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Influence of Salts, Amino Acids and Urea on the Non-enzymatic Browning of the Protein–Sugar System

Chay B. Pham

National Institutes of Biotechnology and Applied Microbiology (BIOTECH),
University of the Philippines at Los Baños, College,
Laguna 4031, Philippines

&

Jean C. Cheftel

Laboratoire de Biochimie et Technologie Alimentaires,
Université Des Sciences et Techniques du Languedoc,
Place E. Bataillon, 34060 Montpellier Cedex, France

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ABSTRACT

Solution, gel and powder mixtures of food model systems were studied for the non-enzymatic browning reaction with or without the potential browning inhibitors sodium chloride, ammonium chloride, glutamic acid, aspartic acid, cysteine and urea at buffer pH 7 under thermal processing treatment. The absorbances (A_{420}) of available lysine, and sugar were determined.

The effect of potential browning inhibitor was dependent on the lysine:glucose ratio, temperature, time and food model system.

The rate of the non-enzymatic browning reaction, and loss of lysine in the lysine-glucose solutions treated with cysteine, urea or NH_4Cl was greatly decreased during heat treatment at 80°C for 180 h, while in the caseinate–glucose gel model system mixed with aspartic acid, urea or cysteine, the brown colour which occurred at 100°C for 2 h was strongly inhibited. The browning inhibition was also consistent in the model powder systems mixed with 1% (w/w) urea or 0·5% (w/w) sodium chloride and 0·5% (w/w) ammonium chloride subjected to different water activities of storage at 30°C for 8 weeks.

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INTRODUCTION

The browning reaction between carbonyl groups from reducing carbohydrates and ε-amino groups of amino acids in food protein–sugar systems during the preparation, processing and storage of foods, caused by heat which is well known as the Maillard reaction, may lead to undesirable colour, flavour and nutritional losses by decreasing essential amino acids. One nutrient on which considerable interest has been focused is lysine, which is made biologically unavailable.

Several authors have researched on non-enzymatic browning (e.g. Wolfrom *et al.*, 1974; Warmbier *et al.*, 1976). The browning Maillard reaction was found to be dependent on the amino acid and sugar which take part in the reaction as well as on reaction conditions such as pH and the molar ratio of amino acid to sugar (Aymard *et al.*, 1978).

This study was designed to investigate the effects of some salts, amino acids and urea on the browning reaction in solution, gel and powder mixture of food model systems.

MATERIALS AND METHODS

Materials

The protein ingredient used was sodium caseinate (90·5% protein, 4% water, pH 6·59) from BEL Industries (Vendôme, France). It was stored at 4°C in the dark until used.

Mono-*N*-dinitrophenyl-lysine hydrochlomonohydrate (DNP-L), fluoro-2,4-dinitrobenzene (FDNB), L-lysine, L-glutamic acid, L-aspartic acid, urea and sodium chloride are from Merck.

Pronase (B-grade) came from Calbiochem, San Diego. Glucose-oxidase, peroxidase, *o*-dianisidine dihydrochloride, Triton X-100 and Tris were purchased from Sigma. All other reagents were of analytical grade.

Food model systems

Model solutions

Ten millilitre solutions (deionized–distilled water) of either: (a) 0·06M lysine, 0·04M glucose and 0·02M potential browning inhibitor(s) or (b) 0·04M lysine, 0·4M glucose and 0·04M potential browning inhibitors were placed in 25 ml test tubes. The initial pH was adjusted to the selected value using 2M NaOH or HCl. The test tubes were sealed with an oxygen gas burner and heated at 80°C for 180 h to assess reaction kinetics. The test tubes were then cooled in

running tap water. The potential browning inhibitors used in this study were the following: NaCl, NH_4Cl, urea, L-glutamic acid, L-aspartic acid and cysteine.

Model gels

Ten millilitre gel model systems were prepared in 25 ml glass vials containing (a) 0·0035M sodium caseinate, 0·22M glucose and 0·37M ammonium chloride or 0·17M urea; (b) 0·0035M sodium caseinate, 0·25M glucose and 0·12M or 0·04M potential browning inhibitor (NaCl, urea, NH_4Cl, glutamic acid, aspartic acid, or cysteine). The pH was adjusted to 7 using 2M NaOH or HCl. The glass vials were capped and then placed in a 100°C water bath.

The gel model systems (a) were studied for changes of colour and lysine retention during the time course of incubation. The systems (b) were studied for comparison of the potential browning inhibitors.

Model powders

Powder mix model systems were prepared as follows: 67·3 g sodium caseinate and 31·7 g glucose were mixed either with (a) 1 g urea or (b) 0·5 g NaCl and 0·5 g NH_4Cl in 100 ml deionized–distilled water and then adjusted to pH 7 with 2M NaOH. These samples were freeze-dried and then ground. The open capsules which contained the powdered samples were placed under vacuum in desiccators over saturated salt solutions (CH_3 COOK: $a_w = 0{\cdot}231$; K_2CO_3: $a_w = 0{\cdot}440$; $CuCl_2$: $a_w = 0{\cdot}680$).

The desiccators were kept at 30°C for 2, 4, 6 and 8 weeks. At the end of the various storage periods, samples were collected for determination of brown colour and available lysine.

Analytical methods

Determination of moisture content

Moisture content of powder mix-model-samples was determined by the method of the AOAC (1975). A 0·5 g sample was dried in the oven at 105°C for 24 h.

Determination of browning

The absorbance of the filtered brown solutions was measured at 420 nm in a Beckman UV spectrophotometer after appropriate dilution in order to bring the A_{420} readings within the 0·1–1·0 range.

For the model gel and powder mix systems, the samples were previously digested with pronase enzyme according to the method of Clark and Tannenbaun (1970). To complete sample digestion for colour extraction, a 10% (w/w) pronase was used. The solutions, digested after incubation at

37°C for 1 h, were centrifuged at 12 500*g* for 15 min. The supernatants were filtered on Millipore filters (0·45 μm). These filtered supernatants were diluted (20 to 200-fold) in order to bring A_{420} readings within the 0·1–1·0 range.

Determination of available lysine
Available protein-bound lysine was determined using the fluorodinitrobenzene (FDNB) procedure of Carpenter as modified by Booth (1971). Absorbance was read at 435 nm. A correction factor of 1·09 was used for the calculation of available lysine.

Determination of free amino acids
The concentration of free amino acids after model solutions were heated was determined with a Technicon Auto Analyser. A 1 ml heated solution was diluted to 10 or 20 ml and 20 μl of the diluted solution was injected for analysis. The standard of 2·5 mM free amino acids solution was also analyzed for comparison.

Determination of sugars
Glucose content in samples was determined using the colorimetric glucose–oxidase peroxidase method of Dahlqvist (1964). Absorbance was read at 412 nm.

RESULTS AND DISCUSSION

Model solutions

The effects of sodium chloride, ammonium chloride, urea, glutamic acid, aspartic acid and cysteine on the brown colour and lysine retention in glucose–lysine model solutions at pH 7, after 180 h of incubation at 80°C are shown in Table 1. In all model solutions, the browning was greatly reduced with potential browning inhibitors. The effectiveness of potential browning inhibitors increased in the order of sodium chloride, glutamic acid, aspartic acid, ammonium chloride, urea to cysteine. Higher lysine and glucose retentions were obtained in the model solutions heated with the potential browning inhibitors. The retention of lysine in the model solution with cysteine was more than twice that of the lysine and glucose solution alone. It is possible that the reaction rate of cysteine with glucose was faster than that of lysine with glucose.

It can also be seen that the potential browning inhibitor depended on lysine concentration in the model solution (Table 2). The inhibition was

TABLE 1

Effect of NaCl, NH_4Cl, Urea, Glutamic Acid, Aspartic Acid and Cysteine on the Brown Colour (A_{420}) and Lysine Retention in Lysine–Glucose Model Solutions of pH 7 kept at 80°C for 180 h[a]

Composition of model solution	*Retention (%)*			A_{420}
	Lysine	*Glucose*	*Potential browning inhibitor*	
0·06M lysine + 0·04M glucose	35	2	—	14·7
0·06M lysine + 0·04M glucose + 0·02M NaCl	51	6	—	8·7
0·06M lysine + 0·04M glucose + 0·02M NH_4Cl	65	5	12[b]	6·9
0·06M lysine + 0·04M glucose + 0·02M urea	78	6	7[c]	5·6
0·06M lysine + 0·04M glucose + 0·02M glutamic acid	62	6	26[d]	7·8
0·06M lysine + 0·04M glucose + 0·02M aspartic acid	63	6	21[e]	7·1
0·06M lysine + 0·04M glucose + 0·02M cysteine	79	14	9[f]	2·9

[a] Average of two replicates.
[b] NH_4Cl.
[c] Urea.
[d] Glutamic acid.
[e] Aspartic acid.
[f] Cysteine.

TABLE 2

Effect of NaCl, NH_4Cl, Urea, Glutamic Acid, Aspartic Acid and Cysteine on the Brown Colour (A_{420}) and Lysine Retention in Lysine–Glucose Model Solutions of pH 7 kept at 80°C for 180 h[a]

Composition of model solution	*Retention (%)*			A_{420}
	Lysine	*Glucose*	*Potential browning inhibitor*	
0·04M lysine + 0·4M glucose	42	90	—	14·5
0·04M lysine + 0·4M glucose + 0·04M NaCl	58	94	—	7·8
0·04M lysine + 0·4M glucose + 0·04M NH_4Cl	64	95	34[b]	6·2
0·04M lysine + 0·4M glucose + 0·04M urea	69	97	29[c]	4·2
0·04M lysine + 0·4M glucose + 0·04M glutamic acid	62	95	37[d]	6·4
0·04M lysine + 0·4M glucose + 0·04M aspartic acid	66	96	34[e]	5·9
0·04M lysine + 0·4M glucose + 0·04M cysteine	79	98	20[f]	2·1

[a] Average of two replicates.
[b] NH_4Cl.
[c] Urea.
[d] Glutamic acid.
[e] Aspartic acid.
[f] Cysteine.

greater in the systems containing less lysine. The retention of lysine is always higher in model solution systems with potential browning inhibitor than in the control lysine–glucose solution. The potential browning inhibitor concentrations decreased during the thermal process treatment with the rate of decrease directly proportional to the retention of lysine. Data presented in Tables 1 and 2 suggest that the reaction rate of browning of model solution was correlated with the interactions between lysine and potential browning inhibitor. Without the use of the potential browning inhibitors, the A_{420} values are almost the same in the model solution whether with 0·04M lysine or 0·04M glucose concentration (Tables 1 and 2). It could be concluded that the browning reaction rate reached a steady state after 180 h incubation at 80°C and was controlled by a low concentration of the reactant.

Model gels

The effectiveness of the potential browning inhibitor was also evaluated in the model gel system. The kinetic data of colour and lysine retention in sodium caseinate–glucose gels with and without ammonium chloride at pH 7 during the time course of incubation at 100°C are shown in Fig. 1. The initial reaction rate of browning in the caseinate with glucose system is almost twice that of the caseinate with ammonium chloride and glucose system. This also corresponded to the decrease in the lysine retention.

The colour was greatly inhibited in the model gel system containing urea (Fig. 2). It is also noticed that the reaction of the caseinate–ammonium chloride–glucose system shows more intense browning than that of the caseinate–urea–glucose system.

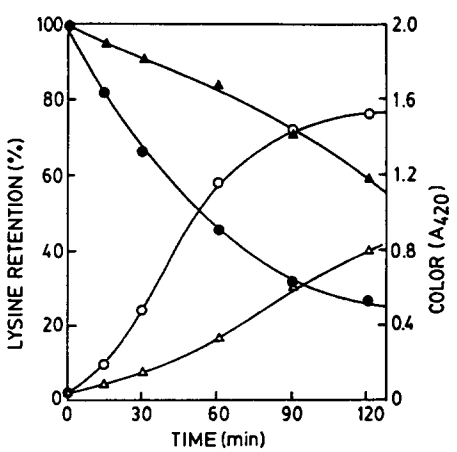

Fig. 1. Effect of ammonium chloride (0·37M) on the colour (A_{420}) and lysine retention in model gel systems of 0·0035M sodium caseinate and 0·22M glucose at pH 7 and temperature of 100°C. Colour (A_{420}): ○, control; △, NH_4Cl. Lysine retention: ●, control; ▲, NH_4Cl.

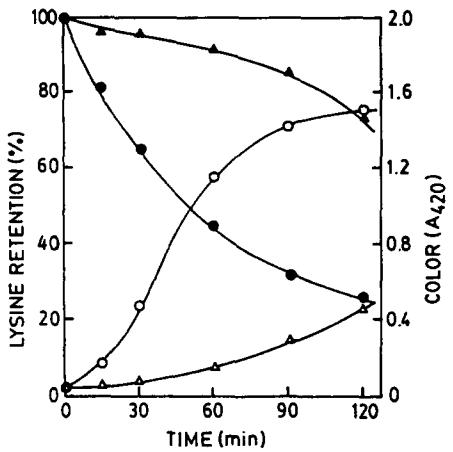

Fig. 2. Effect of urea (0·17M) on the colour (A_{420}) and lysine retention in model gel systems of 0·0035M sodium caseinate and 0·22M glucose at pH 7 and a temperature of 100°C. Colour (A_{420}): ○, control; △, urea. Lysine retention: ●, control; ▲, urea.

TABLE 3
Effect of Potential Browning Inhibitors on the Colour (A_{420}) of Caseinate–Glucose Gel Systems at pH 7 after 2 h of Incubation at 100°C[a]

Composition of model gel	A_{420}
0·003 5M caseinate[b] + 0·25M glucose	1·61
0·003 5M caseinate + 0·25M glucose	
+ 0·12M glutamic acid or	1·10
+ 0·04M glutamic acid	1·34
0·003 5M caseinate + 0·25M glucose	
+ 0·12M aspartic acid or	0·92
+ 0·04M aspartic acid	1·12
0·003 5M caseinate + 0·25M glucose	
+ 0·12M cysteine or	0·45
+ 0·04M cysteine	0·59
0·003 5M caseinate + 0·25M glucose	
+ 0·12M urea or	0·68
+ 0·04M urea	0·80
0·003 5M caseinate + 0·25M glucose	
+ 0·12M NH_4Cl or	1·05
+ 0·04M NH_4Cl	1·31
0·003 5M caseinate + 0·25M glucose	
+ 0·12M NaCl or	1·19
+ 0·04M NaCl	1·29

[a] Average of two replicates.
[b] 25 000 MW caseinate contained 9·34 g available lysine/16 g nitrogen.

The effects of the potential browning inhibitors, glutamic acid, aspartic acid, cysteine, sodium chloride, urea and ammonium chloride on model gels of the caseinate–glucose system were also studied for comparison (Table 3). The highest colour (1·60 A_{420}) is in the caseinate–glucose gel control. Urea and cysteine give very strong inhibition of colour in the caseinate–glucose gel systems.

As the concentration of potential browning inhibitors increased from 0·04M to 0·12M in the gel system, the colour decreased (Table 3).

Model powders

The non-enzymatic browning reaction of the sodium caseinate–glucose powder mixed with urea of pH 7 and stored for 0–8 weeks at 30°C is shown in Fig. 3. It can be seen that the rate of browning reactions for the powder mix model system without urea is higher than those with urea during storage. This may be due to the protein of caseinate complexing with urea, resulting in unavailable ε-amino groups and consequently decreased brown colour.

As the water activity of the powder mix models increased from 0·231 a_w to 0·684 a_w, the brown colour of samples increased (Fig. 3). A linear correlation of colour (A_{420}) and storage time was observed with different water activity values.

A similar trend of colour change in the model powder mix of 0·5% (w/w) NaCl and 0·5% (w/w) NH_4Cl is seen in Fig. 4. However, the colour (A_{420})

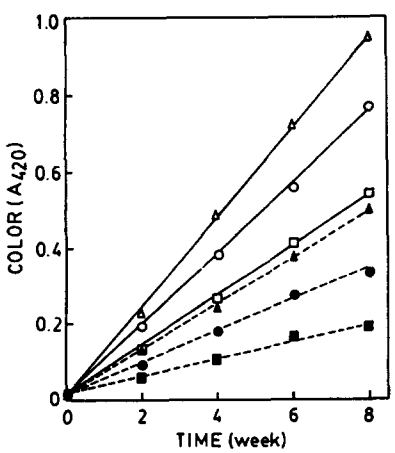

Fig. 3. Colour (A_{420}) in powder mix model of 67·3% sodium caseinate and 31·7% glucose with or without 1% urea, pH 7 during storage at 30°C at different water activities (a_w). With urea: ■, $a_w = 0{\cdot}231$; ●, $a_w = 0{\cdot}440$; ▲, $a_w = 0{\cdot}684$. Without urea: □, $a_w = 0{\cdot}231$; ○, $a_w = 0{\cdot}440$; △, $a_w = 0{\cdot}684$.

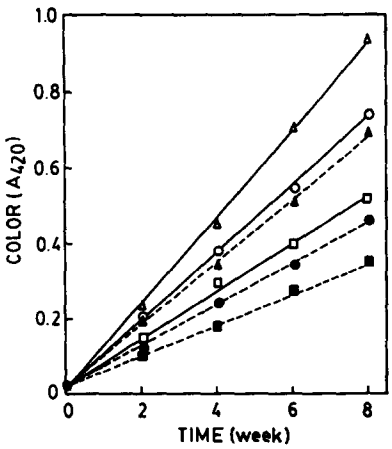

Fig. 4. Colour (A_{420}) in powder mix model of 67·3% sodium caseinate and 31·7% glucose with or without 0·5% NaCl and 0·5% NH_4Cl, pH 7 during storage at 30°C at different water activities (a_w). With NaCl and NH_4Cl: ■, $a_w = 0{\cdot}231$; ●, $a_w = 0{\cdot}440$; ▲, $a_w = 0{\cdot}684$. Without NaCl and NH_4Cl: □, $a_w = 0{\cdot}231$; ○, $a_w = 0{\cdot}440$; △, $a_w = 0{\cdot}684$.

was higher in the model mix with NaCl and NH_4Cl than in the model with urea. This could be related to the high potential browning inhibition of urea.

Additional information about the potential browning inhibitions of urea, sodium chloride and ammonium chloride in powder mix models was observed from the pattern of available lysine in systems stored for different time periods (Table 4). The loss of available lysine was lower in model powders mixed with the potential browning inhibitors than in model mixes of sodium caseinate–glucose. The available lysine retained was 55, 63 and 76%, respectively, in models without potential browning inhibitors, with

TABLE 4

Retention of Available Lysine (%) in Powder Mix Model-Systems (dw) of 67·3% Sodium Caseinate and 31·7% Glucose With and Without (Control) 1% Urea, and 0·5% NaCl and 0·5% NH_4Cl at pH 7 during Storage in Different Water Activities at 30°C

Storage time (weeks)	*Retention of available lysine (%)*								
	0·231 a_w			*0·440 a_w*			*0·684 a_w*		
	Control	*Urea*	*NaCl & NH_4Cl*	*Control*	*Urea*	*NaCl & NH_4Cl*	*Control*	*Urea*	*NaCl & NH_4Cl*
0	100	100	100	100	100	100	100	100	100
2	83	92·5	89·5	80	90·5	87	75·5	85·5	78·5
4	72·5	86	78·5	66·5	82	73	58	74	67
6	64	82	72·5	55	74·5	62·5	47	64	55·5
8	55	76	63	47·5	68·5	55·5	42·5	56·5	47

sodium chloride and ammonium chloride, and with urea at 0·231 a_w after 8 weeks of storage. This is consistent with the results of colour in that the retention of available lysine is higher in powder models mixed with urea, than in those with the combination of sodium chloride and ammonium chloride.

As the water activity increased from 0·231 a_w to 0·684 a_w the retention of available lysine was also decreased according to the effectiveness of potential browning inhibitors.

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