Browning Indicators in Model Systems and Baby Cereals

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In many countries, baby cereals are the first solid food given to 3−4-month-old babies after weaning and to infants aged 6−12 months. Various simple technologies are traditionally used in the processing of cereals, including toasting, hydrolysis, and drying. In this study color and hydroxymethylfurfural (HMF) assays have been used to evaluate heat effects induced during the manufacture of these foods. The baby cereals analyzed were wheat, rice, oat, and four mixtures of flours. No HMF was detected in the raw flours. Toasting the flours increased HMF values by between 1.1 and 4.53 mg/kg and color (ΔE) values by 2.51−9.34. The drying step increased HMF values by between 1.14 and 19.60 mg/kg. High values of HMF coincided with the addition of ingredients containing HMF. Color and HMF contents in sugar−amino acid model systems were much higher than in sugar systems at temperatures >100 °C and low moisture content.

Keywords: *Color; hydroxymethylfurfural; model systems; baby cereals*

INTRODUCTION

The manufacture of baby cereals involves a relatively simple food system in which nonenzymatic browning can readily occur. Chemical reactions that cause the browning of cereal derivatives during manufacturing include the Maillard reaction and caramelization. Both depend on the type of reagent, temperature, water activity, and pH.

Maillard reactions occur slowly in dry food systems at low temperature and relatively slowly in high-moisture foods (Labuza and Saltmarch, 1981). However, in systems with intermediate moisture content, temperatures >50 °C< and pH 4−7 (i.e., in the pH range of food), Maillard reactions are favored (Kroh, 1994), causing changes in color (melanoidins), flavor (aldehydes and ketones), functional properties, and nutritional values (blocking or destruction of lysine) (O'Brien and Morrisey, 1989; Reineccius, 1990). Caramelization depends on the direct degradation of sugars and needs more drastic conditions (temperatures >120 °C pH <3 or >9, and very low A_w) (Kroh, 1994). Sugars and sugar−amino acid mixtures are heated to high temperatures in cereals; thus, Maillard reactions and caramelization may occur simultaneously (Zanoni et al., 1995).

Infant cereals must be processed to improve their dispersibility in liquids and their digestibility, because the pancreas of a 3−4-month-old baby has a limited ability to digest starch (Hardorn et al., 1968; Delachaume-Salem et Sarles, 1970; De Vizia et al., 1975). Furthermore, processing improves sensory qualities and microbial safety (Nout, 1993).

The processing of baby cereals involves toasting, hydrolysis, and drying steps (Gil et al., 1991, 1994). The toasting of the flours is done at temperatures >100 °C, and in these conditions caramelization and Maillard reactions can occur. During hydrolysis at temperatures <100 °C and in aqueous media, the content of reducing sugars increases (Guerra-Hernández et al., 1999). Caramelization reactions are unlikely, whereas the Maillard reaction was observed during the determination of furosine (Guerra-Hernández et al., 1999). During roller-drying the temperatures are >100 °C, the A_w of the product drastically decreases, and the time required is short, but both caramelization and the Maillard reaction can occur. The processing of baby cereals improves sensory qualities and starch digestibility, but the bioavailability of amino acids decreases. Therefore, baby cereals should be manufactured under controlled conditions to ensure products with adequate organoleptic and nutritional properties.

Hydroxymethylfurfural (HMF) is formed by the degradation of hexoses heated in acid solution, even in mild acid solutions (Feather and Harris, 1973; Shallenberger and Mattick, 1983; Kroh, 1994, Berg and van Boekel, 1994), and is also an intermediate product in the Maillard reaction (Hodge, 1953; Berg and van Boekel, 1994; Morales et al., 1997). HMF is a classic index of the browning process in milk, for which two main types are used, free HMF (coming from the degradation of lactulosyllysine through 1,2 enolization in the Maillard reaction) and total HMF (formed by the Maillard reaction and sugar degradation) (van Boekel and Zia-Ur-Rehman, 1987; Morales et al., 1997). In juices (Lee and Nagy, 1988) and honey (Jeuring and Kuppers, 1980) the main pathway is the sugar degradation, due to high sugar concentration and low pH. HMF was used by Acquistucci and Bassotti (1992) and by Resmini et al. (1993) in dried pasta. Our group developed a liquid chromatographic method for HMF determination in commercial samples of baby and breakfast cereals (Guerra-Hernández et al., 1992; García-Villanova et al., 1993).

Brown pigments are formed in the advanced stages of browning reactions. Brown pigment formation is desirable during some types of food processing (baking, cocoa and coffee roasting, cooking of meat) but completely undesirable in others (milk drying, thermal

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Table 1. Composition of Baby Cereal Samples Analyzed

sample	type of cereal	ingredient
single flours	wheat	
	oat	
	rice	
mixture of flours (A)	7 cereals: wheat, rice, barley, rye, oat, corn, and millet	soy
	8 cereals: wheat, rice, barley, rye, oat, corn, millet, and sorghum	soy and honey
	rice, corn	
	rice, corn	soy
mixture of flours (B)	7 cereals: wheat, rice, barley, rye, oat, corn, and millet	soy, biscuit, orange and banana powders, and sugar
	7 cereals: wheat, rice, barley, rye, oat, corn, and millet	soy, orange and banana powders, and sugar
	rice, corn	soy, orange and banana powders, and sugar

treatments for milk stabilization, fruit juices, and tomatoes) (De Man, 1980).

Although there are studies on browning reactions in cereals (mainly in bread and pasta), we have found no data on chemical browning reactions in the processing of baby cereals.

The present work studies sugar and sugar−amino acid model systems heated at temperatures and water contents applied by the industry to process baby cereals. A second aim was to assess the utility in the processing of these foods of (a) HMF measurement by HPLC, which prevents most of the interferences from other furfural compounds observed in traditional colorimetric methods, and (b) color indicators.

MATERIALS AND METHODS

Model Systems. *Reagents.* Pure standards of D-glucose, maltose, glutamic acid (Glu), glutamine (Gln), lysine chlorhydrate (Lys), proline (Pro), and glycine (Gly) were obtained from Sigma (St. Louis, MO). Other reagents were of analytical grade.

System Preparations. Model System A: Effect of Heating on Glucose and Maltose Solids. Glucose or maltose (31.2 g) was placed in the upper level of a desiccator; a Pyrex dish containing water was placed in the lower level to adjust the moisture content to 10%. The sugars were placed on a Petri plate and heated from 30 to 145 °C in a forced-convection oven for 40 min. The relation between temperature and duration of oven heating was linear from 30 to 125 °C, and the time required was 22 min; the time to reach 145 °C was 18 min. The samples were dissolved in phosphate buffer (0.1 M, pH 6.5) at 0.312 g/mL (1.73 M glucose and 0.87 M maltose).

Model System B. Effect of Heating on Glucose and Maltose Dissolved in 0.1 M, pH 6.5, Phosphate Solutions. Ten milliliters of glucose or maltose (0.312 g/mL) was placed in a Pyrex screw-cap vial with PTFE-faced septa and then heated at 55 °C in a water bath for 30, 60, and 90 min.

Model System C: Effect of Temperature on Glucose and Maltose Solids Heated in an Oven and Then Dissolved in Phosphate Buffer and Heated in a Water Bath. Ten milliliters of glucose or maltose phosphate solution (0.312 g/mL) obtained by model system A was heated at 55 °C in the water bath for 30, 60, and 90 min.

Model System D: Effect of Heating on Glucose and Maltose/Amino Acid Solids. Sugars (glucose or maltose) and amino acids (Glu, Gln, Lys, Pro, or Gly) (molar ratio 1:1) were dissolved in deionized water and freeze-dried. The samples were placed in the upper level of a desiccator; a Pyrex dish containing water was placed in the lower level to adjust the moisture content to 10%. The sugar−amino acid mixtures were heated from 30 to 145 °C for 40 min in a forced convection oven. The samples were dissolved to 0.05 M in phosphate buffer (0.1 M, pH 6.5).

Model System E: Effect of Heating on Glucose and Maltose/Amino Acid Dissolved in 0.1 M, pH 6.5, Phosphate Solutions. Ten milliliters of phosphate buffer containing 0.05 M glucose or maltose and 0.05 M amino acid (Glu, Gln, Lys, Pro, or Gly) was placed in a Pyrex screw-cap vial with PTFE-faced septa and then heated at 55 and 100 °C in a water bath for 30 min. All of the above combinations were tested.

Model System F: Effect of Temperature on Glucose and Maltose/Amino Acid Solids Heated in an Oven and Then Dissolved and Heated in a Water Bath. Ten milliliters of 0.05 M glucose or maltose/0.05 M amino acid phosphate solutions obtained by model system D were heated at 55 °C in a water bath for 30, 60, and 90 min.

All of the samples were rapidly cooled under running tap water after the heat treatment and then filtered (0.22 μm Millipore filters) before analysis.

Procedure. *A_{284} and A_{420}.* The solutions obtained from the model systems were measured at 284 and 420 nm in a UV−vis Perkin-Elmer spectrophotometer model Lambda 3B (Norwalk, CT) using as reference the same solution unheated. The sugar−amino acid solutions needed to be diluted before their determination (Table 3 lists the concentrations measured).

HMF. The model system solutions were directly injected into the liquid chromatograph. The conditions are reported in the determination of this parameter in baby cereals.

Samples. Baby cereal samples and ingredients were obtained from a Spanish dietetic products company. The composition of samples used in this study is shown in Table 1. Single and mixed cereal flours were analyzed raw (i.e., before processing), toasted (around 145 °C), hydrolyzed (around 55 °C), and roller-dried. Mixed cereal flours with fruits were also analyzed.

A further study was carried out with wheat, rice, and oat flour samples at two toasting temperatures (140 and 150 °C).

Solid samples were stored at −50 °C until analysis was performed. Hydrolyzed samples were freeze-dried and then stored at −50 °C until their analysis.

HMF Determination. *Reagents.* The clarified solution was composed of 15% potassium ferrocyanide (w/v) (Merck, Darmstadt, Germany) (Carrez I) and 30% zinc acetate (w/v) (Merck) (Carrez II).

A standard stock solution containing 200 mg/L 5-(hydroxymethyl)furfural (Merck) was used to prepare the working standard solutions (0.08−0.6 and 0.015−0.075 mg/L).

Apparatus. The liquid chromatographic system used in this study consisted of a Konic model 500A (Barcelona, Spain) with a 20 μL injection loop chromatograph, a Spherisorb S5 ODS2 (250 mm × 4 mm i.d.) column (Sugelabor, Madrid, Spain), a UV Konic detector model 200 UVIS (Reno, NE) set at 284 nm, and a Hewlett-Packard integrator model 3394A (Avondale, PA). The mobile phase was water/acetonitrile (95:5) (Panreac, Barcelona, Spain).

Procedure. The ground sample (0.4 g) was weighed into a 10-mL centrifuge tube to which 7 mL of deionized water was 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 1 mL each of Carrez I and II solutions. The resulting mixture was centrifuged for 5 min at 5000 rpm. The solution was diluted to a total volume of 25 mL with deionized water. A 2 mL aliquot of this solution was filtered through a 0.45 μm disk filter before injection.

Table 2. Effect of Heating on Solid and Solution Sugars

model system	A_{420}	A_{284}	HMF (mg/L)
model system A[a]			
glucose (powder)	0.014 ± 0.007	0.357 ± 0.0060	0.79 ± 0.04
maltose (powder)	0.014 ± 0.007	0.119 ± 0.028	0.08 ± 0.002
model system B[b]			
glucose (solution)			
30 min	0.006 ± 0.003	0.003 ± 0.001	nd[d]
60 min	0.006 ± 0.003	0.005 ± 0.002	nd
90 min	0.023 ± 0.004	0.028 ± 0.004	nd
maltose (solution)			
30, 60, 90 min	nd	nd	nd
model system C[c]			
glucose (powder + solution)			
30 min	0.017 ± 0.007	0.397 ± 0.050	1.00 ± 0.006
60 min	0.020 ± 0.008	0.401 ± 0.050	1.01 ± 0.005
90 min	0.031 ± 0.004	0.413 ± 0.060	1.01 ± 0.006
maltose (powder + solution)			
30 min	0.009 ± 0.004	0.125 ± 0.030	0.20 ± 0.005
60 min	0.011 ± 0.005	0.120 ± 0.027	0.21 ± 0.005
90 min	0.012 ± 0.005	0.134 ± 0.032	0.21 ± 0.006

[a] Sugar solids heated at 145 °C and then dissolved at 0.312 g/mL (1.73 M glucose or 0.87M maltose). [b] Sugar solutions (0.312 g/mL) heated at 55 °C. [c] Sugar solids heated at 145 °C and then dissolved at 0.312 g/mL and heated at 55 °C. [d] nd, not detected.

Chromatographic Conditions. Twenty microliters of filtered solution was separated in a reversed-phase C_{18} column. The flow rate was 1 mL/min. The HMF was completely separated out in 8 min, and the run time was 15 min.

The HMF dissolution concentrations and the height of the peak obtained were considered as the variables to obtain the linear regression equations. The concentration ranges were 0.08−0.60 and 0.015−0.075 mg/L, with correlation coefficients of 0.9999 for both curves. The linear regression equations used were ($n = 6$) $Y = 2.30 + 697.08X$ and $Y = 0.45 + 305.18X$, respectively, where Y is the peak height and X is the HMF concentration.

The HMF sample concentration was obtained from the calibration curves of the standard solution.

Color Determination. The color of cereal samples was measured using the CIE $L^*a^*b^*$ color system, where L^* is lightness, a^* is redness, and b^* is yellowness. The instrument used was a reflectance spectrophotometer Elrepho 2000 (Datacolor S.A.) (Spain). The colorimetric parameters L^*,a^*,b^* were referred to illuminant D_{65}, and the instrument was calibrated using a $BaSO_4$ standard.

The results are also expressed as $100 - L^*$ and color difference (ΔE) between the raw flours and the heat-treated (toasted and dried) samples according to the equation (Francis and 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.

Statistical Analysis. Analysis with the Student t test and linear regression equation used a Sigma package supplied by Horus Hardware S.A., Madrid, Spain.

RESULTS AND DISCUSSION

Model Systems. The model systems were heated at temperatures applied by the industry for the toasting and hydrolysis of baby cereals. The extent of browning was assessed by the absorbance at 284 nm (a useful measurement for assessing browning extent in systems showing no visible browning) (Karmas et al., 1992; Kroh, 1994) and the HMF by HPLC (Yaylayan and Forage, 1991; Kroh, 1994) and absorbance at 420 nm, a wavelength that is frequently used to determine browning (Karmas et al., 1992; Kroh, 1994).

Sugars. Models A−C contain only glucose and maltose sugars and therefore can exhibit only browning by caramelization. The absorbance values at 420 nm for the model systems A−C (Table 2) were low, with a high standard deviation, and therefore were not evaluated. *Model A* (oven heat treatment) (Table 2) showed 1.5 times more A_{284} intensity for glucose than for maltose at the same molar concentration. The HMF measurement by HPLC was 5-fold more for glucose than for maltose. The HMF detection limit in sugar solutions was 0.003 mg/L.

The heating of disaccharides and trisaccharides at 200 °C for 120 min produces HMF, with the prior breaking of the glucoside bond (Kroh, 1994). The need to break this bond for the maltose in our system would account for the lower reactivity observed.

Model B. The heating of sugar solutions (1.73 M for glucose and 0.87 M for maltose) at 55 °C for 30, 60, and 90 min (Table 2) showed low values of absorbance at 420 and 284 nm for glucose. The maltose solutions showed no absorbance, and HMF was not detected in either sugar.

Model C. Heat treatment of solid sugars and then heating of sugar solutions produced an increase of browning reflected in the HMF and absorbance results at 284 nm.

The browning intensity (A_{284}, HMF) of sugar model systems heated at 145 °C (the temperature used in cereals toasting) was very low, even with the use of high sugar concentrations. The sugar solutions heated at 55 °C (the temperature of cereals hydrolysis) showed no modifications in the browning parameters measured. In these conditions other pathways of sugar degradation reactions could be followed, such as methylglyoxal formation (Homoki-Farkas et al., 1997).

Sugar−Amino Acids. Models D−F contain sugars and amino acids. In these experiments we examined the behavior not only of the caramelization but also of the Maillard reaction.

Model D. Oven heating to 145 °C of glucose−amino acids (Table 3) showed higher values of absorbance units at 284 and 420 nm compared with the maltose−amino acid combinations. The A_{420} values were highest for the glucose−lysine system, whereas the highest A_{284} values were for the glucose−proline system.

Model E. Heating sugar−amino acid (0.05 M) solu-

Table 3. Effect of Temperature on Mixture Sugars and Amino Acids

	model D			model F		
	A_{420}	A_{284}	HMF (mg/L)	A_{420}	A_{284}	HMF (mg/L)
Glu-glucose[a]	0.011 ± 0.001	0.060 ± 0.005	0.10 ± 0.01	0.017 ± 0.002	0.077 ± 0.007	0.10 ± 0.01
Glu-maltose[a]	0.002 ± 0.0001	0.029 ± 0.002	0.07 ± 0.004	0.002 ± 0.0001	0.017 ± 0.001	0.06 ± 0.003
Gln-glucose[b]	0.120 ± 0.005	0.520 ± 0.021	0.34 ± 0.01	0.140 ± 0.006	0.630 ± 0.030	0.34 ± 0.01
Gln-maltose[b]	0.004 ± 0.0002	0.143 ± 0.008	0.07 ± 0.004	0.003 ± 0.0002	0.135 ± 0.007	0.08 ± 0.005
Lys-glucose[c]	0.148 ± 0.016	0.665 ± 0.061	0.24 ± 0.02	0.148 ± 0.016	0.651 ± 0.063	−[d]
Lys-maltose[c]	0.025 ± 0.001	0.174 ± 0.007	0.88 ± 0.04	0.012 ± 0.001	0.163 ± 0.007	0.88 ± 0.04
Pro-glucose[c]	0.126 ± 0.004	0.980 ± 0.032	−	0.128 ± 0.004	0.956 ± 0.031	−
Pro-maltose[c]	0.017 ± 0.002	0.403 ± 0.041	0.16 ± 0.02	0.016 ± 0.002	0.404 ± 0.041	0.16 ± 0.02
Gly-glucose[c]	0.012 ± 0.001	0.116 ± 0.010	0.19 ± 0.02	0.010 ± 0.001	0.122 ± 0.011	0.20 ± 0.02
Gly-maltose[c]	0.018 ± 0.001	0.083 ± 0.004	0.11 ± 0.005	0.019 ± 0.001	0.102 ± 0.006	0.14 ± 0.007

[a] 2×10^{-3} M. [b] 5×10^{-3} M. [c] 5×10^{-4} M. [d] −, not determined due to inadequate resolution.

Table 4. HMF Content (Milligrams per Liter) of Different Model Systems (Sugars and Sugar−Amino Acids) with Equal Concentrations (0.05 M)

	model A	model C	model D	model F
glucose	0.022	0.023		
maltose	0.011	0.012		
Glu-glucose			2.50	2.50
Glu-maltose			1.80	1.50
Gln-glucose			3.40	3.40
Gln-maltose			0.70	0.80
Lys-glucose			24.00	−[a]
Lys-maltose			88.00	88.00
Pro-glucose			−	−
Pro-maltose			16.00	16.00
Gly-glucose			19.00	20.00
Gly-maltose			11.00	14.00

[a] −, not determined due to inadequate resolution

tions at 55 °C for 30 min produced no detectable A_{420}, A_{284}, or HMF. These solutions heated to 100 °C only produced measurable values at 284 nm.

The combination of models D and E (at 55 °C) (*model F*) slightly modified the values of the parameters measured by model D.

The comparison of HMF values between models A and C (sugars) and models D and F (sugar−amino acids) at the same molar concentrations (0.05 M) is shown in Table 4. The HMF values for the sugars were very low (0.011−0.023 mg/L). The systems with amino acids exhibited HMF values ranging from 0.7 mg/L (Gln-maltose) to 88 mg/L (Lys-maltose); the lowest values were for glutamic acid and glutamine. The HMF value for the Lys-glucose system was below that of the Lys-maltose one, which may be accounted for by the higher reactivity of glucose, and thus the Maillard reaction is at a more advanced stage and can be measured at 420 nm (color).

The heating at 55 °C and pH 6.5 for 30 min of sugar and sugar−amino acid solutions previously treated at 145 °C (solid product) did not increase HMF content (Table 4).

The heating of sugars and sugar−amino acids with 10% humidity in the oven from 30 to 145 °C (40 min) produced a concentration of HMF between 32- and 8000-fold less for the sugars versus the sugar amino acid mixtures. In these conditions the Maillard reaction is much more important than caramelization.

Baby Cereals. *Color*. The reproducibility of the color method was studied on a lyophilized sample of the seven cereal flours, and the coefficients of variation (CV) were 14.3, 2.05, and 0.88% for a^*, b^*, and L^*, respectively. Table 5 exhibits the effects of toasting on color values. The color parameters considered by other authors in food cereals were ΔE (Zanoni et al., 1995) for bread

Table 5. Color Parameters of Raw and Toasted Baby Cereals

flour[a]	b^*	a^*	L^*	$100 - L^*$	ΔE
wheat-based					
wheat					
raw	8.90	0.70	94.37	5.63	
toasted	11.20	0.90	92.72	7.28	2.84
7 cereals−soy					
raw	10.20	0.50	93.33	6.67	
toasted	14.20	1.50	90.41	9.59	5.05
8 cereals−soy−honey					
raw	10.40	0.40	93.53	6.47	
toasted	15.70	2.00	89.44	10.56	6.88
rice-based					
rice					
raw	3.50	−0.10	95.74	4.26	
toasted	6.00	0.10	95.64	4.36	2.51
rice−corn					
raw	15.60	1.80	91.65	8.35	
toasted	20.50	2.70	87.95	12.05	6.20
rice−corn−soy					
raw	12.00	0.90	92.92	7.08	
toasted	18.60	3.30	86.77	13.23	9.34
oat-based					
oat					
raw	10.70	0.50	93.23	6.77	
toasted	14.90	1.80	89.60	10.40	5.70

[a] $n = 2$.

surface and $100 - L^*$ (Resmini et al., 1993) for pasta. The color results obtained at the toasting step showed an increase in $100 - L^*$ of between 0.1 for rice and 6.15 for rice−corn−soy sample in comparison with the raw flours. The ΔE increases ranged from 2.51 for rice to 9.34 for rice−corn−soy. The toasted flours with one cereal only (wheat, rice, and oat) showed ΔE values of 2.51, 2.84, and 5.7, respectively. The oat flour reached the greatest browning intensity. The samples with soy in their ingredients showed a greater browning during the toasting step.

A study was carried out at two toasting temperatures (140 and 150 °C) to know the influence of this small temperature difference on browning and to assess the sensitivity of the color index. Table 6 shows the color values of wheat, oat, and rice samples under the following treatments: toasting at 140 °C and at 150 °C and roller-drying after hydrolysis. The browning index (ΔE) increased with heat treatment at 140 and 150 °C in comparison with the raw flours. The browning index of samples heated at 150 °C was higher than that of samples heated at 140 °C. Thus, differences of 10 °C during a short period of toasting produced a browning difference that could be detected by ΔE ($P < 0.05$). When $100 - L^*$ values were considered, oat and rice showed higher values for toasting at 150 °C compared with toasting at 140 °C, but the wheat sample did not. Visual

Table 6. Color Parameters of Baby Cereals Manufactured at Two Toasting Temperatures

sample[a]	*b**	*a**	*L**	100 − *L**	Δ*E*
wheat					
raw	8.10	0.70	94.62	5.38	
toasted 140 °C	9.60	0.80	91.79	8.21	3.20
toasted 150 °C	11.20	0.90	92.65	7.35	3.68
dried	13.80	1.80	87.59	12.41	9.12
rice					
raw	5.10	0.20	95.20	4.80	
toasted 140 °C	7.90	0.20	93.85	6.15	3.13
toasted 150 °C	9.10	0.40	92.80	7.20	4.70
dried	9.10	0.40	92.05	7.95	5.13
oat					
raw	9.10	0.90	90.28	9.72	
toasted 140 °C	12.10	1.50	88.30	11.70	3.64
toasted 150 °C	12.30	1.60	87.74	12.26	4.21
dried	15.80	1.40	83.26	16.74	9.72

[a] $n = 2$.

observation detected very little color difference between samples toasted at 140 °C and those toasted at 150 °C, especially in the wheat samples. The roller-drying process also showed an increase in the browning index, but this was produced by the heating and by the addition of brown-colored ingredients at the previous hydrolysis step.

HMF. *Single Cereal and Mixed Cereal (A) Sample Processing.* Figure 1 shows the HPLC chromatogram of HMF at different steps of the processing of baby oat flour. The identity and purity of the chromatographic peak were confirmed by diode array. The highest increase in HMF was observed at the final step. Table 7 shows the HMF content at the different processing steps in the samples studied.

The precision values of the method for concentrations of 3.25 and 22.8 mg/kg were 2.10 and 2.14%, respectively (Guerra-Hernández et al., 1992).

Raw Samples. No HMF was detected in the raw flours (Table 7). This suggests that the increase of this parameter during heat-processing steps may be a useful indicator.

Toasted Flours. Toasting produced HMF in all of the samples studied, ranging from 1.1 mg/kg for rice to 4.5 mg/kg for rice−corn−soy.

Wheat-based and rice-based baby cereals showed a higher HMF value than single wheat and rice samples. Moreover, the sugar content of mixed flours was also higher (Fernández-Artigas, 1997).

Because the normal industrial temperatures used in the toasting process are around 140−150 °C, the influence of temperature on browning reactions was studied in other baby cereal flour samples (Table 8). Treatment at 150 °C produced a higher HMF value than did treatment at 140 °C. HMF proved to be a sensitive indicator of toasting treatment even when the temperature difference was only 10 °C ($P < 0.01$).

Hydrolyzed Flours. During the hydrolysis process, the content of reducing sugars increases and Maillard reactions can be favored. The mean content of glucose in toasted samples was 0.06% and in hydrolyzed samples was 0.84%. The mean content of maltose was 0.1% for toasted and 4.5% for hydrolyzed samples (Fernández-Artigas, 1997). Hydrolyzed rice and oat flours presented HMF values similar to those of the toasted samples (Table 7) under the conditions of our process (low temperature, high water content, and short duration); that is, the HMF concentrations did not increase. The first stages of the Maillard reaction were observed in

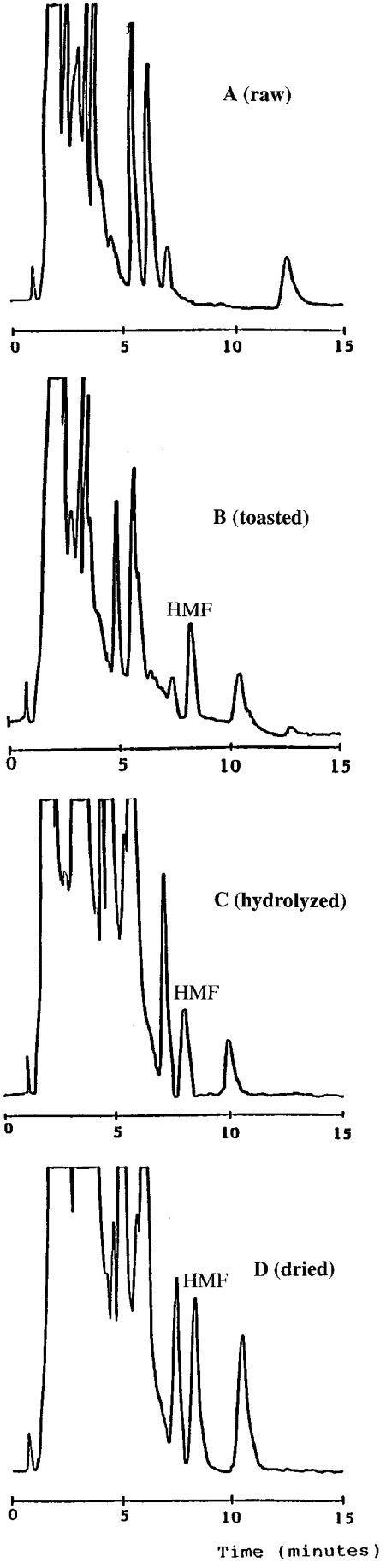

Figure 1. HMF chromatograms of oat baby cereal at the different processing steps.

Table 7. HMF Content of Baby Cereal at Different Processing Steps

	HMF (mg/kg of dry matter)			
sample[a]	raw	toasted	hydrolyzed	dried
wheat- based				
wheat	nd[b]	1.65	16.84	15.93
7 cereals−soy	nd	2.81	19.68	19.60
8 cereals−soy−honey	nd	3.46	13.88	13.49
rice-based				
rice	nd	1.10	1.12	1.64
rice−corn	nd	2.56	15.44	15.98
rice−corn−soy	nd	4.53	15.88	16.18
oat-based				
oat	nd	3.73	3.75	4.29

[a] $n = 2$. [b] nd, not detected.

Table 8. HMF Content of Baby Cereals Manufactured at Two Toasting Temperatures

	HMF (mg/kg of dry matter)			
		toasted		
sample[a]	raw	140 °C	150 °C	dried
wheat	nd[b]	0.94	1.42	22.25
7 cereals−soy	nd	2.02	5.30	23.18
rice	nd	1.44	2.33	2.39
oat	nd	2.70	3.91	5.04

[a] $n = 2$. [b] nd, not detected.

the same samples, because the levels of furosine increased versus the toasting step (Guerra-Hernández et al., 1999). However, as occurred in the sugar and sugar−amino acid model systems, the HMF did not increase, because the hydrolysis conditions do not favor advanced stages of the Maillard reaction. The HMF concentrations of the other samples increased considerably, which must be due to the addition of ingredients with HMF (Table 7).

Dried Flours. The HMF measurement to know the browning reactions during the drying process can only be studied in samples without the addition of ingredients with HMF. HMF increased by between 0.5 and 1 mg/kg in the dried oat sample but showed no change in the rice sample (Tables 7 and 8). The roller-drying process is favorable for browning, especially for the Maillard reaction (Guerra-Hernández et al., 1999). However, the short time applied may have limited the increase in HMF content. The heating at high temperatures for a long time in the model systems showed the highest HMF values for sugar−amino acid systems.

Other drying systems for cereal derivatives (pasta) were studied by Resmini et al. (1993), who found 0.45 mg/kg of HMF after drying for 24 h at 85 °C.

Baby Cereals (Mixed Cereal Flours plus Fruits). There is a high consumption of cereal flours with fruits in Spain. Three different samples were studied: seven cereals plus fruits, seven cereals plus biscuits and fruits, and rice−corn−soy and fruits. HMF results are listed in Table 9. HMF levels determined in different ingredients (biscuits, banana and orange powders) are also shown. The HMF values found in these samples are similar to those in the samples without fruits, approximately ∼15 mg/kg. Our group (Guerra-Hernández et al., 1992) previously reported higher values (65 mg/kg) in a wheat−rice−barley plus fruit sample from another company. If HMF is to be used as an index of heat treatment, the ingredients must be taken into account.

In conclusion, both color and HMF are sensitive indicators for the toasting treatment of flours processed for the production of baby cereals. Sugar−amino acid model systems heated at temperatures used in baby cereal toasting produced a high HMF content versus sugar model systems.

Table 9. HMF Content in Ingredients and Baby Cereals with Fruits

ingredient	HMF (mg/kg dry matter)[a]
biscuits	11.46
banana powder	20.20
orange powder	32.06
cereal flours with fruits	
7 cereals−soy−fruits	12.53
7 cereals−soy−biscuits−fruits	16.07
rice−corn−soy−fruits	16.30

[a] $n = 2$.

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