JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Kinetics of Formation of Three Indicators of the Maillard Reaction in Model Cookies: Influence of Baking Temperature and Type of Sugar

AMÈLIE CHARISSOU, LAMIA AIT-AMEUR, AND INÈS BIRLOUEZ-ARAGON*

Analytical Chemistry Laboratory, Institut National Agronomique Paris-Grignon, 16 rue Claude Bernard, 75005 Paris, France

The Maillard reaction (MR), despite its impact on flavor, color, and texture of cereal products, must be controlled for possible deleterious effects on protein nutritional quality. The present study aims to simultaneously monitor three indicators of the MR reaction (acid-released lysine, furosine, and carboxymethyllysine (CML)) by GC/MS in model cookies and evaluate the effect of formulation and baking temperature. Whereas furosine followed a bell-shape kinetic, indicative of an intermediary compound, CML linearly accumulated, proving to be a good indicator of the advanced MR. Acid-released lysine continuously decreased during baking. A reference baking level was defined to compare differently processed cookies using fluorescence synchronous spectra, highly sensitive to the dough physicochemical properties. Furosine was maximal in glucose-containing cookies, but only accounted for 5−50% lysine blockage, depending on the sugar and baking temperature. High oven temperatures and the use of fructose as the sugar source were associated with lowest the lysine damage and CML formation.

KEYWORDS: Maillard reaction; nutritional value; cookies; lysine; furosine; CML

INTRODUCTION

For as long as food has been cooked, the Maillard reaction (MR) has played an important role in improving the appearance, texture, and taste of foods (*1*). The Maillard reaction is composed by a cascade of consecutive and parallel reaction steps (*2*), which essentially take place in food products containing high levels of sugar and protein or amino acids. The rate of the reaction is proportional to the heat-treatment severity, particularly when a low water activity is reached (optimum between 0.4 and 0.8) (*3*). In this respect, cookies are particularly prone to the MR. Baking is associated with an increase in the temperature in the cookie with a gradient from the inner part to the surface (*4*, *5*). At the same time, water evaporates to reach 4% humidity and its activity decreases to 0.4−0.2, while a brown color and a crumbly texture develop (*6*).

A complex mixture of volatile and nonvolatile compounds derived from the Maillard reaction are formed (*1*) as well as lipid and amino acid degradation products (*7*), which are responsible for the specific taste of cookies. These sensorial parameters are well controlled at the industrial plant to fulfill a constant quality.

However, the MR is also responsible for a loss of nutritional value of foods (*8*), resulting in a decrease of protein digestibility and lysine bioavailability (*9*). This aspect is of particular importance for cereal products where lysine is a limiting amino acid (*10*). Furthermore undesirable compounds, such as acrylamide (*11*, *12*) and carboxymethyllysine (CML) (*13*, *14*, *15*) may be formed with possible carcinogenic (*16*) and pro-inflammatory (*17*) activities. Consequently, a compromise must be found, needing to conveniently monitor these side reactions. This paper aims to illustrate that simultaneous assessment of three pertinent indicators of the MR by GC-MS could be useful to select the formulation and process parameters associated with optimal nutritional quality.

The first source of lysine blockage in cereal products is considered to be the Amadori product, fructosyllysine (FL) (*18*−*20*). This early Maillard product is conventionally quantified using furosine (*21*), which is formed during FL acid hydrolysis. However, in the context of severe heat treatments, such as baking, the Amadori product is further degraded into advanced Maillard products which are responsible for new lysine blockage forms (*22*, *23*). For example, lysylpyrrolaldehyde was described to be formed at high temperature in low moisture food products or models, accounting for significant lysine blockage during the advanced Maillard reaction (*24*, *25*). However, this lysine adduct is further degraded under severe heat treatment. In contrast, CML is considered as a stable advanced Maillard product with low reactivity. On the other hand, the fats added to the dough, despite being poorly unsaturated, may also contribute to lysine blockage by the formation of Schiff bases with the aldehydic compounds formed during secondary heat-dependent lipid peroxidation (*23*, *26*). CML can be produced by degradation of the Amadori compound or by direct reaction of lysine with some dicarbonyl compounds derived from sugar

* Corresponding author: e-mail: birlouez@inapg.fr.

10.1021/jf063024j CCC: $37.00
Published on Web 05/01/2007

or lipid oxidation (*27*). By decreasing the different forms of lysine blockage, an increase in the nutritional value of the cereal protein is expected. Although protein consumption in industrialized countries is almost 2 times higher than that required, controlling the protein quality may be important at least for children and vegetarian subjects who are high cookie consumers.

The simultaneous analysis of lysine, furosine, and CML is possible using GC-MS, following a successive methylation of the carboxylic group and acylation of the amine group of the amino acids (*28*). Such an approach allows a global view on the early (furosine) and advanced (CML) Maillard reaction as well as on the total lysine blockage, whatever the substrate concerned, lipids or sugars. Using the conversion factor between FL and furosine during protein acid hydrolysis, the amount of peptidic lysine specifically blocked by glucose may be calculated, whereas acid-released lysine is indicative of the total damage on this limiting amino acid. By comparison with FL, the proportion between the early and advanced MR can be evaluated. CML as a final and stable product of the MR is indicative of the extent of the advanced MR, including the reaction between lipid peroxides and lysine. Furthermore, per se this compound is of particular interest because of its possible bioactivity (*29*).

Conventionally, CML analysis is performed after a previous reduction of FL, to impede the artefactual conversion of FL into CML during sample acid hydrolysis (*30*). Consequently, no furosine may be detected anymore. However, a previous study (not published) indicates that precipitation and washing of food proteins prior to hydrolysis is sufficient to impede CML formation during acid hydrolysis, despite the presence of high FL concentrations in the sample proteins. So, preliminary reduction was omitted in the present study, allowing simultaneous assessment of furosine and CML.

Different processing conditions, including formulation and baking parameters, may differently influence the relative concentration of the three indicators by accelerating or inhibiting the advanced versus early Maillard reactions, or by favoring other forms of lysine blockage. The aim of this study was to model the evolution of each indicator as a function of baking time for different oven temperatures (200, 250, and 300 °C) and for various type of sugars added to the dough (sucrose, glucose, or fructose), and to compare the nutritional quality of the respective cookies as deduced from these indicators.

EXPERIMENTAL SECTION

1. Materials. A 97% pure lysine standard was purchased from Sigma, and a 99.76% pure furosine dihydrochloride standard and a 95% pure CML standard were purchased from NeoMPS (Strasbourg, France). A sample of 97% pure cycloleucin (CL), used as internal standard, was obtained from Sigma.

2. Sample Preparation and Derivatization. The "model cookies" were prepared by mixing in a container for 4 min wheat flour (60%) with either glucose, sucrose, or fructose syrups (30%) and palm oil (10%). The dough was allowed to rest for 30 min in a drying oven at 25 °C before being rolled in order to obtain 3 mm thick cookies. Three baking kinetics were performed in an oven ((SPAG − ENSIA, France) set at 200 °C, 250 °C, and 300 °C for a total baking time of 16, 10, and 10 min, respectively. The temperature in the core of the cookie was controlled by a sensor inserted horizontally. Cookies were taken every 2 min for analysis. The cookies were crushed and stored at −18 °C until analysis.

Defatting. A quantity of crushed cookie containing 0.5−1 mg proteins was defatted using a modified Folch method (*31*). Three successive extractions were performed by adding 3.8 mL of a chloroform/methanol/water (2/1/0.8, v/v/v) solution followed by centrifugation (15 min, 4000 rpm at room temperature).

Acid Hydrolysis. After addition of the internal standard cycloleucin (50 μg mL^{-1}), the defatted samples were hydrolyzed in 5 mL of 6 M HCl at 110 °C for 18 h. Five hundred microliters was dried under vacuum (Speed Vac, Savant) and further dissolved in distilled water (500 μL), filtered (Nylon 0.22 μm), and dried again.

Derivatization. The derivatization method included an esterification of the carboxylic functions by methanol under strongly acidic conditions followed by an acylation of the amine functions using trifluoroacetic acid anhydride (*32*). The dried sample was derivatized by adding 1 mL of a solution containing 1.46 mL of thionyl chloride in 100 mL of methanol and mixing until total dissolution. Methylation was achieved by heating the sample for 30 min in an oven at 110 °C. After drying under a nitrogen stream, 2 mL of dichloromethane and 400 μL of trifluoroacetic acid anhydride were added. The reaction was total after 1 h at room temperature. The derivatized sample was dried under nitrogen, and 1 mL dichloromethane was added gradually and immediately dried under nitrogen. The dry extract was dissolved in 25 μL of dichloromethane and stored in the freezer. The solution is stable for at least 1 month.

3. GC/MS Quantification. Lysine, furosine, and CML were quantified by selected ion monitoring on a Trace 2000 (Thermo Electron Corporation) gas chromatograph equipped with a DB5-MS capillary column (30 m × 0.25 mm × 0.25 μm) coupled to a Polaris Q mass selective detector. Cycloleucin was used as external standard. The split injection mode was used. The temperature program was 2 min at 70 °C, 5 °C/min to 260 °C, 15 °C/ min to 290 °C, and finally 290 °C for 5 min. Helium was used as carrier at a flow-rate of 1.5 mL min^{-1}. Detection used the electron impact (EI) mode at 70 eV. Data were collected and integrated with Xcalibur software (Thermo Electron Corporation). The quality parameters of the method are described elsewhere.

4. Quantification of the Protein Concentration in the Hydrolyzed Sample. In order to express lysine, furosine, and CML as mg g^{-1}, mg 100g^{-1}, and μg g^{-1} proteins respectively, the amino acid concentration in the protein hydrolysate was quantified by the rapid fluorescamine assay developed by Yaylayan et al. (*33*). Briefly, 20 μL of the filtered hydrolysate was dissolved in 2.2 mL of borate buffer (0.2 M and pH = 8.5) and vigorously mixed with 100 μL of fluorescamine (0.15 g L^{-1} acetone). The fluorescence was measured at λ_{exc} = 390 nm and λ_{em} = 475 nm on a Fluoromax-Spex spectrofluorimeter (ISA, Jobin-Yvon). The external calibration curve used hydrolyzed bovine serum albumin.

5. Assessment of the Baking Level Using Fluorescence Synchronous Spectra. Fluorescence synchronous spectra (FSS) were first used by Zandomenaghi et al. (*34*) to determine the reflectance of flours and monitor their carotenoid content. Actually, registering synchronous spectra from a solid sample allows the recovery of photons from the excitation beam which are either scattered or reflected at the matrix surface (*35*). But a major part of the photons is absorbed by the chromophores present in the solid sample with a probability which is proportional to the light path. So, the percentage of photons absorbed at any excitation wavelength depends on the light path and the sample absorbance. The more porous is the dough, the deeper the excitation light path; moreover, the more colored is the food matrix, the higher absorption. Considering that during

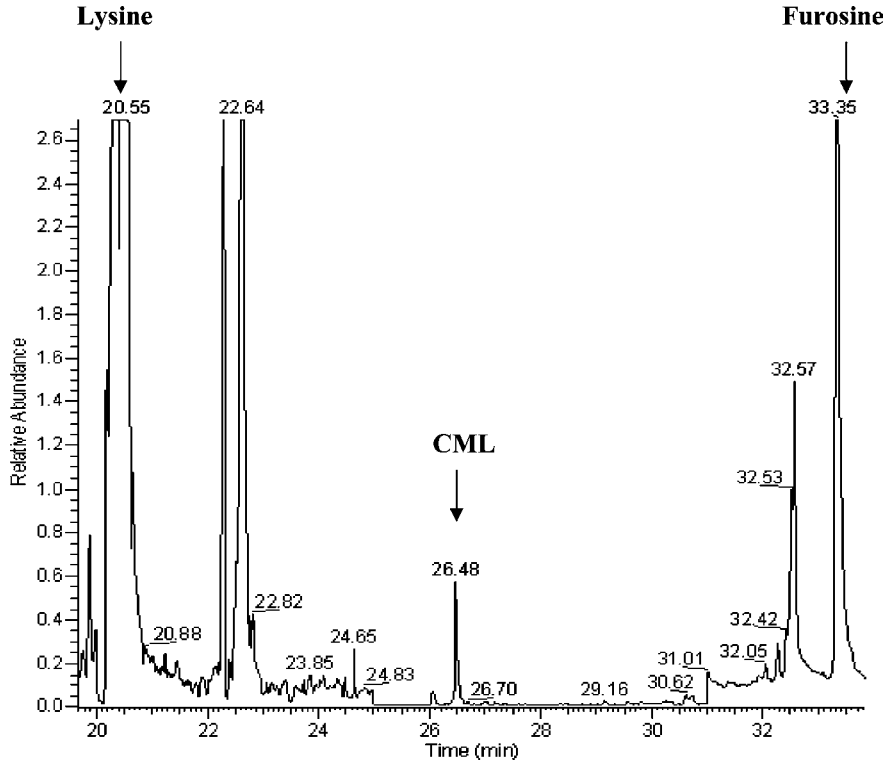

Figure 1. GC-MS chromatogram of lysine, furosine, and CML in a model cookie with SIM detection.

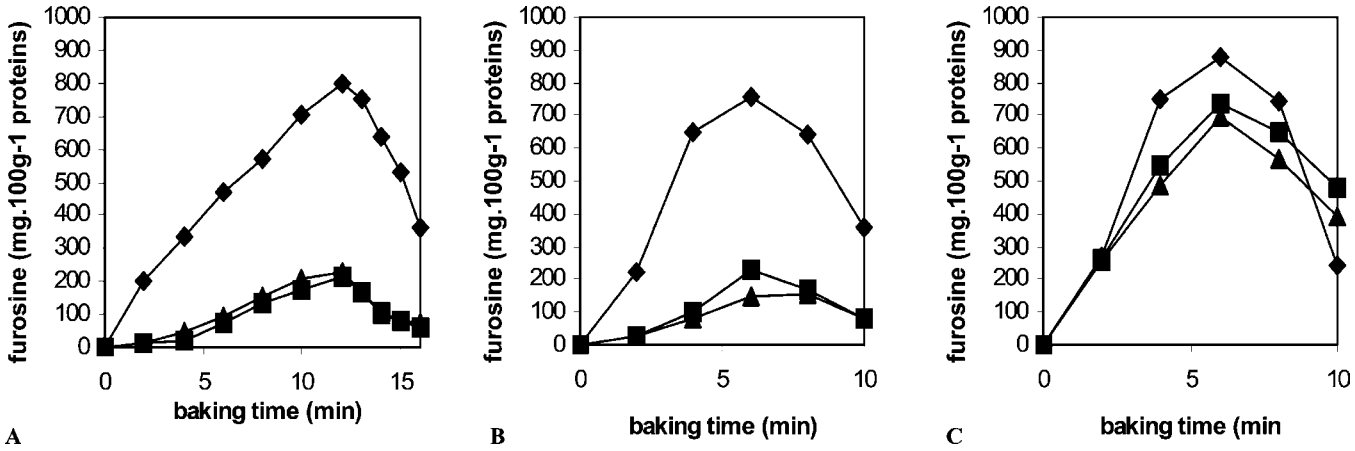

Figure 2. Furosine kinetics in model cookies formulated with different sugars and baked at various temperatures: glucose (lozenge), fructose (triangles), and sucrose (square). A, 200 °C; B, 250 °C; C, 300 °C.

baking both the dough porosity and brown chromophores concentration increase, a higher proportion of photons absorbed and a smaller proportion of photons reflected and scattered are expected. Both phenomena are associated with a drastic fall in the FSS intensity (*36*), explaining why the area under the spectrum is very well correlated to the time−temperature area measured in the core of the cookie (*37*). Therefore, the fluorescence synchronous spectrum area (FSSA) was chosen as an indicator of the baking level. A reference FSSA was defined by the mean value obtained in commercial cookies. Using the regression line obtained between the synchronous spectrum area and baking time ($r^2 > 0{,}95$, $n = 45$; data not shown), the reference baking time was defined for each cookie formula and processing conditions.

RESULTS

1. Simutaneous Lysine, Furosine, and CML Analysis in Cookies. Kinetic Modeling. GC-MS data were processed in the full scan range from *m*/*z* 50 to 450 in order to obtain the mass fragments of the lysine, furosine, CML and internal standard derivatives. The major specific ions were used for selected ion monitoring. The retention times were 20.6, 26.5, and 33.4 min for lysine, CML, and furosine, respectively (**Figure 1**).

CML, furosine, and lysine were modeled in cookies formulated with three different sugars (glucose, fructose, and sucrose) as a function of baking time for three oven temperatures (200 °C, 250 °C, and 300 °C). The levels in the raw dough were 0, 0.105 mg g^{-1} and 35.0 mg g^{-1}, respectively.

Furosine. **Figure 2** shows the typical furosine evolution in the cookies. The Amadori product followed a bell-shape curve. Glucose was associated with a 5 times higher initial furosine formation (0.6 mg g^{-1} min^{-1} at 200 °C) than fructose or sucrose (0.23 and 0.22 mg g^{-1} min^{-1} at 200 °C) (**Figure 2**)), at least for temperatures lower than 250 °C. A similar curve was obtained for fructose- or sucrose-formulated cookies. At 300

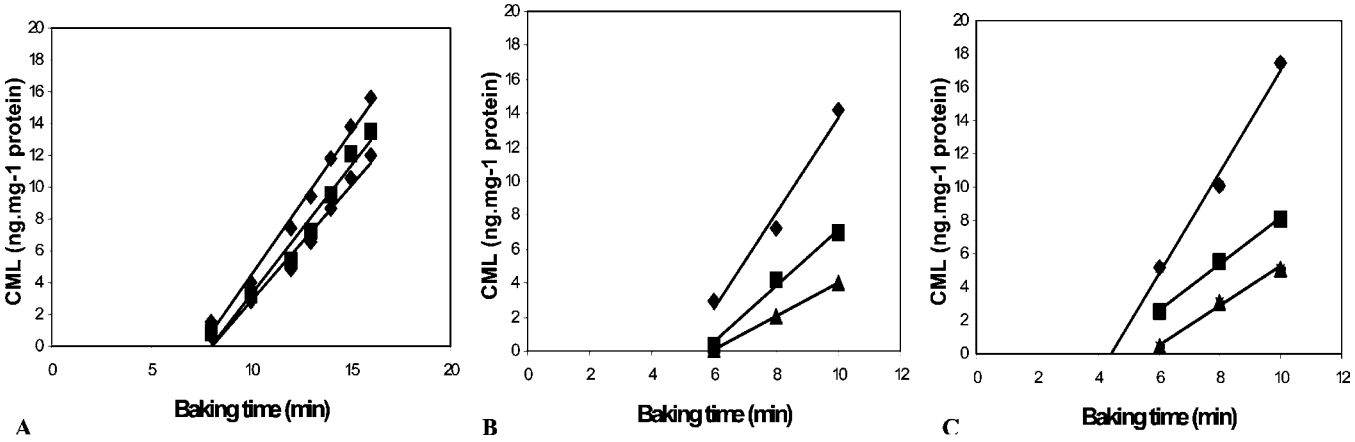

Figure 3. CML kinetics in model cookies formulated with different sugars and baked at various temperatures: glucose (lozenge), fructose (triangles), and sucrose (square). A, 200 °C; B, 250 °C; C, 300 °C.

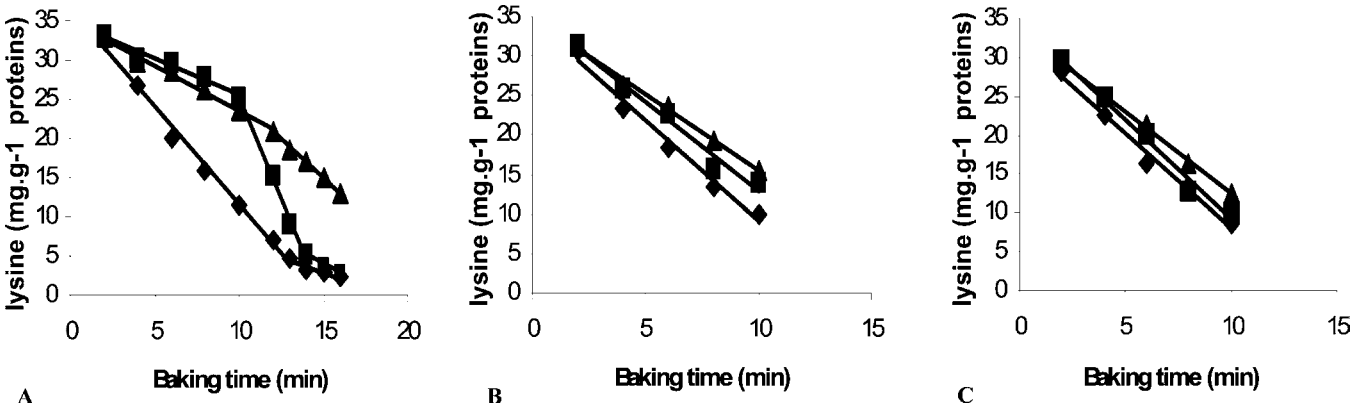

Figure 4. Kinetics of acid-released lysine in model cookies formulated with different sugars and baked at various temperatures: glucose (lozenge), fructose (triangles), and sucrose (square). A, 200 °C; B, 250 °C; C, 300 °C.

°C, the three curves were almost superimposed, demonstrating a strong effect of the oven temperature on the respective isomerization and hydrolysis of fructose and sucrose into glucose. In contrast, oven temperature had little effect on glucose cookies. The main difference observed between glucose-formulated cookies baked at different temperatures was the rate of furosine formation and/or degradation. However, no impact was observed on the maximal furosine concentration.

CML. CML formation followed an apparent zero-order kinetic after a lag time where no CML was detected (**Figure 3**). Except for glucose-containing cookies, the reaction rates were not significantly modified by the oven temperature. But in the presence of glucose, the zero-order kinetic rate increased with oven temperature, going from 1.81 μg g^{-1}protein. min^{-1} at 200 °C to 2.80 μg g^{-1} protein min^{-1} at 250 °C and 3.06 μg g^{-1}. protein min^{-1} at 300 °C. The lag time before CML formation was shortened with the temperature (8, 6, and 4 min at 200, 250, and 300 °C, respectively). Glucose was associated with significantly higher reaction rates, especially at 300 °C, where it was twice that of cookies with fructose or sucrose, the two latters being associated with similar CML formation rates.

Lysine. Lysine decreased rapidly and almost linearly as a function of baking time except at 200 °C, where a more complex decay, with two to three steps, was observed (**Figure 4**). In the presence of sucrose, an initial slow decrease similar to that of fructose-containing cookies (around 1 mg g^{-1} min^{-1} at 200 °C) was followed by a strong drop before a further stabilization, at a level close to that found in glucose cookies. Glucose was always associated with the highest lysine decrease (2.5 mg g^{-1} min^{-1} at 200 °C) and fructose to the lowest one. Between 250 and 300 °C, however, no major effect of the oven temperature or of the sugar source was observed.

2. Comparing the Final Quality of Cookies Depending on the Formulation and Baking Temperature (Table 1). A major difficulty when comparing cookies processed differently is to define a reference baking level, ensuring that all cookies are similarly baked. As indicated in a previous paper (*37*), the heat charge applied to the cookie can be predicted from the synchronous fluorescence spectra, using PLS regression. Indeed, fluorescence synchronous spectra are strongly influenced by the dough color (absorbance probabilility) and microstructure (light scattering probability), two parameters deeply modified during baking. However, the quality of the prediction was not better than that allowed by a simple regression on the fluorescence synchronous spectrum area (FSSA) or on the maximal spectrum intensity. Consequently, FSSA was used to characterize the baking process. A standard baking level was defined as the mean FSSA measured in commercial cookies (*37*). Subsequently the standard baking time was defined for each type of cookies using the FSSA−time curve (not shown). **Table 1** presents the resulting standard baking times calculated for each type of cookie and the associated levels of acid-released lysine, furosine, and CML. Obviously, the standard baking time was inversely proportional to the oven temperature but associated with similar browning at the surface. Furosine levels increased as a function of the temperature, but this increase was particularly evident in fructose- and sucrose-containing cookies when the oven temperature reached 300 °C. In glucose-formulated cookies, the furosine content was almost as high at 200° as at 300 °C. Considering CML, however, the highest concentrations were

Table 1. Simultaneous Assessment of Three Indicators of the Maillard Reaction in Differently Processed Model Cookies

baking time, min	sugar source	oven temp (°C)	furosine (mg g^{-1} P)	CML (μg g^{-1}P)	lysine (mg g^{-1} P)	% total unavailabe lysine[a]	% lysine blocked as FL[b]	% lysine blocked with other products[b]
15	fructose	200	0.83 ± 0.03.	11.3 ± 0.4	15.0 ± 0.5	59.4	5.3	54.1
15	glucose	200	5.27 ± 0.14	13.7 ± 0.3	2.9 ± 0.5	100	33.6	72.3
16	sucrose	200	0.23 ± 0.021	14.4 ± 0.5	2.6 ± 0.5	93.2	1.5	91.7
9	fructose	250	1.20 ± 0.27	4.0 ± 0.2	17.5 ± 0.4	53.2	7.6	45.6
9	glucose	250	5.01 ± 0.17	11.1 ± 0.3	11.5 ± 0.6	80.6	31.9	48.7
11	sucrose	250	0.49 ± 0.22	9.3 ± 0.3	10.7 ± 0.2	70.8	3.1	67.6
7	fructose	300	6.28 ± 0.182	2.0 ± 0.2	18.8 ± 0.3	63.1	39.9	23.2
7	glucose	300	7.78 ± 0.21	8.3 ± 0.2	15.4 ± 0.3	76.9	49.6	27.4
7	sucrose	300	6.87 ± 0.17	4.1 ± 0.4	17.0 ± 0.3	69.9	43.8	26.1

[a] The percentage of total unavailable lysine was calculated by subtracting the acid-released lysine plus that expected to be released from FL (1.66 times the furosine concentration in mmol g^{-1} according to Bujard and Finot (*34*) from the initial lysine concentration in the raw dough (35 mg g^{-1} P)). [b] The percentage of lysine blocked as FL was calculated by multiplying the furosine concentration (mmol g^{-1}) by 3.3 according to Bujard and Finot (*34*). [c] The percentage of lysine blocked by other products was deduced from the difference between *a* and *b*.

found in cookies baked at 200 °C and the lowest in cookies baked at 300 °C. Above 200 °C, glucose was always associated with the highest CML concentration, followed by sucrose and finally fructose. However, at 200 °C, sucrose-formulated cookies contained the highest CML concentration. The content in acid-released lysine decreased inversely to the baking temperature. It was maximal in fructose-containing cookies whatever the oven temperature. A 5 fold difference was observed at 200 °C between fructose and glucose- or sucrose-formulated cookies; this difference dropped to less than 2 fold at 250 °C. In contrast, no major difference between cookies was noticed at 300 °C.

On the basis of these data, the concentrations of total lysine unavailability as well as the proportion blocked under FL and other products were deduced. For this purpose we used the conversion factors determined by Bujard et al. (*38*), indicating that during acid hydrolysis with 6 M HCl at 110 °C for 18 h, 1 mol of FL gives rise to 0.5 mol of free lysine and 0.32 mol of furosine. Considering the initial lysine concentration in the raw cookie dough (35 mg g^{-1} proteins), we calculated the total percentage of lysine blocked (**Table 1**). This value varied from 50 to 100% depending on the sugar and oven temperature. The percentage of lysine blocked as FL increased when the oven temperature rose, in a much faster way in fructose and sucrose-containing cookies than in glucose ones. However, it was always maximal in the presence of glucose, accounting for at least 50% of the lysine blocked (**Table 1**). Two-thirds of unavailable lysine was fructosyllysine in cookies baked at 300 °C. However, at lower temperatures, the great majority of lysine blockage was due to other products than FL, especially in fructose- and sucrose-formulated cookies (**Table 1**).

DISCUSSION

Numerous studies have evaluated the nutritional consequences of the Maillard reaction in heat-treated food products. However, major work was focused mainly on dairy products (*9*, *39*−*41*). Only a few papers examine the impact of the reaction on cereal products (*41*−*45*). Recently Horvatic et al. (*10*) determined the extent of lysine unavailability in cookies and demonstrated a deep degradation of protein quality.

The main consequence of the Maillard reaction is the loss of protein digestibility and lysine availability due to the reaction of the free ϵ-NH_2 groups of the amino acid with reducing sugars or reactive aldehydes (*46*). Furosine is recognized as an indicator of the early step of the reaction with glucose and responsible for most lysine damage in milk products (*21*). Carboxymethyllysine which is formed from oxidative degradation of the Amadori product (*13*−*15*) as well as from the reaction between lysine and glyoxal formed by PUFA or hexose autoxidation (*27*, *47*) is rather considered as an indicator of the advanced Maillard reaction associated with severe heat damage (*13*). Actually, during prolonged or high heat treatment, the Amadori product as well as pyrraline formed by reaction between lysine and sugar degradation products (*29*) are significantly degraded into advanced Maillard products (*23*), some of which are characterized by irreversible interprotein cross-linking and implicated in the crumb texture of cereal products (*48*). The loss of protein digestibility can be explained by the resistance of such cross-links to intestinal protease, as well as direct inhibition of protease activity by advanced Maillard products (*8*, *9*).

In cereal products, where lysine is the limiting amino acid, the Maillard reaction is expected to have a drastic impact on protein nutritional quality. Furthermore, heat treatments applied to cereal products, such as oven baking or extrusion, are rather severe. In addition, the low moisture in such food products is expected to further exacerbate the extent of the reaction (*6*). Up to 10 mg of furosine per gram of protein was found in infant cereals processed by roller-drying after amylolisis, despite the absence of added sugar (*19*), i.e., 10 fold higher levels than those commonly measured in UHT milk (*40*). The furosine concentration is essentially dependent on glucose concentration. In the absence of added sugar, glucose is mainly derived from starch hydrolysis, so that except in the case of roller-drying, a low furosine content (less than 0.05 mg g^{-1} protein) was found in baby cereal products (*21*, *41*). Further storage, however, increased the furosine content especially at high water activities of 0.65, inducing up to 50% loss of available lysine. Another study confirms the strong lysine damage in cookies where reducing sugars are added, indicating levels of lysine blockage as high as 50% (*10*).

The main critical aspect regarding the improvement of the protein quality in cereal products lays on the need for Maillard-associated flavors, color, and texture, for a good acceptance by the consumer. However, we show in this paper that optimization is possible, so that a compromise should be found between the development of sensory and nutritional qualities and safety. Simultaneous monitoring of the kinetics of indicators of the early and advanced Maillard reaction, as well as acid-released lysine allowed optimizing the process conditions to improve the nutritional quality while maintaining the physical aspect of the product. The latter was based on the fluorescence synchronous spectrum area (FSSA), which is indicative of the optical properties of the cookie, itself related to browning and dough microstructure. Despite the interest of pyrraline to account for lysine blockage by degradation products of hexose (*29*) during

the advanced Maillard reaction, the instability of the product in severe heating processes led us to better consider CML which accumulates during the advanced step of the reaction.

Simultaneous analysis of furosine, CML, and acid-released lysine in cookies offers a global view of the extent and advancement of the Maillard reaction. This is possible because no previous reduction was applied before protein acid hydrolysis, despite the recommendation of some authors wondering that some FL could produce artefactual CML during hydrolysis (*30*, *32*). We observed, however, that when isolating and washing the protein pellet prior to hydrolysis a similar CML concentration was found in cookies prepared using or not using a previous reduction step before acid hydrolysis (data not shown). On the basis of the standard FSSA, we defined a standard baking time allowing the comparison of the development of the Maillard reaction in cookies of different formulas and baked at various temperatures.

FL, a product specifically formed from glucose, appeared as a transient product, indicating that the advanced Maillard reaction strongly develops in cookies. However, at the standard baking time, furosine was still near the maximum level or just began to decay. As expected, fructose- and sucrose-containing cookies were generally associated with less FL than glucose ones. Fructose forms the Heyns product during the early step of the MR, which gives no furosine after acid hydrolysis. The presence of furosine in fructose-formulated cookies might derive from either fructose isomerization into glucose or from starch hydrolysis into maltose and glucose. However, in a dough baked without sugar, only trace amounts of furosine were found, indicating that starch is stable below 250 °C (data not shown). At 300 °C, sucrose hydrolysis was total and hexose isomerization was rapid enough to induce almost equimolar concentrations of glucose and fructose in all cookies whatever the initial sugar added to the dough (*37*). Consequently, furosine was formed at similar high levels in all cookies independently of the sugar composition. Unlike furosine, CML was higher at 200 °C than at 300 °C and was maximal in sucrose-containing cookies. Minimal concentrations were obtained in fructose-formulated cookies, especially at 300 °C. The levels of CML found in this study are close to those who were measured by Hartkopf et al. (*15*) using an HPLC method. Up to 100% lysine could be blocked in the final cookie when baked at 200 °C. But the mean level of lysine blockage was mostly around 60−70%, significantly above the levels determined by Horvatic et al. (*10*) on cookies baked at 250 °C (45−50% lysine blockage). However, the analytical method used to evaluate lysine unavailability differed between the two studies as well as the cookie formulation and baking process. At 300 °C, almost 70% lysine was blocked as FL. At such a high temperature, starch and sucrose are completely degraded and hexose isomerization occurs (*37*), so that cookie fructosyllysine concentrations were similar whatever the sugar added. The proportion of FL strongly decreased when the oven temperature decreased. The lower temperature, the reduced fraction of lysine blocked under FL (less than 10% for fructose and sucrose and 30% for glucose at 200 °C). As expected, the proportion of lysine blocked as FL was highest in glucose-based cookies, while it was 4−5 fold and 10−20 fold lower in fructose and sucrose-based cookies, respectively. In the latter cookies, some lysine residues could be blocked by the Heyns product, but at least in sucrose-formulated cookies, where marginal sucrose hydrolysis was observed under 300 °C (*37*), the major part of lysine should be blocked by products other than sugars, for example lipid-derived peroxides. If this hypothesis is correct, one would deduce that lipid peroxidation is strongly enhanced in the presence of sucrose at lower temperatures. Actually when replacing palm oil by rapeseed oil in the dough, a much higher lysine blockage was observed whereas FL decreased (data not shown), which was interpreted as a stronger lipid peroxidation in the omega3-rich oil. In the case of fructose, however, a low level of lysine blockage was observed. The difference with other sugars was especially high at a low temperature (200 °C) where a 5 times higher available amount of lysine was found in fructose-containing cookies. This suggests that small amounts of Heyns product were formed, very few advanced Maillard products, and lipid peroxides, despite a similar CML formation as in glucose- and sucrose-containing cookies. At higher temperatures, CML was lower in fructose- and sucrose- than in glucose-containing cookies, despite the report that ketoses produce CML at least as fast as glucose in model systems (*49*). The behavior of fructose in foods has received little attention and needs to be better understood. CML appeared almost correlated with lysine blocked under forms other than FL in fructose- and sucrose-containing cookies ($r = 0.91$) but not in glucose ones. This could suggest a mechanism essentially based on direct reaction between lysine and some degradation product of fructose, such as glyoxal (*47*). In contrast, CML would mainly derive from FL oxidative degradation in glucose-containing cookies. What must be highlighted is the very low level of lysine blockage explained by CML (ratio between CML and FL ranging between 10^{-4} and 10^{-2}). Consequently, CML is only an indicator of the advanced Maillard reaction.

In conclusion, this paper shows that by simultaneously monitoring furosine, CML, and acid-released lysine in cereal products, a global comprehension of the development of the Maillard reaction is facilitated. This is possible by omitting the reducing step generally used when the sample is hydrolyzed without prior protein isolation. Despite CML being irrelevant as an indicator of lysine damage, its apparent zero-order kinetic allows evaluation of the extent of the advanced Maillard reaction, as compared to other products such as pyrraline, which are not stable at high temperature. Furthermore, CML is considered as indicative of bioactive Maillard products and therefore of the possible side effects of severely heat-treated foods (*17*). As far as cookies are concerned, higher oven temperatures associated with low baking time must be used for minimal lysine damage. Furthermore, fructose appears as a less reactive sugar regarding lysine availability and CML formation. Further work is needed to elucidate the chemical forms of lysine blockage, other than furosine (*18*−*20*) and pyrraline (*24*, *25*), and especially those derived from lipid peroxides. Moreover, the low reactivity of fructose in conditions with low water content and pH and high temperature should be explored.

LITERATURE CITED

(1) Weenen, H. Reactive intermediates and carbohydrate fragmentation in Maillard chemistry. *Food Chem.* **1998**, *62*, 393−401.

(2) Hodge, J. E. Chemistry of browning reactions in model systems. *J. Agric. Food Chem.* **1953**, *1*, 928−943.

(3) Ames, J. M.; Bates, L.; Mcdoulall, D. B. Colour development in an intermediary moisture Maillard model system. In *The Maillard reactions in chemistry, food and health*; Royal Society of Chemistry: London, 1993; pp 120−126.

(4) Manly, D. *Biscuits, cookies and crackers manufacturing manuals*; Woodhead Publishing Limited: Cambridge, England, 1998; pp 15−20.

(5) Wade, P. *Biscuits, cookies and crackers, the principles of the craft*; Elsevier Applied Science: New York, 1988; volume 1, pp 54−81.

(6) Labuza, T. P. The effect of water activity on reaction kinetics of food deterioration. *Food Technol*. **1980**, *34*, 36−41.
(7) Farmer, L. J.; Mottram, D. S.; Whitfield, F. B. Volatile compounds produced in Maillard reaction involving cysteine, ribose and phospholipids. *J. Sci. Food Agric*. **1989**, *49*, 347−368.
(8) O'Brian, J.; Morrisey, P. A. Nutritional and toxicological aspects of the Maillard browning reaction in foods. *Crit. Rev. Food Sci*. **1989**, *28*, 211−248.
(9) Rerat, A.; Calmes, R.; Vaissade.; Finot, P. A. Nutritional and metabolic consequences of the early Maillard reaction of heat treated milk in the pig. *Eur. J. Nutr*. **2005**, *41*, 1−11.
(10) Horvatic, M.; Eres, M. Protein nutritive quality during production and storage of dietetic biscuits. *J. Sci. Food Agric.* **2002**, *82*, 1617−1620.
(11) Grivas, S.; Jägerstad, M.; Lingnert, H.; Skog, K.; Törnqvist, M.; Åman, P. Acrylamide in food-Mechanisms of formation and influencing factors during heating of foods. *Report of the scientific commission of the Swedish Institute for Food and Biotechnology*; 2002, pp 1−22.
(12) Taeymans, D.; Wood, J.; Ashby, P.; Blank, I.; Studer, A. H.; Stadler, R.; Gondé, P.; Van Eijck, P.; Lalljie, S.; Lingnert, H.; Lindblom, M.; Matissek, R.; Muller, D.; Tallmadge, D.; O'brien, J.; Thompson, S.; Silvani, D.; Whitmore, T. A Review of Acrylamide: An Industry Perspective on Research, Analysis, Formation, and Control. *Crit. Rev. Food Sci. Nutr*. **2004**, *44*, 323−347.
(13) Hewedi, M. M.; Kiesner, C.; Meissner, K.; Hartkopf, J.; Erbersdobler, H. J. Effects of UHT heating of milk in an experimental plant on several indicators of heat treatment. *J. Dairy Res.* **1994**, *61*, 305−309.
(14) Golberg, T.; Cai, W.; Peppa, M.; Dardaine, V.; Baliga, B.; Uribarri, J.; Vlassara, H. Advanced glycoxidation end products in commonly consumed foods. *J. Am. Diet. Assoc.* **2004**, *104*, 1287−1291.
(15) Hartkopf, J.; Erbersdobler, H. F. Experiments with sausage meat on the formation of N epsilon-carboxymethyllysine. *Z. Lebensm. Unters. Forsch*. **1995**, *201*, 27−29.
(16) Rice, J. M. The carcinogenicity of acrylamide. *Mutat. Res.* **2005**, *7*, 3−20.
(17) Vlassara, H.; Cai, W.; Crandall, J.; Goldberg, T.; Oberstein, R.; Dardaine, V.; Peppa, M.; Rayfield, E. J. Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *26*, 15596−15601.
(18) Guerra-Hernandez, E.; Corzo, N.; Garcia-Villanova, B. Maillard reaction evaluation by Furosine determination during infant cereal processing. *J. Cereal Sci.* **1999**, *29*, 171−176.
(19) Cardenas, Ruiz, J.; Guerra-Hernandez, E.; Garcia-Villanova, B. Furosine is a useful indicator in pre-baked breads. *J. Sci. Food Agric.* **2004**, *84*, 366−370.
(20) Rada-Mendoza, M.; Garcia-Banos, J. L.; Villamiel, M.; Olano, A. Study on nonenzymatic browning in cookies, crackers and breakfast cereals by maltulose and furosine determination. *J. Cereal Sci*. **2004**, *39*, 167−173.
(21) 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**, *2*, 173−183.
(22) Van Boekel, M. A. J. S. Effect of heating on Maillard reactions in milk. *Food Chem.* **1998**, *62*, 403−414.
(23) Leclere, J.; Birlouez-Aragon, I. The fluorescence of advanced Maillard products is a good indicator of lysine damage during the Maillard reaction. *J. Agric. Food Chem.* **2001**, *49*, 4682−4687.
(24) Henle, T.; Klostermeyer, H. Determination of protein-bound 2-amino-6-(2-formyl-1-pyrrolyl)-hexanoic acid ("pyrraline") by ion exchange chromatography and photodiode array detection. *Z. Lebensm. Unters. Forsch*. **1993**, *196*, 1−4.
(25) Resmini, P.; Pellegrino, L. Occurrence of protein-bound lysylpyrrolaldehyde in dried pasta. *Cereal Chem.* **1994**, *71*, 254−262.
(26) Burcham, P. C.; Kuhan, Y. T. Introduction of carbonyl groups into proteins by the lipid peroxidation product, malondialdehyde. *Biochem. Biophys. Res. Commun.* **1996**, *220*, 996−1001.
(27) Fu, M. X.; Requena, J. R.; Jenkins, T. J.; Litchfield, J. E.; Thorpe, S. R.; Baynes, J. W. The advanced glycation end product, N-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions. *J. Biol. Chem*. **1996**, *271*, 9982−9986.
(28) Büser, W.; Erbersdobler, H.; Liardon, R. Identification and determination of N-ϵ-carboxymethyllysine by gas liquid chromatography (via heptafluorobutyryl-isobutyl esterification). *J. Chromatogr*. **1987**, *387*, 515−519.
(29) Henle, T. Protein-bound advanced glycation endproducts (AGEs) as bioactive amino acid derivatives in foods. *Amino Acids* **2005**, *29*, 313−322.
(30) Hartkopf, J.; Pahlke, C.; Lüdemann, G.; Erbersdobler, H. F. Determination of N-ϵ-carboxymethyllysine by a reversed phase high performance liquid chromatography method. *J. Chromatogr. A*. **1994**, *672*, 242−246.
(31) Folch, J.; Less, M. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. **1957**, *226*, 497−509.
(32) Brand, M. D.; Pamplona, R.; Portero-Otin, M.; Requena, J. R.; Roebuck, S. J.; Buckingham, J. A.; Clapham, J. C.; Cadenas, S. Oxidative damage and phospholipid fatty acyl composition in skeletal muscle mitochondria from mice underexpressing or overexpressing uncoupling protein 3. *Biochem. J*. **2002**, *368*, 597−603.
(33) Yaylayan, V. A.; Huyghues-Despointes, A.; Polydorides, A. A fluorescamine-based assay for the degree of glycation in bovine serum albumin. *Food Res. Intern.* **1992**, *25*, 269−275.
(34) Zandomeneghi, M.; Festa, C.; Carbonaro, L. Front−surface absorbance spectra of wheat flour determination of caretonoids. *J. Agric. Food Chem.* **2000**, *48*, 2216−2221.
(35) Wu, J.; Partovi, F.; Field, M. S.; Rava, R. P. Diffuse reflectance from turbid media: an analytical model of photon migration. *Appl. Opt.* **1993**, 327, 1115−1221.
(36) Ait Ameur, L.; Zude, M.; Trystram, G.; Birlouez−Aragon, I. Hydroxymethylfurfural: an indicative parameter of heat damage in cereal products. *Czech. J. Food Sci*. **2004**, *22*, 99−101.
(37) Ait Ameur, L.; Mathieu, O.; Lalanne, V.; Trystram, G.; Birlouez−Aragon, I. Compared effects of sucrose and hexose on furfural formation and browning in cookies baked at different temperatures. *Food Chem.* **2007**, *101*, 1424−1433.
(38) Bujard, E.; Finot, P. A. Mesure de la disponibilité et du blocage de la lysine dans les laits industriels. *Ann. Nutr. Alim.* **1978**, *32*, 291−305.
(39) Birlouez−Aragon, I.; Moreaux, V.; Nicholas, M; Ducauze, C. J. Effect of iron and lactose supplementation of milk on the Maillard reaction and tryptophan content. *Food Addit. Contam.* **1997**, *14*, 381−8.
(40) Birlouez-Aragon, I.; Pischetsrieder, M.; Leclere, J.; Morales, F. J.; Hasenkopf, K.; Ducause, C.; Rutledge, D. Loss of nutritional quality and chemical safety in heat-processed infant formulas. *Food Chem.* **2004**, *87*, 253−259.
(41) Ferrer, E.; Alegria, A.; Farré, R.; Abellan, P.; Romero, F. Effects of thermal processing and storage on available lysine and furfural compounds content of infant formulas. *J. Agric. Food Chem.* **2000**, *48*, 1817−1822.
(42) Ramirez-Jiménez, A.; Guerra-Hernandez, E.; Garcia-Villanova, B. Evolution of non-enzymatic browning during storage of infant rice cereal. *Food Chem.* **2003**, *83*, 219−225.
(43) Zardetto, S.; Dalla Rosa, M.; Di Fresco, S. Effects of different heat treatments on the furosine content in fresh filled pasta. *Food Res. Intern*. **2003**, *36*, 877−883.
(44) Rufian-henares, J. A.; Delgado-Andrade, C.; Morales, F. J. Analysis of heat-damage indices in breakfast cereals: influence of composition source. *J. Cereal Sci*. **2006**, *43*, 63−69.

(45) Delgado-Andrade, C.; Rufián-Henares, J. A.; Morales, F. J. Lysine availability is diminished in commercial fibre-enriched breakfast cereals. *Food Chem.* **2007**, *100*, 725−731.
(46) Finot, P. A. The absorption and metabolism of modified amino acids in processed foods. *J. AOAC Int*. **2005**, *88*, 894−903.
(47) Thorpe, S. R.; Baynes, J. W. CML: a brief history. *Intern. Congress Ser*. **2002**, *1245*, 91−99.
(48) Gerrard, J. A.; Brown, P. K.; Fayle, S. E. Maillard crosslinking of food proteins. III: the effects of glutaraldehyde, formaldehyde and glyceraldehude upon bread and croissants. *Food Chem.* **2003**, *80*, 45−50.
(49) Ruttak, A.; Erbersdobler, H. F. N-carboxymethyllysine is formed during heating of lysine with ketoses. *J. Sci. Food Agric.* **1995**, 68, 261−263−266.

Received for review October 20, 2006. Revised manuscript received February 19, 2007. Accepted February 28, 2007.

JF063024J