

Evolution of non-enzymatic browning during storage of infant rice cereal

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Abstract

Non-enzymatic browning, during the storage of infant rice cereals, was assessed by determinations of furosine, hydroxymethylfurfural (HMF), colour, and available lysine. Cereal samples were stored at 32 or 55 °C for 1, 3, 6, or 12 months in air or nitrogen atmospheres. Samples were also stored at 25 or 55 °C under modified water activity ($A_w=0.65$) conditions for 1, 2, 3, or 4 weeks. Furosine, HMF and colour are useful indicators in accelerated storage (55 °C) under both normal and elevated A_w conditions. Colour is also useful for controlling storage at 25 and 32 °C. After 1 year of storage, there was a loss in available lysine of 7.14% at 32 °C and 24.5% at 55 °C. Under high A_w (0.65) conditions, this loss was 12.9% at 25 °C and 52.9% at 55 °C after 1 month of storage. Browning showed a slightly greater increase in nitrogen than in air atmosphere.

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1. Introduction

Rice is the second most important cereal in the world in terms of production. Among European countries, the leading producers are Italy and Spain. In Spain, rice is consumed in three forms: brown rice, parboiled rice, or regular-milled white rice (instant rice). Rice flour (generally small or broken grains) is also produced for use in infant foods, breakfast cereals, fermented or baked products and gluten-free foods, and to obtain starch, starch derivatives and additives.

Infant cereals are the first complementary foods given to babies, because they provide an important source of energy and can be easily assimilated. In Mediterranean countries, they form the basis of weaning-feeding from the age of 3–4 months. The introduction of these complementary foods from the age of 4–6 months was recently recommended as a means to prevent intolerance to foods (Molina Font & Maldonado, 2000).

Cereal flours are rich in starch but their protein contents and quality are low. Moreover, some cereals contain gluten, so that their intake at an early age may

favour the development of celiac disease. Rice flour proteins have higher digestibilities and nutritive values than other cereals. The concentration of lysine, a limiting amino acid in cereals, is 4.1 mg/100 g of protein in rice versus 2.3 mg/100 g in wheat. Protein quality, relative to biological value and coefficient efficacy protein (CEP) are higher in rice than in wheat (Caballero Barrigón, 2001).

Cereals introduced at an early age must be highly digestible because pancreatic-amylase is not developed before the fourth month of life. In general, gluten-free flours (rice, corn, or soy) are recommended before 6 months. After that age, wheat flour can be given if the digestive function is normal, rice flour if there is a tendency to diarrhoea or oat flour in cases of costiveness (Molina Font & Maldonado, 2000).

The industrial processing of infant cereals involves toasting and/or boiling, hydrolysis, and drying steps, designed to improve their sensory qualities, digestibility, safety and shelf-life.

Infant cereals have a long shelf-life and can usually be consumed for up to 2 years after their manufacture. The length and conditions of storage and the specific composition of the cereals can all influence the progress of the Maillard reaction that occurs during their processing (Fernández-Artigas, Guerra-Hernández, &

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García-Villanova, 1999; Guerra-Hernández, Corzo, & García-Villanova, 1999).

The Maillard reaction is one of the most important modifications in foods that contain proteins and reducing carbohydrates. It is induced by heating and long storage conditions and can produce a loss in nutritive value (O'Brien & Morrissey, 1989).

Many indices based on Maillard reaction products have been proposed to assess the effects of heat treatment and long storage on foods. Some of these parameters are related to early stages of the Maillard reaction and can be evaluated by the determination of furosine (Erbersdobler & Hupe, 1991).

Hydroxymethylfurfural (HMF) is an intermediate product of the Maillard reaction (Morales, Romero, & Jiménez-Pérez, 1997) and is also formed from the degradation of sugars at high temperature (Kroh, 1994).

Loss of available lysine, the worst nutritional consequence of the Maillard reaction, is particularly significant in cereals, where this amino acid is limiting (O'Brien & Morrissey, 1989). Its measurement has been used to evaluate the effect of heating on protein quality in cereal products (Fernández-Artigas, García-Villanova, & Guerra-Hernández, 1999)

Brown pigments are formed in advanced stages of browning reactions. Colour measurement provides a useful index for evaluating the intensity of browning reactions (Fernández-Artigas, Guerra-Hernández et al., 1999).

There has been some investigation of the effects of processing on the extent of the Maillard reaction in infant cereals (Carratú, Boniglia, Filesi & Bellmonte, 1993; Guerra-Hernández & Corzo, 1996; Guerra-Hernández et al. 1999; Fernández-Artigas, García-Villanova et al., 1999; Guerra-Hernández, García-Villanova & Montilla-Gómez, 1992; Fernández-Artigas, Guerra-Hernández et al., 1999). However, no published study could be found on the effect of long storage periods on the browning of infant cereals.

With this background, the present study was designed to determine the influence of normal and adverse storage conditions (temperature, time, atmosphere and humidity) on the browning of infant rice cereals by measuring the furosine, HMF, lysine loss and colour, and to evaluate the utility of these parameters as damage indicators in the storage of these food products.

2. Materials and methods

2.1. Samples

Infant rice cereal samples were obtained from a dietetic products company. According to the label information, the samples contained 80% rice flour plus sucrose, caramel, vitamins, minerals and flavours. The samples were stored under industrial or laboratory

conditions. Industrial conditions consisted of storage at 32 or 55 °C for 1, 3, 6 or 12 months in air or nitrogen atmospheres. The industry uses a nitrogen atmosphere to preserve the commercial product. The air atmosphere was obtained by cutting open the upper part of the bag containing the cereal and then closing it with adhesive paper. Laboratory conditions were storage at 25 or 55 °C for 1, 2, 3, or 4 weeks in air atmosphere with controlled water activity ($A_w=0.65$). This water activity level was maintained by using the procedure of Sal-march and Labuza (1980), placing samples in a Petri plate on the upper shelf of a desiccator containing saturated sodium nitrite solution.

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

2.2. Furosine determination

2.2.1. Reagents

A standard stock solution containing 1.2 mg/ml of furosine (Neosystem Laboratoire, Strasbourg, France) was used to prepare the working standard solution.

2.2.2. Apparatus

The liquid chromatographic system consisted of a Perkin-Elmer model 250 apparatus (Norwalk, CT) with a Waters plus 717 autosampler (Milford, MA) and Perkin-Elmer model 235 diode array detector (Norwalk, CT). Data were collected by a 1020 software data system (Perkin-Elmer, Norwalk, CT).

2.2.3. Procedure

Furosine determination followed the method of Guerra and Corzo (1996): 150 mg of the sample, weighed with analytical accuracy, were hydrolysed with 4.5 ml of 7.95 M HCl at 110 °C for 24 h in a Pyrex screw-cap vial with PTFE-faced septa. High-purity N₂ gas was bubbled through the solution for 2 min. The hydrolysate was filtered with a medium-grade paper filter. A 0.5-ml portion of the filtrate was applied to a Sep-pak C₁₈ cartridge (Millipore) prewetted with 5 ml of methanol and 10 ml of water, eluted with 3 ml of 3M HCl and evaporated under vacuum (Resmini, Pellegrino, & Batelli, 1990). The dried sample was dissolved in 3 ml of a mixture of water, acetonitrile, and formic acid (95:5:0.2) (Delgado, Corzo, Santa Maria, Gimeno, & Olano, 1992).

2.2.4. Chromatographic conditions

Fifty microlitres of the resulting solution was separated in a reverse phase C₁₈ column (Spherisorb ODSL 5 µm, 250 × 4.6 mm, Phenomenex, Torrance). Duplicate analyses were performed.

The mobile phase consisted of a solution of 5 mM sodium heptanesulphonate with 20% acetonitrile and

0.2% formic acid. The elution was isocratic and the flow rate was 1.2 ml/min. The UV detector was set at 280 nm. Calibration of the chromatographic system for furosine determination was by the external standard method. The calibration was performed by increasing the amount of furosine standard added to a previously hydrolyzed rice flour sample (within the expected concentration range of 0.02–0.4 µg).

2.3. HMF determination

2.3.1. Reagents

Analytical reagent grade chemicals were used. The clarified solution was composed of 15% potassium ferrocyanide (w/v) (Merck, Darmstadt, Germany) (Carrez I) and 30% zinc acetate (w/v) (Merck, Darmstadt, Germany) (Carrez II). A standard stock solution containing 200 mg/l of 5-(hydroxymethyl)furfural (Merck, Darmstadt, Germany) was used to prepare the working standard solutions (0.02–0.5 mg/l).

2.3.2. Apparatus

The liquid chromatographic system consisted of a Konic model 500A (Barcelona, Spain) chromatograph with 20 µl injection loop, a UV Konic detector model 200 UVIS (Reno, NE) set at 284 nm, and a Hewlett-Packard integrator model 3394A (Avondale, PA).

2.3.3. Procedure

HMF determination was performed following the method described by Garcia-Villanova, Guerra-Hernández, Martínez-Gómez, and Montilla (1993). The ground sample (0.4 g) was weighed into a 10-ml tube to which 7 ml of deionised water were then added. The centrifuge tube was shaken vigorously for 1 min and the sample was then centrifuged for 10 min at 5000 rpm. The same procedure was followed twice more. The supernatants were clarified with 0.5 ml each of Carrez I and II solutions. The resulting mixture was centrifuged for 10 min at 5000 rpm. The solution was diluted to a total volume of 25 ml with deionised water. A 2-ml aliquot of this solution was filtered through a 0.2-µm disk filter before injection.

2.3.4. Chromatographic conditions

Twenty microlitres of filtered solution were separated in a reversed-phase C₁₈ (Spherisorb S5 ODS2, 250 × 4 mm i.d., column) (Sugelabor, Madrid, Spain). The mobile phase was water–acetonitrile (95:5) (Panreac, Barcelona, Spain). The flow rate was 1 ml/min. The external standard method was used for the calibration. Duplicated analyses were performed.

2.4. Colour determination

The colour of cereal samples was measured using the CIE $L^*a^*b^*$ colour system, where L^* is lightness, a^* is

redness, and b^* is yellowness. The instrument used was an Elrepho 2000 reflectance spectrophotometer (Datacolor S.A., Spain). The colorimetric parameters L^* , a^* , b^* were referred to illuminant D₆₅, and the instrument was calibrated using a BaSO₄ standard. The results are also expressed as colour difference (ΔE) between the non-stored infant cereal and stored samples according to the following equation (Francis & Clydesdale, 1975):

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

where ΔL = brightness difference, Δa = redness difference, and Δb = yellowness difference.

Duplicate analyses were performed.

2.5. Available lysine determination

2.5.1. Reagents

Analytical reagent grade chemicals were used. The derivative reagent was composed of 3% 1-fluoro-2,4-dinitrobenzene (FDNB) (w/v) (Sigma Chemical Co) in ethanol. A standard stock solution containing 1 mg/ml of *N*-ε-2,4-DNP-L-lysine HCl dissolved in methanol:water (1:4) was used to prepare the working standard solutions (1–10 mg/l) dissolved in 0.01 M sodium acetate pH 5 buffer.

2.5.2. Apparatus

The liquid chromatography used was the same as for the furosine determination.

2.5.3. Procedure

ε-N-DNP-L-Lysine was determined by HPLC, following the method applied to infant cereals by Fernández-Artigas, Garcia-Villanova et al. (1999), with some modifications. A 0.2-g sample was placed in the bottom of a 25-ml Pyrex screw-cap vessel tube with PTFE-faced septa and 1 ml of NaHCO₃ (8%) solution; 1.5 ml of FDNB solution were added. The closed tubes were mechanically shaken for 3 h at room temperature and ethanol was evaporated by immersing them in a 95 °C water bath. The hydrolysis of FDNB derivatized solutions was performed with 3 ml of 8.1 M HCl in an oven at 110 °C for 24 h, after removal of CO₂ by stirring. The hydrolysed solution was filtered and pH 5 was reached with 6 M NaOH and 1 M NaHCO₃; 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 was 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 (1:1) solution, and filtered through a 0.2 µm disc filter. Duplicated analyses were performed.

2.5.4. Chromatographic conditions

Fifty microlitres of filtered solution were separated in a reverse-phase C₁₈ HPLC column (Nova-Pak C₁₈, 250

× 3.9 mm i.d.; Waters) operating at room temperature. The mobile phase was methanol:0.01 M sodium acetate pH 5 (1:1). The elution was isocratic and the flow rate was 1 ml/min. The UV detector was set at 360 nm. The determination of ϵ -DNP-lysine was carried out by external standard method.

2.6. Protein and reducing sugar determinations

Protein determination was carried out by the Kjeldahl method (AOAC, 1990). Reducing sugars were determined by a titrimetric method (AOAC, 1990).

2.7. Statistical analysis

Statistical analysis of data was performed by analysis of variance. The Student's *t*-test was used to compare means, and 99% was regarded as the level of significance.

3. Results and discussion

3.1. General

The manufacture of infant rice cereal from rice flour involves three steps: toasting, α -amylase hydrolysis and drying (Gil, Morales, & Valverde, 1991, 1994). The level of reducing sugars rises during amylolysis (Fernández-Artigas, Guerra-Hernández, & García-Villanova, 2001), and was found to be 6.16% in our samples before their storage. The Maillard reaction can be enhanced by the drying step (Guerra-Hernández et al., 1999) and can continue during the storage of these products, which have a shelf life of 2 years. In accelerated shelf-life studies, the industry uses storage temperatures of 32 °C (maximum room temperature) and 55 °C.

3.2. Furosine

Table 1 shows the changes in furosine during storage under the studied industrial and laboratory conditions.

The initial furosine content in commercial samples before storage was from 515 to 830 mg/100 g protein. Similar values were obtained by Guerra-Hernández et al. (1999) in similar types of sample. The protein content is 4.14%. During storage, the behaviour of the furosine content was the same in either atmosphere (air or nitrogen). At 32 °C, the furosine increased slightly ($P < 0.01$) until 3 or 6 months of storage but decreased at 12 months of storage. Furosine levels were higher in storage at 55 °C than at 32 °C but the behaviour was similar.

Water activity (A_w) also plays an important role in browning development. Our laboratory conditions, with storage of samples at high A_w for short periods, are relevant to countries with high relative humidity. The behaviour of furosine during storage at high A_w was similar to storage under industrial conditions, although the changes in furosine content were more marked, especially at 55 °C. Our results demonstrate that furosine, an indicator of the early stages of the Maillard reaction, can be formed and destroyed under the storage conditions studied. Furosine content can be a useful indicator of browning during short storage periods.

3.3. Hydroxymethylfurfural

This indicator can only be used in infant cereals that do not contain ingredients such as caramel or dehydrated fruits, in which HMF is found. The changes observed in HMF content during storage of infant rice cereal are shown in Table 2. Under industrial conditions, the initial content of HMF was 0.71 mg/kg (non-stored sample); storage at 32 °C significantly increased ($P < 0.01$) HMF content at 1 and 3 months in both air and nitrogen atmospheres. At 55 °C, HMF content increased throughout the 12 month storage ($P < 0.01$), with more marked changes in the nitrogen atmosphere.

Storage at low temperature (25 °C) and high water activity (0.65) did not favour HMF formation during the 4-week study period. However, storage at high temperature (55 °C) and the same A_w (0.65) produced an increased HMF content after the second week of storage.

Table 1
Changes in furosine content (mg/100 g of protein^a) during storage of rice infant cereal

Time (months)	Industrial conditions				Time (weeks)	Laboratory conditions	
	32 °C		55 °C			25 °C/ A_w =0.65	55 °C/ A_w =0.65
	Air	Nitrogen	Air	Nitrogen			
0	830±6.9	830±6.9	830±6.9	830±6.9	0	515±1.8	515±1.8
1	935±2.7	792±9.8	981±4.2	1029±3	1	513±4.3	1481±9
3	953±6.3	906±5.6	1004±2	1178±6	2	375±3.1	1403±1
6	931±6.4	936±0.6	927±9.5	1051±5	3	474±2.4	1338±3
12	851±2.1	699±2.1	776±3.2	754±0.9	4	427±10.0	1311±6

$n=2$.

^a $N \times 5.95$.

Table 2
Changes in HMF content (mg/kg) during storage of rice infant cereal

Time (months)	Industrial conditions				Time (weeks)	Laboratory conditions	
	32 °C		55 °C			25 °C/ $A_w=0.65$	55 °C/ $A_w=0.65$
	Air	Nitrogen	Air	Nitrogen			
0	0.71±0.05	0.71±0.05	0.71±0.05	0.71±0.05	0	1.36±0.05	1.36±0.05
1	1.05±0.02	1.35±0.05	1.56±0.01	1.19±0.02	1	0.91±0.03	0.68±0.02
3	1.38±0.03	1.46±0.06	1.54±0.04	1.50±0.06	2	0.85±0.02	1.47±0.04
6	1.16±0.02	1.47±0.02	1.74±0.02	1.84±0.02	3	0.79±0.03	1.70±0.04
12	1.01±0.02	1.06±0.02	1.86±0.04	2.30±0.03	4	0.85±0.02	2.10±0.07

$n=2$.

In manufacture of infant rice cereal, Fernandez-Artigas, Guerra-Hernández et al. (1999) found HMF values ranging from 0 in untreated rice flour to 1.64 mg/kg after processing (toasting, hydrolysis and drying steps).

3.4. Colour

The reproducibility of the colour method was studied on a non-stored sample of infant rice cereal. The coefficients of variation (CV%) were 0, 1.00 and 0.03% for a^* , b^* and L^* respectively ($n=7$). The colour parameters considered by other authors in cereals were ΔE for bread (Ramírez-Jiménez, García-Villanova, & Guerra-Hernández, 2001; Zanoni, Peri, & Bruno, 1995) and corn and soy products (Konstance et al., 1998), and 100-L for pasta (Resmini, Pellegrino, Pagani & De Noni, 1993), bread (Ramírez-Jiménez, Guerra-Hernández, & García-Villanova 2000) and bakery products (Ramírez-Jiménez, García-Villanova, & Guerra-Hernández, 2000). Fernandez-Artigas, Guerra-Hernández et al. (1999) applied both parameters to processed baby cereals.

In the present study, the b^* and ΔE parameters proved useful for monitoring colour changes during the storage of infant rice cereal (Table 3).

The infant rice cereal stored at 32 °C under industrial conditions showed a small increase in b^* ($P<0.01$) and ΔE values after 12 months of storage. At 55 °C, the

increase in colour values were higher ($P<0.01$); 1 month of storage at 55 °C produced the same colour values ($b^*=6.1$ and $\Delta E=0.72$) as 12 months of storage at 32 °C. These values were slightly higher for storage in nitrogen versus air atmosphere.

The high water activity ($A_w=0.65$) favoured the browning during storage. At 25 °C and $A_w=0.65$, b^* and ΔE were 6.7 ($P<0.01$) and 1.3, respectively, after 4 weeks, higher than the values found at 32 °C and low A_w after 12 months (6.1 and 0.75). Another study of infant rice cereal processing (Fernández-Artigas, Guerra-Hernández et al., 1999) showed an increase in browning index (ΔE) of 2.51 during the toasting step at 140 °C. Similar increases were obtained in the present study when samples were stored in air and nitrogen atmospheres for 6 months at 55 °C (2.39 and 2.82, respectively).

3.5. Available lysine

The changes in available lysine during storage of infant rice cereal are shown in Table 4. A significant loss of available lysine ($P<0.01$) was detected after 6 months of storage at 32 °C in nitrogen atmosphere (5.3%); after 12 months, the lysine loss was 7.14%. Lysine losses were greater at the higher storage temperature (55 °C), reaching 24% after 12 months ($P<0.01$).

Table 3
Changes in colour during storage of rice infant cereal

Time (months)	Industrial conditions								Time (weeks)	Laboratory conditions			
	32 °C				55 °C					25 °C/ $A_w=0.65$		55 °C/ $A_w=0.65$	
	Air		Nitrogen		Air		Nitrogen			b^*	ΔE	b^*	ΔE
b^*	ΔE	b^*	ΔE	b^*	ΔE	b^*	ΔE						
0	5.4	–	5.4	–	5.4	–	5.4	–	0	5.4	–	5.4	–
1	5.4	0.14±0.00	5.6	0.22±0.00	6.1	0.72±0.01	6.2	0.78±0.05	1	5.9	0.54±0.01	14.0	11.9±0.13
3	5.4	0.08±0.02	5.8	0.40±0.00	7.3	1.87±0.05	7.2	1.77±0.05	2	6.2	0.79±0.04	16.4	14.6±0.24
6	5.6	0.23±0.01	5.8	0.41±0.00	7.8	2.39±0.05	8.1	2.82±0.10	3	6.4	0.80±0.10	16.5	15.0±0.04
12	6.1	0.71±0.00	6.2	0.81±0.00	8.8	3.47±0.01	9.2	3.89±0.01	4	6.7	1.31±0.00	17.9	16.5±0.00

$n=2$.

Table 4
Changes in available lysine content during storage of rice infant cereal

Time (months)	Industrial conditions (nitrogen)						Time (weeks)	Laboratory conditions					
	32 °C			55 °C				25 °C/ $A_w=0.65$			55 °C/ $A_w=0.65$		
	µg/g	g/kg protein ^a	Lysine losses (%)	µg/g	g/kg protein ^a	(%)		µg/g	g/kg protein ^a	(%)	µg/g	g/kg protein ^a	(%)
0	756±16.8	17.1	–	756±16.8	17.1	–	0	760±24.9	17.2	–	760±24.9	17.2	–
1	745±4.1	16.9	1.46	664±3.6	15.1	12.2	1	701±12.0	15.9	7.76	471±2.6	10.7	38.0
3	750±4.1	17.0	0.79	627±3.4	14.2	17.1	2	700±3.8	15.9	7.89	408±2.2	9.2	46.3
6	716±3.9	16.2	5.29	613±3.5	13.9	18.9	3	672±3.9	15.2	11.6	398±2.3	9.0	47.6
12	702±3.8	15.9	7.14	571±3.1	12.9	24.5	4	662±21.8	15.0	12.9	358±2.0	8.1	52.9

$n=2$.

^a $N \times 5.95$.

Samples stored under high water activity conditions ($A_w=0.65$) for 1–4 weeks lost 7.72–12.9% of available lysine at 25 °C. No significant losses ($P<0.01$) were detected between 1 and 2 weeks or 3 and 4 weeks. At 55 °C the losses were from 38 to 52.9% ($P<0.01$).

Thus, the loss of lysine is considerably greater under high water activity conditions and these products should not be stored in a humid environment.

We found no reports in the scientific literature on available lysine behaviour during infant rice cereal storage. A study of infant rice cereal processing described available lysine losses of 29 and 36% after toasting and drying steps, respectively (Fernández-Artigas & García-Villanova, 1999).

4. Conclusion

Furosine, mainly generated during the last step of infant cereal manufacture, may be a useful indicator for monitoring browning in accelerated shelf-life studies (55 °C) of short duration.

HMF is a useful indicator for monitoring the browning reaction when infant rice cereal is stored at 55 °C.

ΔE and b^* browning indices are useful when the cereal is stored under adverse conditions, such as high temperature (55 °C) or high humidity, or under normal conditions after 6 months of storage.

Available lysine losses are approximately 7% when the infant rice cereal is stored at 32 °C and almost 25% at 55 °C after 12 months of storage. Under high water activity conditions ($A_w=0.65$), lysine losses after 1 week are similar to those produced after 12 months of storage under normal water activity conditions.

Increases in the browning indicators, furosine, HMF and colour, are slightly higher in nitrogen atmosphere than in oxygen atmosphere.

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