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Liquid Infant Formulas: Technological Tools for Limiting Heat Damage

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In a study considering 15 commercial samples of liquid milk-based infant formulas (MBF) from different manufacturers, the levels of selected molecules, that is, furosine (FUR), galactosyl-β-pyranone (GAP), lactulose (LCT), and lysinoalanine (LAL), have been measured to provide estimation of the heat damage in these products. The ranges of the studied markers were as follows: FUR = 153−600 mg 100 g^{-1} of protein, GAP = 0.5−4.3 mg L^{-1}, LCT = 226−1511 mg L^{-1}, and LAL = 1.0−16.1 mg 100 g^{-1} of protein. The highest levels were found in MBF intended for the youngest babies. Experimental samples were produced in an industrial plant to evaluate the relative contribution of individual technological aspects to the final heat damage. About 90% of both GAP and LCT contents was due to the ultrahigh-temperature sterilization process itself. This effect was more than halved when the pH of the ingredient mixture was adjusted from 7.2 to 6.9 before sterilization or when the product recirculated in the plant was discarded. Up to 60 and 20%, respectively, of the FUR and LAL levels in the finished product were already present in protein ingredients (whey powder, whey protein concentrate). Accurate optimization of processing conditions and scrupulous selection of raw materials proved to be effective means to minimize heat damage in such special food products.

KEYWORDS: Liquid infant formulas; heat damage; Maillard reaction; furosine; lactulose; lysinoalanine

INTRODUCTION

Milk-based infant formulas (MBF), both liquid and powdered, are very sensitive foods in terms of heat damage because of their high lactose content, the heating conditions applied in manufacturing (*1*), the usage of already processed ingredients in the formulation, and their long shelf life (up to 12 months) at room temperature. Taking these aspects into consideration, it is obvious that the overall quality of these food products is crucially related to the extent of heat-induced chemical changes of important nutrients. Whereas heat-labile vitamins can be easily supplemented, irreversible modifications to protein components in MBF may lead to nutritional alteration and induce adverse effects on metabolism and excretion of nursing infants (*2*). The formula gross composition should not be considered relevant from the standpoint of product quality, because the type and level of each nutrient are recommended by international organizations such as the Codex Alimentarius and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) (*3*). Provided the superiority of breast milk, MBF represent the best recognized substitutes. As these last may constitute the sole (in the case of infant formulas, intended for babies 0−4 months old) or principal (follow-on formulas, intended for babies 4−12 months old, or growth milk, for babies 12−36 months old) source of nutrition, the assessment of quality related to protein heat damage in this kind of food is of paramount importance.

The extent of heat damage can be measured by means of suitable chemical indices arising from heat-induced reactions involving protein and/or sugar components. Both furosine (FUR), deriving from the Amadori compounds, mainly lactulosyl lysine, in the early stage of Maillard reaction (MR), and lactulose (LCT), deriving from the lactose isomerization, are widely used as marker compounds for evaluating the extent of heat damage in both sterilized drinking milk (*4*−*7*) and liquid infant formulas (*1*, *8*, *9*). From a nutritional point of view, FUR has been proposed for calculating the amount of bioavailable lysine in heat-processed foodstuffs (*10*), although this approach has been recently criticized (*11*).

When MR takes place extensively, degradation of lactulosyl lysine via the 1-deoxyosone (1-DG) occurs, yielding a variety of advanced glycation end-products (*12*). Among these, galactosyl-β-pyranone (GAP) has been recently measured (*7*) in drinking milk sterilized at ultrahigh temperature (UHT). Indeed, GAP proved to be a very sensitive marker, allowing some technological malpractices (overheating, abuse of milk recirculation in the sterilization plant, recycling of expired drinking milk) in UHT milk processing to be exposed, all resulting in unacceptable high levels of heat damage. No data regarding GAP levels in MBF have been reported in the literature so far.

Much less is known on the impact of lysinoalanine (LAL), a protein cross-linker deriving from β-elimination reactions

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promoted by heat treatment, mainly at alkaline pH, of sulfoproteins, phosphoproteins, or glycoproteins (*13*). Some publications have demonstrated that the presence of molecular cross-links reduces the quality of heat-treated protein, in terms of digestibility and utilization (*14*), and affects mineral status in rats (*15*). Another important significance of LAL derives from its suggested role in inducing nephrocytomegaly, a pathology resulting in kidney damage (*16*). Up to now, this effect of the LAL supply in the diet has been observed only in rats, and its occurrence in infants fed by heat-processed milk formulas remains unclear (*17*).

Only a few authors have investigated the heat damage of liquid MBF, the vast majority of the publications dealing with the powdered ones. Moreover, in the past decade the use of ready-to-feed liquid MBF has become widespread. In fact, although this type of product is more expensive, its use is easier and ensures bacteriological safety not requiring any domestic manipulation. Whereas in-bottle-sterilized formulas have been proved to undergo deep modifications (*1*) that can depress their quality mainly in terms of protein digestibility (*18*), UHT-processed formulas may represent superior products due to the extremely short heating time adopted in the continuous-flow sterilization process (*19*).

Taking this market evolution into account, and because of the importance of providing infant consumers with the best quality food, this research was undertaken to investigate the possibility of improving the manufacturing process of liquid UHT-processed MBF to minimize heat damage in the finished products. To provide more differentiated information regarding the actual levels of heat damage occurring in liquid MBF, commercial samples from the most widespread brands available on the Italian market have been first evaluated by means of the above-reported chemical markers. On this basis, the contribution of both individual raw materials and some technological parameters of the process to the final level of heat damage has been investigated. Finally, the impact of the further increase of heat damage throughout the shelf life has been highlighted.

MATERIALS AND METHODS

Commercial Samples. Fifteen commercial samples of liquid milk-based UHT formulas from five different brands were analyzed within 30 days from manufacturing. The formulas considered in this work were as follows: five samples of infant formula (IF); five samples of follow-on formula (FO); and five samples of growth milk (GR). In addition, multiple packages from single batches of commercial IF of three different brands were stored at room temperature throughout the whole shelf life. A package of each brand was opened every month and analyzed.

Experimental Samples. Samples were obtained from a leading industrial manufacturer of baby foodstuffs. All heat treatments were direct UHT processes performed at 150 °C/5 s in an industrial plant (Rossi Catelli, I; 7000 L/h capacity). The samples included two samples (one IF and one FO), taken both before and after UHT treatment; two samples of IF, adjusted to pH 6.90 and 7.20, respectively, with NaOH, and then UHT treated; two samples of FO formulas, adjusted to pH 6.90 and 7.20, respectively, with NaOH and then UHT treated; and two samples of the same FO formula UHT treated by adopting either 0 or 15% product recirculation in the plant.

The formula's dairy ingredients [pasteurized milk, whey powder, α-lactalbumin-enriched whey protein concentrate (WPC), powdered lactose] were provided by the MBF manufacturer and analyzed.

Methods. The level of FUR was determined by IP-RP HPLC according to ISO 18329-IDF 193 International Standard. An aliquot of 2 mL of MBF was added to 6 mL of 10.6 N HCl, hydrolyzed at 110 °C for 23 h, and then submitted to solid phase extraction (SPE) on a 500 mg C18 cartridge (Millipore, Milford, MA). IP-RP HPLC was performed on a C8 furosine-dedicated column (Alltech, Lokeren, Belgium) with UV detection at 280 nm. The protein content of MBF was determined by Kjeldahl method (ISO 8968-1 IDF 20-1 International Standard) on 2 mL of hydrolysate.

Table 1. Levels of Furosine (FUR), Galactosyl-β-pyranone (GAP), Lactulose (LCT), and Lysinoalanine (LAL) and Related Mean and CV Values in 15 Samples of Commercial Liquid Milk-Based Infant Formulas of Five Different Brands

type of formula	sample code	FUR (mg 100 g $^{-1}$ of protein)	GAP (mg L^{-1})	LCT (mg L^{-1})	LAL (mg 100 g $^{-1}$ of protein)
infant	IF1	310	1.3	971	9.9
	IF2	280	2.8	436	5.8
	IF3	600	4.3	657	4.4
	IF4	380	3.1	1365	15.3
	IF5	400	2.4	1273	16.1
mean		394	2.4	940	10.3
CV		32	58	42	52
follow-on	FO1	270	1.9	1511	11.0
	FO2	180	0.5	400	1.0
	FO3	320	1.8	622	5.3
	FO4	220	1.4	904	5.7
	FO5	298	1.1	1037	7.3
mean		258	1.4	895	6.1
CV		22	41	47	60
growth	GR1	195	1.0	526	3.5
	GR2	182	0.7	226	1.7
	GR3	153	0.8	260	1.3
	GR4	250	1.5	1080	12.7
	GR5	292	1.2	1175	7.7
mean		214	3.5	653	5.4
CV		26	31	69	90
overall mean		289	1.6	830	7.2
CV		55	63	60	62

IDF Standard 147B was used for lactulose determination. After removal of protein and fat, the sample was submitted to chromatographic separation using an Aminex HPX-87 P column (Bio-Rad, Munich, Germany) with refractive index detection. The level of GAP was evaluated by the direct HPLC method of Pellegrino and Cattaneo (*20*). Fifty milliliters of MBF was acidified (2 N HCl) to pH 4.60. Two milliliters of isoelectric whey was submitted to SPE on a 2 g C18 cartridge (Millipore) prewetted with 5 mL of methanol and eluted with 20 mL of water. The eluate underwent HPLC separation using an Aminex HPX-87 P column (Bio-Rad) with UV detection at 257 nm. The level of LAL was determined according to the method of Pellegrino et al. (*21*). One hundred microliters of hydrolysate obtained as described for FUR determination was submitted to derivatization with FMOC. The derivatized sample underwent SPE purification on a 500 mg Amino cartridge (Bakerbond) activated with 10 mL of ACN and washed with a mixture (1:3, v/v) of ACN and 0.1 M boric acid (pH 7.20). Elution was performed using a mixture (1:1, v/v) of ACN and 0.2 M boric acid (pH 9.00). LAL separation was obtained by RP-HPLC using a C18 column (Hypersil AA-ODS, Agilent Technologies, Waldbronn, Germany) with fluorescence detection (excitation at 260 nm, emission at 310 nm). The precision of the determinations was preliminarily assessed. For replicate analyses ($n = 5$) of FUR and LCT, the relative standard deviations (7 and 5%, respectively) complied with those specified for the respective analytical methods. Precision of the GAP and LAL methods was adequate (7 and 8% were the respective relative standard deviations on five replications). The linearity of the GAP concentration ($r > 0.99$) was assessed over the range 0.03−12 mg L^{-1}. All analyses have been performed in duplicate, and mean values are reported.

RESULTS AND DISCUSSION

Heat Damage in Commercial Formulas. The levels of FUR, LCT, GAP, and LAL found in 15 samples of IF, FO, and GR formulas are reported in **Table 1**. A range from 150 to 600 mg 100 g^{-1} of protein (mean value = 289 mg) for FUR levels was observed, whereas those of LCT roughly ranged from 230 to

1500 mg L^{-1}, 830 mg L^{-1} being the mean value. The levels of GAP ranged from 0.5 to 4.3 mg L^{-1}, with a mean value of 1.6 mg L^{-1}, and those of LAL were from 1.0 to 16.1 mg 100 g^{-1} of protein, with a mean value of 7.2 mg 100 g^{-1} of protein. These levels show that formulas from every category considered in this survey have sustained relevant heat damage, the highest values being found on average in IF, that is, in formulas intended for the youngest babies. This aspect should be considered by manufacturers, infant formulas being usually the only food given to newborns, as an alternative to breastfeeding, to meet their dietary requirements.

Furthermore, a great variability of the data could be evidenced, for every chemical marker, by the CV values calculated both overall and within each category of the considered formulas. The effect of storage can be disregarded in this case, the samples being analyzed just a few days after manufacturing. Leclère et al. (*22*) reported that fortification of a whey−lactose system with iron and ascorbate strongly activates the advanced MR, the ascorbate degradation products being 20−100 times more reactive than lactose. The concentrations of iron and ascorbate in our samples, as indicated on the labels, ranged between 0.5 and 1.2 mg 100 mL^{-1} and between 8 and 15 mg 100 mL^{-1}, respectively; however, the levels were very similar within each class. Therefore, the great variability of our data could be reasonably attributed to two main sources: (i) the different processing conditions adopted by manufacturers, including the type of UHT sterilization process (direct or indirect), the time/temperature profile, and also the wide range of sterilizing performances of the various UHT plants (*19*); and (ii) the heat damage that some of the ingredients used in the initial formulation had already suffered.

Processing Conditions. Despite the fact that all of these formulas underwent UHT treatment, in many cases (samples IF3, IF4, IF5, FO1, FO4, FO5, GR4, and GR5) the levels of FUR and/or LCT were comparable to those typical of in-bottle-sterilized drinking milk (*4*, *5*). By using the same analytical method, Birlouez-Aragon et al. (*1*) found values of FUR up to 300 and 380 mg 100 g^{-1} of protein (as mean value + 2 standard deviation) in commercial MBF that had been UHT-processed and in-bottle-sterilized, respectively. The authors report these values to be the result of an extensive degradation of lactulosyl-lysine tacking place in liquid formulas, as proven by the presence of advanced glycation end-products, such as carboxymethyllysine and oxalic acid monoalkylamide.

According to Resmini et al. (*23*), levels of GAP up to 1−1.2 mg L^{-1} are expected for directly processed UHT milk, whereas these can be up to 3.8−4 mg L^{-1} when the indirect process is adopted. In fact, the level of this marker sharply rises only when milk protein is extensively glycated and the subsequent degradation of the Amadori compound takes place. The high levels of FUR in the samples of this survey indicate a relevant extent of MR, but the GAP values mostly fall within the above-reported ranges for UHT drinking milk, thus suggesting that, depending on formula composition, either a degradation pathway of an Amadori compound other than that via 1-DG or degradation of GAP itself could take place. In fact, depending on the type of sugar involved and the pH value (*12*), the Amadori compound can also degrade through the 3-DG pathway, leading to a range of different molecules, lysyl-pyrrolaldehyde (LPA) being the best known among these. We have analyzed these samples using the method of Resmini and Pellegrino (*24*), but no LPA was found.

LAL values determined in the present research are on the lower side of the range (from traces to 50 mg 100 g^{-1} of protein) found by D'Agostina et al. (*25*) in liquid IF analyzed using the same analytical method as we used. At such levels, the molecule is not known to be a health threat for newborns and has been deemed not to be a hazard by several authors (*17*, *26*). Data from Fenaille et al. (*9*) are close to ours, although a different method was used and, consequently, a different recovery rate cannot be excluded.

Table 2. Levels of Furosine (FUR), Galactosyl-β-pyranone (GAP), Lactulose (LCT), and Lysinoalanine (LAL) in Two Experimental Samples of Infant Formula Manufactured by Adopting Different Levels of Product Recirculation in the Sterilization Unit of the Plant

product recirculation	FUR (mg 100 g^{-1} of protein)	GAP (mg L^{-1})	LCT (mg L^{-1})	LAL (mg 100 g^{-1} of protein)
none	200	0.8	702	5.1
15%	273	2.2	1511	11.7

It is noteworthy that, despite the relevant heat damage revealed by the vast majority of the analyzed commercial samples, three of them (FO2, GR2, and GR3) showed very low levels for all of the analyzed markers, comparable to those reported for high-quality UHT drinking milk by Cattaneo et al. (*27*) in a recent survey. The presence of these samples on the market clearly demonstrates that the requested sterility of the packaged products can be achieved in combination with a low heat damage simply by optimizing the whole technological process. In commercial practice, to minimize running costs, manufacturers tend hardly to modify the time/temperature conditions of sterilization that were initially set on their own plant. Taking this fact into account, the effect of nominal time/temperature conditions on the final heat damage of MBF was not considered in the present research.

Among other conditions, the percent amount of the product recirculated in the sterilization plant proved to be a key factor in promoting heat damage of UHT drinking milk (*7*). In UHT sterilization processing, part of the foodstuff to be sterilized is usually recirculated within the plant (i) when a new operation cycle starts, before the target sterilization temperature is reached, and (ii) to keep the balance tank full, thereby ensuring a constant pressure to the filling machine. Although this aspect is often disregarded, the recirculated product is actually submitted to a second heat treatment, the effect of which, in terms of heat damage, is additive at least. In our previous work (*7*) we have demonstrated that a relevant increase in heat damage of UHT milk can be determined by an unjustified level (> 10%) of milk recirculation in the plant. This situation was well highlighted by anomalous (> 4 mg L^{-1}) values of GAP. To confirm this effect in milk-based formulas, two experimental samples of FO formula have been submitted to the same UHT treatment (150 °C/5 s) either without product recirculation or by adopting the 15% recirculation level. The resulting heat damage is clearly evidenced by the data reported in **Table 2**. When the product was partly recirculated, the levels of LCT and LAL were more than doubled; that of FUR increased by only 36%, probably because a partial degradation of lactulosyl-lysine took place in parallel. This hypothesis is consistent with the fact that GAP was 3-fold higher when recirculation occurred and confirmed it to be the most sensitive among the analyzed chemical markers. Data obtained prove the efficacy of the control of recirculation to limit heat damage in MBF as well.

Formulation. Besides the severe heating conditions of sterilization, the typical formulation of MBF makes them particularly susceptible to heat-induced changes. Whey proteins are expected to glycate much more rapidly than casein does (*28*). Therefore, higher levels of FUR can be expected in IF with respect to regular cow's milk due to the higher casein-to-whey protein ratio in the latter. When the pH values of MBF are taken into consideration, the high concentration of lactose (about 7%) is probably much more related to the high levels of LCT than to those of FUR, as will be discussed further. Birlouez-Aragon et al. (*1*) found FUR as

Table 3. Levels of Furosine (FUR), Galactosyl-β-pyranone (GAP), Lactulose (LCT), and Lysinoalanine (LAL) in Samples of Infant Formula (IF) and Follow-on Formula (FO) Sterilized at Different pH Values

type of formula	pH	FUR (mg 100 g^{-1} of protein)	GAP (mg L^{-1})	LCT (mg L^{-1})	LAL (mg 100 g^{-1} of protein)
IF	7.2	314	1.6	971	106
	6.9	322	0.6	635	75
FO	7.2	273	2.2	1551	117
	6.9	271	1.6	1150	106

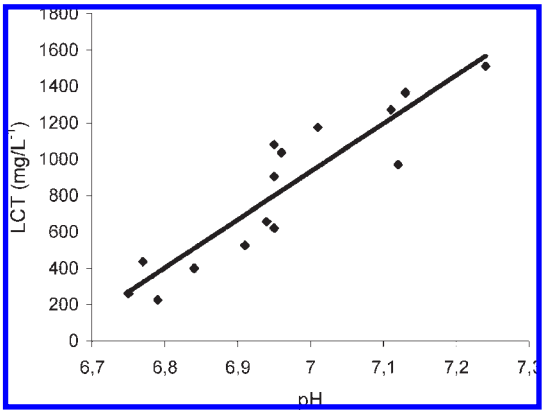

Figure 1. Relationship between lactulose (LCT) content and pH value in commercial liquid MBF of **Table 1**.

well as other heat treatment indicators to be significantly higher in commercial liquid MBF than in similarly processed regular cow's milk. This was hypothesized to be the result of an increased content of lactose and whey protein in some of the samples (probably supplemented with whey powder of poor quality). Lacking unequivocal data on these aspects, we have performed a series of trials to address the most relevant ones by strictly adopting the real processing conditions of MBF industrial manufacturing.

A slight decrease of pH value of the mixture of ingredients had a dramatic effect on the levels of the heat damage chemical markers considered in this study, as shown by the data reported in **Table 3**. For both IF and FO formulas, adjustment of the pH value from 7.2 to 6.9 determined a sharp decrease of GAP, LCT, and LAL values formed upon sterilization. Such variations could be explained by considering that the reactions leading to formation of these compounds are strongly enhanced at alkaline pH (*12*, *13*, *29*). On the contrary, the pH modification did not affect the FUR level, the formation of the Amadori product not being strictly pH-dependent (*12*). Pellegrino et al. (*5*) have already demonstrated for UHT drinking milk that a slight increase of milk pH promotes a sharp increase of LCT formation upon heating. The effect of pH on the level of LCT is clearly evidenced (**Figure 1**) by the strong relationship ($r^2 = 0.836$) existing between the two parameters in the commercial MBF samples of this research. For the same samples, the relationship between LAL and pH was weaker ($r^2 = 0.621$).

The aim of the formulation of infant formulas is to mimic the composition of human milk by modifying the chemical composition of cow's milk. The protein content of human milk (about 1.1%) and the correct casein-to-whey protein ratio (i.e., 40:60 approximately) are usually achieved in MBF by diluting cow's milk with water and by adding whey powder and/or WPC. Moreover, the addition of whey and/or lactose powders raises the lactose content from 5 to 7%, which is the lactose concentration of human milk.

Table 4. Levels of Furosine (FUR), Galactosyl-β-pyranone (GAP), Lactulose (LCT), and Lysinoalanine (LAL) in Some Ingredients for MBF

ingredient	FUR (mg 100 g^{-1} of protein)	GAP (mg 100 g^{-1})	LCT (mg 100 g^{-1})	LAL (mg 100 g^{-1} of protein)
pasteurized milk	6.7	nd[a]	0	0
whey powder	936	0.01	137	5.1
WPC (α-lactalbumin enriched)	205	nd	nd	1.8
lactose powder	nd	nd	0	nd

[a] nd, not determined.

The levels of FUR, GAP, LCT, and LAL were measured in individual ingredients to determine their contribution to the global heat damage of the end-product. Only ingredients expected to show significant heat damage, due to both their composition (presence of both sugar and protein or protein only), pH value, and the severe heating required in manufacturing process (concentration, drying), have been considered. As shown in **Table 4**, whey powder proved to be by far the most heat-damaged ingredient among those utilized in the formulation of MBF. In fact, the relevant contents of lactose (82%) and protein (12%) and the low moisture make this product highly susceptible to MR, which mainly takes place during concentration and spray-drying. Furthermore, due to these favorable conditions, MR in whey powder can easily proceed during storage (*30*); thus, the contribution of this ingredient to the heat damage of the finished MBF can increase progressively. In particular, whey protein may lose solubility and digestibility as a result of structural changes consequent to glycation, cross-linking, coagulation, and interaction with lipids (*18*, *31*, *32*).

In **Table 5** are reported the levels of heat-load markers that we have found by analyzing the mixtures of the ingredients just before and after the UHT heat treatment (150 °C/5 s) in the production chain of IF and FO formulas, respectively. Although the initial mixtures were submitted to only a pasteurization process (90 °C/30 s), aimed to improve solubilization of the ingredients, it is noteworthy that their levels of FUR and LAL were already comparable to those usually found in directly heated UHT milk (*7*).

By comparing the levels of the individual markers determined before and after the UHT treatment, the contribution to heat damage of the finished product given by the raw materials looks to be well described by FUR, this compound being a sensitive marker of the early stage of MR. On the contrary, the levels of GAP, LCT, and LAL in the finished products are mainly determined by the UHT treatment.

Effect of Storage. As already emphasized, liquid MBF usually undergo longer shelf life with respect to regular UHT drinking milk. This can make the keeping time under adverse conditions highly depressive of the quality of these foodstuffs. Only a few papers (*8*, *33*, *34*) report data on the deterioration of liquid MBF during storage. Although all of these papers have evidenced that the levels of specific molecules deriving from heat-induced reactions rise in MBF during storage depending on keeping temperature, comparison among data is difficult because different storage conditions and different analytical methods have been used. The levels of FUR and LCT increased by 15−100% and by 15−70%, respectively, in our samples after about 10 months of storage, the highest percent increase being registered for MBF samples with the lowest initial levels (not shown). Levels of GAP slightly increased in the first 2 months and then remained unchanged (not shown). No data are available in the literature concerning the increase of LAL, as far as we know. LAL accumulated over time, markedly in the first 3−5 months (**Figure 2**), and showed the highest increases (up to

Table 5. Levels of Furosine (FUR), Galactosyl-β-pyranone (GAP), Lactulose (LCT), and Lysinoalanine (LAL) in Samples of Infant Formula (IF) and Follow-on Formula (FO), before and after UHT Treatment, and Relative Contribution of the Formula Ingredients

sample	type of formula	FUR (mg 100 g^{-1} of protein)	GAP (mg L^{-1})	LCT (mg L^{-1})	LAL (mg 100 g^{-1} of protein)
mixture of ingredients before UHT treatment	IF	203	0.06	71	1.9
	FO	104	0.06	33	0.9
finished product	IF	333	1.5	635	9.4
	FO	200	0.8	702	5.1
contribution (%) of the ingredients to final heat damage	IF	61	4	11	20
	FO	52	7	5	18

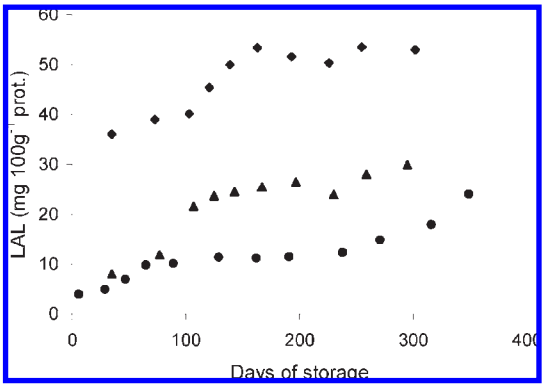

Figure 2. Levels of lysinoalanine (LAL) during storage at room temperature (20 °C) of commercial IF of three differents brands.

500%) among the considered markers. A similar behavior of LAL was evidenced by Cattaneo et al. (7) in UHT milk during 3 months of storage at 25 °C. Interestingly, the amount of LAL formed during the whole storage period was in the range of 20−22 mg 100 g^{-1} of protein in all samples, irrespective of the initial level, with an average daily increase of 0.06−0.08 mg 100 g^{-1} of protein.

The evaluation of heat damage in commercially available liquid MBF has shown that a great variability exists among different products. Our results on experimental samples demonstrate that the level of heat damage in milk-based baby formulas depends on several technological parameters. Besides heating conditions of UHT processing, the percent level of the product recirculated in the plant, the pH of the formulation when heat-sterilized, and the initial heat damage of single protein ingredients play a major role in determining the overall quality of this kind of food.

To minimize heat damage, a quality control scheme based on the determination of one or more of the proposed parameters should be adopted. This approach would help manufacturers in optimizing sterilization conditions on the basis of the actual performances of their own plant, so minimizing the negative effects of heat treatment on the end-product and also saving energy. The parameters studied here, particularly FUR, may also represent useful criteria for selecting raw materials with the best quality in terms of heat damage. Finally, manufacturers should be aware that degradation reactions induced by heat processing continue during storage on the shelf at retail store level and, therefore, the quality of their own products decreases progressively. In this regard, our data suggest that the manufacturers should also reconsider the length of the shelf life targeted for such a sensitive and precious food.

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LITERATURE CITED

(1) Birlouez-Aragon, I.; Pischetsrieder, M.; Leclère, J.; Morales, F. J.; Hasenkopf, K.; Kientsch-Engel, R.; Ducauze, C. J.; Rutledge, D. Assessment of protein glycation markers in infant formulas. *Food Chem.* **2004**, *87*, 253–259.

(2) Puig, C.; Perez, M. D.; Ros, L.; Sanchez, L.; Calvo, M. Effect of processing on the composition of infant formulas. *Milchwissenschaft* **2003**, *58*, 476–480.

(3) Koletzko, B.; Baker, S.; Cleghorn, G.; Neto, U. S.; Gopalan, S.; Hernell, O.; Hock, Q. S.; Jirapinyo, P.; Lonnerdal, B.; Pencharz, P.; Pzyrembel, H.; Ramirez-Mayans, J.; Shamir, R.; Turck, D.; Yamashiro, Y.; Zong-Yi, D. Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. *J. Pediatr. Gastroenterol. Nutr.* **2005**, *41*, 584–599.

(4) Corzo, N.; Delgado, T.; Troyano, E.; Olano, A. Ratio of lactulose to furosine as indicator of quality of commercial milks. *J. Food Prot.* **1994**, *57*, 737–739.

(5) Pellegrino, L.; De Noni, I.; Resmini, P. Coupling of lactulose and furosine indices for quality evaluation of sterilized milk. *Int. Dairy J.* **1995**, *5*, 647–659.

(6) Feinberg, M.; Dupont, D.; Efstatiou, T.; Louapre, V.; Guyonnet, J. Evaluation of tracers for authentication of thermal treatments of milk. *Food Chem.* **2006**, *98*, 188–194.

(7) Cattaneo, S.; Masotti, F.; Pellegrino, L. Effects of overprocessing on heat damage of UHT milk. *Eur. Food Res. Technol.* **2008**, *226*, 1099–1106.

(8) Rossi, M.; Pompei, C. Changes in liquid milk-based infant formulas during long-term storage. *Ital. J. Food Sci.* **1991**, *3*, 49–57.

(9) Fenaille, F.; Parisod, V.; Visani, P.; Populaire, S.; Tabet, J.; Guy, P. A. Modifications of milk constituents during processing: a preliminary benchmarking study. *Int. Dairy J.* **2006**, *16*, 728–739.

(10) Hurrell, R. F.; Carpenter, K. J. The estimation of available lysine in foodstuffs. In *Progress in Food and Nutritional Science, Maillard Reaction in Foods*; Eriksson, C., Ed.; Pergamon Press: Oxford, U.K., 1981: Vol. 5, pp 156−176.

(11) Rutherfurd, S. M.; Moughan, P. J. Development of a novel bioassay for determining the available lysine contents of foods and feedstuffs. *Nutr. Res. (N.Y.)* **2007**, *20*, 3–16.

(12) Ledl, F. Chemical pathways of the Maillard reaction. In *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*; Finot, P. A., Aeschbacher, H. U., Hurrel, R. F., Liardon, R., Eds.; Birkhauser Verlag: Basel, Switzerland, 1990; pp 19−42.

(13) Friedman, M. Chemistry, biochemistry, nutrition and microbiology of lysinoalanine, lanthionine and histidinoalanine in food and other proteins. *J. Agric. Food Chem.* **1999**, *47*, 1295–1319.

(14) Somoza, V.; Wenzel, E.; Weiss, C.; Clawin-Radecker, I.; Grubel, N.; Erbesdobler, H. F. Dose-dependent utilisation of casein-linked lysinoalanine, $N(\varepsilon)$-fructoselysine and $N(\varepsilon)$-carboxymethyllysine in rats. *Mol. Nutr. Food Res.* **2006**, *50*, 833–841.

(15) Sarwar, G.; L'Abbe, M. R.; Trick, K.; Botting, H. G.; Ma, C. Y. Influence of feeding alkaline/heat processed proteins on growth and protein and mineral status of rats. *Adv. Exp. Med. Biol.* **1999**, *459*, 161–177.

(16) Richardson, J. A.; Woodard, J. C. Renal tubular kariocytomegaly, rat. In *Urinary System*; Jones, T. C., Mohr, U., Hunt, R. D., Eds.; Springer Verlag: Berlin, Germany, 1986; pp 189−192.

(17) Langhendries, J. P.; Hurrel, R. F.; Furniss, D. E.; Hischenhuber, C.; Finot, P. A.; Bernard, A.; Battisti, O.; Bertrand, J. M.; Senterre, J. Maillard reaction products and lysinoalanine: urinary excretion and the effects on kidney function of preterm infants fed heat-processed milk formula. *J. Pediatr. Gastroenterol. Nutr.* **1992**, *14*, 62–70.

(18) Rudloff, S.; Lonnerdal, B. Solubility and digestibility of milk proteins in infant formulas exposed to different heat treatments. *J. Pediatr. Gastroenterol. Nutr.* **1992**, *15*, 25–33.

(19) Burton, H. Bacteriology of heat sterilization of milk. In *Ultra-High-Temperature Processing of Milk and Milk Products*; Burton, H., Ed.; Elsevier Applied Science Publishers: Crown House, U.K., 1988; pp 13−43.

(20) Pellegrino, L.; Cattaneo, S. Occurence of galactosyl isomaltol and galactosyl β-pyranone in commercial drinking milk. *Nahrung* **2001**, *3*, 195–200.

(21) Pellegrino, L.; Resmini, P.; De Noni, I.; Masotti, F. Sensitive determination of lysinoalanine for distinguishing natural from imitation Mozzarella cheese. *J. Dairy Sci.* **1996**, *79*, 725–734.

(22) Leclère, J.; Birlouez-Aragon, I.; Meli, M. Fortification of milk with iron-ascorbate promotes lysine glycation and tryptophan oxidation. *Food Chem.* **2002**, *76*, 491–499.

(23) Resmini, P.; Pellegrino, L.; Cattaneo, S. Furosine and other heat-treatment indicators for detecting fraud in milk and milk products. *Ital. J. Food Sci.* **2003**, *15*, 473–484.

(24) Resmini, P.; Pellegrino, L. Occurrence of protein-bound lysylpyrrolaldehyde in dried pasta. *Cereal Chem.* **1994**, *71*, 254–262.

(25) D'Agostina, A.; Boschin, G.; Rinaldi, A.; Arnoldi, A. Updating on the lysinoalanine content of commercial infant formulae and beicost products. *Food Chem.* **2003**, *80*, 483–488.

(26) Corpet, D. E.; Taché, S.; Archer, M. C.; Bruce, W. R. Dehydroalanine and lysinoalanine in thermolyzed casein do not promote colon cancer in the rat. *Food Chem. Toxicol.* **2008**, *46*, 3037–3042.

(27) Cattaneo, S.; Masotti, F.; Rosi, V. Evaluation of some heat-treatment indices in UHT milk marketed in Italy. *Ital. J. Food Sci.* **2008**, *20*, 1–14.

(28) Rufián-Henares, J. A.; Delgado-Andrade, C.; Jiménez-Pérez, S.; Morales, F. J. Assessing nutritional quality of milk-based sport supplements as determined by furosine. *Food Chem.* **2007**, *101*, 573–578.

(29) Andrews, G. R. Formation and occurrence of lactulose in heated milk. *J. Dairy Res.* **1986**, *53*, 665–680.

(30) Sithole, R.; McDaniel, M. R.; Meunier Goddik, L. Rate of Maillard browning in sweet whey powder. *J. Dairy Sci.* **2005**, *88*, 1636–1645.

(31) Resmini, P.; Pellegrino, L. HPLC of furosine for evaluating Maillard reaction damage in skimmilk powders during processing and storage. *IDF Bull.* **1994**, *No. 298*, 31−36.

(32) Mulsow, B.; Jacob, M.; Henle, T. Studies on the impact of glycation on the denaturation of whey proteins. *Eur. Food Res. Technol.* **2009**, *228*, 643–649.

(33) Albalà-Hurtado, S.; Veciana-Nogues, M. T.; Marinè-Font, A.; Vidal-Carou, M. C. Changes in furfural compounds during storage of infant milks. *J. Agric. Food Chem.* **1998**, *46*, 2998–3003.

(34) Guerra-Hernandez, E.; Gomez, C. L.; Garcia-Villanova, B.; Corzo Sanchez, N.; Gomez, J. M. R. Effect of storage on non-enzymatic browning of liquid infant milk formulae. *J. Sci. Food Agric.* **2002**, *82*, 587–592.

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