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Advances in the study of the kinetics of color and fluorescence development in concentrated milk systems

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ABSTRACT

The kinetics of color and fluorescence development in systems containing whole milk powder (WMP), sucrose (S) and water were studied; the influence of three design variables: pH (6, 7 and 8), temperature (105, 122.5 and 140 °C) and WMP/S ratio (R = 0.3, 0.68 and 1.06) being particularly investigated. The Kubelka–Munk index (K/S), luminance or lightness (L), optical density (OD) and fluorescence (F), were measured at different reaction times. Reaction pseudo-order (n) varied between 0 and 1 and reaction rate constants for the development of fluorescence and the color parameters were found to be more dependent on temperature than on R at all measured pH values. Temperature dependence of CP was analyzed through the activation energy (E_a) values. The variables F and OD, which are related to the molecular aspects of the systems, showed the extreme values of E_a, while than K/S and 100/L, related to the macroscopic appearance or reflectance, showed intermediate E_a values and lower dependence with R and pH. When compared to the variables related to brown pigment development, fluorescence development showed a higher relative change, which shows its higher sensitivity as a reaction marker. The variables that most affected color and F development were temperature and protein concentration, respectively.

1. Introduction

The autocatalytic Maillard reaction is initiated from the condensation of carbonyl and amino groups followed by a set of complex reactions that occur in foods during heating and storage (Adhikari and Tappel, 1973; Buera et al., 1987a; Buera et al., 1990a) with consequent product color changes (non-enzymatic browning), aroma and taste. Melanoidin or brown products are the responsible of color changes, which can also be formed by sugar caramelization without the participation of amino groups (Cerrutti et al., 1985; Buera et al., 1990b; Buera et al., 1992; Pauletti et al., 1999; Carabasa-Giribet and Ibarz-Ribas, 2000; Rozycki, 2003).

The rate of the Maillard reaction is known to be affected by several physico-chemical factors, the most studied being: concentration, chemical nature of the reactants (type of amine and carbonyl groups involved); pH; relative humidity; temperature and time of heating (Labuza and Baiser, 1992). Maillard kinetics has been investigated in several sugar–amino-acid systems through the development of fluorescent products (Cerrutti et al., 1985; Buera et al., 1992; Song et al., 2002; Matiacevich and Buera, 2006) and colorimetric parameters (Buera et al., 1987a,c; Buera et al., 1990b). The influence of protein/sugar ratio has also been analyzed (Pauletti et al., 1996; Morales and van Boekel, 1999; Pauletti et al., 1998).

The knowledge of kinetic parameters (rate constant and activation energy) is necessary to predict the extent of a specific chemical reaction and, consequently, its optimization (Pauletti et al., 1999; Carabasa-Giribet and Ibarz-Ribas, 2000). Also, suitable indicators to follow non-enzymatic browning reactions are necessary to be defined. Among the most commonly used indicators are spectrophotometric measurements of the optical density at a particular wavelength (usually 390–500 nm) (Petriella et al., 1985; Carabasa-Giribet and Ibarz-Ribas, 2000). Nevertheless, the absorption at one fixed wavelength may not always be the most reliable way to describe visual color changes in browned food systems (Petriella et al., 1985). The Kubelka–Munk index (K/S), calculated from colorimetric reflectance measurements, can adequately express visually perceived color differences in dairy products such as *dulce de leche* (Buera et al., 1990b; Pauletti et al., 1998; Saguy and Graf, 1999; Rozycki, 2003). The absorption coefficient K indicates the incident light proportion absorbed by the sample (Calvo, 1993) and is related to the number of chromophore groups present in the sample (Pauletti et al., 1998). The formation of advanced non-enzymatic reaction products could also be monitored by the measurements of colorimetric parameters like luminance or lightness (L) or 100/L (Pauletti et al., 1996; Rozycki, 1999, 2003).

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Generally, browning development occurs after an induction period, characterized by the production of flourescent uncolored intermediates. Flourescent compounds are thus considered precursors of brown pigments and allow detecting the progress of the reaction before any visual change has occurred (Labuza and Baiser, 1992). Adhikari and Tappel (1973) and Matiacevich and Buera (2006) showed that the fluorophores are precursors of/but not identical to brown pigments.

Fluorescence from the Maillard reaction is attributed to molecular structures with complex bonds between carbon and nitrogen, such as: –N–C–C–C–N– , and the contribution of sugar caramelization to global fluorescence is negligible in amino-acid containing systems. In fact, samples without amino acids did not develop fluorescence at the studied conditions (Cerrutti et al., 1985; Matiacevich and Buera, 2006).

According to the experimental conditions, the fluorescent compounds are intermediates or end products. Under conditions favorable for the Maillard reaction to occur (neutral pH, high temperature, high reactant concentration) such as those analyzed in the present work, the kinetic constants for the formation of fluorescent products would be higher than those for brown product development, and the accumulation of fluorescent products would occur (Obayashi et al., 1996; Morales and van Boekel, 1999; Tessier et al., 2002; Song et al., 2002; Matiacevich and Buera, 2006). Very few works have been developed at temperatures higher than 100 °C in the range of $a_w = 0.85$ (Pauletti et al., 1992; Paulette et al., 1995; Cano-Ruiz and Richter, 1998; Rozycki, 2003), which correspond to typical processing conditions for some confectionary dairy products (such as *dulce de leche,*toffees and fudges). The objective of the present work was to select the most adequate parameters to describe brown pigment development in a dairy product, and to determine the influence of the involved variables in the experimental design.

2. Material and methods

2.1. Experimental design

The experiments were based on a three-level, two-factor factorial design, with two replicates in the central point (11 runs) (Montgomery, 2001). The complete design was developed at three different initial pH values, in duplicate runs, which resembles a statistical study of three variables at three different levels (Table 1), the three independent variables being: heat-treatment temperature $T = 105$, 122.5, 140 °C), whole milk powder / sucrose ratio ($R = 0.30$, 0.68, 1.06) and initial pH (6 , 7 and 8).

Fluorescence (F), optical density (OD) and the color parameters Kubelka–Munk index (K/S) and luminance (L) of the extracts were determined at each testing time.

The reaction rate constants (k_{CP}) and the global reaction order (n) for each color parameter and fluorescence were determined through the integral kinetic model (Eq. (2)) obtained from the differential model (Eq. (1)) (Rozycki, 1999, 2003):

$$d(\mathrm{CP})/dt = k_{\mathrm{CP}} \cdot (\mathrm{CP})^n \quad (1)$$

where CP represented the evaluated parameter (K/S, 100/L, OD or F); t was the time (min); k_{CP} was the kinetic rate constant for CP(min^{-1}); and n was the reaction order.

$$\mathrm{CP} = [(\mathrm{CP})_o^{1-n} + k_{\mathrm{CP}} \cdot t \cdot (1-n)]^{1/(1-n)} \quad (2)$$

where $(\mathrm{CP})_o$ was the color parameter value at time $t = 0$.

A quadratic polynomial regression model, Eq. (3), was assumed for predicting individual k_{CP} as a function of T and R.

$$k_{\mathrm{CP}} = bk_o + bk_1 \cdot X_1 + bk_2 \cdot X_2 + bk_{11} \cdot X_1^2 + bk_{22} \cdot X_2^2 + bk_{12} \cdot X_1 \cdot X_2 \quad (3)$$

where bk_o, bk_i, bk_{ii}, bk_{ij} were regression coefficients and X_i the coded independent variables, linearly related to T and R.

2.2. Sample preparation

The aqueous systems under study contained whole milk powder (WMP) and sucrose (S) at three different WMP/S ratios $R_1 = 16/5 = 0.30$; $R_2 = 28/42 = 0.68$; $R_3 = 36/34 = 1.06$). The proximate composition (%) of milk powder (Nestlé S.A., Argentina) was: protein 24.95, milk fat 26.50, lactose 39.50, minerals 6.12, and moisture 2.93. The initial solid concentration (WMP + S) was always 70%, the rest being water (30%). Solution pH was adjusted to the design values by adding $Ca(OH)_2$ or lactic acid. Sample solutions were distributed into glass tubes (8 mm internal diameter, 150 mm long and 1 mm thick) sealed by using a flame. Samples were then heat treated at three different temperatures (105 °C, 122.5 °C and 140 °C) in a thermostatic silicone oil bath. During heat treatment, samples were removed from the bath at five different times and cooled at room temperature with cold water (2 °C).

2.3. Colorimetric evaluation

Colorimetric measurements of each sample were made using non-standard white and black cylindrical plastic cells (28 mm diameter, 4 mm high). The reflectance values for 450 nm, 560 nm, and 685 nm were determined to obtain the Kubelka–Munk index K/S, and the Hunter L values, using the SBTR software (Shelf Backing Reflectance Transformation software) appropriate for small samples (Pauletti et al., 1999; Rozycki, 1999, 2003). Measurements were made using a Minolta CM 508d spectrophotometer with illuminant C, at 2° standard observer angle and specular component excluded (Minolta Co. Ltd., Osaka, Japan). In a previous work (Rozycki, 2003) a function of luminance (L) has been found to be a sensitive variable to follow the progress of the non-enzymatic browning development in dairy systems. Total heat treatment time was determined in previous assays, which was the time for the samples to develop the color of standard Argentine ***dulce de leche***, a typical intermediate moisture Latin American product obtained by heating concentrated milk plus sucrose (K/S = 5,100/L = 12 and $a_w = 0.85$).

2.4. Fluorescence and optical density measurements

Samples (3 g each) were exactly weighed, mixed with about 15 mL of 80% ethanol (Park and Kim, 1983) and shaken at 150 agitation cycles per minute, for 30 min. The samples were filtered twice through filter paper in order to obtain a clear solution. Optical density (OD) at 390 and 420 nm was measured in an aliquot of this solution with a Génesis 5 UV–Visible spectrophotometer (Milton Roy, New York, USA). Another aliquot (1 ml) was diluted 10-fold with bi-distilled water and its fluorescence was determined in a Biorad Versafluor fluorimeter TM (Biorad, New York, USA), with excitation maximum at 380 nm and emission maximum at

Table 1
Independent variables and levels used in the experimental design.

Independent variable	Symbol	Codified and non-codified variable levels		
		−1	0	1
[WMP/sucrose] ratio, R (w/w)	X_1[a]	0.30	0.68	1.06
Reaction temperature, T °C)	X_2a	105	122.5	140
pH	X_3a	6	7	8

Codification equations: $X_1 = (R - 0.68)/0.38$; $X_2 = (T - 122.5)/17.5$; $X_3 = (\mathrm{pH}\ 7)$.
[a] WMP = whole milk powder.

465 nm. The standard solution used was quinine sulphate (1 μg/ml in 0.1 N SO_4H_2) (Park and Kim, 1983; Cerrutti et al., 1985), whose fluorescence value was 1435 FU (fluorescence units), as an average of triplicate measurements. The fluorescence of each sample was referred as a percentage of this reference.

2.5. Activation energy determination

The temperature sensitivity of rate constants was analyzed using the Arrhenius equation (Eq. (4)):

$$k_{CP(T)} = k_0 \cdot \exp[-E_a/RT] \quad (4)$$

where, $k_{CP(T)}$ = rate constant for each color and fluorescence parameter; k_0 = pre-exponential factor; E_a = activation energy, [J mol^{-1}]; R = universal gas constant [8.314 J mol^{-1} K^{-1}]; T = absolute temperature, [K]. The E_a for each color parameter was obtained by regression analysis (Singh and Heldman, 1993).

2.6. Statistical analysis

All data were analyzed using Statgraphics Plus 3.0 (Manugistic, Inc., Rockville, Md., USA, 1993) and Statistica (Statistica, Release 4.5, USA, 1993). The data were fitted to the corresponding models, regression analysis and analysis of variance being then carried out ($p < 0.05$).

3. Results and discussion

Table 2a) shows the obtained reaction rate pseudo-constants (k, Table 2a), and global reaction pseudo-orders (n, Table 2b) for each measured parameter at different pHs, obtained by multiple regression analysis of the evolution of color, absorbance in the visible range and fluorescence parameters as a function of reaction time (Eq. 2). The kinetic models obtained were adequate to adjust the changes occurring in those parameters during thermal treatment ($r^2 > 0.97$).

As indicated by the positive signs of the kinetic constants, fluorescence and 100/L increased (luminance decreased), and their values were generally higher than those for K/S and optical density.

For any R, temperature and pH combination, color and fluorescence parameters showed a direct and non-proportional increase during the thermal treatment. Color parameters ranged between 57 and 1.144 for F; 0.2 and 7.5 for the Kubelka–Munk index; 1.9 and 16.7 for 100/L, 0.02 and 1.88 for OD 390 and 0.02 and 1.10 for OD 420.

Table 2a
Reaction rate constants (k) for fluorescence (F), absorbance (OD_{390}, OD_{420}) and color development in aqueous systems with 70% total solids (WMP + sucrose).

Exp. no.	pH 6							pH 7					pH 8				
	T[a]	R[b]	k_F[c]	$k_{K/S}$[d]	$k_{OD\ 390}$	k_{OD420}[f]	$k_{100/L}$[g]	k_F[b]	$k_{K/S}$[d]	k_{OD390}	k_{OD420}[f]	$K_{100/L}$[f]	k_F	$k_{K/S}$[d]	$k_{OD\ 390}$[e]	k_{OD420}[f]	$K_{100/L}$[f]
1	105	0.30	0.0413	0.011	0.002	0.001	0.010	0.076	0.012	0.002	0.001	0.013	0.256	0.021	0.002	0.011	0.046
2	122.5	0.30	0.1329	0.057	0.014	0.016	0.076	0.243	0.078	0.018	0.018	0.172	0.485	0.123	0.025	0.021	0.303
3	140	0.30	0.396	0.209	0.107	0.110	0.195	0.870	0.287	0.110	0.136	0.382	0.980	0.310	0.130	0.170	0.600
4	105	0.68	0.0501	0.012	0.007	0.002	0.015	0.088	0.016	0.009	0.006	0.024	0.390	0.030	0.010	0.012	0.110
5	122.5	0.68	0.2614	0.072	0.029	0.024	0.185	0.426	0.086	0.031	0.030	0.219	0.735	0.151	0.034	0.028	0.610
6	140	0.68	0.6603	0.250	0.120	0.130	0.415	1.363	0.349	0.130	0.150	1.015	1.653	0.380	0.160	0.186	1.130
7	105	1.06	0.1565	0.017	0.008	0.013	0.047	0.228	0.020	0.017	0.016	0.072	0.498	0.038	0.019	0.026	0.151
8	122.5	1.06	0.4963	0.098	0.032	0.031	0.250	0.671	0.112	0.039	0.035	0.400	1.144	0.180	0.041	0.038	0.980
9	140	1.06	1.6802	0.294	0.198	0.150	0.786	2.069	0.418	0.167	0.172	1.455	2.270	0.513	0.189	0.205	1.825
10	122.5	0.68	0.2778	0.073	0.029	0.022	0.196	0.412	0.085	0.030	0.031	0.230	0.747	0.165	0.032	0.028	0.602
11	122.5	0.68	0.2564	0.071	0.031	0.025	0.174	0.436	0.088	0.032	0.030	0.211	0.722	0.140	0.035	0.029	0.621

[a] T = reaction temperature (°C).
[b] R = [WMP/sucrose] ratio.
[c] k_F = reaction rate constant for fluorescence development.
[d] $k_{K/S}$ = reaction rate constant for Kubelka–Munk index.
[e] k_{OD390} = reaction rate constant for development of optical density at 390 nm.
[f] k_{OD420} = reaction rate constant for development of optical density at 420 nm.
[g] $k_{100/L}$ = reaction rate constant for luminance inverse %.

Table 2b
Reaction order (n) for fluorescence (F), absorbance (OD_{390}, OD_{420}) and color development in aqueous systems with 70% total solids (WMP + sucrose). Temperature values are the same as in Table 2a.

Exp. no.	pH 6.0					pH 7.0					pH 8.0				
	n_F[a]	$n_{K/S}$[b]	n_{OD390}[c]	n_{OD420}[d]	$n_{100/L}$[e]	n_F	$n_{K/S}$	n_{OD390}	n_{OD420}	$n_{100/L}$	n_F	$n_{K/S}$	n_{OD390}	n_{OD420}	$n_{100/L}$
1	0.37	0.48	0.19	0.98	0.60	0.63	0.43	0.28	0.28	0.53	0.25	0.01	0.26	0.26	0.03
2	0.50	0.31	0.67	0.02	0.08	0.24	0.01	0.51	0.69	0.04	0.03	0.06	0.39	0.66	0.12
3	0.68	0.51	0.70	0.78	0.64	0.25	0.31	0.93	0.94	0.37	0.76	0.56	0.88	0.94	0.73
4	0.50	0.33	0.62	0.99	0.43	0.26	0.25	0.71	0.73	0.29	0.27	0.26	0.45	0.31	0.43
5	0.33	0.01	0.52	0.60	0.11	0.28	0.02	0.57	0.71	0.12	0.01	0.47	0.37	0.49	0.72
6	0.56	0.20	0.69	0.78	0.21	0.30	0.11	0.54	0.69	0.23	0.30	0.00	0.62	0.96	0.03
7	0.21	0.01	0.49	0.70	0.17	0.10	0.10	0.48	0.62	0.36	0.09	0.13	0.89	0.61	0.37
8	0.29	0.22	0.61	0.80	0.07	0.18	0.06	0.56	0.69	0.17	0.07	0.39	0.50	0.68	0.65
9	0.26	0.03	0.56	0.68	0.15	0.22	0.10	0.63	0.82	0.04	0.38	0.20	0.66	0.84	0.37
10	0.37	0.02	0.48	0.65	0.08	0.31	0.04	0.54	0.63	0.15	0.03	0.41	0.45	0.42	0.65
11	0.31	0.01	0.44	0.69	0.13	0.33	0.03	0.52	0.68	0.17	0.02	0.54	0.33	0.46	0.61

[a] n_F = Reaction order for fluorescence development.
[b] $n_{K/S}$ = Reaction order for Kubelka-Munk Index.
[c] $n_{OD\ 390}$ = Reaction order for development of optical density at 390 nm.
[d] $n_{OD\ 420}$ = Reaction order for development of optical density at 420 nm.
[e] $n_{100/L}$ = Reaction order for luminance inverse %.

Only for very few experiments, the global reaction order governing the kinetics under such conditions was zero, manifested in a linear adjustment of the given parameter and reaction time showed (Table 2b). The remaining experiments showed a reaction order different from zero, mainly between 0 and 0.7, which is in accordance with the results obtained by other authors for only some of these functions, when using similar model systems and studying the complete curve (Buera et al., 1987b; Pauletti et al., 1999; Rozycki, 1999, 2003).

In general, the highest reaction orders were obtained for the absorbance development (at 390 or 420 nm), while the lowest reaction orders corresponded to the reflectance K/S or 100/L parameters.

Fig. 1a shows the evolution of the different measured parameters as a function of the reaction time, for constant conditions and intermediate design variables (pH = 7, $R = 0.68$ and $T = 122.5$ °C). In order to quantify the effect of the design variables (T, R and pH) on k_{CP} for K/S, 100/L, OD and F, the Response surface methodology (RSM) was used (Statistica, 1993), multiple regression model (Eq. 3) being adequate to calculate k_{CP} (Statgraphics 3.0, 1993) for each parameter under study (generally $r^2 > 0.96$).

Eqs. (5)–(19)9 below represent the mathematical models obtained for each measured parameter and at all studied pHs, and they make it possible to predict k_{CP} values for a particular reaction temperature, R and pH. Non-significant regression coefficients ($p > 0.05$) were not included in the models. Table 3 shows the regression statistics.

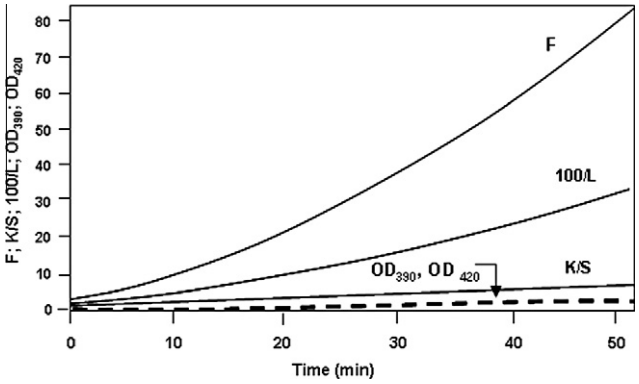

Fig. 1a. Comparison of fluorescence development (F), color functions 100/L and K/S and absorbance of the extracts at 390 (OD_{390}) and at 420 nm (OD_{420}) as a function of reaction time, for selected constant conditions and intermediate design variables (pH = 7; $R = 0.68$ and $T = 122.5$ °C).

k_F , which means that R effects on k_{CP} depended on temperature, particularly for 100/L and F.

R and T dependence on pH can be analyzed through Eqs. (5)–(19).

When comparing the linear term coefficients, by means of their quotient, in the equations that relate K/S with those variables (Eqs. (5)–(7)), a higher influence of R can be observed with a pH increase

$$k_{K/S_{(pH\ 6)}} = 0.0756 + 0.0221 \cdot X_1 + 0.1192 \cdot X_2 + 0.0197 \cdot X_1 \cdot X_2 + 0.0567 \cdot X_2^2 \quad (r^2 = 0.999) \tag{5}$$

$$k_{K/S_{(pH\ 7)}} = 0.0922 + 0.0288 \cdot X_1 + 0.1675 \cdot X_2 + 0.0310 \cdot X_1 \cdot X_2 + 0.0914 \cdot X_2^2 \quad (r^2 = 0.999) \tag{6}$$

$$k_{K/S_{(pH\ 8)}} = 0.1513 + 0.0462 \cdot X_1 + 0.1857 \cdot X_2 + 0.0464 \cdot X_1 \cdot X_2 + 0.0640 \cdot X_2^2 \quad (r^2 = 0.993) \tag{7}$$

$$k_{100/L(pH6)} = 0.2200 + 0.1337 \cdot X_1 + 0.2207 \cdot X_2 + 0.1385 \cdot X_1 \cdot X_2 \quad (r^2 = 0.956) \tag{8}$$

$$k_{100/L(pH\ 7)} = 0.2309 + 0.2101 \cdot X_1 + 0.4572 \cdot X_2 + 0.2538 \cdot X_1 \cdot X_2 + 0.2627 \cdot X_2^2 \quad (r^2 = 0.965) \tag{9}$$

$$k_{100/L(pH\ 8)} = 0.6394 + 0.3343 \cdot X_1 + 0.5415 \cdot X_2 + 0.2801 \cdot X_1 \cdot X_2 \quad (r^2 = 0.998) \tag{10}$$

$$k_{OD390(pH\ 6)} = 0.0250 + 0.0191 \cdot X_1 + 0.0550 \cdot X_2 + 0.0214 \cdot X_1 \cdot X_2 + 0.0486 \cdot X_2^2 \quad (r^2 = 0.973) \tag{11}$$

$$k_{OD390(pH7)} = 0.0292 + 0.0226.X_1 + 0.0681 \cdot X_2 + 0.0211 \cdot X_1 \cdot X_2 + 0.0349 \cdot X_2^2 \quad (r^2 = 0.982) \tag{12}$$

$$k_{OD390(pH\ 8)} = 0.0333 + 0.0253 \cdot X_1 + 0.0746 \cdot X_2 + 0.0104 \cdot X_1 \cdot X_2 + 0.0517 \cdot X_2^2 \quad (r^2 = 0.996) \tag{13}$$

$$k_{OD420(pH\ 6)} = 0.0236 + 0.0112 \cdot X_1 + 0.0565 \cdot X_2 + 0.0070 \cdot X_1 \cdot X_2 + 0.0441 \cdot X_2^2 \quad (r^2 = 0.998) \tag{14}$$

$$k_{OD420(pH\ 7)} = 0.0278 + 0.0128 \cdot X_1 + 0.0624 \cdot X_2 + 0.0203 \cdot X_1 \cdot X_2 + 0.0364 \cdot X_2^2 \quad (r^2 = 0.980) \tag{15}$$

$$k_{OD420(pH\ 8)} = 0.0291 + 0.0213 \cdot X_1 + 0.0869 \cdot X_2 + 0.0709 \cdot X_2^2 \quad (r^2 = 0.998) \tag{16}$$

$$k_{F(pH\ 6)} = 0.4309 + 0.2558 \cdot X_1 + 0.4154 \cdot X_2 + 0.2913 \cdot X_1 \cdot X_2 \quad (r^2 = 0.957) \tag{17}$$

$$k_{F_{(pH\ 7)}} = 0.4468 + 0.2932 \cdot X_1 + 0.6265 \cdot X_2 + 0.1710 \cdot X_1 \cdot X_2 + 0.3761 \cdot X_2^2 \quad (r^2 = 0.987) \tag{18}$$

$$k_{F(pH\ 8)} = 0.7883 + 0.3652 \cdot X_1 + 0.7125 \cdot X_2 + 0.2619 \cdot X_1 \cdot X_2 + 0.2195 \cdot X_2^2 \quad (r^2 = 0.998) \tag{19}$$

For all the studied cases, at a given pH the linear coefficients corresponding to the temperature variable (bk_2), codified (X_2) in the kinetic constant expression, were always higher than those corresponding to R (bk_1), codified (X_1). As a result, within the studied range, and at whatever pH, the kinetic constants k_{CP} showed a higher increase with temperature increase rather than with R increase, OD and K/S being the more affected parameters.

At most of the studied pH, temperature quadratic values were significant ($p < 0.05$). R quadratic values, however, were not significant ($p > 0.05$), not being then included in the regressions.

Values corresponding to the interactions between the variables (X_1X_2) were significant ($p < 0.05$) for $k_{K/S}$, $k_{100/L}$,k_{OD390}, k_{OD420}, and from 7 to 8; temperature influence, however, was higher with a pH increase from 6 to 7. From this point of view, K/S and F show similar behavior as to their variability. The functions 100/L and K/S show the same behavior with respect to temperature, but not to R. The functions OD show the same behavior for both T and R.

Response surface and contour lines, at different pH, show similar shapes for the five parameters selected to follow the system evolution with reaction time. Figs. 1b,1c show the graphic contours corresponding to K/S, which represents the reflectance phenomena, and OD, which is a measure of pigment concentration, at pH 7 (close to that of dairy products). An increase in color development rate (higher k_{CP}) can be observed when R or T increase, this increase being relatively higher as increasing T.

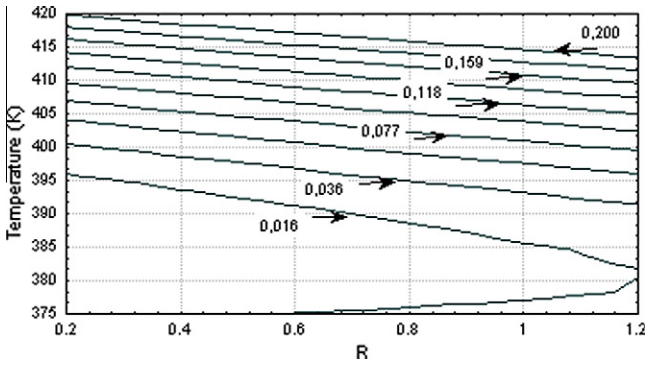

Fig. 1b. Contourn lines temperature vs. R for evolution of the variable k_{OD390} at pH = 7; R is the whole milk powder: sucrose ratio.

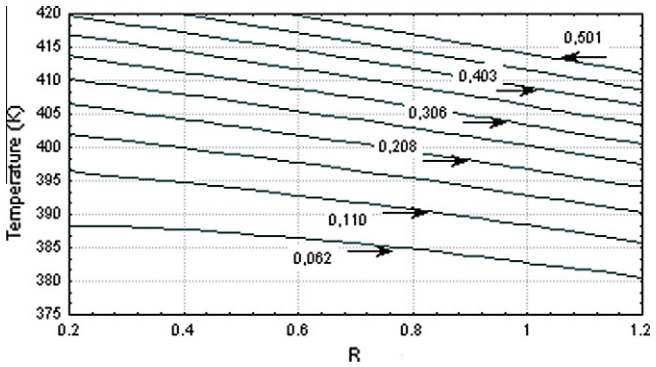

Fig. 1c. Contourn lines temperature vs. R for evolution of the variable $k_{K/S}$ at pH = 7; R is the whole milk powder: sucrose ratio.

From contour line analysis, an approximately "linear" relationship between T and R can be observed by parameterizing the curves in the k_{CP} values, mainly for K/S, T influence being always higher.

Table 3
Analysis of variance of the regression models for the reaction rate constants of fluorescence and color parameters in aqueous systems containing 70% total solids (WMP + sucrose).

Source	DF[a]	Square addition (model) – standard error (coefficients and constant)					
		k_F[b]	$k_{K/S}$b	k_{OD390}b	k_{OD420}b	$k_{100/L}$b	p[c]
pH 6							
Model	3 and 4	1.890**	0.096***	0.037**	0.028***	0.476***	<0.007
Constant	1	0.070**	0.002***	0.009*	0.002***	0.022***	<0.050
Linear term							
X_1	1	0085*	0.001***	0.007*	0.002**	0.027**	<0.050
X_2	1	0.085**	0.001***	0.007***	0.002***	0.027***	<0.005
Interaction							
X_1, X_2	1	0.104*	0.001***	0.008*	0.001*	0.033**	<0.050
Quadratic term							
X_2^2	1	–	0.002***	0.011*	0.003**	–	<0.015
Residue	4 and 5	0.022	0.000	0.001	0.000	0.022	–
Explained variability (r^2)		0.917	0.999	0.973	0.998	0.956	
pH 7							
Model	4	3.838***	0.194***	0.025***	0.026**	1.915**	<0.002
Constant	1	0.065**	0.006***	0.006**	0.007*	0.077*	<0.04
Linear term							
X_1	1	0.046***	0.004***	0.004***	0.005***	0.0541**	<0.002
X_2	1	0.046**	0.004**	0.004**	0.005*	0.054*	<0.02
Interaction							
X_1, X_2	1	0.057*	0.005**	0.005*	0.006*	0.066*	<0.04
Quadratic term							
X_1^2	1	0.080**	0.008***	0.008**	0.008*	0.094*	<0.05
Residue	4	0.051	0.001	0.001	0.001	0.070	
Explained variability (r^2)		0.987	0.998	0.982	0.980	0.965	
pH 8							
Model	3 and 4	3.526***	0.237***	0.041***	0.056**	2.744**	<0.000
Constant	1	0.027**	0.012***	0.004**	0.002*	0.011*	<0.000
Linear term lineal							
X_1	1	0.019***	0.008***	0.003***	0.002***	0.014**	<0.005
X_2	1	0.019**	0.008**	0.003**	0.002*	0.014*	<0.000
Interaction							
X_1, X_2	1	0.023*	0.010**	0.003*	0.003*	0.017*	<0.03
Quadratic term							
X_2^2	1	0.033**	0.014***	0.004**	0.003*	0.017*	< 0.01
Residue	4 and 5	0.009	0.002	0.000	0.000	0.006	
Explained variability (r^2)		0.998	0.993	0.996	0.998	0.998	

X_1 = codified variable for reaction temperature.
X_2 = codified variable for the WPM/sucrose ratio (R).
[a] DF: degrees of freedom.
[b] $k_{F;\ K/S;OD390;OD420;\ 100/L}$ = reaction rate constant for fluorescence and color.
[c] p value for every coefficient.
* Significant at $p \leqslant 0.05$.
** Significant at $p \leqslant 0.01$.
*** Significant at $p \leqslant 0.00$.

Table 4
Activation energy values (E) for different color functions at pH 6–8, at different WMP/sucrose ratios (R) in aqueous systems containing 70% total solids (WMP + sucrose).

R	F[a]	K/S[b]	OD390 [c]	OD420[d]	100/L[e]
E_a (kJ mol^{-1}) – pH 6					
0.30	83.86 (r^2 = 0.999)	110.71 (r^2 = 0.997)	147.05 (r^2 = 0.999)	170.57 (r^2 = 0.995)	109.21 (r^2 = 0.968)
0.68	94.86 (r^2 = 0.982)	112.78 (r^2 = 0.994)	104.96 (r^2 = 0.999)	153.01 (r^2 = 0.994)	124.71 (r^2 = 0.930)
1.06	87.63 (r^2 = 0.999)	105.65 (r^2 = 0.988)	120.74 (r^2 = 0.992)	89.72 (r^2 = 0.964)	104.15 (r^2 = 0.993)
E_a (kJ mol^{-1}) – pH 7					
0.30	158.7 (r^2 = 0.981)	116.2 (r^2 = 0.973)	129.8 (r^2 = 0.975)	154.6 (r^2 = 0.969)	124.9 (r^2 = 0.982)
0.68	101.6 (r^2 = 0.994)	113.5 (r^2 = 0.991)	99.0 (r^2 = 0.976)	108.1 (r^2 = 0.985)	126.0 (r^2 = 0.989)
1.06	81.5 (r^2 = 0.988)	113.0 (r^2 = 0.976)	84.3 (r^2 = 0.991)	87.2 (r^2 = 0.979)	123.7 (r^2 = 0.993)
E_a (kJ mol^{-1}) – pH 8					
0.30	49.61 (r^2 = 0.997)	100.40 (r^2 = 0.975)	152.79 (r^2 = 0.991)	100.10 (r^2 = 0.901)	95.54 (r^2 = 0.943)
0.68	53.5 (r^2 = 0.991)	94.36 (r^2 = 0.982)	102.66 (r^2 = 0.992)	100.35 (r^2 = 0.943)	86.53 (r^2 = 0.941)
1.06 (r^2 = 0.999)	56.11 (r^2 = 0.999)	96.27 (r^2 = 0.992)	84.21 (r^2 = 0.956)	75.64 (r^2 = 0.898)	92.88 (r^2 = 0.934)

[a] F = Fluorescence.
[b] K/S = Kubelka–Munk index.
[c] OD 390 = optical density at 390 nm.
[d] OD 420 = optical density at 420 nm.
[e] 100/L = luminance inverse %.

Eqs. (5)–(19) were used to calculate the kinetic constants for each color function, at 110, 120, 130 and 140 °C, and at each studied pH.

Activation energy values (E_a) were calculated through Arrhenius equation. As Table 4 shows, the E_a corresponding to K/S and 100/L evolution were independent on R values at a given pH value, and showed lower values at pH 8. The E_a corresponding to F, OD_{390} and OD_{420} decreased as increasing R at pH 7. The E_a range for the studied systems was between 49 and 170 kJ mol$^{-1,}$ which is in agreement with that reported by other studies carried out in similar systems (Pauletti et al., 1999; Rozycki, 1999, 2003).

The lowest E_a values corresponded to fluorescence development at pH 8, and the highest ones to OD_{420} at pH 6. Thus, the extreme values of E_a were for the variables that are more related to the molecular properties of the systems. The variables K/S and 100/L, which are related to the macroscopic properties (surface reflectance) presented intermediate values of E_a and were globally less dependent on pH and R.

The ratio R (whole milk to sucrose) has been selected due to its practical implications, since the sucrose concentration is selected according to the milk solids. However, it is to be noted that when R increases not only the protein concentration increases, but also the content of the reducing sugar lactose, which is a key component for the Maillard and caramelization reactions. The ratio between protein and reducing sugar content remained constant in the studied systems. The effect of R is thus the effect of reactants concentration including both protein and reducing sugar. Sucrose, being a non-reducing sugar, has to be first hydrolyzed to participate in Maillard or caramelization reactions (Quintas et al., 2007). However, sucrose hydrolysis is acid-catalyzed and little contribution of sucrose hydrolysis products is expected at the pHs of the studied systems to Maillard or caramelization reactions (Buera et al., 1987a). The pH values were measured as a function of reaction time for each experience. The final pH values depended mainly on the initial pH value. When initial pH was 6.0, the final pH decreased to approximately one unit; when initial pH was 7.0, final pH was approximately decreased to 5.5, and when initial pH was 8.0, final pH fell more than two units, probably due to cracking of sugars that occurs when the pH increases, generating acidic products, formic acid among them (Rozycki, 2003). The observed pH decrease, may favor sucrose hydrolysis on one side, but delays Maillard or caramelization reactions. Nevertheless, the optimum pH for browning development in sucrose systems has been reported to be maximum at pH 5 (Buera et al., 1987b), which is lower than the pH range studied in present work.

What has so far been written shows the extreme complexity of the involved reactions. Since whole milk was employed for the sample preparation, if lipid oxidation products are formed they can react with amino groups forming yellow and fluorescent intermediary products which polymerize into dark brown macromolecules (Pokorny and Sakurai, 2002), thus complicating the pathway of browning reactions. It is known, however, that lipid oxidation is not favored at the experiment conditions: high temperatures and high water contents cause reaction intermediates of reducing character (Manzocco et al., 2001). The different information provided by fluorescence, pigment and color development could be seen in the relative increase of the kinetic coefficients of both reactions, when R increases.

Within the studied temperature range, the increase of the kinetic parameters for pigment development (K/S and 100/L) were less dependent on R (related to milk, or reactants concentration) than the kinetic parameters for fluorescence development.

Fluorescent products are exclusively derived from carbonyl-amino reactions. Consequently, fluorescence development was highly dependent on R which is directly related to milk concentration, the mixture component which contributes with the amino-groups constituents of the systems. The effect was particularly important at lower temperatures within the studied range. Pigment development, on the contrary, was less influenced by milk (protein) concentration within the whole range of temperature studied. It can be thus concluded that caramelization reactions play a significant role in color formation in the studied systems. In agreement with this, Brands and Van Boekel (2001) established that caramelization reactions account for the highest part of the pigments obtained when casein is thermally treated with glucose at the pH of milk.

Table 5
Nine confirming experiences.

Exp. no.	pH	X_1 codif	X_1[a] no codif	X_2 codif	X_2[b] no codif	k_F	$k_{K/S}$	$K_{100/L}$	k_{OD390}	k_{OD420}
Calculated values (Eq. 7) of the specific global reaction rate pseudo-constants for the five functions selected for color and fluorescence development										
1	6	−0.48	+0.50	−0.43	115	0.1896	0.0283	0.0895	0.0056	0.0035
2	6	+0.05	+0.70	+0.43	130	0.6286	0.1389	0.3246	0.0591	0.0568
3	6	+0.58	+0.90	−0.43	115	0.3280	0.0427	0.1681	0.0090	0.0122
4	7	−0.48	+0.50	+0.43	130	0.6097	0.1609	0.3228	0.0497	0.0510
5	7	+0.05	+0.70	−0.43	115	0.2579	0.0379	0.0879	0.0071	0.0079
6	7	+0.58	+0.90	+0.43	130	0.9984	0.2056	0.6612	0.0833	0.0739
7	8	−0.74	+0.40	+0.71	135	0.9970	0.2568	0.6293	0.0881	0.1108
8	8	−0.21	+0.60	−0.71	110	0.3554	0.0489	0.2265	0.0026	0.0013
9	8	+0.84	+1.00	−0.14	120	0.9688	0.1599	0.8115	0.0439	0.0362
Exp. no.	k_F	CV%a	$k_{K/S}$	CV%a	$k_{100/L}$	CV %a	k_{OD390}	CV%a	k_{OD420}	CV%a
Experimental values of the specific global reaction rate pseudo-constants for the five functions selected for color and fluorescence development										
1	0.175	8.01	0.0271	4.69	0.0832	7.55	0.0058	3.51	0.0037	5.55
2	0.671	6.55	0.1412	1.51	0.3130	3.64	0.0630	6.39	0.0545	4.13
3	0.359	9.02	0.0413	4.09	0.1751	4.02	0.0086	4.55	0.0115	5.91
4	0.593	2.78	0.1550	3.74	0.3355	3.79	0.0521	4.72	0.0483	5.44
5	0.269	4.25	0.0394	3.12	0.0901	3.47	0.0068	4.32	0.0082	3.73
6	1.060	5.99	0.2181	5.86	0.6930	4.70	0.0799	4.17	0.0770	4.11
7	1.051	5.21	0.2402	6.78	0.5913	6.31	0.0921	4.33	0.1061	4.43
8	0.341	4.08	0.0510	4.20	0.2391	5.37	0.0025	3.92	0.0014	7.41
9	0.994	2.57	0.1553	3.13	0.7730	4.86	0.0461	4.89	0.0382	5.38

[a] $CV\% = [(k_{F\ calculated} - k_{F\ experimental})/k_{F\ average}] \times 100$.
[b] $k_{F\ average} = [k_{F\ calculated} + k_{F\ experimental}]/2$.

3.1 Confirming experiences

Finally, nine confirming experiences (three at each design pH) were carried out varying reaction temperature and *R*, in order to confirm the global correctness of the modelling approach (Eqs. (5)–(19)) obtained to predict k_{CP}, for the different color functions. Experimental conditions and kinetic parameters *calculated* with those equations are shown in Table 5.

The same table shows the *experimental* values of the constants (mentioned and) obtained under the experimental conditions. A percentage difference (expressed as a function of the coefficient of percentage variation) lower than 10% is observed between the experimental k_{CP} values and those calculated with Eqs. (7)–(19), for the different color functions selected to follow the kinetics of color development and fluorescence. These equations are considered very suitable for quantifying both color and fluorescence in this type of systems.

4. Conclusions

While pigment development in concentrated milk systems depends mainly on reaction temperature, fluorescence development depends on milk solids concentration, and both color and fluorescence kinetic constants are influenced by pH. However, color and fluorescence parameters show a different sensitivity to quantify the different types of reactions, which allows selecting the most appropriate marker for the specific technological conditions and system composition.

The response surface methodology proved to be suitable for the exploratory analysis and quantification of the technological variables normally influencing dairy systems. For a further analysis, the multiple regression method made it possible to select suitable mathematical models which allow the prediction of the system behavior by means of the specific reaction rate constant, under the technological conditions studied. The obtained models allow the control of the different processes and equipment design for a continuous production of concentrated dairy products with specific textural and organoleptic characteristics.

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References

Adhikari, H.R., Tappel, A.L., 1973. Fluorescent products in a glucose–glycine browning reaction. Journal of Food Science 38, 486–488.
Brands, C.M.J., Van Boekel, M.A., 2001. Reactions of monosaccharides during heating of sugar–casein systems: building of a reaction network model. Journal of Agricultural and Food Chemistry 49, 4667–4675.
Buera, M.P., Chirife, J., Resnik, S.L., Lozano, R.D., 1987a. Nonenzymatic browning in liquid model systems of high water activity: kinetics of color changes due to caramelization of various single sugars. Journal of Food Science 52, 1059–1062.
Buera, M.P., Chirife, J., Resnik, S.L., Lozano, R.D., 1987b. Nonenzymatic browning in liquid model systems of high water activity: kinetics of color changes due to reaction between glucose and glycine peptides. Journal of Food Science 52, 1068–1073.
Buera, M.P., Chirife, J., Resnik, S.L., Wetzler, G., 1987c. Nonenzymatic browning in liquid systems of high water activity: kinetics of color changes due to Maillard's reaction between different single sugars and glycine and comparison with caramelization browning. Journal of Food Science 52, 1063–1067.
Buera, M.P., Chirife, J., Resnik, S.L., 1990a. Nonenzymatic nonoxidative browning in hydrolyzed shelf-stable concentrated cheese whey. Journal of Food Science 55, 697–700.
Buera, M.P., Hough, G., Martinez, E., Resnik, S.L., 1990b. Colorimetric, spectrophotometric and sensory color measurements of a dairy product: dulce de leche. Anales de la Asociación Química Argentina 78, 291–299.
Buera, M.P., Resnik, S.L., Petriella, C., 1992. Relative browning rates of fructose and glucose in high water activity systems as a function of amino compound concentration. Lebensmittel, Wissenschaft und Technology 25, 35–37.
Calvo, C., 1993. La medida del color en alimentos traslúcidos: teoría de Kubelka-Munk. Revista Española de Ciencia y Tecnología de Alimentos 33 (6), 597–606.
Cano-Ruiz, M.E., Richter, R.L., 1998. Changes in physicochemical properties of retort-sterilized dairy beverages during storage. Journal of Dairy Science 81, 2116–2123.

Carabasa-Giribet, M., Ibarz-Ribas, A., 2000. Kinetics of colour development in aqueous glucose systems at high temperature. Journal of Food Engineering 44, 181–189.

Cerrutti, P., Resnik, S.L., Seldes, A., Ferro Fontan, C., 1985. Kinetics of deteriorative reaction in model food systems of high water activity: glucose loss, 5-hydroxymethylfurfural accumulation and fluorescence development due to nonenzymatic browning. Journal of Food Science 50, 627–630.

Labuza, T., Baiser, W., 1992. The kinetics of nonenzymatic browning. In: Schwartzber, H. (Ed.), Physical Chemistry of Foods. Marcel Dekker, New York, pp. 595–649.

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M.C., Lerici, C.R., 2001. Review of non-enzymatic browning and antioxidant capacity in processed foods. Trends in Food Science and Technology 11, 340–346.

Matiacevich, S.B., Buera, M.P., 2006. A critical evaluation of fluorescence as a potential marker for the Maillard reaction. Food Chemistry 95, 423–430.

Montgomery, D.C., 2001. Chapter 3: Experiments with a Single Factor: The Analysis of Variance. Design and Analysis of Experiments. I. John Wiley & Sons. pp. 60–125.

Morales, F.J., van Boekel, M.A.J.S., 1999. A study on advanced Maillard reaction in heated casein/sugar solutions: fluorescence accumulation. International Dairy Journal 7, 675–683.

Obayashi, H., Nakano, K., Shigeta, H., Yamaguchi, M., Yoshimori, K., Fukui, M., Fujii, M., Kitagawa, Y., Nakamura, N., Nakamura, K., Nakazawa, Y., Ienaga, K., Ohta, M., Nishimura, M., Fukui, I., Kondo, M., 1996. Formation of crossline as a fluorescent advanced glycation end product in vitro and in vivo. Biochemical and Biophysical Research Communications 226, 37–41.

Park, C.K., Kim, D.H., 1983. Relationship between fluorescence and antioxidant activity of ethanol extracts of a Maillard browning mixture. Journal of the American Oil Chemists' Society 60 (1), 22–26.

Pauletti, M., Calvo, C., Izquierdo, L., Costell, E., 1992. Color y textura del dulce de leche. selección de métodos instrumentales para el control de calidad industrial. Revista Española Ciencia y Tecnología de Alimentos 32, 291–305.

Paulette, M., Castelao, E., Sabbag, N., Costa, S., 1995. Color development rate in dulce de leche. Food Science and Technology International 1, 137–140.

Pauletti, M., Castelao, E., Bernardi, M., 1996. Influence of soluble solids, acidity and sugar on the color of dulce de leche. Food Science and Technology International 2, 45–49.

Pauletti, M., Matta, E.J., Castelao, E., Rozycki, S.D., 1998. Color in concentrated milk proteins with high sucrose as affected by glucose replacement. Journal of Food Science 63, 90–92.

Pauletti, M., Matta, E.J., Rozycki, S.D., 1999. Kinetics of heat-induced browning in concentrated milk with sucrose as affected by pH and temperature. Food Science and Technology International 5, 407–413.

Petriella, C., Resnik, S., Lozano, R., Chirife, J., 1985. Kinetics of deteriorative reactions in model food systems of high water activity: color changes due to nonenzymatic browning. Journal of Food Science 50, 622–626.

Pokorny, J., Sakurai, H., 2002. Role of oxidized lipids in nonenzymic browning reactions. International Congress Series 1245, 373–374.

Quintas, M.A.C., Brandao, T.R.S., Silva, C.L.M., 2007. Modelling colour changes during the caramelisation reaction. Journal of Food Engineering 83, 483–491.

Rozycki, S.D., 1999. M.S. Thesis Food Technology, Facultad de Ingeniería Química, Universidad Nacional del Litoral. Santa Fe, Argentina.

Rozycki, S.D., 2003. Cinética de pardeamiento en sistemas lácteos concentrados: análisis comparativos de modelos. Ciencia y Tecnología Alimentaria 4 (2), 95–108.

Saguy, I.S., Graf, E., 1999. Particle size effects on the diffuse reflectance of a sucrose–caramel mixture. Journal of Food Science 56, 1117–1120.

Singh, R., Heldman, D. (Eds.), 1993. Introduction to Food Engineering, second ed. Academic Press, San Diego, USA.

Song, D.U., Jung, Y.D., Chay, D.O., Lee, K.H., Yang, S.Y., Shin, B.A., Ahn, B.W., 2002. Effect of drinking green tea on age-associated accumulation of Maillard-type fluorescence and carbonyl groups in rat aortic and skin collagen. Archives of Biochemistry and Biophysics 397 (2), 424–429.

Statgraphics Plus 3.0, Copyright 1994–1997. Statistical Graphics Corporation, USA.

Statistica, 1993. Statistica for Windows. Release 4.5 StatSoft , Inc., USA.

Tessier, F., Monnier, V.M., Kornfield, J.A., 2002. Characterization of novel chromophores, fluorophores and cross-links from glyceraldehydes, lysine and arginine. International Congress Series 1245, 303–311.