Browning Indicators in Bread

A. Ramírez-Jiménez, E. Guerra-Hernández,* and B. García-Villanova

Departamento de Nutrición y Bromatología, Facultad de Farmacia. Universidad de Granada, Campus Universitario de Cartuja, 18012 Granada, Spain

Bread is the most important food in the Spanish household and represents the largest proportion of products produced by commercial bakeries. The browning indicators furosine, hydroxymethylfurfural (HMF), and color were determined to evaluate heat effects induced during manufacture of these foods. The breads analyzed were common, special, sliced toasted, and snack breads. Identical sample preparation and HPLC conditions were used to determine HMF in all breads. The precision tested at high and low HMF concentration in breads was 2.60% and 1.57%, respectively. Recovery of HMF was 96.2%. The HMF values ranged from 2.2 to 68.8 mg/kg. Color index (100 − L^*) ranged from 17.0 to 38.2. The linear correlations (r^2) between 100 − L^*/HMF were above 0.70 for common, special, and snack breads. Similar correlation was obtained between 100 − L^*/HMF in a dough baking at different times. The furosine content in common bread ranged between 125 and 208 mg/100 g of protein. No linear correlation was found between furosine and HMF. Moreover, HMF and furosine were also determined in crumb and crust. Levels of HMF had a wide range (0.9−1.76 mg/kg) and furosine was between 43 and 221 mg/100 g of protein.

Keywords: *Bread; furosine; HMF; color*

INTRODUCTION

Breadmaking involves three steps: dough-mixing (flour, water, yeast, and salt), dough fermentation, and baking. During the baking process, the starch is gelatinized and the proteins denatured at an internal temperature of 60−80 °C and then the raw dough is transformed into a light, porous, and readily digestible product (Hui, 1991).

The chemical reactions involved in this process are essentially the Maillard reaction and caramelization. The Maillard reaction is favored in foods with a high protein and carbohydrate content and an intermediate moisture content at temperatures above 50 °C and at a pH of 4−7 (Kroh, 1994), producing changes in color (melanoidins), flavor (aldehydes and ketones), functional properties, and nutritional value (blocking or destruction of lysine) (O'Brien and Morrisey, 1989; Reineccius, 1990). Caramelization (degradation of sugars) needs more drastic conditions (temperatures >120 °C, pH < 3 or pH > 9, and low Aw) (Kroh, 1994). The water content distribution and temperature play an important role in developing the sensory characteristics of these products. During baking, the water content on the surface of the loaf becomes lower than in the middle and this, combined with the high temperature, is one of the factors that makes the crust different from the crumb (Thorvaldsson and Skjöldebrand, 1998).

The early stages of the Maillard reaction can be evaluated by determination of the furosine (ϵ-*N*-(furoylmethyl)-L-lysine) amino acid formed during acid hydrolysis of the Amadori compounds fructosyl-lysine, lactulosyl-lysine, and maltulosyl-lysine produced by reaction of ϵ-amino groups of lysine with glucose, lactose and maltose (Erbersdobler and Hupe, 1991). Furosine determination has been used in cereal to control the processing of pasta (Resmini and Pellegrino, 1994), bakery products (Henle et al., 1995), baby cereals (Guerra-Hernandez et al., 1999), and toasted sliced bread (Ramirez-Jimenez, 1998).

Hydroxymethylfurfural (HMF) is an intermediate product in the Maillard reaction (Berg and van Boekel, 1994; Morales et al., 1997) and is also formed from the degradation of sugars at high temperatures (Kroh, 1994). This indicator has been determined to evaluate the heat effects induced during manufacture of cereal products as commercial baby and breakfast cereals (Guerra-Hernández et al., 1992; Garcia-Villanova et al., 1993), pasta drying (Resmini et al., 1993), baby cereal processing (Fernandez Artigas et al., 1999), and sliced bread toasting (Ramirez-Jimenez, 1998). Brown pigment formation is desirable during breadmaking. The brown color index in solid products has been applied to study the modeling of bread-crust browning kinetics during baking (Zanoni et al., 1995), the toasting of wheat bread (Rychlik and Grosch, 1996; Ramirez-Jimenez, 1998) and the twin-screw extrusion of corn and soy products (Konstance et al., 1998).

The objectives of the present study were to determine the browning intensity by the measurement of the furosine, HMF, and color in differents kind of breads and to evaluate the useful of them in the control of manufacture.

MATERIAL AND METHODS

Samples. Six varieties of common breads were made by a breadmaking company. The bread formula used by the company was wheat flour (50 kg), water (27 kg), baker's yeast (2 kg), NaCl (1 kg), previously fermented dough (5 kg) and additives. Data on the size and shape of the bread and on fermentation and baking conditions are shown in Table 1.

Nine varieties of special breads were made by this breadmaking company. The bread formula used was similar to that

* To whom correspondence should be addressed. Tel: 9582-43867. Fax: 958243869. E-mail: ejguerra@platon.ugr.es.

10.1021/jf9907687 CCC: $19.00
Published on Web 08/02/2000

Table 1. Description, Flours, and Breadmaking Characteristics of Breads

samples	descripn wt (g)	form	flours	fermentation T^a (°C)/ time (min)	baking T^a (°C)/ time (min)	moisture %
white bread						
A	200	stick	baking flour	30−35/50	210/30	28.6
B	30	roll	baking flour	30−35/30	235/16	27.2
C	250		baking flour	double ferment. 30−35/15−20 30−35/30	200/50	30.4
D	1000	large round	baking flour	30−35/50	200/60	33.2
E	200	stick	baking flour	30−35/50	210/30	30.8
F	700	stick	baking flour	30−35/60	200/50	31.8
white bread with fruits						
A	200	stick	baking flour with fruits; orange peel; glucose syrup; sucrose	30−35/45−60	220/15−18	18.6
whole white bread						
A	200	stick	whole white flour-baking flour 1:2	30−35/50	210/35	30.2
B	500	large round	whole white flour-baking flour 1:2	30−35/50	210/50	32.9
mixed-grain bread						
bran bread	200	stick	bran flour (wheat bran; wheat, soy and malt flours; germ wheat; whey)-baking flour 1:1	30−35/20−25	200/30	31.1
mixed-flour bread	200	stick	mixed flour (wheat, corn, sesame, flax, oat, barley, millet whole soy and whole rye flours)-baking flour 1:1	30−35/20−25	200/30	34.3
oat bread	200	stick	oat flour (oat fiber, wheat and oat flakes, wheat flour)-baking flour 1:1	30−35/20−25	200/30	24.5
soy bread	200	stick	soy flour (soy granulated whole, wheat and rye flours)-baking flour 1:1	30−35/20−25	200/30	27.4
whole mixed-flour bread	200	stick	whole mixed flour (wheat, corn, sesame, flax, oat, barley, millet, whole soy and whole rye flours, wheat and oat flakes, oat fibers and granulated soy)-baking flour 1:1	30−35/20−25	200/30	23.2
whole rye bread	200	stick	whole rye flour (whole rye and malt flours, soy lecithin, citric acid, dairy solids and garrafin and guar gums)-baking flour 1:4	30−35/50	210/50	32.9

for the common bread except in the case of the cereal products. Data on bread size and shape, types and proportions of flours and cereal products and on fermentation and baking conditions are shown in Table 1.

Six doughs slightly baked to get stick form proceeding from the same brand and lot were purchased locally. The doughs were packed into plastic bags under vacuum conditions and stored at room temperature. The label information about household baking conditions was 220 °C for 12−15 min. The ingredients listed on the label were wheat flour, water, baker's yeast, salt, enzymes, emulsifiers, and dough-conditioning agent. The weight ranged between 112 and 128 g, and the moisture was 32%. The experiment performed in the laboratory was to bake the different doughs at 190 °C during 10, 15, 20, 25, and 30 min until a distinct intensity of brown color was reached.

Nine different brands of commercial toasted sliced breads were purchased locally. The ingredients listed on the labels were wheat flour, vegetable fat, glucose and sucrose, malt flours, yeast, emulsifiers, and other additives. The weight ranged between 3 and 10 g, and the moisture was 6%.

A commercial case of small toasted sliced breads was purchased locally. The ingredients listed on the label were wheat flour, vegetable oil, yeast, salt, dextrose, malt extract, malted cereals flour (wheat, barley). The weight ranged between 2.5 and 2.8 g, the moisture was 3%, and the pieces had round and thin form and homogeneous brown color.

Four brands of commercial snack bread products were purchased locally. The ingredients listed on the label were wheat flour, whole wheat flour, vegetable oil, malt flour, dough-conditioning agent, emulsifiers, soy lecithin, ascorbic acid, salt, and sucrose. These are common products in Spain. The weight ranged between 1 and 3 g, the shapes included roll, long stick, short stick, and ring forms, and the moisture was 5%.

Color Determination. The color of bread 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^*, and b^* were referred to illuminant D65 and the instrument was calibrated using a $BaSO_4$ standard. The samples were lyophilized prior to the analysis. Analysis was performed on duplicate samples.

HMF Determination. *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).

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 × 40 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 HMF determination was performed following the method described by Garcia-Villanova et al. (1993).

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 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 deionized water. A 2 mL aliquot of this solution was filtered through an 0.2 μm disk filter before injection.

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

The external standard method was used for the calibration. The HMF solution concentrations and the height of the peak obtained were considered as the variables to obtain the linear regression equation. The concentration ranges were 0.02−0.5 mg/L. The linear regression equation used was ($n = 7$)$Y = 292.17X - 0.27$, where Y is the peak height and X is the HMF concentration. The correlation coefficient was 0.9999.

Duplicate samples were analyzed.

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

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

Procedure. Furosine determination was performed following the method described by Guerra and Corzo (1996). A 150 mg of the sample, weighed with analytical accuracy, was hydrolyzed 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 N_2 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 C_{18} cartridge (Millipore) prewetted with 5 mL of methanol and 10 mL of water, eluted with 3 mL of 3 M HCl and evaporated under vacuum (Resmini et al., 1990). The dried sample was dissolved in 3 mL of a mixture of water, acetonitrile, and formic acid (95:5:0.2) (Delgado et al., 1992).

Chromatographic Conditions. Fifty microliters of the resulting solution was separated in a reverse phase C_{18} column. Duplicate samples were analyzed.

The mobile phase consisted of a solution of 5 mM sodium heptanesulfonate 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 adding increasing quantities of furosine standard, within the expected concentration range, to a previously hydrolyzed wheat flour sample. Two calibration curves were constructed by plotting the measured absorbance, expressed in units of area versus micrograms of added furosine. The equations for the two curves were ($n = 8$)$Y = 9\,756\,87\,8.61X - 36\,197.418$ (range 0.0383−0.383 μg)$r^2 = 0.9999$ and $Y = 9\,58\,4\,64\,3.42$ X + 15 901.2 (range 0.0193−0.0958 μg)$r^2 = 0.9999$.

Moisture and Protein Determinations. Moisture was obtained by gravimetric determination (AOAC, 1990, method 925.10). Protein determination was carried out by the Kjeldahl method (AOAC, 1990, method 920.87).

Statistical Analysis. The SPSS 7.5 statistical package (University of Granada, Spain) was applied to study parameter correlations.

RESULTS AND DISCUSSION

Color. The reproducibility of the refractometer color method was studied on commercial snack bread D (n = 7). The coefficient of variation (CV%) of the L^* parameter was 0.30%.

The index was measured in the flours used for breadmaking. Color index (100 − L^*) values ranged between 5.4 and 11.4. The whole flours presented a mean 100 − L^* value of 8.8. Baking flour showed the lowest value and whole mixed and bran flours the highest. The whiteness of flours is conditioned by the milling extraction rate among other factors. The moisture of the flours was around 12%.

The 100 − L^* parameter was considered by Resmini et al. (1993) and Fernandez-Artigas et al. (1999) as browning index in the control of pasta-drying and flour-toasting. The same flour (baking flour) was used in the manufacturer of the white breads (A−F) (Table 1). The 100 − L^* values ranged between 15.9 for white bread F and 22.8 for white bread C (Table 2). White bread F is sliced loaf bread and the crumb volume is proportionally much greater than the crust in comparison to other breads. The high values exhibited for white bread C could be due to the double fermentation of the dough and the longer baking time, which gives rise to a bread with little crumb and substantial crust.

The color values in the special bread are listed in Table 3. The addition of fruits, glucose syrup, and sucrose to the dough (Table 1) caused an appreciable color increase in the white bread with fruits (color index = 38.2) versus white breads A and E of similar size, shape, and breadmaking characteristics.

The other special breads showed 100 − L^* values ranging between 20.1 and 27.8. The lowest value (20.1) corresponded to whole white bread. The bran and whole rye breads showed values around 28, the same as highest value exhibited by bran flour. The color index of whole rye flour was low (8), but the temperature (210 °C) and baking time (50 min) for whole rye bread were higher than for the other special breads (Table 1).

The color values for the commercial toasted and snack breads are given in Table 5. The average 100 − L^* value for snack breads was 19.4 and for sliced toasted breads was 25.7. These values were similar to those for white and mixed grain breads, respectively.

Table 2. Browning Indicators in Different Common Breads

samples	HMF[a]	100 − L^*	furosine[b]
white bread			
A	15.7	17.9	146.3
B	21.8	17.0	125.4
C	68.8	22.8	141.4
D	40.1	18.4	165.4
E	3.4	18.1	177.8
F	11.8	15.9	208.1

[a] mg/kg of dry matter. [b] mg/100 g of protein.

Table 3. HMF and 100 − L^* Values in Special Breads

samples	HMF[a]	100 − L^*
white bread with fruits	51.3	38.2
whole white bread		
A	7.4	20.1
B	23.2	21.5
mixed-grain bread		
bran bread	23.4	27.8
mixed-flour bread	21.1	26.1
oat bread	4.8	21.4
soy bread	18.3	25.7
whole mixed-flour bread	8.7	23.8
whole rye bread	23.4	27.7

[a] mg/kg dry matter.

Table 4. HMF and Furosine in Crumb and Crust of Breads

samples	HMF[a]	furosine[b]
white bread A		
crumb	0.9	55.4
crust	21.4	125.0
white bread D		
crumb	1.7	42.8
crust	176.1	75.5
whole white bread A		
crumb	0.6	78.6
crust	18.3	220.8
whole white bread B		
crumb	2.2	95.0
crust	73.3	170.3

[a] mg/kg of dry matter. [b] mg/100 g of protein.

Table 5. HMF and Color in Commercial Breads

samples	HMF[a]	$100 - L^*$
sliced toasted breads		
A	11.8	24.1
B	13.0	27.9
C	17.3	23.5
D	87.7	27.8
E	38.0	27.2
F	16.2	25.8
G	21.1	28.5
H	47.2	24.9
I	24.6	22.1
snacks		
A	4.4	18.5
B	2.2	24.5
C	10.0	16.8
D	6.8	17.9

[a] mg/kg of dry matter.

HMF. Figure 1 depicts the HPLC chromatograms for the crumb and crust of bread. The same method and chromatographic conditions were applied to different samples with no need for modifications. The identity and purity of the HMF was confirmed by diode array. The precision was tested on breads with a low (white bread E) ($n = 7$) and a high HMF concentration (crust of white bread D) ($n = 7$). The coefficients of variation were 1.57% and 2.60%, respectively.

The accuracy was tested by the addition of HMF and recovery assays on the sample with the lowest HMF level (3.4 mg/kg). The HMF added to sample was ranged between 12.6 and 123.9 mg/kg. The recovery range was between 92.5 and 100% and the average value was 96.2%. The highest accuracy was obtained at values <72 mg/kg. All the samples presented values <72 mg/kg except for the crust of white bread D. No HMF was detected in the flours.

Table 2 shows the HMF content of the common breads. The values ranged from 3.4 mg/kg (white bread E) to 68.8 mg/kg (white bread C). White bread C required longer fermentation and baking times, with the consequent formation of little crumb and considerable crust, and the sugar content was probably elevated and these factors may account for the increased browning. The flour, weight, and manufacturing conditions of white breads A and E were similar (Table 1) but their HMF was different, at 15.7 and 3.4 mg/kg, respectively (Table 2). The dough of bread E had a smaller water content than that of bread A, which could have as much influence as the sugar content on the HMF. The linear correlation between the browning indicators (HMF and $100 - L^*$) for the common breads was $r^2 = 0.7331$.

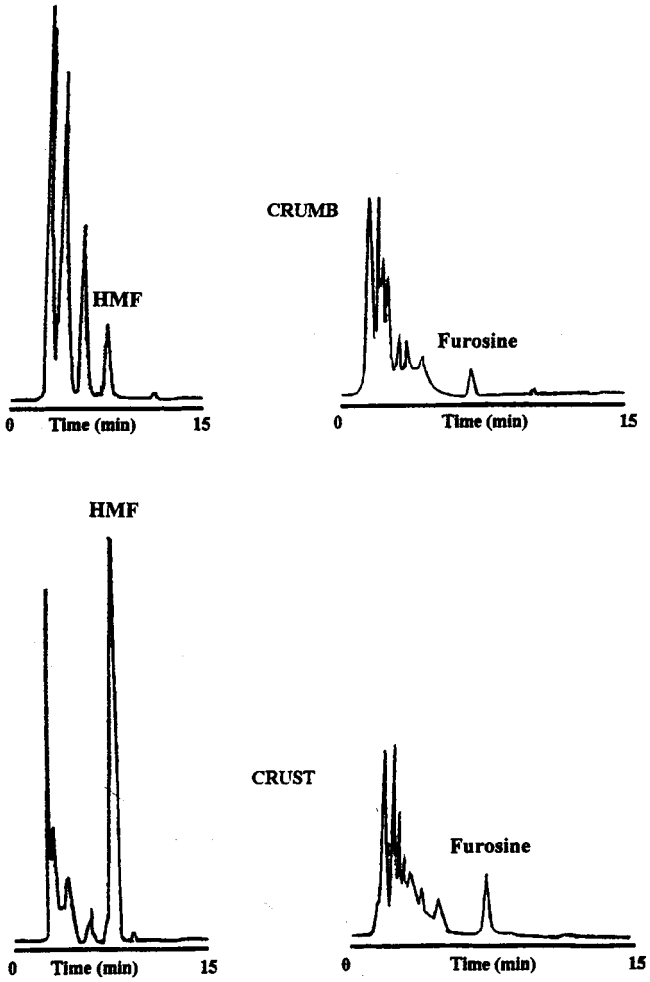

Figure 1. High performance liquid chromatography (HPLC) chromatograms of HMF and furosine of crust and crumb.

The HMF values for the special breads are listed in Table 3. White bread with fruits presented the highest value (51.3 mg/kg) and also gave the highest color results, probably due to the ingredients added.

Whole white breads A and B (Table 3) showed very different HMF levels (7.4 and 23.2 mg/kg). The ingredients of both breads are the same and the higher fermentation and baking times of bread B may account for this difference.

In mixed-grain breads, HMF contents ranged from 4.8 (oat bread) to 23.4 mg/kg (whole rye and brans breads). The oat and whole mixed-flour breads contained <10 mg/kg and the other mixed-grain breads around 20 mg/kg. The linear correlation between HMF and $100 - L^*$ in special bread was $r^2 = 0.8347$.

The differences in HMF content between crumb and crust were studied in four breads (Table 4). HMF levels in crumb were between 0.6 and 2.2 mg/kg and those in crust were notably greater, from 18.3 to the 176.1 mg/kg in white bread D. The fermentation and baking times required to obtain a large bread with a thick crust (white breads D and whole white bread B) are double those required for other breads. This is why between 4 and 8 times more HMF was found in large versus stick breads.

Table 5 shows HMF levels in the nine commercial toasted breads sampled locally, of different shape, weight, and labeled ingredients. They ranged from 11.8 mg/kg (sample A) to 87.7 mg/kg (sample D). The values

Table 6. Behavior of Browning Indicators during Baking Time

time (min)	HMF[a]	100 − L^*	time (min)	HMF[a]	100 − L^*
0	0.06	13.7	20	3.07	17.2
10	0.47	14.1	25	7.38	19.6
15	1.27	16.3	30	19.58	20.6

[a] mg/kg of dry matter.

were generally higher than those for common and special breads since these products are toasted after baking. Sample H, from the company's common bread, contained 47.2 mg/kg of HMF and the HMF before toasting was 15 mg/kg. The correlation between HMF and 100 − L^* was $r^2 = 0.08$. The correlation between products from different brands, with varied ingredients and manufacturing conditions, was not significant.

Table 5 also includes the HMF content of snack breads, whose mean value was 5.8 mg/kg. The manufacturing of these products involves long fermentation and short baking times. These are products of small size (1−3 g) and low moisture content (aproximately 5%). The correlation between HMF and 100 − L^* was $r^2 = 0.7142$.

A new experiment was performed in our the laboratory to confirm some of the results obtained in the commercial breads. Commercial doughs, slightly heated, were baked in the laboratory to demonstrate the behavior of HMF and color indicators in similar doughs at different baking times (Table 6). The 100 − L^* values increased from 13.7 (commercial dough) to 20.6 after 30 min of baking. The correlations between 100 − L^* (color index) and baking time were $r^2 = 0.9357$ and $r^2 = 0.9343$ for linear and exponential, respectively.

The HMF values were from 0.06 mg/kg of initial sample (commercial dough) to 19.6 of bread baked during 30 min at 190 °C. The correlations obtained between HMF and baking time were $r^2 = 0.6523$ and $r^2 = 0.9988$ for linear and exponential, respectively. The linear correlation between HMF and 100 − L^* was $r^2 = 0.7391$ and the exponential correlation $r^2 = 0.9080$.

Another experiment performed on five slice toasted breads from the same lot and color showed the following HMF values: 29.3, 36.0, 36.2, 41.9, and 45.1 mg/kg. Thus, these results suggest that the same products with the same colors can have different HMF values. According to this experiment, HMF is an indicator more sensible than color.

Furosine. The results of furosine determination in the common breads are shown in Table 2. The chromatograms for crust and crumb are depicted in Figure 1. The white breads showed furosine values ranging from 125 mg/100 g of protein for white bread B to 208 mg/100 g of protein for white bread F. No linear correlation was obtained between furosine/HMF ($r = -0.4298$) and furosine/baking temperature ($r = -0.6016$). Breads A and E, of similar form and weight, had a furosine content of 146 and 177 mg/100 g of protein, respectively. The water content the dough of sample E was lower than that of sample A but the water content of bread E was higher, which could be related to a smaller extension of the browning reaction. This can be verified by the higher furosine content (early stage indicator) and lower HMF value for sample E compared with sample A. Table 4 shows the furosine levels of the crumb and crust from the four breads selected for this study (common breads A, D and special breads A, B).

The crumb of the common breads showed lower furosine values (50 mg/100 g of protein) than that of the special breads (78.6 and 95 mg/100 g of protein). The higher baking temperature and/or time of the special breads is related to this increased furosine content. The furosine content was higher in the crust (75−221 mg/100 g of protein) versus the crumb and in the case of the crust, the higher the baking time the lower was the furosine content. In the crust, the high time produces a greater extension of the Maillard reaction and therefore a degradation of furosine. A previous study of toasted sliced bread reported that furosine levels began to descend after 10 min of the toasting process (Ramirez-Jimenez, 1998). No other studies have been found that consider this indicator in bread. The furosine values reported by other authors monitoring the extent of nonenzymatic browning in cereal products are very variable, from 1 mg/100 g of protein in wheat grain (Resmini and Pellegrino, 1991) to 3000 mg/100 g of protein in baby cereals (Guerra and Corzo, 1996). Our values are similar to those observed in pasta and biscuit processing (Resmini and Pellegrino, 1991; Acquistucci et al., 1996), although the sugar contents and the temperatures applied are very different.

In conclusion, the indicators HMF and color increase with the length and temperature of baking process whereas furosine decreases when higher intensity is reached. HMF and color are useful to evaluate the intensity of browning of baking process. Furosine would be a useful indicator to control of commercial precooking doughs. HMF is an indicator more sensible than color, being useful in samples without distinct color.

ACKNOWLEDGMENT

We thank Richard Davies for assisting with the translation into English.

LITERATURE CITED

Acquistucci, R.; Panfili, G.; Marconi, E. Aplication of the microwave hydrolysis to furosine determination in cereal and dairy foods. *J. Agric. Food Chem*. **1996**, *44*, 3855−3857.

Association of Official Analytical Chemists. Official Methods of Analysis of the AOAC, 15th ed.; Methods 925.10, 920.87, 1990, Arlington, VA.

Berg, H. E.; Van Boekel, M. A. J. S. Degradation of lactose during heating of milk. I. Reaction pathways. *Neth Milk Dairy J*. **1994**, *48*, 157−175.

Delgado, T.; Corzo, N.; Santa Maria, G.; Gimeno, M. L.; Olano, A. Determination of furosine in milk samples by ion-pair reversed phase liquid chromatography. *Chromatographia* **1992**, *33*, 374−376.

Erbersdobler H.; Hupe, A. Determination of lysine damage and calculation of lysine bio-availability in several processed foods. *Z. Ernährugswiss.* **1991**, *30*, 46−49.

Fernandez-Artigas, P.; Guerra-Hernandez, E.; Garcia-Villanova, B. Browning indicators in model systems and baby cereals. *J. Agric. Food Chem*. **1999**, *47*, 2872−2878.

García-Villanova, B.; Guerra-Hernández, E.; Martinez Gómez, E.; Montilla, J. Liquid chromatography for the determination of 5-(hydroxymethyl)-2-furaldehyde in breakfast cereals. *J. Agric. Food Chem*. **1993**, *41*, 1254−1255.

Guerra-Hernández, E.; Corzo, N. Furosine determination in baby cereal by ion-pair reversed phase liquid chromatography, *Cereal Chem*. **1996**, *73*, 729−731.

Guerra-Hernández, E.; Corzo, N.; García-Villanova, B. Maillard reaction evaluation by furosine determination during infant cereal processing. *J. Cereal Sci.* **1999**, *29*, 171−176.

Guerra-Hernández, E.; García-Villanova, B.; Montilla-Gómez, J. Determination of hydroxymethylfurfural in baby cereals

by high performance liquid chromatography. *J. Liq. Chromatogr.* **1992**, *15*, 2551−2559.

Henle, T.; Zehetner, G.; Klostermeyer, H. Fast and sensitive determination of furosine. *Z. Lebensm. Unters. Forsch.* **1995**, *200*, 235−237.

Encyclopedia of Food Science and Technology; Hui, Y. H., Ed.; John Wiley & Sons: New York, 1991; Vol. 1, pp 158−176.

Konstance, R. P.; Onwulata, C. I.; Smith, P. W.; Lu, D.; Tunick, M. H.; Strange, E. D.; Holsinger, V. H. Nutrient-based corn and soy products by twin-screw extrusion. *J. Food Sci.* **1998**, *63*, 864−868.

Kroh, L. W. Caramelisation in food and beverages. *Food Chem.* **1994**, *51*, 373−379.

Morales, F. J.; Romero, C.; Jiménez-Pérez, S. Chromatographic determination of bound hydroxymethylfurfural as an index of milk protein glycosylation. *J. Agric. Food Chem.* **1997**, *45*, 1570−1573.

O'Brien, J.; Morrisey, P. A. Nutritional and toxicological aspects of the Maillard browning reaction in foods. *CRC Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 211−248.

Ramirez-Jimenez, A. J. Indicadores de las reacciones de pardeamiento químico en productos panarios. Memory of pharmacy degree of licenciate. The University of Granada, Spain. 1998.

Reineccius, G. A. The influence of Maillard reactions on the sensory properties of foods. In *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*; Finot, P. A., Aeschbacher, H. U., Hurrell, R. F., Liardon, R., Eds.; Birkhäuser Verlag: Basel, Switzerland, 1990; pp 157−170.

Resmini, P.; Pellegrino, L. Analysis of food heat-damage by direct HPLC of furosine. *Int. Chrom. La.* **1991**, *6*, 7−11.

Resmini, P.; Pellegrino, L. Occurrence of protein-bound lysilpyrrolaldehyde in dried pasta. *Cereal Chem.* **1994**, *71*, 254−262.

Resmini, P.; Pellegrino, L.; Batelli, G. Accurate quantification of furosine in milk and dairy products by a direct HPLC method. *Ital. J. Food Sci.* **1990**, *3*, 173−183

Resmini, P.; Pellegrino, L.; Pagani, M. A.; De Noni, I. Formation of 2-acetyl-3-D-glucopyranosylfuran (glucosylisomaltol) from nonenzymatic browning in pasta drying. *Ital. J. Food Sci.* **1993**, *4*, 341−353.

Rychlik M.; Grosch, W. Identification and quantification of potent odorants formed by toasting of wheat bread. *Lebensm.-Wiss. Technol.* **1996**, *29*, 515−525.

Thorvaldsson, K.; Kjjöledbrand, C. Water diffusion in bread during baking. *Lebensm.-Wiss. Technol.* **1998**, *31*, 658−663.

Zanoni, B.; Peri, C.; Bruno, D. Modelling of browning kinetics of bread crust during baking. *Lebensm.-Wiss. Technol.* **1995**, *28*, 604−609.

JF9907687