KINETICS OF COLOR CHANGES DUE TO MAILLARD REACTIONS IN MODEL FOOD SYSTEMS

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ABSTRACT

KINETICS OF COLOR CHANGES DUE TO MAILLARD REACTION IN MODEL FOOD SYSTEMS

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The nonenzymic browning reactions in models, Pekmez and Pekmez containing model systems were studied. The effects of sugars (D-fructose, D-glucose) and type of amino acids (L-glutamine, L-arginine and L-proline) on both 5-HMF accumulation and brown pigment formation in all these systems were investigated at three different temperatures during 10 days period.

It was observed that, 5-HMF accumulation at pH 3.5 and brown pigment formation at pH 6.0 were the highest in Pekmez containing model systems. D-fructose was the major sugar for the formation of 5-HMF and brown pigment in all systems. With fructose as a substrate, 5-HMF and brown pigment formed faster in the presence of L-glutamine than in the presence of L-arginine and L-proline in the systems. Reaction orders were found as 0.5 for 5-HMF accumulation, zero for brown pigment formation in all samples by using the non-linear regression analysis..

Rate constants were determined for 5-HMF accumulation by using non-linear regression analysis and linear regression analysis for brown pigment formation. Activation energies of model systems, Pekmez and Pekmez containing model systems were found in the range of 52.68-265.40 kj/mol for 5-HMF accumulation and 41.46-217.08 kj/mol for brown pigment formation.

Key Words : Nonenzymic browning reaction, 5-Hydroxymethyl furfural (5-HMF), Fructose, Glucose, Glutamine, Arginine, Proline, Pekmez.

To my Daughter

KÜBRA

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Model, Pekmez ve Pekmez içeren model sistemlerinde enzimik olmayan esmerleşme reaskiyonları çalışılmıştır. Şeker (glikoz ve fruktoz) ile amino asit (arginin, glutamin ve prolin) tiplerinin 5-hidroksimetil furfural (5-HMF) birikimi ve esmer pigment oluşumu üzerine olan etkileri bu farklı sistemlerde on gün boyunca üç ayrı sıcaklıkta incelenmiştir.

Pekmez içeren model sistemlerde pH 3,5'da en fazla 5-HMF birkimi ve pH 6,0'da en fazla esmer pigment oluşumu gözlenmiştir. 5-HMF birikimi ve esmer pigment oluşumu açısından bütün sistemlerde D-fruktoz en reaktif şeker olduğu bulunmuştur. D-fruktozun substrat olarak kullanıldığında, L-glutamin içeren sistemlerde 5-HMF birikimi ve esmer piment oluşumu L-arginin ve L-prolin içeren sistemlerden daha fazla olduğu gözlenmiştir. Reaksiyon derecesi, 5-HMF birikimi için 0,5, esmer pigment oluşumunda ise sıfır olarak lineer olmayan regrasyon analizi kullanarak tayin edilmiştir.

Hız sabitleri 5-HMF birikimi için lineer olmayan regrasyon analizi, esmer pigment oluşumunda ise lineer regrasyon analizi kullanılarak bulunmuştur. Aktivasyon enejileri, tüm karışımlarda 5-HMF birikimi için 52,68-265,40 kj/mol, esmer pigment oluşumu için 41,46-217,08 kj/mol değerleri arasında hesaplanmıştır.

ANAHTAR KELİMELER : Enzimik olmayan esmerleşme reaksiyonları, 5-Hidroksimetil furfural, Fruktoz, Glikoz, Glutamin, Arginin, Prolin, Pekmez.

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ABBREVIATION

5-HMF	Hydroxymethly furfural
CO_2	Carbondioxide
ARP's	Amaodri rearrangement products
TPN	Total parental nutrition
n	Reaction order
k	Rate constant
E_a	Activation energy
OD	Optical density
A	Reactant
B	Product
R	Intermediate
cal	Calori
T	Temperature
HPLC	High performance liquid chromatography
GC	Gas chromatography
a_w	Water activity
Glu	D-Glucose
Fru	D-Fructose
Glt	L-Glutamine
Arg	L-Arginine
Pro	L-Proline
Pek	Pekmez mixtures
Mix	Mixture

CHAPTER I
INTRODUCTION

Browning reactions which exhibit a brown discoloration, are one of the most prevalent chemical reactions that occur in foods. Browning reactions in foods are of widespread phenomena which take place during the thermal processing and storage. These reactions occur during the manufacturing the meat, fish, fruit and vegetable products, as well as when fresh fruit and vegetables are subjected to the mechanical injury during the harvesting and transporting.

Browning, to a limited extent, may be considered to be desirable in some food products such as apple juice, potato chips, and french fries. However, in many other food products such as fruits, vegetables, frozen and dehydrated foods, browning is undesirable as it results in off-flavors and in poor appearance. Another significant problem with browning is the proportionate lowering of the nutritive value of the food article. Thus it is important to know the mechanisms of browning reactions, and ways in which browning can be inhibited or controlled in food materials [1].

There are many different pathways and substrates for browning. Oxidation of ascorbic acid in citrus fruits may cause loss of vitamin C with subsequent darkening of the fruits. Sugars can caramelize when exposed to excessively high temperatures. From Maillard-type of browning reactions, reducing sugars and free amines in dehydrated potatoes result in the off-flavor. In dry milk powder, similar Maillard-type browning results in decreased solubility and nutritional loss.

1.1. Types of Browning Reactions

1.1.1. Enzymic Browning

Enzymic browning can be observed on the cut surfaces of light colored fruits and vegetables, such as apples, bananas, and potatoes. Exposure of the cut surface to air results in rapid browning due to the enzymic oxidation of phenols to orthoquinones, which in turn rapidly polymerize to form brown pigments or melanoidins [2].

1.1.1.1. Control of Enzymic Browning

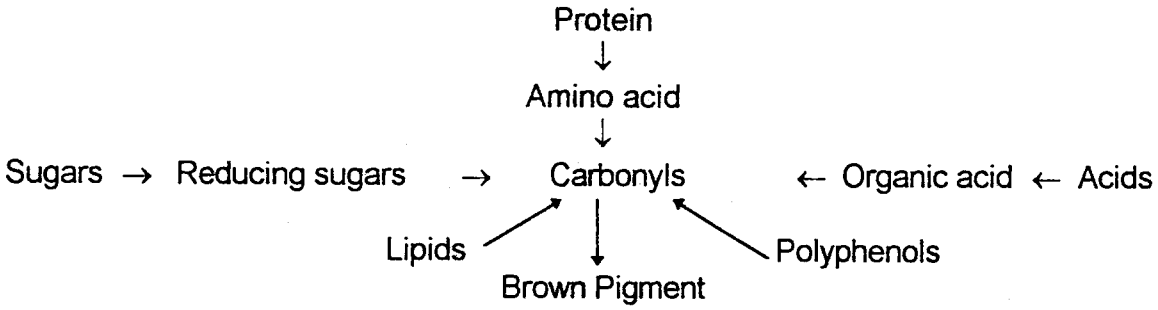
For the most part, Phenolase activity in fruits and vegetables is undesirable because the enzymic browning reactions occur during processing. Consequently, a variety of methods have been developed to inhibit enzymic browning.

Heat treatments, or the application of sulfur dioxide or sulfites, are commonly used methods of inactivating Phenolase. Phenolase activity can be inhibited by the addition of sufficient amounts of acidulants such as citric, malic, or phosphoric acids to yield a pH of 3 or lower. Oxygen can be excluded from the reaction site by such methods as vacumization or immersing the plant tissues in a brine or syrup [2].

1.1.2. Nonenzymic Browning Reactions

Nonenzymic browning reactions occur during the thermal processing and storage of foods. These reactions are very important for foods because of production of flavor, color, and aroma of coffee, caramel, bread, and breakfast cereals. These type of browning reactions must be carefully controlled to minimize excessive browning which could lead to unpleasant changes in the food product during the production and also storage. Some toxic and mutagenic intermediates are formed from the nonenzymic browning reaction [3-5]. Also some intermediates formed to exert considerable antioxidant activity [6-8].

The chemistry of the reactions associated with the development of the pigmented material in the product is very complex. Wedzicha [9] and many authors have been advanced for the mechanism of these reactions. Stadtman [10] reviewed some of these, including the Maillard condensation theory. Simplified schematic presentation of nonenzymic browning reaction occurring during dehydration processes of fruits and vegetables.



Hodge [11] investigated reactions in a model system containing typical component of foods. He noted three kinds of non-enzymic browning exist. These are:

1- Carbonyl amino acid-type which includes the reaction of aldehydes, ketones, and reducing sugars with amino acids, peptides, amines, and proteins (Maillard reactions).

2- Caramelisation type which occurs when polyhydroxycarbonyl compounds (sugars and polyhydroxycarboxylic acids) on their own, provided they are exposed to higher temperatures, will undergo similar reactions by themselves, but then, there can be no interaction with amino compounds and no nitrogen-containing compounds can result.

3- Oxidative reaction-type in which ascorbic acid and Polyphenols are converted to di- or polycarbonyl compounds.

1.2. Sugar-Amine Browning Reactions

The reaction was actually discovered by an English chemist Ling, in 1908, first theorized that color produced in the brewing process came from a reaction between sugars and protein [12]. The man who gave the reaction its name and made it famous was a French chemist Louis Camille Maillard, in 1912. He combined one part of glycine and four parts of glucose in water. He observed that the solution turned progressively darker and that CO_2 was produced. He observed the formation of brown pigments or Melanoidins when heating a solution of with different amino acids and reducing sugars. The reaction was subsequently referred to as the "Maillard reaction" and similar reactions between amines, amino acids, and proteins with sugars, aldehydes, or ketones have also been established. The Maillard reaction appears to be the major cause of browning developing during the heating or prolonged storage of foods.

As it is well known, the rate of coloration, the color produced, and the product properties of the browning reaction (the most characteristic consequences of the Maillard reaction) are strongly dependent on the nature of reactants (types and concentration of the sugars and amino acids), and sugar amino acid ratio, and the reaction conditions, especially pH, and temperature. The browning reaction rates of aldoses in generally are higher than the ketoses, pentoses are higher than the

hexoses. Basic amino acids generally brown more easily than acidic amino acids. Alkaline pH and higher temperatures greatly enhance the reactions and result in changes in the product distribution.

The Maillard reaction is central to food chemistry because of at least five aspects:

1)- Production of color. This may be desirable, as in coffee or bread crust, or undesirable, as in glucose syrup and in many intermediate moisture products. Color is principally produced in final stages.

2)- Production of flavors or off-flavors.

3)- Reduction in nutritional value by involvement of ascorbic acid (a reductone) and lysine (free or bound), an essential and often limiting amino acid. Metal-chelating properties may also be significant.

4)- Toxicity through formation of imidazoles and *N*-nitroso derivatives, e.g. Amadori compounds. The intrinsic is of some concern, but is difficult to study because of their anti-nutritional properties.

5)- Antioxidant properties. These are ought to be due to the reductones formed, but chelation of heavy metals may also be involved.

1.2.1 Mechanism of the Maillard Reaction

The general mechanism of the Maillard reaction was first proposed by Hodge [11] and subsequently reviewed by Ellis [13], Heyns and Poulsen [14], and Reynolds [15-17]. The Maillard reaction is still best summarized by scheme due to Hodge [11].

1.2.1.1 The Initial Stage

The first stage involves a condensation reaction of the free, uncharged α-amino group of the amino acids or proteins and carboxyl group of reducing sugars.

It is generally agreed that the Amadori compounds in the Maillard reaction mixture could exist as enaminol structures. The reducing power of the reaction mixture, probably attributable to such reductones, increases with browning [18].

1. Carbonlyamino reaction

The first stage involves rapidly loss of water. The trivalent nitrogen atom of

amine, which has an unshared electron pair, act as a nucleophile towards the carbonyl group. Bases can catalyze the reaction by removal of a proton from nucleophile converting it from a weak to strong nucleophile [19]. After the removal of water Schiff base produce (usually occur faster than in the first stage) and followed by cyclization to the corresponding *N*-substituted glycosylamine that was extremely unstable and underwent a series of reaction. This reaction is not necessarily restricted to α-amino acids and can involve the participation of other amino groups found in peptides an proteins. This reaction usually requires an acidic catalyst [20]. The concentrations (products) vary in the opposite direction with pH, so the rate of condensation reaches maximum at a weakly acidic pH in the reaction involving aldose and amine.

Song and Chichester [21] reported on the kinetic behavior and mechanism of the Maillard reaction where chemical reactions between the sugars and amino acids present in the fruits and vegetables are involved. The initial reaction takes place between the aldehydic group of a sugar and the amino group of the amino acid, followed by a complex reaction scheme which leads to Melanoidin formation. Sulfur treatment can prevent the initial condensation reaction by forming non-reactive hydroxy-sulfonate sugar derivative.

2. Amadori Rearrangement

The final condensation product, *N*-substituted glycosylamine, that is produced then undergoes the irreversible Amadori rearrangement. The Amadori rearrangement is the isomerization of the, *N*-substituted aldosylamine to a 1-amino-1-deoxy-2-ketose, while the Heyn's rearrangement changes a ketosylamine to a 2-amino-2-deoxyaldose [19]. This condensation reaction is initiated by an attack of a nucleophilic amino nitrogen, with an unshared electron pair, on the carbonyl carbon. The reaction requires an acidic catalyst. At room temperature, the formation of Amadori rearrangement products (ARPs) proceeds slowly, since a tautomeric Schiff to the open-chain form of the reducing sugars is required for the initial reaction occur [22,23]. ARPs are more stable than the glycoylamines in moist, acidic environments, although they are heat sensitive. Upon the heating, the ARPs undergo dehydration and fission and yield colorless reductones as well as fluorescent substance, some of which may be also pigmented [11].

In 1975, Namiki and Hayashi [24] proposed a side reaction in which the initial condensation product may lead directly to browning while bypassing the Amadori rearrangement products. Nevertheless, the Amadori rearrangement products remain an important part of the accepted reaction pathway.

1.2.1.2. The Second or Intermediate stage

The second or intermediate reaction stage involves the removal of the amino groups from the reducing sugar complex to form more reactive compounds. Some of these products are fluorescent and brown pigments may occur at a low concentration The removal of amino groups from the reducing sugar moiety with subsequent dehydration and cyclization, fragmentation, or amine condensations, three general pathways exist during this phase. Under acidic conditions, hydroxymethyl furfural or furfural is produced; under mild to basic conditions, reductones and dehydroreductones result, which can undergo Strecker degradation; and at high temperatures fragmentation products from the Amadori products are produced.

1. Sugar Dehydration

There are two types of sugar dehydration reactions, both of which depend on the pH of the system.

a- Under acidic conditions, the nitrogen is protonated, and enolization involving the C_1 atom favors production of 1,2-eneminol [23]. Dehydration and cyclization produce hyroxymethyl furfural (from hexoses) or furfurals (from pentoses) with the possibility of amino acid regeneration.

b- Under basic conditions, enolization involving the C_3 atom favors the formation of a 2,3-enediol. The sugar chain is dehydrated, with the loss of two molecules of water, and reductones are formed. Conjugated unsaturation is necessary for the stability of the reductones.

2. Sugar Fragmentation

The second reaction with ARPs is fragmentation of the sugar moiety. The accepted mechanism for sugar fragmentation is dealdolization, the reverse of aldol condensation. Amines catalyze this reaction as they catalyze aldol condensation [11].

The products are aldols, amino-free polymers, and free amino compounds.

3. Strecker Degradation

The third and last reaction can occur with ARPs is Strecker degradation. This reaction takes place between a reductones and α-amino acid and liberates carbon dioxide to form an aldehyde with one fewer carbon than the original amino acid. The aldehyde produced from the Strecker degradation is a source of browning since they can condense with themselves, with sugar fragments, with furfurals, or with other dehydrated products to produce brown pigments. This pathway, however, is not major color-producing reaction and is better known as the source of off-flavors associated with Maillard reaction [11].

1.2.3. Final Stage (Pigment Formation)

The final stage of the reaction is the stage where most of the color intermediates and other reactive precursor products (such as enaminol products, low-molecular-weight sugar analogs, and unsaturated carbonyl products) are produced. The chief reaction involved are thought to be aldol condensation, aldehyde-amine polymerization, and formation of heterocyclic nitrogen compounds under amine catalyst such as pyroles, imidazoles, pyridines, and pyrazines [11].

The chemistry at this point is very complex, but it is known that both water-soluble and water-insoluble pigments, called Melanoidins, are produced [15]. The Melanoidins vary widely in molecular weight and contain several discrete chromophores [19]. Melanoidins may be distinguished from one to another on the basis of their molecular weight and solubility. Melanoidins with a molecular weight<500 dalton represent low molecular weight compounds and are soluble in water or organic solvents. Soluble Melanoidins with molecular weights > 12000 daltons can easily be prepared by dialysis of aldose-amino acid reaction mixtures, as the browning reactions proceed the Melanoidins eventually become insoluble in water and precipitate. A general formula $C_{67}H_{76}O_{32}$, (sugar+amino acid)-2~($3H_2O$), has been proposed for Melanoidin based on the consideration that the main pathway of Melanoidin formation involves the reactions of amino compounds with deoxyosones, furfural, and other fragmentation products which accompany the dehydration reaction [25,26]. Rubinsztain

et al. [27] found that basic amino acids and sugars form Melanoidins that have higher molecular weights than those formed by neutral or acidic amino acids. This indicates that cross-linking is probable. The same study revealed that the carboxyl acidity in the Melanoidin structure can be correlated with the relative contribution of the sugar; the higher the sugar:amino acid ratio, the higher the carboxyl group concentration and the higher the concentration of total Melanoidin pigment.

1.3. Factors Influencing the Maillard Reaction

The factors influence the Maillard reaction are type of amino acid, pH, type of sugar, solvent state, sugar:amino acid ratio, and temperature.

1.3.1 Type of Amino acid

Amino acid type which is used or is present in the medium is very important, especially in the Maillard reaction. Some amino acids have two reactive group therefore these type of amino acids rapidly react with the sugars to produce brown pigment. Ashoor and Zent [28] have been classified the amino acids according to the reactivity with the sugars at pH 9.0. They found that lysine, glycine, trytophan, and tyrosine are the most reactive amino acids.

Lysine is often hypothesized as being the most reactive amino acid due to its two reactive amino groups [19]. Despite this logical deduction, numerous studies have provided contradictory results. Wolfrom et al. [29] studied the effect of amino acid type on the extend of browning. They found that, L-arginine and 4-aminobutyric acid are the most intense and they produced rapidly brown pigment formation, followed by glycine, alanine, serine, and L-proline. Aspartic acid, L-glutamic acid, L-glutamine were found to behave similarly to glycine. These amino acids present in orange juice naturally as well as in other foods. Also they found that each amino acid has different induction period.

Massaro and Labuza [30] studied total parental nutrition (TPN) solution at pH 5.5-5.7. They found that tryptophan and cysteine produced high brown pigment than lysine on a molar ratio basis.

1.3.2. pH

pH has a very significant effect on the Maillard reaction. The carbonylamino reaction can occur in acidic or alkaline media, although it is favored under the more alkaline conditions. A number of studies have demonstrated an increase in reaction rate with rise in pH.

The pH dependence of the initial stage of the Maillard reaction can be related to the amount of unprotonated form of the amino acid. The pK_a of the amino acid determines the concentration available in the unprotonated form, which is the form that can react readily with aldose or ketose compounds.

Generally, it has been stated that the rate of browning and substrate loss increases with increasing pH [13], up to a pH of about 10. Ashoor and Zent [28] demonstrated that maximum browning was reached at pH 9 and 10 for three different amino acid.

The relationship between the reaction rate and pH discussed above would therefore render those foods of high acidity less susceptible to this reaction.

1.3.3. Type of sugar

Reducing sugars are essential ingredients in these reactions, providing the carbonyl groups for interaction with the free amino groups of amino acids, peptides, and proteins. The concentration of the acyclic or straight-chain form determines reactivity since it is only the acyclic form with free aldose or hexose group that can react. When a crystalline sugar is dissolved in a suitable solvent, water or other solvent, it undergoes mutarotation, the equilibrium reaction between the α-furanose, β-furanose, β-pyranose, and straight-chain forms.

The initial rate of this reaction is dependent on the rate at which the sugar ring opens to the oxo or reducible form. The amount of the oxo form was much higher for pentoses than hexoses, thus explaining the greater reactivity of pentoses in browning systems. It was found that the order of reactivity was greater for aldopentoses than aldohexoses. It was noted that reducing sugars exerted an inhibitory effect on the hydrolysis of casein by trypsin because of the unavailability of certain essential amino acids resulting from nonenzymic browning reactions [31].

Maillard found that the increasing order of brown pigment formation is: D-

xylose>L-arabinose> hexoses>disaccharides [11]. Fructose did not condense with amino acids in dilute solution although scientists have since confirmed that a definite interaction does take place. D-fructose has also been reported to brown at a much faster rate than glucose during the initial stages of the browning reaction, but it then falls behind. This was confirmed using model systems containing glucose-glycine and fructose-glycine [38]. Sucrose, as a non-reducing sugar, will only participate when the glycosidic bond is hydrolyzed and the reducing monosaccharide constituents released. Hydrolysis of the glycosidic bond in sucrose is facilitated by a low pH, resulting in an increase in the Maillard reaction rate in protein-sucrose systems.

1.3.4. Moisture Content

Labuza and Saltmarch [32] reviewed that the influence of the state of water, as characterized by its thermodynamic availability or water activity (a_w), on the rate of browning and substrate loss in reduced moisture systems. With respect to the browning as a function of a_w, at a_w range of 0.5-0.8 maximum pattern and at a_w between 0-0.5 and 0.8-1.0 minimum pattern was observed as found by McWeeny [33]. Solute mobility is limited below the monolayer due to the higher binding energy and the glassy state that forms [34]. With respect to browning, water can retard the rate of the initial glycosylamine reaction by mass action since water is produced. On the other hand, water may increase deamination reactions in the browning sequence [15] for the production of furfural or hydroxymethyl furfural. For the overall reaction, both liquid and solid model systems containing glycerol had higher browning rates than expected a_w range of 0-0.5, with maximum reaches between 0.41-0.55 a_w range [35,36].

The Maillard reaction proceeds rapidly in solution, although complete dehydration or excessive moisture levels inhibit this process. Browning in milk powder at 40 °C was studied as a function of water activity and lysine over a period of a day. Whose results showed that the loss of lysine paralleled the extent of browning with a maximum between aw of 0.6 and 0.7. An increase in water activity was also shown to cause an increase in the loss of lysine and tryptophan with the concomitant increase in 5-HMF formation and browning [31].

1.3.5. Sugar:Amine Ratio

An excess of reducing sugar over amino compounds promotes the rate of the Maillard reaction [19]. The velocity of the Maillard reaction depends on the different characteristics of the sugars and amino acids, and on the different ratios [19]. Lerici et al. [31] have studied browning kinetics of glucose-glycine model systems at a different molar ratios. They found that increasing the sugar:amino acid ratio decreases the browning rate. Also, the initial stage of the formation of Schiff base depends on the concentration of both sugar and amine. Schiff base formation increases with decreasing the sugar:amino acid ratio.

1.3.6. Temperature

The temperature dependence of the reaction has been demonstrated in number of studies [32,57], where increased rates were reported with rise in temperature. As with other chemical reactions, the temperature dependence of the Maillard reaction has been modeled the Arrhenius equation which the rate constant is exponentially related to the inverse absolute temperature for the simple reaction.

$$A \rightarrow B \qquad (1)$$

The evaluation of the order of the browning reaction was based on the following equations:

The rate of browning is expressed as the formation of B or loss of A:

$$-(dA/dt) = (dB/dt) = k[B]^{n} \qquad (2)$$

where k is the molecular rate constant and n is the order

$$n=0 \qquad B - B_{0} = k\ t \qquad (3)$$

$$n\neq 0 \qquad B^{1-n} - B_{0}^{1-n} = (1-n)\ kt \qquad (4)$$

The temperature dependence of the rate constant may be described by the Arrhenius Equation:

$$k=k_{0} \cdot \exp.^{(-Ea\ /\ RT)} \qquad (5)$$

where k_0 is the absolute rate constant, Ea is the activation energy of browning (kcal/mole), R is gas constant, and T is the temperature of products. From the slope (-Ea / R) of the plot ln k versus 1/T, the activation energy is estimated [32].

Lea and Hannan [37] reported a decrease in the free amino nitrogen for a

casein-glucose system which conformed to the Arrhenius equation over a temperature range of 0-90 °C, where a linear relationship existed between the rate of reaction and temperature over this range using the formation of hydroxymethyl furfural as a measure of progress of the Maillard reaction.

1.3.7 Metals

The formation of metal complexes with amino acids can influence the Maillard reaction. This reaction was catalyzed by copper, and iron, while manganese and tin inhibit the reaction [13]. Using an ovalbumin-glucose mixture Kato et al. [26] examined the effects of Na^{+}, Cu^{2+}, Fe^{2+}, and Fe^{3+} on the rate of browning at 50 °C and 65% relative humidity. They found that browning rate was increased in the presence of Cu^{2+} and Fe^{3+} while Na^{+} had no effect. Fe^{3+} was more effective than the Fe^{2+} in accelerating the browning reaction, because the first stage of the reaction was an oxidation activation and results in reduction of metals.

The more rapid browning of a dried egg white-solid glucose system was attributed by Kato et al. [38] to the presence of trace metals in egg-white.

1.4. 5-HMF Formation

After the formation of the *N*-substituted glycosylamine one can see that there are two branches, one, occurring at lower pH [11, 32], which leads to Melanoidins via 3-deoxyhexosuloses and furfurals, 3-Deoxy-hexosulose (III) is released from the diketoseamino acid, monoketoseamino acid being re-formed and this can react with another sugar molecule in the same way [39]. By elimination of water from 3-deoxyhexosulose, the unsaturated compounds are formed, while under acidic conditions ring closure to hydroxymethyl furfural takes place [40].

Formation of hyroxymethyl furfural in the glucose-glycine browning reaction under the conditions used by Maillard was demonstrated chromatographically by Chichester et al. [41].

In caramelisation reaction, heating of sugars produced hydroxymethyl furfural, which polymerizes easily. The mechanism of sugar dehydration from 1,2-enol to 5-hydroxymethyl-2-furaldehyde (5-HMF) originally described by Anet [42]. Kuster and Temmink [43] investigated the influence of pH and weak acid anions on the

dehydration of D-fructose but were unable to detect 5-HMF formation from D-fructose at pH> 3.9.

1.5 Importance of 5-HMF For Foods

5- Hydroxymethyl furfural (5-HMF) is produced from Amadori product under the acidic condition, optimum pH 3.5 [32]. Amadori product is an intermediate Maillard reaction product. Also 5-HMF is the end product of acid catalyzed hexose degradation [44], and is often used as an index of deteriorative changes in tomato paste [45], honey [46], and fruit products [47]. Its accurate determinations in fruit products are therefore important. In first class fruit jams allowable 5-HMF content is 25 mg /kg [48]. In honey, 5-HMF is used as an adulteration indicator with acid-converted invert syrups [46], or of a heating history. In Pekmez 5-HMF is assessed to indicate the quality parameter [49,50].

In cereal products (especially baby cereals), during processing of baby cereals some amino acids are lost especially lysine, is the most abundant amino acids in the cereal products, is destroyed during the process by Maillard reaction can be followed by 5-HMF determination [19].

In tomato paste, like most of the other foods, browning reactions are occurred during the storage and processing. Thus, color of the tomato paste changes and accumulated 5-HMF determines the quality of tomato products [45]. This is undesirable effect of Maillard reactions. 5-HMF is accumulated from the reaction of the free amino acids and sugars present in tomato products [45].

In grapefruit juice, 5-HMF accumulation and brown pigment formation depend on process time and temperature, and are considered useful abuse indicators [40,51]. Thus, brown pigment formation and 5-HMF accumulation were selected as quality parameters [51,52].

1.6. Determination of 5-HMF

5-HMF can be determined by several methods. A simple method for determination of 5-HMF is spectrophotometric method [47, 53, 54]. This method is based on the reaction of 5-HMF with barbituric acid and p-toluidine forming red complex. Other methods are GC method, HPLC method [55], Ion exchange

chromatography method [56]. In GC method, 5-HMF can be determined by siliylation with hexamethyldisilazane and trimethylchlorosilane [57], procedure is very long thus 5-HMF loss takes place and 5-HMF determination efficiency is very low. In HPLC method and other chromatographic method high rate centrifugation (1 hour at 12000 rpm) is needed. Spectrophotometric method is very simple and accurate.

1.7. Brown Pigment Formation

The final stage of the Maillard reaction is where most of the color intermediates and other reactive precursor products (such as enaminol products, low-molecular-weight sugar analogs, and unsaturated carbonyl products) are produced.

1.8. Effects of Browning on Foods

In general the Maillard reaction leads to a reduction in nutritive value of foods. Sugar-protein compounds may be formed which cannot be readily attacked by the digestive enzymes, or can only be split very slowly. Several essential amino acids (e.g. lysine) of proteins are blocked during the browning reaction and are therefore no longer available to the body. In food technology it is often essential to prevent the Maillard reaction. It is however desirable in some baking processes (browning of bread, crust, manufacture of biscuits, in the brewing of beer, in the roasting of coffee, cereals, potatoes and in frying meat and fish) because here the development of flavor and aroma go hand in hand with browning. It is undesirable, on the other hand, in pasteurization, sterilization and drying processes of milk, fruit juices and egg products [21].

1.9 Nonenzymic Browning in Grape Juice

Rhim et al. [58] studied the kinetics of color change of grape juice generated using linearly increasing temperature. The juice was heated from 60 to 95 °C in 9 h. Kinetic parameters for the color change were determined using Hunter L, a, b, values. Their study demonstrated the use of the linearly increasing temperature method to successfully generate kinetic parameters for describing color changes in grape juice.

Marcy et al. [59] studied the changes in free amino acid and total nitrogen

concentrations during maturation of Muscade grapes (V.rotundifolia).

Sanchez et al. [60] studied the kinetics of browning of sultana grapes. They used a colorimeter to determine the browning. They found that, nonenzymic browning reactions obey pseudo-zero order kinetic.

1.10. Kinetics of Maillard Reactions

A simplified kinetic-based reaction mechanism in foods can be represented as:

$$R + A \rightarrow [RA] + H_2O \rightarrow R^* + A \rightarrow \text{Brown pigment - B}$$

where R is the reducing sugar concentration, A the reactive amine group concentration, RA intermediates, R^* the reactive reducing compound intermediates, and B the brown pigment. The rate of brown pigment formation is related to the formation of R^* by

$$\frac{dB}{dt} = \frac{dR^*}{dt} = k_B * (R)^a * (A)^b \qquad (6)$$

Where k_B is the overall rate constant, which depends on a_w and is inversely proportional to phase viscosity, a, b are specific orders of each reactant usually considered to be equal to 1.

Labuza et al. [32] evaluated the rate constant for browning development from the Maillard reaction (assumed the zero order reaction rate) by using the following equation;

$$\int dB = \int k_B * dt \qquad (7)$$

Integrating both sides of this equation between the limits of B_0 at time zero (t_0) and B at time t gives

$$B - B_o = k_B t \qquad (8)$$

$$B = B_o + k_B t \qquad (9)$$

where B is the brown pigment color concentration developed, and B_o the initial brown pigment concentration. A plot of B vs. t should gives a straight line where slope is equal to k_B and y intercept equal to B_o.

Mizrahi et al. [61] studied at various a_w and temperatures to evaluate k as a function of temperature. This dependence is described by Arrhenius equation (5).

1.11. Aim of Present Study

Nonenzymic browning reactions are often responsible for important color, flavor and nutritional quality changes that occur during storage of foods and limit their shelf-life. The complexities of the color problem are not well understood. Therefore, Maillard browning reactions in foods continue to be an active area of research.

In general, browning reactions are deleterious to the nutritional value of the food concerned, and can occur during processing as well as during storage of food products. It is therefore imperative to arrest these reactions, thereby not only preventing any nutritional changes, but also other changes which might render the food unacceptable to the consumer [1].

5-HMF is often used as an index of deteriorative changes in foods and its accurate determinations in foods are therefore important.

5-HMF content and brown pigment determination in model systems, consisting of glucose, fructose, arginine, proline and glutamine, and Pekmez at two different pH (pH 3.5 and 6.0) were used to estimate the kinetic parameters. Pekmez contains these amino acids and sugars in the flora originally. Koch and Kleesaat [62] found that most nitrogenous compounds in boiled grape juice are mainly composed of amino acids, and there are 18 amino acids. Also they found that the quantity of arginine, proline and glutamine are highest in boiled grape juice. So we decided to study the model system containing the same amino acids and sugars with Pekmez. It was seen from our literature survey that there was no study of two or three amino acids together with sugars to determine the kinetic parameters of 5-HMF accumulation. Also there is no kinetic study with respect to the 5-HMF accumulation and brown pigment formation for Pekmez. Therefore the aims of the present work are,

1- to determine the effects of types of sugars and amino acids on the 5-HMF accumulation and brown pigment formation in the model systems, Pekmez mixtures and Pekmez containing model systems.

2- to examine the effects of the temperature and pH on the 5-HMF accumulation and brown pigment formation.

3- to compare the 5-HMF accumulation and brown pigment formation in Pekmez at pH of 3.5 and pH 6.0.

4- to compare model systems with Pekmez at three different temperatures and

two different pH values.

5- to study the kinetics of model systems, Pekmez mixtures and Pekmez containing model systems by using an accelerated test.

6- to compare the samples with respect to their Ea and rate constants for 5-HMF accumulation and brown pigment formation.

CHAPTER II
MATERIALS AND METHODS

2.1. Materials

2.1.1. Amino acids

The amino acids, L-arginine, L-proline, were purchased from Merck (E-Merck, Germany) and L-glutamine was purchased from (Sigma, USA).

2.1.2. Sugars

The reducing sugars, D-glucose and D-fructose were purchased from the Merck (E-Merck, Germany).

2.1.3. Reagents

Potassium ferrocyanide, zinc acetate, iodine were purchased from Reidel De-Haen (Reidel De-Haen, Germany) and p-toluidine, glacial acetic acid, sodium hydroxide, barbituric acid, phthalate anhydride, 5-hydroxymethly furfural (5-HMF) and isopropyl alcohol (2-propanol) were purchased from the Merck (Merck, Germany), and starch was purchased from Pancreac (Pancreac, Spain).

2.2. Preparation of the Solutions

All of the solutions were prepared by using triple distilled water.

1. Carrez I solution: 15 g potassium ferrocyanide was dissolved in 100 mL water.
2. Carrez II solution: 30 g zinc acetate was dissolved in 100 mL water.
3. Starch: 0.5 g starch was completely dissolved in 100 mL water.
4. 0.1 N Iodine solution: 12.7 g iodine was dissolved in 1L water.
5. Barbituric acid solution: 500 mg barbituric acid was dissolved in 100 mL water.
6. P-toluidine solution: 10 g p-toluidine was dissolved in 70 mL 2-propanol and 10 mL glacial acetic acid was added and made up to 100 mL with 2-propanol.
7. 0.1 M Sodium hydroxide solution: 4 g NaOH was dissolved in 1 L water.
8. 0.1 M phthalate buffer at pH 3.5: 20.42 g phthalate was dissolved in 1 L water and pH was adjusted by the addition of 0.1 M sodium hydroxide solution.
9. 0.1 M phthalate buffer at pH 6.0: 20.42 g phthalate was dissolved in 1 L water

and pH was adjusted by the addition of 0.1 M NaOH.

2.2.1. Standard 5-HMF solution preparation

5-HMF solution was prepared by dissolving 125 mg 5-HMF in 100 mL water.

2.3. Preparation of Model Systems

In order to compare the relative reactivities of amino acids on the formation of both 5-HMF and brown pigment, model systems resembling pekmez were prepared. The types and the concentrations of the amino acids and sugars used were decided by considering the composition of the Pekmez. In order to prepare model system 1 (S1); 9.30 g D-glucose and 8.43 g D-fructose were dissolved and diluted to exactly 30 mL by using 0.1 M phthalate buffer at pH 3.5. Concentrations of D-glucose and D-fructose in model system 1 were 2.150 and 1.950 mol/L respectively. The other model systems (S2-S8) having the compositions given in Table 2.1 were prepared by adding 2 % (w/v) of each amino acid (L-proline, L-arginine and L-glutamine) in the same way.

Table 2.1. Composition of the model systems.

Models Systems	Glucose (mol/L)	Fructose (mol/L)	Proline (mol/L)	Arginine (mol/L)	Glutamine (mol/L)
S1	2.150	1.950	----	----	----
S2	2.150	1.950	0.017	----	----
S3	2.150	1.950	----	0.012	----
S4	2.150	1.950	----	----	0.014
S5	2.150	1.950	0.017	0.012	----
S6	2.150	1.950	0.017	----	0.014
S7	2.150	1.950	----	0.012	0.014
S8	2.150	1.950	0.017	0.012	0.014
S9	2.150	-----	-----	0.012	-----
S10	-----	1.950	-----	0.012	-----
S11	2.150	-----	-----	-----	0.014
S12	-----	1.950	-----	-----	0.014

Model systems resembling the Pekmez (S1-S8) contain two sugars at the same time, thus the effect of each of these sugars on the Maillard reaction were not observed clearly. For that reason single amino acid-sugar model systems (S9-S12) were also prepared by using 0.1 M phthalate buffer at pH 3.5. In order to prepare model 9; 9.30 g D-glucose and 0.06 g L-arginine were dissolved and diluted to exactly 30 mL by using 0.1 M phthalate buffer at pH 3.5. The other single amino acid-sugar models (S10-S12) were prepared by same way. Compositions of the single amino acid-sugar model systems are also given in Table 2.1.

2.4. Preparation of Pekmez Samples

Pekmez used in this study were made by using of boiled grape juice with a pH of 5.0 and Brix 72 °. The boiled grape juice was purchased from the retail market in Gaziantep.

2.4.1. Preparation of Pekmez

Flow diagram given in Figure 2.1 was followed for making the Pekmez. pH of the Pekmez was adjusted to 3.5 by using 0.1 M HCl.

BOILED GRAPE JUICE (pH 5.0, 72 ° Brix)
↓
pH ADUSTMENT
↓
REBOILING at ROTARY EVAPORATOR (up to 72-73 °Brix)
↓
ADDITION OF SOLID PEKMEZ (to solidify the boiled grape juice)
↓
MIXING OF SOLUTION (at 600 rpm for 30 min.)
↓
INCUBATION (at 5 °C for 2 days)

Figure 2.1. Flow diagram for Pekmez preparation

Pekmez at pH 6.0 was made from the boiled grape juice by the same method (Figure 2.1). pH of boiled grape juice was adjusted to 6.0 by the addition of 0.1N NaOH.

2.5. Pekmez Mixtures

After the preparation of the Pekmez, Pekmez mixtures at pH 3.5 and pH 6.0 were prepared as in the Table 2.2. by using the 0.1 M phthalate buffers.

Table 2.2. Composition of the Pekmez mixtures.

Mixtures	Pekmez	0.1 M phthalate buffer
Pure Pekmez	30.00 mL	-------
Pekmez Mix.1	15.00 mL	15.00 mL
Pekmez Mix.2	5.25 mL	24.75 mL

2.6. Pekmez Containing Model Systems

In order to prepare pekmez containing model system 1; 9.30 g D-glucose and 8.43 g D-fructose were taken and dissolved in 24.75 mL of 0.1 M phthalate buffer and 5.25 mL Pekmez was added. The other Pekmez containing model systems 2-8 were prepared by the same way as taking the same concentrations of sugars and amino acids as they are in the model systems (S2-S8). Pekmez containing model systems (S1-S8) were prepared at both pH 3.5 and 6.0 by using of 0.1 M phthalate buffers.

2.7. Methods

2.7.1. Priciple of 5-HMF Determination

Spectrophotometric determination of 5-HMF is based on the reaction of 5-HMF with barbituric acid and p-toluidin forming a red-colored complex. The intensity of the red color is dependent upon the amount of 5-HMF. 5-HMF analysis

was performed by measuring the red color at 550 nm with Spectronic-Bausch 20 spectrophotometer.

2.7.1.1. Preparation of Standard Curve for 5-HMF Analysis

Various concentrations of 5-HMF (0-100 ppm) were prepared by diluting the standard 5-HMF solution with the triple distilled water. Pure $NaHCO_3$ was added into 25 mL of each solution to make it alkaline, and 4 mL of 0.5 % starch solution was added. Solutions were titrated with 0.1 N iodine solution up to blue colour was persisted 15-20 second. 1 mL Carrez I and 1 mL Carrez II solutions were added into titrated solutions. Solutions were diluted to exactly 50 mL by the addition of triple distilled water, then solutions were filtered. 5-HMF analysis was done against blank at 550 nm by using spectrophotometer. Compositions of blank and sample cells are given in Table 2.3. The absorbance values were measured after exactly 3 min at a wavelength of 550 nm. Samples with an absorbance value higher than 0.5 were diluted to get the proper absorbance values.

Table 2.3. Compositions of blank and sample cells for 5-HMF determination.

	Sample cell	Blank cell
Filtrate	2.0 mL	2.0 mL
p-Toludine solution	5.0 mL	5.0 mL
Barbituric acid	1.0 mL	-----
Distilled water	-----	1.0 mL

Standard curve for 5-HMF was drawn by plotting the obtained absorbance values of the standard 5-HMF solution against ppm 5-HMF. Slope of the standard curve was found as;

$A/C = 0.0175$

$C/A = 57.142$

Where A is the absorbance value and C is the concentration.

2.7.1.2. Measurement of 5-HMF in the Mixtures

4.0 g (± 0.1 g) sample was taken and diluted to exactly 25 mL with triple distilled water in a 50 mL volumetric flask. After that 5-HMF analysis was done as given above.

Concentration of 5-HMF in the samples were found from the given equation below:

mg 5-HMF/100g samples = 57.142 (ppm/OD)• A (OD)• cf (mg 5-HMF/ 100 g sample/ppm)

Where A is the absorbance value at 550 nm and cf is the conversion factor (0.625).

2.7.2. Brown Pigment Measurement

1.0 g (± 0.1 g) sample was taken and diluted to 10 mL with triple distilled water. Absorbance values were determined at 420 nm by using the same spectrophotometer. Analysis of the brown pigment formation was done at the same time with the analysis of the 5-HMF accumulation. The samples with an absorbance value higher than 0.5 were diluted.

2.8. Data Analysis

To find the reaction order and rate constant for 5-HMF accumulation in the mixtures nonlinear regression analysis (Marquerdt-Levenberg algorithm) was done by using the foolowing equation ;

$$y = [\ (1-n) \bullet k \bullet t + y_0\ ]^{\ 1\ /\ (1-n)}$$

where y is the concentration, k is the rate constant, n is the reaction order and y_0 is the concentration at time zero.

To find the reaction order and rate constant for brown pigment formation in the samples linear regression analysis was done.

To find the activation energies for both 5-HMF accumulation and brown pigment formation in the samples also linear regression analysis was applied.

CHAPTER III
RESULTS AND DISCUSSION

3.1. Accumulation of 5-HMF and Brown Pigment Formation

3.1.1. Model Systems

D-Glucose and D-fructose are sugars and L-arginine, L-glutamine and L-proline are the most abundant amino acids in the Pekmez [62]. For that reason the model systems that resemble the Pekmez (S1-S8) were prepared with these sugars and amino acids. pH values of the model systems were adjusted to 3.5 which is the optimum pH for the formation of the 5-HMF.

Figures 3.1-3.3 show accumulation of 5-HMF in the model systems at 55, 65 and 75 °C respectively. It is seen from these Figures that 5-HMF accumulation of the models shows a decreasing order of models 8, 7, 6, 5, 4, 3, 2 and 1. The Figures 3.1-3.3 show that 5-HMF accumulation of the model system with L-glutamine was higher than that of model systems with L-arginine and L-proline. The 5-HMF accumulation was found to be higher in the models 2-4 than in the model 1 which is composed of sugars with no amino acids. Thus only hexose degradation occurred in this model system. However, the other model systems having sugars together with amino acids result in the formation of the Maillard reactions through the interaction of amino acids with sugars. 5-HMF accumulation was accelerated by the presence of L-arginine, L-glutamine and L-proline in the model system 8. The highest accumulation of 5-HMF observed in this model system was 49, 133 and 800 mg 5-HMF/100 g sample at 55, 65 and 75 °C respectively.

Brown pigment formation in the model systems was shown in Figures 3.4-3.6. From these Figures order of the models with respect to their brown pigment formation is models 8> 7> 6> 5> 4> 3> 2> 1. Relative ratios of the brown pigment formation of the models by the end of the ten days are calculated and tabulated in Table 3.1 by taking the model system 1 as reference.

5-HMF and brown pigment concentrations in these systems may change due to several factors such as amino acid type, amino acid concentration.

1. Amino acid type

Figures 3.1-3.6 show that the order of the amino acids according to their brown pigment formation is L-glutamine > L-arginine > L-proline (model 4 > model 3 > model 2). Talley and Eppley [64] have found that amino acids such as proline with hydrophobic side chains reacted more slowly than the other amino acids. Addition of two amino acid into model system order of their both 5-HMF accumulation and brown pigment formation were in model systems 7 > 6 > 5. In the presence of L-proline in the model systems 5 and 6, increased brown pigment formation and 5-HMF accumulation but they were not as much as in model system 7. Because, the rate of the sugar consumption of L-proline is higher than that of L-arginine or L-glutamine. This high amount of sugar utilization by L-proline in the first stage of the Maillard reaction may be reduced the available sugar concentration for the other amino acids [11]. In the later stages L-proline reacts more slowly do the rate of reactions in the systems with L-proline is slow.

2. Concentration of Amino Acids

5-HMF accumulation and brown pigment formation were the lowest in model system 1 after ten days at all temperatures, because only hexose degradation occurred in this model. Accumulation of 5-HMF and brown pigment formation increased with time in the model systems having amino acids. 5-HMF accumulation of the model system 2 having 0.017 M amino acid, after ten days, was approximately 1.0, 1.1 and 1.2 times higher than that of model 1 at 55, 65 and 75 °C respectively. The ratio of the brown pigment formation of the model 2 to model 1 was 2.3, 1.1 and 1.8 for the given temperatures. The highest brown pigment formation was observed for the model 8. When the model 8 was compared with model 1 the relative ratios found for brown pigment formation were 8.6, 3.9 and 7.0 and for 5-HMF accumulation were 2.6, 2.5 and 2.5 at 55, 65 and 75 °C respectively.

In this study, various types of amino acids were used in the models. It was observed that increasing in the concentration of amino acids is not a direct parameter in increase of the intermediate and brown pigment formation. As it seen from Table 3.1 the model systems with L-glutamine and L-arginine results in increase

of both 5-HMF and brown pigment formation more than that with the L-proline. However; the addition of L-proline to either of L-glutamine and L-arginine was increased both 5-HMF and brown pigment formation but not as an expected amount. This shows that even if the amount of total amino acids increases, this does not necessarily result in the increase in the product concentration. The reason for that is the different reactivities of amino acids with sugars.

Table 3.1 Relative ratios for both 5-HMF accumulation and brown pigment formation in model systems.

Model Systems	Relative ratio for 5-HMF accumulation			Relative ratio for brown pigment formation		
	55 °C	65 °C	75 °C	55 °C	65 °C	75 °C
S1*	1.00	1.00	1.00	1.00	1.00	1.00
S2	1.03	1.13	1.27	2.25	1.11	1.76
S3	1.15	1.65	1.29	2.75	2.63	2.26
S4	1.27	1.79	1.33	4.00	2.15	2.84
S5	1.38	1.92	1.61	6.80	3.11	3.99
S6	1.43	2.15	2.24	5.00	2.35	3.65
S7	1.49	2.37	2.42	6.00	2.43	4.26
S8	1.76	2.48	2.69	8.60	3.86	7.03

* Selected as a reference model.

Single amino acid sugar model systems were prepared to observe the effects of sugars, D-glucose or D-fructose on L-arginine or L-glutamine on the formation of brown pigment and accumulation of 5-HMF. The concentrations of the components were as in the model systems resembling the Pekmez (S1-S8). Both brown pigment formation and 5-HMF accumulation of L-arginine or L-glutamine was higher than that of L-proline in model systems (S1-S8). Thus these amino acids were chosen to study in single amino acid systems. As it is seen in Table 2.2, model systems (S1-S8) had both D-fructose and D-glucose at the same time and it was difficult to get any idea about the effects of each sugar on the rate of the reaction. For this reason; it was decided to use single sugar systems to observe the effect of each sugar on the rate of reaction.

Accumulation of 5-HMF in single amino acid sugar model systems at pH 3.5 were plotted in Figures 3.7-3.9 at 55, 65 and 75 °C respectively. 5-HMF accumulation in the model systems having L-glutamine-D-fructose was higher than model systems

having L-arginine-D-fructose. In the model systems with D-fructose-L-glutamine, accumulation of 5-HMF was higher about 4.3 times than that in model systems with D-glucose-L-glutamine at all temperatures. The accumulation of 5-HMF in the model system having D-fructose-L-arginine was 3.6, 2.1 and 3.0 times higher than that having D-glucose-L-arginine at 55, 65 and 75 °C respectively.

Brown pigment formation in the single amino acid sugar model systems were plotted in Figures 3.10-3.12 at 55, 65 and 75 °C respectively. The decreasing order of model systems in their brown pigment formation is L-glutamine-D-fructose, L-arginine-D-fructose, L-arginine-D-glucose and L-glutamine-D-glucose.

Brown pigment formation in D-fructose-L-glutamine model system was 10.0, 6.1 and 4.0 times higher than that in D-glucose-L-glutamine model system at 55, 65 and 75 °C respectively. Brown pigment formation in the model system with D-fructose-L-arginine was 2.0, 8.0 and 3.4 times higher than that in the model system with D-glucose-L-arginine at the same temperatures. On the other hand, it was 1.1, 1.2 and 1.2 times higher in the model system with D-fructose-L-glutamine than that with L-arginine-D-fructose.

These results indicate that D-fructose are more reactive than D-glucose with respect to both 5-HMF accumulation and brown pigment formation. Also both 5-HMF accumulation and brown pigment formation depend on the type of sugars rather than the types of amino acids.

When they are considered as the model systems; the most reactive one was D-fructose-L-glutamine model system and it can be explained by following two reasons:

1. Type of sugar

D-fructose was found to be more reactive than D-glucose in the acidic medium (at pH 3.5). This could be because of higher ring opening rate of D-fructose than the D-glucose in the acidic medium [33]. Lee and Nagy [63] and Luh et al [45] have found similar results as D-fructose is more reactive than D-glucose at pH 3.5.

2. Type of amino acids

L-Arginine is a low brown pigments producer and L-glutamine is an intermediate brown pigments producer amino acid at pH 10.0 [28]. L-arginine contains a guanidine group at the 5-position which is less effective in the generating brown pigment [11]. Therefore 5-HMF accumulation and the brown pigment formation in L-glutamine-D-fructose model system were higher than those in L-arginine-D-fructose containing model system.

3.1.2. Pekmez Mixtures

Pekmez mixtures were prepared to observe the effects of Pekmez concentration on the reaction rate and also to make comparison between the Pekmez and Pekmez mixtures.

pH of the Pekmez mixtures was adjusted to 3.5 which is the optimum pH value for the 5-HMF accumulation. Accumulation of 5-HMF in Pekmez mixtures at pH 3.5 is given in Figures 3.13-3.15. These Figures indicate that the decreasing order of the mixtures in their 5-HMF accumulation is; pure Pekmez, Pekmez mixture 1 and Pekmez mixture 2. As it is seen in these Figures final concentrations of the 5-HMF in the Pekmez mixtures are too high when they are compared with the standard values (first quality Pekmez can be coantined maximum 25 ppm of 5-HMF [50]). Brown pigment formation in Pekmez mixtures at pH 3.5 is shown in Figures 3.16-3.18. It was observed from these Figures that decreasing the concentration of Pekmez decreases the formation of brown pigment. When the brown pigment formation of Pekmez and Pekmez mixtures were compared, it was found that the relative ratios of pure Pekmez to the Pekmez mixtures 1 and 2 were 2.0, and 4.9 at 55 °C respectively. These ratios increased with increasing temperature. These results indicate that decreasing the concentration of the Pekmez decreases the brown pigment formation.

The experiments arranged for pH 3.5 were repeated also for pH 6.0. The reason of selecting pH 6.0 was to observe the reaction rate at Pekmez's original pH value. Accumulation of 5-HMF in Pekmez mixtures at pH 6.0 is given in Figures 3.19-3.21. It was found that 5-HMF accumulations after ten days were 20.3, 13.3

and 5.2 mg 5-HMF/ 100 g pekmez in pure Pekmez, Pekmez mixture 1 and Pekmez mixture 2 at 55 °C respectively. Consequently, a decrease in the concentration of the Pekmez leads to decrease the accumulation of 5-HMF. Formation of brown pigment in Pekmez mixtures at pH 6.0 is given in the Figures 3.22-3.24. From these figures decreasing order of the mixtures in their brown pigment formation was pure Pekmez, Pekmez mixture 1 and Pekmez mixture 2, because in the pure Pekmez the concentration of the amino acids and sugars were higher than those in Pekmez mixtures. The relative ratios of the brown pigment formation in the pure Pekmez to Pekmez mixtures 1 and 2 were 2.4 and 14.0 at 75 °C respectively.

The 5-HMF accumulation in the pure Pekmez is very high when it is compared with the standard value of first quality Pekmez that contains maximum 25 ppm 5-HMF. There could be some reasons for this; available reactive intermediates which are produced during the processing of the Pekmez cause to proceed the reaction to further products. Reactant concentration which is a factor of reaction rate is much more higher in Pekmez compared to the boiled grape juice.

When the effect of pH was considered, it was found that the 5-HMF accumulation at pH 3.5 was 2.5 times higher than that at pH 6.0 at 75 °C. This could be because 5-HMF accumulation was favoured in the acidic condition. In contrast to the 5-HMF accumulation the brown pigment formation in the mixtures at pH 6.0 was higher than that at pH 3.5. So, it can be concluded that increasing the pH increases the formation of brown pigments whereas accumulation of 5-HMF is reduced. Ashoor and Zent [28] found that the brown pigment formation increased with increasing the pH. These results indicate that changing the pH changes the routes of the Maillard reaction after the AMPs. Increasing the pH greater than 5 favoured the formation of reductones, dehydroreductones, aldehydes, etc. Decreasing the pH lower than 5 reaction was favoured the formation of 5-HMF. This results indicates that pH is inversely proportional reaction rates for 5-HMF accumulation and brown pigment formation.

3.1.3. Pekmez Containing Model Systems

Accumulation of 5-HMF in Pekmez containing model systems at pH 3.5 is

plotted in Figures 3.25-3.27. From these Figures order of systems with respect to their 5-HMF accumulation was models; 8> 7> 6> 5> 4> 3> 2> 1. When the Pekmez containing model system 8 is compared to systems 1 with respect to their 5-HMF accumulation, it was found that 5-HMF accumulation in system 8 was approximately 2.1 times higher at 75 °C. The formation of the brown pigment is plotted in Figures 3.28-3.30 for the Pekmez containing model systems. These figures indicate that the decreasing order of the systems according to their brown pigment formation was systems 8, 7, 6, 5, 4, 2, 3 and 1. It was calculated that the relative ratio of brown pigment formation of system 8 to system 1 is about 2.2 at 75 °C.

The accumulation of the 5-HMF in Pekmez containing model systems at pH 6.0 is shown in the Figures 3.31-3.33. Figures 3.34-3.36 show the formation of the brown pigment at the given temperatures. As a result of these Figures, decreasing order of systems according to their brown pigment formation was systems 8, 7, 6, 5, 4, 3, 2 and 1. The brown pigment formation in system 8 was approximately 1.8 times higher than that in the system 1 at 75 °C.

It was found that Pekmez containing model systems had higher 5-HMF accumulation and brown pigment formation than the model systems resembling the Pekmez after ten days. This could be because:

a- Pekmez containing model systems have high concentrations of sugars and amino acids.

b- 5-HMF and some reactive intermediates are formed during the production of Pekmez.

c- Pekmez has high concentration of iron which increases the rate of reaction.

When the Pekmez containing model system 8 at pH 3.5 is compared to Pekmez containing model system 8 at pH 6.0 with respect to their 5-HMF accumulation, it was found that the 5-HMF accumulation in Pekmez containing model system 8 at pH 3.5 was about 2.5 times higher. This ratio in the Pekmez containing model system 1 was about 7.5. On the other hand the brown pigment formation in Pekmez containing model systems at pH 6.0 was higher than that at pH 3.5. As a conclusion, 5-HMF accumulation decreased with increasing the pH where the brown pigment formation increased with increasing the pH.

3.2. Reaction Kinetics of 5-HMF Accumulation and Brown Pigment Formation

Reaction orders for the brown pigment formation and 5-HMF accumulation were obtained by applying both linear (LR) and non-linear regression analyses (NLR) between the first and zero order rate based on the work by Labuza and Saltmarch [32]. Reaction orders were determined from the lowest residual sum of squares, RSS, of NLR. As shown in Figure 3.37 and 3.39, the minimum RSS value of rate constant for 5-HMF accumulation is obtained at 0.5 reaction order. In a similar way, the reaction order for the brown pigment formation in the model systems was found as zero (in Figure 3.38 and 3.40). That is why the rate constant calculations for 5-HMF accumulation were based on the reaction order of 0.5 and for brown pigment formation were based on the reaction order of zero for all samples. Rate constants for 5-HMF accumulation were determined from NLR. Rate constants for brown pigment formation were determined from the LR results giving the R^2 which is closest to 1.

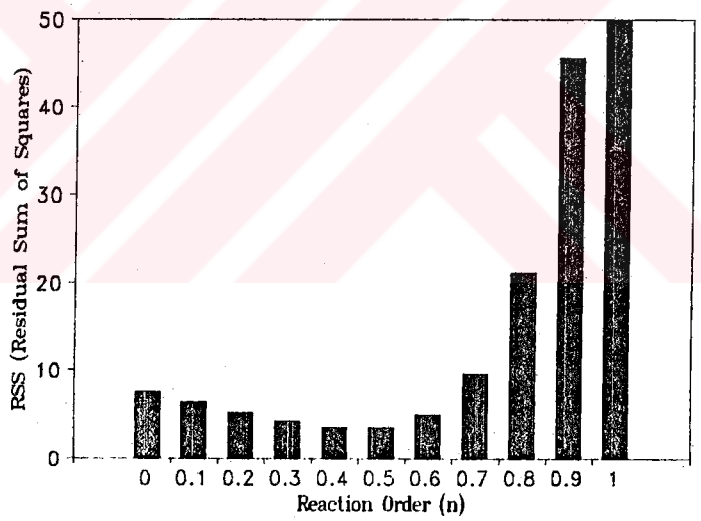


Figure 3.39. Change of RSS with respect to the change in the reaction order for 5-HMF accumulation.

Activation energies of the mixtures were determined from the LR analysis which based on the Arrhenius equation;

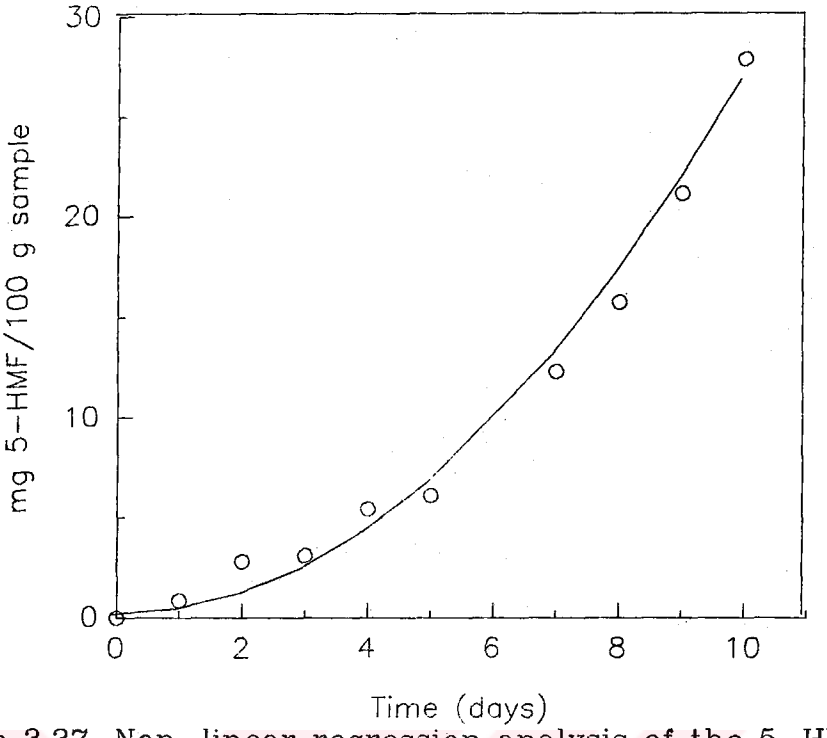


Figure 3.37. Non-linear regression analysis of the 5-HMF accumulation in the Pekmez containing model system 2 as a function of time at pH 3.5 at 55 °C.

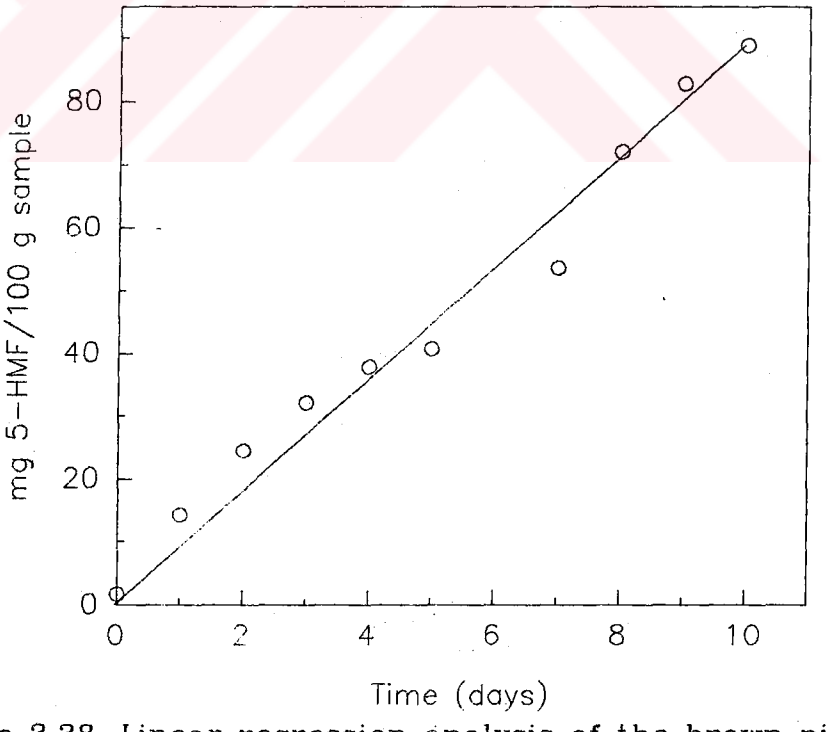


Figure 3.38. Linear regression analysis of the brown pigment formation in the Pekmez containing model system 2 as a function of time at pH 3.5 at 55 °C.

$$k = k_0 * \exp(-Ea/RT)$$

Where k is the rate constant, k_0 is the Arrhenius constant, Ea is the activation energy, R is the gas constant (8.314 kj / mole) and T is the temperature (K). From the slope (-Ea /R) of the plot ln k versus 1/T, the activation energies of the mixtures were determined.

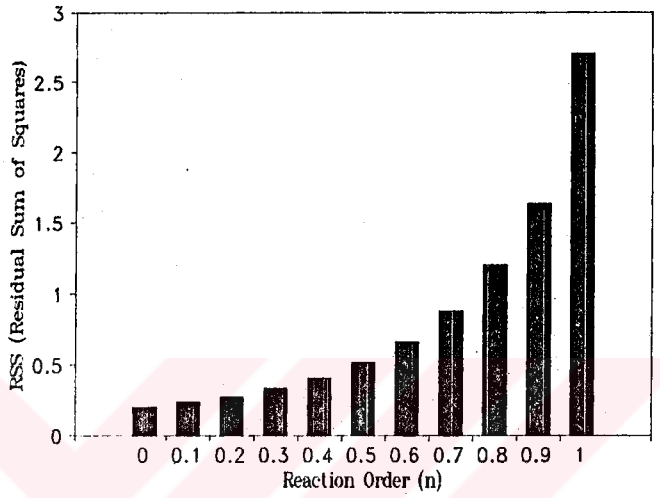


Figure 3.40. Change of RSS with respect to the change in the reaction order for brown pigment formation.

3.2.1. Model Systems

The rate constants (k) and activation energies (Ea) for the 5-HMF accumulation in the model systems were calculated by taking the reaction order as 0.5. The results are given in Table 3.2. The rate constants of model 1, containing only reducing sugars, are the lowest of all. The rate constant increased with the addition of amino acids into model systems. It was found that the rate constants of model 4 containing L-glutamine are higher than those of models 2 and 3 containing L-proline and L-arginine respectively. When the models having two amino acids (5, 6 and 7) are compared, it was found that the rate constants of models 5 and 6 were lower than that of model 7. Reason of this could be due to the

presence of L-proline which reacts much slower than the other amino acids and some interactions are possible between the amino acids, a much less absorbing chromophore is being produced [64].

When the effect of temperature on the rate constant of model systems were considered, it was found that temperature had an accelerating effect on the reaction. The rate constant of model 8 was 1.44 at 55 °C. When the temperature was incraesed to 65 °C it became 2.27. Ratio of rate constants of this model at 75 °C to 55 °C is 3.69. Rate constants for brown pigment formation in model systems are tabulated in Table 3.2. This table indicates that the rate constant for brown pigment formation of the model 1 is the lowest, because in model 1 only sugars are present. Addition of amino acids increased the rate constants. The rate constants of model 4, containing L-glutamine, is higher than that of the model systems 2 and 3, containing L-proline and L-arginine respectively. As it was found for the rate constants of 5-HMF accumulation, rate constants for brown pigment formation increased with increasing temperature. The activation energies for the brown pigment formation are higher than those for 5-HMF accumulation.

Table 3.2. Rate constants and activation energies for 5-HMF accumulation and brown pigment formation in the model systems.

Models Systems	5-HMF Accumulation				Brown Pigment Formation			
	k (day^{-1})			Ea (kj/mol)	k (day^{-1})			Ea (kj/mol)
	55°C	65°C	75°C		55°C	65°C	75°C	
S1	0.95	1.46	3.46	61.17	0.02	0.80	1.66	211.18
S2	1.17	1.55	3.74	54.89	0.05	0.85	2.88	193.21
S3	1.12	1.87	3.76	57.24	0.05	0.25	4.10	208.66
S4	1.18	1.93	3.77	54.99	0.06	1.66	5.04	211.40
S5	1.24	2.18	4.01	55.65	0.08	0.27	6.90	211.28
S6	1.29	2.01	3.92	52.68	0.09	0.30	6.20	201.17
S7	1.37	2.23	5.12	62.43	0.09	1.80	7.52	210.84
S8	1.44	2.27	5.32	61.86	0.13	3.07	12.36	217.08

Rate constants, relative ratios of the rate constants and activation energies of 5-HMF accumulation in the single amino acid-sugar model systems are tabulated in Table 3.3. The relative ratios of the rate constants in Table 3.3 were calculated by selecting the rate constant of models at 55 °C as a reference. The rate constants of

model systems containing D-fructose are higher than those of the model systems containing D-glucose. It was found that D-fructose was approximately twice more reactive than D-glucose. Lee and Nagy [63] have also found that D-fructose was more reactive than D-glucose. The temperature effect on the rate constant can be seen from the Table 3.3 clearly. The increasing temperature increases the rate constant.

The rate constants, relative ratios of the rate constants and activation energies of the brown pigment formation in the single amino acid-sugar model systems are tabulated in Table 3.4. It is seen that the rate constant of D-fructose model systems is higher about 4.5 times than the D-glucose model systems. This result indicates that D-fructose is more reactive for producing the brown pigments.

Table 3.3. Rate constants, relative ratios of the rate constants and activation energies for 5-HMF accumulation in the single amino acid-sugar model systems.

Models	k (day^{-1})			Ratio			Ea (kj/mol)
	55 °C	65 °C	75 °C	55 °C	65 °C	75 °C	
S9	0.48	1.16	3.99	1.00	2.42	8.31	100.4
S10	0.82	1.80	6.92	1.00	2.20	8.44	101.0
S11	0.43	0.78	3.08	1.00	1.82	7.16	93.1
S12	0.89	2.23	8.06	1.00	2.51	9.06	104.4

The rate constant of D-fructose-L-glutamine model systems is higher than that of the D-fructose-L-arginine model systems at given temperatures. Table 3.4 indicates that the activation energies of brown pigment formation in models having L-glutamine are higher than that in models having L-arginine. This could be because of the high reactivity of L-glutamine than L-arginine to form brown pigment under these conditions.

Table 3.4. Rate constants, relative ratios of the rate constants and activation energies for brown pigment formation in the single amino acid-sugar model systems.

Models	k (day^{-1})			Ratio			Ea (kj/mol)
	55 °C	65 °C	75 °C	55 °C	65 °C	75 °C	
S9	0.03	0.09	0.58	1.00	3.00	19.33	71.40
S10	0.07	0.15	1.96	1.00	2.14	27.22	78.42
S11	0.01	0.03	0.53	1.00	3.00	75.75	100.66
S12	0.08	0.19	2.37	1.00	2.38	29.63	79.80

3.2.2. Pekmez Mixtures

The rate constants and activaton energies for 5-HMF accumulation and brown pigment formation in the Pekmez mixtures at pH 3.5 are tabulated in Table 3.5. This Table indicates that order of mixtures concerning the rate constant for both 5-HMF accumulation and brown pigment formation was pure Pekmez > Pekmez mix1> Pekmez mix 2. This could be because of having high concentrations of sugars, amino acids and iron in the pure Pekmez. It can be also observed that temperature had an accelerating effect on the rate constants.

Rate constants and activation energies for 5-HMF accumulation and brown pigment formation in Pekmez mixtures at pH 6.0 are tabulated in the Table 3.6. Same order with that of the results at pH 3.5 was found for both measurements. When the rate constants at pH 3.5 are compared to those at pH 6.0 for the 5-HMF accumulation, Pekmez mixtures at pH 3.5 had higher rate constants than the Pekmez mixtures at pH 6.0. Activation energies of the Pekmez mixtures at pH 3.5 were higher than those of Pekmez mixtures at pH 6.0. This could be because 5-HMF accumulation is favored under acidic condition. It was found that rate constants for

Table 3.5. Rate constants and activation energies for 5-HMF accumulation and brown pigment formation in the Pekmez mixtures at pH 3.5.

Mixtures	5-HMF Accumulation				Brown Pigment Formation			
	k (day^{-1})			Ea (kj/mol)	k (day^{-1})			Ea (kj/mol)
	55 °C	65 °C	75 °C		55 °C	65 °C	75 °C	
Pure Pekmez	2.31	4.57	7.89	58.42	0.39	2.50	10.17	78.06
Pek mix. 1	1.73	3.00	6.54	162.91	0.47	1.15	3.76	49.67
Pek mix. 2	0.98	1.67	3.90	265.40	0.22	0.39	1.25	41.46

Table 3.6. Rate constants and activation energies for 5-HMF accumulation and brown pigment formation in the Pekmez mixtures at pH 6.0.

Mixtures	5-HMF Accumulation				Brown Pigment Formation			
	k (day^{-1})			Ea(kj/mol)	k (day^{-1})			Ea(kj/mol)
	55 °C	65 °C	75 °C		55 °C	65 °C	75 °C	
pure Pekmez	0.65	1.52	4.29	89.83	0.65	1.38	12.77	70.64
Pek mix. 1	0.46	0.62	3.79	99.43	0.74	1.07	5.26	46.58
Pek mix. 2	0.32	0.51	1.89	84.93	0.24	0.39	1.63	45.10

5-HMF accumulation in mixtures at pH 6.0 had about 1.2 times higher than those at

pH 3.5. Reason of this could be because brown pigment formation is favored under alkaline condition.

3.2.3. Pekmez Containing Model Systems

Rate constants of the Pekmez containing model systems at pH 3.5 for 5-HMF accumulation and brown pigment formation are tabulated in Table 3.7. System 1 (PEK+S1), contained Pekmez with only sugars, had the lowest rate constants. The rate constants increased with the addition of the amino acid(s) into systems.

As it is found in model systems L-Proline containing systems, 5 and 6, had low rate constant for 5-HMF accumulation than L-arginine and L-glutamine containing systems 7. The possible for this reasons: L-proline reacts with sugars more slowly than the other amino acids and some interactions between amino acids are possible [64]. 5-HMF accumulation and brown pigment formation rates were accelerated by using reactive amino acids, L-arginine and L-glutamine, together (Pek+S7). It can be seen from the same table that the models with three different amino acids have the highest rate constants for both 5-HMF accumulation and brown pigment formation.

Table 3.7. Rate constants and activation energies for 5-HMF accumulation and brown pigment formation in the Pekmez containing model systems at pH 3.5.

Systems	5-HMF Accumulation				Brown Pigment Formation			
	k (day^{-1})			Ea (kj/mol)	k (day^{-1})			Ea(kj/mol)
	55°C	65°C	75°C		55°C	65°C	75°C	
Control	0.98	1.67	3.90	265.40	0.22	0.39	1.25	41.46
PEK+S1	1.22	2.94	5.74	73.58	0.10	0.43	4.02	175.01
PEK+S2	1.20	3.17	6.38	79.46	0.10	0.47	5.56	190.34
PEK+S3	1.08	3.04	6.89	88.08	0.12	0.83	4.91	176.28
PEK+S4	1.08	3.18	6.99	88.82	0.11	0.82	4.82	177.86
PEK+S5	1.28	3.15	7.12	81.54	0.15	0.85	6.63	180.70
PEK+S6	1.26	3.36	7.71	86.08	0.15	1.05	7.27	184.25
PEK+S7	1.32	3.37	7.98	85.46	0.17	1.46	7.14	177.72
PEK+S8	1.40	3.46	8.30	84.51	0.22	1.52	8.02	170.85

* Control mixture is Pekmez mixture 2 at pH 3.5.

Rate constants of the Pekmez containing model systems at pH 6.0 for 5-HMF accumulation and brown pigment formation are given in Table 3.8. Rate constants of system 1 were the lowest and the rate constant of L-glutamine and L-arginine

containing systems, 3 and 4, were higher than those of L-proline containing system 2. System 7 that contained L-arginine and L-glutamine had higher rate constant values than the other two amino acids added systems 5 and 6. The highest rate constant was observed in the model 8.

When rate constants of Pekmez model mixtures at pH 3.5 and 6.0 are compared with respect to their 5-HMF accumulation and brown pigment formation, it was calculated that the rate constants for 5-HMF accumulation of mixtures at pH 3.5 are about 1.90 times higher than those of mixtures at pH 6.0. However, rate constants for brown pigment formation of mixtures at pH 6.0 were 1.75 times higher than those in the mixtures at pH 3.5. Such a result allows for a conclusion that pH is an effective parameter for rate constant of the 5-HMF accumulation and brown pigment formation.

Table 3.8. Rate constants and activation energies for 5-HMF accumulation and brown pigment formation in the Pekmez containing model systems at pH 6.0.

Systems	5-HMF Accumulation				Brown Pigment Formation			
	k (day^{-1})			Ea (kj/mol)	k (day^{-1})			Ea (kj/mol)
	55°C	65°C	75°C		55°C	65°C	75°C	
Control	0.32	0.51	1.89	84.93	0.24	0.39	1.63	45.10
PEK+S1	0.38	1.05	2.85	95.67	0.15	0.64	4.69	163.15
PEK+S2	0.40	1.22	3.73	106.00	0.17	0.91	4.94	159.95
PEK+S3	0.49	1.44	4.32	103.33	0.17	0.91	4.78	158.41
PEK+S4	0.42	1.55	4.17	109.13	0.28	1.12	5.77	143.53
PEK+S5	0.48	1.53	3.91	99.69	0.26	1.24	6.93	155.79
PEK+S6	0.55	2.00	4.55	100.54	0.27	1.67	6.53	151.47
PEK+S7	0.59	2.10	4.82	99.93	0.29	1.90	7.88	157.01
PEK+S8	0.67	2.22	5.05	96.08	0.49	2.04	9.15	138.93

* Control mixture is Pekmez mixture 2.

When the rate constants for both 5-HMF accumulation and brown pigment formation in the Pekmez containing model systems are compared to the model systems, it was found that the rate constants for both 5-HMF accumulation and brown pigment formation in the Pekmez containing model systems were higher than those in model systems. Reasons of this could be having high concentrations of sugars and amino acids in the Pekmez containing model systems, and also high concentrations of iron and 5-HMF in Pekmez containing model systems which accelerate the reaction [64].

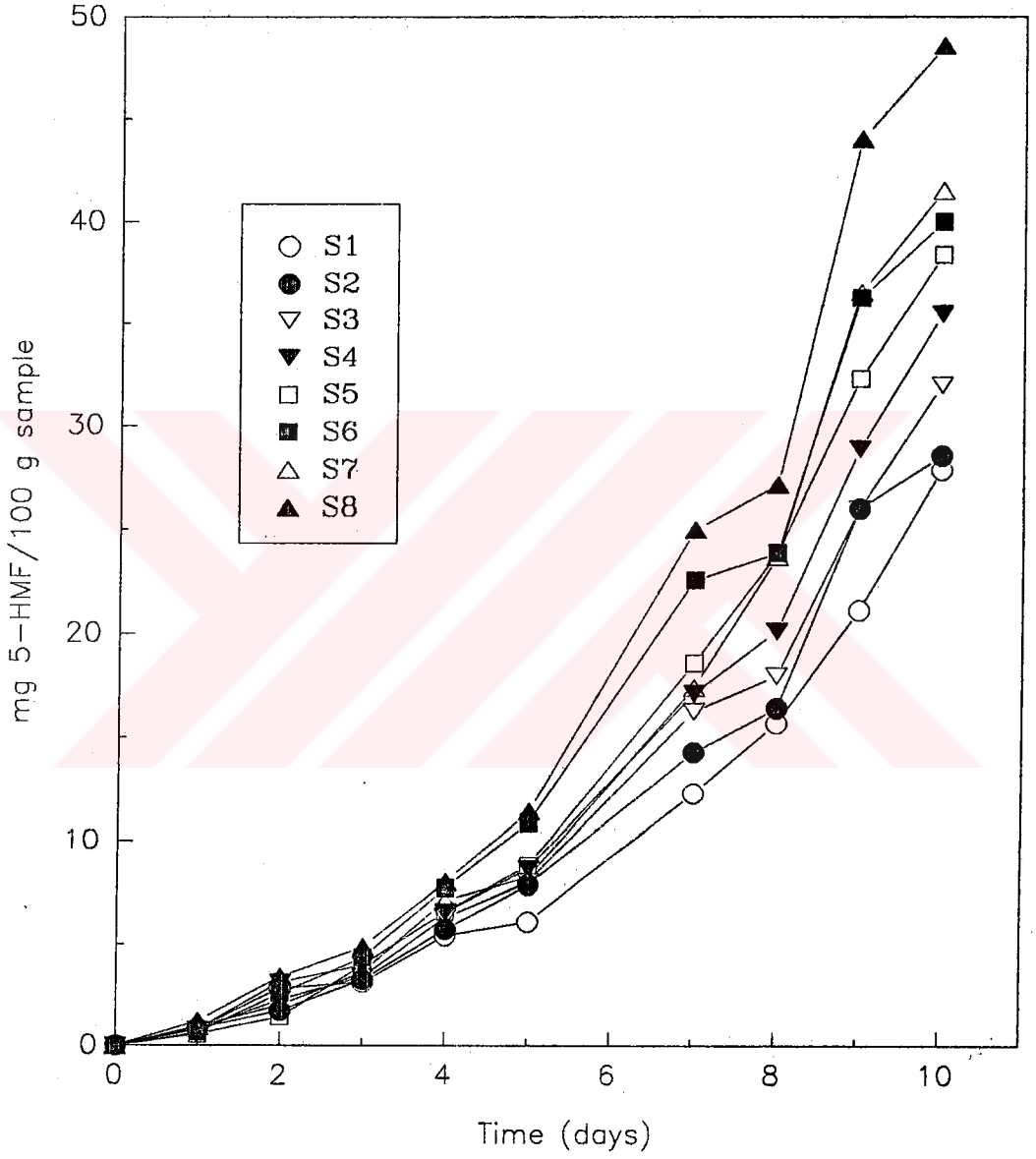


Figure 3.1. Accumulation of 5-HMF in model systems at 55°C.

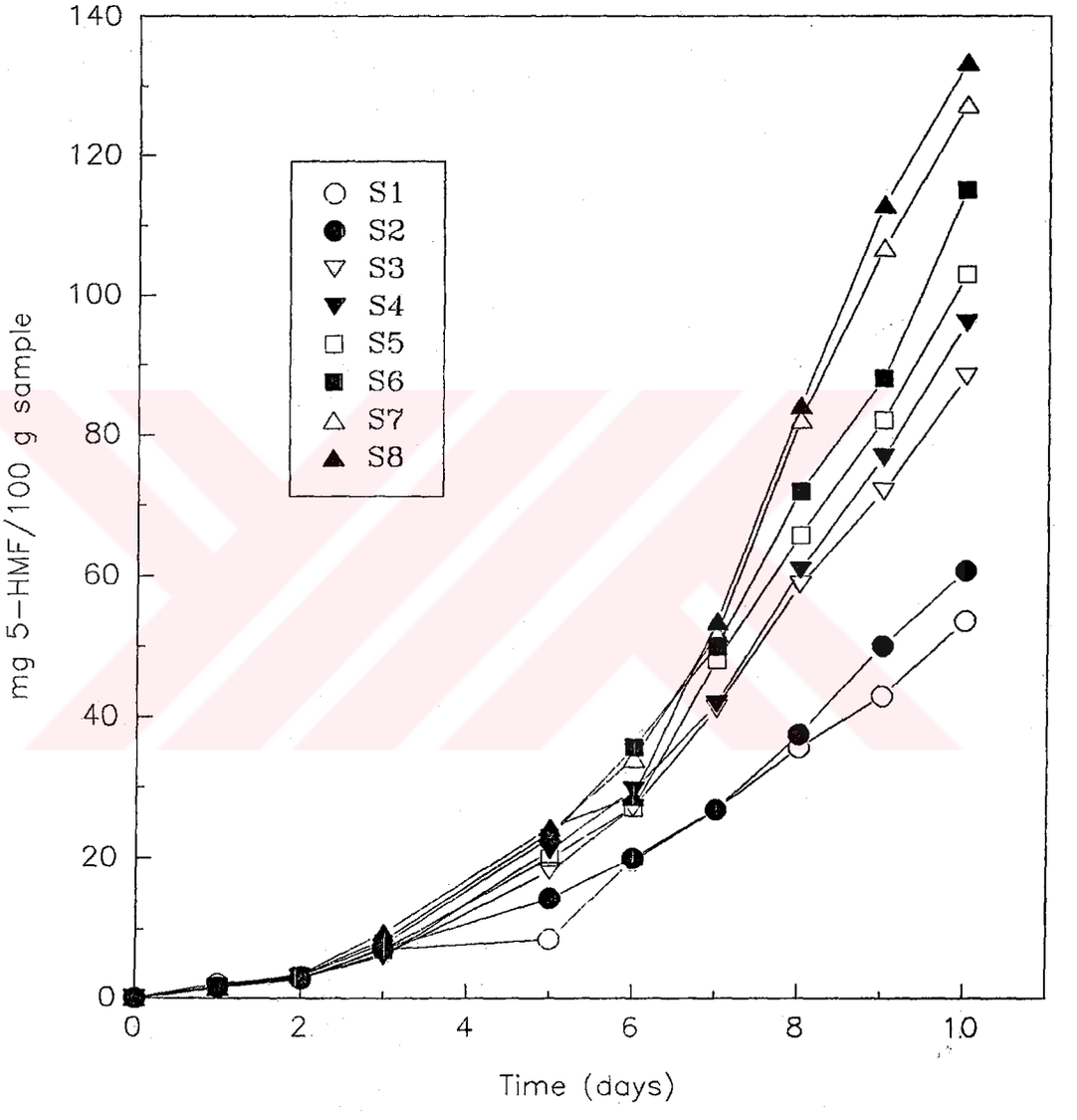


Figure 3.2. Accumulation of 5-HMF in model systems at 65°C.

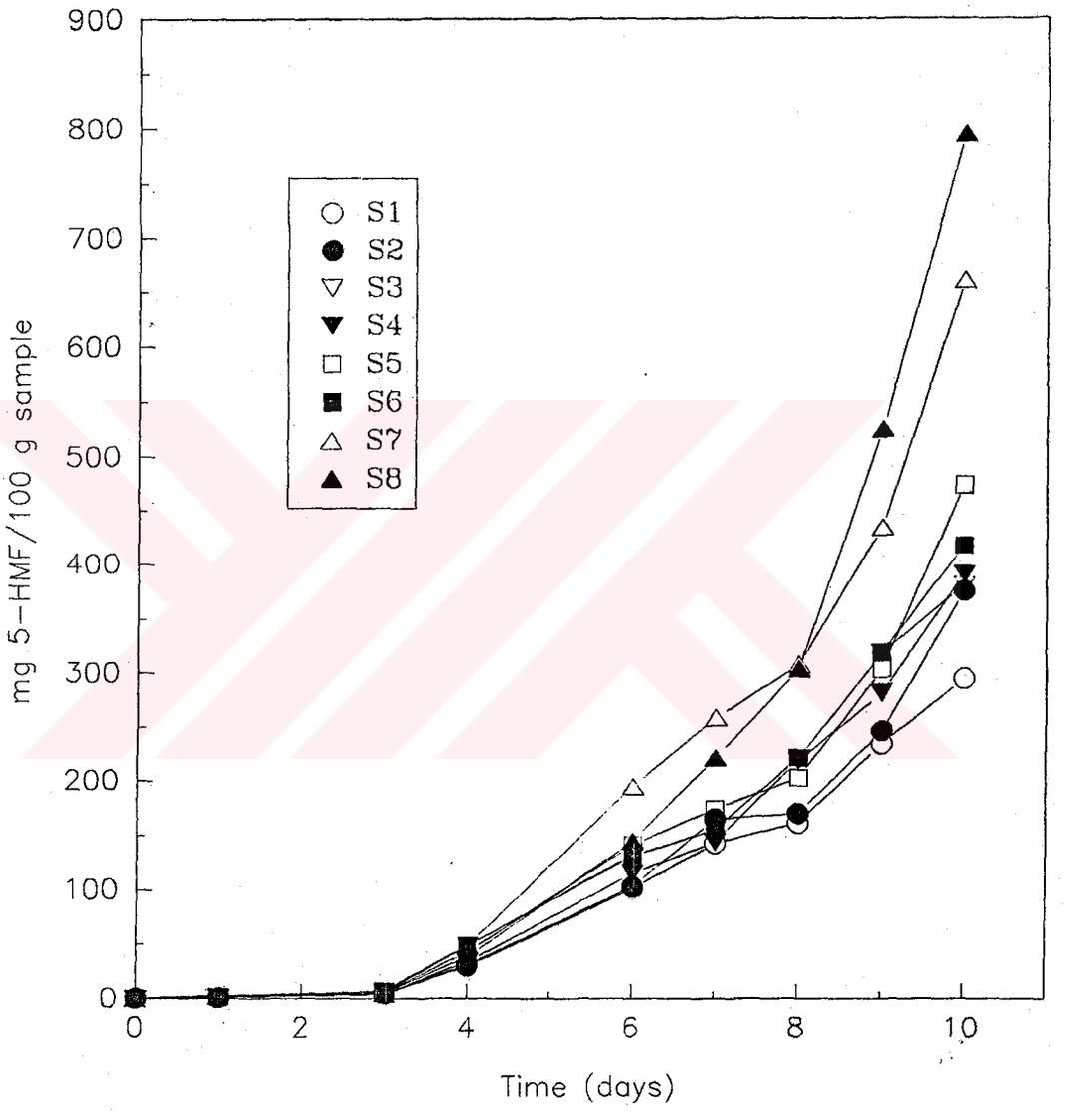


Figure 3.3. Accumulation of 5-HMF in model systems at 75°C.

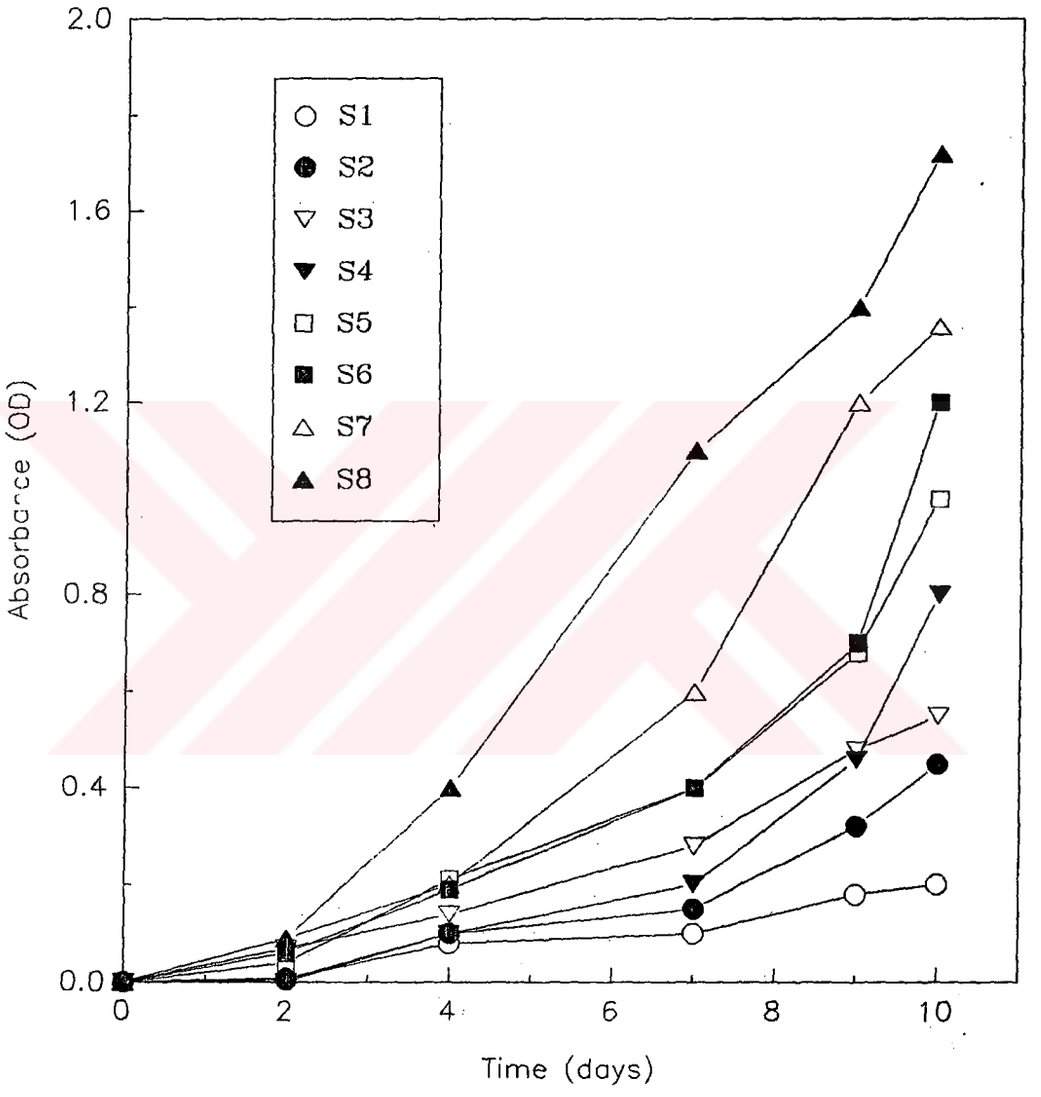


Figure 3.4. Brown pigment formation in model systems at 55°C.

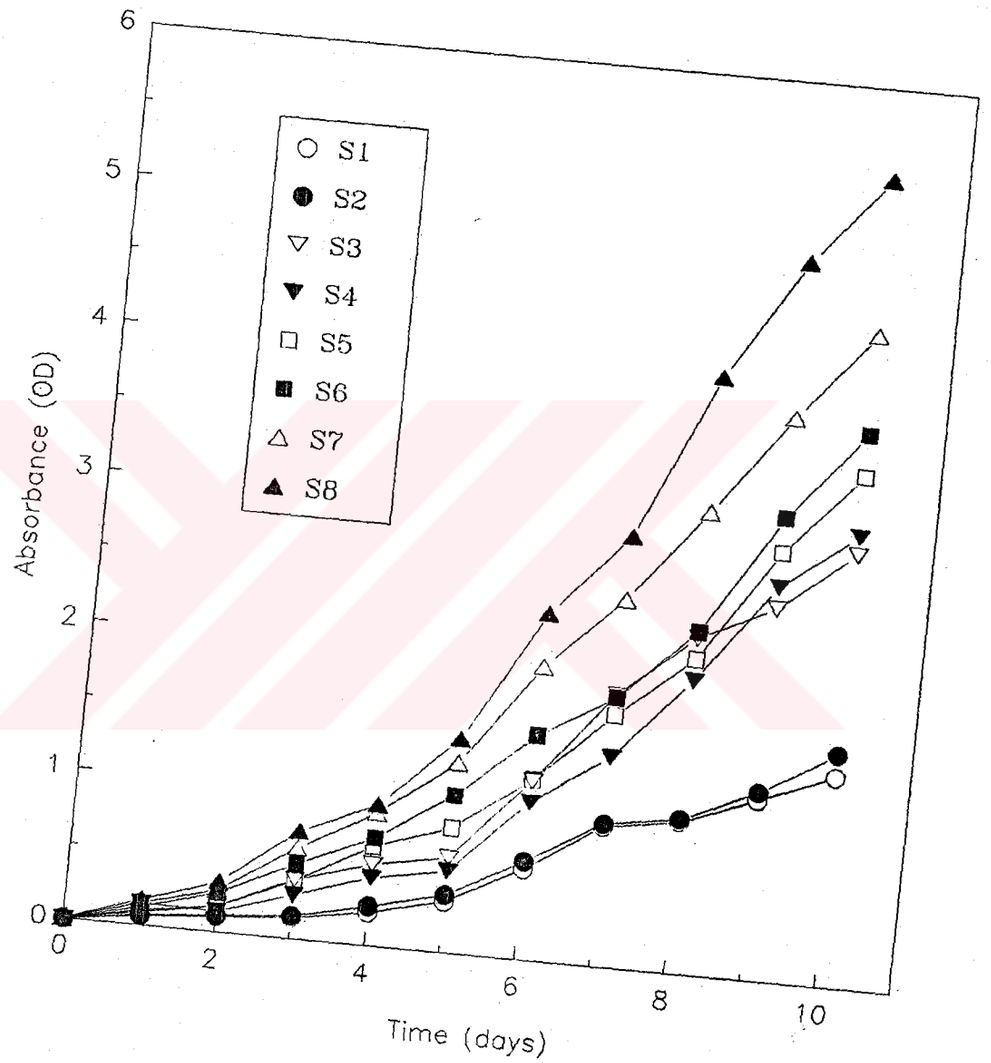


Figure 3.5. Brown pigment formation in model systems at 65°C.

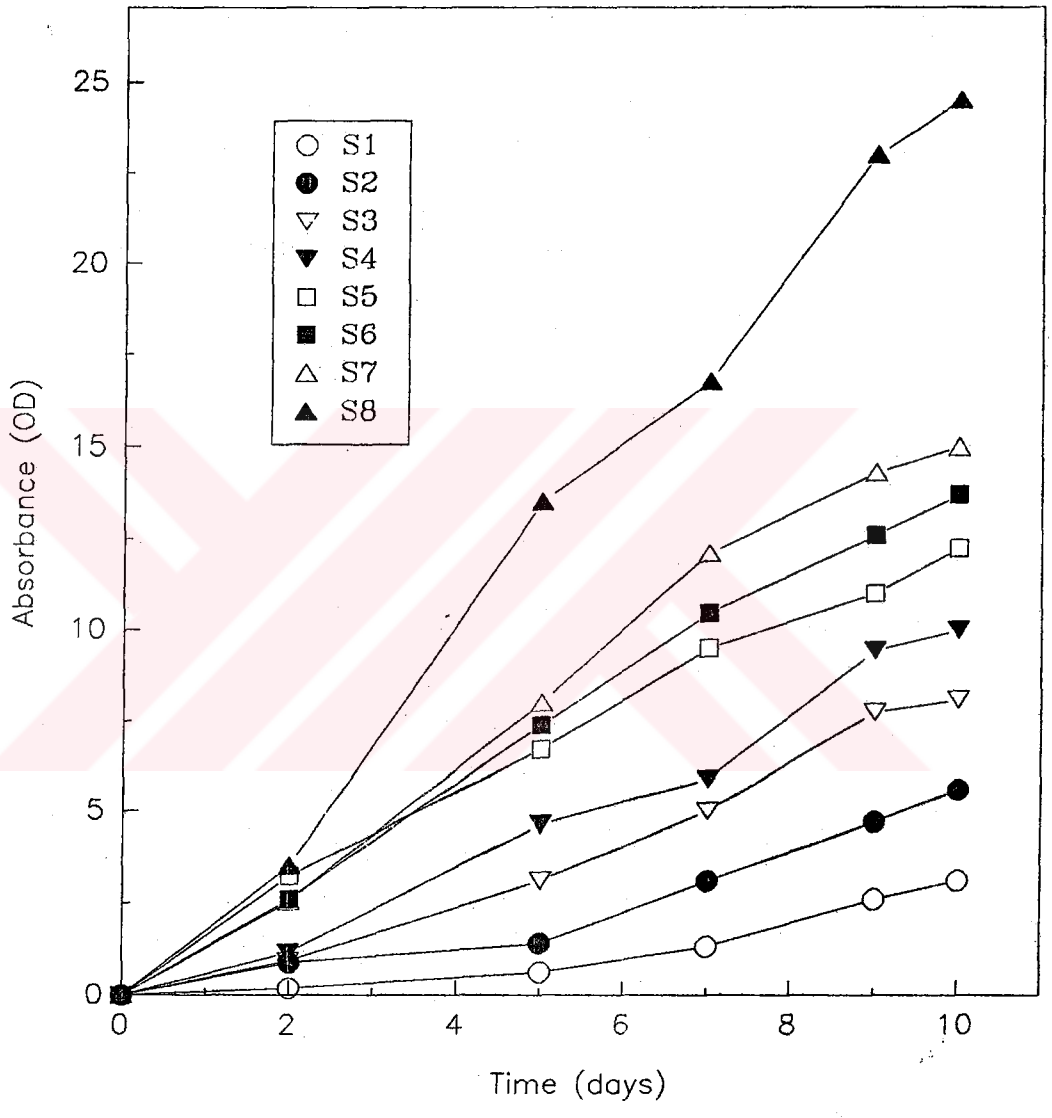


Figure 3.6. Brown pigment formation in model systems at 75 °C.

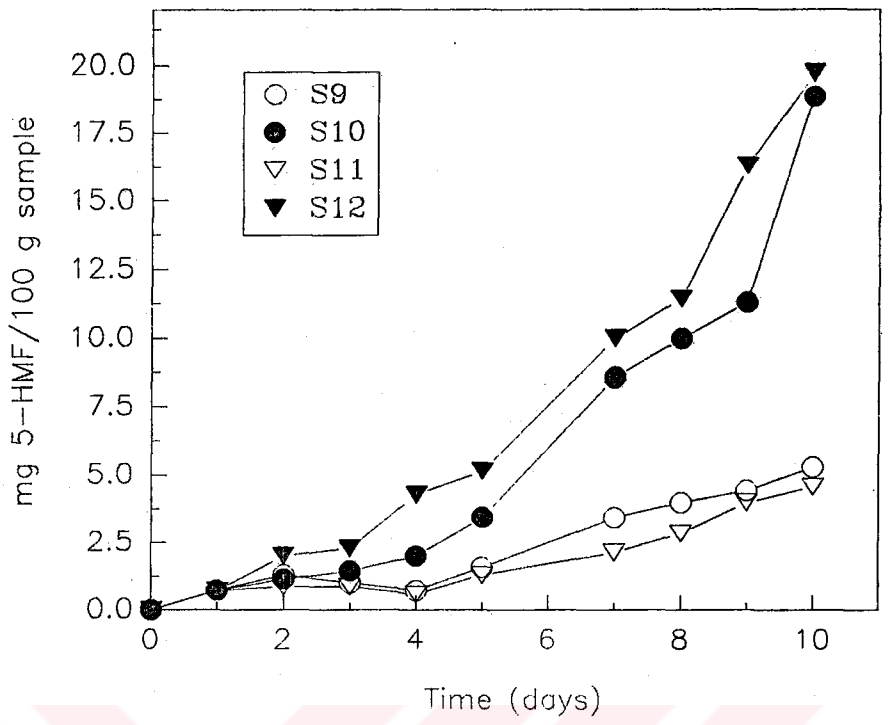


Figure 3.7. Accumulation of 5-HMF in single amino acid-sugar model systems at 55 °C.

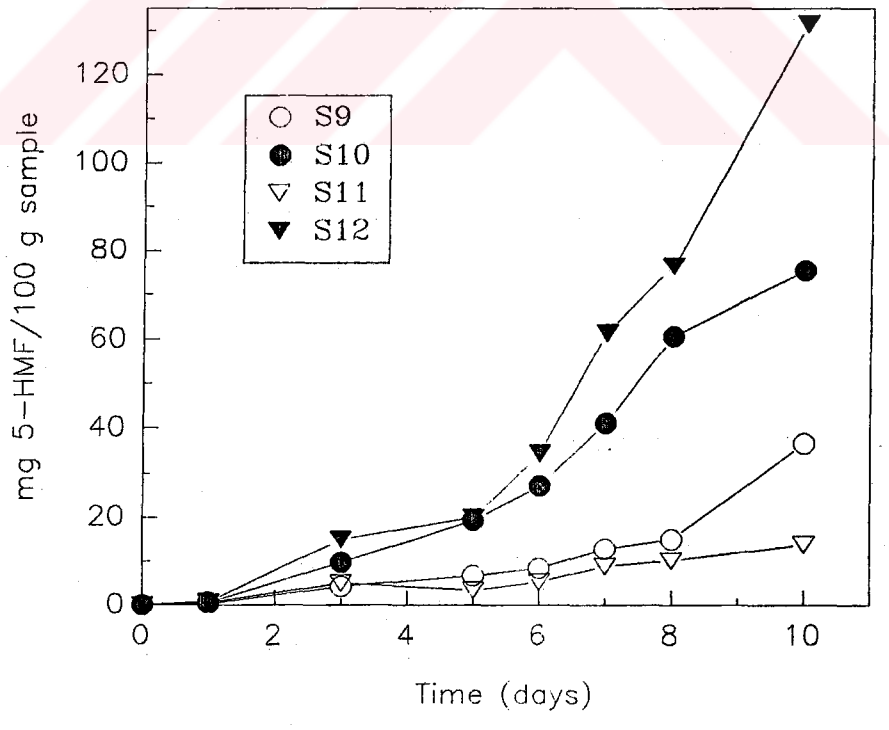


Figure 3.8. Accumulation of 5-HMF in single amino acid-sugar model sytems at 65 °C.

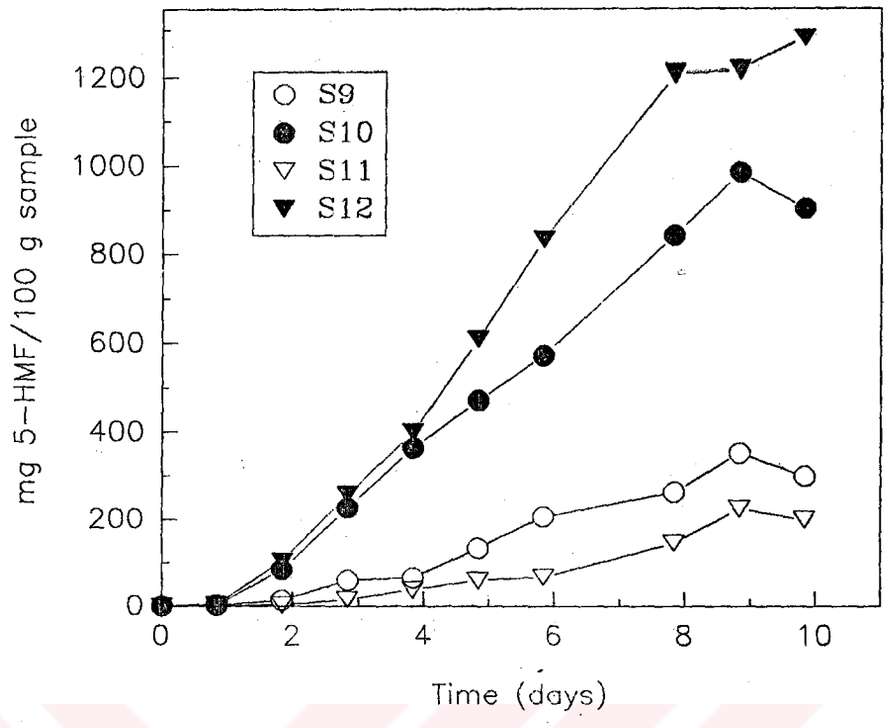


Figure 3.9. Accumulation of 5-HMF in single amino acid-sugar model systems at 75 °C.

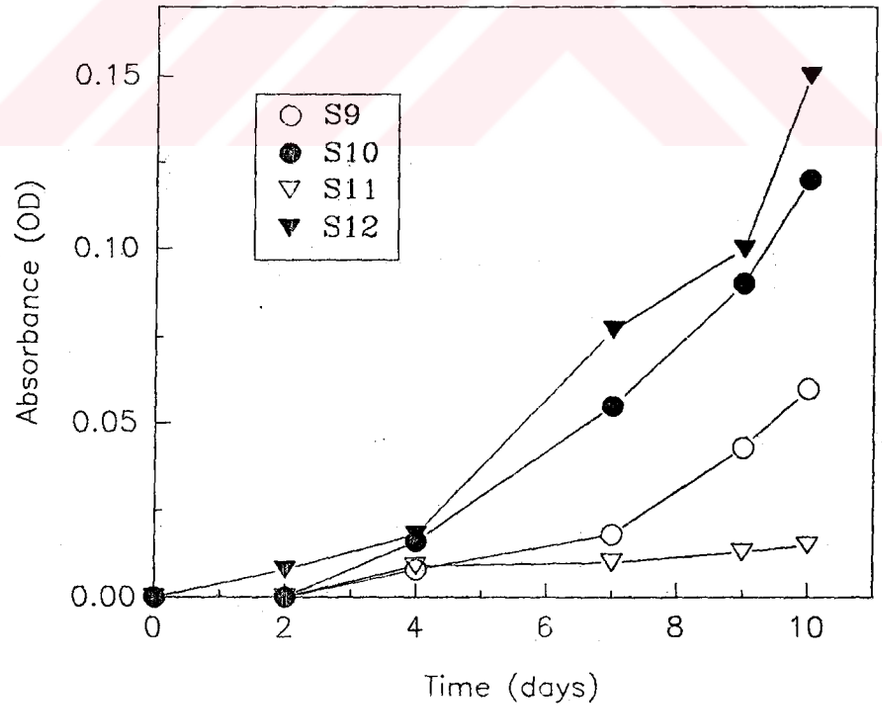


Figure 3.10. Brown pigment formation in single amino acid-sugar model sytems at 55 °C.

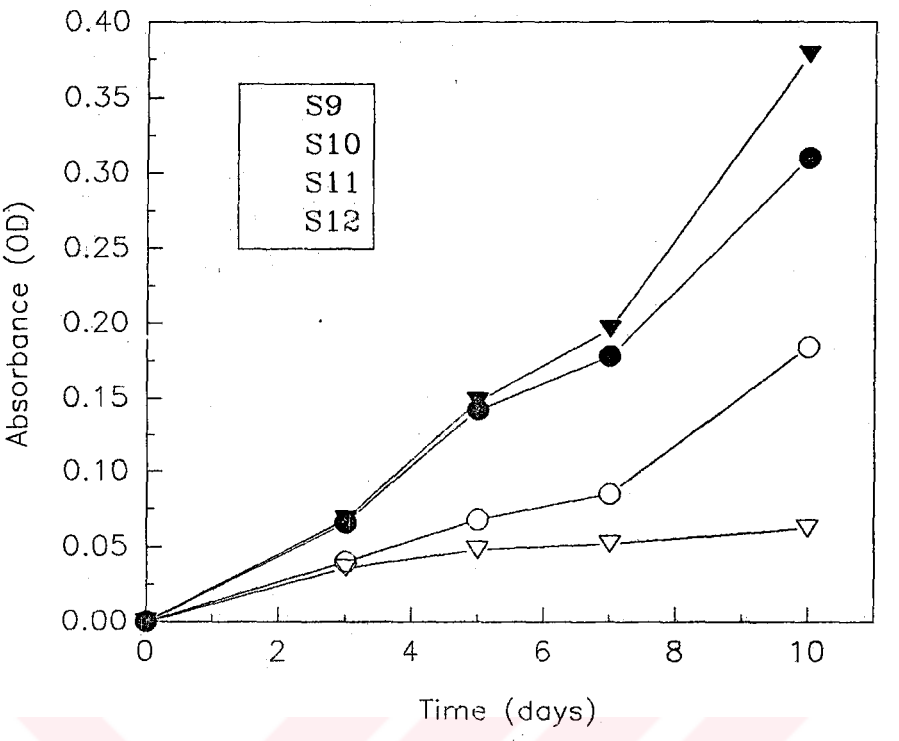


Figure 3.11.Brown pigment formation n single amino acid–sugar model systems at 65 °C.

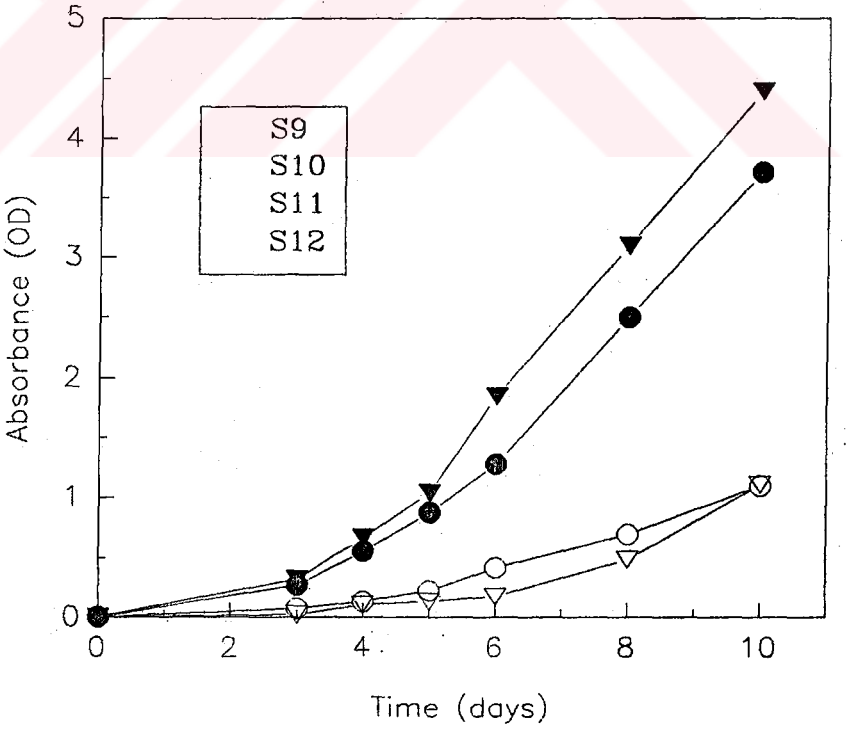


Figure 3.12. Brown pigment formation in single amino acid–sugar model sytems at 75 °C.

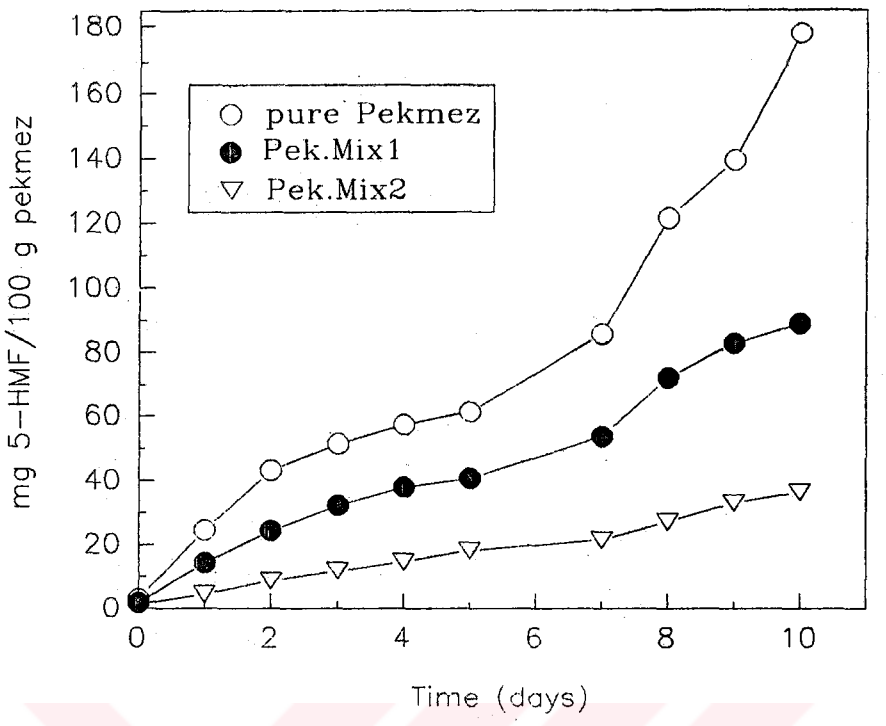


Figure 3.13. Accumulation of 5-HMF Pekmez mixtures at pH 3.5 at 55 °C.

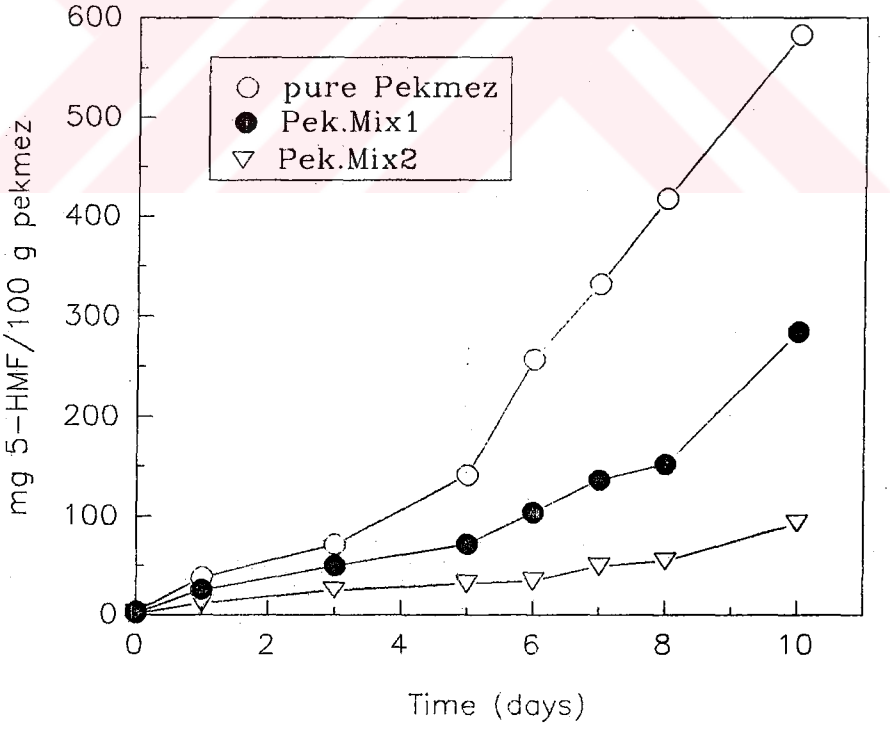


Figure 3.14. Accumulation of 5-HMF in Pekmez mixtures at pH 3.5 at 65 °C.

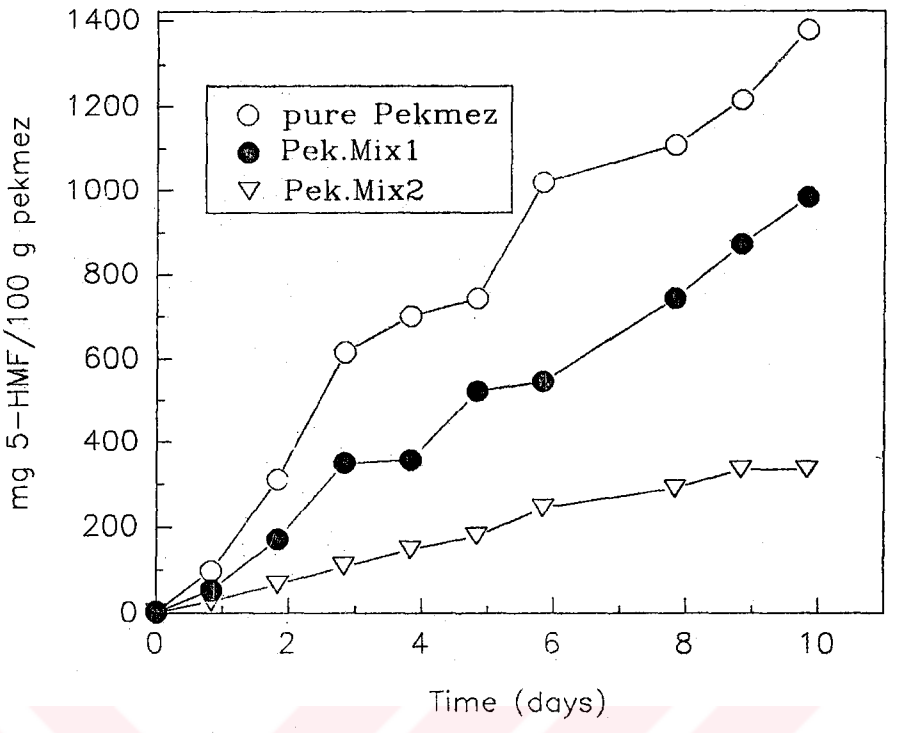


Figure 3.15. Accumulation of 5-HMF in Pekmez mixtures at pH 3.5 at 75 °C.

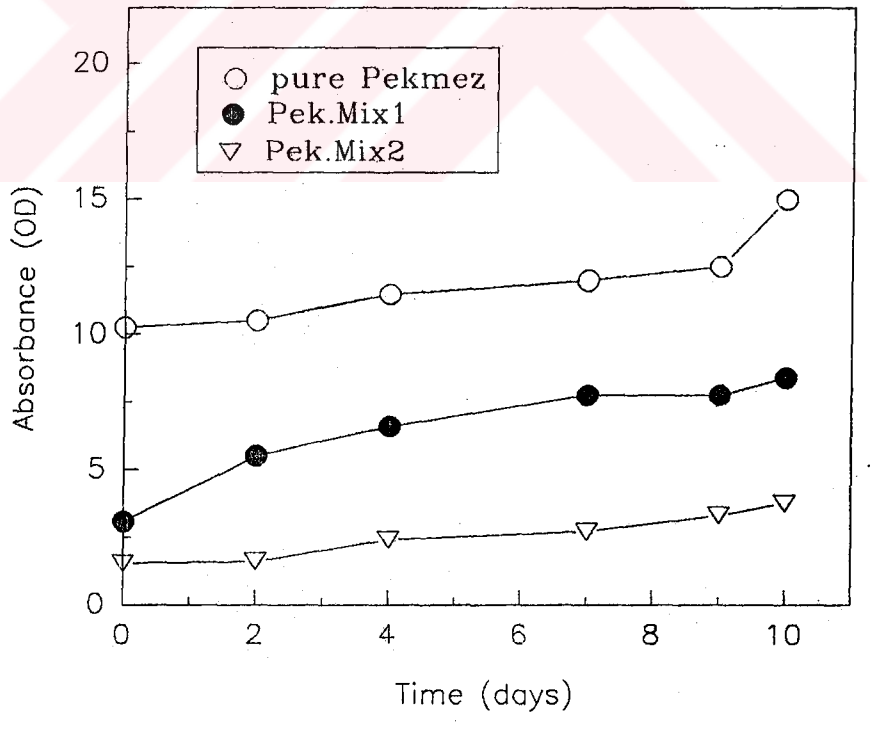


Figure 3.16. Brown pigment formation in Pekmez mixtures at pH 3.5 at 55 °C.

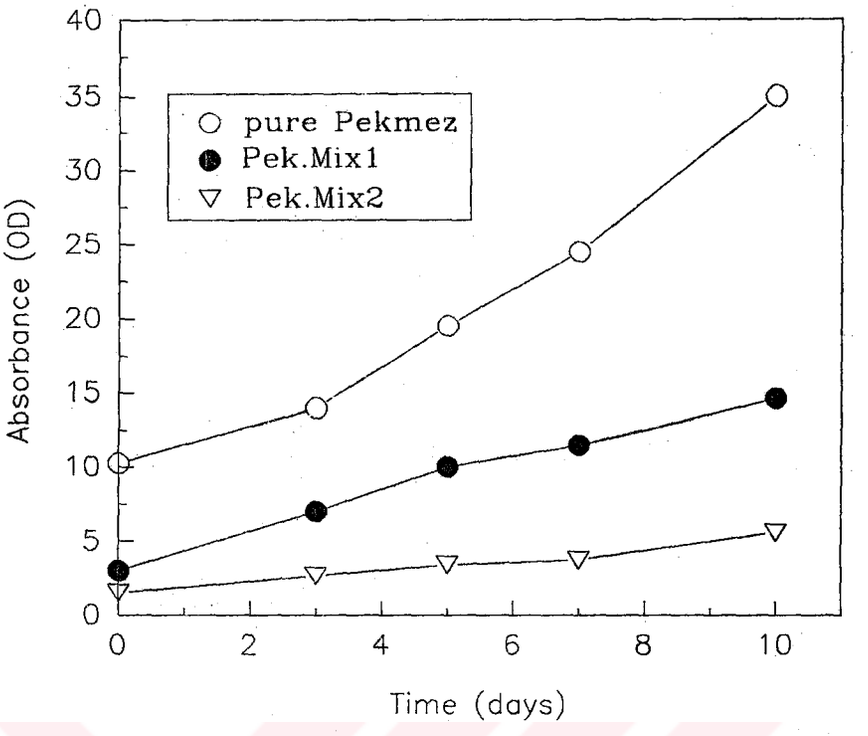


Figure 3.17. Brown pigment formation in Pekmez mixtures at pH 3.5 at 65 °C.

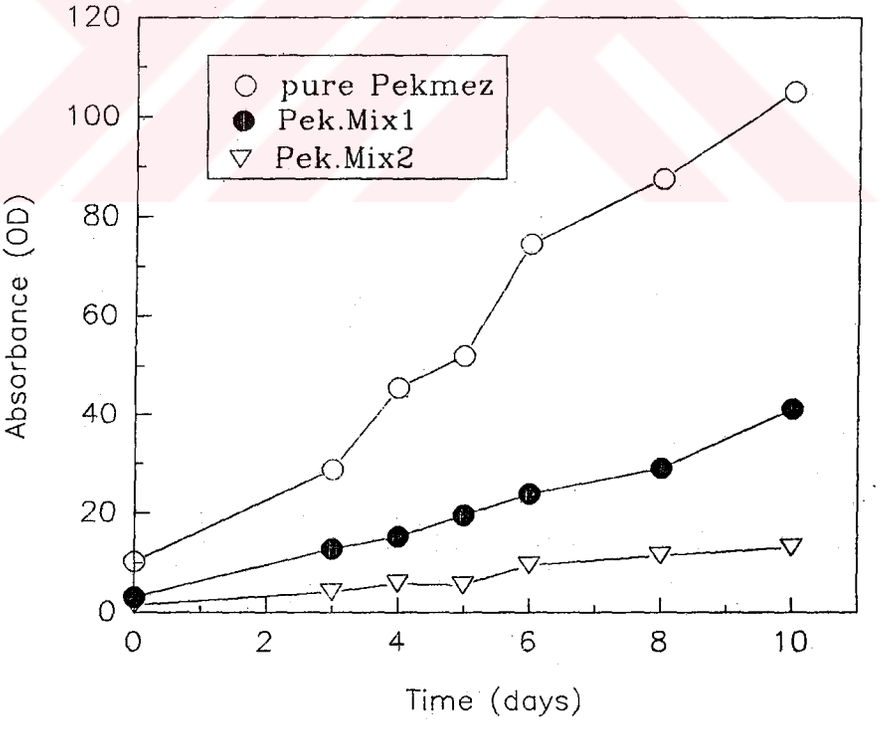


Figure 3.18. Brown pigment formation in Pekmez mixtures at pH 3.5 at 75 °C.

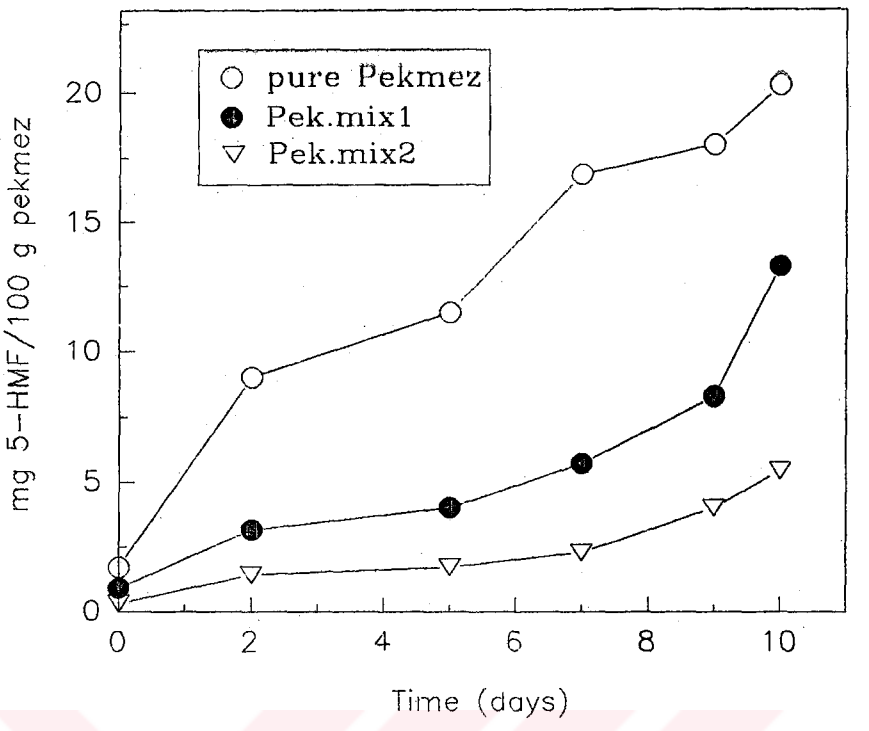


Figure 3.19. Accumulation of 5-HMF in Pekmez mixtures at pH 6.0 at 55 °C.

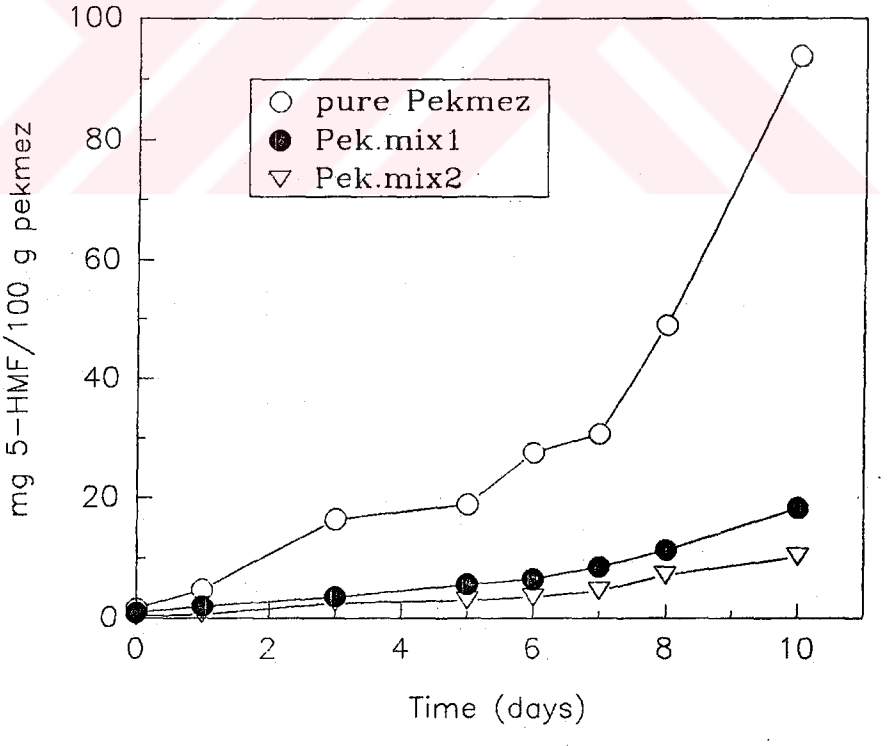


Figure 3.20. Accumulation of 5-HMF in Pekmez mixtures at pH 6.0 at 65 °C.

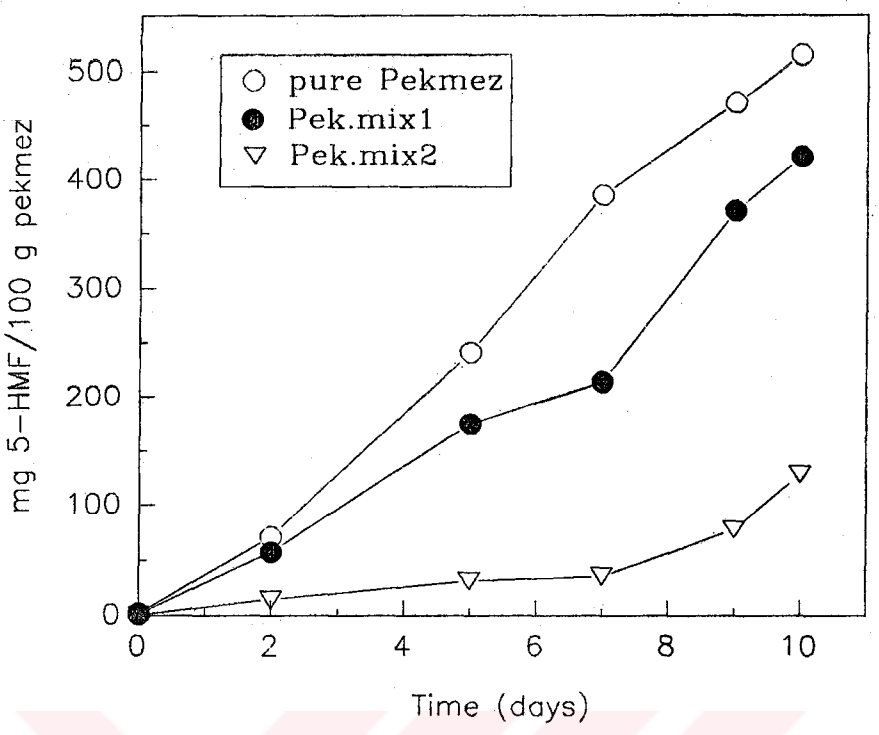


Figure 3.21. Accumulation of 5-HMF in Pekmez mixtures at pH 6.0 at 75 °C.

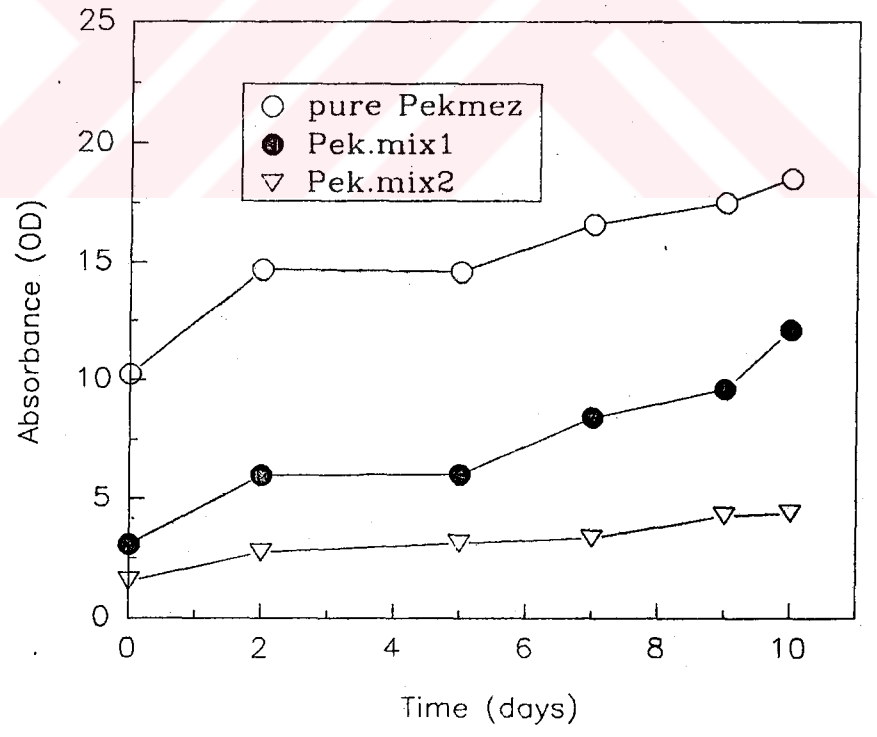


Figure 3.22. Brown pigment formation in Pekmez mixtures at pH 6.0 at 55 °C.

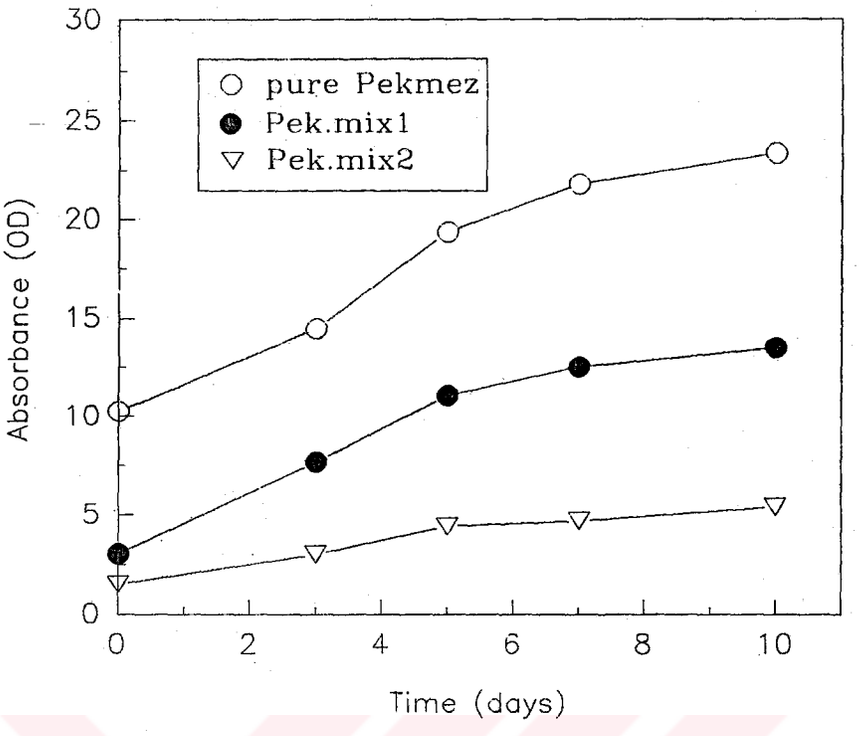


Figure 3.23. Brown pigment formation in Pekmez mixtures at pH 6.0 at 65 °C.

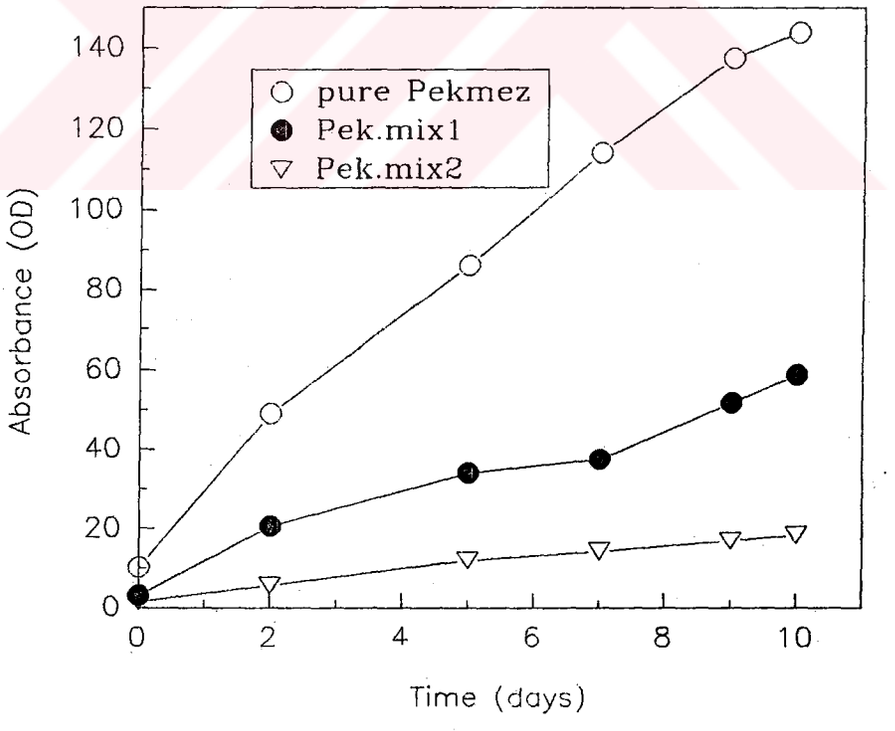


Figure 3.24. Brown pigment formation in Pekmez mixtures at pH 6.0 at 75 °C.

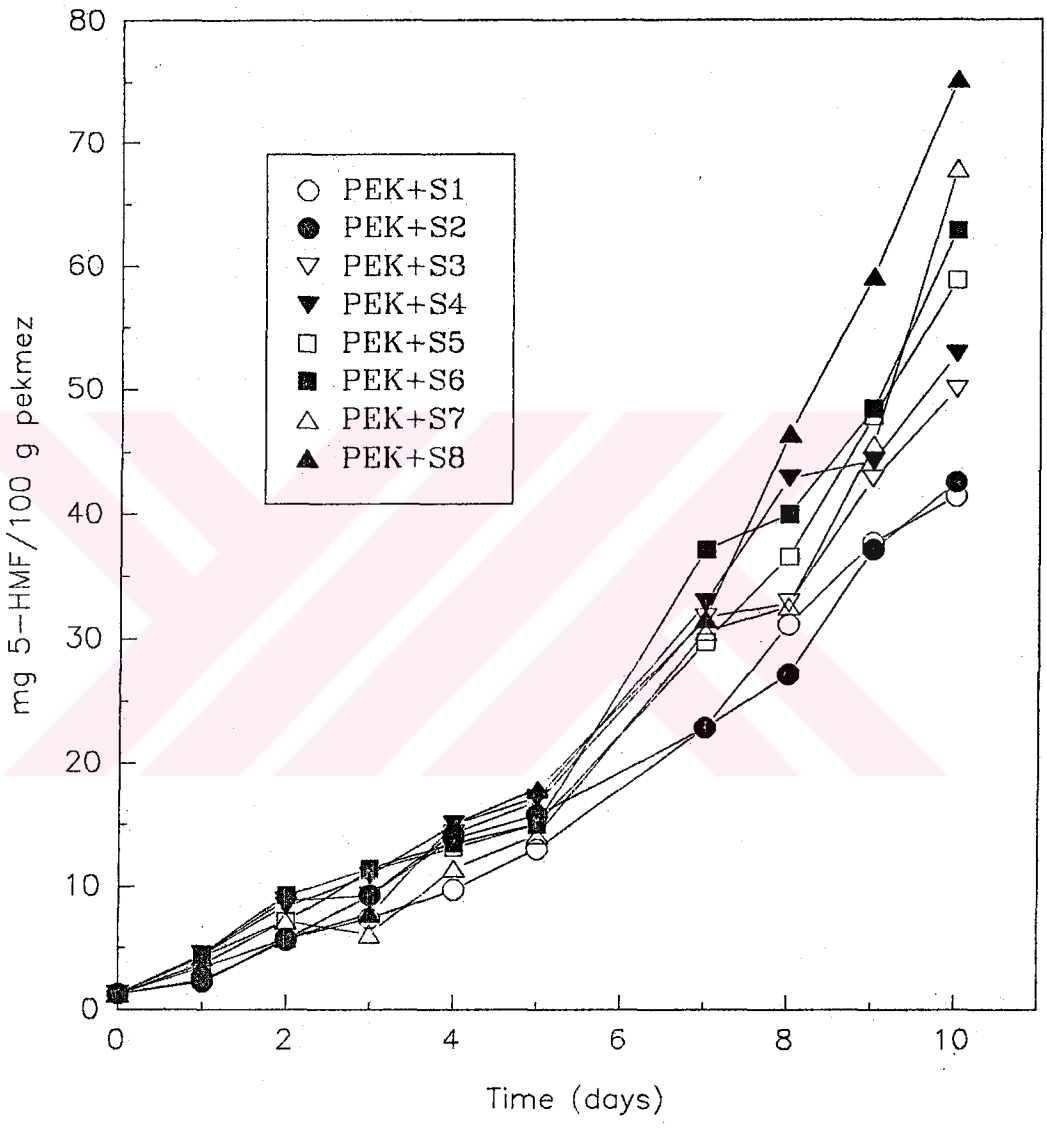


Figure 3.25. Accumulation of 5-HMF in Pekmez containing model systems at pH 3.5 at 55°C.

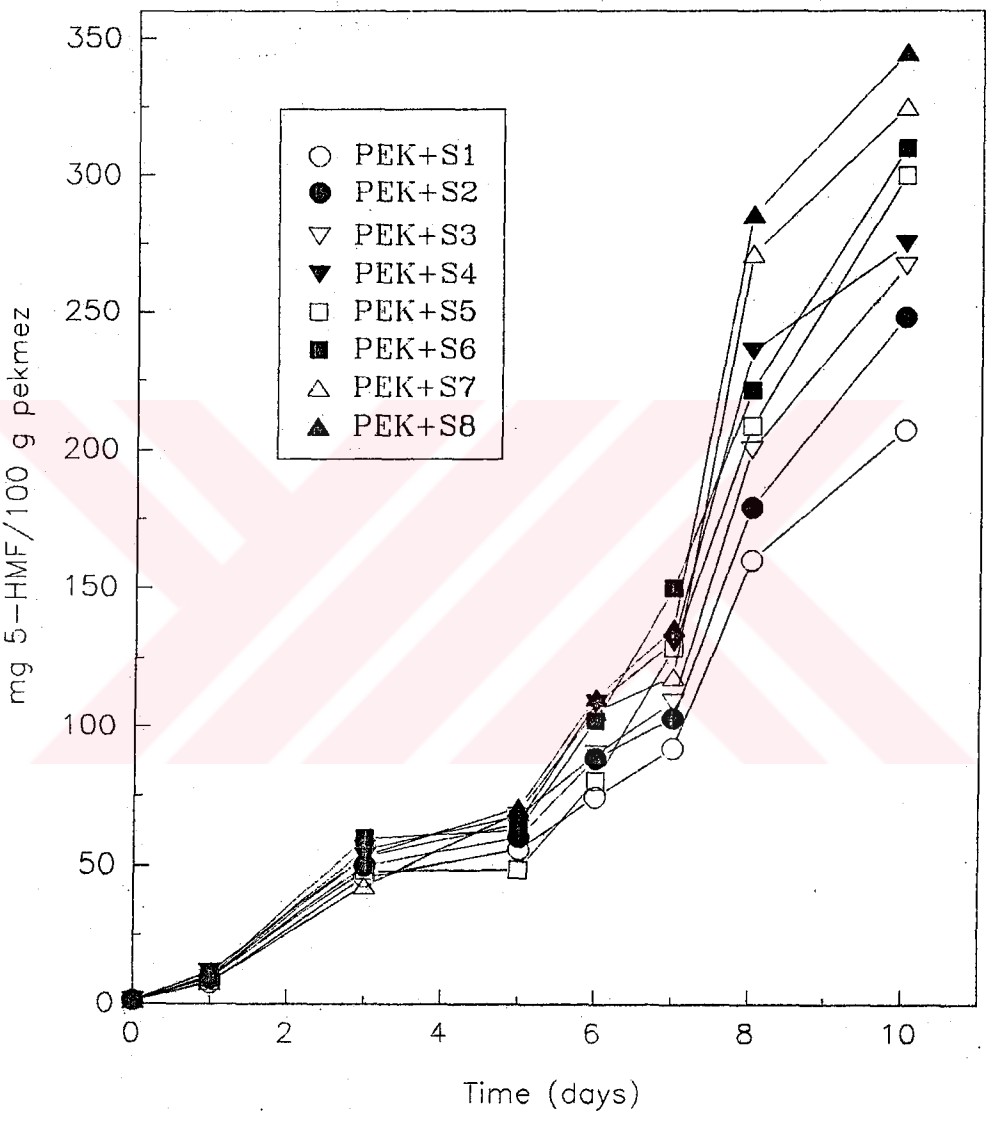


Figure 3.26. Accumulation of 5-HMF in Pekmez containing model systems at pH 3.5 at 65°C.

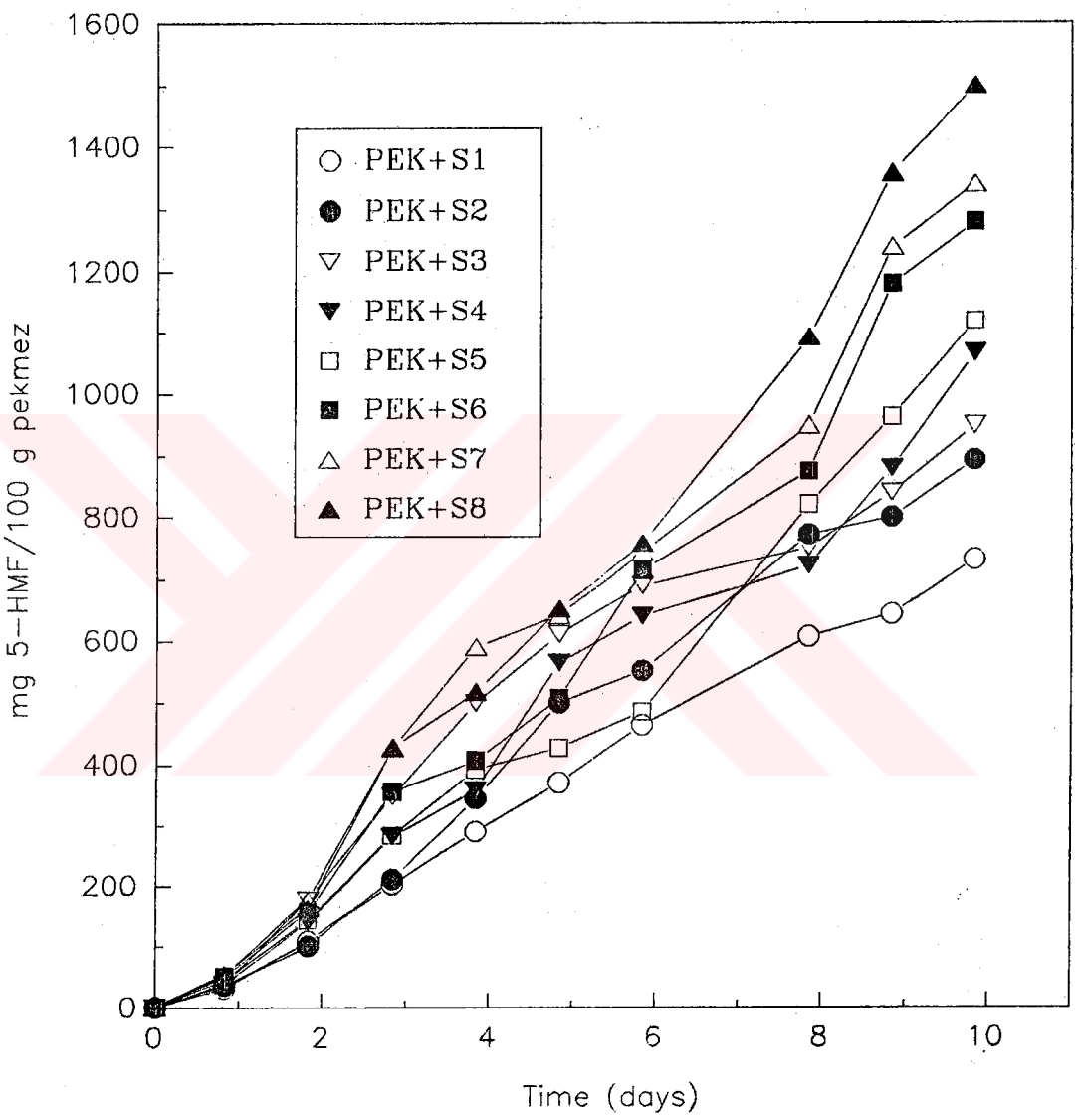


Figure 3.27. Accumulation of 5-HMF in Pekmez containing model systems at pH 3.5 at 75°C.

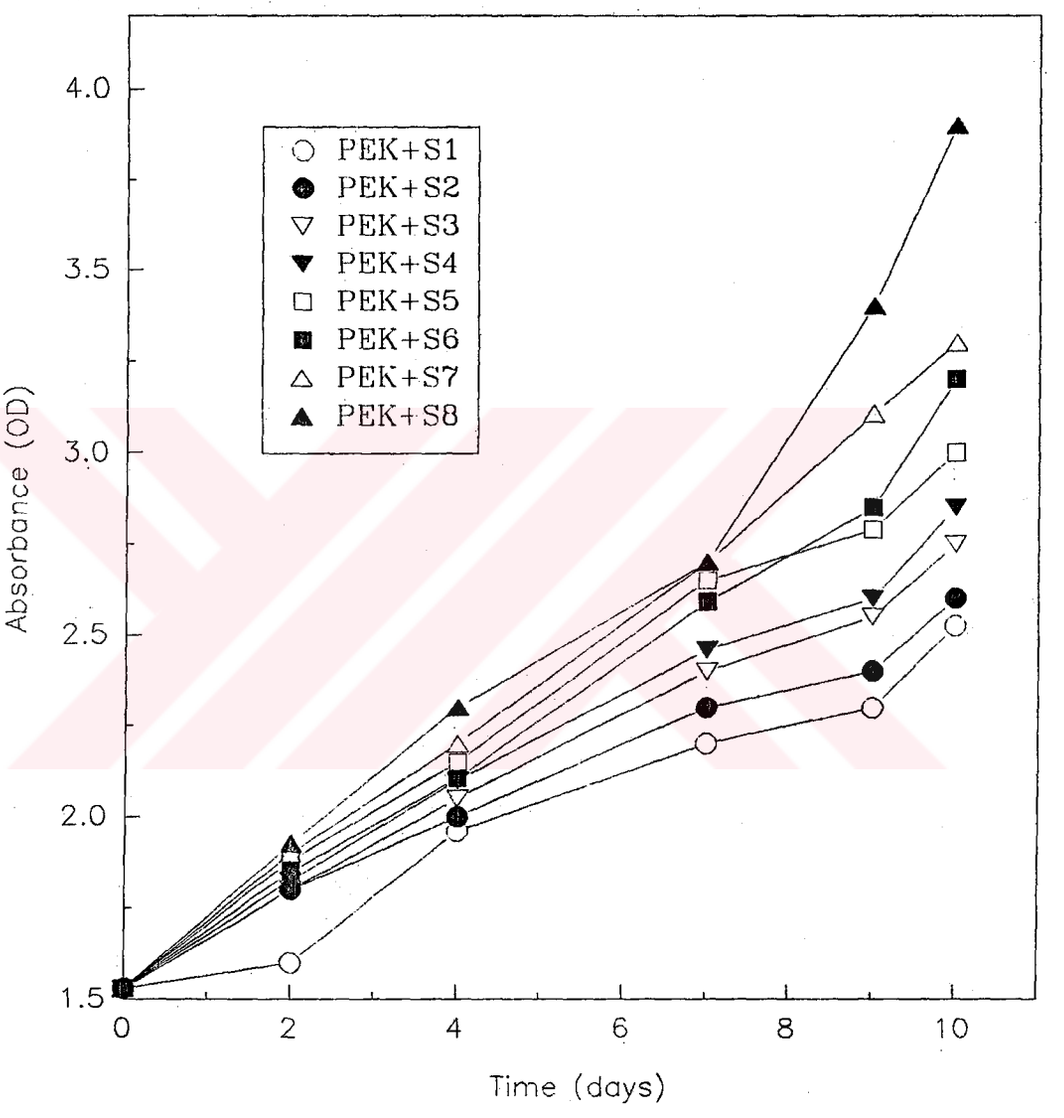


Figure 3.28. Brown pigment formation in Pekmez containing model systems at pH 3.5 at 55 °C.

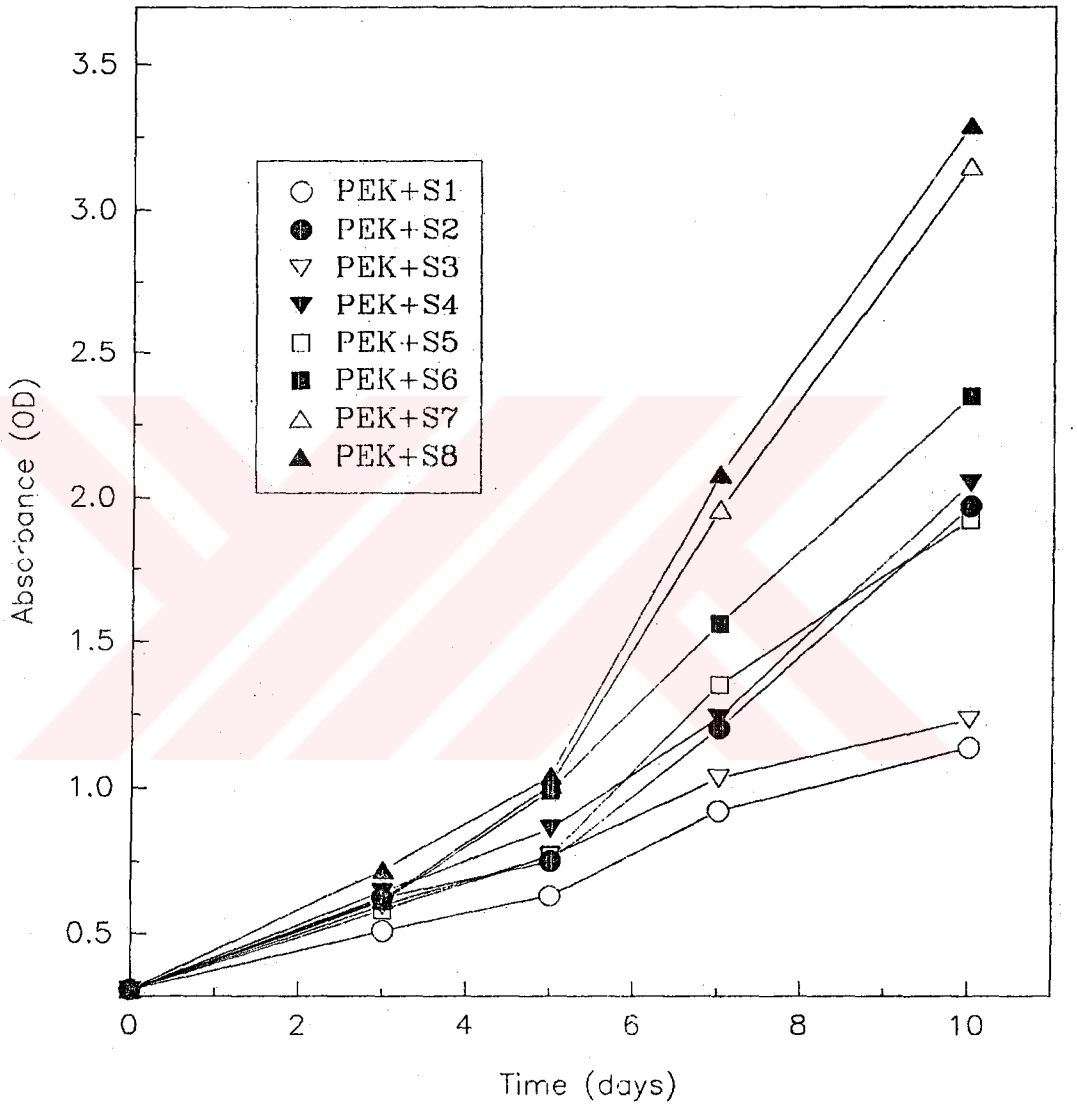
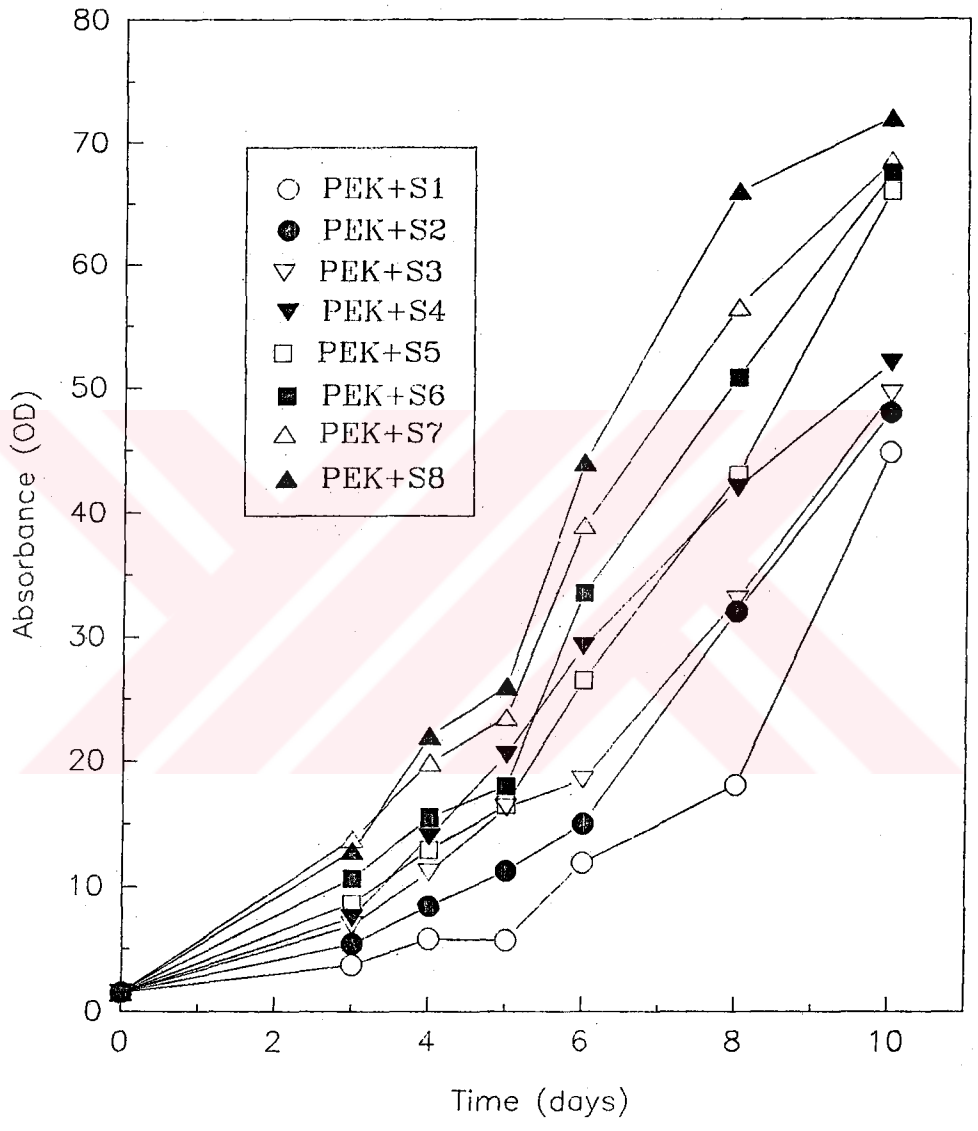


Figure 3.29. Brown pigment formation in Pekmez containing model systems at pH 3.5 at 65°C.



Fiugre 3.30. Brown pigment formation in Pekmez containing model systems at pH 3.5 at 75°C.

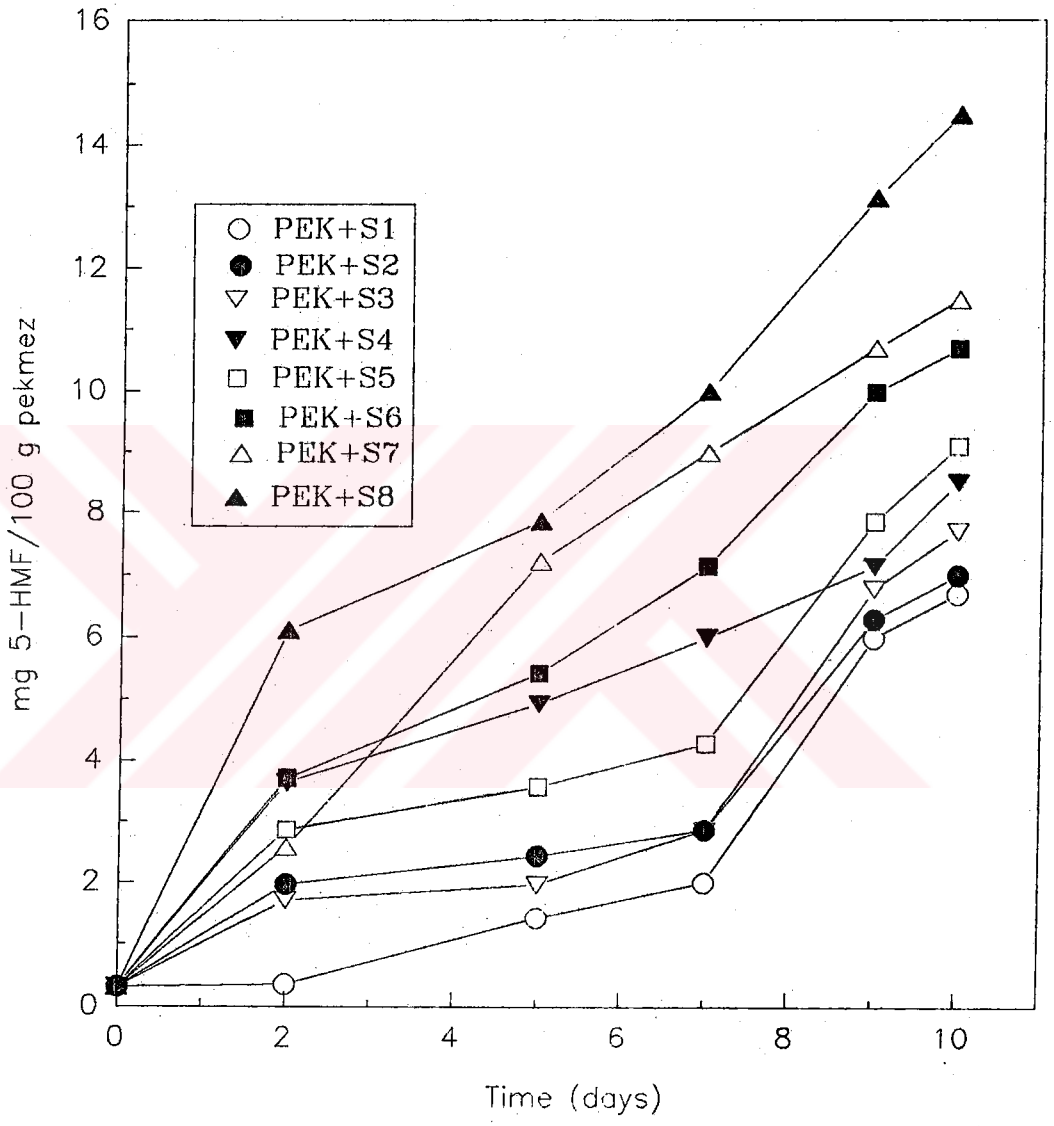


Figure 3.31. Accumulation of 5-HMF in Pekmez containing model systems at pH 6.0 at 55°C.

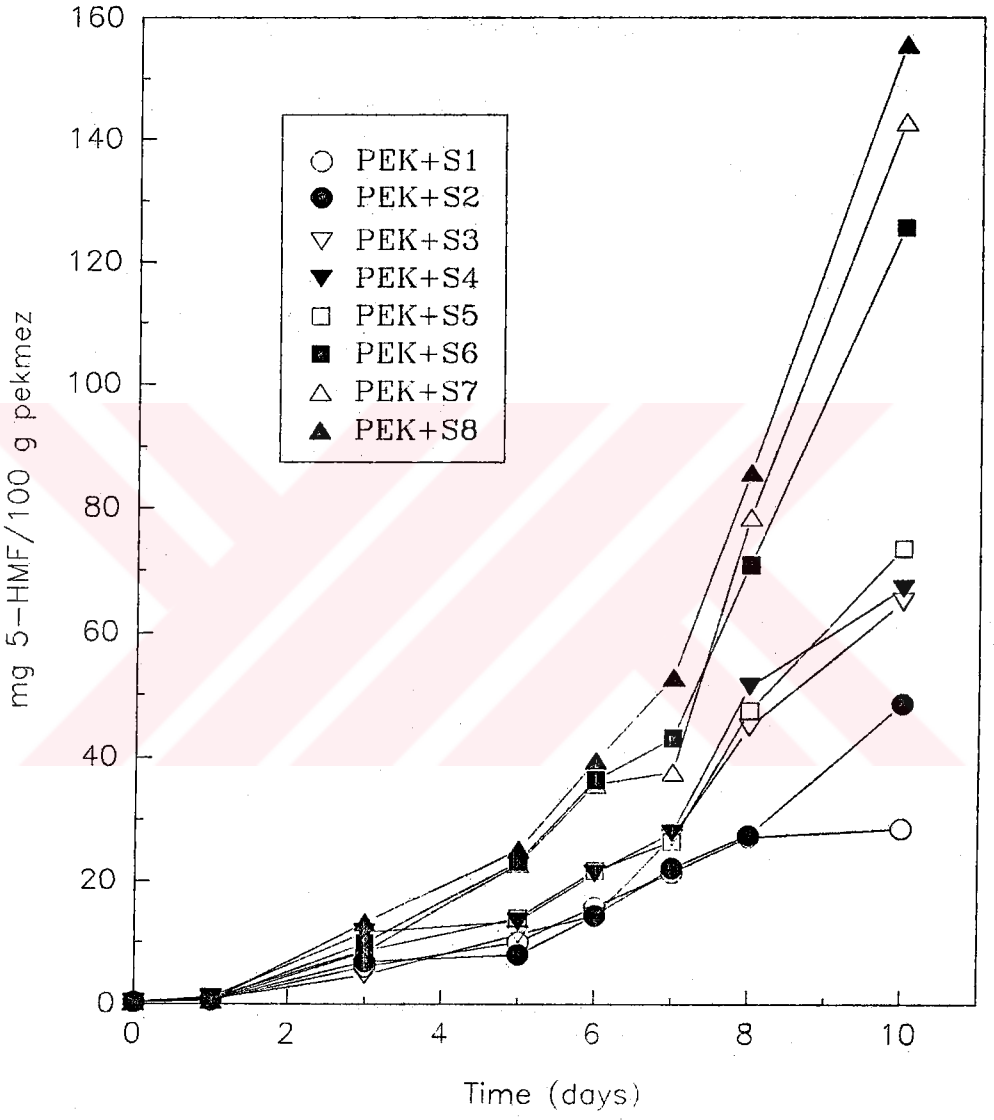


Figure 3.32. Accumulation of 5-HMF in Pekmez containing model systems at pH 6.0 at 65°C.

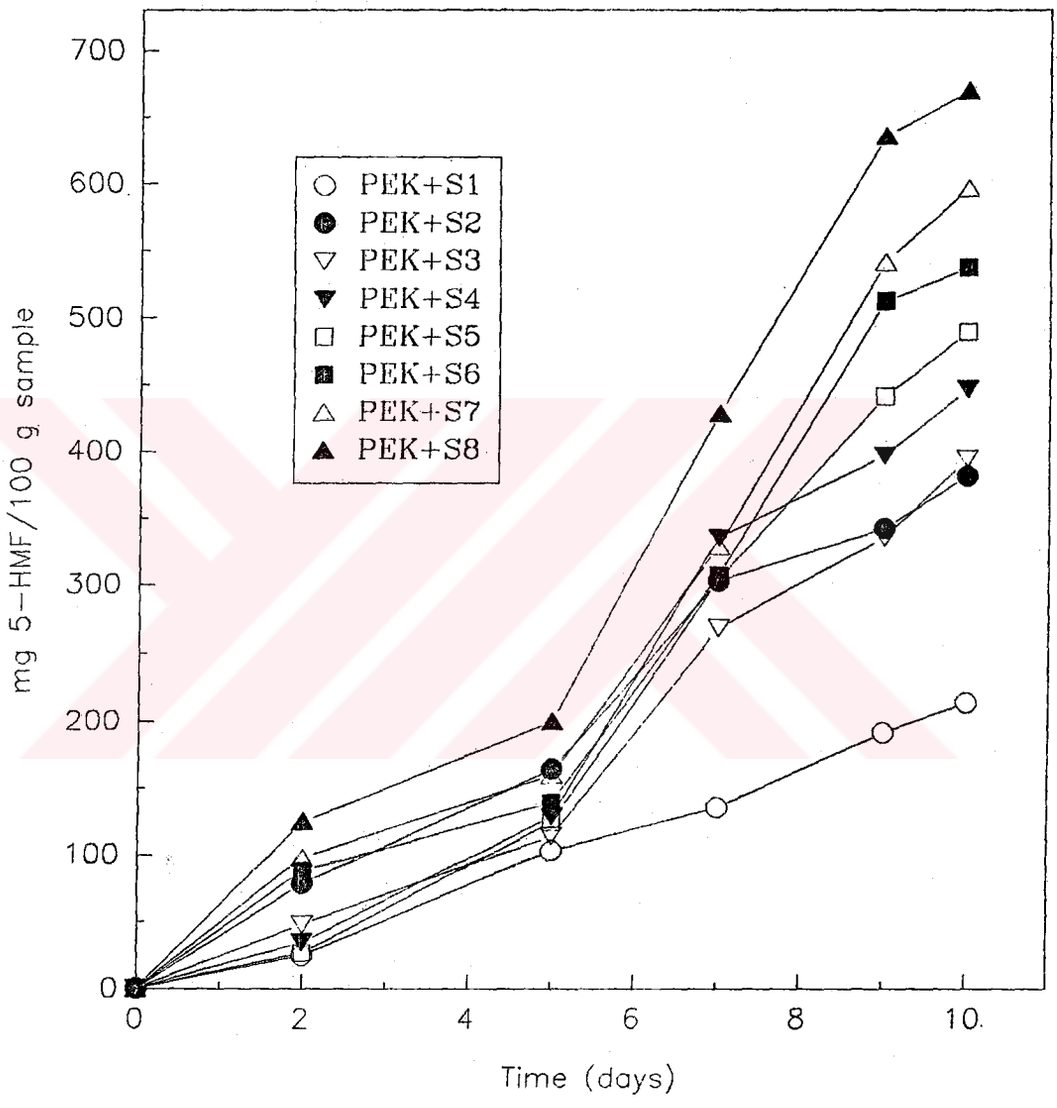


Figure 3.33 Accumulation of 5-HMF in Pekmez conatining model systems at pH 6.0 at 75°C.

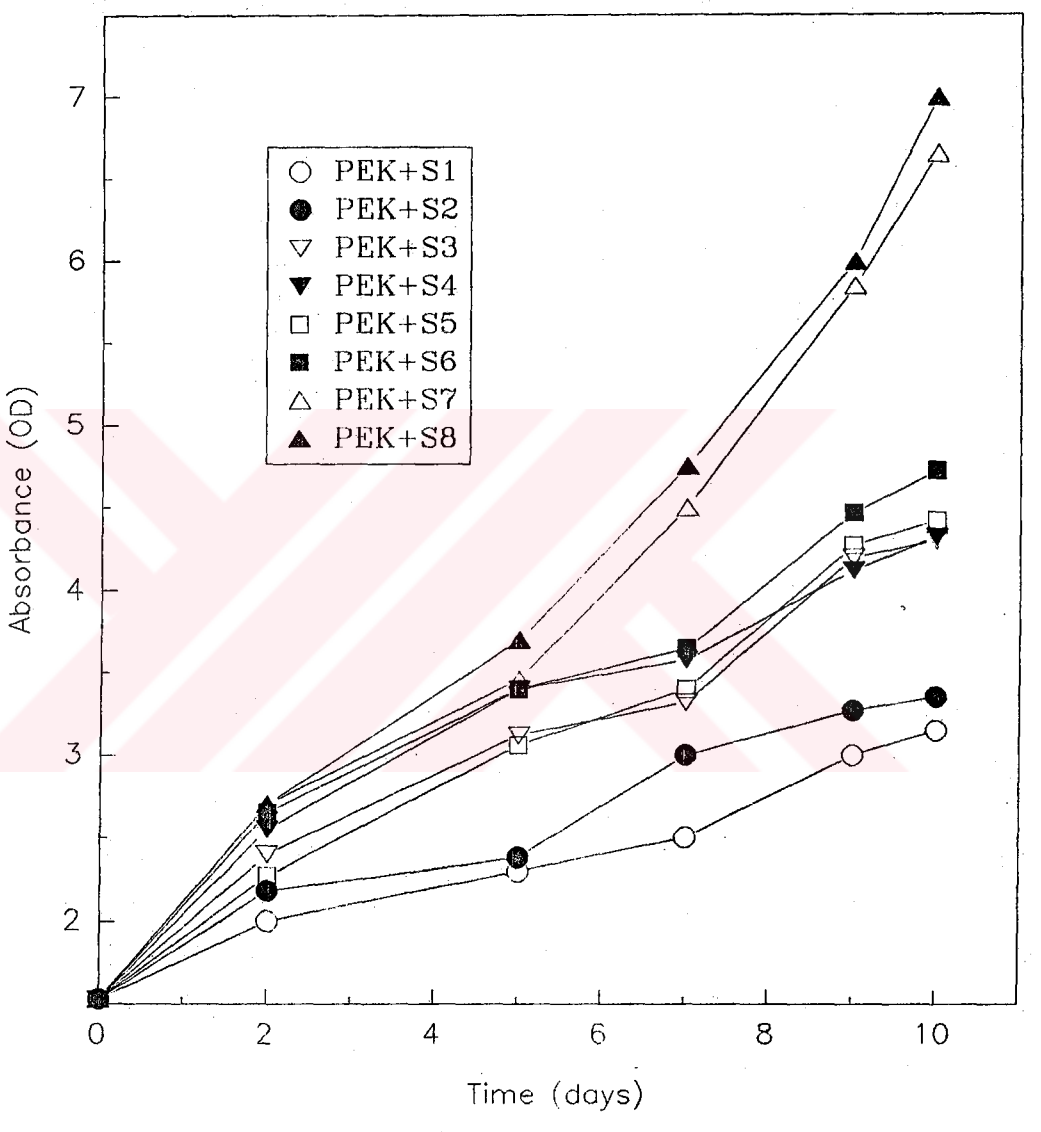


Figure 3.34. Brown pigment formation in Pekmez conatining model systems at pH 6.0 at 55°C.

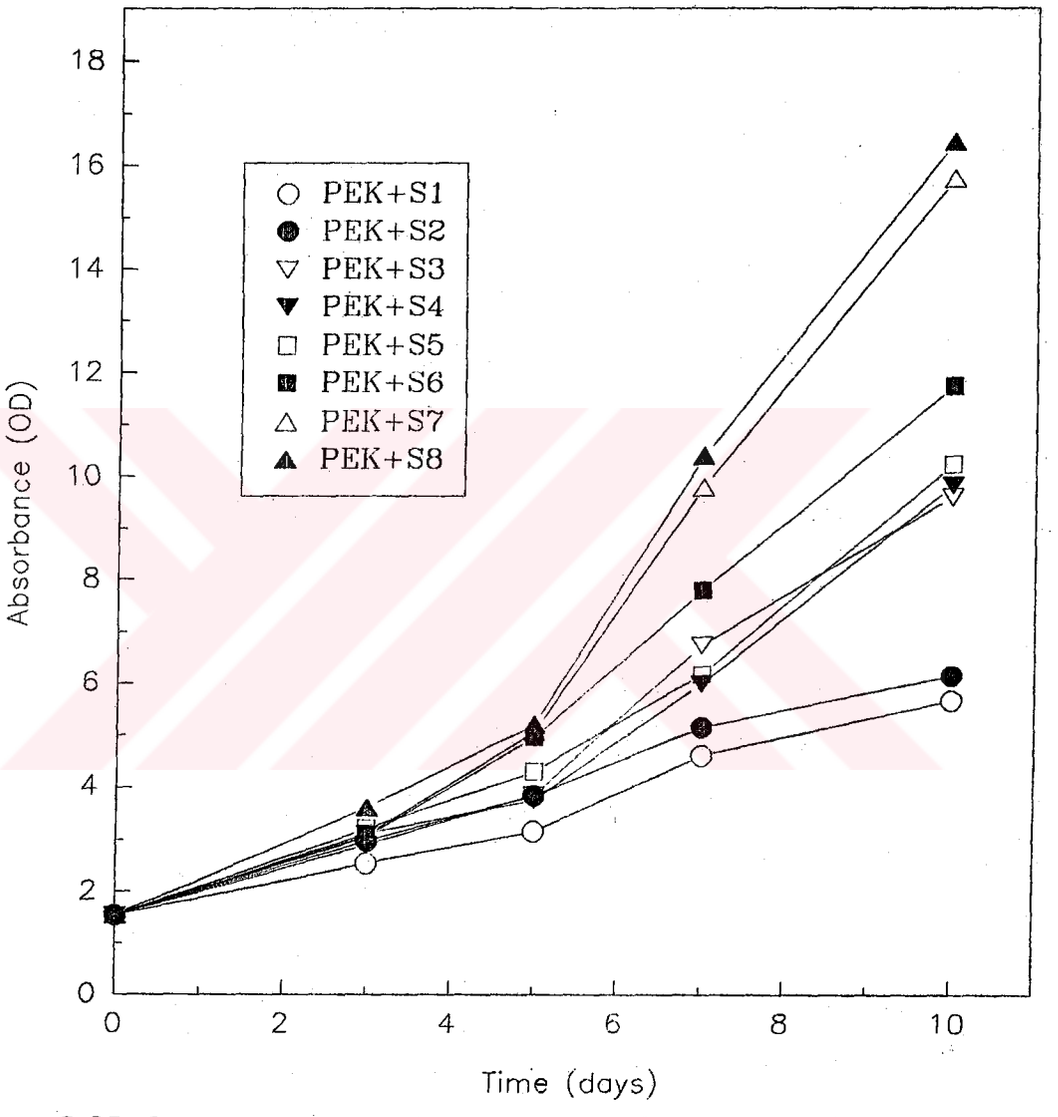


Figure 3.35. Brown pigment formation in Pekmez containing model systems at pH 6.0 at 65°C.

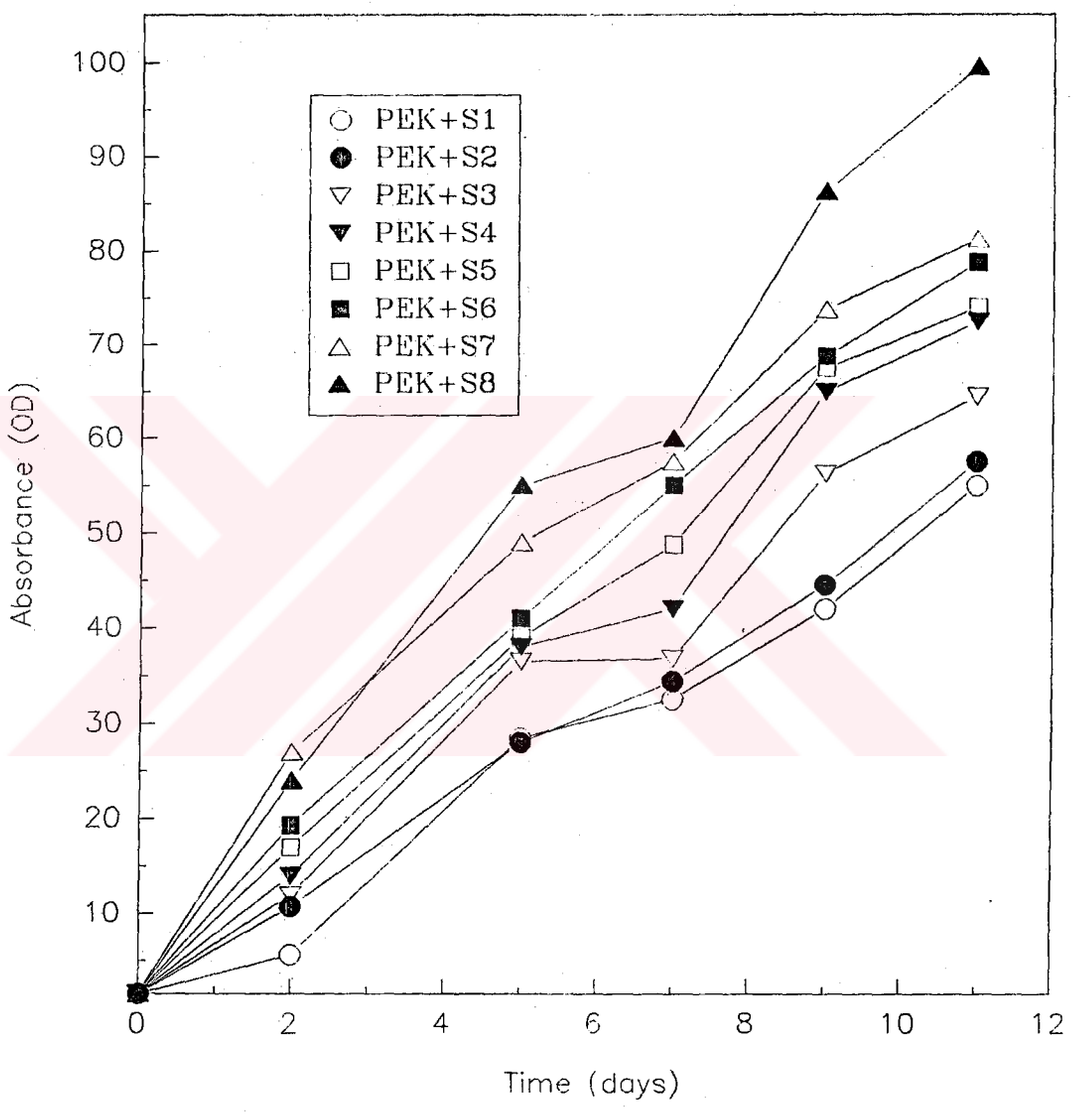


Figure 3.36. Brown pigment formation in Pekmez containing model systems at pH 6.0 at 75°C.

CHAPTER IV
CONCLUSION

The effects of amino acids and sugars on the Maillard reaction has been investigated in model systems, Pekmez, and Pekmez containing model systems according to the both 5-HMF accumulation and brown pigment formation.

It was observed that although both amino acid and sugar types are very important factors effecting the nonenzymic browning reaction, sugar type is more effective factor than amino acid type. D-fructose was the major sugar for the formation of 5-HMF and brown pigment in all systems. With D-fructose as a substrate, 5-HMF and brown pigment formed faster in the presence of L-glutamine than in the presence of L-arginine and L-proline in the systems. Reaction orders were found as 0.5 for 5-HMF accumulation, zero for brown pigment formation in all samples by using the non-linear regression analysis.

Pekmez containing model systems at pH 3.5 had higher 5-HMF accumulation than those at pH 6.0 and Pekmez containing model systems at pH 6.0 had higher brown pigment formation than those at pH 3.5. This indicates that pH is an important parameter for the Maillard reaction increasing the pH of mixture decreases 5-HMF accumulation and increases brown pigment formation.

Reaction orders was determined for 5-HMF accumulation and brown pigment formation from the non-linear regression analysis as 0.5 and zero respectively. Rate constants were determined from the both NLR for 5-HMF accumulation and LR for brown pigment formation by taking these orders.

Temperature played an important role in the acceleration both 5-HMF accumulation and brown pigment formation.

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