Evaluation of the Maillard reaction in infant formulas after opening

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Summary

Infant formula is a substitute for or complement to breast milk. The composition of infant formulas makes them subject to the Maillard reaction, which may be initiated during heat treatment in the manufacturing process and continue during consumer handling or prolonged storage. The Maillard reaction propagation in opened infant formulas was investigated under common consumer storage conditions, at room temperature (24.4 ± 1.3 °C) and refrigerated temperature (3.8 ± 1.8 °C), for 60 days using physico-chemical analysis. In the opened formulas stored at room temperature, free 5-hydroxymethylfurfural (HMF) accumulation, colour changes, decrease in lactose, available lysine and pH, and increase in water activity and moisture were observed. The infant formulas stored at refrigeration temperature showed lower nutritional losses, as well as reduced HMF accumulation.

Keywords

5-hydroxymethylfurfural; heat treatment; nutritional loss; storage; analysis

Breastfeeding without supplementation is recommended for infants from birth to 6 months. It continues to be recommended with supplementation up to the age of 2 or more because of the balanced nutritional composition and immunological protection provided by human milk. Infants fed with breast milk show better development and lower disease risk throughout life [1, 2]. Despite these advantages, breastfeeding practices can be interrupted or altered to include supplementation in cases of postpartum depression, insufficient milk syndrome, social factors (working mothers) or medical recommendations for babies with metabolic disturbances, to name a few causes [2–4]. Under these circumstances, infant formulas are often the only food available. For this reason, the infant formulas must be safe and provide all the nutrients required for child's healthy development.

Most commercial infant formulas are produced using a combination of bovine milk and other components, such as lactose, long-chain polyunsaturated fatty acids, vitamins and minerals [5]. Infant formula production usually involves mixing the ingredients, solubilizing them in water, heat-treating to devitalize pathogenic bacteria, dehydrating in a spray dryer and packaging the powder under aseptic conditions. Beyond heat treatment, it is critical to guarantee the microbiological safety of infant formula products. Doing so can generate nutritional losses and affect the formulas' techno-functional properties including their rehydration degrees, colour modifications and volatile compound formation [6, 7].

Throughout the process of fortifying infant formulas with vitamins, minerals such as iron, and lactose, the products are subjected to heat treatment, drying and storage, which are the major promoters of the Maillard reaction in dairy products [7, 8]. The Maillard reaction occurs when a carbonyl compound (here a reducing sugar) and a protein or amino acid from a free amino group react and form Schiff bases [6, 9, 10]. These bases are chemically unstable and successive reactions promote the formation of a wide variety of hetero-

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cyclic, carboxylic and aliphatic compounds such as dicarbonyls, reductones, Strecker degradation products and furfural compounds, such as 5-hydroxymethylfurfural (HMF) [7, 9, 11].

Particular attention has been paid to HMF because it is an intermediate Maillard reaction compound that can be used as an indicator of heat treatment severity as well as food storage duration and its conditions [8, 12, 13]. Furthermore, studies suggested that HMF at high concentrations has mutagenic, genotoxic and carcinogenic effects. The recommended daily intake for adults should not exceed 2.5 mg by body mass in kilograms (equivalent to 20 $\mu mol \cdot kg^{-1} \cdot d^{-1}$) [14–16].

To summarize, when the Maillard reaction reaches excessive levels, it can make essential amino acids, such as lysine, and reducing sugars, such as lactose, unavailable. It can also produce toxic and dark compounds, HMF and melanoidins, respectively [2, 7, 17].

The Maillard reaction kinetics are primarily influenced by temperature, humidity, pH, reducing sugar type or amine type, and exposure to light and oxygen. Thus, the processing and storage conditions are crucial to determine the propagation levels of the Maillard reaction in infant formulas, which is especially important when the manufacturing and storage process occurs at high temperatures [9, 18, 19].

Taking into account the important role that infant formula plays in child nutrition, the objective of this work was to study the propagation of the Maillard reaction in opened infant formulas stored under ambient and refrigeration conditions.

MATERIALS AND METHODS

Materials

Six commercial powder infant formulas (first stage) from different batches of two brands (brand F1 produced in Argentina and brand F2 produced in Brazil) were acquired in a local market in Viçosa, Brazil. The milk-based formulas had been on the shelf between 2 and 4 months at the time of purchase and their ingredients and nutritional composition are shown in Tab. 1.

The powered infant formulas are produced by dissolving the dried ingredients in pre-heated water or skimmed milk, then pasteurizing and homogenizing the mixture. The preparation is concentrated in a vacuum evaporator and spray dried to form a powder [20].

Oxalic acid (Fmaia, Belo Horizonte, Brazil), trichloroacetic acid (Vetec, São Paulo, Brazil), thiobarbituric acid (Ęxodo Científica, Sumaré, Brazil), sodium dodecylsulfate (Bio-Rad, Hercules, California, USA), *o*-phthaldialdehyde (Sigma-Aldrich, St. Louis, Missouri, USA), ethyl alcohol (Cap-Lab, São Paulo, Brazil), sodium tetraborate (Vetec), 2-mercaptoethanol (Sigma-Aldrich), casein from bovine milk (Sigma-Aldrich) and standard HMF (Sigma-Aldrich) were used. All reagents were of analytical grade (> 99 %) and were used to determine HMF, lactose and lysine levels in the infant formula samples.

Storage and simulation of the conditions of use of infant formulas

Infant formulas from brand F1 and brand F2 were opened and stored at (3.8 ± 1.8) °C or (24.4 ± 1.3) °C for 60 days. Everyday use was simulated by stirring the formulas with a spoon to homogenize them. The formulas stored at refrigeration and room conditions were kept in incubator chambers MOD 344C (FANEM, São Paulo, Brazil) and SL200 (SOLAB, Piracicaba, Brazil), respectively. The storage temperatures and the relative humidity were measured daily with a HigroFlex 5 thermohygrometer (Rotronic, Bassersdorf, Switzerland).

Physical analysis

The dried infant formulas were analysed for moisture using gravimetric analysis, the material's weight loss being evaluated after it was heated in an oven to 105 °C [21]. The water activity (a_w) was measured at 25 °C using an Aqualab meter (Decagon 3TE, Pullman, Washington, USA).

The powders' colours were measured using the CIELAB colour scale, with coordinates L^* (luminosity), a^* (intensity of red and green) and b^* (intensity of yellow and blue), illuminant D65 (Hunter Lab, Reston, Virginia, USA) and observation angle of 10°, in colorimeter Colorquest XE (Hunter Lab). The results were given as total colour difference (ΔE), using Eq. 1.

$$\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)} \quad (1)$$

where ΔE is total colour difference, ΔL^2 is difference in lightness and darkness, Δa^2 is difference in red and green, and Δb^2 is difference in yellow and blue.

All physical analyses were carried out in triplicate.

pH measurement

The infant formulas were reconstituted according to manufacturers' instructions. The pH levels were measured using a pH meter digital PG1800 (Gehaka, São Paulo, Brazil).

Evaluation of the Maillard reaction indicators

Free HMF concentration was measured using a spectrophotometric assay in an acidified medium [22]. This measure reflects the HMF content in the sample at the time of analysis. Five millilitres of the infant formulas were diluted 6× using 5 ml of 0.3 mol·l^{-1} oxalic acid and 5 ml of 0.4 kg·l^{-1} trichloroacetic acid. They were then filtered using filter paper Whatman GR 40 (diameter of 150 mm; GE Healthcare, Chicago, Illinois, USA). Volumes of 4 ml of the filtrate and 1 ml of 0.5 mol·l^{-1} thiobarbituric acid were added to a capped test tube and placed in a water bath at 40 °C for 30 min. After cooling the solution to room temperature, read-

Tab. 1. List of ingredients and nutritional composition of infant formulas.

Brand F1		
Ingredients	Nutritional composition per kilogram	
Whey protein, vegetable oils (palm oil, canola oil, coconut oil, sunflower oil), lactose, skimmed milk powder, galacto-oligosaccharides, maltodextrin, fructooligosaccharides, calcium carbonate, *Mortierella alpina* oil, fish oil, potassium chloride, potassium citrate, calcium caseinate, sodium L-ascorbate, L-ascorbic acid, magnesium carbonate, taurine, choline chloride, dibasic potassium phosphate, ferrous sulfate, L-tryptophan, disodium salt of uridine 5-monophosphate, cytidine 5-monophosphate, zinc sulfate, myo-inositol, tribasic calcium phosphate, adenosine 5-monophosphate, disodium salt of inosine 5-monophosphate, DL-α-tocopheryl acetate, nicotinamide, guanosine 5-monophosphate disodium salt, cupric gluconate, calcium D-pantothenate, ascorbyl palmitate, DL-α-tocopherol, thiamine chloride hydrochloride, retinyl acetate, pyridoxine hydrochloride, *N*-pteroil-L-glutamic acid, potassium iodate, manganese sulfate, phytomenadione, sodium selenite, cholecalciferol, D-biotin, cyanocobalamin, mono- and diglyceride emulsifiers of fatty acids and lecithin and concentrated mixture of tocopherols	Energy [kJ]	20250
	Carbohydrates [g]	530
	Proteins [g]	98
	Total fats [g]	260
	Linoleic acid [g]	40.00
	α-linolenic acid [g]	6.86
	DHA [g]	0.52
	ARA [g]	0.91
	Fibres [g]	58.00
	FOS [g]	6.00
	GOS [g]	52.00
	Sodium [g]	1.35
	Calcium [g]	4.10
	Iron [g]	0.06
	Potassium [g]	4.90
	Chloride [g]	3.40
	Phosphorus [g]	2.03
	Magnesium [g]	0.34
	Zinc [g]	0.04
	Manganese [mg]	0.73
	Copper [mg]	2.92
	Selenium [mg]	0.11
	Iodine [mg]	0.88
	Vitamin A [mg]	4.53
	Vitamin D [mg]	0.09
	Vitamin E [g]	0.08
	Vitamin K [mg]	0.33
	Vitamin B1 [mg]	3.65
	Vitamin B2 [mg]	6.86
	Vitamin B6 [mg]	2.94
	Vitamin B12 [mg]	0.01
	Vitamin C [g]	0.68
	Niacin [mg]	31.00
	Pantothenic acid [mg]	24.00
	Folic acid [mg]	0.87
	Biotin [mg]	0.11
	Taurine [g]	0.38
	L-Carnitine [g]	0.08
	Choline [g]	0.91
	Inositol [g]	0.32
	Nucleotides [g]	0.24

Brand F2		
Ingredients	Nutritional composition per kilogram	
Demineralized whey, lactose, palm olein, skim milk, palm kernel oil, canola oil, galactooligosaccharides, corn oil, mineral salts (calcium citrate, potassium citrate, calcium chloride, sodium phosphate, magnesium chloride, sodium chloride, ferrous sulphate, zinc sulfate, cupric sulfate, manganese sulfate, potassium iodide, sodium selenate), fructooligosaccharides, maltodextrin, fish oil, vitamins (sodium L-ascorbate, DL-α-tocopheryl acetate, nicotinamide, calcium D-pantothenate, thiamine mononitrate, retinyl acetate, pyridoxine hydrochloride, riboflavin, *N*-pteroil-L-glutamic acid, phylloquinone, D-biotin, cholecalciferol, cyanocobalamin), L-phenylalanine, *Mortierella alpina* oil, taurine, myo-inositol, L-histidine, nucleotides (cytidine 5-monophosphate, disodium salt of uridine 5-monophosphate, adenosine 5-monophosphate, guanosine 5-monophosphate disodium salt), choline bitartrate, L-carnitine, soya lecithin, potassium hydroxide and citric acid.	Energy [kJ]	21200
	Carbohydrates [g]	550
	Proteins [g]	93
	Total fats [g]	280
	Linoleic acid [g]	41.00
	α-linolenic acid [g]	4.75
	DHA [g]	0.53
	ARA [g]	0.53
	Fibres [g]	30.00
	FOS [g]	3.00
	GOS [g]	27.00
	Sodium [g]	1.30
	Calcium [g]	3.20
	Iron [g]	0.06
	Potassium [g]	4.80
	Chloride [g]	3.50
	Phosphorus [g]	1.70
	Magnesium [g]	0.63
	Manganese [mg]	1.10
	Iodine [mg]	1.20
	Copper [mg]	4.00
	Selenium [mg]	0.14
	Zinc [g]	0.06
	Vitamin A [mg]	4.60
	Vitamin D [mg]	0.10
	Vitamin E [g]	0.07
	Vitamin K [mg]	0.45
	Vitamin C [g]	0.85
	Vitamin B1 [mg]	6.00
	Vitamin B2 [mg]	9.00
	Niacin [g]	0.04
	Vitamin B6 [g]	3.30
	Vitamin B12 [mg]	0.01
	Folic acid [mg]	0.76
	Pantothenic acid [g]	0.04
	Biotin [mg]	0.14
	Choline [g]	0.55
	Inositol [g]	0.55
	Taurine [g]	0.33
	L-Carnitine [g]	0.10
	Nucleotides [g]	0.16

DHA – docosahexaenoic acid, ARA – arachidonic acid, FOS – fructooligosaccharides, GOS – galactooligosaccharides.

ings were made using a UV-visible spectrophotometer UV-5100 (Global Trade Technology, Monte Alto, Brazil) at 443 nm and plotted against a standard curve of HMF (2–55 μmol·l^{-1}).

Available lysine level was measured using a spectrophotometric assay with *o*-phthaldialdehyde (OPA) [23, 24]. The infant formulas were dissolved in 0.1 kg·l^{-1} sodium dodecyl sulfate to the amount where they contained 50 μg of protein. A volume of 50 μl of sample solution was added to 2 ml of OPA reagent. The absorbance was measured at 340 nm and plotted against a standard curve of purified casein (0.003–0.01 kg·l^{-1}) dissolved in a 0.1 mol·l^{-1} sodium tetraborate buffer solution at pH 9.0. The lysine content in the purified casein was 72.4 g.kg^{-1}. The interference of free amino groups of amino acids, small peptides and amines was verified in the supernatant of samples dissolved in the sodium tetraborate buffer solution at pH 9.0. Since this interference was considered negligible, the samples were subsequently treated with 0.1 kg·l^{-1} trichloroacetic acid solution.

Lactose in the infant formulas was determined using an enzymatic kit for lactose/D-glucose (R-Biopharm, Darmstadt, Germany) according to manufacturers' instructions. This method is based on the hydrolysis of lactose to D-galactose and D-glucose with β-galactosidase, followed by determination of D-glucose.

All chemical analyses were carried out in triplicate.

Statistical analysis

The results were evaluated by analysis of variance (ANOVA), with a post hoc t-Student test as a means of comparison, for a significance level of 5 %, using Assistat statistical assistance software beta version 7.7 (Federal University of Campina Grande, Campina Grande, Brazil).

RESULTS AND DISCUSSION

Maillard reaction development in opened infant formulas stored at room temperature

Maillard reaction product formation is induced by heat treatment during infant formula production [6, 12, 25]. As previously described, excess HMF accumulation may present toxigenic and mutagenic properties that could be harmful to infant and new-born in the long term. The furfurals may cause DNA damage and growth inhibitors formation, in addition to cytotoxic, genotoxic, mutagenic and carcinogenic activities. It was reported that furfural compounds increase the number of strand breaks in duplex DNA by reaction with AT base pairs [26]. These potential risks justify HMF quantification in infant formulas.

Reconstituted infant formulas of two different brands were evaluated for HMF concentration just after opening, approximately 120 μmol·l^{-1} HMF being detected in the samples of both brands (Fig. 1A). However, HMF concentrations were expected to increase or decrease over time, depending on the everyday storage conditions simulated for the formulas [8, 13, 27, 28].

To test our hypothesis, formulas of brand F1 and brand F2 were first evaluated in everyday room temperature conditions at (24.4 ± 1.3) °C, with a relative humidity of (62.5 ± 5.0) %. The formulas were stirred daily. Once an infant formula package is opened, manufacturers recommend consumption within 30 days. Our experiments extended to 60 days to simulate also the situation when consumers disregard the recommended consumption times.

Thirty days after the formulas were opened, HMF concentration significantly increased by approximately 10 % for both brands (Fig. 1A). From 30 days to 60 days, HMF concentration increased by 14 % only for brand F2, while that of brand F1 remained constant (Fig. 1A). These results were in concordance with those of other authors who reported 20 % to 30 % increase in HMF concentration in infant formula after 90 days of storage [11, 13, 27, 29].

Intermediate compounds such as HMF polymerize throughout the Maillard reaction and form complex brown pigments (melanoidins), which are responsible for undesirable browning sometimes found in infant formulas. Thus, HMF concentration is known to strongly correlate with the colour of milk [30]. Brand F1 showed some colour variation 60 days after opening, even though the variation was not visually noticeable. No significant colour variation was observed for brand F2's formula (Fig. 1B). Others authors also found colour variation in infant formulas and, in some cases, increased colour variation after 15 days of storage was reported [7, 13, 27].

The accumulation of HMF compounds formed by the Maillard reaction results in lowered amounts of other formula components, such as lactose and lysine, both of which are precursors to the Maillard reaction. According to brand F1 and brand F2 manufacturers, lactose was the only reducing sugar present in the formulas. In the initial phases of the Maillard reaction, lysine can react with lactose and becomes blocked. Studies also suggest that lysine blockage due to the Maillard reaction is more intense in infant formulas with higher contents of lactose and proteins. [31].

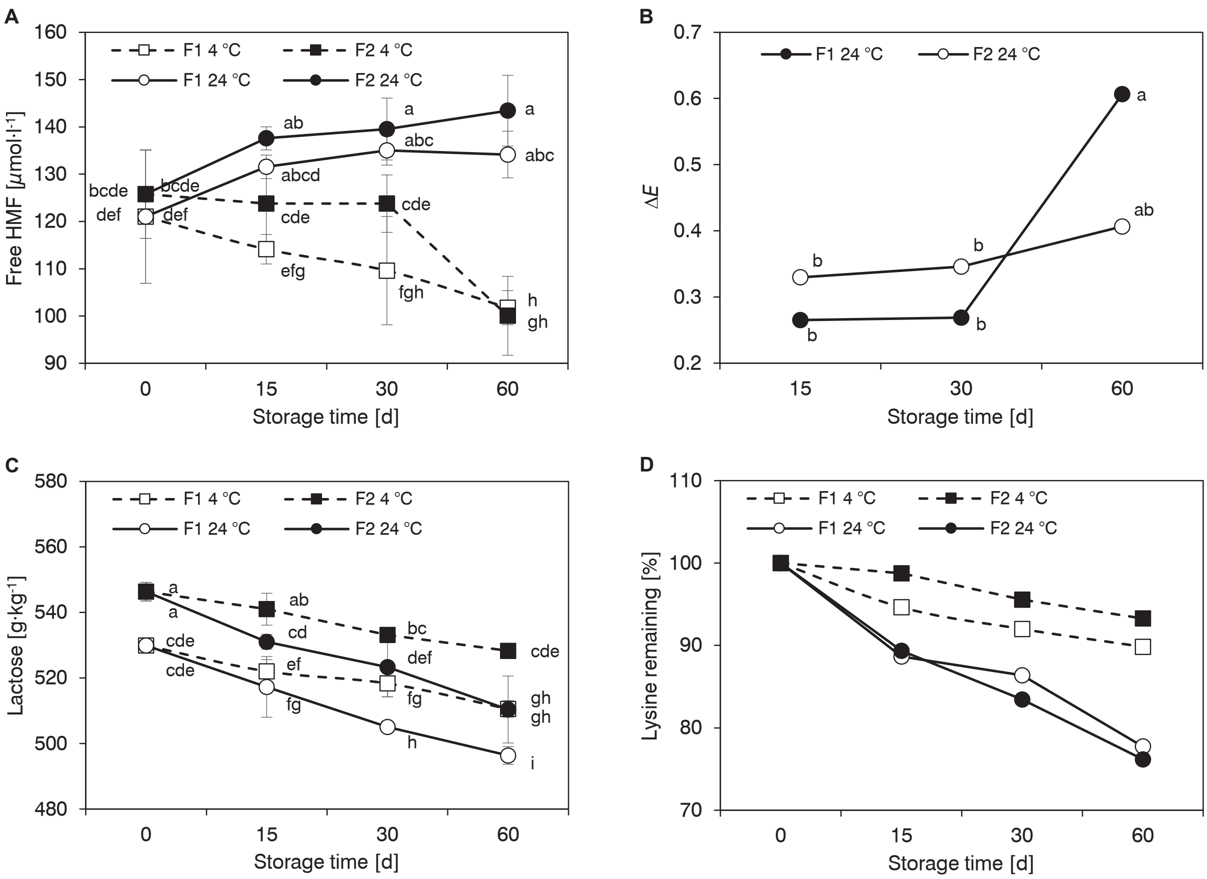

Fig. 1. Maillard reaction indicators evaluated in infant formulas stored at 24 °C and 4 °C.

A – hydroxymethylfurfural, B – colour variation, C – lactose, D – lysine remaining.
Means followed by equal letters in the same line do not differ at the 5 % level of significance.
HMF – hydroxymethylfurfural is expressed as micromoles per litre of reconstituted formulas. ΔE – colour variation expressed as the colour coordinates of powdered formulas. Content of lactose is expressed as gram per kilogram of powdered formulas. Lysine remaining is expressed as percentage of lysine remaining in powdered formulas.

Lactose is essential for infant nutrition, once it stimulates the development of intestinal microbiota, acts as a source of energy and has been linked to optimal infant brain development [32]. In the same vein, lysine is required for fatty acid metabolism and calcium absorption in bones and connective tissues. Lysine deficiency can cause kidney stones, nausea, loss of appetite, fatigue, slow growth, anemia, and other physiological disorders [25].

Fifteen days after the infant formulas were opened, significant reduction in lactose and lysine levels was found in both brand F1 and brand F2 infant formulas (Fig. 1C and Fig. 1D). Lactose content dropped from 540 g to 500 g per kilogram of formula after 60 days of storage, for a total loss of 7 %. Lysine levels dropped by 24 % over the same period (Fig. 1C and 1D).

Lysine loss of 10–35 % can be attributed to infant formula processing alone [3]. Statistically significant loss of available lysine (of approximately 30 %) was found in heat-treated infant formulas compared to the lysine levels of raw cows' milk [6]. Additional losses (12–19 %) occured over 6 months in formulas stored at room temperature [29].

In addition to the reduction of lactose and lysine levels, a decrease in pH by 0.3 units was observed in the infant formulas after 60 days of storage (Fig. 2A). This result matches those of other authors who also observed a a decrease in pH by 0.3 and 0.4 units [27, 33, 34]. The slight pH reduction is also related to the Maillard reaction following Amadori product degradation, which leads to formic acid formation during product storage [9, 33].

In addition to causing nutritional losses and browning, the Maillard reaction releases water molecules that can significantly alter a_W and moisture content of infant formulas over time [35]. Even with a minimal increase in moisture content, water is known to have a strong plasticizing effect that reduces the glass transition temperature of powder. This induces degradative changes in dried milk products, such as stickiness, caking, collapse and acceleration of non-enzymatic browning [36].

Furthermore, once opened, dairy powders can absorb more water molecules. This leads to additional increase in moisture. Increased water content can boost Maillard reaction kinetics that then accelerate nutritional losses and browning processes [37]. Losses of lysine at aw of 0.44 (whey powder), 0.3–0.6 (skim milk powder) and 0.3–0.7 (whey protein concentrate) were reported [38].

Browning rates begin to increase when a_W approaches a value between 0.2 and 0.3, reaching a maximum at a_W of 0.5–0.8 [7, 39]. In this work, the water activity of the infant formulas studied ranged from 0.22 to 0.35 (Fig. 2B). Increase in humidity of 39.2 % and 41.7 % were noted also in this study for brand F1 and brand F2, respectively (results not shown). It can, therefore, be stated that a_W and relative humidity favour the Maillard reaction rates, which helps to explain HMF accumulation over time for both brands (Fig. 1A).

Maillard reaction development in refrigerated opened infant formulas

In the previous section, the effects of the Maillard reaction on opened infant formulas stored at room temperature were demonstrated. However, once both the environment temperature and humidity are lowered, the reaction rates should decrease accordingly. In other words, refrigerating the opened infant formulas can attenuate the nutritional losses and browning that may occur. To investigate this hypothesis, opened infant formulas of brand F1 and brand F2 were stored at (3.8 ± 1.8) °C at a relative humidity of (16.6 ± 4.8) % for 60 days simulate conditions of consumer use.

Contrary to what was observed when the infant formulas were stored at room temperature, HMF levels dropped to ~100 μmol·l^{-1} for reconstituted formulas for both brands after 60 days of refrigerated storage (Fig. 1A). However, lactose and lysine levels of the formulas continued to decrease by 3.5 % and 8.5 %, respectively, on average (Fig. 1C and Fig. 1D). These results can be explained by the simultaneous formation of HMF molecules from the Maillard reaction along with the degradation of HMF molecules via oxidation or other transformations, such as decarboxylation, hydration or reduction [40–42]. Therefore, the rate of HMF generation could be estimated to be lower than the degeneration rate when the infant formulas were stored at low temperatures. This resulted in the decrease in HMF levels over time (Fig. 1A).

Conversely, lactose and lysine molecules in the infant formulas studied continued to react and generate HMF molecules, albeit at a lower rate (Fig. 1C and Fig. 1D). A minor decrease in HMF levels was previously reported at 6 °C in infant formulas [43]. Other authors demonstrated that by reducing the storage temperature of lactose-hydrolysed skim milk powder from 20 °C to 4 °C, lysine loss was drastically reduced from 55 % to 3 % [44].

No colour variation was observed in the refrigerated infant formulas as there was no sufficient melanoidin formation. The infant formulas' pH levels were similar to those stored at room tem-

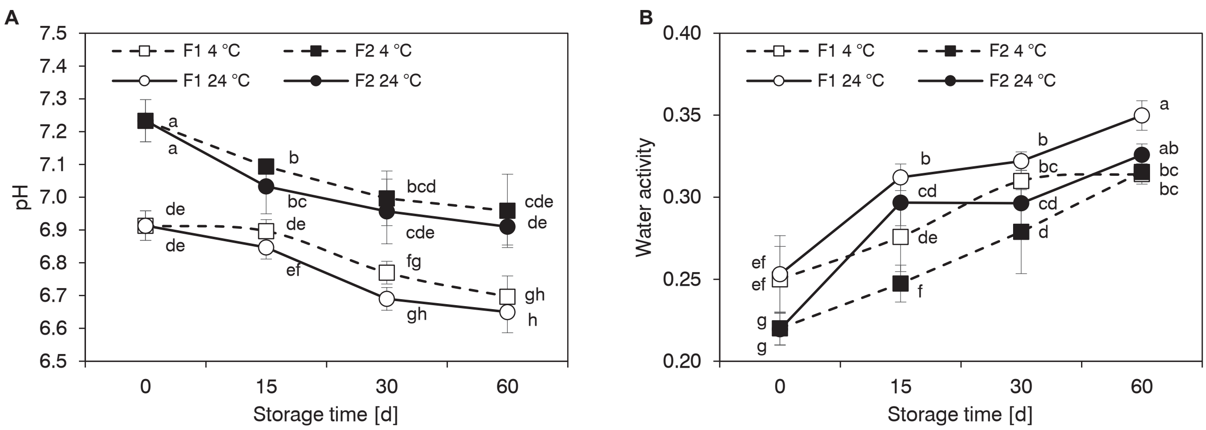

Fig. 2. Other indicators evaluated in infant formulas stored at 24 °C and 4 °C.

A – pH of reconstituted formulas, B – water activity of powdered formulas.
Means followed by equal letters in the same line do not differ at the 5 % level of significance.

perature (Fig. 2A). However, powders stored at ~4 °C showed lower gain in a_w (Fig. 2B) and moisture after 60 days.

The Maillard reaction in infant formulas

Based on our findings and on previously published research, we propose the following hypothesis for Maillard reaction propagation in opened infant formulas. In infant formulas where lactose is the only reducing sugar present, lactose reacts with other compounds during the initial stages of the Maillard reaction. Lactose reacts with proteins and amino acids such as lysine, arginine, methionine, tryptophan and histidine, to form Schiff bases [25, 45]. Of all the amino acids that react during the Maillard reaction, lysine's increased reactivity should be singled out because its side chain contains a free ε-amino group [9]. Note that during the reaction between lactose and lysine, one molecule of water is released. This can alter a_w and moisture of the infant formula powder. Due to the unstable nature of Schiff bases, additional isomerization (Amadori re-arrangement) occurs in an Amadori product (lactulosyl-lysine), under slightly acidic conditions. At this stage, the amino acid becomes nutritionally unavailable and its digestibility is also reduced [7, 10].

When subjected to prolonged heat or storage, the Amadori products undergo an isomerization reaction called 1,2-enolization, in which the ketone group of carbon 2 is broken down and a new double bond is formed between carbons 1 and 2. The Amadori products in their isomerized form are hydrolysed, releasing lysine and a molecule that is degraded under acid or neutral conditions. This degradation forms furfural compounds such as HMF and galactose [9]. Another molecule of water is released during this reaction, which can then alter a_w and moisture of powder. The furfurals can be produced by the Maillard reaction and by lactose isomerization, known as the Lobry De Bruyn-Alberda van Ekenstein transformation [41]. Amadori product degradation also generates formic acid, which is responsible for the slight decrease in pH in stored dairy products. Formic acid was reported as a main degradation reaction product for the Maillard reaction of lactose [46].

Because the initial steps of the Maillard reaction depend on environmental temperatures, refrigerating the opened infant formulas reduces both formation rates of Maillard reaction intermediates and water gain (Fig. 1A and Fig. 2B). A zero-order reaction is observed for HMF accumulation in milk systems [47, 48]. Accumulation is then expected to occur at a lower speed when the formula is stored at 4 °C. When storage temperature is reduced twice, the reaction rate is expected to decrease by 9 to 16 fold compared to that originally observed, since the temperature coefficient (Q_{10}) of the reaction is 3–4 [31]. Moreover, the substantial decrease in HMF accumulation due to refrigeration was previously attributed to the possibility of loss of HMF generated due to decarboxylation or other transformations, such as oxidation, hydration, reduction and reactions to form intermediates such as 2-furaldehyde, 2-furylmethyl ketone and 5-methyl-2-furaldehyde [40]. Hence, several authors have recommended milk storage at ≤ 4 °C. They went on to specify that refrigeration should be required for milk that had been lactose-hydrolysed in order to limit protein damage [49–51].

Finally, the compounds generated are cyclic and highly reactive. This results in polymerization of the formed intermediate compounds with carbohydrates and proteins to form high molecular weight compounds and the formation of dark pigments, known as melanoidins, which cause undesirable colour changes in some dairy products [7].

CONCLUSIONS

For a 6-month infant weighing 7.5 kg who consumes 1 l of infant formula each day, the worst case HMF consumption scenario to come out of our study (for brand F2 infant formulas stored at room temperature for 60 days) would total 19 μmol HMF per day. The recommended maximum consumption of HMF for adults of 20 $\mu mol \cdot kg^{-1} \cdot d^{-1}$ would allow an infant to safely intake 150 μmol of HMF per day. By considering that the maximum consumptions of HMF for adults and infants are similar, it can, therefore be said that after 60 days of storage, HMF levels in infant formulas do not pose a significant health risk, although the nutritional losses that regard other components that were not analysed may limit the extent of product validity. However, it should be kept in mind that no recommended daily intake of HMF for infants has yet been determined, despite the health risk the compound may pose at high doses. Therefore, the ideal HMF intake for the infant may be much lower than 20 $\mu mol \cdot kg^{-1} \cdot d^{-1}$.

Refrigerated infant formulas showed lower lactose and lysine loss while maintaining the powders' original colour even after 60 days. Thus, refrigerating the opened infant formulas is recommended in order to avoid physico-chemical changes resulting from extreme conditions of processing, transport and storage that can happen.

Future research will be focused on studying more sensitive infant formula components, such as vitamins and fatty acids, in order to determine the best storage conditions and expiration dates for infant formulas.

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