ELSEVIER

Carbohydrate Polymers 37 (1998) 225–229

Carbohydrate Polymers

Determination of sugars, and some other compounds in infant formulae, follow-up milks and human milk by HPLC-UV/RI

I.M.P.L.V.O. Ferreira*, A.M.P. Gomes, M.A. Ferreira

CEQUP/Laboratório de Bromatologia da Faculdade de Farmácia da Universidade do Porto, Rua Aníbal Cunha 164, 4050 Porto, Portugal

Received 8 December 1997

Abstract

In the present study the composition of sugars, uric and orotic acids in infant formulae and follow-up milks commercially available on the local market is reported. The levels found are compared with Portuguese and European Standards and with human and bovine milk composition. Fifty samples including all such products were analysed, using a rapid and accurate HPLC procedure developed for that purpose, which allowed simultaneous determination of lactose, glucose, galactose, saccharose, maltose, uric and orotic acids in less than 15 min, by HPLC using refractive index and UV detectors connected in series.

Similar concentrations of lactose and uric acid were obtained for infant formulae, follow-up milks and human milk. The concentrations of orotic acid found in infant formulae and follow-up milk solutions were similar to those determined for bovine milk. No orotic acid was detected in human milk.

Keywords: Sugars; Organic acids; Infant formulae; Follow-up milks; Bovine milk; Human milk; HPLC-UV/RI

1. Introduction

Progressive attempts have been made by the industry to bring the composition of infant formulae closer to that of the human milk. Follow-up milks are given to infants after 4–6 months of age in order to make the transition from human or infant formulae to bovine milk. However, the composition of these artificial milk formulae, relative to some components, namely the sugars, does not correspond to that of genuine bovine milk from which they are originally prepared. Therefore, European and Portuguese regulations (91/5/EC Directive, J.O.E.C., no. L 175/35, 4.7.91 and Port no. 541/93, 25/6, respectively) establish the type and respective limits of carbohydrates which can be added to meet the necessary nutritional requirements. Other endogenous milk compounds such as uric and orotic acids appear naturally in these formulae and because their levels can be good indicators of the quality of bovine milk used their quantification is also important.

The objective of our research was to evaluate the composition of sugars, uric and orotic acids in infant formulae and follow-up milks commercially available on the local market, and compare the levels found with Portuguese and European Standards and with human and bovine milk samples. To this end, a precise, reproducible, rapid and economic analytical procedure was required. Undoubtedly, the method of choice for analysing sugars is HPLC with its accuracy, separation abilities and rapidity. It appeared more than 20 years ago, but remains the most widely used technique as is attested by the great number of published reviews (Jandera and Churacek, 1974; Pirisino, 1984; Menuier et al., 1986; Churms, 1990; Herbreteau, 1992). HPLC methods have also been employed to analyse uric and orotic acids in dairy products (Marsili et al., 1981-Navder et al., 1990). The developed method allowed simultaneous determination of lactose, glucose, galactose, saccharose, maltose, uric and orotic acids in less than 15 min, by HPLC using refractive index and UV detectors in series. The chromatographic separation was obtained with an amina-bonded silica column (Spherisorb NH_2), a commonly used system for mono and disaccharide separation. The use of ion-pair formation of uric and orotic acids allowed their simultaneous determination with sugars using the same column for separation of all different groups of compounds.

* Corresponding author.

0144–8617/98/$ - see front matter
PII: S0144-8617(98)00064-2

2. Materials and methods

2.1. Reagents and solutions

All reagents used were (p.a.) obtained from Merck. The standards for the orotic and uric acids were from Sigma. The water used for chromatography analysis had a resistance greater than 15 MΩ. It was filtered through a membrane of 0.45 μm porosity and subsequently degassed. The acetonitrile (Lichrosolv) used was Merck ''gradient grade''.

2.2. Apparatus

The chromatographic analyses were carried out in a Gilson, high performance liquid chromatograph equipped with a type 305 pump and a type 7125 Rheodyne Injector with a 20 μl loop. A Gilson variable wavelength UV/VIS detector 118, a 132 RI detector and Gilson 712 HPLC System Controller Software were also used.

2.3. Chromatographic conditions

The UV detector, set at 280 nm, was used for quantification of uric and orotic acids. The RI detector was used for quantification of sugars. These detectors were connected in series. The chromatographic separation was achieved with a Spherisorb NH_2 chromatographic column, 5 μm, 250 mm $\times$ 4.6 mm i.d. The mobile phase used was acetonitrile/HCL 0.01 M (84:16). The analyses were performed isocratically at a flow rate of 1.0 ml/min and at room temperature.

2.4. Sampling

Fifty samples were assayed, and included 18 infant formulae samples based on bovine milk proteins, 13 follow-up milks, two lactose-free infant formulae, five breast milks and 12 bovine milks.

2.5. Sample preparation

Approximately 1.5 g of previously homogenized infant formula or follow-up milk powder was dissolved in 2 ml of warm distilled water. Subsequently, 0.5 ml of 5% oxalic acid (w/v) and 5 ml of 95% ethanol were added and the resulting mixture was homogenized for 5 min. Finally, the volume was adjusted to 20 ml with deionized water and allowed to stand for 10 min. The supenant formed was injected into the 20 μl loop with a 5 ml syringe filter holder containing 0.2 μm membranes filters. The same procedure of sample preparation was used for human and bovine milk except that 5 ml and 10 ml of sample were used, respectively, and they were not dissolved in warm distilled water.

Complete triplicate analyses were performed on all samples to enable calculation of average deviations, which were useful as a measure of the extraction and chromatographic reproducibility.

2.6. Statistical analysis

Data are represented as the mean $\pm$ standard deviation. Analysis of variance was used to determine the effects of type of brand, on the one hand, and type of formulation on the other, on the lactose, orotic acid and uric acid concentrations. Fisher's protected least significant difference *t*-test (PLSD) at the 5% significance level was applied to all experimental results to assess intrapair significant differences. All statistical analyses were done with the Statview™ 4.0 statistical package (Abacus concepts, Berkeley, CA).

3. Results and discussion

Under the chromatographic conditions described above, a linear relationship between the concentration of sugars and the refractive index was obtained. The same occurred between the concentration of uric and orotic acids and the UV absorbance at 280 nm. This linearity was maintained over the concentration range of 0.5–30.0 g/l for sugars, 1.0–18.0 mg/l for uric acid, and 0.5–20.0 mg/l for orotic acid. The detection limits were 0.20 g/l for glucose, galactose and lactose, 0.10 g/l for saccharose and 0.35 g/l for maltose. For uric and orotic acids the detection limits were 0.5 and 0.1 mg/l, respectively. The correlation coefficient for each standard curve invariably exceeded 0.999 for all compounds.

The analytical results for sugars and organic acids in infant formulae samples and follow-up milks are reported in Table 1Table 2, respectively.

Lactose was detected as the single sugar present in all, except two, infant formulae. The two exceptions referred to lactose-free infant formulae use for lactase-deficient infants and contained maltose and glucose instead. The data pertaining to the evaluated sugars, uric acid and orotic acid indicate differences among the commercial brands of infant formulae which are confirmed by the high *F*-values ($p < 0.001$), obtained from the statistical analysis for lactose ($F = 31.45$), uric acid ($F = 166.15$) and orotic acid data ($F = 85.77$) for all infant formulae. Maximum levels of lactose, i.e. 56–58 g/100 g were obtained for 50% of the commercial brands analysed, followed by 33% which registered lactose contents within the range 54–56 g/100 g and the remaining 27% reported lactose contents below 50 g/100 g (Table 1). It is interesting to note that although brand 8 showed the lowest lactose content (35.9 g/100 g infant formula powder), well below the mean levels (50.7 g/100 g) found, such apparent deficiency was compensated for by the presence of glucose polymers as referred to on the label. A similar observation was reported for the two lactose-free infant formulae assayed wherein the energy source was provided by the monosaccharides, glucose and maltose, as mentioned above (Table 1) and by polysaccharides or glucose polymers, as acknowledged from the respective labels.

Table 1
Sugars, uric and orotic acids composition of infant formulae samples[1]

Samples	Glucose (g/100 g)	Galactose (g/100 g)	Saccharose (g/100 g)	Maltose (g/100 g)	Lactose (g/100 g)	Uric acid (mg/100 g)	Orotic acid (mg/100 g)
1	N.D.	N.D.	N.D.	N.D.	54.2 ± 2.3^{a}	6.80 ± 0.11^{a}	26.7 ± 1.0^{a}
2	N.D.	N.D.	N.D.	N.D.	58.2 ± 1.1^{b}	8.02 ± 0.07^{b}	25.1 ± 0.9^{a}
3	N.D.	N.D.	N.D.	N.D.	45.6 ± 2.2^{c}	6.10 ± 0.09^{c}	21.8 ± 1.1^{b}
4	N.D.	N.D.	N.D.	N.D.	54.4 ± 0.8^{a}	10.8 ± 0.10^{d}	37.6 ± 1.7^{c}
5	N.D.	N.D.	N.D.	N.D.	$55.8 \pm 0.9^{a,b}$	5.12 ± 0.9^{e}	15.1 ± 0.8^{d}
6	N.D.	N.D.	N.D.	N.D.	$56.1 \pm 2.0^{a,b}$	4.87 ± 0.21^{e}	19.7 ± 1.3^{e}
7	N.D.	N.D.	N.D.	N.D.	58.1 ± 0.7^{b}	5.12 ± 0.09^{e}	$28.1 \pm 0.7^{a,f}$
8	N.D.	N.D.	N.D.	N.D.	35.9 ± 1.2^{d}	7.10 ± 0.07^{f}	24.8 ± 0.8^{a}
9	N.D.	N.D.	N.D.	N.D.	54.7 ± 0.6^{a}	10.2 ± 0.60^{g}	$27.3 \pm 1.0^{a,f}$
10	N.D.	N.D.	N.D.	N.D.	$56.8 \pm 0.9^{a,b}$	7.22 ± 0.19^{f}	35.1 ± 0.5^{g}
11	N.D.	N.D.	N.D.	N.D.	54.9 ± 0.9^{a}	8.81 ± 0.19^{h}	16.9 ± 1.1^{d}
12	N.D.	N.D.	N.D.	N.D.	55.0 ± 1.2^{a}	9.22 ± 0.27^{h}	$27.1 \pm 0.8^{a,f}$
13	N.D.	N.D.	N.D.	N.D.	47.6 ± 1.3^{c}	6.10 ± 0.29^{c}	31.7 ± 0.8^{h}
14	N.D.	N.D.	N.D.	N.D.	$57.4 \pm 0.9^{a,b}$	11.8 ± 0.18^{i}	$27.6 \pm 1.2^{a,f}$
15	N.D.	N.D.	N.D.	N.D.	$55.5 \pm 0.9^{a,b}$	7.12 ± 0.19^{f}	16.2 ± 0.7^{d}
16	N.D.	N.D.	N.D.	N.D.	$56.2 \pm 2.1^{a,b}$	5.80 ± 0.11^{c}	$26.9 \pm 0.2^{a,f}$
17	N.D.	N.D.	N.D.	N.D.	58.2 ± 1.2^{b}	7.42 ± 0.17^{f}	$21.2 \pm 0.7^{b,e}$
18	N.D.	N.D.	N.D.	N.D.	52.6 ± 1.9^{a}	6.10 ± 0.19^{c}	$20.4 \pm 1.0^{b,e}$
19[2]	2.36 ± 1.2	N.D.	N.D.	19.3 ± 0.4	N.D.	N.D.	N.D.
20[2]	1.97 ± 0.9	N.D.	N.D.	25.8 ± 1.1	N.D.	N.D.	N.D.

[1]Values are expressed as mean ± standard deviation of three determinations.
[2]Infant formulae lactose free. N.D.—not detected.
[a–i]Means in columns without common superscripts are significantly different ($p < 0.05$); $n = 3$.

Normal biochemical metabolic processes in bovine milk account for the production of uric and orotic acids and are a likely explanation for the presence of these compounds (especially orotic acid) in infant formulae which are, in general, mostly based on bovine milk. Concentrations varied between 4.72 and 11.9 mg/100 g and between 14.5 and 38.8 mg/100 g, for uric acid and orotic acid, respectively. The greater variability among commercial brands (as apparent from the high *F*-values) may reflect different modifications made to the bovine milk (by the manufacturer) employed in the formulation of the different infant formulae.

With respect to the follow-up milks, the prevailing carbohydrate was still lactose but these also contained other sugars, such as maltose, saccharose and traces of glucose and galactose (Table 2), at levels allowed by regulations (EEC Directive 91/321). In fact, a good correlation between lower levels of lactose (< 48 g/100 g follow-up milk powder) and the detection of other carbohydrates was observed. Brands containing lactose within the range of 34–48 g/100 g (Table 2) also contained, either saccharose (brand 22) or glucose and maltose (brand 28) or a combination of the three monosaccharides (brand 21). Brands 23 and 29

Table 2
Sugars, uric and orotic acids composition of follow-up milk samples[1]

Samples	Glucose (g/100 g)	Galactose (g/100 g)	Saccharose (g/100 g)	Maltose (g/100 g)	Lactose (g/100 g)	Uric acid (mg/100 g)	Orotic acid (mg/100 g)
21	0.51 ± 0.03	< 0.3	7.12 ± 0.24	3.40 ± 0.10	47.8 ± 1.2^{a}	8.67 ± 0.13^{a}	23.1 ± 0.3^{a}
22	N.D.	N.D.	5.18 ± 0.71	N.D.	46.7 ± 1.3^{a}	4.17 ± 0.09^{b}	14.2 ± 0.7^{b}
23	0.53 ± 0.4	N.D.	N.D.	0.61 ± 0.08	26.7 ± 0.5^{b}	10.9 ± 0.20^{c}	34.1 ± 1.1^{c}
24	N.D.	N.D.	N.D.	N.D.	56.4 ± 0.9^{c}	11.8 ± 0.19^{d}	34.6 ± 1.1^{c}
25	N.D.	N.D.	N.D.	N.D.	52.8 ± 0.7^{d}	5.19 ± 0.19^{e}	25.1 ± 0.5^{d}
26	N.D.	N.D.	N.D.	N.D.	56.2 ± 1.0^{c}	4.99 ± 0.20^{e}	15.7 ± 1.2^{b}
27	N.D.	N.D.	N.D.	N.D.	52.1 ± 0.8^{d}	6.12 ± 0.07^{f}	$24.1 \pm 0.5^{a,d}$
28	2.53 ± 0.03	N.D.	N.D.	4.93 ± 0.08	33.9 ± 1.5^{e}	6.16 ± 0.02^{f}	22.8 ± 0.5^{a}
29	0.82 ± 0.08	N.D.	N.D.	N.D.	24.7 ± 0.6^{b}	15.6 ± 0.70^{g}	27.3 ± 1.0^{e}
30	N.D.	N.D.	N.D.	N.D.	49.8 ± 0.9^{f}	9.12 ± 0.19^{a}	25.1 ± 0.6^{d}
31	N.D.	N.D.	N.D.	N.D.	54.9 ± 0.9^{c}	7.41 ± 0.19^{h}	36.4 ± 1.2^{f}
32	N.D.	N.D.	N.D.	N.D.	53.0 ± 1.1^{d}	10.2 ± 0.3^{i}	37.1 ± 0.5^{f}
33	N.D.	N.D.	N.D.	N.D.	57.6 ± 1.3^{c}	6.10 ± 0.29^{f}	21.7 ± 0.7^{a}

[1]Values are expressed as mean ± standard deviation of three determinations. N.D.—not detected.
[a–i]Means in columns without common superscripts are significantly different ($p < 0.05$); $n = 3$.

Table 3
Sugars, uric and orotic acids composition of bovine milk and human milk samples[1]

Samples	Glucose (g/100 ml)	Galactose (g/100 ml)	Saccharose (g/100 ml)	Maltose (g/100 ml)	Lactose (g/100 ml)	Uric acid (mg/100 ml)	Orotic acid (mg/100 ml)
34[a]	N.D.	N.D.	N.D.	N.D.	4.62 ± 0.23	2.06 ± 0.01	4.02 ± 0.07
35[a]	N.D.	N.D.	N.D.	N.D.	4.68 ± 0.11	1.92 ± 0.02	5.21 ± 0.18
36[a]	N.D.	N.D.	N.D.	N.D.	4.56 ± 0.21	2.10 ± 0.02	4.80 ± 0.42
37[a]	N.D.	N.D.	N.D.	N.D.	4.85 ± 0.07	1.88 ± 0.10	4.67 ± 0.07
38[a]	N.D.	N.D.	N.D.	N.D.	4.58 ± 0.09	1.51 ± 0.09	5.11 ± 0.08
39[a]	N.D.	N.D.	N.D.	N.D.	4.69 ± 0.13	2.27 ± 0.02	3.08 ± 0.03
40[a]	N.D.	N.D.	N.D.	N.D.	4.68 ± 0.10	2.22 ± 0.04	6.31 ± 0.04
41[a]	N.D.	N.D.	N.D.	N.D.	4.36 ± 0.09	2.11 ± 0.08	4.79 ± 0.21
42[a]	N.D.	N.D.	N.D.	N.D.	4.87 ± 0.05	1.78 ± 0.01	2.27 ± 0.51
43[a]	N.D.	N.D.	N.D.	N.D.	4.52 ± 0.09	1.41 ± 0.05	6.10 ± 0.18
44[a]	N.D.	N.D.	N.D.	N.D.	4.69 ± 0.21	2.16 ± 0.02	3.22 ± 0.09
45[a]	N.D.	N.D.	N.D.	N.D.	4.54 ± 0.10	1.99 ± 0.01	4.21 ± 0.09
46[b]	N.D.	N.D.	N.D.	N.D.	7.02 ± 0.23	0.82 ± 0.07	N.D.
47[b]	N.D.	N.D.	N.D.	N.D.	6.68 ± 0.15	0.92 ± 0.25	N.D.
48[b]	N.D.	N.D.	N.D.	N.D.	7.24 ± 0.23	0.80 ± 0.01	N.D.
49[b]	N.D.	N.D.	N.D.	N.D.	7.85 ± 0.06	0.89 ± 0.01	N.D.
50[b]	N.D.	N.D.	N.D.	N.D.	7.58 ± 0.08	0.81 ± 0.01	N.D.

[1]Values are expressed as mean ± standard deviation of three determinations.
[a]Bovine and [b]Human milk samples. N.D.—not detected.

contained relatively low levels of lactose (Table 2), but no other monosaccharides were detected. These levels were within the minimum allowable limit (1.8 g/100 kcal) for lactose, but below the minimum allowable limit (7 g/100 kcal) for carbohydrate content. Nevertheless, the addition of polysaccharides or glucose polymers as referred to by the labels of these specific brands total the necessary carbohydrate contribution.

A large variability among commercial brands for uric acid ($F = 301.8$) and orotic acid ($F = 162.7$) was also reported for the follow-up milks assayed. Concentrations varied between 4.10 and 16.1 mg/100 g and between 13.7 and 37.5 mg/100 g for uric acid and orotic acid, respectively. Further, analysis of variance showed that the effect of type of formulation, infant formula or follow-up milk, was not significant for uric acid ($p = 0.226$) and orotic acid ($p = 0.479$). On the other hand, lactose content was affected significantly ($p = 0.0008$) by the type of formulation; follow-up milks reported a higher percentage of brands with lactose contents below 50 g/100 g compared to that reported by the infant formulae.

Human milk samples reported higher concentrations of lactose ($p < 0.05$) than bovine milk counterparts (Table 3), which is in good agreement with literature values (Worthington-Roberts, 1985).

Significant differences between the levels of uric acid in bovine milk and in human milk were obtained ($p < 0.05$); bovine milk contained, in general, levels twice as high as those reported in human milk. In contrast with bovine milk, where its occurrence is a result of normal biochemical metabolic processes as discussed above, no orotic acid was detected in human milk.

In order to make the comparison between results obtained for infant formulae and for human milk possible, it was necessary to consider the labels associated with the infant formulae. The standard dilution recommended on all labels was the use of 13 g of infant formula powder to prepare 100 ml of reconstituted milk, the factor which we used to convert our original data. Upon conversion, we concluded that infant formulae solutions contained an average of 6.99 ± 0.72 g/100 ml of lactose and 0.96 ± 0.26 mg/100 ml of uric acid. No significant ($p > 0.05$) differences between these and the levels obtained for human milk were reported. This is a desirable observation because infant formulae should closely approach human milk in nutrient composition. The average concentration of orotic acid in infant formulae was 3.25 ± 0.81 mg/100 ml, but, as discussed above, such a component was not detected in human milk.

4. Conclusions

The levels found for sugars in infant formulae and follow-up milks randomly purchased from the Portuguese market fell within the Portuguese and European legislation, and were in close agreement with the contents mentioned on the respective labels. Similar concentrations of lactose and uric acid were obtained for infant formulae and human milk. The concentrations of orotic acid found in human formulae and follow-up milk solutions were similar to those determined for bovine milk.

Acknowledgements

This work received financial support from Junta Nactional de Investigação Científica Projecto PBIC/C/TPR/

2564/95. Infant formulae samples were generous gifts from Nutrícia and Mead Johnson.

References

Churms, S.C. (1990). J. Chromatogr., *500*, 555.
Herbreteau, B. (1992). Analusis, *20*, 355.
Jandera, P., & Churacek, J. (1974). J. Chromatogr., *98*, 55.
Marsili, R.T., Ostapenko, H., Simmons, R.E., & Green, D.E. (1981). J. Food Sci., *46*, 52.
Menuier, A., Caude, M., & Rosset, R. (1986). Analusis, *14*, 363.
Navder, K.P., Huang, R.F.S., Fryer, E.B., & Fryer, H.C. (1990). J. Food Sci., *55*, 585.
Pirisino, J.F. (1984). Food Sci. Technol., *11*, 159.
Worthington-Roberts, B.S., 1985. Lactation and human milk: nutritional considerations. In *Nutrition in Pregnancy and Lactation* (pp. 187–240). Times Mirror, Mosby College Publishing.