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Effects of temperature and pH on the kinetics of caramelisation, protein cross-linking and Maillard reactions in aqueous model systems

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Abstract

Solutions of either glucose, bovine serum albumin (BSA), casein, glucose–BSA or glucose–casein were heated at 60 to 100 °C at pH 8.0 and pH 9.7, and the kinetics of the reaction markers (disappearance of the glucose and amino groups, development of UV absorbance and browning, and protein reticulation) were monitored. All the markers were enhanced at increasing temperatures, and, except for the disappearance of the free amino group and protein polymerisation, at alkaline pH levels. The loss of the amino group occurred in parallel with increase in polymerisation. The two proteins reacted in line with their amino content and solubility. The activation energy (E_a) of the amino group loss in the protein–glucose mixture was around 100 kJ per mol and that in the protein heated alone was higher. The E_a associated with the disappearance of glucose was around 90 kJ per mol. The E_a of UV absorbance and browning processes, which showed parallel time courses, were found to range from 90 to 150 kJ per mol.

Keywords: Maillard reaction; Caramelisation; Activation energy; Protein cross-linking

1. Introduction

In 1912, Louis Camille Maillard working at the Sorbonne in Paris discovered the browning process in a glucose–glycine mixture, and he soon suspected the great future impact of this process in organic chemistry, food science, biology and geology (Maillard, 1916). The research carried out on this topic during the last century has generated a large body of data supporting this author's original predictions. Reviews are to be found in the proceedings of symposia on this subject published from 1979 (Eriksson, 1981) to 2004 (Baynes et al., 2005) as well as in the recent COST action (Somoza, 2005). In food, the spontaneous interactions occurring between carbonyls and amines, mainly in the form of reducing sugars and the amino groups of proteins, respectively, lead to the formation of a wide range of products which can have either deleterious or beneficial effects, since the physical and chemical conditions (temperature, water activity, pH, concentrations, etc.) can vary considerably. These effects include the development of attractive or unattractive odours and colours, the loss of protein nutritional value, digestive incompatibility and potential toxicity. *In vivo*, at a temperature of 37 °C, similar or different products to those present in food can develop and accumulate, and the resulting reactions are enhanced under high glucose and carbonyl stress conditions, in particular.

Most of the information available so far on Maillard reactions was based on the use of model systems in which less attention was paid to the proteins involved than to the free amino acids. More than half a century ago, Lea and Hannan (1949) published some valuable kinetic data on the influence of temperature, pH and water activity on the Maillard reaction in a glucose–casein model system. In subsequent kinetic studies, model systems such as those based on soy proteins (Jokinen, Reineccius, & Thompson, 1976; Thompson, Wolf, & Reineccius, 1976), egg albumin

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(Kato, Matsuda, Kato, & Nakamura, 1989; Tanaka, Kimiagar, Tung-Ching, & Chichester, 1977) and milk (Brands & Van Boekel, 2002; Morales & Van Boekel, 1998) were used. In these studies, the proteins were rarely tested alone under similar conditions to those occurring in the presence of reducing sugar, and the pH levels were below 7.0.

In our previous studies (Ajandouz & Puigserver, 1999; Ajandouz, Tchiakpe, Dalle ore, Benajiba, & Puigserver, 2001), we investigated the contribution of caramelisation reactions, especially those occurring under alkaline conditions, to non-enzymatic browning reactions in sugar and sugar–amino acid model systems, by monitoring the reactants and UV–visible absorbance. In the present study, glucose, protein and glucose–protein aqueous systems were heated at 60–100 °C at pH 8.0 and pH 9.7. The kinetics of the reaction markers (disappearance of the glucose and amino groups, increasing UV absorbance and browning levels, and protein reticulation) were monitored. The activation energy values obtained from the experimental data are discussed with reference to the literature on the topic. The kinetics of the Maillard reactions, caramelisation and protein reticulation processes occurring simultaneously under various pH and temperature conditions have never been previously investigated, to our knowledge.

2. Material and methods

2.1. Reagents

BSA, casein, trinitrobenzenesulfonic acid (TNBS) and glucose were purchased from Fluka. All the other chemicals were of the purest grade commercially available, and all the buffer solutions were filtered through 0.45 μm Millipore HA filters before use.

2.2. Heating procedure

Solutions containing either glucose (0.2 M), protein (BSA or casein: 5 mg per ml) or glucose–protein were heated in screw-cap tubes at 60, 70, 80 or 100 °C at pH 8.0 and pH 9.7 (using 0.2 M sodium phosphate or sodium borate buffer, respectively). At pH 8.0, the solutions were heated for 240 min at 60 °C, 180 min at 70 °C, 120 min at 80 °C and 40 min at 100 °C. At pH 9.7, the solutions were heated for 100 min at 60, 70 and 80 °C, and for 10 min at 100 °C. After the heating procedure, the tubes were cooled in ice. Part of the heated solution was used directly for absorbance and final pH measurements, while the remainder was stored at −20 °C for glucose and amino group loss determinations and electrophoresis analysis. Each kinetic point was measured at least in duplicate and mean values were used to express the kinetic parameters. The mean deviation in the experimental data did not exceed 12%.

2.3. Absorbance measurements

The absorbance of the heated solutions was measured using a DU 640 Beckman spectrophotometer at 294 nm and 420 nm, as markers during the intermediate and final stages of the reactions, respectively (Ajandouz et al. 2001). Appropriate dilutions were prepared in order to obtain an absorbance value of less than 1.5.

2.4. Glucose loss measurements

The remaining undegraded glucose was monitored using high performance anion exchange chromatography-pulsed amperometric detection (Dionex Corporation, Sunnyvale, Ca). Glucose was eluted under isocratic conditions from the CarboPac PA-100 analytical anion exchange column (250 × 4 mm) equipped with an IonPac AG4A-SC guard column (25 × 4 mm), connected to a gold working electrode cell. The eluent (5 mM sodium acetate containing 0.1 M NaOH), was delivered at a rate of 1 ml per min by a Dionex GP 40 gradient pump. Samples were injected by a Spectra System AS 3500 autosampler from Thermo Electron (Fremont, Ca) and the detection was carried out with an ED 40 electrochemical detector. The area under the eluted peak was integrated using PeakNet chromatography software, based on a 0–250 pmol glucose calibration chart.

2.5. Free amino group determination

The free amino groups were determined using TNBS, as described by Fields (1971). One millilitre of protein (0.2–2.0 mg per ml in 0.1 M sodium borate containing 0.1 M NaOH) was reacted with 20 μl of TNBS (1 M in water) for 5 min. The reaction was stopped by adding two volumes of 1.5% sodium sulfite (1.5 mM in water) and 98.5% of dibasic sodium phosphate (0.1 M). The absorbance was measured at 420 nm.

2.6. Electrophoresis

Polyacrylamide gel-sodium dodecyl sulfate electrophoresis (PAGE-SDS) was performed using a Mini Protean electrophoresis cell (Bio rad, Hercules, CA), as described by Laemmli (1970). Polyacrylamide gels of 10% and 12% were used with BSA and casein, respectively. The samples were first heated for 3 min at 100 °C in the presence of 0.1% SDS and 50 mM dithiotreitol, and then subjected to PAGE-SDS. Migration was performed at an initial current of 20 mA for 45 min. The proteins were then stained with Coomassie Brillant Blue R-250.

3. Results and discussions

3.1. Loss of glucose

Kinetic plots of the disappearance of glucose heated alone and in the presence of casein at pH 8.0 at temperatures

between 60 and 100 °C are given in Fig. 1. This temperature range has not been frequently used in previous studies on non-enzymatic browning reactions involving proteins, nor have alkaline conditions been previously tested. The plots in Fig. 1 show that depending on the temperature, glucose disappears variably linearly during the heating, which suggests that either zero or higher order reactions may be involved. Similar kinetic behaviour was found to occur in a heated glucose–BSA model system at pH 8.0 and in the three heated model systems (glucose, glucose–casein, and glucose–BSA) at pH 9.7 (not shown). The rate constants of glucose disappearance were deduced from the slopes of the curves in the initial stages ($r^2 > 0.9$). At pH 9.7, the glucose disappeared from the three model systems at rates which were 3.5- to 6-fold higher than at pH 8.0, and the temperature was found to have very little effect on this process. The rate constants of the disappearance of glucose heated alone were equal to or higher than those of glucose heated after adding proteins. The Arrhenius plots obtained using these rate constants were linear, with correlation coefficients higher than 0.98. The activation energy values obtained were around 90 kJ per mol: these values were slightly affected (by less than 10%) by the pH and amino groups (Table 1). These slight effects might suggest that the net "catalytic" effect of the heating may increase with amino group availability but decrease with hydroxide ion availability, although the latter factor also enhances glucose disappearance *via* isomerisation, the opening of the pyranose ring and degradation, as described by De Bruijn, Kieboom, and Van Bekkum (1986).

In fact, little attention has been paid so far to the behaviour of the reducing sugar in studies on the Maillard reaction, although some authors, using mixtures of amino acids or proteins and reducing sugars, have reported that the carbonyl disappeared faster than its counterpart, the amino group (Warmbier, Schnickels, & Labuza, 1976; Ajandouz & Puigserver 1999; Ajandouz et al., 2001; Brands & Van Boekel, 2001). Vernin et al. (1992) have suggested that the fast disappearance of glucose may be due to the formation of diglycosylamines, in line with findings by Lee, Sherr, and Koh (1984), who reported that difructosyl-lysine accounted for more than one quarter of the blocked lysine, when a glucose–lysine mixture was refluxed for several hours in 80% methanol. In addition, although the glucose disappears, the amino group may be regenerated from the Schiff base or glycosylamine intermediates (Hodge, 1953; Baisier & Labuza, 1992).

Table 1
Activation energies associated with the disappearance of glucose, in heated glucose and glucose–protein aqueous model systems at pH 8.0 and pH 9.7

	Activation energy (kJ per mol)		
	Glucose	Glucose + casein	Glucose + BSA
pH 8.0	93	85	87
pH 9.7	96	93	92

It is also worth noting that the activation energy values shown in Table 1 are lower than that (105 kJ per mol) reported by Warmbier et al. (1976) in a glucose–casein model system treated under lower pH, temperature and water activity conditions, as well as being lower than the value of 100 kJ per mol suggested by Morales and Van Boekel (1998), based on their studies on milk based systems. The question still remains to be answered, however, as to what the effects of the amino groups may be on the first chemical changes, which the reducing sugar underwent.

3.2. Amino group loss

Fig. 2 shows the kinetics of the disappearance of the free amino groups in heated BSA and BSA–glucose model systems at pH 8.0 and pH 9.7. At pH 8.0, the amino groups disappeared in both the absence and presence of glucose, but this occurred more rapidly in the latter case. However, at pH 9.7, only a slight loss of amino groups occurred in BSA heated alone, except at 100 °C, whereas in the presence of glucose, the losses were quite similar to those measured at pH 8.0. Similar tendencies were observed in

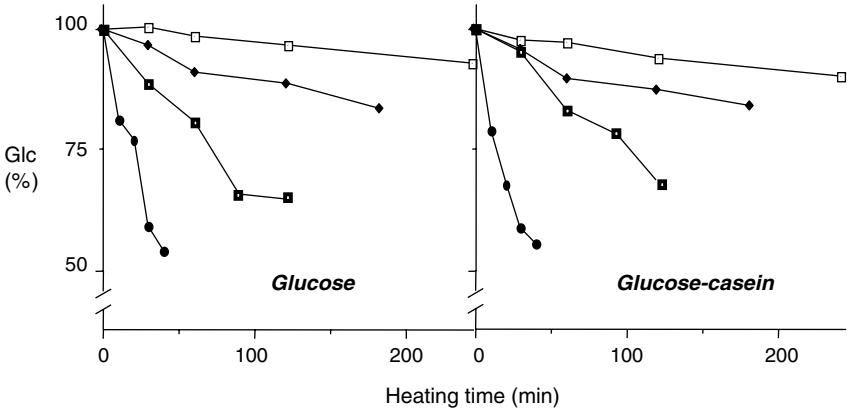

Fig. 1. Kinetics of glucose loss in heated aqueous glucose (Glc) and glucose–casein model systems at pH 8.0, at temperatures ranging from 60 °C to 100 °C. (60 °C: □, 70 °C: ♦, 80 °C: ■, 100 °C: ●).

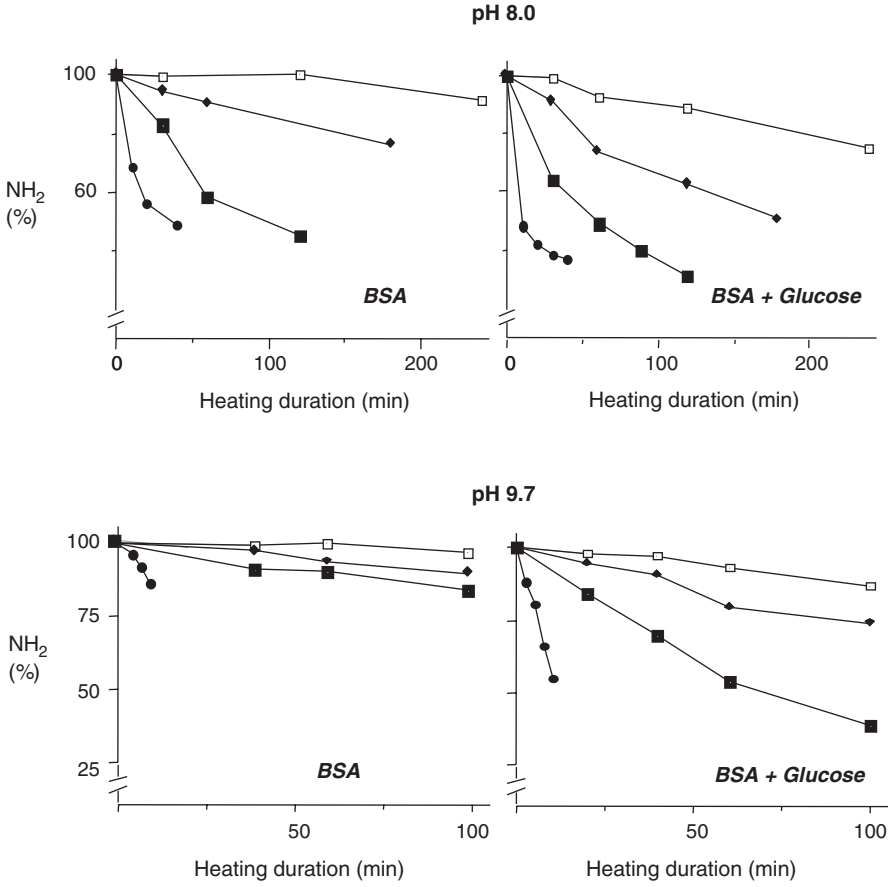

Fig. 2. Kinetics of free amino group loss in BSA and BSA–glucose heated at pH 8.0 and pH 9.7. (60 °C: □, 70 °C: ♦, 80 °C: ■, 100 °C: ●).

casein and casein–glucose model systems, but casein is obviously less reactive than BSA (data not shown).

Amino group losses have been previously reported to occur in proteins heated alone (Erbersdobler & Anderson, 1983; Smith & Friedman, 1984). The lost amino groups probably originate largely from the side chains of lysine residues, but guanidino groups of arginine may also be involved, as found to occur in casein heated with glucose at temperatures ranging between 37 and 60 °C (Lea & Hannan, 1951). Alpha-amino groups at the N-terminus of the proteins are also possible candidates for interacting with glucose.

As regards the amino group losses occurring at pH 8.0, BSA heated alone was 4-fold more reactive than casein at 60 °C, but the two proteins were found to show almost the same reactivity at 100 °C, on the basis of the plots obtained during the first stages of heating. The fact that heating had more pronounced effects on the casein amino group was probably due to the disturbance of micelle structures, rendering lysine available for reticulation. At the same pH level, in the presence of glucose, the rate of amino group disappearance was 2- to 3-fold and 2- to 16-fold higher in BSA and casein, respectively, than in its absence: the lower the temperature, the higher the enhancing effects became. The contribution of the Maillard reaction to amino group loss seems to decrease as the heating temperature increases. The question therefore arises as to what the real contribution of the Maillard reaction may be to the lysine losses occurring in food.

In the presence of glucose, no marked differences in the rates of amino group loss were observed between pH 8.0 and pH 9.7 in either in BSA (Fig. 2) or casein (not shown). We previously reported the occurrence of a dramatic increase in the free amino acid losses between pH 8.0 and pH 10.0 in aqueous reducing sugar–amino acid models heated at 100 °C (Ajandouz & Puigserver, 1999; Ajandouz et al., 2001). The plots obtained were consistent with the deprotonation of the amino groups, as the base form (NH_2) is the reactive form, according to Hodge (1953). Here, at pH 9.7, the amino groups of lysine and arginine residues are still largely in the protonated form, which at least partly explains why no high additional loss of amino groups occurs between pH 8.0 and pH 9.7.

The loss of amino groups obeyed both zero and higher order kinetics (Fig. 2), as described in other glucose–protein model systems, although these were heated under lower moisture conditions (Jokinen et al., 1976; Labuza & Saltmarch, 1981; Thompson et al., 1976). The slopes at the origin ($r^2 > 0.9$) were used to calculate activation energy values ($r^2 > 0.97$). These values are shown in Table 2, along with data published in the literature, giving the analytical methods used to determine the free amino

Table 2
Activation energy E_a associated with the loss of free amino groups in protein-reducing sugar model systems and in some kinds of food

System	a_w or (% H_2O)	T (°C)	Analytical method	E_a (kJ per mol)	References
Casein pH 8.0	~1.0	60–100	TNBS	143	Present study
BSA pH 8.0	//	//	//	116	//
Glucose+					
Casein pH 8.0	//	//	//	106	//
BSA pH 8.0	//	//	//	102	//
Casein pH 9.7	//	//	//	105	//
BSA pH 9.7	//	//	//	92	//
Casein pH 6.7	0.7	0–70	Van Slike	121	Lea and Hannan (1949)
Soy proteins	0.33–0.93	30–80	FDNB	119	Jokinen et al. (1976)
Soy proteins	(6–18)	80–130	FDNB	147	Thompson et al. (1976)
Ribose + BSA	−14.1	85–145	FDNB	130	Carpenter et al. (1962)
Lactose + casein	0.33–0.98	37–60	OPA	156-117	Malec et al. (2002)
HM	(3.9–11.9)	85–145	FDNB	113	Carpenter et al. (1962)
NFDM	0.4 and 0.6	20–40	Sanger	46 and 39	Ben-Gara and Zimmerman (1972)[a]
Pasta	0, 50	45–80	MB, FDNB	31	Fabriani and Frantoni (1972)[a,b]
Rice + lysine	(15–20)	115–184	TNBS	52	Tsao et al. (1978)

[a] E_a was calculated based on the data published by the authors quoted.
[b] Loss of lysine was determined in soluble proteins (albumins and globulins). FDNB: l-fluoro-2,4-dinitrobenzene; HM: herring meal; MB: microbiological; NFDM: non-fat dry milk; OPA: *o*-phtaldialdehyde.

groups available, as the analytical method may account largely for the discrepancies between various data published in the literature.

Some general conclusions can be drawn from these data, although the physico-chemical conditions and the analytical methods used differ. The E_a of casein heated alone at pH 8.0 is higher than that of BSA, probably because of the higher energy cost of the deformation of casein micelles. But both of these values are higher than those obtained when the proteins were heated in the presence of glucose, which shows the net catalytic effect of the reducing sugar on the disappearance of amino groups. Our E_a values were somewhat lower than those obtained on other reducing sugar–protein model systems (Carpenter, Morgan, Lea, & Parr, 1962; Jokinen et al., 1976; Lea & Hannan, 1951; Malec, Pereyra Gonzales, Naranjo, & Vigo, 2002; Thompson et al., 1976), and the factors such as pH and water activity may possibly have contributed to this difference, although it is difficult to allot a specific score to each factor. The pH values at which previous studies were performed were below neutral and these values probably decreased during the reaction. It is therefore possible that the activation energy involved in the disappearance of amino groups in reducing sugar–protein mixtures may decrease as the pH increases, and this suggests that less amino group consumption may occur in the case of the advanced reaction products. Malec, Pereyra Gonzales, Naranjo, and Vigo (2002), have also reported that increasing the water activity decreases the activation energy (Table 2), which is in line with our data. A similar tendency was observed by Ben Gara and Zimmerman (1972) in non-fat dry milk. However, Thompson et al. (1976) and Jokinen et al. (1976) did not observe any effect of water activity on the activation energy in a heated glucose–soy protein model system. In all these cases, as shown in Table 2, the activation energy values corresponding to amino group disappearance in food systems (Ben-Gara & Zimmerman, 1972; Fabriani & Frantoni, 1972; Tsao, Frey, & Harper, 1978) are much lower than those observed in reducing sugar–protein model systems, which suggests that the nutritional loss assessments based on these model systems are underestimated. Herring meal (Carpenter, Morgan, Lea, & Parr, 1962) does not show this tendency, but in this meal, the reducing sugar content can be assumed to be lower, and non Maillard reticulation reactions are therefore likely to make an important contribution to these losses.

3.3. Reticulation

Fig. 3 shows the electrophoretic behaviour of the heated proteins in the presence and absence of glucose. At pH 8.0, the casein and BSA bands gradually disappeared with time, whether or not glucose was present, and polymeric species having heavier molecular masses were concomitantly formed. The reticulation process was faster in the presence than in the absence of glucose. Casein heated alone was distinctly less reactive than BSA. These structural changes in the protein are in good agreement with the amino group losses. Comparisons between the amino acid compositions of BSA and casein showed that the latter protein contains about 2-fold higher lysine residues than the former, whereas BSA contains about 50% more arginine residues. TNBS test showed that the free amino group content was about 15% greater in casein than in BSA; this difference is smaller than was to be expected on the basis of the amino acid compositions, but the reactivity of TNBS to arginine and lysine side chain amino groups is presumably not the same. In addition, BSA contains 17 disulfide bridges, whereas casein contains none. It is therefore conceivable, in line with the report of Finley (1989), that heating may induce the breakage of disulfide bridges in BSA, leading to the unfolding of the protein and rendering cross-linking

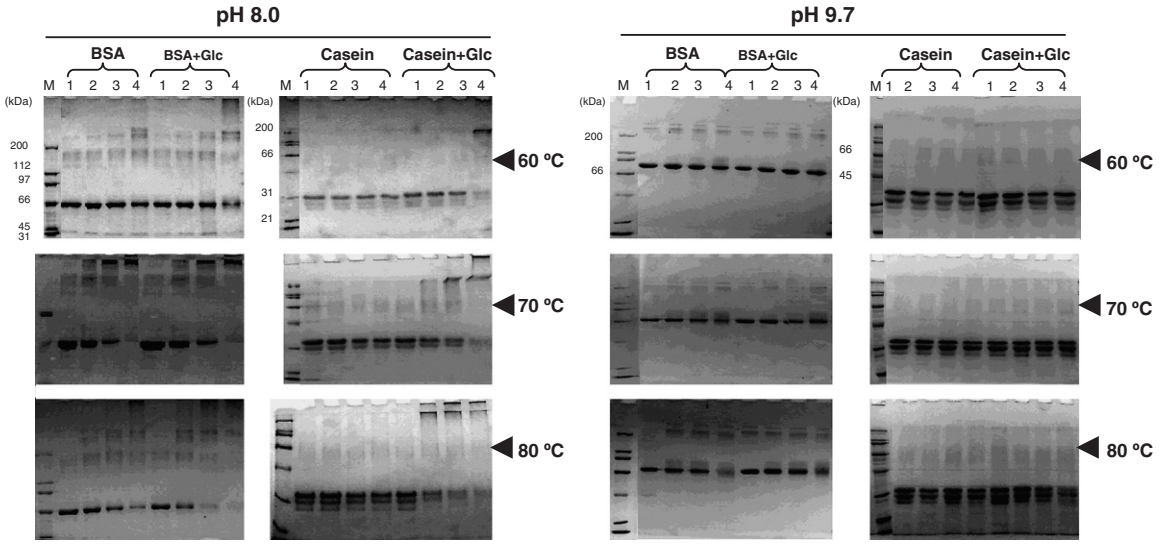

Fig. 3. Electrophoretic behaviour of BSA and casein heated alone or in the presence of glucose at pH 8.0 or pH 9.7. Lines 1, 2, 3 and 4 correspond to heating times. At pH 8.0 : 0, 30, 60 and 240 min (60 °C); 0, 30, 60 and 180 min (70 °C) and 0, 30, 60 and 120 min (80 °C), respectively. At pH 9.7: 0, 20, 40 and 100 min for 60 °C, 70 °C and 80 °C. M: mass markers. Glc: glucose. With each gel, equal quantities of proteins were loaded, ranging from 3 to 6 μg in the case of BSA and 8 to 12 μg in that of casein.

sites, including sulfhydryl groups, more accessible. On the other hand, electrostatic interactions occurring in the micelle structures of casein may hinder the cross-linking reactions and the concomitant loss of amino groups. The data presented in Fig. 3 are in agreement with those published by Easa et al. (1996) who reported that the molar mass of BSA increased up to 2000 kg per mol after 10 min and remained constant thereafter, when a solution of BSA (initial pH 6.3) was heated at 95 °C, whereas in the presence of xylose, the molar mass of the protein continued to increase up to 8000 kg per mol. Hofmann (1998) has also reported that in an aqueous casein–glucose model system (pH 7.0) heated for 4 h at 95 °C, the molecular weights of more than 40% of the proteins were greater than 100 kDa. The cross-linking of casein was found to develop in parallel with the colour intensity, which indicated that chromophoric substructures originating from carbohydrates are incorporated into these oligomers. Hofmann (1998) has suggested that these substructures may be covalently linked to reactive chains of lysine, arginine and cysteine. It can be seen from Fig. 3 that at pH 8.0, the protein–protein reticulation process is particularly enhanced in the absence of glucose. In the case of lysine, the loss of amino groups in proteins heated alone is likely to be mainly due to the formation of glutamyl- and aspartyl-lysine isopeptides (Otterburn, 1989), since lysinoalanine formation requires more severely alkaline conditions (Friedman, 1999), although high rates of lysinoalanine formation have also been reported to occur in a heated acetylcasein–glucose system at 121 °C and pH 7 (Pellegrino, Van Boekel, Gruppen, Resmini, & Pagani, 1999).

At pH 9.7, whether or not glucose was added, BSA underwent only slight reticulation and no changes were observed in casein and casein–glucose model systems during heating, excepting at 100 °C, where some loss of the protein bands occurred (not shown). The lack of reticulation observed at pH 9.7 in protein–glucose models was therefore not in agreement with the loss of amino groups. The question arises as to why fewer amino groups disappear at pH 9.7 than at pH 8.0. This in fact suggests that in heated protein–glucose mixtures at moderate to severe alkaline pH levels, the proteins may undergo less reticulation and serve rather as "catalysts" of the processes generating advanced brown products, including those containing nitrogen, as the amino groups gradually disappear.

Whatever the case may be, these findings show what a useful tool electrophoresis can be for exploring protein–protein and protein–carbohydrate interactions. As Fayle et al. (2001) have pointed out, this analytical method has been underused in studies on the Maillard reaction.

3.4. UV absorbance and browning

The kinetics of UV absorbance and browning in heated glucose and glucose–BSA model systems at pH 8.0 are shown in Fig. 4. The kinetic behaviour of glucose–casein is similar to that shown in Fig. 4, and the initial rate constants of UV absorbance and browning follow similar tendencies (not shown). The similarities observed between glucose and glucose–protein in terms of the kinetic development of the UV-absorbing and brown compounds are in agreement with the statement made by Mauron (1981) that free amino groups of proteins mainly have catalytic effects on chemical reactions rather being specific to the reducing sugar. The UV absorbance of glucose accounts for 25–80% of that of glucose–protein mixtures, although the browning accounts for only 7–55%. In both cases, the percentage increases with temperature. The

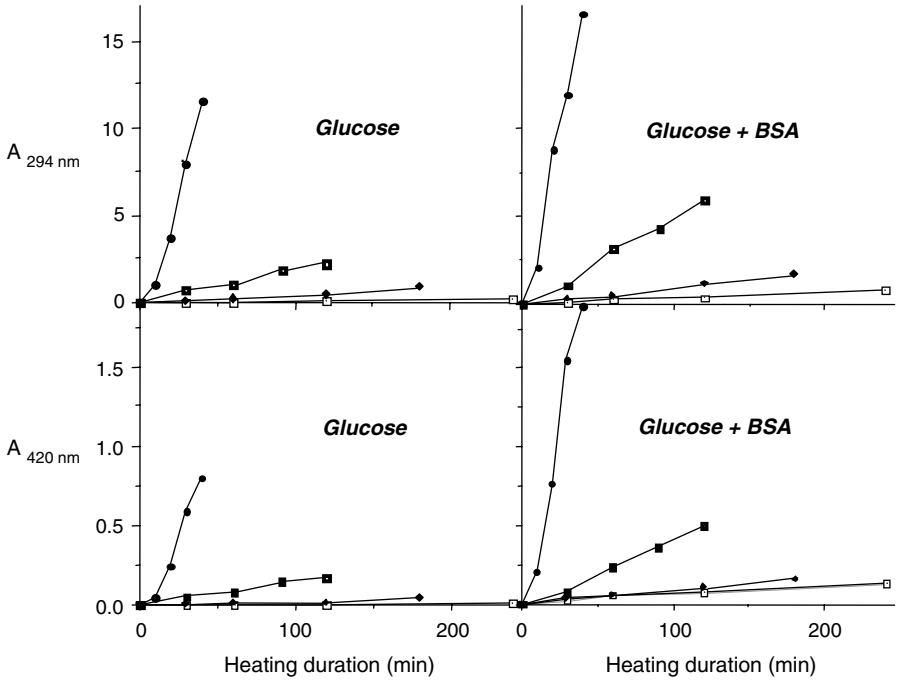

Fig. 4. Kinetics of UV absorbance and browning in glucose and glucose–BSA heated at pH 8.0. (60 °C: □, 70 °C: ♦, 80 °C: ■, 100 °C: ●).

higher values recorded during the intermediate stages (UV absorbance) may be due to the high reactivity of the di- and polycarbonyl compounds generated during these stages. In our previous study (Ajandouz & Puigserver, 1999) using glucose and glucose–lysine solutions heated at 100 °C and pH values ranging from 4.0 to 7.0, the caramelisation reaction was found to account for 40–62% and 10–36% of UV absorbance and browning, respectively. The percentage increases with the pH value. The higher percentages recorded in glucose–protein model systems are in agreement with the enhancing effect of alkaline pH on caramelisation.

At pH 9.7, in all the models studied here, the UV absorbance and browning rate constants obtained were 10- to 50-fold higher than those measured at pH 8.0. The rates of caramelisation occurring in the intermediate and final stages show the same pattern as at pH 8.0. The fact that the accelerating stage, which occurs from pH 8.0 to pH 9.7, decreases with increasing temperatures, suggests that as the temperature is increased, some switch may occur from alkalinity to heating as the main factor enhancing non-enzymatic browning.

The initial rate constants ($r^2 > 0.92$) of UV absorbance and browning were used to calculate the activation energies of the three model systems at the two pH values. The browning values are given in Table 3, along with relevant data from the literature. The UV absorbance E_a values obtained were fairly similar to the browning E_a values: 152, 128 and 129 kJ per mol at pH 8.0, and 123, 107 and 101 kJ per mol at pH 9.7, in the case of glucose, glucose–casein and glucose–BSA, respectively. This means that UV absorbance can be used to control browning in food, as it can be measured before browning ocurrs.

Table 3
Activation energy (E_a) associated with the browning process in glucose, glucose–protein and milk-based systems[a]

System	T (°C)	Analytical method	E_a (kJ mol^{-1})	References
Glucose pH 8.0	60–100	A_{420}	164	Present study
Glucose pH 9.7	//	A_{420}	126	//
Glucose+				
Casein pH 8.0	//	A_{420}	120	//
Casein pH 9.7	//	A_{420}	92	//
BSA pH 8.0	//	A_{420}	130	//
BSA pH 9.7	//	A_{420}	95	//
Lactose + casein	110–150	A_{420}	125	Morales and Van Boekel (1998)
Glucose + casein	110–150	A_{420}	121	Brands and Van Boekel (2001)
Lactose + casein	90–130	Kinetic modelling[b]	71–159	Brands and Van Boekel (2002)

[a] All the milk-based systems were formed in aqueous solution, pH 6.7.
[b] Multiresponse modelling methods were used to simulate 15 steps in non-enzymatic browning reactions, including isomerisation and degradation reactions, formation of Amadori products, formation of advanced Maillard reaction products and melanoidins. A_{420}: absorbance at 420 nm.

Overall, the values obtained here are in the 80–160 kJ per mol range taken by Bluestein and Labuza (1975) to correspond to browning, which is in good agreement with the range calculated by Brands and Van Boekel (2002) in a lactose–casein model system. The E_a value obtained here with a heated glucose–casein system at pH 8.0 is similar to those previously obtained on heated glucose–casein and lactose-casein at pH 6.7 (Morales & Van Boekel, 1998; Brands & Van Boekel, 2001), which suggests that the pH has little effect in the 6.7–8.0 range. In our studies, the values obtained with glucose heated alone were found to be one fifth to one fourth higher than those obtained with glucose–protein mixtures at either pH 8.0 or pH 9.7. On the other hand, the 20–30% decrease in E_a recorded here from pH 8.0 to pH 9.7 reflects the catalytic effects of hydroxide ions on both the dehydration/degradation reactions involving the reducing sugar (De Bruijn et al., 1986), and on the polymerisation reactions involving the subsequently formed reactive intermediates (Hodge, 1953). The activation energy values associated with browning in food and food model systems have been mainly determined so far in substances with a low or intermediate moisture content (Labuza & Saltmarch, 1981; Reynolds, 1963). The E_a was generally found to increase with the water activity. Little attention has been paid so far to the effects of pH, especially under alkaline conditions, on the non-enzymic browning reactions, although alkaline treatments are usually used in plant protein extraction processes to obtain textured protein foods, protein concentrates and isolates (Whitaker & Feeney, 1983). A need has therefore arisen for well-designed experiments specifically addressing the effects of the pH on these chemical events.

4. Conclusion

Using simple model systems, it was attempted here to draw up an overall picture of non-enzymatic browning reactions, based on kinetic studies performed at various stages in this process using appropriate analytical methods. Kinetic studies are an excellent means of predicting and managing food browning processes, as mentioned by Van Boekel (2001). The kinetic constants of the two proteins tested here were in good agreement with their amino group contents and their solubility with all the markers used. The data obtained clearly show that protein polymers grow continuously while being heated from 60 to 100 °C at moderately alkaline pH levels, although the high activation energy associated with amino group loss decreases when glucose is added. This finding extends our knowledge of non enzymatic browning reactions (Negroni, D'Agostina, & Arnoldi, 2001) as far as the pools of products generated are concerned, although it may not be really possible to define strict limits in the complex chains of chemical reactions involved in these processes. In food, protein-protein polymers may contribute to the texture but they will probably be of little nutritional value, in comparison with monomeric proteins.

Surprisingly, the reticulation reaction was found to be inhibited when the pH level was increased from 8.0 to 9.7. Further studies are required on this point, and it will be necessary to determine the behaviour of these proteins in lower pH ranges. It is also worth noting that the amino group losses occurring under specific conditions are much more severe in food systems than in model systems (Table 2).

The activation energy values associated with the disappearance of glucose in all the present model systems at both pH levels studied were around 90 kJ per mol. This value is lower than that associated with amino group loss (i.e., around 100 kJ per mol in glucose–protein model systems), which indicates that caramelisation occurs readily during the initial stages of these reactions. Brands and Van Boekel (2001) have established that caramelisation accounts for most of the mass of the products obtained in heated casein-reducing sugar models under milk pH levels. Based on the present data, it seems likely that the amino groups may have strictly catalytic effects at increasing alkalinity levels and contribute less to the brown products, the formation of which is strongly enhanced at alkaline pH levels.

The pH and the presence of amino groups were found to have more marked effects on UV absorbance and browning, since the E_a values ranged in this case from 90 to 150 kJ per mol. The intermediate and final stages in the reaction clearly showed parallel patterns. It is also worth noting that the E_a values obtained in a glucose–casein system at pH 8.0, which ranged from 120 to 125 kJ per mol, were similar to those determined in the same model system at pH 6.7 (Table 3).

Finally, it was difficult to draw any general conclusions about the effects of water activity on Maillard reactions based on amino group loss measurements, because of the discrepancies between the activation energy values published in the literature. A similar problem arose as far as the effects of the pH were concerned, but in this case, the reason was the lack of data on this topic in the literature.

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