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Ratio of Maltose to Maltulose and Furosine as Quality Parameters for Infant Formula

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Nonenzymic browning reactions in commercial infant formulas were evaluated through their furosine content as well as the isomeric disaccharides formed during processing. Lactulose was observed only in samples containing appreciable amounts of lactose, whereas maltulose was present in all samples due to the isomerization of maltose. Because formation of maltulose depends on the initial amount of maltose present, the ratio maltose/maltulose was used for comparative purposes. The ratio maltose/maltulose varied within a wide range, 27−167; therefore, low values in maltose/maltulose ratio may indicate severe processing conditions during manufacture, whereas high values may indicate mild processing conditions. Variable amounts of furosine content in samples with similar maltose/maltulose ratios may be attributed to different conditions used during storage. Levels of furosine higher than those reported for milk powder were detected in most studied samples. Determination of both furosine and maltose/maltulose ratio would yield information retrospectively about the heat treatment applied during processing and the storage conditions of commercial infant formula.

KEYWORDS: Maltulose; furosine; infant formula

INTRODUCTION

Nonenzymic browning reactions that may take place during processing or storage of foods can cause adverse effects on color and appearance, decrease of nutritional value, changes in functional properties, and formation of toxic compounds.

Carbonyl and free amino acids groups are mainly involved in these complex reactions; thus, foods containing reducing carbohydrates and proteins or free amino acids are prone to nonenzymic browning.

The composition of infant formula includes as major components carbohydrates (54−61 g/100 g of product) and proteins (11−15 g/100 g of product). Depending on the type of infant formula, sugars such as corn syrup solids, lactose, sucrose, or starch have been successfully used as sources of carbohydrates (*1*−*3*). The principal source of proteins used in infant formula elaboration is bovine milk; thus, caseins and whey proteins are usually incorporated. In addition, specialized formulas have been developed using soybean protein for infants intolerant of milk proteins (*2*).

Infant formula elaboration includes different steps (blending of components, pasteurization, concentration, spray-drying, storage) that have a great influence in their final quality. For liquid infant formula more severe heat treatment such as sterilization (UHT or in bottle) must be applied. These treatments can cause reactions and/or interactions between constituents, resulting in a loss of nutritive value. This is very important because infant formulas sometimes are the only source of infant nutrition during the first months of life.

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Because infant formulas may contain high levels of carbohydrates and proteins, the Maillard reaction plays an important role during manufacture from the point of view of losses in nutritive value (*4*, *5*). Besides, carbohydrates can suffer isomerization and degradation reactions as has been observed in different carbohydrate-containing foods (*6*). Maltose present in infant formula may be isomerized to maltulose during heat processing and also contributes to furosine formation through the Maillard reaction.

Heat-induced damage in infant formula composition can be evaluated using different chemical indices. Losses of available lysine and hydroxymethylfurfural (HMF) and furfural compound contents (as a measurement of the Maillard reaction) have been determined in powdered and liquid infant formulas (*7*−*10*). The early stages of the Maillard reaction, through furosine determination, have been also studied during thermal processing and storage of infant formulas (*4*, *9*−*11*). The relationship between blocked lysine and carbohydrate composition in commercial infant formula has been also studied by Evangelisti et al. (*12*).

The formation of isomeric carbohydrates during heat processing of foods containing reducing sugars has been related to the intensity of the heat treatment applied, and the levels of these compounds have been proposed to evaluate heat damage in processed foods (*13*, *14*). Thus, lactulose, an isomer of lactose used as a thermal index to differentiate heated milks, has been detected and quantified in powdered and liquid infant formulas (*1*, *5*, *9*, *10*, *15*, *16*).

The presence of maltulose, an isomer of maltose, has been detected in different types of foods (bread and honey) (*17*, *18*), and the ratio of maltose to maltulose has been proposed for the

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Table 1. Compositions of the Commercial Powdered Infant Formulas Analyzed

		carbohydrates[a]		orotein	
sample	pH (after reconstitution)	total content (g/100 g of product)	composition (g/100 g of product)	content[b] (g/100 g of product)	source[a]
1	6.88	61.5	lactose (41.3) starch (7.5) maltodextrins (12.7)	11.2	milk proteins
2	6.83	57.1	lactose (43.0) maltodextrins (13.4)	11.6	milk proteins
3	6.58	56.8	lactose (44.7) maltodextrins (12.1)	14.5	milk proteins
4	6.76	55.8	lactose (10.2) maltodextrins (45.6)	11.0	casein/whey proteins, 40:60
5	6.54	54.1	sugars (25.6) lactose (20.5) glucose (1.1) maltose (4.0) polysaccharides (28.4)	11.9	whey protein hydrolysate
6	6.99	50.6	lactose (38.7) maltodextrins (11.9)	15.5	milk proteins
7	6.53	55.3	lactose (19.2) starch (9.9) maltodextrins (26.0)	10.9	whey protein hydrolysate
8	6.54	54.0	maltodextrins (54.0)	11.2	100% free amino acids
9	6.75	52.8	maltodextrins (52.8)	12.9	soy
10	7.14	55.6	sugars (10.4) glucose (5.4) maltose (4.9) polysaccharides (44.7)	12.1	casein/whey proteins, 60:20
11	6.73	53.6	maltodextrins (53.6)	14.2	whey protein hydrolysate

[a] Information on the package label. [b] Calculated by Kjeldahl (*20*).

first time as an indicator to assess the heat treatment during manufacture and to monitor storage of enteral formulas (*19*).

Because studies on changes of the carbohydrate fraction during processing of infant formula are scarce (*1*, *3*), the objective of this work was to study mono- and disaccharide compositions of commercial samples and the feasibility of using maltulose content alone or in combination with furosine for quality evaluation of infant formula.

MATERIALS AND METHODS

Samples. Eleven commercial powdered infant formulas were collected from local pharmacies, and their compositions (taken from information on the package label) are shown in **Table 1**. Powdered samples were reconstituted in water at 10% w/v prior to chromatographic analysis.

Analytical Determinations. Sample preparation and analytical determinations were realized in duplicate.

Protein Determination. Protein determination was carried out according to the Kjeldahl method of the AOAC (*20*).

HPLC of Furosine. Analysis of furosine was performed according to an ion-pair RP-HPLC method (*21*), using a chromatographic system composed of a model 250 pump (Perkin-Elmer), an oven (Kariba), a UV detector ($\lambda = 280$ nm) (SM 4000 LDC Analytical), and a model 406 interface (Beckman). Furosine separation was carried out using a C_8 (Alltech furosine-dedicated) column (250 × 4.6 mm i.d.) and a linear binary gradient at a flow rate of 1.2 mL/min.

Before HPLC analysis, a sample preparation was realized. Powdered infant formula (0.5 g) was hydrolyzed with 8 mL of 8 N HCl at 110 °C for 24 h in a screw-capped Pyrex vial with a PTFE-faced septum. The hydrolysates were filtered through Whatman no. 40 filter paper, and 0.5 mL of the filtrate was applied to a previously activated Sep-Pak C_{18} cartridge (Millipore). Furosine was eluted with 3 mL of 3 N HCl, and 50 μL of this volume was injected in the chromatograph.

Quantitation was performed according to the external standard method using a commercial standard of pure furosine (Neosystem Laboratories, Strasbourg, France).

Gas Chromatographic Analysis of Carbohydrates. Analysis of mono- and disaccharides in infant formula was performed by GC, following the method of Garcia-Baños et al. (*22*). Chromatographic analysis was performed with a Hewlett-Packard (Avondale, PA) model 6890 gas chromatograph furnished with a split injector, a flame ionization detector, and a 25 m × 0.25 mm i.d. fused silica capillary column coated with OV-101. Nitrogen at a flow rate of 0.5 mL/min was used as carrier gas. The injector and detector temperatures were 280 and 300 °C, respectively. The oven temperature was programmed from 180 to 280 °C at a heating rate of 2 °C/min, held for 1 min, then programmed to 300 °C at a heating rate of 10 °C/min, and held for 15 min. The split ratio was 1:40.

Quantitative analysis was performed following an internal standard method using *myo*-inositol and trehalose as standards of mono- and disaccharides, respectively.

To eliminate proteins, fat, and carbohydrates of high molecular mass, sample preparations of infant formulas before chromatographic analysis must be carried out. Two milliliters of reconstituted powdered formula was gently mixed with ~10 mL of methanol in a 25 mL volumetric flask, which was filled to volume by the addition of methanol. After mixing again, the mixture was held for several hours at room temperature and centrifuged at 10000 rpm at 10 °C. Four milliliters of supernatant was mixed with 1 mL of *myo*-inositol solution (1 mg/mL) and 1 mL of trehalose solution (1.2 mg/mL) (internal standards), evaporated under vacuum, and transformed into their trimethylsilylated oximes (TMS-oxime) (*23*). All of the precipitations were performed in duplicate.

Lactulose determination was done by means of gas chromatography of the trimethylsilyl derivatives of the free carbohydrate fraction using a Sigma 3B gas chromatograph equipped with a 3 m × 1.0 mm i.d. stainless steel column (Chrompack, Middelburg, The Netherlands) packed with 2% OV-17 on nonsilanized 120/140 Volaspher A2 (Merck, Darmstadt, Germany), following the method described by de Rafael et al. (*24*).

Before analysis, infant formula samples (2.5 mL) were gently mixed with ~10 mL of ethanol in a 25 mL volumetric flask; so that denatured protein particles would not get stuck above the volume mark, the flasks

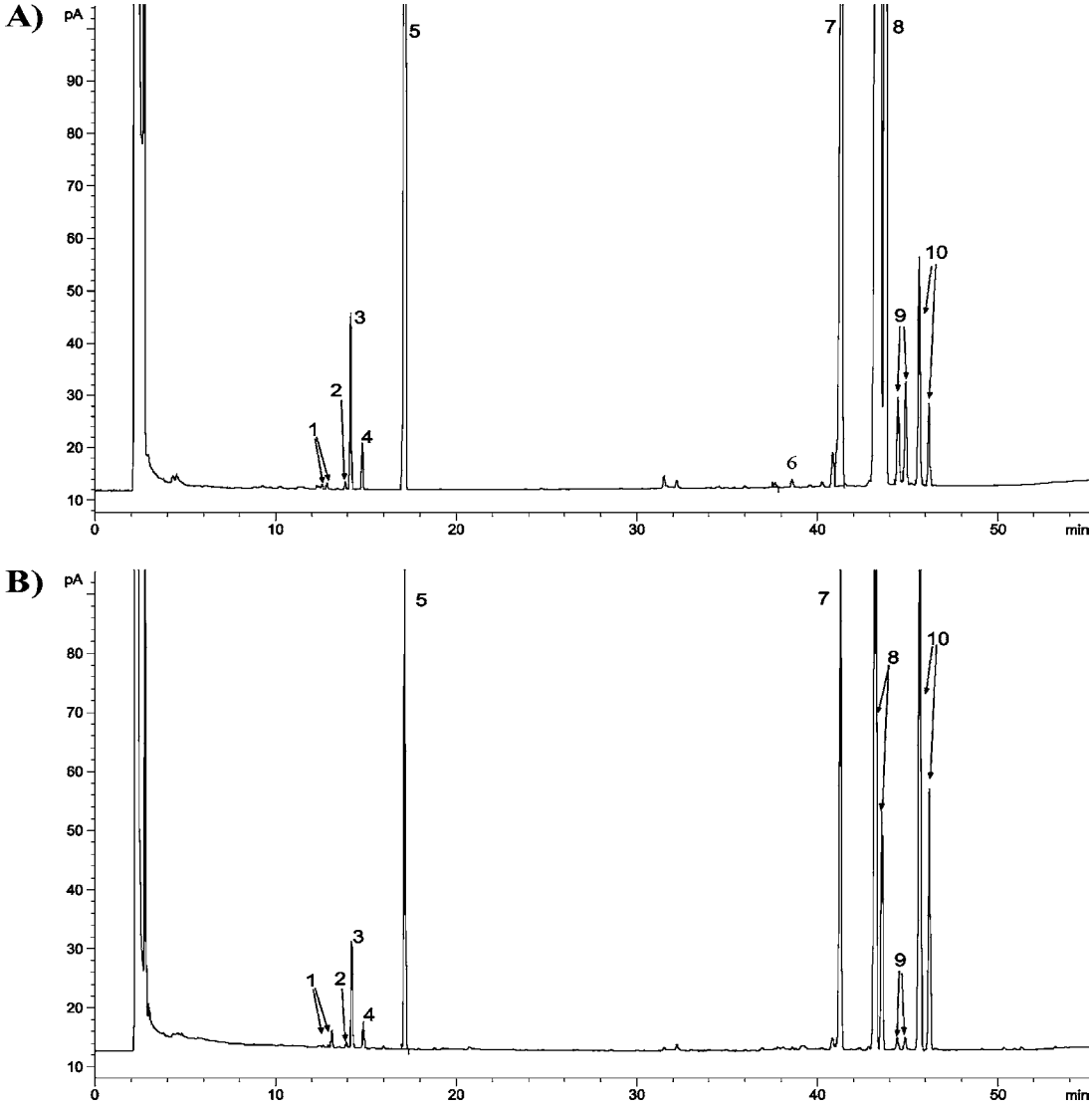

Figure 1. GC profiles of trimethylsilyl oxime derivatives of carbohydrates of (**A**) standard solution and (**B**) infant formula (sample 7): 1, fructose; 2, galactose; 3, glucose; 4, galactose + glucose; 5, *myo*-inositol (internal standard for monosaccharides); 6, sucrose; 7, trehalose (internal standard for disaccharides); 8, lactose; 9, maltulose; 10, maltose.

were filled to volume by adding ethanol. After mixing again, the mixture was kept for 48 h at room temperature to allow lactose precipitation. Four milliliters of supernatant was mixed with 1 mL of 0.05% of phenyl-β-glucoside and evaporated under vacuum at room temperature. The mixture was converted to trimethylsilyl derivatives using *N*-trimethylsilylimidazole.

RESULTS AND DISCUSSION

Figure 1A shows a chromatogram of the GC analysis of a mixture of carbohydrate standards: fructose, glucose, galactose, *myo*-inositol (internal standard), sucrose, threhalose (internal standard), lactose, maltulose, and maltose. The minor peaks of galactose and glucose overlap, but the major peaks of these carbohydrates were well resolved, and the area of each was proportional to its concentration, so these monosaccharides were quantified using only areas of major peaks.

The repeatability of the GC method was also calculated using infant formula sample 8, prepared five times and analyzed on the same day and on different days (**Table 2**). It was acceptable for all reducing sugars. Also, the accuracy of the complete methodology was checked by spiking known concentrations of glucose, maltulose, and maltose in an infant formula (sample 2), and good recoveries were obtained for the three sugars studied (**Table 3**).

Table 2. Repeatability of the Analytical Method Illustrated by Analysis of an Infant Formula (Sample 8) Prepared Five Times and Analyzed on the Same Day and Prepared One Time and Analyzed on Different Days

	concn (mg/100 g of product)		concn (mg/100 g of product)	
carbohydrate	same day (five preparations)	RSD[a] (%)	different days (one preparation)	RSD (%)
fructose[b]	39.5	5.6	41.7	3.3
glucose	727.5	1.8	712.0	0.4
maltose[c]	2767.3	4.1	2673.9	2.9
maltulose	89.1	1.7	86.7	1.4

[a] Relative standard deviation. [b] *myo*-Inositol, internal standard for monosaccharides. [c] Trehalose, internal standard for disaccharides.

The optimized GC method was applied to determine mono- and disaccharide contents of commercial infant formulas. **Figure 1B** shows the chromatogram obtained for sample 7. Fructose, glucose, galactose, sucrose, lactose, maltulose, and maltose were present in most of the samples analyzed. The individual contents

Table 3. Recovery for GC Analysis of Glucose, Maltose, and Maltulose Spiked in an Infant Formula (Sample 2)

	concn (mg/100 g of product)			
carbohydrate	initial	added	recovered	recovery (%)
glucose	390.1	1214.5	1501.2	93.55
		3036.3	3185.5	92.97
		4250.9	4236.3	91.28
maltulose	13.9	886.0	803.3	89.30
		443.0	412.3	90.24
		221.5	216.6	92.01
maltose	664.7	6607.0	7361.3	101.23
		4404.7	4953.1	97.70
		2202.3	2842.8	99.15

in each sample are shown in **Table 4**. In formulas containing lactose (samples 1−7), this sugar was the major component, ranging from 7152 to 44044 mg/100 g of product, with the exception of sample 4, which presented similar levels of lactose and maltose.

Samples 8−11 showed maltose and glucose as major carbohydrates. These samples correspond to formulas without lactose, except sample 11, containing 1584 mg/100 g of product.

Sucrose was detected in low amounts in five infant formulas. Glucose and low levels of fructose were detected in all studied samples. Small amounts of galactose were detected in samples containing lactose, similar to those obtained by Troyano et al. (*25*) in commercial powdered milks.

All samples presented variable amounts of maltose in a range between 459 and 7449 mg/100 g of product; this may be attributted to the presence of maltodextrins in their composition. Variable amounts of these carbohydrates were also obtained by Scott and Hatina (*1*) and Kaine and Wolnik (*3*).

Maltulose was found in all samples analyzed in a range between 12.6 and 842.8 mg/100 g of product. The presence of this carbohydrate has not been previously reported in infant formulas, and the wide variability of maltulose levels found could be attributable to differences in processing conditions and/or differences in maltose levels present in maltodextrins (*19*). The high content of maltulose present in sample 7 could be possible due to overprocessing.

Because the formation of maltulose depends on the initial amount of maltose present, the ratio of maltose to maltulose has been previously proposed as an indicator of maltose isomerization during processing of foods containing maltose (*19*). Three samples showed levels of maltose/maltulose ratio from 80 to 167, which may indicate mild processing conditions. The rest of the samples presented values lower than 65, which may correspond to more severe processing.

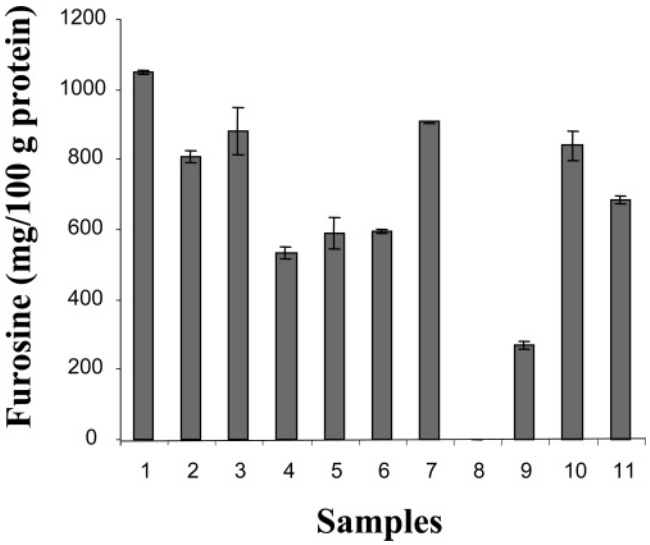

Figure 2. Furosine contents in the studied commercial infant formulas.

Lactulose was also determined in the studied samples and was detected in only five samples (samples 1−3, 6, and 7), at levels of 23−45 mg/100 g of product; these values are in agreement with those found by Puig et al. (*5*), Gonzales et al. (*16*), and Park et al. (*26*). Scott and Hatina (*1*) did not find lactulose in any powdered infant formula samples analyzed. According to previous studies, isomerization of lactose is predominant over the Maillard reaction during sterilization or ultrahigh-temperature (UHT) treatment of milk; however, the Maillard reaction is more noticeable than lactose isomerization during storage of milk powders (*27*).

A study of the Maillard reaction through furosine determination was also carried out, and the levels found in commercial infant formula are shown in **Figure 2**. Furosine was absent in sample 8. The nitrogen fraction of this sample consists of a synthetic mixture of free amino acids enriched with taurine. It is also the sample with the lowest amount of reducing mono- and disaccharides. The low content of reducing carbohydrates and the presence of large amounts of free amino acids that compete with lysine for carbonyl groups for Maillard reaction may hinder the formation of furosine. The rest of the samples, with the exception of sample 9, showed values of furosine higher than those reported in high-heat-treated milk powder (*27*, *28*) but similar to previously reported levels in infant formula (*4*, *9*, *10*, *29*, *30*). Studies on the furosine content of freshly produced milk powder (*31*) showed values ranging from 55 to 350 mg/100 g of protein, and concentrations up to 600 mg/100 g of protein were found in some powders after storage for long

Table 4. Carbohydrate Contents of the Commercial Infant Formulas Studied

	carbohydrate (mg/100 g of product)								
sample	fructose	galactose	glucose	sucrose	lactose	lactulose	maltose	maltulose	Ma/Mu[a]
1	9.8	74.3	182.6		32898	45.4	459.1	12.6	36.4
2	10.6	59.0	394.1	23.7	39274	23.7	584.7	14.7	39.8
3	14.3	49.0	761.8	16.7	40902	25.1	632.0	13.6	46.5
4	24.1	50.8	654.5		7152		7269	152.2	47.7
5	23.6	119.0	1967.8		14145		4369	26.1	167.4
6	11.5	96.7	153.1	8.4	44044	23.1	594.2	22.2	26.8
7	29.4	97.8	2812.9		21856	43.4	2544	842.8	3.0
8	39.5		727.5				2767	89.1	31.0
9	37.5		4105.8				5462	67.6	80.8
10	59.5		4597.6	48.9			5276	81.5	64.7
11	27.3	20.3	1995.0	3.8	1584		7449	58.6	127.1

[a] Maltose/maltulose ratio.

periods. The relatively high level of furosine found in the present study indicates that the Maillard reaction has a considerable effect on the lysine provided by infant formula.

The high levels of furosine detected in some infant formulas may be attributable to excessive heat treatment during processing or abusive storage conditions. High values of the maltose/maltulose ratio detected in samples 5 and 11 indicate that they have been thermally processed under relatively mild conditions, so high levels of furosine present in these samples are mostly a consequence of storage. On the other hand, in sample 7 a low value of the maltose/maltulose ratio and a high furosine content may be due to excessive heat treatment.

The present results indicate that the determination of both furosine and the maltose/maltulose ratio would yield information retrospectively about the heat treatment applied during processing and the storage conditions of commercial infant formulas. Storage under controlled temperature appears to be a feasible way of extending the shelf life and preserving the quality of infant formulas.

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