INVESTIGATION OF THE MAILLARD REACTION WITH DERIVATOGRAPH

By

F. ÖRSI

Department of Biochemistry and Food Technology, Technical University, Budapest

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1. Introduction

The Maillard reaction is the most important of the chemical changes caused by the heat treatment applied in food technological operations. In this reaction primarily the carbohydrate and amino acid components of foodstuffs react with each other. This process decreases the nutrition and biological value of the product, and according to recent publications toxic, or at least physiologically not neutral compounds are formed. [1] The initial steps of the reactions have already been determined in aqueous solutions and in foodstuffs, but there are still several unsettled problems. [2, 3]

Formerly, we have successfully applied thermal analysis for studying the reactions of some essential amino acids (methionine, triptophan and lysine), and we determined the main stages of the reactions and the optimum sugar — amino acid ratio. [4, 5, 6]

This paper deals with the continuation of this study in two directions. Namely, we investigated the reaction of cystine and cysteine amino acids with glucose, and carried out the kinetic analysis of the lysine — glucose reaction.

2. Experimental

2.1. *Materials and methods*

The amino acids and sugars used in the investigation were Reanal products.

Investigations by derivatograph

The measurements were carried out with a modified Paulik—Paulik—Erdey derivatograph (MOM, Budapest, Hungary). The scheme of the instrument is shown in Fig. 1. The temperature control unit of the derivatograph was replaced by an LP 839 temperature controller produced by Chinoin, which permitted to achieve simpler and more reliable the required temperature program.

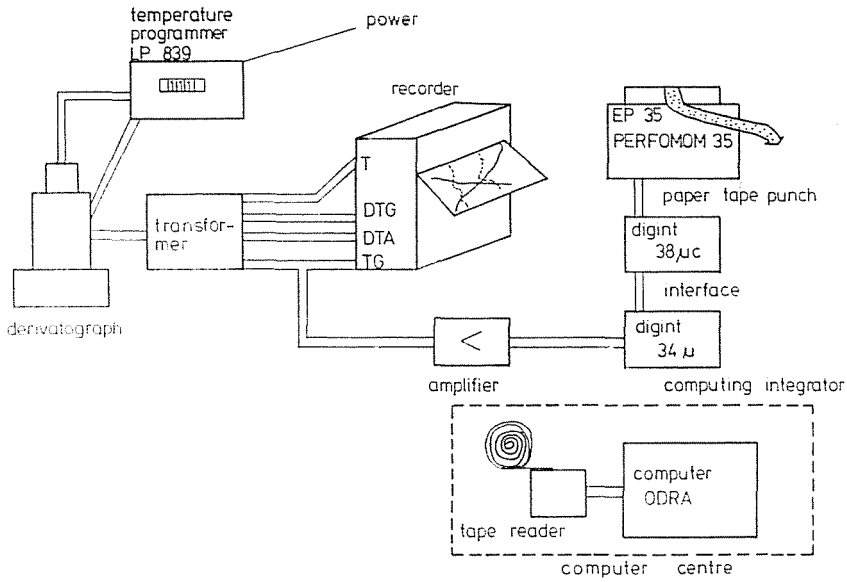

Fig. 1. Modified Derivatograph-computer off line system

With the use of a preamplifier (produced by Chinoin) and a digint 34 μ integrator and a digint 38 μ interface, the thermogravimetric (TG) curves were digitized and the data punched on a MOM paper tape unit. Through the keyboard of the interface auxiliary informations, necessary for the evaluation of the thermal curves, could also be punched onto the paper tape. For the off-line processing of the paper tapes obtained by this system an ALGOL-60 program was developed and run on the ODRA computer of the university. The details of the program will be given elsewhere.

From the weight loss data measured at regular intervals (adjustable between 0.25 and 1 min) and the auxiliary information (initial and final temperature and weight respectively, heating rate) the program can reproduce the TG and DTG curves, determine and separate the steps of the TG curve, and determine the kinetic parameters (activation energy and rate constant) of the steps.

Always 100 mg sample was weighted in the largest Pt crucible of the derivatograph and was heated at a heating rate of 10 °C/min from room temperature to 700 °C.

In all cases, TG, DTG, DTA and T curves were recorded with a four-channel dot recorder produced by MOM, and were simultaneously punched on paper tape. The DTA and DTG curves were recorded with sensitivities set to 1/5. The reference crucible was always left empty.

By means of a vacuum pump, air was passed through the oven at a rate of 0.5 dm^3/min in order to remove pyrolitic and combustion products.

2.2. *Results*

2.2.1. *Reaction between glucose and cystine*

Figures 2 and 3 show the thermal curves of the pure compounds. The thermal curves of glucose have already been investigated in detail and interpreted. [5] Decomposition starts only after melting, above 165 °C.

With L-cystine no change can be observed up to 215 °C. An endothermic reaction takes place between 215 and 305 °C, with a maximum rate at 265 °C. The process is accompanied by a 80% weight loss corresponding to the sublimation of cystine. From 305 to 650 °C the pyrolyzed residues (20%) burn in an exothermic process. The rate of the process is determined primarily by the diffusion rate of air.

In Fig. 4 the thermal curves of a 10:90 mixture of cystine and glucose are shown. Unlike the amino acids investigated so far, cystine enters the reaction only after the melting of glucose (DTA peak at 165°). The reaction is indicated by a higher weight loss in the first step than in the heat treatment of pure glucose. Up to 165 °C neither the DTA nor the TG curve shows changes. The first endothermic stage of the reaction proceeds until 280 °C, with 44.9% weight loss, which is more than double of the loss observed with pure glucose. Above 212 °C pure cystine also sublimes but cystine could not be detected among the volatile products of the reaction.

The reaction becomes exothermic in the 280—415 °C region, due to the heat released during the burning of pyrolytic decomposition products. Under nitrogen atmosphere this stage of the reaction remains endothermic.

The 415—600 °C region corresponds to the burning of the solid products formed in pyrolysis.

The measurements were also carried out with glucose-L-cystine mixtures of compositions: 95 + 5, 90 + 10, 85 + 15, 80 + 20, 70 + 30, 50 + 50, 40 + 60, 30 + 70, 20 + 80 and 10 + 90; by connecting the ranges of weight losses read from the TG curves at the same temperature, composition — weight loss isotherms were constructed. From the isotherms, shown in Fig. 5, the changes and weight losses may be read as a function of temperature.

Unexpectedly caramelization and Maillard reaction, could not be distinguished and, therefore, they have a common area in the diagram. By relating the weight loss measured in this stage to the amount of glucose, a constant value, $44 \pm 3\%$ was obtained which did not depend on composition, and it dropped to 19.5% only with pure glucose. Another extreme value was observed with 10% cystine content. According to my assumption a part of glucose decomposes at this composition through caramelization and the amount of volatile products formed in the Maillard reaction is added to this weight loss.

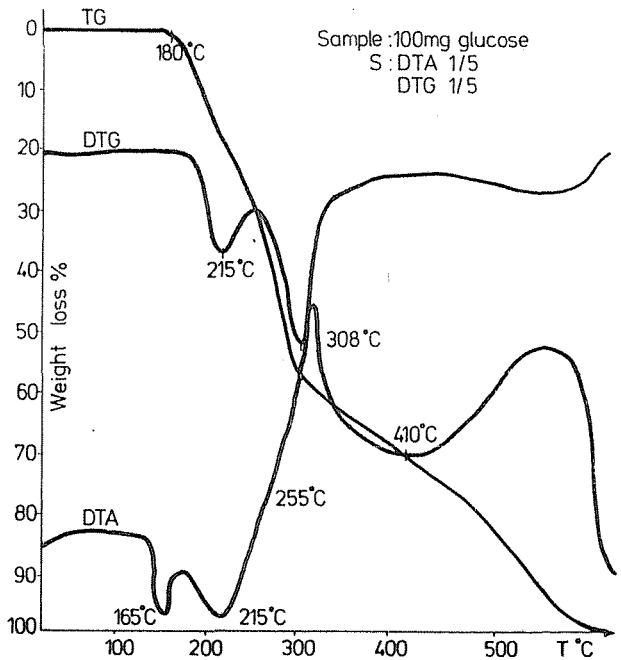

Fig. 2. TG, DTG and DTA curves of glucose

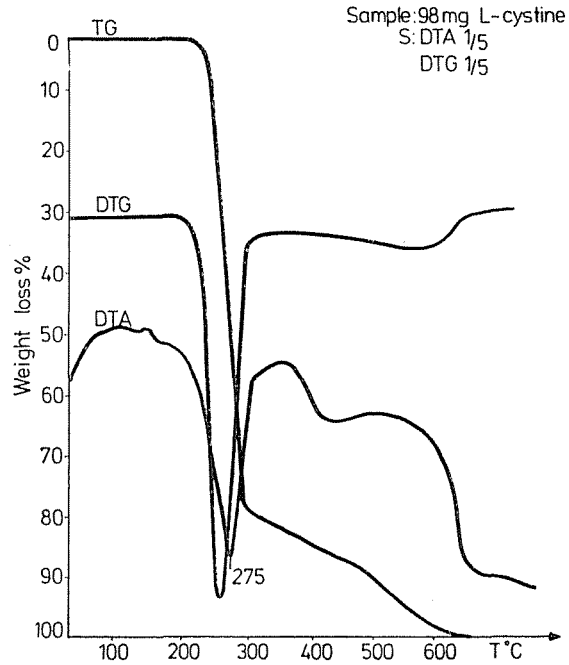

Fig. 3. TG, DTG and DTA curves of L-cystine

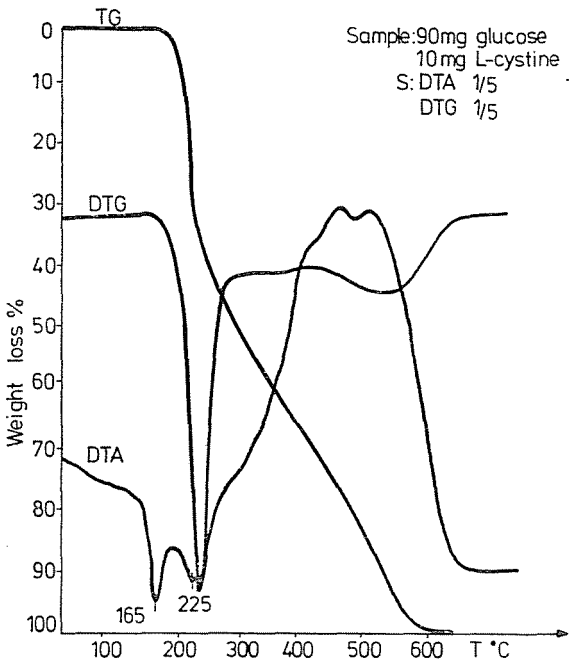

Fig. 4. TG, DTG and DTA curves of a mixture of 10 mg L-cystine and 90 mg glucose

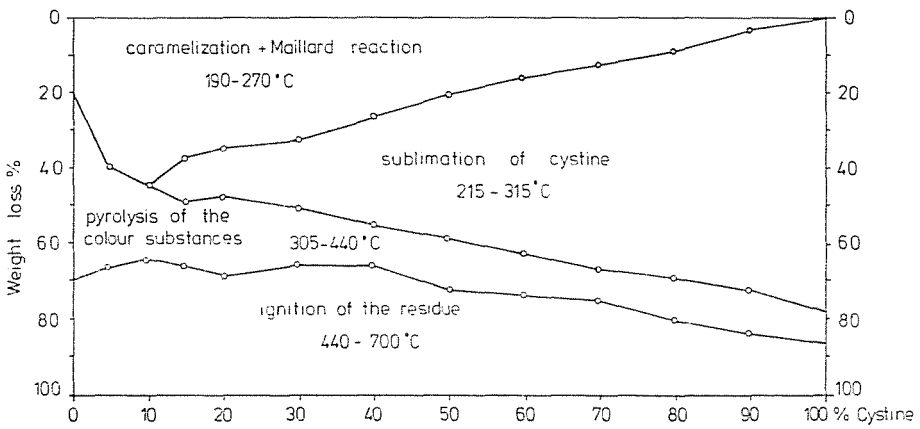

Fig. 5. The composition-weight loss isotherms of glucose-cystine systems

The second step of the process may be observed only in samples which contain excess amino acid, and it is correlated to its sublimation. Consequently, the amino acid excess does not take part in the reaction, and leaves the reaction mixture by sublimation, like methionine. By relating the sublimed amount to the amino acid content of the mixture, a monotonously decreasing

curve is obtained in function of composition (Fig. 6); the change is particularly steep around 10% cystine. Sublimation cannot be observed with samples of 5 and 10% cystine content. This indicates that the equivalent cystine concentration must be in this range. In turn, this suggests that 1 mole of cystine reacts with nearly 10 moles of glucose before it is built irrecoverably into the reaction

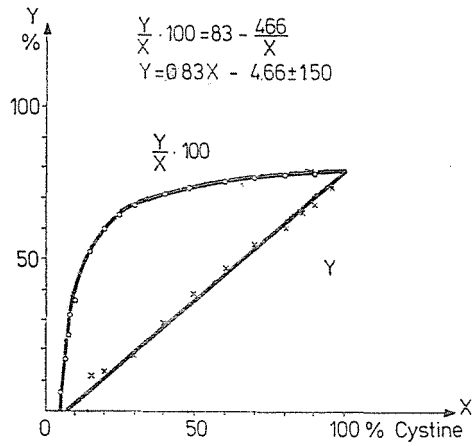

Fig. 6. The sublimed absolut (Y) and relative (Y/X) amount of excess cystin vs. its proportion in the mixture

product. Consequently, it hardly decomposes in the Strecker degradation, and not only the amino but also the carboxy or even the disulfide groups may take part in the reaction with glucose. The accurate value is not easy to determine since caramelization and the Maillard reaction proceed simultaneously.

The pyrolytic stage between 205 and 440 °C rapidly decreases as increasing amounts of glucose enter the reaction, then it remains constant or slowly decreases.

The last stage of the reaction, the burning of residues, takes place in exothermic reaction. The product of the Maillard reaction yields here the largest weight loss, and the rate of burning is lower than with the pyrolytic products of pure amino acids.

On the basis of the results it can be concluded that cystine is very active from the aspects of Maillard reaction, but the reaction begins at higher temperatures. Presumably, the reaction does not take place in solid phase, and the Maillard reaction starts only when glucose melts and dissolves cystine. The weight loss of 44% observed in the Maillard reaction and related to the weight of glucose hardly exceeds the elimination of 4 moles of water pro glucose molecules. This indicates changes of primarily dehydration character.

2.2.2. *Reaction of glucose with cysteine*

The thermal decomposition of cysteine shows a very complex feature (Fig. 7). Neither weight nor enthalpy change can be observed up to ca 70 °C.

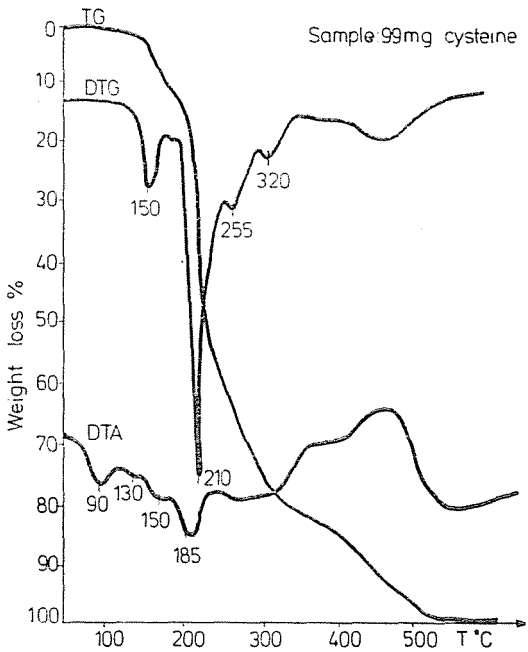

Fig. 7. TG, DTG and DTA curves of L-cysteine

Between 70 and 130 °C, with peak temperature 90 °C, cysteine HCl melts, causing a weight loss of ca. 2%. Since the hydrochloric acid salt of cysteine used in the experiments forms relatively large crystals, it may be assumed that after melting the water content of the included mother liquor evaporates in this stage.

The next step of the decomposition takes place from 130—190 °C with a weight loss of 11%. It is followed by a step in the 190—230 °C range with a weight loss of 42%.

In the next two steps of decomposition, causing smaller weight losses, the cysteine sample starts to become brown and intense formation of sulfur-containing products can also be observed. In the 230—286 °C region 18% and then in the 286—325 °C region 6% weight losses were measured.

In the final two exothermic steps the pyrolytic residues burn: between 325 and 421 °C a loss of 6.5% and between 421 and 550 °C a loss of 12.5% takes place.

Figure 8 shows the thermal curves of a mixture of 95 mg glucose and 5 mg cysteine. It can be seen that even this small amount of cysteine causes significant changes. Whereas in the case of pure glucose weight loss started only above 197 °C, in the mixture it occured already at 140 °C i.e. already below the melting point of glucose (165 °C); this can be due to the fact that cysteine melts at 90 °C and thus a reaction may take place between the melt and the solid phase. The weight loss in the 140—190 °C region is 14.5%, which

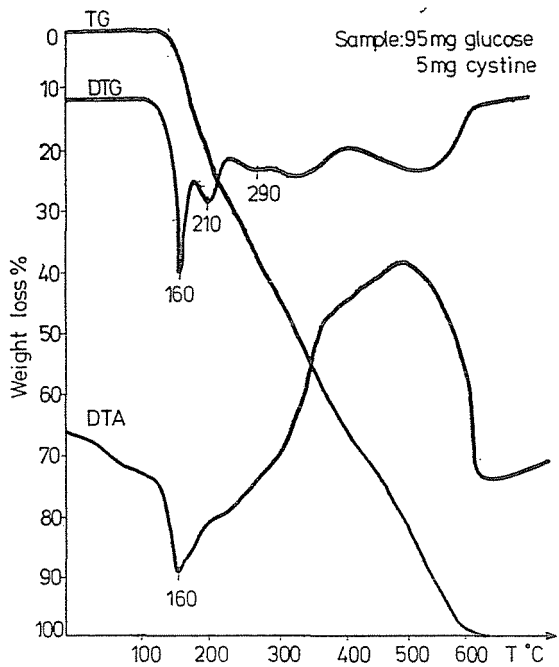

Fig. 8. TG, DTG and DTA curves of a mixture of 5 mg cysteine and 95 mg glucose

indicates a strong catalytic effect, since the amount of pure cysteine present in the system accounts for only 0.5% of the total weight loss in this region, and for 0.6% if it reacts with glucose with water elimination. The remaining weight loss is, therefore, due to the catalytic decomposition of glucose.

The presence of amino acid also changes the direction of decomposition. The largest weight loss of pure glucose is connected with the pyrolytic processes taking place in the 275—425 °C temperature range, of which the most important one is the pyrolytic decomposition of the reversion polysaccharide. In glucose with 5% cysteine content the weight loss of this stage decreased substantially, indicating the effect of Maillard reaction. This is always represented in the suppression of the formation of reversion polysaccharides and in the formation of brown colouring substances already at low temperatures.

In the 245—310 °C region the colouring substance becomes insoluble, but with a much less weight loss. In some zones even the caramelization of glucose may take place.

In the 310—440 °C region the pyrolytic decomposition of the colouring substance takes place as well, but even if the two steps are combined, the weight loss does not reach that observed for pure glucose. The amount of pyrolytic residues is significantly higher than in the case of pure cysteine, but

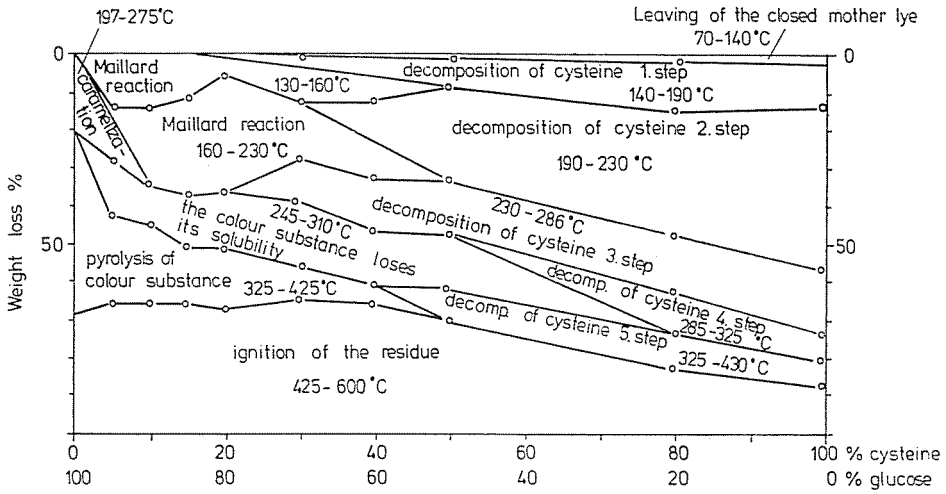

Fig. 9. The composition — weight loss isotherms of glucose — cysteine systems

it is also higher than that of glucose. For the detailed investigation of the processes, reaction mixtures of varying compositions were prepared, and they were studied in a similar manner, by derivatograph.

The weight losses read from the TG curves are shown in Fig. 9 as a function of composition. The circles indicating the start and end points of the weight losses belonging to the same temperature or arising from the same process in the various mixtures are joined by lines. The resulting diagram contains weight loss isotherms as a function of composition, since the temperature along the curves is approximately constant. The area enclosed by the curves indicates the realization of a given process.

The weight loss isotherms of the glucose — cysteine reaction are very complex as can be seen in Fig. 8. The complexity arises from the facts that the decomposition of cysteine starts already at 130—150 °C, i.e. in the region of the decomposition of sugar, and thus almost any stage of the decomposition of glucose or of the glucose — cysteine Maillard reaction overlaps a parallel step of cysteine decomposition. The separation and distinction of these steps is not always possible.

Above 50% cysteine content most of the decomposition stages may be assigned to the decomposition of cysteine, and only the insolubilization of colouring substance extends into it, as shown by the figure. If, however, the weight losses are subjected to more through analysis, the influence of sugar may be detected in the subsequent weight losses.

In mixtures containing more than 50% glucose, the decomposition steps may be assigned to sugar decomposition and sugar — amino acid interaction.

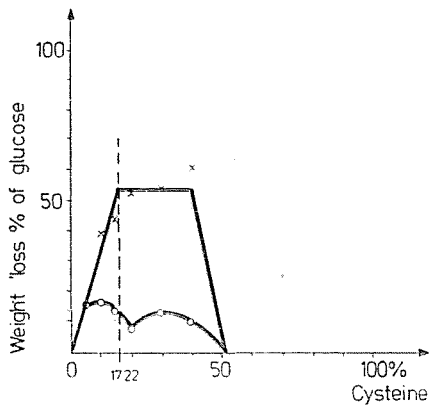

Fig. 10. The weight losses measured in the first step (0), and in the first two steps (+) related to glucose vs. proportion of cysteine in the mixture

The steps of cysteine decomposition do not extend beyond the composition corresponding to 15% cysteine content.

The stage attributable to the Maillard reaction consists of two steps. The first one is in the 130—160 °C region, i.e. before the melting of glucose, and the second one takes place after the melting of glucose. The two steps can be assumed to differ in that at the lower temperature the reaction takes place on the surface of glucose grains floating in molten cysteine, whereas at higher temperatures the reaction also extends to molten glucose. A second explanation for the two steps is that cysteine has two active centres activated at different temperatures.

On plotting the weight losses observed in the first stage of the reaction in function of composition a curve with two maxima is obtained; the two maxima are at cysteine contents of 10 and 30% (Fig. 10). If the weight losses observed in the two steps are related to the amount of glucose and plotted against the amount of cysteine, the curve shown in Fig. 10 can be obtained. Circles denote the weight loss occurring in the first step, crosses indicate the sum of the weight, losses of the two steps. It is clear that initially the weight loss increases with the amount of cysteine, then reaches a constant value of 54% at a cysteine content of 17.2%. This means that 1 mole amino acid

forms an equilibrium mixture with 4 moles of glucose. This result differs from those obtained for the amino acid — glucose systems studied so far, and indicates the second strongest catalytic effect on sugar decomposition after the effect of cystine. This is presumably in correlation with its relatively low melting point.

Consequently, it can be stated that the sulfur-containing amino acids nvestigated are active components of the Maillard reaction under the given ıxperimental conditions, and they show no protecting effect.

The chromatographic analysis of the reaction mixtures revealed a very cumplex composition already in the middle stage of the Maillard reaction; a oore detailed analysis of the results is in progress.

2.2.3. *Kinetic investigation of the glucose-lysine reaction*

In addition to a static analysis, thermal curves make it possible to determine the kinetic parameters of the reaction. In this paper the kinetic analysis of the glucose-lysine reaction is presented on basis of earlier investigations. The thermal curves divided into sectons, were analysis by an iteration process suggested by Briscal and based on the method of Zsakó.

The investigations were extended to pure glucose, pure lysine and to the following glucose-lysine mixtures: 90 + 10, 10 + 90 and 70 + 30. The latter mixture corresponds to the optimum ratio.

Table 1 shows the kinetic parameters obtained for the section of Maillard reaction, or to the first decomposition sections of the pure substances.

Table 1

The kinetic parameters of the Maillard reaction of glucose-lysine mixtures

Glucose: lysine ratio	T_{max}	Activation energy kJ/mol	Preexponentional factor	k_{150}	$\frac{k_{150}}{C_{LYS}}$
100 : 0	215	140	5.60×10^{13}	7.0×10^{-4}	—
90 : 10	175	119	0.77×10^{13}	300×10^{-4}	0.30
70 : 30	150	111	0.21×10^{13}	909×10^{-4}	0.30
10 : 90	153	83	0.0012×10^{13}	1388×10^{-4}	0.15
0 : 100	273	223	1.45×10^{21}	0.002×10^{-4}	0.2×10^{-7}

It can be seen that at 150 °C, corresponding to the first decomposition step, neither glucose nor lysine decomposes to significant extent. The activation energy of their decomposition is considerably higher as well. The activation energy of samples containing glucose in excess is also higher than that of mixtures with lysine excess.

The rate constant calculated for 150 °C (k_{150}) depends on the concentration of amino acid, and increases proportionally to the amount of amino acid up to the optimum ratio. Above this ratio it does not increase, and thus the ratio k_{150}/C_{LYS} decreases in the samples containing amino acid in excess (k_{150} does not increase, only C_{LYS}).

The activation energy obtained with samples containing glucose in excess is similar to the activation energy of the caramelization of pure glucose measured by other methods. In the case of amino acid excess this parameter is similar to the activation energy of the Maillard reaction determined by other methods.

The results of the kinetic investigation of the further decomposition steps indicated that the decomposition of lysine in the presence of glucose required higher activation energy, which can be traced back to the missing amount of lysine consumed in the reaction of the two components. In certain, apparently similar stages just the elastic changes of kinetic parameters indicated that the actual situation is a superposition of different processes.

Summary

In order to complete our previous studies of the reaction between glucose and lysine the thermal curves were subjected to kinetic analysis; it has been found that the kinetic parameters permit more refined and deeper conclusions to be drawn, and also confirm the conclusions of the static analysis of the thermal curves.

Sulfur-containing amino acids, cystine and cysteine, have very active, catalytic effect on the Maillard reaction under the given experimental conditions. 1 mole cysteine can react with nearly 4 moles, 1 mole cystine with nearly 10 moles of glucose, and catalyze its conversion into colouring substances. This is in disagreement with the results of certain authors which suggest conclusions on some protecting effect of sulfur containing amino acids.

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Dr. Ferenc ŐRSI H-1521 Budapest