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A kinetic model for the reaction of tryptophan with glucose and mannose—the role of diglycation in the Maillard reaction

V. A. Yaylayan & N. G. Forage

McGill University, Macdonald Campus, Department of Food Science and Agricultural Chemistry, 21,111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, Canada, H9X 1CO

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The kinetics of the reaction of tryptophan with excess mannose and glucose were studied at two temperatures (110°C and 140°C) under identical conditions. The rate of disappearance of tryptophan and the rate of accumulation of the Amadori rearrangement product (ARP) formed were followed by RP-HPLC, over a period of 6 h. Since both sugars produce the same ARP, any changes in the kinetic parameters of the reaction can be attributed to the differences in the sugars, such as mutarotation rates and contents of open-chain forms. Kinetic analysis indicated that the first-order rate constant for the disappearance of tryptophan in the mannose system was 1·3 times that of glucose at 110°C, and 1·8 times that at 140°C. However, the rate of accumulation of ARP was generally much lower in the mannose system than in the glucose system. The differences in the rates of tryptophan disappearance can be attributed to the higher percentage of the acyclic form in mannose and its higher rate of mutarotation; the differences in the rate of ARP accumulation can be explained by the ARP formed in the mannose/tryptophan system reacting further with mannose to produce the diglycated Amadori product (diARP), whereas in the glucose/tryptophan system glucose reacts with the ARP at a much slower rate. It seems that the diglycated amino acid provides a more efficient pathway for browning.

INTRODUCTION

In considering the kinetics of the Maillard reaction it is important to define, accurately, the system under study and the terms used to characterize the rates of the different reactions involved. Non-enzymatic browning has special significance in food processing since it affects the sensory properties of the food product. Numerous factors are involved in determining the rate of browning, such as pH, water activity, temperature, sugar/amino acid ratio and the type of amino acid and sugar (Labuza & Schmidl, 1986; Kaanane & Labuza, 1989). From a mechanistic point of view, it is also interesting to know the relationship between the rate of browning and the rates of formation and/or loss of different reactants and intermediates involved in the Maillard reaction, in particular the key intermediate Amadori rearrangement product (ARP). Temperature plays an especially important role in proposing a kinetic model: at ambient or near ambient temperatures equilibrium conditions may prevail, while at higher temperatures these conditions may be upset by the availability of much faster decomposition pathways for the intermediates, which might prevent the attainment of such an equilibrium. Thus, a kinetic model proposed based on observations at ambient temperature does not apply to systems studied at higher temperatures. Hashiba (1982), for example, studied the effect of the reducing power of various sugars on the extent of browning with glycine at 120°C. The amounts of glycine consumed were found to be directly proportional to the intensity of browning; however, the amounts of ARP accumulated did not correlate with the extent of browning. In addition, sugars that had the highest concentration of the acyclic forms produced more browning when heated with glycine, but

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their corresponding ARPs produced the least amount of browning when heated alone. The lack of correlation between the rate of accumulation of ARP and the rate of browning produced during the Maillard reaction is expected, since ARPs should decompose and produce brown colour precursors or monomers such as pyrylium ions (Yaylayan & Lachambre, 1990) and their concentration should reach a maximum value before polymerization starts. Labuza and Massaro (1990), on the other hand, studied the rate of browning at 4°C and 30°C; at these temperatures, the amino acids showed an initial decrease in concentration followed by regeneration and attainment of steady-state equilibrium after 6 weeks of incubation at 30°C; hence, no correlation was found between the rate of amino acid loss and browning at lower temperatures than those used in Hashiba's (1982) studies. Takeoka *et al.* (1979) studied the rate of loss of tryptophan from a 6 molar excess glucose solution in methanol at 72°C. Under these conditions the amino acid concentration declined steadily and after 5·5 h had mostly disappeared, without reaching a steady-state concentration; this means that at temperatures higher than at least 30°C the rate of amino acid loss is always higher than the rate of its regeneration from the ARP and these two rates become equal only at lower temperatures. A similar result was obtained in our study, which was conducted at temperatures above 100°C.

The observation by Hashiba (1982) that sugars with the highest concentration of the acyclic forms produced more browning when heated with glycine (but their corresponding ARPs produced the least amount of browning when heated alone) led us to investigate the kinetics of the reaction of tryptophan with glucose and mannose. Mannose and glucose being epimers, the only difference in their structure is the orientation of the C-2 hydroxyl group. During the Maillard reaction these sugars react with amino acids and undergo Amadori rearrangement to produce 1-amino acid-1-deoxy-2-ketoses, thus losing the chirality of C-2 in the process. Mannose and glucose therefore produce the same Amadori compound when reacted with the same amino acid. Since mannose mutarotates faster than glucose, and contains more acyclic form of the sugar, a comparison of the reaction rates of these two sugars under the same conditions can enable us to elucidate the relationship between the reactivity of the sugars and (i) the amount of ARPs (formed and accumulated) and (ii) the extent of browning.

Amino acids, being primary amines, have more or less the same nucleophilicities—everything else being equal, such as the amounts of protonated form of the amino group of the amino acid. The decisive factor in determining the rate of amino acid consumption and hence the rate of browning (assuming the amino acids do not decompose under experimental conditions, as is the case with tryptophan) is the reactivity of the sugars involved. In considering the reactivity of sugars in the Maillard reaction two factors are important; the amount of acyclic form (which correlates with the reducing power of the sugar) and the rate at which the open-chain form interconverts with cyclic forms (mutarotation rate). Although sugars having higher rates of mutarotation do not necessarily have higher contents of acyclic forms, such higher rates of mutarotation might expose the amino acid to higher concentrations of open-chain forms *per unit time* and hence speed up the rate of loss of amino acid. Studies on the browning rate during the Maillard reaction indicate that sugars with the highest concentrations of the open-chain form cause browning at the fastest rates (Burton & McWeeny, 1963). These sugars also have the fastest rates of mutarotation (Lippich, 1932). However, this does not mean that the rate of ARP formation or the rate of browning should increase in the same ratio as the contents of open-chain forms when we compare two different sugars, unless they form the same ARP (as in the case of mannose and glucose) and the reactions are performed under identical conditions, especially temperature, which is known to increase the content of acyclic forms (Isbell & Pigman, 1969).

The kinetics of accumulation of ARP are complicated and will not be attempted in this paper. More detailed analysis of the tryptophan/mannose and glucose systems is underway in this laboratory to calculate the differences in the rates of formation of ARP and its accumulation when mannose is used instead of glucose. In this paper, however, a general kinetic model will be proposed for the Maillard reaction to explain qualitatively the rate of accumulation of ARP during the Maillard reaction. The model stipulates the reaction of ARP with a second mole of sugar, if the sugar is reactive enough, to form diglycated product which can provide an efficient pathway of decomposition and browning and, at high temperatures, can regenerate tryptophan, further complicating the rate of accumulation of ARP.

MATERIALS AND METHODS

L-Tryptophan, D-glucose, D-mannose, hydroxymethylfurfural (HMF), maltol and indole were purchased from Aldrich Chemical Company (Wisconsin, USA) and used without further purification; all the solvents were of HPLC grade (BDH, Chicago, USA). Mobile phases were degassed by application of vacuum with gentle agitation for 5 min. Samples were loaded via a Rheodyne injector with a 20-μl loop. A C-18 Ultrasphere, 5 μm, 2·0 mm × 150 mm column from Beckman or C-18 Lichrosphere, 100-RP-18, 5 μm, 2·0 mm × 150 mm column mounted with a guard column Licro CART 4-4 from Merck, was used in the analysis. The column was operated at ambient temperature. Repeated injections of 20 μl of the sample were needed to achieve stable peaks for the Amadori product. Water was obtained

from a Milli-Q reagent grade water system (Millipore Corp., Bedford, Massachusetts, USA). The wavelength used to detect the Amadori compound and its decomposition products was 280 nm. Retention time and quantifications represent the average of triplicate injections.

Instrumentation

The modular HPLC system used was a Beckman System Gold consisting of a variable wavelength UV detector model 166 and a 110B solvent delivery module controlled by a NEC laptop computer that was connected to a Shimadzu CR-18 integrator–printer operated on 10-mV scale.

Synthesis of tryptophan Amadori products (ARP)

The Amadori product of L-tryptophan with D-glucose was synthesized according to the procedure of Sgarbieri *et al.* (1973); 5·0 g D-glucose and 0·6 g of L-tryptophan were dissolved in 80 ml of methanol and refluxed for 5 h. The concentrate obtained after rotary evaporation was diluted with 3–4 ml of water, then directly applied to a cellulose column (44 mm × 550 mm). The column was packed with Whatman CF_{11} fibrous powder suspended in water-saturated *n*-butanol and eluted with the same solvent. Aliquots of 20 ml were collected and stored in the refrigerator overnight to allow crystallization, and the crystals were purified twice with *n*-butanol

General procedure for kinetic analysis

Two sets of six sealed ampoules, each containing 0·2 mg tryptophan and 0·66 mg glucose or mannose dissolved in 0·5 ml water, were mixed for 1 min. The initial pH of the glucose/tryptophan system was 5·1, while that of the mannose/tryptophan system was 5·0. The ampoules were then sealed using an oxygen–air flame. The reaction mixtures were heated in a pre-equilibrated dri-bath (Thermolyne) for a period of 6 h at 110°C for the first set and at 140°C for the second set. One ampoule was removed at the end of each hour, diluted with water to 2 ml, and filtered using a 0·45-μm membrane filter (type HA, from Millipore, Waters Scientific), before injection on to the column.

Identification of the ARP peak

The ARP peak was identified by direct comparison of its retention time with that of a synthetic sample under identical conditions, using different compositions of mobile phases and spiking the samples with a standard. The purities of the peaks were ensured by wavelength ratioing.

Kinetic analysis

The kinetics of the reaction of 0·2 mg L-tryptophan and 0·66 mg D-glucose or D-mannose in 0·5 ml water at 110°C and 140°C were studied for a period of 6 h, as described above. The contents of the reaction mixtures were analysed by RP-HPLC using a mobile phase consisting of 68% methoxyethanol, 30% phosphoric acid (0·01M) and 2% methanol; the flow rate was 0·5 ml min^{-1}. Disregarding the actual mechanism or the number of steps involved, a pseudo-first-order reaction was assumed in determining the kinetics of disappearance of tryptophan; that is $\ln R/R_0 = -kt$, where R is the concentration of the reactant (mol $litre^{-1}$), R_0 is the concentration of reactant and t is the time (h). Regression analyses were performed using a standard non-linear regression model (Lotus 1-2-3 software package).

RESULTS AND DISCUSSION

The products formed from the reaction of tryptophan with glucose or mannose and their retention times are shown in Table 1. In an earlier study (Yaylayan & Forage, 1991) the kinetics of formation of the compounds shown in this table from the decomposition of the tryptophan ARP were examined when the ARP was heated alone. The first-order rate constant for the disappearance of ARP in the absence of sugar was found to be dependent on the temperature and the

Table. 1. Retention times (min) of the ARP and its decomposition products

Compound	t_R[a]
HMF[b]	2·1
Maltol	2·6
diARP[c]	2·9
Indole	3·7
ARP[d]	4·7
TRP[e]	7·4

[a] 68% Methoxyethanol, 30% phosphoric acid (0·01M) and 2% methanol; flow rate 0·5 ml min^{-1}.
[b] Hydroxymethylfurfural.
[c] Diglycated Amadori rearrangement product.
[d] Amadori rearrangement product.
[e] Tryptophan.

Table 2. First-order rate constants[a] for the disappearance of TRP–ARP

	1·0 mg TRP–ARP 0·65 ml^{-1} water	1·0 mg TRP–ARP 0·75 ml^{-1} water
$k^{110°C}$	**0·52**	**0·36**
r^2	0·97	0·97
$k^{140°C}$	**1·83**	**1·69**
r^2	0·81	0·96

[a] k (h^{-1}).

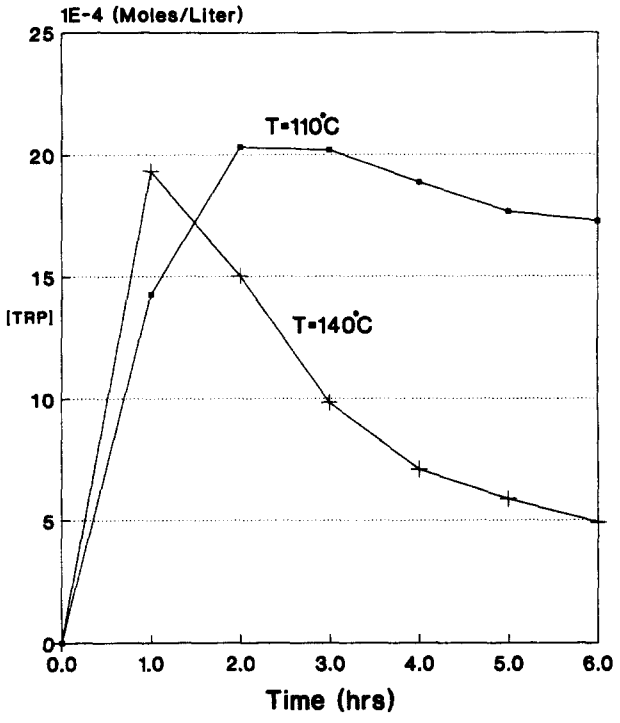

Fig. 1. The plot of concentration versus time for the generation of tryptophan from its Amadori product.

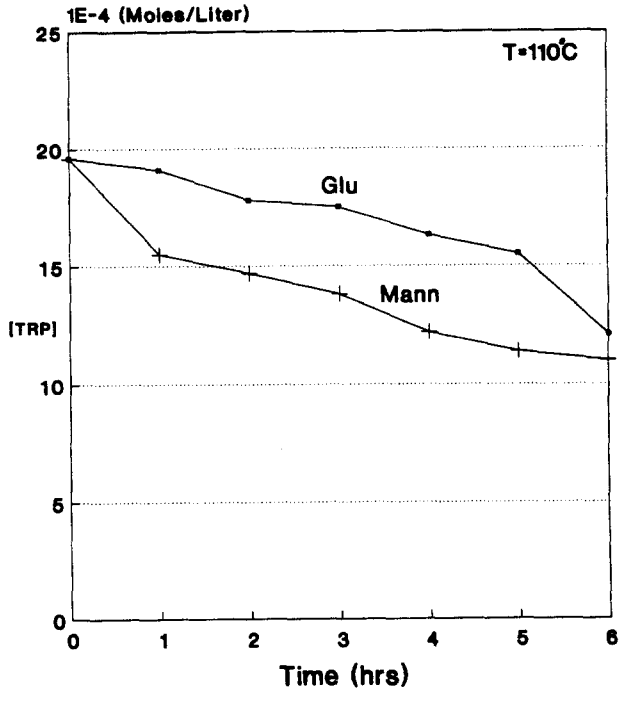

Fig. 2. The plot of concentration versus time for the disappearance of tryptophan from glucose/tryptophan and mannose/tryptophan reaction mixtures, at 110°C.

water content of the reaction mixture (see Table 2). However, the rate of accumulation of tryptophan (regenerated from ARP) showed a nonlinear behaviour, reaching a maximum concentration after 1 h of heating at 140°C and between 2 h and 3 h when ARP was heated at 110°C (Yaylayan & Forage, 1991) (see Fig. 1), which indicated that, after reaching a certain concentration, tryptophan started to react with carbonyl-containing compounds generated from the decomposition of ARP.

In the present study, the pseudo-first-order rate constants for the disappearance of tryptophan from glucose and mannose mixtures were calculated (see Table 3) and the amounts of ARP accumulated were measured at two different temperatures, over a period of 6 h, to study the effect of the reactivity of the sugar (contents of acyclic forms) and its relation to the rates of tryptophan disappearance and the accumulation of the ARP. Although there are conflicting reports in the literature concerning the contents of acyclic forms of mannose and glucose (Kaanane & Labuza, 1989), there is agreement that the mannose content of the acyclic form is at least twice that of glucose at ambient temperature. It is

Table 3. First-order rate constants[a] for the disappearance of tryptophan from glucose/mannose reaction mixtures

	Glucose		Mannose	
	k_1	r^2	k'_1	r^2
110°C	**0·07**	0·85	**0·09**	0·94
140°C	**0·31**	0·85	**0·60**	0·92

[a] k (h^{-1}).

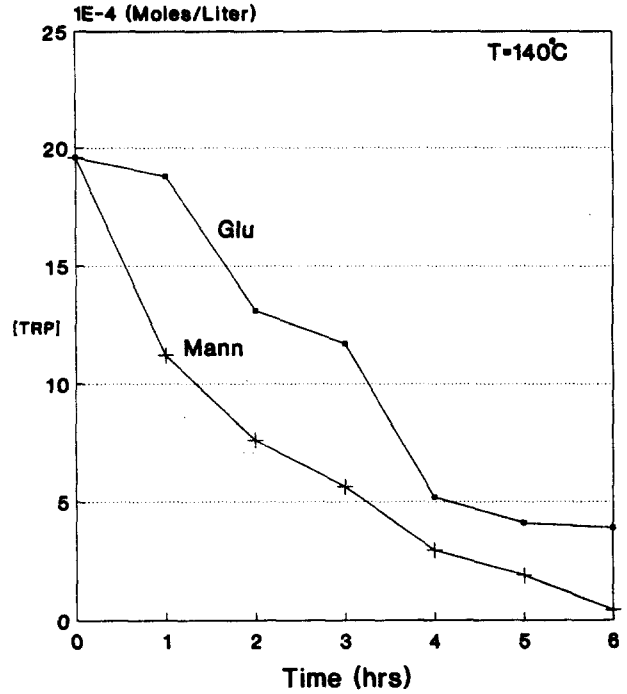

Fig. 3. The plot of concentration versus time for the disappearance of tryptophan from glucose/tryptophan and mannose/tryptophan reaction mixtures, at 140°C.

expected, therefore, that tryptophan should disappear from the reaction mixture of mannose at a faster rate than from the glucose mixture. According to Table 3 (see also Figs 2 and 3), this is indeed the case; tryptophan disappears 1·3 times faster in the mannose system at 110°C and 1·8 times faster at 140°C, assuming first-

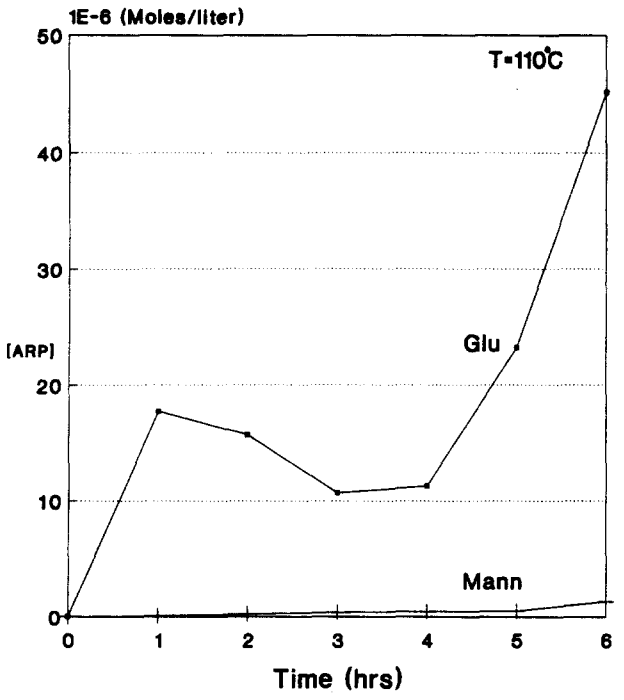

Fig. 4. The plot of concentration versus time for the accumulation of Amadori rearrangement product in the glucose/tryptophan and mannose/tryptophan reaction mixtures, at 110°C.

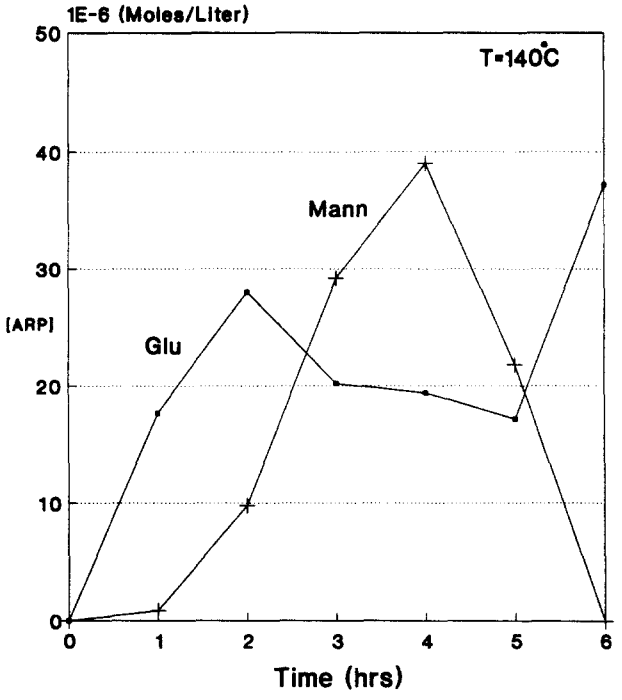

Fig. 5. The plot of concentration versus time for the accumulation of Amadori rearrangement product in the glucose/tryptophan and mannose/tryptophan reaction mixtures, at 140°C.

order kinetics. Since the two reactions were run under identical conditions, the only reason for the higher rate of disappearance of tryptophan from the mannose system is that it reacted with mannose at a faster rate due to the higher content in mannose of the acyclic form. Consequently, it is expected that the mannose system should accumulate ARP at a faster rate, since the temperature and the structure of ARP is the same in both systems. However, inspection of Figs 4 and 5 indicates that, generally, this is not the case. In addition, the ac-

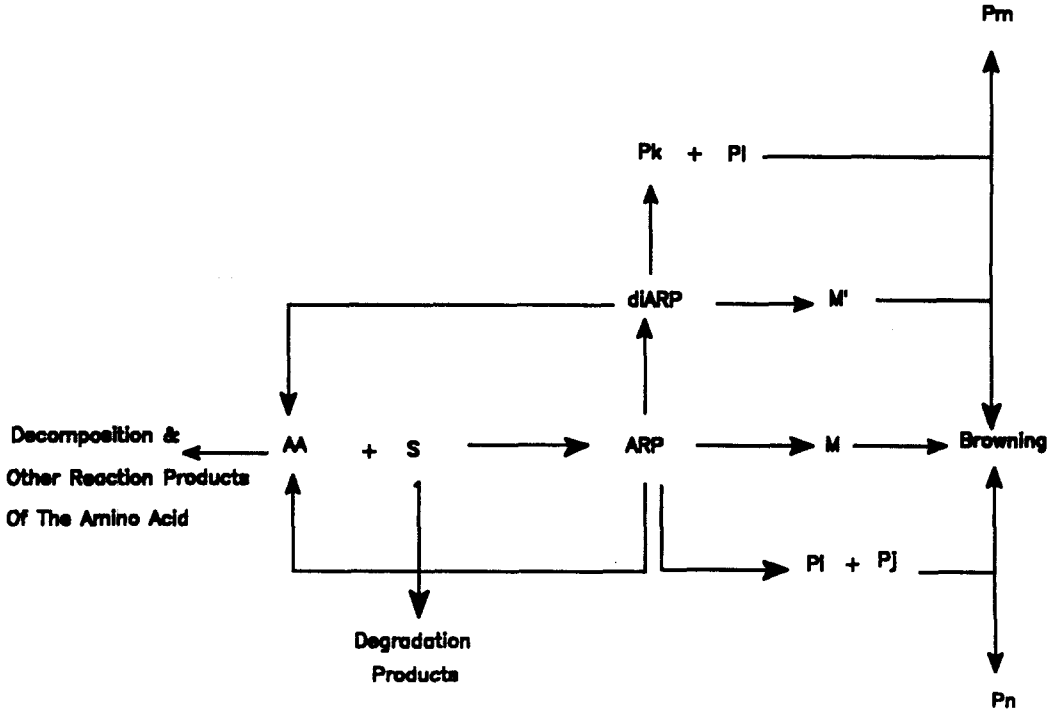

Fig. 6. General kinetic model for the reaction of amino acids with sugars. AA, amino acid; S, sugar; M and M', monomers; ARP, Amadori rearrangement product; diARP, diglycated Amadori rearrangement product; Pi, Pj, Pk, Pl, Pm and Pn are sets of different products.

cumulation of ARP in the glucose system exhibited a nonlinear behaviour. These observations can be explained qualitatively, by proposing a general kinetic model for the Maillard reaction as shown in Fig 6. This model differs from the one proposed by Hodge (1953) in that it stipulates the formation of diglycated ARP when more reactive sugars are used. The amino groups of the ARPs are secondary amines and, hence, less nucleophilic than the primary amino groups of the amino acids; consequently, more reactive sugars are required to form significant amounts of diglycated product.

Formation of diglycated Amadori product (diARP)

To verify that ARPs react with more reactive sugars, the reactions of the tryptophan ARP with glucose and mannose were studied at 110°C, using the same procedure as outlined above; in both systems, after 30 min of heating, a peak appeared at the retention time of 2·9 min, which increased in intensity after 90 min and after increasing the molar ratio of the sugar/ARP from 1 to 3. Since this peak was absent from the separately heated solutions (at 110°C) of the ARP and the sugars, it can only be produced as a result of their interaction. Since diARP is more polar than ARP, it is expected that diglycated product should elute faster (2·9 min) than monoglycated product (4·7 min) on a reversed phase column. In addition, the intensity of the peak in the ARP/mannose system was three times that of the ARP/glucose system at the end of 90 min of heating, indicating that the reactivity of the sugar is important in forming this product. Comparison of the chromatograms of the reaction mixtures of the tryptophan/glucose and tryptophan/mannose systems shows that the peak at the retention time of 2·9 min is absent from the tryptophan/glucose reaction mixture at both temperatures, whereas in the tryptophan/mannose system, at 110°C, the peak gradually forms and disappears after 3 h and at 140°C after 1 h.

The effect of the reactivity of the sugars and the reaction temperature on the rate of accumulation of the ARP

At ambient temperature, mannose mutarotates 1·7 times faster than glucose and contains approximately twice the amount of acyclic form as glucose (Labuza & Schmidl, 1986). As the temperature is increased, the mutarotation rates and the concentrations of the acyclic forms will also increase (Pigman & Isbell, 1968); however, there are few data on the values at higher temperatures. Hashiba (1982), for example, showed that the reducing power of glucose and, hence, the content of the open-chain form, increases as temperature is increased, especially between 60 and 100°C, and that different sugars exhibited different rates of increase in their contents of acyclic forms as the temperature was increased.

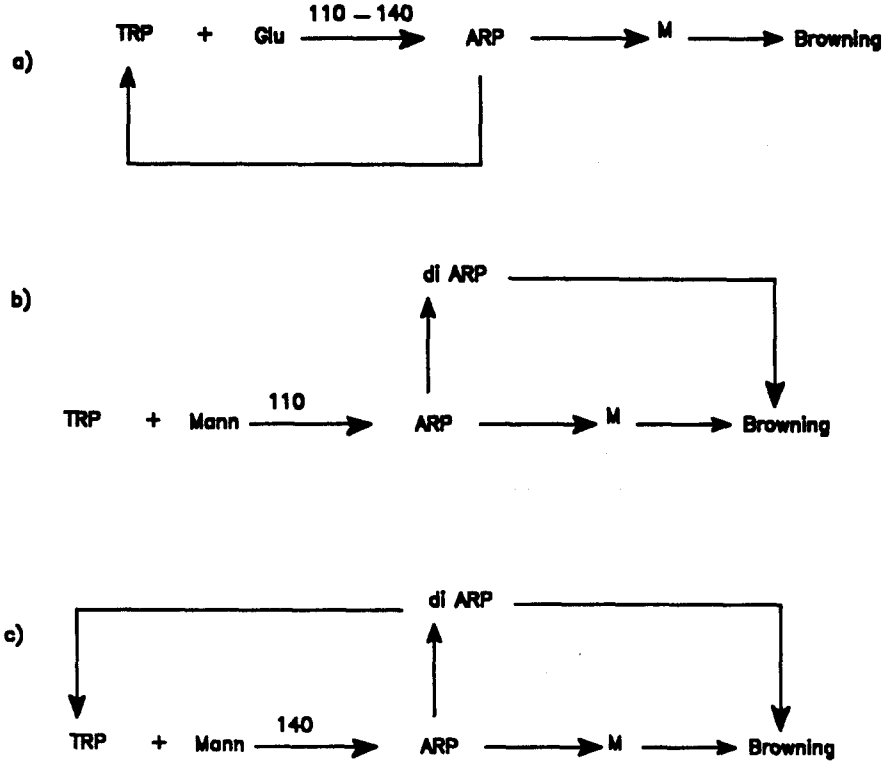

Fig. 7. (a) Simplified kinetic model for the reaction of tryptophan with glucose, at 110 and 140°C; (b) simplified kinetic model for the reaction of tryptophan with mannose at 110°C; (c) simplified kinetic model for the reaction of tryptophan with mannose at 140°C. TRP, tryptophan; Mann, mannose; Glu, glucose; M, monomer; ARP, Amadori rearrangement product; diARP, diglycated Amadori rearrangement product.

Since the rate of disappearance of tryptophan from the mannose system was higher, the mannose system was expected to accumulate ARP at a faster rate than glucose as the rate of decomposition of the ARP is the same in both systems; however, this was not the case. There must be other reaction pathways available to the ARP in the mannose system that remove it from the reaction mixture, in addition to its decomposition which is common to both systems. ARPs derived from primary amines are known to react with another molecule of a reducing sugar to form the diglycated products (diARP) (Burton & McWeeny, 1964); these can decompose more readily in water than monoglycated products (Gottschalk, 1972), producing reactive intermediates. Since mannose is more reactive than glucose, the ARP formed in the mannose/tryptophan system might react further with the free mannose forming the diglycated product (since the reactions were done in excess sugar), whereas the ARP formed in the glucose/tryptophan system can survive longer and hence accumulate faster. A simplified version of the general kinetic model proposed for the Maillard reaction and shown in Fig. 6 can help explain the experimental observation that the rate of accumulation of ARP did not follow the rate of disappearance of tryptophan in the tryptophan/mannose and tryptophan/glucose mixtures when the two systems were compared. Figures 4 and 5 show the rate of accumulation of ARP at 110°C and at 140°C, respectively.

The behaviour of the accumulation rate of the ARP at both temperatures in the tryptophan/glucose system (Fig 4 and 5) can be explained by the simplified kinetic model of the Maillard reaction shown in Fig 7(a). At both temperatures, the initial rate of ARP formation is faster than the rate of its decomposition; hence, the concentration of the accumulated ARP reaches a maximum. The rate of the decomposition of ARP becomes dominant after 1 h of heating at 110°C and after 2 h at 140°C. However, since tryptophan can be regenerated from the decomposition of ARP this can trigger an increase in the rate of ARP formation, making the rate of ARP formation higher than its rate of decomposition again, causing the second rate maximum seen in Fig. 4 for glucose. In the case of the tryptophan/mannose system, due to the presence of the more reactive sugar, the ARP reacts at 110°C with the sugar to form diARP rather than regenerate tryptophan (see Fig. 7(b)). That is, the rate of tryptophan generation from the ARP is not significant enough, which causes the rate disappearance of ARP to be much faster; hence, the increase in the rate of ARP accumulation is much slower than in the case of the glucose system. Consequently the reaction slowly reaches a maximum value and is expected to begin to decline sometime after 6 h. However, at 140°C, the rate of regeneration of tryptophan from the diARP becomes significant (see Fig. 7(c)). This makes the rate of ARP formation faster than its decomposition, which causes the rate of ARP accumulation to reach a maximum value after 4 h of heating, and then begin to decline; after 6 h, it almost disappears. It is also noteworthy that the diARP peak in the tryptophan/mannose system at 140°C forms and disappears before ARP reaches its maximum concentration, indicating a relationship between the two events.

Browning rates

The formation of the diglycated product might partially explain Hashiba's (1982) observation that sugars with the highest concentration of the acyclic forms produced more browning when heated with the amino acids but their corresponding ARPs produced the least amount of browning when heated alone; that is, ARPs of the more reactive sugars brown at slower rates when heated alone than when heated in the presence of their respective sugars. In the absence of the reactive free sugar, the diglycation of the ARP is prevented and, hence, the more efficient pathway of browning is absent. The mannose/glycine system browns 1·6 times faster than the corresponding glucose solution with glycine at 120°C (Hashiba, 1982). This increased rate of browning can be attributed to the presence of a more efficient pathway of decomposition of ARP; that is, browning through the diglycated product. After the formation of the ARP, in the presence of the more reactive sugar (mannose), the ARP can also react with mannose to form diARP, in addition to undergoing decomposition. Since the diglycated products are more reactive, they decompose more readily to produce the brown colour.

CONCLUSION

The extent of Maillard browning depends on the presence of reactive free sugars and on the formation of Amadori products. Amadori products tend to react with a second molecule of sugar to produce diglycated products if the sugar is reactive enough, in addition to undergoing decomposition reactions. In the presence of less reactive sugars, only Amadori products tend to decompose. Diglycated Amadori products are known to be far more reactive than Amadori products in producing brown colour.

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