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Effect of storage conditions and inclusion of milk on available lysine in infant cereals

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Abstract

Infant cereals have a prolonged shelf-life. Available lysine was determined in order to ascertain the stability of lysine during storage for different periods under varied temperature and water activity conditions. Studies were undertaken on wheat-based (''7 cereals'' brand) and rice-based (gluten-free) infant cereals (with and without the inclusion of milk powder). Samples of all cereals were monitored during 1, 3, 6 or 12 months of storage at 32 or 55 °C. Samples of ''7 cereals'' and ''7 cereals + milk'' were also stored at 25 or 55 °C for 1, 2, 3 or 4 weeks under modified a_w. A gradual decrease in available lysine during storage was observed at all temperatures and times studied. Lysine losses at 32 °C reached values of 4.26% (wheat-based) and 5.47% (rice-based) in infant cereals without milk after 12 months of storage and at 55 °C, 11.2 and, 19.4%, respectively. For the same storage time, infant cereals with milk exhibited higher losses: 8.09 and 10.5% at 32 °C and 21.4 and 23.2% at 55 °C. Increased a_w during the storage of ''7-cereals'' (with and without milk) samples caused a considerably greater loss of lysine.

Keywords: Available lysine; Infant cereals; Storage

1. Introduction

Infant cereals are an important energy source for the nutrition of infants in Mediterranean countries and form the basis of their weaning-feeding from the age of 3–4 months.

In the past, Spanish infants were given cereal-based food that was processed at home by toasting and boiling cereal flours. Nowadays, infant cereals are processed by dietetic product manufacturers in large-scale factories. These products undergo toasting and/or boiling, hydrolysis and drying processes to improve their sensory qualities, digestibility, safety and shelf-life (Gil, Morales, & Valverde, 1991, 1994).

Technological processes, applied to infant cereals, can cause modifications in their composition. The heating process induces one of the most important modifications, the Maillard reaction, which involves amino acids (mainly lysine) and reducing carbohydrates (Guerra-Hernández, Corzo, & García-Villanova, 1999) and can produce a loss of nutritive value (Fernandez-Artigas, García-Villanova, & Guerra-Hernández, 1999; Henle, Walter, Krause, & Klostermeyer, 1991).

The ε-amino group of protein-bound lysine can react with glucose, maltose or lactose to form Amadori products, which are not susceptible to attack by proteolytic enzymes during digestion (Finot, Deutsh, & Bujard, 1981). Loss of available lysine, which is the most negative nutritional consequence of the Maillard reaction, is particularly significant in cereals, in which this amino acid is limiting (O'Brien & Morrisey, 1989).

The evaluation of available lysine by the biological analysis of growing rats is a laborious process with a high coefficient of variation and has been replaced by faster methods, such as evaluation of plasma amino acids, in vitro enzymatic determination, microbiological measurement and mainly chemical methods (Lettelier & Cuq, 1991). Several chemical methods have been developed to measure nutritionally-available lysine, understood as those lysine units that have not combined with other food ingredients and that still have free reactive ε-amino groups (Hurrell & Carpenter, 1981). Diverse reagents are used that present variable specificity for the ε-amino group of lysine. The method using 1-fluoro-2,4-dinitrobenzene (FDNB) is the most widespread (Carpenter, 1960). Chromatography has been used to

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separate ε-DNP-lysine from interfering amino acids and other compounds (Rabasseda, Rauret, & Galceran, 1988).

Commercial infant cereals are mostly composed of cereal flours (either with gluten or gluten-free) or legume flours (e.g. soy). The protein quantity and quality (Oyeleke, Morton, & Bender, 1985) of the latter are enhanced by their lysine content, a limiting amino acid in most staples. Among other ingredients included in these cereals are sucrose, glucose or fructose syrup, honey, powdered fruit, biscuits, minerals, vitamins and flavours. Infant cereals are commercialized with or without powdered milk in their formulation. The consumer must reconstitute these products by the addition of water or milk before use.

Infant cereals have a long shelf-life and can usually be consumed until two years after their manufacture. Storage duration and conditions, as well as the particular composition of these cereals, affect the progress of the Maillard reaction that is initiated during their processing (Fernández-Artigas et al., 1999; Guerra-Hernández et al., 1999). With this background, the present study was undertaken in order to study the influence of the length, temperature, and conditions of storage on the available lysine content of infant cereals, and to evaluate the effect of including powdered milk.

2. Materials and methods

2.1. Samples

Infant cereal samples, with and without powdered infant milk, were obtained from a dietetic products company. They comprised four types: "7 cereals" (brand containing wheat, rice, barley, maize, rye, oat, millet and soy flours), "7 cereals + milk" (including powdered milk), "gluten-free cereals" (containing rice and maize flours), and "gluten-free cereals + milk". According to the label information, the milk-free cereals contained 80% flours, as well as sucrose, caramel, vitamins, minerals and flavours. The infant cereals with milk contained approximately 40% powdered infant milk (follow-up formula).

The samples were stored under industrial or laboratory conditions. *Industrial conditions* involved storage at 32 or 55 °C during 1, 3, 6 or 12 months under a nitrogen atmosphere. The industry uses a nitrogen atmosphere to preserve the commercial product. *Laboratory conditions* involved storage at 25 or 55 °C for 1, 2, 3 or 4 weeks in an air atmosphere with controlled water activity ($a_w = 0.65$). To maintain this water activity level, the moisture of the cereals was controlled by the procedure described by Salmarch and Labuza (1980), placing the samples in a Petri plate on the upper shelf of a desiccator containing saturated sodium nitrite solution.

The samples were analysed before their storage and again after different storage conditions. Solid samples were stored at −50 °C until their analysis.

2.2. Apparatus

The liquid chromatograph used was a Perkin-Elmer 250 model with a Waters 717 automatic injector and Perkin-Elmer 235 UV diode array detector. The integrator-computer used was a Perkin-Elmer Nelson with program 1020.

2.3. Reagents

All chemicals were of analytical grade.

- *Standards* were N-ε-2,4-DNP-L-lysine HCl and N α-acetyl-L-lysine (Sigma Chemical Co).
- *Stock standard solution* was 1 mg/ml of N-ε-2,4-DNP-L-lysine HCl, dissolved in methanol:water (1:4).
- Working standard solutions (1–10 mg/l) were prepared by diluting stock standard solutions in 0.01 M sodium acetate, pH 5, buffer.
- *Derivative reagent* was 1-fluoro-2,4-dinitrobenzene (FDNB) solution (Sigma Chemical Co) in 3% v/v, ethanol.

2.4. Procedure

ε-NDP-lysine was determined by HPLC, following the method applied to infant cereals by Fernandez-Artigas et al. (1999) with some modifications. A 0.2 g sample was placed in the bottom of a 25 ml Pyrex screw-capped tube with PTFE-faced septa and 1 ml of $NaHCO_3$ (8%) solution; 1.5 ml of FDNB solution were added. The closed tubes were shaken mechanically for 3 h at room temperature and ethanol was evaporated by immersing them in a 95 °C water bath. The FDNB derivate solutions were hydrolyzed with 3 ml of 8.1 M HCl in an oven at 110 °C for 24 h, after the removal of CO_2 by stirring. The hydrolysed solution was filtered and adjusted to pH 5 with 6 M NaOH and 1 M $NaHCO_3$; the volume was adjusted with methanol:0.01 M sodium acetate, pH 5, buffer (1:1) solution to 25 ml, and 3 ml of this solution were cleaned with diethyl ether (three times), removing the ether with Pasteur pipette and nitrogen. The solution was then diluted to 25 ml with methanol:0.01 M sodium acetate, pH 5, buffer (1:1) solution, and filtered through an 0.2 μm disc filter.

2.5. Chromatographic conditions

Fifty microlitres of filtered solution were separated on a reverse-phase C_{18} HPLC column (Nova-Pak C_{18},

250×3.9 mm id; Waters) operating at room temperature. The mobile phase was methanol:0.01 M sodium acetate, pH 5, buffer (1:1). The elution was isocratic and the flow rate was 1 ml/min. The UV detector was set at 360 nm. The run time was 15 min and ε-DNP-lysine was completely separated in 5 min. ε-DNP-lysine was determined by the external standard method. The concentration range was 1-10 mg/l, with a correlation coefficient (r^2) of 0.9998. The linear regression equation used was ($n=10$) $y=2.698\times10^{10}$ x-204490.

2.6. Additional determinations

Proteins were determined by the Kjeldahl method (AOAC, 1990). Reducing sugar determination was carried out by the Schoorl method (Snell & Ettre, 1971).

3. Results and discussion

3.1. Preliminary studies

The same method and chromatographic conditions were applied to infant cereals, with and without milk. The identity of ε-DNP-lysine was confirmed by concordance of the spectra of the ε-DNP-lysine standard with those of the samples.

The reproducibility of the method was tested on "7 cereals" samples ($n=7$): the mean (±S.D.) was 1.68±0.004 g/kg of sample and the coefficient of variation was 0.24%. *The precision* was also tested on "7 cereals" ($n=7$): the mean value was 1.73±0.02 g/kg and the coefficient of variation was 1.16%. This level of precision allows detection of variations in available lysine during infant cereal storage. *The recovery* was determined by the usual addition procedure, using *N*-α-acetyllysine as the standard. This compound has the *N*-α-amino group blocked (e.g. lysine in proteins). *N*-α-acetyllysine was added to the gluten-free cereals sample, at four levels from 0.40 to 1.61 g/kg. The recovery ranged between 95.9 and 97.5% (Table 1) and the mean value was 96.7±0.66 for a range of 1.56–2.77 g/kg. *Detection limits* were assayed in a sample of gluten-free cereal. The limits, calculated as twice the background noise, were 0.002 g/kg of sample and 0.050 g/kg of protein.

Table 1
Recovery of available lysine in the "gluten-free cereal" samples[a] (g/kg sample)

Added	Total	Detected[b]	Recovered%
0.40	1.56	1.51	96.8
0.81	1.97	1.89	95.9
1.21	2.37	2.29	96.6
1.61	2.77	2.70	97.5
			96.7±0.66

[a] Cereal contained 1.16 g/kg sample.
[b] Mean of two samples with two injections per sample.

3.2. Available lysine in infant cereals

3.2.1. Available lysine in infant cereals stored at 32 °C

Studies of available lysine were carried out on samples of "7-cereals" and "gluten-free cereals", with and without milk, stored under industrial conditions at 32 °C in commercial packs in a nitrogen atmosphere (Table 2). In "7-cereals" samples, storage caused a gradual decrease in available lysine, with lysine losses of 1.06% at 1 month and 4.26% at 12 months; the "7-cereals + milk" samples showed similar losses at 1, 3 and 6 months but at 12 months the loss (8.09%) was twice that in the sample without milk. The samples with milk had a higher reducing sugar content (15.1%) than samples without milk (4.8%). Moreover, the available lysine content in "7-cereals" samples was 1.8 and 4.2 g/kg in "7-cereals + milk".The available lysine content also decreased in "gluten-free cereals" during storage, with a loss of 5.47% at 12 months. The loss of available lysine

Table 2
Available lysine content of infant cereals stored at 32 °C

Infant cereals, time (months)	Available lysine		Lysine losses[c] (%)
	g/kg sample	g/kg protein	
7 Cereals			
0	1.78±0.010	18.8[a]	
1	1.76±0.010	18.6	1.06
3	1.74±0.010	18.4	2.13
6	1.73±0.010	18.3	2.66
12	1.71±0.009	18.0	4.26
7 Cereals-milk			
0	4.15±0.023	30.9[b]	
1	4.10±0.023	30.5	1.29
3	4.07±0.022	30.3	1.94
6	4.01±0.022	29.9	3.24
12	3.81±0.021	28.4	8.09
Gluten-free cereals			
0	1.16±0.017	20.1[b]	
1	1.16±0.017	20.1	0.00
3	1.15±0.017	20.0	0.50
6	1.13±0.016	19.5	2.99
12	1.10±0.016	19.0	5.47
Gluten-free cereals + milk			
0	6.18±0.034	42.7[b]	
1	6.12±0.034	42.2	1.17
3	6.08±0.033	42.0	1.64
6	5.79±0.032	40.0	6.32
12	5.53±0.030	38.2	10.5

[a] $N\times5.70$.
[b] $N\times6.25$.
[c] Obtained from g of available lysine/kg of protein.

in "gluten-free cereals + milk" was 10.5% at 12 months. The samples with milk had a higher reducing sugar content (16.2%) than samples without milk (3.9%) and the available lysine was 1.2 and 6,2 g/kg respectively. The greater the content of available lysine in the samples and the greater the reducing sugars, the greater was the loss of lysine after 12 months of storage.

Infant cereals with included milk powder are reconstituted with water. The available lysine content of "gluten-free cereals (rice-corn) (6.2 g/kg) was approximately 1.5-fold that of "7-cereals (4.2 g/kg)", while both types had similar protein contents.

3.2.2. Available lysine in infant cereals stored at 55 °C

Infant cereals are stored by industry at drastic temperatures (55 °C) for accelerated stability studies. Lysine losses were greater at 55 °C than at 32 °C (Table 3). Greater lysine losses were found in the infant cereals with milk than in those without. After 12 months of storage, the lysine loss was 11.2 and 19.4% in "7-cereals" and "gluten-free cereal", respectively. In "gluten-free cereals", the lysine loss after one year at 32 °C (5.2%) was the same as the loss after one month at 55 °C. Infant cereals with milk showed a lysine loss of 21.4 and 23.2% for "7-cereals + milk" and "gluten-free cereals + milk", respectively. The loss of lysine increased gradually with the length of storage.

Although the infant cereals were dried products with very low water activity (3% water) the storage produced lysine losses.

Table 3
Available lysine content of infant cereals stored at 55 °C

Infant cereals, time (months)	Available lysine		Lysine losses (%)[c]
	g/kg sample	g/kg protein	
7 Cereals			
0	1.78±0.010	18.8[a]	–
1	1.75±0.002	18.5	1.60
3	1.74±0.001	18.3	2.66
6	1.69±0.001	17.9	4.79
12	1.58±0.005	16.7	11.2
7 Cereals + milk			
0	4.15±0.023	30.9[b]	–
1	–	–	–
3	3.76±0.007	28.0	9.39
6	3.71±0.003	27.6	10.7
12	3.26±0.010	24.3	21.4
Gluten-free cereals			
0	1.16±0.017	20.1[b]	
1	1.10±0.009	19.0	5.47
3	1.06±0.027	18.5	7.96
6	1.03±0.047	17.8	11.4
12	0.93±0.008	16.2	19.4
Gluten-free cereals + milk			
0	6.18±0.034	42.7[b]	
1	5.75±0.060	39.7	7.03
3	5.29±0.042	36.5	14.5
6	4.86±0.032	33.6	21.3
12	4.74±0.033	32.8	23.2

[a] $N \times 5.70$.
[b] $N \times 6.25$.
[c] Obtained from g of available lysine/kg of protein.

3.2.3. Available lysine in infant cereals stored at 25 °C/$a_w = 0.65$

The length and temperature of storage are critical to the progress of the Maillard reaction (Hurrell, Finot, & Ford, 1983; Van Mill & Jans, 1991), and water activity (a_w) also plays an important role in its development. The study design allowed us to estimate the influence of high a_w during short storage periods, relevant to some household storage conditions and/or storage in areas of high relative humidity. An environment of $aw = 0.65$ was generated using saturated sodium nitrite solution in a glass desiccator, producing a humidity of 8–9% in "7 cereals" and "7 cereals + milk" samples.

Table 4 shows the available lysine content of "7-cereals" samples stored at 25 °C in an environment of $a_w = 0.65$ for 1–4 weeks. The lysine loss ranged from 0 to 6.01% for "7-cereal" and 5.11–12.6% for "7-cereal + milk". Thus, after 4 weeks of storage under these conditions, the loss of available lysine in the sample with milk was twice that of the sample without milk.

The storage of commercial "7-cereals" sample at 32 °C showed a 1.06% loss of available lysine after 1 month, compared with a loss of 6.01% after 1 month at 25 °C/$a_w = 0.65$. The "7-cereals + milk" samples showed similar behaviour. Higher water activity ($a_w = 0.65$) considerably increased the loss of lysine, even at room temperature (25 °C).

Table 4
Available lysine content of infant cereals stored at 25 °C/$a_w = 0.65$

Infant cereals, Time (weeks)	Available lysine		Lysine losses (%)[c]
	g/kg sample	g/kg protein	
7 cereals			
0	1.74±0.026	18.3[a]	–
1	1.71±0.013	18.1	1.09
2	1.74±0.010	18.3	0.00
3	1.73±0.013	18.2	0.55
4	1.65±0.016	17.2	6.01
7 cereals + milk			
0	3.72±0.047	27.7[b]	–
1	3.53±0.012	26.3	5.11
2	3.38±0.017	25.2	9.03
3	3.30±0.003	24.6	11.2
4	3.25±0.001	24.2	12.6

[a] $N \times 5.70$.
[b] $N \times 6.25$.
[c] Obtained from g of available lysine/kg of protein.

3.2.4. Available lysine in infant cereals stored at 55 °C/ $a_w = 0.65$

Storage at high temperature and high water activity produced a notable loss of lysine (Table 5). The lysine loss in the "7-cereals" sample ranged from 25.2% at 1 week to 61.7% at 4 weeks. The loss in "7-cereals + milk" samples was even greater; 43.8% at 1 week to 76.3% at 4 weeks. When the original available lysine content and reducing sugars were higher, the lysine loss was greater. The available lysine contents in samples, expressed as g/kg protein, with and without milk, were similar after the first week.

We found no reports, in the scientific literature, on the stability of available lysine in infant cereal products during storage. Studies of infant cereal processing reported losses of 14 and 32% of available lysine after toasting and drying steps, respectively, in "gluten-free cereals" (Fernandez-Artigas et al., 1999). Few studies have been published on available lysine in infant milk during storage. Albala-Hurtado, Veciana-Nogués, Mariné-Font, and Vidal-Carou (1998), using the FDNB method, found no changes in available lysine during storage of infant formula at 20, 30, or 37 °C for up to 9 months. However, other authors (Ferrer, Alegría, Farré, Abellán, & Romero, 2000; Guerra-Hernández, Leon, Corzo, García-Villanova, & Romera, 2000), using the OPA method, reported a reduction in reactive lysine in infant formulae after storage under various time/temperature conditions. Ferrer et al. (2000) reported losses of 12–19% after storage of formulae for 6 months at 20–37 °C, while Guerra-Hernandez et al. (2002) reported losses of around 8% after 3 months of storage at 20 °C. Studies of powdered milk showed losses of available lysine after storage at 30 °C (El & Kavas, 1997; Hurrell et al., 1983; Van Mill & Jans, 1991). A 10% increase in the water content of powdered milk caused higher losses (37%) of available lysine after storage for 2 months at 37 °C (Hurrell et al., 1983).

In conclusion, the inclusion of milk in commercial infant cereals doubles the loss of available lysine after storage at room temperature (25 and 32 °C), compared with the milk-free products. Greater water activity in infant cereals, with or without milk, increases the loss by 6–10 times for the same storage period (1 month). The loss of available lysine was a maximum of 10% at 32 °C and of 23% at 55 °C in commercial samples after 1 year of storage.

Table 5
Available lysine content of infant cereals stored at 55 °C/$a_w = 0.65$

Infant cereals, time (weeks)	Available lysine		Lysine losses (%)[c]
	g/kg sample	g/kg protein	
7 Cereals			
0	1.74±0.026	18.3[a]	–
1	1.30±0.004	13.7	25.1
2	1.10±0.001	11.6	36.6
3	0.95±0.019	10.0	45.4
4	0.67±0.081	7.0	61.7
7 Cereals + milk			
0	3.72±0.047	27.7[b]	–
1	2.09±0.001	15.6	43.7
2	1.67±0.023	12.5	54.9
3	1.54±0.006	11.5	58.5
4	0.88±0.008	6.6	76.2

[a] $N \times 5.70$.
[b] $N \times 6.25$.
[c] Obtained from g of available lysine/kg of protein.

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