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Maillard reaction during storage of powder enteral formulas

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Abstract

Mono- and disaccharide compositions and Maillard reaction development in four powder enteral formulas were determined. Also, a study of the changes in this carbohydrate fraction as well as the progress of Maillard reaction during storage of two samples, under different temperature and time conditions and water a activity of 0.44, was carried out.

Variable amounts of fructose, glucose, lactose, sucrose, maltose and maltulose were detected in the studied samples. Furosine (ε-2-furoylmethyl lysine), was present in the four enteral samples studied whereas 2-furoylmethyl alanine (2-FM-Ala) and α-2-furoylmethyl lysine were only present in two samples.

Storage at 30 and 50 °C produced slight changes in carbohydrate composition. At the two temperatures assayed the same level of carbohydrate was found at the end of storage period studied. The maltose/maltulose ratio did not suffer notable changes under moderately-severe conditions of storage. Furosine, 2-FM-Ala and α-2-furoylmethyl lysine content increased during storage, and the formation of 2-furoylmethyl arginine was detected in one stored sample.

Results seem to indicate that 2-furoylmethyl derivatives of lysine, alanine and arginine could be used as good indicators of storage conditions of powder enteral formulas. Moreover, the maltose/maltulose ratio may be a quality indicator of the processing conditions during manufacture of commercial enteral products.

Keywords: Powder enteral formulas; Maltulose; Furosine; Storage

1. Introduction

Enteral products are widely used in both hospitals and nursing homes as complete nutritional products or as dietary supplements (Lowry, Fly, Izquierdo, & Baker, 1989). Their composition can include carbohydrates, proteins, fats, vitamins and mineral components. Carbohydrates are the main source of calories and they are incorporated in the form of corn syrups, maltodextrins and glucose oligosaccharides (Krause & Mahan, 1984). Proteins can be incorporated in the form of whole protein, protein hydrolyzate or free amino acids, giving rise to polymeric, oligomeric or elemental enteral formulations, respectively.

Elaboration of powder enteral formula implies several steps, including sterilization and spray-drying processes. These treatments may produce different changes in their constituents and a decrease of bioavailability of necessary nutrients for the health of the patient (Lowry et al., 1989). The carbohydrate fraction can undergo different reactions, such as isomerization and degradation (Lowry & Baker, 1989) or may react with proteins (Maillard reaction). Enteral formulas are very liable to undergoes these reactions, due to the high reducing carbohydrate content present in their composition.

In spite of the importance of maintaining nutritional content in enteral formula, limited data exist on modifications of constituents during manufacture and storage. Lowry et al. (1989) studied the effect of heat-processing on protein quality during sterilization of commercial liquid enteral formula, protein quality and bioavailability remaining unaltered. Frías and Vidal-Valverde (2001) found a decrease of vitamin content (thiamine, A and E) during storage of liquid enteral formulas.

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In a previous work, we reported the presence of maltulose, an isomer of maltose, in commercial liquid enteral formulations (García-Baños, Olano, & Corzo, 2000) and the suitability of the maltose/maltulose ratio as an indicator, to assess heat treatment during manufacture and to monitor product storage (García-Baños, Olano, & Corzo, 2002).

As a measurement of the extent of Maillard reaction, Rufián-Henares, García-Villanova, and Guerra-Hernández (2002a) and Rufián-Henares, Guerra-Hernández, and García-Villanova (2002b) studied furosine (ϵ-2-furoylmethyl lysine) formation, loss of OPA reactivity and fluorescence intensity during processing and storage of liquid enteral formula. The presence of a high level of furosine in whey proteins, used as an ingredient limited its use as a heat treatment indicator and they proposed the loss of OPA reactivity and fluoresence intensity measurement as thermal indices to control enteral formula processing.

The objective of this work was to study changes in the mono- and disaccharide fraction as well as Maillard reaction development during storage of powder enteral formulas, to identify chemical indicators for quality control of commercial samples.

2. Materials and methods

2.1. Samples

Four commercial powder enteral formulas (Samples 1–4) were purchased at local pharmacies and their compositions (reported on the package) are shown in Table 1. The samples were analyzed within the expired date. Before analysis, samples were reconstituted, following instructions given by producers (22–25 g/100 ml).

Storage assays were carried out after taking aliquots (6 g) of samples 1 (oligomeric) and 4 (elemental). Samples were equilibrated to $a_w = 0.44$ in a desiccator over saturated K_2CO_3 solution, using the method of Labuza and Saltmarch (1981), and then stored at 30 °C for 6 months. Samples were taken at 0, 3 and 6 months of storage. Moreover, sample 1 was stored at 50 °C for 14 days, sampling was at 0, 7 and 14 days.

Table 1
Composition (% w/w) of powder enteral formulas

Enteral formula	Type	Composition (%)		
		Carbohydrates	Protein	Fat
1	Oligomeric	61.1[a]	16.4[c]	15.1
2	Oligomeric	73.9[a]	14.2[c]	3.9
3	Elemental	54.6[b]	19.2[d]	17.4
4	Elemental	75.9[b]	12.6[d]	7.4

[a] Maltodextrins.
[b] Mono-, di- and polysaccharides.
[c] Whey protein hydrolyzate.
[d] α-Lactalbumin hydrolyzate.

2.2. Synthesis of 2-furoylmethyl amino acids

2-FM derivatives of alanine (2-FM-Ala), arginine (2-FM-Arg) and lysine (α-2-FM-Lys) were synthesized according to del Castillo, Corzo, and Olano (2000).

2.3. Analytical determinations

Sample preparation and analytical determinations were carried out in duplicate.

2.3.1. Protein determination

Protein determination was carried out by the Kjeldahl method (AOAC, 1990).

2.3.2. HPLC analysis of 2-furoylmethyl amino acids

Analysis of 2-furoylmethyl derivatives was performed by an ion-pair RP-HPLC method (Resmini, Pellegrino, & Batelli, 1990) using a C_8 column (250 mm × 4.6 mm i.d.) (Alltech furosine – dedicated) thermostated at 35 °C, using a linear binary gradient. A Dionex chromatograph (DX-300) and a variable wavelength detector at $\lambda = 280$ nm (LTD Analytical, SM 4000) were used. Acquisition and processing of data were achieved in an HPChem Station (Hewlett–Packard).

HPLC analysis was performed as previously; 2 ml of reconstituted powder enteral formula were hydrolyzed, under inert conditions, with 6 ml of 10.6 N HCl at 110 °C for 24 h in a screw-capped Pyrex vial with PTFE-faced septa. The hydrolyzate was filtered through Whatman No. 40 filter paper, and 0.5 ml of the filtrate was applied to an activated Sep-pak C_{18} cartridge (Millipore). 2-Furoylmethyl amino acids (2-FM-AA) were eluted with 3 ml of 3 N HCl, and 20 μl of this volume were injected into the column.

Quantitative analysis was performed by the external standard method using a commercial standard of pure furosine (Neosystem Laboratories, Strasbourg, France).

2.3.3. HPLC–MS identification of 2-furoylmethyl amino acids

To check the identity of 2-FM-AA formed, HPLC–MS analysis was performed (del Castillo, Sanz, Vicente, & Corzo, 2002) using a Hewlett–Packard 1100. This instrument, which consists of an HPLC HP Series 1100 equipped with a diode array detector (DAD) coupled to a quadrupole HP-1100 mass detector, was used in the electrospray positive mode (API-ES). Samples (100 μl) were injected into a C_8 column (4.6 × 250 mm, 5 μm) (Alltech), maintained at 32 °C. The mobile phase was acetic acid (2%) in water and elution was under isocratic conditions at a flow rate of 1 ml/min. The DAD signal was recorded at 280 nm. Mass spectrometer values of needle potential, gas temperature, drying gas and nebulizer pressure were adjusted to 4000 V, 330 °C, 10 l/min and 50 psi, respectively. A fragmentor potential of 80 V

Table 2
Content of carbohydrates (mg/100 g product), pH values (after reconstitution in water)[a] and maltose/maltulose ratio found in commercial powder enteral formulas ($n = 2$)

Samples	pH	Carbohydrate content (mg/100 g product)						
		Fructose	Glucose	Sucrose	Lactose	Maltose	Maltulose	Ma/Mu[b]
1	6.55	36.5 (7.0)[c]	663 (3.8)	n.d.[d]	449 (9.0)	3563 (2.3)	150 (4.2)	23.7
2	6.46	27.9 (8.6)	792 (0.9)	n.d.	345 (2.4)	4018 (1.8)	114 (7.8)	35.4
3	4.91	128 (7.6)	795 (3.8)	28986 (5.7)	489 (0.1)	1282 (2.4)	24.5 (7.5)	52.3
4	7.63	7040 (5.4)	8398 (6.2)	20114 (1.1)	tr[e]	2525 (1.2)	87.3 (6.5)	28.9

[a] Degree of reconstitution: according to indications of manufacturer (22–25 g/100 ml).
[b] Ma/Mu, maltose/maltulose ratio.
[c] RSD, relative standard deviation (%).
[d] n.d., not detected.
[e] tr, traces.

was selected, since it provided the best sensitivity with reference compounds. Scan range was from 50 to 500 u.

2.3.4. GC analysis of mono- and disaccharides

Analysis of mono- and disaccharides in powder enteral formulas was performed by GC, following the method of García-Baños et al. (2000). Chromatographic analysis was performed in a Hewlett–Packard (Avondale, PA, USA) model 6890 gas chromatograph provided with a split injector, 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. 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 and held 1 min, then programmed to 290 °C at a heating rate of 10 °C/min and held for 15 min. The split ratio was 1:40.

To eliminate proteins, fat and carbohydrates of high molecular mass, sample preparations of enteral formulas before chromatographic analysis must be carried out. 0.8 ml of reconstituted enteral formulation was dissolved in 1 ml of an internal standard solution of myo-inositol and trehalose (1 mg/ml). After mixing, 0.9 ml of this solution was placed on a 2 cm diameter column containing 5 g of a 50/50 (w/w) mixture of activated charcoal and Celite (Swallow & Low, 1990). Then 2 ml of water were added and mono- and disaccharides were eluted with 15% (v/v) ethanol at a flow rate of 2 ml/min at room temperature. The collected fraction (120 ml) was dried in vacuum and converted to the corresponding silyl oxime derivatives (Li & Schumann, 1981) for chromatographic analysis.

3. Results and discussion

3.1. Commercial powder enteral formulas

The content of mono- and disaccharides as well as pH values of reconstituted powder enteral formulas are shown in Table 2. pH values observed in the reconstituted samples, 1–3, agree with those previously found for liquid enteral formulas by García-Baños et al. (2002) with the exception of sample 4 which showed a high pH value (7.63). In oligomeric samples 1 and 2, sucrose was absent since the only source of carbohydrates was maltodextrins; thus mono- and disaccharide composition were similar to those reported for liquid enteral formulas (García-Baños et al., 2000, 2002). In elemental samples, 3 and 4, sucrose was the main constituent of the carbohydrate fraction. The high content of glucose and fructose found in sample 4 may be due to the use of hydrolyzed syrups. The presence of lactose in the studied samples may be attributed to the use of milk proteins as ingredient.

Maltose (Table 2) was present in a wide range (from 1282 to 4018 mg/100 g product). Also, presence of maltulose has been detected in variable amounts (24.5–150 mg/100 g product); this fact could be due to the different conditions used for elaborating of powder enteral formula. Sample 3 presented a low value of maltulose, probably as a consequence of the low pH of the sample. García-Baños et al. (2002) showed that maltulose formation during heating of enteral formulas increased with increasing pH. Maltulose can be present in the maltodextrins used as ingredient but can be also formed during the elaboration process of enteral formulas.

The level of maltulose found for reconstituted powder enteral formulas (5.4–33 mg/100 ml) was lower than those previously reported for liquid enteral formulas (12–107 mg/100 ml) (García-Baños et al., 2000).

The maltose/maltulose ratio found for powder enteral samples ranged from 23.7 to 52.3, being considerably higher than the mean values reported for commercial liquid formulations (García-Baños et al., 2000). These differences may be attributed to the sterilization process undergone by the liquid samples.

Presence of 2-FM-AA as a measure of early stages of the Maillard reaction in enteral formulas was also studied and the results are shown in Table 3. Furosine

Table 3
Content of 2-FM-AA in the commercial powder enteral formulas studied ($n = 2$)

Enteral formulas	2-Furoylmethyl-amino acid (mg/100 g protein)		
	2-FM-Ala	α-2-FM-Lys	Furosine
1	14.2	23.5	207
2	–[a]	–	306
3	–	–	163
4	tr[b]	18.2	85.4

[a] Not detected.
[b] tr, traces

(2-FM-Lys) was detected in all studied enteral formulas in variable amounts, ranging from 85.0 to 305.8 mg/100 g protein. Samples 3 and 4 presented the lowest furosine values, attributable to the use of mild processing conditions or a better quality of the protein used as ingredient, since furosine may be formed not only as a consequence of the processing or storage conditions of enteral formulas, but also may be already present in the protein prior to the manufacture of the enteral formula (Rufián-Henares et al., 2002a, 2002b).

Small amounts of 2-FM-AA, other than furosine in samples 1 and 4 (Table 3) were observed. Identification of these compounds was acheived by HPLC–MS analysis and by comparison of their retention times with the previously synthesized compounds (del Castillo et al., 2002). Compounds were identified as 2-FM-Ala (m/z 198) and α-2-FM-Lys (m/z 255). These results suggest that the Maillard reaction could take place during processing of the formula, involving free amino acids or *N*-terminal amino acids from protein hydrolyzates in their manufacture.

3.2. Changes during storage of powder enteral formulas

Changes experienced by the mono- and disaccharide fraction during storage at 30 °C of powder enteral samples 1 and 4 are shown in Table 4. A decrease of total carbohydrates was observed at the end of the storage period. According to previous studies, the Maillard reaction is the predominant reaction in food with low moisture content (Labuza & Saltmarch, 1981; Olano & Martinez-Castro, 1989). Thus, most of the observed carbohydrate decrease may be due to their participation in the Maillard reaction.

During storage at 50 °C (Table 5), the loss of carbohydrate proceeds considerably faster than at 30 °C; after 14 days of storage, the decrease of total carbohydrates was similar to that attained after 6 months of storage at 30 °C.

The maltose/maltulose ratio suffered a slight decrease (13.5% and 16.6% for samples 1 and 4, respectively) during storage at 30 °C. Storage of sample 1 at 50 °C produced a decrease of 26.6%. García-Baños et al. (2002) also observed a diminution in this ratio during storage of liquid enteral formulas.

The evolution of the Maillard reaction during storage of sample 1 and 4 was also studied by 2-FM AA determination. Formation of these compounds in sample 1

Table 4
Changes of carbohydrate fraction during storage at 30 °C and $a_w = 0.44$ of two powder enteral formulas ($n = 2$)

Samples	Storage (months)	Carbohydrates (mg/100 g product)						
		Fructose	Glucose	Sucrose	Lactose	Maltose	Maltulose	Ma/Mu[a]
1	0	36.5 (7.0)[b]	663 (3.8)	–[c]	449 (9.0)	3563 (2.3)	150(4.2)	23.7
	3	40.8 (1.0)	529 (1.4)	–	381 (0.9)	3255 (1.5)	164 (0.9)	19.8
	6	35.8 (0.1)	418 (1.4)	–	369 (1.7)	2952 (1.0)	144 (0.1)	20.5
4	0	7040 (5.4)	8398 (6.2)	20,114 (1.1)	–	2525 (1.2)	87.3 (6.5)	28.9
	3	7300 (8.9)	8546 (4.2)	18,640 (3.6)	–	2631 (5.0)	98.4 (3.2)	26.7
	6	6591 (3.6)	8080 (2.9)	18,196 (1.1)	–	2325 (1.0)	96.6 (2.5)	24.1

[a] Ma/Mu, maltose/maltulose ratio.
[b] RSD, relative standard deviation (%).
[c] Not detected.

Table 5
Changes of carbohydrate fraction (mg/100 g product) during storage of sample 1 at 50 °C and $a_w = 0.44$ ($n = 2$)

Storage (days)	Carbohydrates (mg/100 g product)					
	Fructose	Glucose	Lactose	Maltose	Maltulose	Ma/Mu[a]
0	36.5 (7.0)[b]	663 (3.8)	449 (9,0)	3563 (2.3)	150 (4.2)	23.7
7	40.4 (4.2)	496 (2.1)	336 (6.9)	3272 (1.9)	168 (0.7)	19.4
14	39.6 (5.5)	419 (2.8)	282 (1.0)	2896 (0.7)	166 (0.4)	17.4

[a] Ma/Mu, maltose/maltulose ratio.
[b] RSD, relative standard deviation (%).

is shown in Fig. 1. Increases of 2-FM-Ala, α-2-FM-Lys and furosine were observed at both temperatures studied. Furosine was the most abundant 2-FM-AA, showing levels of 579 and 874 mg/100 g protein at the end of storage at 30 and 50 °C, respectively.

During storage of elemental formula (sample 4) at 30 °C (Fig. 2), an increase of 2-FM-AA content was observed. Furosine reached a value of 221 mg/100 g protein at the end of the storage period lower than that found in sample 1. Besides, formation of the 2-FM derivative of arginine was detected after 3 months of storage. Identity of this compound was checked by HPLC–MS (del Castillo et al., 2002).

These preliminary results show that, during storage of powder enteral formula under moderately severe conditions, the isomerization reaction is not favored and only under extremely severe storage conditions were any considerable changes of the maltose/maltulose ratio detected. Since the maltose/maltulose ratio is not altered during storage under appropriate conditions, could be used as a quality indicator of the processing during manufacture of commercial powder samples. In addition, furosine and 2-FM derivatives of alanine, arginine and α-lysine increased during storage; therefore, these compounds may be useful indicators of storage conditions of powder enteral formulas. However, further studies of a larger number of samples are needed in order to assess the feasibility of these parameters as quality indicators of powder enteral formulas.

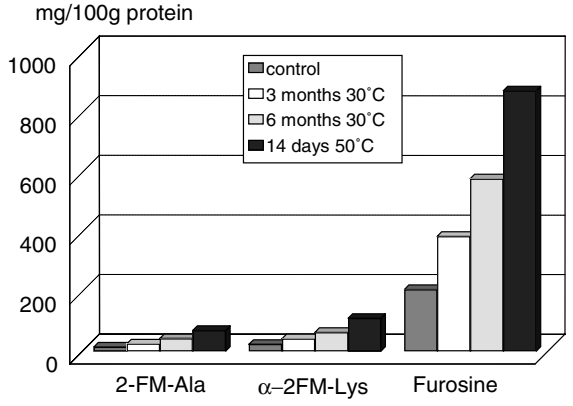

Fig. 1. Changes of 2-FM-AA content (mg/100 g protein) of sample 1 during storage at 30 °C (for 6 months) and 50 °C (for 14 days).

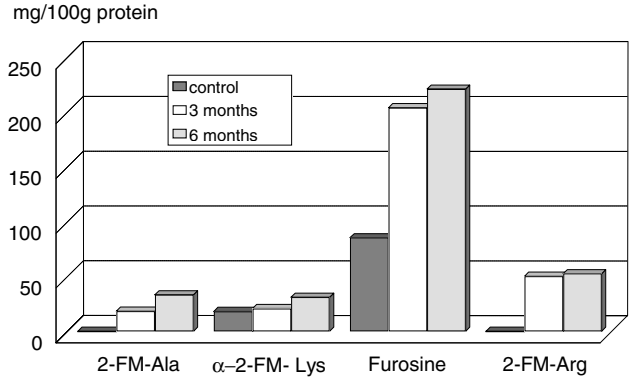

Fig. 2. Changes of 2-FM-AA content (mg/100 g protein) of sample 4 during storage at 30 °C for 6 months.

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