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A SPECTROPHOTOMERIC INVESTIGATION OF THE RADIATION INDUCED MAILLARD'S REACTION IN THE MODEL SYSTEM OF FRUCTOSE-ALANINE *)

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Key words: monenzymic browniną, fructose-alanine, irradiation.

The effects of γ-irradiation on the browning reaction in the model system of fructose-alanine solution were investigated. The irradiation was followed by the heating of the solution at 100°C to develop the brown colour. The run of the reaction was determined spectrophotometrically by measuring the absorbance at 450 nm. The optical spectra of irradiated and heated solutions of fructose-alanine exhibit a broad absorption maximum at about 280 nm.

INTRODUCTION

Increasing interest in food products processed by irradiation has led to a greater need for fundamental information about nonenzymic browning. A number of works has been done on the browning reaction of sugars with amino acids, peptides or amines [2, 4, 6, 10, 13, 17]. In general, the condensation of reducing sugars and amino compounds known as the Maillard's reaction is initiated by heating of the sample during several hours at higher temperatures. It has been recently found that the browning reaction is strongly accelerated by γ-irradiation [5, 7, 8, 15]. Therefore, there is a need to explain the influence of radiation upon the course of amino-carbonyl reaction, first in the model system consisting of reducing sugar and amino acid in solution, and then in natural systems. The effects of γ-irradiation on the antioxidative activity

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developed in amino acid — sugar reaction were investigated by Fujimaki et al. [5]. They found that the browning measured by the increase of absorbance at 420 nm for glucose-glycine solution was enhanced by irradiation, especially at the initial step of browning. The extent of browning depended on the kind of sugars. Fructose, sorbose and sucrose were more reactive than other sugars [7]. On the other hand, the decomposition of sugar by irradiation was reduced if equimolar amounts of amino acids were present during irradiation of aqueous solution of trehalose [1], which as a nonreducing disaccharide did not undergo browning reaction. At present little is known about the acceleration of the Maillard's reaction by γ-irradiation. Kawakishi et al. [8] suggest that irradiation of sugar solution generates some reactive intermediates which react with amino acids and promote the browning reaction.

In the present study, experiments were made to explain the effects of irradiation on the model system containing fructose and alanine. The irradiation of the aqueous solution of fructose-amino acid was followed by a heating process.

MATERIALS AND METHODS

D-Fructose, puriss, from Koch-Light Laboratories, and DL-alanine, puriss, from Reachim were used. Aqueous solution containing alanine (0.1 M) and fructose (0.3 M) at pH 4.9 were irradiated with ^{60}Co rays in the presence of oxygen with a dose from $0.5 \cdot 10^4$ to $3.5 \cdot 10^4$ J/kg. After irradiation the samples were heated in a boiling water bath to allow the browning reaction to proceed. The intensity of brown colour was deter-

Table Time needed to develop the brown colour corresponding to the absorbance equal to 0.05 nmeasured at 450 nm

Dose $\times 10^{-4}$	J/kg	0.0	0.5	1.0	2.0	3.5
$t^{0.05}_{450\,nm}$	min	120	17	11	6	5

mined by measuring the absorbance at 450 nm. Optical spectra were recorded at room temperature with a Beckman DK-2A spectrophotometer for the wavelength range between 200 and 550 nm. Measurements were carried out both for heated and nonheated solutions. In order to obtain solutions of different pH, appropriate quantities of 0.1 M sodium hydroxidate or hydrochloric acid were added. The pH values were checked with a precision pH-meter using a combined glass electrode.

RESULTS

The effects of γ-irradiation on the Maillard's reaction were investigated on the model system of fructose — alanine solutions. The irradiation was followed by heating the samples at 100°C to develop the brown colour, because at lower temperatures an aminocarbonyl reaction proceeds very slowly.

The run of browning reaction was examined spectrophotometrically by measuring the absorbance of the solutions at 450 nm. The increase of absorbance at 450 nm of fructose-alanine solutions irradiated with different doses and subsequently heated up to 7 hours is shown in Fig. 1.

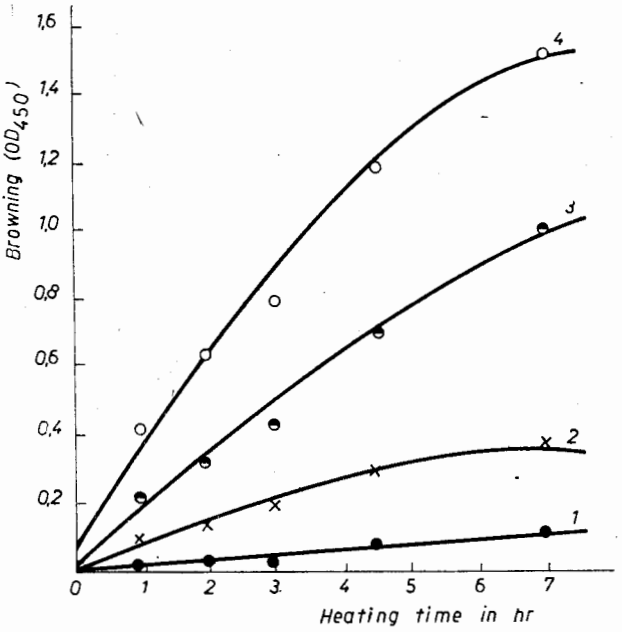

Fig. 1. Development of browning in alanine-fructose solutions irradiated and subsequently heated at 100°C. The intensity of browning was measured spectrophotometrically at 450 nm; doses: 1 — not irradiated, 2 — 0.5×10^4 J/kg, 3 — 2×10^4 J/kg, 4 — 3.5×10^4 J/kg

It may be seen from Fig. 1 and table 1 that the time needed to develop the brown colour, which corresponds to the absorbance equal to 0.05 measured at 450 nm, is much shorter for the samples irradiated with higher doses.

The optical absorption spectra recorded in the ultraviolet and visible regions for the irradiated and nonheated, and irradiated and heated during 2 hours solutions of fructose — alanine are presented in Fig. 2a and 2b, respectively. The nonirradiated and nonheated sample was used as a blank solution. The spectra of the samples without heating exhibit two absorption bands at about 215 nm and about 270 nm, which are

typical for the products of fructose as well as of other carbohydrates radiolysis [3, 11]. The changes of the absorption spectra upon the heating of the irradiated solutions are illustrated in Fig. 2b and Fig. 3.

A broad absorption maximum occurs at about 280 nm, the intensity of which increases with heating time. Also, the absorption in the range from 350 nm to 500 nm increases in intensity. The brown colour of the heated solutions is obviously caused by the extension of the tail-end absorption into the violet region of the spectrum. No absorption maximum appears in the visible region of the spectrum.

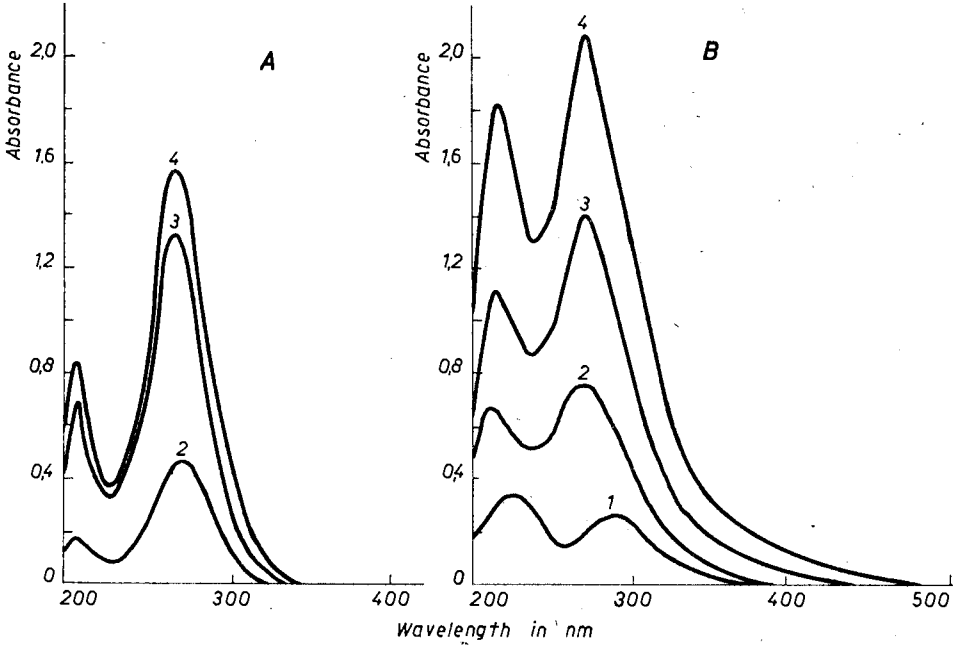

Fig. 2. Optical absorption spectra of irradiated fructose-alanine solutions without (A) and with (B) subsequent heating during 2 hours at 100°C. The solutions were 10 times diluted prior to measurement; doses as in Fig. 1

The effect of preirradiation of fructose or alanine solution alone on the browning reaction with the nonirradiated second component of the mixture is shown in Fig. 4. It should be noted that the irradiation of fructose solution followed by heating with nonirradiated alanine leads to larger increase of the absorbance at 450 nm than the irradiation of alanine solution followed by the heating with nonirradiated fructose. On the other hand the heating of the irradiated mixture of fructose-alanine developes markedly more intensive browning than that of the irradiated solutions of individual components. These experimental results led to the conclusion that the products of fructose radiolysis are more reactive towards alanine during heating than the parent sugar. The similar effect was observed for the irradiated glucose-glycine system [5].

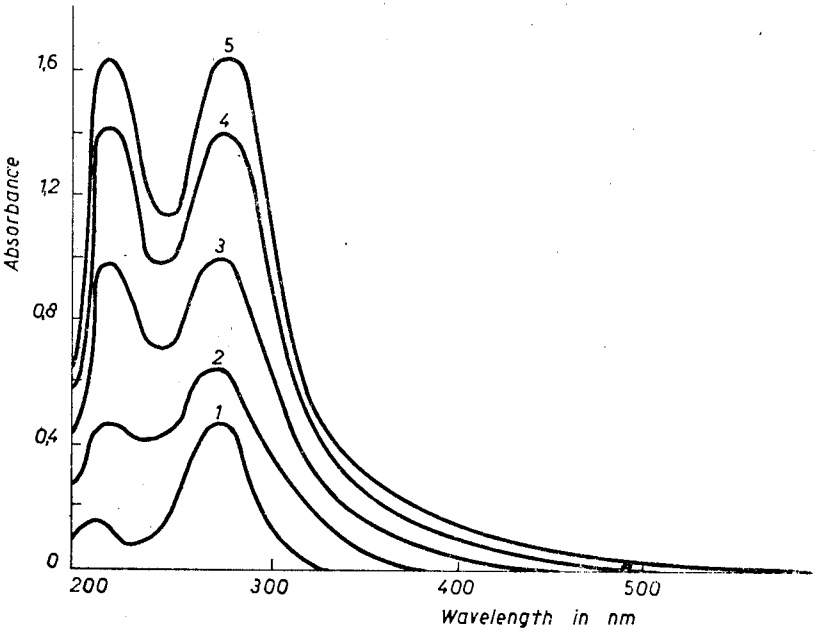

Fig. 3. Effect of heating on the absorption spectra of fructose-alanine solution irradiated with a dose 0.5×10^4 J/kg; the solutions were 10 time diluted; heating times: 1 — not heated, 2 — 1 hr, 3 — 3 hrs, 4 — 4.5 hrs, 5 — 7 hrs

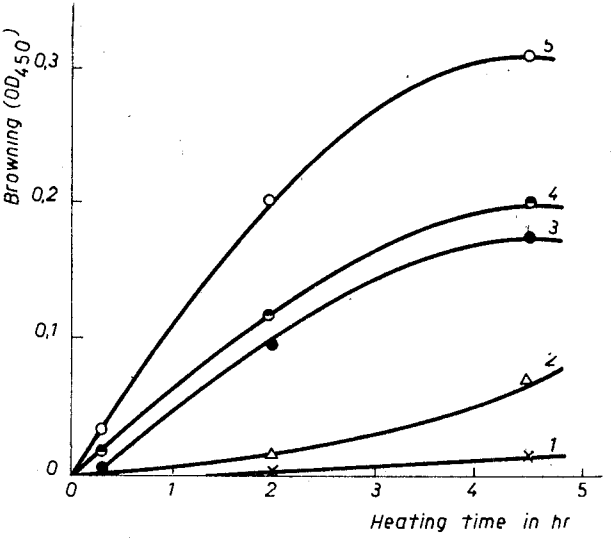

Fig. 4. Development of browning in different fructose-alanine solutions measured spectrophotometrically at 450 nm after heating: 1 — nonirradiated mixture of fructose-alanine, 2 — fructose solution irradiated with a dose 1.25×10^4 J/kg and heated with nonirradiated alanine solution, 3 — alanine solution irradiated with a dose 1.25×10^4 J/kg and heated with nonirradiated fructose solution, 4 — separately irradiated with a dose 1.25×10^4 J/kg fructose and alanine solutions and mixed prior to heating, 5 — mixture of fructose-alanine irradiated with the dose 1.25×10^4 J/kg and heated at 100°C

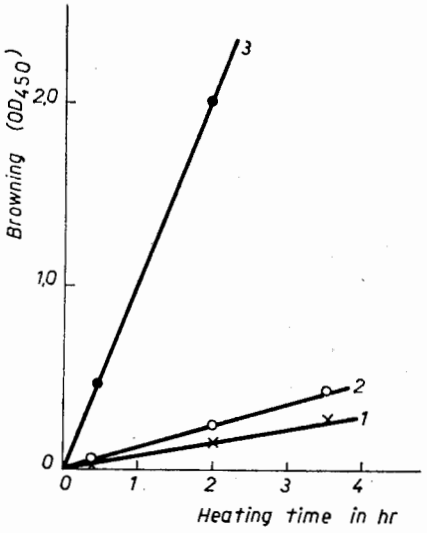

Fig. 5. Effect of pH on the browning in fructose-alanine solutions; dose: 1.25× ×10^4 J/kg, 1 — pH 3.9, 2 — pH 4.5, 3 — pH 9.0

The amino-carbonyl reaction between fructose and alanine was enhanced in alkaline solution (pH 9.0) in comparison with the acidic solutions (pH 3.0 and 4.9) as is shown in Fig. 5. The observation can be explained by the base catalyzed degradation of fructose during heating at 100°C [14].

DISCUSSION

Nonenzymic browning involves a complex of reactions which take place when sugars are heated with amino compounds. In many cases the early stages of reaction lead to the formation of low molecular weight compounds. In the next step the brown high molecular weight material (melanoidins) is formed [10]. The results presented in this paper can be considered as restricted to the first stage because the heating time used was too short to produce the polymeric products.

Our results confirmed that the irradiation accelerates the browning reaction which proceeds during heating of fructose-alanine solutions. The heating of the irradiated samples was found to be necessary, because at a low temperature the reaction ran very slowly. During irradiation of aqueous solution of sugar and aliphatic amino acid mixture the radiolytic degradation of both components occurs. In the presence of oxygen the radiolysis of fructose leads to the formation of different ketocompounds, namely, glucosone, 5-ketoglucose, glucuronolactone, arabinose, glyoxal and dihydroxyaceton [11]. These compounds are known to take part in Maillard's reaction with amino acids [9, 12], and they are responsible

for acceleration of browning in irradiated system (cf. Fig. 4). It was shown that amino acids more rapidly reacted with the radiolysis products of glucose than with the parent sugar [8]. During heating, fructose and their radiolysis products react with alanine giving N-substituted compounds, for example, fructosylamine, which are unstable. The degradation of these compounds leads to the formation of various products which exhibit the absorption in ultraviolet and visible regions [10, 16].

CONCLUSIONS

1. Development of the browning of fructose-alanine solutions during heating was markedly accelerated when the solution was preirradiated. The increase of intensity of brown colour was proportional to the radiation dose.

2. During heating, markedly more intensive browning was developed for the irradiated mixture of fructose-alanine than that of nonirradiated sample or when only one component of the mixture was irradiated. On the other hand, the irradiation of fructose solution followed by the heating with nonirradiated alanine led to a larger increase of the absorbance at 450 nm than the irradiation of alanine solution followed by the heating with nonirradiated fructose.

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SPEKTROFOTOMETRYCZNE BADANIA INDUKOWANYCH RADIACYJNIE REAKCJI MAILLARDA W UKŁADZIE MODELOWYM FRUKTOZA — ALANINA

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Streszczenie

Badano wpływ promieniowania gamma ^{60}Co na przebieg reakcji nieenzymatycznego brunatnienia w układzie modelowym fruktoza — alanina. Wodne roztwory o stężeniach 0,3 M fruktozy i 0,1 M alaniny napromieniano dawkami od 0 do $3{,}5\times10^4$ J/kg w temperaturze 20°C i w obecności powietrza. W celu przyspieszenia reakcji Maillarda napromienione roztwory ogrzewano we wrzącej łaźni wodnej w przedziałach czasu od kilku minut do 7 godz. Intensywność barwy badanych roztworów fruktozy i alaniny mierzono spektrofotometrycznie przy 450 nm. Równocześnie wykonywano pomiary widm absorpcyjnych w zakresie ultrafioletu i światła widzialnego. Promieniowanie jonizujące wyraźnie przyspiesza przebieg reakcji brunatnienia w roztworach fruktozy i alaniny, o czym może świadczyć fakt, że czas potrzebny do uzyskania barwy odpowiadającej absorbancji 0,05 dla próby nienapromienionej wynosił 120 min, a dla próby napromienionej dawką $0{,}5\times10^4$ J/kg tylko 17 min. Dla wyższych dawek czas ten był jeszcze krótszy. Napromienione roztwory fruktozy i alaniny wykazywały charakterystyczne widma absorpcyjne w zakresie ultrafioletu. W wyniku ogrzewania następowało poszerzenie maksimów absorpcji i przesunięcie pasma absorpcji w stronę zakresu widzialnego, przy czym nie powstawały nowe maksima w tym zakresie. Stwierdzono, że produkty radiolizy fruktozy odpowiedzialne są za przyspieszenie reakcji nieenzymatycznego brunatnienia. Istotny wpływ ma również pH roztworów. W środowisku alkalicznym szybkość reakcji Maillarda jest wyższa niż w kwaśnym.