DETERMINATION OF HYDROXYMETHYL FURFURAL IN FOODS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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By

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ABSTRACT

DETERMINATION OF HYDROXYMETHYL FURFURAL IN FOODS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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MSc. in Food Engineering

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Two different HMF determination methods, which are spectrophotometric and HPLC were compared in randomly selected food products. It was observed that the HPLC method gives higher sensitivity and more accurate results for the determination of HMF in foods. In this study, HMF contents of various food products including dried and concentrated food, honey, jam, cereal product, beverage, vinegar, and halvah, were determined by HPLC method. The HMF concentrations of analysed food products showed wide variability and ranged between 0 and 3500 ppm. The HMF concentrations were found to be ranged within 0.3-3500 ppm for dried and concentrated foods, 0.5-27 ppm for honey, 4.2-80 ppm for jams, 0.7-29 ppm for cereal products, 0-20.3 ppm for beverages, 0-7 ppm for vinegars and 48.6-168 ppm for halvah. The highest HMF concentration was found in the boiled juices. The total solid/soluble solids contents of food products ranged within the values of 13-88 % for concentrated and dried food products, 80.5-89 ^{0}Brix for honey, 65.5-77 ^{0}Brix for jams, 55-97 % for cereal products, 2-16.5 ^{0}Brix for beverages, 5-7 ^{0}Brix for vinegars and 97.5- 98 % for halvah. When HMF concentrations of food products were evaluated in dry basis, the HMF concentration ranged within 0.5-5000 ppm. According to the experimental results it was observed that the HMF concentration of food products increases with excessive thermal treatments.

Key words: hydroxymethyl furfural (HMF), nonenzymic browning reactions, dried and concentrated foods, cereal products, beverage

ÖZ

GIDALARDAKİ HİDROKSİMETİL FURFURALIN YÜKSEK PERFORMANSLI SIVI KROMATOGRAFİ İLE BELİRLENMESİ

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İki farklı HMF belirleme metodu olan spektrofotometrik ve HPLC metodları, rastgele seçilen gıda ürünleri için karşılaştırılmıştır. Gıdalarda HMF belirlenmesinde HPLC metodunun daha hassas ve doğru sonuçlar verdiği gözlenmiştir. Bu çalışmada, kurutulmuş ve konsantre gıdalar, bal, reçel, hububat ürünü, içecek, sirke ve helva gibi ürün gruplarının HMF içerikleri HPLC metodu ile belirlenmiştir. Analiz edilen gıda maddelerinin HMF konsantrasyonları çeşitlilik göstermekde ve 0 ile 3500 ppm aralığında değişmektedir. HMF konsantrasyonlarının, kurutulmuş ve konsantre gıdalar için 0.3-3500 ppm, ballar için 0.5-27 ppm, reçeller için 4.2-80 ppm, hububat ürünleri için 0.7-29 ppm, içecekler için 0-20.3 ppm, sirkeler için 1-7 ppm ve helvalar için 48.6-168 ppm aralığında değişdiği bulunmuştur. En yüksek HMF konsantrasyonu pekmezler için bulunmuştur. Gıda ürünlerinin toplam kuru madde/çözünen kuru madde içerikleri, kurutulmuş ve konsantre gıdalar için % 13-88, ballar için 80.5-89 ^{0}Brix, reçeller için 65.5-77 ^{0}Brix, hububat ürünleri için % 55-97, içecekler için 2-16.5 ^{0}Brix, sirkeler için 5-7 ^{0}Brix ve helvalar için % 97.5-98 değerleri arasında değişmektedir. Gıda maddelerinin HMF konsantrasyonları kuru bazda değerlendirildiğinde, HMF konsantrasyonu 0.5-5000 ppm aralığında değişmektedir. Deneysel sonuçlara göre gıda ürünlerinin HMF konsantrasyonunun fazla ısıl işlemle arttığı gözlenmiştir.

Anahtar kelimeler: hidroksimetil furfural (HMF), enzimik olmayan esmerleşme reaksiyonları, kurutulmuş ve konsantre gıdalar, hububat ürünleri, içecekler

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LIST OF ABBREVIATIONS

ANOVA	: Analysis of Variance
AOAC	: Association of Official Analytical Chemists
a_w	: Water Activity
GC	: Gas Chromatography
IFFJP	: International Federation of Fruit Juice Producers
HMF	: Hydroxymethyl Furfural
HPLC	: High Performance Liquid Chromatography

CHAPTER I

INTRODUCTION

The color of foods can change considerably during transportation, processing and storage or due to mechanical distruption. The color changes, varying from pink to brown, are result of browning reactions. These reactions are vitally important, since they affect the original color, flavor and nutritive value of foods.

Browning reactions in foods are responsible for the development of valued flavors in some cases, and for quality deterioration in others. It 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 poor appearance (Eskin, 1990). These reactions are also important that, they may decrease the nutritive value of food products through the loss of essential amino acids and reduction in protein solubility. Therefore, it is important to know the mechanism of browning reactions for preserving both the original color, flavor and the nutritive value of the foods.

1.1 Types of Browning Reactions

Browning of foods is due to oxidative and nonoxidative reactions. Oxidative or enzymic browning is a reaction between oxygen and a phenolic substrate catalyzed by polyphenol oxidase (Fennema, 1976). This is the common browning that occurs in cut apples, bananas, pears and it does not involve carbohydrates. Nonoxidative or nonenzymic browning is of widespread importance in foods. It involves caramelization of carbohydrates and the interaction of proteins or amines with carbohydrates. The latter reaction is the Maillard reaction. Oxidation of ascorbic acid in citrus fruits may cause loss of vitamin C with subsequent darkening of the fruit. Maillard type browning of reducing sugars and free amines in dry milk powder results in decreased solubility and nutritional loss.

1.1.1 Enzymic Browning

Enzymic browning is a phenomenon which occurs in many fruits and vegetables, such as potatoes, mushrooms, apples and bananas. When the tissue is bruised, cut, peeled, diseased or exposed to any abnormal conditions it rapidly darkens on exposure to air as a result of the conversion of phenolic compounds to brown melanoidines (Eskin, 1990). 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 (Fennema, 1976). For this type of browning to occur, three components are essential: enzyme, substrate and oxygen. In the presence of oxygen, highly active phenolases oxidize orthophenolic substrates to form the colored quinone compounds. The various enzymes that catalyze the oxidation of phenols are commonly known as phenolases, polyphenol oxidases, tyrosinases or catecholases.

Mostly, enzymic browning of vegetables and fruits are undesirable because it occurs during processing. For this reason, various methods have been developed to inhibit enzymic browning. Inhibitors of polyphenol oxidase activity can be provided by elimination of substrates (O_2 and polyphenol) or inactivation of enzyme. Heat treatments, or the application of sulfur dioxide or sulfites and lowering the pH of the system (pH <3) by addition of acidulants such as citric acid, malic or phosphoric acid are commonly used methods of inactivating enzyme. The simplest method of controlling enzymic browning is exclusion of oxygen from system by vacumization or immersing the plant tissue in a brine or syrup (Fennema, 1976). Among the most effective and safest inhibitors of enzymatic browning are L-ascorbic acid and its isomers and derivatives. Addition of L-ascorbic acid reduces quinone structures. Reduction of quinone compounds by L-ascorbic acid is accompanied by a decrease of enzyme activity. In some cases, L-ascorbic acid, D-iso-ascorbic acid or its derivatives have been used in combination with sodium benzoate, sulphur dioxide, citric acid, and sodium or calcium chloride.

1.1.2 Nonenzymic Browning Reactions

Nonenzymic browning is one of the most prevalent chemical reactions that occur in foods . The importance of this reaction in the production of food is simply explained by its contributing to the flavor, color and aroma of coffee, caramel, bread and

breakfast cereals. Nonenzymic browning reactions occur during the thermal processing and storage of foods. Careful control must be exercised to minimize excessive browning which could lead to unpleasant changes in the food product. Also, some toxic and mutagenic intermediates are formed from the nonenzymic browning reaction such as imidazoles, N-nitroso derivatives, Hydroxymethyl furfural (HMF), and melanoidines. N-nitrosoamines, the most hazardous of the cancinogens produced by mainly from secondary amines and nitrous acid (Namiki, 1988).

Nonenzymic browning reactions are responsible for deteriorations of food in most cases and for the development of food in some cases. It is often difficult to make meaningful comparison of the results of different studies, even when the reactants are same. Because of the wide differences in reaction condition, concentration and type of reactant conditions, three major sets of conditions have to be thought for comparison (Danehy, 1986).

- Low moisture-high temperatures for relatively short periods of time. These are the conditions which develop flavors and colors that otherwise would not appear at all, e.g. in the roasting of coffee beans, cocoa beans, nuts, grains.
- Low moisture-moderate temperatures for relatively long periods of time. These are the conditions that produce deterioration, e.g. off-flavors and unwanted colors on storage of foods where it is desired to retain the properties as originally packaged.
- High moisture over a wide range of temperatures (20-110 ^{0}C). This set of conditions is the least relevant to food conditions, except for beverages, where browning is not usually important.

There are three types of nonenzymic browning reactions in food stuffs.

1. Reactions of amino groups and carbonyl groups present in foods such as reaction of aldehydes, ketones and reducing sugars with amino acid, peptides, amines, and proteins (Maillard reactions).
2. Caramelization reactions which forms caramel flavor and brown color, are caused from degradation of polyhydroxycarbonyl compounds (sugars and polyhydroxycarboxylic acid) when they exposed to higher temperatures.
3. Oxidative reaction in which ascorbic acid and polyphenols are converted to di- or polycarbonyl compounds.

1.1.2.1 Maillard reaction

The reaction between sugars and amino acids was firstly described by Ling, in 1908; he considered color formation in beer. The Maillard reaction takes its name from the French chemist, Louis Camille Maillard, who examined the reaction between glycine and glucose. He had made a very simple experiment with glucose and glycine; he heated the solution of glucose and glycine and observes color changes from yellow to dark brown. Maillard also used the term melanoidins to describe the brown-black pigments formed in the reaction and found that CO_2 was released as a product at the end of reaction. This reaction essentially covers all those reactions involving comounds with amino groups and carbonyl groups present in foods. These include amines, amino acids and proteins intereacting with sugars, aldehydes and ketones, as well as products of lipid oxidation (Namiki, 1988; Friedman, 1996). The Maillard reaction appears to be most significant browning reaction for several types of foods. It has a great significance for foodstuffs with the following aspects.

- Formation of color: Controlled browning is desirable in caramel production, chocolate manufacture, beer production, bread making or undesirable as in glucose syrup and in many intermediate moisture products (Danehy, 1986).
- Formation of falvors and off-flavors (Hodge, 1967; Hodge, 1972; Eichner and Ciner-Doruk, 1981): The reaction is desired frequently in domestic cooking (the roasting or frying of meat) to produce desirable aromas and flavors (O'Brien and Morrisey, 1989).
- Reduction in nutritional value: The Maillard reaction may seriously reduce the nutritional value of foods through the destruction of essential amino acids and by involvement of ascorbic acid (O'Brien and Morrisey, 1989).
- Production of antinutritive and toxic compounds: The nutritive value of food may be considerably reduced due to the formation of colorless Amadori compounds which are regarded as only form of bound amino acid (Namiki, 1988).
- Formation of reductones can behave as an antioxidant and also chelation of heavy metals may also be involved.

i) Mechanism of Maillard reaction

The first general rewiev was proposed by Hodge (1953) and the original reaction sequence propsed by Hodge still remains valid. Hodge (1953) divided his scheme of

browning into three stages as shown in Figure 1.1. The initial step is that of glycosylamine formation (A) and subsequent rearrangement (B). The intermediate stage comprises dehydration (C), either by the loss of three molecules of water to furfurals or by loss of three molecules of water to give reductones; fission (D), mainly by dealdolisation, Stecker degradation (E), the interaction of amino acids with dicarbonyl compounds, be they furfurals, fission products, dehydroreductones, or Stecker aldehydes, into high molecular weight products, either nitrogen free (F) or nitrogenous (G).

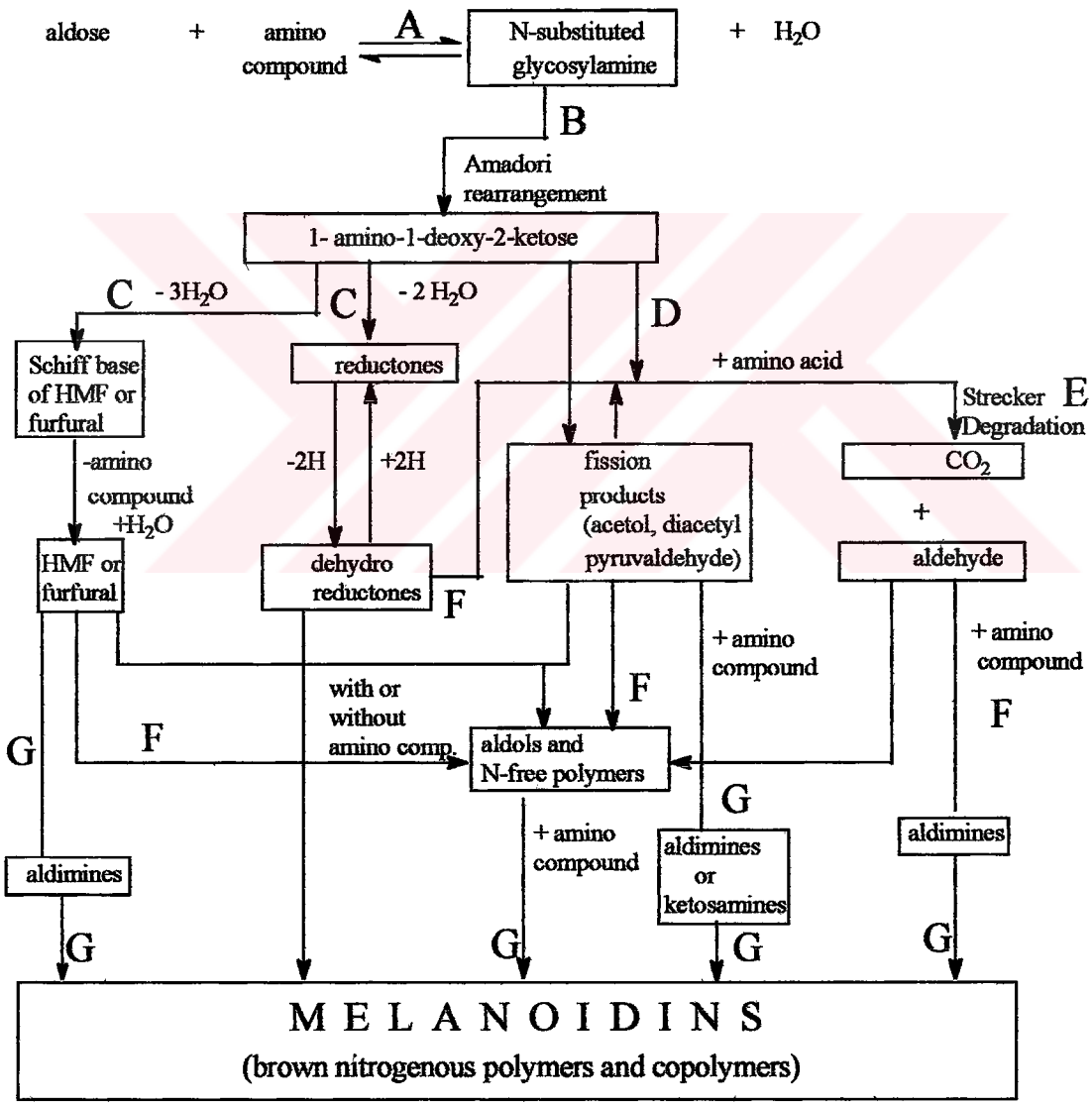

Figure 1.1 Hodge's view of browning pathways

Stage 1

The initial stage involves the sugar-amine condensation and the Amadori rearrangement. This part of reaction chains has been well-defined and no browning phenomenon is obtained from this stage (Hodge, 1953; Danehy, 1986; Namiki, 1988; Labuza *et al.*, 1992).

a) Carbonylamino reaction

The first step in the Maillard reaction is condensation between the free amino groups of amino acid or proteins and the carbonyl groups of reducing sugars. This is the result of nucleophilic attack by the $-NH_2$ group of the amino acid on the electrophilic carbonyl groups of sugar. This is mainly an amine-asisted dehydration reaction of sugar. The condensation product rapidly losses water as a product and it is converted into a schiff base, then cyclizes into N-substituted glycosylamine (Figure 1.2). Schiff base usually occur faster than the first combination step, since the increase in the nucleophilic strenght of the amine increases the rate of the carbonyl-amine reaction. This reaction is reversible and requires acidic catalyst. (Namiki, 1988).

Condensation reaction is not necessarily restricted to α-amino acids and can involve the participation of other amino groups found in peptides and proteins. The reaction rate is mainly dependent on the isoelectric point of amino group being reacted. When the pH of medium is above the isoelectric point of amino group, basic amino acids are formed and so the rate of condensation reaches maximum.

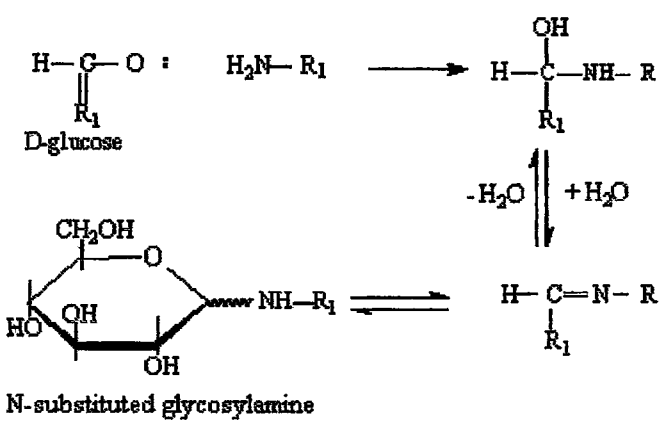

Figure 1.2 Reaction of aldose with amino compound to give N-substituted glycosylamine

b) Amadori rearrangement

It is an acid-base catalysed conversion of the N-substituted glycosylamine to an N-substituted 1-amino-1-deoxy-2-ketose. N-substituted glycosylamine is very unstable, thus attacked by various rearrangements (Figure 1.3). The transition from an aldose to a ketose sugar derivative is referred as the amadori rearrangement that involves protonation of nitrogen at carbon-1. In the case of ketones and amines, ketosylamines are formed which then undergo the heyns rearrangement to form 2-amino-2-deoxy aldoses by protonation of the oxygen at carbon-6. Weak acid catalyzes the amadori reaction where it has significant effect on protonation of schiff base and subsequent proton schift constitue. Whether there is no added acid in the medium, amino acids serve as their own acid catalysts, so the reaction rate is rapid in the absence of acid (Namiki, 1988).

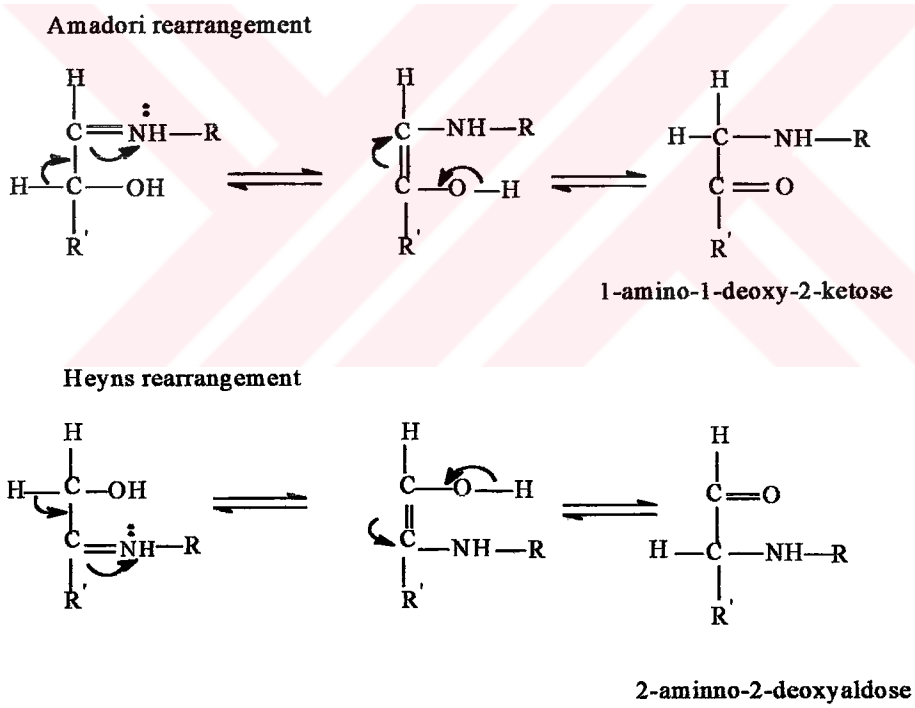

Figure 1.3 Amadori and Heyns rearrangements. R'= $(CHOH)_3CH_2OH$ and R represents either an alkyl group or amino acid moiety (for glucose derivative)

Inhibition of Amadori reaction prevent production of colored substances, termed as melanoidins. This realizes the importance of amadori reaction for Maillard reaction (Eichner and Ciner-Doruk, 1981a; Feather, 1985). The amadori rearrangement is considered to be key step in the formation of major intermediates for the browning reaction (Davies and Labuza, 1997). These are various heterocyclic compounds, with

important flavor characteristics, and intermediates that cross-link proteins in food. The importance of the decomposition of Amadori products is not limited to the formation of flavor, color and melanoidin formation but also there is carcinogen and antioxidant effect (Friedman, 1996).

Stage 2 (Intermediate stage)

The second stage involves sugar dehydration and fragmentation and amino acid degradation via strecker degradation reaction in which the carbonyl compounds react with amino acids. The amadori products degrade via several pathways, depending on the pH of the reaction solution, the basicity of the amine attached to the sugar, and the temperature. Two of these proceed by 2,3 and 1,2 enolisation of the amadori compound to give a variety of products including α-dicarbonyl compounds. 2-Furaldehydes are distinctive end product of the first branch, where as C-methyl aldehydes, keto-aldehdes, ketols, and reductones are products of second. The third pathway is the strecker degradation reaction (Hodge, 1967). Browning and flavor are considered to be caused by additional reactions of these active intermediates with amino compounds (Namiki, 1988).

a) Sugar dehydration

Sugar dehydration reactions depend on the pH of system (Figure 1.4). In acidic media, an enolization of the amadori product gives the 1,2 enodiol form, which is followed by the elimination of hydroxyl group at C-3 and subsequent deamination at C-1 yields the 3-deoxyosulose after water addition. 3-deoxyosulose then degrades to form colored products and flavor compounds by dehydration and translocation reactions. Most important ones are hydroxymethyl furfural and furfural. Hydroxymethyl furfural is quality criteria for most of food products and indicates level of browning (Lee and Nagy, 1988a).

In basic media, enolization of 1-amino-2-ketose occurs at 2,3 position to irreversibly produce 2,3-enediol. This undergoes a series of changes including the loss of the amine from C-1 to form a methyldicarbonyl intermediates (pyruvaldehyde, hydroxydiacetyl, diacetyl).

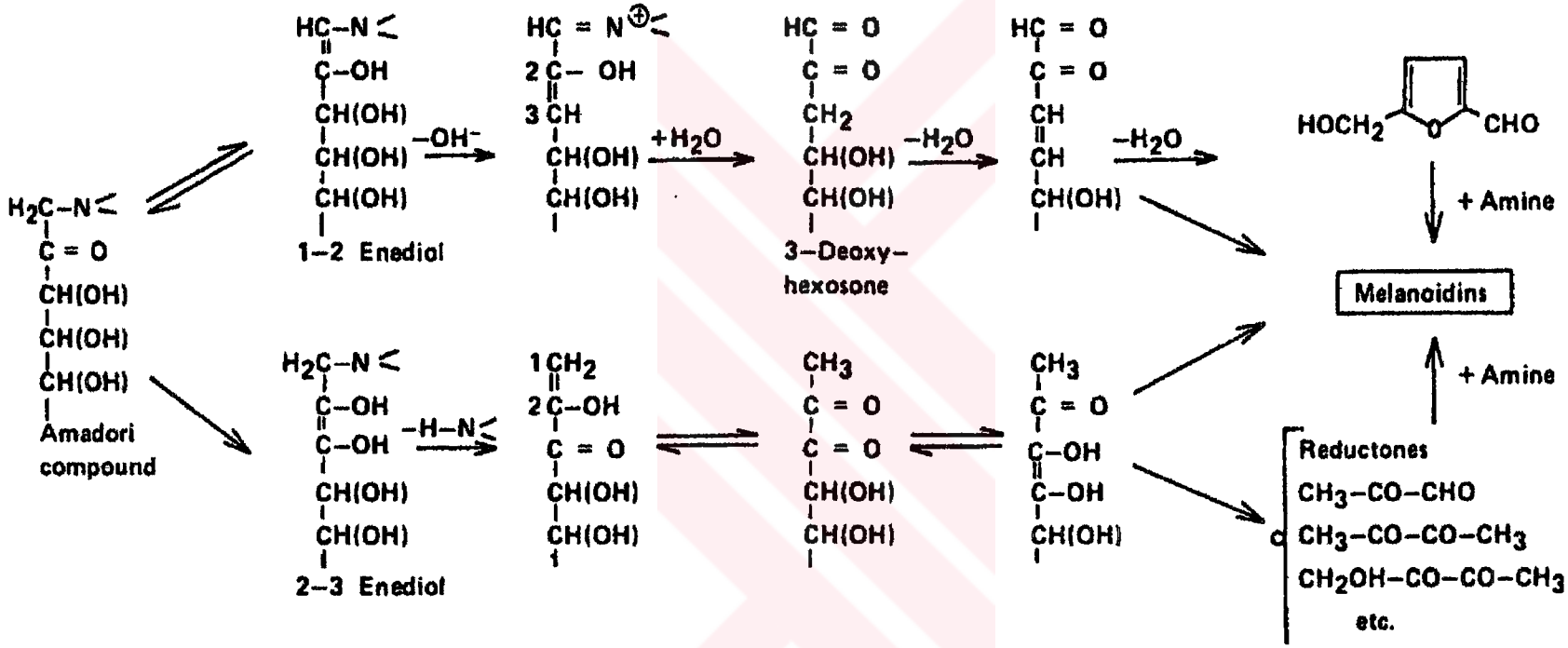

Figure 1.4 Decomposition of amadori products

b) Sugar fragmantation

Subsequent to the formation of the amadori products, alternative pathways available for the next stage in the browning sequence. The second reaction with amadori rearrangement products is fragmantation of the sugar moiety. The subsequent reactions involve aldol condensation and polymerization reactions under amine catalyst and products formed are classified as aldols, amino-free poymer and free amino compounds (Labuza and Baisier, 1992).

c) Strecker degradation

At high temperatures, the third reaction that occurs with Amadori rearrangement products is strecker degradation (Figure 1.1). The strecker degradation of amino acids involves their oxidative degradation by carbonyl compounds or reductones which arise from the degradation of ketosamines. In this degradation reaction, amino acids first react with products of schiff bases and undergo acid catalyzed decarboxylation. The new schiff base is easily hydrolyzed to form an amine and aldehyde. The aldehyde produced from the stecker degradation is considered to be directly responsible from browning, because they can condense with themselves, with sugar fragments, with furfurals, or with other dehydrated products through production of brown pigment. The fact that different aromas are produced by the same reaction mixtures heated to different temperatures shows that cross reactions between the sugar carbonyl compounds and the strecker aldehydes probably occur (Hodge, 1967). This reaction is charachterized by the production of CO_2 and the transamination reaction which is important for the incorporation of nitrogen into melanoidins. The aldehydes formed are important as auxillary flavor compounds. This pathway, however is not the major color producing reaction and is better known as the source of off-flavors associated with Maillard browning (Hodge, 1953).

Stage 3 (Melanoidin formation)

The final products formed in the Maillard reaction are polymers or melanoidins. The formation of melanoidins is result of polymerization of the highly reactive intermediates that are formed during the advanced Maillard reaction. Most of color intermediates and other reactive precursor products such as enaminol products, low molecular weight sugar anologs and unsaturated carbonyl products are produced. Aldol condensation, aldehyde-amine polymerization, and the formation of

heterocyclic nitrogen compounds such as pyroles, imidizoles, pyridines and pyrazines are basic reactions that lead to the formation of melanoidins at this stage (Feather, 1985; Namiki, 1988).

The structure of melanoidin is considered to consist mainly of repeating aromitized moiety because of its dark brown color. Melanoidins are generally insoluble in water, organic solvents and vary widely in molecular weight and have featureless electronic spectra in the visible region with absorbance decreasing as wavelenght increases (O'Brien and Morrisey, 1989). The molecular weight of these compounds increases as browning proceeds, untill they become insoluble high molecular weight substances.

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. It was showed that elemental analyses of polymers (carbon, hyrogen and nitrogen) resulted that amino acid was combined into the polymer (Feather, 1985). The nature of the heterogenous melanoidin structure makes it hard to seperate individual compounds. There is evidence of heterocyclic compounds in melanoidin structure. It was observed that basic amino acids and sugars form melanoidins that have high molecular weights than those formed by neutral or acidic amino acids. This indicates that cross-linking is probable (Namiki, 1988).

ii) Factors influencing the Maillard reaction

The extent of reaction is controlled by many factors. The factors influence the Maillard reaction are reactant type, sugar:amine ratio, temperature, moisture content, pH, metals.

1) Reactant type

a) Type of amino acid

The nature of the amino compound affects the rate of Maillard browning. Some amino acids have two reactive groups, so they rapidly react with the sugars moiety. Lysine is hypothesized as being the most reactive amino acid due to its two reactive amino groups (O'Brien and Morrisey, 1989).

Most studies have been carried out on the relative reactivities of free amino acids. Ashoor and Zent (1984) suggested a classification of amino acids in three groups depending on the extent of browning when reacted with glucose at different pH, and 121^{0}C for 10 minutes. The most reactive amino acids were lysine, glycine, trytophan and tyrosine. Wolform *et al.* (1974) studied the effect of amino acid type on extent of browning. They found that, L-arginine and 4-aminobutyric acid are the most intense for browning and they are followed by glycine, alanine, serine, and L-proline. Aspartic acids, L-glutamic acid, L-glutamine were found to behave similarly to glycine. Labuza and Massaro (1990) studied browning of model systems composed of glucose and either lysine, trytophan, and cysteine alone or in combination. They observed that the molar rate of browning with all amino acids combined was significantly less than that of sum of the individual rates, indicating interactions and inhibiton.

In general, the amount of browning has been shown to increase with chain length of the peptide. However, the amount of browning is not proportional to the conversion of amino acid or peptides, as the degree of browning depends on the type of melanoidin formed during the Maillard reaction. When the degree of sugar decomposition was measured; it was appeared that small peptides (up to 3 amino acid groups) were more reactive than the corresponding amino acids. Melanoidins from peptides exhibited a darker degree of color than those from amino acids (Davies and Labuza, 1997).

b) Type of sugar

Reducing sugars are essential in these reactions, providing the carbonyl groups for intereacting with the free amino groups of amino acids, peptides, and proteins (Eskin, 1990). Low molecular weight compounds tend to be more reactive than the high molecular weight compounds as a result of greater steric hindrance in latter. Accordingly, aldopentoses are generally more reactive than aldohexoses and monosaccharides are more reactive than di- or oligosaccharides. Aldoses in general appear to be more reactive than ketoses, appearently a consequence of the more sterically hindered carbonyl group of ketoses (O'Brien and Morrisey, 1989).

Reactivity of sugars is dependent both on the concentration of acyclic or straight-chain form, thus only the acylic form with free aldose or hexose group can react. For sugars, the rate of reaction depends on the rate at which the sugar ring opens to the reducible, open-chained form and this increases with increasing pH. However, the relative concentration of acyclic sugar present does not complately explain the differences between reactions observed for the different sugars. The relative rates of browning of glucose and fructose also depend on the extent to which the reaction mixture is buffered. In buffered media, the rate of browning of fructose with amino acids is greater than that of glucose (Davies and Labuza, 1997). Buera *et al.* (1992) showed that, browning in glucose containing systems showed a stronger dependence on the amino compound concentration than fructose solutions. Fructose browned faster than glucose in low amino compound concentration systems.

It was observed that the increasing order of brown pigment formation is: D-xylose>L-arabinose>hexoses>disaccharides (Hodge, 1953). For hexoses, the order of reactivity is D-galactose>D-mannose>D-glucose. Reducing disaccharides are considerably less reactive than their corresponding monomers. D-fructose has been reported to brown at a much faster rate than glucose during the initial stages of browning reaction, but it then falls behind. This was confirmed using model systems containing glucose-glycine and fructose-glycine (Lee and Nagy, 1990).

2) Sugar: Amine ratio

The extent of browning seems to vary according to the sugar:amine ratio. It was found that, at a molar ratio of 1:1 the development of color in a glycine-glucose reaction system was higher than at a ratio of 10 sugar to 1 amino group (Wolfrom *et al.*, 1974). O'Brien and Morrisey (1989) stated that, an excess of reducing sugar over amino compound promotes the rate of Maillard browning, since there are mechanistic differences in the destruction of sugar compared to amino acid.

However, Warmbier *et al.* (1976a) found that, the browning rate increased to a maximum at a ratio of one glucose to 3 lysine. Since, the initial step of formation of the schiff base is dependent on the concentration of both sugar and amino acid. Schiff base formation increases with decreasing the sugar:amino acid ratio. Labuza and Baisier (1992) showed that the ratio of amino acid to reducing sugar can effect the rate of browning. The effect of increasing the amino concentration show a greater

increase in browning than that of increasing the sugar content on a molar basis and the increase for both is greater than the relative concentration increase.

3) Temperature

Since the Maillard reaction consist of several reaction steps, each with a possibly different temperature sensitivity, it strongly depends on temperature which reaction route preveails (Boekel, 2001). In general, the rate of browning increases with rise in temperature. Temperature affects the activities of the reactants. The active form of the sugar is considered to be the open chain, the concentration of which increases with temperature. At high temperatures, mainly Strecker degradation take place and different flavors are formed at different temperatures. The lenght of heating is also important as the formation of melanoidins usually occurs at a rate which increases in proportion to the square of the reaction time at any given temperature and different flavors are formed depending on the extent of reaction (Davies and Labuza, 1997).

The effect of temperature is best defined by the temperature dependence of the rate constant as shown by the Arhenius type equation.

$$k = k_0 (\exp[-Ea/R*T]) \qquad (1.1)$$

where k_0 is the absolute rate constant, Ea is the activation energy of browning (kcal/mole), R is universal gas constant (8.314 kJ/kg mol.K) and T is the temperature of products in K. From the slope (-Ea/R) of the plot ln k versus 1/T, the activation energy is estimated.

4) Moisture content

The Maillard reaction proceeds rapidly in intermediate moisture levels, although complete dehydration or excessive moisture levels inhibit this process (Eichner and Karel, 1972). For the first stage of the Maillard reaction to occur, water is essential. Thus the rate of this reaction is dependent on the amount of free water available as related to water activity. The region where the maxima occurs is usually near a_w of 0.65-0.70 (Labuza and Saltmarch, 1981). Water content exerts its influence by controling the liquid phase viscosity, by dissolution, concentration or dilution of reactants (O'Brien and Morrisey, 1989).

The effect of water is complex and dependent on the presence of various water binding agents among other factors. In the intermediate moisture range of foods, the reaction rate becomes maximum due to increased mobility of reactants. However, reduction in reaction rate is observed both at high and low moisture levels. At higher moisture levels, the lower reaction rate has generally been attributed to dilution of reactants. Since water is produced in the reaction, it is likely that the a_w of mass action also leads to a decreased rate of reaction at high moisture levels (O'Brien and Morrisey, 1989). On the other hand, mobility of reactants is lowered by increasing diffusion resistance of system at lower moisture content (Eichner and Karel, 1972). Overall effect of water content can be modified by various substances like humectants such as glycerol. Eichner and Karel (1972) and Warmbier (1976b) found that both liquid and solid systems containing glycerol had nonenzymic browning rate maximas in the a_w range 0.41-0.55.

Maillard reaction is also influenced by physicochemical state of food system that is amorphous, crystalline, or other factors such as fat content (O'Brien and Morrisey, 1989). At low water activity, an amorphous food system absorbs much water in the spaces between the molecules, while in a crystalline system absorption of water can take place only at the surface crystal lattice. The amorphous phase absorbs water until the molecules acquire sufficient mobility and space to form a crystal lattice. As crystallization is initiated, water is expelled and may become trapped in localized areas within the food. This water is then available for intereaction with other food components, and affects the rate of the Maillard reaction unless it evaporates (Karmas *et al.*, 1992).

5) pH

Both the initial pH of the product and the buffering capacity of the system, influence the rate and direction of the Maillard reaction. The rate of browning is low at acid pH values and increases with increasing pH to a maximum at a pH of 10 (Ashoor and Zent, 1984). It was observed that, reaction generally has a minimum pH at 3 and optimum pH above 7. At a pH<3 and pH>9, other nenenzymic reactions complete with the Maillard reaction. The decrease in reaction rate at high pH values due to a deficiency of H^+ ions which are required to catalyze both the Amadori compound is also influenced by pH. For example, degradation take place via the 1,2-E pathway at low pH, while the 2,3-E route is favored at alkaline pH values.

The pH dependence of the initial step of the reaction can be related to the amount of unprotonated amine present which is controlled by the following equilibrium:

$$-NH_3^+ \longleftarrow pH>7 \longrightarrow -NH_2 + H^+$$

At the pK_a of the amine group, by definition, half the amine is present as the protonated NH_3^+ state preventing electron transfer. So, the rate of the Maillard reaction is lower at a pH lower than pKa of the reactive amino group that can easily react with aldose or ketose compounds (Labuza and Baisier, 1992). The rate of the reaction is also dependent on the concantration of the acylic sugar present. The amount of acylic sugar increases with increasing pH and so increases the rate of reaction (Davies and Labuza, 1997).

Influence of buffer: The role of buffer in nonenzymic reactions has been shown to increase the rate of browning for sugar-amino acid systems as a result of their influence on the ionic environment in which the reaction takes place (Eskin, 1990). The Maillard reaction forms H^+ ions, so decreasing the pH system which results decrease in the reaction rate. Thus, to control the pH of system during experimental studies (eg. model studies) buffers should be used. Buffers have been shown to increase the rate of browning.

6) Metals

The formation of metal complexes with amino acids can influence the Maillard reaction. This reaction is catalyzed by copper and iron, while manganase and tin inhibit the reaction (Eskin, 1990). Kato *et al.* (1981) reorted that, browning rate was increased in the presence of Cu^{+2} and Fe^{+3} in ovalbumin-glucose mixture, while Na^+ had no effect. Besides, Fe^{+3} was more effective than the Fe^{+2} in the accelerating the browning reaction, which suggested that the first step of reaction was an oxidation activation that results reduction of metals.

iii) Consequences of the Maillard Reaction in food

Maillard reaction affects nutritional and sensory properties of food during storage and processing. Apart from the brown discoloration, the reaction also decreases nutritional value and solubility, creates off-flavors, and induces textural changes.

Color: The most characteristic consequences of the Maillard reaction is formation of brown color. Browning has been used as a measurable symptom of the Maillard reaction and extent of browning provides a quantitative indicator of the extent of chemical reaction. In many instances, it is highly desirable part of food processing such as in the processing of bread or pastries. However, Maillard browning is detrimental to product quality if consumers find the browned product unappealing such as fruit products (Fayle and Gerrard, 2000). Browning mainly occurs during final stage of Maillard reaction where most of the color intermediates and other reactive precursors (such as enaminol products, low molecular weight sugar anologs, and unsaturated carbonyl products) are produced. The chief reactions responsible from brown color are thought to be aldol condensation, aldehyde-amine polymerization, and formation of heterocyclic nitrogen compounds such as pyrroles, imidazoles, pyridines, and pyrazines (Hodge, 1953). Polymerization of products from the second step and copolymerization with amino compounds yield the colored products (Warmbier *et al.*, 1976b). Generally, it is easy to measure extent of browning in foodstuffs with different methods such as image analysis or by measurement of intensity of color and rarely by photometric detection (Fayle and Gerrard, 2000).

Aroma and Flavor: Flavor production by nonenzymatic browning in foods proceeds mainly from reactions of reducing sugars with amines, amino acids, peptides, and proteins. The Maillard reaction plays a role in forming distinctive flavors of many foods although off-flavor is favored in some products through the aldehyde and sulfur compound formation. Hundred of volatile products have been identified in real or model food systems and may be classified into three groups.

1. Simple sugar dehydration/fragmentation products: furans, pyrones, cyclopentones, carbonyls and acids.
2. Simple amino acid degradation products; aldehydes and sulphur compounds.
3. Volatiles produced by further reaction; pyroroles, pyridines, imidizoles, pyrazines, oxasoles, thiazoles and aldol condensation products (O'Brien and Morrisey, 1989).

Browned flavors include caramelized sugar aromas; food aromas that have been described variously as toasted, baked, nutty, or roasted; corny and amine-like aromas from cooked grains and meals; and both desirable and undesirable burnt aromas and bitterish tastes of roasted malt, nuts, coffee, chicory, cocoa, meats, fruits, and vegetables. The objectionable burnt and bitter flavors of overheated or long-stored dehdrated foods also falls within this class (Hodge, 1972). Amadori compounds (1-amino-1-deoxy-2-ketose) are important nonvolatile precursors in the formation of browned flavor compounds from Maillard reactions (Hodge, 1967). Strecker degradation of aldehydes, 2-furaldehydes and methyl ketones contribute to bread, peanut, cocoa, and roasted aromas. Most of the acyclic sugar degradation products have a sharp, penetrating pungency that hinders an evaluation of their possible contribution to caramel flavor (Hodge, 1967). Acceptable browned aromas are provided by compounds from the classes of alkyl-(or acyl-) substituted pyrazines, dihydropyrazines, pyrrolines, piperideines, enolic dihydrofuranones, enolic dihydropyrones, and enolic pyrones (Hodge, 1967).

Texture: Maillard reaction can result cross linking of protein that has predefined effects on food texture. It has been demonstrated that heating proteins and reducing sugars together reduces the solubility of proteins, and that the proteins are covalently cross linked by Maillard chemistry.

Nutritional quality: The Maillard reaction may seriously reduce the nutritional value of foods through the destruction of essential amino acids, the production of antinutritive and toxic compounds, decrease in digestibility and inhibition of proteolytic and glycolytic enzymes. Even in foods where no browning or off-flavor is evident, the nutritive value may be considerably reduced due to the formation of colorless Amadori compounds. Amadori compounds are generally regarded as having no nutritional value. Nevertheless, it has long been known that Amadori compounds are superior as sources of nitrogen than the complete absence of amino acids (O'Brien and Morrisey, 1989). Lysine destruction is the most significant consequence of the Maillard reaction in most of foods especially cereal based products (Garcia-Villanova *et al.*, 1993; Ames *et al.*, 1997; Fernandez-Artigas *et al.*, 1999; Ramirez-Jimenez *et al.*, 2000a). Depending on the severity of heat treatment, significant losses of valuable amino acids such as sulfur amino acids, arginine, trytophan occurs. Aggregation of proteins on heating is known to decrease protein

digestibility and loss of valuable amino acids such as lysine. There are several available methods for measuring chemically available lysine residue. In addition to the obvious effect of structural changes in protein on digestibility, certain products of the Maillard reaction may also inhibit digestive enzyme activity (O'Brien and Morrisey, 1989).

Freidman (1996) summarized chemistry, biology, pathology of browning products and the impact on human nutrition and health. Beneficial and mutagenic Maillard products including antimutagens, antioxidants, antibiotics, carcinogens were well described. Mutagenic and carcinogenic products in cooked protein-rich foods are formed by several mechanisms, including carbohydrate caramelization, protein pyrolysis, amino acid/creatinine reactions, and amino-carbonyl reactions, in which free amino groups condense with reducing sugars to produce brown melanoidins, furans, carbolines, and a variety of other heterocyclic amines. N-nitrosoamines, the most hazardous of the carcinogens produced by the interaction of food components, are produced mainly from secondary amines and nitrous acid. Since the Maillard reaction produces a number of secondary amines including the amadori products, the possibility of mutagen formation can not be ignored (Namiki, 1988). Besides, there are many bioactive compounds, some of which may be beneficial such as antioxidant properties at the end of Maillard reaction (Namiki, 1988). The antioxidative activity observed in Maillard reaction is assumed to be present mainly in the reductones due to their reducing activity and metal chelating ability.

1.1.2.2 Caramelization reaction

It is known that sugars, polysaccharides, polyhydroxycarboxylic acids, reductones, α-dicarbonyl compounds, and quinones will undergo browning in the absence of amino compounds. Such decompositions, even in the absence of catalysts, occur mainly at high temperatures not often encountered in normal food processing (Hodge, 1953). Direct heating of carbohydrates, particularly sugars and sugar syrup, produces a complex group of reactions termed as caramelization. When sugars are heated above their melting points they darken to a brown coloration under alkaline or acidic condition. The produced flavor; changes from mild, caramel-like and sweet to burning bitter. Caramelization products are obtained form strongly heated food surfaces like baking and roasting of bread, the processing of foods with high sugar content such as jams and certain fruit juices or in wine production Caramel colors,

ie, ammonia caramel, ammonia sulphite caramel, and the caustic caramel are the most widely used additives and are found as coloring agents in a wide range of foods and beverages (Kroh, 1994).

First of all, sugars undergo intramolecular rearrangements during the thermally induced caramelization. Figure 1.5 shows a sequence of sugar degradation reactions. The first step involves the stepwise conversion of D-glucose to D-fructose D-mannose, referred as the Lobry de Bruyn-Albedra van Eckenstein transformation. It is followed by dehydration or β elimination, dicarboxylic cleaving, retro-aldol reaction and later, aldol condensation and, finally, a radical reaction.

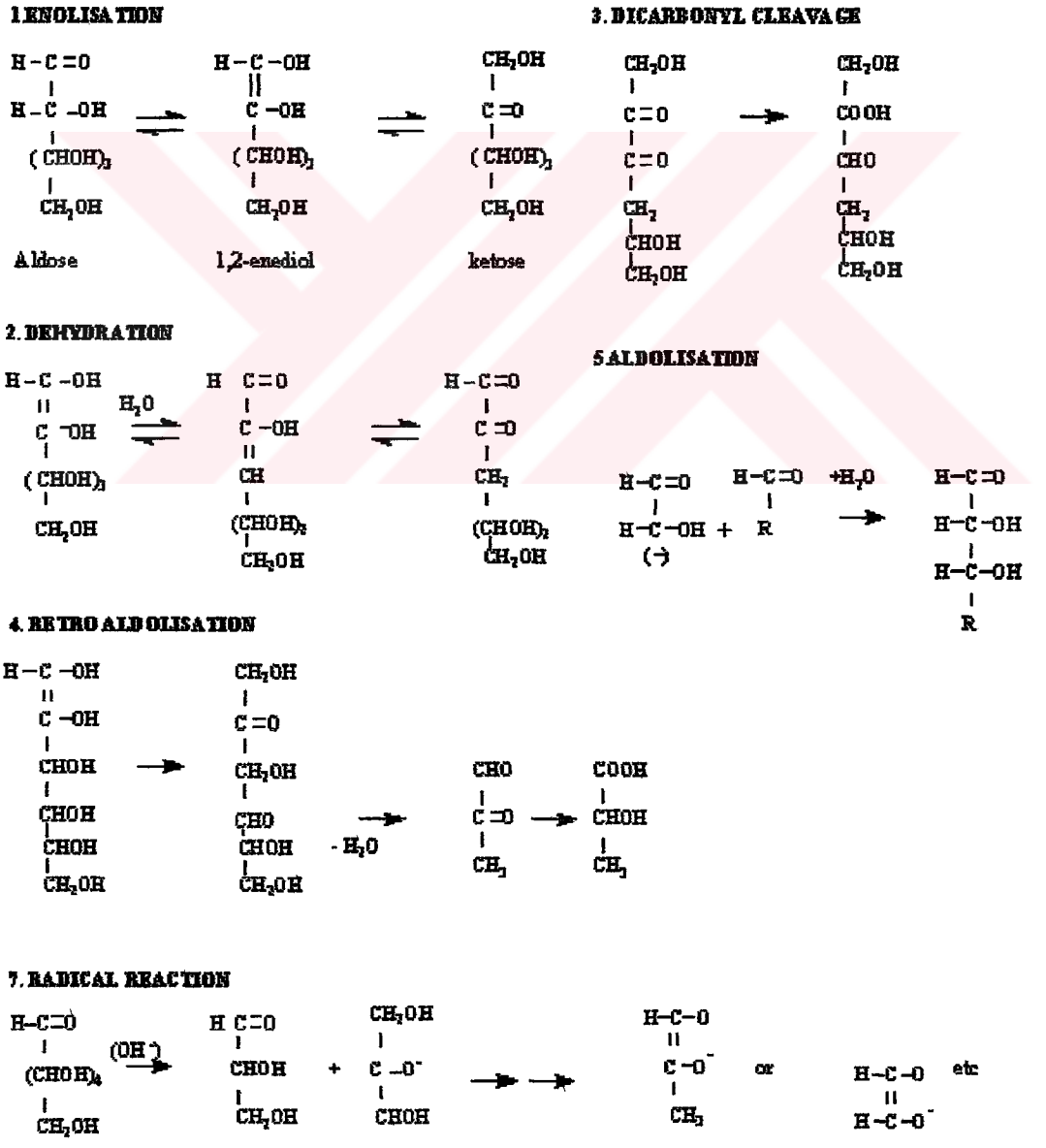

Figure 1.5 Sugar degradation reactions

The enolization reaction is of particular importance because it initiates the subsequent chain of events. These reactions give rise to aliphatic sugar degradation products which can react to produce oxygen heterocyclic and carboxylic compounds via aldol condensation. Transformation of reducing sugars of the Lobry de Bruyn type occur both in alkaline and in acid media. Furfural formation is strongest in highly acidic media (below pH 3), but it occurs to a measurable extent at elevated temperatures even in the pH range 6 to 6.7 (Hodge, 1953).

Some typical components of caramel flavor are shown in Figure 1.6. According to conditions in which carmelization take place, compounds produced from reactions changes. Influencing parameters are temperature, acidic or basic environment.

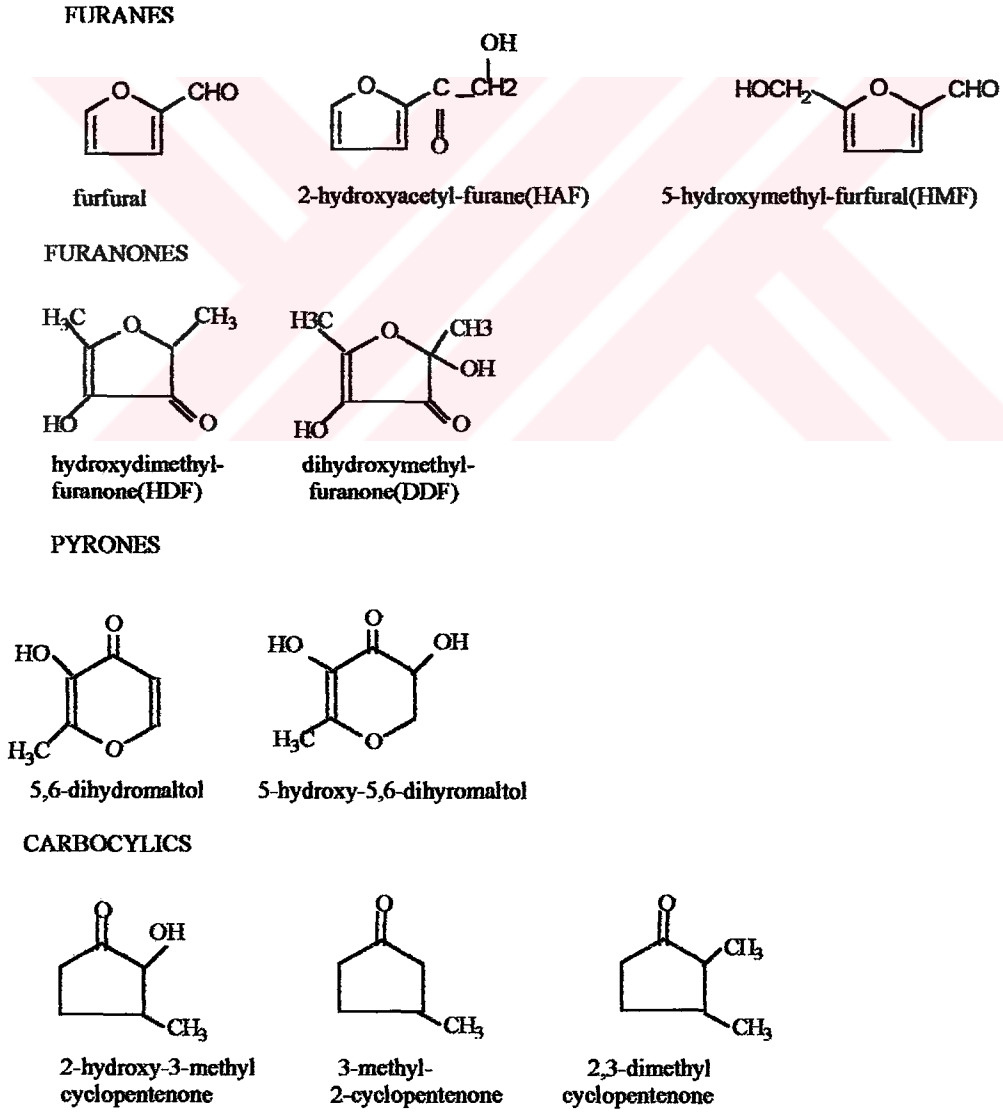

Figure 1.6 Typical caramel aromatics

While the thermally and /or acid-induced dehydration and cyclization reactions dominate, there is an increased tendency for cleavage of the carbon chain of the sugar under basic conditions. Thus, depending on the reaction conditions derivatives of furan such as hydroxymethyl furfural (HMF), hydroxyacetyl furan (HAF), furanones such as hydroxymethyl furanone (HDF), dihydroxymethyl furanone (DDF), and pyranones are produced. Derivatives of cyclopentenane are examples of carboxylic products (Kroh, 1994). These aromatics are not only responsible from caramel color but also responsible from typical caramel flavor (Hodge 1972).

The visible sign of a caramelization reaction is the formation of a brown product that is caramel. Typical structure of caramel is shown in Figure 1.7. However, structural characteristics of caramel and which stage it occurs are still studied like polymers obtained from Maillard reaction. It was concluded that, HMF, furfural and HAF are considered to be precursors of such regular polymers. The HMF concentrations measured in solvent extractors of caramel colors are significantly greater than that of the comparable volatile compounds, as the reaction proceeds. Thus, the caramelization reaction could be followed kinetically by the measurement of HMF concentration and such measurement could be introduced into the control of food processing operations (Lee and Nagy, 1988a).

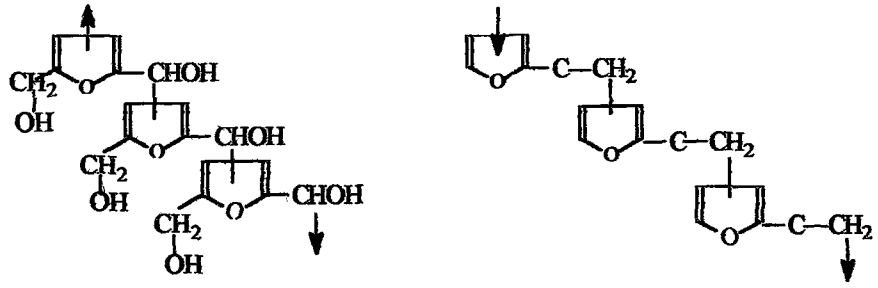

Figure 1.7 Selected hypothetical polymer structure of sugar caramel

1.1.2.3 Ascorbic acid oxidation

Browning in citrus juices, especially concentrates and more specificially in lemon and grape fruit concentrates, has been found to be caused by the decomposition of ascorbic acid. This kind of browning takes place only after the bulk of ascorbic acid has dissappeared. In fact, if ascorbic acid is heated with an acid, it is finally transformed into furfural with the formation of CO_2.

The mode of decomposition of ascorbic acid is mainly oxidative, but nonoxidative ascorbic acid decomposition also occurs. Ascorbic acid is most stable at pH 6, when anaerobically decomposed at pH 2, 4, or 6. Furfural is the main product of the reaction under strongly acidic condition. The most important factors in the oxidative decomposition of ascorbic acid are pH and the presence of metal ions. Decomposition is rapid in an alkaline medium and is much slower at a pH less than 7 in the absence of metal catalyst (Namiki, 1988). Amino acids accelerate the ascorbic acid breakdown, and in the presence of amine it is dehydroascorbic acid that is the reactive intermediate in the pathway to furfural and brown pigment production. Studies showed that L-ascorbic acid decomposition resulted in furaldehyde and hydroxymethyl furaldehyde formation (Roig *et al.*, 1999). It was observed that, when ascorbic acid breaks down by acid catalysis, furfural is the main end product. It was also concluded that acid catalysis is functional even under oxidative conditions and that ascorbic acid is partially responsible for orange juice discoloration (Handwerk and Coleman, 1988).

There were many decomposition products of ascorbic acid degradation which were mainly dehydroascorbic acid, 2,3-diketogulonic and oxalic acids. Besides the browning of ascorbic acid by the interaction of various decomposed carbonyl compounds, the reaction of carbonyl products (notably α-dicarbonyl compounds) with amino compounds leads to the active formation of pigments and flavors.The reaction of Ascorbic acid in fruit juices and concantrates are very much dependent on pH and concantration of juices, browning process being inversly proportional to the pH over a range of 2-3.5. Those juices with a higher pH are less susceptible to browning such as orange juice with a pH of 3.4. Below pH 4.0, browning is due primarily decomposition of ascorbic acid to furfural (Eskin, 1990).

1.2 Analysing the Nonenzymic Browning Reactions in Foods

Several changes in the properties of foods have been attributed to the nonenzymic browning reactions. These include changes in color, particularly browning and, to a lesser extent, fluorescence; production of aroma and flavor compounds; production of bioactive compounds, both beneficial and toxic; loss of nutritional quality, especially of proteins; and changes in texture. In order to correlate changes in each of individual properties of a particular food with, quantitative methods are required for each property of interest. Developing a method to monitor the concentration of a

single chemical compounds, or group of compounds, is substantially easier than monitoring a complex mixture in its entirety. In many cases, the monitoring of particular molecule, or biomarker are useful methods for determination of extent of reactions (Fayle and Gerrard, 2000). Methods to measure the extent of Maillard reactions in food systems include chemical methods, immunochemical methods, mammalian growth bioassays, and submammalian growth bioassays. Many of these methods are related to the individual steps and products of the reaction (O'Brien and Morrisey, 1989).

Most of the chemical methods used to evaluate the nutritional quality of heated protein foods are based on the measurement of available lysine. A variety of reactions are employed with variable specificity for the ε-amino group of lysine: fluorodinitrobenzene (FDNB) method, guanidation, trinitrobenzene sulfonic acid (TNBS) method, borohydrate reduction, dye-binding, succinylation. When protein bound or free ε-fructose-lysine and/or ε-lactulose-lysine is subjected to acid hydrolysis, it is hydrolyzed to yield lysine and two unique amino acids termed furosine and pyridosine. Thus, by measuring the furosine content after acid hydrolysis, one can calculate the quantity of lysine present as bound lysine in the form of lactul-lysine or fructose-lysine. As a method of assessing lysine destruction in a food, this method is more direct than the methods which measure reactive lysine and is most specific chemical method available for detecting early Maillard reactions. More recently, furosine content is determined by easier method, HPLC. Chiang (1983) developed an HPLC method to measure furosine in heated and stored foods. This HPLC method complements the amino acid analysis method and appears to be fast, simple, and economical. Morales *et al.* (1996) studied heat-induced changes of commercial milk by measuring both Hydroxymethyl furfural and available lysine content. Advanced Maillard reaction can be followed by determination of ε-pyrrole-lysine whose concentration increased dramatically with prolonged heat treatment and it is a better indicator of long-term storage at elevated temperatures than furosine (Friedman, 1996).

Considerable progress has been made using concept of odor activity, which is a parameter relating to the threshold concentration at which a compound must be present before its odor is detectable. Once these parameters are defined and quantified, chyanges in flavor and aroma during Maillard chemistry can be followed

(Fayle and Gerrard, 2000). Determination of Strecker degradation volatiles has also been exploited as an indicator of Maillard reactions in foods. Eichner and Ciner-Doruk (1981) proposed the determination of isovaleraldehyde, the Stecker degradation product of leucine as a suitable early indication of sensory changes caused by Maillard reactions in dried foods.

Nonenzymic browning reactions in foods reactions can be followed by HMF determination. HMF determination was generally made by colorimetric thiobarbituric acid method, and more recently HPLC and GC techniques.

Many useful and rapid methods are available for determining the color of heat-treated foods. At the simplest level, browning of a whole food can be monitored by visual inspection and comparison to standart samples, or by image analysis. Food extracts and model systems, presuming that they can be made from non-turbid solutions, can be monitored as they brown by following the absorbance at a chosen wavelenght (typically 420-460 nm). While brown color correlates well with heat treatment, it is not a sensitive criterion of the extent of Maillard reactions as compared with other methods. More recently, technique of capillary electrohoresis has been used to separate melanoidins (Royle *et al.*, 1998).

The use of bioassays to assess the loss of amino acid residues may be misleading unless the susceptible aminoacid is limiting in the first instance. Recently, attempts to develop a rapid assay to follow Maillard reactions in food proteins have focused on changes in the antigenic determinants of the glycosylated proteins. Microbiological evaluation of protein quality has been in use for long-term using the protozoan *Tetrahymena pyriformis* (O'Brien and Morrisey, 1989).

1.3 Hyroxymethyl Furfural (HMF)

1.3.1 HMF Formation in Foods

HMF is the end product of acid-catalyzed hexose dehydration. It is an indicator of the Maillard reaction and potential browning. In fresh foods, HMF level is close to zero. However, it is found to be a significant level in processed foods and it is often used as a quality indicator (Lee and Nagy, 1988a).

In food stuffs, HMF is resulted from occurance of nonenzymic browning reactions. Thus, there should be nitrogen containing compound, carbonhydrate source and

suitable environment. For the Maillard reaction, it occurs when N-substituted glycosylamine is degraded to 3-deoxyhexosuloses under acidic condition. By elimination of water from 3-deoxyhexosulose, the unsaturated compounds are formed, ring closure of hydroxymethyl furfural take place. In caramelization reaction, derivative of furan such as hydroxymethyl furfural (HMF) and hydroxyacetyl furan (HAF) formed. Significant concentrations of HMF can be measured after thermal or acid-catalyzed caramelization of D-glucose. The HMF concentration measured in solvent extracts of caramel colors is also significantly greater than that of the comparable volatile compounds as the reaction proceeds (Kroh, 1994).

1.3.2 Significance of HMF Formation for Foodstuffs

HMF is a recognized indicator of deterioration for a wide range of foods containing carbonhydrates such as honey, jam, pekmez, fruit juices and concentrates, alcoholic beverages, cereal products, tomato paste, vinegar and it is formed as a result of excessive heating or storage in nonadequate condition.

Jam

During manufacture of jams, severe heat treatment is applied to destroy spoilage organisms in jams. Furthermore, they are usually stored for long periods of time. HMF formation is judged to be the most useful method for assesing the effectiveness of heat treatments in destroying spoilage organisms in jams and fruit products (Rada-Mendoza *et al.*, 2002).

There are mainly two possible ways for the formation of HMF in jam; conversion of aminodeoxy ketose to HMF during Maillard reaction and decompostion of hexoses to HMF at high temperatures. It was reported that HMF formation in jam influenced by reducing sugar and amino acid content, pH and both heating temperature and time (Ekşi and Velioğlu, 1990).

Heat treatment of jam can be made with vacuum type processing or in open tank. The differences in processing types of jam expose variable HMF contents of jam in market. According to Turkish Standart Institue, HMF content of first class jam must not exceed 25 ppm and 50 ppm for second class jam (Anonymus, 1983).

Honey

There is low HMF value in fresh honey and a high amount in honey that has been heated, stored in nonadequate conditions or adultered with invert syrup (Nozal *et al.*, 2001; Costa *et al.*, 1999). Sugars represent the largest portion of honey composition (95-99 % of the honey solids) and it mainly consists of fructose and glucose. The protein content of honey is normally less than 0.5 % and small proportions of honey proteins are enzymes.

Honey is submitted to thermal treatments for two different reasons: 1) to modify its tendency to crystallization or delay its appearance and 2) to destroy the microorganisms which contaminate it (Tosi *et al.*, 2002). Owing to the low pH value of honey (about 3.9) and its high fructose and glucose content, the heating has to be mild (e.g. 65^0C for 30 sec). However, honey is generally subjected to the higher temperatures during industrial treatments and usually stored at room temperature for very long periods (Villamiel *et al.*, 2001).

Adulteration techniques of honey based on two different principles; dilution of honey by water addition, and extension with sugar and syrups (e.g. corn syrup, high fructose corn syrup (HFCS)), other adulterations are due to bee feeding with sugars and sugar syrup (Anklam, 1998). The European quality standarts allow a maximum of 40 mg HMF per kg of honey. Extremely high HMF(>500 ppm) values demonstrate an adulteration with invert syrup (Lo Coco *et al.*, 1996).

Pekmez

Pekmez, named as boiled juice, is a traditional food that is produced widely from grape, mulbery and fig. It is produced by concentration of juice up to 70-80 % soluble solids content. According to Turkish Standard Institute, boiled grape juice or pekmez is defined as, viscous or solid product which is obtained by concentrating grape or raisin must under vacuum or open system and by addition of some addditives to reduce its acidity (Anonymus, 1983).

In traditional production, obtained must is mixed with pekmez earth and heated, and then it is clarified from pekmez earth and concentrated in open tanks. In modern methods, heated must is treated with pekmez earth as a clarifying agent and $CaCO_3$ to reduce its acidity and simultaneusly cooled and matured. Then, it is filtrated and concentrated under vacuum evaporation and matured. In Turkey, most of pekmez in

markets are produced by traditional method. There are many differences between products that is produced by traditional and modern methods because of difference in heating processes such that in modern methods boiled juice are treated with both lower temperature and heating time. Batu (1991) observed that, traditionally produced pekmez has darker color, lower pH and higher acidity and HMF values with respect to modern methods. Furthermore, total sugar content of this pekmez is reduced by 12.56 % amount by caramelization of sugars.

Browning is an important quality criterion for pekmez like most of concentrated fruit juices. Nonenezymic browning of boiled grape juice was studied by Bozkurt *et al.* (1998). They observed brown pigment formation and HMF accumulation in boiled grape juice and its model systems during storage. Batu (1991) observed change in chemical structure of pekmez produced by both traditional and modern methods. Göğüş and Eren (1997) studied effect of processing type on pekmez quality by measuring both brown pigment and HMF concentration. Recently, nonezymic browning during storage of white hard grape pekmez was studied by Tosun *et al.* (2003). Most important non-enzymic browning reactions that occur in pekmez are caramelization reaction and Maillard reaction. Nonenzymic browning reactions produce unpleasant changes by production of undesirable color, odor and flavor and brown pigment formation is most important result of nonenzymic browning in pekmez.

Brown pigment formation is strongly correlated with the formation of Hydroxymethyl furfural (Bozkurt *et al.*, 1998). Under acidic conditions, non-enzymic browning reactions are followed by formation of intermediate product HMF and finally formation of brown pigments. HMF is quality indicator for most of food products and also it is used as one of the quality parameters of pekmez (Göğüş and Eren, 1997). High concentration of HMF found in pekmez show adulteration with sugar syrup. There are two classes of pekmez defined by Turkish Standart Institue, designated as first class and second class pekmez and also liquid or solid pekmez according to type. Maximum allowable HMF content of pekmez is 25 ppm for first class pekmez and 50 ppm for second classs pekmez (Anonymus, 1983).

Beverages

Thermal treatments such as pasteurization or concentration and unsuitable storage temperatures affect the quality of fruit juices through nonenzymic browning reactions. Fruit juices undergo flavor, taste, color and nutritional changes when stored at warm temperatures and/or for prolonged periods of time (Marcy and Rouseff, 1984; Lee and Nagy, 1988a; Lee and Nagy, 1988b; Lo Coco *et al.*, 1997). Nonenzymic browning occurs as a result of sugar caramelisation, sugar-amine reaction (Maillard reaction) and ascorbic acid oxidation. In the case of citrus juices, Maillard reaction has minor importance because of high acidity involved (pH 3-4) (Lee and Nagy, 1988b). However, acid catalyzed thermal decomposition of the reducing sugars and ascorbic acid degradation are important elements of citrus browning (Handwerk and Coleman, 1988; Roig *et al.*, 1999; Göğüş *et al.*, 2000). Roig *et al.* (1999) studied nonenzymic browning of aseptically filled citrus juices during storage time and observed that nonenzymic browning was mainly due to carbonyl compounds formed from L-ascorbic acid degradation. Handwerk and Coleman (1988) concluded necessary pathways and role of reactants for nonenzymic browning of citrus juices.

Hydroxymethyl furfural and furfural are the principal degradation products of nonenzymic browning, both are useful indicators of quality deterioration. HMF has been correlated with color changes in fruit juices, while furfural is widely accepted as an indicator of flavor changes (Marcy and Rouseff, 1984; Lee and Nagy, 1988b; Gomis *et al.*, 1991; Lo Coco *et al.*, 1997). This compound is a precursor of browning pigments, so the increase of HMF during storage seems to be related to darkening of juice. The HMF content is important because it indicates the degree of heating of the treated products during processing and quantity of this compound is considered as a quality parameter in fruit juices (Garza *et al.*, 1999). The detection and quantitative determination of this component becomes important as aseptic processing and packaging of fruit juices assert themselves. Aseptic packaging allows for higher temperatures during distribution and storage without microbial storage, but off-flavors and loss of nutritional quality develop as citrus products are exposed to these conditions (Marcy and Rouseff, 1984; Lo Coco *et al.*, 1994; Lo Coco *et al.*, 1997).

Nonenzymic browning reactions more readily take place in concentrated fruit juices rather than in fresh juices. Because, the rate of the reaction depends on the amount of

total soluble solids present in the juice or concentrate (Toribio and Lozano, 1986; Göğüş *et al.*, 2000). The International Federation of Fruit Juice Processors (IFFJP) recommends a maximum concentration of 5-10 ppm HMF in fruit juices and 25 ppm in cocentrates (IFFJP, 1964). There is large body of work where HMF is evaluated as an indicator of nonenzymic browning in different juices. Toribio and Lozano (1986) studied the heat induced browning of apple juice concentrate at high temperature. Lee and Nagy, (1988b) studied the quality changes and nonenzymic browning of grape fruit juice during storage. Browning of citrus juices was studied by Roig *et al.* (1999), they observed that nonenzymic browning of citrus juices was mainly due to oxidation of ascorbic acid present. Furthermore, many authors developed methods of HMF determination for citrus juices and fruit concentrates (Marcy and Rouseff, 1984; Lee *et al.*, 1986; Mijares *et al.*, 1986; Lo Coco *et al.*, 1994; Nicoletti *et al.*, 1996).

Nonenzymic browning of alcoholic beverages mainly results reduction in organoleptic properties of these products, such as formation of off-flavors. Many attempts have been made to identify and measure off-flavouring compounds and nonenzymic browning intermediates in these products (Lo Coco *et al.*, 1995; Madigan *et al.*, 1998; Shimuzu *et al.*, 2001).

Beer is a fresh product produced from natural ingredients and, with a few exceptions, its flavor will deteriorate with prolonged storage (Madigan *et al.*, 1998). There is direct relationship between organoleptic charachteristics of beer and browning intermediates that is released during high temperature treatments. Several candidates were identified, one of the most important was hydroxymethyl furfural. Although the concentration of HMF typically found in beer is not thought to be significant in terms of overall beer flavor, the presence of this compound almost invariably results from exposure of the beer to high temperature (Madigan *et al.*, 1998). It is useful indices of flavor instability and analysis of this compound may provide useful device for the detection of high temperature stress of packaged beer. Schimuzu *et al.* (2001), who observed dramatic increase during wort boiling and storage, have reported behaviour of HMF formation during brewing process. They also showed that active oxygen and low pH in finished beer enhance the formation of HMF during storage. Effect of storage time and temperature on HMF formation was studied by Madigan *et al.* (1998), who described simple HPLC method for HMF determination.

Formation of HMF in vinegar was studied by Theobald *et al.* (1998) and they noted that quantitiy of HMF in this products were changed according to types Depending on their HMF content, the vinegars colud be divided into four groups: samples with no, low, medium, and high HMF concentrations. It has been demostrated that, HMF content also could be considered as a good indicator for the vinegar age (Theobald *et al.*, 1998).

Cereal Product

Cereal products have appropriate potential to formation of browning reactions, since they are generally exposed to high temperature during processing. Maillard and cramelization reactions in these products produce changes in color (melanoidins), flavor (aldehydes and ketones), functional properties and nutritional value (blocking or destruction of lysine) (O'Brien and Morrisey, 1989). Chemical reactions that cause the browning of cereal derivatives during manufacturing include the Maillard reaction and caramelization. These reactions is desirable in some cases, especially in bread making, it enhances the color of final products (Ramirez-Jimenez *et al.*, 2000a). On the other hand, extreme reactions may lead decrease in nutritional value and excessive browning in final product. Nutritionally destruction of lysine is the most significant consequence and is often greatest importance in those foods in which this amino acid is limited (O'Brien and Morrisey, 1989).

Moisture content and heating temperature are most important factors that influencing the development of sensory quality of cereal products. According to condition of process applied, different affects are observed. It was reported that, during baking the water content on the surface of the loaf becomes lower than in the middle. This effect is combined with high temperature baking and creates difference between crust and crumbs (Ramirez-Jimenez *et al.*, 2000a). During extrusion cooking of breakfast cereals, high temperature (>180 0C) and shear (>100 rpm) in combination with low moisture (<%15), starch and non-reducing sugars are hydrolyzed and Maillard and caramelization reaction are favored (Garcia-Villanova *et al.*, 1993).The browning reactions in these products can be followed by HMF determination. This indicator has been analyzed in baby cereals (Guerra-Hernandez *et al.*, 1992; Fernandez-Artigas *et al.*, 1999), in breakfast cereals (Garcia-Villanova *et al.*, 1993), in bakery products (Ramirez-Jimenez *et al.*, 2000b), in pasta and bread (Resmini *et al.*, 1993; Ramirez-Jimenez *et al.*, 2000a).

Advanced Maillard reactions may be followed by HMF determination. Thus, measurement of HMF concentration could be introduced into the control of food processing operations (Lee and Nagy, 1988a).

1.3.3 Methods of HMF Determination

There are different methods for the HMF determination. The classical methods for the quantitative determination of HMF in food products are based on colorimetric measurements. The commonly employed colorimetric method is thiobarbituric acid method, which is based on reactions of reagents barbituric acid and p-toludiene with HMF and formation of red-colored complex, whose intensity is proportional to concentration of HMF.

Chromatographic techniques used include ion exchange chromatography, gas chromatography and more recently high performance liquid chromatography using UV detection at 280-285 nm. Kim *et al.* (1992) developed method for determination of HMF by UV detection at 285 nm for several types of foods including juices, honey, syrup, tomato paste, grape juice concentrate and dehydrated pear. Micellar electronic chromatography was used for seperation and determination of HMF in fruit juices (Corradini and Corradini, 1992). Lee *et al.* (1986) developed method for determination of furfurals and HMF in citrus juices of Furhermore, reversed phase chromatorgraphy was used for the determination of HMF by Nicoletti *et al.* (1996). Ames *et al.* (1997) compared different chromatographic methods including isoelectric focusing, capillary zone electrophoresis, ion-exchange HPLC, and reversed phase HPLC for seperation of Maillard reaction products in a model extrusion cooked cereal product. They found that, reversed-phase HPLC is a useful method for determination of HMF. Recently, Lo Coco *et al.* (1996) developed a HPLC method which based on formation of the 2,4-dinitrophenylhydrazones of carbonyl compounds for determination of HMF. For the gas chromatography, the molecules were partitionated between a carrier gas (the mobile phase) and a liquid phase (the stationary phase). In the case of liquid chromatography, molecules are separated by partitionating between a solvent (the mobile liquid phase) and solid support (stationary phase), usually in the form of a packed column. High pressure or high performance liquid chromatography (HPLC), is the most common general method applied to the separation of molecules from mixtures . A basic instrument

consist of a solvent reservoir, pump, gradient chamber, injection port, column, detector, fraction collector and recorder (Pomeranz and Meloan, 2000). HPLC method offer improved accuracy, sensitivity and specifity as compared to other methods especially colorimetric methods (Porretta, 1992).

For the chromotoghrapic analysis, presence of interfering peaks complicates the chromatographic separation of HMF, sample treatment such as distillation, extraction or clarification is needed before injection. For liquid chromotoghraphy, the sample must be in a solvent that is compatable with the column to be used for the separation and free from the contaminants that may damage the column. Certain foods, particularly beverages and other liquid products, may be amenable to direct analysis by liquid chromotoghraphy, for example soya sauce, beer and fruit juice. However, the majority of foods do not yield so readily detailed analysis. Typical procedures for extracting molecules from food stuffs include homogenising the sample with an appropriate solvent, and centrifuging the resulting slurry to remove remainig solid matter. The supernatant liquid constituents a crude extract of Maillard reaction products, along with many other molecules that were present in the food. The simple extraction allow direct comparison with the analysis of model systems, typically of a sugar and an amino acid, where direct analysis of the resulting mixture is often possible, or where solvent extraction is only sample preparation that is required (Pomeranz and Meloan, 2000).

Some foods require the specific removal of substances that interfere with the analysis. For example, in the analysis of Maillard reaction products in beetroot, a method has been developed to reduce the background level of sucrose prior to analysis (Fayle and Gerrard, 2000).

1.4 Aim of Present Study

Heat processing such as sterilization, pasteurization, frying, roasting or drying is the most common way of preserving food and making it edible. Under adequate conditions, foods retain their expected organoleptic and nutritional properties. However, overprocessing may cause deteriorative reactions and decrease in nutritional value. Nonenzymic browning reactions are one of the significant deterioarative reactions limiting the storage life of processed foods. They contribute color, flavour and nutritional quality changes in foodstuffs during processing and

storage time. Therefore, further investigations on nonenzymic browning are still required to clarify the numerous and complex reaction mechanisms and the nature and composition of compound formed.

Hydroxymethyl furfural is a recognized indicator of nonenzymic browning, and it is often used as an index of deteriorative changes in foods. The HMF formation in food products generally changes according to composition, processing type and storage condition.

In this project, it was intended to analyze several types of food products with respect to HMF formation. These products, which were obtained from different firms, were cereal products such as biscuits, breakfast cereals, breads; drinks such as fruit juices and concentrates, alcoholic beverages, citrus juices and vinegar; pastes; dried fruit products such as red pepper and dried fruits; sugar based products such as honey, jams and boiled juices and halvah. So, the aims of present work are;

- to determine the HMF content of various food products
- to determine HMF content of similar type food products that obtained from different firms.
- to coordinate food products according to applied processes, especially heat treatment, and observe effect of processing condition on HMF formation in these products.

CHAPTER II

MATERIALS AND METHODS

2.1 Material

2.1.1 Reagents

Clarifiying agents; potassium ferrocyanide (Carrez I, $K_4Fe(CN)_63H_2O$) and zinc acetate (Carrez II, $Zn(CH_3CO_2)2H_2O$), $NaHCO_3$ and iodine were obtained from Riedel De-Haen (Riedel De-Haen, Germany). Pure hydroxymethyl furfural standard was obtained from Sigma (Steinhein, Switzerland). HPLC grade methanol, p-toludine, glacial acetic acid, barbituric acid, isopropyl alcohol (2-propanol) and hexane were obtained from Merck (Darmstad, Germany) and starch was obtained from Pancreac (Pancreac, Spain).

2.1.2 Samples

Total number of samples were 132 and collected under fourteen main groups. These were cereal products (28), jams (13), pekmez or boiled fruit juice (17), honey (10), fruit juices (13), concentrated fruits juices (7), carbonated beverages (6), dried fruits (4), alcoholic beverages (7), vinegar (3), pastes (12), red pepper (5) and halvah (7).

Cereal products were classified in 7 groups; salty crackers (4), stick crackers (4), cream-filled biscuits (3), sweet biscuits (4), breakfast cereals (3), bread types (6) and pasta (4). Biscuit products and breakfast cereals were belong to 11 different firms. Bread samples were white bread (3), crisp bread (1), pitta bread (2).They were obtained from local bakeries. HMF determination was made in whole bread for white and crisp bread and in crust part for pitta bread.

Commercially produced thirteen jam samples from various types of fruits including strawberry (2), cherry (4), apricot (3), rose (3), blackberry (1) were collected from local markets and they were belong to five different firms. Ten samples of non-processed (5) and processed (5) honey which were obtained from local producers and markets were analysed.

In the case of beverages, totally 25 samples were analysed, they were classified as fruit juices, carbonated beverages, beer and wine. Twelve samples of fruit juices were obtained from four different firms based on different fruit sources. These were orange (2), cherry (3), apricot (4) and peach (3). Six samples of carbonated beverages obtained from six different firms were analysed. In the case of alcoholic beverages, totally seven samples were analysed. These were five samples of beer and two sample of wine. In addition to drinks, three samples of vinegar were analysed.

For the concentrated and dried foods; 7 samples of fruit concentrates, 17 samples of boilde grape juices or pekmez, 12 samples of tomato and paprika pastes, 5 samples of red pepper and 4 samples of dried fruits were analysed. Seven samples of fruit concentrates from various types of fruits including cherry, strawberry, apple, orange, peach, briar rose and apricot were analysed and they were obtained from one firm. Pekmez (boiled fruit juice) samples were grouped as commercially (3) and traditionally produced pekmez (14) and they were obtained from local producers and markets. Also, they were grouped according to fruit sources; mulberry (4), grape (9), pomegranate (4).Twelve samples of pastes including tomato pastes (6) and paprika paste (6) were collected from local producers and markets. They were also grouped as commercially (3) and traditionally produced pastes (9). Five samples of red pepper obtained from local producers were analysed. In the case of dried fruits, four type of sample including two types of raisins (Sultana and Dımışkı types), apricot and fig were analysed.

Commercially produced five different halvah samples were analysed. Additionally, sugar syrup and sugar syrup-soapworth mixture were analysed to discuss effect of process condition on HMF formation during halvah production.

2.2 Preparation of Samples for HPLC

In order to find HMF content of each sample by high performance liquid chromatography, the sample should be extracted in a suitable solvent. Thus, preparation of sample was needed prior to experiment and different procedures were applied to the samples.

2.2.1 Cereal Products and Red Peppers

These products were prepared by method described by Guerra-Hernandez *et al.* (1993). A 0.4 gr portion of ground sample was weighed into a 10 mL centrifuge tube to which 7 mL of triple distilled water added. The centrifuge tube was shaken vigorously for 1 min and the sample was then centrifuged for 10 min at 2500xg. The supernatant was collected into 50 mL erlenmeyer flask. The same procedure was followed twice more. Supernatants was mixed in 50 mL erlenmeyer flask and clarified with 0.5 mL each of Carrez I and Carrez II solutions. The resulting mixture was centrifuged for 10 min at 2500xg and supernatant was separated. The supernatant was diluted to 15 mL by using triple distilled water and homogenized.

2.2.2 Honey, Jam, Pekmez and Paste Samples

Sample was homogenized by stirring with a spatula. Ten gram of sample was placed in 100 ml flask and 2 mL of each of Carrez I and Carrez II reagents was added. Then, the volume was taken up to mark with triple distilled water. After standing 5 min, the mixture was filtered through coarse filter paper and centrifuged for 10 min at 2500xg. 2 mL of homogenized supernatant was used for analysis.

2.2.3 Beverages

For carbonated beverages, degassing was made to eliminate bubbles from the sample. 50 mL sample was taken into 100 mL flask and 2 mL of each of Carrez I and Carrez II was added. After homogenizing the mixture, volume was taken up to a mark with sample and stand for 5 min. Then, it was filtered through coarse filter paper and centrifuged for 10 min at 2500xg. 2 mL of clear supernatant of this solution was used for analysis.

2.2.4 Dried Fruits

Ten gram of dry sample was put in 100 mL flask and 50 mL triple distilled water was added. After standing for 1 night, the mixture was pulped with homogenizator (Heidalph, Germany). Then 2 mL of each of Carrez I and Carrez II solution was added and volume was taken up to mark with water and stand for 5 min. Resulting mixture was filtered through filter paper and centrifuged for 10 min at 2500xg. 2 mL of homogenized supernatant was used for analysis.

2.2.3 Halvah

In order to eliminate contamination of column being used and interfering peaks in the final chromatogram, samples containing high amount of oil were extracted from its oil part prior to common preparation step. For this reason, oil was extracted with hexane by cold extraction method. Approximately 10 gr samples was put in erlenmeyer flask, then 50 mL pure hexane was added and mixed with sample in magnetic stirrer for 30 min. After standing for 15 min, hexane-oil mixture was decanted and the solid portion was decanted with hexane twice following the same procedure. Excess hexane was removed from sample in vacuum oven at 30 ^{0}C under vacuum pressure. Then the sample was prepared according to method described by Guerra-Hernandez *et al.*, (1993).

2.3 Preparation of Solutions

All solutions were prepared by using triple distilled water. Preparation of solutions for spechtrophotometric method were as follows:

1. Carrez I solution: 37.5 gr potassium ferrocyanide was dissolved in 250 mL water.
2. Carrez II solution: 75 gr zinc acetate was dissolved in 250 mL water.
3. Starch solution: 0.5 gr starch was complately dissolved in 100 mL water by heating.
4. Iodine solution: 12.7 gr pure granular iodine was dissolved in 1000 mL water.
5. Barbituric acid solution: 500 mg barbituric acid was dissolved in 100 mL water. This solution can be used for one week.
6. p- toludine solution: 10 gr p-toludiene was dissolved in 50 mL isopropanol and treated with 10 mL glacial acetic acid. After that the solution was made up to 100 mL with 2-propanol. The solution should be prepared daily.

Standard HMF solution was prepared by dissolving 250 mg HMF standard in 100 mL water. HPLC grade methanol:water (10:90 v/v) mobile phase was prepared as follows:100 mL HPLC grade methanol was mixed with 900 mL triple distilled water. Then, the mixture was filtered through Whatman # 42 filter paper and degassed in ultrasonic bath.

2.4 Methods

2.4.1 HMF Determination by Spectrophotometric Method

Spectrophotometric determination of HMF is based on the reaction of HMF with barbituric acid and p-toludiene forming a red-colored complex. The intensity of red color is dependent upon the amount of HMF and measured by using Novaspech II spectrophotometer (Pharmacia Biotech., England).

2.4.1.1 Procedure

A ten gr of sample was put into 100 mL graduated flask and made up to mark with triple distilled water. 10 mL of homogenized aliquot, that was taken from prepared dilution, was made weakly alkaline by adding small amount of pure $NaHCO_3$. The solution was then treated with 1.6 mL of 0.5% starch solution followed by dropwise addition of 0.1 N iodine solution until a blue color of starch-iodine complex persisted for 15-20 seconds. After that, volume was made up to 23 mL with distilled water and 1 mL of each of Carrez I and Carrez II reagents were added. After filtration through filter paper, 2 mL of filtrate were pipetted into each of 2 flask (designated as; s for sample and b for blank). Before the measurement, the sepectrophotometer should be calibrated by means of blank solution. Blank solution was prepared as follows: 5 mL of p-toludine and 1 mL distilled water were added to flask b, the solutions were mixed, transferred to 1 cm cell and absorbance was measured. Then the absorbance of sample solution was determined. 5 mL p-toludine solution and 1 mL of barbituric acid solution was added quickly to the sample flask and absorbance was measured in 1 cm cell. The absorbance value was determined at 550 nm wavelength during 3 min time interval. Samples with an absorbance value higher than 0.5 were diluted to get proper absorbance values. The amount of HMF of the sample was calculated by using standard curve. Analyses were carried out in duplicate.

2.4.1.2 Preparation of standard curve of HMF for spectrophotometric analysis

Various concentrations of HMF solutions (5-50 ppm) were prepeared by diluting the stock solution of HMF with triple distilled water. Same procedure was carried out for all solutions as described in procedure part. Standart curve for HMF was drawn with obtained absorbance values versus HMF concentration of each solutions by using package programme, Sigma plot (version 4.0). Figure 2.1 shows standart curve of HMF with a correlation coefficient of 0.99.

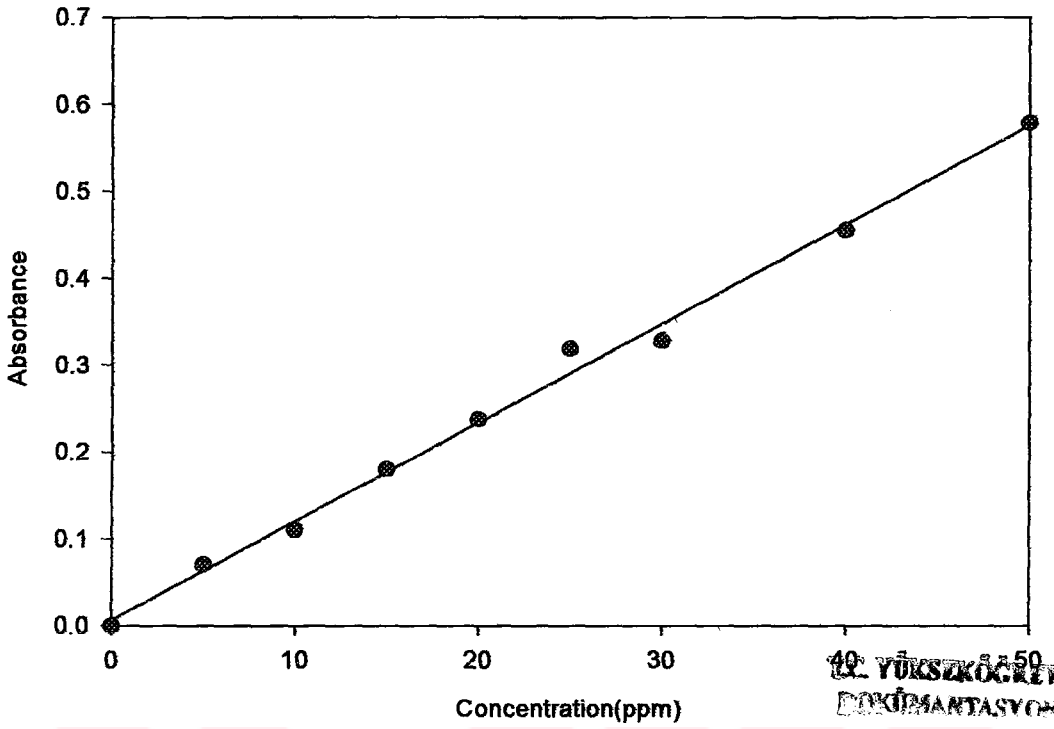

Figure 2.1 Calibration curve of HMF determination by spectrophotometric method

2.4.2 HMF Determination by High Performance Liquid Chromatography

2.4.2.1 Apparatus

The chromatographic system used in this study consisted of a Schimadzu high performance liquid chromatography equipped with a LC-10 AD VP model quatred pump (Schimadzu, Japan), Hewlett Packard 1100 series UV detector (Hewlett Packard, Japan), Waters-Novapak C-18 column (150 mm long, 3.9 mm inner diamater), Waters-Novapak C-18 guard column, 20 µl injection port (Rheodyne, Japan) and software.

2.4.2.2 Chromatographic condition

All samples were filtered through disk filter (Minisart, Sartorius) with 0.2 µm pore size before injecting to the HPLC. 20 µl of the resulting solution was injected into a C-18 column. Analyses were carried out in duplicate.

The mobile phase consisted of a solution of methanol:water (10:90 v/v) mixture. The elution was isocratic and the flow rate of mobile phase was 1 mL/min. The UV detector was set at 280 nm. The peak area obtained from final chromatogram was calculated by Borwin (version 1.2) package programme.

2.4.2.3 Preparation of standard curve of HMF for HPLC analysis

Linear regression method was used to determine HMF content of samples. Working standard HMF solutions were prepeared from stock solution (250 ppm) by dilution with triple distilled water. These were 3, 6, 9, 12, 15, 18, 21, 24, 27, 30 ppm concentrations and injected into HPLC under the same condition with samples.

The Sigma plot (version 4.0) was used to obtain the linear regression equation. The concentration of HMF solution and the peak area obtained were considered as the variables for this equation. The linear regression equation used was (n=11) Y= 80198*X +24.265, where Y is the peak area and X is the HMF concentration of solutions and correlation coefficient of linear regression equation was 0.99 and was shown in Figure 2.2. The HMF content of sample solution was obtained from the calibration curve of standard solution. The sample solutions were diluted prior to the measurement by HPLC when it is required.

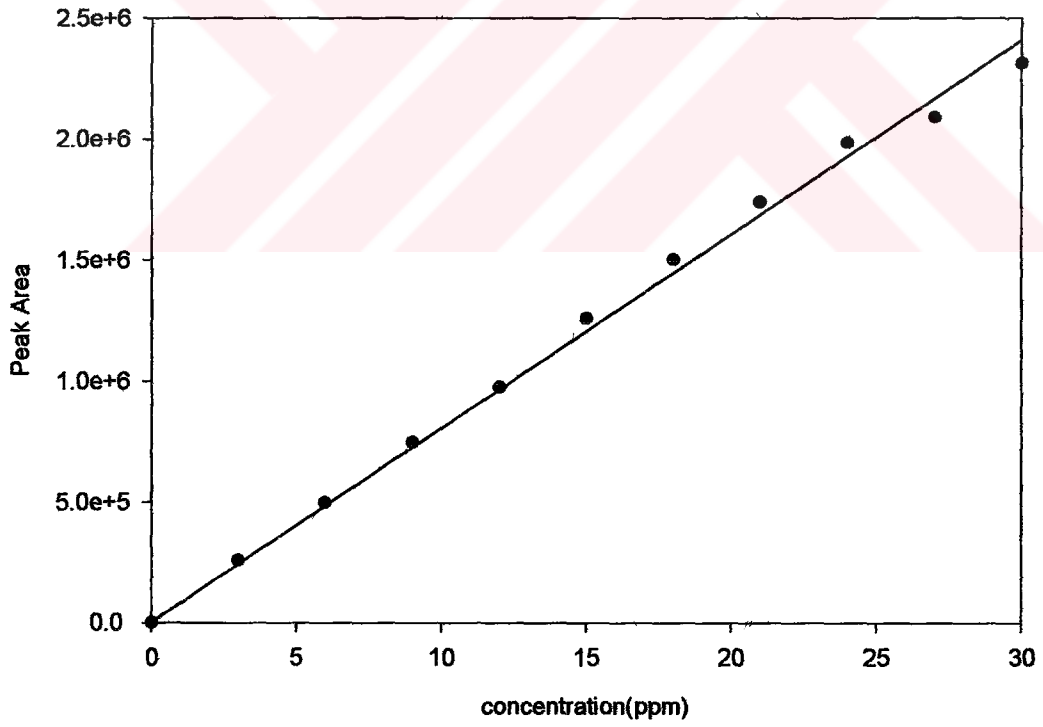

Figure 2.2 Calibration curve of HMF determination by HPLC method

2.4.2.4 Determination of recovery of HMF

The recovery of HMF from samples was determined by standard HMF addition method. A 25 ppm of standard HMF solution was added to the sample. Then, the sample obtained was proceed as described in the sample preperation section. Then percent recovery of HMF from the sample was calculated.

2.4.3 Determination of Moisture Content

Moisture was determined by Infrared drying method (Sartorius, Germany). Before drying process, sample was ground and homogenized. 1 gr homogenized sample was taken for analysis. In the case of oily products, oil part was removed by cold extraction method prior to measurement. This method was carried out to determine moisture content of cereal product, red pepper, paprika paste, halvah and dried fruit. Optimum drying condition that was determined for each sample was given below Table 2.1.

Table 2.1 Drying conditions for samples by infrared drying method

Sample Type	Drying Condition
Cereal Products	5 min at 110 ^{0}C, 8 min at 135 ^{0}C, 7 min at 105 ^{0}C
Paprika paste Red Pepper	10 min at 120 ^{0}C, 5 min at 135 C, 5 min at 105 ^{0}C
Halvah	8 min at 115 ^{0}C, 7 min at 125 ^{0}C, 5 min at 105 ^{0}C
Dried Fruit	8 min at 110 ^{0}C, 5 min 130 ^{0}C, 7 min 110 ^{0}C

2.4.4 Determination of Soluble Solid Content

Soluble solids content of samples was determined by refractometry following the AOAC (1990) method. This method was used for honey, pekmez, jam; fruit juices and concentrates, carbonated beverages, alcoholic beverages, vinegar; tomato paste and carried out at 25 ^{0}C.

2.5 Statistical Analysis of Data

Statistical analysis of data was made by using SPSS (version 10.0) package programme at 95 % confidence interval. One-way ANOVA with Duncan's multiple range tests was used to compare experimental data.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Comparison of Spectrophotometric and Chromatographic Results of HMF Content

Various analytical methods have been developed for the determination of HMF in foodstuffs. Spectrophotometric methods have been used for many years and are often the official method for the determination of HMF. However, these methods have some disadvantages such as the instability of the color complex formed, the time required and the use of hazardous chemicals (Marcy *et al.*, 1984; Mijares *et al.* 1986). The chromatographic techniques used are based on ion exchange chromatography, gas chromatography, micellar electrokinetic chromatoghraphy and more recently high performance liquid chromatography using UV detection at 280-285 nm (Lee *et al.*, 1986; Kim *et al.*, 1992; Corradini and Corradini, 1992; Ames *et al.*, 1997; Nicoletti *et al.*, 1996; Lo Coco *et al.*, 1997). HPLC method offer improved accuracy, sensitivity and specificity as compared to other methods especially colorimetric methods (Marcy *et al.*, 1984; Mijares *et al.* 1986; Porretta, 1992).

Chromatographic conditions determined for HPLC method were given in previous chapter. Under the condition adopted, the characteristic chromatographic peak of HMF appeared after 2.6 min. Figure 3.1 and Figure 3.2 show HPLC chromatograms of standard HMF solution and boiled grape juice sample, respectively. The HMF concentrations of samples were determined from the calibration curve of standard solutions. The lowest HMF limit that can be detected by HPLC was found as 0.01 ppm.

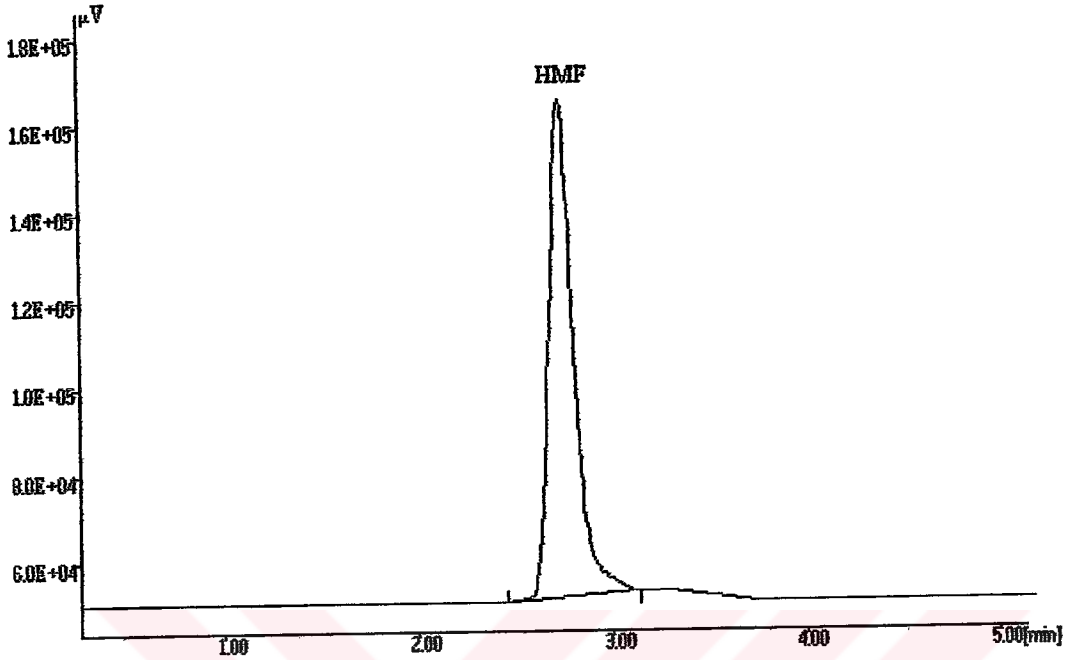

Figure 3.1 HPLC chromatogram of standard HMF solution

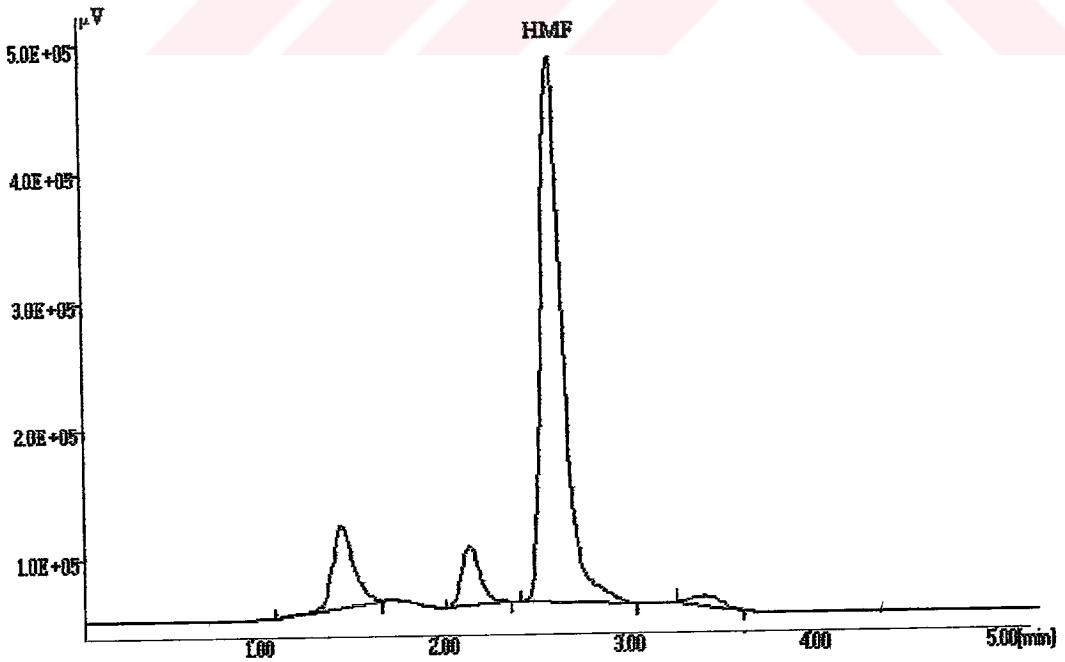

Figure 3.2 HPLC chromatogram of boiled grape juice

Comparison of spectrophotometric method with HPLC method was made with randomly selected 13 samples of honey, pekmez and jams. In order to find any difference between methods, the results were subjected to the one-way ANOVA test within 95 % confidence interval (Table A1). HMF concentrations obtained from these two methods are given in Table 3.1.

Table 3.1 HMF concentrations for honey, jam and pekmez samples determined by spectrophotometric and HPLC methods [a]

Sample number	Spectrophotometric method (ppm)	HPLC method (ppm)
1	0.0	4.2
2	4.0	5.0
3	5.0	6.8
4	7.0	8.7
5	11.0	12.8
6	15.0	12.6
7	16.7	18.4
8	20.8	24.6
9	24.0	26.8
10	24.6	27.0
11	28.0	31.0
12	28.0	32.0
13	29.0	38.5

[a] Values for both methods are the means of two analyses

As it is seen in Table 3.1, HMF concentrations determined from two methods were significantly different from each other ($p<0.05$). The percent recoveries of HMF were 99.3 and 84.5 with the HPLC and spectrophotometric methods, respectively. The obtained results were in agreement with those reported by Porretta and Sandei (1991). They determined maximum HMF recovery of 83 % in tomato pastes which have several concentrations.

Figure 3.3 shows HMF concentrations determined by HPLC and spectrophotometric methods. HPLC results mostly higher than corresponding spectrophotometric results for each sample. As mentioned before, spectrophotometric methods require strict control of reaction time and temperature to achieve stable, reproducible absorbance readings. The first important limitation to the spectrophotometric method is the difficulty in following accurately the colorimetric reaction over time due to the fact that maximum color intensity is obtained within an interval of 1-4 min, so its

optimum recording is not always possible when large quantities of samples are to be analyzed (Porretta and Sandei, 1991). Furthermore, lower concentration of HMF level (up to 0.01 ppm) can be detected in samples by HPLC method. It was stated that spectrophotometric method of HMF determination is limited by inconsistency and low sensitivity when applied to dairy products (O'Brien and Morrisey, 1989). It can be concluded that HPLC method gives higher sensitivity and more accurate results for HMF determination with respect to spectrophotometric method. Accordingly, in this study HPLC method was used for the determination of HMF content of various food products.

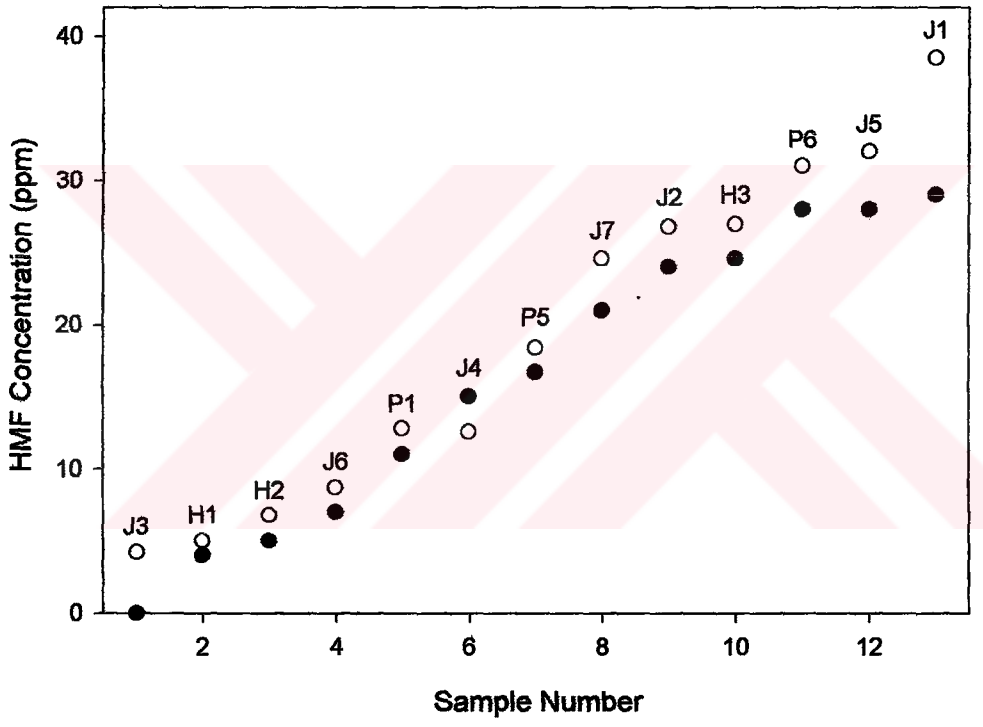

Figure 3.3 HMF concentrations determined by HPLC and spectrophotometric methods (●, Spectrophotometric method; ○, HPLC method; H, Honey; J, Jam; P, Pekmez)

When the sepectrophotometric HMF results were compared with those of HPLC, it was found that the results were in agreement within the defined concentrations (r = 0.977). The plot gives straight line for the results (Figure 3.4). The linear regression equation of correlation line was found as;

$$HMF_{spectrophotometric} = -1.01 + 0.9364\ HMF_{HPLC}$$

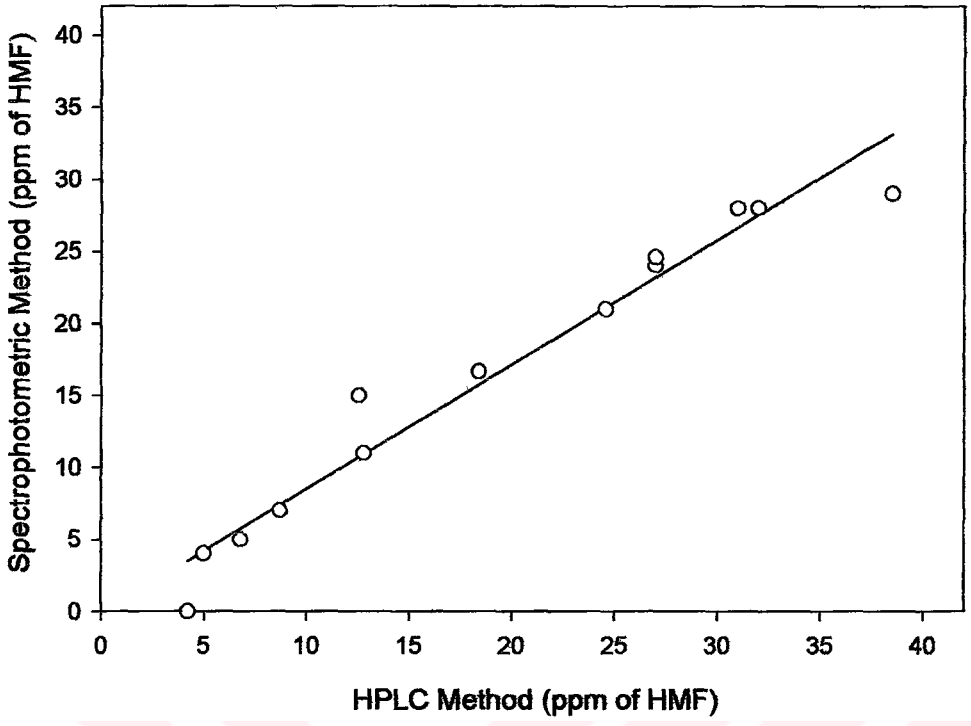

Figure 3.4 Correlation between the spectrophotometric and the HPLC method

3.2 Hydroxymethyl furfural Contents of Foods

3.2.1 HMF Content of Concentrated and Dried Food Products

Nonenzymic browning is especially critical in dried and concentrated foods. Maximum browning has been observed at water activities between 0.3 and 0.7, depending on the type of food (Eichner, 1975). In this study, HMF contents of several dehydrated foods including fruit juice concentrates, boiled grape juices or pekmez, tomato and paprika pastes, red peppers and dried fruits, were determined by HPLC method.

Fruit Juice Concentrates

It has been reported that increasing the concentration significantly enhanced browning reactions (Labuza *et al.*, 1980; Toribio and Lozano, 1984). Nonenzymic browning reactions more readily take place in concentrated fruit juice rather than in fresh juice. Because the rate of the reaction depends on the amount of total soluble solids present in the juice or concentrate (Toribio and Lozano, 1986).

Seven different samples of fruit juice concentrates were examined for the determination of HMF content. Table 3.2 shows soluble solid and HMF contents (dry basis) of samples analysed by HPLC method. Total soluble solid content in fruit juice concentrates mainly represents sugar content that composed of sucrose, glucose and fructose. The sugars participated directly in the browning reactions and yielded HMF. HMF, which indices formation of nonenzymic browning reactions was detected in all analysed samples. Recovery of HMF from samples was determined by standard addition method and found as 98 % and results were corrected according to 100 % recovery.

Table 3.2 Soluble solids and HMF contents of fruit juice concentrate samples (HPLC method)

Sample Identification	Sample number	Production type	Soluble Solids Content (^{0}Brix)	HMF content in dry basis (ppm)
Strawberry Concentrates	FC_1	Commercial Production	65	0.5
Briar-rose Concentrates	FC_2	Commercial Production	13	3.5
Peach Concentrates	FC_3	Commercial Production	14	3.6
Apricot Concentrates	FC_4	Commercial Production	21	7.0
Cherry Concentrates	FC_5	Commercial Production	60	5.0
Orange Concentrates	FC_6	Commercial Production	65	5.4
Apple Concentrates	FC_7	Commercial Production	66	6.8

FC, Fruit Juice Concentrate

During concentration of fruit juices or dehydration of fruits, HMF content may probably increased by both temperature and water activity effects. The effect of water activity on rate of HMF formation in these systems can be explained by dilution and diffusion at low and high water activities, respectively. At high water activity dilution of reactants may take place. On the other hand, the reaction rate is decreased at low water activity due to an increasing diffusion resistance which lowers the mobility of sugars and the organic acids that catalyzes the reaction (Resnik and Chirife, 1979). The rate of reaction is maximum at critical water activity value that generally ranged between 0.3-0.7 for most foods. Thus, this critical water activity range was probably reached during concentration of initial juice. High

temperature of dehydration process may probably increase HMF content of final products as well. Garza *et al.* (1999) observed significant increase in HMF content during thermal treatment of peach puree at several high temperatures including 85, 90 and 98 ^{0}C.

HMF concentration of analysed samples changes between 0.4 and 4.5 ppm. Gomis *et al.* (1991) developed HPLC method for controlling and determination of HMF formation in pasteurized apple juice and juice concentrates. Figure 3.5 shows HMF contents of analysed fruit juice concentrates with respect to their types. Higher HMF contents of apple, cherry and orange juice concentrates were significantly different ($p<0.05$) than these of others (Table A2). It was noted that citrus juices more readily subjected to ascorbic acid oxidation (Lee and Nagy, 1988a).

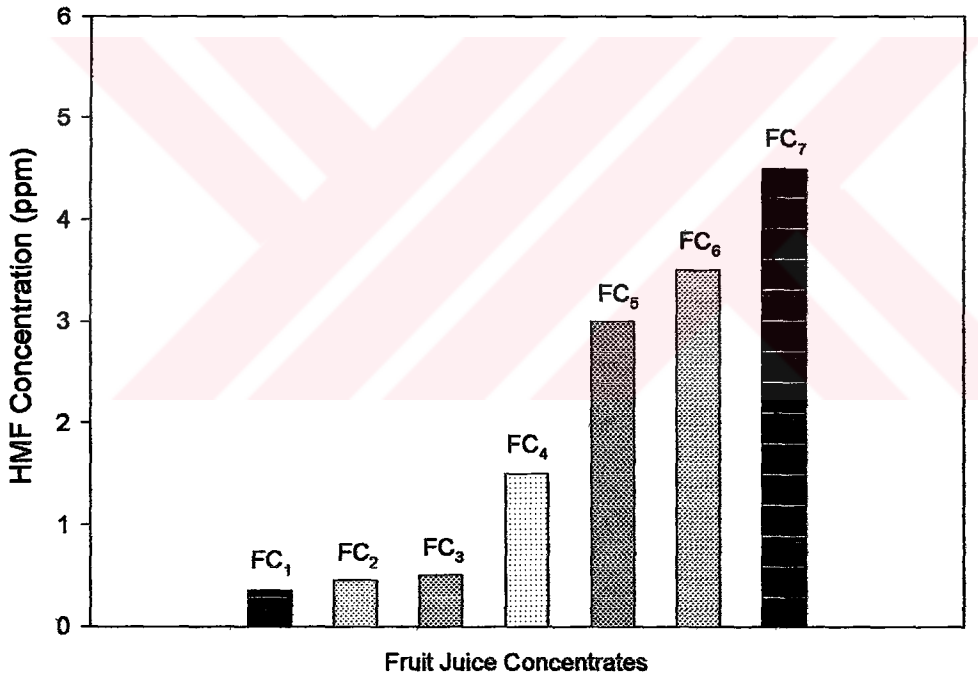

Figure 3.5 HMF concentrations of fruit concentrates determined by HPLC method (FC, fruit juice concentrate)

HMF concentrations of all analysed samples were appropriate to the standard HMF limit that should be max 25 ppm for fruit concentrates (IFFJP, 1964). So, the possible high content of HMF was not observed in these products. This is a clear indication of a proper process of the fruit juice concentrates examined. The further risk of increase in the browning products will be lower for concentrates produced under similar proper conditions (low temperature, vacuum treatment, etc.). If these concentrates

stored properly as well, they will be reached to the final consumer in its good condition from the standpoint of the browning.

Pekmez

Pekmez is produced by concentration of fruit juice up to 70-80 % soluble solids content. Browning reactions may occur in high sugar containing system of pekmez during processing and storage. It also contains significant amount of amino acids. For example, boiled grape juice contains eight different amino acids; major ones are arginine, glutamine and proline, and high composition of D-glucose and D-fructose (Bozkurt *et al.*, 1998). Caramelization of pekmez takes place by degradation of sugars at high temperatures during boiling. Maillard reaction occurs between amino acids and reducing sugars present in pekmez (Bozkurt *et al.*, 1998, Batu, 2001).

Seventeen samples of pekmez were examined for the determination of HMF content by HPLC. Soluble solids and HMF contents (dry basis) of analysed samples are seen in Table 3.3. Highly variable HMF concentrations, changing from 13.5 ppm to 3500 ppm, were obtained for analysed samples. These were 12.8-152 ppm for boiled mulberry juice, 31-200 ppm for boiled grape juice and 514-3500 ppm for boiled pomegranate juice. The results were in agreement to the findings of Velioğlu and Artık (1993). Their findings showed that HMF concentration was in a range of 13.4-236 ppm for several types of pekmez. HMF results found in this study were significantly different from each other ($p<0.05$) with respect to type of processes and to type of pekmez ($p<0.05$). Soluble solid content of samples were in the range of 64-79 brix. Recovery of HMF from samples was determined by standard HMF addition method and found as 99.3 % and obtained results were corrected according to 100 % recovery.

When HMF concentration of samples was compared with Turkish standards (Anonymous, 1983), two commercially produced samples of boiled mulberry juice and boiled grape juice with 12.8 ppm and 18.4 ppm HMF, respectively were in the limits of first class pekmez. Two samples with HMF concentrations of 31 and 32 ppm were in the limits of second class pekmez. When the results of all analysed samples are considered, only 24 % of the samples are in the limits of HMF concentration of the referred standard. This is a clear indication of excessive

nonenzymatic browning (caramelization and/or Maillard) in significant number of pekmez samples.

Table 3.3 Soluble solids and HMF contents of boiled juices (HPLC method)

Sample Identification	Sample number	Production type	Soluble Solid content (^{0}Brix)	HMF content in dry basis (ppm)
Boiled Mulberry Juice	BJ_1	Commercial Production	73	17.0
Boiled Mulberry Juice	BJ_2	Traditional Production	74	43.0
Boiled Mulberry Juice	BJ_3	Traditional Production	78	144.0
Boiled Mulberry Juice	BJ_4	Traditional Production	67	226.0
Boiled Grape Juice	BJ_5	Commercial Production	64	28.7
Boiled Grape Juice	BJ_6	Commercial Production	69	45.0
Boiled Grape Juice	BJ_7	Traditional Production	77	72.2
Boiled Grape Juice	BJ_8	Traditional Production	78	88.0
Boiled Grape Juice	BJ_9	Traditional Production	78	112.8
Boiled Grape Juice	BJ_{10}	Traditional Production	74	166.9
Boiled Grape Juice	BJ_{11}	Traditional Production	79	180.0
Boiled Grape Juice	BJ_{12}	Traditional Production	78	201.0
Boiled Grape Juice	BJ_{13}	Traditional Production	77	260.0
Boiled Pomegranate Juice	BJ_{14}	Traditional Production	72	714.0
Boiled Pomegranate Juice	BJ_{15}	Traditional Production	69	1420.0
Boiled Pomegranate Juice	BJ_{16}	Traditional Production	68	3641.0
Boiled Pomegranate Juice	BJ_{17}	Traditional Production	70	5000.0

BJ, Boiled Juice

During concentration process of pekmez by traditional or commercial method, HMF content of pekmez may be increased by both concentration and temperature effect. Effect of water activity on nonenzymic browning was well described for several foods having various moisture contents (Warmbier *et al.*, 1976b; Eichner, 1975; Labuza and Saltmarch, 1981; Cerrutti *et al.*, 1985). In the intermediate moisture range of foods, the reaction rate becomes maximum due to increased mobility of reactants (Warmbier *et al.*, 1976b). Bozkurt *et al.* (1998) observed increase in HMF concentration of pekmez with increase in soluble solid content. During the production of juice concentrates, the concentration is not constant, but changes as a function of time. Therefore, the juice samples pass through the critical water activity range of browning reactions to be made concentrates. Because the reaction rate is maximum at a water activity range of 0.4-0.7, the HMF content might be increased

especially during concentration in this range. In addition to this water activity effect, the juice samples might be also more sensitive to the temperature at the increased concentrations.

As mentioned earlier, the type of processing also affects the final HMF content of pekmez. Traditionally produced samples had higher HMF values with respect to commercially produced ones. This may be due to higher temperature and longer time evaporation process of traditional method. Bozkurt *et al.* (1998) showed that the effect of temperature on both HMF accumulation and brown pigment formation was significant in pekmez. Furthermore, high acidity of pekmez in traditional process may cause high values of HMF formation, since formation of HMF is favored in acidic conditions (Batu, 2001; Bozkurt, 1996). In traditional method of pekmez production, longer time of evaporation process is necessary to meet desired soluble solid content (about 70 brix). During this time HMF content of pekmez was probably increased, since the formation of HMF in pekmez is significantly dependent on the time of process (Göğüş and Eren, 1997). Batu (1991) found HMF level of 32.2 ppm in boiled grape juice that was produced under vacuum. On the other hand he found 681.4 ppm HMF content in boiled grape juice produced by traditional method. He concluded that, high amount of HMF values in traditionally produced pekmez is due to higher temperature and longer processing time .

Figure 3.6 shows HMF concentrations of pekmez samples. It can be seen that HMF concentrations of boiled grape and mulberry juices are in a range of 12.8-200 ppm, while higher range of HMF concentrations (514-3500 ppm) was obtained in boiled pomegranate juice. This variation may be due to difference in composition of raw material. Boiled pomegranate juices are highly acidic and thus, formation of HMF in those juices might be higher with respect to boiled grape and mulberry juices. As indicated previously, the formation of HMF in pekmez is highly dependent on pH (Bozkurt, 1996; Batu, 2001).

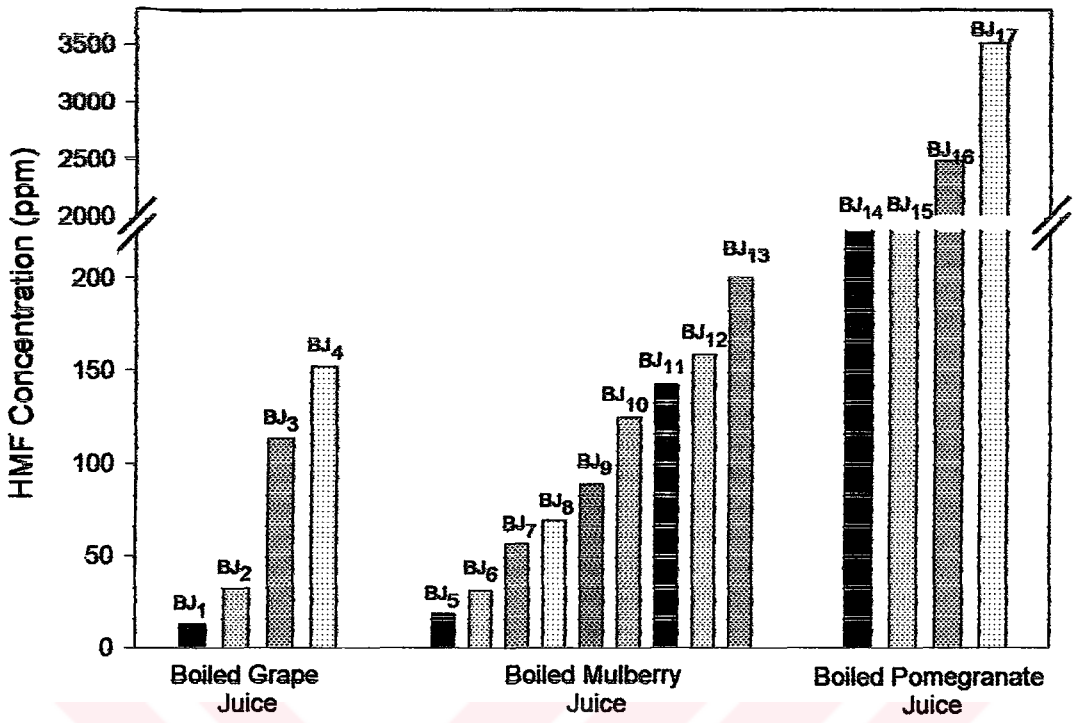

Figure 3.6 HMF concentrations of boiled juice samples determined by HPLC method (BJ, boiled juice)

Tomato and Paprika Pastes

Thermal treatments during the manufacturing process can affect the quality of fruit products through nonenzymic browning reactions. Tomato and paprika pastes are commercially produced by concentrating the fruit juices/purees under vacuum pressure. In traditional method, obtained fruit juices are concentrated by draining of serum part of juice through the filter like a cheese-cloth (only for tomato paste). After draining for two days, resulting paste is evaporated under hot sunshine by spreading of the pastes on slabs (Peter, 1973). Nonenzymic browning reactions in these products can be followed by HMF determination. Poretta and Sandei (1991), proposed method of HMF determination in tomato products by HPLC.

Twelve samples of tomato and paprika pastes were analysed for determination of HMF content. HMF concentration in analysed samples fell within the range of 0.4-18 ppm. Table 3.4 lists the solid and HMF contents (in dry basis) of samples. While solid contents of paprika pastes ranged within 55-61 %, soluble solids content of tomato pastes changed from 29 to 53 %. Wider range of HMF concentrations were obtained when HMF content of analysed samples was evaluated in dry basis and it was 0.7-62

ppm. Recovery of HMF was determined by standard HMF addition method and was found as 97 % and the HMF results were corrected according to 100% recovery.

Table 3.4 Total/soluble solids and HMF contents of tomato and paprika pastes (HPLC method)

Sample Identification	Sample number	Production type	Total solid content (%/^{0}Brix)	HMF content in dry basis (ppm)
Paprika Paste	P_1	Traditional Production	57 [a]	0.7
Paprika Paste	P_2	Traditional Production	61 [a]	1.0
Paprika Paste	P_3	Traditional Production	60 [a]	5.0
Paprika Paste	P_4	Traditional Production	58 [a]	6.9
Paprika Paste	P_5	Traditional Production	55 [a]	11.0
Paprika Paste	P_6	Commercial Production	55 [a]	0.9
Tomato Paste	T_1	Traditional Production	54 [b]	6.7
Tomato Paste	T_2	Traditional Production	53 [b]	10.4
Tomato Paste	T_3	Traditional Production	55 [b]	10.9
Tomato Paste	T_4	Traditional Production	54 [b]	11.3
Tomato Paste	T_5	Commercial Production	30 [b]	50.0
Tomato Paste	T_6	Commercial Production	29 [b]	62.0

P, Paprika paste; T, Tomato paste; [a], Total Solid Content; [b], Soluble Solid Content

There was a significant difference ($p<0.05$) between the HMF concentration of the two different types of pastes. HMF concentrations of the pastes are shown in Figure 3.7. HMF concentrations in wet basis ranged within 3.6-18 ppm for tomato pastes and 0.3-6 ppm for paprika pastes. According to Duncan's multiple range test at 95 % confidence interval, tomato pastes had higher HMF content with respect to paprika pastes. In tomatoes, 50-65 % of the total solid is the reducing sugars, mainly glucose and fructose in almost in equal proportions (Peter, 1973). Because of high sugar content and low pH of system, tomato pastes may give suitable conditions for occurrence of caramelization reactions and subsequently formation of accumulation product, hydroxymethyl furfural. It was indicated that when sugars are heated in the presence of acids, particularly organic acids, they yield furan compounds such that hexoses yielding HMF as the main furan derivative (Resnik and Chirife, 1979).

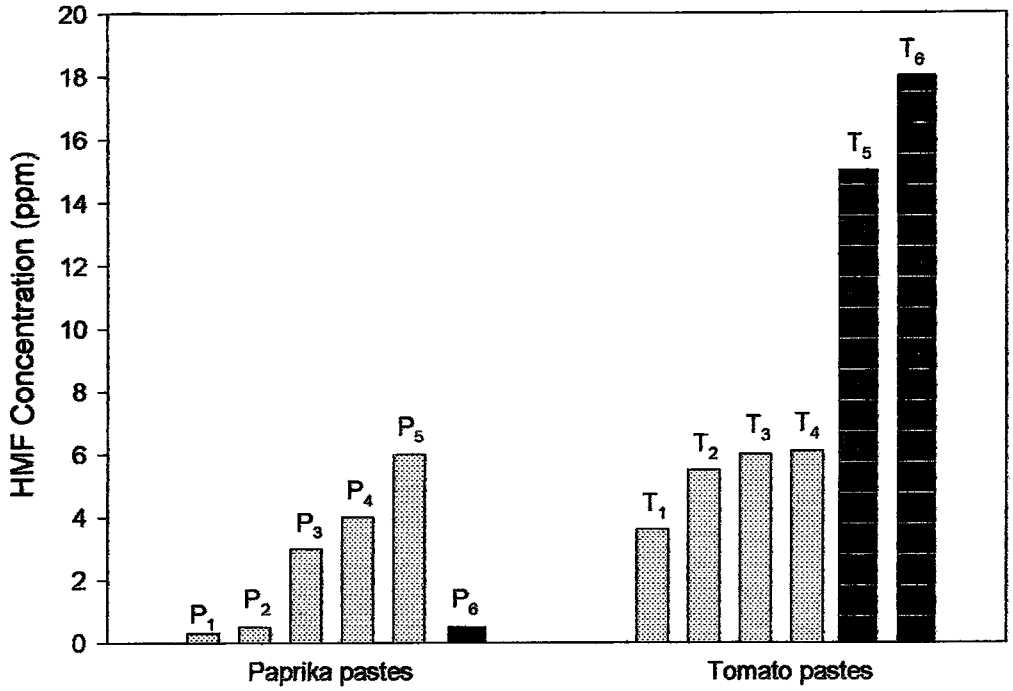

Figure 3.7 HMF concentrations of pastes determined by HPLC method (P, paprika paste; T, tomato paste, black bar shows commercially produced pastes)

The effect of processing on HMF contents is significant ($p<0.05$) for tomato pastes and non-significant ($p<0.05$) for paprika pastes. Various heat treatments are applied in commercial production of tomato and paprika pastes in which color has major importance. During breaking of tomatoes, high break temperatures may darken color of final product due to caramelization of sugars. Besides, during concentration of tomato and/or paprika puree, nonenzymic browning reactions may excessively take place due to high evaporation temperature and concentration through the critical water activity range at which maximum browning may take place. It was observed that change in moisture content of system can cause increased concentration or dilution of the reacting species, then influences the reaction rate (Eichner and Karel, 1972). Evaporation under partial vacuum pressure takes place at a much lower temperature; in consequence, the resulting paste retains most of the color and flavor of fresh fruit. In traditional process, tomato and/or paprika paste is subjected to the lower temperature with sun-drying method. Thus, there was low probability of nonenzymic browning in traditionally produced paste. However, traditionally produced pastes might be more susceptible to the enzymic browning because of lack of enzyme inactivation and possibly the color formation of this type is higher compared to the commercial one.

Red Peppers and Dried Fruits

In conventional manufacturing of dried ground red pepper and drying of fruits, sun-drying is widely used method to meet optimum moisture content at which deteriorative reactions become minimum. Five samples of red pepper and four samples of dried fruit (Apricot, Fig, Raisins) were analysed for determination of HMF content. HMF concentrations changed within 0.4-2.5 ppm for red peppers (Figure 3.8) and 0.4-17.3 ppm for dried fruits (Figure 3.9). Table 3.5 shows total solid and HMF content of samples (in dry basis). Total solid contents of all samples changed from 82 to 88 %. Recovery of HMF from red peppers and dried fruits were 97 % and 96 %, respectively. The results were corrected according 100 % recovery.

Table 3.5 Total solid and HMF contents of red peppers and dried fruits (HPLC method)

Sample Identification	Sample number	Production type	Total solid content (%)	HMF content in dry basis (ppm)
Red Pepper	R_1	Traditional Production	84	0.6
Red Pepper	R_2	Traditional Production	88	0.8
Red Pepper	R_3	Traditional Production	88	2.3
Red Pepper	R_4	Traditional Production	88	2.5
Red Pepper	R_5	Traditional Production	88	2.9
Dried Apricot	D_1	Traditional Production	83	0.4
Dried Fig	D_2	Traditional Production	82	0.5
Raisin	D_3	Traditional Production	87	3.4
Raisin	D_4	Traditional Production	85	20.3

R, Dried Ground Red Pepper; D, Dried Fruit

There was no significant difference ($p<0.05$) between the HMF concentrations of the dried ground red peppers obtained from different sources (Figure 3.8). During conventional production of dried ground red pepper, harvested red peppers are threshed and cleaned prior to the drying. Then, cleaned peppers are dried under the hot sunshine by spreading of the peppers on slabs. After reaching to the 12-13 % moisture content, the dried red peppers are ground by manual or mechanical grinding mills. The HMF results obtained for analysis of dried ground red pepper and traditionally produced paprika pastes were comparable, since both sources of raw material and processing steps were similar. Thus, when average HMF contents in dry

basis were compared, paprika pastes had significantly higher HMF concentration ($p<0.05$) with respect to the dried ground red peppers.

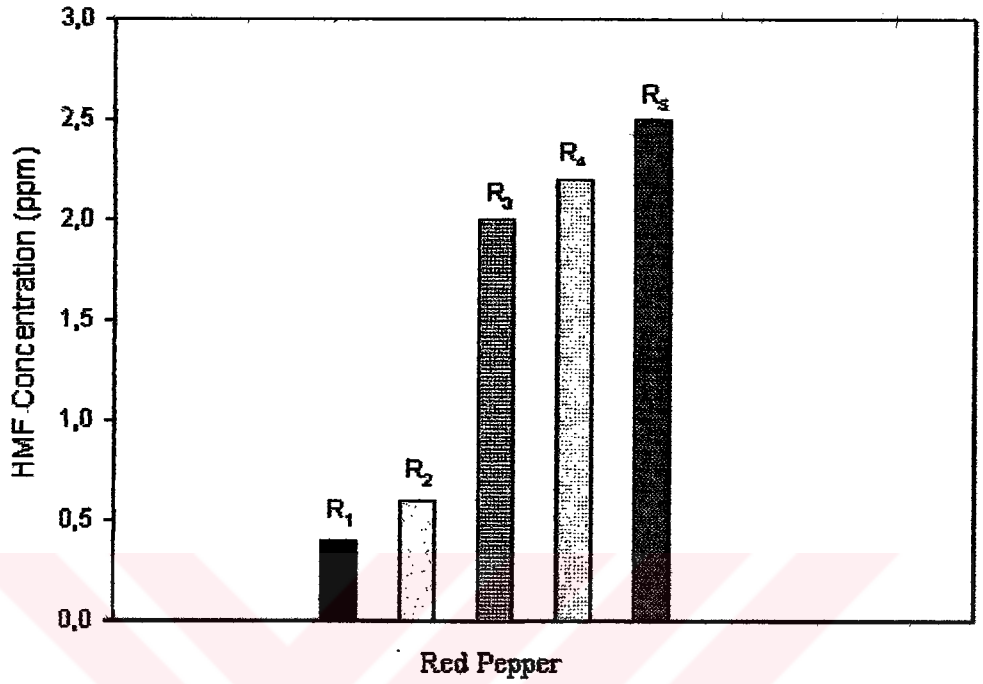

Figure 3.8 HMF concentrations of dried ground red peppers determined by HPLC method (R, red pepper)

Highly variable HMF concentrations were obtained from analysis of dried fruits (Figure 3.9). There were very low amounts of HMF in dried fig and apricot samples, while significant amount of HMF was determined from the analysis of two raisin samples. The HMF concentration of raisin samples were 3 ppm and 17.3 ppm for D_3 (Sultana type) and D_4 (Dımışkı Type), respectively. Higher amount of HMF in these samples may be due to high sugar contents of raisins. The composition of grape juice has been reported by Velioğlu and Artık (1993). The juice contains high amounts of glucose and fructose in almost equal quantities, no sucrose and very small amounts of protein. During sun-drying, nonenzymic browning reactions may lead to the formation of the HMF in those products. The difference in HMF concentrations of D_3 and D_4 samples might be due to the drying time. Since the sizes of these two samples are significantly different from each other and this difference possibly affected the rate of drying and dependently the rate of browning reactions.

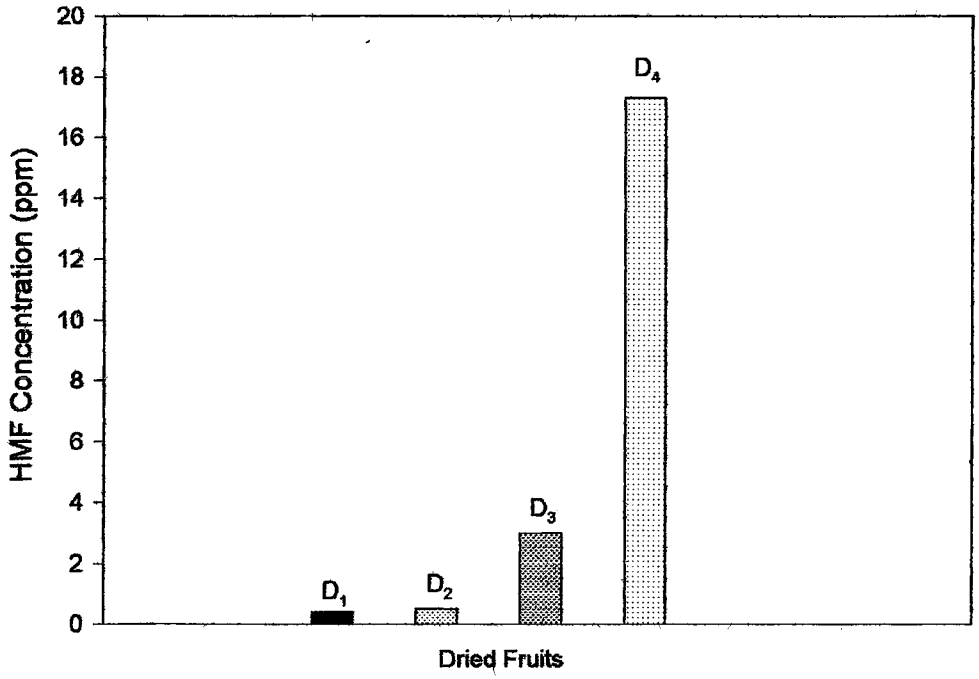

Figure 3.9 HMF contents of dried fruits by HPLC method (D, dried fruit)

When average HMF concentrations of each type of dried and concentrated products (fruit concentrates, pekmez, tomato and paprika pastes, dried ground red pepper and dried fruits) were statistically compared, it was seen that nonenzymic browning is significantly different ($p<0.05$) in samples. HMF concentrations in pekmez samples were the highest in all with an average value of 727 ppm. Pekmez is highly susceptible to the nonenzymic browning because of its high sugar content and excessive thermal treatment used during manufacturing. Thus, it is necessary to improve processing conditions to prevent excessive browning in pekmez.

3.2.2 HMF Content of Honeys

Honey is the natural sweet substance produced by honey bees from nectar of blossoms and from secretions of living parts of plants. Sugars represent the largest portion of honey composition (95-99 % of the honey solids) and it mainly consists of fructose and glucose (Anklam, 1998). As mentioned earlier, the processing of honey may lead to changes due to caramelization of carbohydrates. In acid medium of honey, dehydration of carbohydrates is favored; leading to the formation of HMF and other furfural compounds (Villamiel *et al.*, 2001). In particular the amount of HMF, which is produced by action of the normal honey acidity on fructose at room

temperature, increases notably owing to thermic treatments and/or storage at improper temperatures (Lo Coco *et al.*, 1996).

Ten samples of honey including processed and non-processed honeys were analysed for determination of HMF. Soluble solid and HMF content of the samples (in dry basis) were given in Table 3.6. Soluble solids content of samples was changed from 80 to 89 ^{0}Brix. Recovery of HMF from the samples was 99.3 % and HMF results were corrected to 100 % according to obtained recovery.

Table 3.6 Soluble solids and HMF contents of honey (HPLC method)

Sample Identification	Sample number	Production type	Soluble solid content (^{0}Brix)	HMF content in dry basis (ppm)
Honey	H_1	Non-processed	83	0.6
Honey	H_2	Non-processed	85	1.0
Honey	H_3	Non-processed	80	1.2
Honey	H_4	Non-processed	83	1.6
Honey	H_5	Non-processed	83	3.8
Honey	H_6	Processed	88	5.6
Honey	H_7	Processed	89	7.7
Honey	H_8	Processed	83	8.7
Honey	H_9	Processed	83	28.6
Honey	H_{10}	Processed	86	31.3

H, honey

HMF in honeys fell within the range of 0.5-27 ppm. HMF concentrations of all honey samples were below the allowable limit of 40 ppm with maximum HMF value of 27 ppm. Mendes *et al.* (1998) stated that, HMF is recognized parameter used for the evaluation of honey freshness. Thus, all analysed samples were classified as fresh honey when HMF value was used as a criterion. But, two processed honey samples, with HMF content of 23.7 ppm and 27 ppm, may probably have lower shelf life with respect to the others, since HMF content of these honeys may have exceed limiting value during storage.

Figure 3.10 shows the HMF concentrations of non-processed and processed honey samples. Processed honey samples had significantly higher HMF values ($p<0.05$) with respect to non-processed ones. This was probably due to thermal treatments of processed honey such as pasteurization and liquefaction. Tosi *et al.* (2001) studied the thermal treatment effects on HMF formation in honey. They found that HMF formation in honey was accelerated by both higher temperature and longer

processing time. Thus, the use of thermal treatments during processing of honey should be optimized to meet better quality characteristics for honey.

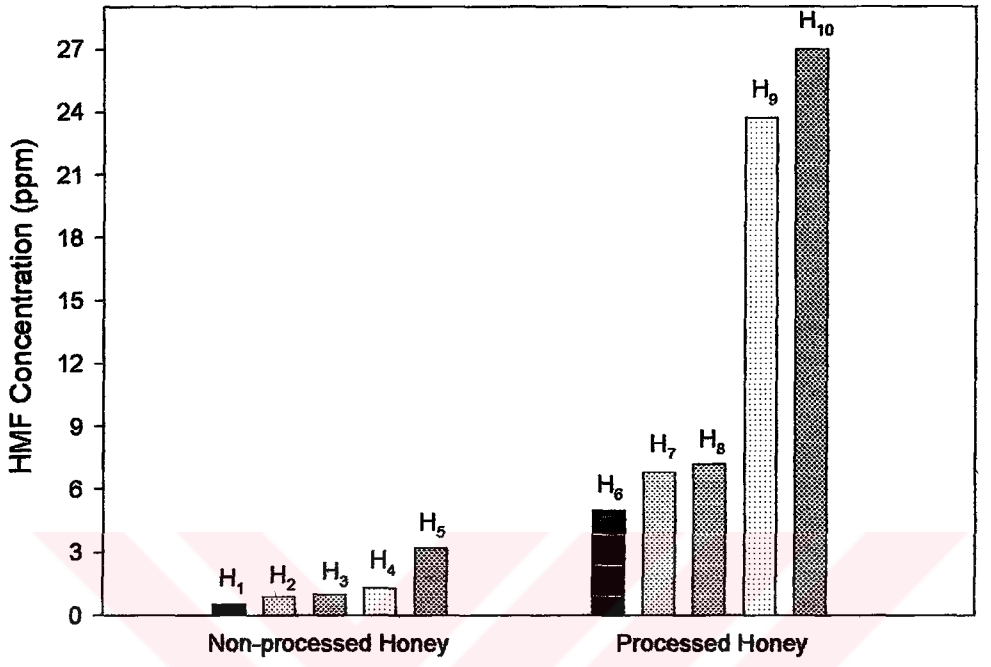

Figure 3.10 HMF concentrations of honeys determined by HPLC method

3.2.3 HMF Content of Jams

The HMF content of 13 commercially produced jam samples were determined in order to evaluate their processing conditions, differences in various types, and also confrontation to standards. HMF concentrations of samples changed from 4.2 to 80 ppm (Figure 3.11). Soluble solids content of all jam samples changed from 66 to 77 ^{0}Brix. These data were in agreement with those previously reported by Rada-Mendoza *et al.* (2002). Recovery of HMF from the samples was 99 % and given results were corrected to 100 % recovery.

In samples included HMF, which indices the occurrence of nonenzymic browning, was detected. Table 3.7 shows both HMF content (in dry basis) and soluble solids content of jams. There are mainly two possible ways of formation of HMF in jam; conversion of aminodeoxy ketose to HMF by the Maillard reaction and decomposition of hexoses to HMF at high temperatures (Ekşi and Velioğlu,1990)

Table 3.7 Soluble solids and HMF contents of commercial jams (HPLC method)

Sample Identification	Sample number	Production type	Soluble Solid content (^{0}Brix)	HMF content in dry basis (ppm)
Blackberry jam	J_1	Vacuum Process	66	6.4
Apricot jam	J_2	Vacuum Process	67	36.7
Apricot jam	J_3	Vacuum Process	67	47.8
Apricot jam	J_4	Open Tank Process	75	44.0
Cherry jam	J_5	Vacuum Process	69	38.8
Cherry jam	J_6	Vacuum Process	67	47.8
Cherry jam	J_7	Vacuum Process	71	54.2
Cherry jam	J_8	Vacuum Process	76	81.6
Strawberry jam	J_9	Vacuum Process	66	19.0
Strawberry jam	J_{10}	Vacuum Process	77	49.0
Rose jam	J_{11}	Vacuum Process	70	12.4
Rose jam	J_{12}	Vacuum Process	66	75.8
Rose jam	J_{13}	Open Tank Process	77	105.9

J, jam

According to statistical analysis, average HMF concentrations of each jam type showed a significant difference ($p<0.05$) with respect to the others. Average HMF concentrations were 40 ppm for cherry jams, 30 ppm for apricot jams, 46.7 ppm for rose jams, 25 ppm for strawberry jams and 4 ppm for blackberry jam. It was reported that fruit content and sugar composition of jams affected the HMF concentration (Rada-Mendoza *et al.*, 2002). Thus, variations in HMF concentrations of different jam types may be due to this effect.

HMF concentrations of jams also found to be significantly different ($p<0.05$) with respect to their firms. The considerable variations of HMF concentrations found in the analysed samples may be an indication of differences in the processing conditions. Ekşi and Velioğlu (1990) found similar results for different types of jams. Figure 3.11 shows HMF content of analysed jam samples. When HMF concentrations of samples were compared with Turkish standards (Anonymous, 1983), it was found that six samples are first class, five samples are second class and two samples are out of the class. The samples which do not obey the Turkish standards were cherry and rose jams with 62 and 81.5 ppm HMF concentrations, respectively. When the results of all analysed samples are considered, 84.6 % of the jam samples are in the limits of HMF concentration of the referred standard.

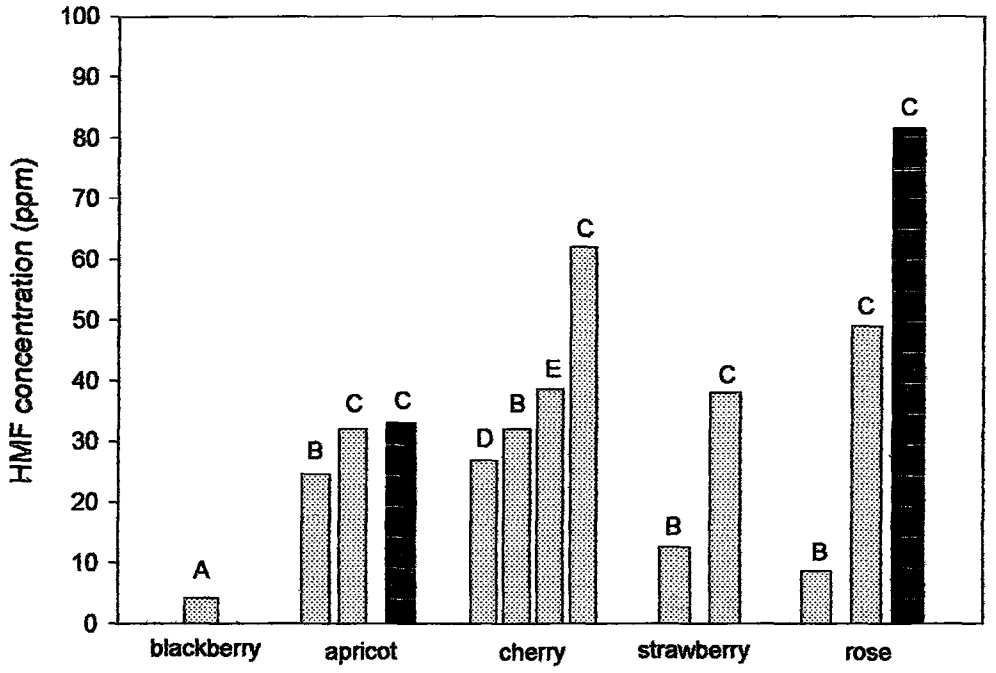

Figure 3.11 HMF concentrations of jams determined by HPLC method (letters (A-E) show different firms, grey and black bars show vacuum and open tank processes, respectively)

Soluble solid content has a significant importance in commercial jam making process from the standpoint of the microbiological stability. Generally, desired soluble solids content is obtained by concentration with the evaporation and/or addition of thickening agents such as pectin. During concentration, HMF concentrations of jam possibly affected by processing time in such a way that longer heat treatment process lead to the increase in the final HMF concentration. It was reported that the HMF concentration of jam was significantly dependent on thermal treatment time (Ekşi and Velioğlu, 1990; Rada-Mendoza *et al.*, 2002). In this study, the jam samples having higher soluble solids content had generally higher HMF concentrations with respect to the others. According to this, during concentration of these jams, longer heating time might be used to obtain desired soluble solids content and consistency in the final product and consequently may cause higher HMF concentrations in these jams.

As mentioned earlier, the type of processing also affects the final HMF concentrations of the jam. The HMF concentrations of two jam samples which treated with open tank process were found to be 38.5 ppm and 81.5 ppm for J_4 and J_{13}, respectively. Higher concentration of HMF in those jams was possibly due to the type of processing applied. It is known that open tank process has higher temperature and longer processing time with respect to vacuum type process (Ekşi and Velioğlu, 1990). The HMF results obtained for samples J_{12} and J_{13} were comparable, since raw material of both products and the brand name that was obtained were the same, but only the type of jam manufacturing process applied was different. In vacuum type process, HMF concentration of sample J_{12} was found as 49 ppm, on the other hand it was found as 81.5 ppm for J_{13} which treated by open tank method. Additionally, it has been reported by Ekşi and Velioğlu (1990) that there is no cooling process following the pasteurization step during the commercial jam production. It may also increase amount of HMF formation in these jams. High amount of HMF formation was reported in fruit juices that were cooled by themselves (Ekşi and Velioğlu, 1990).

3.2.4 HMF Content of Cereal Products

Cereal products have a good potential for the formation of browning reactions, since they are generally exposed to high temperature during processing. The browning reactions in these products can be followed by HMF determination (Guerra-Hernandez *et al.*, 1992; Garcia-Villanova *et al.*, 1993; Resmini *et al.*, 1993; Fernadez-Artigas, 1999; Ramirez-Jimenez *et al.*, 2000a; Ramirez-Jimenez *et al.*, 2000b).

In this study, HMF concentrations of seven groups of cereal products were determined. Moisture content of all samples changed from 3 to 47 %. This value was 4.5-6.7 % for salty crackers, 3-7.4 % for stick crackers, 4.9-5.4 % for cream filled biscuits, 2.5-7.4 % for sweet biscuits, 3-3.5 % for breakfast cereals, 22-47 % for breads (pitta and crisp bread, crust part for white bread). Table 3.8 shows total solids and HMF contents (in dry basis) of cereal samples.

Table 3.8 Total solid and HMF contents of cereal products (HPLC method)

Sample Identification	Sample number	Production type	Total Solid Content (%)	HMF content in dry basis (ppm)
Salty cracker	C_1	Fabricated product	95.5	27.0
Salty cracker	C_2	Fabricated product	95.0	4.1
Salty cracker	C_3	Fabricated product	94.0	1.1
Salty cracker	C_4	Fabricated product	93.0	5.4
Cream-filled biscuit	C_5	Fabricated product	93.0	24.0
Cream-filled biscuit	C_6	Fabricated product	93.0	0.8
Cream-filled biscuit	C_7	Fabricated product	92.5	1.5
Stick cracker	C_8	Fabricated product	97.0	6.8
Stick cracker	C_9	Fabricated product	93.5	5.4
Stick cracker	C_{10}	Fabricated product	92.5	1.3
Stick cracker	C_{11}	Fabricated product	93.0	1.7
Sweet biscuit	C_{12}	Fabricated product	97.0	30.0
Sweet biscuit	C_{13}	Fabricated product	93.0	2.1
Sweet biscuit	C_{14}	Fabricated product	94.0	2.6
Sweet biscuit	C_{15}	Fabricated product	95.0	6.0
Breakfast cereal	C_{16}	Fabricated product	97.0	20.0
Breakfast cereal	C_{17}	Fabricated product	96.5	5.2
Breakfast cereal	C_{18}	Fabricated product	97.0	4.5
White bread	C_{19}	Local product	74.0	5.2
White bread	C_{20}	Local product	76.0	7.0
White bread	C_{21}	Local product	77.5	7.2
Pitta bread	C_{22}	Local product	55.0	6.0
Pitta bread	C_{23}	Local product	56.0	8.0
Crisp bread	C_{24}	Fabricated product	93.0	28.0
Pasta Product	C_{25}	Fabricated product	86.0	-
Pasta product	C_{26}	Fabricated product	87.0	-
Pasta Product	C_{27}	Fabricated product	85.0	-
Pasta Product	C_{28}	Fabricated Product	86.5	-

C, Cereal

Recovery of HMF from samples was determined according to standard HMF addition method. The recoveries ranged from 67.7 to 97.3 % and average recovery was determined as 85.2 % for all samples. The lower recovery of HMF for these products was probably due to absorption of HMF within the protein matrix. Ramirez-Jimenez (2000b) found HMF recovery of bakery products changed from 90.1 to 97.8 %. Table 3.9 shows HMF recoveries for each group of samples.

Table 3.9 Recoveries of HMF from cereal product samples

Sample	HMF in original sample (ppm)	Total (original+added) HMF (ppm)	Found (ppm)	% Recovery
Stick cracker	2.5	27.5	19.0	69.0
Salty cracker	11.6	36.6	35.0	95.6
Sweet biscuit	5.0	31.0	21.0	67.7
Breakfast cereal	9.0	34.0	29.3	86.0
Crisp bread	4.9	29.9	27.3	91.3
Bread	4.0	29.0	26.2	90.5
Cream-filled biscuits	23.0	48.0	46.8	97.3

Figure 3.12 shows change in HMF concentration of samples with respect to different groups. Wide range of HMF concentration was found from samples ranged from 0.7 ppm to 29 ppm. In the case of pasta samples, there was no detectable amount of HMF concentration. Statistical analysis of data showed significant differences ($p<0.05$) between the firms. The differences between the HMF concentrations may be due to the variations in the processing conditions (heat treatment, and etc.), differences in composition of samples and additives.

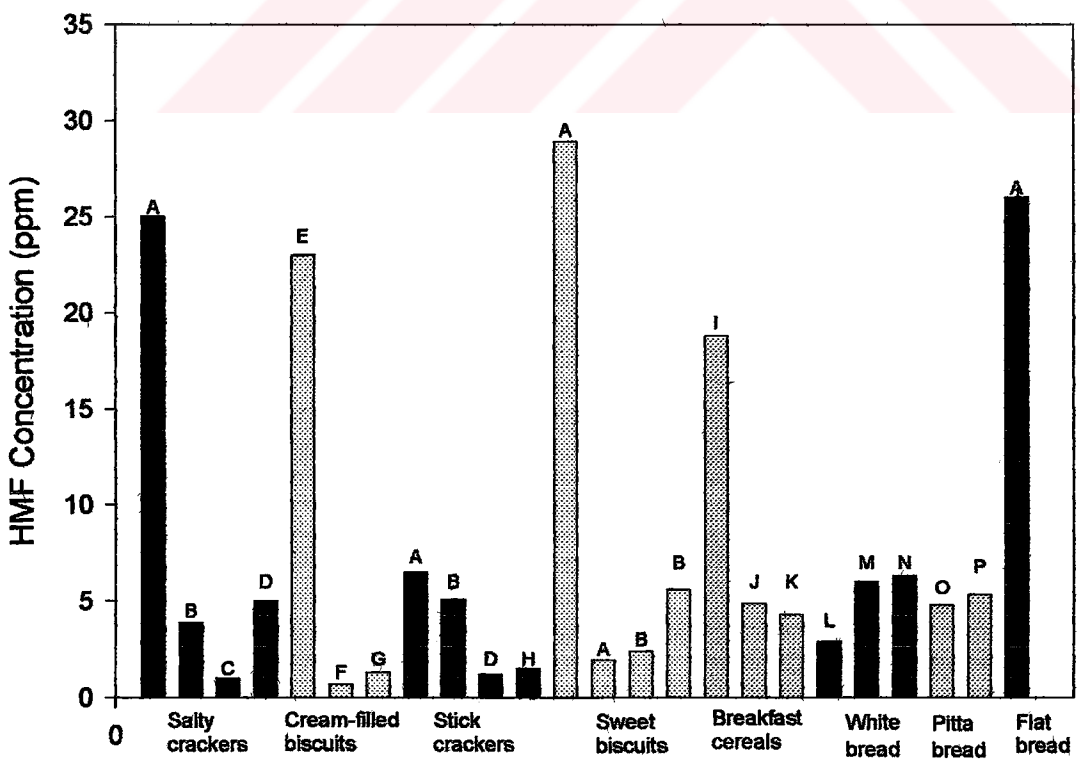

Figure 3.12 Change in HMF concentrations for various cereal samples determined by HPLC method (letters (A-P) show different firms)

According to Ramirez-Jimenez *et al.* (2000a), HMF and color were useful indicators to evaluate the intensity of browning of baking process. HMF contents of bakery products have shown wide variability (Ramirez-Jimenez *et al.*, 2000b). In this study, the highest HMF contents were found in breakfast cereals, salty crackers and sweet biscuits. This may be explained as follows. Breakfast cereals include both high amount of proteins and reducing sugars together and may give suitable conditions for the Maillard reaction to take place. Furthermore, higher concentrations of HMF in breakfast cereals may be due to the extrusion cooking method which is a high temperature application process used during manufacturing of breakfast cereals. Maillard reaction is favored in foods with high protein and carbohydrate content and intermediate moisture content at temperatures above 50 ^{0}C (O'Brien and Morrisey, 1989). Garcia-Villanova *et al.* (1993) found range of HMF values for breakfast cereals between 3.7 to 193.3 ppm. HMF concentrations of breakfast cereals ranged from 4.3 ppm to 19 ppm in this study. Our results are in agreement with those found in literature.

Higher concentrations of HMF were found in salty crackers and sweet biscuits. The concentrations ranged from 1 to 25 ppm for salty crackers and 2.5 to 29 ppm for sweet biscuits. These two types of products are good sources of reducing sugars, thus caramelization reactions may take place during high temperature and long time baking process. Caramelization needs more drastic conditions such that temperatures above 120 ^{0}C, pH<3 or pH>9 and low a_w (Kroh, 1994). During baking, water content of surface becomes lower and combined with high temperature and may result caramelization at the surface. HMF concentrations of cream-filled biscuits and stick crackers changed from 1.3 to 23 ppm and 1.2 to 6.5 ppm, respectively.

There was no significant difference ($p<0.05$) between HMF concentrations of white and pitta breads. Ramirez-Jimenez *et al.* (2000a) found that HMF contents of various bread types changed from 2.2 to 87.7 ppm. The obtained HMF concentrations in this study ranged from 3.5 to 6.5 ppm for white and pitta breads. Water content distribution and temperature play an important role in developing the sensory characteristics of these products (Ramirez-Jimenez *et al.*, 2000b). Caramelization reactions mainly take place at surface of breads by degradation of sugars at high temperature. During baking moisture content of surface is reduced by heating effect and may lead to increase in caramelization rate. White breads had higher HMF

concentrations with respect to pitta breads, since the HMF determination of white breads were carried on crust part, although that of pitta breads were carried out on whole bread. Besides, HMF concentrations of white breads were also showed a significant difference ($p<0.05$) with respect to each other. This may be due to the variations in heat treatments during manufacturing. HMF concentration of crisp bread was found as 26 ppm. Ramirez-Jimenez *et al.* (2000a) found that HMF concentrations of sliced toasted breads changed from 11.8 to 87.7 ppm. Higher HMF concentration found for the crisp bread compared to the other cereal products may be due to the differences in processing conditions and composition. It has also lower moisture content and higher baking temperature with respect to the other types.

Several studies showed that nonenzymic browning reactions, especially Maillard reaction, are strongly enhanced in pasta products (Resmini *et al.*, 1993; Resmini *et al.*, 1996). Although there was high susceptibility of browning in pasta products, no detectable quantity of HMF was determined in the analysis of pasta samples. The results were in agreement with those reported by Resmini *et al.* (1993). He reported that there may be a lack of or trace amounts (<0.1 ppm) of HMF in commercially dried pasta. In pasta products, the early Maillard reaction can occur between reducing sugars and amino group of available lysine under high- temperature drying conditions as well as equilibrium relative humidity values ranging from 12 to 30 % (Sensidoni *et al.*, 1999). A water-soluble furanic compound was identified in dried pasta samples, arising from the advanced Maillard reaction between maltose and free amino acids, called 2-acetyl-3-D-glucopyranosylfuran (AGPF), and a lysylpyrrolaldehyde (LPA), recognised as the main derivative of protein-bound lysylketoses degradation. It was found that the higher the drying temperature the higher the formation of these compounds, which, therefore, can be used as indices for evaluating the extent of the Maillard reaction in dried pasta. N-(2-furoylmetyl)-L-lysine (furosine) and 2-acetyl-3-D-glucopyranosylfuran (AGPF) have been proposed as markers of the early and advanced Maillard reaction. (Resmini *et al.*, 1993).

As a result, HMF content of cereal products showed a wide range with respect to the type of products. HMF contents of cereal products may be used to get an idea about the period and strength of the heat treatment applied during processing.

3.2.5 HMF Content of Beverages

In this part of the study, four different groups of samples including fruit juices, carbonated beverages, alcoholic beverages and vinegars were analysed. Nonenzymic browning has been considered one of the major causes of quality and color loss during the processing and storage of these products.

Twenty five different samples of beverages and 3 samples of vinegars were examined for the determination of HMF content. Analysed beverages included twelve samples of fruit juices, six samples of carbonated beverages and seven samples of alcoholic beverages. Table 3.10 shows soluble solids and HMF contents (in dry basis) of samples.

Table 3.10 Soluble solids and HMF contents of beverages and vinegars (HPLC method)

Sample Identification	Sample number	Production type	Soluble Solids Content (^{0}Brix)	HMF Content in Dry Basis (ppm)
Apricot Juice	FJ_1	Commercial Production	15.5	3.9
Apricot Juice	FJ_2	Commercial Production	13.3	22.6
Apricot Juice	FJ_3	Commercial Production	15.3	21.6
Apricot Juice	FJ_4	Commercial Production	15.9	22.0
Peach Juice	FJ_5	Commercial Production	13.3	18.8
Peach Juice	FJ_6	Commercial Production	15.5	25.8
Peach Juice	FJ_7	Commercial Production	16.5	38.0
Cherry Juice	FJ_8	Commercial Production	15.5	23.2
Cherry Juice	FJ_9	Commercial Production	15.5	22.6
Cherry Juice	FJ_{10}	Commercial Production	13.3	75.0
Orange Juice	FJ_{11}	Commercial Production	13.3	19.5
Orange Juice	FJ_{12}	Commercial Production	14.4	32.0
Cola	CB_1	Commercial Production	2.0	35.0
Cola	CB_2	Commercial Production	2.1	38.0
Cola	CB_3	Commercial Production	3.0	33.0
Cola	CB_4	Commercial Production	8.0	49.0
Cola	CB_5	Commercial Production	11.0	59.0
Cola	CB_6	Commercial Production	12.0	86.0
Red Wine	AB_1	Commercial Production	10.0	-
Red Wine	AB_2	Commercial Production	11.0	-
Beer	AB_3	Commercial Production	8.5	79.0
Beer	AB_4	Commercial Production	8.0	107.5
Beer	AB_5	Commercial Production	7.7	157.0
Beer	AB_6	Commercial Production	7.5	240.0
Beer	AB_7	Commercial Production	8.5	239.0
Vinegar	V_1	Commercial Production	7.0	-
Vinegar	V_2	Commercial Production	5.0	20.0
Vinegar	V_3	Commercial Production	6.0	113.0

FJ, Fruit Juice; CB, Carbonated Beverage; AB, Alcoholic Beverage; V, Vinegar

HMF concentrations of all analysed samples showed wide variability for each group and changed from 0 to 20.3 ppm. Soluble solids content of samples ranged within 13.3 to 16.5 for fruit juices, 2.1 to 12 for carbonated beverages, 7 to 11 for alcoholic beverages and 6 to 7 for vinegars. When HMF concentrations of samples were evaluated in dry basis, wider range of HMF concentrations was obtained and it was 3.9-240 ppm. Average HMF concentrations obtained from analysis of each group were found as 4 ppm for fruit juices, 3.9 ppm for carbonated beverages, 7.2 ppm for alcoholic beverages and 2.7 ppm for vinegars. When average HMF concentrations of were statistically compared, the HMF concentration is significantly different ($p<0.05$) in samples except fruit juices and carbonated beverages. The maximum HMF concentration was found for the alcoholic beverages. Recovery of HMF from samples was determined by standard addition method and average recovery was found as 98 %.

Fruit Juices

Figure 3.13 shows change in HMF concentrations of fruit juice samples with respect to types and firms. As shown, HMF was detected in all analysed samples, indicating the occurance of this compound as the degradation product of nonenzymic browning reactions. Lee and Nagy (1988b) reported that HMF is a precursor of browning pigments, and the formation of this browning intermediate is probably as a result of by-product production from sugars and ascorbic acid or from amino acids and sugars.

The HMF concentrations of analysed fruit juices changed from 0.6 ppm to 10 ppm. Similar results were reported by Yuan *et al.* (1998). Although the HMF concentrations found in all samples did not exceed limiting HMF concentration that should be max 10 ppm for fruit juices (Anonymous, 1989), two samples, with HMF concentrations of 6.3 ppm and 10 ppm, were susceptible to the excessive browning. All analysed samples had shelf life of one year and they were analysed in fourth month of their shelf life. It can be said that the HMF concentrations of these two samples may be increased by any increase in temperature of storage or through the end of storage time. Cerrutti *et al.* (1985) studied accumulation of HMF in model system simulating the high water activity foods. They observed that HMF accumulation was significantly dependent on temperature. Lee and Nagy (1988b) reported the formation of nonenzymic browning intermediates in grape fruit juices

during storage and they observed that more HMF is formed by increasing both storage periods and temperature.

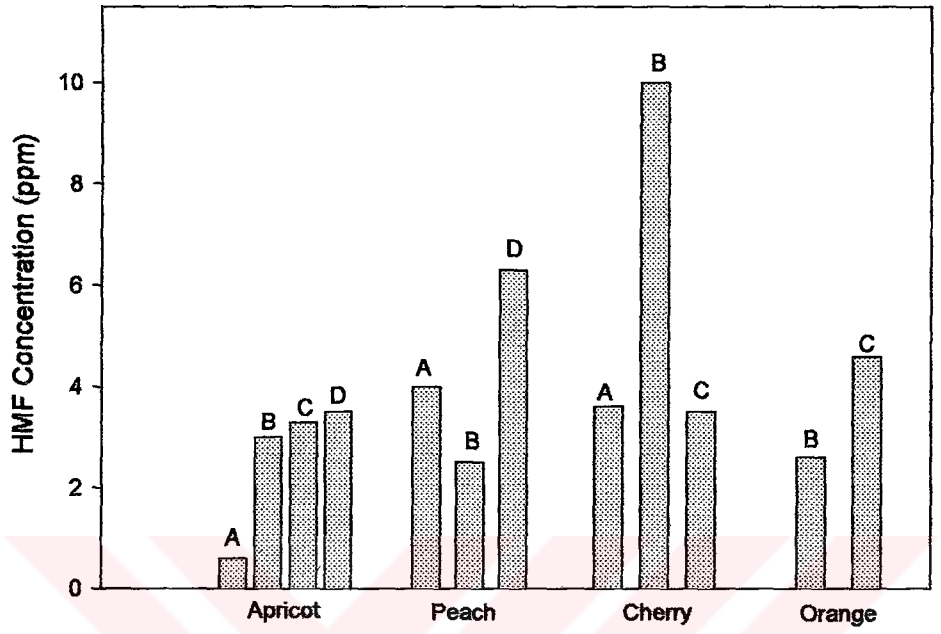

Figure 3.13 HMF concentrations of fruit juices determined by HPLC method (letters (A-D) show different firms)

Statistical analysis of data showed significant difference ($p<0.05$) with respect to the types of beverages. When average HMF concentrations were compared, HMF concentration of cherry juice was higher than that of the other juice samples. High concentration of HMF was probably due to the high acidity of cherry juice. It was reported that acid-catalyzed thermal decomposition of reducing sugars to reactive intermediates is an important element of citrus nonenzymic browning (Handwerk and Coleman, 1988). Large amounts of organic acids and their salts, mainly citric acid, create favorable conditions for degradation of sugars during processing and upon subsequent storage (Lee and Nagy, 1990). Kroh (1994) concluded that, caramelization of sugars is effected by heat and it is catalyzed by acids and bases. Furthermore, difference in HMF concentrations of juice samples may be due to variations in composition of samples. Toribio and Lozano (1986) stated that, rate of nonenzymic browning reaction depended not only on environmental conditions, such as temperature and water content, but also on the chemical composition and more critically on the total amino acids.

HMF concentrations of the samples also changed with respect to the types of firms. As mentioned earlier, this difference may be due to effect of processing conditions such as pasteurization, evaporation or deaeration. Nonenzymic browning of peach puree was studied by Garza *et al.* (1999). They observed the effect of heat treatment on HMF formation and obtained necessary kinetic parameters for the reactions. They found that HMF concentration increased with increase in both treatment time and temperature. The rate of nonenzymic browning reactions is also dependent on the soluble solids content and the type of sugar present in juice (Toribio and Lozano, 1986; Lee and Nagy, 1990). Citrus juices mainly contain sucrose, fructose, glucose, but a number of other sugars are present in small amounts (Handwerk and Coleman, 1988). In a sugar-catalyst model system (Lee and Nagy, 1990), simulating grapefruit juice, fructose was major potential source for the formation of HMF. Fructose was 36 times faster than the glucose, whereas, sucrose was 18 times faster than glucose in the formation of HMF. Thus, the importance of fructose in browning of citrus juices should be considered. Arena *et al.* (2001) studied thermal damage in blood orange juice and in its model system. It was concluded that the HMF formation in blood orange juice depends only on sugar concentration and thus, during the thermal processing of blood orange juice the Maillard reactions can be excluded.

Carbonated Beverages

In the case of carbonated beverages, HMF concentrations of six cola samples were determined. Highly variable HMF concentrations were obtained and the values changed from 0.7 to 10.3 ppm. Yuan *et al.* (1998) found HMF concentration of cola sample as 9.5 ppm. Figure 3.14 shows change in HMF concentrations of different cola samples. Formation of HMF in those products was probably due to the caramelization of sugars at high temperatures during syrup-making process, which is main processing step for these type of products. The quantities of sugar degradative products are increased by acid catalyzed thermal treatments of sugars. Lee and Nagy (1990) observed accelerating effects of citric acid on HMF formation. Furthermore, during processing of these products, caramels added as a coloring agent may also increase the HMF content. Caramel colors, i.e. ammonia caramel, ammonia sulphite caramel, and caustic caramel are the most widely used food additives and are found as coloring agents in a wide range of foods and beverages (Kroh, 1994).

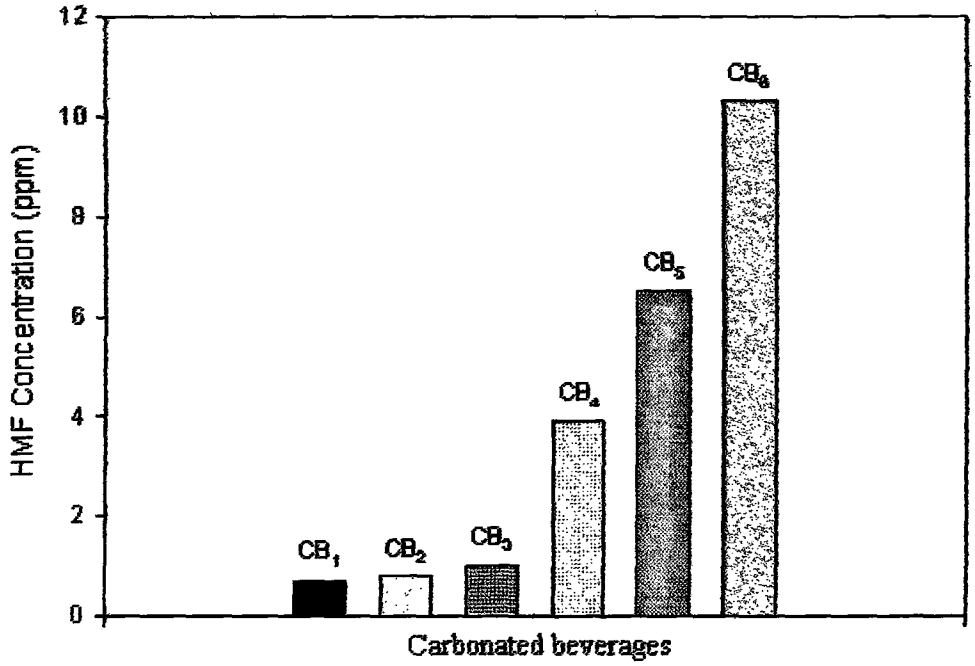

Figure 3.14 HMF concentrations of carbonated beverages determined by HPLC method (CB, Carbonated Beverage)

Soluble solids content of samples showed wide variation as well and they ranged from 2.1 to 12 brix. According to obtained data, samples with higher soluble solids content generally included higher concentration of HMF. As mentioned previously, the formation of HMF in cola mainly occurs during syrup making process. During processing of cola; prepared sugar syrup, water and cola additive were mixed to each other with a predefined concentration. According to dilution rate of syrup and cola additive with water, the soluble solids content and HMF concentration of final cola changed.

Alcoholic Beverages

Seven samples of alcoholic beverages were examined for the determination of HMF; five samples were beer and two samples were red wine. Change in HMF concentrations of various alcoholic beverage samples is shown in Figure 3.15. HMF concentration of analysed beers was significantly different ($p<0.05$) from each other. This variability relates most probably to differences between raw materials used in the production of the different beer types, and in particular to the degree of kilning or roasting of the barleys and malts used. Since, HMF is known to be an intermediate in the Maillard reactions that take place during kilning or roasting of beer (Madigan *et al.*, 1998). Shimizu *et al.* (2001) studied concentrations of HMF at various stages of

the brewing process and after the beer had been stored. The HMF concentration did not increase in the mashing process but increased dramatically during wort boiling and wort clarification and storage. The increase accelerated at higher temperatures. Processing conditions also effect the formation of HMF in beer, such as temperature, pH and amount of active oxygen.

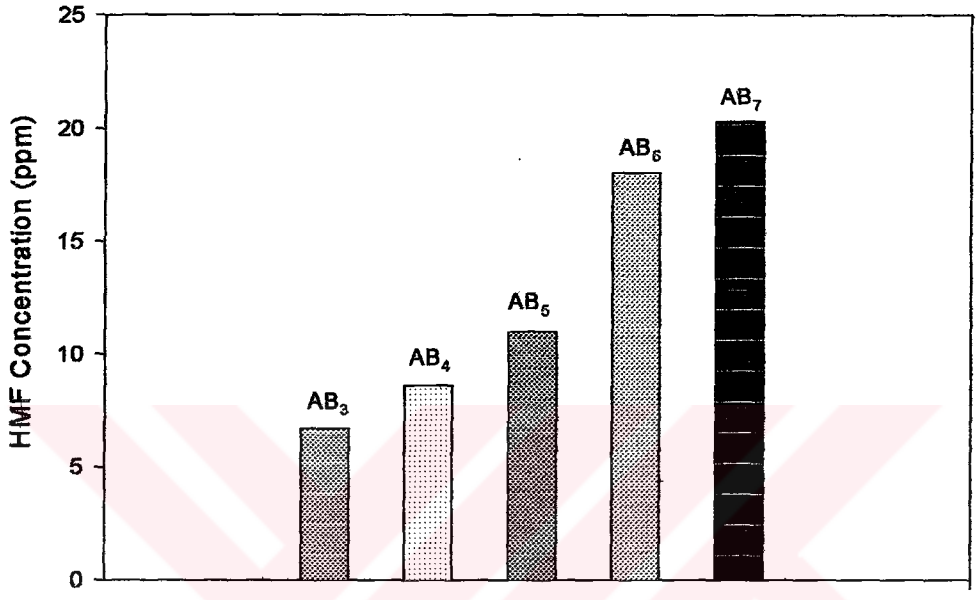

Figure 3.15 HMF concentrations of alcoholic beverages determined by HPLC method (AB, alcoholic beverages)

It was reported that HMF and furfural are index compounds for beer staling (Shimizu *et al.*, 2001). The HMF results for beer samples changed from 6.7 to 20.3 ppm. Soluble solids content of whole samples were changed from 7.5 to 8.5 brix. These results were in agreement with previous ones obtained by Madigan *et al.* (1998). They studied formation of HMF during storage of beer at various temperatures. They found that initial HMF content of fresh beers were ranged from 0.72 to 2.19 ppm, but considerable increase in HMF content was observed in beer samples that stored at 37 and 60^{0}C. According to this, it can be said that analysed beer samples may be stored at higher temperatures than recommended during storage time. Thus, kinetics of HMF formation in beer may be useful for estimation of average temperature at which given beer has been stored.

It was suggested that controlling HMF formation during brewing might provide useful clues for improving the flavor stability of beer. Because there is a correlation between the organoleptically determined degree of staling flavor and HMF contents in beer (Schimuzu *et al.*, 2001). High level of HMF concentration in analysed samples may also exhibit staling flavor in beer. The concentration of furanic aldehydes, HMF and furfural, serves to provide an estimate of the level of staleness in beer (Madigan *et al.*, 1998). Refrigerated storage and/or transportaion of beer during marketing may be useful way of retarding nonenzymic browning of beer and stale flavor.

No detectable concentration of HMF was observed in the wine samples. Yuan *et al.* (1998) found HMF concentration of red wine as 1.3 ppm, while they found 1 ppm of HMF concentration for white wine by HPLC method. Kroh (1994) studied kinetics of HMF formation in model system representing dessert wine. Determination of HMF content was important for brandies, since it may be useful for measurement of added caramel into commercial brandies (Mir *et al.*, 1992).

Vinegar

Depending on the origin of food-matrix, there are different types of vinegar. Three vinegar samples were analysed, all of them were produced from red grape. HMF was present only in two samples and the concentrations were 1 ppm and 7 ppm. HMF concentrations of vinegars were highly variable and changed according to the source of vinegar (Theobald *et al.*, 1998). Highly favourable data was obtained in this study from this point of wiev. Generally grape wine is produced by microbiological fermentation of concentrated red or white grape juice. During processing or storage, it is expected to increase HMF concentration of sugar containing samples. On the other hand, there are limiting amounts of sugars for nonenzymic browning reaction and it was probably due to fermentation of available sugar. According to classification of Theobald *et al.* (1998), analysed samples replaced in low HMF concentration vinegars. These groups cover the types of red wine vinegar, white wine vinegar, table vinegar with caramel and HMF concentration of these groups changed from 0 to 10.5 ppm. The results obtained in this study in agreement with those reported by Theobald *et al.* (1998). Regarding HMF content, it should be stated that vinegar does not contribute considerably in comparison to other foods.

3.2.6 HMF Content of Halvah

Halvah is a traditional product which is produced by mixing of sugar syrup with tahini. For the preparation of sugar syrup, sucrose is firstly mixed with water and heated in open tank up to 130 ^{0}C. After that, the syrup is transferred into the another tank and soapwort is added. Syrup is concentrated in this tank up to desired consistency and immediately mixed and kneaded with the tahini. Nonenzymic browning reactions may take place in halvah during syrup preparation and further storage of final product.

Totally, five halvah samples were analysed for the determination of HMF content. Sugar syrup and sugar syrup-soapwort mixture were also analysed to discuss the effect of processing conditions on HMF formation during the production of halvah. Table 3.11 lists the total solid and HMF contents (in dry basis) of halvah, syrup and syrup-soapworth mixture. Recovery of HMF was found as 93 % and given HMF results were corrected according to 100 % recovery.

Table 3.11 Total solid and HMF contents of halvah (HPLC method)

Sample Identification	Sample number	Production type	Total solid content (%)	HMF content in dry basis (ppm)
Halvah	H_1	Commercial Production	98.0	49.0
Halvah	H_2	Commercial Production	97.7	126.0
Halvah	H_3	Commercial Production	98.0	151.0
Halvah	H_4	Commercial Production	97.6	165.0
Halvah	H_5	Commercial Production	97.5	173.3
Syrup	S	Commercial Production	-	-
Syrup-Soapwort Mixture	SS	Commercial Production	-	-

H, Halvah; S, Syrup; SS, Syrup-Soapworth Mixture

HMF concentrations of halvah samples ranged from 48.6 to 168 ppm. Sample H_1, syrup and soapworth-syrup mixture were immediately obtained from manufacturer during production. The HMF concentration of sample H_1 was 48.6 ppm, whereas that of syrup and soapworth-syrup mixture was found as 167.8 ppm and 178.7 ppm, respectively (Figure 3.16). According to these results, it can be said that HMF formation in halvah was probably due to caramelization of sugars at high temperatures during syrup making process. During thermal treatment of syrup, sugars degraded to its derivatives in the presence of acids, minerals and amino acids (Lee and Nagy, 1990).

As shown in Figure 3.16, all halvah samples contained excessive amounts of HMF except H_1. Sample H_1 was a fresh product that was immediately obtained after production. Other halvah samples (H_2-H_4) were obtained from local market during their storage. It can be seen that HMF content of halvah significantly increased during storage as well. As a result it can be said that, nonenzymic browning reactions excessively took place in halvah during production and storage. HMF content may be useful indicator to evalaute amount of browning in these products. However, there is no standard about limiting values of HMF in halvah according to Turkish Standard Institute.

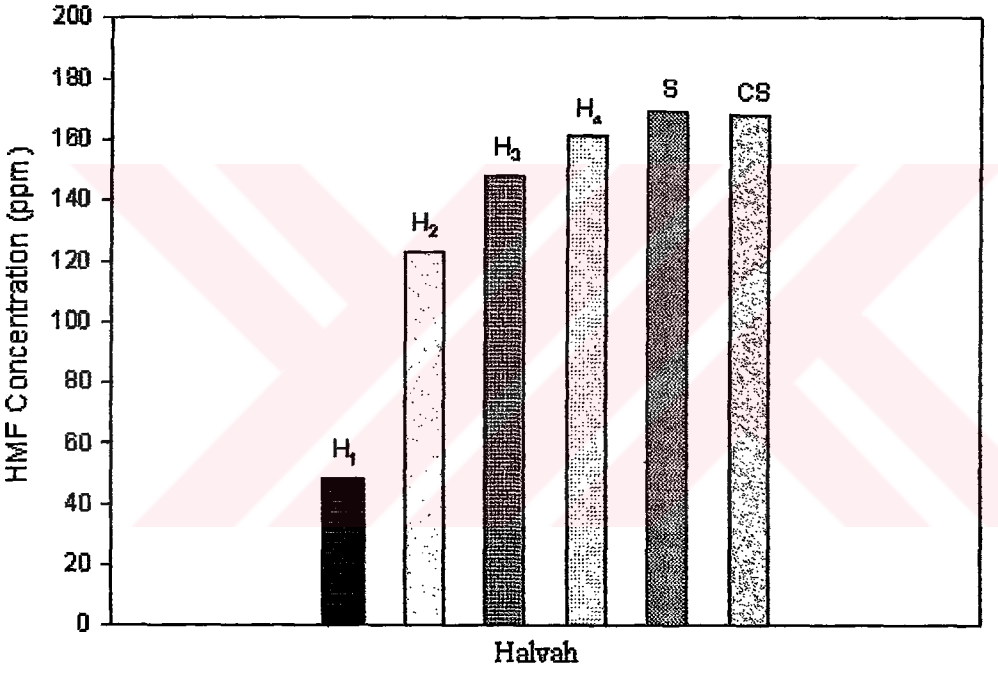

Figure 3.16 HMF concentrations of halvah samples,syrup and syrup-soapworth mixture determined by HPLC method (H, Halvah; S, Syrup; SS, Syrup-Soapworth Mixture)

CONCLUSIONS

The following conclusions can be made after the examination of experimental results.

1. The HPLC method gives higher sensitivity and more accurate results for the determination of HMF in food products with respect to spectrophotometric method.
2. Most of analysed food products (dried and concentrated foods, honey, jams, cereal products, beverages, vinegars and halvah) included the HMF which indices the occurance of nonenzymic browning reactions. The HMF concentrations showed wide variability and ranged between 0 and 3500 ppm.
3. The HMF concentration of individual food products ranged within 0.3-3500 ppm for concentrated and dried food products, 0.5-27 ppm for honey, 4.2-80 ppm for jams, 0.7-29 ppm for cereal products, 0-20.3 ppm for beverages, 0-7 ppm for vinegars and 48.6-168 ppm for halvah.
4. The total solid/soluble solids contents of food products were found to be ranged within 13-88 % for concentrated and dried food products, 80.5-89 brix for honey, 66-77 brix for jams, 55-97 % for cereal products, 2-16.5 brix for beverages, 5-7 brix for vinegars and 97.5- 98 % for halvah. When HMF concentrations of food products were evaluated in dry basis, wider range of HMF concentrations was obtained and it was 0-5000 ppm.
5. The effect of processing type on HMF content was significant for boiled juices, honey, jam and tomato and paprika pastes. The higher HMF concentration was found in open type processed boiled juices and jams with respect to vaccum type. Processed honeys had significantly higher HMF values than the non-processed ones. Commercially produced tomato pastes had higher HMF concentrations with respect to traditionally produced ones.

REFERENCES

Ames, J.M., Arnoldi, A., Bates, L., Negroni, M. (1997). Analysis of methanol-extractable nonvolatile Maillard reaction products of a model extrusion-cooked cereal product. *Journal of Agriculture and Food Chemistry*, **45**, 1256-1263.

Anklam, E. (1998). A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry*, **63**, 549-562.

Anonymous, (1983). TSE No: 3631, Pekmez, Turkish Standard Institute, Ankara

Anonymous, (1983). TSE No: 3958, Sour Cherry Jam, Turkish Standard Institute, Ankara

Anonymous, (1989). TSE No: 1535, Orange Juice, Turkish Standard Institute, Ankara

Association of Official Analytical Chemists (AOAC), (1990). *Official methods of Analysis of the Association of Official Analytical Chemists* (15 th ed.) Washington, DC.

Arena, E., Fallico, B., Maccarone, E. (2001). Thermal damage in blood orange juice: kinetics of 5-hydroxymethyl-2-furancarboxaldehyde formation. *International Journal of Food Science and Technology*, **36**, 145-151.

Ashoor, S.H. and Zent, J.B. (1984). Maillard browning of common amino acids and sugars. *Journal of Food Science*, **49**, 1206-1207.

Batu, A. (1991). Farklı iki yönteme göre elde edilen kuru üzüm pekmezinin kimyasal bileşiminde oluşan değişmeler üzerinde bir araştırma. *Cumhuriyet Üniversitesi Tokat Ziraat Fakültesi Dergisi*, 7, 179-190.

Batu, A. (2001, Şubat 2). Pekmez üretim ve denetimindeki geleneksel problemler. *Gıda*, pp. 78-81.

Boekel, M.A.J.S. (2001). Kinetics aspects of the Maillard reaction: a critical review. *Nahrung*, **45**, 150-159.

Bozkurt, H. (1996). *Kinetics of color changes due to Maillard reaction s in model systems.* A MSc Thesis in Food Engineering Department, University of Gaziantep. Gaziantep: Gaziantep University Press.

Bozkurt, H., Göğüş, F., Eren, S. (1998). Nonenzymic browning reactions in boiled grape juice and its models during storage. *Food Chemistry*, **64**, 89-93.

Buera, M.P., Resnik, S.L., Petriella, C. (1992). Relative browning rates of fructose and glucose in high water activity systems as a function of amino compound concentration. *Lebensmittel-Wissenschaft Untersuchung-Technology*, **25**, 35-37.

Cerrutti, P., Resnik, S.L., Seldes, A., Fontan, C.F. (1985). Kinetics of deteriorative reactions in model food systems of high water activity: glucose loss, 5-hydroxymethylfurfural accumulation and fluorescence development due to nonenzymatic browning. *Journal of Food Science*, **50**, 627-630.

Chiang, G.H. (1983). A simple and rapid high performance liquid chromatographic procedure for determination of furosine, lysine-reducing sugar derivative. *Journal of Agriculture and Food Chemistry*, **31**, 1373-1374.

Corradini, D. and Corradini, C. (1992). Separation and determination of 5-hydroxymethyl-2-furaldehyde and 2-furaldehyde in fruit juices by micellar electrokinetic capillary chromatography with direct sample injection. *Journal of Chromatography*, **624**, 503-509.

Costa, L.S.M., Albuquerque, M.L.S., Trugo, L.C., Quinteiro, L.M.C., Barth, O.M., Riberio M., De Maria, C. A. B. (1999). Determination of non-volatile compounds of different botanical origin Brazillian honeys. *Food Chemistry*, **65**, 347-352.

Danehy, J.P. (1986). Advances in food research. In Academic Press Inc., *Maillard reaction: nonenzymatic browning in food systems with special reference to the development of flavor*.

Davies, C.G.A. and Labuza, T.P. (1997). The Maillard reaction application to confectionery products. http://www.fsci.umn.edu/Ted_Labuza/PDF_files

Eichner, K. and Karel, M. (1972). The influence of water content and water activity on the sugar-amino browning reaction in model systems under various conditions. *Journal of Agriculture and Food Chemistry*, **20**, 218-223.

Eichner, K. (1975). Water relations of foods. In R.B. Duckworth (Ed.), *The influence of water content on non-enzymic browning reactions in dehydrated foods and model systems and the inhibition of fat oxidation by browning intermediates*.

Eichner, K. and Ciner-Doruk, M. (1981). Early indication of the Maillard reaction by analysis of reaction intermediates and volatile decomposition products. *Progress in Food and Nutrition Science*, **5**, 115-135.

Ekşi, A. and Velioğlu, S. (1990, Mayıs 5). Hidroksimetilfurfural (HMF)açısından ticari reçellerin durumu. *Gıda Sanayii*, pp. 30-34.

Eskin, N.A.M (1990). *Biochemistry of foods.* (2nd ed.) California: Academic Press. Inc.

Fayle, S.E. and Gerrard, J.A. (2002). RSC food analysis monographs. In P.S. Belton (Ed.), *The Maillard reaction.*

Feather, M.S. (1985). Chemical changes in food during processing. In T. Richardson (Ed.), *Some aspects of the chemistry of nonenzymatic browning (The Maillard Reaction).*

Fennema, R.O. (1976). *Principles of Food Science*. New York: Marcel Dekker Inc.

Fernandez-Artigas, P., Guerra-Hernandez, E., Garcia-Villanova, B. (1999). Browning indicators in model systems and baby cereals. *Journal of Agriculture and Food Chemistry*, **47**, 2872-2878.

Friedman, M. (1996). Food browning and its prevention: an overview. *Journal of Agricultural and Food Chemistry*, **44**, 631-653.

Garcia-Villanova, B., Guerra-Hernandez, E., Martinez-Gomez, E., Montilla, J. (1993). Liquid chromatography for the determination of 5-(hydroxymethyl)-2-furaldehyde in breakfast cereals. *Journal of Agriculture and Food Chemistry*, **41**, 1254-1255.

Garza, S., Ibarz, A., Pagan, J., Giner, J. (1999). Nonenzymatic browning in peach puree during heating. *Food Research International*, **32**, 335-343.

Gomis, B., Alvarez, M.D.G., Naredo, L.S., Alonso, J.J.M. (1991). High-performance liquid chromatographic determination of furfural and hydroxymethyl furfural in apple juice and concentrates. *Chromatographia*, **32**, 45-48.

Göğüş, F., Eren, S. (1997, Şubat 2). Pekmez imalatı esnasında esmerleşme reaksiyonlarının hızının belirlenmesi. *Gıda Teknolojisi*, pp. 34-39.

Gögüs, F., Düzdemir, C., Eren, S. (2000). Effects of some hydrocolloids and water activity on nonenzymic browning of concentrated orange juice. *Nahrung*, **44**, 438-442.

Guerra-Hernandez, E., Garcia-Villanova, B., Montilla-Gomez, J. (1992). Determination of hydroxymethyl furfural in baby cereals by high performance liquid chromatography. *Journal of Liquid Chromatography*, **15**, 2551-2559.

Handwerk, R.L. and Coleman, R.L. (1988). Approaches to the citrus browning problem. *Journal of Agriculture and Food Chemistry*, **36**, 231-236.

Hodge, J.E. (1953). Chemistry of browning reactions in model systems. *Agriculture and Food Chemistry*, **1**, 928-943.

Hodge, J.E. (1967). Chemistry and physiology of flavors. In H.W. Schultz, E.A. Day, L.M. Libbey (Eds.), *Origin of flavor in foods. Nonenzymatic browning reactions.*

Hodge, J.E., Mills, F.D., Fisher, B.E. (1972). Compounds of browned flavor derived from sugar-amine reactions. *American Association of Cereal Chemists*, **17**, 34-40.

International Federation of Fruit Juice Producers (IFFJP), (1964). *Analysen-analyses*. Switzerland

Karmas, R., Buera, M.P., Karel, M. (1992). Effect of glass transition on rates of nonenzymatic browning in food systems. *Journal of Agriculture and Food Chemistry*, **40**, 873-879.

Kato, H. And Tsuchida, H. (1981). Estimation of melanoidin structure by pyrolysis and oxidation. *Progress in Food Science and Nutrition*, **5**, 147-153.

Kim, H.J. and Richardson, M. (1992). Determination of 5-hydroxymethylfurfural by ion-exclusion chromatography with UV detection. *Journal of Chromotography*, **593**, 153-156.

Kroh, L.W. (1994). Caramelisation in food and beverages. *Food Chemistry*, **51**, 373-379.

Labuza, T. and Saltmarch, M. (1981). Water activitiy: Influences on food quality. In Academic Press. Inc., *The nonenzymatic browning reaction as affected by water in foods.*

Labuza, T.P. and Massaro, S.A. (1990). Browning and amino acid loss in model total parenteral nutrition solutions. *Journal of Food Science*, **55**, 821-826.

Labuza, T.P. and Baisier, W.M. (1992). Physical chemistry of foods. In H. Schwartzberg (Ed.), *The kinetics of nonenzymatic browning.*

Lee, H.S., Rouseff, R.L., Nagy, S. (1986). HPLC determination of furfural and 5-hydroxymethyl furfural in citrus juices. *Journal of Food Science*, **51**, 1075-1076.

Lee H.S. and Nagy, S. (1988a, November 42). Relationship of sugar degradation to detrimental changes in citrus juice quality. *Food Technology*, pp. 91-94, 97.

Lee H.S. and Nagy, S. (1988b). Quality changes and nonenzymic browning intermediates in grapefruit juice during storage. *Journal of Food Science*, **53**, 168-172.

Lee, H.S. and Nagy, S. (1990). Relative reactivities of sugars in the formation of 5-hydroxymethylfurfural in sugar-catalyst model systems. *Journal of Food Processing and Preservation*, **14**, 171-178.

Lo Coco, F., Valentini, C., Novelli, V., Ceccon, L. (1994). High performance liquid chromatographic determination of 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde in processed citrus juices. *Journal of Liquid Chromatography*, **17**, 603-617.

Lo Coco, F., Valentini, C., Novelli, V., Ceccon, L. (1995). Liquid chromatographic determination of 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde in beer. *Analytica Chimica Acta*, **306**, 57-64.

Lo Coco, F., Valentini, C., Novelli, V., Ceccon, L. (1996). High-performance liquid chromatographic determination of 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde in honey. *Journal of Chromatography A*, **749**, 95-102.

Lo Coco, F., Novelli, V., Valentini, C., Ceccon, L. (1997). High performance liquid chromatographic determination of 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde in fruit juices. *Journal of Chromatographic Science*, **35**, 578-583.

Madigan, D., Perez, A., Clements, M. (1998). Furanic aldehyde analysis by HPLC as a method to determine heat-induced flavor damage to beer. *Journal of American Society of Brewing Chemists*, **56**, 146-151.

Marcy, E.J., Rouseff, R.L. (1984). High performance liquid chromatographic determination of furfural in orange juice. *Journal of Agriculture and Food Chemistry*, **32**, 979-981.

Mendes, E., Proença, E.B., Ferreira, I.M.P.L.V.O., Ferreira, M.A. (1998). Quality evaluation of Porteguese honey. *Carbohydrate Polymers*, **37**, 219-223.

Mijares, R.M., Park, G.L., Nelson, D.B., Mciver, C. (1986). HPLC analysis of HMF in orange juice. *Journal of Food Science*, **51**, 843-844.

Mir, M.V., Granados, J.Q., Serrana, H.L., Martinez, M.C.L. (1992). High performance liquid chromatography determination of furanic comounds in commercial brandies and caramels. *Journal of Liquid Chromatography*, **15**, 513-524.

Morales, F.J., Romero, C., Jimenez-Perez, S. (1996). Evaluation of heat-induced changes in Spanish commercial milk: hydroxymethylfurfural and available lysine content. *International Journal of Food Science and Technology*, **31**, 411-418.

Namiki, M. (1988). Advances in food research. In Academic Press. Inc., *Chemistry of Maillard reactions: Recent studies on the browning reaction mechanism and the development of antioxidants and mutagens.*

Nicoletti, I., Corradini, C., Cogliandro, E., Corradini, D. (1996). Identification and dosage of 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde in beverages by reversed phase chromatography with a microbore column. *Journal of Liquid Chromatography and Related Technology*, **19**, 1241-1254.

Nozal, M.J., Bernal, J.L., Toribio, L., Jimenez, J.J., Martin, M.T. (2001). High-performance liquid chromatographic determination of methyl anthranilate, hydroxymethyl furfural and related compounds in honey. *Journal Chromatography A*, **917**, 95-103.

O'Brien, J. and Morrisey, P.A. (1989). Nutritional and toxicological aspects of the Maillard browning reaction in foods. *Critical Reviews in Food Science and Nutrition*, **28**, 211-235.

Peter, G.G. (1973). *Tomato paste and other tomato products.* London: Food Trade Press. Ltd.

Pomeranz Y.and Meloan, C.E. (2000). Food analysis, theory and practise (3th ed.). In Aspen Publisher Inc., *High-performance liquid chromatoghraphy and ion chromatoghraphy.*

Porretta, S. and Sandei, L. (1991). Determination of 5-(hydroxymethyl)-2-furfural (HMF) in tomato products: proposal of a rapid HPLC method and its comparison with the colorimetric method. *Food Chemistry*, **39**, 51-57.

Porretta, S. (1992). Chromatographic analysis of Maillard reaction products: review. *Journal of Chromatography*, **624**, 211-219.

Rada-Mendoza, M., Olano, A., Villamiel, M. (2002). Determination of hydroxymethylfurfural in commercial jams and in fruit-based infant foods. *Food Chemistry*, **79**, 513-516.

Ramirez-Jimenez, A., Guerra-Hernandez, E., Garcia-Villanova, B. (2000a). Browning indicators in bread. *Journal of Agriculture and Food Chemistry*, **48**, 4176-4181.

Ramirez-Jimenez, A., Garcia-Villanova, B., Guerra-Hernandez, E. (2000b). Hydroxymethylfurfural content of selected bakery products. *Food Research International*, **33**, 833-838.

Resmini, P., Pellegrino, L., Pagani, M.A., De Noni, I. (1993). Formation of 2-acetyl-3-d-glucopy-ranosylfuran (glucosylisomaltol) from nonenzymatic browning in pasta drying. *Italian Journal of Food Science*, **4**, 341-353.

Resmini, P., Pagani, M.A. and Pellegrino, L. (1996, August 48). Effect of semolina quality and processing conditions on nonenzymatic browning in dried pasta. *Food Australia*, pp. 362-367.

Resnik, S. and Chirife, J. (1979). Effect of moisture content and temperature on some aspects of nonenzymatic browning in dehydrated apple. *Journal of Food Science*, **44**, 601-605.

Roig, M.G., Bello, J.F., Rivera, Z.S., Kennedy, J.F. (1999). Studies on the occurance of nonenzymatic browning during storage of citrus juices. *Food Research International*, **32**, 609-619.

Royle, L., Bailey, R.G., Ames, J.M. (1998). Separation of Maillard reaction products from xylose-glycine and glucose-glycine model systems by cappilary electrophoresis and comparison to reverse phase HPLC. *Food Chemistry*, **62**, 425-430.

Sensidoni, A., Peressini, D. and Pollini, C.M. (1999). Study of the Maillard reaction in model systems under conditions related to the industrial process of pasta thermal VHT treatment. *Journal of the Science of Food and Agriculture*, **79**, 317-322.

Shimizu, C., Nakamura, Y., Miyai, K., Araki, S., Takashio, M., Shinotsuka, K. (2001). Factors affecting 5-hydroxymethyl furfural formation and stale flavor formation in beer. *Journal of American Society of Brewing Chemists*, **59**, 51-58.

flavor formation in beer. *Journal of American Society of Brewing Chemists*, **59**, 51-58.

Theobald, A., Müller, A., Anklam, E. (1998). Determination of 5-hydroxymethyl furfural in vinegar samples by HPLC. *Journal of Agriculture and Food Chemistry*, **46**, 1850-1854.

Toribio, J.L. and Lozano, J.E. (1984). Nonenzymatic browning in apple juice concentrate during storage. *Journal of Food Science*, **49**, 889-892.

Torbio, J.L. and Lozano, J.E. (1986). Heat induced browning of clarified apple juice at high temperatures. *Journal of Food Science*, **51**, 172-175.

Tosi, E., Ciappini, M., Re, E., Lucero, H. (2002). Honey thermal treatment effects on hydroxymethyl furfural content. *Food Chemistry*, 77, 71-74.

Tosun, İ., Üstün, N.Ş. (2003). Nonenzymic browning during storage of white hard grape pekmez (zile pekmezi). Food Chemistry, **80**, 441-443.

Velioğlu, S. and Artık, N. (1993, Nisan 4). Bazı pekmez örneklerinin standarda (TS 3792) uygunluğunun belirlenmesi üzerine araştırma. *Standard*, pp. 51-54.

Villamiel, M., Castillo, M.D., Corzo, N., Olano, A. (2001). Presence of furosine in honeys. *Journal of the Science of Food and Agriculture*, **81**, 790-793.

Warmbier, H.C., Schnickels, R.A., Labuza, T.P. (1976a). Nonenzymatic browning kinetics in an intermediate moisture model system: effect of glucose to lysine ratio. *Journal of Food Science*, **41**, 981-983.

Warmbier, H.C., Schnickles, R.A., Labuza, T.P. (1976b). Effect of glycerol on nonenzymatic browning in a solid intermediate moisture model food system. *Journal of Food Science*, **41**, 528-531.

Wolform, M.L., Kashimura, N., Horton, D. (1974). Factors affecting the Maillard browning reaction between sugars and amino acids. Studies on the nonenzymic browning of dehydrated orange juice. *Journal of Agriculture and Food Chemistry*, **22**, 796-800.

Yuan, J.P., Chen, F. (1998). Separation and identification of furanic compounds in fruit juices and drinks by high performance liquid chromatography photodiode array detection. *Journal of Agriculture and Food Chemistry*, **46**, 1286-1291.

APPENDICES

Table A1 ANOVA (one-way) for comparison spectrophotometric and HPLC methods

Sample Number		Sum of Squares	Df	Mean Square	F	P
1	Between Groups	17.682	1	17.82	712800	
	Within Groups	5.0 E-05	2	2.5 E-05		0.000
	Total	17.682	3			
2	Between Groups	1.000	1	1.000	200000	
	Within Groups	1.0 E-04	2	5.0 E-05		0.000
	Total	1.000	3			
3	Between Groups	3.240	1	3.240	64800	
	Within Groups	1.0 E-04	2	5.0 E-05		0.000
	Total	3.240	3			
4	Between Groups	2.890	1	2.890	57800	
	Within Groups	1.0 E-04	2	5.0 E-05		0.000
	Total	2.890	3			
5	Between Groups	3.240	1	3.240	64800	
	Within Groups	1.0 E-04	2	5.0 E-05		0.000
	Total	3.240	3			
6	Between Groups	90.250	1	90.250	1805000	
	Within Groups	1.0 E-04	2	5.0 E-05		0.000
	Total	90.250	3			
7	Between Groups	5.954	1	5.954	119072	
	Within Groups	1.0 E-04	2	5.0 E-05		0.000
	Total	5.954	3			
8	Between Groups	2.924	1	2.924	58482	
	Within Groups	1.0 E-04	2	5.0 E-05		0.000
	Total	2.924	3			
9	Between Groups				320000	
	Within Groups	16.000	1	16.000		
	Total	1.0 E-04	2	5.0 E-05		0.000
		16.000	3			
10	Between Groups	12.960	1	12.960	259200	
	Within Groups	1.0 E-04	2	5.0 E-05		0.000
	Total	12.960	3			
11	Between Groups	7.840	1	7.840	156800	
	Within Groups	1.0 E-04	2	5.0 E-05		0.000
	Total	7.840	3			
12	Between Groups	5.760	1	5.760	115200	
	Within Groups	1.0 E-04	2	5.0 E-05		0.000
	Total	5.760	3			
13	Between Groups	9.000	1	9.000	180000	
	Within Groups	1.0 E-04	2	5.0 E-05		0.000
	Total	9.000	3			

Table A2 Duncan's Multiple Range Test for fruit concentrates

		Subset for alpha 0.05				
Sample Type	N	1	2	3	4	5
Strawberry	2	0.35				
Briar rose	2	0.45				
Peach	2	0.50				
Apricot	2		1.55*			
Cherry	2			3.05*		
Orange	2				3.45*	
Apple	2					4.55*

* Significant difference at the 0.05 level