition of formulae), it is difficult to compare distinct formulae, and the amount of furfural compounds may differ. Nevertheless, a comparison of the evolution of furfural contents in the same formula during shelf life could be a useful indicator of changes caused by the MR.

The aims of this study were to validated an HPLC-diode array detection (DAD) method that separates furfurals from components such as proteins, fat and other interference macromolecules for the qualitative and quantitative analysis of potential and free furfural compounds (HMF, F, FMC and MF) in milk-based formula. We used this method and monitored the evolution of these compounds in an experimental FPW stored at 25 and 37 °C, during shelf life. In addition, this study aims at obtaining more information on furfural formation, formula stability, and the usefulness of furfural compound analysis to evaluate deterioration in this product.

2. Experimental

2.1. Reagents and standards

The chemicals used for sample preparation were of analytical reagent grade: acetonitrile HPLC-grade (SDS, Peypin, France), oxalic acid dihydrate >95.5% and trichloroacetic acid (TCA) >99.5% (Fluka, Buchs, Switzerland). Deionised water was purified through a Milli-Q system (Millipore, Bedford, MA, USA). Standards of 5-hydroxymethyl-2-furaldehyde (HMF), 2-furaldehyde (F), 2-furyl-methyl ketone (FMC) and 5-methyl-2-furaldehyde (MF) were >99% pure and were purchased from Fluka (Buchs, Switzerland).

2.2. Instrument

The HPLC system used consisted of a Hewlett-Packard HP 1050 series controller pump degassing device, a Waters 717 plus autosampler and a DAD HP 1040 M series II HPLC detection system. The HP 1090 Win Chemstation system was used for data acquisition.

Separation was performed on a Tracer ODS-2 C_{18} column (150 mm × 4.6 mm), with a 5 μm particle size (Teknokroma, Barcelona, Spain).

2.3. Chromatographic conditions

Separation was performed at 30 °C using a mixture of acetonitrile–water (4.5:95.5, v/v) as the mobile phase and a flow rate of 1 ml/min.

Detection was made at 284 nm for HMF, 277 nm for F, 274 nm for FMC and 293 nm for MF. The injection volume was 20 μl.

2.4. Samples

The method can be applied to any kind of milk-based formula (i.e. IFs, FPW, etc.). Here we tested an experimental FPW, which, according to the label, contained milk powder, animal fat, fructose, sucrose, minerals and artificial aroma (53.7% carbohydrate, 20.2% fat and 18.1% protein: casein/serum protein: 80/20), Vitamin A (2200 μg/100 g), Vitamin C (1800 mg/100 g), and minerals mg/100 g: Na (250), Ca (2000), P (1600), Mg (620); and a commercial IF (58% carbohydrate, 26% fat and 12% protein: casein/serum protein 40/60), whose ingredients were whole milk powder, lactose, minerals and vitamins. Both formulae were obtained from a firm in Barcelona, Spain.

2.5. Storage

To evaluate the evolution of furfurals only in FPW during shelf life, we kept the product in a storage chamber at 25 °C or 37 °C from production until 15 months.

2.6. Measurement of furfural compounds

Free and potential furfurals were measured by RP-HPLC-DAD, with a slight modification of the Albalá-Hurtado method [29]. Potential furfurals include free furfurals, furfurals bound to proteins (like Amadori products) and those formed from precursors. The procedure was as follows.

Potential furfurals: 2 g of formula powder was mixed with 10 ml of 0.2 N oxalic acid (freshly prepared) in a sealed tube covered with parafilm to prevent evaporation. The tube was heated in a water-bath system at 100 °C for exactly 25 min. It was then left to cool at room temperature and 3 ml of 40% (w/v) TCA solution was added. The mixture was stirred for 5 min. It was then centrifuged at 4000 rpm for 15 min. The supernatant phase was passed through a paper filter and col-

lected in a 25 ml volumetric flask. Ten milliliters of 4% (w/v) TCA was added to the solid residue. This was then mixed thoroughly for 10 min and then centrifuged again. The supernatant was filtered and added to the flask, and the solid phase was discarded. The solution was made up to 25 ml with 4% TCA in the volumetric flask. The mixture was then filtered through a 0.45 μm nylon filter before HPLC analysis.

Free furfurals: The sample was prepared as above but heating in the water-bath system was omitted.

Furfurals were identified by retention times and by their characteristic spectra. They were quantified by interpolation in a calibration curve in the range 0.05–2 μg/ml for F, FMC, MF and 0.05–5 μg/ml for HMF.

2.7. pH

The pH of the samples was measured in a pH meter micro-pH 2000 with a glass electrode (Crison Instruments, Barcelona, Spain). Following the manufacturer's instructions, the FPW was reconstituted with cold water (10–15 °C; 15 g in 200 ml) and after of the samples had reached room temperature the pH values were measured.

2.8. Statistical analysis

For statistical analysis, we used a one-way analysis of variance (ANOVA) and multiple comparisons using the Tukey HSD procedure for each furfural and temperature of storage, in order to detect differences in the FPW along storage time at 25 and 37 °C. The level of statistical significance was set at 5% for all analyses. We performed statistical analysis using the SPSS package for Windows version 11 (SPSS, Chicago, IL, USA).

3. Results and discussion

3.1. Validation of proposed method

Before the method proposed here, first we used a Spherisorb ODS-2 C_{18} column 250 mm × 4.6 mm, 5 μm particle size [27,29]. A pool of standards for furfural compound (HMF, F, FMC, MF) was prepared and injected into the HPLC system. The time required for a single sample injection was about 30 min at room temperature. To observe a relationship between the column temperature and the time required for the detection of each furfural peak, in order to reduce the analysis time, we tested with 25, 30, 35, 50, 60, 70, 80 and 85 °C. Besides we want to know if the column temperature affect the quantity of furfural detected due to the lability of these compounds. We injected the pool of standards three times at each column temperature. A linear relation in each compound ($r^2 \geq 0.99$) was observed between the temperature of analysis and detection. In addition, the RSDs of the amount of each compound detected at all the temperatures tested were less than 0.81. No statistical differences were found between temperatures of analysis and furfural detection ($p > 0.05$). This observation indicates that the increases in column temperature did not affect the amount of compound. Nevertheless, when we prepared the extraction of furfural compounds from an IF and a FPW sample, the increase in temperature did not allow the reliable quantification of HMF because this compound eluted by joining to the matrix leftovers. We then used a shorter column, an ODS-2 C_{18} (4.6 mm × 150 mm), and repeated the analyses. The optimum temperature that allowed the separation of the furfural peaks was 30 °C. However, the HMF peak eluted by joining to a minimal residue, which interfered with the analysis of this compound. We examined the mobile phase using slight variations of water–acetonitrile (90:10, 93:7, 95.5:4.5 and 96:4), and observed that a 95.5:4.5 ratio gave the best result. The time required for each HPLC analysis was about 15 min.

3.1.1. Linearity

Under the chromatographic conditions tested, a linear relationship was verified in the range 0.05–2 μg/ml for F, FMC, MF and 0.05–5 μg/ml for HMF of standard solutions, by analysis of variance of the regression (r^2). For all these compounds, the r^2 values were >0.999 at seven levels. The concentration of HMF in the standard solution was higher than that of the other furfurals because HMF is the main furfural compound in milk-based formulae.

3.1.2. Sensitivity

To determine the sensitivity of the method, the detection limit (DL) and the quantification limit (QL) was studied following the USP criteria [34]. These two limits were determined by the chromatographic noise obtained by repeated analysis of a blank through the system, which was injected under the HPLC conditions described. This is the most common method used to estimate sensitivity in chromatographic procedures. The method showed acceptable sensitivity (Table 1).

3.1.3. Precision

Six replicate measurements were performed on the same day to evaluate repeatability. For reproducibility, eight determinations with the same formula were made on different days. FMC and MF (1 ppm) were added to each sample. The RSDs for HMF, F, FMC and MF were satisfactory according to Horwitz (Table 1) [35].

3.1.4. Recovery

Standards of HMF, F, FMC and MF were added (1 μg/ml of each) to milk-based formula that had been analyzed previously. The six replicate analyses showed acceptable recoveries (Table 1).

3.2. pH analysis

Given that pH can enhance the formation of furfural compounds either by lactose isomerization (Lobry De Bruyn-Alberda van Ekenstein transformation, L-A) or by Amadori

Table 1
Validation method of furfural determination in milk-based formulae by HPLC-DAD

Analytical parameter	HMF	F	FMC	MF
Detection limit (μg/100 g)	32.07	0.32	5.66	18.58
Quantification limit (μg/100 g)	49.05	15.17	22.13	32.11
Repeatability (RSD, %)	1.05	2.30	1.66	1.38
Reproducibility (RSD, %)	2.68	4.28	3.51	3.32
Recovery (%, $n = 6$)	96.32 ± 2.58	96.24 ± 4.12	94.46 ± 3.32	98.71 ± 1.80

Table 2
Potential and Free HMF contents in stored formulae for pregnant women

Sample	Storage (months)	Potential HMF			Free HMF		
		μg/100 g	μg/100 ml	RSD (%)	μg/100 g	μg/100 ml	RSD (%)
FPW, 25 °C	0	902.81 ± 10^{i,1}	67.71 ± 0.7	1.13	379.80 ± 3.7^{i,1}	28.49 ± 0.2	0.98
	5	1040.83 ± 29 ^{j,3}	78.06 ± 2.1	2.80	392.75 ± 2.7^{i,1}	29.46 ± 0.2	0.70
	9	1142.84 ± 10 ^{k,2}	85.71 ± 0.7	0.87	443.47 ± 8.2^{j,3}	33.26 ± 0.6	1.86
	12	1426.10 ± 17 ^{l,5}	106.96 ± 1.3	1.20	356.73 ± 1.4^{i,1}	26.75 ± 0.1	0.42
	15	1562.30 ± 18 ^{m,4}	117.17 ± 1.3	1.15	315.34 ± 9.5^{k,4}	23.65 ± 0.7	3.04
FPW, 37 °C	0	902.81 ± 10 ^{a,1}	67.71 ± 0.7	1.13	379.80 ± 3.74^{a,1}	28.49 ± 0.2	0.98
	5	1199.31 ± 1.8^{b,2}	89.95 ± 0.1	0.16	547.78 ± 33.3^{b,2}	41.08 ± 2.5	6.08
	9	1180.66 ± 9.8^{b,2}	88.55 ± 0.7	0.83	558.19 ± 13.7^{b,2}	41.86 ± 1.1	2.46
	12	1521.12 ± 13^{c,4}	114.08 ± 1.1	0.92	319.75 ± 11.2^{c,4}	23.98 ± 0.8	3.52
	15	2618.66 ± 60^{d,6}	196.40 ± 4.5	2.32	1166.77 ± 65^{d,5}	87.51 ± 4.9	5.64

Values are expressed as mean ± standard deviation ($n = 4$). No coincidence in the superscript letters indicates a significant difference ($p < 0.05$) with the storage time of the same column. No coincidence in the superscript numbers indicates a significant difference ($p < 0.05$) with the temperature of storage.

compounds formation [26], the pH of the reconstituted FPW samples was measured each month of storage. No differences were observed in the evolution of pH values at 25 °C or 37 °C. The average pH over the 15 months of storage was 8.01 ± 0.15 and 8.11 ± 0.18, respectively. These values are slightly basic and could enhance the formation of Amadori compounds with subsequence formation of furfurals during determination.

3.3. Furfural contents

Heating at 100 °C not only released HMF but prolonged heating at this temperature also induced the formation of this compound. Therefore, the conditions of hydrolysis used in this method prevented HMF formation. These conditions were evaluated previously [29], and no furfural compounds were detected in raw milk samples. This observation implies that furfural content in milk-based formula depends on the heating process during manufacture and/or on changes caused by storage conditions.

The chromatograms of furfural in a standard solution, IF and FPW are given in Fig. 2. Potential and free furfural compounds (HMF, F and HMF + F), expressed as μg/100 g powder sample and μg/100 ml of reconstituted sample from FPW at 25 and 37 °C are reported in Tables 2–4.

Other studies refer to "total furfurals" instead of "potential furfurals". We believe that the term "total furfurals"

Table 3
Potential and free F contents in stored formulae for pregnant women

Sample	Storage (months)	Potential F			Free F		
		μg/100 g	μg/100 ml	RSD (%)	μg/100 g	μg/100 ml	RSD (%)
FPW, 25 °C	0	128.40 ± 2.6^{i,1}	9.63 ± 0.2	2.07	61.34 ± 4.0^{i,1}	4.60 ± 0.3	6.65
	5	216.61 ± 13^{j,3}	16.25 ± 1.1	6.15	63.78 ± 1.2^{i,1}	4.78 ± 0.1	1.91
	9	162.55 ± 7.7^{i,5}	12.19 ± 0.5	4.79	89.40 ± 0.4^{j,3}	6.70 ± 0.1	0.43
	12	249.84 ± 5.5^{j,2}	18.74 ± 0.4	2.21	84.95 ± 1.4^{j,3}	6.37 ± 0.1	1.65
	15	345.36 ± 5.6^{k,4}	25.90 ± 0.4	1.64	82.49 ± 2.3^{j,3}	6.19 ± 0.2	2.85
FPW, 37 °C	0	128.40 ± 2.6^{a,1}	9.63 ± 0.2	2.07	61.34 ± 4.0^{a,1}	4.60 ± 0.3	6.65
	5	274.87 ± 4.4^{b,2}	20.62 ± 0.3	1.63	123.12 ± 3.7^{b,2}	9.23 ± 0.2	3.06
	9	265.49 ± 4.7^{b,2}	19.91 ± 0.3	1.79	135.19 ± 6.6^{b,2}	10.14 ± 0.4	4.48
	12	328.06 ± 7.0^{c,4}	24.67 ± 0.5	2.13	106.68 ± 3.8^{c,4}	8.00 ± 0.3	3.62
	15	515.96 ± 13^{d,6}	38.70 ± 0.9	2.50	243.30 ± 6.4^{d,5}	18.25 ± 0.5	2.66

Values are expressed as mean ± standard deviation ($n = 4$). No coincidence in the superscript letters indicates a significant difference ($p < 0.05$) with the storage time of the same column. No coincidence in the superscript numbers indicates a significant difference ($p < 0.05$) with the temperature of storage.

Table 4
Potential and free HMF + F contents in stored formulae for pregnant women

Sample	Storage (months)	Potential HMF + F			Free HMF + F		
		μg/100 g sample	μg/100 ml sample	RSD (%)	μg/100 g sample	μg/100 ml sample	RSD (%)
FPW, 25 °C	0	$1031.21 \pm 12.8^{i,1}$	77.34 ± 0.9	1.24	$441.14 \pm 7.8^{i,1}$	33.09 ± 0.6	1.77
	5	$1257.44 \pm 42^{j,3}$	94.31 ± 3.1	3.38	$456.52 \pm 1.5^{i,1}$	34.24 ± 0.1	0.34
	9	$1305.39 \pm 2.2^{k,4}$	97.90 ± 0.2	0.17	$532.87 \pm 7.9^{j,3}$	39.96 ± 0.6	1.48
	12	$1675.93 \pm 22^{l,6}$	125.69 ± 1.7	1.35	$441.67 \pm 2.9^{i,1}$	33.13 ± 0.2	0.65
	15	$1907.68 \pm 23^{m,5}$	143.08 ± 1.7	1.21	$397.83 \pm 8.8^{i,1}$	29.84 ± 0.7	2.21
FPW, 37 °C	0	$1031.21 \pm 12.8^{a,1}$	77.34 ± 0.9	1.24	$441.14 \pm 7.8^{a,1}$	33.09 ± 0.6	1.77
	5	$1474.18 \pm 6.3^{b,2}$	110.56 ± 0.5	0.43	$670.90 \pm 37^{b,2}$	50.32 ± 2.7	5.52
	9	$1446.14 \pm 5.6^{b,2}$	108.46 ± 0.4	0.35	$693.38 \pm 7.7^{b,2}$	52.00 ± 0.6	1.11
	12	$1850.08 \pm 20^{c,5}$	138.76 ± 1.5	1.08	$426.43 \pm 9.6^{a,1}$	31.98 ± 0.7	2.25
	15	$3134.62 \pm 47^{d,7}$	235.00 ± 3.5	1.52	$1410.07 \pm 59^{d,4}$	105.75 ± 4.4	4.21

Values are expressed as mean ± standard deviation ($n = 4$). No coincidence in the superscript letters indicates a significant difference ($p < 0.05$) with the storage time of the same column. No coincidence in the superscript numbers indicates a significant difference ($p < 0.05$) with the temperature of storage.

can lead to confusion because it could be taken as the sum of the total furfurals present in a sample, for example HMF + F + FMC + MF, and not the potential of these compounds in terms of their precursors. Therefore, we refer to "potential furfurals" when referring to the sum of free furfurals, plus the furfurals bound to proteins such as Amadori products, furfural from reductor sugars and the furfurals from precursors.

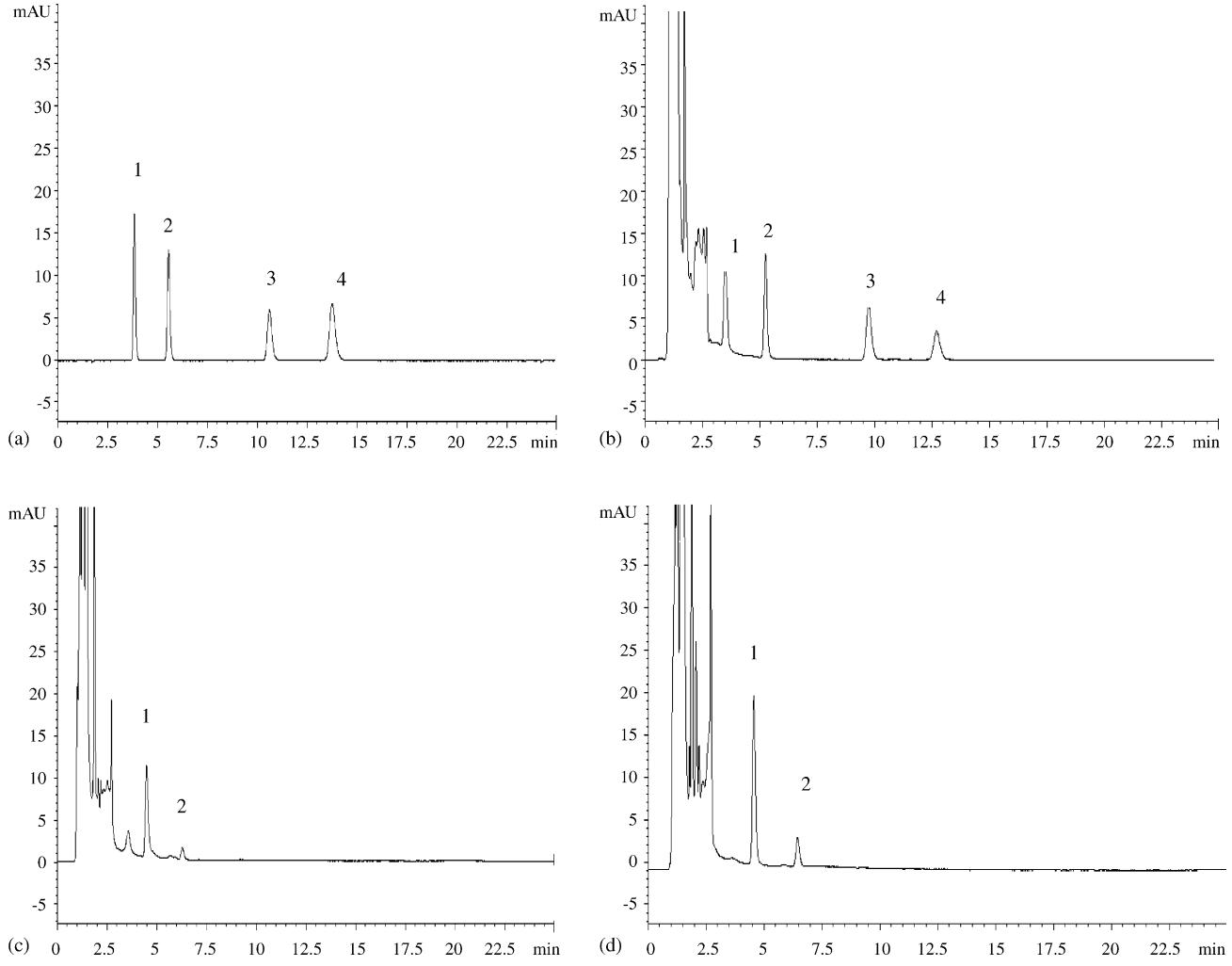

Fig. 2. Typical chromatograms of furfural determination by the HPLC-DAD method. See conditions in Section 2.3. Furfural peaks: 1, HMF; 2, F; 3, FMC; 4, MF. (a) Furfural standards; (b) recoveries; (c) infant formula and (d) formula for pregnant women.

It has been reported that the first furfural compound formed during the MR is HMF, and F, MF and FMC are products of the most advanced states of the reaction, or are formed by inter-conversion as a result of greater heating or longer storage periods [30]. To detect FMC and MF compounds and to corroborate their formation in milk-base formulae at advances stages of the MR, we stored IF and FPW at 47 °C, and then analyzed potential and free furfurals at 10, 30, 50, 100 and 120 days (data not show). Neither FMC nor MF was formed. This result is consistent with other studies that did not detect either of these compounds in IF or milk [26,27,29,30,36].

To our knowledge, no study has analyzed furfural compounds in FPW, which are specific formulae for adults. Since furfural formation is the result of many factors such as composition of formulae, thermal treatments during manufacturing and storage, etc., the comparisons of different milk-based formulae is complicated. However, comparison of furfural content in different milk-based formulae could be useful, in spite of these limitations.

The potential furfural value (HMF + F) found at point zero in the FPW was 1031.21 μg/100 g (Table 4). Other authors have reported, [26] 601.95 μg/100 g for an adapted IF (casein/serum 40/60) and 1362.03 μg/100 g for a follow-up formula (casein/serum 80/20). The FPW used in this study also had a casein/serum ratio of about 80/20. This could be explained because of the reactivity of the ε-amino group of lysine from casein is higher than that from serum as reported previously [12,26].

In general, the values of potential HMF in the FPW were slightly lower than those reported by Ferrer et al. [26] in follow-up formulae with the same casein/serum protein ratio (80/20), but distinct composition. Nevertheless, the values of potential and free F were higher in FPW than in the adapted formulae.

In addition, the values of potential and free HMF and F in the FPW were slightly lower than those reported in liquid and powdered IF by Albalá-Hurtado et al. [29], except in free HMF and F from liquid infant milk which have the lowest values, respectively.

At zero point, the level of potential HMF in the FPW was 67.71 μg/100 ml of reconstituted sample, which is similar to the concentration reported in several UHT milk samples [30].

3.4. Furfural evolution

In the FPW, distinct evolution of furfurals was observed with respect to storage at 25 and 37 °C. In general, HMF and F concentrations were greater with increased storage time, and this increment was higher in the FPW stored at 37 °C. This observation can be explained because storage at inadequate temperatures, such as at 37 °C, favor the MR. The sugar content of the FPW differed from that of the IFs. In addition to lactose, the FPW contained fructose and sucrose [37]. The reductor sugars such as lactose and fructose can favor the MR.

3.4.1. Potential furfurals

At the beginning of the study the potential HMF in the FPW was 902.81 μg/100 g, and after 15 months of storage at 25 °C increased to 1562.30 and to 2618.66 μg/100 g at 37 °C, respectively (Table 2). Similar results were observed for the potential F, whose initial content was 128.40 μg/100 g and at the end of 15 months of storage reached 345.36 and 515.96 μg/100 g at 25 and 37 °C, respectively. These results indicate that the storage temperature affects the MR; the greater the temperature, the faster the MR.

The potential HMF and F in the FPW increased with extended storage and higher temperatures. However, these increases were not regular (Tables 2 and 3). Other authors [26] have reported that the potential HMF and F contents vary in an irregular way with the storage time and temperature. These observations can be explained by the fact that HMF reaches a state of equilibrium between destruction by oxidation and formation from precursors [38].

3.4.2. Free furfurals

At zero point, free HMF in the FPW reached 28.49 μg/100 ml of reconstituted sample, which is slightly lower or higher than those reported for several UHT milk samples (in the range 7.40–65.22 μg/100 ml) [30].

The contents of free HMF in the FPW did not follow a regular pattern. At the beginning of the study, it was 379.80 μg/100 g, and at the end of 15 months of storage dropped to 325.34 and 1166.77 μg/100 g at 25 and 37 °C, respectively. Moreover, HMF concentrations fluctuated throughout storage (Table 2). Free F was 61.34 μg/100 g and after 15 months of storage reached 82.49 and 243 μg/100 g at 25 and 37 °C, respectively. Increasing values were observed during storage, and a decrease was detected only at 12 months of storage in samples at 25 and 37 °C.

Many studies have addressed the chemical changes that are produced by thermal process. The search for compounds induced by heating and the concentration of these as possible indicators of the heat treatment or product deterioration, such as furfural compounds, continues. The question whether or not HMF and F are really suitable indicators has not yet been satisfactorily answered.

4. Conclusions

The HPLC-DAD method used in this study is relatively simple and reproducible for measuring furfural compounds in milk-based formulae. It is suitable for routine analysis and shows acceptable precision, recovery and sensitivity.

HMF was the main furfural compound detected in the FPW, followed by F. Levels of furfurals were higher in FPW stored at 37 °C than at 25 °C. The levels found before storage were, for free HMF and F: 379.80 ± 3.7 and 61.34 ± 4 μg/100 g; and for potential HMF and F were 902.81 ± 10 and 128.40 ± 2.6 μg/100 g of sample, respec-

tively. No formation of FMC and MF were detected in any of the formulae. At present, there are no established limits for furfural compounds concentrations in milk-based formulae. In the case of IFs the recommendation is that the amount of unavailable lysine (or blocked), such as Amadori compounds, should be as low as possible [39]. This recommendation could be extended to other milk-based formulae.

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