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Investigation of the contribution of radicals to the mechanism of the early stage of the Maillard reaction

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Involvement of radical reactions in the mechanism of the Maillard reaction is demonstrated. Intermediate Maillard reaction product interactions with the stable 2,2-diphenyl-1-picryl-hydrazinyl radical, and also electron transfer, followed spectrophotometrically with phenanthrolin, allowed the contribution of these reactions in the Maillard system to be explored. It was, thus, possible to estimate which reaction mechanism (ionic or radical) dominates the early stage of the Maillard reaction, depending on the milieu and the reactants in a model system.

The pH value has a significant influence on the participation of radical reactions. Whereas at pH 5 an ionic mechanism seems to be predominant, at increasing pH values reactions proceed with an increasing amount of radical participation. The influence of the amino acids used is marginal, especially at high pH values. In the glycine model, the amount of ionic reactions rises on changing the pH value from 8 to 7.

The sugar influence on the reaction mechanism depends particularly on the ability to form sugar fragments and on the molar ratio of sugar to amino acid.

INTRODUCTION

It is well known from the literature (Kroh *et al.*, 1989; Namiki & Hayashi, 1981; Zeise *et al.*, 1991) that in the early stage of the Maillard reaction radical mechanisms play an important role in the formation of Maillard reaction products. These may include radical reaction pathways for the formation of brown products which differ from the classical HODGE sequence (Hodge, 1953):

sugar + amino acid → glucose amine →
Amadori compound → aldimine/ketimine →
desoxyosulose → melanoidin.

For the postulated mechanism involving radicals, the formation of pyrazine radicals from amino components and sugar degradation products are preferred (Namiki & Hayashi, 1981), but it has also been reported that, depending on the reaction conditions, the formation of C_2-, C_3- and C_4-sugar fragmentation radicals (Calle *et al.*, 1992; Korth *et al.*, 1986) is possible.

Thus, not much is presently known about the contribution of milieu conditions for radical alternatives to ionic pathways in the general mechanism of the Maillard reaction.

MATERIALS AND METHODS

Materials

All the reagents were of analytical grade: D-glucose, D-fructose, maltose and the solvent chloroform were purchased from Merck (E. Merck, Darmstadt, Germany); D,L-glycine, D,L-phenylalanine and *o*-phenanthroline were purchased from Fluka (Fluka Chemie AG, Buchs, Switzerland); and 2,2-diphenyl-1-picryl-hydrazinyl (DPPH), of practical grade, was also from Fluka.

Maillard reaction mixture

Carbohydrate (0.03 mol) and amino acid (0.03 mol) were dissolved in 300 ml of deionized water and heated under reflux. The pH value (5, 7 or 8) was monitored during heating by a pH-measuring sterilizable electrode and corrected to constant values by adding 0.1 M HCl or

0.1 M NaOH. The amount depends on the initial value and the quantity needed for correction (5–10 ml during the total heating time).

Detection with DPPH

DPPH reagent

About 100 mg DPPH was dissolved in 100 ml chloroform to give a final absorption at 520 nm between 1.8 and 2.0.

Every 20 or 30 min, a 1-ml sample was taken from the Maillard reaction mixture (see above), rapidly cooled to room temperature and mixed with 3 ml of the DPPH solution. After shaking for 1 h, the absorption of the organic layer was measured at 520 nm.

Detection with phenanthroline

Detection reagents

$FeCl_3$ (241.5 mg) dissolved in 250 ml of deionized water and *o*-phenanthroline (120 mg) dissolved in 100 ml water were used as detection reagents.

Every 20 or 30 min, a 5-ml sample was taken from the Maillard reaction mixture (see above) and 4 ml of each reagent solution was added. The pH value was corrected to 3–4 with 0.1 M acetic acid. Then the solution was brought to a final volume of 25 ml with deionized water and allowed to stand in the dark for 30 min. The absorption was measured at 505 nm against a sample that contained only the reagents.

Preparation of model melanoidins used for ESR spectroscopy

The preparation of model melanoidins was carried out in accordance with the description in the literature (Cämmerer & Kroh, 1995).

Electron spin resonance (ESR) spectroscopy

The ESR spectra of the free radicals were measured on an EPR spectrometer E_4 (Fa. Varian, USA) under the following instrument conditions: field sweep 3230 T; scan range 100 mT, time constant 0.3 s; modulation amplitude 2 mT; microwave power 20 mW; scan time 480 s: microwave frequency 9085 GHz.

The standard for line intensity and resonance frequency was Cr^{3+} in MgO. The dialysed model melanoidins were dried and, for ESR measurement, 50 mg of sample in a circular quartz tube (ID, 1.5 mm) was placed directly in the resonator.

Spectrophotometry

The absorbances were measured at the wavelength described with a spectrophotometer Spectronic 601 (Milton Roy Company, Oostende, Belgium).

RESULTS AND DISCUSSION

With the help of ESR spectroscopy it is also possible to identify radicals in dynamic processes, using an extensive amount of measurement. An alternative and a more simple solution is to detect radicals by chemical methods which also deliver information about the possible radical mechanisms in the Maillard reaction

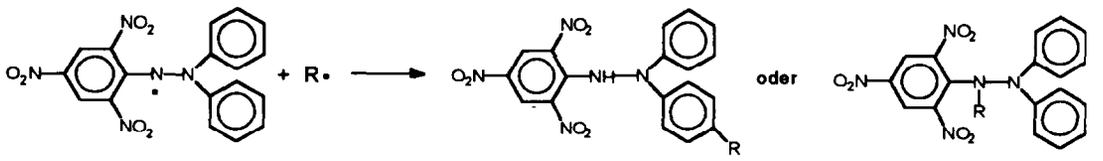
We added intensively coloured, stable 2,2-diphenyl-1-picryl-hydrazinyl radical (DPPH) as a radical quencher to the Maillard model system and followed a possible radical combination reaction spectrophotometrically (Scheme 1).

Because of their steric hindrance, a reaction of DPPH molecules with each other is not possible. As a result, a reaction of the DPPH radical has to be based either on a combination of the DPPH with radicals formed during the Maillard reaction (I) or on a charge transfer in suitable Maillard reaction products or precursors (II) perhaps initiated by DPPH. These reactions are spectrophotometrically detectable by a decrease in the absorption at 520 nm.

For this reason, the results of the DPPH investigations alone are not clear evidence of the radical mechanism of the Maillard reaction.

Distinguishing between radical reactions and electron transfer processes in the early stage of the Maillard reaction is possible with the help of the redox system Fe^{3+}/Fe^{2+}.

In an acidic solution, Fe^{2+} ions form a stable red-coloured complex with phenanthroline which could be easily quantified by spectrophotometry (Scheme 2).



DPPH• + R• ⟶ DPPH - R (I)

DPPH• + R - OH ⟶ DPPH + e^- + H^+ + RO•

⟶ DPPH - H (II)

Scheme 1

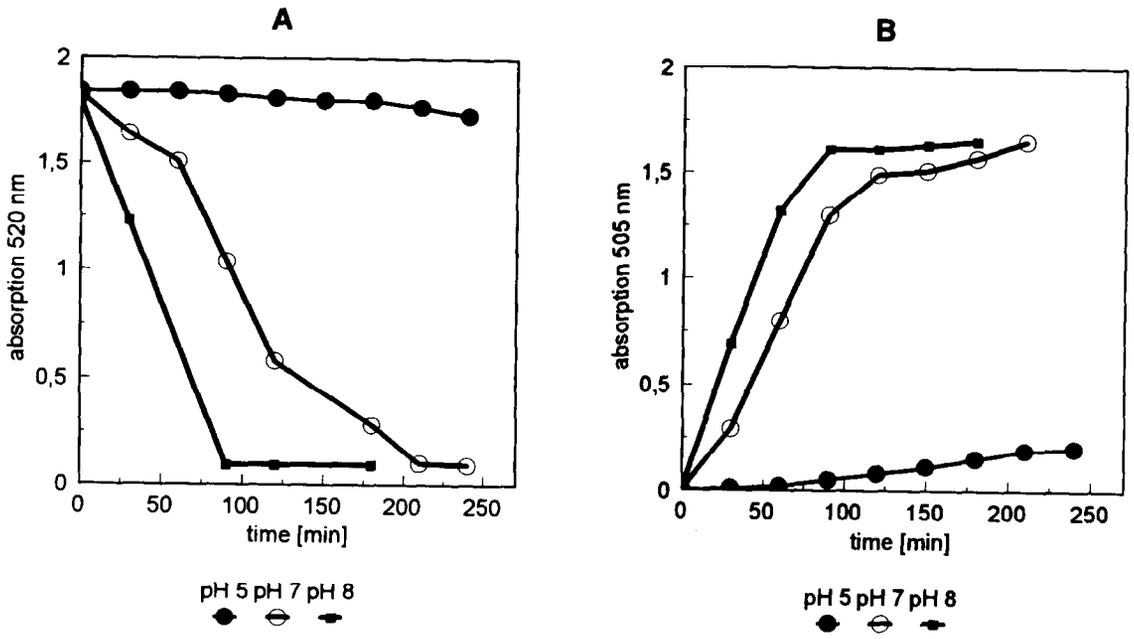
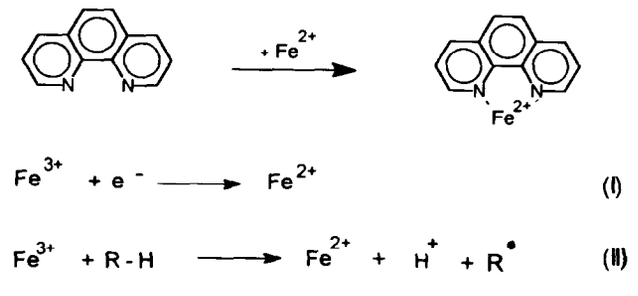


Fig. 1. Detection methods for the contribution of radical and ionic reactions to the Maillard reaction mechanism in a glucose–glycine (1:1) model system. (A) Consumption of DPPH depending on pH during the Maillard reaction. (B) Formation of phenanthroline–ferro(II) complex depending on pH during the Maillard reaction.



Scheme 2

If Fe^{3+} ions are added to a Maillard reaction solution, the ions will take part in the electron transfer processes and will be reduced to Fe^{2+} (I), the formation of which can be detected by the formation of the red phenanthroline-ferro(II) complex. Furthermore, it is also possible that Fe^{2+} generates radicals by abstraction of protons or by redox processes with suitable Maillard reaction products (sugar, reductones, phenols) involving the reduction of Fe^{3+} itself to Fe^{2+} (II).

By comparison of these two methods, and with the help of calibration curves, it is possible to give quantitative figures for the proportion of radical to ionic mechanisms taking place simultaneously in the Maillard reaction, and to clarify the influence of the reaction conditions and the kind of reactants on this ratio.

Influence of the pH value on the radical mechanism

Hayashi & Namiki (1986) suggested two different mechanisms for the formation of brown products during the Maillard reaction depending on the pH value. At lower pH values, the main pathway for melanoidin production should be the Amadori rearrangement and osone formation. At basic pH values, the formation of C_2-sugar fragmentation products, identified by some workers as precursors for pyrazinium radicals in the early stage of the Maillard reaction (Namiki & Hayashi, 1981), takes place very easily. Therefore, a preferred radical mechanism is postulated for this pH region.

Based on our investigations, we were able to demonstrate and quantify the contribution of radical reactions to the Maillard reaction mechanism depending on the pH value.

Both detection methods described show strong pH dependence in the Maillard glucose–glycine model (Fig. 1). With increasing pH value, the consumption of added DPPH radicals increases strongly (Fig. 1A) and the electron transfer processes quantified using phenanthroline also increase (Fig. 1B).

With the help of calibration curves in model Maillard systems, a large number of values may be obtained from these two figures and the part of each—ionic mechanism and ionic together with radical mechanism—can be calculated.

The difference between the reactions detected with DPPH and the alternative ionic redox mechanism describes the percentage of the radical part in the Maillard reaction (Fig. 2).

Whereas, at pH 5, the ionic reactions strongly dominate the mechanism and a significant participation of radicals may only be observed after a longer reaction period (about 100 min), at pH values of 7 and above the contribution of radical reactions increases to about 60 or 80% from the beginning. But there is no correlation between the amount of radicals which participate in the reaction and the browning of the solution measured at 430 nm in the early stage of the Maillard reactions. In contrast, with pH 8, where a high radical participation

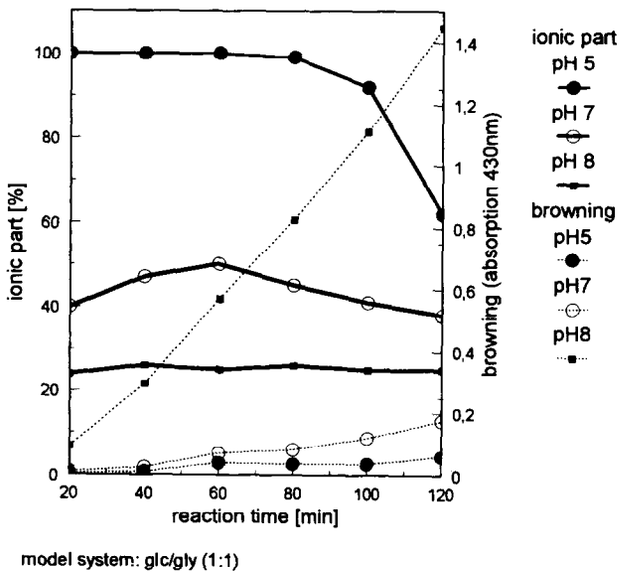


Fig. 2. The influence of the pH value on the contribution of ionic reactions to the Maillard reaction mechanism and on browning in a glucose–glycine (1:1) model system.

and a high browning intensity are detectable, at pH 7 a high radical contribution causes only a low browning under the reaction conditions investigated. For the glucose–glycine model, radical reactions are preferably included at basic and neutral pH values of the Maillard reaction mechanism.

At the same time these radicals seems to be very unstable at these pH values and react very rapidly in the final stages to give brown products where they can be detected by ESR spectroscopy with comparatively low intensities (Table 1).

Furthermore, no direct correlation between the radical properties of the high molecular melanoidins formed in the final phase and the radical reaction mechanisms taking place in the early stage of Maillard reaction could be confirmed.

Influence of amino acid and sugar on the proportion of radical reactions in the mechanism

It is known that the structure of the amino acid and the conformation of the sugar have an important influence on the pathways and the kinetics of the Maillard reaction, and the formation of brown products (Westphal & Kroh, 1985; Westphal *et al.*, 1985). These characteristics are also important for explanation of the amount of participation of radical reactions in the general Maillard reaction mechanism.

Table 1. ESR spectroscopic parameters of glucose–glycine (1:1) melanoidins

Conditions	Linewidth [G]	Intensity
100°C/10 h/pH 5	8.5	11.67
100°C/10 h/pH 7	9	4.19

Amino acids

Based on investigations of the glucose–glycine and glucose–phenylalanine models, the amount of radical reactions in the early stage of the Maillard reaction at pH 8 seems not to be influenced significantly by the type of amino acid (Fig. 3). But the radical participation in the glucose–glycine model is lower than in the glucose–phenylalanine model.

Whereas, in the glucose–glycine model, the ionic part increases with decreasing pH, the radical reactions in the glucose–phenylalanine model remain constantly high. This is possibly due to a good stabilization of the radicals by interaction with the π-electron sextet of phenylalanine or the formation of benzyl radicals from the amino acid (Papadapoulou & Ames, 1994).

Some more investigations should make clear whether such differences in the reaction mechanism of both the amino acids investigated are based on a differing reactivity or on different stabilization means of radicals formed or if the amino acid used can force the reaction mechanism in one of the alternative directions.

Sugar

At pH 7, the three sugar–glycine model systems show considerable differences in the reaction pathways (Fig. 4).

This figure shows that the maltose–glycine system has a complete ionic reaction pathway without a marginal radical part, even after a long reaction time.

During the whole reaction time in the glucose–glycine model, the radical participation is at about 60%, whereas in the fructose system the reaction begins ionically and the radical proportion rises strongly during the reaction time. Obviously, no correlation with the browning intensity measured spectrophotometrically at 430 nm was found. A difference between maltose and glucose in the radical participation in the early stage of the Maillard reaction results in an equal browning.

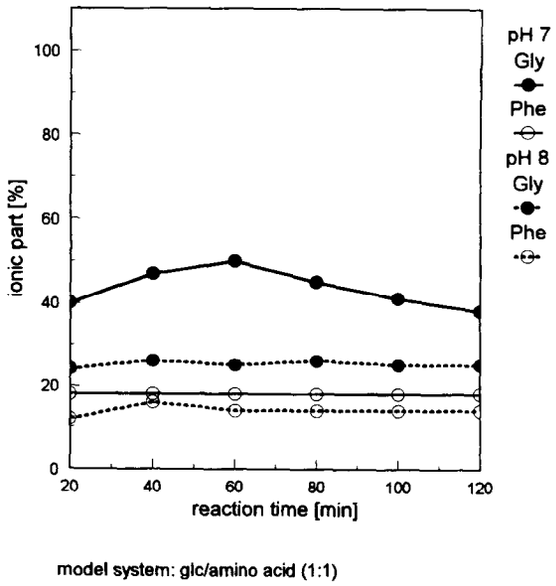


Fig. 3. The influence of the amino acid on the contribution of ionic reactions to the Maillard reaction mechanism and on browning in a glucose–amino acid (1:1) model system.

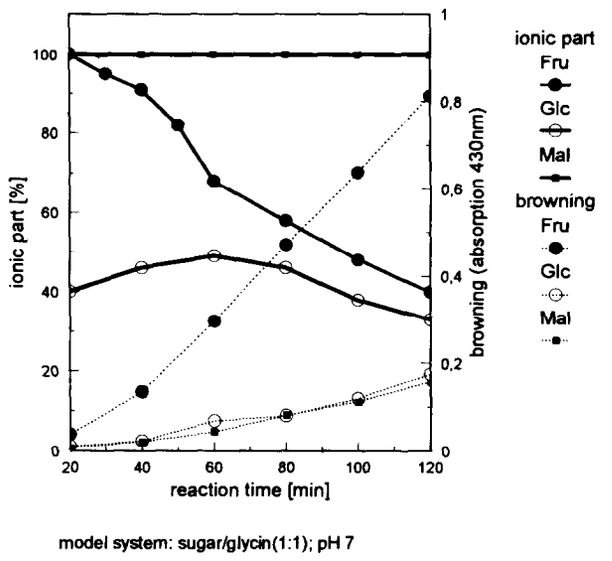


Fig. 4. The influence of the kind of sugar on the contribution of ionic reactions to the Maillard reaction mechanism and on browning in a sugar–glycine (1:1) model system.

If we assume that radicals are formed in the early stage preferred for C_2- or C_3-sugar fragments (Hayashi & Namiki, 1986), the explanation for our observations will rest on the different fragmentation behaviour of several sugars. Whereas maltose is able to form sugar radicals probably only after cleavage of the C_2–C_3 bond in the reducing part, with or without previous hydrolysis of the glycosidic bond, glucose and fructose are able to form C_2- and C_3-fragments easily by retroaldol reaction (Calle *et al.*, 1992).

We would normally expect that fructose reacts in basic milieu with a higher rate because of known rapid enolization reactions (de Bruijn *et al.*, 1986).

C_2- and C_3-imine and diimine are also described as radical precursors (Hayashi & Namiki, 1986) which could be formed in the early stage of the Maillard reaction by retroaldol reactions from glycosylamines or Amadori compounds. The several paths for the formation of C_2- or C_3-fragments are mostly determined by the type and stereochemistry of the amino acid, by the molar ratio of the reactants and the pH value.

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