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A critical evaluation of fluorescence as a potential marker for the Maillard reaction

Silvia B. Matiacevich *,[1], M. Pilar Buera [2]

Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 1428 Buenos Aires, Argentina

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Abstract

During storage of foods and biological systems, fluorescent products are developed through the Maillard reaction, along with the brown pigments. Fluorescent products have been proposed as early indicators of this reaction. The aim of present work was to compare the kinetics of fluorescence and pigment development in order to define adequate early markers. Model glucose–aminoacid systems were prepared in several salts and buffers and stored at 55 °C. Pigment and florescence development was evaluated as a function of time. The results showed that, under unfavourable conditions for the reaction (low pH, presence of retardant salts), fluorescence was detected after important colour changes had occurred. However, under favourable conditions for the reaction (neutral pH, accelerating salts) fluorescent products could be considered as adequate markers because they sensitively reflected early steps of the reaction. Compositional factors and/or environmental conditions are the key factors for defining the performance of fluorescence as an adequate early marker.

Keywords: Maillard reaction; Fluorescent intermediates; Fluorescent AGEs; Non-enzymatic browning

1. Introduction

The non-enzymatic modification of amino-compounds evolves from their initial condensation with carbonyl-containing compounds. The Schift basis formed rearrange to form Amadori adducts (Hodge, 1953). Following this initial stage, the reaction diverges into the complex network of the Maillard reaction towards advanced glycation end-products (AGEs). Developments of fluorescence, brown pigments and cross-linking products are observed in vitro (Chace, Carubelli, & Nordquist, 1991; Fu et al., 1994) during the course of the reaction. When the reaction takes place in biological systems and foods as a cause of condensation of systems between amino groups of proteins and carbonyl groups of reducing sugars or products from lipid oxidation, it is of paramount importance to predict damage of sensitive biomolecules. The occurrence of this reaction in vivo has profound adverse effects on the functionality of biomolecules and biological structures, and it has been investigated in agricultural, medical and food sciences in relation to aging and illnesses such as diabetes. Fluorescent products have been proposed as early indicators of the Maillard reaction (Adhikari, 1973; Baker & Bradford, 1994; Leclère & Birlouez-Aragon, 2001; Park & Kim, 1983; Pearce, 1950; van Boekel, 2001). However, advanced products (chromophores and AGEs) are also fluorophores and fluorescence has been used as a measure of the level of AGE-modified proteins in vitro (Morales & van Boekel, 1997; Obayashi et al., 1996; Pongor, Ulrich, Bencsath, & Cerami, 1984; Tessier, Monnier, & Kornfield, 2002). Friedman and Kline (1950), Pearce (1950), Overby and Frost (1951) and

* Corresponding author. Tel.: +54 11 4576 3397; fax: +54 11 4576 3366.
E-mail addresses: silviam@di.fcen.uba.ar (S.B. Matiacevich), pilar@di.fcen.uba.ar (M.P. Buera).
[1] Research fellow of Consejo Nacional de Investigaciones, Científicas y Técnicas de la República Argentina (CONICET).
[2] Member of (CONICET).

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Adhikari (1973) showed that the fluorophores are precursors of the brown pigments but are not identical to them. In view of this non-identity and of the fact that inhibition of pigment formation does not affect the formation of fluorescent products (Burton, Mc Weeny, Pandhi, & Biltcliffe, 1962; Burton, Mc Weeny, & Biltcliffe, 1962a, Burton, Mc Weeny, & Biltcliffe, 1962b) and resultant deterioration of nutritional quality, they proposed the measurement of fluorescence as a more useful variable than browning development for assessing nutritional quality. The rate of the Maillard reaction and the nature of its products are governed by the immediate chemical environment of the reactants defined by the chemical composition of the system (water content, pH, presence and type of buffer salts, temperature and exposure to light) (Labuza & Baiser, 1992; Petriella, Chirife, Resnik, & Lozano, 1988).

The aim of present work was to compare the kinetics of fluorescence and pigment development during the Maillard reaction under different conditions, in order to define suitable markers to sensitively assess the degree of reaction at early stages for each particular case, accounting for the time/temperature effects.

2. Materials and methods

2.1. Model systems

The model systems studied consisted of solutions containing sugar (D-glucose, 0.28 M (5% m/v) (Merck)), and amino acid (L-glycine 0.7 M (Mallinckrodt)) in citrate 0.1 M (pH 5); phosphate or acetate 0.1 M (both at pH 5 or pH 6.84) and salts, NaCl or LiCl, at 0.278 M, and $MgCl_2 \cdot 2\,H_2O$ at 0.28 and 0.14 M (all salts were from Merck or Mallinckrodt, analytical grade). Controls, without salts and without amino acid, were also prepared. The sugar:salt ratio was selected as 1:1 since early work by Rendleman (1966) showed that most carbohydrate–metal complexes have 1:1 ratio. Previous experiments on systems similar to those used in the present work Petriella, Resnik, Lozano, and Chirife (1985) had also been performed at that sugar:salt ratio.

Aliquots of each system in vials (5 ml capacity) were sealed using hermetic covers and stored at different selected temperatures. The vials were held at constant temperatures (±0.5 °C) in an air circulating oven at 55 °C. Since the storage of samples was performed at 55 °C, no special care was taken about microbial growth. Accordingly, none of the samples presented any turbidity during storage.

2.2. Determination of pH

The measurement of pH in each system, initial and final was performed with surface electrode (Mettler Toledo 427, Switzerland) and pH meter (Mettler Delta 320, Switzerland).

2.3. Water activity (a_w) measurement

The a_w of the samples, at initial and final storage times, was measured an Aqualab, (Decagon Devices, Pullman, Washington).

2.4. Colour measurements

Colour measurement, at the different times, was performed to follow the progress of non-enzymatic browning in the model systems with time. Huidrobo and Simal (1985) proposed a simplified method of four selected coordinates to evaluate the colour parameters of yellow to yellow-brown samples, which was employed to obtain the CIE (Comission Internationale de I'Eclairage) tristimulus values X, Y and Z, according to the following equations:

$$X = T_{625} \cdot 0.42 + T_{550} \cdot 0.35 + T_{445} \cdot 0.21, \quad (1.1)$$

$$Y = T_{625} \cdot 0.2 + T_{550} \cdot 0.63 + T_{495} \cdot 0.17, \quad (1.2)$$

$$Z = T_{495} \cdot 0.24 + T_{445} \cdot 0.94, \quad (1.3)$$

where T_{625}, T_{495}, T_{445}, T_{550} are the transmittance values at 625, 495, 445, 550 nm, respectively.

Transmittance values at 445, 495, 550 and 625 nm were measured in a UV–VIS Shimatzu 1620 spectrophotometer. The function browning index "Br" which has been proposed as a suitable measure of visual browning (Buera & Resnik, 1990) was calculated (following Eq. (1.4))

$$\mathrm{Br} = 100 * (x - 0.31)/0.172, \quad (1.4)$$

where

$$x = X/(X + Y + Z) \quad (1.5)$$

and Br_0 is the colour measurement of system without thermal treatment.

From eight measurements on five different samples stored at the same time under the same conditions, the standard deviation for colour functions was evaluated and, applying the t factor, the confidence interval was estimated to be 3%.

2.5. Fluorescence measurements

The samples were diluted in order to have systems with absorbance values lower than 0.1 (Skoog, Holler, & Nieman, 2001) at the wavelength for excitation. The diluted mixtures were employed for the determination of fluorescence.

The characterization of the fluorescence emission spectra was performed with a luminescent spectrofluorimeter Aminco-Bowman Serie 2. The emission corre-

sponding to 0.8 μg quinine sulfate/ml in 0.1 N H_2SO_4 (BDH) was defined as 40% of the fluorescence scale, exciting at 365 nm.

Fluorescence measurements for the kinetic studies were determined on a fluorimeter C.G.A. Model MINOR 501. The fluorescence of the mixture at the successive stages was compared with that of 10 μg quinine sulfate/ml of 0.1 N H_2SO_4 (BDH), for which fluorescence was arbitrarily taken as 100 fluorescence units (UF). The instrument allowed selecting among five levels of sensitivity. For most of the samples, the third sensitivity level was enough to perform the measurements adequately.

The excitation and emission maxima wavelengths of the studied systems were 365 and 440 nm, respectively.

Fluorescence units were calculated following Eq. (1.6):

$$\mathrm{UF} = \frac{\mathrm{UF}_x \cdot f \cdot d - b}{m}, \tag{1.6}$$

where UF_x is the fluorescence units of the each mixture at the successive stages; f is the sensitivity factor of the instrument; d is the dilution factor; b is the origin ordinate of calibration curve = −0.09, and m is the slope of calibration curve = 0.159.

Interval confidence for fluorescence values was calculated by measuring eight replicates of five different samples, treated under the same conditions, and was in the range 1–10 %.

3. Results

The measured water activity values for all systems were in the range 0.98–0.99. Slight differences of a_w in this range do not affect the reaction rates of pigment or fluorescence development significantly (Cerrutti, Resnik, Seldes, & Ferro Fontán, 1985) and the present studies can be considered as performed at constant a_w values.

Brown pigment development was observed during storage at 55 °C, at a rate dependent on the composition of the systems. As known, the visual colour changes over time were: uncoloured → yellow → golden → cinnamon → reddish brown (Burton et al., 1962). Fig. 1 shows a CIE chromaticity diagram of all the samples measured. The observed chromatic changes along time followed exactly the browning curve described for the Maillard reaction (Buera, Petriella, & Lozano, 1986) in all cases. Therefore, the composition of the systems affected the kinetics of the reaction without affecting their chromatic characteristics.

It has previously been shown, in model systems (Adhikari, 1973; Leclère & Birlouez-Aragon, 2001; Masaki, Okano, & Sakurai, 1999; Park & Kim, 1983) and in biological samples (Baker & Bradford, 1994; Davies et al., 1998; Murthy & Sun, 2000; Murthy, Liang, Kumar, & Sun, 2002; Rufian-Henares, Guerra-Hernandez, & García-Villanova, 2002), that fluorescent products from the Maillard reaction have a maximum of excitation at wavelengths between 340 and 370 nm, and that the wavelengths of maximum emission are between 420 and 450 nm. The fluorescence from these products is clearly distinguishable from that of tryptophan in proteins (for which the wavelengths for maximum excitation and emission are 290 and 336 nm, respectively). A wavelength of 365 nm was thus selected to excite the samples. Fig. 2 shows the emission spectra of systems with and without salts, after 0 or 26 days of incubation a 55 °C. The wavelength of maximum emission of fluorescent products was 445 nm in all cases, in agreement with the above-mentioned authors. The spectral characteristics were independent of the salt or buffer present in the media. The fluorescence intensity at 440 nm was negligible at zero time of incubation and increased with increasing incubation time. A Raman peak, produced for water dispersion close to 420 nm, was also observed, and did not interfere with measurements of the fluorescent products. In agreement with Cerrutti et al. (1985), samples without amino acids did not develop fluorescence under the studied conditions. Thus, caramelization, due to the reaction of sugars in the systems, was not considered to contribute to development of fluorescence. Although the chemical structure of the fluorescent products was only described for a few of those compounds, all the available structures indicated that they always have one or more nitrogen atoms

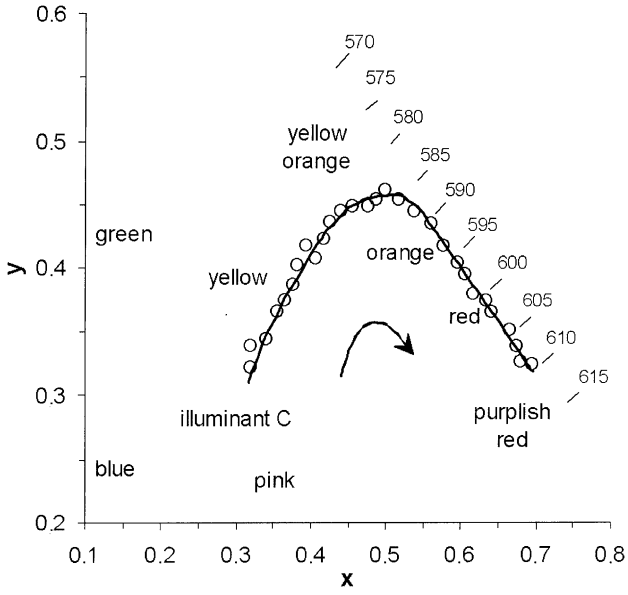

Fig. 1. CIE chromatic diagram. The line on diagram indicates the chromatic changes of pigments over time due to the Maillard reaction for all analyzed systems. The time increase is indicated by the arrow. The numbers on the right side of the diagram indicate the wavelength (in nm) of the pure chromatic colours.

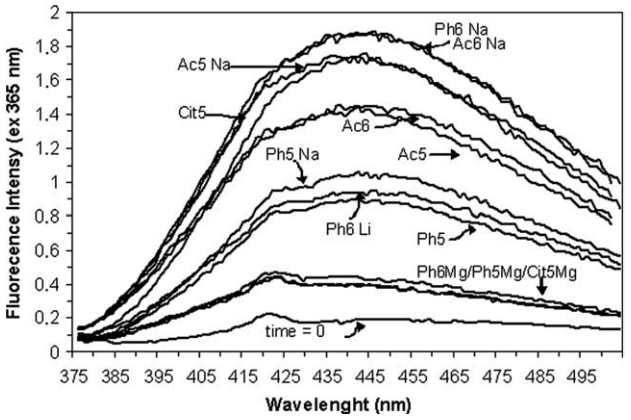

Fig. 2. Emission spectra of fluorescent Maillard reaction products from glucose–glycine systems in different buffers with and without salts (excitation wavelength was 365 nm). The measurements were performed at 0 days (time = 0) or after 26 days of incubation at 55 °C for all systems. Ph6: phosphate buffer, 0.1 M, pH 6.84. Ph5: phosphate buffer, 0.1 M, pH 5. Cit5: citrate buffer, 0.1 M, pH 5. Ac5: acetate buffer, 0.1 M, pH 5. Ac6: acetate buffer 0.1 M, pH 6.84 Mg, Li or Na: samples with $MgCl_2$, LiCl or NaCl, respectively.

originating from the reactant amino-compounds (Chio & Tappel, 1969; Obayashi et al., 1996; Pongor et al., 1984; Shipanova, Glomb, & Nagaraj, 1997).

Typical curves were observed for both fluorescence and browning development (Buera, Chirife, Resnik, & Lozano, 1987; Cerrutti et al., 1985; Park & Kim, 1983; Pongor et al., 1984; van Boekel, 2001). The curves showed an initial stage of low reaction rate (sometimes referred to as "induction period"), followed by an almost linear evolution with time. At long storage times, the curves for browning development levelled off due to a saturation effect, while fluorescence continued increasing, although at a lower rate than in the previous period. Fig. 3 shows the development of browning and fluorescent products as a function of reaction time for

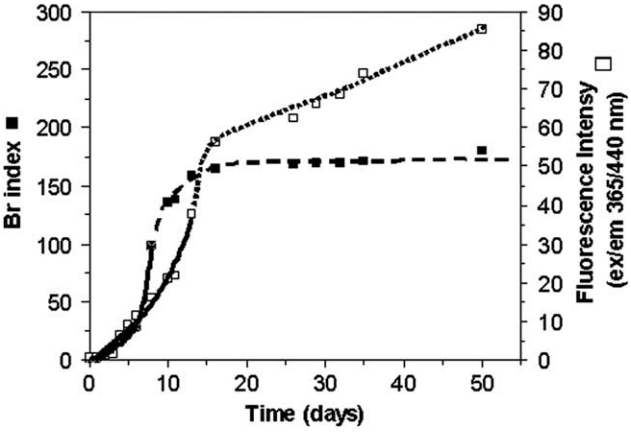

Fig. 3. Brown and fluorescent product development as a function of time at 55 °C in glucose–glycine–phosphate buffer, pH 6.84, system. Symbols represent experimental values. Full lines are the result of fitted curves by Eq. (3), with fitting parameters given in Table 1. Dotted lines are trends out of fitting.

a system in which the fluorescent compounds can accumulate (glucose–glycine–phosphate buffer, 0.1 M, pH 6.84) during storage at 55 °C. At short storage times (when the browning index was lower than 50), the measurements of fluorescence or absorbance were equality sensitive for following the reaction. However, at high reaction times (>15 days), when the saturation of colour was produced, the measurement of fluorescence was more sensitive.

Petriella et al. (1985) and Buera et al. (1987) proposed a mixed order kinetics model to describe the first part of curves of this shape, including induction and quasi-linear evolution periods. In order to compare the kinetics of browning and fluorescence development, the mixed order kinetics model was employed. The reaction rate can be expressed as:

$$\frac{\partial \phi}{\partial t} = k\phi^n, \tag{1}$$

where ϕ is the parameter which changes with time (Br index or fluorescent units, in our case), k is the rate coefficient and n is the reaction order. If it is considered that the inverse of the rate coefficient is the resistance of the system to react, and it can be composed of the sum of two independent resistances, the following equation applies:

$$\frac{1}{\partial \phi/\partial t} = \frac{1}{K_0} + \frac{1}{K_1}, \tag{2}$$

where K_0 and K_1 are the zero-order and first-order kinetic coefficients, respectively. When is small (short reaction times), the second term prevails and the reaction order is close to 1; when ϕ is high, the second term can be neglected and an expression of zero-order is obtained. Eq. (2) may be integrated to give

$$t = \frac{1}{K_0} \cdot (\phi - \phi_0) + \frac{1}{K_1} \cdot \ln(\phi - \phi_0). \tag{3}$$

The kinetic parameters K_0 (units of time^{-1}), and K_1 were determined by a computer programme, through non-linear regression analysis, which minimizes the square differences between predicted and observed values. It is to be noted that the model allows fitting the main part of the curve at which colour and/or fluorescence develop, excluding the last saturation part of low or zero rate of development (shown in Fig. 3). Table 1 shows the kinetic coefficients for the analyzed systems. Eq. (3), proposed for browning development, proved to be useful for describing the experimental data of fluorescence measurements along time, as shown in Fig. 3. The rate coefficients, K_0 and K_1, for fluorescence and browning development strongly increased with increasing pH. Also, the systems containing phosphate buffer had a faster colour and fluorescence development. The accelerating effect of phosphate on browning reactions has previously been observed by several authors (Bell,

Table 1
Kinetic coefficients (K_0 and K_1) for the formation of fluorescent products (F) and brown pigments (Br) in glucose–glycine systems with different buffers, with or without chlorides of Na, Li and Mg

	System	Salt	K_0	K_1	R^2
F	Citrate, pH 5	-	3.2 ± 0.4	0.25 ± 0.03	0.985
	Phosphate, pH 5	-	1.1 ± 0.1	0.5 ± 0.1	0.981
	Phosphate, pH 6.8	-	1.9 ± 0.2	3 ± 6	0.981
	Acetate, pH 5	-	0.1 ± 0.02	0.15 ± 0.08	0.992
		-	1.3 ± 0.5	0.5 ± 0.1	0.98
	Acetate, pH 6.8	NaCl	3.9 ± 0.6	0.4 ± 0.1	0.995
		$MgCl_2$	0.4 ± 0.1	0.08 ± 0.02	0.98
		LiCl	2.8 ± 0.5	0.04 ± 0. 03	0.985
Br	Citrate, pH 5	-	1.3 ± 0.3	0.10 ± 0.01	0.984
	Phosphate, pH 5	-	1.9 ± 0.2	0.24 ± 0.03	0.989
	Phosphate, pH 6.8	-	11 ± 1	3 ± 2	0.99
	Acetate, pH 5	-	1.3 ± 0.3	0.14 ± 0.01	0.992
		-	1.2 ± 0.1	0.29 ± 0.006	0.99
	Acetate, pH 6.8	NaCl	2.9 ± 0.2	0.43 ± 0.07	0.99
		$MgCl_2$	0.6 ± 0.1	0.20 ± 0.07	0.97
		LiCl	2.6 ± 0.3	0.18 ± 0.01	0.994

1997; Bobbio, Bobbio, & Revisan, 1973; Potman & Van Wijk, 1989; Saunders & Jervis, 1966). Bell and Wetzel (1995) suggested that, in contrast to citrate and acetate buffers, the increased kinetic reaction in phosphate buffer was due to the ability of phosphate anion to simultaneously donate and accept the protons necessary in the complex series of reactions leading to non-enzymatic browning.

Fig. 4 shows the relative impact of both kinetic coefficients shown in Table 1 for the systems in acetate buffer at pH 6.84, with and without chlorides of Na, Mg or Li. As discussed previously, the rate coefficient, K_1, is related to the initial stage of the reaction. The Na-containing systems had the higher K_1 values and they presented a short induction period, as compared with the Li- or Mg-containing systems. On the other hand, the systems containing Mg and Li had similar K_1 values, and they showed similar degrees of reaction at the first stages of the reaction. However, the K_0 coefficient for Li-containing systems was higher than that of Mg systems, and the reaction for the Li systems occurred at a higher rate in the second part of the curves. Besides, the Li- and Na-containing systems had similar values of K_0 but, as the coefficient K_1 was lower for the Li-containing system, the reaction was delayed at the first stages in this system. These results illustrate that both kinetic coefficients should be considered to determine the effect of a given component (salt or buffer) on the global reaction under given conditions.

When the kinetics of browning and fluorescence development were compared, it was observed that, in the systems at the lowest pH studied (4), the browning products appeared before fluorescence was detectable (Fig. 5(a)). In the systems at higher pH, the fluorescence products were detected before any visible change (Fig. 5(b)). The results observed in Fig. 4(a) and (b) indicated

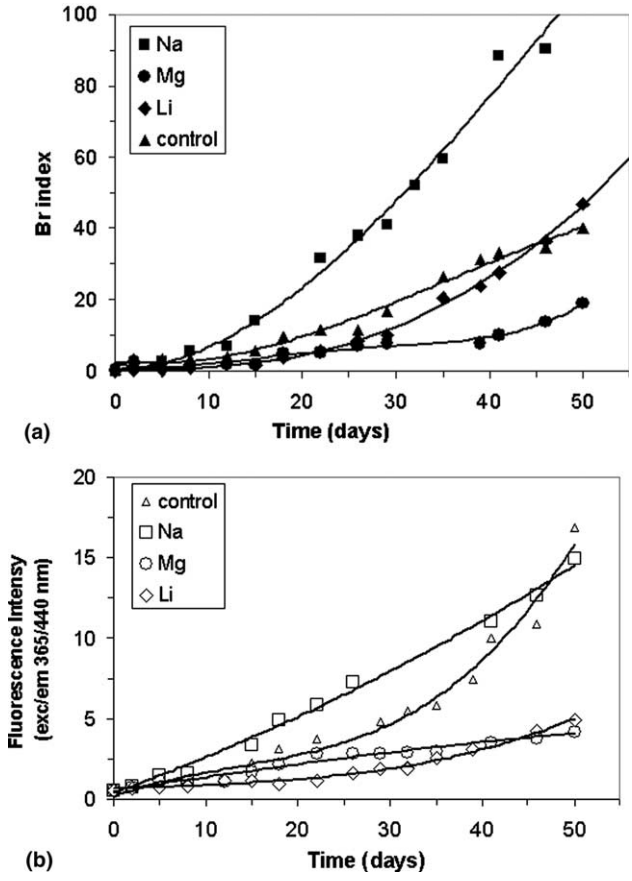

Fig. 4. Browning index (Br) evolution (a) and Fluorescence intensity (b) as a function of time at 55 °C in glucose–glycine systems at pH 6.84 (acetate buffer 0.1 M). (■, ○, ◆): experimental values for systems containing sodium (Na), magnesium (Mg) or lithium (Li) chlorides, respectively. (—) fitted curve with Eq. (3), with fitting parameters given in Table 1.

that the accumulation of fluorescent products over time depends on the pH of the systems. It is notable that, if fluorescent compounds are intermediate products of the reaction, they are not always detectable before the brown end-products. It can be thus suggested that they would be consumed at a higher rate than they are formed, and they would not accumulate in the reaction medium. On the other hand, one can speculate that fluorescent and brown products are formed by different pathways, in parallel reactions.

The ratio (K_{1Br}/K_{1Fluo}) was calculated as a measure of the relative rate of both reactions at the first stage (“induction period”), and is shown in Table 2. When this ratio was close to 1, the lag periods for both reactions were similar. When the ratio was lower than 1, the browning development was slower than fluorescence development and when it was higher than 1 the opposite was observed. As analyzed previously, for the systems of pH 4 the browning development occurred at a faster rate at the initial stages of the reaction. Besides, the Mg salt, acting as retardant (as shown in Fig. 4 and Table 1),

Table 2
Ratio between the rate coefficients for browning (K_{1Br}) and fluorescence (K_{1F}) development at the first stages of the reaction, for systems of different composition treated at 55 °C

System Buffer 0.1 M	Salt	K_{1Br}/K_{1Fluo}	First detectable index
Acetate, pH 6.84	-	3.5	Br
Acetate, pH 5	-	0.93	Either F or Br
Acetate, pH 6.84	NaCl	0.8	F
Acetate, pH 6.84	$MgCl_2$	2.4	Br
Phosphate, pH 6.84	-	0.6	F
Phosphate, pH 5	-	4.1	Br
Phosphate, pH 4	-	25	Br
Citrate, pH 5	-	0.4	F
Citrate, pH 4	-	8.7	Br

Br, browning development; F, fluorescence development.

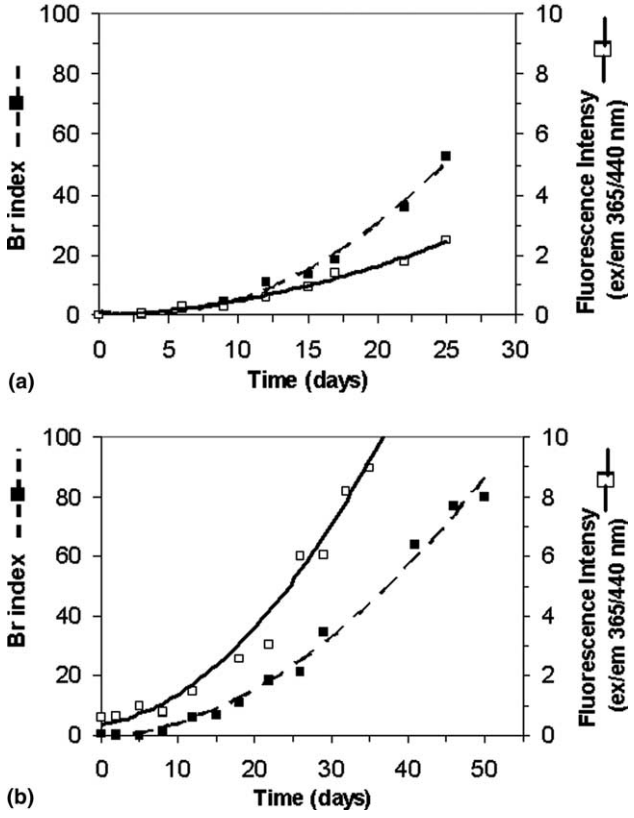

Fig. 5. Browning index (Br) and Fluorescence intensity as a function of reaction time in system glucose–glycine, citrate buffer, a pH 4 (a) or pH 5 (b).

promotes a browning development faster than fluorescence development in the first stages (see Fig. 5).

Several authors have proposed that, at early stages, while the systems still do not show any colour, fluorescence measurements are more sensitive for following the development of the Maillard reaction. Accordingly, fluorescence measurements would offer the possibility of analyzing the kinetics of the reaction when there are still no visible changes. As observed in the present work, when the reaction occurs under disadvantageous conditions (low pH or presence of retardant salts), the measurements of brown pigments are more sensitive than fluorescence measurements in the early stages of the reaction. It can be proposed either, that, under these conditions fluorescent compounds are consumed faster (in spite of the low reaction rate for colour development), or that their formation is less favourable.

Burton et al. (1962a, 1962b) observed that, in glucose–amino acid systems, the browning inhibition through the use of sulphide did not affect the development of fluorescent compounds and that the nutritional deterioration was still produced in systems in which colour development was delayed. In cases such as those presented by Burton et al. (1962a, 1962b), the measurement of fluorescence would be more useful than browning for assessing nutritional quality and it can be proposed as a very suitable marker. However, as shown in present work, depending on the composition of the systems, brown pigment development may appear before fluorescent compounds, and fluorescence development is not a sensitive indicator. Therefore, fluorescent compounds can not be considered as universal early indicators of the Maillard reaction.

4. Discussion

A number of kinetic studies have been carried out on the Maillard reaction. The majority of these studies have investigated the rate of browning in the visible range in liquid model systems, at high water activities and at high temperatures (Ajandouz & Puigserver, 1999; Buera et al., 1987; Fox, Loncin, & Weiss, 1983; Petriella et al., 1985). Various authors reported that the simplest model for the mathematical prediction of the browning and fluorescent development rates of the Maillard reaction is that of a zero-order kinetic reaction. However, this approach describes the experimental data only after an initial induction period, indicating a more complex reaction (Buera et al., 1987; Morales & van Boekel, 1997; Petriella et al., 1988). It was observed in the present work, that the induction periods for the formation of brown pigments and fluorescent compounds were different, and a mixed order kinetics model allowed the comparison between both indexes, at both the first and later stages of the reaction.

Friedman and Kline (1950), Overby and Frost (1951), Adhikari (1973) and Labuza (1994) have shown that fluorescent and coloured compounds were not identical and that the peak of fluorescence preceded the increase in pigment formation, indicating that the fluorophores may be precursors of the brown pigments. All these works have been performed under favourable conditions for the reaction to occur: in the presence of phosphate buffer and at neutral pHs (7), and they are in agreement with the results of the present work. In advanced stages of the reaction, when there is saturation of colour, the

measurement of fluorescence is more sensitive for following the reaction.

It was also observed here that, under disadvantageous conditions for the Maillard reaction (low pH, presence of retardant buffers or salts) the determination of fluorescent products is not a suitable method for sensitively following the degree of reaction at the initial stages, since absorbance in the visible range was first detected.

The following schemes of the reaction are proposed to explain the present results:

(a) Fluorophores and pigments are formed in consecutive reactions (this is the generally accepted picture)

If the limiting rate process is the transformation of the fluorescent products into pigments,

$$\text{Reactive} \rightarrow \underset{\uparrow}{\text{Fluorescent products}} \rightarrow \underset{\text{Limiting}}{\text{Pigments}}$$

the kinetic constant $K_{\text{fluorescence}}$ would be higher than K_{browning}, and accumulation of fluorescent products would occur, and this is what we observed under advantageous conditions for the reaction. Most of the papers on the Maillard reaction have analyzed this situation, and it is thus understandable that the fluorescent products were proposed as early markers, to follow the kinetics of the reaction when there are no visible changes (Baisier & Labuza, 1992; Friedman & Kline, 1950; Labuza, 1994; Overby & Frost, 1951). It is notable that a decrease of fluorescence was never observed, as would be expected if the fluorescent compounds were truly intermediates in the reaction.

(b) The production of fluorophores and pigments are parallel reactions.

$$\text{Reactive} \rightarrow \text{Fluorescent products.}$$

$$\text{Reactive} \rightarrow \text{Pigments.}$$

According to our results and those which consider fluorescent products as end products (Morales & van Boekel, 1997; Obayashi et al., 1996; Song et al., 2002; Tessier et al., 2002), under favourable conditions, the kinetic constant for the formation of fluorescent products would be higher than that for brown product development. Under disadvantageous conditions, the global reaction would be slower, but the pigment development would be favoured.

It is also possible that some reactions occur in parallel and some others consecutively, depending on the conditions under which the reaction takes place. Fluorescence measurements are easy to perform, rapid, accurate and require only very small quantities of sample but fluorescent products are not always the best markers for the Maillard reaction. The index that most conveniently reflects the degree of reaction should be defined for each particular system. The possible involved mechanisms by which fluorescence is formed, need to be investigated.

The functional damage in foods can be due to colour/flavour development, texture changes (due to protein cross-linking) or nutritional damage; in biological systems it is manifested, for example, by protein cross-linking, enzyme inactivation, health deterioration or seed viability decrease. The fast developing marker has the advantage of providing a tool to follow early changes, even when quality has not changed, and it is still possible to take corrective actions. This paper underlines the importance of having reliable early markers to follow a reaction from the first stages, when there is still no noticeable quality change. When the kinetics of fluorescence development and pigment formation were analyzed, it was observed that, in systems exposed under unfavourable conditions for the reaction, fluorescence appearance occurred at later stages, when other important biomolecules have suffered profound changes. In this latter case, fluorescence development should not be considered as a convenient marker. It is clear, however, that, in systems exposed under favourable conditions (high temperatures, and/or at neutral pH and/or in the presence of highly reactive species), the fluorescent products were detected before any visible colour or nutritional change and they can be considered as adequate markers of the reaction. Such is the case for milk products treated at high temperatures, for which fluorophores were observed to be good early indicators of damage (Leclère & Birlouez-Aragon, 2001).

As discussed in the paper, which one of the two parameters (browning or fluorescence) develops at an earlier stage, depends on the environmental conditions and on the system composition.

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