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Perikles Markakis

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A STUDY OF THE SUGAR-AMINE REACTION THROUGH MODEL SYSTEMS

By

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ABSTRACT

A STUDY OF THE SUGAR-AMINE REACTION
THROUGH MODEL SYSTEMS

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Karim Nafisi-Movaghar

The extent of the browning resulting from the interaction of ten amino acids (alanine, arginine, aspartic acid, glutamic acid, glycine, lysine, methionine, phenylalanine, serine and α-amino-n-butyric acid) with two sugars (glucose and fructose) was measured as a function of pH, temperature, duration of the reaction, and concentration of reactants. The main findings are summarized as follows:

Increasing the pH of the system from 6 to 8 increased the rate of browning (absorbance at 540 nm), though not linearly;

The extent of browning was less for fructose than glucose while reacting with amino acids, except in the model systems containing amounts of glycine less than 0.031 M, at pH 8.0;

Fructose was found to caramelize more readily than glucose;

Among the amino acids tested, lysine and arginine were the most active in the production of brown color when

reacting with glucose and fructose. However the activity of arginine at pH 6 with fructose was nearly nil;

At pH 7 the reactivity of arginine with fructose was greater than that of lysine with fructose;

Glutamic acid and aspartic acid appeared to suppress the caramelization of glucose and fructose;

The addition of glutamic acid or aspartic acid to model systems containing lysine and glucose or lysine and fructose diminishes the color developed by these systems. At a 1:1 molar ratio of lysine to glutamic acid, the inhibition of browning is greater when the total concentration of lysine and glutamic acid is higher. Impregnating freeze-dried potato slices with aspartic or glutamic acid solutions resulted in less brown discoloration upon frying the slices;

The activation energy of the glucose-lysine reaction was 13,331 cal/mole, and for the fructose-lysine reaction 10,210 cal/mole;

The Q_{10} for the same reactions were 1.87 for glucose-lysine and 1.61 for fructose-lysine.

To Terry for all her patience

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INTRODUCTION

The carbonyl-amine interaction which results in the formation of brown pigments in foods has attracted the attention of many investigators since the first pertinent observations of Maillard in 1912. This aptly named browning reaction is desirable (even obligatory) in some foods (e.g., cakes, bread, coffee, and beer) as it results in both an attractive color and a pleasant flavor. In other cases, browning is not desirable because it results in discoloration (e.g., in dried or canned fruits and vegetables, white wine and dried eggs). Furthermore, the nonenzymatic browning of foods may affect the nutritional value of foods by reducing the availability of certain amino acids; certain products of the Maillard reaction may be even hazardous for health.

To prevent browning reactions, physical methods, such as the control of the moisture content and storage temperature of foodstuffs, has been used. Chemical methods have also been investigated. Sulfur dioxide has been used in the prevention of the browning of dried fruits for centuries. Sorbitol, a substitute for glucose, has been reported to cause less browning. An aluminum salt of

phytic acid has been considered as a retardant of non-enzymatic browning.

A careful review of the literature indicated that a systematic investigation of the reaction of different amino acids with the two most common reducing sugars, i.e., glucose and fructose, under varying conditions. A comparison of fructose to glucose in terms of their potential for causing non-enzymatic browning seemed especially to be necessary in view of the large quantities of high fructose corn syrups that are being used currently in the food industry (1.5 billion pounds in 1973).

In the present study, the reactivity of glucose and fructose with ten individual amino acids was investigated under a variety of experimental conditions. During the course of those experiments, the observation was made that aspartic acid and glutamic acid failed to react and even inhibited the reaction of the other amino acids. Consequently, these two dicarboxylic amino acids were then tested as browning inhibitors in a food commodity, fried potatoes.

LITERATURE REVIEW

Non-Enzymatic Browning

The formation of brown pigments (melanoidins) while heating a solution of glucose and glycine was first observed in 1912 by the French chemist L. C. Maillard. Similar discolorations have been observed when nitrogen-containing compounds such as ammonia, amines, amino acids, peptides, and proteins react with sugars, aldehydes or ketones. Browning occurs also during heat pyrolysis of sugars, which is a different phenomenon though the appearance is the same. It is now known that the Maillard "carbonylamine" reaction, a major cause of browning during the prolonged storage or heat processing of food, involves a condensation between the α-amino groups of the amino acids or proteins and the carbonyl group of the reducing sugars.

After the first interaction of the amino group with the carbonyl group, a series of complex and continuous reactions occur. The final products of these reactions are colored, soluble and insoluble pigments called melanoidins.

A. Production of N-Substituted Glycosylamine

The condensation product of an amine and an opened ringed form of sugar is one molecule of water and

Schiff's base. Schiff's base has never been isolated from the reaction medium (Figure 1). The stoichiometry of the reaction is one amine to one sugar (Hannan and Lee, 1952). However, reactions of up to 6 moles of amine with one mole of sugar have also been reported (Erickson, 1953). Subsequent cyclisation of Schiff's base produces N-substituted glycosylamine (Figure 2). The initial reaction is reversible, but irreversible reactions (not chemically irreversible) soon follow (Haugaard et al., 1951).

D-Glucose Schiff's base

Figure 1. Schiff's base production from condensation of carbonyl and amino groups.

B. Production of Amadori Compound

N-substituted glycosylamine goes through a series of rearrangements in the presence of protons with an isomerization often called the Amadori rearrangement (Figure 3). N-substituted 1-amino 1-deoxy-2-ketose is called the Amadori product.

The "Amadori" rearrangement involves the transition from an aldose to a ketose sugar derivative. Amadori rearrangement products are more stable than the original glycosylamines. Reaction products up to this stage are

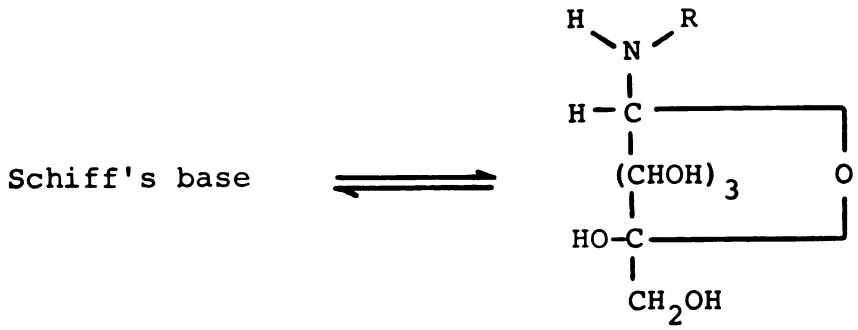


Figure 2. Production of glycosylamine from Schiff's base cyclization.

colorless. The color starts to be produced after the third stage, which is the production of melanoidins from the Amadori compound.

C. Formation of Pigments

The "Amadori" rearrangement products undergo a browning decomposition in aqueous solutions. The rate of browning is highly enhanced in the presence of amines. The reaction pathway and its mechanism after the formation of ketose are not clearly understood. Reactions at this stage involve many condensations and polymerization reactions, giving brown pigments or melanoidins at the final stage of reaction.

The main reactions occurring are thought to be aldol condensations, the formation of heterocyclic nitrogen compounds such as pyrroles, pyridines, imidazoles, and aldehyde-amine polymerization (Hodge, 1953).

A simplified pathway of melanoidin formation originating from Amadori compounds is shown in Figure 4

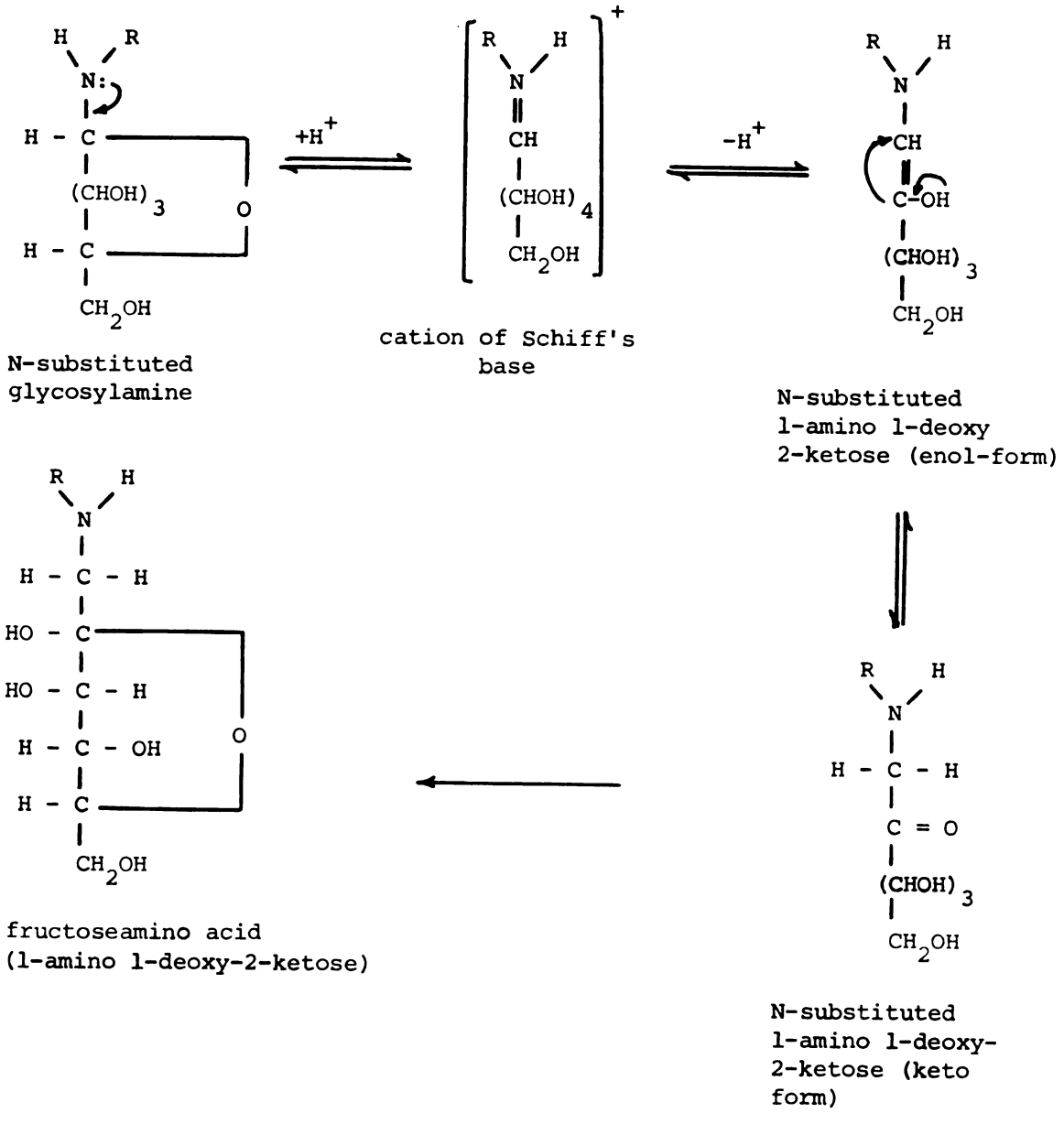


Figure 3. The Amadori rearrangement.

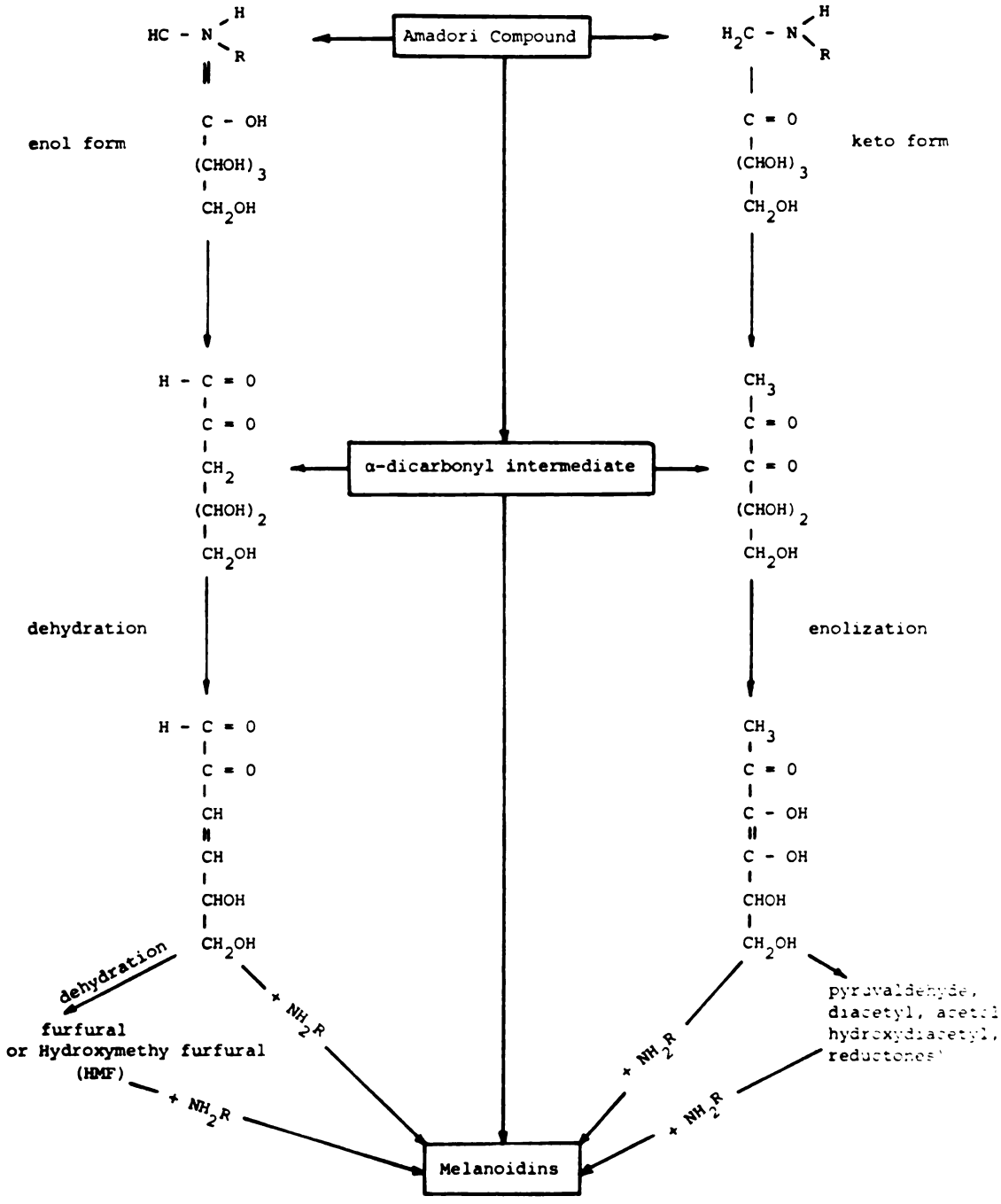


Figure 4. Melanoidins formation from Amadori compounds.

(Markakis, 1979). Melanoidins can originate either from the keto form or the enol form of the Amadori compound.

1. From the Keto Form

The keto form of the "Amadori" rearrangement product decomposes to a methyl-α-dicarbonyl derivative by the elimination of the amino group. The dicarbonyl compound can undergo various enolizations and ketolizations to yield numerous isomerized intermediates. The intermediates either condense with amino compounds (aminated) to form melanoidins, or break down first, then react with amino compounds and produce melanoidins.

2. From the Enol Forms

Deamination of the enol form of the Amadori compound yields 3-deoxy-α-dicarbonyl intermediates. Dicarbonyl intermediates are subjected to varying degrees of molecular dehydration. Furfural (2-furaldehyde) and 5-hydroxymethyl-2-furaldehyde (HMF) could be the product of this dehydration. The products of this dehydration condense with amines and produce melanoidins.

In summary, in the "Amadori" rearrangement, a 1-amino 1-deoxy 2-ketose type molecule is formed from an N-substituted glycosyl amine. The Amadori compound is a turntable for the reaction which can progress in three different ways:

1. Reactions producing fission products, small carbonyl molecules, such as pyruvaldehyde, diacetyl, acetol and hydroxy-diacetyl.
2. Severe dehydration results in furfurals and 5-hydroxymethylfurfurals.
3. Moderate dehydration produces reductones and dehydro-reductiones.

Some of the Maillard reaction products are water soluble and others are water insoluble. The soluble products are called premelanoidins and the insoluble ones melanoidins. Figure 5 shows a general outline of the reactions (Adrian, 1974 and 1973).

Strecker Degradation

Strecker degradation is not primarily concerned with pigment production; however, it provides reducing compounds essential for brown color formation. The Strecker degradation is more important for flavor development than for browning. It involves the degradation of α-amino acids and the formation of a corresponding aldehyde containing one carbon atom less, which is lost as a carbon dioxide. The degradation is carried out by α-dicarbonyl compounds (Figure 6).

According to Schonberg, et al. (1948), only carbonyl compounds containing the structure,

$$- \overset{O}{\overset{\|}{C}} - [- C = C -]_n - \overset{O}{\overset{\|}{C}} -$$

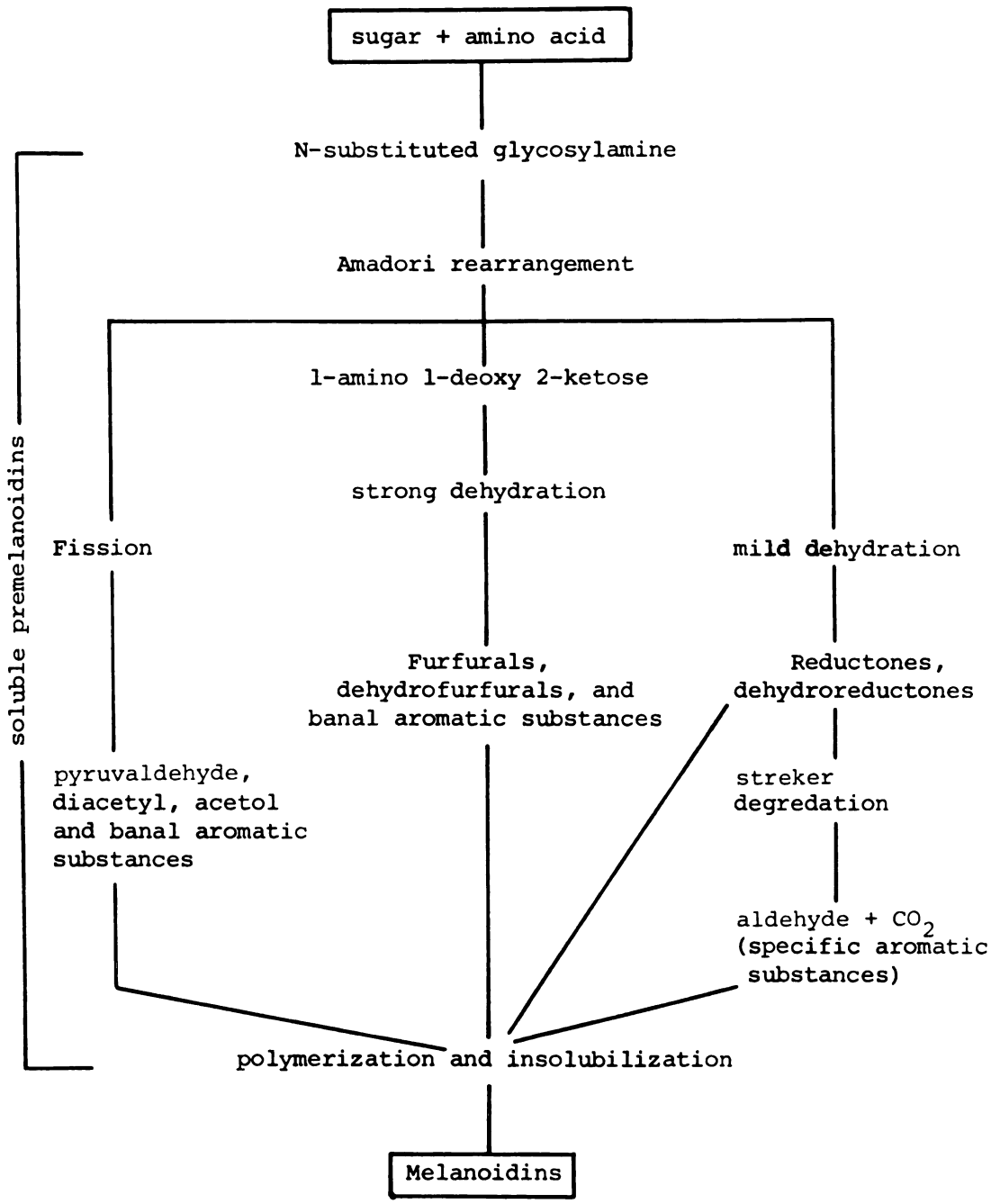


Figure 5. General overview of the Maillard reaction.

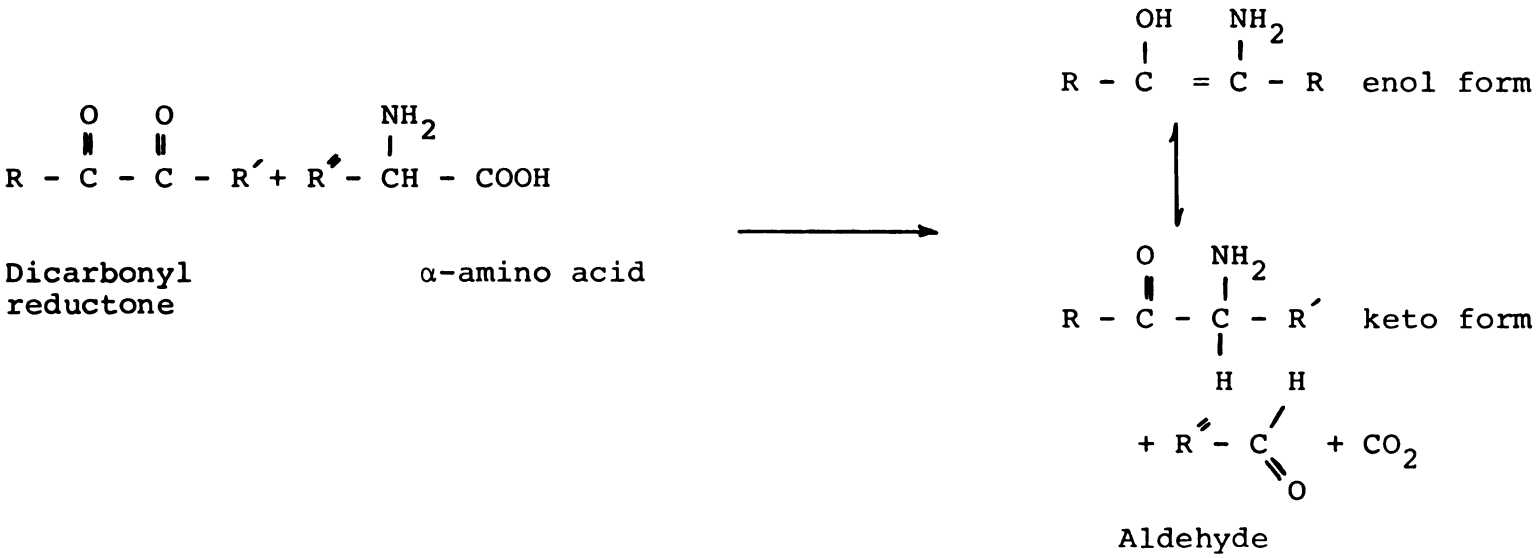
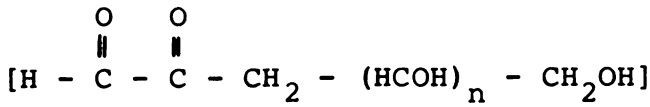
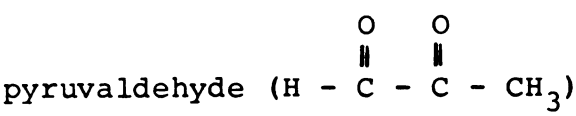


Figure 6. An example of Strecker degradation reaction.

where n is zero and/or an integer, can initiate the degradation.

If the α-dicarbonyl compound is an osone such as 3-deoxyosone,





the α-amino nitrogen is not lost in the reaction but only transferred to the α-position of the osone moiety (Hodge, 1959).

Caramelization

Caramelization is another example of nonenzymatic browning. It is the degradation of sugars either in a solution or dry while in the absence of nitrogen-containing compounds. This degradation is an acid, base or salt catalyzed process.

Many substances discovered during sugar degradation are also found during Maillard reactions, so the two processes should be clearly distinguished. The distinction between sugar degradations and the Maillard reaction is possible upon the black precipitate collected at the end of the reaction. Sugar decomposition produces nitrogen-free precipitation, whereas the Maillard reactions generate nitrogen-containing products of variable nitrogen content.

Alkaline and acidic degradation of sugars such as starch, sucrose, glucose, and fructose, follow a common pathway, and the following steps have been proposed:

a. Acid degradation:

First, 1,2- enol is formed from the corresponding sugars, then it is followed by a molecular dehydration in a series of stages to 5-(hydroxymethyl)-2-furaldehyde (HMF). Hydroxymethyl furaldehyde is a precursor of the pigment (Wolfrom et al., 1948). The intermediates of these reactions are 3-deoxyaldos-2-ene, 3-deoxyosulose, and osulos-3-ene (Figure 7).

b. Base degradation:

The effect of aqueous alkali is very complex. It involves isomerizations, fragmentations, and intramolecular oxidation and reduction. Isomerization occurs by way of an enediol intermediary. Therefore, a solution of glucose in an alkali media yields fructose and mannose, and finally 1,2-enediol. These reactions are known as the Lobry de Bruyn-Alberta van Ekenstein transformations, as shown in Figure 8 (Kearslay, 1975).

The fragmentation is followed after the 1,2-enol formation. It involves the scission of the parent sugar into smaller fragment molecules, such as dihydroxy acetone, glyceraldehyde, formic, acetic, and lactic acid (Figure 9). Following the fragmentation, condensation between ketones and aldehydes generates polymers and browning products.

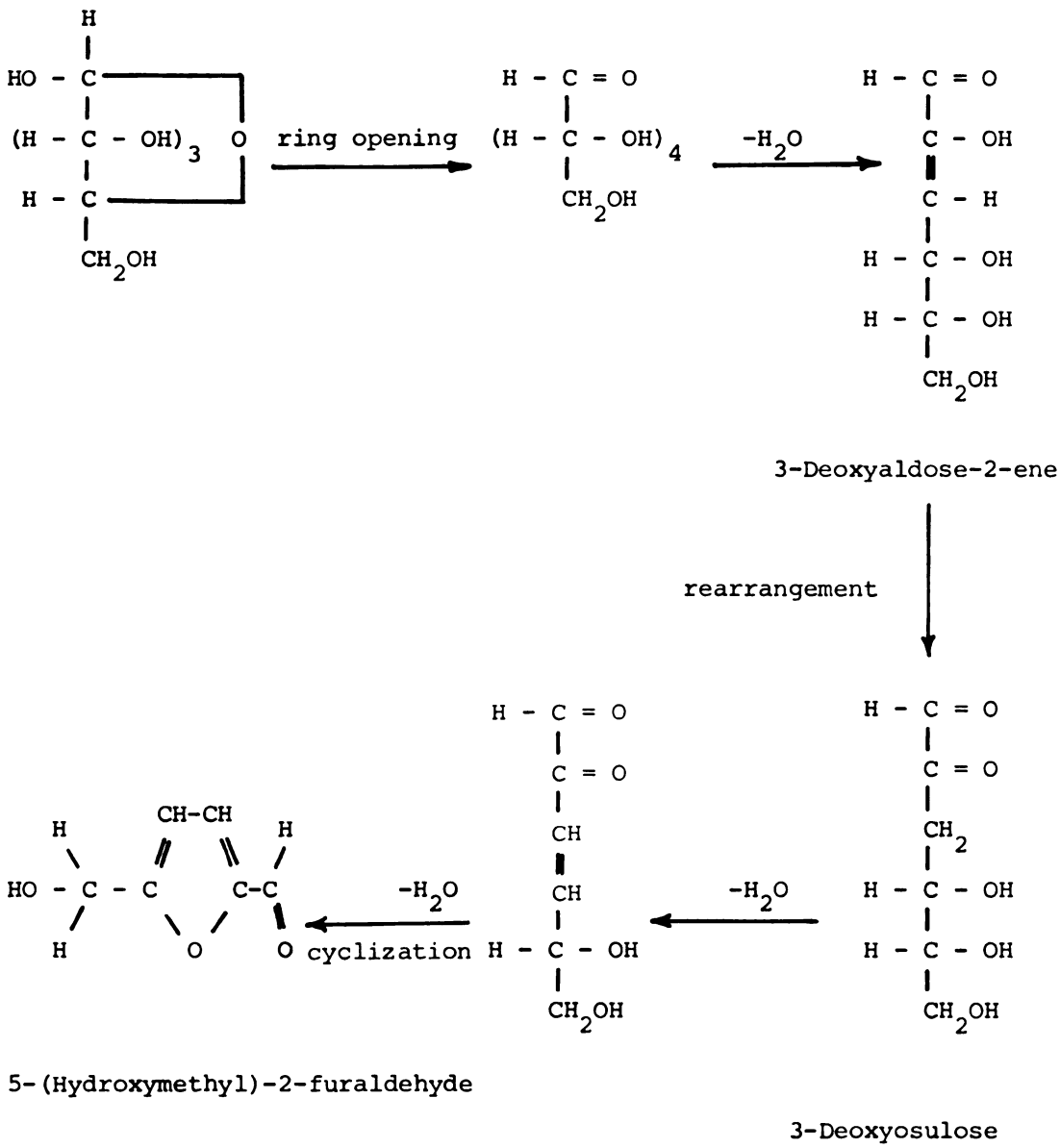


Figure 7. Acid degradation reactions of D-glucose.

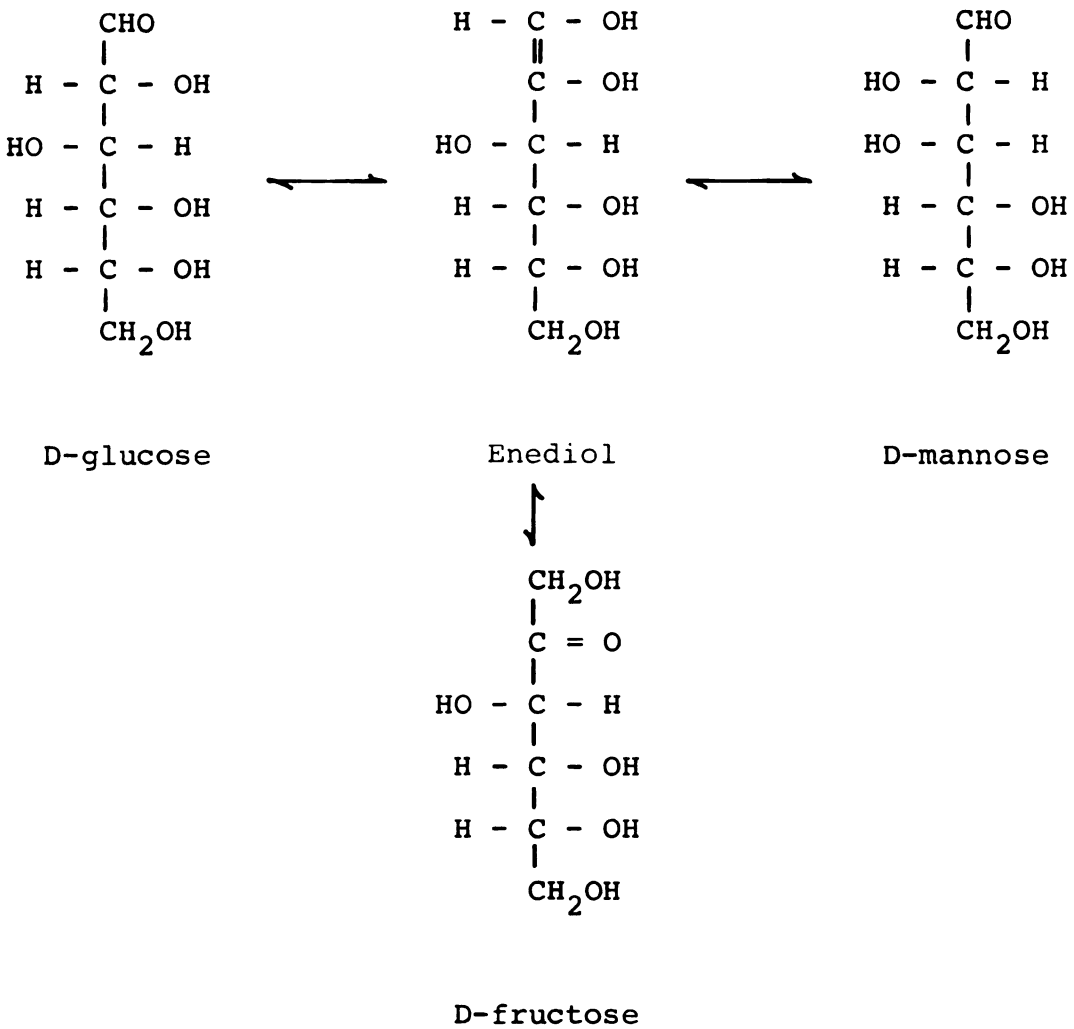


Figure 8. Isomerization reactions (Lobry de Bruyn and Alberta van Ekenstein isomerization).

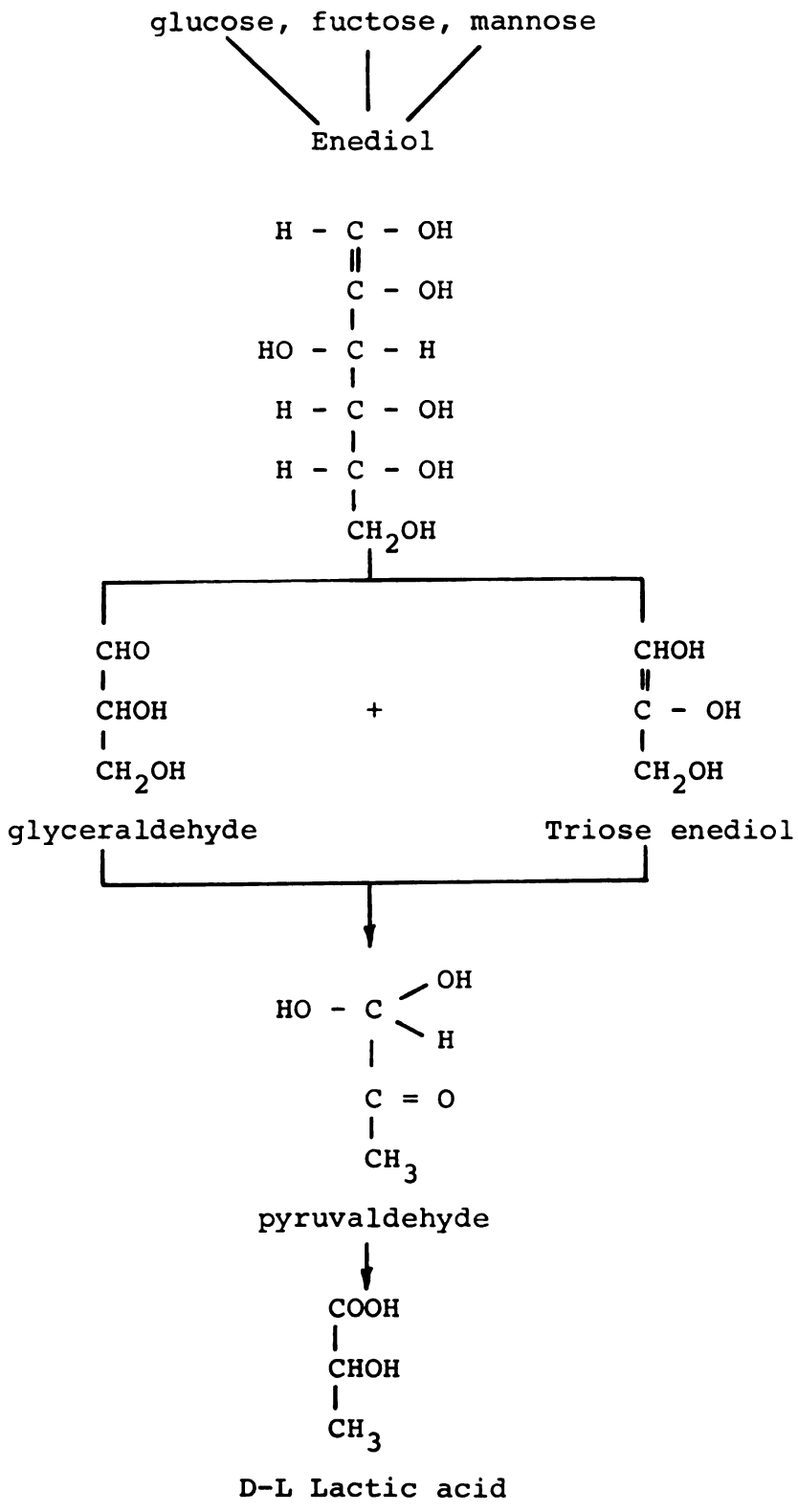


Figure 9. Degradation of enediol to lactic acid.

Factors Affecting the Maillard Reaction

The color formation in sugar-amino acid mixtures has been chosen as an index to determine the effect of various factors in contributing to the color development. Various factors such as temperature, pH, and water content can highly affect the extent and browning rate.

The Maillard reaction has been shown to have a high temperature coefficient. An increase in thermal energy not only helps to break down sugars, but it also increases the rate of collision between the fragments produced during degradation. Activation energy (E_a) and time for 50% of the reaction to take place ($t_{1/2}$) for polylysine-glucose systems are reported to be 22 kcal/mole, and 400 min. respectively (Hansen, et al., 1979). The activation energy for ketosamine fructose-glycine formation is 26 kcal/mole (Reynolds, 1959). In model systems, the rate of browning increases 2 to 3 times for each 10°C increase of temperature (Shallenberger, 1975).

An increase in pH favors the Maillard reaction. The rate of the reaction is extremely high at the alkaline side of the pH. An example of alkaline food is the egg. In order to obviate browning and discoloration during the preparation of egg powders, the pH is lowered by the deliberate addition of acid prior to dehydration. Willits et al. (1958), observed that heating glucose with DL-alanine for 20 minutes at 114°C in an aqueous solution up to pH 6 failed to cause measurable browning. The greatest effect of pH is

with solutions containing diamino acids and the least effect is with the α-amino acids (Underwood, 1959).

The browning reaction in solutions follows the law of mass action, i.e., the rate of the reaction increases by increasing the concentration of the reactant(s). The browning reactions in foodstuffs are more complex. The water activity, (a_w), in the system affects the browning rate. At a very low and a high water activity, the browning rate is minimal due to the solubility limitation and the dilution effect, respectively. The water activity that affects the maximum rate of browning varies for different foods. Pea soup mix at a 70% relative humidity has shown a maximum rate of browning (Labuza, 1970). During the dehydration of diced potatoes, at a 15% moisture level (dry basis), the rate of browning is reported to be maximum. Increasing the moisture level to 33% causes the browning rate to decrease 1.5 fold. Similarly, decreasing the moisture level to 4.9% will cause a 6 fold decrease in the browning rate (Hendel et al., 1955). In a model system (described by Hannan and Lea, 1952), consisting of α-N-acetyl-L-lysine and glucose, the rate of loss of the amino groups reached maximum at 40% relative humidity.

Inhibition of the Browning Reactions

Non-enzymatic browning is usually considered as a deteriative process, particularly by food processors. The loss of the nutritive value of food cannot be compensated

for by adding the lost nutrients to the food during processing, because the non-enzymatic browning may continue even during storage. Because of the complexity of the food systems, controlling the browning is not without problems either. Primarily the process can be slowed down by simply decreasing the temperature, controlling the moisture content, and by gas packing the food, or by controlling the pH.

Non-enzymatic reactions have high activation energies; therefore, lowering the temperature will slow down the reactions. Non-enzymatic browning is accelerated at a high pH value. Therefore, decreasing the pH reduces the browning rate. The processing and storage of food at certain moisture levels in which the non-enzymatic reaction is minimized is another way to control the non-enzymatic browning.

All of these ways seem theoretical, and most of the time not practical. Certain dehydrated or highly acidic foods are not marketable. Keeping food at low temperatures or refrigerated all of the time is not economical, and processing particular foods below the required temperature is not acceptable.

Chemical Methods of Inhibition

A wide variety of inhibiting chemicals have been used to retard non-enzymatic browning such as,

1. Aluminum salt of phytic acid, introduced by Mitsui Totsau Chemicals, Inc., 1976 (Japanese patent).

2. Sorbitol.

Sorbitol has been reported to cause less browning (British patent, 1966). This is due to a conversion of the aldehyde groups to alcohol and consequently, a loss of reducing power. On the other hand, other properties such as viscosity, sweetness, and osmotic pressure remain unchanged (Kearsley, 1978). Hydrogenation lowers the sweetness and hygroscopicity of glucose and renders it less fermentable.

3. Calcium Chloride.

A 0.02%-0.84% calcium chloride solution retards considerably non-enzymatic browning in potatoes (Simon et al., 1955). The inhibitory effect is suggested to be due to the blocking of the amino group. Simon concluded that this blockage might be due to the chelate compound formed with α-amino carboxylic acid. It is also anticipated that the application of an excessive amount of calcium salt may affect the texture of potatoes; consequently, a longer cooking time is required. A positive sinergestic effect was also reported by Simon when the calcium chloride was used along with sulfite.

4. Hydrogen Peroxide.

According to Pokorney et al. (1973), hydrogen peroxide hinders, but not entirely stops, the formation of brown pigment resulting from the interaction of glycine and

diacetyl. Actually, hydrogen peroxide destroys the amino acid rather than blocking it.

$$NH_2 - CH_2 - COOH + H_2O_2 \rightarrow NH_4OH + HCHO + CO_2$$

Pokorny et al., reported that hydrogen peroxide also bleaches the brown pigments.

5. Butylhydroxyanisole (BHA)

BHA has been shown to retard the oxidation of lipids by interfering with the free radical formation. It has also been shown to reduce amino acid and carbohydrate destruction caused by thermal stress (Magna and Monte, 1977).

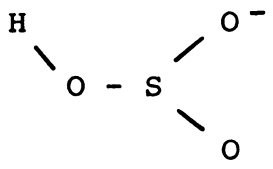
6. Mercaptans

Mercaptans are reducing agents, and they can prevent non-enzymatic browning (Joslyn and Braverman, 1954). Hodge (1953) suggested that the inhibition might be due to the ability of mercaptans to reduce reductones and eliminate the active dehydro reductones. Ingles (1963) reported that the mercapto derivatives of reducing sugars when reacting with amino acid form an imperceptible color compared with non-derivatized sugars. The use of mercaptans is limited because of its unpleasant odor.

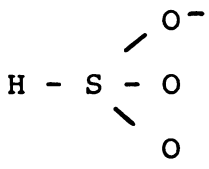
7. Sulfur Dioxide

Sulfur dioxide is the most widely and oldest compound used to retard non-enzymatic browning. It is also used as a preservative, a reducing agent, and for the prevention of enzymatic browning. It is used in the form of its alkali metal sulfites, bisulfites, or as sulfurous acid.

Sulfur dioxide has two series of salts, sulfite ($SO_3^{=}$) and bisulfite (HSO_3^-). Two tautomers of bisulfite are known.



(a)



(b)

At high pH, form (b) dominates to form (a) (Cotton and Wilkinson, 1966). Form (b) has more reducing power than form (a).

Sulfur dioxide is a very strong reducing agent, and has a bleaching character. It can be added to aldehydes and to ketones, and it similarly reacts with reducing sugars and unsaturated carbonyls which are the products of sugar dehydration and reductones.

Ingles (1959) prepared carbonyl bisulfite by reacting bisulfite ion with aldose sugars, such as glucose, galactose, mannose, xylose, and arabinose, at pH 4 (Figure 10). The sulfonated product would release sulfur dioxide upon acid hydrolysis.

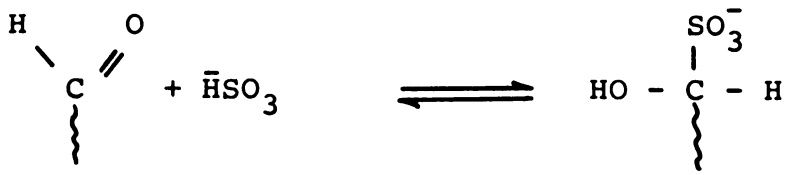


Figure 10. Reaction of aldo sugars with bisulfite ion.

Conjugated unsaturated carbonyl compounds, which are important in the development of chromophors, react with an excess of bisulfite both at the olefinic and carbonyl group at 60°C (McWeeny and Burton, 1962, and 1963). Bronislaw and McWeeny (1974) identified 4-sulphopentosulose in dehydrated, sulphited cabbage.

Ingles (1966) reported bisulfite reactions with glucose at pH 6.5. The sulfonic acid derivative (4-sulfohexosulose) is obtained either by adding it to the unsaturated osone or by substitution in 3-deoxyglucose (Figure 11).

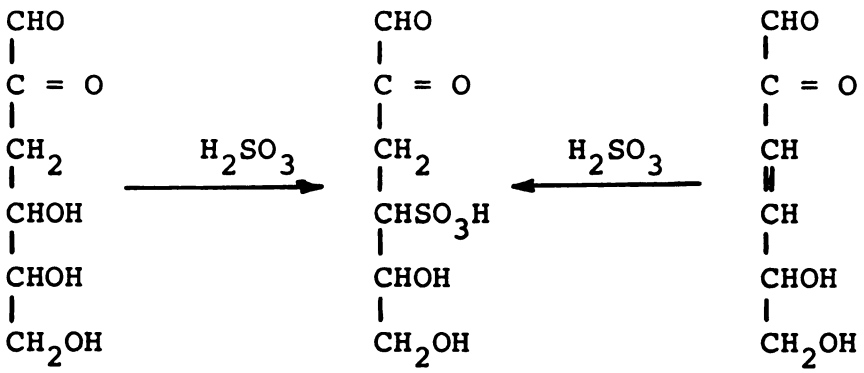


Figure 11. Sulfonation of sugar derivatives by sulfur dioxide to produce 4-sulfohexosulose.

The sulfonic acid derivative obtained at this pH is very stable, and upon reaction with acid or base, even in the Monier-Williams distillation, does not release its sulfur dioxide.

According to Hodge (1953), the amine derivative of carbonyl disulfite prepared by Ingles (1959) may have a role

in inhibiting those browning reactions dependent upon the Amadori rearrangement.

The use of sulfur dioxide for the prevention of non-enzymatic browning works alright, but its safety for human consumption is now being questioned. To prevent the browning of white wine, Safar (1976) fermented must pressed from grapes with sulfur dioxide, producing yeast such as Saccharomyces ellipsoideus and S. carlsbergensis. Safar states that wine produced in this way does not pose the harm for humans that the sulfur dioxide apparently does.

Nutritional Aspects of the Maillard Reaction

The most important aspect of the Maillard reaction is its role in the nutritional value of foodstuffs. It reduces the nutritional value of the free amino acids, proteins, and sugars. Its products also are suspected to be toxic.

A. Amino Acids

An amino acid linked to a sugar is resistant to enzymatic hydrolysis. But the regeneration of the initial sugar and the amine by means of chemical hydrolysis is possible. Therefore, from a nutritionist point of view, the amino acid is destroyed right from the beginning of the Maillard reaction (Cook et al., 1951; Evans and Butts, 1948) and from a chemist point of view it is "blocked."

The behavior of amino acids in browning reactions is different. The closer the amino group to the carboxylic group, the less active is the amino acid in brown color

production. Lysine generates six times more intense color compared to norleucine, having only one α-amino group. Similarly, γ-amino butyric acid contributes 10 times more color compared to its αisomer (Underwood et al., 1959).

The chain length of the amino acids has an effect on the coloring of the sugar solution. The longer the chain length in an homogeneous series, the more intense the color development (Lento et al., 1958).

Friedman and Kline (1950) reported that the biological value of food will be reduced as a result of the reaction of amino acids with glucose. However, during food experiments Friedman and Kline observed that the amino acid-glucose complex is more available to microorganisms than to rats.

B. Proteins

The interaction of sugars and proteins is less understood because of the complexity of their systems. The destruction of proteins by the Maillard reaction varies with respect to the amino acids representing the proteins, e.g., first the N terminal of the protein is attacked, then the basic amino acid side chain, particularly the lysine, is damaged. The sulfur containing amino acid, methionine, is destroyed last by the Maillard reaction. When soybean protein was autoclaved alone, there was no evidence of destruction; however, up to 97% less methionine was liberated by enzymic digestion in vitro from protein autoclaved with sucrose or glucose (Evans et al., 1949b). Evans et al.

(1948), also reported that adding sucrose to soybean protein, and autoclaving it, causes about a 50% destruction of lysine level.

Among the protein-bounded amino acids, lysine is reported to be the most sensitive to the Maillard reaction, and it can become a protein limiting-factor after the Maillard reaction occurs (Adrian, 1972). This is particularly important in processed milk (Greaves et al., 1933; Mauren et al., 1955; McDonald, 1966; and Payne-Betha et al., 1959). Lysine is the first limiting amino acid in cereals. A slight heat treatment of cereal products is enough to block and may destroy a large amount of lysine. Bread crust and toasted bread has much less nutritional value than untoasted bread (Palamidis and Markakis, 1979; Rosenberg and Rehdenburg, 1951). In vegetables, the protein destruction is fairly low. This is because of the lack of active forms of carbohydrates. Among vegetable products, cotton meal-cake seems to be more labile to heat. A 20 min. autoclaving of cotton meal-cake reduces its biological value (B.V.) by 25% (Balica et al., 1959).

C. Sugars

Reducing sugars are severely damaged by the Maillard reaction (Allen et al., 1973; Haugaard et al., 1951). Hannen et al., (1952) reported that in a model system consisting of α-N-acetyl-L-lysine and D-glucose, at relative humidity of 60% the loss of glucose reached a maximum.

The initial hydroxyl configuration and the speed of dehydration are important in the browning reaction. Burton (1963) suggests that the rate of production of brown pigment is closely related to the initial configuration of the aldose.

According to Cantor (1942), the order of reactivity of the aldohexose series are in the ascending order of glucose, mannose and galactose. In ketose/amines, the initial chromophoric development is greater than for aldoses (Burton et al., 1963).

In general:

a. Short chain sugars have greater reactivity. If a hexose destroys 42% of lysine in solution, an equimolar pentose causes 70% destruction of lysine in the same solution. On the basis of their reactivity, pentoses are more reactive than hexoses, followed by disaccharides.

b. The reactivity of sugars also depends on the type of protein involved. A hexose reacting with lactalbumin destroys 64% of lysine moiety, whereas the same hexose destroys only 59% lysine of soybean globuline.

c. Different isomers of the same sugar behave differently when reacting with the same amino acids (Rubenthaler et al., 1963).

Foods low in carbohydrates are subject to less nutritional damage from the Maillard reaction. In foods high in carbohydrates, most of the available amino acids and proteins are destroyed by the carbonyl amino interaction.

This somehow would explain the reason for the low sensitivities of legumes to heat damage. According to Evans and Butts (1949), heating soybeans alone will cause a 3% reduction of its lysine; however, the addition of sugar may decrease the lysine content up to 47%. Schroeder et al. (1961), report that adding 5% glucose to meat, and autoclaving it at neutral pH for 30 minutes reduces its digestible lysine by 11%.

Sugars with an alcohol group may not cause Maillard reaction (Pomerant et al., 1962). Polyhydric alcohols in general and glycerol in particular may contribute to the browning reaction when they are used as humactants in intermediate moisture meats (Obanu et al., 1977). Obanu's results do not rule out the possible mild oxidation of polyhydric alcohols.

D. Toxicity

The production of melanoid substances during Maillard reaction not only occurs at the expense of nutrients, such as sugar, proteins, and amino acids, but also the melanoidins affect the utilization of the nutrient. Melanoidins inactivate amylolytic malt enzymes (Zabrodskii et al., 1960). To obviate this, Zabrodskii suggested fermentation at a temperature which considerably lowers the rate of sugar-amino reactions. Melanoidins modify an enzymatic proteolysis in vitro performed with pepsin and trypsin.

Some commercial solutions designed for parenteral nutrition contain sugar-amino acid complex due to sterilization of the solutions. The presence of these complexes resulted in a 2 to 5-fold increase in the urinary excretion of Zu, Cu and Fe in both adult and infant subjects.

Stegnik et al. (1977) reported that premelanoidins can transfer across the placenta during infusion into the Rhesus monkey. Premelanoidins are not only antinutritional, but toxic as well. The toxicity of the premelanoidins varies with the amino acids involved to produce them. Among the amino acids, lysine premelanoidins are the most toxic.

Reductones are common products of sugar pyrolysis and the Maillard reaction. Reductones become more toxic when they are bound to an amino group. For mice the LD_{50} is 850 mg/living weight for nitrogen-free hexose reductones, whereas the LD_{50} value is reduced to 300 mg/kg, if hexose reductones are methylaminated (Ambrose et al., 1961). Premaloidins provoke histological disturbances in the liver.

E. Flavor Produced by the Amino-Carbonyl Reaction

The products of the Maillard reaction cannot totally be condemned, because they are also responsible for desirable color such as in bread crust, cookies, and for pleasant flavors, such as in roasted nuts, coffee, potato chips, and many other food products. So flavor production could be included as a positive effect of the Maillard reaction, at least from the consumer's point of view.

The sources of aroma could be either from furfurals of sugar pyrolysis or aldehydes derived from amino acids during Strecker degradation. The aldehydes formed from degradation of alanine, glycine, leusine, methionine, and phenylaline are thought to be acetaldehyde formaldehyde, isoraleraldehyde, methional, and phenylactaldehyde respectively (Johnson et al., 1966; and Self et al., 1963). The development of flavors depends on the nature of amino acids which react with sugars; however, the exact stage of Maillard reaction, water content, pH, and temperature are also responsible for them (Adrian, 1973; and Herz et al., 1960).

Bondarovicy et al. (1967), report that the aroma complex of coffee consists of more than two hundred constituents. The volatile components responsible for the aroma of ground coffee were identified by Merritt et al. (1963), as aldehydes, ketones, esters, heterocyclic compounds, sulfur compounds, alcohols, and nitriles. Thus they concluded that heterocyclic compounds, particularly 2-methyl furan and furan, are among the significant compounds in the complex which make up typical coffee aroma.

According to Wiseblatt et al. (1960), the compounds responsible for bread flavor are acetaldehyde, acetone, crotanaldehyde, diacetyl, formaldehyde, furfural, hexanone-2, heptanone-3, isobutyraldehyde, 2-methylbutanal, methylglyoxal, methylketone, n-valeraldehyde, and pyruvic acid. Volatile aldehydes formed by non-enzymatic browning

reactions during baking are a major factor in bread flavor. Linko, Y.-Y. et al. (1963), examined free amino acid content in the crust and crumb of baked bread. They observed a decrease in amino acid content in the crust compared with the crumb regardless of the type of sugar used. This fact would explain the different flavors in the two parts. Linko et al. concluded that the decrease in free amino acids in crusts, together with the formation of several aldehydes, suggest the importance of Maillard-type browning in flavor production. A gradual loss of carbonyl compounds from the crust parallels the aging of bread. In fresh bread a few amino acids are especially responsible for the flavor, e.g., degradation product from leucine (isovaleraldehyde) (Branes et al., 1948). The products of the reaction between glycerol and proline also generate a bread-like aroma (Hunter et al., 1966).

Methylbutanal and methional, products of the Strecker degradation of methionine, are responsible for cheddar cheese flavor (Day et al., 1960). It is proper to mention that flavor production by the Maillard reaction is not desirable in all cases. This again depends on the reaction condition, for instance, the heating casein mixed with 3% lactose for 40 hrs. at 80°C produces an objectionable gluey flavor (Ranshaw et al., 1969).

F. Other Aspects of the Maillard Reaction (Antioxidant Property

The products of amino acid sugar browning not only take part in the color and flavor development of foodstuffs, but also show antioxidative activity. The antioxidant activity is believed to be due to the reductones formed in the browning reaction between the reducing sugars and amino acids (Griffith et al., 1957).

Cooney et al. (1958), suggested a possible mechanism of antioxidation by reductones in oil. Cooney states there are at least four possible reactions for the mechanism of antioxidation in oils (Figure 12):

1. The air oxidation of the endiols to produce α-dicarbonyl compounds;
2. The spontaneous reduction of the α-bicarbonyl compounds enediols;
3. The spontaneous oxidation of the α-dicarbonyl compounds which is independent of oxygen to produce deep-colored products;
4. The oxidation-reduction reactions between the colored compounds and fat peroxides.

Reductones are believed to react with precursors of peroxide-forming compounds rather than directly with peroxides. The features of the reductone-treated oils are long induction periods, slow absorption of oxygen, and low rates of peroxide development (Evans et al., 1958).

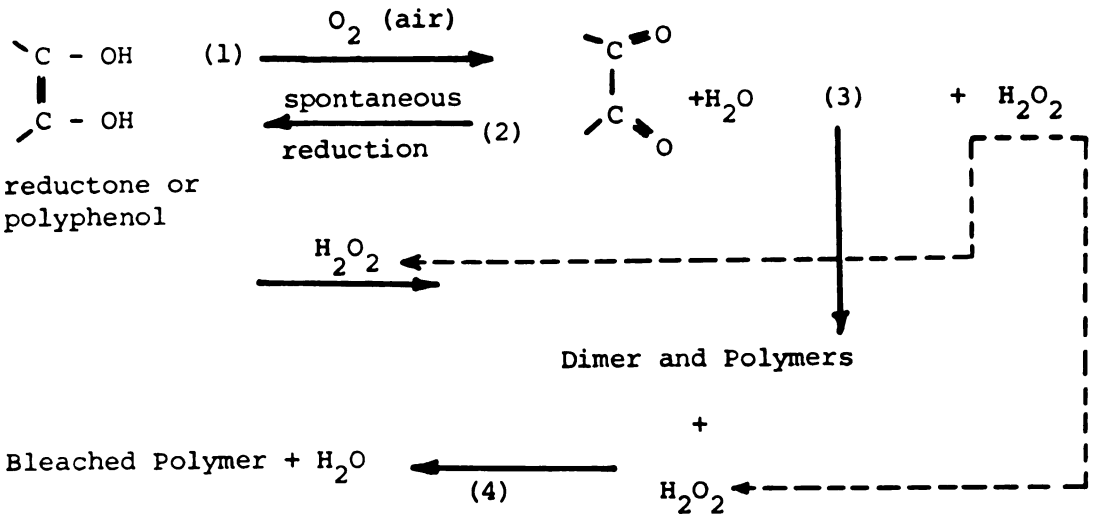
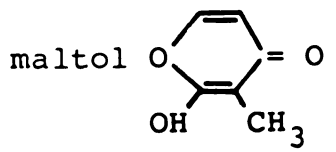
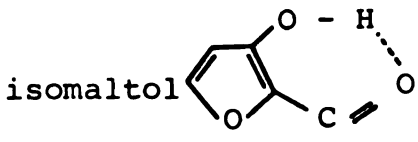
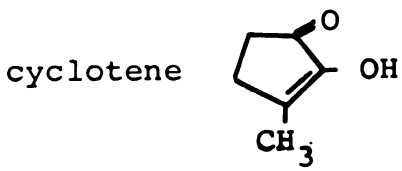


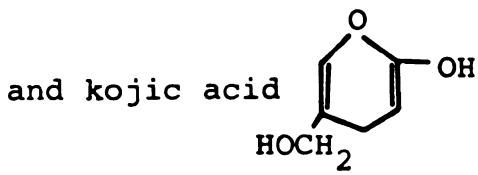
Figure 12. Possible mechanism of antioxidation by reductones in oil (from Cooney et al., 1958).

Alcoholic solutions of some dehydro reductones, such as









at a level of 20-100 ppm have shown antioxidative activity when spread on toasted cereals (Anderson et al., 1963).

A parallel correlation between the color intensity of browning solutions and antioxidant activity is shown in a model system of xylose, glycine, and linoleic acid (Kirigaya, 1968). The author indicated that the antioxidant ability of the model system increases, as the color of the

solution becomes more intense. However, after storing a browning reaction solution, the reductones were decreased, whereas both the color intensity and the antioxidant activity remained constant, meaning that melanoidins have an antioxidant ability also.

The products of the reaction between dihydroxyacetone (DHA) with different amino acids have exhibited a very potent antioxidant activity, followed by xylose and glucose. Among the amino acids reacting with DHA, methionine, leucine, isoleucine and valine give browning products having more potent antioxidant ability than butylated hydroxyanisol (BHA) (Itoh et al., 1975).

METHODS AND MATERIALS

A. Materials

1. Amino Acids and Amine

The amino acids used in this study, along with their molecular weight, are listed below:

Amino Acid	F.W.
Alanine	89.1
Arginine	174.2
L-aspartic acid	133.1
L-glutamic acid	147.1
glycine	75.1
lysine monohydrochloride	146.2
methionine	149.2
phenylalanine	165.2
serine	105.1
D-L-α-amino-n-butyric acid	103.1

All of the amino acids were of reagent grade and were purchased from the Sigma Chemical Company, except for glutamic acid which was obtained from the Nutritional Biochemicals Company.

2. Ampoule-holder

An ampoule holder with the dimensions of l = 20, w = 14, h = 30 cm was built from plexiglass (Figure 13). The ampoule holder is composed of two levels. The first level is fixed to the base. The second level is removable. At each level 43 holes of 2 cm diameter (diameter of one ampoule) were drilled; therefore, the ampoule holder had a total capacity of a maximum of 86 ampoules.

3. Ampoules

Approximately 3,000 ten-milliliter ampoules were obtained from the Fisher Scientific Company.

4. Phosphate Salts

Sodium phosphate, dibasic (FW 142.0) and sodium phosphate, monobasic (FW 137.99) were obtained from the Baker and Mallinckrodt Companies.

5. Reducing Sugars

D-glucose and D-fructose (FW 180.16) were obtained from both the Mallinckrodt Company and the Sigma Chemical Company.

B. General Procedure for Obtaining Data for Reaction Rates

1. Phosphate Buffers

Phosphate buffer solutions of pH 6, 7, and 8 with an ionic strength of 0.2 were prepared by dissolving the

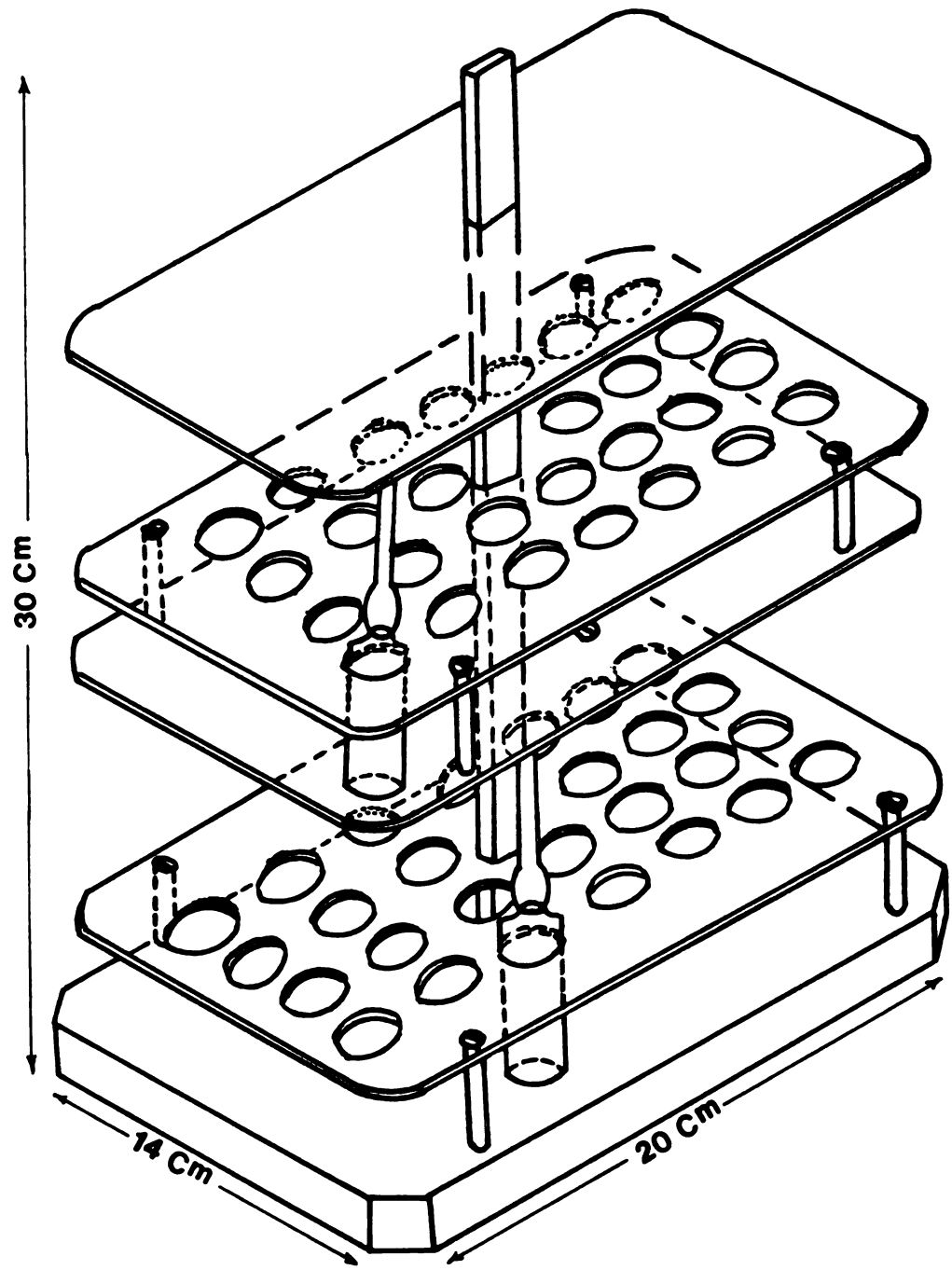


Figure 13. An ampoule holder made of plexiglass. Capacity = 86 ampoules.

proper amount of sodium phosphates in deionized distilled water.

2. Amino Acid Solutions

Stock solutions containing 0.05 moles of each amino acid per liter were made, using phosphate buffers. The stock solutions were made fresh prior to each experiment.

3. Reducing Sugars

Five portions of 1.8016 g (0.01 mole) of D-glucose and/or D-fructose were weighed on an analytical balance and transferred into five 25 mL volumetric flasks. The sugar content in each volumetric flask after dissolving them in buffer was 0.4 M.

4. Final Sample Preparations

From the stock solution of amino acids 20, 18, 16, 14, or 12 mL aliquots were transferred into each one of the individual five 25 mL volumetric flasks containing reducing sugars. The final concentrations of amino acids in the five flasks were 0.040, 0.036, 0.032, 0.028, and 0.024 M respectively. They were then made to volume with the proper buffer solution. From each volumetric flask, an aliquot of 8 mL was transferred into each of three (triplicate) 10 mL ampoules. This step of sample transfer is very crucial since even a very minute amount of sample adhering to the neck of the ampoule could greatly affect the results of the experiment. The ampoules were then

sealed in a flame. If there was any small quantity of the sample adhering to the neck of the ampoule, with the high temperature of hot flame, browning reactions would have proceeded and the results would be very unreliable. The sealed ampoules were then transferred into the ampoule holder and immersed into a water bath at 60°C for 58 hours (Figure 14).

Controls were prepared in exactly the same manner as the samples except that amino acids were absent from them. After 58 hours of reaction, the ampoules were pulled out of the water bath, immediately cooled down to room temperature, and then their color intensity was measured against the blanks. A flow chart of sample preparation is shown in Figure 15.

Table 1 shows the amino acids, sugars, and pH at which each experiment was performed with both glucose and fructose for 58 hours at 60°C.

C. Preparation of Samples Containing Glutamic and Aspartic Acids

Stock solutions of glutamic and aspartic acid were prepared in the same way as the other amino acids. The stock solutions contained 0.05 moles of aspartic and glutamic acid each per liter.

Aliquot in the amounts of 20, 18, 16, 14, 12, and 6 mL of the stock solutions were mixed with the same volume of other amino acids, and then added to each of the five 25 mL volumetric flasks containing 1.8016 grams of reducing sugars. The other steps of the procedure were the same with

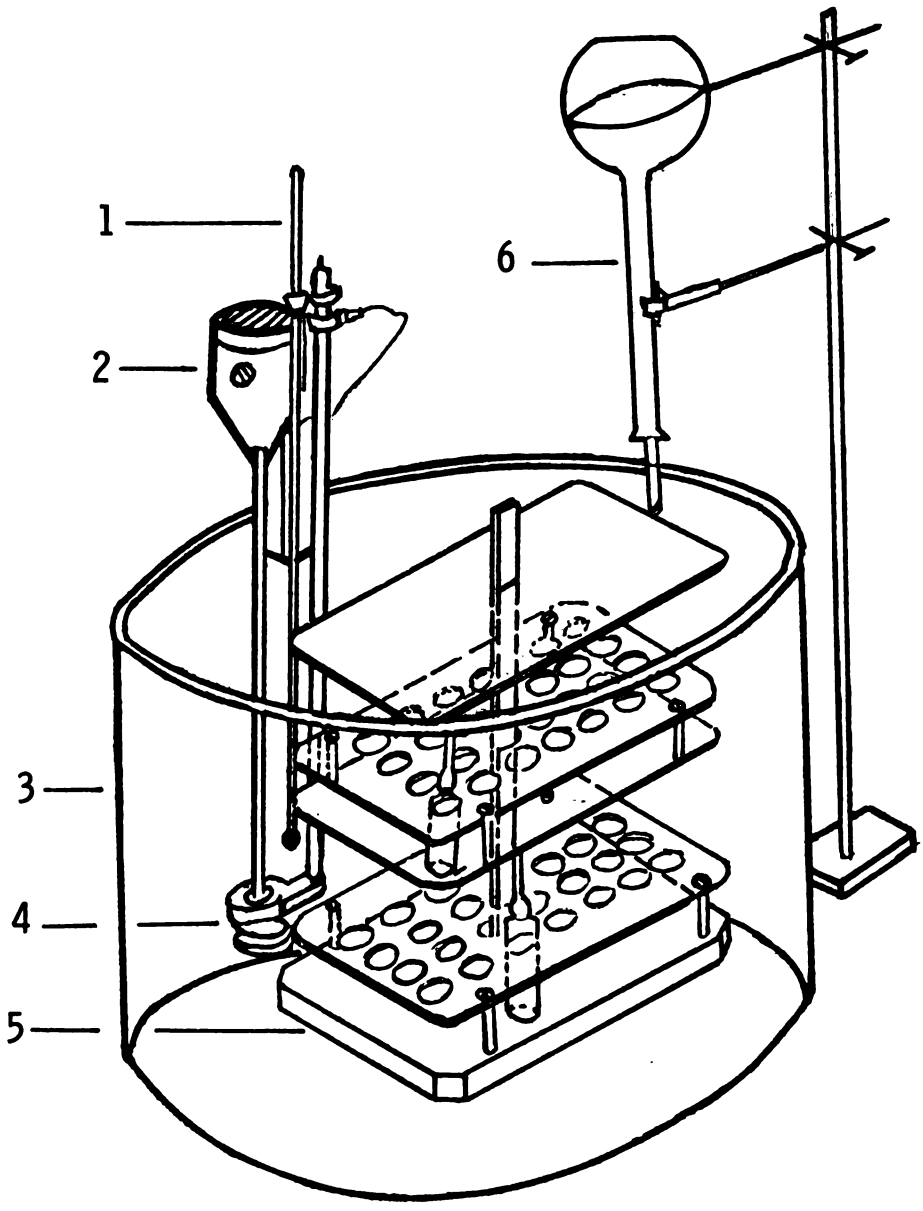


Figure 14. Assembly of the sample processing: 1. thermometer, 2. agitator, 3. water bath, 4. heater with thermostat, 5. samples, and 6. a 2 liter volumetric flask to keep the water level of the bath constant.

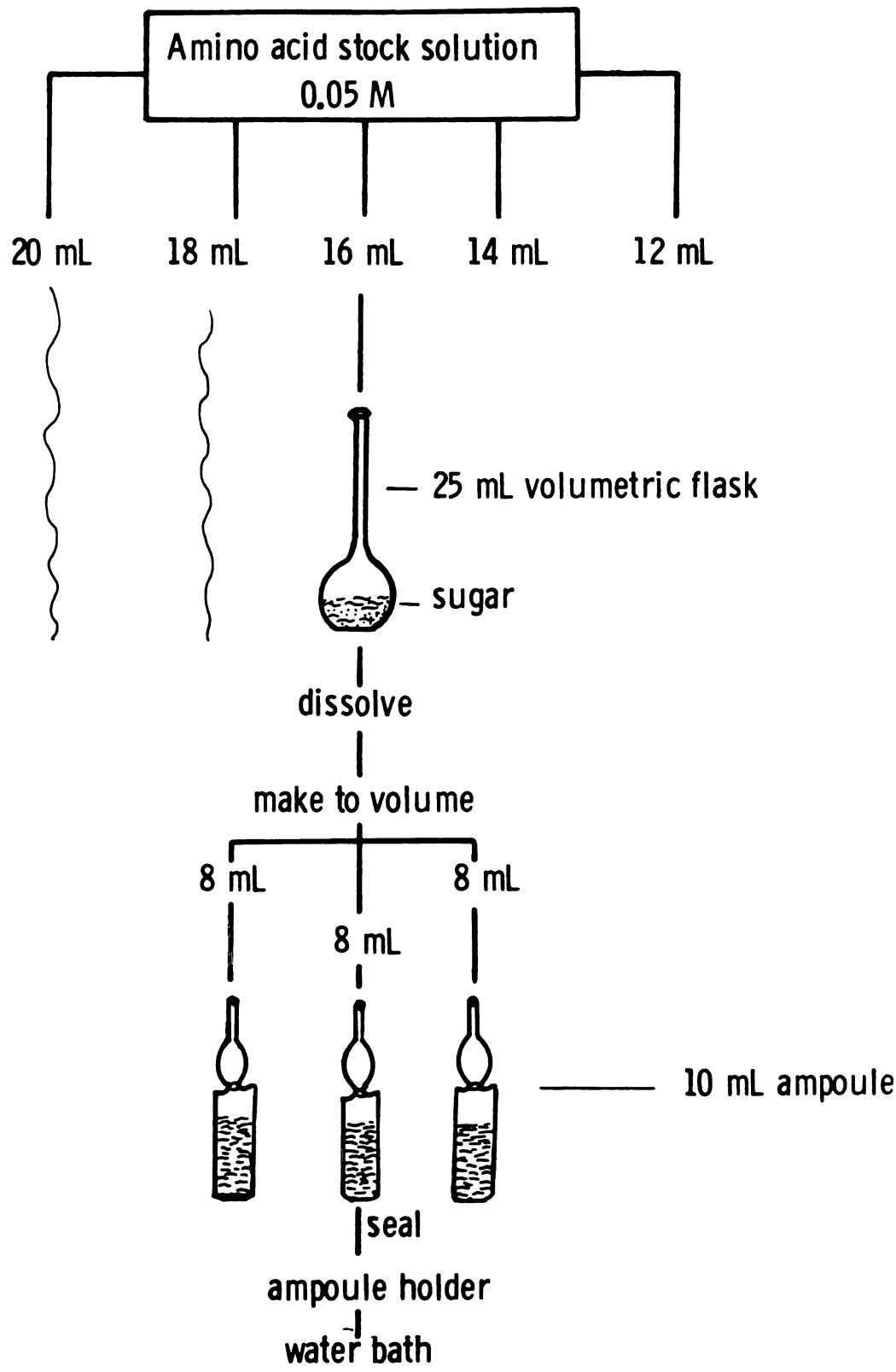


Figure 15. Flowchart of sample preparation.

Table 1.--Amino acid, sugar, and pH combinations in sugar-amino acid reaction for 58 hours at 60°C.

Amino Acid or Amine	pH					
	6		7		8	
	Glu	Fr	Glu	Fr	Glu	Fr
Alanine	-[a]	-	+[b]	+	+	+
Arginine	+	+	+	+	+	+
Glycine	+	+	+	+	+	+
Lysine	+	+	+	+	+	+
Methionine	-	-	+	+	+	+
Phenylalanine	-	-	+	+	-	-
Serine	-	-	+	+	-	-
α-amino-n-butyric acid	-	-	+	+	+	+

[a](-) means the reaction was not performed either due to low solubility of the amino acid, or the reaction did not generate enough color to be measured.

[b](+) means the reaction was performed.

those described in Figure 15. The reaction mixtures along with the pH of the reactions are shown in Table 2.

Table 2.--Amino acid or mixture of amino acids (1:1 M ratio), sugar and pH combinations in inhibited browning reactions performed at 60°C for 58 hours.

Amino Acids	pH			
	7		8	
	Glu	Fr	Glu	Fr
Aspartic Acid	+	+	+	+
Lysine and Aspartic Acid	-	-	+	+
Glutamic Acid	+	+	+	+
Lysine and Glutamic Acid	-	-	+	+

D. Procedure for Determining the Time Dependence of the Browning Reactions

The following procedure was used only for lysine, because lysine was the only amino acid that could generate enough color with both fructose and glucose.

1. Phosphate Buffer Solutions

A phosphate buffer with pH 8 and ionic strength 0.2 was prepared as before.

2. Mixture of Glucose and/or Fructose with Lysine Solution

A 36.032 g of glucose and/or fructose was placed in a 500 mL volumetric flask. Then 3.6539 g of lysine monohydrochloride was added to it, dissolved in the buffer

solution, and the volume was made to 500 mL. This solution contains 0.4 and 0.04 mole/L of reducing sugar and lysine monohydrochloride, respectively.

3. Reducing Sugar Solution

A 18.016 g of glucose and/or fructose was placed in a 250 mL volumetric flask, dissolved in the buffer solution, and made to volume. This solution was used as a control.

4. The solutions of the sample and the control were transferred into a buret. The tips of the burets were attached to a very narrow tygon tube which, in turn, was attached to a capillary tube to facilitate the transfer of the solution into the ampoules without the solution adhering to the neck of the ampoules.

5. A set of 80 ten mL ampoules were prepared. In 60 of them the mixture of sugar-lysine was added (8 mL per each ampoule). Into the 20 remaining ampoules only the reducing sugars were transferred (8 mL in each ampoule). Filling the ampoules with the exact quantity of sample is important, although theoretically any sample size can be used. But during the experiment, inconsistencies in the results were observed. The difficulty was corrected after keeping the head space in the ampoules steady for all of the samples. Apparently, different sizes of head space result in different amounts of evaporation with consequent changes in the concentration of the solutions and in the rates of the browning reaction.

6. The ampoules were sealed and placed in the water bath at 60, 55, and 50°C. The ampoules were not placed in the ampoule holder this time, because of the difficulty of removing them after each time interval. A net was placed on the surface of the water bath. This facilitated the periodic removal of the ampoules.

7. From the water bath, ampoules were withdrawn at selected intervals of time, and placed in a freezer. The time intervals between successive samplings at the beginning of the reaction were slightly shorter.

8. After all of the ampoules were pulled out of the bath (approximately 80 hours of maximum reaction time), they were broken and analyzed similarly to the others mentioned before.

E. Test for the Retardation of Browning with Aspartic Acid and Glutamic Acid

Approximately 6 lbs. of potatoes of one variety were washed thoroughly in cold water, peeled and sliced to a 2 mm thickness with a meat slicer. The slices were immediately blanched in water for 3 minutes, then frozen at 0°F. The frozen potatoes were placed in a freeze drier (Virtis Model No. 10-145 MR-BA). The freeze dried potatoes were packed in plastic bags and stored in the freezer for further use.

1. A few slices of the freeze-dried potatoes were ground to a flour. From this flour, two 5 g portions were used in the following experiment. One portion was mixed with 30 mL of phosphate buffer pH 8.0, and the mixture was

transferred to a 50 mL ampoule, which was then sealed in the flame. The second portion of flour was mixed with 20 mL phosphate buffer pH 8.0 and 10 mL of a solution containing 7.5 g/L glutamic acid; the mixture was transferred to a 50 mL ampoule and sealed in the flame. The sealed ampoules were placed in 110°C oven overnight.

2. Two 10 g portions of freeze-dried slices of equal diameter and thickness were used in the following experiment. One portion was carefully mixed with 10 mL of 7.5 g/L of glutamic acid in phosphate buffer pH 8. The other portion was carefully mixed with 10 mL of buffer solution of pH 8 (freeze-dried potatoes absorb the solutions fairly readily). They were separately deep-fat-fried at 210°C for 7 minutes. The potatoes were then cooled and their color measured both with the Hunter Color Difference Meter and with the Spectronic 505.

F. Physical Measurements

Ampoules containing samples and blanks were broken and their absorbance was measured either directly in a 1 cm path length cell or diluted first (if it was necessary) and then measured at 450 nm. The Beckman Model DU and Model 24 spectrophotometers were used for measurements. The spectrophotometers were calibrated for wavelength with a didymium filter. The absorption spectra of the glucose-lysine system are shown in Figure 16. Over a period of time, the increase in absorbance at 282 nm is larger than that at other

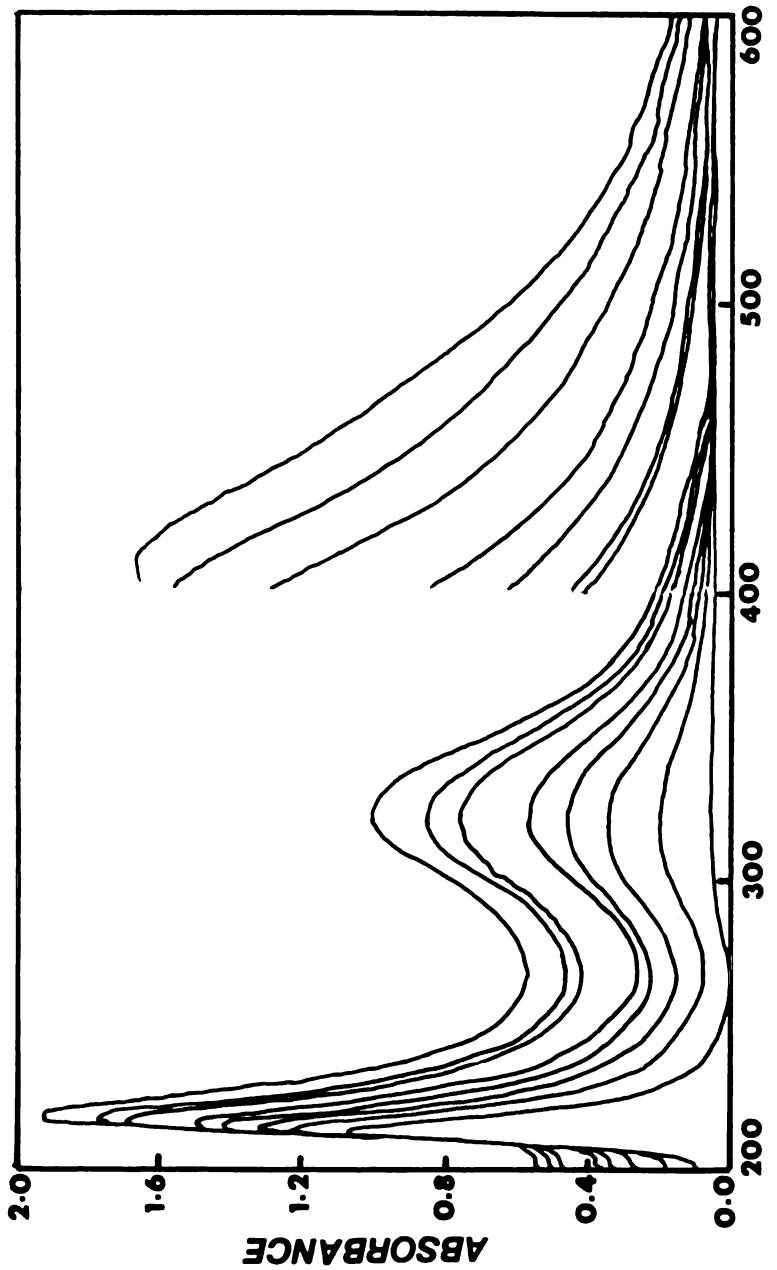


Figure 16. Absorption spectra of a model system containing 0.4 M glucose and 0.04 M lysine in phosphate buffer, pH 8, and ionic strength 0.2 after several reaction periods.

wavelengths. However, there is no proportionality between absorbance at 282 nm and absorbance at 450 nm, the latter being used in measuring browning (Figure 17). Therefore, the absorbance at the lower wavelength cannot be used as a measure of browning.

For the comparison of the color of treated and untreated fried potatoes, a Hunter Lab Color/Difference Meter Model D25-2 was used. The instrument was calibrated with the standard tile No. C_2-6007 (Gardner Laboratory, Inc.). The values of a_L, b_L, and L for this tile were -1.9, 25, and 78.4, respectively.

The reflectance was measured with the Bausch and Lomb 505 spectrophotometer. The instrument was checked with magnesium carbonate and "black body" for 100% and 0.0% line adjustment. The spectra were recorded from 400 to 700 nm wavelength.

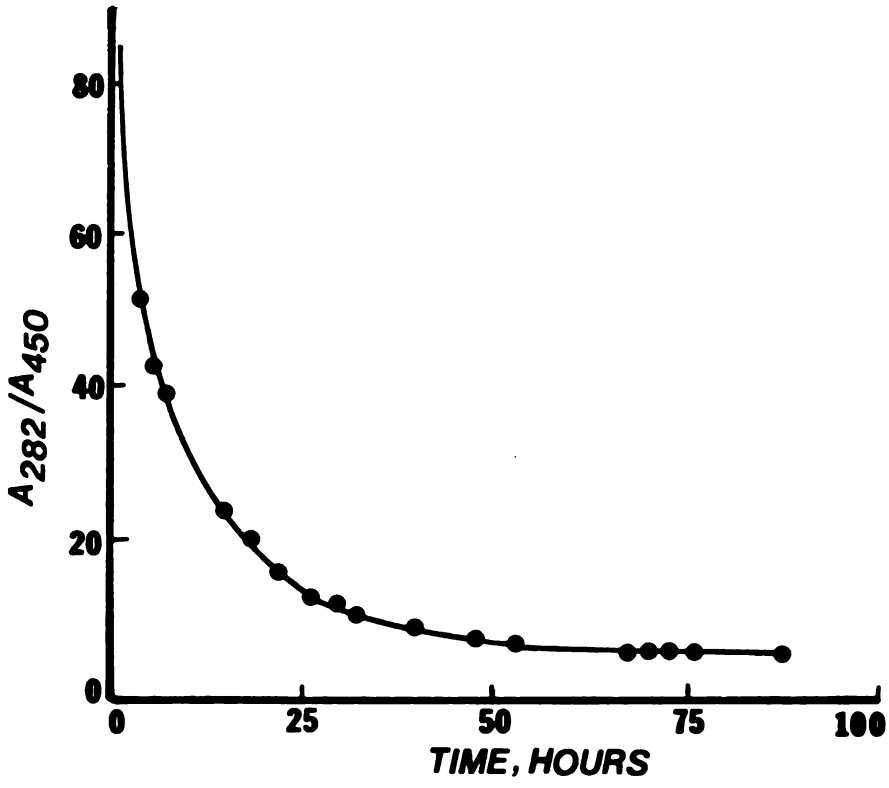


Figure 17. The ratio of absorbance at 282 nm to absorbance at 450 nm versus time for the reaction between lysine and glucose in phosphate buffer pH 8, ionic strength 0.2 at 60° C.

RESULTS AND DISCUSSION

I. Amino Acids

Both essential and nonessential amino acids were included in this study. Among the essential amino acids, lysine, methionine, phenylalanine, and arginine were tested. Among the nonessential amino acids, alanine, aspartic acid, glutamic acid, glycine, and serine were investigated.

A. Amino Acids-Glucose Systems at pH 8

In these systems, the glucose concentration was kept constant at 0.4 M, while the amino acid concentration varied in the range 0.024 to 0.04 M.

1. Glycine-Glucose

Glycine structurally is the simplest amino acid. The lower its concentration in the glycine-glucose system, the lower the browning rate. A graph of concentration versus color intensity is shown in Figure 18. The relationship between the color development and glycine concentration is given by the equation,

$$Y = 20.2\ X = 0.0498,$$

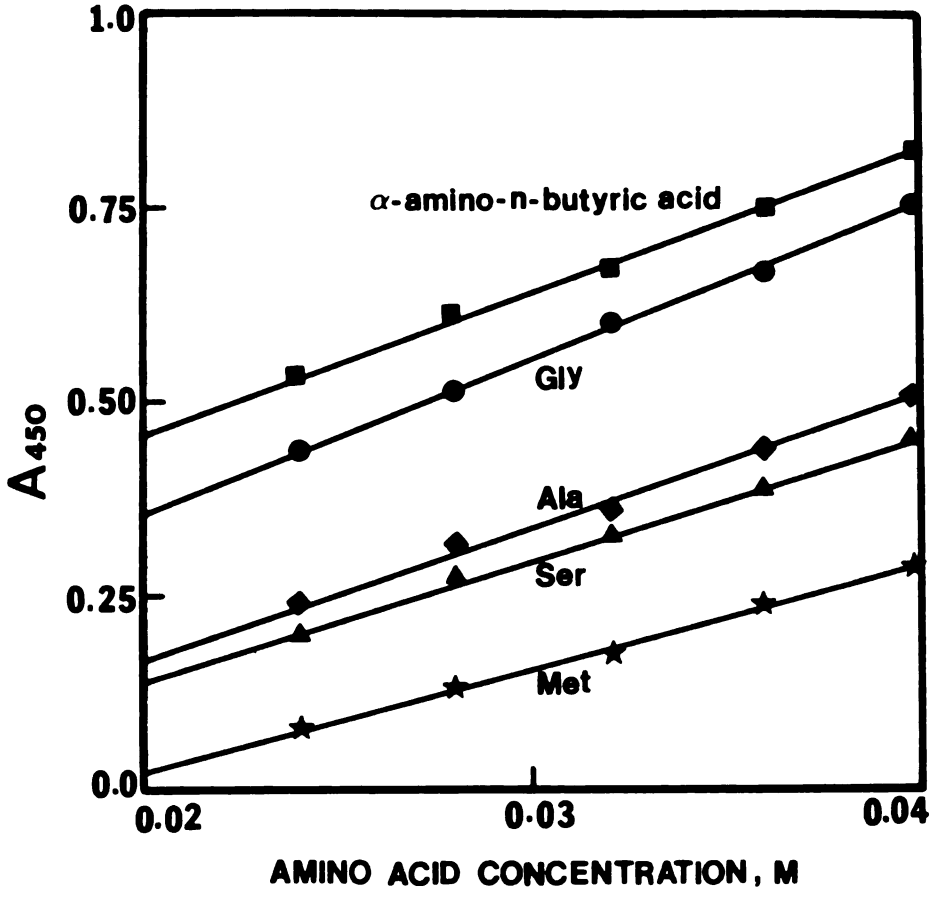


Figure 18. Color formation by the reaction of α-amino-n-butyric acid, glycine, serine, alanine, and methionine (0.02 to 0.04 M) with glucose (0.4 M) at pH 8, ionic strength 0.2, for 58 hours at 60° C.

where Y is absorbance at 450 nm, and X is glycine concentration in M. The coefficient of correlation, r = 0.9988, shows an excellent correlation between glycine concentration and color intensity.

2. Alanine-Glucose

In alanine, a methyl group is substituted for one of the side chain hydrogen atoms of glycine. The color developed in the alanine-glucose system is less intense than that of the glycine-glucose system. The effect of the length of the side chain in browning should be interpreted cautiously, because α-amino-n-butyric acid with a side chain longer than alanine and glycine showed more color development at this particular pH (Figure 18). While the order of reactivity of these three amino acids with glucose, and under the conditions of this experiment, is α-amino-n-butyric acid > glycine > alanine, the slope representing color change with amino acid concentration places them in a different order: glycine > α-amino-n-butyric acid > alanine.

3. Phenylalanine-Glucose

Phenylalanine behaves differently than glycine, alanine, and most other amino acids. The relationship between color development and amino acid concentration is not linear. Color development is affected by amino acid concentration much more strongly at higher levels of amino acid concentration (Figure 19).

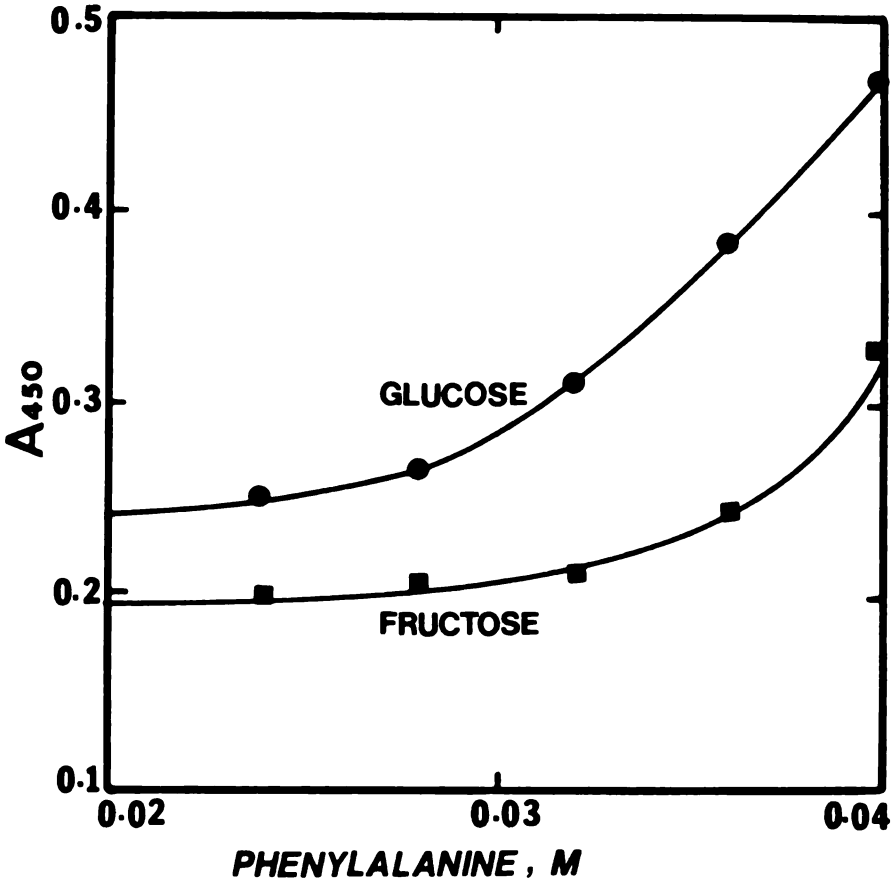


Figure 19. Color formation by the reaction of phenylalanine with glucose (0.4 M) and fructose (0.4 M) at pH 8, ionic strength 0.2 for 58 hours at 60° C.

4. Serine-Glucose

Serine produces less color than alanine, glycine and α-amino-n-butyric acid under the same conditions of reaction with glucose (Figure 18).

5. Methionine-Glucose

Methionine shows very little tendency to interact with glucose (Figure 18). Paton et al. (1948), in determining the nutritive availability of the amino acids, observed a minimal loss of methionine due to the Maillard reaction, compared to other amino acids.

6. Lysine and Arginine-Glucose

Lysine and arginine will be discussed separately as they react with glycine in somewhat different fashion.

B. Amino Acids-Glucose Systems at pH 7

As anticipated, the rate of color formation was less intense at pH 7 than at pH 8. Again linear relationships were obtained between color and amino acid concentration as shown in Figures 20 and 21. The slopes, the y-intercepts, and the correlation coefficients of these relationships appear in Table 3.

C. Amino Acids-Glucose Systems at pH 6

At pH 6 the above mentioned amino acids produce very little color in their reactions with glucose. The linear relationship between color and glycine concentration

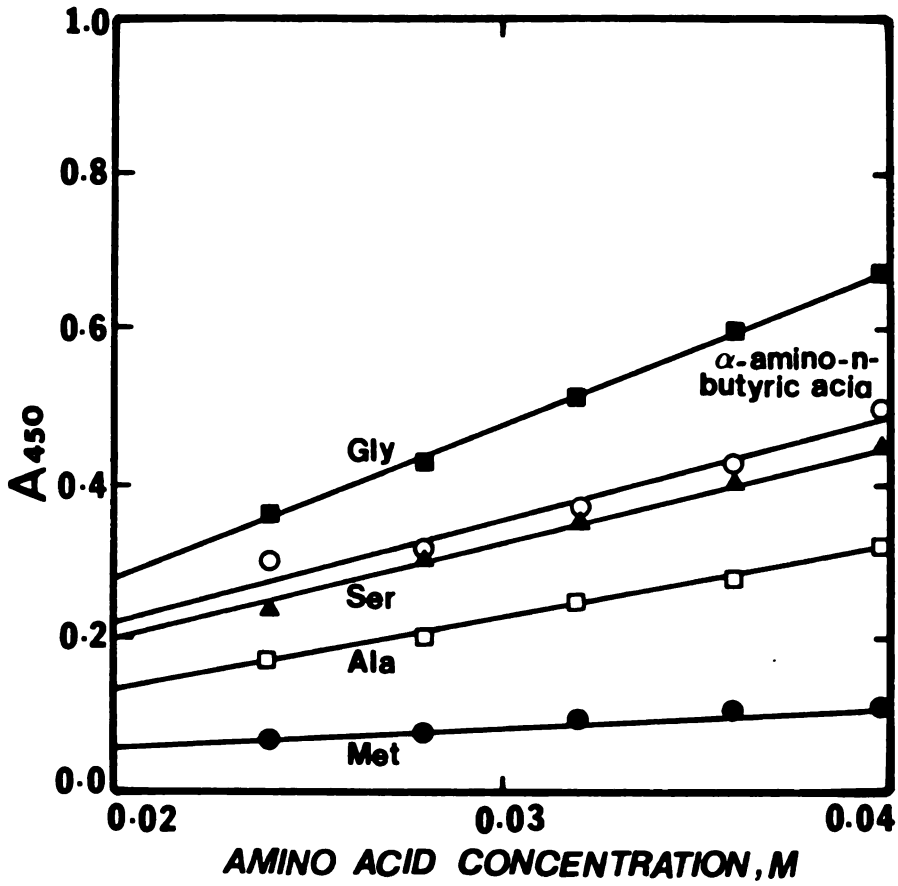


Figure 20. Color formation by the reaction of α-amino-n-butyric acid, glycine, serine, alanine, and methionine (0.02 to 0.04 M) with glucose (0.4 M) at pH 7, ionic strength 0.2, for 58 hours at 60° C.

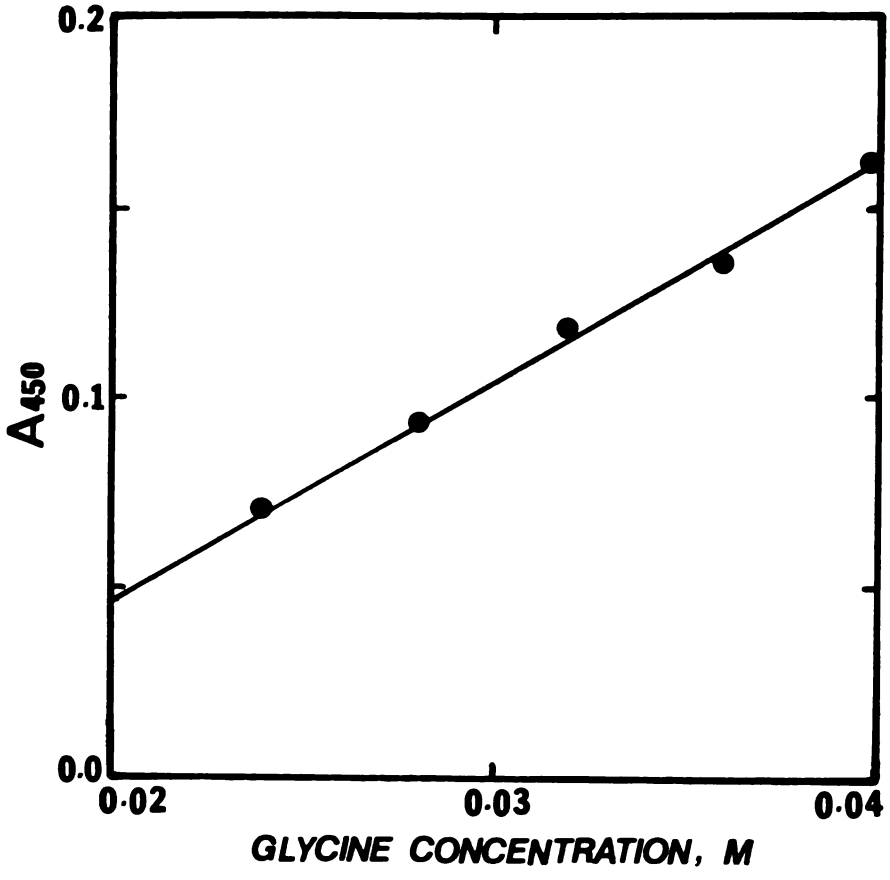


Figure 21. Color formation by the reaction of glycine with glucose (0.4 M) at pH 6, ionic strength 0.2 for 58 hours at 60° C.

Table 3.--Slopes, y-intercepts, and correlation coefficients (r) of the linear regressions between color (A_{450}) and amino acid concentration for the reaction of glucose with several amino acids.

Amino Acid	pH 8			pH 7		
	Slope	y-intercept	r	Slope	y-intercept	r
Glycine	20.20	-0.0498	0.9988	20.25	-0.132	0.991
Alanine	16.70	-0.1612	0.9989	9.37	-0.056	0.9939
α-amino-n-butyric acid	18.62	+0.0850	0.9997	13.47	-0.049	0.9520
Serine	15.05	-0.1540	0.9972	12.48	-0.055	0.9947
Methionine	13.47	-0.2496	0.9949	3.05	-0.006	0.9957

is shown in Figure 21, and can be described by the following regression equation and correlation coefficient,

$$Y = 5.825\ X = 0.072 \text{ and } r = 0.9944.$$

II. Sugars

In spite of the wealth of data for glucose, there has been little attention paid to the role of fructose in browning in the past. The role of fructose is important today because of the high utilization and demand for fructose in the food industry.

A similar series of experiments to those of glucose and various amino acids was performed with fructose and the same amino acids. The results are shown in Figures 22, 23, and 24. In all cases, the amount of color produced by the fructose-amino acid reaction was lower than that of the corresponding glucose-amino acid interaction (Figure 25). The slopes, the y-intercepts, and the correlation coefficients appear in Table 4. Lewis and Lea (1950) reported that the loss in free amino-N in the casein-glucose reaction was twice that observed in the casein-fructose reaction, at 25°C.

III. Lysine and Arginine Interaction with Reducing Sugars

Lysine and arginine are discussed separately because (a) of their basic side chains, and (b) their different behavior in interacting with reducing sugars. Lysine is a diamino amino acid with its second amino group in

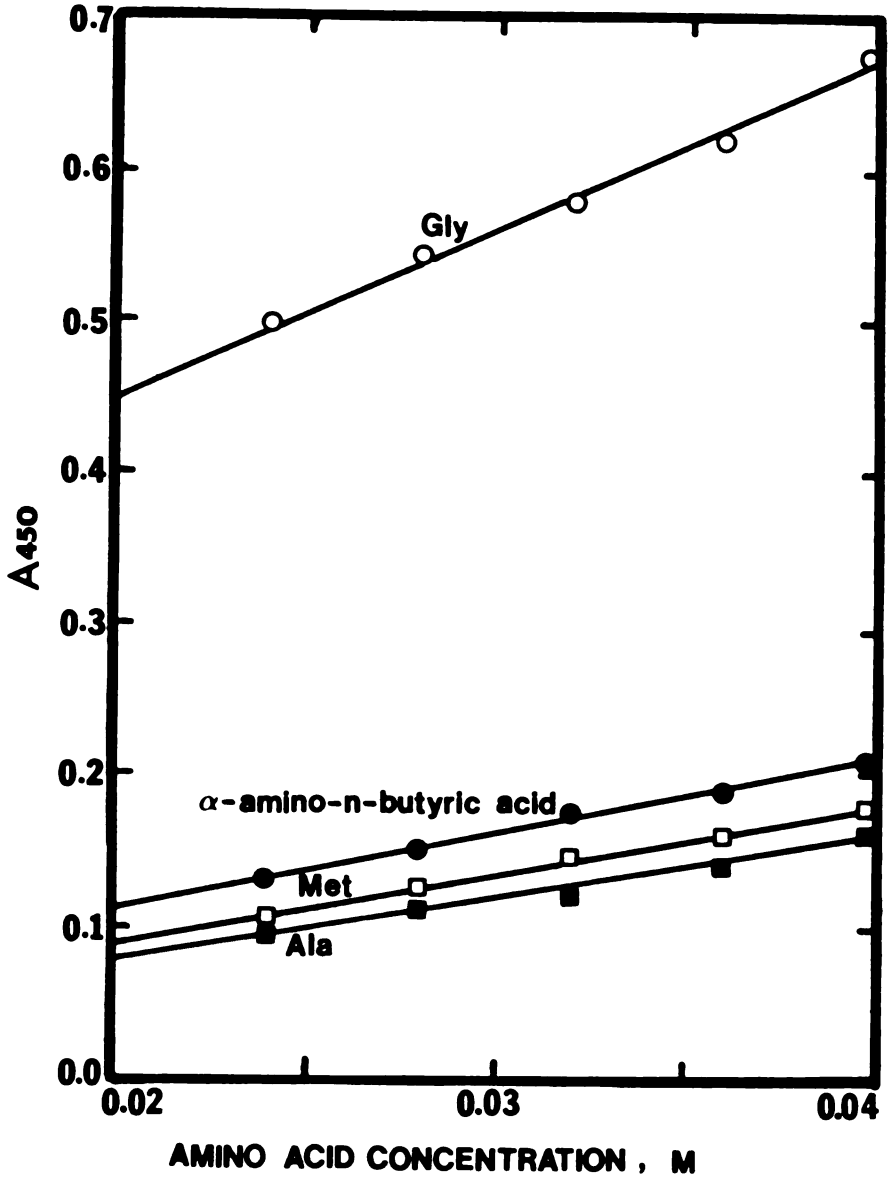


Figure 22. Color formation by the reaction of glycine, α-amino-n-butyric acid, methionine, and alanine (0.02 to 0.04 M) with fructose (0.4 M) at pH 8, ionic strength 0.2 for 58 hours at 60° C.

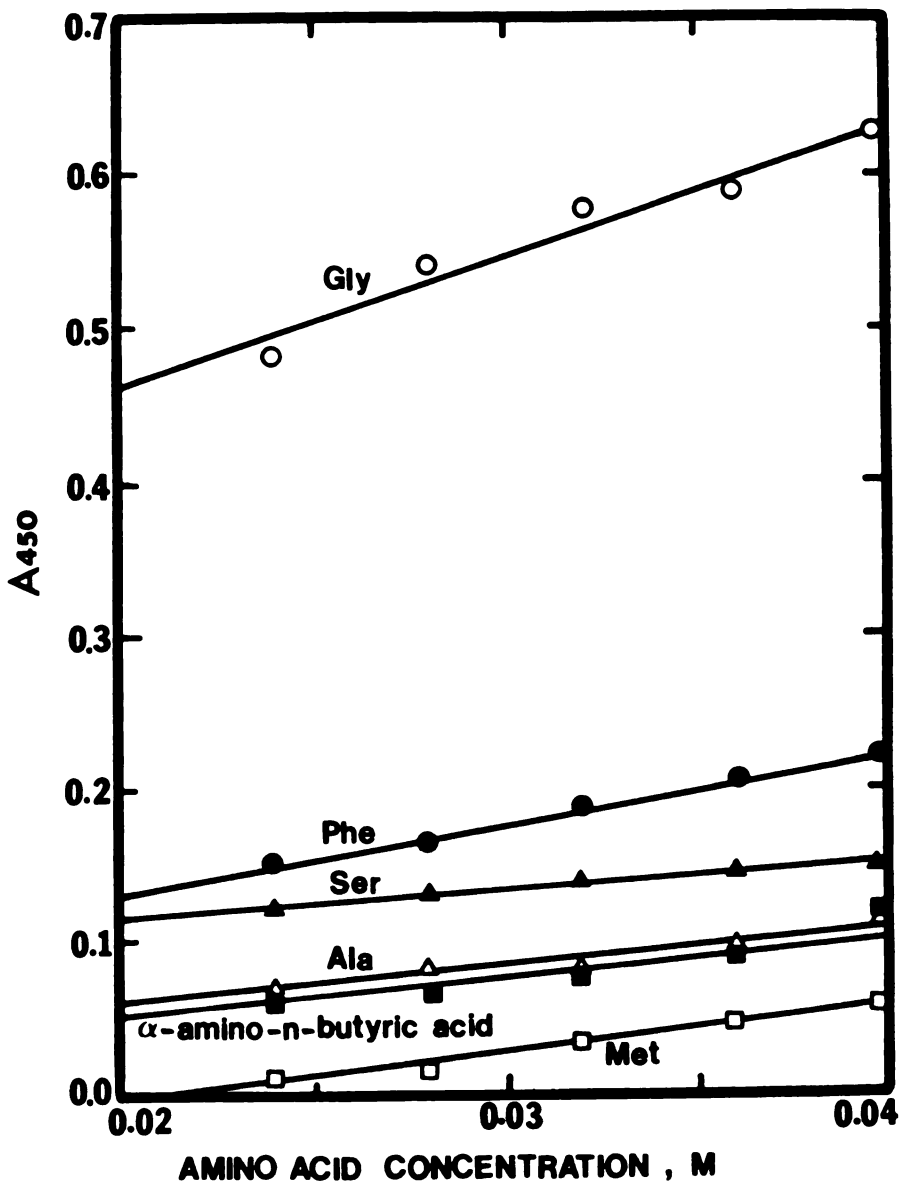


Figure 23. Color formation by the reaction of glycine, phenylalanine, serine, alanine, α-amino-n-butyric acid, and methionine (0.02 to 0.04 M) with fructose (0.4 M) at pH 7, ionic strength 0.2 for 58 hours at 60° C.

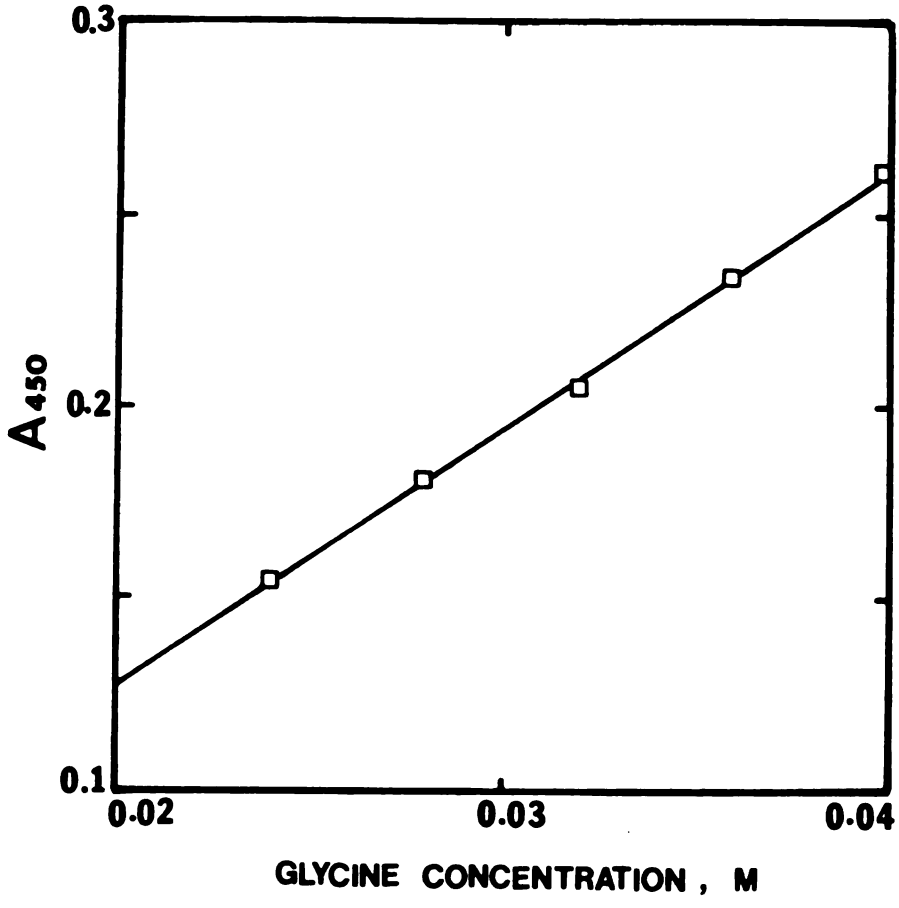


Figure 24. Color formation by the reaction of glycine with fructose (0.4 M) at pH 6, ionic strength 0.2 for 58 hours at 60° C.

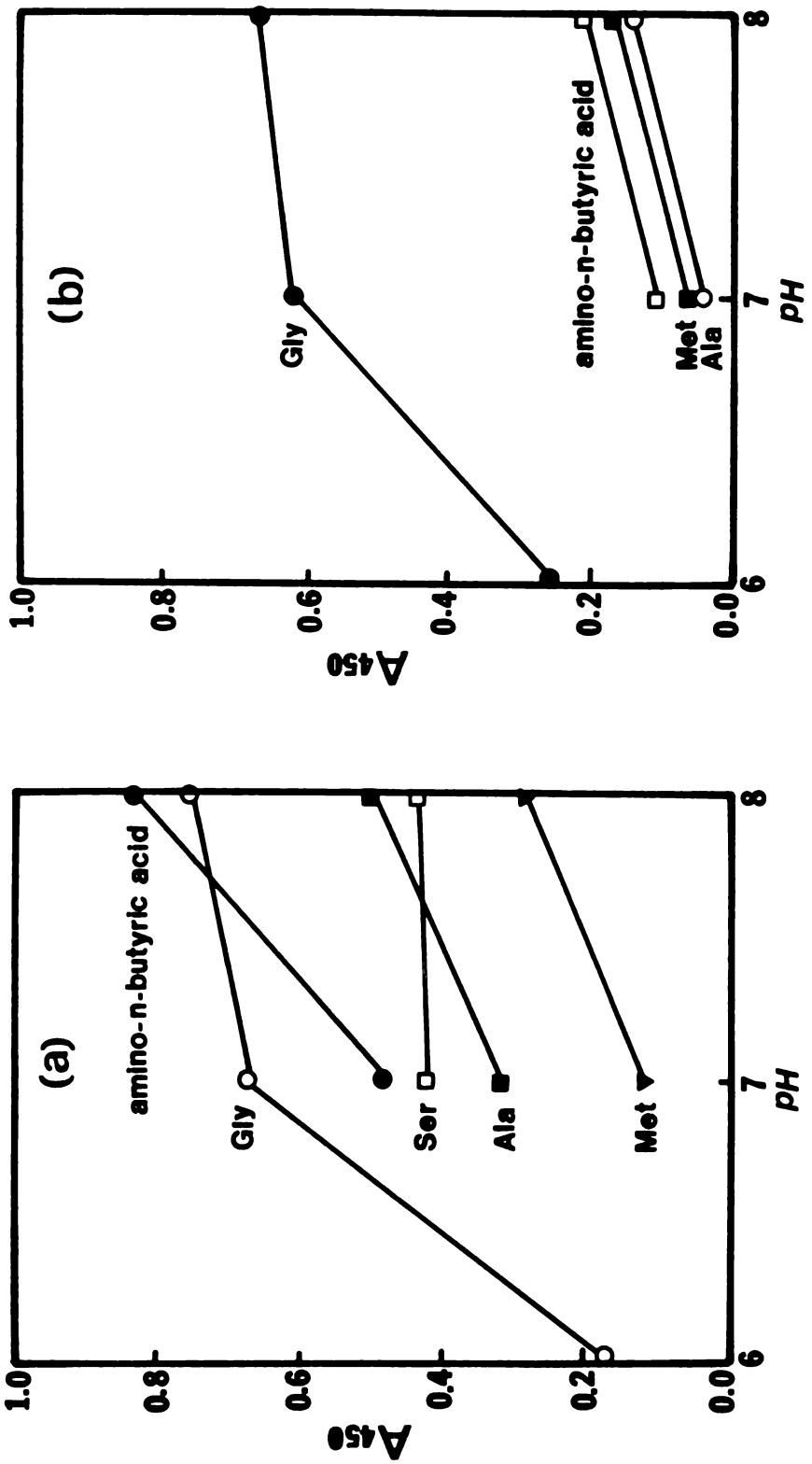


Figure 25. Effect of pH on the color development in the reaction between (a) glucose (0.4 M) and (b) fructose (0.4 M) with different amino acids (0.04 M) at 60° C, ionic strength 0.2 and 58 hours.

Table 4.--Slopes, y-intercepts, and correlation coefficients (r) of the linear regressions between color (A_{450}) and amino acid concentration for the reaction of fructose with several amino acids.

Amino Acid	pH 8			pH 7		
	Slope	y-intercept	r	Slope	y-intercept	r
Glycine	11.30	+0.2238	0.9929	8.62	+0.2870	0.9688
Methionine	4.52	-0.0022	0.9986	3.35	-0.0738	0.9891
Alanine	4.05	-0.0026	0.9848	2.60	+0.0060	0.9939
Phenylalamine	-	-	-	4.60	+0.037	0.9986
α-amino-n-butyric acid	4.82	+0.0174	0.9981	3.72	-0.0356	0.0737
Serine	-	-	-	1.62	+0.0848	0.9903

epsilon position. Both the α and ε-amino groups of lysine are active in the browning reaction. However, the ε-amino group contributes more to the brown color of the Maillard reaction than the α-amino group does.

In monoamino acids the color development caused by the Maillard reaction is higher when the amino groups are located further away from the carboxylic group. This is true for amino acids containing two to four carbon atoms. However, for four to six carbon atom amino acids the opposite is true (Lento et al., 1957).

Lysine is the most reactive amino acid among the ones which were tested here, and perhaps among all amino acids.

The rate of color production in the fructose-amino acid systems, as it was observed for fructose-lysine systems, was higher than that of glucose-amino acid systems at the early stage of browning, but at later stages of the reaction, the glucose-lysine dominates fructose. This might be perhaps caused by the shorter induction period that was observed for fructose (Figure 31). The color produced by the lysine-glucose and lysine-fructose systems is graphically presented in Figure 26. The slopes, y-intercepts, and correlation coefficients for the same reactions are shown in Table 5.

The reaction of some primary aliphatic and aromatic amines with a number of simple carbohydrate derivates was studied by Beacham and Dull (1951). These authors concluded

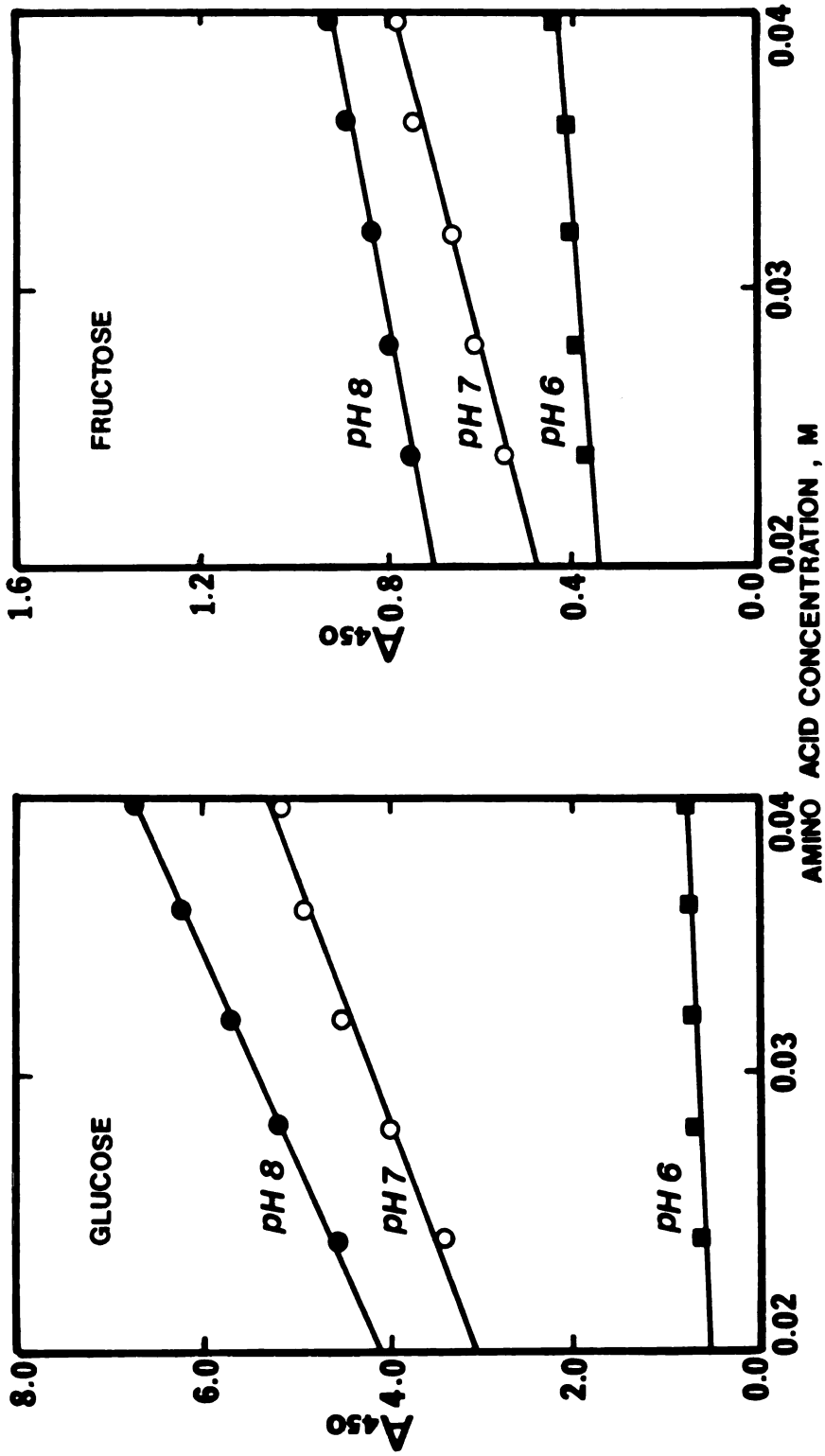


Figure 26. Color formation by the reaction of lysine with glucose (0.4 M) and fructose (0.4 M) at pH 6, 7, and 8, ionic strength 0.2 after 58 hours at 60° C.

Table 5.--Slopes, y-intercepts, and correlation coefficients (r) of the linear regressions between color (A_{450}) and amino acid concentration for the reactions of lysine with glucose and fructose.

pH		Glucose	Fructose
6	slope	12.0	4.5
	y-intercept	+0.318	+0.265
	r	0.991	0.9949
7	slope	110.00	15.0
	y-intercept	+0.91	+0.19
	r	0.9918	0.9917
8	slope	133.75	10.25
	y-intercept	+1.44	0.508
	r	0.9981	0.9967

that the order of effectiveness in the brown color production in most cases is parallel to the increase of the basicity of the amines. However, Willits et al. (1958) suggested that the reactivity of lysine is not due to its basicity, but to a lysine-alkaline pH synergism, since arginine and histidine, other basic amino acids, had no positive effect on browning.

Arginine contains a guanidinum group at the 5 position and it is reported that only the α-amino group of arginine is active (Shallenberger and Birch, 1974). Comparing these two amino acids reveals the following:

First, despite the Beachman and Dull (1951) report, there is a significant interaction between reducing sugars and arginine (Figures 27 and 28). The color developed by the reaction of arginine with glucose is second in

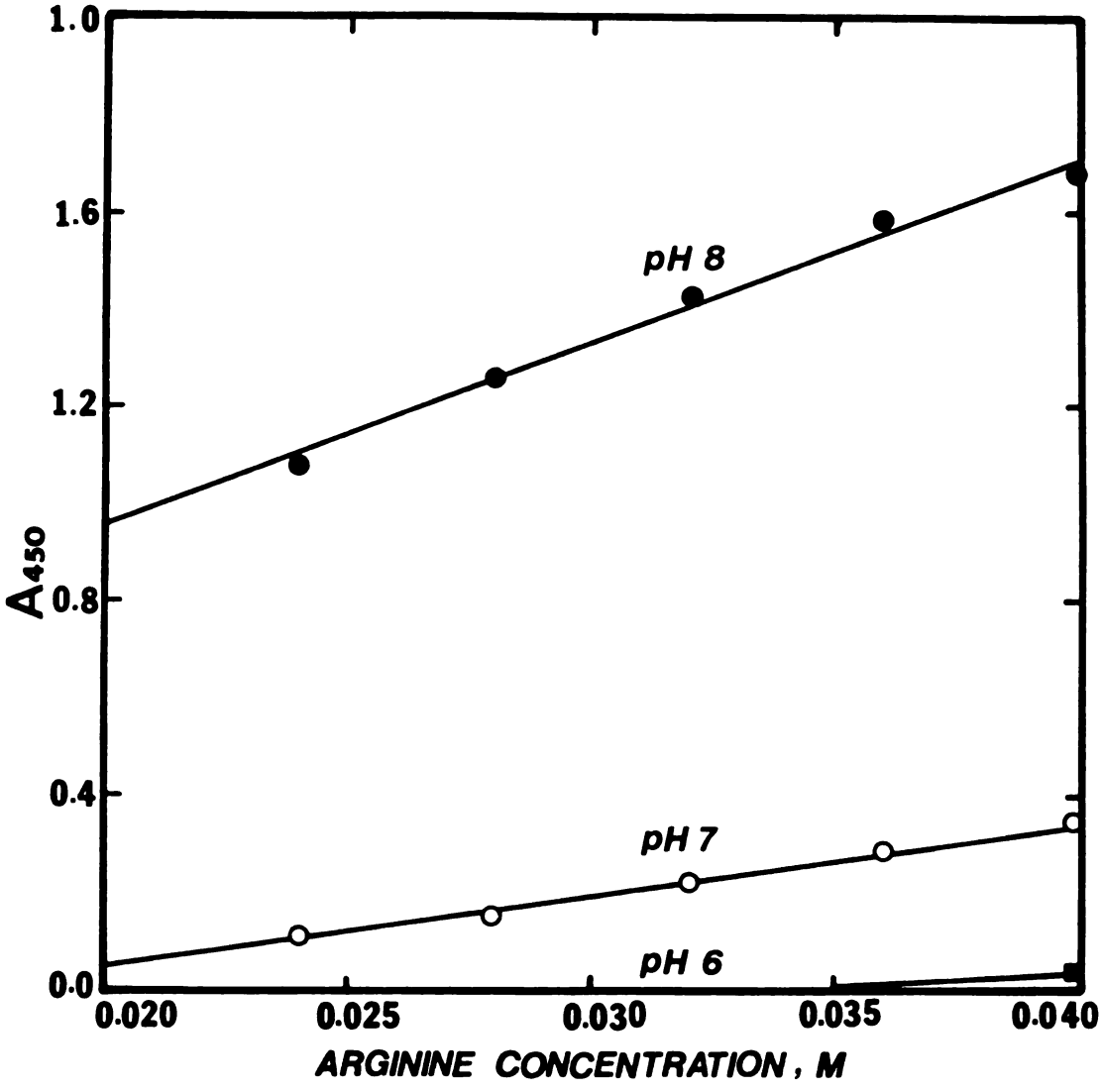


Figure 27. Color formation by the reaction of arginine with fructose (0.4 M) at pH 6, 7, and 8, ionic strength 0.2, for 58 hours at 60° C.

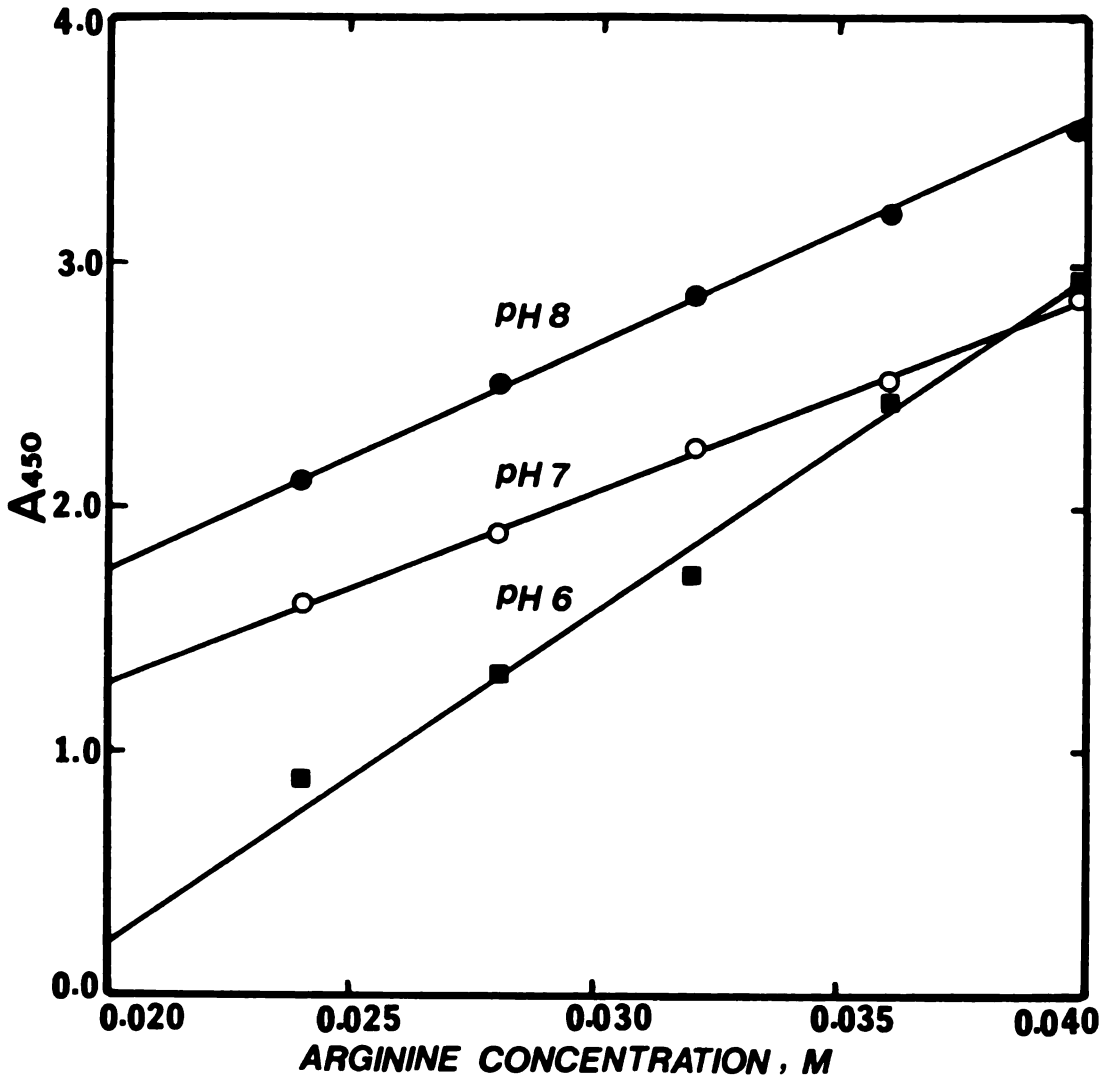


Figure 28. Color formation by the reaction of arginine with glucose (0.4 M) at pH 6, 7, and 8, ionic strength 0.2, for 58 hours at 60° C.

intensity only to that of lysine-glucose, among the amino acids tested.

Second, at pH 8 the color of the arginine-fructose system is more than that of the lysine-fructose system.

Third, in the arginine-glucose interaction more color is produced at pH 7 than at pH 6 or 8, under the conditions of this experiment (Figure 29).

Fourth, in the reaction between fructose and arginine, there was no significant color development at pH 6. The slopes, y-intercepts, and correlation coefficients for the reactions between arginine-glucose and arginine-fructose are shown in Table 6.

Table 6.--Slopes, y-intercepts, and correlation coefficients (r) of the linear regressions between color (A_{450}) and amino acid concentration for the reactions of arginine with glucose and fructose.

pH		Glucose	Fructose
6	slope	132.2200	-
	y-intercept	-2.3934	-
	r	0.9953	-
7	slope	89.1000	38.2200
	y-intercept	+0.0032	+0.1808
	r	0.9989	0.9952
8	slope	77.6200	14.8000
	y-intercept	-0.2460	-0.2512
	r	0.9993	0.9979

The complete analysis of variance is shown in Tables 7 and 8. This analysis indicates that not only all independent variables (amino acids, concentrations of amino

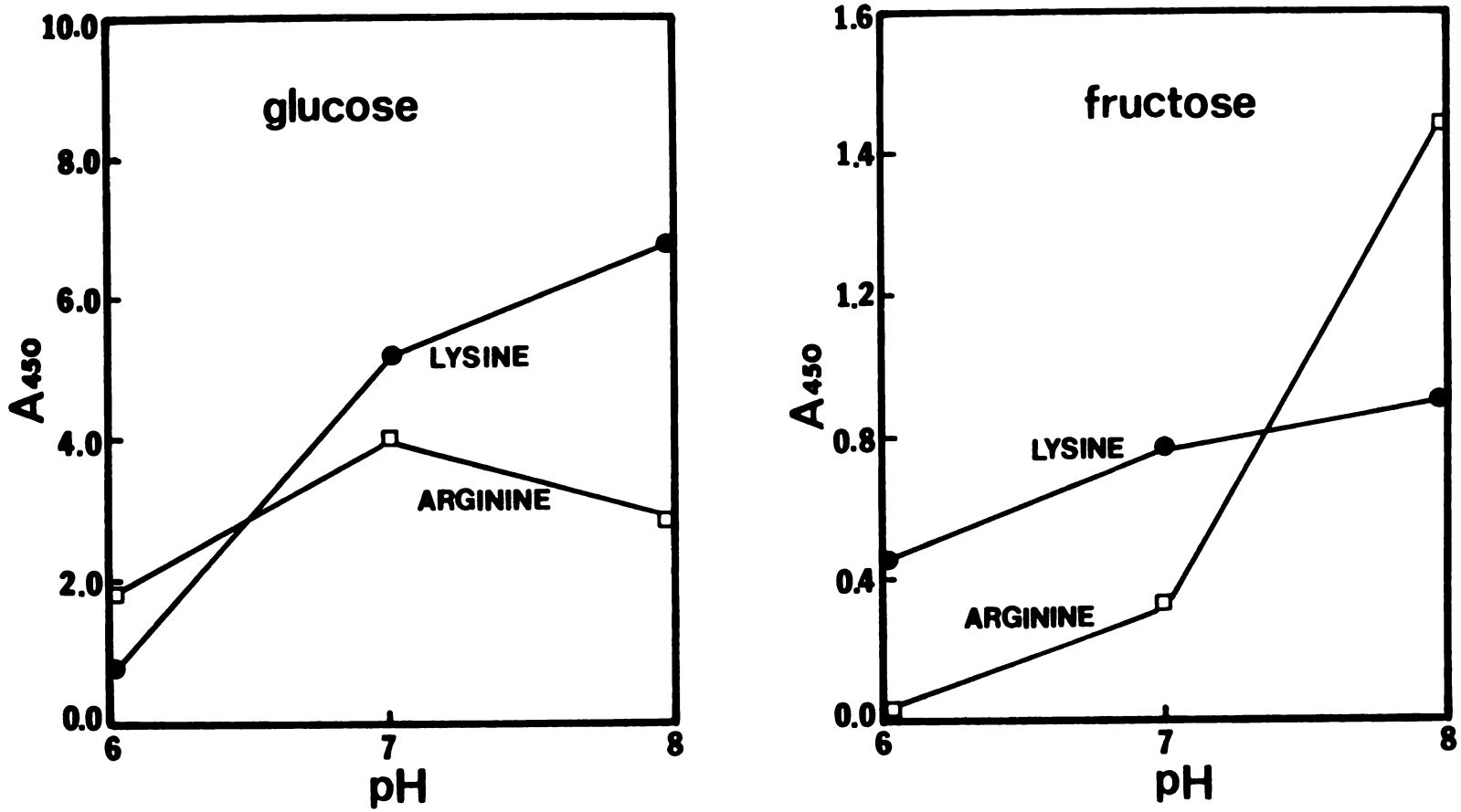


Figure 29. Effects of pH on color development in the systems glucose -arginine, glucose-lysine, fructose-arginine, fructose-lysine, after 58 hours at 60° C. Concentration: sugars 0.4 M, amino acids 0.04 M.

Table 7.--Analysis of variance showing the effect of the nature of amino acid, amino acid concentration, pH, and their interactions on the color produced by the reaction of fructose with lysine and arginine.

Source of Variation	Degree of Freedom	Sums of Squares	Mean Squares	F-Ratio	F-table (1%)
Nature of Amino Acid (A)	1	0.1984	0.1984	2834.28	7.0771
Amino Acid Concentration (B)	4	0.5654	0.1413	2018.57	3.6491
pH (C)	2	4.4845	2.2422	32031.42	4.9774
AB	4	0.0463	0.0115	164.28	3.6491
AC	2	13.8677	0.9338	99054.28	4.9774
BC	8	0.1207	0.0150	214.28	2.8233
ABC	8	0.2417	0.0314	448.57	2.8233
Error	60	0.0045	0.00007		

Table 8.--Analysis of variance showing the effect of the nature of amino acid, amino acid concentration, pH, and their interactions in the reaction of glucose with lysine and arginine.

Source of Variation	Degree of Freedom	Sums of Squares	Mean Squares	F-Ratio	F-table (1%)
Nature of Amino Acid (A)	1	31.1876	31.1876	17326.44	7.0771
Amino Acid Concentration (B)	4	27.2189	6.8047	3780.39	3.6491
pH (C)	2	119.8715	59.9357	33297.66	4.9774
AB	4	0.0338	0.0084	4.66	3.6491
AC	2	68.9227	34.4613	19145.16	4.9774
BC	8	1.2608	0.1576	87.55	2.8233
ABC	8	5.4543	0.6817	378.72	2.8233
Error	60	0.10885	0.0018	-	-

acids, and pH), but also all their interactions had a statistically significant (99%) effect on the color produced by these reactions.

IV. Temperature Dependence of the Reaction Rate

The rate of color development in the reaction between glucose and fructose with lysine was followed at pH 8 and three temperatures, 60°, 55°, and 50°C. The plots of absorbance at 450 nm versus time of reaction for glucose and fructose are shown in Figures 30 and 31. An induction period is visible in both plots. The induction periods for the glucose-lysine reaction appears longer than those for the fructose-lysine reaction.

Rate constants (k) for the reactions lysine-glucose, and lysine-fructose were calculated at 50°, 55°, and 60°C from the first order rate equation

$$\ln (a/a - x) = kt,$$

in which a is the absorbance at ∞ time, and x the absorbance at time t in minutes. Temperature dependence of the rate constants was characterized from the Arrhenius equation

$$\log k = \log A - (E_a/2.303\ RT),$$

where E_a is activation energy (cal/mole), R the gas constant (1.987 cal degree^{-1}), and T is the temperature (K°).

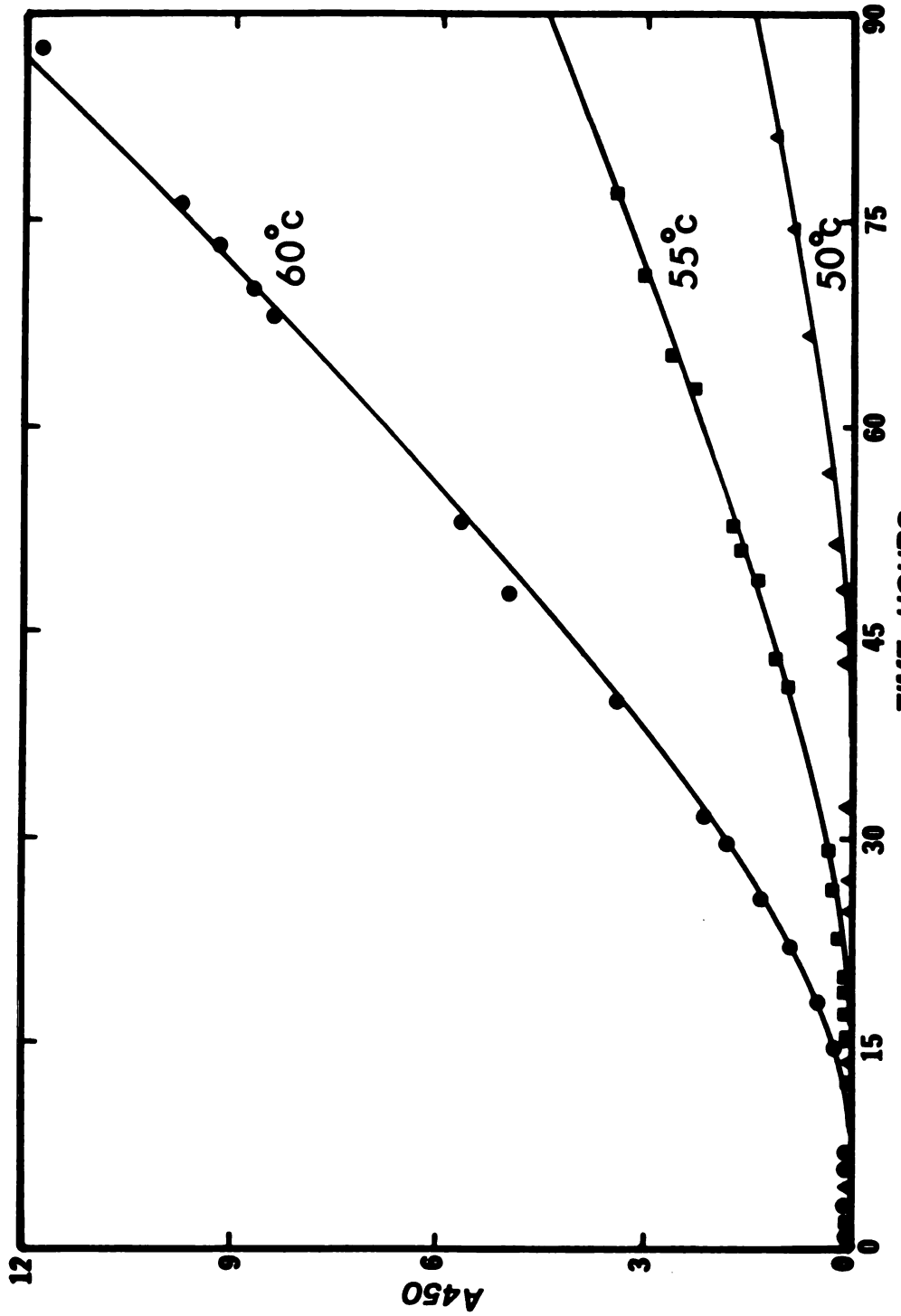


Figure 30. Color formation during the reaction of lysine (0.04 M) with glucose (0.4 M) at pH 8, ionic strength 0.2, at 50°, 55°, and 60° C.

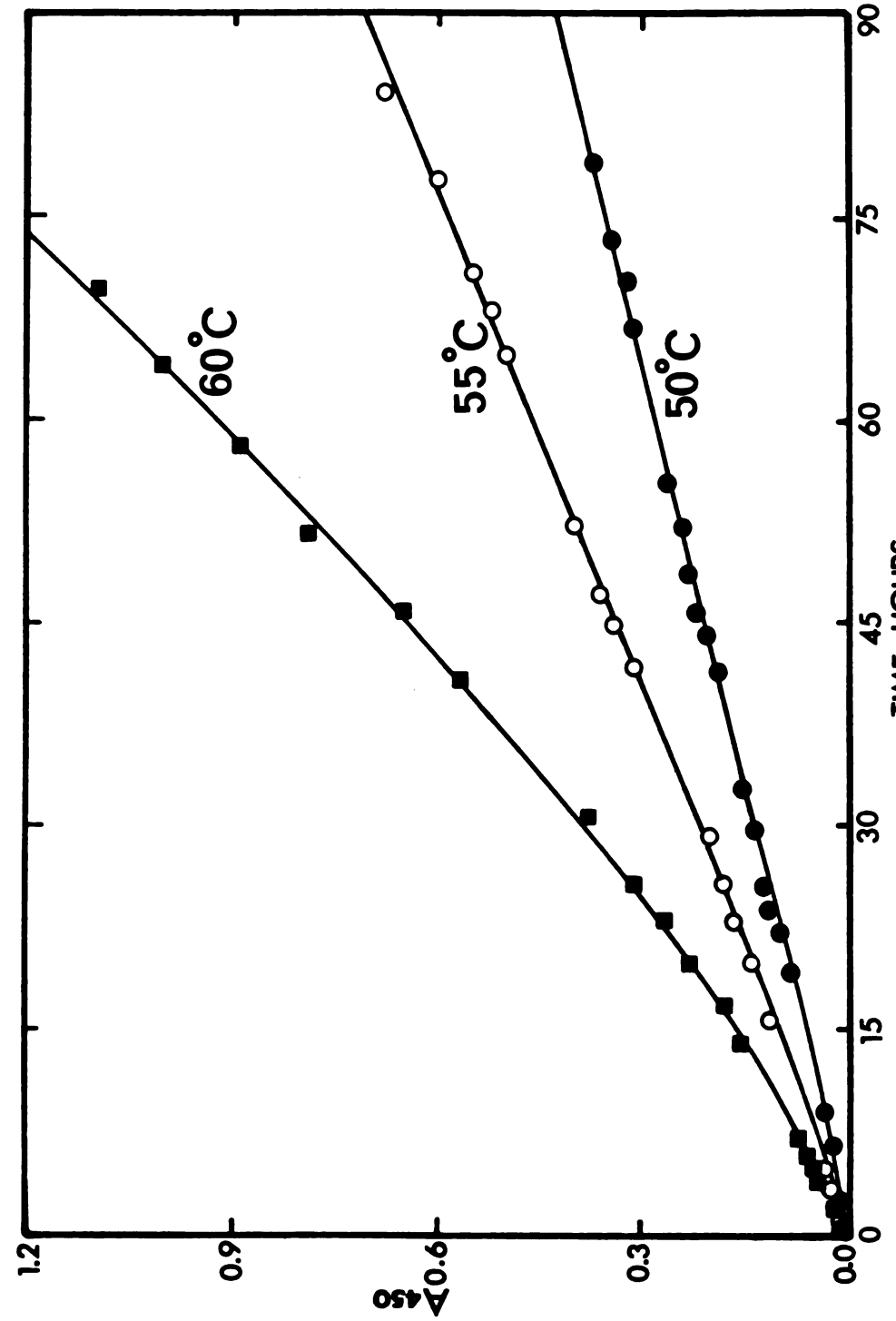


Figure 31. Color formation during the reaction of lysine (0.04 M) with fructose (0.4 M) at pH 8, ionic strength 0.2, at 50°, 55°, and 60° C.

The Q_{10} values were calculated from the equation

$$\log Q_{10} = 10\ E_a/T_2.T_1.R,$$

where T_2 and T_1 are 333 and 323°K respectively.

The results are shown in Table 9. An Arrhenius plot of lysine-glucose and lysine-fructose data is shown in Figure 32. The results indicate that the Q_{10} value for glucose is higher than that for fructose by a factor of 1.15, meaning the higher sensitivity of the glucose-lysine reaction to temperature compared to the fructose-lysine reaction.

Table 9.--Kinetic data for the lysine-glucose, and lysine-fructose reaction.

Reaction	Temp °C	k* x 10^3 min^{-1}	$t_{1/2}$ min	E_a cal/mole	Q_{10}
lysine-glucose	50	0.300	2310	13,331	1.87
	55	0.410	1690		
	60	0.550	1260		
lysine-fructose	50	0.382	1823	10,210	1.61
	55	0.449	1575		
	60	0.611	1136		

*k = first order rate constant, $t_{1/2}$ = time for 50% of the reaction to take place; E_a = activation energy; Q_{10} = change in reaction rate for each 10°C change in temperature.

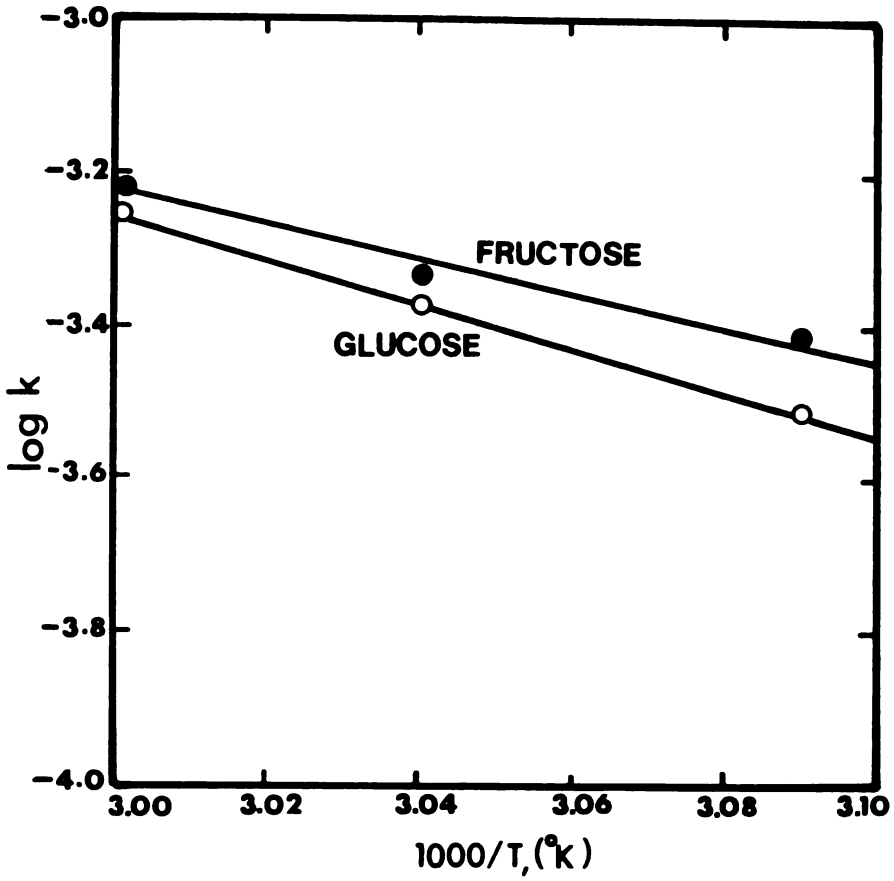


Figure 32. Arrhenius plot of the lysine-glucose and lysine-fructose reaction data.

V. Inhibition of Caramelization

When glutamic acid or aspartic acid was added to a glucose or fructose solution and the mixture was heated at 60° for 58 hours, it was noticed that more color was developed in the blank, that is the heated sugar alone, than in the sugar-amino acid mixture. This can be considered as inhibition of sugar caramelization (Table 10). An anomaly was observed in the 0.024 M glutamic acid 0.4 M glucose system, in which a slight increase in absorbance occurred over the glucose blank. The caramelization inhibition was much greater in the fructose-containing systems than in the glucose ones.

Although the mechanism of this inhibition is not known, the following may be suggested as possible explanations:

i. The products of the glutamic or aspartic acid interaction with fructose or glucose may be very stable and therefore not subject to the fragmentation and polymerization reaction leading to caramel formation.

ii. Aspartic acid or glutamic acid may interact with the fragments of the dehydrated sugars. The products of these interactions may be stable, and therefore prevent caramel color.

iii. The two carboxyl groups of the dicarboxylic amino acid may protect stereochemically (caging) the amino group from reacting with the carbonyl of the sugars (Figure 33).

Table 10.--Effect of various concentrations of glutamic and aspartic acids on the caramelization of fructose (0.4 M) and glucose (0.4 M), at 60°C and 58 hours reaction time in buffer solution of pH 8.

Glu, M	Fructose + Glu, A_{450}	Glucose + Glu, A_{450}	Asp, M	Fructose + Asp, A_{450}	Glucose + Asp, A_{450}
0.000	+0.410	+0.090	0.000	+0.410	+0.090
0.024	-0.084*	+0.098	0.024	-0.205	+0.021
0.028	-0.060	+0.065	0.028	-0.192	0.000
0.032	-0.031	+0.040	0.032	-0.172	-0.011
0.036	-0.003	+0.012	0.036	-0.158	-0.022
0.040	+0.020	+0.002	0.040	-0.140	-0.037

*(-) means that the blank (heated sugar alone) absorbed at 450 nm more than the sample. In this case, the blank cuvette was placed in the sample position and vice versa.

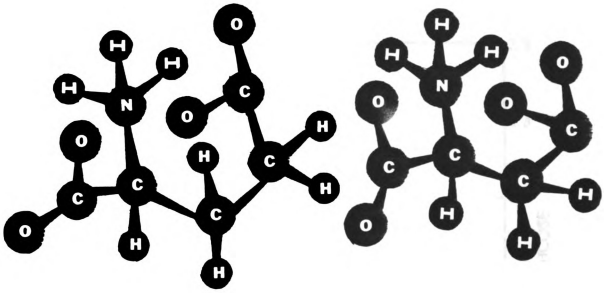


Figure 33. Molecular structure of (a) glutamic and (b) aspartic acids.

iv. Another possible cause for the resistance of glutamic or aspartic acid to browning might be due to hydrogen bonding, or dipole-dipole interaction, or both, between the charged amino group and the carboxylic groups.

VI. Inhibition of Sugar-Amine Browning by Glutamic and Aspartic Acids

A. Glutamic Acid

Figure 34 shows the inhibitory effect of glutamic acid on lysine-glucose and lysine-fructose systems. The molar ratio, lysine:glutamic acid, was the same (1:1) in both the fructose and glucose-containing systems. At higher concentrations of both amino acids, the color development

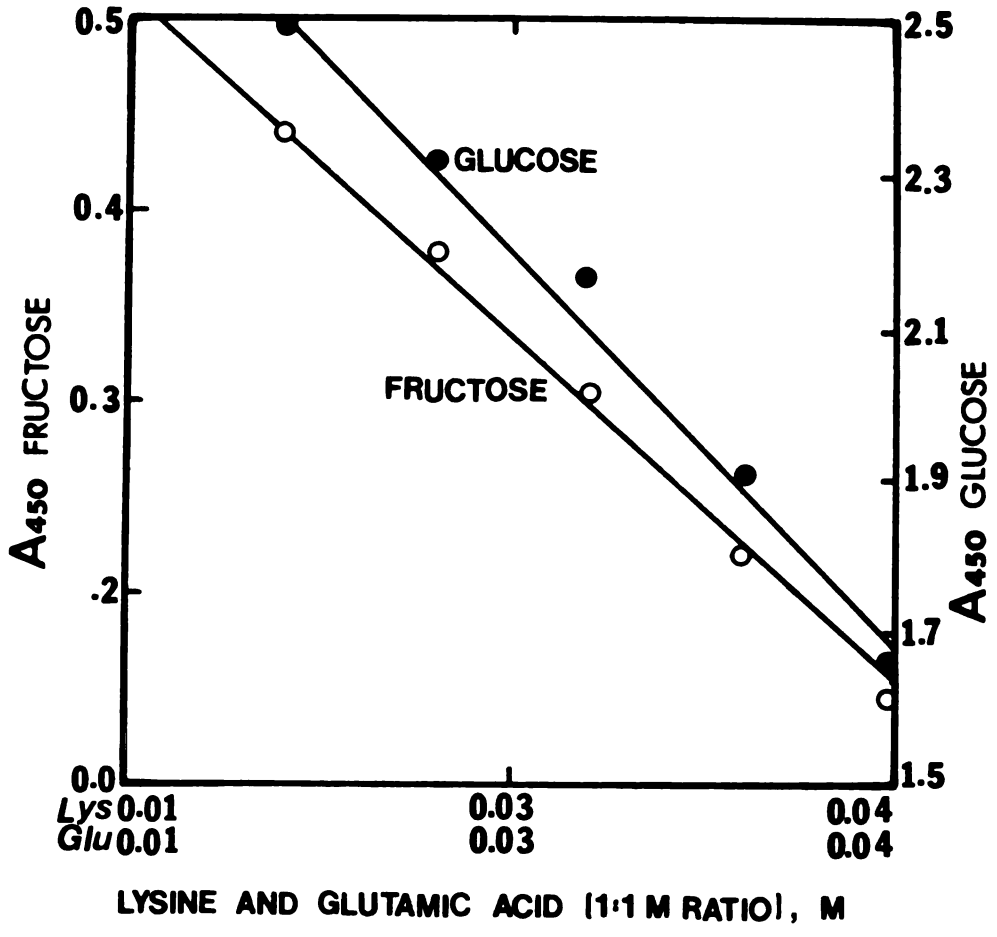


Figure 34. Effect of glutamic acid concentration (0.02 to 0.04 M) on the retardation of the browning reaction between lysine (0.02 to 0.04 M)-fructose (0.4 M) and lysine (0.02 to 0.04 M)-glucose (0.4 M) at pH 8, ionic strength 0.2, after 58 hours reaction time at 60° C.

was lower than that observed at lower concentrations of amino acids. Apparently, this becomes more evident from the results shown in Figures 35 and 36, in which increasing concentrations of glutamic acid over constant concentrations of lysine result in decreasing browning.

B. Aspartic Acid

Results similar to those of glutamic acid were observed when aspartic acid was added to lysine-sugar systems (Figures 37 and 38). The mechanism of this inhibition is unknown. One may speculate that aspartic and glutamic acids interfere with the reducing sugar-lysine reaction in a way similar to that of the inhibition of caramelization by these amino acids. Aspartic and glutamic acids may react with one or more of the many intermediate products formed during melanoidin formation (Figure 4) and thereby prevent the production of the final brown compounds.

C. Inhibition of Non-Enzymatic Potato Browning by Aspartic and Glutamic Acid

When the ampoules containing potato flour with or without glutamic acid were examined after overnight heating at 110°C, it was observed that the ampoules which did not contain glutamic acid had exploded, while the others were intact. The explosion was probably due to the formation of CO_2, which is regularly produced in sugar-amine reactions. The ampoules containing glutamic acid were less dark and must have had little or no gas, as no explosion occurred.

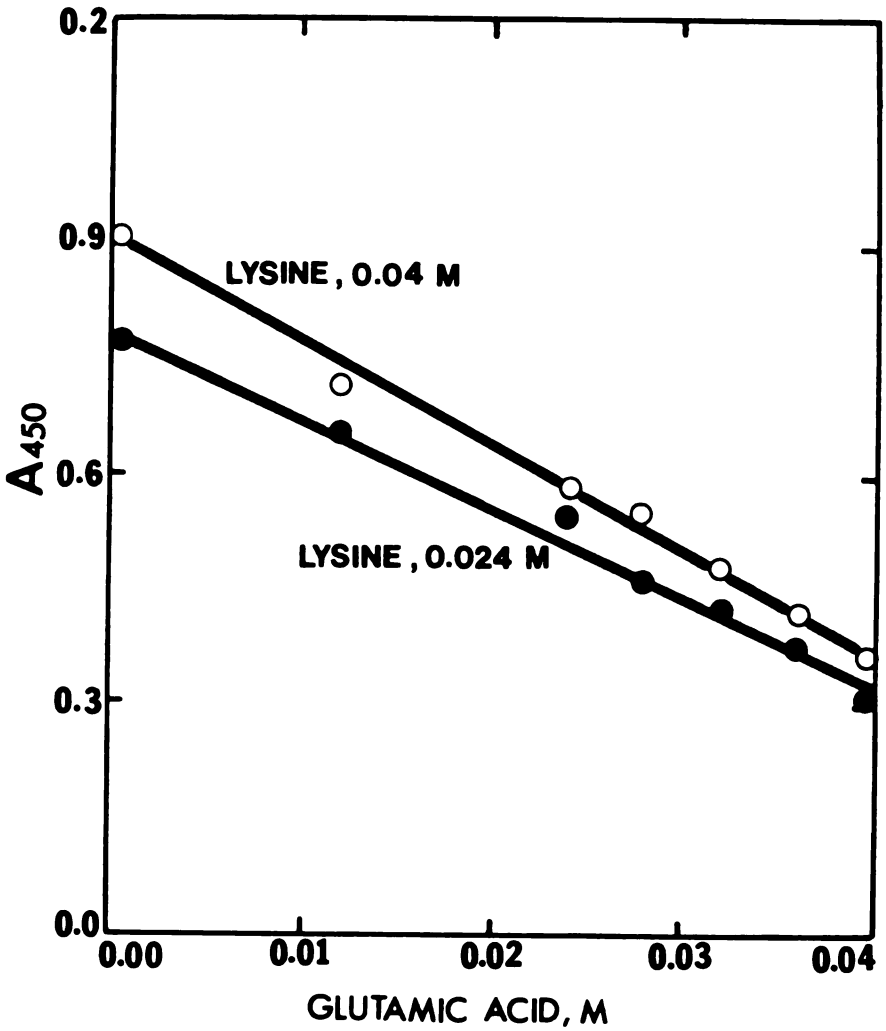


Figure 35. Effect of lysine concentration on color development in the glutamic acid-fructose system at pH 8, ionic strength 0.2, after 58 hours reaction time, and at 60° C.

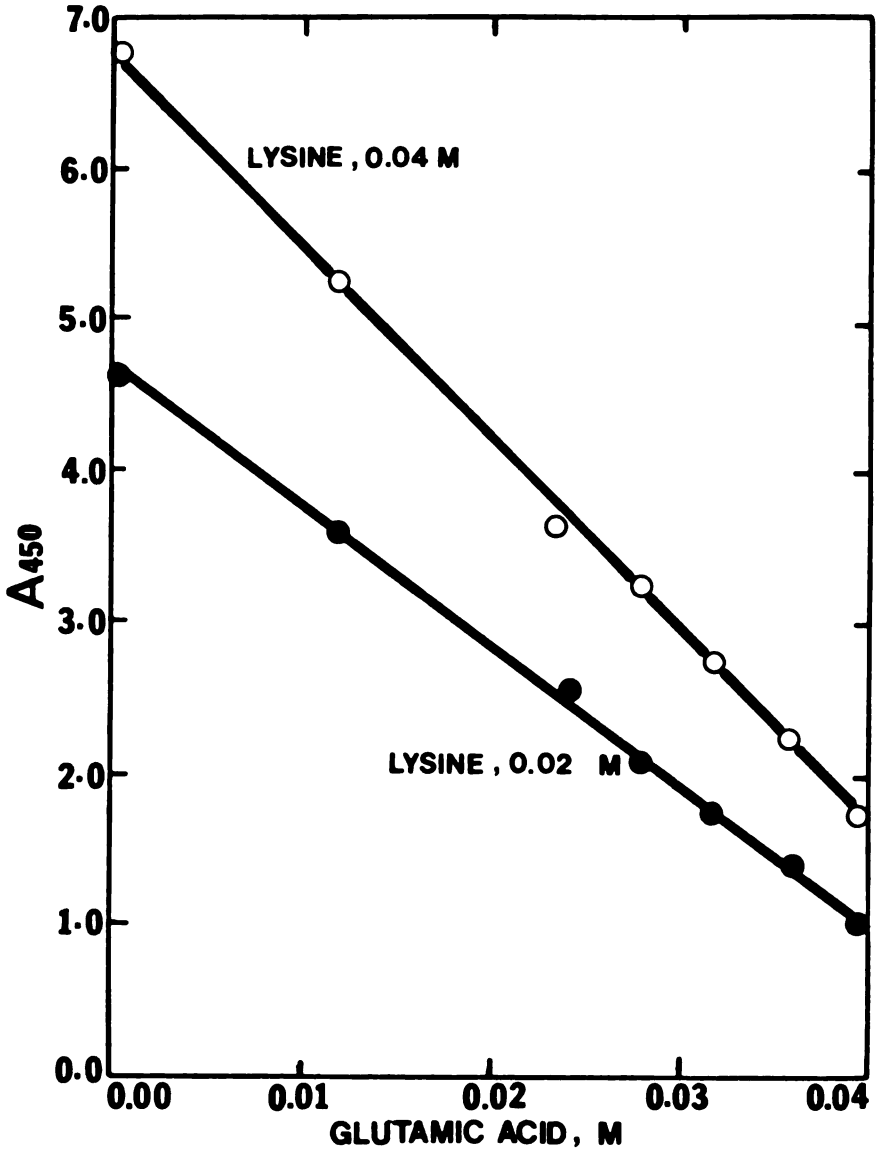


Figure 36. Effect of lysine concentration on color development in the glutamic acid-glucose system at pH 8, ionic strength 0.2, after 58 hours reaction time, and at 60° C.

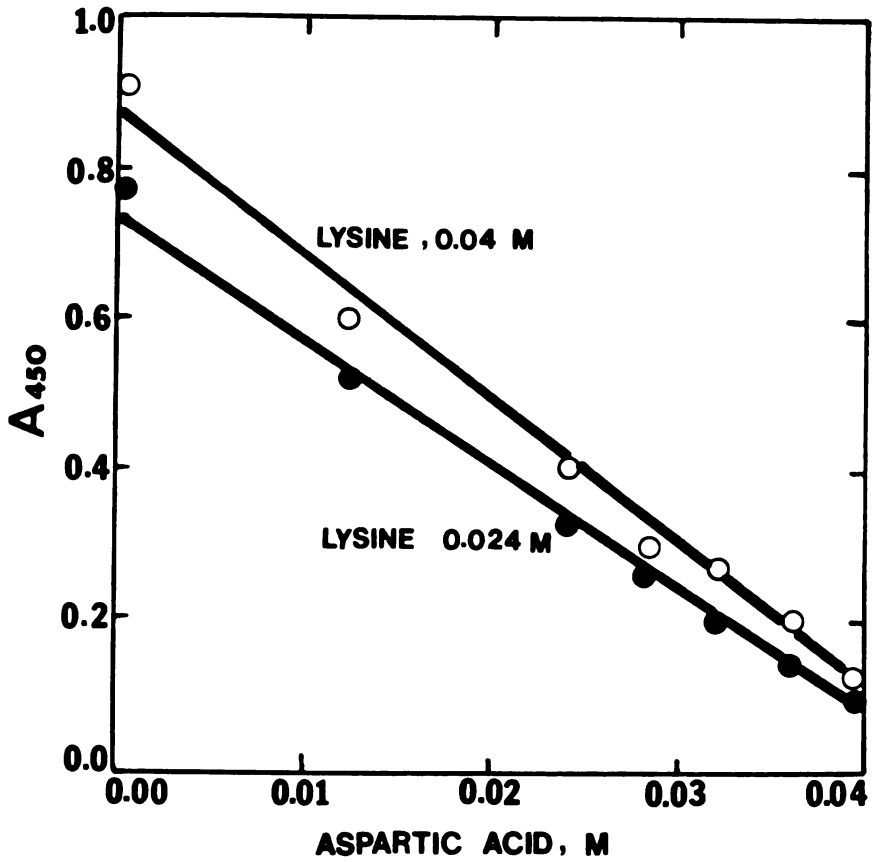


Figure 37. Effect of lysine concentration on color development in the aspartic acid-fructose system at pH 8, ionic strength 0.2, after 58 hours reaction time, and at 60° C.

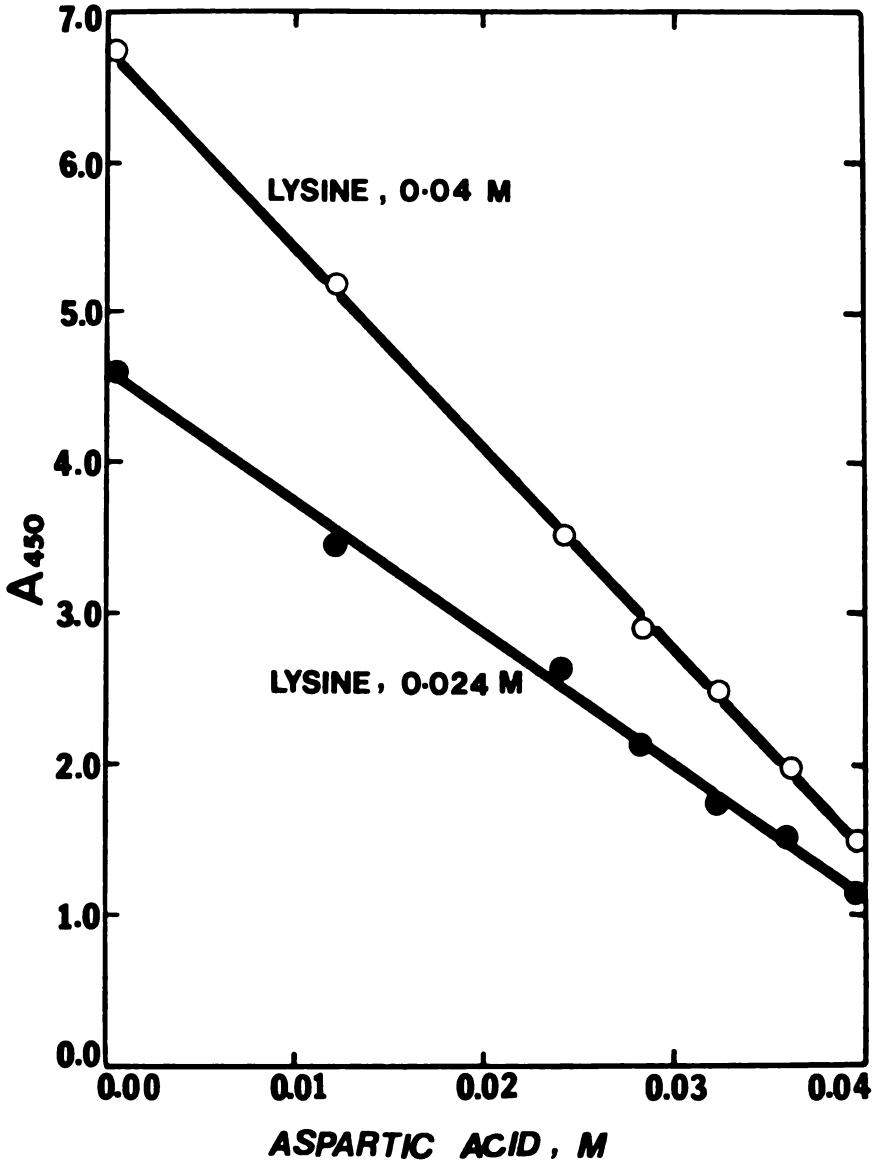


Figure 38. Effect of lysine concentration on color development in the aspartic acid-glucose system at pH 8, ionic strength 0.2, after 58 hours reaction time, and at 60° C.

Apparently, glutamic acid inhibited the browning reaction in the heated potato flour.

The results of the color measurement of the sliced fried potatoes treated or nontreated with glutamic acid and aspartic acids were summarized in Table 11 and Figure 39. In Table 11 the L-value, which is a measure of the lightness in color, clearly indicates that the samples treated with glutamic or aspartic acid are less dark than the untreated samples.

The reflectance spectra depicted in Figure 39 show that the treated samples reflect more light in almost all of the visible region, confirming the results obtained by the Hunter method of color measurement. It must be noted that usually these color differences were small immediately after frying, but they were amplified after 24 hours of standing at room temperature. The instrumental color evaluation was performed after the 24 hours of storage.

Should glutamic and aspartic acids prove to inhibit the sugar-amino reaction in a number of commodities in which non-enzymatic browning is undesirable, a rather novel way of preventing this browning may be in sight. These amino acids not only are non-toxic, as SO_2 is suspected to be when added to foods, but they are also nutrients. Furthermore, monosodium glutamate is known to be a flavor enhancer.

Table 11. Hunter color difference values for fried potatoes treated or untreated with aspartic and glutamic acid prior to frying.

Replicate*	Untreated	Treated with Glutamic Acid	Treated with Aspartic Acid
	L	L	L
1	39.0	44.2	47.6
2	38.3	44.7	44.6
3	38.2	43.0	45.3
4	39.0	43.0	47.0
5	37.9	43.7	46.8
6	36.7	43.9	46.6
7	39.5	43.6	47.2
Average	38.4 $\pm$ 0.57**	43.7 $\pm$ 0.57	46.4 $\pm$ 1.00

*The values for each replicate is the average of 3 numbers obtained by rotating each sample three times at an angle of 120°.

**The differences of treated from untreated samples are significant at the 99% probability level. The difference between treated samples is not significant.

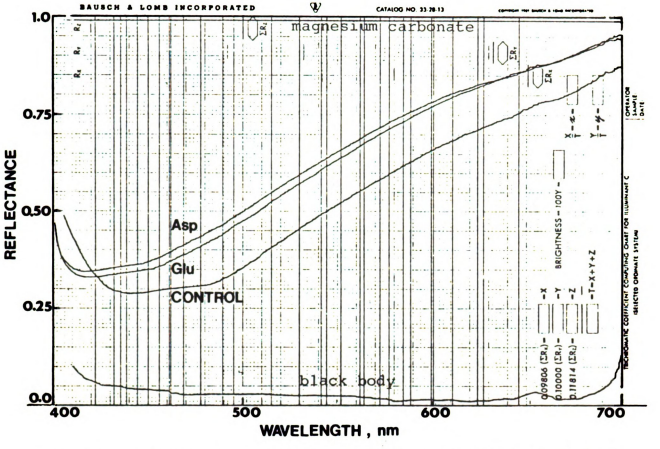


Figure 39. Reflectance spectra of untreated and treated (with glutamic and aspartic acids) fried potatoes at 210° C for 7 minutes.

SUMMARY AND CONCLUSION

The interaction between sugars, amino acids and proteins may result in undesirable discoloration in foods (browning) and concommitant loss of nutritional value. In order to better understand the nature of this discoloration, the interaction between glucose and fructose on one hand, and alanine, arginine, aspartic acid, glutamic acid, glycine, lysine monohydrate, methionine, phenylalanine, serine, and α-amino-n-butyric acid on the other hand was studied through model systems. These systems were solutions of sugars and amino acid in phosphate buffers, exposed to higher than ambient temperature for defined periods of time.

The main findings of this study are as follows:

A. Sugars

1. Fructose contributes less than glucose to browning while reacting with amino acids, except in the model systems containing less glycine than 0.031 M, at pH 8.0. Fructose also shows a shorter induction period compared to glucose, so that the rate of discoloration at the initial stages of browning is higher than that of glucose; as the reaction advances, however, the browning of glucose systems is greater than that of the corresponding fructose systems.

2. Fructose is more readily caramelized compared to glucose.

B. Amino Acids

1. The rate of the browning reactions in most cases was increased by increasing the pH value from 6 to 8. However, the relationship between reaction rate and pH was not linear. In the arginine-glucose system, the reaction proceeded the fastest at pH 7.

2. Among the amino acids tested, lysine and arginine were the most active in the production of brown color when reacting with glucose and fructose. However, the activity of arginine at pH 6 with fructose was nearly nil.

3. At pH 7 the reactivity of arginine with fructose is greater than that of lysine with fructose.

4. Glutamic acid and aspartic acid not only do not contribute to the browning in the particular reaction condition, but also retard caramelization and the Maillard reaction. Impregnating freeze-dried potato slices with aspartic or glutamic acid solutions resulted in less brown discoloration upon frying the slices.

C. Activation Energy

The activation energy for the reaction between glucose and fructose with lysine was 13,331 cal/mole for glucose-lysine and 10,210 cal/mole for fructose-lysine. The Q_{10} for the same reactions were 1.87 for glucose-lysine and 1.61 for fructose-lysine.

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