T9

QUANTIFICATION OF FUROSINE AND HYDROXYMETHYLFURFURAL IN SPANISH POWDERED INFANT FORMULAS (IFs)

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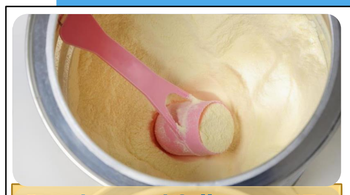
INTRODUCTION

The **manufacture** of infant formulas (IFs) includes different steps such as blending of components, homogenization, pasteurization and spray-drying, along with **storage** having a great influence in their final **quality** [1]. In processes involving heat, reactions and/or interactions between constituents can give rise to a loss of nutritive value being this very important because IFs sometimes are the only source of **infant nutrition** during the first months of life. **Maillard reaction** (MR) is one of the main reactions causing deterioration of proteins during processing or storage of foods. Because IFs may contain high level of **carbohydrates and proteins**, MR plays an important role during elaboration from a nutritional point of view. Evaluation of the initial steps of MR provides very valuable information for processing control, since the reaction can be followed before any important nutritional damage takes place. Different compounds have been selected as indicators of different stages of MR being the **furosine** (2-furoylmethyl lysine) one the most used, in processed foods, as an index of **early stages** of MR. Besides, **hydroxymethylfurfural** (HMF) has been used as indicator of **advanced stages** as a result of excessive heating or storage [2].

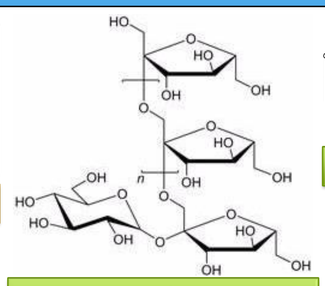
OBJECTIVE

The aim of this study was to assess the thermal damage of protein, by the measurement of **furosine** and **HMF**, in commercial **prebiotic-enriched infant formulas (IFs),** representatives of the most popular marketed in Spain. Also, **influence of storage time (8 and 15 months)** at **room temperature** on **MR** has been studied.

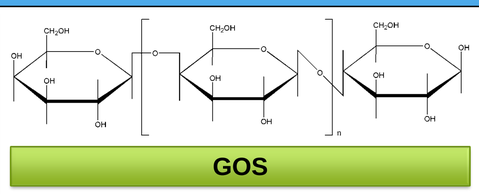
MATERIALS AND METHODS



Starting and follow-up IFs



Inulin



GOS

PREBIOTIC-ENRICHED IFs

- **Fructooligosaccharides, FOS** (n=3)
- **Galactooligosaccharides, GOS** (n=7)
- **GOS/FOS** (n=6)

IFs WITHOUT PREBIOTICS

- **With lactose (L)** (n=4)
- **Lactose-free (LF)** (n=4)

Protein from different origin:

- Whey
- Whey protein hydrolysate
- Milk
- Soy

STORAGE

Most representative IFs (n=10)

- **L**
- **LF**
- **FOS**
- **GOS**
- **GOS/FOS**

Room temperature, **8-15** months

CHARACTERIZATION

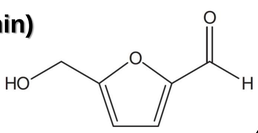
- Dry matter (**DM**): 94.5 - 99.2%
- Water activity (a_w): 0.07 - 0.28
- **pH**: 6.67 - 7.22
- **Protein** content (Kjeldhal method, 6.25 factor): 8.3 -16.0%

HMF ANALYSIS [4]

- **Sample preparation:** Precipitation of fat and protein with Carrez reagents → **supernatants**
- **HPLC-UV determination:**

ACE5 C_{18} column at 25ºC
(250 mm × 4.6 mm × 5 µm)
Detection UV: λ 283 nm

MOBILE PHASE (1.0 mL/min)
A: Methanol/water (5:95)
B: Methanol/water (80:20)
GRADIENT ELUTION

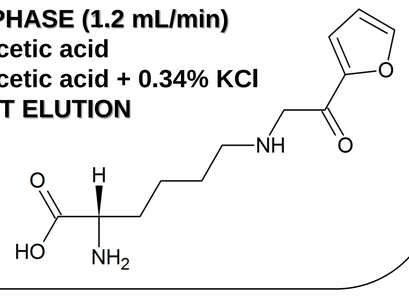


FUROSINE ANALYSIS [3]

- **Sample preparation:**
  Acid hydrolysis (HCl 8 N,110 ºC, 23 h)
  SPE extraction of furosine (activated Sep- Pack C_{18})
- **Ion pair RP-HPLC-UV determination:**

Furosine dedicated C_8 column at 35ºC
(250 mm × 4.6 mm × 5 µm)
Detection UV: λ 280 nm

MOBILE PHASE (1.2 mL/min)
A: 0.4% acetic acid
B: 0.4% acetic acid + 0.34% KCl
GRADIENT ELUTION



RESULTS AND DISCUSSION

FUROSINE CONTENT

- **Wide range** found in IFs: 94 - 1226 mg/100 g protein, probably attributable to differences in protein and sugar content and/or heat treatment
- There was no statistically significant difference between the groups of IFs studied
- **No significant differences were found** between IFs containing **proteins** from **different origin**

HMF CONTENT

- **Range**: 0.6 - 5.1 mg/100 g
- **Significant differences** between **GOS/FOS** IFs and **L** and **GOS** IFs

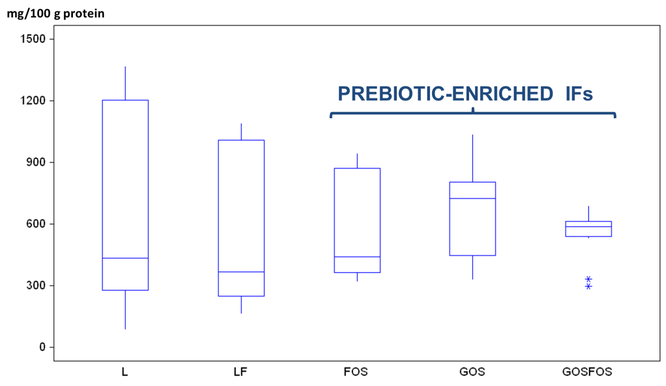


Figure 1. Furosine levels found in **IFs** with lactose (**L**), lactose-free (**LF**) and **prebiotic-enriched (FOS**, **GOS** and **GOS/FOS IFs**).

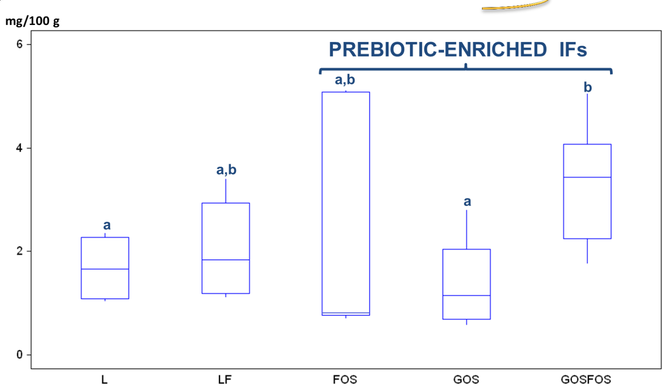


Figure 2. HMF levels found in **IFs** with lactose (**L**); lactose-free (**LF**) and **prebiotic-enriched** (**FOS**, **GOS** and **GOS/FOS IFs**).
[a,b] **Statistically significant differences between IFs.**

IFs STORAGE

- **HMF**: **Few changes** were observed. HMF **increased significantly** in **1** sample and **decreased** in **1** sample
- **Furosine**: **Overall increase** of furosine content was found during storage.
  In some IFs furosine decreased due to formation of **advanced MR compounds.**

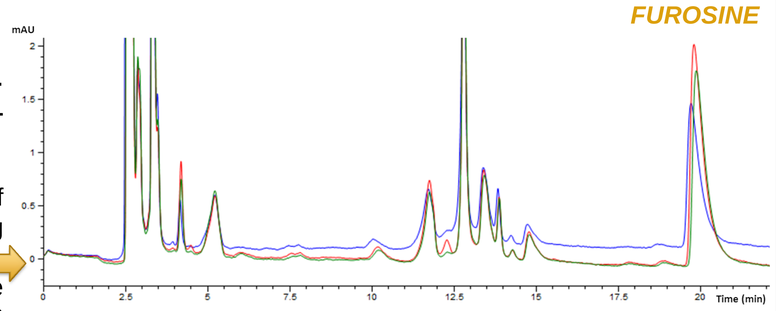


Figure 3. RP-HPLC-UV profile of furosine found in commercial IF at 0 (**blue**), 8 (**green**) and 15 (**red**) months.

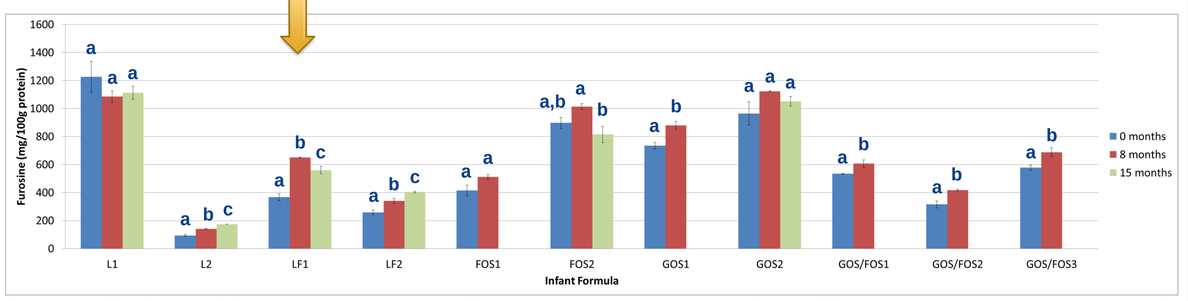


Figure 4. Evolution of furosine content (mg/100g protein) during storage (8 and 15 months) at room temperature of **IFs** with lactose (**L**), lactose-free (**LF**) and **prebiotic-enriched IFs** (**FOS**, **GOS** and **GOS/FOS**).
[a,b,c] **Statistically significant differences of furosine content during storage.**

CONCLUSIONS

- **Great variability** in **furosine** and **HMF** levels was found in commercial IFs .
- **Scarce differences** in **furosine** and **HMF** contents between **IFs with** and **without prebiotics** were observed.
- The **high levels** of **furosine** detected in some infant formulas may be attributable to **excessive heat treatment** during processing.
- **Storage** at room temperature of IFs **did not produce** important changes in the **content of furosine and HMF.**
- The use of these **thermal indicators** (furosine and HMF) allows establish **quality of enriched-prebiotics IFs.**

References

[1] V.Morales, A. Olano, N. Corzo *Journal of agricultural and food chemistry* **52(22)** (2004) 6732-6736. [2] M. Corzo-Martínez, N. Corzo, M. Villamiel, M.D. del Castillo, *Food Biochemistry and Food Processing.* B.K. Simpson (Ed.). John Wiley & Sons (2012) 56-83. [3] P. Resmini, L. Pellegrino, G. Battelli, *Italian Journal of Food Science* **2(3)** (1990) 173-183. [4] M. Rada-Mendoza, M.L. Sanz, A. Olano, M. Villamiel, *Food Chemistry* **85(4)** (2004) 605-609.

Acknowledgements

Funding from the Spanish MINECO (AGL2014-53445-R), ALIBIRD-CM S-2013/ABI-272 and Spanish Danone Institute. Carlos Sabater thanks his FPU Predoc contract from Spanish MECD (FPU14/03619).