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Kinetics of the Maillard Reaction Between Lysine and Some Reducing Sugars

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SUMMARY

The extent of nonenzymic browning in the model systems lysine - glucose, lysine - fructose and lysine - lactose was measured at temperatures 323, 333, 343 and 363 K spectrophotometrically. In each system, browning followed zero - order kinetics The calculated activation energies were 116.6, 153.1 and 162.5 kJ mol^{-1} for lysine - fructose, lysine - glucose and lysine - lactose systems, respectively. Results proved that between the temperatures 323 - 363 K, the reactivity of sugars under examination towards lysine in the Maillard reaction devreased in the order fructose, glucose and lactose.

ÖZET

Lisin - glukoz, lisin - fruktoz ve lisin - laktoz model sistemlerinin 323, 333, 343, 353 ve 363 K'deki enzimsiz kararma tepkimeleri her model sistemde sıfır derece kinetiği gösterdi. Aktivasyon enerjileri lisin - fruktoz sistemi için 116.6 $kJmol^{-1}$, lisin - glukoz sistemi için 153.1 $kJmol^{-1}$ ve lisin - laktoz sistemi için 162.5 $kJmol^{-1}$ olarak hesaplandı Sonuçlar 323 - 363 K arasında, lisin ile olan Maillard tepkimesinde şeker reaktivitesinin fruktoz, glukoz ve laktoz sırasıyla azaldığını gösterdi.

INTRODUCTION

Stability of foods during storage depends on many factors such as T, pH, a_w, light, presence of O_2. Each of these factors have different effects on various deteriorative reactions in foods. Browning is one of these deteriorative reactions which is encountered guite often in foods. Since it generally changes the color, flavor, appearance and nutritional values of foods, browning plays an important role in the acceptance of a food product by consumers. Occurence of browning reactions are sometimes desirable as in the cases of coffee roasting, bread toasting and potatoes frying. But in many cases, such as fruits, vegetables, frozen and dehydrated foods, browning is an undesirable process.

Browning reactions may be broadly divided into two categories as enzymic and nonenzymic. Enzymic browning reactions take place in many fruits and vegetables such as potatoes, apples, bananas and peaches when the tissue is cut, peeled, bruised and diseased. These reactions are catalyzed by the enzyme polyphonel oxidase in the presence of air. Hence when the injured tissue comes into contact with air, a rapid darkening occurs. (1) Nonenzymic browning reactions, on the other hand, do not require enzyme. Three main reactions may be included in this group : Maillard reaction, caramelization and ascorbic acid oxidation. Caramelization is the name given to the process of darkening when sugars are heated to very hing temperatures. Under controlled conditions, this is a required color change for the production of caramel which finds uses in candy industry.

Browning as a result of ascorbic acid oxidation is observed mainly in citrus fruits. Ascorbic acid oxidation occurs in the presence of oxygen and leads to the formation of furfural, derivatives of furfural and carbon dioxide. Maillard reaction, which is the most important nonenyzmic browning reaction in foods, includes the reaction of reducing sugars with amino acids, peptides and proteins. Rate of Maillard reaction is dependent on temperature, pH, water activity and reactant concentration. Final reaction products are brown melanoidin pigments. In addition to this color change, various off - flavorn may be producedand nutritional value of the food is decreased due to the unavailability of the bound amino acids.

There is a great number of experimental work perfromed in order to elucidate the nature of Maillard reaction. Ashoor and Zent

(2) have classified the amino acids into three groups as high browning producing amino acids lysine, glycine, tryptophan, tyrosine; intermediate browning producing amino acids proline, leucine, isoleucine, alanine, hydroxyproline, phenylalanine, methionine, valine; and low browning producing amino acids histidine, threonin, aspartic acid, arginine, glutamic acid and cysteine. It has been established that Maillard reaction is one of the main degradative reactions occurring at a maximum rate in intermediate moisture foods which have an a_w range of 0.60 - 0.85. Addition of glycerol or other liquid humectants, however, seems to decrease this a_w range at which maximum browning occurs. (3) The rate of Maillard reaction has been reported by several workers to follow a zero - order kinetics. (3 - 6). pH of the medium has an obvious effect on the rate of this reaction, higher pH values being more preferred than the lower ones (7). Ashoor (2), in a study of the effect of pH on Maillard browning of lysine, alanine and arginine with glucose and fructose, has demonstrated that the rate of reaction has increased until the optimum pH of 10 and then decreased at higher pH values. Research in this area also indicates the role of the concentration of reactants in the degree of nonenzymic Maillard browning. (8, 9, 6).

The primary step in the Maillard type nonenzymic browning reactions is the condensation of the α - amino groups of amino acids or proteins with the carbonyl groups of reducing sugars to form carbonylamino compounds which is followed by the Amadori rearrangement leading to the production of ketose sugar derivatives. The mechanism of the rest of the reactions which causes the brown colored pigment formation is not well - defined. The Amadori compound might be rearranged into 5 - hydroxymethyl - 2 - furaldehyde in one pathway, while the second pathway requires the formation of C - methyl reductones and α - dicarbonyls. Both of these pathways lead to the production of melanoidins when the reaction products react with amines. A third pathway is the Strecker degradation of α - amino acids in the presence of α - dicarbonyls or other conjugated dicarbonyl compounds (1).

The aim of this study was to compare the reactivities of the reducing monosaccharides glucose, fructose and the reducing disaccharide lactose towards the amino acid lysine in the nonenzymic Maillard browning reaction by determining the activation energies for their interaction.

MATERIALS AND METHODS

In the present study, the occurrence of nonenzymic Maillard type browning reactions was examined in three model systems, namely, glucose - lysine, fructose - lysine and lactose - lysine. α - amino acid L - Lysine was purchased from SIGMA Chemical Company. D (—) fructose was bought MERCK. D (+) glucose and α - lactose were obtained from OXOID. All solutions were prepared by double distilled water. Carbonate buffer of pH 10 was also used. For the absorbance measurements, a Beckman Model 24 Spectrophotometer was used.

In order to find the best experimental conditions, various preliminary tests were performed at varying concentrations of reactants by changing the pH and measuring the maximum absorbance of the brown color produced. A pH of 10 seemed to be most suitable for our purposes when the initial concentration of each reactant was 0.5 M. Maximum absorbance of colored solutions was found to be at 313 nm. Hence 313 nm was chosen as the wavelength of absorbance measurements in experiments. Stock solutions (0.5 M) of each of the glucose, fructose, lactose and lysine were prepared for the study.

PROCEDURE

Reactions of the three model systems, separately, were followed at five different temperatures; 323, 333, 343, 353 and 363 K.

Into each of the five small balloons, with 10 ml volume, the following solution was introduced : 1 ml of the 0.5 M lysine stock solution, 1 ml of the 0.5 M sugar stock solution and 3 ml of the carbonate buffer, pH 10. Balloons were then tightly capped and placed in a water bath maintained at the desired constant temperature which is controlled to $\pm$ 0.5°C. After the thermal equilibrium was reached, balloons were removed from the

water bath at 30 min., intervals, and quickly cooled with tap water. Absorbance of the cooled solution was measured at 313 nm, using double distilled water as the reference solution.

RESULTS AND DISCUSSION

For all model systems studied, intensity of brown color formation at each temperature increased as the reaction time was lengthened. Browning also intensified noticably as the temperature was increased. Figures 1 and 2 are the examples of increasing absorbance values at a constant temperature for the fructose - lysine, glucose - lysine and lactose - lysine systems by increasing reaction times. As seen in Figures 1 and 2, data suggest zero - order kinetics, representing a linear relationship between absorbance values and reaction times. Date at other temperatures also gave similar linear relationships. Therefore, the rate constant for brown color formation in the three model systems were calculated by assuming a zero - order rate as reported earlier. (10). They are obtained from the slopes of linear plots of measured absorbance values versus time for each temperature condition tested and are given in Table 1.

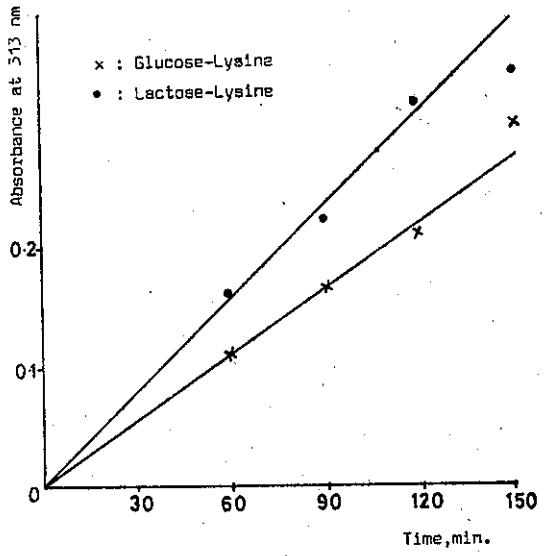

Fig. 1. Brown color formation in Glucose-Lysine and Lactose-Lysine model systems at 323 K.

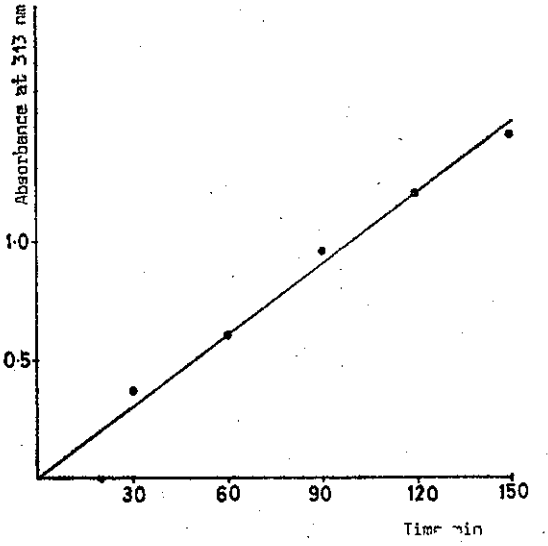

Fig. 2. Brown color formation in Fructose-Lysine model system at 323 K.

Table 1. Rate constants for brown color formation
Rate constant (Absorbance min^{-1})

Model system	323 K	333 K	343 K	353 K	363 K
Fructose - Lysine	9.95×10^{-3}	3.69×10^{-2}	1.49×10^{-1}	—	1.29
Glucose - Lysine	1.83×10^{-3}	2.51×10^{-2}	6.94×10^{-2}	5.14×10^{-1}	1.18
Lactose - Lysine	2.70×10^{-3}	7.00×10^{-2}	—	1.014	3.293

Lacking rate constants in Table 1 for fructose - lysine and lactose - lysine at temperatures 353 K and 343 K, respectively, could not be determined due to some experimental misfortunes.

It is shown in Table 1. that at the lowest temperature under study, the value of the rate constant for the fructose - lysine system is the largest. Those of glucose - lysine and lactose - lysine systems are relatively close to each other. This tells the ease of reaction at 323 K between fructose and lysine compared to the other two systems. However, as the temperature is increased, the value of the rate constant for the lactose - lysine system shows more increase when the changes in other rate constant are considered. Thus at 363 K, the values of the rate constants are 130, 645, and 1220 times larger than the corresponding values at 323 K for the fructose - lysine, glucose - lysine, and lactose - lysine systems, respectively.

Activation energies were obtained from the plots of natural logarithm of rate constants versus reciprocal of temperatures according to the Arrhenius model. The Arrhenius plots of the three model systems are shown in Figures 3, 4 and 5. Tablo 2 represents the values of activation energies calculated from the slopes of Arrhenius plots by the method of least squares. Correlation coefficient for each model system is also included in the same table.

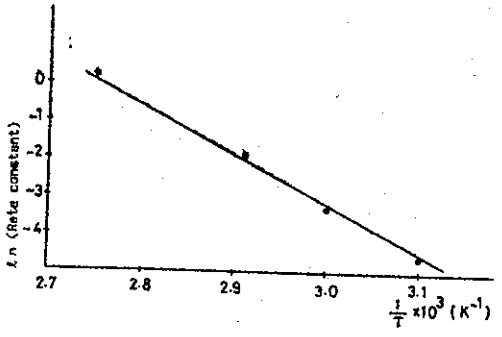

Fig. 3. Arrhenius plot for browning of Fructose - Lysine model system.

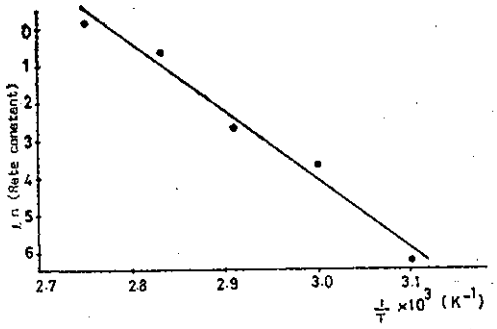

Fig. 4. Arrhenius plot for browning of Glucose - Lysine model system.

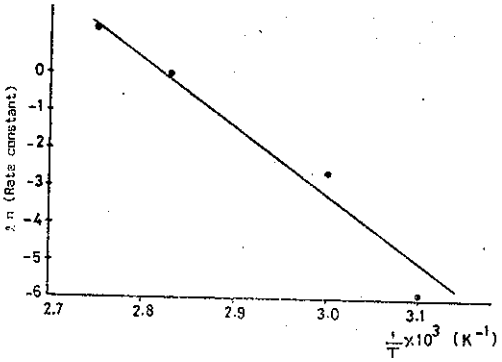

Fig. 5. Arrhenius plot for browning of Lactose - Lysine model system.

Tablo 2. Activation energies for brown color formation

Model system	Activation energy (kJmol^{-1})	r^2
Fructose - Lysine	116.6	0.999
Glucose - Lysine	153.1	0.989
Lactose - Lysine	162.5	0.984

The value of activation energy for the fructose - lysine system is the smallest while that of the lactose - lysine is the largest one. Glucose - Lysine system seems to have an intermediate activation energy value between the other two systems. These results are in agreement with the values previously reported. (11 - 13) It is known that the initial rate of browning of a reducing sugar with amino acids is dependent on the rate at which the sugar's ring opens and reducing group becomes available for reaction. Therefore, pentoses are expected to undergo browning reactions more

repidly than hexoses which inturn show browning at a faster rate than reducing disaccharides. Fructose, being a ketohexose, forms N-substituted 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-mannose and 1-amino-1-deoxy-D-glucose derivatives and hence it participates in browning reactions more easily compared to glucose. The rate of enolization process is also faster in fructose compared to that of glucose. Therefore, the lower activation energy of the browning process in fructose-lysine system is actually an expected result from these considerations. This activation energy of 116.6 kJ mol^{-1} for the fructose-lysine system compared to 153.1 kJ mol^{-1} for the glucose-lysine system may of course change during the later stages of browning or at different temperature ranges since the reaction mechanism might change under different reaction conditions. The highest value of activation energy obtained in the present study is due to the browning in the lactose-lysine system. α-Lactose is named as O-B-D-galactopyranosyl-(1 $\rightarrow$ 4) - α-D-glucopyranose. Hence, reducing group of only the glucose portion remains free. Its reducing property is less than those of fructose and glucose. Most probably the mechanism of browning is different in the presence of lactose compared to monosaccharides at these high temperatures. As a result, this study showed that the Maillard reaction between lactose and lysine is more temperature-dependent than the reactions of glucose and fructose with lysine in the temperature range 323-363 K.

Maillard type browning reactions need more research for the understanding of the mechanisms involved. Some applications related to food products are in progress in our laboratories.

KEY WORDS

Kinetics of Maillard reaction, Nonenzymatic browning reactions, Lysine, Glucose, Fructose, Lactose.

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