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Assessment of protein glycation markers in infant formulas

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Abstract

The typical formulation of infant formulas (IF) may enhance damage of proteins during spray drying or sterilization. Lactose reacts with amino groups of proteins resulting in the formation of lactulosyllysine (LL). The latter is further degraded to give advanced glycation end-products (AGEs). Furthermore, oxidation reactions, which are catalyzed by iron can further promote AGE formation. In this study, six parameters for protein modification were measured for 41 commercially available IF samples: lysine blockage, tryptophan degradation and LL formation by HPLC, the AGEs N^{ε}-carboxymethyllysine (CML) and oxalic acid monoalkylamide (OMA) by ELISA. The FAST index was used as a rapid method for monitoring AGEs. IF showed increased lysine loss (6-fold), LL formation (2–3-fold) and AGEs levels (3–5 times) (CML, OMA, and FAST index) compared to similarly treated cow's milk, indicating more severe protein modifications in the former. Further studies on the technological processes are required to minimize heat-induced damage of IF during manufacturing.

Keywords: Infant formulas; Advanced glycation end-products; Protein glycation; Lactulosyllysine; Carboxymethyllysine; Fluorescence; Nutritional quality

1. Introduction

The composition of mature human milk, which covers the nutritional needs of healthy infants up to 5–6 months from birth, is used as the standard for manufacturing milk-based infant formulas (IF). Hence, the major components of raw cow's milk (CM) (proteins, carbohydrates, lipids), as well as the minor components (minerals and vitamins), must be adjusted to mirror the human milk levels.

The carbohydrate content of infant formulas (essentially lactose) is set at levels comparable to human milk (60–70 $g\,l^{-1}$). The protein content is typically 2–3 times higher than that of breast milk. Iron, due to its very low bioavailability (Garcia, Alegria, Barbera, Farre, & Lagarda, 1998), is set at levels nearly 10 times those found in human milk and 25 times that of CM. IF are also typically enriched in vitamin C, not only for nutritional purposes, but also to increase iron intestinal absorption (Gillooly et al., 1984).

To ensure microbiological safety and to extend the shelf-life period, IF constituents are blended, pasteurised, homogenised, concentrated, spray-dried or heat-sterilised, and canned in modified atmosphere containers (N_2/CO_2). The sterilisation, through UHT (130–140 °C, 3–6 s), in some cases followed by in-bottle sterilization (110 °C, 10 min), or spray-drying in towers, where temperature at the surface of milk droplets reaches 70–80 °C, leads to major changes in the initial composition of the infant formula. To date, these changes are only partially characterized.

Of particular significance is the reaction of lactose with lysine side chains of the milk proteins in the Maillard reaction. The first stable product of this reaction is

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lactulosyllysine (LL), which leads to a loss of nutritional value due to blockage of the essential amino acid lysine. This loss of nutritional value is further compounded through decreased digestibility. It has recently been shown that, aside from lactose, ascorbic acid is also a very potent protein glycation agent (Leclère & Birlouez-Aragon, 2002). Prolonged thermal treatment results in further degradation of the primary reaction products, yielding advanced glycation end-products (AGEs). Recently it was shown that, in diabetic patients, a diet rich in AGEs leads to the induction of inflammatory mediators (Vlassara et al., 2002).

Furthermore, pro-oxidative conditions, such as the presence of iron/ascorbic acid (Almaas, Rootwelt, Oyasaeter, & Saugstad, 1997), can promote AGE formation and also tryptophan degradation (Puscasu & Birlouez-Aragon, 2002).

The purpose of this study was to investigate how the typical composition and processing of IF influence protein modifications and protein damage.

Therefore we examined 41 samples of commercially available infant formulas from Germany, France and Spain. The products were either in liquid (UHT and in bottle sterilized; L-IF) or powdered form (spray-dried, P-IF). Nutritional damage was evaluated by the analyses of lysine blockage and of tryptophan degradation after enzymatic hydrolysis of the milk proteins. The early glycation product, lactulosyllysine, was measured after conversion into furosine, and two specific AGE structures, CML and OMA were determined by ELISA. The results were compared to similar processed milk products (UHT milk and milk powder). The fluorimetric FAST assay was used as a rapid method to monitor the quality of processed formulas (Birlouez-Aragon, Sabat, & Gouti, 2002).

2. Materials and methods

2.1. Chemicals

Hexane, acetonitrile and hydrochloric acid were from PROLABO. Enzymes for hydrolysis, thymol, norleucine, sodium hydroxide, bovine serum albumin (BSA), tetramethylbenzidine, and anti-rabbit IgG peroxidase conjugate (from goat) were from Sigma (France). 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate and eluent for amino-acid analysis were from Waters (France).

2.2. Milk samples

Forty-one commercial infant formulas (IF) were purchased in France, Spain and Germany from the most representative national and international manufacturers. Twenty-four of the IF were liquid formula (L-IF, 19 UHT sterilised, and 5 in-bottle sterilised), and 17 P-IFs. Most IF ($n = 30$) had casein/whey ratios of 80/20, similar to CM, and 11 samples had modified casein/whey ratios of 60/40, 50/50, and 40/60, near the human milk ratio of 40/60. Seventeen of the formula were IF for infants up to 4–6 months (10 powders, 2 UHT sterilised, five in-bottle sterilised), 12 were follow-on IF for infants 4–12 months (7 powders, 5 UHT sterilised) and 12 were growth milk IF for 10 months to 3 years (UHT sterilised). Seven UHT-treated CM samples (L-CM) and 7 milk powder's (P-CM) (5 medium-heat and 2 low-heat treated) were purchased in France. Two milk samples treated at 65 °C were the gift of ARILAIT (France) and were used to measure the native lysine content of CM.

2.3. Amino acid analysis after enzymatic hydrolysis

Milk samples were enzymatically hydrolysed as described by Henle, Walter, and Klostermeyer (1991) by successively incubating the lipid-free milk samples for 24 h with pepsin, pronase E and prolidase and finally with aminopeptidase. To ensure total hydrolysis of lysine, seven different raw and pasteurised CM samples were measured. A mean amount of 86.0 mg g^{-1} lysine was recovered (expected level is 83 mg g^{-1}).

Enzymatically (Enz-Lys) and acid-hydrolysed (Ac-Lys) lysine were quantified by reversed-phase HPLC-fluorescence using the AccQTag method, as described by Cohen and Michaud (1993). After derivatization by 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC), lysine was separated by gradient HPLC on a AccQ-Tag^{TM} C_{18} column (Waters) at 37 °C. The lysine concentration in low (65 °C) heat-treated milk samples was determined as 88.7 ± 0.1 mg g^{-1} protein. For whey-enriched formulas the FAO/WHO (1990) concentrations of 99 mg g^{-1} for whey and 83 mg g^{-1} for casein were used to calculate the expected lysine level of protein concentration indicated by the manufacturer.

Enzymatically-released tryptophan (Enz-Trp) was quantified by reversed-phase HPLC-fluorescence on a C_{18} Spherisorb reverse-phase column, as described previously (Puscasu & Birlouez-Aragon, 2002). For comparison, the Trp concentrations of raw CM (14.8 mg g^{-1}) and whey (16.8 mg g^{-1}) were taken from FAO/WHO (1990).

Total protein content was quantified by the official Kjeldahl procedure and the pH 4.6 soluble protein fraction was quantified by the Lowry method.

2.4. Fluorescence of advanced Maillard products and soluble tryptophan method

This method is based on the simultaneous determination of Trp fluorescence (λ_{exc} 290 nm; λ_{em} 340 nm) (FW) and of the fluorescent advanced Maillard products

(λ_{exc} 330 nm ; λ_{em} 420 nm) (FAST index) in the acid soluble milk fraction (acetate buffer at pH 4.6), as described by Birlouez-Aragon et al. (2002). The ratio between FW (in counts of photon emitted per second, cps) and the protein concentration (in $g\,l^{-1}$) (FW $prot^{-1}$) is an indication of the degradation of the amino acid in the oxidative-sensitive acid-soluble protein.

2.5. Determination of lactulosyllysine after conversion into furosine

The samples (1.5 ml, containing ~45 mg protein) were hydrolyzed in the presence of 8 ml of 8 M HCl under nitrogen at 110 °C for 23 h in a screw-cap Pyrex-vial. Furosine was measured by ion-pair HPLC with a C8 Alltech Furosine-dedicated column maintained at 35 °C after solid-phase separation in a pre-wetted Sep-pak C18 cartridge, as described by Resmini, Pellegrino, and Battelli (1990).

2.6. CML and OMA determination

OMA contents were measured by ELISA, as described in the literature (Hasenkopf, Übel, & Bordiehn, 2001). The samples were diluted 1:10 prior to analysis. The results represent the mean values of five determinations. Since the antibody affinities to free and protein-bound antigen are different, units were defined for quantification. One unit is equal to the response which was obtained for 1 ng synthesised OMA-β-lactoglobulin. OMA-β-lactoglobulin was prepared according to the literature (Hasenkopf et al., 2001). The modification rate was $49.8 \pm 3.5\%$, as determined by TNBS (Fields, 1972) and GC/MS (Hasenkopf, Rönner, Hiller, & Pischetsrieder, 2002). Thus, 1 ng of OMA-β-lactoglobulin equals 0.4 pmol OMA. CML determinations were carried out with a non-commercial ELISA developed by Roche Diagnostics GmbH (Germany), using the monoclonal anti-CML antibody 4G9 from Alteon (Ramsey NJ, USA) (Mellinghoff, Reininger, & Wuerth, 1997). The streptavidine-coated microtitre plates were coated with biotinylated BSA-AGE at room temperature for 1 h. Unless otherwise noted, the wells were washed 3 times with washing buffer after each step. Fifty microlitres of standard (6-(*N*-carboxymethylamino)caproate, 0–140 ng/ml), or samples (diluted 1:250) in water plus 50 μl of horseradish peroxidase-labeled CML antibody, were added. The plates were incubated for 1 h at room temperature. One hundred microliter of ABTS® substrate were added and incubated for 30 min. Absorption was measured at 405 nm. Samples were analyzed in triplicate. The CML content of the samples was quantified using the calibration curve of the standard. The results are given in ng CML · mg^{-1} protein and are the mean values of three determinations.

2.7. Statistical analysis

Comparisons of means and value distributions were done by parametric and non-parametric tests using Origin 5.0 (Microcal). A principal components analysis (PCA) was performed on the data matrix composed of column-centered and column-standardised values for the eight variables measured on the 55 milk samples. PCA (Vandeginste, Massart, Buydens, DeJong, Lewi, & Smeyers-Verbeke, 1998) is a multivariate statistical method which decomposes a data matrix with n rows (samples) and p columns (variables) into the product of a scores matrix, with n rows (samples) and $d < p$ columns (principal components), and a loadings matrix, with $d < p$ rows (principal components) and p columns (variables). The scores are the positions of the samples in the space of the principal components and the loadings are contributions of the original variables to the PCs. All PCs are mutually orthogonal, and each successive PC contains less of the total variability of the initial data set. Usually only a limited number $d < p$ of PCs are retained as the variability in the others is due to noise. These analyses were done using CATS, a statistical program developed in-house and validated using Matlab.

3. Results

3.1. General

Seven parameters of protein modifications have been determined in 41 commercial IF and in similarly processed CM. The loss of the essential amino acids lysine and tryptophan was determined by HPLC and early Maillard reaction products were quantified by HPLC after conversion into furosine. Furthermore, two structurally-defined AGE compounds, CML and OMA, were determined by ELISA. The FAST method allowed us to obtain the two parameters, FAST index and FW/protein, indicators of the global advanced Maillard reaction and of protein degradation in acid-soluble proteins respectively (Puscasu & Birlouez-Aragon, 2002).

3.2. Lysine and tryptophan loss

Amino acid composition of the IF was measured after enzymatic digestion of the proteins. Thus, tryptophan degradation and partial release of lysine from LL expected from acidic hydrolysis was avoided. Table 1 shows the levels of lysine loss in IF during sterilisation or drying, compared to similarly processed CM. L-IF with a whey to casein ratio similar to CM showed a 6-fold higher damage of lysine (20.7%) than to the CM samples (3.7%; $p < 10^{-5}$). In the L-IF, which were enriched with whey, lysine blockage tended to be higher

Table 1
Lysine loss (%) in P-IF and L-IF formulas compared to similarly composed CM

	L-IF [80/20] ($n = 19$)	P-IF [80/20] ($n = 11$)	L-IF [60–40]/[50–50] ($n = 5$)	P-IF [60–40]/[50–50] ($n = 6$)
Mean	20.7	16.9	25.2	30.4[a]
Standard	7.7	8.0	10.0	7.9
Quartile 1	16.8	10.9	21.4	26.5
Median	20.3	18.8	24.2	29.4
Quartile 3	21.3	20.3	31.6	36.8

Note: Lysine loss is calculated, as indicated in Section 2, by comparison with the lysine concentration measured in low-heat L-CM for 80/20 composed IF, and by comparison with the expected value for other formulations, using the whey and casein lysine concentrations given in FAO/WHO, 1990. Brackets indicate the whey-to-casein ratio of the formula. UHT L-CM samples had a mean lysine concentration of 85.4 ± 2.5 mg g^{-1} ($n = 7$), mean loss of 3.7% and medium-heat spray-dried P-CM had a mean lysine concentration of 74.8 ± 0.8 mg g^{-1} ($n = 3$; mean loss of 15.7%).
[a] Difference between whey-enriched and 80/20 formulas is significant ($p = 0.007$).

(25.2%) than in the other L-IF (NS; $p = 0.28$). In powdered regular IF (P-IF), lysine blockage (16.9%) was slightly, but not significantly higher than in CM powder (15.7%). However, in the P-IF, in which the whey to casein ratio was adjusted, lysine was significantly more degraded (30.4%) than in regular P-IF ($p < 0.01$). Thus, liquid and powder IF had similar lysine losses which were 6- to 10-fold higher than that of regular liquid UHT CM.

The total tryptophan contents of L-IF, with non-adjusted whey to casein ratio, and of P-IF were similar to the value of similarly processed CM (Table 2). L-IF with adjusted whey to casein ratio, tended to higher tryptophan degradation (13.8%) than adjusted P-IF ($p = 0.13$). Tryptophan degradation was also measured in the acid soluble protein fraction, which is particularly sensitive to oxidation (Table 3). A trend to higher tryptophan degradation (+30%) was observed in L-IF compared to P-IF ($p = 0.089$) and CM samples ($p = 0.057$). When quantified by means of fluorescence (FW/protein) the difference between powder and liquid IF was highly significant ($p < 10^{-7}$), as also was the difference between powder and liquid CM (1.07 ± 0.19 and 0.60 ± 0.16, respectively, $p < 10^{-5}$), indicating a specific degradation of tryptophan in the acid-soluble proteins of sterilised samples.

3.3. Formation of lactulosyllysine and AGEs

The formation of the early glycation product LL was measured after its convertion into furosine. Furthermore, the AGEs, CML and OMA, were determined by ELISA. The FAST method is a rapid fluorimetric test which allows simultaneous evaluation of advanced protein glycation and protein oxidation.

Furosine was significantly higher (2.6-fold, $p < 10^{-5}$) in L-IF than in similarly processed and composed L-CM, but the difference between P-IF and P-CM was not significant. The whey liquid and powdered enriched formulas had 50% higher furosine concentrations than the respective IF ($p < 0.05$). Thus, furosine was 3 and 2 times higher in adjusted liquid and powder formulas, respectively, than in regular sterilised CM ($p < 10^{-3}$). The mean furosine concentration was 1.4 times higher in powders than in liquid IF samples ($p < 0.05$) (Fig. 1).

AGE concentrations, measured as CML and OMA, were 3- and 8-fold higher in L-IF than in L-CM samples, and 2.5 and 5 times higher in P-IF compared to milk powder ($p < 10^{-3}$ to 10^{-5}). Among the infant formulas, the liquid formulas showed significantly higher damage than the powders ($p < 10^{-2}$ to 10^{-3}). A global 2.5 times increase in fluorescent AGEs was observed in IF compared to similarly processed CM ($p < 0.01$), the levels

Table 2
Tryptophan concentration in P-IF and L-IF infant formulas and in CM samples A-in total milk proteins

	L-IF [80/20] ($n = 19$)	P-IF [80/20] ($n = 11$)	L-IF [60/40]/[50–50] ($n = 5$)	P-IF [60–40]/[50–50] ($n = 6$)
Tryptophan (mg g^{-1})	15.8 ± 2.1	16.4 ± 0.7	14.4 ± 2.2	16.7 ± 2.1

Note: Brackets is indicated the whey-to-casein ratio of the formula. UHT L-CM samples had a mean Trp concentration of 14.8 ± 1.3 mg g^{-1} ($n = 7$) (similar to the concentrations given by FAO/WHO (1990) of 14.8 mg g^{-1}). The expected Trp concentration in adjusted IF is 16.8 mg g^{-1} FAO/WHO (1990).

Table 3
Tryptophan concentration in P-IF and L-IF infant formulas and in CM samples B- in acid-soluble proteins

	L-CM ($n = 7$)	L-IF ($n = 18$)	P-IF ($n = 16$)
Acid soluble protein (g l^{-1})	3.3 ± 0.9	3.0 ± 0.8	2.8 ± 1.5
Tryptophan (mg l^{-1} protein)	33.4 ± 16.1[a]	23.5 ± 9.1[a,b]	34.5 ± 25.5[b]
FW/protein (cps g^{-1})	0.60 ± 0.16	0.50 ± 0.22[b]	0.94 ± 0.22[b]

[a] Comparison between L-CM and L-IF ($p = 0.057$ for Trp in mg l^{-1}).
[b] Comparison between L-IF and P-IF ($p = 0.089$ for Trp in mg l^{-1} and $p < 10^{-7}$ for FW/protein).

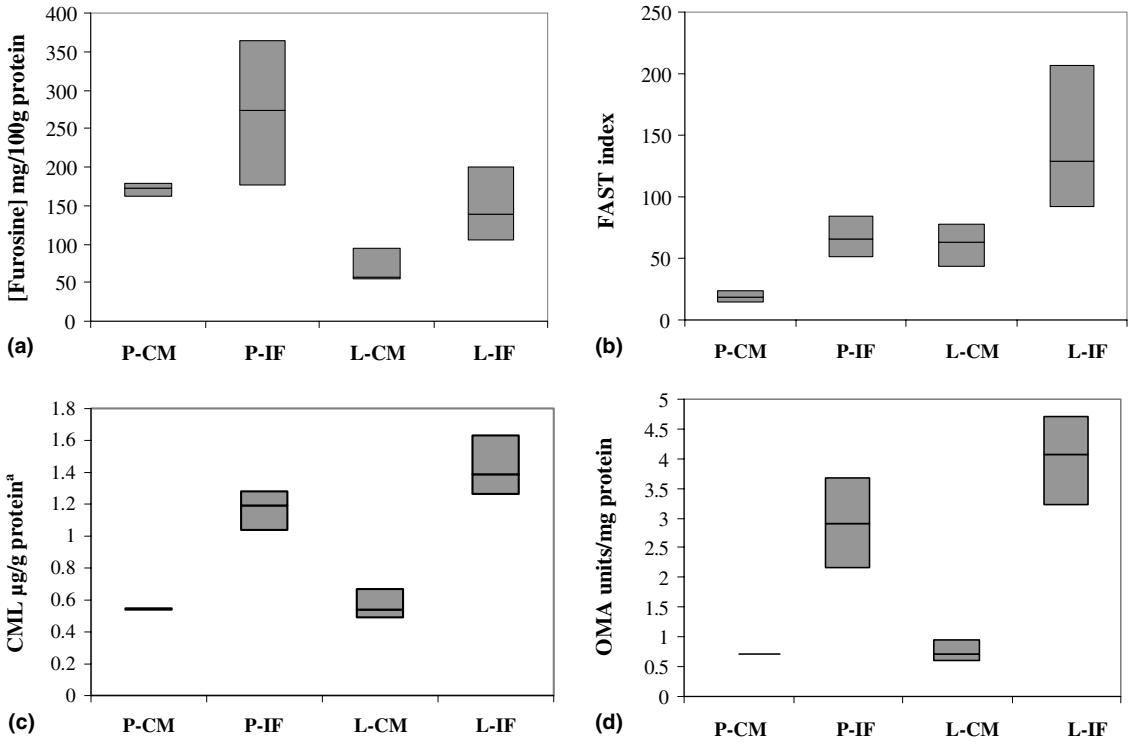

Fig. 1. Median and first/third quartiles for the different indicators of the Maillard reaction in powder CM (P-CM), liquid CM (L-CM), P-IF, and L-IF.

being 2.3 times higher in liquid sterilised than in spray-dried powders ($p < 0.01$). A significantly higher protein damage of in-bottle sterilised L-IF compared to UHT L-IF ($p < 0.01$) was revealed by the indicators OMA, CML and furosine (Table 4). The increase in the FAST index of sterilised L-IF was not significant.

The samples were statistically analysed all together by applying a principal component analysis (PCA) to the different variables. The PC1–PC2 scores plot in Fig. 2(A) shows a clear discrimination (along the horizontal PC1 axis) between infant formulas and CM and (along the PC2 vertical axis) between sterilised liquids and powders. The area occupied by the infant formula samples in the PC1–PC2 scores plot is much greater than that occupied by the CM samples, indicating a much higher variability of these samples. As can be seen in the loadings plot (Fig. 2(B)), the PC1 axis (40% of the variability) is due to a combination of CML, OMA, FAST index and furosine as significant positive contributions. On the other hand, FW/protein (FAST indicator for Trp degradation in the acid-soluble proteins) and the lysine protein content make significant negative contributions. The enzymatic tryptophan (Enz-Trp) content does not make a significant contribution to PC1. The PC2 axis was composed of a combination of the lysine content (Enz-Lys) and the FAST index in the positive direction and of FW/protein and furosine as negative contributions (Fig. 2(B)).

4. Discussion

Infant formulas are produced from CM, which is modified to mimic human milk and address the specific nutritional needs of the infant. The bacteriological

Table 4
Indicators of the Maillard reaction in UHT ($n = 19$) and in-bottle sterilised ($n = 5$) L-IF samples

		Mean	Standard deviation	Quartile 1	Median	Quartile 3
FAST No units	UHT	162	103	96.7	129	206
	Sterilised	228	139	136	167	241
CML ($\mu g\,g^{-1}$)	UHT	1.39[a]	0.21	1.24	1.38	1.43
	Sterilised	1.68	0.24	1.61	1.76	1.79
OMA (Units mg^{-1})	UHT	4.14[a]	1.42	3.22	4.09	4.70
	Sterilised	6.51[a]	1.09	6.61	6.68	7.24
Furosine (mg 100 g^{-1})	UHT	159[a]	69.2	105	139	201
	Sterilised	252	65.0	212	244	289

[a] Significant difference at the 0.01 level between UHT and in-bottle sterilised L-IF samples.

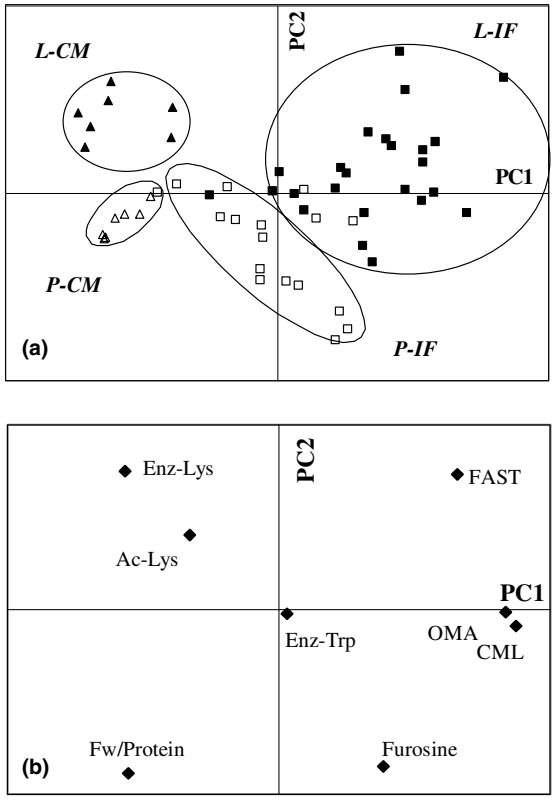

Fig. 2. Principal component analysis of L-IF and P-IF infant formulas, as well as similarly processed CM (L-CM and P-CM). (A) PC2 scores plot for the 55 milk samples and (B) PC2 loadings plot of the eight centred and standardised variables. The eight variables were analysed as indicated in Section 2: furosine (mg 100 g^{-1} protein), FAST index, fluorescence of pH 4.6-soluble tryptophan divided by the soluble protein content (FW $prot^{-1}$) carboxymethyllysine (CML, μg g^{-1} protein), oxalic acid monoalkylamide (OMA, units mg^{-1} protein), acid- or enzymatically-hydrolysed lysine (Ac-Lys and Enz-Lys, mg g^{-1} protein) and enzymatically-hydrolysed tryptophan (Enz-Trp, mg g^{-1} protein).

safety of these formulas is extremely well controlled and ensured, either by sterilization or spray-drying, followed by storage under modified atmospheric conditions. However, the chemical changes that take place during this heat treatment are not fully known.

In this study, 41 IF were analysed, which are representative of the products on the French, Spanish and German market. Several parameters of heat damage, which occur during manufacturing, were measured: modification of the essential amino acids lysine and tryptophan, furosine formation as a marker for LL, and the AGEs CML and OMA. Furthermore, the FAST index was determined, which is a rapid method to measure simultaneously, formation of fluorescent AGEs and tryptophan degradation in acid-soluble proteins.

Liquid infant formulas showed 6 times higher lysine loss (20%), due to 2–3 times more formation of lactulosylysine and AGEs, than similarly treated CM (4%). Increased lactose levels have been shown to promote lysine blockage through early glycation reaction (Birlouez-Aragon, Moreaux, Nicolas, & Ducauze, 1997). Some IF are supplemented with honey, glucose and other ingredients, which have been shown to magnify the extent of non-desired glycation reactions. Other formulas substitute lactose with maltodextrin, a carbohydrate with lower glycation potential; the result is a reduction in the glycation level (Evangelisti, Calcagno, & Zunin, 1994). Highest lysine degradation and formation of LL was measured in infant formulas (up to 50%) which were enriched with whey protein, possibly because of the poor quality of the whey used (most powder whey contain very high concentrations of furosine, data not shown). The damage was also significantly more severe in in-bottle sterilised IF than for UHT IF. Liquid infant formulas showed significantly higher AGE modifications than powdered formulas, but the latter had more LL than liquid formulas. This accumulation can be explained by the low water activity (0.4–0.8) attained during the drying process, which favours early glycation reactions. On the other hand, the high temperatures, which are applied for sterilisation, enhance oxidation and degradation reactions, explaining the almost 3 times higher levels in specific or globally fluorescent AGEs in liquid formulas. Processing of infant formulas leads to oxidative degradation of vitamin C (up to 50%, AFSSA, France). This loss is compensated by supplementation of vitamin C before processing. It was shown, however, that oxidation products of vitamin C are very potent precursors for AGE formation (Leclère & Birlouez-Aragon, 2002).

Another important factor is iron, which is added at concentrations 10 times higher than in human milk and 25 times than in CM. Metal ions, particularly in the presence of ascorbic acid, catalyze oxidation reactions (Almaas et al., 1997). Therefore they strongly activate AGE formation and catalyze degradation of nutrients. Tryptophan degradation was not significant in infant formulas, despite a specific degradation in the acid-soluble protein fraction of liquid sterilised milk samples.

From this study, it can be concluded that, compared to regular CM products, IF are particularly prone to protein degradation and modifications during thermal treatment, depending on the product's specific formulation. The loss of the essential amino acid lysine in IF is compensated by higher protein concentrations in the formulas (16–22 $g\,l^{-1}$), which is about twice as much as in human milk (8–10 $g\,l^{-1}$). Thus, nutritional lysine deficiency is not expected in formula-fed infants. However, excess of protein in infant nutrition should be avoided, because it leads to excessive urea production and excretion (Lönnerdal & Hernell, 1998). Furthermore, increased protein intake leads to higher exposure of AGEs. A maximum daily uptake of 7 mg of CML and 20 μg of OMA kg^{-1} body weight was calculated in IF-fed infants (based on 1 l milk per day for a 6 kg infanct), as compared to 0.13 mg kg^{-1} and 0.2 μg kg^{-1} body weight for an adult ingesting 0.5 l UHT-milk per day

(mean weight 60 kg). The physiological relevance of food derived AGEs is not fully clear. However, some studies suggest that AGEs are partially resorbed (Koschinsky et al., 1997; Šebeková, Krajčovičová-Kudlá čková, & Schinzel, 2001). Furthermore, it was shown that a diet rich in AGEs induces inflammatory mediators and decrease insulin sensitivity, in both animals and humans (Hofmann et al., 2002; Lin et al., 2002; Vlassara et al., 2002). Although these physiological effects could not be related to a specific AGE-structure, there is growing evidence that, at least in vitro, CML triggers cellular reactions (Boulanger et al., 2002; Kislinger et al., 1997; Miyahara et al., 2002). Further studies are therefore required to evaluate the physiological relevance of AGEs ingested in infant formulas. Nonetheless, it is important to search for means to minimize protein damage during processing of infant formulas.

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