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MONITORING SELECTED QUALITY INDICATORS OF POWDERED INFANT MILK FORMULAS

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Key words: infant milk formula, Maillard reaction, vitamins, HPLC

This work was addressed to monitor some quality indicators content in commercial powdered infant milk formulas. Furosine, furfural compounds, vitamins (B_1, B_2, B_6, and C) were determined in starting and follow-up infant milk formulas. Contents of furosine, total and free furfural compounds, and B group vitamins were assayed by RP-HPLC with UV detection. The contents of furfural (F), free and total hydroxymethylfurfural (HMF) in the starting infant milks were lower than in the follow-up formulas, whereas free and total F contents were not detectable in the starting formulas. The content of free and total F in follow-up formulas ranged from 0.77 to 1.86 μmol/100 g and from 2.70 to 7.08 μmol/100 g, respectively. Free and total HMF contents in starting milks ranged from 1.22 to 1.47 μmol/100g and from 5.79 to 8.34 μmol/100 g, respectively. The content of free and total HMF in follow-up milks ranged from 2.11 to 8.20 μmol/100g and from 19.86 to 84.89 μmol/100g, respectively. Similarly, the furosine content in starting infant milks (ranged from 53.47 to 57.57 mg/100g) was lower than in the follow-up infant milks (ranging from 70.94 to 96.14 mg/100g). There were higher levels of B group vitamins in the follow-up milk than in the starting infant samples. The content of vitamin C was higher in starting formulas then in the follow-up formulas. These values indicated that there was no significant difference between the results obtained and the labelled levels. These results show that the type and contents of proteins and carbohydrates in commercial powdered infant milk formulas (starting milk, follow-up milk) play an important role in the course of the Maillard reaction.

INTRODUCTION

Human milk is recognised as the best nutrition for infants. Mothers' milk provides all the nutrients essential for normal growth and for infants' digestive conditions. Although human milk is the first choice for the infant, milk substitutes play an indispensable role in infant nutrition when breastfeeding is not possible, desirable or sufficient.

The industry has developed considerable technological resources to bringing up the composition of infant formulas closer to that of human milk. Hence, the major components of raw cow's milk (proteins, carbohydrates, lipids), as well as the minor components (minerals and vitamins), must be adjusted to mirror the human milk levels. Milk-based formulas can be based on any appropriate blend of proteins, carbohydrates, fats, minerals and vitamins. Milk powders are usually free-flowing agglomerates formed by spray drying [Birlouez-Aragon *et al.*, 2004].

The stability control of the powdered infant milk formulas is very important, because of a number of components that may interact. Infant milks in powdered form are highly sensitive to Maillard reactions, as they contain a relatively high concentration of lactose and proteins with a high lysine level. Besides the high temperature applied during the manufacturing process, the packaging conditions and their storage for long periods of time are also critical to the progress of the Maillard reaction, while water activity (a_w) would also play an important role in its promotion. The Maillard reaction may seriously reduce the nutritive value of foods through the destruction of essential amino acid and the production of antinutritive and toxic compounds [Ferrer *et al.*, 2000; Guerra-Hernandez *et al.*, 2002; Albalá-Hurtado *et al.*, 1998]. Furosine is formed during acid hydrolysis of an Amadori compound generated during the early stage of the Maillard reaction in foods. The furosine content is a good indicator to evaluate the extent of the Maillard reaction and can be used in order to calculate the percentage of blocked lysine [Pizzoferrato *et al.*, 1998] Hydroxymethylfurfural (HMF) is another indicator of the reaction, still it is an intermediate or final product [Albalá-Hurtado *et al.*, 1998]. Vitamins are essential for the normal health and growth of the infants and hence, there is a need to assay the concentrations of each type of vitamins present in infant foods. Ascorbic acid, the B group vitamins and other vitamins are added to infant milk formulas. However, they are sensitive to heat, oxygen and light, therefore their determination can be useful to know the stability in heating and storage [Albalá-Hurtado *et al.*, 1997; Antonelli *et al.*, 2002].

Infant formulas are sometimes the only food given to infants, and just because, they must fulfill all their nutritional needs. Therefore, the aim of this work was to monitor furosine, HMF (free and total), furfural, ascorbic acid and B group vitamins contents in commercial powdered infant milk formulas to control the quality of commercial samples.

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MATERIAL AND METHODS

Material. Seven different types of powdered infant milk (starting milk, follow-up milk) were purchased in the local market and derived from two production dates. The commercial spray-dried infant formulas were produced by two different producers. The samples were analysed within the expiry date. The composition of the infant milks studied as given on the label was as follows: starting infant formulas - proteins (casein/serum proteins, 40/60) 10–11%, carbohydrates (lactose) 57–58%, lipids 26–28%, vitamin C 48–52 mg), follow-up infant formulas – proteins (casein/serum proteins, 80/20) 15–16%, carbohydrates (lactose, saccharose, maltodextrine and other) 55–60%, lipids 18–27%, and vitamin C 56–85 mg.

Analytical methods. Sample preparation and analytical determinations were carried out in duplicate. Before analysis, samples were reconstituted (10–12.5 g/100 mL), following instructions according to the Polish standard PN-78/A--86030 [1978].

Chemicals. Enzymes used: taka-diastase from *Aspergillus oryzae* (Fluka, catalogue No.86247), acid phosphatase from potato type II (Sigma-Aldrich, catalogue No.P3752). All reagents were analytical or HPLC grade, as required, and were obtained from Sigma-Aldrich (St. Louis, MO, USA), and Merck (Darmstadt, Germany).

Furosine. Sample preparation and HPLC analysis were carried out following the method of Resmini *et al.* [1990] and Henle *et al.* [1995]. A 2 mL sample was hydrolysed with 6 mL of 10.6 HCL at 110°C for 23 h in a closed glass vial. After cooling, the hydrolysate was filtrated through medium-grade filter paper. The filtrate (2 mL) was dried in vacuo and dissolved in 3 mL of the mobile phase, and 50 L was injected into the chromatograph. A Pye Unicam LC 3XP (Cambridge, UK) chromatograph was used. Furosine was quantified by ion-pair RP-HPLC, using a Lichrospher 100 ODS column (125×4 mm I.D.). The column was eluted isocratically at ambient temperature. The mobile phase consisted of a solution of 7.5 mmol pentane (7 mmol hexane) sulphonic acid in 5% aqueous ethanol with pH adjusted to 3.0 with concentrated propionic acid. Flow rate was 1 mL/min. UV detection was performed at 280 nm. Injection volume was 50 μL. Calibration was performed by the external standard using a commercial pure furosine (Neosystem Laboratories, Strasburg, France).

Free and total furfural compounds: HMF (hydroxymethylfurfural), F (furfural), FMC (furylmethylketone) and MF (methylfurfural). Sample preparation was based on the method described by Ferrer *et al.* [2002]. For the total furfurals determination 10 mL reconstituted infant formula was mixed with 5 mL oxalic acid in a sealed tube. The tube was heated in a boiling water bath for 60 min. After cooling to room temperature, 5 mL of a 40% (w/v) TCA solution was added and stirred thoroughly for 5 min. After stirring, the mixture was filtered and used for the chromatographic determination. To determine the free furfurals the sample was prepared as described earlier for total furfurals but omitting the heating in the boiling bath. Determinations were carried out using an HPLC technique according to the method proposed by Ferrer *et al.* [2002]. A liquid chromatograph HP 1050 Hewlett Packard (Waldbronn, Germany) coupled with a computer with chromatographic software ChemStation version A.00.33 (Microsoft Corporation, Germany) and a MicroSph LC18 5 μm column (150×4 mm I.D.) were used. Separations were carried out isocratically at room temperature using a mixture of acetonitrile/deionised water (5:95, v/v) at a flow-rate of 1 mL/min as the mobile phase. Detections were carried out at wavelengths of λ=284 nm for HMF and F, at 274 nm for FMC and at 293 nm for MF. The injection volume was 20 μL. Peak identification was based on the retention time, by comparison of the ratios of peaks with those of the standard compounds purchased from Sigma-Aldrich (St.Luis, MO, USA).

Vitamin C. Total vitamin C content was determined by the Tilmans method [PN-78/A-86030:1978].

Vitamins B_1, B_2, B_6. The samples (5 g) of powdered infant milks were finely ground. Samples were submitted to hydrolysis with hydrochloric acid followed by taka-diastase (for vitamins B_1, B_2), and with phosphatase (for vitamin B_6) according to the procedure described by Ndaw *et al.* [2000]. Chromatographic separations of all vitamins were performed on a reversed-phase C_{18} column Lichrospher RP 18 (5.0 μm particle size, 250×4.6 mm I.D.). Separation by ion pair chromatography was accomplished isocratically with acetonitrile/0.05 μmol/L potassium dihydrogen phosphate (10:90, v/v) containing 0.3×10^3 μmol/L sodium octane sulfonate as a mobile phase. The mobile phase was then adjusted to pH 2 with orthophosphoric acid. The separation was performed at 30°C at a flow rate of 1 mL/min, the UV--detection was carried out at 260 nm for thiamine hydrochloride (B_1), at 268 nm for riboflavin (B_2) and at 290 nm for vitamin B_6 (pyridoxamine phosphate, pyridoxine hydrochloride, pyridoxamine dihydrochloride). The injection volume was 50 μL. Peaks identification was based on the retention time, by comparison of the ratio of UV spectra with that of standard commercial compounds (Sigma-Aldrich). Separation conditions were used after Arella *et al.* [1996], and Bergaentzlé *et al.* [1995].

Water content (%). The content of water was determined according to the Polish Standard PN-78/A-86030 [1978].

Statistical analysis. Data were analysed with the Student's t-test at a significance level of $p<0.05$, as described by Szczepaniak [2002].

RESULTS AND DISCUSSION

The furfural compounds can be formed in processed food during thermal processing or storage at inappropriate temperatures [Ferrer *et al.*, 2005]. Although the analytical method used enables detecting MF and FMC, we did not detect them in any of the samples analysed. The HMF contents in the infant milk formulas are presented in Table 1.

Much the same results was found by Ferrer *et al.* [2005] in infant powdered formulas. The F and HMF contents in the starting infant milks were lower than in the follow-up formulas. Both formulas were subjected to the same thermal treatments and the same quality raw cow's milk was used, which was declared by the producers. Therefore, the statistically significant differences ($p<0.05$) in the contents of furfural compounds between the starting and follow-up milks may result from differences in their composition (Table 1). The starting infant milk contained only lactose. According to the label, the follow-up formula had lactose, saccharose and maltodextrine. Several authors studied the effect of sugar type on lysine losses during the thermal treatment. Albalá-Hurtado *et al.* [1998] found more HMF and F in infant formulas containing lactose and other sugar than in those having only lactose. The casein/serum protein ratios of the formulas analysed differed as well: 40/60 and 80/20 in starting and follow-up formulas, respectively, as given on the label. Ferrer *et al.* [2002] reported that in the case of milk, the reacting amino groups are mainly the lysine residues in milk proteins, and it seems that the reactivity of lysine residues from casein is higher than from serum proteins. Opposite results were obtained by Albalá-Hurtado *et al.* [1998] who reported high furfural content with a low casein/serum protein ratio in infant milks. Though opinions presented by several authors are different, it is essential that the type and contents of proteins and carbohydrates play an important role in the available lysine content and in the HMF and F contents.

Drying and sterilisation are thermal processes commonly used in the manufacture of infant milk formulas. The technological process induces one of the most important modifications, the Maillard reactions, which involves amino acids (mainly lysine) and reducing carbohydrates and can produce a loss of nutritive value. Loss of available lysine is the most negative nutritional consequence of the Maillard reaction in infant milk formulas. The determination of furosine generated from the acid hydrolysis of Amadori compounds formed during the early stages of the Maillard reaction has been used for the evaluation of lysine loss in infant milks. The furosine content is a good indicator of the Maillard reaction extent and can be used to calculate the decrease of bioavailable lysine. According to Bujard & Finot [1978], the lysine units in milk-based products, which occur in the form of lactulosyl-lysine (blocked lysine), generate on acid-hydrolysis under specified conditions, approximately 40% of lysine, 32% of furosine and 28% of pyridosine. The blocked lysine calculation was done using relations established by Finot *et al.* [1981]. Milk powders normally have higher furosine content than liquid milks because of a low water activity and relatively mild thermal conditions. The furosine contents in the infant milk samples (Table 1) are within the same range as those described by Henle *et al.* [1995], Villamiel & Corzo [2000] and Guerra-Hernandez *et al.* [2002] in powdered infant formulas. In our study, the furosine content was much lower than that received by Sarriá *et al.* [2001] and it was quite satisfactory from the nutritional standpoint. The furosine content in starting infant milks was significantly lower than in the follow-up infant milks ($p<0.05$). Probably the type and contents of proteins and carbohydrates in the two kinds formulas (starting, follow-up) can also influence the furosine content. Pereyra Gonzáles *et al.* [2003] reported that available lysine content of samples with casein and glucose was about 40% lower than that in other formulas. Therefore, probably glucose and other sugars are considerably more reactive than lactose and could cause a greater loss of available lysine upon the Maillard reaction.

Structurally, ascorbic acid is a sugar acid, a γ-lactone and an enediol. It is unstable and is easily oxidised to dehydro-L-ascorbic acid. Upon heating foods, ascorbic acid behaves in the some way as reducing sugars in the Maillard reaction. Its degradation products react with amino acids, peptides, and lipids (or their degradation products) and give rise to

TABLE 1. Composition of various powdered infant milks.

Compound	Concentration *						
	Infant milk						
	Starting from 1 to 5 month	Starting from the born	Follow-up from 5 month	Follow-up above 4 month	Follow-up above 12 month	Follow-up from 1 to 3 year	Follow-up from 1 to 3 year (with honey)
Furosine (mg/100g)	53.47±3.8	57.57±2.5	70.94±3.7†	96.14±3.4†	86.80±4.2†	80.27±4.1†	70.94±6.8†
HMF free	1.47±0.1	1.22±0.1	2.11±0.2	2.62±0.2	2.73±0.3	3.28±0.2	8.20±0.5†
HMF total (μmol/100g)	5.79±2.1	8.34±1.8	19.86±3.1†	21.88±4.4†	25.64±5.2†	28.75±6.1†	84.89±9.3†
F free	nd$^{\Delta}$	nd$^{\Delta}$	1.86±0.1	nd$^{\Delta}$	1.05±0.3	0.77±0.1	nd$^{\Delta}$
F total (μmol/100g)	nd$^{\Delta}$	nd$^{\Delta}$	3.36±0.5	nd$^{\Delta}$	2.70±0.4	7.08±2.1	nd$^{\Delta}$
Vitamin C (mg/100g)	78.50±7.8	75.33±5.9	74.62±8.1	68.47±6.7	67.86±7.1	66.71±6.1	48.58±6.3
Water content (%)	2.31±0.3	2.49±0.2	1.99±0.1	2.28±0.4	2.46±0.1	1.79±0.3	1.84±0.1

HMF: hydroxymethylfurfural, F: furfural, * Mean ± standard deviation, Δ nd: not detectable, † Values are significantly different at $p<0.05$, Student's test

TABLE 2. Content of B group vitamins in various powdered infant milks.

Vitamin	Concentration* (labelled) (μg/g)						
	Infant milk						
	Starting from 1 to 5 month	Starting from the born	Follow-up from 5 month	Follow-up above 4 month	Follow-up above 12 month	Follow-up from 1 to 3 year	Follow-up from 1 to 3 year (with honey)
B_1	6.3±0.2 (7.2)	5.2±0.1 (4.0)	10.3±0.2 (7.8)	9.9±0.2 (9.7)	6.8±0.1 (7.2)	10.3±0.2 (9.7)	10.3±0.2 (9.7)
B_2	9.0±0.5 (7.8)	7.6±0.3 (6.9)	13.5±0.5 (11.0)	7.5±0.3 (6.9)	11.2±0.3 (11.0)	13.9±0.3 (11.0)	13.1±0.5 (11.0)
B_6 (total)	5.2±0.1 (3.9)	4.8±0.1(5.0)	12.1±0.2 (9.6)	5.3±0.1 (5.0)	9.6±0.2 (9.6)	18.8±0.3 (14.0)	19.6±0.3 (14.0)

* Mean ± standard deviation

a large number of products *via* non-enzymatic browning reactions [Vernin *et al.*, 1998]. Therefore furfural components can be formed by ascorbic acid degradation or Maillard reaction. In some infant milks higher amounts of furfural components correspond quite well with lower ascorbic acid content (Table 1). This may indicate the degradation of the ascorbic acid pathway.

The water-soluble B vitamins are a broad group of organic compounds that are essential for normal health and growth of a child. Among the B group vitamins, B_1, B_2 and B_6 are the most important ones. They have different chemical structure and serve different specific and biological functions in the metabolism. Losses of vitamins are produced during food processing and storage. Fortification of infant milk formulas with these vitamins is intended to compensate for the loss of these compounds due to the heat treatment to which they are subjected during manufacture [Albalá-Hurtado *et al.*, 1997]. Thus, vitamin fortification enables us to meet the nutritional requirements of the infant formulas submitted to rigorous quality control analysis. Table 2 shows the results obtained in the present paper and the contents labeled by the manufacturers. Usually higher levels of vitamins were observed in the follow-up formulas in comparison to the starting infant milks. The statistical analysis indicated that there were no differences between the labelled and the experimental results ($p<0.05$).

CONCLUSIONS

The starting and follow-up infant formulas did not behave in the same way with regard to the browning reactions. They differ in the carbohydrates' fraction, the presence or lack of whey serum as a component and the protein content. Therefore, the type and contents of proteins and carbohydrates play an important role in the course of the Maillard reaction. The contents of Maillard compounds (F, HMF and furosine) in the analysed starting and follow-up formulas was low and did not involve any risk for the consumer. No maximum content limits have been established for these compounds in these products. However, the European Society of Paediatry Gastroenterology and Nutrition recommends that the amount of blocked lysine be as low as possible for preterm infants [ESPGAN, 1987].

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WYBRANE WSKAŹNIKI JAKOŚCI ODŻYWEK MLECZNYCH W PROSZKU

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Celem pracy było określenie poziomu wybranych wskaźników jakości odżywek mlecznych dla dzieci. Badania przeprowadzono na siedmiu różnych produktach modyfikowanego mleka w proszku zakupionych w lokalnym markecie. Badane produkty podzielono na dwie grupy: mleko początkowe i następne. W analizowanych produktach oznaczono poziom wskaźników reakcji Maillarda: furozyny, HMF (5-hydroksymetylofurfural), oraz innych związków furfuralu (F – furfural, FMC – furylometyloketozyna, MF – metylofurfural) przy zastosowaniu wysokosprawnej chromatografii cieczowej (HPLC). Ponadto w analizowanych produktach oznaczono zawartość witaminy C, B_1, B_2 i B_6. Poziom witaminy C określono przy zastosowaniu metody Tilmansa a zawartość witamin z grupy B metodą HPLC.

Przeprowadzone badania wykazały, że zawartość HMF (wolny i ogółem) w mleku początkowym była kilkakrotnie niższa niż poziom tego związku w mleku następnym (tab. 1). Podobną prawidłowość zaobserwowano w przypadku zawartości F (furfural) w tych produktach (tab. 1). Natomiast przeprowadzone badania nie wykazały obecności w analizowanych produktach innych związków furfuralu (FMC, MF). Stwierdzono również, że mleko początkowe wykazywało niższą zawartość furozyny niż mleko następne (tab. 1). Na podstawie otrzymanych wyników oraz składu badanych odżywek podanych przez producentów na opakowaniach stwierdzono, że na przebieg reakcji Maillarda w produktach początkowych i następnych miał wpływ ilościowy i jakościowy skład sacharydów i białek w tych produktach. Skład ten był, bowiem różny dla tych dwóch typów produktów (mleko początkowe i następne). Na podstawie analizy zawartości witamin w badanych odżywkach stwierdzono zgodność ich poziomu z ilościami deklarowanymi na opakowaniach przez producentów (tab. 2).

Wyniki przeprowadzonych badań wykazały, więc większe zmiany w obrębie składników mleka następnego niż początkowego, a co za tym idzie większe obniżenie się ich jakości i wartości odżywczej na skutek intensywniejszego przebiegu reakcji Maillarda w wyniku procesu produkcyjnego, transportu i przechowywania. Miało to związek przede wszystkim z różnym składem produktów początkowych i następnych. Przeprowadzone badania potwierdziły, więc, że przebieg reakcji Maillarda może mieć inny zakres i kierunek ze względu na różny skład nawet tak podobnych produktów. Ponadto należy podkreślić, że poddane analizie mleczne produkty modyfikowane charakteryzowały się zadowalająca jakością i na ogół zgodnością poziomu badanych składników z ilością deklarowaną przez producentów na opakowaniach.