INVESTIGATION OF CHEMICAL REACTIONS IN HAZELNUT INDUCED BY ROASTING

FINDIKTA KAVURMA İLE MEYDANA GELEN KİMYASAL REAKSİYONLARIN İNCELENMESİ

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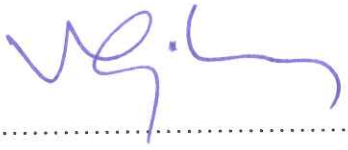
Supervisor ..... 

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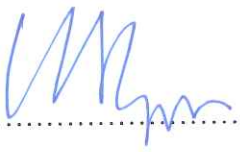
Member ..... 

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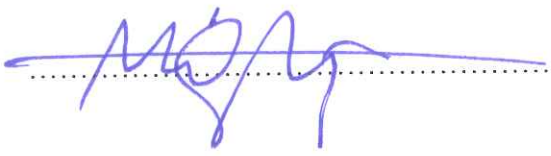
Member ..... 

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19 / 06 /2017

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NESLİHAN TAŞ

ABSTRACT

INVESTIGATION OF CHEMICAL REACTIONS IN HAZELNUT INDUCED BY ROASTING

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High lipid content, proteins and their reactive amino side chains, sugars including the reducing sugars as well as vitamins, minerals and the other constituents make hazelnut a complex reaction medium. The constituents of this reaction medium are precursors of many chemical reactions induced by roasting. Among the chemical reactions proceeding during roasting of hazelnuts; Maillard reaction, sugar degradation and lipid oxidation are the most prominent ones. As a consequence of these reactions, not only desirable properties like color, flavor and aroma are improved but also unavoidably undesirable compounds like 5-hydroxymethylfurfural and advanced glycation end products or toxigenic compounds like furan are formed. In order to understand the proceeding of these reactions during roasting of hazelnuts in depth, the formation and elimination of the main compounds of Maillard reaction, sugar degradation and lipid oxidation should be introduced, quantified and explained by a kinetic approach. The objective of this PhD thesis is the investigation of the chemical reactions occurring during roasting of hazelnuts. To achieve this aim, the proximate composition and profiles of constituents of hazelnuts belong to fourteen Turkish hazelnut varieties harvested in two consecutive years were evaluated at first. Then, the formation of Maillard reaction and sugar degradation products as well as their common products was determined at different roasting temperatures and times. Effect of oil on the formation of Maillard reaction products, early glycation and

advanced glycation end products, was also investigated by heating hazelnut resembling model reaction mixtures under the conditions of hazelnut roasting. Finally, a multiresponse kinetic model was proposed, after testing many of them, by using the data of roasted hazelnuts and the important reaction steps of Maillard reaction and caramelization were revealed.

At the beginning of this study, proximate composition of hazelnut varieties was determined as well as their profile analysis. By doing so, each component taking part in the chemical reactions during roasting was entirely identified. The lipid content of hazelnuts was found to be higher than the other constituents and rich in mostly oleic acid, followed by linoleic acid and saturated fatty acids namely palmitic and stearic acid. Hazelnut protein was found to contain all essential amino acids and 20% of total amino acids were arginine, glutamic and aspartic acid. The most predominant sugar in hazelnut was sucrose, constituted of 80% of total sugars. The others were glucose, fructose, raffinose, stachyose and myo-inositol. Phytic and malic acids were the organic acids found in hazelnut. Hazelnut was found to be rich in both water-soluble vitamins like panthotenic acid and nicotinic acid and lipid-soluble vitamins like alpha-tocopherol. Potassium, magnesium, calcium, sodium, manganese, zinc, iron and copper were the minerals found in hazelnut. Hazelnut skin, as a part of hazelnut, was found to be rich in phenolic compounds, 74% of which were in conjugated soluble form. Gallic and ferulic acids were the phenolic acids found in hazelnut skin.

Common products of Maillard reaction and sugar degradation quantified in roasted hazelnuts were alpha-dicarbonyl compounds including, 3-deoxyglucosone, 1-deoxyglucosone, 3,4-dideoxyglucosone, glyoxal, methylglyoxal and dimethylglyoxal, as well as 5-hydroxymethylfurfural and furan. An early glycation product, fructosyllysine, and an advanced glycation product, carboxymethyllysine, as well as acrylamide were the Maillard reaction products analyzed in roasted hazelnuts. 3-deoxyglucosone, glyoxal and methylglyoxal reached to the highest concentrations when compared with the other dicarbonyl compounds during roasting of hazelnuts. The maximum furosine concentration was almost four times higher than carboxymethyllysine concentrations of hazelnuts. Acrylamide, furan and 5-hydroxymethylfurfural were found to be not a great concern as process contaminants in roasted hazelnuts. The effect of oil on the formation of early and advanced glycation products during roasting of hazelnuts were enlightened by heating hazelnut resembling mixtures. Fructosyllysine was found to degrade immediately after its

formation within 15 min, indicating the predominance of advanced glycation products during prolonged roasting. The concentration of pyrraline, among the advanced glycation end products, was the highest indicating that the modification of lysine was mostly achieved by 3-deoxyglucosone. The concentration of other advanced glycation end product was carboxymethyllysine>formyline>MP-lysine>maltosine. MP-lysine, as an advanced lipation end product, was the only advanced stage product whose concentration increased depending on the amount of oil.

A multiresponse kinetic model of Maillard reaction and caramelization occurring during roasting of hazelnuts was proposed. Isomerization of glucose and fructose, sucrose degradation, 5-hydroxymethylfurfural formation, reaction of sugars and amino acids, degradation of Amadori/Heyns product, formation and degradation of 3-deoxyglucosone and 1-deoxyglucosone, formation of glyoxal, methylglyoxal, dimethylglyoxal and their further reactions were included in the proposed model. Model discrimination was performed and explained until to find the best model fits to the experimental data. The important reaction steps in the proposed model were found to be as isomerization of glucose and fructose through 1,2-enolization, formation of 5-hydroxymethylfufural from the fructofuranosyl cation which was formed via sucrose degradation, Amadori product formation from reaction of glucose and amino acids, glyoxal formation through glucose oxidation as well as methylglyoxal and dimethylglyoxal formation from 1-deoxyglucosone. The reactions occurring during roasting of hazelnuts leaded to the proceeding of Maillard reaction to the advance and final stages with the contribution of caramelization reaction as revealed by both mass balance of the compounds and the color of the hazelnuts.

Keywords: hazelnut, roasting, Maillard reaction, sugar degradation, advanced glycation end products, multiresponse kinetic modeling

ÖZET

FINDIKTA KAVURMA İLE MEYDANA GELEN KİMYASAL REAKSİYONLARIN İNCELENMESİ

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Yüksek lipid içeriği, proteinler ve onların reaktif amino yan zincirleri, indiren şekerleri de içeren şekerler ve ayrıca vitaminler, mineraller ve diğer bileşenler fındığı kompleks bir reaksiyon ortamı haline getirmektedir. Bu reaksiyon ortamının bileşenleri, kavurmadan kaynaklanan birçok kimyasal tepkimenin öncüleridir. Fındığın kavrulması sırasında meydana gelen kimyasal reaksiyonlar arasında en önemlileri Maillard reaksiyonu, şeker degradasyonu ve lipit oksidasyonudur. Bu reaksiyonların sonucunda, sadece renk, tat ve koku gelişimi gibi istenilen özellikler gelişmez aynı zamanda kaçınılmaz olarak 5-hidroksimetilfurfural ve ileri glikasyon son ürünleri gibi istenmeyen bileşikler veya furan gibi toksijenik bileşikler oluşur. Bu reaksiyonların fındık kavurma sırasında nasıl ilerlediğini tam olarak anlamak için, Maillard reaksiyonu, şeker degradasyonu ve lipit oksidasyonu ana bileşiklerinin oluşumu ve eliminasyonu ortaya koyulmalı, miktarları belirlenmeli ve kinetik bir yaklaşımla açıklanmalıdır. Bu doktora tezinin amacı fındıkta kavurma sırasında gerçekleşen kimyasal reaksiyonların araştırılmasıdır. Bu amaca ulaşmak için, öncelikle birbirini takip eden iki hasat yılına ait on dört Türk fındık türünün kompozisyon ve bileşenlerinin profil analizleri değerlendirilmiştir. Ardından, farklı kavurma sıcaklık ve sürelerinde Maillard reaksiyonu ve şeker degradasyonu ürünlerinin oluşumu ve ayrıca onların ortak ürünleri belirlenmiştir. Fındık benzeri model reaksiyon karışımlarının fındık kavurma koşullarında ısıtılmasıyla yağın Maillard reaksiyonu

ürünleri, erken glikasyon ve ileri glikasyon son ürünleri oluşumu üzerine etkisi araştırılmıştır. Son olarak, pek çok model test edildikten sonra, kavrulmuş fındıkların verileri kullanılarak bir kinetik model ileri sürülmüş ve Maillard reaksiyonu ve karamelizasyon reaksiyonlarının önemli reaksiyon basamakları ortaya çıkarılmıştır.

Bu çalışmanın başında, fındık türlerinin kompozisyon ve ayrıca profil analizleri belirlenmiştir. Böyle yapılarak, kavurma sırasında gerçekleşen reaksiyonlarda yer alan her bir bileşen bütünüyle belirlenmiştir. Fındıkların lipit içeriğinin diğer bileşenlere göre daha fazla olduğu ve çoğunlukla oleik asit bakımından zengin olduğu, onu linoleik asit ve doymuş yağ asitlerinin yani plamitik ve stearik asitin izlediği bulunmuştur. Fındık proteininin bütün esansiyel amino asitleri içerdiği ve toplam amino asitlerinin %20'sinin arjinin, glutamik, ve aspartik asitten oluştuğu bulunmuştur. Fındıktaki en baskın şeker, toplam şekerlerin %80'ini oluşturan, sukrozdur. Diğerleri glukoz, fruktoz, rafinoz, stakiyoz, ve miyo-inositoldür. Fitik ve malik asit fındıkta bulunan organik asitlerdir. Fındığın hem pantotenik asit ve nikotinik asit gibi suda çözünen vitaminler hem de alfa-tokoferol gibi yağda çözünenen vitaminler bakımından zengin olduğu bulunmuştur. Potasyum, magnezyum, kalsiyum, sodyum, manganez, çinko, demir, ve bakır fındıkta bulunan minerallerdir. Fındık zarının, fındığın bir kısmı olarak, %74'ü konjuge çözünür formda olan fenolik bileşiklerce zengin olduğu bulunmuştur. Gallik asit ve ferulik asit fındık zarında bulunan fenolik asitlerdir.

Kavrulmuş fındıklarda miktarı belirlenen Maillard reaksiyonu ve şeker degradasyonu ortak ürünleri 3-deoksiglukozon, 1-deoksiglukozon, 3,4-dideoksiglukozon, glioksal, metilglioksal ve dimetilglioksali içeren alfa-dikarbonil bileşikleri, ve ayrıca 5-hidroksimetilfurfural ve furandır. Bir erken glikasyon ürünü olan fruktozillizin ve ileri glikasyon ürünü olan karboksimetillizin ve ayrıca akrilamid kavrulmuş fındıklarda analiz edilen Maillard reaksiyonu ürünleridir. 3-deoksiglukozon, glioksal ve metilglioksal diğer dikarbonillere kıyaslandığında fındıkların kavrulması sırasında en yüksek konsantrasyonlara ulaşmıştır. Fındıkların maksimum furozin konsantrasyonu, karboksimetillizin konsantrasyonlarından neredeyse dört kat daha fazla bulunmuştur. Kavrulmuş fındıklarda akrilamid, furan ve 5-hidroksimetilfurfuralın proses kontaminantı olarak büyük bir endişe yaratmadığı bulunmuştur. Fındıkların kavrulması sırasında yağın erken ve ileri glikasyon ürünleri oluşumu üzerine etkisi fındık benzeri karışımların ısıtılmasıyla aydınlatılmıştır. Fruktozillizinin oluştuktan 15 dakika sonra, ileri glikasyon ürünlerinin uzun kavurma sırasında baskın olduğunu gösterecek şekilde, hemen degrade

olduğu bulunmuştur. İleri glikasyon son ürünleri arasında piralin konsantrasyonu, lizin modifikasyonunun çoğunlukla 3-deoksiglukozon tarafından gerçekleştirildiğini gösterir şekilde, en yüksektir. Diğer ileri glikasyon son ürünlerinin konsantrasyonu karboksimetillizin>formilin>MP-lizin>maltozin şeklindedir. Bir ileri lipasyon ürünü olarak MP-lizin, konsantrasyonu yağ miktarına göre artan tek ileri aşama ürünü olmuştur.
Fındık kavurma sırasında gerçekleşen Maillard reaksiyonu ve karamelizasyonun çok-değişkenli bir kinetik modeli ileri sürülmüştür. Glukoz ve fruktozun izomerizasyonu, sukroz degradasyonu, 5-hidroksimetilfurfural oluşumu, şekerler ve amino asitlerin reaksiyonu, Amadori/Heyns ürününün degradasyonu, 3-deoksiglukozon ve 1-deoksiglukozon oluşumu ve degradasyonu, glioksal, metilglioksal, dimetilglioksal oluşumu ve onların ileri reaksiyonları ileri sürülen modelde yer almıştır. Model diskriminasyonu, deneysel verilere en uyumlu model eğriler bulunana kadar, gerçekleştirilmiş ve açıklanmıştır. İleri sürülen modeldeki en önemli reaksiyon basamaklarının glukoz ve fruktozun 1,2-enolizasyonla izomerizasyonu, sukroz degradasyonu yoluyla oluşan fruktofuranozil katyon üzerinden 5-hidroksimetilfurfural oluşumu, glukoz ve amino asitlerin reaksiyonundan Amadori ürününün oluşması, glukoz oksidasyonuyla glioksal oluşması ve ayrıca 1-deoksiglukozondan metilglioksal ve dimetilglioksal oluşması olduğu bulunmuştur. Hem bileşenlerin kütle denkliği hem de fındıkların rengiyle ortaya çıkarıldığı gibi, fındıklarda kavurma sırasında meydana gelen reaksiyonlar karamelizasyon reaksiyonunun da katkısıyla Maillard reaksiyonunun ileri ve final aşamalarına ilerlemesine öncülük etmiştir.

Anahtar Kelimeler: fındık, kavurma, Maillard reaksiyonu, şeker degradasyonu, ileri glikasyon son ürünleri, çok-değişkenli kinetik modelleme

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CONTENTS

LIST OF TABLES

page

LIST OF FIGURES

page

SYMBOLS AND ABBREVIATIONS

Symbols

E_a	Activation energy
k	Reaction rate constant
R	Gas constant
T	Temperature
t	time

Abbreviations

AA	Amino acids
ABTS	2,2-azino-bis/3-ethyl-benothiazoline-6-sulfonic acid
AGEs	Advanced glycation end products
ALEs	Advanced lipoxidation end products
AOAC	Association of Official Analytical Chemists
AP	Amadori product
APCI-MS	Atmospheric pressure chemical ionization mass spectrometry
Cis/trans-BPP-lysine	(Z)/(E)-1-(5-amino-5-carboxypentyl)-4-butyl-3-(pent-1-en-1-yl) pyridin-1-ium
CEL	Carboxyethyllysine
CML	Carboxymethyllysine
DAD	Diode array detector
DETAPAC	Diethylenetriaminepentaacetic acid
DMG	Dimethylglyoxal
DOLD	3-deoxyglucosone-methyl-dimer
1-DG	1-Deoxyglucosone
3-DG	3-Deoxyglucosone
4-DG	4-deoxy-hexo-2,3-diulose
3,4-DG	3,4-Dideoxyglucosone
DNA	Deoxyribonucleic acid
ECN	Equivalent carbon number
EFSA	European Food Safety Authority
ESI-MS	Electrospray ionization-mass spectrometry

FAME	Fatty acid methyl ester
FDP-lysine	N-ε-(3-formyl-3,4-dehydropiperidino) lysine
FFC	Fructofuranosyl cation
FID	Flame ionization detector
FRU	Fructose
Formyline	6-(2-formylpyrrol-1-yl)-L-nor-leucine
GAE	Gallic acid equivalent
GC-MS	Gas chromatography mass spectrometry
GLC	Glucose
GO	Glyoxal
GOLD	Glyoxal-methyl-dimer
HFBA	Heptaflourobutyric acid
HHE	4-Hydorxy-2-hexenal
HLB	Hydrophilic-lipophilic-balanced
HMF	5-Hydroxymethylfurfural
HMW	High molecular weight
HNE	4-Hydroxy-trans-2-nonenal
HP	Heyns product
HPD	Highest posterior density
HPLC	High performance liquid chromatography
INT	Intermediate
L-	Linoleic acid
Maltosine	6-(3-hydroxy-4-oxo-2-methyl-4(1H)-pyridin-1-yl)-L-norleucine
MCX	Mixed mode cation exchange reversed phase
MDA	Malondialdehyde
MGO	Methylglyoxal
MRM	Multiple reaction monitoring
MRPs	Maillard reaction products
MP-lysine	2-amino-6-(3-methylpyridin-1-ium-1-yl) hexanoic acid
MOLD	Methylglyoxal-methyl-dimer
O-	Oleic acid
ONE	4-oxo-trans-2-nonenal
P	Product

P-	Palmitic acid
2-PPL	2-amino-6-(2-pentyl-1H-pyrrol-1-yl) hexanoic acid
Pyrraline	6-(2-formyl-5-hydro-xymethyl-1-pyrrolyl)-L-norleucine
QUENCHER	QUick, Easy, New, CHEap, Reproducible
RID	Refractive index dedector
S-	Stearic acid
SUC	Sucrose
Trolox	6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

INTRODUCTION

Hazelnut (*Corylus avellana L.*) is being taken attention because of its distinctive composition. It contains high amounts of lipids (around 60%), followed by proteins (14%), moisture (4%), ash (2%) and sugar (1.4%) [1] as well as vitamins and minerals [2-6]. Lipid fraction is rich in unsaturated fatty acids, in particular oleic acid (80%) and linoleic acid (13%) and low in saturated fatty acids, which are palmitic (5%) and stearic acids (2%) [4, 7]. Proteins of hazelnut contain almost all essential amino acids and rich in arginine and leucine [4]. A variety of sugars including fructose, glucose, sucrose, myo-inositol, raffinose and stachyose are also present in hazelnut [8]. The content of these constituents and their profiles are important not only because of providing health benefits but also because of being the reactants of many chemical reactions. However, the chemical reactions and their mechanisms and the causative reactants in the hazelnut are still not known entirely. For that reason, the main material to be studied in this thesis is the hazelnut which will behave like a reaction medium.

Roasting is the most commonly used process for hazelnuts which is applied at temperatures between 100 and 160°C for 10 to 60 min [9]. Roasting could induce many chemical reactions like Maillard reaction, sugar degradation and lipid oxidation in hazelnuts. These reactions could result in the formation of desirable flavor and color as well as the formation of undesirable thermal process contaminants such as acrylamide, furan and 5-hydroxymethylfurfural throughout complex chemical mechanisms [10, 11]. Lipid oxidation could contribute these reactions by causing the formation of carbonyl compounds which could behave like reducing sugars in Maillard reaction [12]. Carbonyl compounds originating from both lipid oxidation and sugar degradation reactions could modify protein side chains resulted in both nutritional losses and formation of lipation or glycation of proteins [13, 14].

Hazelnut is suitable for the proceeding of the mentioned reactions under the conditions of roasting. The detailed composition and profile analysis of each constituent of hazelnut is necessary in order to identify the reaction medium clearly. Moreover, little is known about the changes in the concentration of reactants in hazelnuts, namely lipids, sugars and amino acids and formation of process contaminants, carbonyl compounds especially α-dicarbonyl compounds, early and advanced glycation end products during roasting of hazelnuts. Therefore, the aim of this thesis was firstly identifying composition of the lipid and non-

lipid fractions of Turkish hazelnut varieties together with their skin characteristics, secondly quantifying the changes in the composition of hazelnuts and the concentrations of Maillard reaction and sugar degradation products during roasting of hazelnuts, thirdly understanding the reaction mechanisms and important reaction steps of Maillard reaction and sugar degradation during roasting of hazelnuts by performing multiresponse kinetic modeling.

Considering the mentioned context this thesis is presented as four chapters:

Chapter 1 gives the general information about the composition of hazelnut and its skin, roasting and roasting induced chemical reactions particularly Maillard reaction, sugar degradation, and lipid oxidation and as well as multiresponse kinetic modeling of chemical reactions.

Chapter 2 identifies the composition of fourteen Turkish hazelnut varieties belong to two consecutive harvest years together with their skin characteristics in order to understand the possible reactants of the chemical reactions induced by roasting. The results reported in this chapter have been published in the following articles;

Taş, N.G., Gökmen, V., Profiling triacylglycerols, fatty acids and tocopherols in hazelnut varieties grown in Turkey, *Journal of Food Composition and Analysis*, 44, 115-121, **2015**.

Taş, N.G., Gökmen, V., Bioactive compounds in different hazelnut varieties and their skins, *Journal of Food Composition and Analysis*, 43, 203-208, **2015**.

Chapter 3 discusses the changes in the concentration of reactants, namely amino acids and sugars, and formation of Maillard reaction, sugar degradation and lipid oxidation products under different roasting conditions of hazelnuts. It also includes the changes in the concentration of early and advanced glycation products in the hazelnut resembling model systems during heating.

Chapter 4 discusses the important steps of reaction by model discrimination, rate constants of the reaction steps of Maillard reaction and sugar degradation in order to understand the contribution of these reactions to the formation of reaction products during roasting.

The results reported in Chapter 3 and 4 have been published in the following article;

Taş, N.G., Gökmen, V., Maillard reaction and caramelization during hazelnut roasting: A multiresponse kinetic study, *Food Chemistry*, 221, 1911-1922, **2017**.

1 GENERAL INFORMATION

1.1 Hazelnut

Hazelnut (*Corylus avellana* L.) is one of the most economically important commodity for Turkey and contributes to the total world production with its 450000 tones of in-shell hazelnut production [15]. Italy is the second leader country in the production of hazelnut followed by Georgia, the USA and Azerbaijan [15]. Acı, Cavcava, Çakıldak, Foşa, Ham, İncekara, Kalınkara, Kan, Karafındık, Kargalak, Kuş, Mincane, Palaz, Sivri, Tombul, Uzun Musa, Yassı Badem, and Yuvarlak Badem are the 18 hazelnut varieties grown in Turkey [8]. These varieties are classified based on their quality as 'Giresun' or 'Levant' quality. Tombul is the only variety known as Giresun quality and the others are Levant quality [16]. The other classification is based on the shape of hazelnut varieties. Sivri and İncekara are classified as 'pointed', Yuvarlak Badem and Yassı Badem are classified as 'long' and the other varieties are classified as 'round' [17, 18].

Hazelnut has a unique compositional characteristic with its lipids, carbohydrates, dietary fibers, organic acids, vitamins, minerals, phytosterols, and antioxidant phenolics [4, 19, 20]. Lipid is the most predominant fraction of hazelnut (55.01-64.85 g/100g) [21] and it is followed by carbohydrate (12.1-21.1 g/100g) [22] and protein (11.51-15.48 g/100g) [23]. Hazelnut has moisture content ranging from 3.17 to 4.32 g/100g [21] and ash content ranging from 2.4 to 3.4 g/100g [22]. In addition to be a good source of energy, lipid fraction of hazelnut has also important effects on human nutrition and health because of its triacylglycerol, fatty acid, tocopherol, and tocotrienol profile and bioactive components like phytosterols [7, 16, 24].

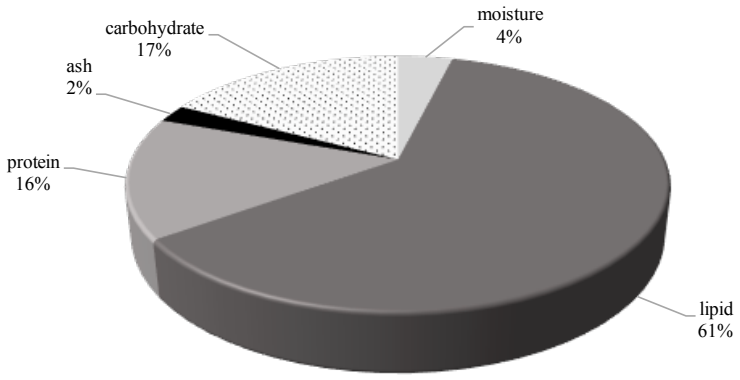


Figure 1.1. Proximate composition of hazelnut, adopted from Alasalvar et al [19]

Triacylglycerols account for the 95-98% of the vegetable oils [25]. Distribution of fatty acids in the triacylglycerols is substantial from many aspects. Triacylglycerol composition could be considered as the fingerprint of the oils which helps detection of adulterations and determination of authenticity [26, 27]. Additionally, distribution of fatty acids in triacylglycerols may be specific for a group of oil as in the case of vegetable oils which have unsaturated fatty acids in *sn*-2 position [28]. Furthermore, triacylglycerol profile specifies the physical properties of oils [27]. Hazelnut lipids are composed of mostly non-polar (98.8%) constituents, like many vegetable oils, and triacylglycerols are the major contributors of this non-polar fraction [25, 29]. The triacylglycerols of crude hazelnut oils were reported as OOO (61-77.5%), OOL (10.5-22.8%), POO (6.4-11%), SOO (1.63-5.69%), and OLL (0.87-5.54%) [3]. The remaining triaclyglycerols, which are LLL, PLL, POL, PPL, POO, PPO, SOO and PSO, only contributed 0.62-2.65% [3]. The presence of these triacylglycerols in hazelnut oil indicates abundant presence of oleic acid in hazelnut oil along with a high unsaturated to saturated fatty acid ratio [16, 30].

Oleic (C18:1) (79.4%), linoleic (C18:2) (13%), palmitic (C16:0) (5.4%), stearic (C18:0) (1.8%), palmitooleic (C16:1) (0.36%), and linolenic (C18:3) (0.06%) acids are the fatty acids reported in hazelnuts [4]. Percentage distribution of fatty acids in hazelnuts may vary depending upon the variety, harvest time and year, geographical and growing conditions, season, climate, soil type and the conditions of storage among many other factors [22, 31-33]. Unsaturated fatty acids are 92.8%, polyunsaturated fatty acids are 13.1% and saturated fatty acids 7.2% of the total fatty acids of hazelnuts [4]. Ratio of unsaturated to saturated fatty acids ranges from 11.6 to 16.4 in oil of Turkish hazelnut varieties with a mean value of 13.1 while it is 5.7, 4.4, 2.9 for walnut, peanut and pistachio oils, respectively [34]. Having high proportion of unsaturated/saturated fatty acids makes hazelnut oil an alternative for addition into processed foods to improve nutritional quality [4, 35]. However, high concentrations of oleic and linoleic acids may result in the formation of oxidative rancidity [36].

Lipid fraction of hazelnut also contains tocols such as tocopherols and tocotrienols, which exert health beneficial effects [16, 37] and act as antioxidant by scavenging free radicals [38-40]. Hazelnuts were reported to contain seven tocol isoforms, which are α-, β-, γ-, δ-tocopherols and α-, β-, γ-tocotrienols, with a total content of 51.31 mg/100 g hazelnut oil [16]. α-Tocopherol is the most abundant tocopherol in hazelnuts, which is almost 80% of total tocols, followed by γ-, β-, and δ-tocopherols [4, 16]. Tocotrienols account for only

1.02% of total tocols [16]. Among phytoserols in hazelnuts, β-sitosterol is the most abundant one [16, 22]. Phytosterols have been known not only with their effects on reducing the certain types of cancers [41, 42] and cardiovascular diseases [43] but also with their positive effects on immune system [44].

The non-lipid fraction of hazelnut contains mainly proteins and amino acids, carbohydrates including sugars and organic acids, as well as vitamins and minerals. Hazelnuts are a good source of protein (17.4 g/100g) both because of the amount of protein they have and the profile of amino acids [4]. Hazelnuts contain essential and non-essential amino acids. Arginine is the most predominant essential amino acid (2 g/100g) and leucine (1.2 g/100g) is the second abundant one [4]. Among the non-essential amino acids, glutamic acid (2.8 g/100 g) has the highest concentration followed by aspartic acid (1.5 g/100g) [4]. The concentration of cysteine and methionine are lower than the other amino acids comparatively [1, 4].

Hazelnuts have a variety of sugars including non-reducing and reducing sugars. Sucrose, glucose, fructose, raffinose, stachyose [8, 45], and myo-inositol [8] are the sugars present in hazelnuts, whose total concentration ranges from 1.99 to 4.94 g/100g [8]. Sucrose represents the almost 80% of sugars followed by stachyose (5-10%) and the other sugars (3-4%) [45]. Oxalic, malic, citric, maleic, lactic, succinic and acetic acids are the organic acids found in hazelnuts [8]. Malic acid is the abundant organic acid in hazelnut, which is 80% of the total organic acids [45]. Additionally, hazelnuts contain condensed tannins (3.99-40.56 mg catechin equivalent/100g) and gallic acid (0.159-0.871 mg/100g) [8].

Total ash content of hazelnuts ranges from 1.87 to 2.72% [4]. Hazelnuts are a good source of minerals and contain potassium, magnesium, manganese, calcium, iron, zinc, sodium and copper. Among these minerals, potassium has the highest concentration (637.14 mg/100g) followed by magnesium (143.59 mg/100g) while copper (0.65 mg/100g) and sodium (0.70 mg/100g) have the lowest [2]. The vitamins found in hazelnuts are niacin, vitamin B_1, vitamin B_2, vitamin B_6, ascorbic acid, folic acid and retinol, whose concentrations range between 0.05 and 3.25 mg/100g [4].

Hazelnut could be consumed with its skin intact or the skin could be removed after roasting. Hazelnut skin constituents 2.5% of the hazelnut kernel [46] and the skin is one of the most valuable part of the hazelnut because of its high antioxidant activity exerting phenolic compounds [47, 48]. Hazelnut skin is especially rich in flavanols which are

catechin, epicatechin, epicatchin gallate, gallocatechin, and gallocatechin gallate [49]. Quercetin-3-O-rutinoside is the flavonol, procyanidin B_1 is the tannin, ferulic and coumaric acids are hydroxycinnamic acids, gallic and protocatechuic acids are the hydroxybenzoic acids found in hazelnut skin [50, 51]. Therefore, consumption of hazelnuts with their skin could be better for health. However, as the hazelnuts are generally consumed after being roasted, the brown skin removed might arise as an important by-product of the hazelnut industry.

1.2 Roasting

Hazelnuts are generally used in the products such as snacks and confectionaries including chocolates, pastries and ice cream after being roasted [8, 22]. Roasting is generally performed at 100-160°C for 10-60 min until the desirable color is obtained. The roasted hazelnuts could have a variety of colors including whitened, golden yellow, dark and very dark roasts [9]. Roasting is not only important for the color of hazelnuts but also important for the formation of aroma and flavor compounds. Pyrrolines like 2-acetyl-1-pyrroline (pop-corn) and 2-propionyl-1-pyrroline (pop-corn), thiols like 2-furfurylthiol (coffee-like) and 2-thenylthiol (sulfury-smelling), aldehydes like (*Z*)-2-octenal (fatty, green), (*Z*)-2-nonenal (fatty), and (*Z*)-2-decenal (fatty) were reported to have highest odor activities in roasted hazelnuts [52]. Roasting also causes microstructural changes which lead to the formation of desirable textural changes such as crispness and crunchiness [53]. Some microstructural changes in extremely liked roasted hazelnuts (roasted at 165°C, 1 m/s, 25 min) were reported to be cell wall separation, formation of intercellular spaces, disruptions in cytoplasmic network and aggregation of proteins [53]. A two-step roasting, low temperature moist conditions with steam injection followed by dry roasting, was suggested to control the structural changes of hazelnuts by limiting the thermal destabilization of cell wall materials [54]. Additionally, roasting causes denaturation of proteins, inactivation of enzymes like lipase and lipoxygenase as well as microorganism [55]. Moreover, formation of antioxidant activity exerting compounds is also possible during roasting of hazelnuts especially the formation of melanoidins via Maillard reaction [56]. However, it should be noted that there is a balance in the thermal degradation of natural antioxidants in nuts and formation of new compounds through Maillard reaction having antioxidant activity [57].

In addition to providing some desirable properties, roasting may also trigger some undesirable changes mostly because of the chemical reactions originating from the compositional characteristics of hazelnuts. Maillard reaction, sugar degradation and lipid

oxidation are some of the important chemical reactions leading to changes in the composition of hazelnuts. Maillard reaction could cause decreases in the concentration of total amino acids and the concentration of essential amino acids like lysine leading to nutritional losses [58, 59]. Protein glycation and formation of AGEs are other consequences of the Maillard reaction [60]. Sugar degradation products like α-dicarbonyl compounds could take part in the progress of Maillard reaction by reacting with amino acids to form AGEs [61]. Carbonyl compounds derived from lipid oxidation could also behave like carbonyl compounds derived from sugar degradation and react with nucleophilic amino groups [12]. Lipid oxidation products contribute to the formation of biogenic amines by decarboxylation of amino acids as also in the case of acrylamide formation [12, 62]. One of the undesirable consequences of roasting induced reactions is the formation of potentially toxic compounds like acrylamide, furan and 5-hydroxymethylfurfural (HMF). Acrylamide, as a possible human carcinogen, is formed in nuts depending on the asparagine content and roasting temperature applied [11]. Furan is a potentially hazardous compound which is formed in hazelnuts from precursors such as polyunsaturated fatty acids (linoleic acid), amino acids (threonine and alanine) and sugars [63]. HMF is also formed during roasting of hazelnuts depending on the roasting time and temperature [10]. In addition to blockage of lysine and formation of undesirable compounds including potentially toxic compounds, roasting could also affect the loss of vitamins like thiamine and riboflavine [58]. Roasting induced chemical reactions of nuts are summarized in Figure 1.2.

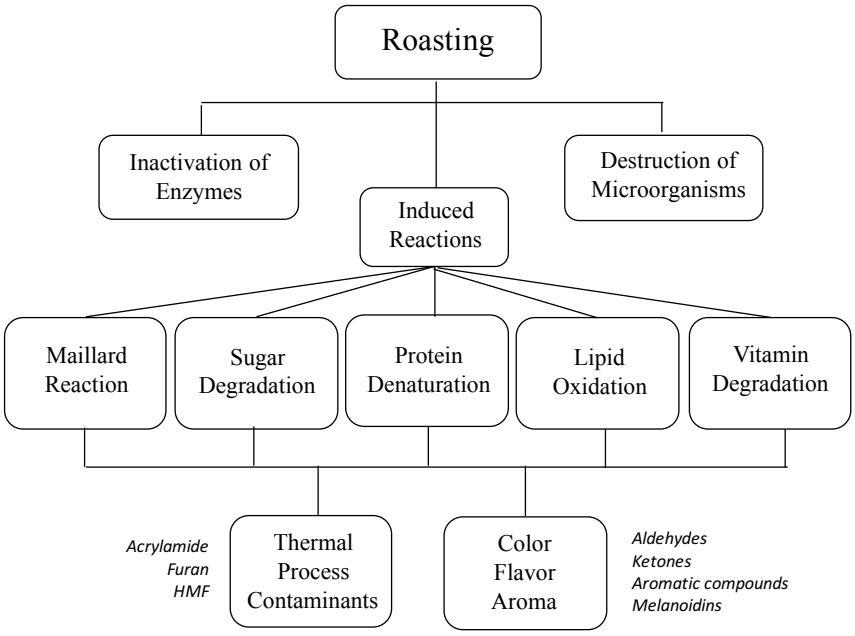


Figure 1.2. Chemical reactions occurring in nuts during roasting, adopted from [55].

1.3 Roasting Induced Reactions

1.3.1 Maillard Reaction

Maillard reaction was discovered by Louis-Camille Maillard at the year of 1912 by chance during heating amino acids and sugars which resulted in brown color [64]. Since that time Maillard reaction took interest of many researchers and quite a lot numbers of studies have been performed in the past century. Maillard reaction also takes the interests of the food industry a lot as it plays an important role in the improvement of aroma, taste and color. However, a bunch of reaction products including toxic and mutagenic compounds like acrylamide, furan, and heterocyclic amines are also formed in the Maillard reaction [65-67]. Additionally, Maillard reaction decreases the nutritional value of proteins and the bioavailability of essential amino acids including lysine and arginine [68, 69]. Consumption of diets rich in Maillard reaction products (MRPs) affects the protein digestibility in a negative way [70] and modulates intestinal microbioata in vivo depending on the chemical structure and amount of the product consumed [71]. Apart from the development of sensorial properties, the other positive side of the Maillard reaction is its association with the increased antioxidant activity [72]. Considering the positive and negative sides of the Maillard reaction, controlling Maillard reaction becomes an important issue in the food industry especially for the ones applying thermal processes such as roasting, baking and cooking. The reactants and the factors influencing the Maillard reaction and the effects of Maillard reaction from the viewpoint of chemistry, food technology, nutrition and health are summarized by Namiki [73] (Table 1.1).

Table 1.1. The reactants and the factors influencing the Maillard reaction and the effects of Maillard reaction, adopted from Namiki [73]

Reactants: Amino acid, peptide, protein, amine, ammonia + Reducing sugar, carbonyl compounds (from oxidation of fatty acids, ascorbic acid, and polyphenols)	
Influencing factors: pH, temperature, moisture content, heavy metal ions, oxygen, light, sulphite, and other constituents	
Chemistry	Browning reaction mechanism Isolation and identification of intermediate products Structure and properties of melanoidins
Food technology aspects	Flavour development Physicochemical quality changes Antioxidant Control of browning
Nutritional aspects	Loss of amino acids (Lysine, arginine etc.) Loss of nutritive value Antinutritive properties Metal ion chelation
Toxicology	Mutagen formation Antimutagens
In vivo	Diabetic diseases Aging, etc.

The complex nature of the reactions in biological systems and foods is explained in a simplified way by Namiki [73] (Table 1.2). The functional groups of the structural units (amino acids, monosaccharides, fatty acids and alcohols) of proteins, polysaccharides and lipids are limited to four groups which are -COOH, -OH, $-NH_2$ and -CHO. In enzymatic reactions, polymerization of the monomers occurs in one step and it is reversible. However, in case of the non-enzymatic reaction of -CHO and $-NH_2$, the situation is completely different then the enzymatic reaction. The first step of the reaction is reversible which is formation of glycosyl-amino products. However, its product rearranges via Amadori rearrangement to form ketosyl-amino products which undergo irreversible dehydration, rearrangement and scission reactions resulting in decomposed and polymerized products like flavor compounds or melanoidins [73].

Table 1.2. Reactions of structural functional units in foods, adopted from Namiki [73]

Functional groups:	
–COOH, −OH, $–NH_2$, –CHO (–CO) : (–SH, $–PO_3H$, etc.)	
Reactions between functional groups:	
Enzymatic	
RCOOH + R'$–NH_2$ ↔ RCONHR'	Peptide, protein
RCOOH + R'–OH ↔ RCOOR'	Fat
RCHO + R'–OH ↔ R–OR'	Glycoside
Non-enzymatic	
RCHO + R'$–NH_2$ ↔ RCH=NR' → Amadori product → Scission products → MRPs	
→ Scission products → MRPs	

The chemistry of Maillard reaction is very complex and the Maillard reaction is a reaction network containing various reaction pathways. These reactions are influenced by each other and can occur simultaneously [74, 75]. Today, Maillard reaction is classified in three stages as early, advanced and final stages based on the works of Amadori [76], Heyns [77] and Hodge [78] and many others [61].

In the early stage of the Maillard reaction, condensation of an aldose sugar with an amine forms Schiff base and consequently it rearranges to Amadori compound (1-amino-1-deoxy-ketose) that is the first stable product of the Maillard reaction. In case of ketose sugars, the analogous product formed is called as Heyns compound (2-amino-2-deoxy-aldose) [79].

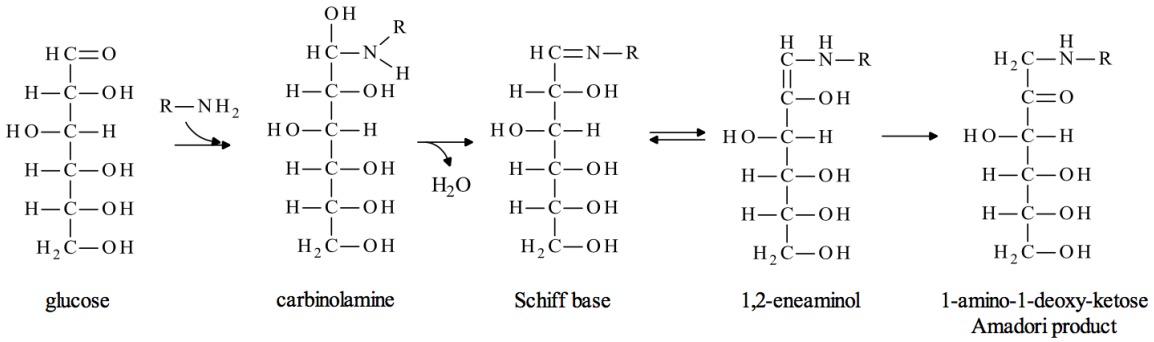


Figure 1.3. Formation of Schiff base and Amadori rearrangement product from aldose sugars, adopted from [80]

Under the controlled acid hydrolysis conditions, Amadori product degrades to furoyl derivatives which could be determined quantitately [81]. N-ε-fructosyllysine, namely furosine, is known as the early stage marker of the Maillard reaction and it is the chemical indicator of Amadori product [82]. N-ε-fructosyllysine, N-ε-lactulosyllysine, and tagatosyllysine are the Amadori products which form from the reaction of lysine with fructose, lactose, and galactose, leads to the formation of furosine under the controlled acid hydrolysis [83]. Content of furosine has been used as an indicator of heat treatment of many products including milk products [84], cookies [85], pasta [86], jams and fruit based infant foods [87]. However, depending on the conditions of the process, heat treatment may lead to the degradation of Amadori product and decreases in the content of furosine as in the case of cocoa roasting [88]. Furosine is used for the evaluation of quality of foods during storage [89]. Content of furosine could also be used for the calculation of percentage of blocked lysine [82].

The advanced stage of the Maillard reaction involves the reaction from degradation of Amadori product to until the formation of brown nitrogenous compounds [78]. Degradation of Amadori product, which is not very stable compound, results in the formation of α-dicarbonyl compounds [61, 90]. Amadori product mainly undergoes 1,2-enolization or 2,3-enolization depending on the pH of the medium. 1,2-enolization predominates at pH<7 resulting in the formation of HMF from hexose sugars and furfurals from pentose sugars. In case of pH>7, 2,3-enolization of Amadori product dominates leading to the formation of reductones, and fission products like diacetyl and acetol [91]. In the 1,2-enolization reaction, an intermediate called 1,2-eneaminol is formed from ketoseamines. Through this intermediate 3-deoxyglucosone (3-DG) is formed via dehydration reactions resulted in the release of amino acid to the medium again. In the 2,3-enolization reaction, fructoseamine forms 2,3-enediol and this intermediate decomposes to 1-deoxyglucosone (1-DG) by retro-aldol reaction [92].

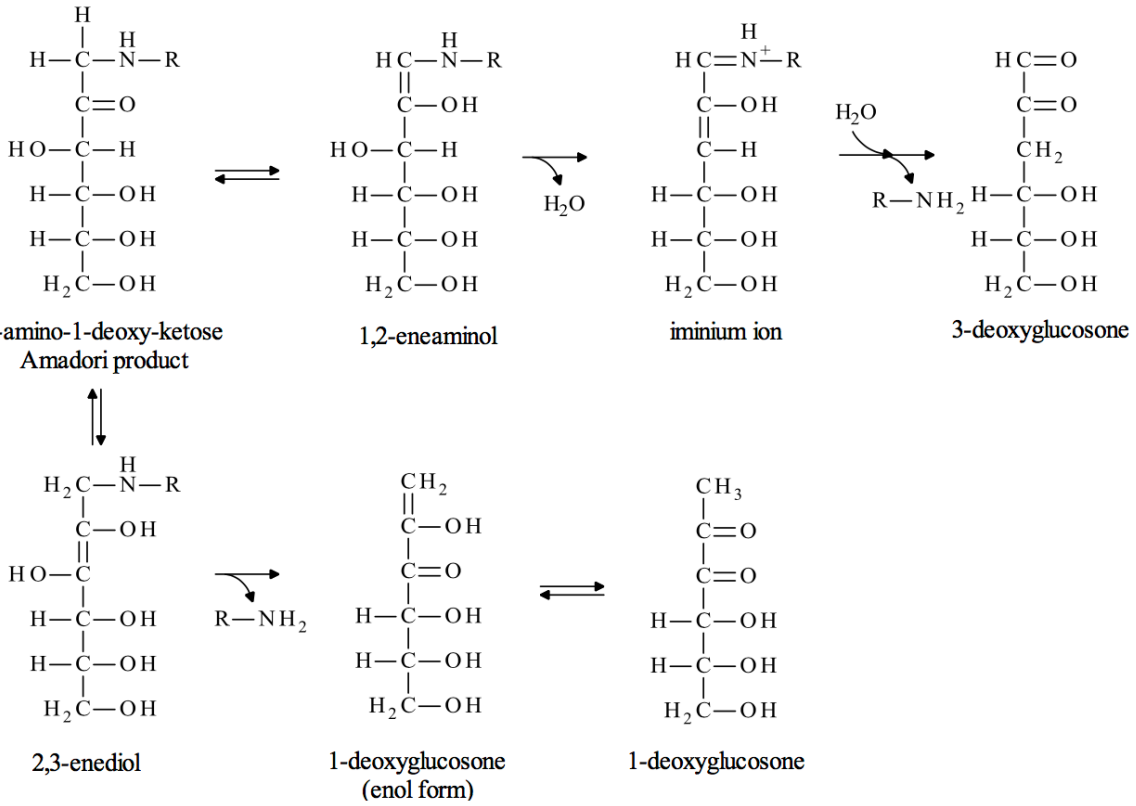


Figure 1.4. Formation of 3-deoxyglucosone and 1-deoxyglucosone from through degradation of Amadori product, adopted from [92]

3-DG, 1-DG, methylglyoxal and glyoxal are the most important representatives of α-dicarbonyl compounds [90, 93]. Their formation is based on retro aldol reactions, α- and β-dicarbonyl cleaveage [94]. It is remarkable that presence of amines is not always necessary in the formation of α-dicarbonyl compounds, they could be formed also by degradation of sugars in the caramelization process [95]. Formation mechanisms of these α-dicarbonyl compounds are mentioned in the following section of this chapter in more detail. In addition to degradation of sugars and Amadori products, α-dicarbonyl compounds could either be formed in early stages of the Maillard reaction from Schiff base, condensation product of sugar and amino compound, through the Namiki pathway [96]. Another possible formation mechanism of α-dicarbonyl compounds is the oxidation of especially polyunsaturated fatty acids which could lead to the formation of glyoxal and methylglyoxal [97]. It should be mentioned that α-dicarbonyl compounds are common products of Maillard reaction, sugar degradation and lipid oxidation reaction. However, it is hard to distinguish the origin of these compounds in a complex food matrix. There is only a restricted number of multiresponse kinetic studies dealing with the possible

predominant pathways in the formation of α-dicarbonyl compounds in both model systems and foods [98-101]. This is because the reactivity of these compounds is very high and therefore difficult to analyze in food matrixes. For that reason, it is necessary to use a trapping agent like o-phenylendiamine to derivatize and make α-dicarbonyl compounds quantifiable [102]. Degen et al [103] performed a comprehensive analysis of α-dicarbonyl compounds, including 3-DG, 3-deoxygalactosone, and methylglyoxal in foods like bakery products, pasta, beverages and spreads. They found that balsamic vinegar, fruit juices and cookies are rich in 3-DG while methylglyoxal is in minor quantities in the foods except manuka honey [103]. 3-Deoxygalactosone is also relevance in many foods even they have no galactose [103]. Additionally, baked products like cookies have been shown to have glyoxal and methylglyoxal [101, 104], baby foods including vegetable puree, fruit puree, puddings and infant formula have also been shown to contain 3-DG, 1-DG, and glucosone [105].

α-Dicarbonyl compounds could present in coffee and cocoa also as a result of balance in their formation and degradation depending upon the roasting conditions [88, 106] and processes applied before roasting [88]. α-Dicarbonyl compounds are not only present in processed foods but also formed *in vivo* and they are responsible for the dicarbonyl stress in the body. Dicarbonyl stress contributes cell and tissue dysfunctions during diseases and aging and is described by the increased protein and DNA modifications [107].

Among α-dicarbonyl compounds, 3-DG has special interest as it leads to the formation of HMF, mechanisms of which is given in the following section of this chapter. HMF is formed from both Maillard reaction [108] and caramelization [109]. In the formation of HMF through Maillard reaction, positively charged amino group helps the shift of the equilibrium in the side of enol resulted in the formation of glucosulose-3-ene which further cyclodehyrate to form HMF [110]. HMF concentration is being used as an indicator of applied thermal load to carbohydrate-rich products during processes [111]. It is also known as the marker of quality deterioration, indicating the excess heat treatment or inadequate storage in many foods that contain carbohydrates [112]. Although it is a useful marker from many aspects, presence of HMF in foods is undesirable as there are still concerns about potential health risks. In case of bioactivation of HMF in vitro to 5-sulfoxymethylfurfural through sulfonation of its allylic hydroxyl group, the resulting ester has been shown to induce mutagenic and genotoxic effects [113]. Additionally, HMF

molecule has carbonyl group, allylic hydroxyl group and furan ring that could react with amines to form Schiff base and Michael addition reactions. These functional sites of the molecule could affect its activity and its role in the body [112].

α-Dicarbonyl compounds are also important in the proceeding of the Maillard reaction as they lead to formation of protein/peptide bound amino acids in the final stage [61]. The term 'glycation' is originated from the reactions the 'glycosylation' *in vivo*. Glycation is the term used for the reactions of reducing carbohydrates and amines. Analogously, a new term was suggested for the reactions of carbonyl compounds derived from lipid peroxidation and amines which is 'lipation' [14]. The side chains of lysine and arginine are responsible from the glycation reactions derived by α-dicarbonyl compounds and the resultant products are called as advanced glycation end products (AGEs) [61]. The AGEs, for instance CML, pyrraline, pentosidine, or pronyl-lysine are considered to be useful heat treatment markers of the advanced stage of the Maillard reaction [82].

N-ε-carboxymethyllysine (CML) was the first identified AGE in lens proteins and collagens [114] as well as in lysine and glucose mixtures [115]. There are different mechanisms identified in the formation of CML. One of them is the reaction of glyoxal, whether it is originated from lipid oxidation or glucose autoxidation, with the lysine residues [116]. The other possibility of CML formation is Namiki pathway [96], in which reducing sugars react with an amine to form a Schiff base at the early stage. Then, sugar moiety of Schiff base splits off yielding glycoladehyde alkylimine. Finally, oxidation of this compound generates glyoxal which further reacts with lysine residues on proteins to form CML [117]. Another pathway is the oxidation of N-ε-fructosllysine to form CML [114]. It should be noted that during thermal processing, CML formation could proceed from one or more of the pathways simultaneously, depending on the compositional characteristics of food and processing conditions especially temperature [118]. CML concentrations have been determined in a wide range of foods including dairy, fish and meat, bakeries, cereals, pasta, coffee, fruit and vegetables. Dairy products (5143.7 mg/kg protein) have extremely high CML concentration compared to cereals (281.3 mg/kg protein), sweet and snacks (340.1 mg/kg protein), bread and biscuits (178.4 mg/kg protein), fish and meat (44.5 mg/kg protein), fruit and vegetables (26.6 mg/kg) [119].

Pyrraline (6-(2-formyl-5-hydro-xymethyl-1-pyrrolyl)-L-norleucine), as an acid labile AGE, is formed by Paal-Knoor reaction via addition of 3-DG to the lysine residues and

subsequent dehydration [120, 121]. Identification of pyrraline was performed in heated skim milk for the first time [122]. Later, the concentration of pyrraline was determined in various food products and pyrraline concentrations were found to be very low for milk products and highest for bakery products, i.e. 242.6 mg/kg protein of wheat bread [103, 123]. Pyrraline is one of the dominating AGEs in food products due to its high concentrations [61]. Pronyl-lysine, which is similar to pyrraline structurally, is formed via the reaction of acetylformoin with the lysine residues and it was detected in bread crusts [124]. Lysine derivatives having a pyrrole moiety, like pyrraline, could also form as a result of modification by a lipid peroxidation product, 4,5-epoxy- 2-alkenals [125].

Maltosine (6-(3-hydroxy-4-oxo-2-methyl-4(1H)-pyridin-1-yl)-L-norleucine), a glycated amino acid with a 3-hydroxy-4(1H)-pyridinone structure, is formed as a result of the reaction between oligosaccharides derived carbonyl compounds and lysine side chains [126]. Quantification of maltosine in foods has been recently performed by Hellwig et al [127] with a board range of food items including milk, bakery, and pasta products, corn flakes, coffee powder, brewing malt. It was reported that the highest concentrations were in bread samples ranging from 0.1 to 4.2 mg/kg. The wheat bread crust had the maximum concentration (up to 19.3 mg/kg) and was responsible for the 0.4% of lysine modification [127].

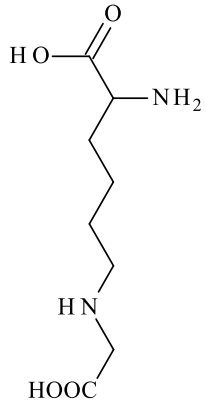
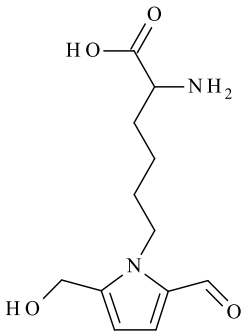
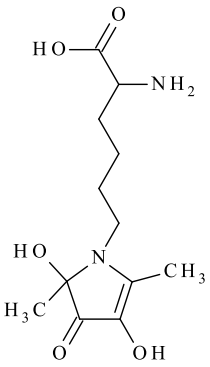
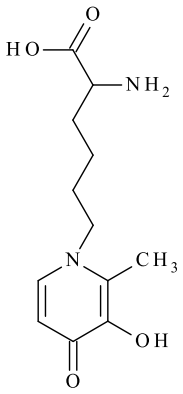
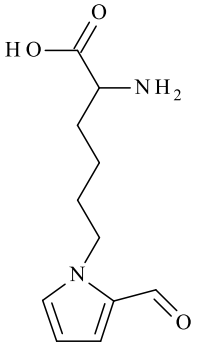
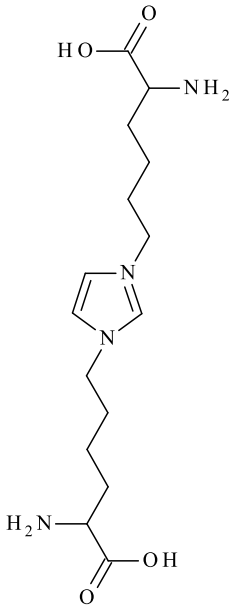
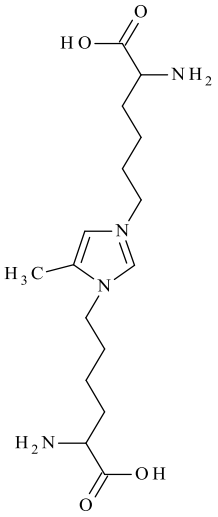
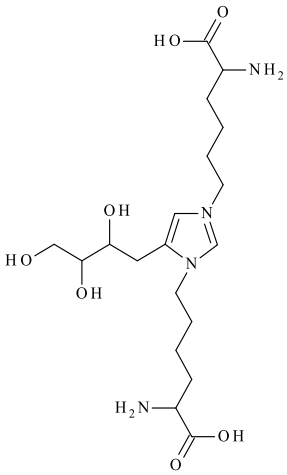
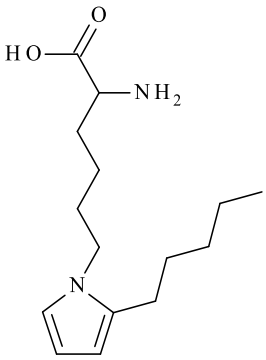
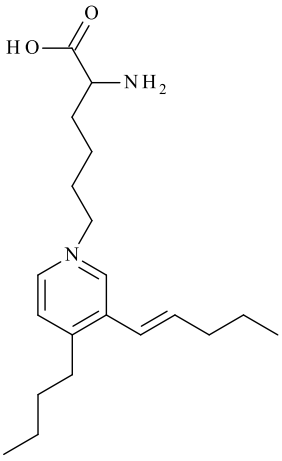
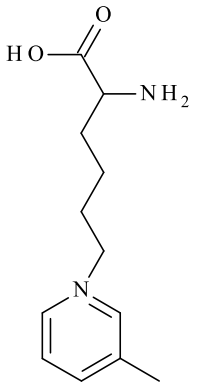
Formyline (6-(2-formylpyrrol-1-yl)-L-nor-leucine), has possibility to form from either predominant precursor, 3-deoxypentosone and pentose sugars, or from lysine and degradation products of dissacharides and glucuronic acid [128]. The formyline concentrations in breakfast cereals, pasta and bakeries were found up to 34.8 mg/kg. Formyline was also quantified in milk and whey products in low concentrations [123].

Pentosidine is formed from one lysine and arginine residue linked by a C_5 precursor originated from carbohydrate degradation [129]. The modification of the guanidino side chains of arginine by methyglyoxal results in the formation of imidazolinones, namely MG-H1 [130]. Argypyrimidines, mostly formed *in vivo*, are formed from the reaction of arginine side chains and methylglyoxal [131]. Lysine dimers form with the reaction of two lysine side chains with two molecules of α-dicarbonyl compound namely glyoxal, methylglyoxal or 3-DG resulting in the GOLD (glyoxal-methyl-dimer), MOLD (methylglyoxal-methyl-dimer) and DOLD (3-deoxyglucosone-methyl-dimer) [132]. These compounds have been found in bakery products with their arginine or lysine crosslinks

which are GODIC, MODIC, DODIC [133].

Modifications of protein side chains by lipid peroxidation products, namely lipation reactions, have been studied in peanut samples in recent years [14, 134, 135]. After heating the native protein extract with trans-2-heptenal following by an acid hydrolysis, two isomeric pyridinium derivaties, cis- and trans-BPP-lysine ((Z)- and (E)-1-(5-amino-5-carboxypentyl)-4-butyl-3-(pent-1-en-1-yl) pyridin-1-ium) were quantified, explaining the 80% of the total lysine modification [14]. The other pyridinium quantified in peanut samples was, 2-PPL (2-amino-6-(2-pentyl-1H-pyrrol-1-yl) hexanoic acid), concentration of which increased with roasting time [134]. MP-lysine (2-amino-6-(3-methylpyridin-1-ium-1-yl) hexanoic acid) forms from the reaction of lysine side chains and acrolein [136]. It was quantified in peanut samples and its concentration increased with roasting. The concentration of MP-lysine in peanut samples was up to 10.2 mg/kg [135].

Table 1.3. Molecular structures of some of the advanced glycation end products and advanced lipation products

			
CML	Pyrraline	Pronyllysine	Maltosine
			
Formyline	GOLD	MOLD	DOLD
			
2-PPL	*trans*-BPP-lysine	MP-lysine	

The reactivity of α-dicarbonyl compounds is essential in the formation of aroma and flavor compounds during Maillard reaction. α-Dicarbonyl compounds could react with amino acids to form a Strecker aldehyde, which has one less carbon atom than its amino acid, by deamination and decarboxylation [137].

Cyclization, dehydration, retroaldolization, rearrangement, isomerization and condensation reactions that take place in advanced stage of the Maillard reaction, lead to the formation of brown nitrogenous compounds, known as melanoidins at the final stage. The structure of melanoidins is still not known as the reactions taking place during melanoidin formation are very complex [138, 139]. In the production of low moisture foods (processing conditions, T>150°C and t<2h) such as bread, biscuits, cocoa, and coffee, oligo- and polysaccharides react from their reducing ends as a complete molecule [140]. Additional side chain reactions produce high molecular weight (HMW) melanoidins having carbohydrate skeleton [140]. In case of the reaction of carbohydrate degradation products with proteins HMW melanoidins having protein, skeleton is formed. This is more relevant in high moisture content foods like beer and wine where storage and fermentation is applied (processing conditions, T<50°C and t>30 days) [56].

1.3.2 Sugar Degradation

Sugar degradation reactions are characterized with an initial isomerization reaction [80]. Sugar isomerization and sugar degradation reactions are the key reactions happening during caramelization [141]. Depending on the conditions of the reaction, sometimes isomerization and degradation reactions could be more important than Maillard reaction from a quantitative point of view as in the case of heated milk. At the heating temperature of 100-150°C, the activation energy of isomerization was reported to be 130 kJ/mol while the activation energy of early Maillard reaction was 100 kJ/mol which indicates that sugar isomerization/degradation reactions are more noticeable in heated milk at temperatures higher than 100°C [142].

Monosaccharides in neutral and alkaline aqueous medium undergo reversible reactions like ionization, mutarotation and enolization [141, 142]. The interconversion of carbohydrates into their epimers via enolization is called as ‘Lobry de Bruyn-Alberda van Ekenstein transformation’ [143]. Enolization of D-glucose to D-fructose happens via the formation of 1,2-enediol. The reaction resulting in the formation of 1,2-enediol is called as 1,2-enolization. Enolization is a reversible reaction and could lead to the formation of D-

mannose and D-fructose, which are epimers of D-glucose, from D-glucose producing a mixture of all three sugars. D-fructose may proceed to form 2,3-enediol via the reaction 2,3-enolization forming D-psicose in a small extent [80].

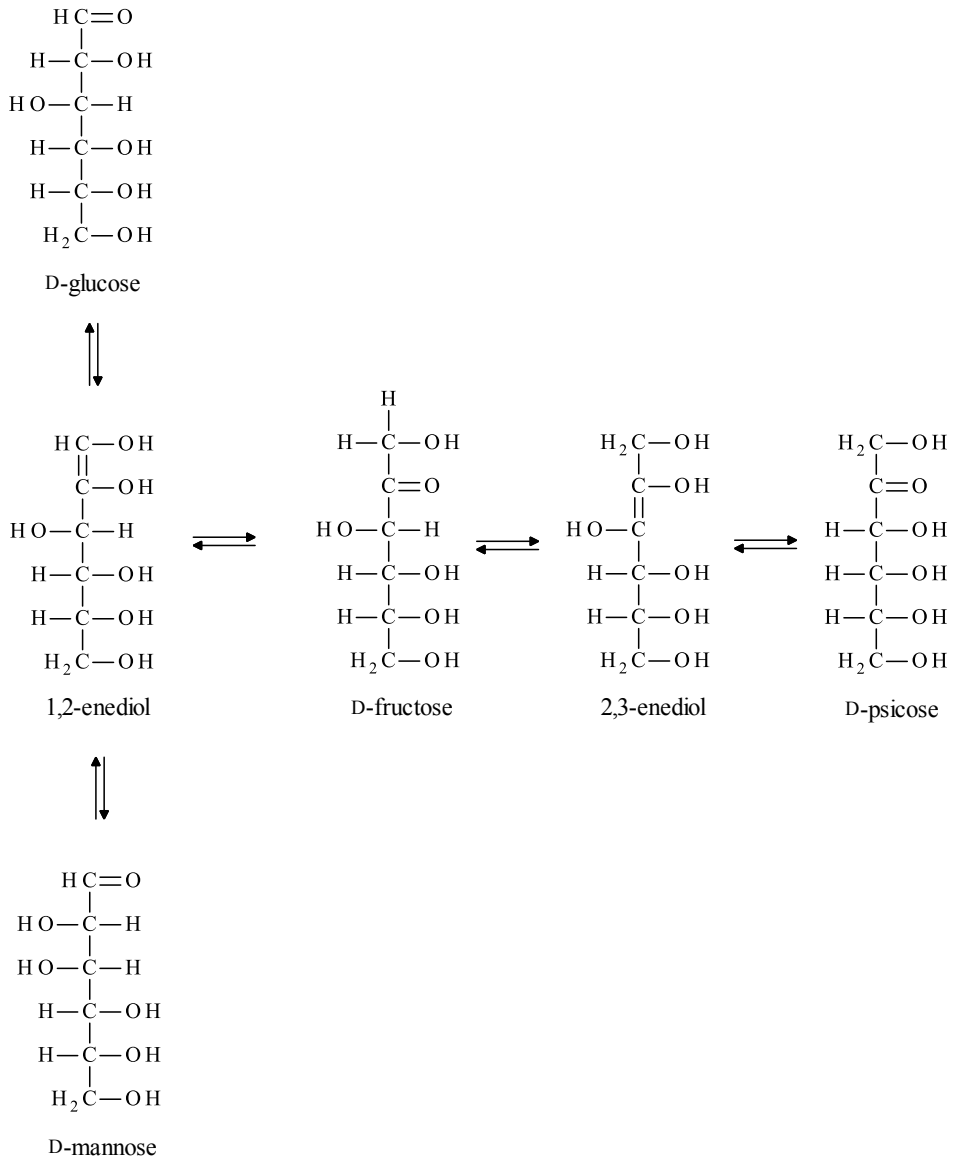


Figure 1.5. The Lobry de Bruyn-Alberda Ekenstein transformation including 1,2- and 2,3-enolization of monosaccharides, adopted from [80]

Osuloses are the principal compounds which form during caramelization reactions. They contributes to the flavor of caramel by giving rise to the formation of volatile compounds [109]. One molecule of water elimination from D-glucose or D-fructose gives rise to the formation of 3-DG. After opening of the furanoid ring of 3-DG, enolization and removal of one more molecule of water produce 3,4-dideoxyglucosone (3,4-DG), which is a cytotoxic

compound and the most biologically active product among the other glucose degradation products [92, 144]. Elimination of one molecule of water from 3,4-DG or totally 3 molecules of water from hexose sugars results in the formation of HMF, which is also a cytotoxic compound [92, 111]. HMF formation from sucrose, glucose and fructose was investigated and compared with the HMF formation from 3-DG under dry heating conditions by Perez Locas and Yaylayan [145]. According to their study, sucrose and fructose generated 4.5 and 2.5 fold more HMF compared to 3-DG while glucose generated 0.16 fold. They found that 3-DG is not the principal precursor in the formation of HMF. Sucrose and fructose as the highest HMF generating precursors were found to produce HMF through fructofuranosyl cation while glucose produce through 3-DG (Figure 1.6). Further confirmations of generation of more HMF from glycosidically linked fructose compared to free fructose were performed by them using raffinose, stachyose and lactose. Lactose which is lack of glycosidically linked fructose was found to exhibit less efficiency in the formation of HMF compared to raffinose and stachyose. Overall, they concluded that under thermal treatments, glycosidic bond of sucrose could easily cleave to form glucose and fructofuranosyl cation which has ability to be quickly converted into HMF or could react with amino compounds to form Heyns product by reaarangement of fructofuranosyl amine. Glucose originated from this cleavage could further react to form HMF through 3-DG in a smaller extent [145]. Antal and Mok [146] also found that hydrolysis of sucrose produces fructofuranosyl cation which is able to produce high amounts of HMF in aqueous medium at high temperatures.

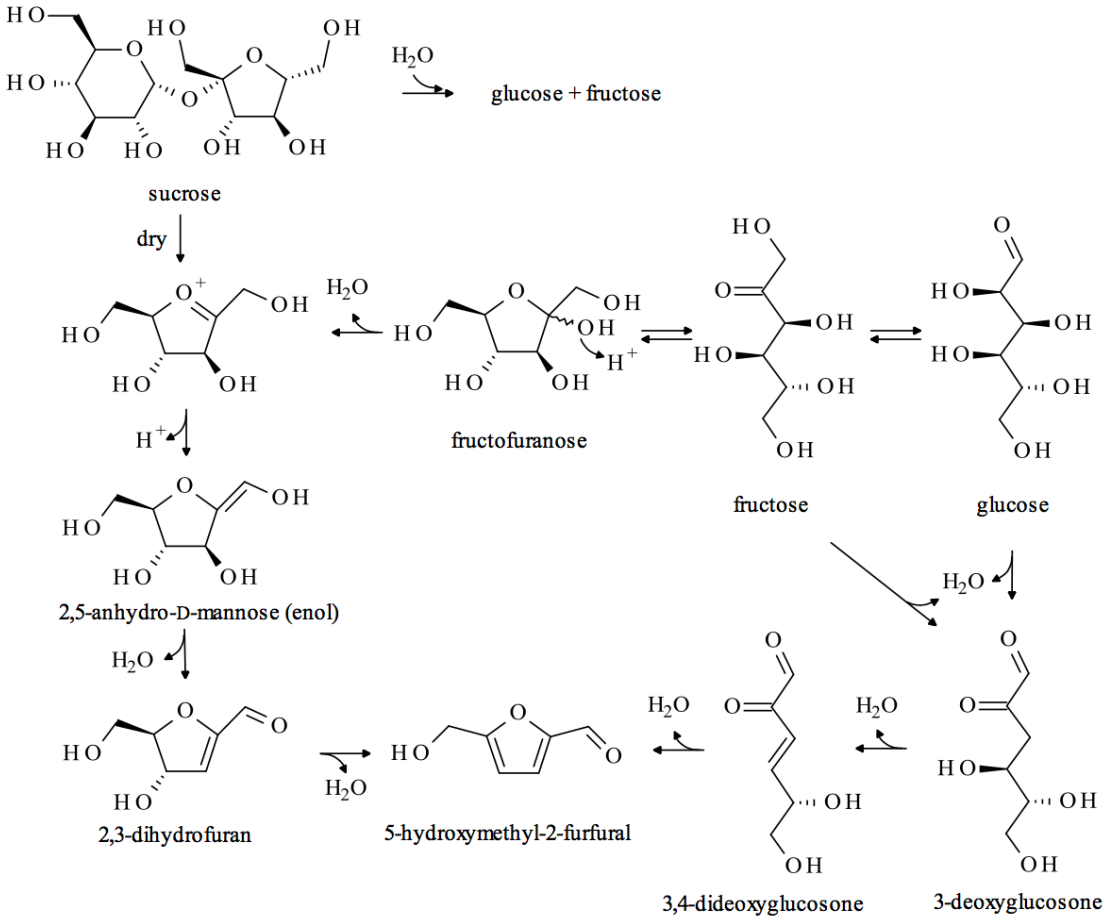


Figure 1.6. HMF formation through sucrose degradation and through dehydration of 3-DG, adopted from Perez Locas and Yaylayan [145]

2,3-enolization of a 2- or 3-ketose could lead to the formation of 1-DG or 4-deoxy-hexo-2,3-diulose (4-DG) [80, 141]. The reactivity of 1-DG was reported to be higher than 3-DG [147]. Higher reactivity of α-dicarbonyl compounds could lead to the formation of shorter chain α-dicarbonyl compounds, which have still high reactivity and analysis of which require a trapping agent like o-phenylenediamine [102, 110]. Retro-aldolization and β-cleavage of 3-DG, 1-DG or corresponding monosaccharide are the effective mechanisms in the formation of α-dicarbonyl compounds [110]. The fragmentation of α-dicarbonyl compounds is also favorable under the conditions of food processing although it is highly favorable under alkaline conditions [95]. Glyoxal (C_2), methylglyoxal (C_3), and dimethylglyoxal (C_4) are some of the shorter chain α-dicarbonyl compounds which were reported to be formed in cookies prepared with different flours during baking by both with the contribution of caramelization and Maillard reaction [101]. Formation of methylglyoxal and glyoxal in cookies were also reported by Arribas-Lorenzo and Morales [104]. Additionally, vinegars and soy sauce were found to be a rich source of

methylglyoxal [103]. Hollnagel and Kroh [95] identified glyoxal, methylglyoxal and dimethylglyoxal as a result of the non-enzymatic browning reaction of D-glucose, D-fructose, maltose and maltulose. They suggested that monosaccharides form more dicarbonyl compounds than disaccharides and glucose forms more dicarbonyl compounds than fructose.

Weenen [110] suggested that glyoxal formation could not be explained by only degradation of Amadori product or deoxyosones. It could form directly form aldose sugars or from imines by retro-aldolization and a following oxidation [110]. Formation of glyoxal from directly glucose by retro-aldol scission was also proposed by Thornalley et al [90]. Addition of glycine to the reaction medium containing glucose and o-phenylenediamine was reported to change the glyoxal concentration in only a small extent after heating [95].

Formation of methylglyoxal was suggested to form from intermediates that arise during early stages of Maillard reaction by cleavage of Schiff base followed by formation of free radical, C_2 and C_3 compounds [148]. Hollnagel and Kroh [95] were reported the formation of methylglyoxal from hexose sugars through 1-DG by the cleavage of the bonds between C_3 and C_4. On contrary to the formation of methylglyoxal through 1-DG, formation of methyglyoxal through retro-aldol fragmentation of 3-DG was also proposed [90]. Its formation was reported to be not dependent on the presence of oxygen in the reaction medium [102].

Dimethylglyoxal was reported to mostly originate from an isomerization product of 1-DG however another possible way suggested for dimethylglyoxal formation was the reaction of 3-DG with alanine or cyclohexylamine [110]. Formation of dimethylglyoxal enhanced in the presence of amines in the Maillard reaction as addition of glycine to the glucose and o-phenylenediamine reaction system produced more dimethylglyoxal [95].

1.3.3 Lipid Oxidation

Lipid oxidation is a radical chain reaction which could be divided into three phases as initiation, propagation, and termination [149]. The initiation step of the reaction starts with the formation of a fatty acid free radical (R•) and hydrogen radical (H•). At the beginning, an initiator might be necessary like heat, ultraviolet, or radiation to break down the covalent bond between C-H. This breakdown could also happen in the presence of transition metal ions or another free radical. In the propagation step, reaction of fatty acid free radical (R•) with oxygen gives rise to the formation of peroxy radicals (ROO•) and

further reaction of peroxy radicals with fatty acids (RH) results in the formation of hydroperoxides (ROOH) and a new fatty acid free radical (R•). In the termination step of the reaction, two reactive species like fatty acid free radical (R•) and peroxy radicals (ROO•) come together to form a rather stable compound. The accumulation of hydroperoxides continue until the rate of decomposition become higher than the rate of formation [80]. Hydroperoxides and their radicals could participate in reactions resulting in the formation of secondary oxidation products like cyclic and endo peroxides, epoxy and hydroxyl acids, aldehydes, hydrocarbons, oxoacids or polymerized molecules [80]. Fragmentation of hydroperoxides and endoperoxides produces reactive carbonyl species, among which α,β-unsaturated aldehydes including 4-hydroxy-trans-2-nonenal (HNE), 4-hydroxy-2-hexenal (HHE) and acrolein, dialdehydes including malondialdehyde (MDA) and glyoxal, and keto aldehydes including 4-oxo-trans-2-nonenal (ONE) and methylglyoxal are the most reactive ones [132, 150, 151].

These reactive carbonyl species could react with food constituents like proteins, saccharides, vitamins and phenolic compounds. Cysteine, cystine and methionine are the amino acids which are prone to reactions with lipid oxidation products. For instance, reaction of cysteine with 13-hydroperoxyoctadeca-9,11-dienoic acid, an oxidation product of linoleic acid, results in the formation of (*E*)-9-(S-cysteinyl)-10,13-dihydroxyoctadec-11-dienoic acid [80]. The products of lipid oxidation could also be able to react with amino acids to produce Strecker aldehydes which are the same compounds produced by the reaction of amino acids with α-dicarbonyl compounds and hydroxylcarbonyls. The mechanism of Strecker aldehyde formation from amino acids and lipid oxidation products involves the imine formation as a first step. Then the imine undergoes an electronic reaarangement and decarboxylation to produce a new imine, which is the precursor of Strecker aldehyde. The amino acid may also follow another route where imine undergoes to a different electronic rearrangement and produce an imine without decarboxylation which results in the formation of α-keto acids [12]. Lipid oxidation products are able to decarboxylate amino acids to produce biogenic amines as also in case of decarboxylation of asparagine in the presence of alkadienals like 2,4-decadienal [152]. Nucleophilic side chains of proteins, which are cysteine, lysine, arginine and histidine residues, could be modified by lipid oxidation products forming ALEs [150]. The precursors of both ALEs and AGEs have electrophilic characters so they could react with nucleophilic sides of the molecules [132]. ALEs that are formed by reactive carbonyl species of lipid oxidation are

MDA-Lys, HNE-Lys, carboxymethyllysine (CML), carboxyethyllysine (CEL), hydroimidazolenes, GOLD, lysine-MDA-lysine, and S-carboxymethyl-cysteine [150, 153]. α,β-Unsaturated aldehyde compounds are able to react with primary or side chain amino group of lysine to form Schiff base [132]. They could also react with thiol of cysteine, imidazole of histidine and epsilon amino of lysine in the protein side chains to form Michael adducts [132]. HNE and ONE, which are α,β-unsaturated aldehydes, react with amino compounds to form Michael adducts and different from HNE, ONE could also react with arginine [154]. The reactivity of amino acids toward HNE follows the order Cys>>His>Lys while ONE follows the order of Cys>>His>Lys>Arg, being more reactive than HNE towards thiols and amines [154]. HNE is able to form pyrroles as a result of its reaction with primary amines [155]. Acrolein is another α,β-unsaturated aldehyde and the most electrophilic one which makes it more reactive towards protein side chains [151]. It both naturally occurs in foods and is formed during heating oils [156]. One of the major adducts which is formed as a result of the reaction between two acrolein and one lysine side chain is N-ε-(3-formyl-3,4-dehydropiperidino) lysine (FDP-lysine) [157]. N-ε-(3-methylpyridinium) lysine (MP-lysine) is another adduct of acrolein whose formation is explained by Schiff base formation first. Then, an imine is formed from the reaction between the Schiff base and one more acrolein molecule which is followed by an oxidation step and intramolecular cyclization [136].

Dicarbonyl compounds are able to form ALEs very quickly because of their two carbonyl groups both are capable of withdrawing electrons [132]. In case of lipid-derived glyoxal and methylglyoxal, the first step of the reaction is the formation of carbinolamine and reaarangements to form heterocyclic crosslinks [132]. Glyoxal and methylgyoxal could be formed due to lipid oxidation, especially polyunsaturated fatty acids like linoleic and linolenic acids [97]. Their formation is believed to be from further reaction of α,β-unsaturated aldehydes occurring during lipid oxidation [158]. Both glyoxal and methyglyoxal could react with lysine, arginine and cysteine side chains producing AGEs [132], formation of which from lysine and arginine are mentioned in Maillard reaction in this chapter. MDA is a dicarbonyl compound, which could react with reactive side chains of proteins, nucleic acids and phospholipids, formed through cleavage of alkenals and 2,4-alkedienals [80]. Some of the products of the reaction between MDA and lysine are N-ε-(prop-2-enal)lysine (MDA-lysine), N-α-(prop-2-enal)lysine and lysine-MDA-lysine, conjugated crosslinks in proteins [159].

Repetitive aldol condensation of lipid oxidation products could lead to the proceeding of nonenzymatic browning. The Schiff bases of lipid carbonyls and amino groups polymerize by aldol-condensation leading to the formation of high molecular weight brown molecules which are unstable and degrade/dehydrate to produce volatiles [12]. Another mechanism for the proceeding of browning is polymerization of 2-(1-hydroxyalkyl)pyrroles which happens spontaneously and results in the formation of compounds from dimers to higher polymers [12].

Lipid oxidation products have also effects on the compounds other than proteins or amino compounds. Free radicals originating from lipid oxidation could initiate the oxidation of saccharides [80]. Vitamins and many of the phenolic compounds, which have role as antioxidants, could prevent oxidation of other compounds via free radicals by being oxidized [80].

Lipid oxidation products including α,β-unsaturated aldehydes, dicarbonyl compounds and their further reaction products, ALEs and AGEs, take the attention because of their possible negative health effects [97, 150, 151, 160]. One of the hazardous compounds originating from polyunsaturated fatty acids that creates health risks is furan. Furan could be formed from 4-hydroxy-2-butenal through cyclization followed by formation of 2,5-dihydro-2-furanol and further dehydration. Although formation of furan creates concerns, it has a positive side that the precursor, 4-hydroxy-2-alkenal, is more toxic than volatile furan [161]. During the formation of furan, 4-hydroxy-2-butenal not only originates from lipid oxidation but also through dehydration of 2-deoxyaldotetrose which is formed via aldol condensation of acetaldehyde and glycolaldehyde or from ascorbic acid [161, 162].

1.4 Multiresponse Kinetic Modeling of Chemical Reactions

To control the quality of foods, changes in foods over time should be controlled during processing or storage. Quality indicators may differ depending on the type of the reaction which may be microbiological, physical, biochemical or chemical [163]. The relevant changes in microbiological reactions could be the growth of microorganisms, physical reactions may be related with heterogeneous structure of foods, biochemical reactions could be related with endogenous enzymes in foods and chemical reactions are mainly lipid oxidation, sugar degradation and Maillard reaction [59]. Kinetic description of the reactions is necessary to express the quality parameters as quantitative as possible [164].

A uniresponse kinetic model, describes the changes in the concentration of one product with time. However, it does not give information about the mechanism of the reaction. A simple example was given by Van Boekel [164] to show the strength of the multiresponse models against uniresponse models,

$$A \xrightarrow{k} B$$

If A decomposes to form B, the loss in the concentration of A should be equal to the concentration of B formed. However, in a uniresponse model as we only measure the concentration of A, it will not be possible to understand whether a complete conversion is achieved or not. In the multiresponse kinetic modeling of the same reaction, concentration of B will also be considered and measured. If the concentration B is less than its expected concentration (complete conversion of A to B), we will have an idea of revising the model by considering B could further react to form another product or decomposition of A may proceed more than one pathway. As could be understood from the given example, multiresponse kinetic models help to understand mechanisms of reactions. In case of kinetic modeling of complex reactions, changes in one response of a variety of chemical reactions could not be linked with the underlying mechanism [165].

In the multiresponse modeling of complex systems, the main reactants and products of the reactions should be identified and to understand that all the reactants and products are identified, a mass balance should be applied. Then, defining co-products of the same reaction, differentiating the main and secondary reaction routes, and specifying the critical parameters of the process as well as the effect of concentrations of reactants are important considerations before proposing a model mechanism [59, 166].

Once the main reactants and products have been identified, each reaction step should be expressed as differential equations. However, it will be difficult to solve these equations analytically. The data obtained by solving the differential equations of the proposed model needs to be fitted to experimentally obtained data [166]. If the model fit obtained is not well compatible with the experimental data, it will be necessary to revise the model until the most satisfactory model is obtained [164, 165, 167].

Reaction rate constants and activation energies of the reactions are generally the model parameters tested [98, 99, 167, 168]. Consistence temperature dependence might be another criteria for the model is acceptable or not [165]. The temperature dependence of

the reaction rate constants (k) to the temperature (T) is generally explained by Arrhenius equation

$$k = k_0 \times exp\left(\frac{-E_a}{RT}\right)$$

where k_0 is the frequency factor, R is the gas constant (8.314 J/mol K) and E_a is the activation energy (J/mol). However, as the temperature ranges studied are narrow for applying Arrhenius equation, Martins and Van Boekel [165] reparametrized the Arrhenius equation to obtain better precision [163]. The reparametrized Arrhenius equation

$$k = k_b \times exp\left(\frac{E_a}{RT_b}\left(1 - \frac{T_b}{T}\right)\right)$$

involves k_b, which is the reaction rate constant at a reference temperature and T_b, is the reference temperature.

Multiresponse modeling of chemical reactions, especially Maillard reaction and caramelization, has been studied so far in many different model systems [165, 169-174]. Heating of sugar reactants which are lactose, lactulose, galactose, protein bound lactulosyllysine in milk like systems together with caseinate in the presence of milk salts was performed in order to predict the behavior of reactants in a quantitative way by van Boekel [169]. The results of that study showed that isomerization/degradation and Maillard reaction were the main reactions during heating of milk like system and isomerization/degradation was more important. In another study of Brands and van Boekel [170], kinetic modeling of reactions in disaccharide-casein systems during heating at 120°C and pH 6.8 was performed. Two main pathways were suggested for the degradation of disaccharides which help to quantitative prediction of reactions during heating of disaccharide-casein systems. The first one was isomerization of lactose to lactulose and its further degradation to galactose and formic acid as well as isomerization of maltose to maltulose and its further degradation to glucose and formic acid. The other pathway was Maillard reaction between aldose sugars and casein resulting in the formation of colored compounds. Martins and van Boekel [165] proposed a multiresponse kinetic model for Maillard reaction in aqueous glucose/glycine model system. According to their model, D-fructose, N-(1-deoxy-D-fructos-1-yl)glycine, 1-DG, 3-DG, HMF, methylglyoxal, formic and acetic acids were the reactants, intermediates and end products of the system. As a result of that study, organic acids were stable end products, acetic acid was the indicator of

Maillard reaction at pH 6.8, 3-DG took role in color formation and degradation of N-(1-deoxy-D-fructos-1-yl)glycine to glucose and glycine was not important from a quantitative point of view. Martins et al [171] also proposed a reaction mechanism for the degradation of N-(1-deoxy-D-fructos-1-yl)glycine in aqueous model systems at different temperatures and pH conditions. A 20°C increase in temperature or 1.3 unit decrease in pH was reported by them to have same effect in the degradation of N-(1-deoxy-D-fructos-1-yl)glycine and accordingly in the release of glycine. Together with glycine, acetic acid was the main product of the system and mannose was the dominant sugar at pH 5.5 while glucose was dominant at pH 6.8. Martins et al [172] also reported that 1,2-enolization was favored in the degradation of N-(1-deoxy-D-fructos-1-yl)glycine at lower pH values while 2,3-enolization was favored at higher pH values. A kinetic model of formation of CML, one of the AGEs, in aqueous model system of sugar and casein was proposed by Nguyen et al [173]. They suggested that CML did not originate from reducing sugars and it was formed from Maillard reaction via Amadori product. A multiresponse kinetic study of another AGE was the formation and elimination of protein-bound pyrraline in the Maillard reaction in lysine-glycine/glucose model systems including equimolar glucose-peptide, glucose excess or peptide excess systems [174]. It was suggested that isomerization of glucose to fructose was dominant rather than glucose to mannose in the equimolar and excess reactant systems. Elimination of peptide-bound pyrraline was more difficult in the excess peptide containing system and caramelization was negligible in peptide excess and equimolar systems [174].

There are only a few studies dealing with the multiresponse modeling in real foods [99, 175-177] as multiresponse kinetic modeling of Maillard reaction and caramelization is a challenging task because of the complexity of real food systems. A kinetic model for acrylamide and HMF formation in biscuits was proposed [176] and effect of sugar type on the formation of acrylamide and HMF had been investigated by Nguyen et al [175]. HMF concentration was higher in biscuits with glucose and fructose while acrylamide was higher in biscuits with glucose which also had the highest asparagine concentration. According to their proposed mechanisms, HMF formed via caramelization and acrylamide followed specific amino acid route [175]. A kinetic model for the acrylamide formation was also proposed during frying of commercial French fries in order to understand the effect of each stage of Maillard reaction on the concentration of acrylamide in the final product [177]. A kinetic study describing the formation of α-dicarbonyl compounds during

Maillard reaction and caramelization was also proposed in heated glucose/wheat flour system, which has the complexity of real foods [99]. According to the findings of this study, formation of 1-DG was found to be mainly from degradation of Amadori product. 3-DG was originated from glucose itself and degradation of Amadori product. Glyoxal was formed from glucosone, methylglyoxal and diacetyl was formed from 1-DG and fructose was the main precursor of HMF formation [99]. There is still a lot to do in the area of multiresponse modeling of chemical reactions in both model systems and real foods.

2 COMPOSITIONAL CHARACTERISTICS OF HAZELNUTS

2.1 Introduction

Hazelnuts are challenging food matrixes with their sugar, amino acids, vitamins, minerals and high content of lipids. All these constituents of hazelnuts act individually and together as reactants during chemical reactions. To understand the chemistry behind the chemical reactions, it is necessary to identify and quantify the reactants. For that reason, constituents of hazelnuts, which are the reactants of the chemical reactions, have been identified and quantified in detailed. Despite the studies being done on the topic of hazelnut composition, it is believed that a compact study including compositional characteristics with their profile analysis of Turkish hazelnut varieties harvested in two consecutive years will contribute much to the literature.

In this chapter, lipid and non-lipid fraction of 14 hazelnut varieties have been identified and quantified. Proximate composition analysis, profile of amino acids, sugars and organic acids, water soluble vitamins and minerals have been identified as the non-lipid part of the hazelnuts. After extraction of hazelnut oil, profile of triacylglycerol, free fatty acid, and tocopherols have been determined to identify lipid part of the hazelnut varieties. Additionally, as a part of hazelnut, when consumed intact, phenolic profile of skins of hazelnut varieties have been identified.

2.2 Material and Methods

2.2.1 Chemicals and Consumables

Hexane (≥95%), 2-propanol (99.5%), acetonitrile (≥99.9%), methanol (≥99.9%), acetone, ethyl acetate, diethyl ether and water were purchased from Sigma Aldrich (Steinheim, Germany). Formic acid (98%), potassium hydroxide, sodium carbonate, sodium nitrite, aluminium chloride, sodium hydroxide, potassium hexacyanoferrate (II) trihydrate, zinc sulphate heptahydrate, strontium chloride hexahydrate, hydrochloric acid (37%), nitric acid (65%) and sulfuric acid (95-97%) were supplied from Merck (Darmstadt, Germany). All sugar (≥99%), organic acid (≥95%), amino acid (≥97%) and vitamin (≥98%) standards were purchased from Sigma Aldrich (Steinheim, Germany). Standard solutions (1000 µg/mL) of minerals were purchased from Chem-Lab NV (Zedelgem, Belgium). Supelco 37 component fatty acid methyl ester mix (10 mg/mL in methylene chloride, in varied concentrations), triacylglycerols (all ≥99%) (tripalmitin (PPP), 1,2-dioleoyl-3-palmitoyl-rac-glycerol (OOP), 1,3-dipalmitoyl-2-linoleoyl-glycerol (PLP), 1,3-dipalmitoyl-2-oleoyl-

glycerol (POP), 1,2-dilinoleyl-3-palmitoyl-rac-glycerol (LLP), glyceryl trioleate (OOO) and tocopherols (α-tocopherol (≥96%), β-tocopherol (≥96%), γ-tocopherol (≥96%), δ-tocopherol (≥90%)) were purchased from Sigma Aldrich (Steinheim, Germany). Standards of gallic acid, ferulic acid, catechin, potassium peroxy disulphate, ABTS (2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid)), Folin–Ciocalteu reagent were also obtained from Sigma Aldrich (Steinheim, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Fluka Chemie AG (Buchs, Switzerland). Syringe filters (nylon, 0.45 μm) were supplied by Waters (Millford, MA, USA).

2.2.2 Hazelnut Samples and Their Skins

A total of 14 hazelnut (*Corylus avellana* L.) varieties (Acı, Çakıldak, Foşa, İncekara, Kalınkara, Kan, Kargalak, Kuş, Palaz, Sivri, Tombul, Uzun Musa, Yassı Badem, Yuvarlak Badem), which were grown in the collection orchard of Hazelnut Research Institute in Giresun, which is located at the northern east coast of Turkey, were used for the analysis. The latitude of the collection orchard is 40° 54’ 32.26’’ N and the longitude is 38° 21’ 0.73’’ E. In the collection orchard of Hazelnut Research Institute, randomly selected hazelnuts were collected by hand at the first week of August when their green leafy covers were turned to pale and the moisture content was decreased to 30%. Then, they were exposed to sun drying approximately for 3-5 days to decrease their moisture content to 6%. After removal of green leafy covers, each hazelnut varieties with their brown hard shell were packed in three separate bags, each of them containing 2 kg hazelnuts, and immediately sent for analysis at the harvest years of 2013 and 2014. Until the analysis, hazelnut samples were kept at -20°C. Before analysis, hazelnut hard shells were cracked and hazelnut skins were scraped manually. About 2.5 g of hazelnut skin was obtained by scrapping 100 g of hazelnut from each bag. Hazelnut skins, belong to the hazelnut varieties of the harvest year 2013, were collected in order to perform phenolic profile analysis. A 100 g portion of hazelnut from each bag and the hazelnut skins belong to each variety were finely grounded with a coffee mill. Three independent samples were analyzed from each hazelnut varieties and their skins and all analytical measurements were performed duplicate.

2.2.3 Analysis of Proximate Composition

Proximate composition of hazelnut varieties was determined by using the methods of Association of Official Analytical Chemists (AOAC) [178]. Hazelnut samples were dried at 105°C in an oven to a constant weight in order to determine moisture contents (AOAC

925.10). Total oil content was determined gravimetrically after Soxhelet extraction with hexane for 10 h (AOAC 948.22). Kjeldahl method was used to find out total nitrogen content and protein content was calculated from total nitrogen content value by using conversion factor of 6.25 (AOAC 984.13). Total ash content of hazelnut varieties was determined after ashing with a gradual temperature increase (250°C-650°C) and obtaining a constant weight (AOAC 923.03). Carbohydrate content of hazelnut varieties was calculated by subtraction of the total percentage of other constituents from 100%.

2.2.4 Defatting of Samples

After grinding, 5 g of hazelnut samples was defatted with hexane by using a Soxhelet extractor at 55°C for 10 h. The residual hexane was removed with a vacuum rotary evaporator at 40°C at about 15 min. The samples were placed in a flow cabined at room temperature in order to remove the residual hexane completely. They were stored at -18°C until the analyses of amino acids, sugars, organic acids and water-soluble vitamins were performed. Extracted hazelnut oils were kept at -20°C in tubes flushed with nitrogen until the chemical analyses were performed. The hazelnut oils were used for determination of triacylglycerol, fatty acid, and tocopherol profiles of Turkish hazelnut varieties.

2.2.5 Analysis of Amino Acids

Amino acids were analyzed after acid hydrolysis. First, 50 mg of defatted sample was weighted into glass tubes and 5 mL of 8 N HCl was added onto it. After nitrogen gas flushing to the headspace, screw caps were closed tightly. The tubes were kept at 110°C for 23 h until all amino acids in proteins were totally hydrolyzed. Then, 100 µL of hydrolyzate was transferred to another glass tube and dried under a gentle nitrogen stream. The final residue was redissolved in 1 mL of the mixture of acetonitrile:water (1:1, v/v) and filtered through a 0.45 µm filter into a vial. Analysis was performed according to the method described by Kocadağlı et al [179].

2.2.6 Analysis of Sugars and Organic Acids

A quantity of 0.3 g of defatted sample was extracted with deionized water in triple stages (5 mL, 2.5 mL, 2.5 mL). Extracts were combined in a test tube and centrifuged at 7500 x *g* for 3 minutes. Then, 1 mL of the supernatant was transferred to a test tube and 50 µL of Carrez I and Carrez II were added into it. The tubes were vortexed for 2 minutes and centrifuged at 7500 x *g* for 3 minutes. After then, 1 mL of extract was passed through preconditioned Waters Oasis HLB cartridges and collected into a vial after dropping first 8

drops. Sugar analysis was performed by using RID as described by Kocadağlı and Gökmen [99]. Organic acid analysis was performed concurrently by using DAD at 210 nm.

2.2.7 Analysis of Water Soluble Vitamins

A quantity of 0.3 g of defatted hazelnut was extracted with deionized water in triple stages (5 mL, 2.5 mL, 2.5 mL). Extracts were combined in a test tube and centrifuged at 7500 x *g* for 3 minutes. The supernatant was diluted with acetonitrile to maintain a final acetonitrile to water ratio of 1:1 (v/v). After centrifugation at 7500 x *g* for 3 minutes, the supernatant was filtered through 0.45 μm filter into a vial. Analysis was performed according to the method described by Yılmaz and Gökmen [180].

2.2.8 Analysis of Minerals

Analysis of sodium and potassium minerals were performed by using flame emission spectroscopy according to AOAC method 956.01 and calcium, magnesium, manganese, copper, zinc, iron was analyzed by using atomic absorption spectroscopy according to AOAC method 975.03 [178].

2.2.9 Analysis of Triacylglycerols

Hazelnut oil was dissolved in 2-propanol:acetonitrile (50:50, v/v) to obtain 1% (w/v) solution. After filtering through 0.45 μm filter, the solution was collected in a vial. Analysis was performed with an Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump, a oven and a diode array detector at 210 nm. The chromatographic separation of triacylglycerols was performed on an Inertsil ODS-4 (5 μm, 4.6 x 250 mm) column. Elution was performed by using the mixture of 2-propanol:acetonitrile (50:50, v/v) at a flow rate of 1 mL/min at 30°C. The working solutions of triacylglycerols (1%, w/v) were prepared in the elution mixture and injected into the column to determine their specific retention times. Identification of the peaks was performed tentatively and supported by the data reported in the literature that was obtained from APCI-MS analysis of acylglycerols [25, 181, 182]. In these studies, triacylglycerols were separated by using reverse phase HPLC and a C18 column resulted with some co-eluted triacylglycerol peaks, especially triacylglycerols having same equivalent carbon numbers (ECN), similar to the chromatograms obtained from this study. Hazelnut oil showed almost the same typical fingerprint chromatograms in both APCI-MS based analysis and in this study. However, APCI-MS based studies gave more information about the position of fatty acids in triacylglycerols without using a standard. In addition to

comparison with typical fingerprint chromatograms previously reported [25, 181, 182], standard working solutions of triacylglycerols, ECN and fatty acids' composition were also considered in the evaluation of triacylglycerols' composition of hazelnut oils. For the quantification of triacylglycerols, co-eluted peaks were separated by dropping a perpendicular line between valleys to the baseline and the area under the peaks were determined [183]. The concentration of triacylglycerols in hazelnut oil was calculated and expressed as percentages of glyceryl trioleate (OOO%).

2.2.10 Analysis of Fatty Acid Composition

Methyl esters of extracted hazelnut oils were prepared as described by Yılmaz and Gökmen [180]. Two hundred mg of hazelnut oil was weighted into test tubes. Ten mL of hexane and 100 µL of 2 N KOH in methanol was added. After vortexing, all extracts were centrifuged at 6000 x *g* for 3 min. Supernatants were diluted five times with hexane and taken into a vial after filtering through a 0.45 µm filter. Analysis was performed at Agilent 6890N gas chromatography system (GC) (Agilent, Waldbronn, Germany) coupled to flame ionization detector (FID) and a capillary column. Temperature of back inlet and detector was 250°C and 260°C. Separation was performed on Agilent J&W 122-2362 DB-23 (60 m x 0.25 mm x 0.25 µm) capillary column. The column temperature was at 160°C initially for 5 min and increased to 220°C with a rate of 5°C/min and held isothermal for 8 min. The flow rate of helium was 1 mL/min. Injection volume was 1 µL with a split ratio of 1:50. Standards of FAMEs were also analyzed under the same conditions. Fatty acid compositions of hazelnut oils were expressed as percentages of their relative areas. Methyl esters of palmitic, stearic, oleic and linoleic acids constituted >98% of the all fatty acid methyl esters found in hazelnut oil samples. Percentage areas of the minor FAMEs (<1%) were not reported.

2.2.11 Analysis of Tocopherol

A 0.5 g of hazelnut oil or hazelnut skin was weighed into test tubes and tocopherols were extracted with 2.5 mL of 2-propanol:methanol mixture (50:50, v/v) and centrifuged at 6000 x *g* for 3 min. The procedure was repeated for 3 times and supernatants were collected and combined. After passing through 0.45 µm filter, the extracts were collected in vials. Analysis of tocopherols was performed as described by Yılmaz and Gökmen [184].

2.2.12 Separation of Phenolic Fractions of Hazelnut Skins with Alkaline Hydrolysis

Soluble free, soluble conjugated and insoluble bound phenolic compounds were extracted as described by Moore et al [185]. First, 0.25 g of hazelnut skin was extracted with 4 mL of methanol/acetone/water (7:7:6, v/v/v) and centrifuged at 6000 x *g* for 3 min. Extraction with 4 mL of methanol/acetone/water (7:7:6, v/v/v) was repeated for 5 times and supernatants of each step were collected and combined in a test tube (extract A). Pellet obtained after this extraction was kept for the analysis of insoluble bound phenolic compounds.

A quantity of 10 mL of the extract A was transferred to another tube and 10 mL of 4 N NaOH was added onto it in order to release soluble conjugated phenolic compounds (extract B). A quantity of 7 mL of 4 N NaOH was added onto pellet simultaneously to release insoluble bound phenolic compounds (extract C). Then, both tubes were left for 4 h in the shaker at the room temperature.

After then, 5 mL of extract from A, B and C were transferred to test tubes and the pH was adjusted to 2, by using 6 N HCl, in all tubes. A quantity of 5 mL of dietyl ether:ethyl acetate (1:1, v/v) was added into these tubes, vortexed for 2 minutes and centrifuged at 6000 x *g* for 2 min. The same extraction procedure with 5 mL of dietyl ether:ethyl acetate (1:1, v/v) was repeated for four times. Supernatants were collected at each step and extracts were combined. Then, 5 mL of combined extract were dried by using N_2 at 30°C. After then, phenolic compounds were redissolved in 1.5 mL of methanol and kept at -20°C until the analysis were performed. As extract B contained both soluble free and soluble conjugated phenolic compounds, soluble conjugated phenolic compounds were calculated by subtracting the amount of soluble free phenolic compounds obtained from the analysis of extract A.

2.2.13 Analysis of Total Phenolic Content of Hazelnut Skins

Total phenolic content analyses were performed according to the Folin-Ciocalteu method [186] by using appropriate extracts.

2.2.14 Analysis of Individual Phenolic Acids of Hazelnut Skins

An Agilent 1200 HPLC system consisting of a DAD, quaternary pump, autosampler, and column oven was used for the analysis (Agilent Technologies, Waldbronn, Germany). Before analysis, extracts were filtered by using a 0.45 μm nylon syringe filters and taken into vials. Phenolic acids were separated on a Waters Atlantis C18 column (250 mm x 4.6

mm id., 5 μm) (Milford, MA, USA) by using 1% formic acid in water (A) and 1% formic acid in acetonitrile (B) at a flow rate of 1 mL/min with the following gradient program: linear gradient elution from 10 to 20% B, 0-10 min; linear gradient elution from 20 to 40% B, 10-20 min; linear gradient elution from 40 to 10% B, 20-25 min and isocratic elution of 10% B, 25-30 min. The column temperature was 30°C and injection volume was 10 μL. Analyses were performed by using diode array detector at 280 nm.

2.2.15 Analysis of Total Flavonoid Content of Hazelnut Skins

Total flavonoid content was determined according to the method described by Zhishen et al [187] by using appropriate extracts.

2.2.16 Analysis of Total Antioxidant Capacity

Total antioxidant capacity measurements were performed according to the QUENCHER method described by Serpen et al [188] by using ABTS solution.

2.2.17 Statistical Analysis

Data was given as mean ± standard deviation. Significance of difference between varieties was analyzed by using One-way ANOVA Duncan's test ($p<0.05$) by using SPSS Version 17.0. Differences between harvest years were determined by t-test using Excel ($p<0.05$).

2.3 Results and Discussion

2.3.1 Proximate Composition

Proximate compositions of hazelnut varieties harvested in the year of 2013 and 2014 are given in Table 2.1. Oil constituted the largest part of the hazelnuts, followed by proteins, carbohydrates and ash. Foşa (60.4 g/100g) and Yuvarlak Badem (58.1 g/100g) had the lowest oil contents in harvest years of 2013 and 2014, respectively. Acı was found to contain the highest amount of oil in both harvest years (69.9 g/100g in 2013 and 68.9 g/100g in 2014). Total oil content of hazelnut varieties changed significantly ($p<0.05$) depending upon the harvest years for the varieties Kargalak, Palaz, İncekara, Sivri, Foşa, Yuvarlak Badem, Kuş and Uzun Musa. Köksal et al [4] determined the total oil contents of seventeen Turkish hazelnut varieties harvested in 2002 and the values ranged between 56.07 g/100g and 68.52 g/100g. They also reported that Cavcava variety had the lowest oil content while Kalınkara had the highest. Kıralan et al [21] found the total oil contents of Turkish hazelnut varieties harvested in the years of 2009-2010 ranged from 55.01 to 63.26 g/100g that were relatively lower compared to the total oil contents presented herein.

Table 2.1. Proximate composition of Turkish hazelnut varieties harvested in 2013 and 2014 (g/100g)

	Moisture		Oil		Protein		Carbohydrate		Ash	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Kargalak	2.41±0.11^{a}	4.97±0.16^{f*}	67.6±0.76^{b,c}	60.5±2.49^{a,b*}	15.1±0.36^{a,b}	20.0±0.11^{f,g*}	10.9±1.09^{b}	10.4±2.45^{a,b,c,d}	4.23±0.01^{e}	4.15±0.01^{b,c,d*}
Palaz	2.44±0.05^{a}	3.66±0.01^{a,b*}	68.0±1.26^{b,c}	65.2±1.75^{c,d,e*}	16.2±0.27^{c,d}	17.8±0.64^{c,d*}	9.07±1.57^{a,b}	9.04±2.44^{a,b,c,d}	4.38±0.01^{f}	4.23±0.05^{c,d*}
İncekara	2.47±0.03^{a}	4.01±0.02^{c,d,e*}	68.2±0.18^{b,c}	62.7±3.41^{b,c*}	14.8±0.13^{a,b}	16.7±0.11^{a,b,c*}	10.3±0.32^{a,b}	12.7±3.38^{d}	4.21±0.02^{e}	3.90±0.11^{a,b*}
Sivri	2.38±0.10^{a}	4.08±0.06^{d,e*}	68.4±0.70^{c,d}	66.0±0.22^{c,d,e*}	16.5±0.11^{d}	18.0±0.27^{d,e*}	8.63±0.96^{a,b}	7.90±0.57^{a,b,c}	4.02±0.00^{b,c,d}	3.99±0.03^{a,b,c}
Yassı Badem	2.54±0.01^{a,b}	3.64±0.10^{a,b*}	68.1±1.04^{c,d}	67.1±0.11^{d,e}	15.0±0.08^{a,b}	16.1±0.18^{a*}	10.1±1.13^{a,b}	9.09±0.18^{a,b,c,d}	4.29±0.01^{e}	4.02±0.02^{a,b,c*}
Foşa	2.75±0.31^{b,c}	4.11±0.15^{e*}	60.4±0.61^{a}	63.5±0.35^{b,c,d*}	21.9±0.89^{g}	17.8±0.17^{c,d*}	10.5±1.82^{a,b}	10.4±0.39^{a,b,c,d}	4.42±0.01^{f}	4.19±0.02^{c,d*}
Kalınkara	2.34±0.08^{a}	5.36±0.07^{g*}	68.8±0.49^{c,d}	66.2±2.58^{c,d,e}	14.5±0.36^{a}	17.4±0.33^{b,c,d*}	10.5±0.47^{a,b}	7.33±2.79^{a,b}	4.09±0.04^{d}	3.77±0.19^{a*}
Yuvarlak Badem	2.84±0.09^{c}	6.77±0.22^{h*}	66.2±1.10^{b}	58.1±0.35^{a*}	17.6±0.14^{e}	20.3±0.04^{g*}	9.97±0.51^{a,b}	10.9±0.30^{b,c,d*}	4.07±0.02^{d}	3.96±0.13^{a,b,c}
Kuş	2.46±0.09^{a}	5.09±0.07^{f,g*}	68.3±1.21^{c,d}	63.9±0.97^{b,c,d*}	15.1±0.00^{a,b}	16.0±1.35^{a}	10.9±0.04^{b}	11.2±0.12^{b,c,d*}	3.97±0.03^{a,b,c}	3.79±0.18^{a}
Çakıldak	2.52±0.02^{a,b}	3.98±0.05^{c,d,e*}	68.2±0.84^{c,d}	67.5±0.21^{d,e}	16.7±0.12^{c,d}	16.5±0.58^{a,b}	8.58±1.32^{a,b}	7.73±0.37^{a,b,c}	4.00±0.00^{b,c,d}	4.31±0.05^{d*}
Kan	2.55±0.55^{a,b}	3.74±0.09^{b,c*}	67.2±1.60^{b,c}	66.6±1.14^{c,d,e}	18.3±0.69^{f}	19.0±0.24^{e,f}	7.57±2.80^{a}	6.80±1.24^{a,b}	4.04±0.01^{c,d}	3.85±0.04^{a*}
Uzun Musa	2.52±0.14^{a,b}	5.03±0.29^{f*}	67.9±0.49^{b,c}	61.2±2.13^{a,b*}	17.0±0.32^{d,e}	17.8±0.13^{c,d*}	8.58±0.51^{a,b}	11.9±1.98^{c,d*}	4.01±0.00^{b,c,d}	4.03±0.27^{a,b,c}
Acı	2.51±0.12^{a,b}	3.40±0.10^{a*}	69.9±1.69^{d}	68.9±1.90^{e}	15.5±0.08^{b,c}	17.3±0.22^{b,c,d*}	8.14±1.38^{a,b}	6.47±1.88^{a}	3.92±0.11^{a,b}	3.89±0.09^{a,b}
Tombul	2.53±0.02^{a,b}	3.80±0.03^{b,c,d*}	68.4±1.03^{c,d}	66.8±1.50^{d,e}	16.3±0.64^{c,d}	18.2±0.50^{d,e*}	8.88±0.46^{a,b}	7.01±2.07^{a,b}	3.90±0.09^{a}	4.22±0.04^{c,d*}

Superscript letters in each column indicate statistically significant difference ($p<0.05$) according to Duncan's test. (*) indicates statistically significant difference ($p<0.05$) according to t-test. Data are expressed as mean±standard deviation. Three independent samples were analyzed from each hazelnut varieties and all analytical measurements were performed duplicate.

Oil contents of nineteen Portuguese hazelnut cultivars were reported to range from 59.3 to 69.0 g/100g, which were almost in the same range with Turkish hazelnut varieties harvested in 2013 and 2014 [22]. Moreover, total oil contents of Spanish hazelnut cultivars, ranging from 57.22 to 64.1 g/100g, were lower than the total oil contents of Turkish hazelnut varieties found herein [32].

Protein contents of hazelnuts were 14.5-21.9 g/100g and 16.0-20.3 g/100g in the harvest years of 2013 and 2014, respectively. Kalınkara and Kuş were the varieties that had the lowest protein content while Foşa and Yuvarlak Badem had the highest in harvest years 2013 and 2014, respectively. Kuş, Çakıldak and Kan were the only varieties whose protein content did not change significantly ($p>0.05$) in the harvest year 2014. The protein content of Kalınkara harvested in 2002 was also found to be lowest with 11.7 g/100g and the protein content was highest in Yuvarlak Badem and Cavcava with 20.8 g/100g but they were lower than the highest protein content found herein [4]. Compared to the protein contents of Turkish hazelnut varieties, Portuguese hazelnut varieties were reported to have lower protein contents ranging from 9.3 to 12.7 g/100g [22]. Carbohydrate contents were 7.57-10.9 g/100g in 2013 and 6.47-12.7 g/100g in 2014. Ash contents ranged between 3.90 g/100g (Tombul) and 4.42 g/100g (Foşa) in 2013, and between 3.77 g/100g (Kalınkara) and 4.31 g/100g (Çakıldak) in 2014. Differences between the ash contents of hazelnut varieties were found significant ($p<0.05$). Significant differences ($p<0.05$) were also observed in the ash contents between harvest years except for the varieties Sivri, Yuvarlak Badem, Kuş, Uzun Musa and Acı.

2.3.2 Amino Acid Profile of Hazelnut Varieties

Amino acid compositions of Turkish hazelnut varieties harvested in 2013 and 2014 are given in Table 2.2 and Table 2.3, respectively. Glx (glutamine plus glutamic acid), Arg, and Asx (asparagine plus aspartic acid) were found to be the most dominant amino acids in Turkish hazelnuts. These three amino acids constituted of at least 20% of the total amino acids. Venkatachalam and Sathe [1] also found these three amino acids as the most abundant amino acids in hazelnuts. Ruggeri et al [189] reported that glutamic acid, arginine and aspartic acid account for about 40% of protein of Italian hazelnuts, which was higher than in Turkish hazelnut varieties analyzed here. Individual contribution of the other amino acids was not exceeded 4% of the total amino acids in Turkish hazelnuts. Moreover, hazelnuts were found to contain all essential amino acids (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val) except Trp. Their total concentrations were 19.3-29.5 g/100g protein and

22.4-27.5 g/100 g protein in 2013 and 2014, respectively. It should be noted here that Trp wsa degraded during acid hydrolysis conditions. Alasalvar et al [19] reported Trp as the least abundant amino acid in Tombul variety. Among the essential amino acids, Arg was found to be the highest (>6.72 g/100g) and Met was the lowest (<0.56 g/100g). The second dominant essential amino acid was either Phe or Leu depending upon the variety of hazelnuts.

Significant differences were found between the concentrations of individual amino acids in both harvest years ($p<0.05$). Compared to 2013, the most remarkable change was observed in Kargalak, Palaz and Yassı Bademm with significant differences in the concentrations of almost all amino acids ($p<0.05$). Met was the only amino acid whose concentrations showed significant change in almost all hazelnut varieties ($p<0.05$). The concentrations of Asx, Glx, Gly, Lys and Ser did not change significantly in most of the hazelnut varieties depending upon the harvest year ($p>0.05$).

Table 2.2. Amino acid composition of Turkish hazelnut varieties harvested in 2013 (g/100 g hazelnut protein)

	Kargalak	Palaz	İncekara	Sivri	Yassı Badem	Foşa	Kalınkara	Yuvarlak Badem	Kuş	Çakıldak	Kan	Uzun Musa	Acı	Tombul
Ala	2.72 ±0.01^{b,c,d}	2.49 ±0.16^{a,b}	2.84 ±0.15^{c,d}	2.54 ±0.15^{a,b,c}	2.66 ±0.06^{b,c,d}	2.54 ±0.12^{a,b,c}	2.52 ±0.12^{a,b,c}	2.48 ±0.17^{a,b}	2.92 ±0.06^{d}	2.75 ±0.27^{b,c,d}	2.31 ±0.02^{a}	2.51 ±0.04^{a,b}	2.54 ±0.02^{a,b,c}	2.72 ±0.19^{b,c,d}
Arg	9.21 ±0.38^{c,d}	7.56 ±0.05^{a}	9.13 ±0.36^{c,d}	8.25 ±0.68^{a,b}	8.43 ±0.05^{a,b,c}	8.07 ±0.09^{a}	7.83 ±0.39^{a}	7.92 ±0.59^{a}	9.09 ±0.17^{b,c,d}	9.41 ±0.58^{d}	7.80 ±0.19^{a}	7.84 ±0.07^{a}	8.13 ±0.06^{a}	9.09 ±0.46^{b,c,d}
Asx	7.56 ±0.23^{d,e,f}	6.55 ±0.23^{a,b}	7.83 ±0.31^{f}	6.79 ±0.48^{a,b,c}	7.17 ±0.07^{b,c,d,e,f}	6.87 ±0.33^{a,b,c,d,e}	7.00 ±0.30^{a,b,c,d,e}	6.71 ±0.39^{a,b}	7.82 ±0.03^{f}	7.49 ±0.53^{c,d,e,f}	6.40 ±0.03^{a}	6.70 ±0.02^{a,b}	6.85 ±0.05^{a,b,c,d}	7.57 ±0.40^{e,f}
Glx	15.2 ±0.34^{d,e}	13.0 ±0.72^{a,b}	15.2 ±0.52^{d,e}	13.6 ±0.90^{a,b,c}	14.2 ±0.24^{b,c,d,e}	13.3 ±0.35^{a,b}	12.9 ±0.40^{a,b}	12.9 ±0.95^{a,b}	14.3 ±0.05^{b,c,d,e}	15.4 ±0.95^{e}	12.7 ±0.03^{a}	12.7 ±0.48^{a}	13.8 ±0.20^{a,b,c,d}	14.9 ±0.89^{c,d,e}
Gly	2.96 ±0.04^{b,c,d}	2.52 ±0.13^{a}	2.98 ±0.16^{c,d}	2.74 ±0.33^{a,b,c,d}	2.74 ±0.08^{a,b,c,d}	2.77 ±0.06^{a,b,c,d}	2.59 ±0.10^{a,b}	2.53 ±0.25^{a}	3.00 ±0.03^{d}	2.91 ±0.27^{a,b,c,d}	2.53 ±0.08^{a}	2.66 ±0.00^{a,b,c,d}	2.61 ±0.05^{a,b,c}	2.91 ±0.19^{a,b,c,d}
His	1.61 ±0.06^{d,e}	1.36 ±0.02^{a,b}	1.66 ±0.03^{e}	1.46 ±0.15^{b,c,d}	1.53 ±0.00^{c,d,e}	1.42 ±0.07^{a,b,c}	1.47 ±0.07^{b,c,d}	1.46 ±0.05^{b,c}	1.67 ±0.00^{e}	1.53 ±0.10^{c,d,e}	1.30 ±0.00^{a}	1.44 ±0.03^{b,c}	1.39 ±0.03^{a,b,c}	1.54 ±0.07^{c,d,e}
Leu	2.95 ±0.12^{c,d}	2.65 ±0.27^{a,b,c,d}	2.98 ±0.15^{d}	2.60 ±0.12^{a,b,c}	2.70 ±0.06^{a,b,c,d}	2.57 ±0.15^{a,b}	2.65 ±0.12^{a,b,c,d}	2.62 ±0.24^{a,b,c}	2.90 ±0.05^{b,c,d}	2.85 ±0.17^{b,c,d}	2.42 ±0.04^{a}	2.56 ±0.01^{a,b}	2.62 ±0.13^{a,b,c,d}	2.87 ±0.20^{b,c,d}
Ile	2.08 ±0.11^{b,c,d}	1.87 ±0.22^{a,b,c}	2.14 ±0.10^{d}	1.85 ±0.06^{a,b,c}	1.96 ±0.02^{a,b,c,d}	1.87 ±0.08^{a,b,c}	1.84 ±0.10^{a,b,c}	1.88 ±0.08^{a,b,c}	1.91 ±0.09^{a,b,c,d}	2.09 ±0.14^{c,d}	1.73 ±0.00^{a}	1.79 ±0.02^{a}	1.81 ±0.08^{a,b}	2.07 ±0.19^{b,c,d}
Lys	2.74 ±0.05^{f,g}	2.30 ±0.01^{a,b,c,d}	2.71 ±0.02^{e,f,g}	2.32 ±0.25^{a,b,c,d}	2.59 ±0.02^{d,e,f,g}	2.29 ±0.13^{a,b,c}	2.34 ±0.15^{a,b,c,d}	2.45 ±0.20^{b,c,d,e}	2.78 ±0.02^{g}	2.57 ±0.19^{c,d,e,f,g}	2.13 ±0.06^{a}	2.43 ±0.11^{b,c,d,e}	2.23 ±0.04^{a,b}	2.47 ±0.07^{b,c,d,e,f}
Met	0.24 ±0.06^{a,b}	0.26 ±0.10^{a,b}	0.26 ±0.00^{a,b}	0.25 ±0.08^{a,b}	0.23 ±0.02^{a,b}	0.22 ±0.04^{a,b}	0.16 ±0.00^{a}	0.17 ±0.06^{a}	0.15 ±0.06^{a}	0.24 ±0.00^{a,b}	0.18 ±0.01^{a}	0.14 ±0.02^{a}	0.34 ±0.08^{b}	0.22 ±0.02^{a,b}
Phe	3.03 ±0.03^{b,c,d}	2.58 ±0.08^{a}	3.22 ±0.09^{d}	2.79 ±0.26^{a,b}	2.98 ±0.04^{b,c,d}	2.80 ±0.08^{a,b,c}	2.83 ±0.14^{a,b,c}	2.75 ±0.25^{a,b}	3.30 ±0.03^{d}	3.17 ±0.26^{d}	2.55 ±0.08^{a}	2.83 ±0.11^{a,b,c}	2.73 ±0.06^{a,b}	3.13 ±0.13^{c,d}
Pro	2.41 ±0.02^{c,d}	2.13 ±0.11^{a,b}	2.45 ±0.11^{d}	2.16 ±0.22^{a,b,c}	2.31 ±0.00^{b,c,d}	2.12 ±0.10^{a,b}	2.13 ±0.13^{a,b}	2.11 ±0.15^{a,b}	2.43 ±0.00^{d}	2.36 ±0.14^{b,c,d}	1.95 ±0.04^{a}	2.17 ±0.02^{a,b,c}	2.12 ±0.02^{a,b}	2.35 ±0.09^{b,c,d}
Ser	3.66 ±0.05^{b,c,d,e}	3.19 ±0.16^{a,b}	3.85 ±0.15^{d,e}	3.28 ±0.35^{a,b,c}	3.53 ±0.01^{a,b,c,d,e}	3.54 ±0.37^{a,b,c,d,e}	3.31 ±0.09^{a,b,c}	3.45 ±0.30^{a,b,c,d}	3.96 ±0.13^{e}	3.71 ±0.31^{c,d,e}	3.12 ±0.03^{a}	3.40 ±0.07^{a,b,c,d}	3.35 ±0.10^{a,b,c}	3.72 ±0.11^{c,d,e}
Thr	2.19 ±0.09^{c,d}	1.90 ±0.09^{a,b}	2.26 ±0.09^{d}	1.94 ±0.19^{a,b}	2.07 ±0.07^{a,b,c,d}	1.95 ±0.09^{a,b,c}	1.93 ±0.09^{a,b}	1.96 ±0.14^{a,b,c}	2.28 ±0.04^{d}	2.18 ±0.13^{c,d}	1.84 ±0.04^{a}	1.98 ±0.04^{a,b,c}	1.90 ±0.09^{a,b}	2.13 ±0.11^{b,c,d}

Tyr	1.55 ±0.11^{a,b}	1.33 ±0.01^{a,b}	1.59 ±0.03^{a,b}	1.41 ±0.18^{a,b}	1.43 ±0.01^{a,b}	1.36 ±0.10^{a,b}	1.28 ±0.03^{a}	1.55 ±0.35^{a,b}	1.73 ±0.37^{b}	1.57 ±0.15^{a,b}	1.26 ±0.02^{a}	1.40 ±0.08^{a,b}	1.68 ±0.22^{a,b}	1.62 ±0.07^{a,b}
Val	3.04 ±0.13^{e,f}	2.54 ±0.10^{a,b,c}	3.15 ±0.16^{f}	2.71 ±0.18^{a,b,c,d}	2.82 ±0.05^{c,d,e}	2.66 ±0.09^{a,b,c,d}	2.67 ±0.09^{a,b,c,d}	2.46 ±0.09^{a}	2.79 ±0.18^{b,c,d,e}	2.69 ±0.16^{a,b,c,d}	2.47 ±0.10^{a,b}	2.62 ±0.09^{a,b,c,d}	2.39 ±0.25^{a}	2.88 ±0.11^{d,e,f}

Superscript letters in each raw indicate statistically significant difference ($p<0.05$) according to Duncan's test.
Data are expressed as mean±standard deviation.
Three independent samples were analyzed from each hazelnut varieties and all analytical measurements were performed duplicate.

Table 2.3. Amino acid composition of Turkish hazelnut varieties harvested in 2014 (g/100 g hazelnut protein)

	Kargalak	Palaz	İncekara	Sivri	Yassı Badem	Foşa	Kalınkara	Yuvarlak Badem	Kuş	Çakıldak	Kan	Uzun Musa	Acı	Tombu
Ala	2.00 ±0.52^{a*}	3.04 ±0.04^{b*}	3.03 ±0.33^{b}	2.95 ±0.36^{b}	3.09 ±0.07^{b*}	2.86 ±0.08^{b*}	2.76 ±0.03^{b*}	2.49 ±0.22^{a}	2.96 ±0.57^{b}	3.16 ±0.07^{b*}	2.61 ±0.32^{a}	2.86 ±0.41^{b}	3.02 ±0.23^{b*}	2.64 ±0.21^{a,}
Arg	6.72 ±0.77^{a*}	9.25 ±0.18^{b,c*}	8.97 ±1.45^{b,c}	8.33 ±0.44^{a,b,c}	9.00 ±0.09^{b,c*}	8.40 ±0.84^{a,b,c}	8.14 ±0.31^{a,b,c}	7.11 ±1.14^{a,b}	8.31 ±1.83^{a,b,c}	9.70 ±0.58^{c}	7.48 ±0.16^{a,b*}	8.18 ±1.51^{a,b,c}	8.96 ±0.27^{b,c*}	7.78 ±0.35^{a,b,}
Asx	4.61 ±0.29^{a*}	7.32 ±0.12^{b*}	6.99 ±1.04^{a,b}	6.73 ±0.75^{a,b}	7.13 ±0.04^{a,b}	6.68 ±0.25^{a,b}	6.97 ±0.27^{a,b}	6.41 ±0.38^{a,b}	7.75 ±1.34^{b}	8.05 ±0.11^{b}	6.53 ±1.00^{a,b}	7.34 ±1.21^{b*}	7.73 ±0.88^{b}	6.41 ±0.70^{a,b}
Glx	10.1 ±4.00^{a*}	15.4 ±1.01^{b*}	15.6 ±2.23^{b}	14.5 ±0.98^{b}	16.5 ±0.41^{b*}	14.5 ±0.56^{b*}	15.5 ±0.57^{b*}	12.9 ±0.99^{a,b}	15.7 ±2.82^{b}	16.3 ±0.15^{b}	13.2 ±1.42^{a,b}	15.1 ±1.96^{b}	15.3 ±1.76^{b}	13.7 ±1.24^{a,}
Gly	2.02 ±0.57^{a*}	3.12 ±0.22^{b*}	3.15 ±0.48^{b}	2.85 ±0.18^{b}	3.00 ±0.09^{b*}	3.05 ±0.55^{b}	2.64 ±0.04^{a,b}	2.36 ±0.26^{a,b}	2.81 ±0.59^{a,b}	3.01 ±0.00^{b*}	2.45 ±0.16^{a,b}	2.74 ±0.31^{a,b}	2.90 ±0.14^{b*}	2.80 ±0.33^{a,}
His	1.03 ±0.32^{a*}	1.48 ±0.02^{b*}	1.44 ±0.21^{b}	1.42 ±0.07^{b}	1.61 ±0.04^{b*}	1.46 ±0.00^{b}	1.50 ±0.02^{b}	1.31 ±0.14^{a,b}	1.57 ±0.36^{b}	1.67 ±0.05^{b}	1.28 ±0.07^{a,b}	1.48 ±0.21^{b}	1.50 ±0.06^{b*}	1.29 ±0.09^{a,b}
Leu	2.27 ±0.47^{a*}	3.25 ±0.13^{b,c*}	3.10 ±0.38^{b,c}	3.14 ±0.45^{b,c*}	3.42 ±0.02^{b,c*}	2.76 ±0.41^{a,b}	3.17 ±0.02^{b,c*}	2.75 ±0.31^{a,b}	3.20 ±0.52^{b,c}	3.58 ±0.12^{c*}	2.79 ±0.25^{a,b*}	3.01 ±0.29^{a,b,c*}	3.17 ±0.12^{b,c*}	2.67 ±0.26^{a,}
Ile	1.66 ±0.36^{a*}	2.45 ±0.11^{b,c*}	2.32 ±0.30^{b,c}	2.20 ±0.32^{b,c}	2.40 ±0.06^{b,c*}	1.97 ±0.23^{a,b}	2.20 ±0.03^{b,c*}	1.91 ±0.24^{a,b}	2.28 ±0.40^{b,c}	2.61 ±0.06^{c*}	2.00 ±0.15^{a,b*}	2.10 ±0.08^{a,b,c*}	2.32 ±0.10^{b,c*}	1.96 ±0.16^{a,}
Lys	2.16 ±0.18^{a,b*}	2.68 ±0.01^{b,c*}	2.57 ±0.41^{a,b,c}	2.52 ±0.26^{a,b,c}	2.58 ±0.02^{a,b,c}	2.42 ±0.05^{a,b,c}	2.36 ±0.01^{a,b,c}	2.05 ±0.25^{a*}	2.51 ±0.57^{a,b,c}	2.85 ±0.16^{c*}	2.16 ±0.06^{a,b}	2.34 ±0.21^{a,b,c}	2.50 ±0.01^{a,b,c*}	2.22 ±0.23^{a,}
Met	0.18 ±0.06^{a}	0.27 ±0.01^{a,b}	0.28 ±0.01^{a,b*}	0.45 ±0.07^{b,c,d*}	0.40 ±0.05^{b,c,d*}	0.49 ±0.11^{c,d*}	0.34 ±0.06^{a,b,c*}	0.38 ±0.01^{a,b,c,d*}	0.41 ±0.04^{b,c,d*}	0.53 ±0.01^{c,d*}	0.39 ±0.05^{b,c,d*}	0.53 ±0.22^{c,d*}	0.56 ±0.13^{d*}	0.40 ±0.03^{b,c,}
Phe	2.03 ±0.81^{a*}	3.22 ±0.03^{b,c*}	3.10 ±0.44^{b,c}	3.20 ±0.45^{b,c}	3.39 ±0.01^{b,c*}	2.95 ±0.07^{a,b,c*}	3.06 ±0.17^{b,c*}	2.42 ±0.76^{a,b}	3.19 ±0.65^{b,c}	3.51 ±0.06^{c*}	2.72 ±0.07^{a,b,c*}	2.76 ±0.19^{a,b,c}	3.32 ±0.25^{b,c*}	2.83 ±0.30^{a,b}
Pro	1.67 ±0.48^{a*}	2.52 ±0.11^{b,c*}	2.34 ±0.37^{a,b,c}	2.53 ±0.35^{b,c}	2.76 ±0.09^{b,c*}	2.31 ±0.27^{a,b,c}	2.60 ±0.10^{b,c*}	2.37 ±0.17^{a,b,c*}	2.78 ±0.51^{b,c}	2.91 ±0.17^{c*}	2.37 ±0.29^{a,b,c*}	2.71 ±0.36^{b,c*}	2.66 ±0.48^{b,c}	2.11 ±0.22^{a,}
Ser	2.40 ±0.10^{a*}	3.69 ±0.12^{b*}	3.70 ±0.46^{b}	3.55 ±0.24^{b}	3.68 ±0.03^{b*}	3.32 ±0.06^{a,b}	3.38 ±0.11^{a,b}	3.04 ±0.20^{a,b*}	3.84 ±0.85^{b}	3.92 ±0.04^{b}	3.04 ±0.32^{a,b}	3.56 ±0.70^{b}	3.58 ±0.30^{b}	3.19 ±0.30^{a,b}
Thr	1.38 ±0.60^{a*}	2.03 ±0.04^{a,b*}	1.96 ±0.25^{a,b*}	2.13 ±0.22^{b}	2.39 ±0.02^{b*}	2.01 ±0.05^{a,b}	2.18 ±0.07^{b*}	1.88 ±0.24^{a,b}	2.39 ±0.53^{b}	2.42 ±0.06^{b*}	1.95 ±0.22^{a,b}	2.21 ±0.21^{b}	2.23 ±0.39^{b*}	1.78 ±0.24^{a,b}

Tyr	1.02 ±0.44^{a*}	1.68 ±0.00^{b*}	1.66 ±0.18^{b}	1.79 ±0.04^{b*}	1.78 ±0.02^{b*}	1.66 ±0.14^{b*}	1.62 ±0.01^{b*}	1.41 ±0.16^{a,b}	1.77 ±0.35^{b}	1.93 ±0.01^{b*}	1.56 ±0.17^{b*}	1.64 ±0.53^{b}	1.83 ±0.06^{b}	1.64 ±0.07^{b}
Val	1.87 ±0.53^{a,b*}	2.33 ±0.04^{a,b*}	2.38 ±0.10^{a,b*}	2.26 ±0.05^{a,b*}	2.82 ±0.01^{b}	1.98 ±0.26^{a,b*}	2.19 ±0.47^{a,b}	1.59 ±0.74^{a*}	2.39 ±0.85^{a,b}	2.63 ±0.09^{a,b}	1.76 ±0.32^{a*}	1.93 ±0.69^{a,b}	2.02 ±0.15^{a,b*}	2.19 ±0.31^{a,b}

Superscript letters in each raw column indicate statistically significant difference ($p<0.05$) according to Duncan's test. (*) indicates statistically significant difference ($p<0.05$) according to t-test. Data are expressed as mean±standard deviation. Three independent samples were analyzed from each hazelnut varieties and all analytical measurements were performed duplicate.

2.3.3 Profiles of Sugars and Organic Acids of Hazelnut Varieties

Hazelnuts were found to contain a range of saccharides including fructose, glucose, sucrose, raffinose, stachyose and a polyol myo-inositol. Sugar contents of Turkish hazelnut varieties harvested in 2013 and 2014 are given in Table 2.4, respectively. Independent of the harvest year, sugar amounts in different hazelnut varieties were in the following order; sucrose > fructose > glucose > stachyose > raffinose > myo-inositol with minor exceptions. A typical chromatogram indicating the sugars in hazelnuts is given in Figure 2.1.

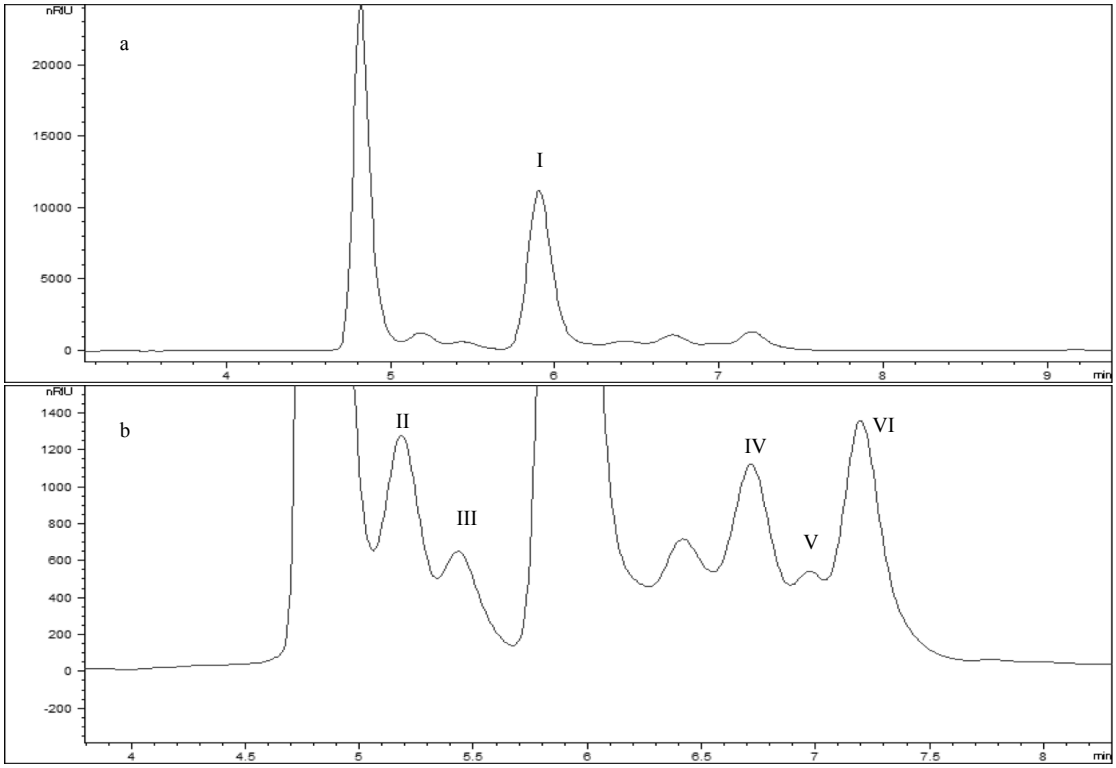


Figure 2.1. A chromatogram showing sugar profile of Turkish hazelnuts, a) sucrose (I), b) stachyose (II), raffinose (III), glucose (IV), myo-inositol (V), fructose (VI)

Table 2.4. Sugar and organic acid contents of Turkish hazelnut varieties harvested in 2013 and 2014 (g/100g hazelnut)

	Stachyose		Raffinose		Sucrose		Glucose		Fructose		Myo-inositol		Phytic acid		Malic acid	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Kargalak	0.28 ±0.07^{c,d}	0.25 ±0.01^{c,d}	0.17 ±0.05^{c,d,e}	0.20 ±0.01^{c,d}	3.00 ±0.75^{a,b,c}	1.99 ±0.02^{a,b,c*}	0.73 ±0.24^{d}	0.55 ±0.02^{d}	1.04 ±0.31^{c,d}	0.60 ±0.00^{c,d*}	0.11 ±0.02^{c}	0.09 ±0.00^{c}	1.03 ±0.18^{b,c,d}	0.71 ±0.12^{d*}	0.17 ±0.04^{a,b}	0.21 ±0.02^{a,b}
Palaz	0.25 ±0.03^{b,c,d}	0.21 ±0.01^{b,c,d*}	0.12 ±0.00^{a,b,c,d}	0.14 ±0.01^{a,b,c,d*}	3.28 ±0.29^{a,b,c}	2.16 ±0.07^{a,b,c*}	0.23 ±0.02^{a,b}	0.28 ±0.02^{a,b*}	0.65 ±0.04^{a,b,c}	0.36 ±0.04^{a,b,c*}	0.08 ±0.00^{b}	0.08 ±0.00^{b}	0.90 ±0.14^{a,b,c}	0.50 ±0.01^{a,b,c*}	0.26 ±0.02^{b,c}	0.36 ±0.00^{a,b,c*}
İncekara	0.19 ±0.02^{a,b,c}	0.31 ±0.00^{a,b,c*}	0.14 ±0.01^{b,c,d}	0.20 ±0.00^{b,c,d*}	2.35 ±0.07^{a,b}	2.60 ±0.04^{a,b*}	1.08 ±0.12^{f}	0.37 ±0.06^{f*}	1.22 ±0.08^{d}	0.41 ±0.06^{d*}	0.13 ±0.00^{c,d}	0.08 ±0.00^{c,d*}	0.83 ±0.01^{a,b}	0.45 ±0.01^{a,b,c*}	0.34 ±0.02^{c,d,e}	0.52 ±0.02^{c,d*}
Sivri	0.22 ±0.03^{a,b,c}	0.28 ±0.01^{a,b,c*}	0.11 ±0.00^{a,b,c}	0.22 ±0.01^{a,b,c*}	3.30 ±0.20^{a,b,c}	1.53 ±1.80^{a,b,c}	0.25 ±0.00^{a,b}	0.51 ±0.02^{a,b*}	0.46 ±0.03^{a,b}	0.58 ±0.02^{a,b*}	0.12 ±0.00^{c}	0.09 ±0.00^{c*}	1.13 ±0.09^{b,c,d,e}	0.55 ±0.08^{b,c*}	0.44 ±0.03^{e}	0.84 ±0.05^{f*}
Yassı Badem	0.29 ±0.05^{c,d}	0.43 ±0.02^{c,d*}	0.14 ±0.01^{b,c,d}	0.23 ±0.00^{b,c,d*}	3.49 ±0.64^{a,b,c}	2.95 ±0.03^{a,b,c}	0.38 ±0.17^{b,c}	0.16 ±0.00^{b,c*}	0.39 ±0.06^{a,b}	0.16 ±0.01^{a,b*}	0.08 ±0.01^{a,b}	0.08 ±0.00^{b}	1.39 ±0.35^{e,f}	0.51 ±0.04^{a,b,c*}	0.14 ±0.03^{a}	0.18 ±0.01^{a*}
Foşa	0.16 ±0.01^{a,b}	0.34 ±0.00^{a,b*}	0.09 ±0.01^{a,b}	0.20 ±0.01^{a,b*}	2.22 ±0.05^{a,b}	3.01 ±0.05^{a,b*}	0.41 ±0.01^{b,c}	0.36 ±0.03^{b,c*}	0.62 ±0.02^{a,b}	0.40 ±0.03^{a,b*}	0.03 ±0.00^{a}	0.06 ±0.02^{a*}	0.64 ±0.01^{a}	0.45 ±0.01^{a,b,c*}	0.30 ±0.00^{c,d}	0.44 ±0.01^{c*}
Kalınkara	0.41 ±0.07^{e}	0.31 ±0.07^{e*}	0.21 ±0.02^{e}	0.23 ±0.06^{e}	6.45 ±1.21^{d}	2.89 ±0.59^{d*}	0.49 ±0.02^{c}	0.47 ±0.11^{c}	0.55 ±0.07^{a,b}	0.57 ±0.12^{a,b}	0.15 ±0.01^{d}	0.11 ±0.01^{d*}	0.90 ±0.17^{a,b,c}	0.47 ±0.03^{a,b,c*}	0.90 ±0.15^{f}	0.86 ±0.28^{f}
Yuvarlak Badem	0.30 ±0.03^{c,d}	0.25 ±0.01^{c,d*}	0.14 ±0.01^{b,c,d}	0.19 ±0.01^{b,c,d*}	3.68 ±0.22^{a,b,c}	3.22 ±0.26^{b,c*}	0.77 ±0.00^{d,e}	0.16 ±0.02^{d,e*}	1.05 ±0.05^{c,d}	0.25 ±0.02^{c,d*}	0.14 ±0.00^{d}	0.09 ±0.01^{d*}	1.63 ±0.05^{f}	0.36 ±0.01^{a*}	0.32 ±0.02^{c,d,e}	0.33 ±0.02^{a,b,c}
Kuş	0.41 ±0.04^{e}	0.28 ±0.04^{e*}	0.18 ±0.06^{d,e}	0.18 ±0.01^{d,e}	4.67 ±0.33^{c}	2.94 ±0.30^{c*}	0.30 ±0.06^{a,b,c}	0.17 ±0.02^{a,b,c*}	0.41 ±0.07^{a,b}	0.16 ±0.02^{a,b*}	0.13 ±0.02^{c,d}	0.05 ±0.00^{c,d*}	0.83 ±0.01^{a,b}	0.43 ±0.04^{a,b,c*}	0.40 ±0.02^{d,e}	0.30 ±0.07^{a,b,c*}
Çakıldak	0.35 ±0.01^{d,e}	0.34 ±0.00^{d,e}	0.13 ±0.00^{b,c,d}	0.21 ±0.01^{b,c,d*}	3.58 ±0.05^{b,c}	1.13 ±0.02^{b,c*}	0.39 ±0.01^{b,c}	1.01 ±0.05^{b,c*}	0.77 ±0.05^{b,c}	1.08 ±0.07^{b,c*}	0.04 ±0.01^{a}	0.06 ±0.01^{a*}	1.30 ±0.05^{d,e,f}	0.48 ±0.03^{a,b,c*}	0.36 ±0.00^{c,d,e}	0.51 ±0.09^{c,d*}
Kan	0.13 ±0.10^{a}	0.21 ±0.01^{a}	0.07 ±0.04^{a}	0.15 ±0.01^{a*}	1.81 ±1.80^{a}	2.18 ±0.14^{a}	0.77 ±0.00^{d,e}	0.20 ±0.01^{d,e*}	0.40 ±0.05^{a,b}	0.21 ±0.01^{a,b*}	0.03 ±0.01^{a}	0.07 ±0.00^{a*}	0.88 ±0.17^{a,b,c}	0.42 ±0.03^{a,b,c*}	0.33 ±0.00^{c,d,e}	0.32 ±0.01^{a,b,c}
Uzun Musa	0.14 ±0.02^{a}	0.28 ±0.00^{a*}	0.10 ±0.02^{a,b}	0.21 ±0.01^{a,b*}	2.66 ±0.17^{a,b}	2.56 ±0.10^{a,b}	0.96 ±0.00^{e,f}	0.59 ±0.00^{e,f*}	0.64 ±0.03^{a,b,c}	0.67 ±0.00^{a,b,c}	0.04 ±0.00^{a}	0.07 ±0.00^{a*}	0.88 ±0.13^{a,b}	0.39 ±0.00^{a,b*}	0.25 ±0.05^{b,c}	0.70 ±0.02^{d,e*}
Acı	0.23 ±0.03^{a,b,c}	0.20 ±0.01^{a,b,c}	0.12 ±0.02^{a,b,c}	0.18 ±0.02^{a,b,c*}	3.48 ±0.47^{a,b,c}	2.34 ±0.14^{a,b,c*}	0.15 ±0.07^{a}	0.35 ±0.02^{a*}	0.32 ±0.04^{a}	0.38 ±0.00^{a*}	0.07 ±0.01^{b}	0.08 ±0.02^{b}	0.88 ±0.07^{a,b,c}	0.46 ±0.01^{a,b,c*}	0.28 ±0.04^{b,c,d}	0.36 ±0.01^{a,b,c*}
Tombul	0.15 ±0.03^{a,b}	0.35 ±0.06^{a,b*}	0.10 ±0.03^{a,b,c}	0.21 ±0.02^{a,b,c*}	3.65 ±0.89^{b,c}	2.65 ±0.37^{b,c}	0.15 ±0.03^{a}	0.26 ±0.04^{a*}	0.35 ±0.08^{a,b}	0.30 ±0.05^{a,b}	0.04 ±0.01^{a}	0.11 ±0.00^{a*}	1.24 ±0.28^{c,d,e}	0.58 ±0.19^{c,d*}	0.26 ±0.06^{b,c}	0.40 ±0.11^{b,c*}

Superscript letters in each column indicate statistically significant difference ($p<0.05$) according to Duncan's test. (*) indicates statistically significant difference ($p<0.05$) according to t-test.

Sucrose content showed large variations, which was highest in Kalınkara (6.45g/100g) and lowest in Kan (1.81 g/100g) in 2013. Similar patterns were observed for other sugars in these two varieties. For example, stachyose (0.41 g/100g), raffinose (0.21 g/100g) and myo-inositol (0.15 g/100g) were found in the highest amounts in Kalınkara. However, Kan contained the lowest amounts of stachyose (0.13g/100g), raffinose (0.07 g/100g) and myo-inositol (0.03 g/100g). Yuvarlak Badem contained the highest amount of sucrose (3.22 g/100g) while Çakıldak had the lowest amount (1.13 g/100g) in 2014. In 2014, the highest amount of glucose (1.01 g/100g) and fructose (1.08 g/100g) was found in Çakıldak. İncekara, Foşa, and Yuvarlak Badem were varieties that showed significant differences in the amounts of all sugars between harvest years of 2013 and 2014 ($p<0.05$). Cerbulis [190] reported sucrose, glucose, fructose, stachyose, raffinose and myo-inositol as the major sugars in Turkish hazelnuts. Alasalvar et al [19] quantified these sugars in Turkish Tombul hazelnut. They reported sucrose (2.67 g/100g) as the most abundant sugar, followed by stachyose (0.48 g/100g), raffinose and fructose (both 0.14 g/100g), glucose (0.11 g/100g) and myo-inositol (0.04 g/100g). Alasalvar et al. [8] reported total sugar content of eighteen Turkish hazelnut varieties between 1.99 (Kan) and 4.94 g/100g (Uzun Musa). They also found that sucrose is responsible for 80-90% and stachyose constitutes of 5-13% of the all sugars while the other sugars were in low amounts. Cristofori et al [45] found the same sugars except myo-inositol in 24 hazelnut varieties originated mostly from Italy and collected from other countries. According to average values of their three harvest year results sucrose ranged from 3.98 g/100g in Tonda Rossa to 5.95 g/100g in Tonda Gentile Romana and comprised of the 80% of the sugars. Moreover, stachyose was responsible for the 5-10% of the total sugars, followed by raffinose, glucose and fructose [45].

Turkish hazelnuts were found to contain phytic and malic acids in both harvest years (Table 2.4). On contrary to previous studies [8, 19, 45], phytic acid was found as the predominant organic acid in hazelnuts in the present study. The amount of phytic acid ranged between 0.64 g/100g (Foşa) and 1.63 g/100g (Yuvarlak Badem) in harvest year 2013 while it ranged from 0.36 g/100g (Yuvarlak Badem) to 0.71 g/100g (Kargalak) in harvest year 2014. Yassı Badem was found to contain the lowest amount of malic acid (0.14 and 0.18 g/100g, respectively) in both harvest years while Kalınkara had the highest with 0.90 g/100g in 2013 and 0.86 g/100g in 2014.

2.3.4 Water-Soluble Vitamins of Hazelnut Varieties

Hazelnut varieties were found to be high in pantothenic acid and nicotinic acid. They were also found to contain pyridoxal, biotin, thiamine and nicotinamide, but in relatively small quantities (Table 2.5). Their amounts in different hazelnut varieties were in the following order; pantothenic acid > nicotinic acid > pyridoxal > biotin > thiamine > nicotinamide. Pantothenic acid was lowest in Kan (0.83 mg/100g) and highest in Çakıldak (1.60 mg/100g) in harvest year 2013. Nicotinic acid was not detected in Yuvarlak Badem while highest in Kargalak (0.79 mg/100g). Alasalvar et al [19] reported pantothenic acid content of Turkish Tombul as 1.12 mg/100g which is in accordance with the results obtained in this study. The amounts of pantothenic acid and nicotinic acid were found to be significantly higher in most of the varieties in harvest year 2014 ($p<0.05$). Pantothenic acid ranged from 0.81 mg/100g (Kan) to 2.63 mg/100g (Yassı Badem) and nicotinic acid was not detected in Kan while the highest concentration was 1.73 mg/100 g in Kalınkara in harvest year 2014. On contrary to these findings, niacin was reported as the most predominant vitamin in hazelnuts followed by vitamin B_1, B_2, B_6 [2, 4]. Folic acid and ascorbic acid were also found in hazelnuts in small quantities [4, 19].

Table 2.5. Water-soluble vitamin contents of Turkish hazelnut varieties harvested in 2013 and 2014 (µg or mg/100g hazelnut)

	Pantothenic acid (mg/100g)		Nicotinic acid (mg/100g)		Pyridoxal (µg/100g)		Biotin (µg/100g)		Thiamine (µg/100g)		Nicotinamide (µg/100g)	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Kargalak	1.48 ±0.23^{b,c}	2.19 ±0.07^{b*}	0.79 ±0.07^{f}	1.33 ±0.00^{d,e*}	48.0 ±1.00^{b,c,d}	nd	33.0 ±5.00^{a,b}	42.0 ±7.00^{a,b*}	23.0 ±3.00^{a,b}	57.0 ±2.00^{d*}	6.0 ±0.30^{a,b,c}	25.0 ±3.00^{a,b*}
Palaz	1.38 ±0.09^{b,c}	2.18 ±0.01^{b*}	0.72 ±0.03^{e,f}	1.55 ±0.05^{f*}	32.0 ±21.0^{a,b}	20.0 ±0.20^{a,b}	30.0 ±4.00^{a}	66.0 ±25.0^{a,b*}	21.0 ±10.0^{a,b}	46.0 ±2.00^{a,b,c,d*}	3.0 ±0.00^{a,b}	23.0 ±10.0^{a,b*}
İncekara	1.25 ±0.03^{a,b,c}	2.12 ±0.12^{b*}	0.55 ±0.06^{b,c,d}	1.22 ±0.03^{b,c,d*}	57.0 ±34.0^{b,c,d,e}	24.0 ±15.0^{a,b,c}	35.0 ±5.00^{a,b}	72.0 ±36.0^{b}	19.0 ±4.00^{a}	38.0 ±4.00^{a,b,c*}	11.0 ±4.00^{c,d}	25.0 ±0.00^{a,b*}
Sivri	1.19 ±0.02^{a,b,c}	2.32 ±0.14^{b*}	0.65 ±0.06^{d,e}	1.35 ±0.05^{e*}	50.0 ±32.0^{b,c,d}	48.0 ±21.0^{c,d}	35.0 ±12.0^{a,b}	78.0 ±17.0^{b*}	17.0 ±5.00^{a}	54.0 ±1.00^{c,d*}	11.0 ±7.00^{c,d}	36.0 ±2.0^{a,b,c*}
Yassı Badem	1.37 ±0.11^{b,c}	2.63 ±0.10^{b*}	0.46 ±0.03^{b}	1.21 ±0.06^{b,c,d*}	50.0 ±39.0^{b,c,d}	64.0 ±4.00^{d,e}	32.0 ±16.0^{a,b}	66.0 ±22.0^{a,b*}	19.0 ±4.00^{a,b}	30.0 ±1.00^{a,b*}	9.0 ±7.00^{b,c}	23.0 ±5.00^{a,b*}
Foşa	0.85 ±0.01^{a}	2.12 ±0.01^{b*}	0.44 ±0.02^{b}	1.26 ±0.05^{c,d,e*}	67.0 ±2.4^{b,c,d,e}	42.0 ±2.00^{a,b,c*}	26.0 ±0.20^{a}	48.0 ±43.0^{a,b}	30.0 ±2.00^{b,c}	41.0 ±3.00^{a,b,c,d*}	11.0 ±0.50^{c,d}	49.0 ±4.00^{a,b,c*}
Kalınkara	1.47 ±0.18^{b,c}	2.43 ±0.21^{b*}	0.63 ±0.02^{c,d,e}	1.73 ±0.10^{g*}	84.0 ±9.30^{c,d,e,f}	9.0 ±6.50^{a*}	36.0 ±0.10^{a,b}	38.0 ±6.00^{a,b}	35.0 ±1.00^{c}	49.0 ±1.00^{b,c,d*}	12.0 ±1.00^{c,d}	29.0 ±3.00^{a,b*}
Yuvarlak Badem	1.21 ±0.01^{a,b,c}	1.89 ±0.20^{a,b*}	nd	1.10 ±0.02^{b*}	nd	43.0 ±4.00^{b,c,d*}	36.0 ±3.20^{a,b}	54.0 ±19.0^{a,b}	19.0 ±0.00^{a}	27.0 ±0.00^{a*}	nd	54.0 ±19.0^{b,c,d*}
Kuş	1.29 ±0.01^{b,c}	2.32 ±0.07^{b*}	0.71 ±0.03^{e,f}	1.10 ±0.04^{b*}	59.0 ±3.00^{b,c,d,e}	135.0 ±30.0^{f*}	35.0 ±6.00^{a,b}	84.0 ±44.0^{b}	24.0 ±5.00^{a,b}	27.0 ±2.00^{a}	12.0 ±0.50^{c,d}	84.0 ±44.0^{d*}
Çakıldak	1.60 ±0.09^{c}	1.76 ±1.30^{a,b}	0.46 ±0.12^{b}	0.87 ±0.00^{a*}	85.0 ±3.60^{c,d,e,f}	46.0 ±0.00^{b,c,d*}	37.0 ±2.00^{a,b}	46.0 ±0.00^{a,b*}	24.0 ±4.00^{a,b,c}	49.0 ±27.0^{b,c,d}	17.0 ±3.20^{d}	46.0 ±0.00^{a,b,c*}
Kan	0.83 ±0.05^{a}	0.81 ±0.00^{a}	0.55 ±0.01^{b,c,d}	nd	92.0 ±2.30^{d,e,f}	nd	33.0 ±10.0^{a,b}	14.0 ±0.00^{a*}	19.0 ±8.00^{a}	nd	11.0 ±0.20^{c,d}	14.0 ±0.00^{a*}
Uzun Musa	1.11 ±0.13^{a,b}	2.12 ±0.00^{b*}	0.64 ±0.05^{c,d,e}	1.23 ±0.11^{c,d,e*}	102 ±2.40^{e,f}	86.0 ±7.00^{e*}	31.0 ±3.30^{a}	69.0 ±22.0^{a,b*}	47.0 ±4.00^{d}	39.0 ±1.00^{a,b,c,d*}	17.0 ±3.20^{d}	69.0 ±22.0^{c,d*}

Acı	1.22 ±0.12^{a,b,c}	1.59 ±1.00^{a,b}	0.04 ±0.00^{a}	1.30 ±0.00^{c,d,e*}	42 ±3.00^{a,b,c}	237.0 ±0.30^{g*}	49.0 ±3.30^{b}	41.0 ±0.00^{a,b*}	79.0 ±0.00^{e}	36.0 ±5.00^{a,b,c*}	28.0 ±2.00^{e}	26.1 ±0.00^{a,b}
Tombul	1.14 ±0.09^{a,b}	1.59 ±1.10^{a,b}	0.51 ±0.11^{b,c}	1.19 ±0.00^{b,c*}	121 ±33.0^{f}	129.0 ±0.00^{f}	38.0 ±6.00^{a,b}	29.0 ±0.00^{a,b*}	26.0 ±5.00^{a,b,c}	34.0 ±9.00^{a,b}	17.0 ±1.10^{d}	39.7 ±0.00^{a,b,c*}

Superscript letters in each column indicate statistically significant difference ($p<0.05$) according to Duncan's test. (*) indicates statistically significant difference ($p<0.05$) according to t-test. Data are expressed as mean±standard deviation. Three independent samples were analyzed from each hazelnut varieties and all analytical measurements were performed duplicate. nd: not determined.

2.3.5 Mineral Profile of Hazelnut Varieties

Mineral contents of Turkish hazelnut varieties are given in Table 2.6. Among the minerals, potassium was the most predominant, followed by magnesium, calcium, sodium, manganese, zinc, iron and copper. The amount of potassium ranged from 0.88 g/100g (Çakıldak) to 1.21 g/100g (Palaz) in 2013, while it was between 0.86 g/100g (Sivri) and 1.65 g/100g (Kargalak) in 2014. Açkurt et al [2] also reported potassium as the most abundant mineral in Turkish hazelnuts with a mean concentration of 0.64 g/100g. They also reported magnesium as the second highest mineral followed by calcium, and copper as the least mineral in parallel with the findings of present study. On contrary, potassium was found to be the second abundant mineral followed by phosphorous, calcium and magnesium by other researchers [3, 5].

There were significant changes in the amounts of mineral elements of Sivri, Kargalak, Palaz, Kalınkara, Çakıldak, and Tombul between the harvest years ($p<0.05$). These changes have been attributed to the harvest year, climate, composition of soil and watering [2-4].

Table 2.6. Mineral contents of Turkish hazelnut varieties harvested in 2013 and 2014 (mg or g/100g hazelnut)

	K (g/100g)		Mg (mg/100g)		Ca (mg/100g)		Na (mg/100g)		Mn (mg/100g)		Zn (mg/100g)		Fe (mg/100g)		Cu (mg/100g)	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Kargalak	1.15 ±0.00^{e,f,g}	1.65 ±0.13^{b*}	193 ±1.00^{a,b}	161 ±10.0^{b,c*}	73.0 ±6.00^{c,d}	92.0 ±10.0^{c*}	29.0 ±2.00^{a,b}	13.0 ±0.00^{b,c,d*}	2.12 ±0.07^{a}	3.75 ±0.37^{a,b*}	4.91 ±3.3^{b}	1.81 ±0.11^{c,d}	4.05 ±2.3^{b}	1.36 ±0.15^{b,c,d*}	2.01 ±0.00^{d}	1.23 ±0.08^{c,d*}
Palaz	1.21 ±0.01^{g}	1.31 ±0.04^{a,b*}	219 ±12.0^{b,c,d}	162 ±0.00^{b,c*}	77.0 ±8.00^{d}	95.0 ±10.00^{c*}	29.0 ±7.00^{a,b}	18.0 ±5.00^{d*}	3.87 ±0.07^{f}	8.03 ±0.39^{c*}	3.23 ±0.07^{a}	1.88 ±0.06^{d*}	2.29 ±0.9^{a}	1.56 ±0.09^{d}	2.08 ±0.00^{d}	1.62 ±0.02^{d*}
İncekara	1.11 ±0.05^{e,f,g}	1.26 ±0.24^{a,b}	214 ±5.00^{b,c,d}	165 ±21.0^{c*}	69.0 ±4.00^{b,c,d}	77.0 ±11.0^{b,c}	25.0 ±1.00^{a,b}	16.0 ±8.00^{c,d}	3.50 ±0.03^{d,e}	3.89 ±0.64^{a,b}	3.60 ±0.00^{a,b}	1.69 ±0.37^{b,c,d*}	3.08 ±0.09^{a,b}	0.99 ±0.21^{a,b,c,d*}	2.21 ±0.08^{d}	0.89 ±0.24^{a,b,c*}
Sivri	0.94 ±0.05^{a,b,c}	0.86 ±0.03^{a*}	219 ±12.0^{b,c,d}	108 ±0.00^{a,b,c*}	59.0 ±2.00^{b}	51.0 ±0.00^{a,b*}	32.0 ±1.00^{a,b}	9.00 ±1.00^{a,b,c*}	3.51 ±0.13^{d,e}	3.00 ±0.17^{a*}	3.69 ±0.46^{a,b}	0.94 ±0.08^{a*}	3.34 ±0.04^{a,b}	0.61 ±0.16^{a*}	2.18 ±0.03^{d}	0.60 ±0.06^{a,b*}
Yassı Badem	1.09 ±0.05^{d,e,f,g}	1.28 ±0.21^{a,b}	234 ±6.00^{d}	161 ±41.0^{b,c*}	77.0 ±10.0^{d}	91.0 ±22.0^{c}	29.0 ±6.00^{a,b}	14.0 ±4.00^{b,c,d*}	5.44 ±0.14^{h}	4.53 ±1.5^{a,b}	3.33 ±0.08^{a,b}	1.35 ±0.40^{a,b,c,d*}	3.35 ±0.06^{a,b}	0.91 ±0.05^{a,b,c,d*}	2.71 ±0.02^{f}	0.95 ±0.25^{b,c*}
Foşa	1.19 ±0.03^{f,g}	1.33 ±0.48^{a,b}	227 ±4.00^{c,d}	139 ±49.0^{a,b,c*}	56.0 ±4.00^{a,b}	65.0 ±20.0^{a,b,c}	26.0 ±3.00^{a,b}	12.0 ±0.00^{a,b,c,d*}	3.55 ±0.02^{e}	3.12 ±1.2^{a}	3.63 ±0.01^{a,b}	1.41 ±0.39^{a,b,c,d*}	3.23 ±0.05^{a,b}	1.42 ±0.09^{b,c,d*}	2.45 ±0.13^{e}	0.82 ±0.5^{a,b,c*}
Kalınkara	1.11 ±0.01^{e,f,g}	1.13 ±0.05^{a}	199 ±8.00^{a,b,c}	133 ±7.00^{a,b,c*}	61.0 ±9.00^{b,c}	73.0 ±0.00^{a,b,c*}	26.0 ±3.00^{a,b}	7.00 ±2.00^{a,b*}	2.71 ±0.19^{c}	5.41 ±0.46^{b*}	2.40 ±0.06^{a}	1.30 ±0.14^{a,b,c,d*}	2.39 ±0.04^{a,b}	0.84 ±0.20^{a,b,c*}	1.61 ±0.07^{b,c}	0.56 ±0.12^{a,b*}
Yuvarlak Badem	1.07 ±0.08^{c,d,e,f}	1.13 ±0.12^{a}	180 ±6.00^{a}	100 ±10.0^{a,b*}	46.0 ±2.00^{a}	45.0 ±7.00^{a}	23.0 ±3.00^{a}	8.00 ±1.00^{a,b*}	3.27 ±0.26^{d,e}	2.97 ±0.41^{a}	2.93 ±0.16^{a,b}	1.02 ±0.02^{a,b*}	2.82 ±0.40^{a,b}	0.72 ±0.08^{a,b*}	1.60 ±0.13^{b,c}	0.43 ±0.12^{a,b*}
Kuş	1.02 ±0.07^{b,c,d,e}	0.94 ±0.37^{a}	205 ±12.0^{a,b,c}	94.0 ±44.0^{a*}	57.0 ±2.00^{a,b}	68.0 ±32.0^{a,b,c}	28.0 ±1.00^{a,b}	7.00 ±2.00^{a,b*}	2.56 ±0.09^{b,c}	3.62 ±1.8^{a,b}	3.07 ±0.9^{a,b}	1.28 ±0.58^{a,b,c,d*}	2.92 ±0.40^{a,b}	1.42 ±0.06^{c,d}	1.60 ±0.04^{b,c}	0.47 ±0.4^{a,b*}
Çakıldak	0.88 ±0.02^{a}	1.21 ±0.15^{a,b*}	195 ±0.00^{a,b}	140 ±30.0^{a,b,c*}	58.0 ±4.00^{a,b}	75.0 ±1.00^{b,c*}	25.0 ±4.00^{a,b}	8.00 ±1.00^{a,b*}	4.84 ±0.17^{g}	4.01 ±1.6^{a,b}	3.66 ±0.02^{a,b}	1.55 ±0.43^{a,b,c,d*}	4.00 ±0.27^{b}	1.50 ±0.04^{c,d*}	1.75 ±0.13^{c}	0.54 ±0.4^{a,b*}
Kan	0.97 ±0.08^{a,b,c,d}	0.89 ±0.01^{a}	203 ±2.00^{a,b,c}	106 ±15.0^{a,b,c*}	59.0 ±4.00^{b,c}	59.0 ±0.00^{a,b}	23.0 ±1.00^{a}	10.0 ±3.00^{a,b,c*}	2.41 ±0.11^{b}	2.75 ±0.47^{a}	2.99 ±0.09^{a,b}	1.47 ±0.02^{a,b,c,d*}	2.79 ±0.12^{a,b}	0.97 ±0.26^{a,b,c,d*}	1.69 ±0.07^{b,c}	0.27 ±0.02^{a*}
Uzun Musa	0.93 ±0.14^{a,b}	1.18 ±0.17^{a,b*}	182 ±34.0^{a}	154 ±23.0^{b,c}	64.0 ±6.00^{b,c}	71.0 ±6.00^{a,b,c}	30.0 ±0.00^{a,b}	11.0 ±5.00^{a,b,c,d*}	4.10 ±0.13^{e}	4.41 ±1.1^{a,b}	3.09 ±0.00^{a,b}	1.77 ±0.08^{c,d*}	3.27 ±0.05^{a,b}	1.50 ±0.19^{c,d*}	1.68 ±0.19^{b,c}	0.60 ±0.12^{a,b*}
Acı	0.90 ±0.02^{a,b}	0.99 ±0.13^{a}	179 ±5.00^{a}	109 ±15.0^{a,b,c*}	56.0 ±1.00^{a,b}	49.0 ±7.00^{a}	26.0 ±6.00^{a,b}	6.00 ±2.00^{a*}	3.45 ±0.04^{d,e}	2.63 ±0.27^{a*}	3.42 ±1.10^{a,b}	1.31 ±0.30^{a,b,c,d*}	3.30 ±0.06^{a,b}	1.35 ±0.20^{b,c,d*}	1.29 ±0.03^{a}	0.81 ±0.21^{a,b,c*}
Tombul	0.93 ±0.03^{a,b,c}	1.22 ±0.08^{a,b*}	186 ±7.00^{a}	122 ±19.0^{a,b,c*}	63.0 ±5.00^{b,c}	74.0 ±7.00^{a,b,c*}	34.0 ±7.00^{b}	7.00 ±1.00^{a,b*}	3.22 ±0.02^{d}	3.04 ±0.59^{a}	2.43 ±0.01^{a}	1.16 ±0.14^{a,b,c*}	2.55 ±0.08^{a,b}	0.99 ±0.26^{a,b,c,d*}	1.51 ±0.12^{b}	0.59 ±0.20^{a,b*}

Superscript letters in each column indicate statistically significant difference ($p<0.05$) according to Duncan's test. (*) indicates statistically significant difference ($p<0.05$) according to t-test. Data are expressed as mean±standard deviation. Three independent samples were analyzed from each hazelnut varieties and all analytical measurements were performed duplicate.

2.3.6 Triacylglycerol Profile of Hazelnut Varieties

Triacylglycerol profile of hazelnut oils was almost identical in the chromatograms of all hazelnut varieties, which could be considered as the fingerprint of hazelnut oil. A typical chromatogram illustrating the triacylglycerol profile of hazelnut oil was shown in Figure 2.2. The following triacylglycerols were determined in hazelnut oils; LLL, OLL-LLP, OOL, OLP-PLP, OOO, POO-POP-SLO and SOO. Triacylglycerols that have same ECN number were co-eluted in the column. Identification of the co-eluted triacylglycerols was only possible tentatively with HPLC analysis. In this study, retention times of POO, PLP, POP, LLP and OOO was determined with the standard solutions of these triacylglycerols. Additionally, triacylglycerols that could not be identified with standards were enlightened by taking account the typical chromatograms of hazelnut oils that were identified previously with APCI-MS analysis [25, 181, 182]. Triacylglycerol composition of hazelnut oil was also compatible with the fatty acid profile, which provided an additional confirmation.

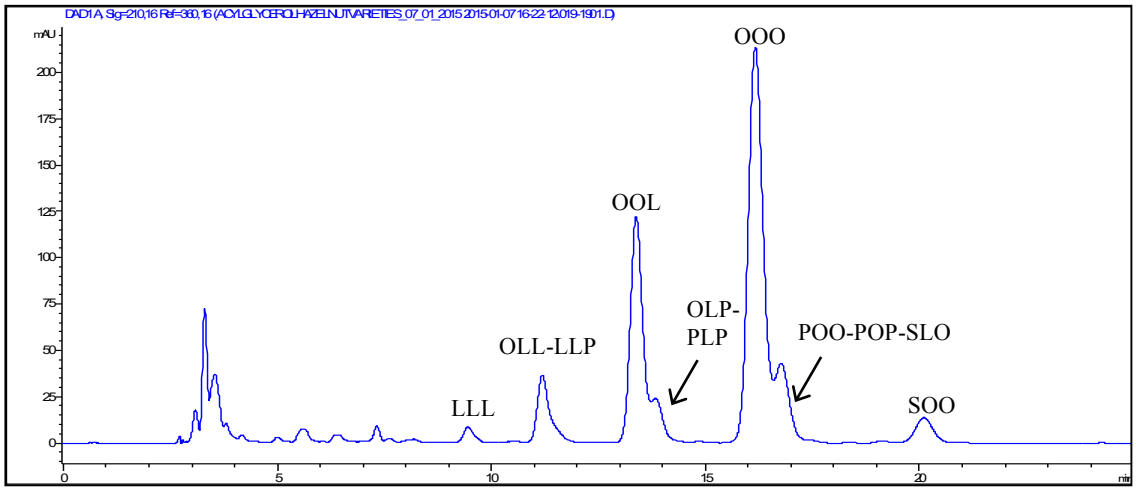


Figure 2.2. A sample chromatogram of triacylglycerols in hazelnut oil

Concentrations of triacylglycerols found in different hazelnut varieties harvested in year 2013 were given as percentages of glyceryl trioleate equivalent (OOO%) in Table 2.7. Hazelnuts predominantly contained oleic acid in triacylglycerols and less linoleic, palmitic and least stearic acid. OOO (45.1-59.6%) was found to be the richest triacylglycerol based on average values of all varieties, followed by OOL (25.5-75.6%), OLL-LLP (7.20-41.3%), POO-POP-SLO (6.34-13.5%), OLP-PLP (4.56-10.6%), LLL (1.42-9.20%) and SOO (1.68-4.45%). OOL was found to be the highest triacylglycerol in Kalınkara, Yuvarlak Badem, Foşa and Tombul varieties. Kargalak variety was found to be the richest

in OOO concentration among all varieties harvested in 2013. In addition to that, SOO concentration was least in most varieties except for Kargalak, Sivri, Çakıldak, Kan and Uzun Musa that contained LLL in least amounts. In general, there were significant differences between hazelnut varieties in their triacylglycerol concentrations in harvest year 2013 ($p<0.05$).

As given in Table 2.8, concentrations of triacylglycerols in hazelnut varieties harvested in 2014 followed similar order with the hazelnuts harvested in 2013; OOO (39.3-60.2%)> OOL (30.6-60.0%)> OLL-LLP (10.2-35.0%)> POO-POP-SLO (5.55-10.2%)> OLP-PLP (4.17-11.0%)> LLL (2.85-7.78%)> SOO (1.61-4.01%). OOL was found to be dominant in İncekara, Foşa, Kalınkara, Yuvarlak Badem, and Uzun Musa varieties although OOO was dominant in the rest of the varieties. Acı variety was found to have the highest amount of OOO. SOO was found to be the least triacylglycerol in hazelnut varieties although LLL was in lowest amounts in Palaz, Sivri, Kan, Çakıldak and Acı varieties. Considering the consecutive harvest years, triacylglycerol concentrations of Sivri, Kalınkara and Kuş did not change significantly ($p<0.05$). Palaz and Yuvarlak Badem showed significant differences in only one triacylglycerol (OLP-PLP and SOO) while the other varieties showed significant differences at least in three triacylglycerols ($p<0.05$).

It has been previously reported that predominant triacylglycerol was OOO (61.0-77.5% in relative percentages) followed by OOL, POO, SOO and OLL in decreasing order in the prime quality (Tombul) and four second quality (Yassı Badem, Sivri, Karafındık and Ham) native Turkish hazelnut varieties [3]. Their results did not compare well with our results as it was found in this study that OLL-LLP was in higher amounts than POO and SOO. Kıralan et al [21] determined 12 triacylglycerols in 19 Turkish hazelnut varieties that were OOO, OOP, OOL, SOO, OLL, PLO, LLL, POP, PPL, POS, PPP and PLL. They reported OOO as the most dominant triacylglycerol with a minimum of 57.86% (Foşa) and maximum of 68.99% (Bolu). Additionally, they found OOL as the second dominant triacylglycerol with values ranging between 9.21-14.75% [21]. OOO was also found to be the most dominant triacylglycerol followed by OOL considering the average values of all hazelnut varieties herein. However, the variation between varieties in triacylglycerol values was higher (OOO (45.1-59.6%) and OOL (25.5-75.6%) in harvest year 2013, OOO (39.3-60.2%) and OOL (30.6-60.0%) in harvest year 2014). OOO was dominant triacylglycerol in most varieties although OOL was dominant in Foşa, Kalınkara and

Yuvarlak Badem in both harvest years.

Table 2.7. Triacylglycerol profile of hazelnut oils obtained from hazelnut varieties harvested in 2013 (glyceryl trioleate %)

Variety	LLL	OLL-LLP	OOL	OLP-PLP	OOO	POO-POP-SLO	SOO
Kargalak	1.60±0.42^{a}	7.37±0.10^{a}	25.5±0.09^{a}	4.56±0.03^{a}	59.6±0.02^{g}	9.96±0.34^{f}	3.76±0.13^{f}
Palaz	4.04±0.00^{e}	16.43±0.28^{e}	35.3±0.06^{c}	8.25±0.30^{e}	51.1±0.13^{d}	13.43±0.35^{i}	3.79±0.08^{f}
İncekara	3.05±0.10^{c}	15.72±0.05^{d}	44.8±0.14^{f}	7.19±0.13^{d}	51.3±0.05^{d}	9.49±0.43^{e,f}	2.92±0.02^{d}
Sivri	2.93±0.12^{b,c}	14.65±0.32^{c}	39.4±0.09^{d}	6.06±0.0.04^{c}	53.6±0.38^{e}	8.97±0.23^{d,e}	4.17±0.00^{g}
Yassı Badem	2.54±0.08^{b}	15.59±0.21^{d}	47.5±0.20^{g}	6.75±0.29^{d}	58.6±0.53^{g}	8.48±0.20^{c,d}	2.11±0.15^{b}
Foşa	8.62±0.03^{g}	28.77±0.11^{h}	47.9±0.20^{g}	8.29±0.10^{e}	45.1±0.06^{a}	8.17±0.06^{b,c}	3.73±0.01^{f}
Kalınkara	9.20±0.10^{h}	41.27±0.48^{j}	75.6±0.41^{i}	10.58±0.17^{f}	47.4±0.09^{b}	6.34±0.17^{a}	1.68±0.02^{a}
Yuvarlak Badem	5.87±0.13^{f}	27.34±0.18^{g}	52.3±0.36^{h}	10.32±0.07^{f}	49.8±0.11^{c}	7.89±0.22^{b,c}	3.72±0.12^{f}
Kuş	3.87±0.13^{d,e}	18.67±0.18^{f}	45.1±1.40^{f}	7.20±0.19^{d}	49.4±0.82^{c}	7.70±0.14^{b}	2.39±0.02^{c}
Çakıldak	1.58±0.11^{a}	8.38±0.02^{b}	27.2±0.02^{b}	4.63±0.15^{a}	58.6±0.05^{g}	11.53±0.22^{h}	4.37±0.08^{g,h}
Kan	1.42±0.06^{a}	7.20±0.15^{a}	26.9±0.21^{b}	5.14±0.48^{b}	58.6±0.55^{g}	13.47±0.09^{i}	4.21±0.15^{g}
Uzun Musa	2.96±0.07^{b,c}	14.11±0.05^{c}	40.4±0.07^{d}	7.13±0.16^{d}	55.4±0.67^{f}	10.70±0.26^{g}	3.34±0.11^{e}
Acı	3.49±0.06^{d}	15.45±0.57^{d}	41.9±1.11^{e}	6.30±0.29^{c}	54.2±1.28^{e}	9.13±0.48^{e}	3.73±0.07^{f}
Tombul	8.58±0.49 ^{g}	31.44±0.27^{i}	52.8±0.42^{h}	10.26±0.02^{f}	46.7±0.35^{b}	10.68±0.02^{g}	4.45±0.20^{h}

Superscript letters in each column indicate statistically significant difference ($p<0.05$) according to Duncan's test. Three independent samples were analyzed from each hazelnut varieties and all analytical measurements were performed duplicate. Data are expressed as mean±standard deviation. LLL (glyceryl trilinoleate), OLL (1-oleoyl-2,3-dilinoleoyl-glycerol), LLP (1,2-dilinoleoyl-3-palmitoyl-glycerol), OOL (1,2-dioleoyl-3-linoleoyl-glycerol), OLP (1-oleoyl-2-linoleoyl-3-palmitoyl-glycerol), PLP (1,3-dipalmitoyl-2-linoleoyl-glycerol), OOO (glyceryl trioleate), POO (1-palmitoyl-2,3-dioleoyl-glycerol), POP (1,3-palmitoyl-2-oleoyl-glycerol), SLO (1-stearoyl-2-linoleoyl-3-oleoyl-glycerol), SOO (1-stearoyl-2,3-dioleoyl-glycerol)

Table 2.8. Triacylglycerol profile of hazelnut oils obtained from hazelnut varieties harvested in 2014 (glyceryl trioleate %)

Variety	LLL	OLL-LLP	OOL	OLP-PLP	OOO	POO-POP-SLO	SOO
Kargalak	4.70±0.44^{a,b,c,d}*	16.3±1.29^{a,b}*	35.2±2.93^{a,b}*	6.46±0.68^{a,b,c}*	50.3±5.33^{a,b,c,d}	8.63±1.25^{b,c,d}	3.84±0.71^{e,f}
Palaz	3.61±0.41^{a,b,c}	15.2±1.83^{a,b}	35.7±4.63^{a,b,c}	5.96±1.05^{a,b}*	51.8±7.55^{b,c,d}	10.13±1.71^{d}	4.01±0.71^{f}
İncekara	6.08±0.36^{b,c,d,e}*	29.1±1.80^{c,d}*	58.9±2.38^{f}*	8.40±0.41^{b,c,d,e}*	51.6±2.13^{b,c,d}	7.94±0.00^{b,c,d}*	2.97±0.24^{c,d,e}
Sivri	3.18±0.20^{a,b}	16.1±0.71^{a,b}	41.3±2.57^{a,b,c,d,e}	5.99±0.24^{a,b}	54.7±3.62^{c,d}	9.07±0.16^{b,c,d}	3.62±0.38^{e,f}
Yassı Badem	3.08±0.11^{a,b}*	17.4±0.04^{a,b}*	47.7±0.61^{b,c,d,e,f}	6.25±0.41^{a,b}	56.1±0.72^{d}*	8.02±0.60^{b,c,d}	2.39±0.35^{a,b,c}
Foşa	6.45±0.26^{c,d,e}*	28.5±1.11^{c,d}	53.5±1.77^{e,f}*	9.02±0.49^{c,de}	48.9±1.29^{a,b,c,d}*	8.44±0.24^{b,c,d}	3.23±0.09^{c,d,e,f}*
Kalınkara	7.59±1.98^{d,e}	33.9±8.54^{d}	59.9±14.55^{f}	9.46±2.54^{d,e}	39.3±10.07^{a}	5.55±1.29^{a}	1.79±0.54^{a,b}
Yuvarlak Badem	6.47±0.97^{c,d,e}	28.9±3.88^{c,d}	52.6±7.06^{d,e,f}	9.47±1.47^{d,e}	41.7±5.68^{a,b}	7.49±0.91^{a,b,c}	2.42±0.41^{a,b,c}*
Kuş	4.62±0.63^{a,b,c,d}	19.5±2.86^{a,b,c}	41.3±5.81^{a,b,c,d,e}	6.50±0.89^{a,b,c}	42.6±5.92^{a,b,c}	7.02±1.44^{a,b}	2.59±0.19^{b,c,d}
Çakıldak	3.23±0.45^{a,b}*	15.0±1.39^{a,b}*	38.4±2.73^{a,b,c,d}*	6.02±0.55^{a,b}*	52.3±3.47^{b,c,d}	9.81±0.98^{c,d}	3.24±0.16^{c,d,e,f}*
Kan	3.08±0.02^{a,b}*	15.1±0.35^{a,b}*	40.5±1.40^{a,b,c,d,e}*	6.17±0.38^{a,b}	57.9±2.68^{d}	10.16±0.77^{d}*	4.01±0.17^{f}
Uzun Musa	7.78±0.41^{e}	35.0±1.21^{d}	60.0±12.51^{f}	10.95±2.16^{e}	42.4±3.52^{a,b}*	7.39±0.50^{d,b}*	1.61±0.13^{a}*
Acı	2.85±0.20^{a}*	10.2±0.54^{a}*	30.6±2.63^{a}*	4.17±0.27^{a}*	60.2±6.25^{d}	9.31±1.50^{b,c,d}	3.96±0.26^{f}
Tombul	5.91±0.24^{a,b,c,d,e}*	25.1±1.41^{b,c,d}*	50.2±3.37^{c,d,e,f}	7.56±0.43^{b,c,d}*	52.1±2.96^{b,c,d}	8.79±0.34^{b,c,d}*	3.45±0.17^{d,e,f}*

Superscript letters in each column indicate statistically significant difference ($p<0.05$) according to Duncan's test. * indicates statistically significant difference ($p<0.05$) according to t-test. Three independent samples were analyzed from each hazelnut varieties and all analytical measurements were performed duplicate. Data are expressed as mean± standard deviation. LLL (glyceryl trilinoleate), OLL (1-oleoyl-2,3-dilinoleoyl-glycerol), LLP (1,2-dilinoleoyl-3-palmitoyl-glycerol),OOL (1,2-dioleoyl-3-linoleoyl-glycerol), OLP (1-oleoyl-2-linoleoyl-3-palmitoyl-glycerol), PLP (1,3-dipalmitoyl-2-linoleoyl-glycerol), OOO (glyceryl trioleate), POO (1-palmitoyl-2,3-dioleoyl-glycerol), POP (1,3-palmitoyl-2-oleoyl-glycerol), SLO (1-stearoyl-2-linoleoyl-3-oleoyl-glycerol), SOO (1-stearoyl-2,3-dioleoyl-glycerol)

2.3.7 Fatty Acid Profile of Hazelnut Varieties

Fatty acid profiles of hazelnut varieties harvested in 2013 were given in Table 2.9. Total oil content of hazelnut varieties ranged between 60.4% (Foşa) and 69.9% (Acı) in harvest year 2013 (Table 2.1). Kıralan et al [21] reported the total oil content of 19 Turkish hazelnut varieties between 55.01% (Kargalak) and 64.85% (İncekara). Bacchetta et al [191] reported average oil content of hazelnut cultivars originated from different countries as follows: Italian (60.8%), Slovenian (59.3%), Portuguese (58.2%), Greek (56.8%), Spanish (55.9%) and French (51.5%). A typical chromatogram illustrating the profile of fatty acids in hazelnut oils was given in Figure 2.2.

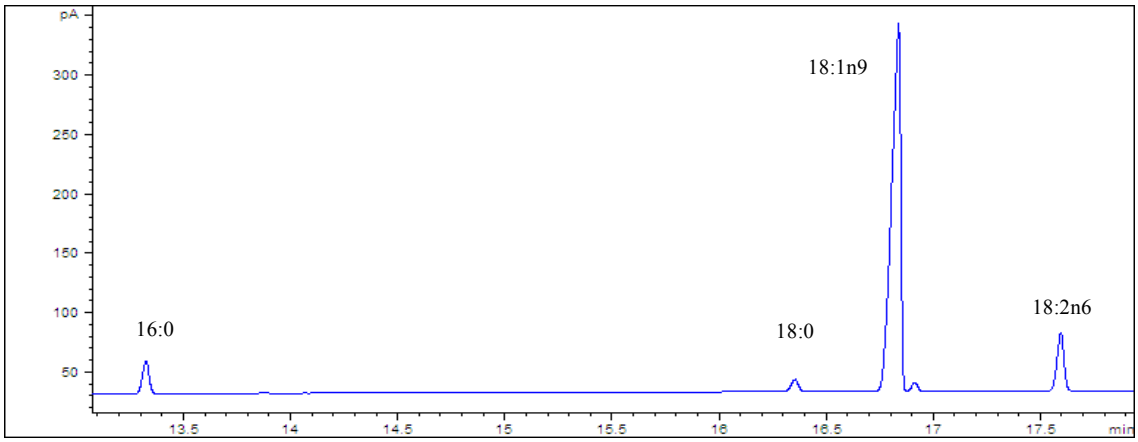


Figure 2.3. GC-MS chromatogram of the fatty acid profile of hazelnut oil

The most abundant fatty acids in hazelnut oil was monounsaturated fatty acids, namely oleic acid, ranging from 74.0 to 83.5% followed by polyunsaturated fatty acids, namely linoleic acid, ranging from 6.39 to 16.0%. Palmitic acid (4.59-7.08%) and stearic acid (2.08-4.61%) were the saturated fatty acids that constituted of 8.86% of total fatty acids. Kargalak variety had the highest oleic acid content among other varieties harvested in 2013, which is in accordance with triacylglycerol results as it had also the richest OOO concentration. Significant differences were found between fatty acid concentrations of hazelnut varieties especially in oleic and linoleic acid concentrations ($p<0.05$). Taking into account the ratio of unsaturated fatty acids to saturated fatty acids, Kalınkara variety had the highest ratio of 12.2 while Uzun Musa variety had the lowest ratio of 8.2.

Table 2.10 gives the fatty acid profiles of hazelnut varieties harvested in 2014. Total oil content of hazelnut varieties ranged between 58.1% (Yuvarlak Badem) and 68.9% (Acı) (Table 2.1). Similar to harvest year of 2013, oleic acid was highest ranging from 75.9 to 85.7%, followed by linoleic acid (5.49- 16.0%), palmitic acid (4.81 to 6.12%) and stearic

acid (1.43-3.08%) in hazelnuts harvested in 2014. Acı variety had the highest oleic acid content among all, which is in accordance with its triacylglycerol profile as it had also the richest OOO concentration. Concentrations of palmitic, stearic, oleic and linoleic acid did not significantly change according to harvest years in Yuvarlak Badem, and Kuş varieties ($p>0.05$). On the other hand, there were significant differences in the concentration of at least one of the fatty acids in the remaining hazelnut varieties depending upon the harvest year ($p<0.05$).

Table 2.9. Fatty acid profile of hazelnut oils extracted from hazelnut varieties harvested in 2013 (%)

Variety	Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid	Total Saturated	Total Unsaturated	Unsaturated/Saturated
Kargalak	5.88±0.01^{a,b}	2.79±0.00^{a}	83.5±0.13^{i}	6.76±0.08^{b}	8.67±0.01^{b,c,d}	90.2±0.21^{b,c,d,e,f}	10.4±0.0^{d,e}
Palaz	6.54±0.01^{b}	3.08±0.04^{a,b}	81.8±0.08^{h}	7.28±0.08^{c}	9.62±0.05^{e}	89.1±0.16^{b}	9.3±0.0^{b,c}
İncekara	5.68±0.17^{a,b}	2.50±0.03^{a}	79.5±0.01^{d,e}	11.03±0.14^{i}	8.18±0.20^{a,b,c}	90.5±0.15^{c,d,e,f}	11.1±0.2^{e,f}
Sivri	6.03±0.11^{a,b}	2.79±0.10^{a}	79.7±0.08^{d,e}	10.18±0.00^{g}	8.82±0.22^{c,d,e}	89.9±0.08^{b,c,d}	10.2±0.0^{c,d,e}
Yassı Badem	5.57±0.48^{a,b}	2.08±0.06^{a}	81.5±0.46^{g,h}	9.61±0.12^{f}	7.65±0.54^{a}	91.1±0.58^{e,f}	11.9±0.9^{f,g}
Foşa	5.69±0.02^{a,b}	3.12±0.04^{a,b}	74.0±0.13^{a}	15.99±0.08^{l}	8.81±0.07^{c,d,e}	90.0±0.21^{b,c,d,e}	10.2±0.1^{c,d,e}
Kalınkara	5.34±0.04^{a,b}	2.16±0.02^{a}	76.7±0.14^{b}	14.39±0.05^{k}	7.49±0.06^{a}	91.1±0.19^{f}	12.2±0.0^{g}
Yuvarlak Badem	5.90±0.04^{a,b}	3.06±0.36^{a,b}	77.7±0.60^{c}	11.75±0.19^{j}	8.96±0.40^{c,d,e}	89.4±0.79^{b,c}	10.0±0.5^{b,c,d}
Kuş	5.52±0.08^{a,b}	2.38±0.01^{a}	80.2±0.03^{e,f}	10.47±0.05^{h}	7.89±0.08^{a,b}	90.7±0.08^{d,e,f}	11.5±0.1^{f,g}
Çakıldak	4.59±0.26^{a}	4.61±0.26^{b}	83.1±0.14^{i}	6.39±0.06^{a}	9.19±0.52^{d,e}	89.5±0.20^{b,c}	9.7±0.0^{b,c,d}
Kan	6.29±0.03^{a,b}	2.98±0.08^{a,b}	80.7±0.12^{f,g}	8.81±0.07^{d}	9.27±0.11^{d,e}	89.5±0.20^{b,c}	9.7±0.1^{b,c,d}
Uzun Musa	7.08±0.58^{b}	3.68±0.63^{a,b}	79.1±1.22^{d}	8.75±0.19^{d}	10.75±1.21^{f}	87.9±1.41^{a}	8.2±1.1^{a}
Acı	5.80±0.04^{a,b}	3.19±0.01^{a,b}	80.7±0.24^{f,g}	9.11±0.26^{e}	8.99±0.05^{c,d,e}	89.8±0.50^{b,c,d}	10.0±0.0^{b,c,d}
Tombul	6.13±0.03^{a,b}	3.58±0.08^{a,b}	79.0±0.13^{d}	10.11±0.06^{g}	9.72±0.11^{e}	89.1±0.19^{b}	9.2±0.1^{b}

Superscript letters in each column indicate statistically significant difference ($p<0.05$) according to Duncan's test.
Three independent samples were analyzed from each hazelnut varieties and all analytical measurements were performed duplicate.
Data are expressed as mean± standard deviation.

Table 2.10. Fatty acid profile of hazelnut oils extracted from hazelnut varieties harvested in 2014 (%)

Variety	Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid	Total Saturated	Total Unsaturated	Unsaturated/Saturated
Kargalak	5.79±0.02^{f,g}*	3.08±0.03^{g}*	82.3±0.02^{c,d}*	7.81±0.04^{b}*	8.87±0.05^{f,g}*	90.1±0.06^{a,b}*	10.2±0.1^{a,b}*
Palaz	6.12±0.04^{h}*	3.05±0.03^{g}	82.4±0.06^{c,d}*	7.32±0.01^{a,b}	9.17±0.06^{g}*	89.7±0.06^{a}*	9.8±0.1^{a}*
İncekara	5.06±0.02^{a,b}*	2.15±0.05^{c}*	79.4±0.03^{b}*	12.07±0.08^{d}*	7.21±0.06^{b}*	91.5±0.12^{f,g}*	12.7±0.1^{e}*
Sivri	5.43±0.01^{c,d,e}*	2.63±0.07^{e,f}	82.7±0.18^{c,d}*	8.01±0.09^{b}*	8.07±0.08^{c,d}*	90.7±0.27^{c,d,e}*	11.2±0.1^{c,d}*
Yassı Badem	4.81±0.02^{a}	1.76±0.01^{b}*	83.4±0.05^{d}*	8.90±0.03^{b,c}*	6.57±0.02^{a}	92.3±0.08^{h}	14.0±0.0^{g}*
Foşa	5.39±0.01^{c,d}*	2.44±0.09^{d,e}*	79.0±0.12^{b}*	11.98±0.00^{d}*	7.83±0.09^{c}*	91.0±0.12^{d,e}*	11.6±0.1^{d}*
Kalınkara	5.20±0.01^{b,c}*	1.65±0.02^{b}*	75.9±0.04^{a}*	15.97±0.06^{e}*	6.85±0.03^{a,b}*	91.9±0.10^{g,h}*	13.4±0.1^{f,g}*
Yuvarlak Badem	5.70±0.22^{d,e,f}	2.34±0.19^{c,d}	78.8±2.38^{b}	11.87±2.09^{d}	8.04±0.41^{c,d}*	90.6±4.47^{c,d}	11.3±0.1^{c,d}*
Kuş	5.73±0.27^{e,f,g}	2.32±0.15^{c,d}	78.7±2.25^{b}	11.95±1.96^{d}	8.04±0.42^{c,d}	90.6±4.20^{c,d}	11.3±0.2^{b}
Çakıldak	6.02±0.06^{g,h}	2.59±0.10^{e,f}	82.5±0.15^{c,d}*	7.64±0.03^{a,b}*	8.61±0.17^{e,f}*	90.1±0.18^{b}*	10.5±0.2^{b,c}*
Kan	5.71±0.00^{e,f}*	2.67±0.06^{f}*	83.3±0.00^{d}*	7.23±0.06^{a,b}*	8.38±0.06^{d,e}*	90.5±0.06^{b,c}*	10.8±0.1^{e,f}*
Uzun Musa	5.69±0.36^{d,e,f}	1.43±0.19^{a}	76.3±1.59^{a}	14.87±2.12^{e}*	7.12±0.55^{b}*	91.1±3.70^{e,f}*	12.8±1.1^{d}*
Acı	5.29±0.02^{b,c}*	2.61±0.02^{e,f}*	85.7±0.14^{e}*	5.49±0.08^{a}*	7.90±0.04^{c}*	91.2±0.22^{e,f}*	11.5±0.1^{c,d}*
Tombul	5.43±0.00^{c,d,e}*	2.56±0.02^{e,f}*	80.5±0.04^{b,c}*	10.42±0.02^{c,d}*	8.00±0.03^{c,d}*	90.9±0.07^{c,d,e}*	11.4±0.0^{a,b}*

Superscript letters in each column indicate statistically significant difference ($p<0.05$) according to Duncan's test.
* indicates statistically significant difference ($p<0.05$) according to t-test.
Three independent samples were analyzed from each hazelnut varieties and all analytical measurements were performed duplicate.
Data are expressed as mean± standard deviation.

Comparing to 2013, unsaturated/saturated fatty acid ratio was higher in hazelnuts harvested in 2014. Yassı Badem variety was found to have the highest ratio of 14.0 and Palaz variety had the lowest ratio of 9.8 in 2014. Venkatachalam and Sathe [1] revealed that regardless of the type of nut, oleic acid and linoleic acid contributes most to the monounsaturated and polyunsaturated fatty acid content of the nut oils. They reported oleic acid as the most abundant fatty acid in hazelnut oil (83.0%), followed by linoleic acid (7.60%), palmitic acid (5.80%), stearic acid (3.14%), linolenic acid (0.24%), palmitoleic acid (0.15%). Köksal et al [4] reported fatty acid profile of Turkish hazelnut varieties similar to our results although they identified trace amounts of palmitoleic acid (0.36%) and linolenic acid (0.062%). They also reported unsaturated/saturated fatty acid ratio in Turkish hazelnut varieties ranging from 11.1 to 16.4. Bacchetta et al [191] reported the major fatty acid as oleic acid in 75 hazelnut oil belong to different cultivars with an average value of 80.63%. They found an inverse correlation between oleic acid and linoleic acid content (average 10.57%). Palmitic acid and stearic acid were reported to be the saturated fatty acids of the hazelnut oils with an average value of 5.95% and 2.48%, respectively [191]. Matthaus and Ozcan [192] did not reported stearic acid on contrary to our study while they found oleic (76.3-82.6%), linoleic (6.5-14.0%) and palmitic (%.7-6.5%) acids in two hazelnut varieties grown in Turkey and one variety purchased from Germany.

2.3.8 Tocopherol Composition of Hazelnut Varieties

Individual tocopherols and total tocopherol contents of hazelnut oils obtained from hazelnut varieties harvested in 2013 and 2014 were given in Table 2.11. The mean α-tocopherol content of all hazelnut varieties was 23.7 mg/100g oil for harvest year of 2013, while it was 14.3 mg/100g oil for 2014. α-Tocopherol content was found to be 7.6 and 3.1 times higher than β+γ-tocopherol content in harvest year 2013 and 2014, respectively. Parcerisa et al [181] also reported that β- and γ-tocopherol (total 2.6 mg/100g oil) as the minor components of hazelnut oil compared to α-tocopherol (33.4 mg/100 g oil). α-Tocopherol was found to be responsible for the minimum 75.2% and maximum 96.5% of the total tocopherols in Kuş and Kargalak varieties harvested in 2013, respectively. A decrease in the contribution of α-tocopherol to the total tocopherols was observed in harvest year 2014 (ranged from 64% in Yuvarlak Badem to 94% in Kalınkara). Ciemniewska-Zytkiewicz et al [193] reported α-tocopherol as the most abundant tocopherol in Polish hazelnut cultivars, Katalonski and Webba Cenny, accounting for the

90-92% of total tocopherol content, followed by γ-tocopherol (4.3-7%) and β-tocopherol (2.4-4.1%). Total tocopherol content of Tombul variety was the highest with 41.2 mg/100g oil, and Kan variety was the lowest with 19.4 mg/100g oil in 2013. The total tocopherol contents changed significantly for most of the hazelnut varieties in 2014 ($p<0.05$), ranging from 13.6 to 22.9 mg/100g oil, except for the varieties of Palaz, Yuvarlak Badem, Kuş and Kan.

Alasalvar et al [3] reported total tocopherol contents of five native hazelnut varieties between 36.3 and 46.9 mg/100g oil, most of which is α-tocopherol ranging from 21.4 to 34.5 mg/100g. Benitez-Sanchez et al [194] compared α-tocopherol contents of various oils and found that after sunflower oil (40.3-93.5 mg/100g) and maize oil (23-57.3 mg/100g), hazelnut oil counted third among the oils tested ranging from 32.9 to 44.8 mg/100g oil which was higher than the results obtained herein. The reason for that might be extraction of hazelnut oil from hazelnut varieties after removal of their skin where most of the tocopherols concentrated.

Concentrations of (β+γ)-tocopherol, α-tocopherol and total tocopherols found in skin of hazelnut varieties are given in Table 2.12**.** A total ion chromatogram of hazelnut skin and extracted ion chromatograms indicating α-tocopherol and (β+γ)-tocopherol are presented in Figure 2.3. α-Tocopherol was also the most abundant tocopherol in hazelnut skin. α-Tocopherol was found to be ranging from 168.2 to 443.8 μg/g. (β+γ)-tocopherol concentration of hazelnut skins was almost five times higher than hazelnuts and ranged from 57.9 to 197.7 μg/g skin. Total tocopherol content of hazelnut skins was lowest in Uzun Musa with 226.1 μg/g and highest in Kargalak with 593.5 μg/g, that was approximately two fold higher than hazelnuts. Tocopherol content of hazelnut skin oil was found to be 2770 μg/g [49] that was almost ten times higher than tocopherol contents of hazelnut skins detected herein.

Venkatachalam and Sathe [1] suggested that genetic factors and environmental conditions could change the distribution of fatty acids in triacylglycerols. Alasalvar et al [16] reported geographic origin, climate, harvesting year, storage conditions, culture conditions and soil type as the effective factors changing the tocopherol and tocotrienol composition of hazelnut oils. Cristofori et al [45] stated that temperature rises during growing seasons, mainly summer months, caused the changes in fatty acid composition. In this study, the difference in triacylglycerol, tocopherol and fatty acid content of hazelnut varieties could

be attributed to the genetic factors and the difference between harvest years mostly affected from climate changes, as the other conditions were same for the all hazelnut varieties.

Table 2.11. Tocopherol profile of hazelnut oils obtained from hazelnut varieties harvested in 2013 and 2014 (mg/100g oil)

Variety	Harvest year 2013			Harvest year 2014		
	β+γ-tocopherol	α-tocopherol	Total tocopherols	β+γ-tocopherol	α-tocopherol	Total tocopherols
Kargalak	0.94±0.22^{a}	25.0±0.51^{c,d}	25.9±0.73^{a,b}	1.11±0.13^{a}	16.4±2.88^{c,d}*	17.5±3.01^{a,b,c}*
Palaz	3.18±0.45^{b,c,d}	19.0±1.47^{a,b}	22.2±1.92^{a,b}	5.37±0.19^{e,f}*	13.2±1.88^{a,b,c,d}*	18.6±2.07^{a,b,c,d}
İncekara	3.87±0.21^{b,c,d}	19.7±0.66^{a,bc}	23.6±0.87^{a,b}	5.34±0.28^{e,f}*	11.7±1.39^{a,b,c}*	17.0±1.67^{a,b,c}*
Sivri	3.66±0.00^{b,c,d}	21.3±0.06^{a,b,c,d}	25.0±0.06^{a,b}	4.06±0.32^{c}	9.56±0.66^{a}*	13.6±0.98^{a}*
Yassı Badem	1.93±0.00^{a,b}	21.7±1.82^{a,b,c,d}	23.6±1.83^{a,b}	3.16±0.03^{b}*	12.2±0.48^{a,b,c,d}*	15.3±0.51^{a,b}*
Foşa	1.99±0.12^{a,b}	25.7±1.24^{d}	27.7±1.36^{b,c}	5.08±0.26^{d,e}*	14.7±0.56^{b,c,d}*	19.8±0.82^{b,c,d}*
Kalınkara	1.16±0.17^{a}	26.4±1.79^{d}	27.5±1.96^{b,c}	1.08±0.05^{a}	17.3±2.09^{d}*	18.4±2.14^{a,b,c,d}*
Yuvarlak Badem	1.97±0.80^{a,b}	24.1±6.09^{b,c,d}	26.1±6.89^{a,b}	5.68±0.48^{e,f}*	10.0±1.74^{a,b}*	15.7±2.22^{a,b}
Kuş	6.99±0.28^{e}	21.2±4.48^{a,b,c,d}	28.2±4.76^{b,c}	6.55±0.38^{g}	16.3±2.35^{c,d}	22.9±2.73^{d}
Çakıldak	4.32±0.21^{c,d}	19.9±0.09^{a,b,c}	24.3±0.30^{a,b}	4.38±0.07^{c,d}	15.0±0.90^{b,c,d}*	19.4±0.97^{b,c,d}*
Kan	2.89±0.59^{a,b,c}	16.5±0.53^{a}	19.4±1.12^{a}	6.14±0.74^{f,g}*	12.8±0.58^{a,b,c,d}	19.0±1.64^{b,c,d}
Uzun Musa	3.81±0.34^{b,c,d}	23.0±2.19^{b,c,d}	26.8±2.53^{b}	3.01±0.76^{b}	12.1±0.00^{a,b,c*}	15.1±0.76^{a,b}*
Acı	1.97±0.01^{a,b}	32.0±1.01^{e}	34.0±1.02^{c}	1.19±0.00^{a}*	13.6±0.40^{a,b,c,d}*	14.8±0.40^{a,b}*
Tombul	4.90±0.05^{d}	36.3±0.24^{e}	41.2±0.29^{d}	6.03±0.04^{f,g}*	16.0±0.43^{c,d}*	22.0±0.47^{c,d}*

Superscript letters in each column indicate statistically significant difference ($p<0.05$) according to Duncan's test. * indicates statistically significant difference ($p<0.05$) according to t-test. Three independent samples were analyzed from each hazelnut varieties and all analytical measurements were performed duplicate. Data are expressed as mean± standard deviation.

Table 2.12. Concentration of tocopherols in hazelnut skins (μg/g)*

	Hazelnut Skin		
	β+γ tocopherol	α- tocopherol	Total tocopherols
Kargalak	149.6±2.3^{d}	443.9±8.7^{h}	593.5±11.0^{f}
Palaz	150.8±1.1^{d}	250.0±0.4^{c,d,e}	400.7±0.8^{d,e}
İncekara	146.7±3.2^{c,d}	295.6±1.1^{d,e,f}	442.3±4.3^{e}
Sivri	197.7±0.9^{e}	357.5±19.1^{f,g}	555.2±18.2^{f}
Yassı Badem	143.4±12.9^{c,d}	293.7±11.0^{d,e,f}	437.1±23.9^{e}
Foşa	75.9±5.8^{a,b}	372.0±9.4^{g}	447.9±15.2^{e}
Kalınkara	73.8±3.4^{a,b}	346.3±7.3^{f,g}	420.0±3.9^{e}
Yuvarlak Badem	79.9±2.4^{b}	236.7±22.5^{b,c,d}	316.6±24.9^{b,c}
Kuş	126.8±2.6^{c}	200.7±3.8^{a,b,c}	327.5±1.2^{b,c,d}
Çakıldak	146.5±11.4^{c,d}	256.6±39.2^{c,d,e}	403.1±50.6^{d,e}
Kan	78.4±15.5^{a,b}	172.6±27.1^{a,b}	251.0±42.6^{a,b}
Uzun Musa	57.9±0.5^{a}	168.2±3.8^{a}	226.1±3.2^{a}
Acı	79.4±7.1^{a,b}	312.9±9.5^{e,f,g}	392.3±16.6^{c,d,e}
Tombul	89.1±0.9^{b}	207.0±0.6^{a,b,c}	296.1±1.6^{a,b}

*Superscript letters in each column indicate statistically significant difference ($p<0.05$) according to Duncan's test. Three independent hazelnut skin and hazelnut sample were analyzed with two analytical measurements.

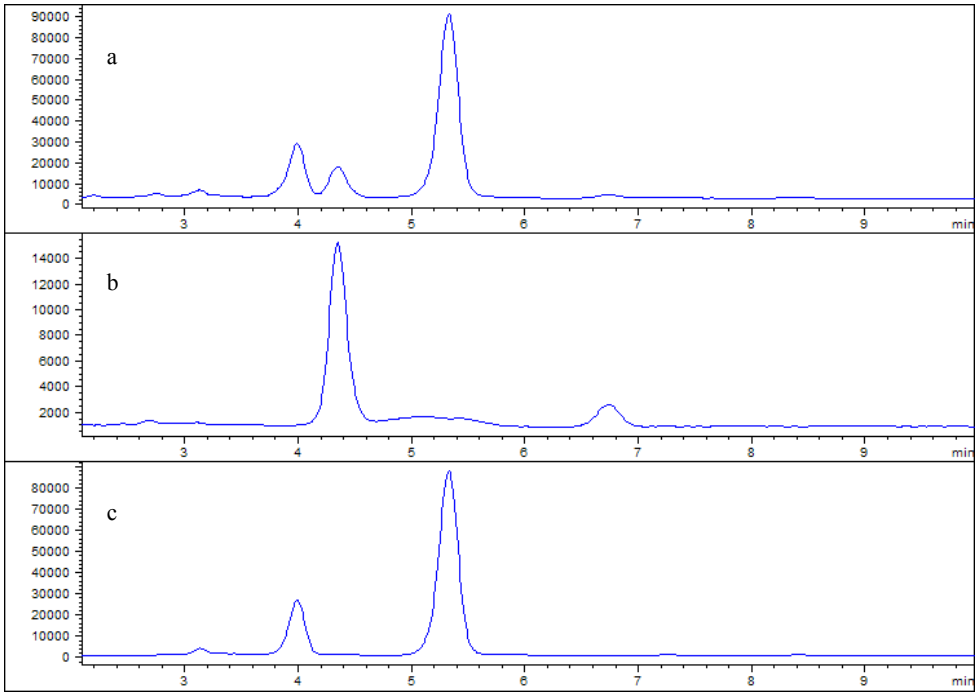


Figure 2.4. a) Total ion chromatogram of tocopherols of hazelnut skin. Extracted ion chromatograms of hazelnut skin indicating b) beta-tocopherol (m/z 417.7) and gamma-tocopherol (m/z 417.7), c) alpha-tocopherol (m/z 431.7)

2.3.9 Soluble Free, Conjugated Soluble, Insoluble Bound and Total Phenolic Contents of Hazelnut Skins

Soluble free, conjugated soluble, insoluble bound and total phenolic contents of hazelnut skins belong to fourteen hazelnut varieties are shown in Table 2.13. Soluble free phenolic compounds of skins of fourteen hazelnut varieties were found 17.3 mg GAE/g skin on an average that was 15% of the total phenolic compounds. Among hazelnut varieties, Yassı Badem had the lowest soluble free phenolic compounds with 7.1 mg GAE/g skin while Çakıldak had the highest with 30.6 mg GAE/g skin, respectively. Conjugated soluble phenolic compounds, comprising of 74% of total phenolics with an average value of 84 mg GAE/g skin, were considerably higher than soluble free and insoluble bound phenolics. Concentration of conjugated soluble phenolic compounds was lowest in Foşa with 30.1 mg GAE/g skin and highest in Çakıldak with 155.2 mg GAE/g skin, respectively. Insoluble bound phenolics comprised of the 11% of the total phenolic compounds with an average value of 11.8 mg GAE/g skin. Insoluble bound phenolics were lowest in Acı with 7.6 mg GAE/g and highest in Uzun Musa with 29.4 mg GAE/g. Total phenolic compounds ranged between 51.9 and 203.1 mg GAE/g skin among the varieties.

Table 2.13. Concentration of soluble free, conjugated soluble, insoluble bound and total phenolic compounds of hazelnut skins (mg GAE/g)*

	Soluble Free Phenolics	Conjugated Soluble Phenolics	Insoluble Bound Phenolics	Total Phenolics
Kargalak	18.8±4.9^{d}	122.9±18.2^{d,e}	9.5±2.0^{a}	151.2±21.1^{f}
Palaz	14.4±1.5^{b,c,d}	103.9±11.3^{d}	9.4±0.1^{a}	127.7±9.7^{e}
İncekara	17.0±2.9^{d}	73.2±11.2^{c}	11.8±2.5^{a,b}	102.0±5.8^{d}
Sivri	15.2±5.0^{c,d}	57.0±1.1^{b,c}	8.2±1.4^{a}	80.4±2.4^{b,c,d}
Yassı Badem	7.1±1.2^{a}	45.8±0.4^{a,b}	8.0±0.4^{a}	60.9±0.5^{a,b}
Foşa	11.1±0.1^{a,b,c}	30.1±3.8^{a}	10.8±2.0^{a}	51.9±2.0^{a}
Kalınkara	24.4±1.5^{e}	67.0±6.5^{b,c}	9.1±1.6^{a}	100.6±4.9^{c,d}
Yuvarlak	9.8±0.7^{a,b}	74.2±9.4^{c}	9.3±0.9^{a}	93.4±9.2^{c,d}
Kuş	17.1±1.4^{d}	107.3±7.0^{d,e}	7.7±0.8^{a}	132.0±7.7^{e,f}
Çakıldak	30.6±0.6^{f}	155.2±3.3^{f}	17.3±2.4^{c}	203.1±1.5^{h}
Kan	27.6±1.9^{e,f}	44.7±8.2^{a,b}	10.9±0.2^{a}	83.2±6.1^{b,c,d}
Uzun Musa	16.8±0.4^{d}	127.3±17.5^{e}	29.4±0.8^{d}	173.5±17.1^{g}
Acı	16.1±0.8^{c,d}	54.2±8.3^{b,c}	7.6±1.7^{a}	77.9±9.2^{b,c}
Tombul	15.7±1.3^{c,d}	111.0±8.1^{d,e}	15.4±2.3^{b,c}	142.2±11.7^{e,f}

*Superscript letters in each column indicate statistically significant difference ($p<0.05$) according to Duncan's test. Three independent hazelnut skin sample were analyzed with two analytical measurements.

Previous works revealed the total phenolic content of roasted hazelnut skins as 233 mg CE/g skin of Tombul variety [49], or ranging between 41 and 127 mg polyphenols/g of roasted hazelnut skin varieties from Turkey, Italy and Chile [51], and 181.51 mg CE/g of medium roasted hazelnut skin and 190.88 mg CE/g of high roasted hazelnut skin [195]. Pelvan et al [48] found 1.78-2.46 mg GAE/g total phenolic content in natural hazelnut varieties and suggested to consume hazelnuts with their skins as there was a significant loss in total phenolics together with antioxidant values, condensed tannins and free and bound phenolic acids after removal of skin.

2.3.10 Soluble Free, Conjugated Soluble, Insoluble Bound and Total Flavonoid Content of Hazelnut Skins

Soluble free, conjugated soluble, insoluble bound and total flavonoid content of hazelnut skins is given in Table 2.14.

Table 2.14. Concentration of soluble free, conjugated soluble, insoluble bound and total flavonoid compounds of hazelnut skins (mg CE/g)*

	Soluble Free Flavonoids	Conjugated Soluble Flavonoids	Insoluble Bound Flavonoids	Total Flavonoids
Kargalak	3.4 ± 0.0^{c}	52.4 ± 4.1^{c}	$3.4\pm0.4^{a,b}$	59.2 ± 3.73^{d}
Palaz	$2.6\pm0.8^{a,b,c}$	$48.1\pm2.8^{b,c}$	$3.5\pm0.10^{a,b}$	$54.1\pm1.88^{c,d}$
İncekara	$2.4\pm0.1^{a,b,c}$	$42.9\pm1.4^{a,b,c}$	$4.2\pm0.40^{a,b}$	$49.5\pm1.25^{b,c,d}$
Sivri	$2.0\pm0.2^{a,b}$	$35.5\pm0.4^{a,b,c}$	2.7 ± 0.71^{a}	$40.2\pm0.15^{a,b,c}$
Yassı Badem	1.7 ± 0.1^{a}	$30.4\pm1.3^{a,b}$	2.7 ± 0.50^{a}	$34.8\pm1.91^{a,b}$
Foşa	$2.0\pm0.5^{a,b}$	25.5 ± 1.0^{a}	$3.4\pm0.27^{a,b}$	30.9 ± 0.39^{a}
Kalınkara	3.3 ± 0.4^{c}	$44.0\pm0.5^{b,c}$	$3.3\pm0.27^{a,b}$	$50.7\pm1.25^{b,c,d}$
Yuvarlak	$2.6\pm0.5^{a,b,c}$	$41.4\pm2.4^{a,b,c}$	$3.0\pm0.10^{a,b}$	$47.0\pm2.87^{a,b,c,d}$
Kuş	$2.6\pm0.1^{a,b,c}$	50.9 ± 3.7^{c}	2.7 ± 0.41^{a}	$56.2\pm4.08^{c,d}$
Çakıldak	4.5 ± 0.2^{d}	82.4 ± 2.3^{d}	6.7 ± 0.95^{b}	93.6 ± 2.41^{e}
Kan	4.3 ± 0.1^{d}	$40.1\pm3.7^{a,b,c}$	$5.6\pm0.10^{a,b}$	$50.0\pm3.75^{b,c,d}$
Uzun Musa	$2.8\pm0.6^{b,c}$	81.7 ± 3.4^{d}	27.9 ± 5.68^{c}	112.4 ± 2.86^{f}
Acı	$2.9\pm0.5^{b,c}$	$38.3\pm2.5^{a,b,c}$	$2.9\pm0.18^{a,b}$	$44.0\pm3.14^{a,b,c,d}$
Tombul	$2.3\pm0.5^{a,b}$	49.4 ± 1.3^{c}	$5.3\pm0.60^{a,b}$	$57.0\pm1.31^{c,d}$

*Superscript letters in each column indicate statistically significant difference ($p<0.05$) according to Duncan's test. Three independent hazelnut skin sample were analyzed with two analytical measurements.

Most of the flavonoids were found in conjugated soluble form in hazelnut skin that was 85% of the total flavonoids. In parallel with the results of conjugated phenolic compounds, Foşa was the least conjugated flavonoid containing variety with 25.5 mg CE/g and Çakıldak was the highest with 82.4 mg CE/g. Insoluble bound flavonoids comprised of the 10% of the total flavonoids with an average 5.5 mg GAE/g skin. Besides, least flavonoid-containing fraction was found to be soluble free with an average of 2.8 mg CE/g skin. Total flavonoid content of hazelnut skins was ranged between 30.9 and 112.4 mg CE/g skin, which were almost 60% of the total phenolic compounds. Catechin, epicatechin, epicatechin gallate, gallocatechin, epigallocatechin gallate [49], procyanidin and procyanidin dimers and trimers, procyanidin dimer gallate were the flavan-3-ols identified in aqueous extracts of hazelnut skins [51]. Besides, quercetin, myricetin, quercetin-3-O-rhamnoside, myricetin rhamnoside, kaempherol rhamnoside were the flavonols and phloretin 2'-O-glucoside was the dihydrochalcone detected in methanolic extracts of hazelnut skin [51]. Because combination of methanol/acetone/water was used for the

extraction of skins of hazelnut variteties and alkaline hydrolysis performed, flavonoids in all forms could be extracted from hazelnut skins.

2.3.11 Characterization of Soluble Free, Conjugated Soluble and Insoluble Bound Phenolic Acids of Hazelnut Skins

Gallic acid and ferulic acid were the phenolic acids characterized in natural hazelnut skins (Table 2.15). Gallic acid was found in both soluble free and conjugated soluble fractions while ferulic acid was detected in conjugated soluble and insoluble fractions. Both phenolic acids were found to be dominant in conjugated soluble fraction. Soluble free phenolic acids found in skin of Kargalak was lowest with 0.18 mg/g while Uzun Musa was highest with 0.5 mg/g skin although there was no significant difference between varieties ($p>0.05$). Gallic acid found in conjugated form ranged between 0.5 and 3.7 mg/g skin. Ferulic acid in conjugated soluble form was approximately 8 fold higher than insoluble bound form. Ferulic acid ranged from 1.2 to 2.1 mg/g skin in conjugated form and 0.11 to 0.39 mg/g skin in insoluble bound form. Total phenolic acids determined in hazelnut skins were minimum of 3.0 and maximum of 8.3 mg/g skin. Pelvan et al [48] reported the total phenolic acid contents of natural hazelnuts ranged from 62.1 mg/g to 143.1 mg/g. Özdemir et al [49] found 0.79 mg gallic acid/g skin in soluble free fraction of roasted hazelnut skin.

Table 2.15. Concentration of soluble free, conjugated soluble, insoluble bound and total phenolic acids of hazelnut skins (mg/g)*

	Soluble Free Phenolic Acids	Conjugated Soluble Phenolic Acids		Insoluble Bound Phenolic Acids	Total Phenolic Acids
	Gallic Acid	Ferulic Acid	Gallic Acid	Ferulic Acid	
Kargalak	0.18±0.03^{a}	2.1±0.01^{a}	3.7±0.25^{c,d}	0.20±0.03^{a,b}	6.2±0.3^{a,b}
Palaz	0.30±0.13^{a}	1.7±0.10^{a}	3.5±0.92^{c,d}	0.27±0.01^{a,b,c}	5.8±1.2^{a,b}
Incekara	0.33±0.08^{a}	1.8±0.12^{a}	1.7±0.05^{a,b,c}	0.22±0.09^{a,b,c}	4.1±0.5^{a}
Sivri	0.41±0.12^{a}	1.6±0.01^{a}	1.1±0.01^{a,b,c}	0.16±0.02^{a,b}	3.3±0.2^{a}
Yassı Badem	0.28±0.15^{a}	1.8±0.10^{a}	0.7±0.04^{a,b}	0.22±0.01^{a,b,c}	3.0±0.3^{a}
Foşa	0.49±0.13^{a}	1.9±0.3^{a}	0.5±0.03^{a}	0.23±0.03^{a,b,c}	3.1±0.5^{a}
Kalınkara	0.50±0.13^{a}	1.5±0.17^{a}	1.6±0.07^{a,b,c}	0.18±0.01^{a,b}	3.7±0.4^{a}
Yuvarlak Badem	0.35±0.13^{a}	2.0±0.47^{a}	1.9±0.16^{a,b,c}	0.26±0.09^{a,b,c}	4.5±0.9^{a}
Kuş	0.34±0.10^{a}	2.0±0.25^{a}	2.7±0.40^{a,b,c}	0.19±0.01^{a,b}	5.2±0.4^{a,b}
Çakıldak	0.48±0.10^{a}	1.5±0.25^{a}	3.3±0.34^{b,c,d}	0.39±0.09^{c}	5.6±0.4^{a,b}
Kan	0.41±0.03^{a}	2.2±0.23^{a}	0.6±0.18^{a,b}	0.14±0.03^{a,b}	3.4±0.3^{a}
Uzun Musa	0.51±0.03^{a}	1.7±0.09^{a}	5.8±1.91^{d}	0.31±0.09^{b,c}	8.3±1.8^{b}
Acı	0.22±0.03^{a}	2.0±0.39^{a}	1.5±0.10^{a,b,c}	0.11±0.02^{a}	3.8±0.9^{a}
Tombul	0.25±0.08^{a}	1.2±0.10^{a}	3.10±0.44^{a,b,c}	0.27±0.09^{a,b,c}	4.9±0.4^{a}

*Superscript letters in each column indicate statistically significant difference ($p<0.05$) according to Duncan's test. Three independent hazelnut skin sample were analyzed with two analytical measurements.

2.3.12 Total Antioxidant Capacity of Hazelnuts and Hazelnut Skins

Total antioxidant activity of hazelnut and hazelnut skins are expressed as Trolox equivalent antioxidant capacity in Table 2.16. Total antioxidant capacity of hazelnut varieties ranged from 5.4 to 8.8 µmol Trolox equivalent/g hazelnut. Total antioxidant capacity of hazelnut skins, with an average value of 878 µmol Trolox equivalent/g, was more than 100 times higher than hazelnuts. Among varieties, skin of Foşa was found to contain lowest amount of antioxidants with 309 µmol Trolox equivalent/g while skin of Çakıldak had highest antioxidant capacity with 1375 µmol Trolox equivalent/g. Total antioxidant capacity of hazelnut skins were found to be in accordance with total phenolic compounds. Locatelli et al [195] was found total antioxidant capacity of medium roasted

defatted skins as 1.10 mmol Trolox equivalent/g and 0.94 mmol Trolox equivalent/g for high roasted defatted hazelnut skins. Antioxidant capacity of hazelnut skin was 10 times higher than even from the most antioxidant rich cereal, buckwheat [188]. In addition to that, antioxidant rich foods like cinnamon, dark chocolate (70% cocoa), blueberry, black filtered coffee and green tea were reported to have antioxidant capacity of 984, 134, 82, 28 and 25 μmol Trolox equivalent/g or mL, respectively [196]. Moreover, 1 gram of the most antioxidant rich hazelnut skin, belong to variety Çakıldak, could compensate about 1.4 g of cinnamon, 10 g of dark chocolate, 16.7 g of blueberry, 49.1 mL of black filtered coffee or 55 mL of green tea. However, these values could change among varieties as one gram of the least antioxidant containing skin variety, Foşa, compensated 0.3 g of cinnamon, 2.3 g of dark chocolate, 3.8 g of blueberry, 11.0 mL of black filtered coffee or 12.4 mL green coffee.

Table 2.16. Total antioxidant capacity of hazelnuts and hazelnut skins (μmol Trolox equivalent/g)*

	Hazelnut	Hazelnut Skin
Kargalak	$7.8\pm0.5^{a,b,c}$	1153 ± 120^{g}
Palaz	$7.1\pm0.2^{a,b,c}$	$887\pm3^{d,e,f}$
İncekara	$7.5\pm1.5^{a,b,c}$	$875\pm37^{d,e,f}$
Sivri	$8.0\pm1.4^{b,c}$	$775\pm139^{c,d,e}$
Yassı Badem	$6.5\pm1.1^{a,b,c}$	$462\pm50^{a,b}$
Foşa	$6.6\pm0.5^{a,b,c}$	309 ± 14^{a}
Kalınkara	$7.0\pm0.3^{a,b,c}$	$934\pm14^{e,f}$
Yuvarlak	$6.2\pm0.2^{a,b}$	$706\pm17^{c,d}$
Kuş	$8.3\pm1.1^{b,c}$	$613\pm56^{b,c}$
Çakıldak	$6.8\pm0.8^{a,b,c}$	1375 ± 171^{h}
Kan	5.4 ± 0.5^{a}	$952\pm117^{e,f}$
Uzun Musa	$5.9\pm0.3^{a,b}$	1343 ± 233^{h}
Acı	$7.7\pm1.5^{a,b,c}$	$881\pm24^{d,e,f}$
Tombul	8.8 ± 1.5^{c}	$1027\pm171^{f,g}$

*Superscript letters in each column indicate statistically significant difference ($p<0.05$) according to Duncan's test. Three independent hazelnut skin and hazelnut sample were analyzed with two analytical measurements.

2.4 Conclusion

In conclusion, proximate composition and particularly the characteristics of the non-lipid and lipid part of fourteen hazelnut varieties were comprehensively evaluated for two consecutive harvest years, 2013 and 2014. The data reported here provides in depth information on the contents of amino acids, water-soluble vitamins, mineral elements, sugars and organic acids in hazelnut varieties grown in Turkey. Glutamic acid, arginine and aspartic acid were the most predominant amino acids. Individual amino acid profiles showed significant differences depending upon the harvest year ($p<0.05$). Sucrose concentration was the highest followed by fructose, glucose, stachyose, raffinose and myo-inositol, respectively. Phytic acid was predominant organic acid in all varieties, followed by malic acid. Independent of the variety, hazelnuts were rich in pantothenic acid, nicotinic acid, pyridoxal, biotin, thiamine, nicotinamide. Pantothenic and nicotinic acid were significantly higher in most of the varieties in harvest year 2014. Potassium was the most predominant mineral, followed by magnesium, calcium, sodium, manganese, zinc, iron and copper, respectively. Hazelnut varieties were found to be rich in oleic acid containing triacylglycerols, namely OOO and OOL, while they contain lower amounts of stearic acid

containing triacylglycerols, namely SOO, independently of the harvest years. Significant statistical differences in most of triacylglycerols were determined considering hazelnut varieties and harvest years ($p<0.05$). Hazelnut varieties were found very rich in monounsaturated fatty acids, oleic acid (more than 74%), followed by linoleic (more than 5.5%), palmitic and stearic acids. Unsaturated/saturated fatty acid ratio was found to be relatively higher than most of the vegetable oils. All hazelnut varieties were found to contain α-, β-, and γ-tocopherol, but not δ-tocopherol. Taking the total tocopherol contents into account, Tombul, which is the most commercialized hazelnut variety in Turkey, was found to be the richest among others for both harvest seasons. Hazelnut skin could be considered as a good source of bioactive compounds in comparison to its kernel. Bioactive profile and distribution of bioactive compounds in skin of hazelnut varieties was enlightened in detail. Phenolic compounds, flavonoids and phenolic acids were found to be rich in conjugated soluble fraction. Total phenolic compounds were lowest in skin of Foşa and highest in skin of Çakıldak. Total flavonoid content of hazelnuts was 60% of total phenolic compounds. Moreover, hazelnut skin was found to contain two times higher amount of tocopherols, especially α-tocopherol, than hazelnut itself. Total antioxidant capacity of hazelnut skin is much higher (>100 times) than hazelnut and many other foods previously reported. Total antioxidant capacity was in parallel with total phenolic compounds and the skin of Foşa was the least antioxidant containing variety and Çakıldak was the highest. Although bioactive profile of hazelnut varieties changed in a wide range, hazelnut skins belong to all varieties were known to have positive effects on health. Therefore, their consumption while intact with hazelnut kernel or as an ingredient of other foods should be taken into consideration. Identification of compositional characteristics not only allowed to understand the reactants in the hazelnut but also helped to reveal nutritional quality of Turkish hazelnut varieties and their changes with the harvest year.

3 EFFECT OF ROASTING ON THE FORMATION OF COMMON PRODUCTS OF MAILLARD REACTION, SUGAR DEGRADATION AND LIPID OXIDATION PRODUCTS

3.1 Introduction

Chemical reactions that are responsible for the changes in hazelnuts during roasting are Maillard reaction, caramelization and lipid oxidation. The nature of hazelnuts and the roasting conditions are suitable for the proceeding of these reactions. The reactions and the products occurring in the hazelnuts during roasting are summarized in Figure 3.1.

In the early stages of Maillard reaction, reducing sugars condense with an amino compound to form a Schiff base and consequently it rearranges to Amadori/Heyns product [78, 79]. The early stage of the Maillard reaction could be tracked with the formation of Amadori product of lysine, fructosyllysine, which is measured as furosine [82]. Later, in the advanced stage of Maillard reaction, Amadori compound degrades leading to formation of reactive α-dicarbonyl compounds such as 1-, 3- and 4-deoxyhexulose [197]. Fragmentation of these hexuloses and degradation of Amadori product result in the formation of shorter chain α-dicarbonyl compounds such as glyoxal, methylglyoxal and diacetyl [90, 95, 110, 117]. Further contribution of α-dicarbonyl compounds could lead to modification of protein bound reactive side chains of amino acids, that will be resulted in the formation of AGEs or advanced lipation products. CML, pyrraline, formyline, and maltosine are some of the AGEs and MP-lysine, 2-PPL, and cis/trans-BPP-lysine are some of the advanced lipation products quantified in food products [14, 119, 123, 127, 134, 135]. In the final stage of Maillard reaction, amino compounds and sugar fragments condense to form brown nitrogenous compounds called melanoidins [78]. Acrylamide is one of the process contaminants forms in the Maillard reaction from its precursor amino acid, asparagine [66].

Caramelization involves both sugar isomerization and sugar degradation reactions. Isomerization of monosaccharides generally starts with enolization followed by sugar degradation reactions [109]. Some of the products, including α-dicarbonyl compounds and HMF, which form during caramelization reactions are also common in Maillard reaction [95, 112]. In addition to the contribution of monosaccharides, disaccharides could also contribute to the formation of these common products. Degradation of sucrose could form HMF through fructofuranosyl cation [145]. Moreover, 3-DG is known to dehydrate to form 3,4-DG and consequently HMF [146]. Furan, as a toxic process contaminant, which

could originate from the degradation of glucose, fructose and lactose directly or in the presence of amines [198, 199].

Lipid oxidation also contributes to the formation of reactive carbonyl compounds which could promote Maillard reaction during hazelnut roasting. Among the reactive α-dicarbonyl compounds, glyoxal and methyglyoxal are known to be form through oxidation of especially polyunsaturated fatty acids [97]. Although these dicarbonyl compounds form during lipid oxidation, caramelization and Maillard reaction, it is hard to distinguish them in a complex food matrix. Oxidation of polyunsaturated fatty acids might also be responsible from the formation of furan [199].

As there are more than one pathway in the formation of the reaction products in hazelnuts during roasting, quantification of the reactants and products first could be a sensible way to understand the underlying mechanisms.

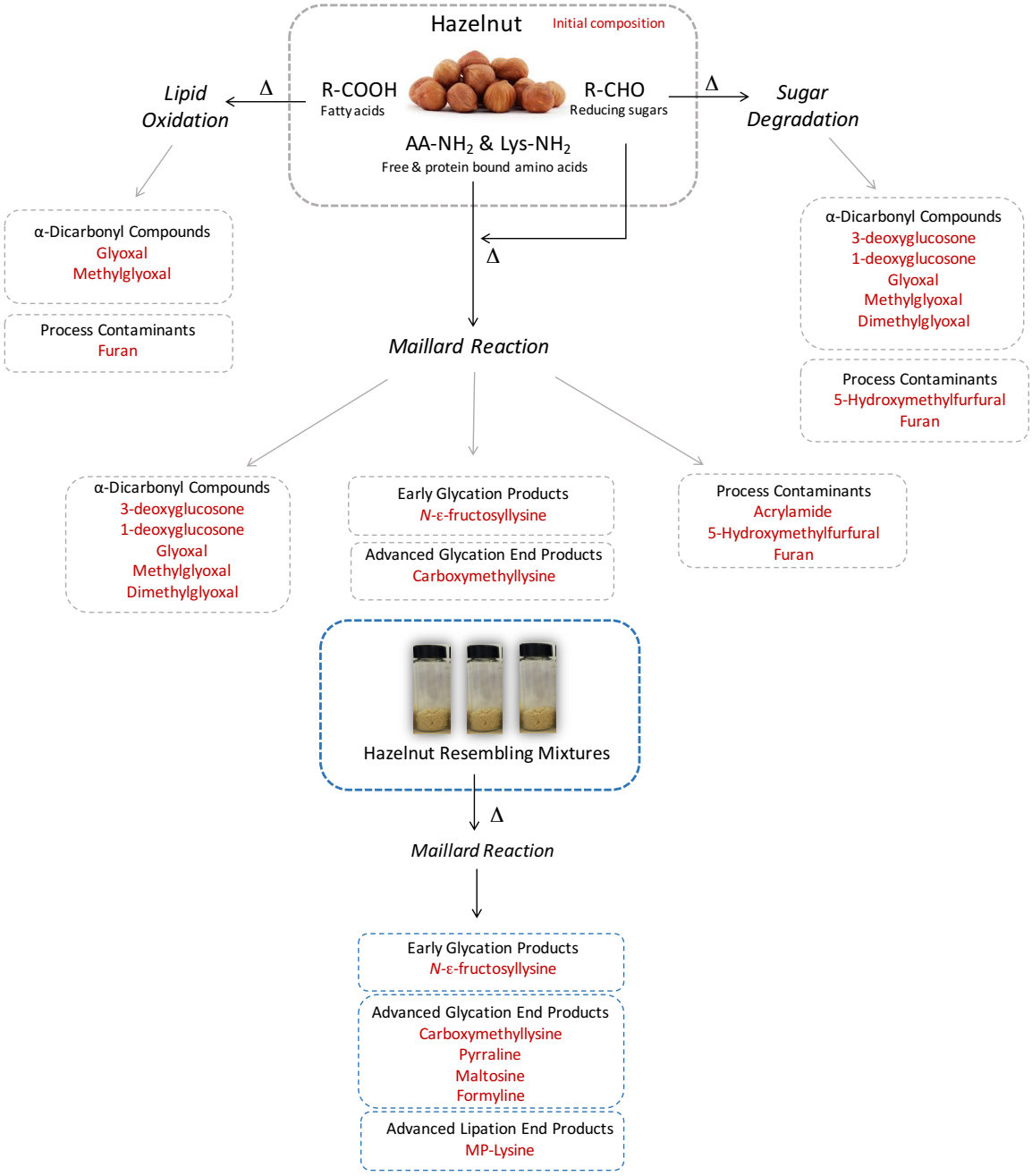


Figure 3.1. Chemical changes in hazelnuts during roasting

3.2 Materials and Methods

3.2.1 Chemicals and Consumables

High purity (>99%) sucrose, glucose, and fructose were purchased from Sigma-Aldrich (Diesenhofen, Germany). All amino acids (>98%) were purchased from Merck Co. (Darmstadt, Germany). HMF (98%) was purchased from Acros (Geel, Belgium). Furan (99%) was purchased from Merck (Darmstadt, Germany). Acrylamide, 3-deoxyglucosone (75%), quinoxaline (99%), 2-methylquinoxaline (97%), 2,3-dimethylquinoxaline (97%), o-phenylenediamine (98%), diethylenetriaminepentaacetic acid (DETAPAC) (98%), sodium

borohydride powder (≥98%), methanol, acetonitrile, and hexane were obtained from Sigma-Aldrich (Steinheim, Germany). Disodium hydrogen phosphate anhydrous and sodium dihydrogen phosphate dehydrate, potassium hexacyanoferrate (Carrez I), zinc sulfate (Carrez II), sodium hydroxide, sodium carbonate, boric acid, hydrochloric acid (37%), sulfuric acid (95-98%) were supplied from Merck (Darmstadt, Germany). Formic acid (98%) was purchased from JT Baker (Deventer, The Netherlands). Furosine standard was obtained from Neosystem Laboratoire (Strasbourg, France). Heptafluorobutyric acid was obtained from Alfa Aesar (Karlsruhe, Germany). Nonafluoropentanoic acid was purchased from Sigma-Aldrich (Taufkirchen, Germany). Pepsin (10 FIP-U/mg protein), and pronase E (4.000 PU/ mg protein), prolidase (106 U/mg protein), and aminopeptidase M (28 U/mg protein) were purchased from Merck (Darmstadt, Germany). N-benzoylglycyl-L-phenylalanine was purchased from Bachem (Bubendorf, Switzerland). Ammonium formate was obtained from Grüssing (Filsum, Germany). Ultra-pure water was used throughout the experiments (Milli Q-System, Millipore, Millford, MA, USA). Syringe filters (nylon, 0.45 μm), Oasis HLB and OASIS MCX cartridges were supplied by Waters (Milford, MA, USA). The previously synthesized standards of formyline, pyrraline, maltosine and MP-lysine were used at the laboratory of Prof. Dr. Thomas Henle at Technical University of Dresden.

3.2.2 Roasting of Hazelnuts

Hazelnuts (*Corylus avellana L.*) of Turkish variety Tombul were supplied from a local manufacturer (Giresun, Turkey). Five grams of unshelled hazelnuts were roasted in an oven at 150, 160, 170°C for 15, 30, 60, 90, 120 min. The selected roasting conditions represented both industrial and extreme conditions. As soon as the hazelnuts were taken out of the oven, they were placed to a freezer (-18°C). After taken out to the room temperature, hazelnut skins were easily removed by hand. Then, hazelnuts without skins were finely grounded and kept frozen at -18°C prior to analysis.

3.2.3 Defatting of Hazelnuts for Preparation of Oil:Non-fat Hazelnut Mixtures

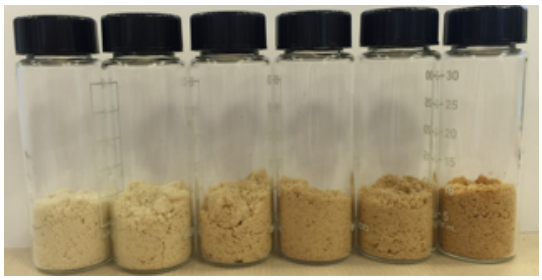
After grinding, 500 g of hazelnut was defatted by five times extraction with totally 2.5 L of hexane. The residual hexane was removed with a vacuum rotary evaporator at 40°C at about 15 min. The defatted hazelnut was put in a flow cabinet at room temperature for removal of the residual hexane completely. After then, non-fat hazelnut and hazelnut oil were obtained for the preparation of oil:non-fat hazelnut mixtures.

3.2.4 Preparation of Oil:Non-fat Hazelnut Mixtures

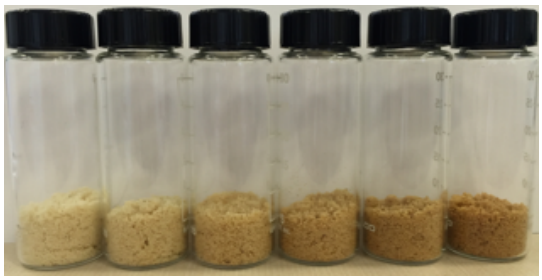
Non-fat hazelnut was mixed with three types of oils, which were hazelnut oil, paraffin oil and sunflower oil. The mixtures were prepared in the ratio of 1:1 and 2:1 (w/w) by mixing 30 g of oil with 30 g non-fat hazelnut and 60 g of oil with 30 g non-fat hazelnut, respectively. For hazelnut and paraffin oils, both 1:1 and 2:1 mixtures were prepared while only 1:1 ratio was prepared for sunflower oil. After the oil and non-fat hazelnut was taken into a mortar, they were mixed well to make a homogenous reaction medium.

3.2.5 Heating of Oil:Non-fat Hazelnut Mixtures

A 3 g portions of hazelnut oil:non-fat hazelnut (1:1), hazelnut oil:non-fat hazelnut (2:1), paraffin oil:non-fat hazelnut (1:1), paraffin oil:non-fat hazelnut (2:1) and sunflower oil: non-fat hazelnut (1:1) mixtures were weighted into glass headspace vials to perform roasting. The vials were placed into the oven without their caps and only one vial was placed at each roasting. Heating was performed twice for each mixture at 150°C for 15, 30, 45, 60, and 90 min, respectively. After heating, the vials were placed to -18°C and kept frozen until the analyses were performed.



(a)



(b)

Figure 3.2. Non-treated and hazelnut oil:non-fat hazelnut mixtures heated at 150°C for 15, 30, 45, 60, and 90 min (a) 1:1 (b) 2:1

3.2.6 Extraction

Ground hazelnut sample (0.50 g) was weighted into tubes and triple extraction was

performed by using 10 mL water (5, 2.5, 2.5 mL). The tube was vortexed for 3 min and centrifuged at 5000 x *g* for 5 min at each step of extraction. Then, supernatants were transferred to another tube and centrifuged at 5000 x *g* for 3 min. The extracts were used for the analysis of sugars, free amino acids, HMF, α-dicarbonyl compounds, and acrylamide.

3.2.7 Acid Hydrolysis

For the analysis of protein bound amino acids and furosine in either hazelnut samples or oil: non-fat hazelnut mixtures, 50 mg of hazelnut or mixture was weighted into a glass tube and 5 mL of 8 N HCl was added onto it. After nitrogen gas flushing to the headspace, screw caps were closed tightly. The tubes were kept at 110°C for 23 h until all the amino acids were totally hydrolyzed.

For the analysis of MP-lysine in oil:non-fat hazelnut mixtures, 80 mg of mixture was weighted into glass tubes and 4 mL of 6 N HCl was added onto it. The tubes were immersed into a sand bath in an oven at 110°C and kept there for 23 h. Then, the hydrolysates were filtered through filter papers in order to have a clear acid hydrolysate.

3.2.8 Enzymatic Hydrolysis

Enzymatic hydrolysis for the analysis of formyline, pyrraline and maltosine was performed as described previously elsewhere [123, 127]. A 20 mg portion from the oil:non-fat hazelnut mixtures was put into glass tubes and suspended with 1 mL of 0.02 N HCL containing thymol. Then, 50 µL of pepsin (1 FIP-U of pepsin) was added onto it and the caps of the glass tubes were closed. The samples were left for incubation for 24h at 37°C. At the end of incubation, 250 µL of TRIS buffer and 50 µL pronase E (400 PU) were added. 24 h later, 20 µL prolidase (1 U) and 4 µL aminopeptidase (0.4 U) were added. After 24 h, lyophilization was performed and the samples were reconstituted with 1 mL of distilled water. Then, the samples were filtered through 0.45 µm nylon filters.

3.2.9 Determination of Protein Content

Protein content of oil:non-fat hazelnut mixtures was determined with Kjeldahl method [178] by using the factor 6.25.

3.2.10 Analysis of Sugars

A part of extract of hazelnut samples was clarified by using Carrez I and Carrez II solutions and then the tubes were centrifuged at 5000 x *g* for 5 min. Before analysis, extracts were filtered through a 0.45 µm nylon syringe filters and taken into vials. Analysis

of sugars was performed as described by Kocadağlı and Gökmen [99].

3.2.11 Analysis of Free Amino Acids and Protein Bound Lysine

For the analysis of free amino acids of hazelnut samples, a part of the extract was mixed with an equal volume of acetonitrile and centrifuged at 5000 x *g* for 3 min. After then, the mixture was passed through a 0.45 μm nylon filter and collected in a vial.

For the analysis of protein bound lysine in hazelnut samples acid hydrolysis was performed as mentioned above. Then, 100 μL of hydrolyzate was transferred to a glass tube and dried under a gentle stream of nitrogen. The final residue was redissolved in 1 mL of the mixture of acetonitrile:water (1:1, v/v) and filtered through a 0.45 μm filter into a vial.

The analysis of free amino acids and protein bound lysine was performed as described by Kocadağlı et al [179]. The total amino acids of roasted hazelnut samples were expressed as mg/kg hazelnut and the it was the sum of concentration of free amino acids and protein bound lysine. Protein bound lysine was expressed as mg/kg protein.

3.2.12 Analysis of 5-Hydroxymethylfurfural

The extracts of hazelnut samples were passed through a 0.45 μm nylon filter and collected in vials. Analysis was performed as described by Kocadağlı et al [200].

3.2.13 Analysis of α-Dicarbonyl Compounds

Derivatization of α-dicarbonyl compounds was carried out with o-phenylenediamine according to a previously described procedure with minor modifications [103]. Two hundred μL of the extract of hazelnut samples was diluted with 800 μL of the mixture of acetonitrile:water (5:3, v/v), and centrifuged at 5000 x *g* for 5 min to precipitate colloidal particles. The derivatization of 0.5 mL supernatant was performed by adding 150 μL of 0.5 M sodium phosphate buffer (pH 7) and 150 μL of 0.2% o-phenylenediamine in 10 mM DETAPAC. The mixture was immediately filtered through 0.45 μm nylon syringe filter and kept at room temperature in the dark for 2 h prior to HPLC-ESI-MS measurement. Analysis was performed as described previously by Kocadağlı and Gökmen [105].

3.2.14 Analysis of Acrylamide

Acrylamide was extracted from hazelnut matrix by using a multiple stage extraction strategy as described by Gökmen et al [201]. The analysis of acrylamide was performed as previously described by Kocadağlı et al [202].

3.2.15 Analysis of Furan

A set of roasting experiments was also performed for furan analysis. After roasting, hazelnuts were left at the room temperature for an hour and grounded. Then, 0.7 g of hazelnut samples were weighted in headspace vials and the caps were screwed immediately. Furan content of hazelnut samples was determined as previously described by Mogol and Gökmen [203].

3.2.16 Analysis of Furosine

A hundred μL of acid hydrolyzates were transferred to centrifuge tubes. Then, the hydrolyzates in the centrifuge tubes were dried under nitrogen gas. After then, the content was dissolved with 1 mL deionized water, passed through a pre-conditioned OASIS HLB cartridge and collected in a vial. Analysis of furosine was performed as described by Gökmen et al [204].

3.2.17 Analysis of Carboxymethyllysine

CML extraction was performed as described by Charissou et al [205] with slight modifications. Twenty mg of ground hazelnut sample was weighted in a glass tube and 100 μL of water was added onto it. Then, 450 μL of sodium borate buffer (0.2 M prepared by adjusting the pH of 0.2 M boric acid to pH 9.2 with 0.2 M NaOH) and 500 μL of sodium borohydride (1 M prepared by using 0.1 M NaOH) were added into tube. The tubes were incubated at room temperature for 4 hours in order to turn fructosyllysine to hexitol lysine. After then, 2 mL of 8 N HCl was added to tubes and the tubes were tightly closed with their caps under the stream of nitrogen gas. Hydrolysis was performed at 110°C for 24 hours. Twenty μL of hydrolyzates were transferred to glass tubes and dried under nitrogen gas until to obtain complete drynesss. Then, the content was dissolved with 1 mL of water and passed through a preconditioned OASIS HLB cartridges. After discarding the first 8 drops of the eluent, the rest was collected in vials. The CML analyses were performed as described by Akıllıoğlu and Gökmen [206].

3.2.18 Analysis of Pyrraline

Pyrraline analysis of oil:non-fat hazelnut mixtures was performed as described previously [123]. A Kanuer WellChrom HPLC system (Kanuer, Berlin, Germany) consisting of a K-1001 pump, with online degasser, a column, and a K-2700 UV dedector was used for the analysis. Chromatographic separation was performed on a stainless steel column filled with Eurosphere 100 C-18 material and the guard column (5 x 4 mm) of the same material.

The injection volume was 50 μL. The column temperature was 30°C. As mobile phase; (A) 5 mM heptaflourobutyric acid (HFBA), 5 mM ammonium formate (pH 2.8) in H_2O and (B) mixture of 60% acetonitrile and 40% 5 mM HFBA, 5 mM ammonium formate (pH 2.8) in H_2O solution (Eluent A). The gradient was as follows: initially 3% B and linearly increased to 40% B in 2 min, linear between 2 and 16 min with 40% B, decreased to initial conditions linearly in 19-22 min and stay at initial conditions (3% B) for 5 min. Flow rate was 1 mL/min. The absorbance was recorded at 297 nm. Quantification was performed based on the external calibration. Standards of pyrraline were added to the clear enzymatic hydrolysis extracts of each sample.

3.2.19 Analysis of Formyline and Maltosine

Maltosine and formyline analysis of oil:non-fat hazelnut mixtures were performed as described previously by Hellwig et al [127]. The analysis was performed on the HPLC 1200 Series (Agilent Techologies, Böblingen, Germany), containing a binary pump, an autosampler, a column oven, a degasser and a diode array dedector. The HPLC system was coupled to a Triple Quad Mass Spectrometer Agilent 6410. Chromatographic separation was performed on Zorbax 300 SB-C18 column (5 x 2.1 mm, 3.5 μm) (Agilent Technologies, Germany). The injection volume was 10 μL and the column temperature was 30°C. Mobile phases were (A) 10 nm nonafluoropentanoic acid in water and (B) 10 nm nonafluoropentanoic acid in acetonitrile. To obtain a well separation in the column, a gradient program was used with a total time of 32 min. The gradient was 5% B initially, linearly increased to 50% B in 25 min and then 85% B in 1 min. After 5 min with 85% B, decreased to 5% B in 1 min to the initial conditions.

Measurements were performed in MRM mode and the detector was operated in positive ionization mode. The capillary voltage was 4000 V and the capillary temperature was 350°C. The fragmentor voltage used for the maltosine (m/z 255) was 120 V. The collision energies of its product ions were 10 eV for 255>130 and 255>126, and 20 eV for 255>84.The fragmentor voltage for formyline (m/z 225) was 80V. The collision energies of its product ions were 10 eV for 225>161 and 20 eV for 225>134. The fragmentor ion m/z 84 was used for the quantification of maltosine while m/z 126 and 130 were used for confirmation. For the quantification of formyline, the fragmentor ion, m/z 134, was used and m/z 161 was used for quantitation. External calibration was performed by addition of standards of maltosine and formyline to the clear extracts of enzymatic hydrolysis. Data

were collected and evaluated by using Mass Hunter B.02.00 (Agilent Technologies, Germany).

3.2.20 Analysis of MP-Lysine

The analysis of MP-lysine was performed as described by Globisch et al [135]. One mL of the acid hydrolyzates of oil:non-fat hazelnut mixtures was dried by using vacou SpeedVac vacuum concentrator (Thermo Fischer Scientific, Waltham, USA). Then, redissolved in 280 µL 10 nM nonafluoropentanoic acid in H_2O: 10 nM nonafluoropentanoic acid in acetonitrile (50:50, v/v) and 20 µL 3 pmol N-benzoylglycyl-L-phenylalanine in H_2O:methanol (50:50, v/v). After then, the samples were filtered through a 0.45 µm nylon filter before subjected to analysis.

An Agilent 1200 series HPLC system (Agilent Technologies, Böblingen, Germany) coupled with an Agilent 6410 triple quadrupole mass spectrometer was used to determine the concentration of MP-lysine in oil:non-fat hazelnut mixtures. Chromatographic separation was performed on Eurosphere-100-5 C18 column (250 x 3 mm, 5 µm) (Knauer, Berlin, Germany). Injection volume was 10 µL and the temperature was 30°C. The mobile phase was (A) 10 nm nonafluoropentanoic acid in water and (B) 10 nm nonafluoropentanoic acid in acetonitrile. A gradient program was applied as follows: 2% B initially, increased to 27% B in 20 min linearly and increased from 27% to 90% B between 20 and 25 min, remained at 90% B for 10 min, then decreased to 2% in 2 min. The total run time was 37 min and the flow rate was 0.38 mL/min. The measurements were performed in MRM mode in positive ionization mode. The capillary voltage was 4000 V and the source temperature was 350°C. Fragmentor voltage for MP-lysine (m/z 223.1) was 129 V, the collision energies of precursor ions were 11 eV for m/z 223.1>94.1 and 20 eV for 223.1>84.1. N-benzoylglycyl-L-phenylalanine was used as internal standard. The precursor ion voltage for N-benzoylglycyl-L-phenylalanine (m/z 327.1) was 90V and the collision energies for product ions were 4 eV for 327.1>166.1 and 32 eV for 327.1>105.1. For the quantitation of MP-lysine and the internal standard N-benzoylglycyl-L-phenylalanine, the signals of the m/z 223.1>84.1 and m/z 327>166.1 were used, respectively. Quantification was performed by addition of MP-lysine standard and 3 pmol of N-benzoylglycyl-L-phenylalanine in redisolved hydrolysate matrix.

3.2.21 Analysis of Color

Color of hazelnuts was measured by a computer vision based image analysis technique as

given previously [207]. The surface color of hazelnuts was given as L^* (lightness), a^* (redness), and b^* (yellowness) values together with the images of the hazelnuts.

3.2.22 Statistical Analysis

Data was given as mean ± standard deviation. All measurements were performed twice.

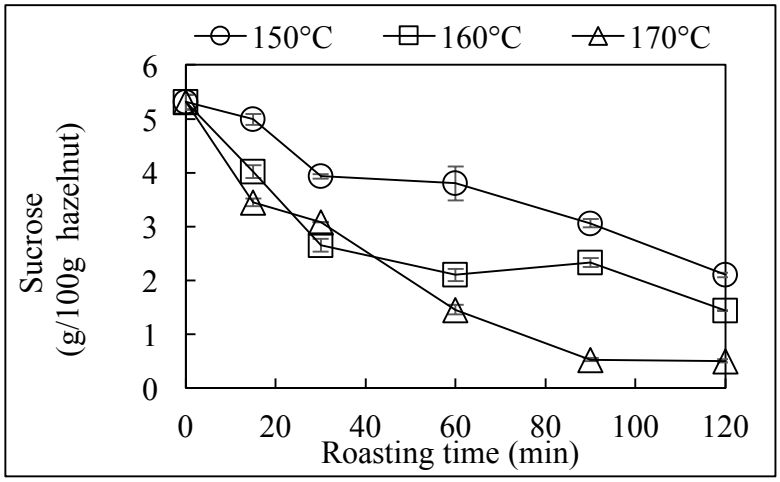
3.3 Results and Discussion

3.3.1 Roasting Induced Changes in Hazelnut

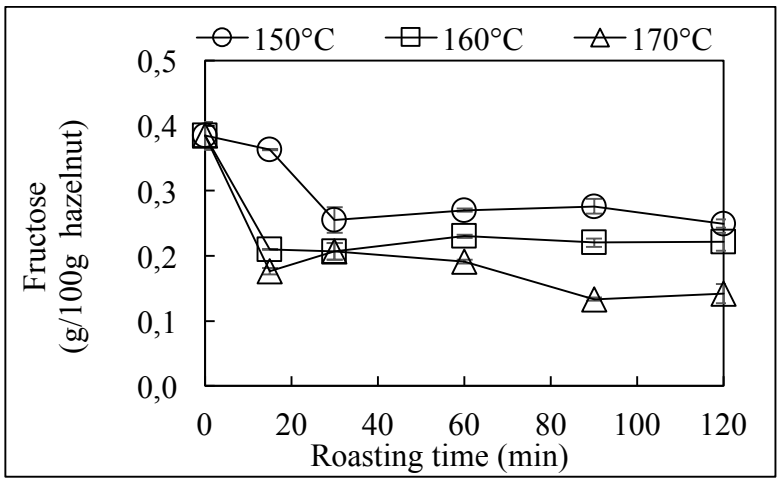
3.3.1.1 Degradation of Sugars and Amino Acids

Reactants in hazelnuts which are involved in Maillard reaction and sugar degradation reactions are mainly sugars and amino side chains of protein bound amino acids as well as free amino acids themselves. Lipids, especially unsaturated fatty acids, are prone to oxidation and they trigger the formation of lipid oxidation products. However, fatty acid composition of hazelnuts was found not to change significantly ($p>0.05$) at high degrees of roasting (data not given). That does not mean lipid oxidation is not responsible from any of the reactions in hazelnuts. Lipid oxidation may not be quantifiable by the change in fatty acid composition.

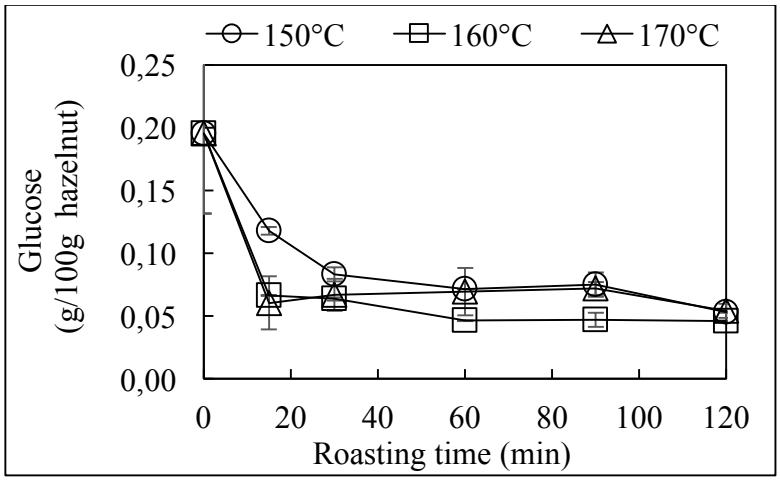
Sugars are the reactants of both Maillard reaction and sugar degradation. Sucrose is the most predominant sugar in hazelnuts that represents 80-90% of total sugars [8]. Initial sucrose content of hazelnuts was 5.5±0.1 g/100 g dry weight (dw). The loss of sucrose was 60, 70 and 90% after roasting at 150, 160 and 170°C for 120 min. The other sugars in hazelnuts were fructose and glucose with an initial concentration of 0.4±0.02 g/100 g dw and 0.2±0.06 g/100 g dw, respectively. There was a significant ($p<0.05$) decrease in the concentration of fructose and glucose after 30 min of roasting at 150°C (0.26±0.02 and 0.08±0.01 g/100 g dw, respectively), after 15 min of roasting at 160°C (0.21±0.01 and 0.07±0.01 g/100 g dw, respectively) and 170°C (0.18±0.01 and 0.06±0.02 g/100 g dw, respectively). Changes in the concentration of sucrose, fructose and glucose were given in Figure 3.3.



(a)



(b)



(c)

Figure 3.3. Changes in the concentrations of (a) sucrose, (b) fructose and (c) glucose during roasting (g/100g hazelnut)

Amino acids and side chains of proteins could participate a variety of reactions in foods. Maillard reaction is one of the most important reactions that amino compounds take parts. They could be involved in many other reactions such as Strecker degradation, acrylamide and furan formation, pyrolysis, and formation of heterocyclic amines [80]. Total free amino acids and protein bound lysine concentrations of raw hazelnuts were 2112±49 mg/kg dw and 5401±50 mg/kg dw, respectively. Changes in the concentration of individual free amino acids and protein bound lysine with roasting time and temperature were presented in Table 3.1. The reason for only addition of protein bound lysine to the total amino acids was the decrease in its concentration with roasting temperature and time. The concentration of total amino acids was 7513±87 mg/kg dw of hazelnut. A decrease of 68, 81 and 85% in total amino acids after roasting at 150, 160 and 170°C for 120 min was noted (Figure 3.4). The initial degradation rates of glucose and fructose were higher than the initial degradation rate of total amino acids. A possible explanation for this initial gradual decrease of total amino acids could be the regeneration of amino acids via the degradation of Amadori/Heyns product also reported by other researchers [99, 172].

Table 3.1. Changes in the concentration of individual free amino acids and protein bound lysine (mg/kg hazelnut) during hazelnut roasting

	Non-treated	150°C 15 min	150°C 30 min	150°C 60 min	150°C 90 min	150°C 120 min	160°C 15 min	160°C 30 min	160°C 60 min	160°C 90 min	160°C 120 min	170°C 15 min	170°C 30 min	170°C 60 min	170°C 90 min	170°C 120 min
Ala	359 ±3.1	261 ±2.1	161 ±1.5	65 ±0.7	42 ±0.5	47 ±0.6	278 ±1.6	115 ±3.3	27 ±2.2	29 ±0.8	16 ±1.1	191 ±1.1	39 ±4.3	24 ±2.4	12 ±2.1	21 ±2.8
Arg	205 ±1.2	135 ±4.6	52 ±1.5	21 ±1.3	11 ±2.5	15 ±2.1	60 ±1.0	42 ±1.6	10 ±0.9	9.1 ±0.5	6.7 ±1.1	162 ±1.9	19 ±0.4	6.9 ±2.9	8.5 ±0.5	6.2 ±1.8
Asn	35 ±1.1	18 ±1.4	18 ±1.4	6.2 ±0.2	3.6 ±0.3	4.0 ±0.4	35 ±2.1	3.8 ±0.0	2.2 ±0.0	2.3 ±0.0	1.9 ±0.7	11 ±0.6	1.5 ±0.6	3.2 ±0.9	3.0 ±0.1	3.2 ±0.8
Asp	115 ±2.1	91 ±0.6	70 ±2.1	31 ±0.0	19 ±0.1	18 ±0.2	113 ±6.5	40 ±2.2	16 ±0.1	17 ±0.3	10 ±0.6	69 ±1.2	38 ±3.4	16 ±1.5	21 ±1.7	17 ±1.2
Gln	37 ±2.3	3.6 ±0.4	1.4 ±0.1	1.1 ±0.1	1.1 ±0.0	1.2 ±0.2	2.3 ±0.3	1.1 ±0.1	0.9 ±0.0	0.9 ±0.0	0.8 ±0.0	10 ±9.5	1.0 ±0.0	1.0 ±0.2	0.9 ±0.1	0.9 ±0.0
Glu	388 ±20.1	438 ±9.8	155 ±1.5	51 ±2.2	43 ±0.6	32 ±0.9	241 ±4.9	64 ±2.8	22 ±1.9	31 ±1.3	29 ±1.7	220 ±24	49 ±5.0	22 ±3.0	23 ±0.9	26 ±1.4
Gly	89 ±3.3	46 ±0.6	26 ±2.1	14 ±1.0	9.7 ±0.3	9.9 ±0.5	54 ±1.9	27 ±0.1	13 ±0.7	14 ±1.7	8.4 ±1.2	35 ±1.3	20 ±3.4	13 ±2.0	6.5 ±0.5	13 ±0.9
His	37 ±0.4	17 ±0.0	6.9 ±0.1	3.2 ±0.0	2.1 ±0.1	2.2 ±0.1	14 ±0.0	5.4 ±0.0	2.3 ±0.1	1.9 ±0.2	1.8 ±0.2	10 ±2.1	3.6 ±0.2	2.3 ±0.2	2.0 ±0.0	2.1 ±0.0
Leu +Ile	211 ±5.2	77 ±0.3	16 ±0.4	9.0 ±0.1	2.5 ±0.1	2.9 ±0.5	49 ±2.3	2.7 ±1.3	3.0 ±0.8	0.5 ±0.4	0.2 ±0.0	146 ±137	6.7 ±1.2	0.2 ±0.0	0.3 ±0.1	0.8 ±0.4
Lys	63 ±2.3	23 ±1.3	12 ±0.4	6.6 ±0.1	5.3 ±0.1	6.1 ±0.1	19 ±0.8	12 ±0.4	7.5 ±0.8	5.6 ±0.0	5.0 ±0.0	20 ±7.1	7.6 ±0.2	6.0 ±0.1	5.1 ±0.1	6.1 ±0.5
Met	23 ±0.4	5.6 ±0.7	1.7 ±0.0	0.6 ±0.0	0.6 ±0.0	0.7 ±0.2	2.8 ±0.2	0.7 ±0.0	0.5 ±0.0	0.5 ±0.1	0.4 ±0.0	32 ±32	0.5 ±0.0	0.5 ±0.0	0.4 ±0.0	0.5 ±0.1
Phe	69 ±0.3	23 ±1.8	17 ±0.6	11 ±0.0	6.6 ±0.1	5.9 ±0.1	49 ±0.3	11 ±0.6	3.9 ±0.0	3.0 ±0.1	3.4 ±0.1	94 ±75	5.5 ±0.4	2.9 ±0.0	3.1 ±0.0	3.9 ±0.2
Pro	66 ±0.9	40 ±0.6	22 ±0.4	6.3 ±0.9	5.7 ±0.3	2.1 ±0.7	31 ±0.8	15 ±0.3	2.0 ±1.5	1.9 ±0.2	1.3 ±0.1	27 ±0.3	3.0 ±1.1	1.7 ±0.3	1.5 ±0.0	2.1 ±0.1
Ser	70 ±1.1	38 ±0.9	15 ±0.8	9.1 ±0.3	4.0 ±0.1	6.0 ±0.4	35 ±1.2	21 ±0.6	8.1 ±0.3	6.3 ±0.7	4.0 ±0.4	24 ±2.5	15 ±1.9	7.0 ±0.8	5.1 ±0.2	4.4 ±0.3
Thr	62 ±0.4	38 ±0.3	13 ±0.5	6.4 ±0.0	4.5 ±0.2	5.1 ±0.1	25 ±0.1	12 ±0.6	5.1 ±0.3	4.8 ±0.1	4.4 ±0.2	16 ±1.1	8.9 ±1.0	4.2 ±0.3	3.8 ±0.0	4.3 ±0.2

Trp	54 ±1.2	43 ±1.9	25 ±0.3	12 ±0.1	8.8 ±0.4	7.7 ±0.7	33 ±1.2	9.7 ±0.4	5.9 ±0.0	5.7 ±0.1	5.3 ±0.1	25 ±4.2	6.4 ±0.5	4.2 ±0.0	4.2 ±0.1	4.9 ±0.3
Tyr	51 ±1.1	32 ±1.1	14 ±0.1	7.3 ±0.1	5.4 ±0.2	5.9 ±0.1	25 ±0.9	13 ±0.2	5.7 ±0.2	4.2 ±0.1	4.1 ±0.2	50 ±35	5.5 ±0.1	3.8 ±0.0	5.2 ±0.0	4.4 ±0.1
Val	102 ±0.9	55 ±0.8	28 ±0.9	11 ±0.2	4.0 ±0.0	4.1 ±0.0	51 ±1.7	21 ±0.4	3.7 ±0.5	1.6 ±0.0	1.0 ±0.1	43 ±13	7.9 ±0.9	0.8 ±0.1	1.3 ±0.1	2.0 ±0.3
Protein bound Lys	5207 ±36	4777 ±16	3546 ±242	2592 ±52	2318 ±25	4452 ±40	3526 ±100	2634 ±263	1736 ±23	1380 ±114	3798 ±27	2782 ±46	1467 ±52	1056 ±55	1360 ±57	932 ±62

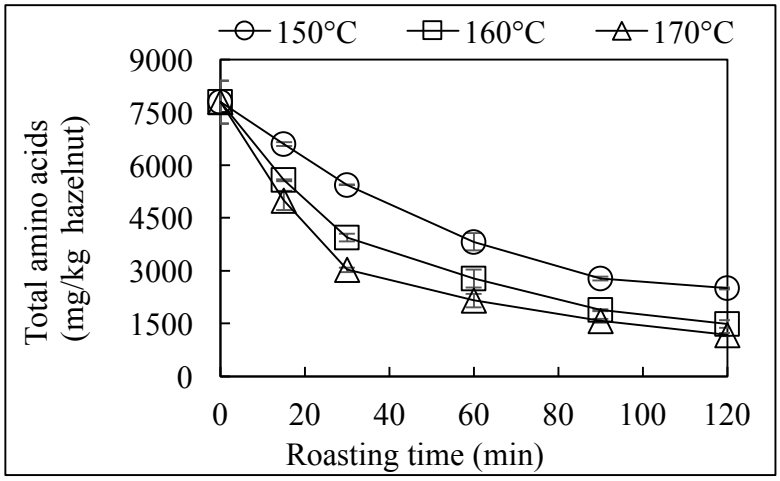


Figure 3.4. Changes in the concentration of total amino acids during roasting of hazelnuts (mg/kg hazelnut)

3.3.1.2 Formation of α-Dicarbonyl Compounds

α-Dicarbonyl compounds form mostly from Maillard reaction and sugar degradation. 3-DG originates from both glucose and its Amadori product, fructosyllysine via 1,2-enolization [78, 90]. The removal of one molecule water from glucose results in the formation of 3,4-DG and further dehydration forms HMF, whose formation is also possible from sucrose [78, 146, 208]. Formation of 1-DG could be via 2,3-enolization from fructose or ketoamines by decomposition of bound amino acids [80]. Dimethylglyoxal forms from 1-DG through degradation [95].

The roasting process induced the formation of 3-DG, 3,4-DG, and 1-DG in hazelnuts. To the best of our knowledge, there is no study regarding the formation of 3-DG in roasted hazelnuts in the literature. However, the levels of 3-DG concentration in roasted hazelnuts were relatively low in comparison to processed foods i.e. honey, jam, jellies and sweeteners, vinegars, candies and cookies [103]. Additionally, staples like bread, cooked pasta and potatoes were reported to contain 45, 1.2, and 6.9 mg/kg of 3-DG, respectively [103]. The concentration of 3-DG gradually increased with time during roasting at 150 and 160°C reaching the content of 6.7±0.1 and 6.1±0.1 mg/kg dw, respectively, within a roasting time of 120 min. However, the kinetics of 3-DG formation/elimination in hazelnuts was different at 170^{o}C. The concentration of 3-DG reached to an apparent maximum of 5.4±0.1 mg/kg dw within a roasting time of 60 min at 170^{o}C, then decreased gradually.

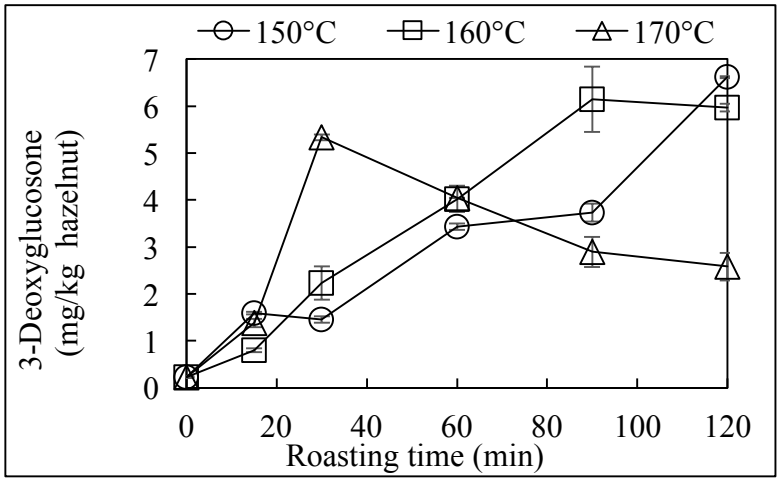


Figure 3.5. Formation of 3-deoxyglucosone during roasting of hazelnuts (mg/kg)

Formation/elimination of 3,4-DG in hazelnuts followed a similar trend with 3-DG, although a short lag phase was observed during roasting. The concentrations of 3,4-DG were approximately 5 times lower than those of 3-DG. Among the deoxyhexuloses formed in roasted hazelnuts, 1-DG was the least abundant one. The maximum concentrations of 1-DG in hazelnuts roasted at 150, 160 and 170°C were 0.22±0.01, 0.31±0.03, and 0.27±0.01 mg/kg dw, respectively.

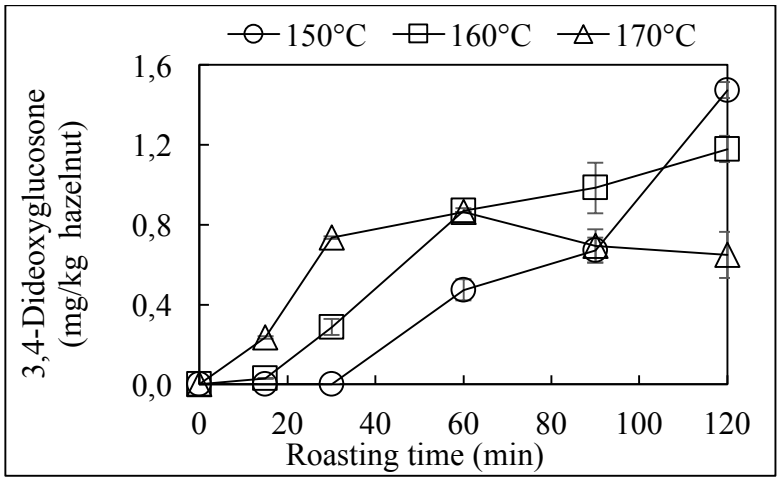


Figure 3.6. Formation of 3,4-dideoxyglucosone during roasting of hazelnuts (mg/kg hazelnut)

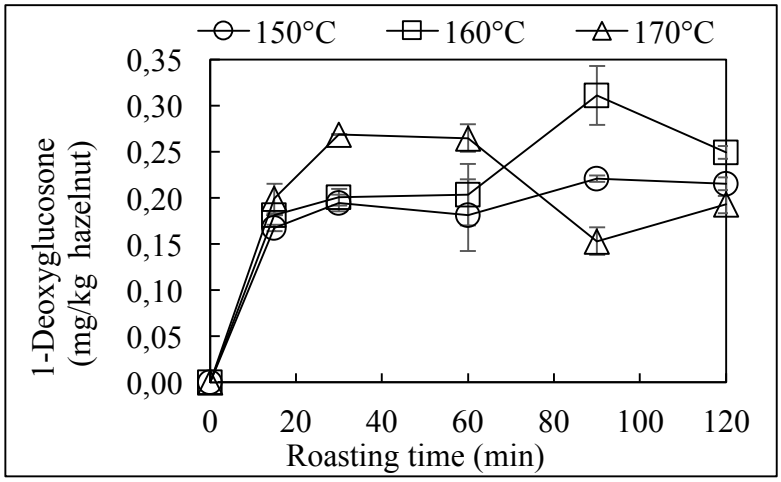
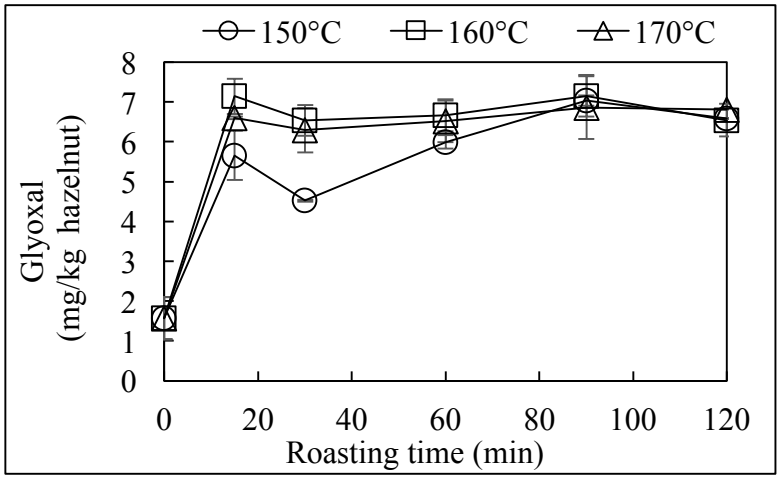


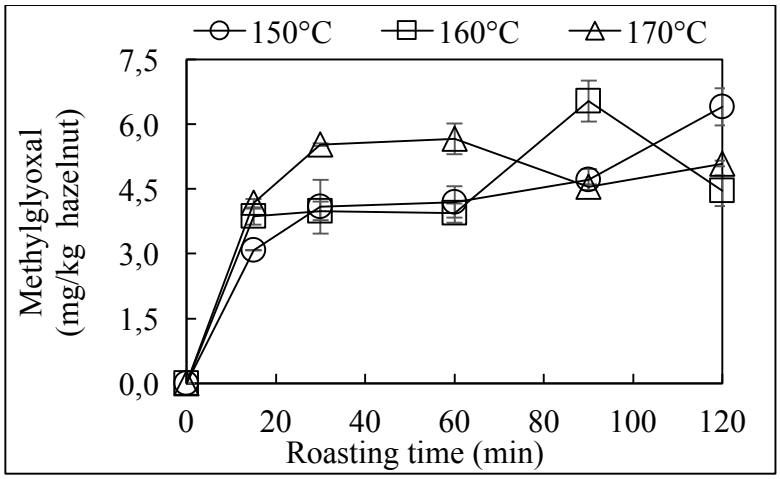
Figure 3.7. Formation of 1-deoxyglucosone during roasting of hazelnuts (mg/kg hazelnut)

Glyoxal, methylglyoxal and dimethylglyoxal were α-dicarbonyl compounds found in roasted hazelnuts that had shorter chain. Among these shorter chain dicarbonyl compounds glyoxal and methyglyoxal are the common products of sugar degradation, Maillard reaction and lipid oxidation [95, 97]. Glyoxal was the only α-dicarbonyl compound that was found also in raw hazelnuts (1.7±0.6 mg/kg dw). Its concentration increased up to 4 times after 15 min of roasting and did not change during prolonged roasting at all roasting temperatures. Methylglyoxal levels reached a maximum content at 6.6±0.5 mg/kg dw in hazelnuts roasted at 160°C for 90 min. Dimethylglyoxal formed at all roasting temperatures and increased to a certain extent, but there were no significant differences ($p>0.05$) in the concentration of dimethylglyoxal after roasting at 150 and 160°C for 120 min.

After heating at 200°C for 1 h, methylglyoxal and glyoxal formation in olive oil where the fatty acids profile is similar to hazelnut's oil, was reported as 0.61±0.03 mg/kg and around 0.5 mg/kg, respectively [97]. Methylgyoxal and glyoxal originated from hazelnut oil during roasting could not be expected to be higher than the values reported for heat treated (200°C 1 h) olive oil. It was notable that methylglyoxal and glyoxal concentrations of olive oil were almost 10 times lower than the maximum values found in hazelnuts. Therefore, most of the methylglyoxal and glyoxal formed in hazelnuts during roasting was considered to be originated from fractions other than lipids.



(a)



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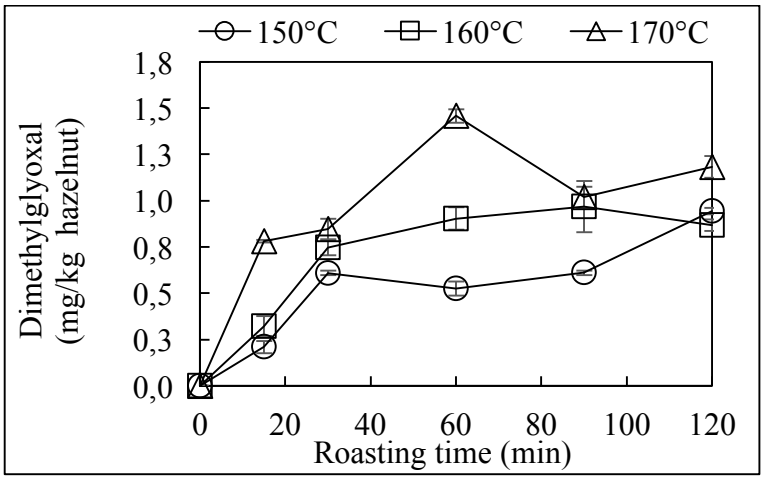


Figure 3.8. Formation of (a) glyoxal, (b) methylglyoxal and (c) dimethylglyoxal during roasting of hazelnuts (mg/kg hazelnut)

3.3.1.3 Formation of 5-Hydroxymethylfurfural

Formation of HMF in hazelnuts during roasting followed a typical kinetic pattern. Increase in the temperature during roasting accelerated the formation of HMF. The concentration of HMF reached to 104±0.5, 238±1.9, and 278±0.7 mg/kg dw after 120 min at 150, 160 and 170°C, respectively. Fallico et al [10] also reported a similar increase in the concentration of HMF in hazelnuts during roasting. Dietary intake of HMF was estimated for 1.6 mg/person/day by European Food Safety Authority [209]. Thus, a portion of hazelnut (30 g) might contain more than estimated value of HMF if roasting time at high temperatures is prolonged. However, this is not the case in the industry as the undesirable flavor compounds and color will be formed during high temperature long time treatments. Considering the generally used thermal treatment by industry at 145°C for 15 min, HMF will not be a potent risk.

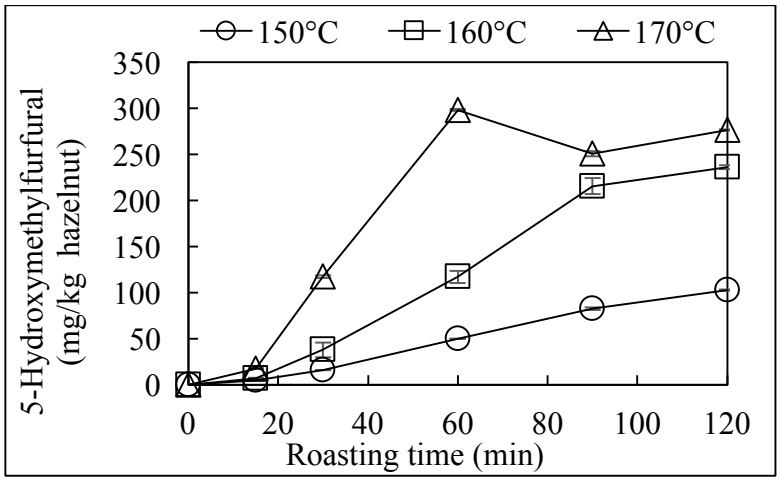


Figure 3.9. Formation of HMF during roasting of hazelnuts (mg/kg hazelnut)

3.3.1.4 Formation of Furan

Furan content of hazelnuts was increased with roasting time at 140°C, 150°C, 160°C and 170°C, respectively. Furan was reached to 0.5 ng/g at 140°C and 1.5 ng/g at 170°C after roasting for 60 min, respectively. According to Scientific Report of EFSA on furan levels in food [210], the highest levels were in coffee categories (45 ng/g for brewed coffee and 3660 ng/g in roasted coffee beans) and the lowest levels were in baby foods with a mean value of 3.2 ng/g.

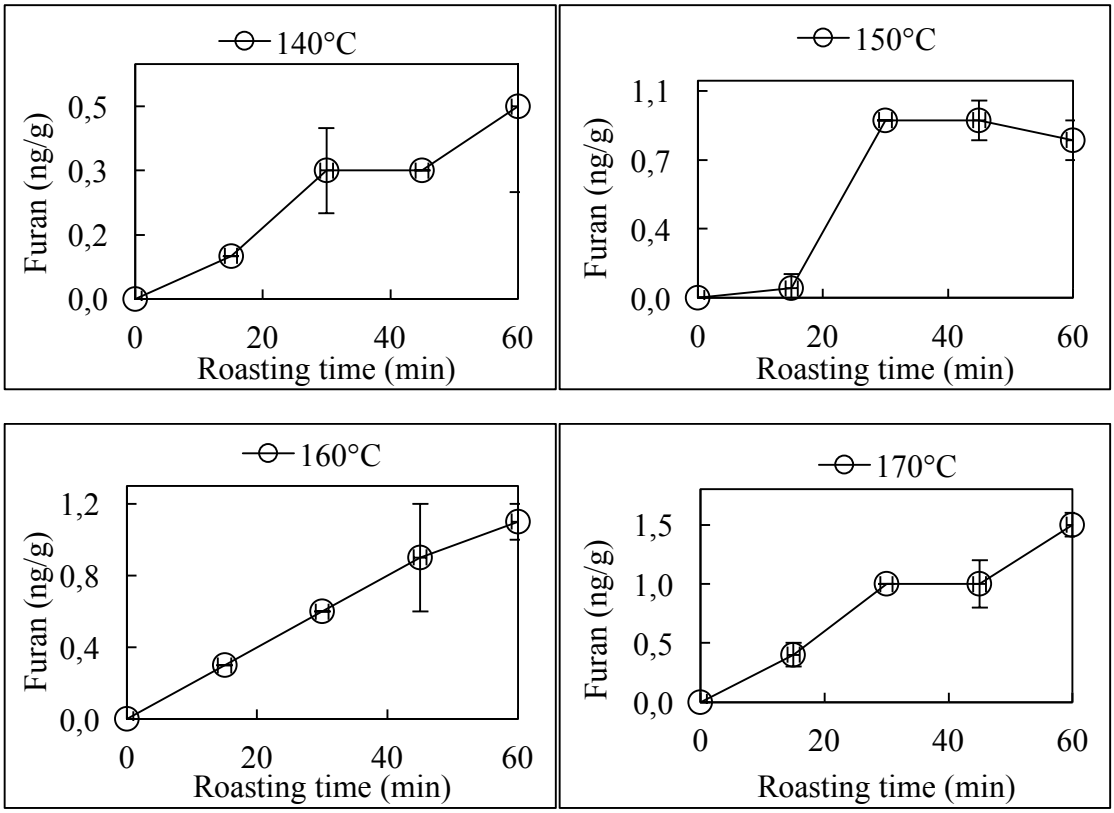


Figure 3.10. Formation of furan during roasting of hazelnuts (ng/g hazelnut)

A correlation between oxidation time and 5-pentylfuran formation was reported in olive oils [211]. Similarly, furan was proposed to be form from a highly toxic 4-hydroxy-2-butenal, which originates from the hemolytic cleavage of PUFA hydroperoxides [161]. Thermal degradation of glucose and fructose could also lead to the formation of furan [198] as well as vitamins (thiamine) [212]. Serine and cysteine are the direct precursors of furan and they could generate furan even in the absence of sugars while aspartic acid, alanine and threonine need the presence of the sugars [161]. It could be expected from roasted hazelnuts to have high furan contents as hazelnuts have all precursors except cysteine. However, highly volatile furan, as a contaminant, does not cause a concern in roasted hazelnuts as the furan concentration remaining in the hazelnut matrix was very low.

3.3.1.5 Formation of Acrylamide

Acrylamide, a possible carcinogen, was found to be not a concern in roasted hazelnuts. Acrylamide could not be detected in any of the roasted hazelnut samples independent of the roasting temperature and time. The reason for that was the asparagine, the precursor of acrylamide [66], was one of the least abundant amino acids in hazelnuts (Table 3.1). Amrein et al [11] reported 14-22 μg/kg acrylamide in roasted hazelnuts. They also

determined the acrylamide content of almonds during light roasting at 145°C (20-260 μg/kg) and dark roasting at 165°C (up to 1500 μg/kg). As the sugar contents of almonds and hazelnuts were found to be almost same, they concluded that asparagine was the limiting factor in the formation of acrylamide in hazelnuts, whose concentration was 40 times less in hazelnuts than found in almonds.

3.3.1.6 Formation of Early and Advanced Stage Markers of Maillard Reaction During Roasting of Hazelnuts

Furosine was determined as the early glycation marker of Maillard reaction in hazelnuts during roasting. The furosine concentration of hazelnuts reached to a maximum value of 449±15 mg/kg protein in hazelnuts roasted at 150°C for 15 min. The maximum concentrations were 392±35 mg/kg protein and 366±79 mg/kg protein at 160°C and 170°C for 15 min, respectively. There was no significant difference ($p>0.05$) in the maximum furosine concentrations of hazelnuts during roasting. After reaching to a maximum value within 15 min, furosine followed a decreasing trend during prolonged roasting times. The difference between roasting at 150°C and 170°C became significant ($p<0.05$) at roasting times higher than 15 min, indicating that degradation or oxidation of Amadori product increased more at higher temperatures during prolonged roasting.

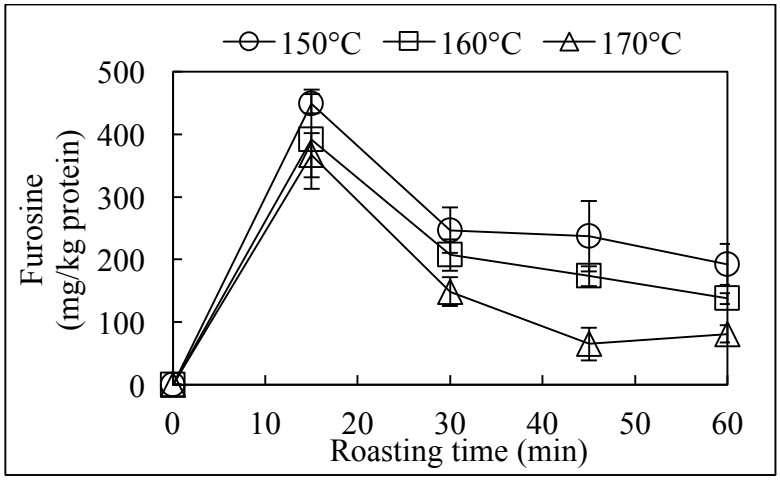


Figure 3.11. Formation of furosine during roasting of hazelnuts (mg/kg protein)

CML, as an indicator of advanced glycation, was found to increase during roasting of hazelnuts at 150°C and reached to 106±0.0 mg/kg protein at the end of 60 min of roasting. On the other hand, it reached to a maximum content of 100±27 mg/kg protein and 132±27 mg/kg protein at 160°C and 170°C after 45 and 30 min of roasting, respectively. Furosine concentrations of roasted hazelnuts were almost 4 times higher than CML concentrations.

Accordingly, Wellner et al [60] reported the furosine and CML concentrations of commercial roasted peanuts (n=5) to be 129-267 mg/kg protein and 50-77 mg/kg protein, respectively. Zhang et al [213] determined the CML concentrations of almonds during roasting. They performed roasting either at low temperature for long time or at high temperature for short time at different roasting temperatures (129-182°C) and times (3.2-70 min). They found the average CML values of each roasting temperature and reported that average total CML level of almonds roasted at 182°C was lower than average total CML values obtained at the lower temperatures.

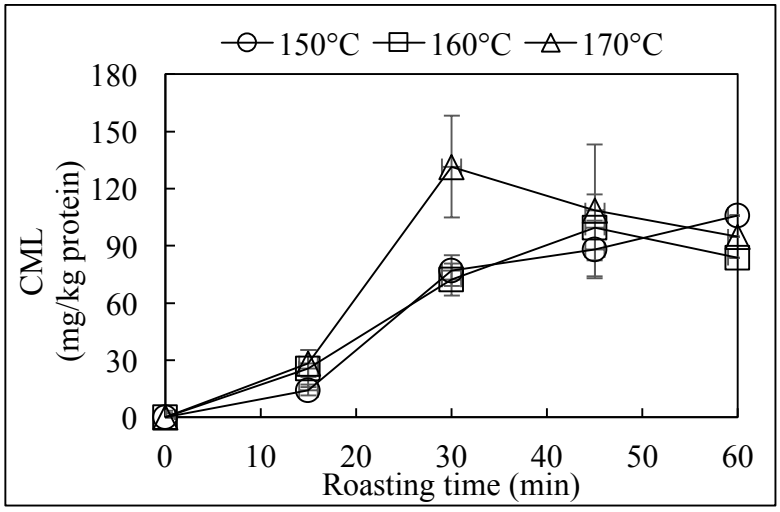
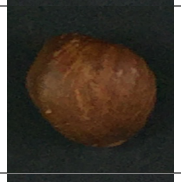
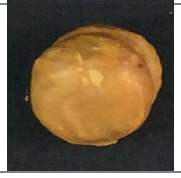
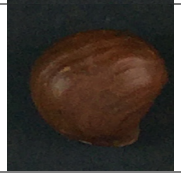


Figure 3.12. Formation of CML during roasting of hazelnuts (mg/kg protein)

3.3.1.7 Changes in Color of Hazelnuts During Roasting

The compounds formed in the early and advanced stages of the Maillard reaction are colorless compounds but in the final stage of Maillard reaction, high molecular weight brown colored compounds known as melanoidins are formed also with the contribution of sugar dehydration and lipid oxidation [78, 80]. Changes in the color of hazelnuts were expressed as *L*, a*, b** values and given in Table 3.2. *L** (lightness) values of hazelnuts decreased with increased roasting temperature and time, *a** (redness) values increased with roasting temperature and time at 150°C and 160°C, although it did not change with increased roasting time at 170°C after 30 min of roasting. The values of *b** (yellowness) increased to a certain roasting time at 150°C and 160°C and then decreased although it always decreased at 170°C with increased roasting time. It was obvious that from the color of the hazelnuts, formation of brown colored pigments was highly dominant at higher roasting temperatures and prolonged roasting times during roasting of hazelnuts.

Table 3.2. Changes in color values (*L*, a*, b**) of hazelnuts during roasting

	150°C		160°C		170°C	
15 min		*L** 76.4±0.8 *a** -0.5±0.0 *b** 31.2±0.3		*L** 80.7±0.7 *a** 5.3±0.1 *b** 44.5±0.4		*L** 70.5±0.2 *a** 9.1±0.0 *b** 46.3±0.0
30 min		*L** 77.1±0.4 *a** 4.5±0.0 *b** 38.1±0.1		*L** 75.1±0.0 *a** 7.5±0.0 *b** 46.4±0.0		*L** 51.0±0.2 *a** 17.8±0.0 *b** 41.3±0.0
60 min		*L** 69.7±0.1 *a** 13.5±0.0 *b** 50.7±0.1		*L** 58.5±0.1 *a** 20.3±0.0 *b** 47.6±0.0		*L** 27.8±0.0 *a** 15.4±0.1 *b** 23.0±0.1
90 min		*L** 65.8±0.0 *a** 14.8±0.0 *b** 46.4±0.0		*L** 41.8±0.7 *a** 20.2±0.1 *b** 36.0±0.4		*L** 26.5±0.4 *a** 14.7±0.0 *b** 18.3±0.0
120 min		*L** 60.9±0.0 *a** 17.8±0.0 *b** 46.6±0.0		*L** 45.9±0.0 *a** 21.3±0.0 *b** 34.8±0.0		*L** 24.0±0.1 *a** 13.8±0.0 *b** 17.7±0.1

3.3.2 In Depth Investigation of the Effect of Heating on the Formation of Early Glycation Products and Advanced Glycation End Products in Hazelnut Resembling Model System

The oil:non-fat hazelnut mixtures were prepared in order to understand the effect of oil on the formation of early and advanced glycation products in hazelnuts. To simulate the composition of hazelnut, two part of hazelnut oil was mixed with one part of non-fat hazelnut (2:1) and to understand the effect of oil, amount of oil was reduced to half in (1:1) mixtures. Sunflower oil was also used in (1:1) mixtures to understand the effect of fatty acid composition, which is richer in unsaturated fatty acids. Paraffin oil was used in the mixtures as control because of its saturated fatty acid content.

N-ε-fructosyllysine, Amadori product of lysine, was determined in reaction mixtures as an early glycation marker of Maillard reaction. Furosine concentrations of oil:non-fat hazelnut mixtures (1:1) during heating were given in Figure 3.13. Furosine concentration of (1:1) mixtures was found to almost doubled after 15 min of heating. The highest concentration of furosine (333±22 mg/kg protein) was found at hazelnut oil:non-fat hazelnut (1:1) mixture. After 30 min of heating, furosine concentration of oil:non-fat hazelnut mixtures

slightly decreased and remained almost not changed during prolonged heating except for sunflower oil mixtures heated at 60 and 90 min. Wellner et al [60] reported a maximum lysine modification by fructosyllysine in peanuts after roasting at 160°C for 20 min. They did not observe an increase with higher roasting temperature or time.

Hazelnut oil:non-fat hazelnut (1:1) mixtures followed the same trend with paraffin oil:non-fat hazelnut (1:1) mixtures. Additionally, hazelnut oil:non-fat hazelnut (2:1) mixtures were again followed the same trend with paraffin oil:non-fat hazelnut (2:1) except for the heating time of 15 min, where furosine concentration of hazelnut oil:non-fat hazelnut (2:1) reached to a maximum value of 326±14 mg/kg protein (Figure 3.14). Amount of hazelnut oil was found to be not effective in the formation furosine in hazelnuts as could be clearly understood from the (1:1) and (2:1) hazelnut oil:non-fat hazelnut mixtures.

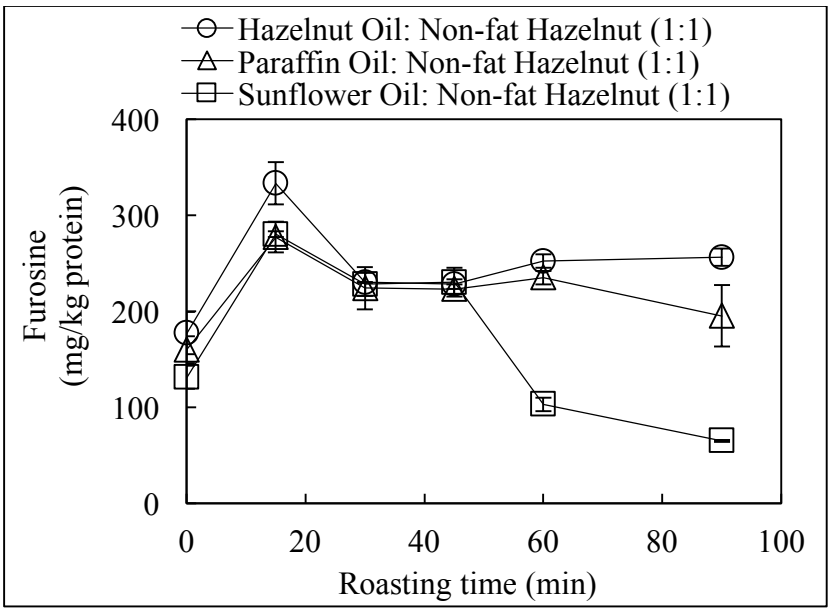


Figure 3.13. Formation of furosine in oil:non-fat hazelnut (1:1) mixtures during heating (mg/kg protein)

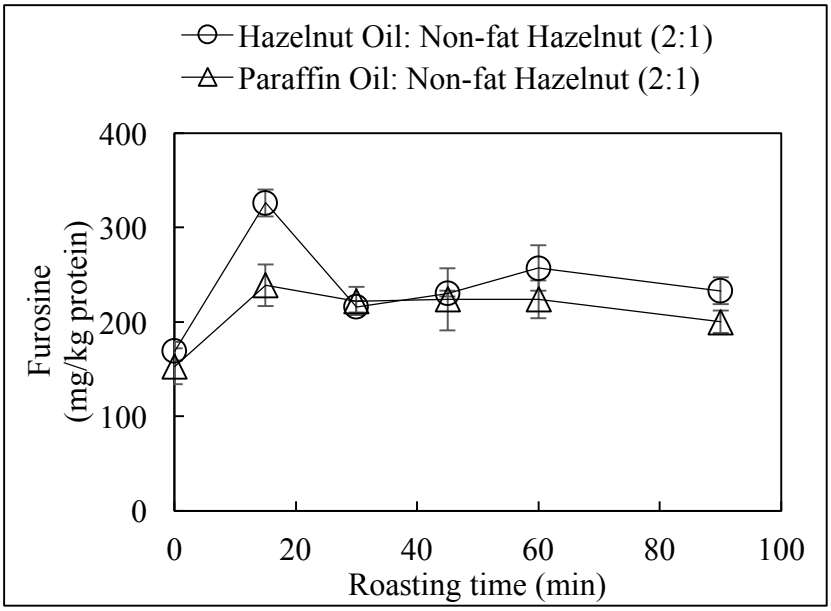


Figure 3.14. Formation of furosine in oil:non-fat hazelnut (2:1) mixtures during heating (mg/kg protein)

CML was found to be increased during heating of both (1:1) and (2:1) reaction mixtures and its concentration reached to a plateu in the (2:1) reaction mixtures. The CML concentration of hazelnut oil:non-fat hazelnut (1:1) and hazelnut oil:non-fat hazelnut (2:1) was reached to 325±56 mg/kg protein and 286±38 mg/kg protein after heating for 90 min, respectively. CML could be not only formed from oxidation of Amadori product of lysine but also formed from the reaction of glyoxal and amino side chains of proteins [114, 116, 117]. However, if the reaction of glyoxal, originating from oil, with lysine side chains was the effective mechanism for the formation of CML in hazelnuts, concentration of CML could be expected to be in higher concentrations in hazelnut oil:non-fat hazelnut (2:1) mixtures. It could be stated that oil did not give rise to the formation of CML during roasting of hazelnuts.

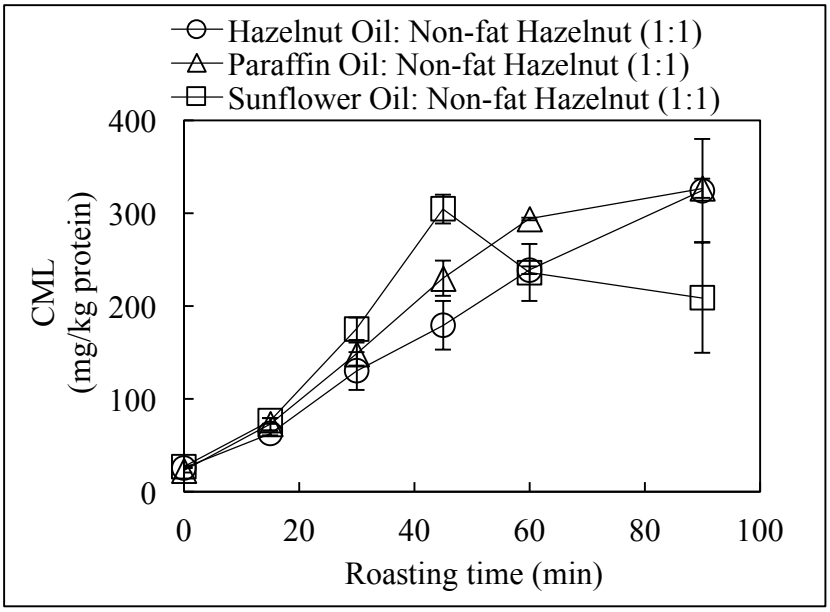


Figure 3.15. Formation of CML in oil:non-fat hazelnut (1:1) mixtures during heating (mg/kg protein)

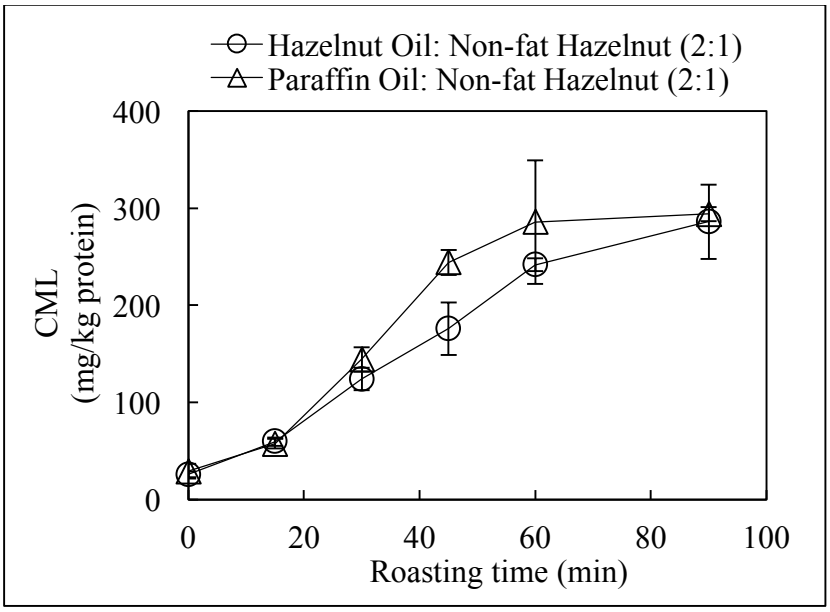


Figure 3.16. Formation of CML in oil:non-fat hazelnut (2:1) mixtures during heating (mg/kg protein)

Pyrraline, an AGE formed from the reaction of 3-DG and amino side chains of lysine, concentration of hazelnut oil:non-fat hazelnut (1:1) and (2:1) mixtures increased to 392±80 mg/kg protein and 386±117 mg/kg protein after heating for 90 min, respectively. Pyrraline was reported to be to up to 361 mg/kg protein in commercial roasted peanuts, 340-382 mg/kg protein in peanut puffs, 268-357 mg/kg protein in peanut butters and not detected in unroasted peanuts [60]. It was noted that significantly higher amounts of pyrraline than fructosyllysine, indicating the predominance of advanced stage, was formed during

roasting of peanuts [60]. Pyrraline concentration of skim milk powder (1450 and 3150 mg/kg protein) and bread crust (3250 and 3680 mg/kg protein) were extremely high, followed by crackers (1320 mg/kg protein), whey powder (850 and 1150 mg/kg protein), crisp bread (280 and 480 mg/kg protein), sterilized milk (not detectable-260 mg/kg protein) and Italian spaghetti dried at high temperatures (30 and 50 mg/kg protein) [214]. The pyrraline formation in oil:non-fat hazelnut mixtures was found to be moderate compared to the pyrraline concentrations of various foods. Pyrraline formation of oil:non-fat hazelnut mixtures followed the same trend and the concentrations did not remarkably change depending on the oil used in the mixture during heating (Figure 3.17).

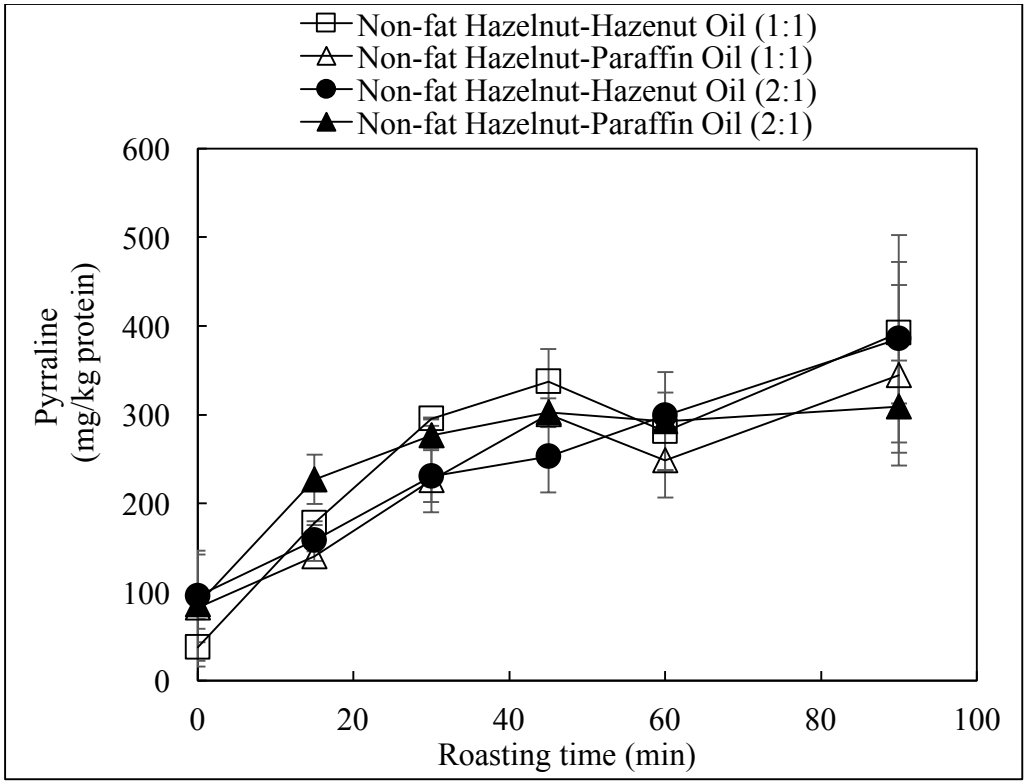


Figure 3.17. Formation of pyrraline in oil:non-fat hazelnut (1:1) and (2:1) mixtures during heating (mg/kg protein)

Formyline was found to increase during heating of oil:non-fat hazelnut mixtures. The concentration of formyline reached up to 46.5 mg/kg protein. The contribution of formyline was found to be lower than CML and pyrraline to the modification of proteins in the advanced stage of the Maillard reaction. There was apparently no contribution of oil to the formation of formyline in hazelnuts during roasting (Figure 3.18 and Figure 3.19).

The formyline levels found in the various food products including milk products (UHT milk, cream milk, yoghurt, whey and milk powder, evaporated milk) ranged from not

detected to 0.3 mg/kg, in bread products (wheat bread, 1 mm crust of wheat bread, white bread crumb, rye bread and its 1 mm and 5 mm crusts, crisp bread, breakfast cereals, etc.) from not detected to 34.8 mg/kg, in pasta products from not detected to 3.4 mg/kg and in beer retentate between 2.8 and 3.9 mg/kg [123].

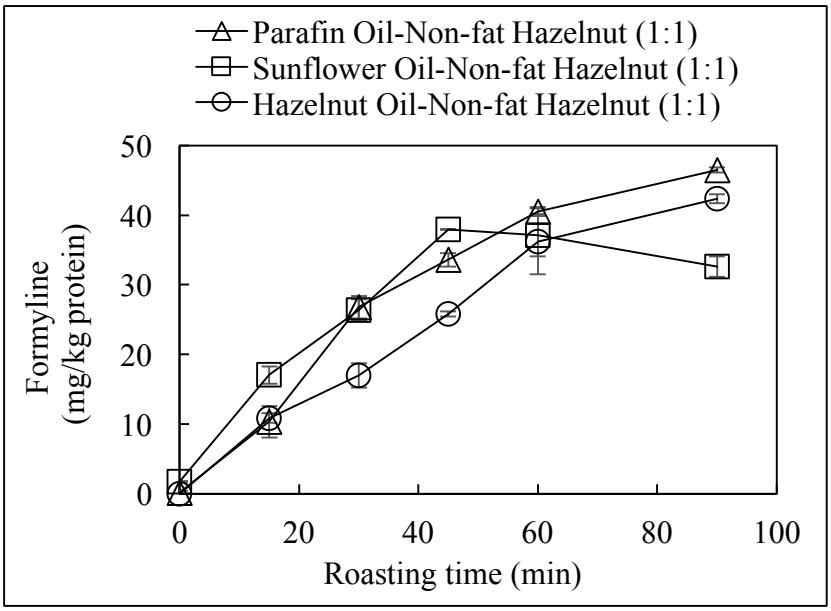


Figure 3.18. Formation of formyline in oil:non-fat hazelnut (1:1) mixtures during heating (mg/kg protein)

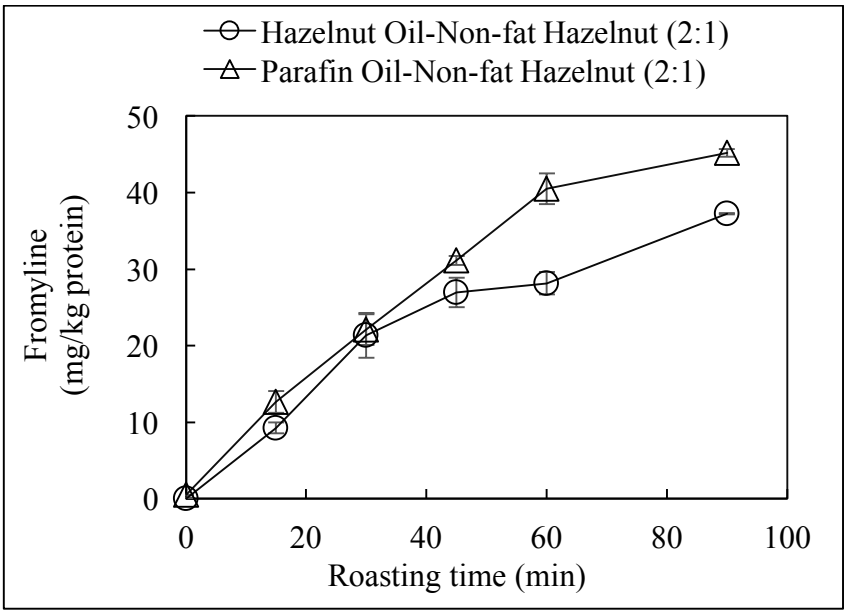


Figure 3.19. Formation of formyline in oil:non-fat hazelnut (2:1) during heating (mg/kg protein)

Maltosine was formed in the reaction mixtures in relatively lower concentrations than the other AGEs. Maltosine concentration of hazelnut oil:non-fat hazelnut mixtures (1:1) and

(2:1) reached to 2.86±0.06 and 3.36±1.42 mg/kg protein at the end of 90 min of heating at 150°C, respectively. Maltosine concentrations of various food products including milk, bakery, and pasta products, biscuits, snacks, cakes, and caramel candies, which were reported by Hellwig et al [127], were higher than the maltosine concentrations found in oil:non-fat hazelnut mixtures during heating. Same as the other AGEs, oil did not contribute to the formation of maltosine in hazelnuts as could be seen clearly during the heating of reaction mixtures.

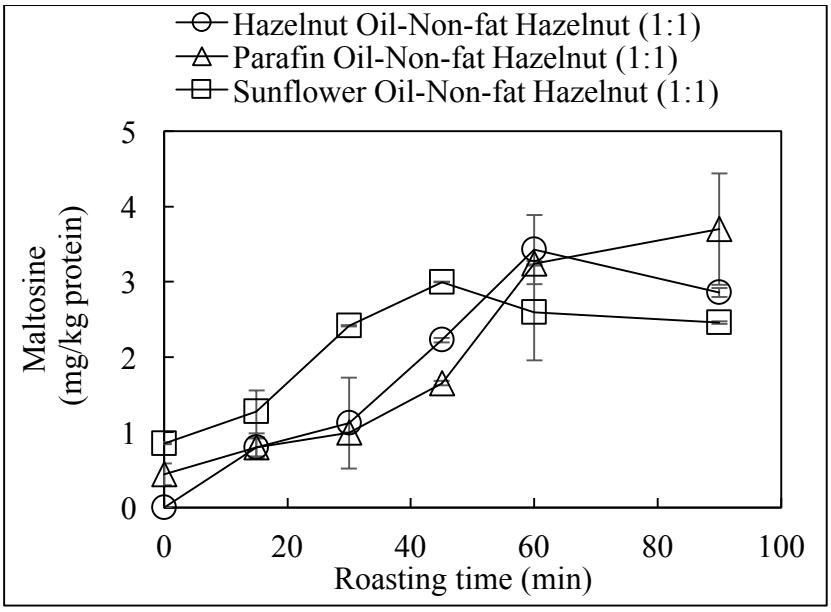


Figure 3.20. Formation of maltosine in oil:non-fat hazelnut (1:1) mixtures during heating (mg/kg protein)

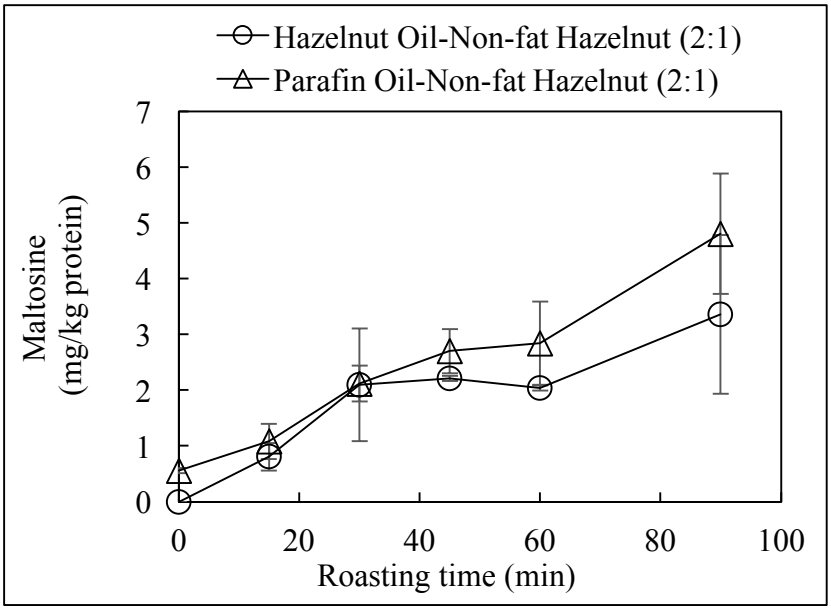


Figure 3.21. Formation of maltosine in oil:non-fat hazelnut (2:1) mixtures during heating (mg/kg protein)

MP-lysine is an advanced lipation end product formed from the reaction of acrolein with amino side chains of lysine [135]. Acrolein could be form both from glycerol and hydroperoxides of unsaturated fatty acids [156, 215]. The other sources of acrolein could be glucose and threonine via the formation of 2-hydroxypropanal or methionine through its Strecker aldehyde [160]. MP-lysine concentration of hazelnut oil:non-fat hazelnut (1:1) and (2:1) mixtures increased to 2.9±0.4 mg/kg protein and 5.2±1.1 mg/kg protein at the end of 90 min heating at 150°C, respectively. Increase in oil amount resulted in the increase in the concentration of MP-lysine. Sunflower oil:non-fat hazelnut mixture (1:1) reached to a MP-lysine concentration of 22.2±1.5 mg/kg protein at the end of 90 min heating at 150°C, which was ten times higher than the concentration in hazelnut oil:non-fat hazelnut (1:1) reaction mixture. Interestingly, MP-lysine concentration of paraffin oil:non-fat hazelnut (1:1) and (2:1) mixtures were 4.5±1.8 mg/kg protein and 8.3±1.3 mg/kg protein at the end of 90 min of heating, which were higher than the concentrations in hazelnut oil:non-fat hazelnut reaction mixtures. The highest concentration of MP-lysine in paraffin oil containing mixtures compared to hazelnut oil containing mixtures might be the glycerol content of paraffin oil which could arise from its production process. Having low concentrations of MP-lysine in hazelnut oil:non-fat hazelnut mixtures might be related to high tocopherol content of hazelnut oil which makes it not susceptible to oxidation and its production by extraction at room temperature. It was observed that oxidation of sunflower oil was inevitable as it contains more PUFA than hazelnut oil. Overall, hazelnut oil was

found to be effective in the modification of lysine in the advanced stage of Maillard reaction by formation of MP-lysine, whose formation increased with the prolonged heating time.

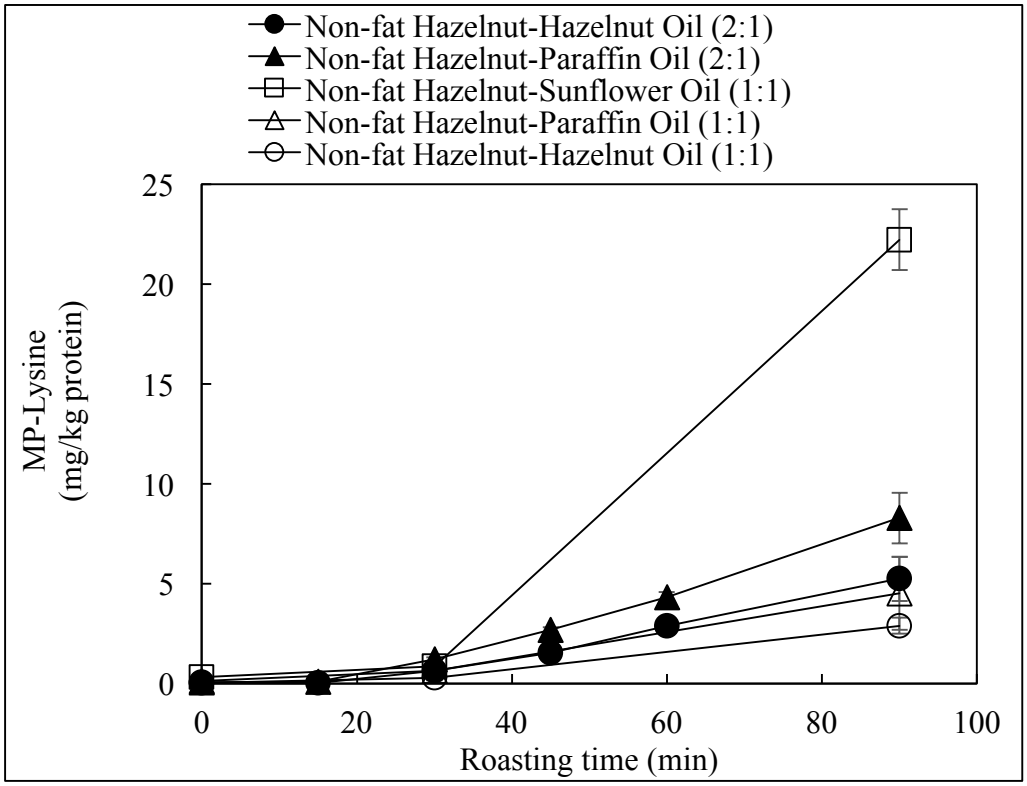


Figure 3.22. Formation of MP-lysine in oil:non-fat hazelnut (1:1) and (2:1) mixtures during heating (mg/kg protein)

3.4 Conclusion

In conclusion, sugars, amino acids and lipids were the reactants found in hazelnuts leading to the formation of Maillard reaction, sugar degradation and lipid oxidation. Sucrose was the most predominant sugar and more than half amount of sucrose was lost at the end of 150°C and it was almost completely consumed at the end of roasting at 170°C. Not only sucrose but also glucose and fructose contents of hazelnuts decreased immediately after roasting even at shorter roasting times. Lysine in proteins of hazelnuts was modified with the reactive carbonyl compounds, like reducing sugars, α-dicarbonyl compounds and lipid oxidation products, in the reaction medium during roasting of hazelnuts. Lysine was the only amino acid whose side chains had been modified as the concentration of arginine in proteins did not change with roasting (data not shown). Formation of α-dicarbonyl compounds in hazelnuts during roasting was shown for the first time. 3-DG, 1-DG, 3,4-DG were quantified as the longer chain α-dicarbonyl compounds originating from both

Maillard reaction and sugar degradation. Dimethylglyoxal, which could form from both Maillard reaction and sugar degradation, was one of the shorter chain α-dicarbonyl compounds quantified during hazelnut roasting. Glyoxal and methylglyoxal were the other shorter chain α-dicarbonyl compounds which could be formed from either lipid oxidation or Maillard reaction and sugar degradation. Among the α-dicarbonyl compounds formed in hazelnuts during roasting, 3-DG, glyoxal and methylglyoxal were the predominant ones. Further reactions of α-dicarbonyl compounds lead to the propagation of the Maillard reaction and therefore their presence in the hazelnuts during roasting may trigger modification of lysine side chains more. To understand the progress of early glycation, its marker furosine was quantified during roasting of hazelnuts. Furosine formed rapidly within 30 min of roasting at all roasting temperatures and then decreased due to degradation of fructosyllysine or oxidation of fructosyllysine. CML concentrations of hazelnuts were lower than the furosine concentrations during roasting of hazelnuts. In the final stage of the Maillard reaction with contribution of sugars and lipid oxidation products, more colored compounds were formed resulted in the remarkable changes in the *L*, a*, b** values of hazelnuts during roasting. From the viewpoint of safety, HMF, as a process contaminant, was found to reach high concentrations (300 mg/kg) in hazelnuts during roasting. However, considering the industrial roasting conditions, the daily consumption and its dietary intake, the HMF levels of roasted hazelnuts were found not to be a great concern. Acrylamide was the other process contaminant, which was below the limit of quantification in roasted hazelnuts as the precursor amino acid asparagine was one of the least abundant amino acids in hazelnuts. Furan was another process contaminant quantified in hazelnuts. Although the presence of precursors like PUFA, sugars, and amino acids which might lead to the formation of furan in hazelnuts, the processing conditions and volatility of the furan could be the reason of its low concentrations in roasted hazelnuts.

Formation of fructosyllysine (measured via furosine), advanced glycation end products (CML, pyrraline, formyline, maltosine) and an advanced lipation end product (MP-lysine) was investigated by using hazelnut resembling oil:non-fat hazelnut mixtures to understand the effect of oil on the extend of lysine modification. It was found that in the formation of fructosyllysine, CML, pyrraline, formyline and maltosine in hazelnuts during roasting, oil did not play a key role as the concentrations of the mentioned compounds did not change significantly even the amount of oil was changed. The early glycation marker,

furosine, was highest in the 15 min of heating and then decreased slightly, indicating the either oxidative cleavage or degradation of fructosyllysine. The concentrations of advanced glycation and lipation end products were in the order of pyrraline>CML>formyline>MP-lysine>maltosine. MP-lysine was found to increase with the amount of oil and within the longer roasting times, suggesting it as a marker of advanced lipation in hazelnuts during roasting.

4 MULTIRESPONSE KINETIC MODELING OF MAILLARD REACTION AND CARAMELIZATION

4.1 Introduction

The reaction products formed during roasting of hazelnuts could not be originated from just one reaction pathway. The formation and degradation pathways of the reactants, intermediates and products of Maillard reaction, sugar degradation and lipid oxidation were mentioned in Chapter 1. Foreseeing the possible intermediates and products of the reactions as well as their formation and degradation pathways helps specifying the compounds that are necessary to be analyzed and quantified. Quantification of these compounds, as many as possible, is important for kinetic studies. Therefore, the reactants, intermediates and products occurring during roasting of hazelnuts were quantified at different roasting temperatures and roasting times as presented in Chapter 3.

Multiresponse kinetic modeling gives an insight to the mechanism of the reactions that happen simultaneously as it considers all the reactants and products at the same time [59, 167]. From the scientific point of view, it helps to understand the chemistry taking place in the food which has been under investigation. From the engineering point of view, it helps to predict the changes in the food in the future quantitatively as well as to control the processing conditions and the properties of food [216]. Although using multiresponse kinetic models in foods has advantages mentioned here, multiresponse modeling of changes in foods is a hard task to deal with as the food is a mixture of components that causes inevitable interactions especially during processing [216]. To understand the reactions taking place during hazelnut roasting at selected time and temperatures; determination of main reactants and products, proposing a reaction mechanism, building a kinetic model according to the proposed mechanism and fitting of the kinetic models to the data should be performed. By doing so, an insight to the reaction mechanism could be obtained together with the determination of kinetic parameters.

Impact of roasting on lipid fraction of hazelnut at different roasting time and temperatures had been studied before [28]. The triacylglycerol and fatty acid composition of lipid fraction were reported to be basically identical with raw hazelnuts. Only modest decreases in phytosterol and vitamin E contents was determined even after roasting at higher temperatures used by the industry [28]. Although content of proteins, free amino acids and sugars are comparatively lower than lipid fraction of hazelnuts, significant changes in these

fractions resulted in the formation of caramelization and Maillard reaction products depending upon the roasting time and temperature were observed (Chapter 3). The aim of multiresponse modeling was to understand contribution of Maillard reaction and caramelization during roasting of hazelnuts. Therefore, sugars and amino acids were selected as the main reactants of these reactions [110, 165], HMF was included in the reaction network as it was found in respectively high concentrations in roasted hazelnuts [10] and dicarbonyl compounds were selected as important common intermediates both in the Maillard reaction and caramelization.

4.2 Materials and Methods

4.2.1 Analysis of Moisture Content and pH

Ground hazelnut samples (2 g) were dried at 105°C in an oven to a constant weight in order to determine moisture contents (AOAC 925.10) [178].

Ground hazelnuts (0.5 g) were suspended with 10 mL distilled water and shaken vigorously for 10 min. After centrifugation at 5000 x *g* for 3 min, the pH of supernatants was measured using a pH meter (MeterLab PHM210, France).

4.2.2 Analysis of Reactants and Products of Maillard Reaction and Caramelization Products during Roasting of Hazelnuts

Analysis of reactants and products of Maillard reaction and caramelization was performed as described in the previous chapters. Analysis of sugars and amino acids was performed as described in Chapter 2. Analysis of α-dicarbonyl compounds including 3-DG, 1-DG, glyoxal, methylglyoxal, dimethylglyoxal, and HMF were performed as given in Chapter 3.

4.2.3 Kinetic Data Analysis

Kinetic modeling of Maillard reaction and caramelization was performed with the concentration data of reactants and products of hazelnuts during roasting at 150, 160 and 170°C for 15 to 120 min. The reactants were sugars (sucrose, glucose, and fructose) and amino acids (free amino acids and protein bound lysine), and the products were α-dicarbonyl compounds (3-DG, 1-DG, glyoxal, methylglyoxal and dimethylglyoxal) and HMF.

A kinetic model was proposed comprising of Maillard reaction and caramelization reactions occurring during hazelnut roasting by involving the specified reactants and products (Figure 4.1).

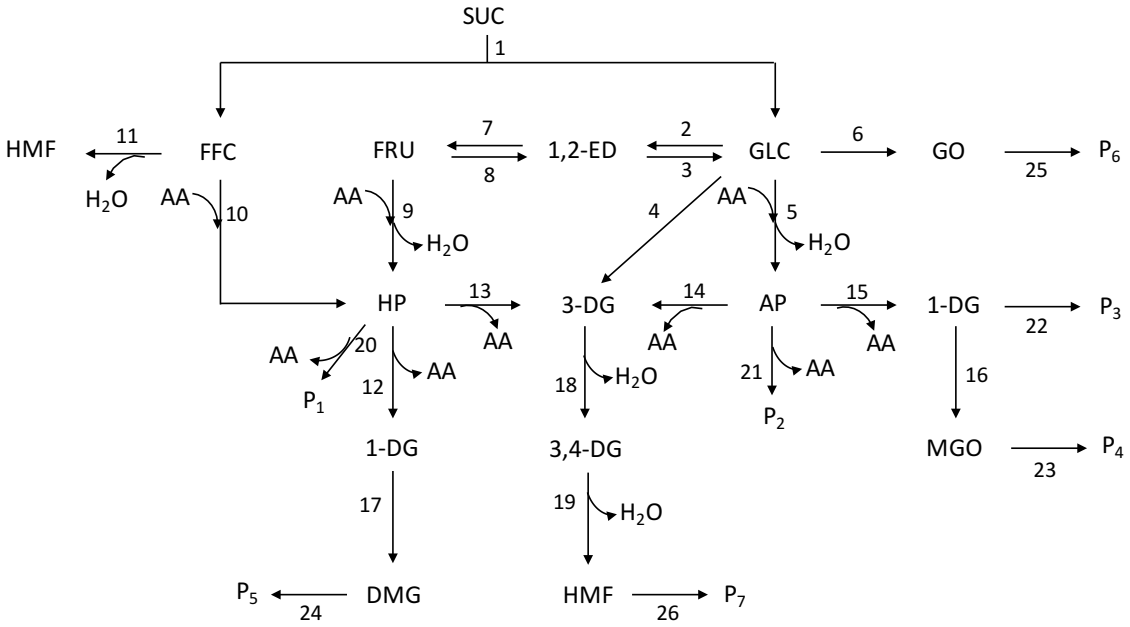


Figure 4.1. Proposed kinetic mechanism for the Maillard reaction and caramelization during hazelnut roasting. SUC, sucrose; GLC, glucose; FRU, fructose; FFC, fructofuranosyl cation; 1,2-ED, 1,2-enediol; AP, Amadori product; HP, Heyns product; 1-DG, 1-deoxyglucosone; 3-DG, 3-deoxyglucosone 3,4-DG, 3,4–dideoxyglucosone; GO, glyoxal; MGO, methylglyoxal; DMG, dimethylglyoxal; HMF, 5-hydroxymethyl-2-furfural; AA, total amino acids; P, products.

Each step of the reaction network given in Figure 4.1 was represented by an ordinary differential equation, which was characterized by a reaction rate constant (k) as follows:

$$\frac{d[SUC]}{dt} = -k_1[SUC]$$

$$\frac{d[FRU]}{dt} = k_7[1,2-ED] - k_8[FRU] - k_9[FRU][AA]$$

$$\frac{d[GLC]}{dt} = k_1[SUC] + k_3[1,2-ED] - (k_2+k_4+k_6)[GLC] - k_5[GLC][AA]$$

$$\frac{d[3-DG]}{dt} = k_4[GLC] + k_{13}[HP] + k_{14}[AP] - k_{18}[3-DG]$$

$$\frac{d[1-DG]}{dt} = k_{12}[HP] + k_{15}[AP] - (k_{16}+k_{17}+k_{22})[1-DG]$$

$$\frac{d[MGO]}{dt} = k_{16}[1-DG] - k_{23}[MGO]$$

$$\frac{d[GO]}{dt} = k_6[GLC] - k_{25}[GO]$$

$$\frac{d[HMF]}{dt} = k_{11}[FFC] + k_{19}[3,4-DG] - k_{26}[\mathrm{HMF}]$$

$$\frac{d[3,4-DG]}{dt} = k_{18}[3-DG] - k_{19}[3,4-DG]$$

$$\frac{d[FFC]}{dt} = k_{1}[SUC] - k_{11}[FFC] - k_{10}[FFC][AA]$$

$$\frac{d[AP]}{dt} = k_{5}[GLC][AA] - (k_{14} + k_{15} + k_{21})[AP]$$

$$\frac{d[HP]}{dt} = k_{9}[FRU][AA] + k_{10}[FFC][AA] - (k_{12} + k_{13} + k_{20})[HP]$$

$$\frac{d[AA]}{dt} = -k_{5}[GLC][AA] - k_{9}[FRU][AA] - k_{10}[FFC][AA] + (k_{12} + k_{13} + k_{20})[HP] + (k_{14} + k_{15} + k_{21})[AP]$$

$$\frac{d[DMG]}{dt} = k_{17}[1-DG] - k_{24}[DMG]$$

$$\frac{d[1,2-ED]}{dt} = k_{2}[GLC] + k_{8}[FRU] - (k_{3} + k_{7})[1,2-ED]$$

$$\frac{d[P_1]}{dt} = k_{20}[HP]$$

$$\frac{d[P_2]}{dt} = k_{21}[AP]$$

$$\frac{d[P_3]}{dt} = k_{22}[1-DG]$$

$$\frac{d[P_4]}{dt} = k_{23}[MGO]$$

$$\frac{d[P_5]}{dt} = k_{24}[DMG]$$

$$\frac{d[P_6]}{dt} = k_{25}[GO]$$

$$\frac{d[P_7]}{dt} = k_{26}[HMF]$$

To understand the reaction mechanism, a set of differential equations compiled and solved by numerical integration. Numerically solved equations were fitted to the experimental data obtained at different roasting time-temperatures. Numerical integration and determination of the reaction rate constants were performed by Athena Visual Studio software version 14.2 (Athena Visual Inc.) with non-linear regression using determinant criterion [163]. For the evaluation of kinetic models, posterior probability criterion [217] was used to find the best model fit to the experimental data.

Temperature dependence of the reaction rate constants was indicated by means of activation energies, E_a (kJ/mol), by using the reparametrized Arrhenius equation

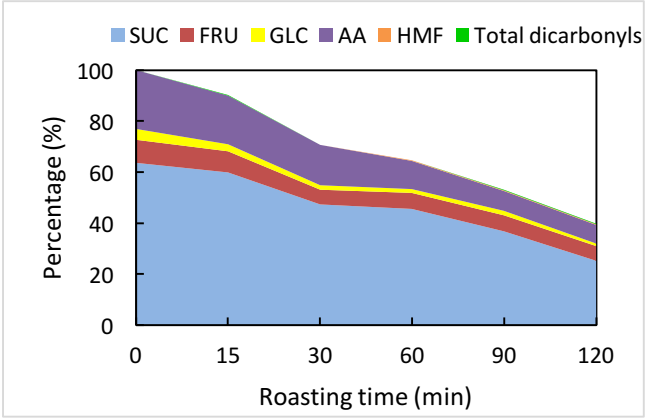
$$k = k_b \times exp\left(\frac{E_a}{RT_b}\left(1 - \frac{T_b}{T}\right)\right)$$

where R is the universal gas constant (8.3145×10^{-3} kJ/mol K), T is the temperature concerned, k_b is the reaction rate constant at a reference temperature T_b of 160°C. The reaction rate constant (k) in the differential equations of each reaction step was replaced with the reaction rate constant determined by the reparametrized Arrhenius equation and the data at 150, 160, and 170°C were fitted to experimental data all at once.

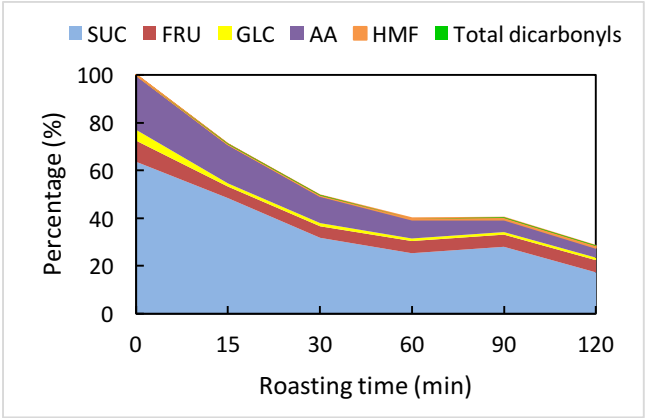
4.3 Results and Discussion

4.3.1 The Mass Balance

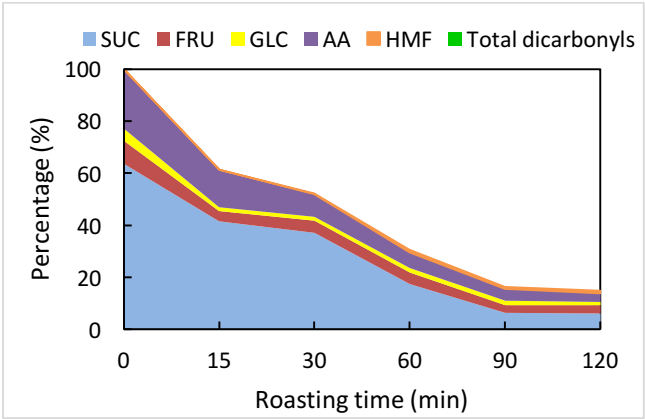
The mass balances of reactants and products indicating the relative proportion of individual compounds (%) at each roasting time for all roasting temperatures were demonstrated in Figure 4.2.



(a)



(b)



(c)

Figure 4.2. Mass balance of reactants and products during hazelnut roasting (%) (a)150°C (b)160°C (c)170°C.

Total moles of the reactants and products decreased gradually during roasting at all roasting temperatures. At 150°C, 90% of the total moles of these compounds was recovered after 15 min roasting while the recovery was 39% at the end of 120 min. The

total moles of reactants and products was 71% and 62% after roasting for 15 min, 29% and 15% after roasting for 120 min at 160 and 170°C, respectively. This gradual decrease in the total moles of the reactants and products indicated the importance of advanced and final stages of the Maillard reaction together with the progress of caramelization. The total molar loss of determined compounds during biscuit baking was also attributed to the significance of not quantified compounds occurring during intermediate and advanced stages of Maillard reaction [175]. Melanoidins are one of these final stage products that form as a result of condensation and polymerization reactions of sugar fragments with amino compounds and the structure of which have not been completely identified, yet [59, 78].

4.3.2 Kinetic Modeling

Kinetic modeling of real foods during heat treatments is a challenging task as the water, reactants and intermediates are not homogenously distributed. The changes in water and temperature profile in time bring the necessity of the incorporation of the heat and mass transfer coefficients into the model [176]. However, this is not the case for hazelnuts as the moisture content of raw hazelnuts is very limited (Table 4.1).

Table 4.1. Changes in pH and moisture content of hazelnuts during roasting

	Moisture (%)	pH
Non-treated	3.63±0.04	6.61±0.00
150°C 15 min	1.89±0.04	6.40±0.04
150°C 30 min	1.62±0.10	6.40±0.02
150°C 60 min	1.59±0.08	6.39±0.01
150°C 90 min	1.48 ±0.08	6.33±0.01
150°C 120 min	1.11±0.06	6.32±0.02
160°C 15 min	1.48±0.08	6.41±0.03
160°C 30 min	1.11±0.06	6.43±0.00
160°C 60 min	1.14±0.04	6.21±0.04
160°C 90 min	1.17±0.07	6.22±0.04
160°C 120 min	1.01±0.08	6.20±0.04
170°C 15 min	1.27±0.06	6.38±0.08
170°C 30 min	1.13±0.05	6.31±0.00
170°C 60 min	1.05±0.04	6.17±0.05
170°C 90 min	0.99±0.05	6.01±0.05
170°C 120 min	0.97±0.09	6.09±0.04

Correspondingly, the temperature of hazelnuts could rapidly reach to isothermal conditions. Specific heat (c_p) value of hazelnuts were reported as 1994 J/kg K [218] which is almost equal to specific heat value of oil and half of the specific heat value of water. According to the thermal analysis and experiments performed by Demir et al [218], it took around 4-6 min for hazelnuts to reach to the oven temperature (150-170°C). However, in our study it could take less time for hazelnuts to reach the oven temperature as there was no thermocouples incorporated which let hazelnuts to be placed to the oven as fast as possible. Hence, incorporation of heat and mass transfer coefficients to the model equations were not considered.

Model discrimination for (i) 1,2-enolization during sugar isomerization reaction, (ii) sucrose degradation and HMF formation through reactive fructofuranosyl cation, (iii) reaction of amino acids with α-dicarbonyl compounds was done in order to find the best model fitting to the experimental data. The comprehensive reaction mechanism of Maillard reaction and caramelization during roasting of hazelnuts was given in Figure 4.3.

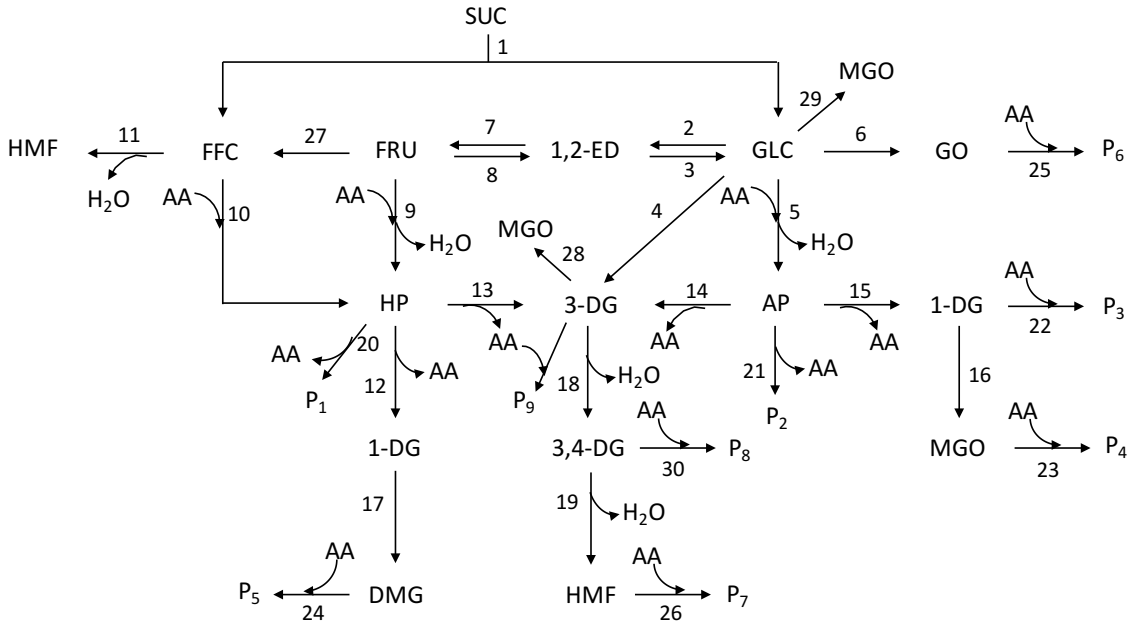


Figure 4.3. Comprehensive kinetic mechanism for the Maillard reaction and caramelization during hazelnut roasting. SUC, sucrose; GLC, glucose; FRU, fructose; FFC, fructofuranosyl cation; 1,2-ED, 1,2-enediol; AP, Amadori product; HP, Heyns product; 1-DG, 1-deoxyglucosone; 3-DG, 3-deoxyglucosone 3,4-DG, 3,4–dideoxyglucosone; GO, glyoxal; MGO, methylglyoxal; DMG, dimethylglyoxal; HMF, 5-hydroxymethyl-2-furfural; AA, total amino acids; P, products

The proposed reaction mechanism of Maillard reaction and caramelization of hazelnuts during roasting (Figure 4.1) was obtained by simplification of the comprehensive model

given in Figure 4.3. Further simplification of this comprehensive model until to obtain the proposed model (Figure 4.1) was discussed in the following sections. Additionally, after the proposed model in Figure 4.1 was obtained, the importance of some of the reaction steps (1,2-enediol formation and HMF formation through sucrose degradation) was evaluated by excluding those steps from the proposed mechanism one by one. However, it should be mentioned that until the models in Figure 4.3 and finally Figure 4.1 was proposed, several models including more simple ones had been tested.

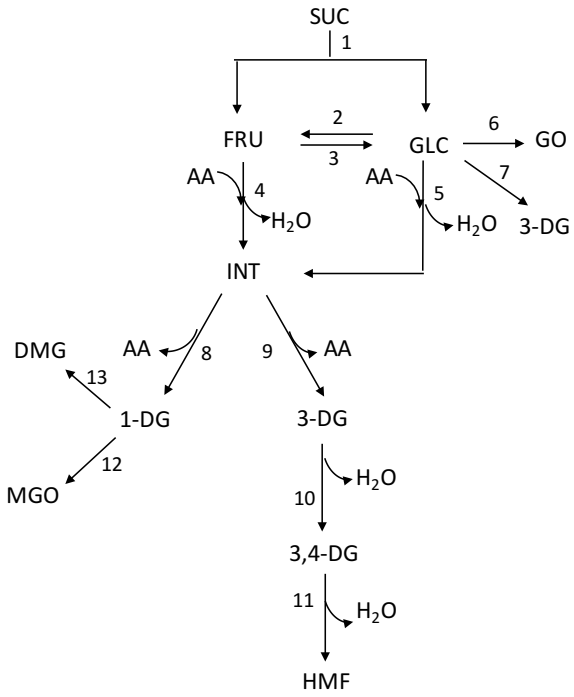


Figure 4.4. A simple model (model 1) proposed for the Maillard reaction and caramelization of hazelnuts during roasting. SUC, sucrose; GLC, glucose; FRU, fructose; INT, intermediate; 1-DG, 1-deoxyglucosone; 3-DG, 3-deoxyglucosone; 3,4-DG, 3,4-dideoxyglucosone; GO, glyoxal; MGO, methylglyoxal; DMG, dimethylglyoxal; HMF, 5-hydroxymethylfurfural; AA, total amino acids

The model 1 given in Figure 4.4 involves as less reaction steps as possible to have less parameters and a simpler model. The reaction steps of hydrolysis of sucrose to glucose and fructose, interconversion of glucose and fructose by not including the intermediate 1,2-enediol, formation of an intermediate (INT) instead of Amadori or Heyns products from the Maillard reaction of glucose and fructose, formation of 1- DG and 3- DG from the INT, formation of 3-DG from glucose, formation of methylglyoxal and dimethylglyoxal through degradation of 1-DG, glyoxal formation from glucose, and HMF formation through 3-DG were incorporated to the model. After the model equations were settled and solved, the predicted data for model 1 (Figure 4.4) was obtained and they were compared with

experimental data at 150°C as given in Figure 4.5. As could be clearly seen from Figure 4.5, concentrations of sucrose, glucose, HMF, 1-DG, methylglyoxal, and dimethylglyoxal could not be estimated well. Therefore, a revision in the model became necessary.

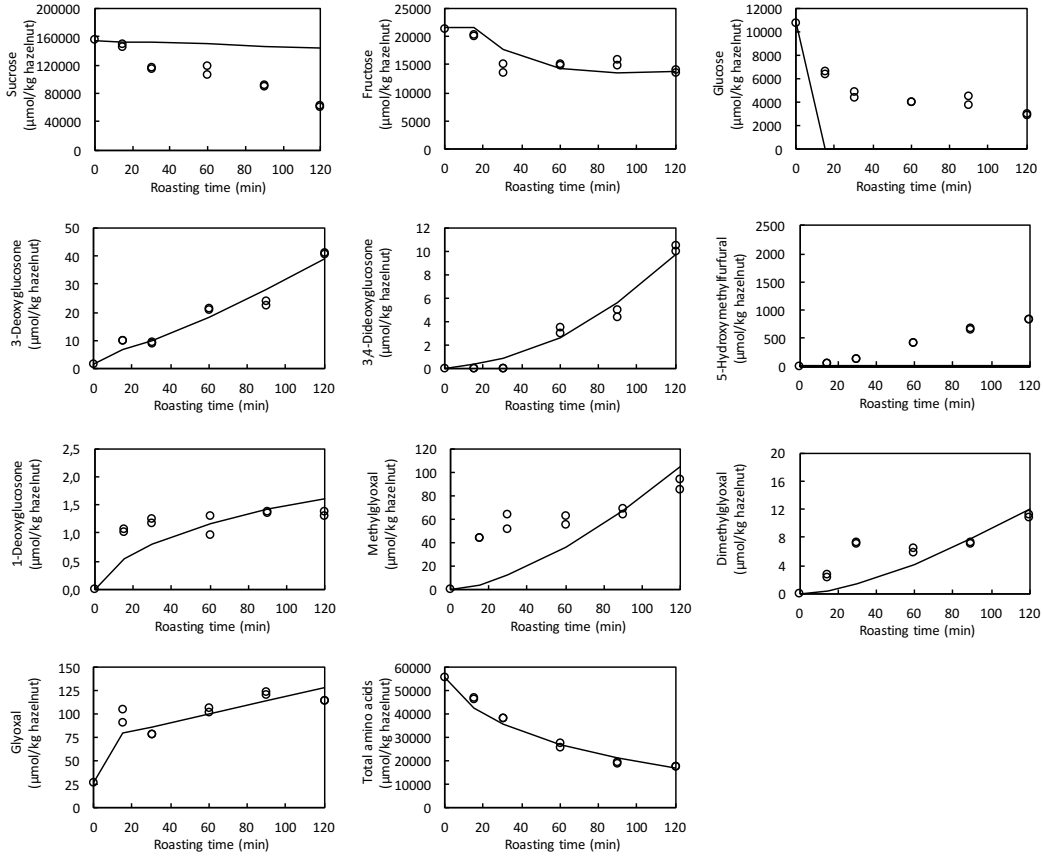


Figure 4.5. Kinetic model fit, obtained according to kinetic model 1, to the experimental data at 150°C

Because sucrose, glucose and HMF were the ones whose concentrations could not be well estimated in the previous model, model 1, their degradation and formation pathways were reconsidered. In the model 2 given in Figure 4.6, instead of hydrolysis of sucrose to glucose and fructose, its degradation to glucose and fructofuranosyl cation was considered to be involved in the reaction network as this pathway was suggested to be more relevant under dry heating conditions [145]. As well as degradation of sucrose to fructofuranosyl cation and glucose, formation of HMF through fructofuranosyl cation was also involved in the model 2.

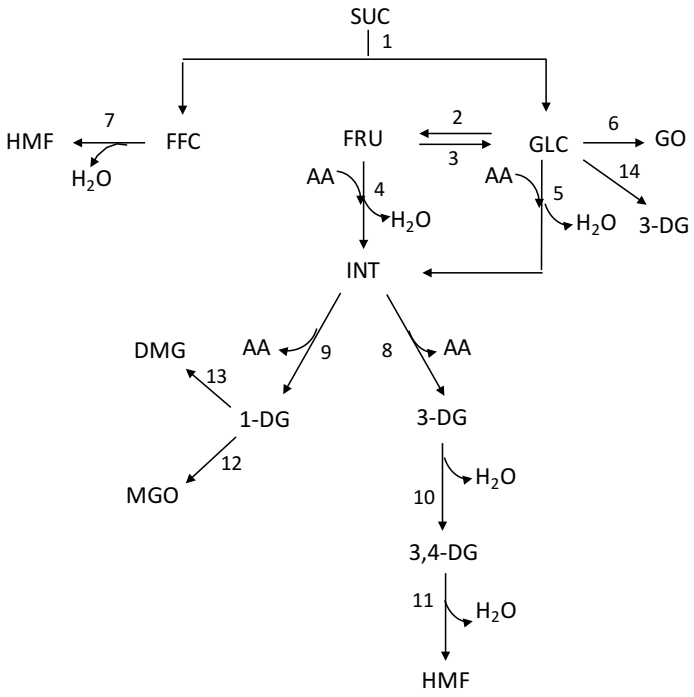


Figure 4.6. Model 2 proposed for the Maillard reaction and caramelization of hazelnuts during roasting. SUC, sucrose; GLC, glucose; FRU, fructose; FFC, fructofuranosyl cation; INT, intermediate; 1-DG, 1-deoxyglucosone; 3-DG, 3-deoxyglucosone; 3,4-DG, 3,4-dideoxyglucosone; GO, glyoxal; MGO, methylglyoxal; DMG, dimethylglyoxal; HMF, 5-hydroxymethylfurfural; AA, total amino acids

The predicted concentrations of reactants and products according to the model 2 were compared with the experimental data at 150°C and given in Figure 4.7. Involvement of degradation of sucrose to the glucose and fructofuranosyl cation resulted in the well prediction of concentrations of sucrose and glucose. However, concentration of fructose could not be estimated in this case. At the same time, the predicted concentration values of 1-DG, methylglyoxal, dimethylglyoxal did not change remarkably. Additionally, the predicted values of glyoxal were lower than the predicted values according to model 1.

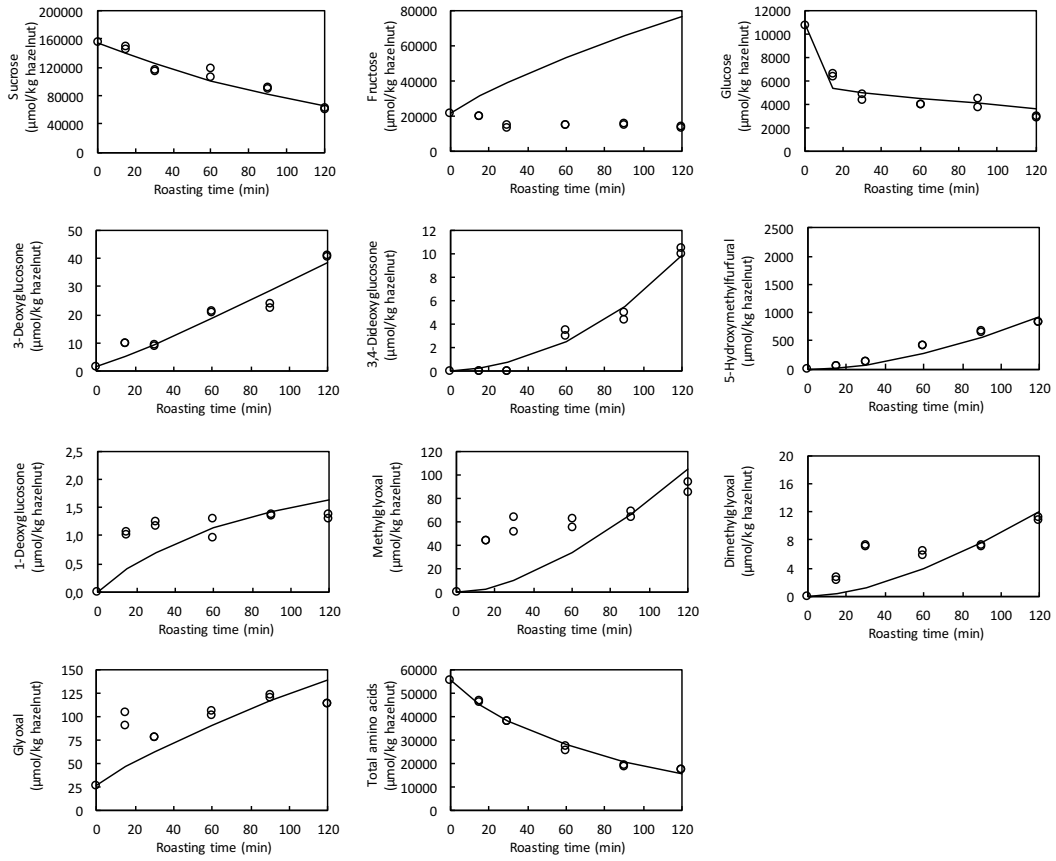


Figure 4.7. Kinetic model fit, obtained according to kinetic model 2, to the experimental data at 150°C

As the concentrations of most of the dicarbonyl compounds could not be estimated by the models given above, a new model (model 3) including the further reactions of dicarbonyl compounds was proposed (Figure 4.8). In this model, further reactions of dicarbonyl compounds, which may give rise to the formation of a variety of products, were added to the reaction network. The added products were indicated with 'P'. Moreover, in the way of a more complex mechanism, reaction of fructofuranosyl cation with amino acids was also included in the model, which could give rise to the formation of Heyns product [145].

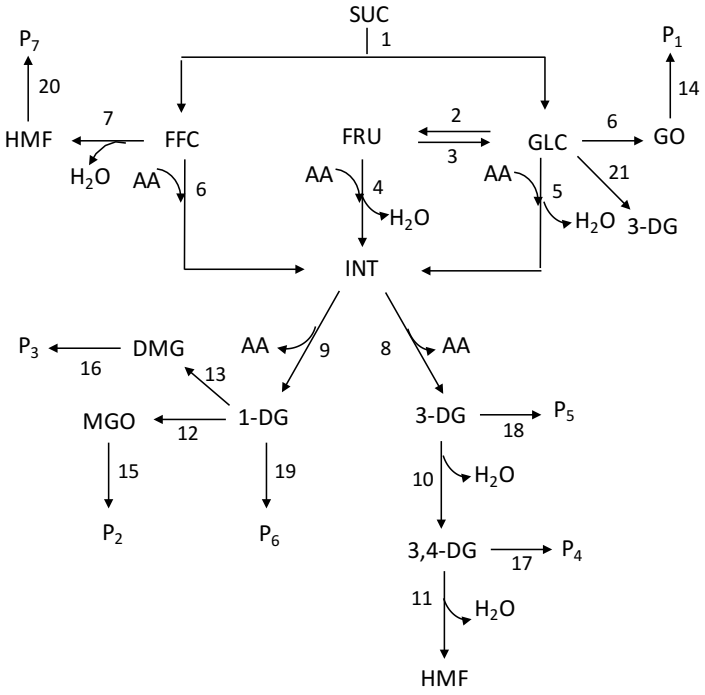


Figure 4.8. Model 3 proposed for the Maillard reaction and caramelization of hazelnuts during roasting. SUC, sucrose; GLC, glucose; FRU, fructose; FFC, fructofuranosyl cation; INT, intermediate; 1-DG, 1-deoxyglucosone; 3-DG, 3-deoxyglucosone; 3,4-DG, 3,4-dideoxyglucosone; GO, glyoxal; MGO, methylglyoxal; DMG, dimethylglyoxal; HMF, 5-hydroxymethylfurfural; AA, total amino acids; P, products

Involvement of products in the reaction steps of dicarbonyl compounds helped to obtain a promising reaction mechanism. As could be seen in Figure 4.9, the predicted concentration data of reactants and products were almost well fitted with their experimental data at 150°C, except for the data of total amino acids.

To test the effect of amino acids on the further reactions of dicarbonyl compounds, reactions of amino acids with individual dicarbonyl compounds were added to the new proposed model (model 4) (Figure 4.10). However, the predicted data of amino acids obtained according to the model 4 were not compatible with the experimental data at 150°C. A sudden decrease in the predicted concentration of total amino acids within 15 min was observed as well as a prediction of less glyoxal concentration and a prediction of a constant concentration of 1-DG after 15 min (Figure 4.11). Therefore, the reaction network was considered to be entirely revised after several attempts and earnings by going more detail in reactions with addition of new reaction steps.

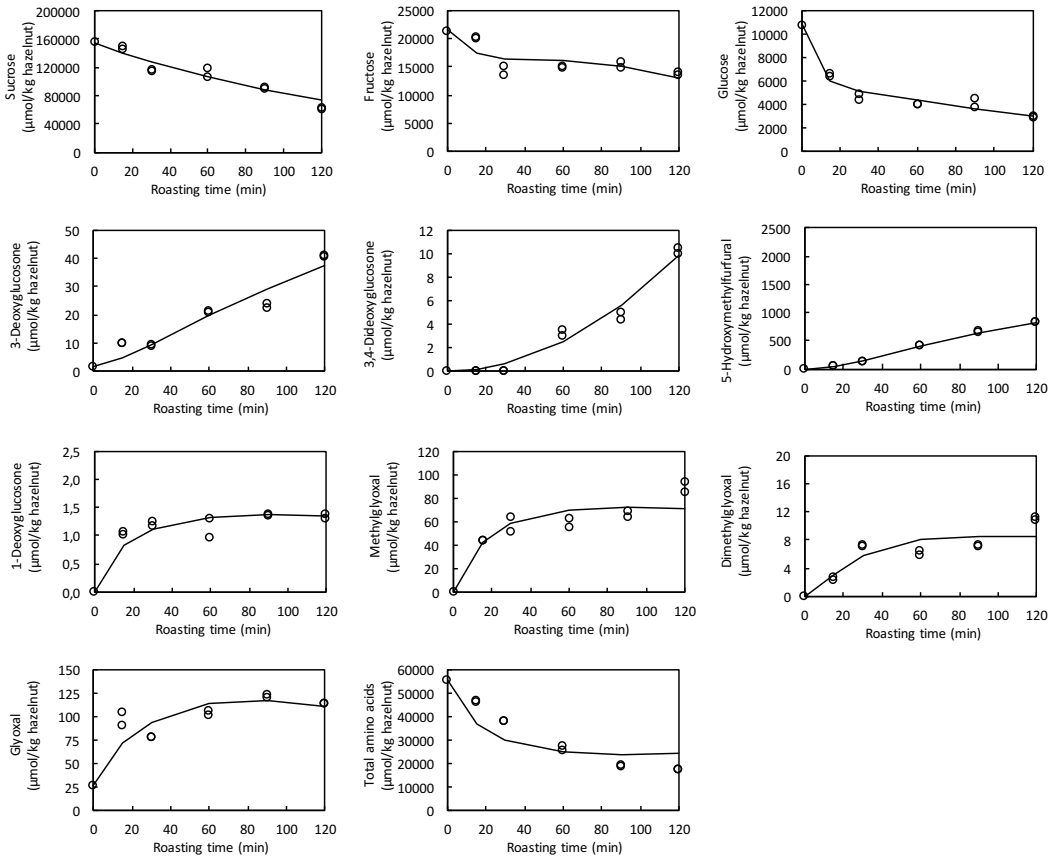


Figure 4.9. Kinetic model fit, obtained according to kinetic model 3, to the experimental data at 150°C

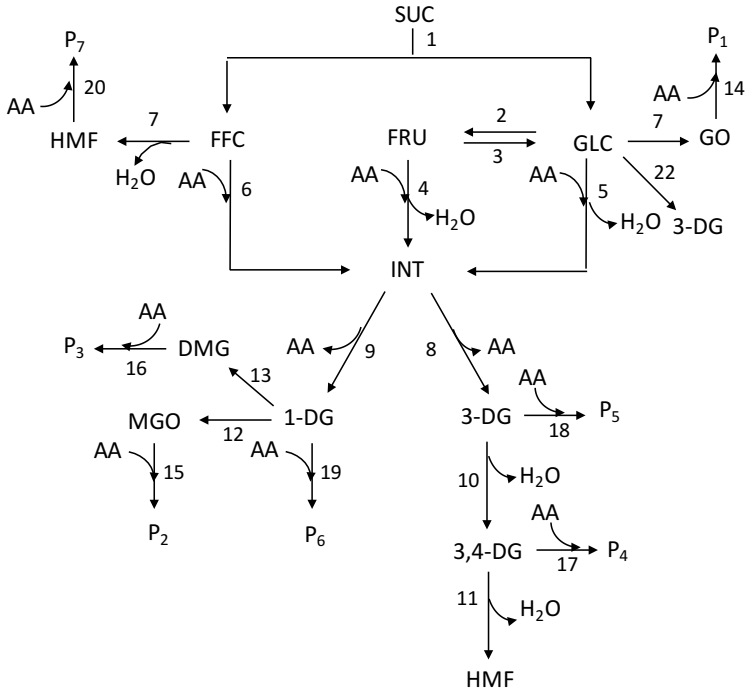


Figure 4.10. Model 4 proposed for the Maillard reaction and caramelization of hazelnuts during roasting

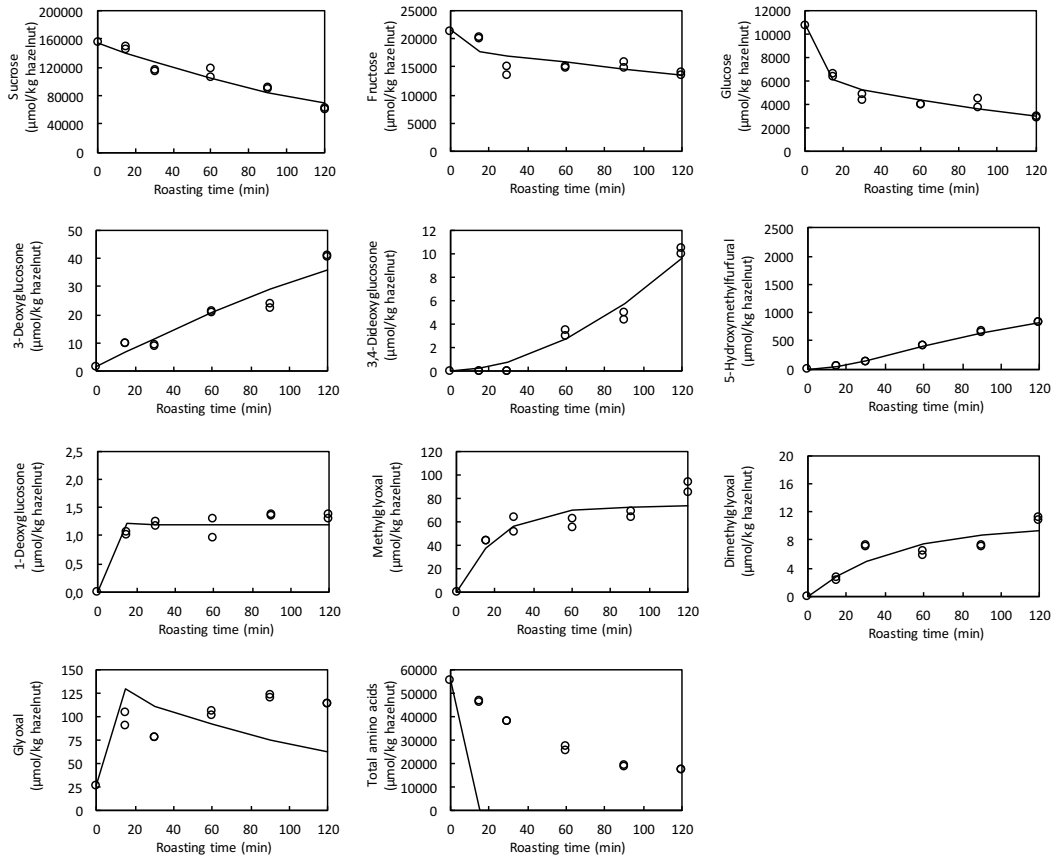
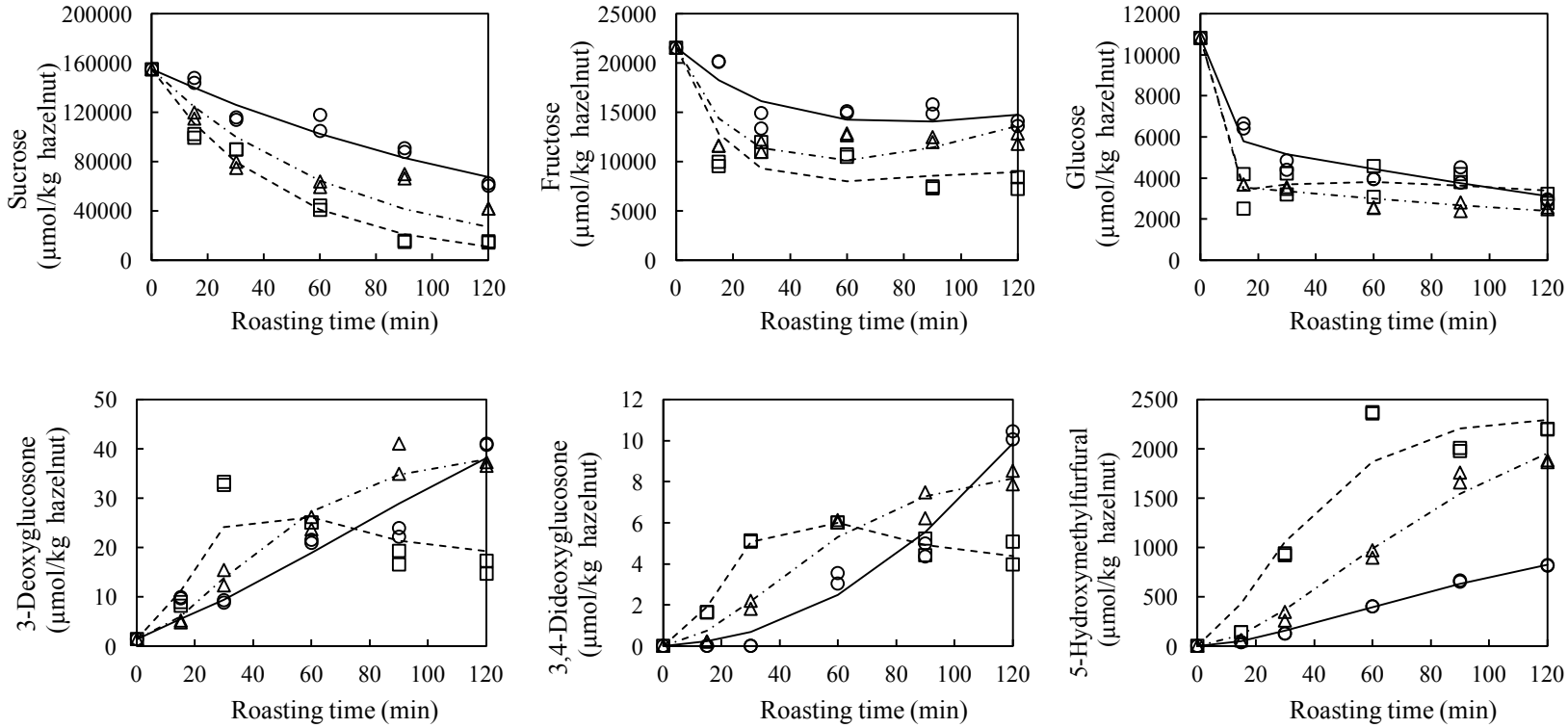


Figure 4.11. Kinetic model fit, obtained according to kinetic model 4, to the experimental data at 150°C

The reaction steps added to model 4, to obtain a more comprehensive model, were formation of fructofuranosyl cation from fructose, 1,2-enediol formation in the interconversion of glucose and fructose, formation of 3-DG and methylglyoxal from glucose, formation of Heyns product with the reaction of amino acids and fructose, formation of Amadori product with the reactions of amino acids and glucose, degradation of Amadori and Heyns products to 1- and 3-DG, as well as further reactions of dicarbonyls with amino acids as given in Figure 4.3. Finally, by a few simplifications in the comprehensive reaction network (Figure 4.3), which will be discussed in the following sections, the proposed reaction network was obtained (Figure 4.1).

The reactions included in the proposed reaction (Figure 4.1) network were: sucrose degradation, isomerization reaction between glucose and fructose, Amadori/Heyns product formation, HMF formation through fructofuranosyl cation and 3-DG pathway, α-dicarbonyl compound formation via degradation of Amadori/Heyns product or sugar degradation reactions, and elimination reactions of α-dicarbonyl compounds and HMF.

The elementary reactions of the proposed model were expressed as differential equations. The predicted data and rate constants of each reaction at 150, 160 and 170°C were obtained by solving these differential equations simultaneously. The fits of the predicted data to experimental data were satisfactory at all roasting temperatures for all measured compounds (Figure 4.12).


Sucrose (µmol/kg hazelnut)
Roasting time (min)
Fructose (µmol/kg hazelnut)
Roasting time (min)
Glucose (µmol/kg hazelnut)
Roasting time (min)
3-Deoxyglucosone (µmol/kg hazelnut)
Roasting time (min)
3,4-Dideoxyglucosone (µmol/kg hazelnut)
Roasting time (min)
5-Hydroxymethylfurfural (µmol/kg hazelnut)
Roasting time (min)

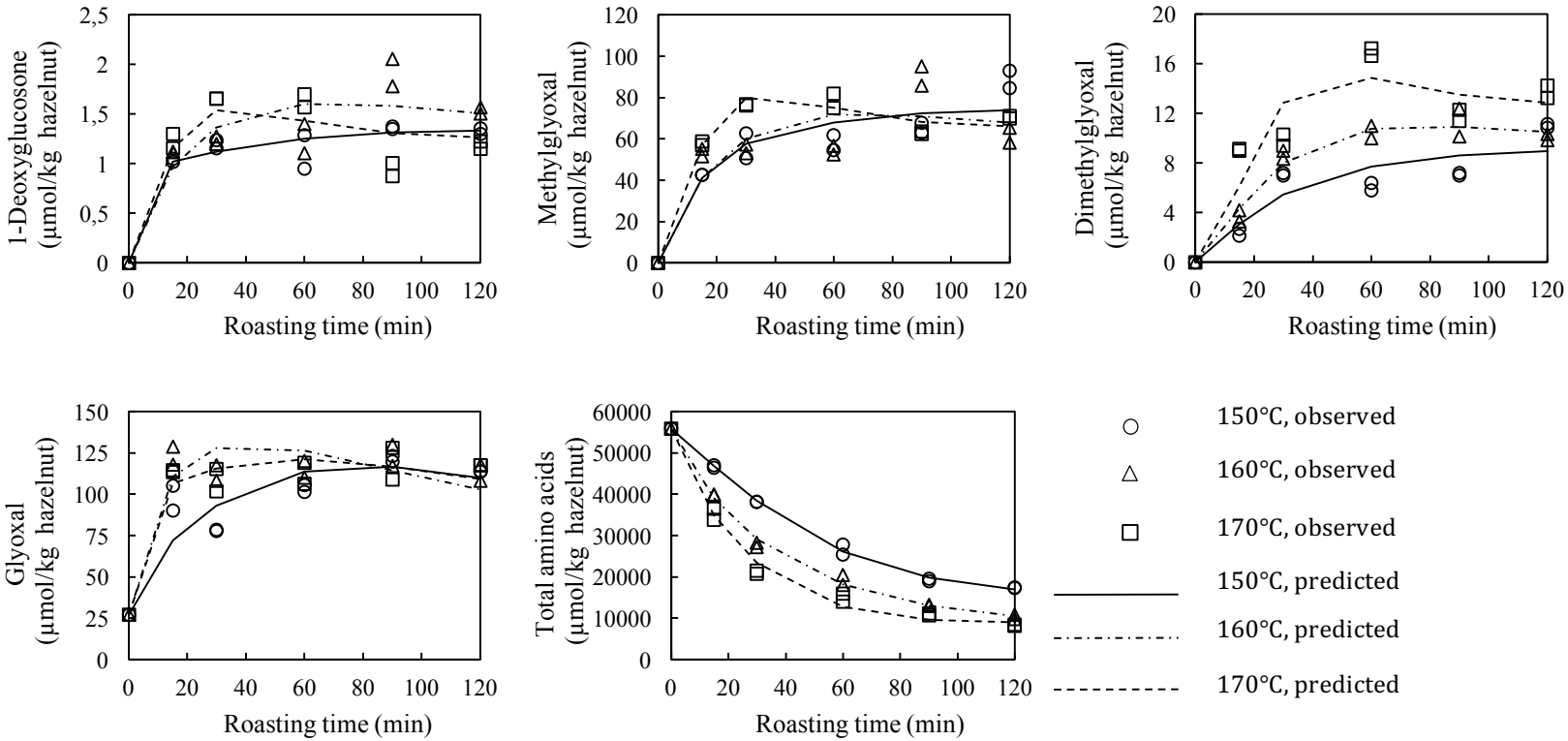


Figure 4.12. Kinetic model fit (lines) to the experimental data (symbols) of reactants and products during hazelnut roasting.

Table 4.2. Reaction rate constants with 95% Highest Posterior Density (HPD) intervals at different temperatures according to the proposed kinetic model in Figure 4.1 for Maillard reaction and caramelization during roasting of hazelnuts.

			150 °C		160 °C		170 °C	
Elementary reaction step		**Rate constant**	***k***	**HPD**	***k***	**HPD**	***k***	**HPD**
1	SUC→GLC+FFC	$min^{-1}\times10^3$	6.9	±0.8	15	±3.1	22	±2.0
2	GLC→1,2-ED	$min^{-1}\times10^3$	141	±27.0	473	±131	698	±178
3	1,2-ED→GLC	$min^{-1}\times10^3$	0	±0	8.5	±3.6	28	±8.3
4	GLC→3-DG	$min^{-1}\times10^3$	0.03	±0.02	0	±0	0	±0
5	GLC+AA→AP	$kg\times\mu mol^{-1}\times min^{-1}\times10^3$	0.0009	±0.0007	0.003	±0.001	0.009	±0.001
6	GLC→GO	$min^{-1}\times10^3$	0.6	±0.2	2.5	±0.9	9.2	±1.0
7	1,2-ED→FRU	$min^{-1}\times10^3$	1.3	±0.9	1.8	±0.7	4.2	±1.9
8	FRU→1,2-ED	$min^{-1}\times10^3$	0	±0	0	±0	41	±14
9	FRU+AA→HP	$kg\times\mu mol^{-1}\times min^{-1}\times10^3$	0.00023	±0.00007	0.00062	±0.00015	0	±0
10	FFC+AA→HP	$kg\times\mu mol^{-1}\times min^{-1}\times10^3$	0.00094	±0.00028	0.00027	±0.00030	0.00004	±0.00002
11	FFC→HMF	$min^{-1}\times10^3$	0.58	±0.13	0.57	±0.19	2.02	±1.32
12	HP→1-DG	$min^{-1}\times10^3$	0.23	±0.18	0.85	±0.58	267	±177
13	HP→3-DG	$min^{-1}\times10^3$	0.009	±0.004	0.022	±0.030	12	*ind*[b]*
14	AP→3-DG	$min^{-1}\times10^3$	0	±0	0.62	±0.03	0	±0
15	AP→1-DG	$min^{-1}\times10^3$	3.47	±3.12	3.51	±1.33	0.56	±0.58
16	1-DG→MGO	$min^{-1}\times10^3$	7012	±5510	33016	*ind*[b]*	47920	±33240
17	1-DG→DMG	$min^{-1}\times10^3$	371	±241	895	±581	1073	±618
18	3-DG→3,4-DG	$min^{-1}\times10^3$	4.27	±0.57	29.4	±26.1	88.1	±22.7
19	3,4-DG→HMF	$min^{-1}\times10^3$	0	±0	134	±127	390	±111
20	HP→P_1	$min^{-1}\times10^3$	11	±1.4	4.7	±5.6	59	*ind*[b]*
21	AP→P_2	$min^{-1}\times10^3$	140	±122	21.2	±34.4	5.24	±1.75
22	1-DG→P_3	$min^{-1}\times10^3$	122	*ind*[b]*	0	*ind*[b]*	404	*ind*[b]*
23	MGO→P_4	$min^{-1}\times10^3$	126	±106	737	±113	918	±639
24	DMG→P_5	$min^{-1}\times10^3$	54	±41	130	±90	106	±65
25	GO→P_6	$min^{-1}\times10^3$	18	±8.3	61	±25	290	*ind*[b]*
26	HMF→P_7	$min^{-1}\times10^3$	12	±3.7	21	±11	103	±63.7

The reaction rate constants, given in ±95% HPD, of elementary reaction steps in the proposed model were presented for each roasting temperature (Table 4.2). The reaction rate constants of most of the elementary reaction steps were found within a ±95% HPD interval. However, reaction rate constants of some steps (k_{13}, k_{16}, k_{20}, k_{22}, k_{25}) showed a higher uncertainty and could not be estimated in this interval. The reason for that might be the involvement of compounds like Heyns product or degradation products of dicarbonyl compounds, that could not be quantified analytically, in the model. However, exclusion of these compounds from the model, as given in Figure 4.4-Figure 4.12, did not give better solutions.

The temperature dependence of elementary reactions during hazelnut roasting was

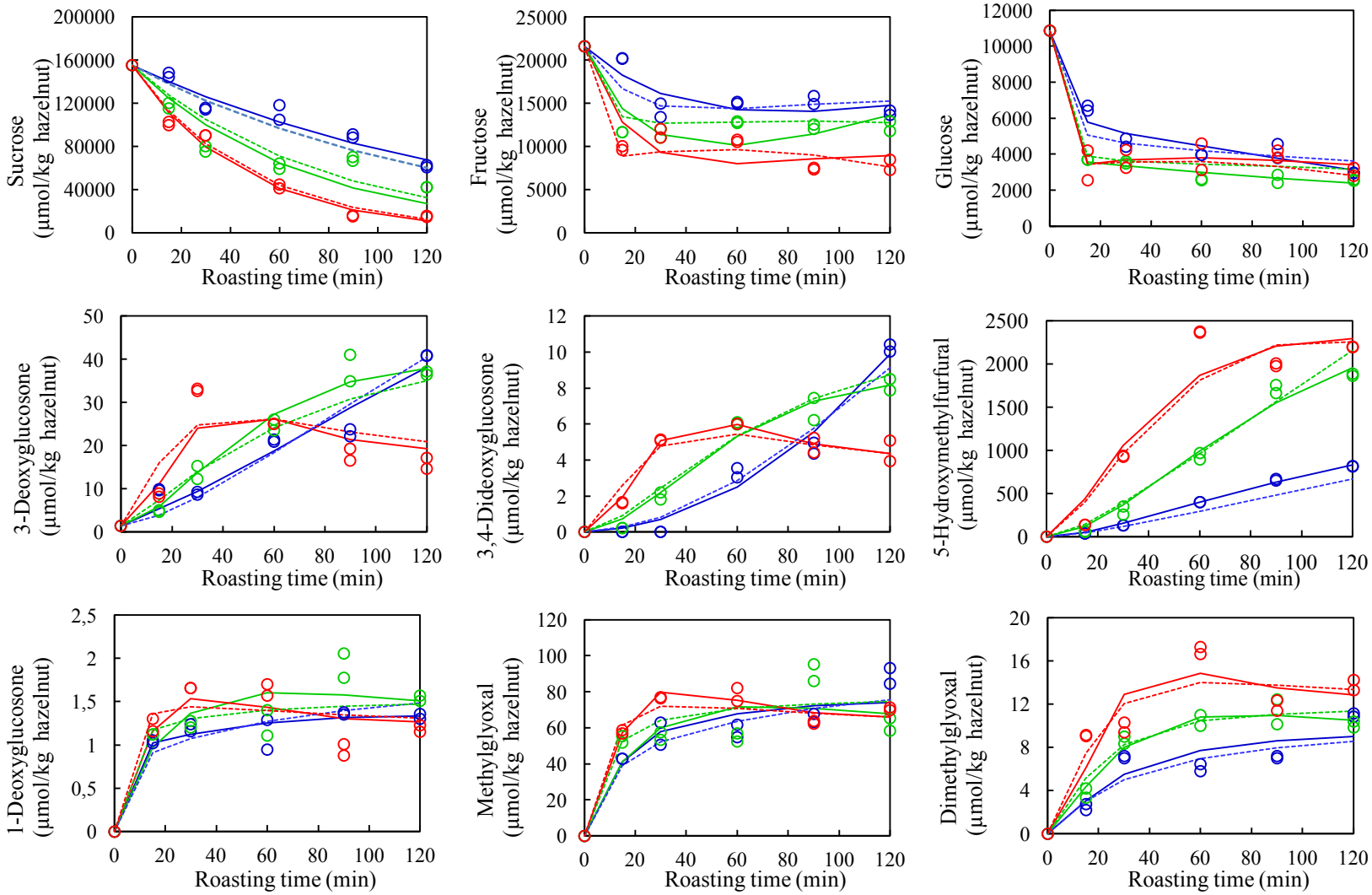
determined by the activation energies (E_a) and reaction rate constants (k_b) at reference temperature of 160°C (Table 4.3).

Table 4.3. Optimal estimates with 95% Highest Posterior Density (HPD) intervals for the reparametrized Arrhenius equation according to the proposed kinetic model (Figure 4.1) for roasting of hazelnuts.

Elementary reaction steps		Reaction rate constant unit	k_b	HPD	E_a (kJ/mol)	HPD
1	SUC→GLC+FFC	$min^{-1}\times10^3$	13.1	±1.1	77	±17
2	GLC→1,2-ED	$min^{-1}\times10^3$	1631	±2242	272	±165
3	1,2-ED→GLC	$min^{-1}\times10^3$	1005	*ind*[b]*	8.5	±3.6
4	GLC→3-DG	$min^{-1}\times10^3$	0.0023	±0.0400	53	±347
5	GLC+AA→AP	$kg\times\mu mol^{-1}\times min^{-1}\times10^3$	0.0044	±0.0004	100	±17
6	GLC→GO	$min^{-1}\times10^3$	2.89	±2.17	223	±110
7	1,2-ED→FRU	$min^{-1}\times10^3$	5422	±8603	0	±0
8	FRU→1,2-ED	$min^{-1}\times10^3$	2056	*ind*[b]*	290	±231
9	FRU+AA→HP	$kg\times\mu mol^{-1}\times min^{-1}\times10^3$	0.0023	±0.0002	96	±19
10	FFC+AA→HP	$kg\times\mu mol^{-1}\times min^{-1}\times10^3$	0.0025	±0.0031	18	±231
11	FFC→HMF	$min^{-1}\times10^3$	1.11	±1.17	117	±188
12	HP→1-DG	$min^{-1}\times10^3$	2.41	±2.37	16	±68
13	HP→3-DG	$min^{-1}\times10^3$	0.010	±0.008	182	±89
14	AP→3-DG	$min^{-1}\times10^3$	0.018	±0.009	0	±0
15	AP→1-DG	$min^{-1}\times10^3$	0.32	±0.28	0	±0
16	1-DG→MGO	$min^{-1}\times10^3$	10904	±9553	24	22
17	1-DG→DMG	$min^{-1}\times10^3$	570	±217	41	±16
18	3-DG→3,4-DG	$min^{-1}\times10^3$	22.9	±8.12	23	±8
19	3,4-DG→HMF	$min^{-1}\times10^3$	88	±40	186	±50
20	HP→P_1	$min^{-1}\times10^3$	660	±537	0	±0
21	AP→P_2	$min^{-1}\times10^3$	0.034	*ind*[b]*	706	±115
22	1-DG→P_3	$min^{-1}\times10^3$	0	*0*	0	±0
23	MGO→P_4	$min^{-1}\times10^3$	214	±195	26	±22
24	DMG→P_5	$min^{-1}\times10^3$	73	±31	0	±0
25	GO→P_6	$min^{-1}\times10^3$	88	±68	215	±111
26	HMF→P_7	$min^{-1}\times10^3$	0.01	*ind*[b]*	1174	±92

The activation energies of elementary reaction steps were found to range between 0-1174 kJ/mol with six zero and a few respectively quiet high values. However, it was reported that the activation energies of most of the chemical reactions were in the order of 120 kJ/mol [142] and this was confirmed for some of the reaction steps of Maillard reaction in an aqueous model system of glucose/glycine [165]. The inconsistent activation energy values of elementary steps of Maillard reaction and caramelization during hazelnut roasting might be explained with the effect of different complex mechanisms rather than the temperature dependence defined by the Arrhenius equation that made an over

simplification [59]. The model fits at 150, 160 and 170°C were obtained all at once during parameter estimation by using the reparametrized Arrhenius equation (Figure 4.5). On the other hand, it should be expressed that these findings represented a temperature range of 150-170°C and it might be better to study in a wider temperature range, by including lower temperatures (100-120°C), to explain Arrhenius behavior of the reactions.


Sucrose (µmol/kg hazelnut)
Fructose (µmol/kg hazelnut)
Glucose (µmol/kg hazelnut)
3-Deoxyglucosone (µmol/kg hazelnut)
3,4-Dideoxyglucosone (µmol/kg hazelnut)
5-Hydroxymethylfurfural (µmol/kg hazelnut)
1-Deoxyglucosone (µmol/kg hazelnut)
Methylglyoxal (µmol/kg hazelnut)
Dimethylglyoxal (µmol/kg hazelnut)
Roasting time (min)

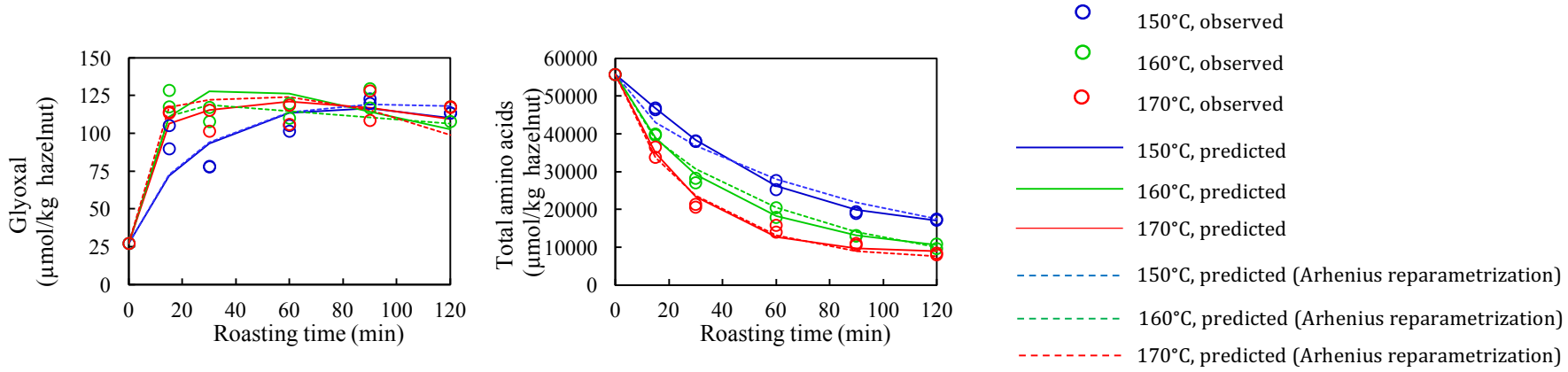
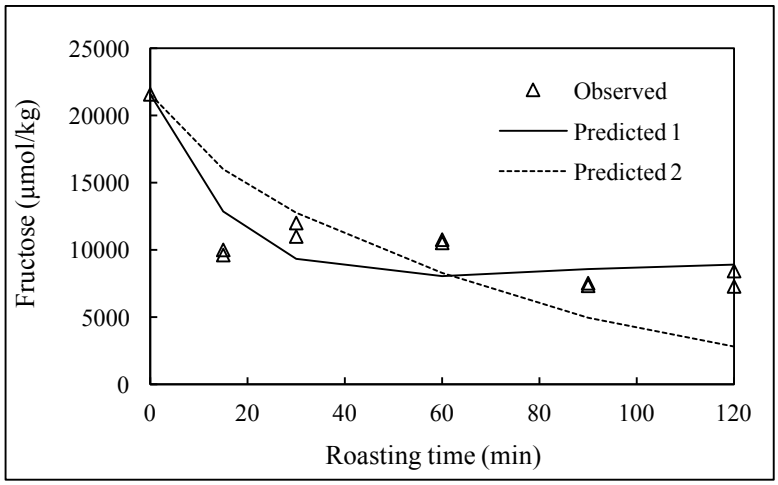


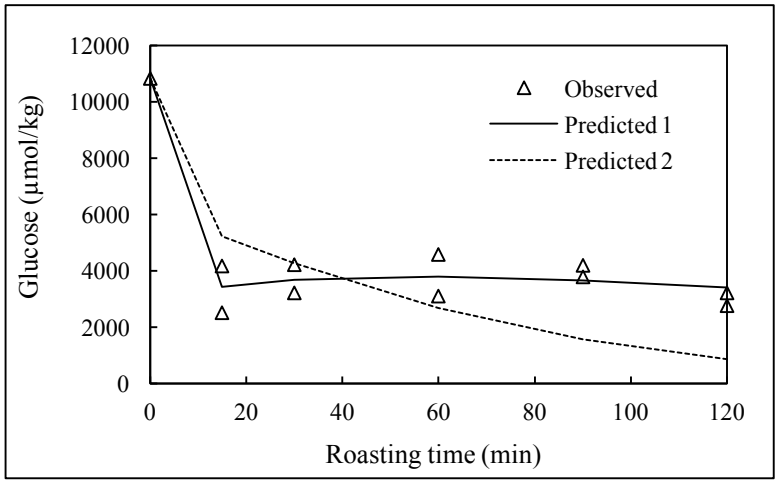
Figure 4.13. Comparison of fit of the kinetic model in Figure 4.1 to the individually obtained experimental data of reactants and products when reparametrization was not used and after performing reparametrization of the Arrhenius equation. Symbols indicate experimental data; full lines, the predicted values without using the Arrhenius equation; dashed lines, the predicted values after reparametrization of Arrhenius equation. Blue color symbols and lines designate 150; green, 160; and, red, 170°C.

4.3.3 1,2-Enolization in the Interconversion of Glucose and Fructose

In the 1,2-enolization reaction of glucose, the main product was reported to be fructose [141, 165]. Additionally, mannose could also form via epimerization of glucose through 1,2-enediol in parallel to glucose-fructose interconversion [141]. During roasting of hazelnuts, conversion of glucose to fructose or fructose to glucose via 1,2-enolization was not observed as an increase in their concentration and mannose formation could not be detected. The reason for that was obviously the immediate and simultaneous reactions of sugars other than 1,2-enolization under the conditions of hazelnut roasting. To demonstrate the importance of 1,2-enolization reaction during roasting, this reaction step was omitted from the proposed model (Figure 4.1). Differential equations were settled up again by including direct conversion of glucose to fructose and fructose to glucose and the model was tested with the experimental data. It was shown that when 1,2-enolization step was excluded from the model, the concentrations of both fructose and glucose were not estimated well and continuously decreased during roasting (Figure 4.14).



(a)



(b)

Figure 4.14. Model fits showing importance of presence of 1,2-enediol during interconversion of glucose and fructose during hazelnut roasting at 170°C. Symbols indicate experimental data; straight line (predicted 1), model fit according to Figure 4.1; dashed line (predicted 2), the model prediction of (a) fructose and (b) glucose when 1,2-enediol was omitted.

However, this was not the case as the reactions of fructose and glucose was rapid at the beginning of the roasting and their concentration did not change significantly in prolonged roasting times. Therefore, it could be stated that 1,2-eneolization is one of the rate determining steps in Maillard reaction and caramelization during roasting of hazelnuts. 1,2-enolization was also reported to be one of the primary reaction steps in Maillard reaction by other researchers [99, 165]. It was previously suggested in a glucose/wheat flour system that 1,2-enediol formation in interconversion of glucose-fructose becomes significant in the presence of amino acids because acyclic sugars primarily react with amino acids instead of enolization [99]. Contrary to that, in the absence of amino acids conversion of glucose to fructose proceeds very fast that involvement of 1,2-enolization step in the kinetic model is not necessary [99]. In case of hazelnut roasting, 1,2-enolization reaction was also found to be important and necessary reaction step proving that caramelization was not the only reaction.

4.3.4 Formation of Amadori/Heyns Product

Condensation reaction of glucose/fructose with an amino compound, following dehydration and rearrangement results in the formation of Amadori/Heyns product in the early stages of Maillard reaction [79]. These reaction steps were reduced to one reaction step (AP formation k_5, HP formation k_9) in order not to increase number of unknown

parameters. Another possible formation pathway of Heyns product is via the fructofuranosyl amine which forms as a reaction of fructofuranosyl cation and an amine [145]. Although reactions leading to formation of Amadori/Heyns product were involved in the proposed model, Amadori/Heyns product could not be measured experimentally.

The estimated reaction rate constants of Amadori product formation from glucose-amino acid reaction (k_5) were almost 5 times higher than the reaction rate constants of Heyns product formation through the reaction of fructose-amino acids (k_9) at roasting temperatures of 150 and 160°C (Table 4.2). The reason for the higher rate of Amadori product formation than Heyns product formation could be attributed to the higher chemical reactivity of aldose sugars [219]. Although the melting behavior was found to be effective in the reactivity of sugars under the conditions of low moisture [220], roasting temperatures in this study were higher than the melting temperature of both fructose and glucose. The estimated reaction rate constant of Heyns product formation through fructofuranosyl cation-amine reaction (k_{10}) was almost the same with the Amadori product formation at 150°C. However, when the roasting temperature increased, it was decreased gradually where the rate constant of Amadori product formation was increased. Additionally, Heyns product formation through fructose-amine reaction became less important during hazelnut roasting at 170°C. Glucose was found to contribute more than fructose and fructofuranosyl cation to the early stage of the Maillard reaction during hazelnut roasting especially at higher roasting temperatures.

4.3.5 Degradation of Sucrose and 5-Hydroxymethylfurfural Formation

The low moisture content of raw hazelnuts makes the reaction medium to reach high temperatures immediately. These conditions are suitable for sucrose degradation which proceeds by the cleavage of glycosidic bond. During heating under low moisture conditions, the cleavage of the glycosidic bond resulted in the release of a free glucose molecule and a very reactive intermediate, fructofuranosyl cation [145, 146]. Another possibility of fructofuranosyl cation formation was reported as formation from free fructose which was more difficult under dry heating conditions [145, 221]. This cation could easily dehydrate to form HMF because of its cyclic form or react with amines to form fructofuranosyl amine which could rearrange to form Heyns product [145]. In the proposed model of Maillard reaction and caramelization during hazelnut roasting, glucose and fructofuranosyl cation formation through sucrose degradation was included. Contribution of free fructose to the fructofuranosyl cation formation was not included in

the model (Figure 4.1) as the rate constants (k_{27}) were found to equals to zero in the comprehensive model (Figure 4.3) which means it was kinetically less significant under these conditions.

HMF not only forms through dehydration of fructofuranosyl cation but also forms via dehydration over acyclic intermediates that are 3-DG and 3,4-DG. However, the latter was reported to be less efficient in comparison to dehydration through fructofuranose ring intact [145]. The role of fructofuranosyl cation in HMF formation during hazelnut roasting was tested by excluding this reaction step from the proposed model. The predicted values of HMF were found to be far below the experimental values and indicated only the contribution of 3-DG pathway (Figure 4.15).

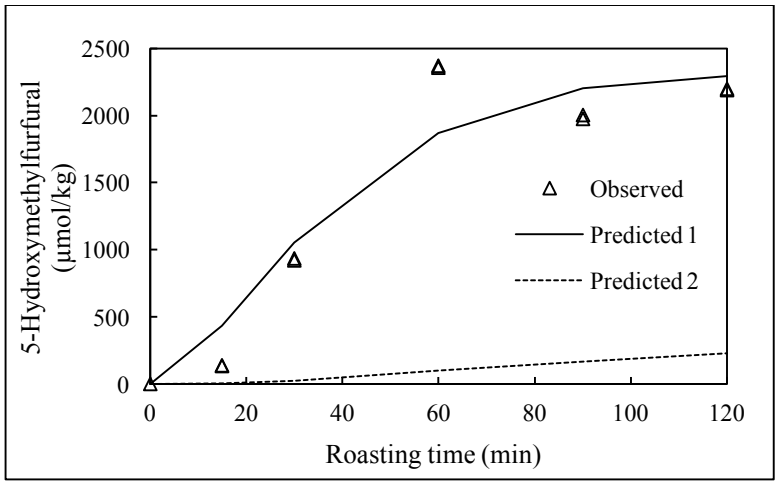


Figure 4.15. Model fit indicating the model prediction when 5-hydroxymethylfurfural formation from fructofuranosyl cation was omitted during roasting at 170°C. Symbols indicate experimental data; straight line (predicted 1), model fit according to Figure 4.1; dashed line (predicted 2), 5-hydroxymethylfurfural formation when formation through fructofuranosyl cation was omitted.

Therefore, contribution of HMF formation through dehydration of fructofuranosyl cation was found to be crucial compared to formation through 3-DG pathway during roasting of hazelnut. The reason for less contribution of 3-DG on HMF formation was due to lower reaction rate constants of 3-DG formation from glucose, Heyns product and Amadori product (k_4, k_{13}, and k_{14}) (Table 4.2). The rate constants of the 3,4-DG formation from 3-DG (k_{18}) and HMF formation from 3,4-DG (k_{19}) were higher than the rate constants of HMF formation through fructofuranosyl cation (k_{11}). The lower reaction rate constants of HMF formation through fructofuranosyl cation could be attributed to the fact that concentration of fructofuranosyl cation could not be measured because of experimental restrictions and the reaction steps of HMF formation through fructofuranosyl cation was

reduced to one dehydration step for simplification of the proposed model.

The reaction rate constants of HMF formation from 3,4-DG was found to be almost 5 times higher than the rate constant of 3,4-DG formation from 3-DG. In the HMF formation through 3-DG pathway, HMF formation from 3,4-DG was found to be the fast step and 3,4-DG formation from 3-DG was found as the rate determining step.

4.3.6 Degradation of Amadori/Heyns Product and Formation of α-Dicarbonyl Compounds

3-DG and 1-DG are formed by degradation of Amadori product with the release of amino acids [172]. The rate of formation of 3-DG through degradation of Heyns product was significantly increased (±95% HPD) with an increase in the roasting temperature (Table 4.2). However, it should be mentioned that Heyns product degradation to 3-DG could not be estimated in the 95% HPD interval at roasting temperature of 170°C. The formation rate of 3-DG through degradation of either Amadori product or glucose was estimated at only one roasting temperature which might indicate that these reaction steps were not as important as degradation of Heyns product during hazelnut roasting. In addition to that Amadori product was found to play an important role in the formation of 1-DG at 150 and 160°C while Heyns product degradation rate was found to be higher at 170°C which pointed out importance of this step at higher roasting temperatures. Moreover, the rate of both 3-DG and 1-DG formation from Heyns product showed a significant increase whereas their formation through Amadori product significantly (±95% HPD) decreased at 170°C.

Methylglyoxal was proposed to be formed from hexoses by cleavage of C_3-C_4 bond of 1-DG by Hollnagel and Kroh [95]. Weenen [110] was reported methylglyoxal formation through retro-aldolization of both 1- and 3-DG and stated 3-DG as the precursor. In the comprehensive model (Figure 4.3), methylglyoxal formation through glucose, 3-DG, and 1-DG was tested. When all three formation pathways were included (k_{16}, k_{28}, k_{29}), the comprehensive model did not fit well to experimental data and gave rise to need for simplification of the model (Figure 4.3**)**. Each formation pathway was excluded from the comprehensive model one by one and the best model fit was obtained when only formation through 1-DG (k_{16}) was included as given in Figure 4.1. This could be attributed to the higher reactivity of 1-DG in comparison to 3-DG [147]. Formation of methylglyoxal from 3-DG was also reported to be quantitatively less important in a proposed kinetic model of Maillard reaction and caramelization in glucose/flour system [99]. In parallel to the findings of that study, methylglyoxal formation was found to predominate from

degradation of 1-DG in hazelnuts during roasting. The rate constants of methylglyoxal formation through 1-DG (k_{16}) during hazelnut roasting were the highest reaction rate constants of the all steps of the proposed model with 7, 33 and 48 min^{-1} at 150, 160 and 170°C, respectively (Table 4.2). These reaction rate constants of methylglyoxal formation through 1-DG were comparatively higher than the reaction rate constants reported for Maillard reaction and caramelization in glucose/flour system [99]. Methylglyoxal and glyoxal originated from lipids might be resulted in over-estimated formation reaction rate constants (k_{16} and k_6, respectively) or higher degradation rates (k_{23} and k_{25}, respectively) (Table 4.2). However, this discrepancy would not be significant as the concentration of methylglyoxal and glyoxal originated from lipids will not expected to be more than 10% of their total concentration.

Dimethylglyoxal is the other short chain α-dicarbonyl compound that originates from 1-DG [110]. The formation of dimethylglyoxal was only considered through degradation of 1-DG in the proposed model of Maillard reaction and caramelization during hazelnut roasting. Its formation rate was found to increase with increased roasting temperature as in the case of methylglyoxal formation through 1-DG. However, the reaction rate constants of dimethylglyoxal formation was around 20, 40 and 45 folds lower at 150, 160 and 170°C than methylglyoxal formation although they were still high compared to most of the reaction steps in the proposed reaction network. Hollnagel and Kroh [95] reported an enhanced formation of dimethylglyoxal in the presence of amino compounds due to amino catalyzed rearrangement and subsequent pathways in the Maillard reaction.

Glyoxal was reported to form from glucose itself via retro-aldol scission [90]. Additionally, it was stated that the formation of glyoxal was only influenced a little (0.28%) from the addition of glycine into the glucose [110]. The reaction rate constants of glyoxal formation through glucose was comparatively lower than the reaction rate constants of methylglyoxal and dimethylglyoxal. On the other hand, it is possible to say that degradation of glucose to glyoxal was quantitatively more important than its degradation to 3-DG.

4.3.7 Reaction of Amino Acids with α-Dicarbonyl Compounds

Reactions of α-dicarbonyl compounds with amino acids are important in the Maillard reaction as they take part in the formation of aroma and colour via Strecker degradation [222]. α-Dicarbonyl compounds are also substantial as the starting material of

polymerization reactions leading to the formation of carbohydrate-based melanoidins [223]. Binary reaction of α-dicarbonyl compounds with amino acids, Strecker degradation, was included in the comprehensive model (Figure 4.3) to monitor the changes during hazelnut roasting. In this case, the fit of amino acids was not well compatible with the experimental data (Figure 4.16) which indicated these reactions might not be quantitatively important under this reaction conditions.

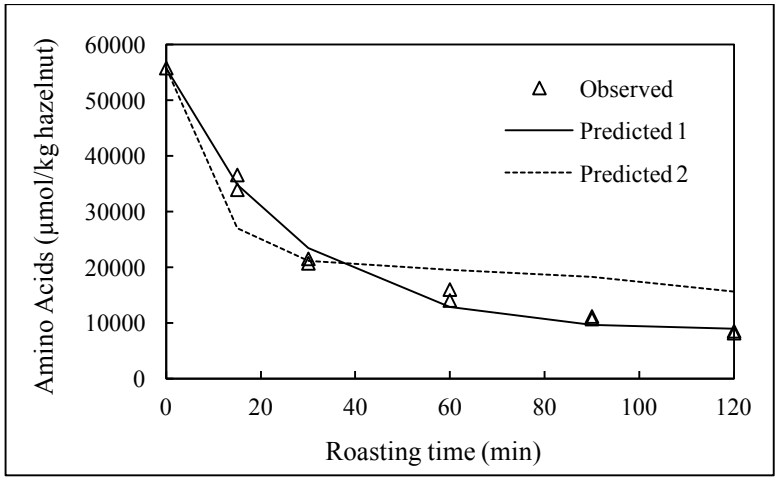


Figure 4.16. Model fit indicating the necessity of not including reactions of dicarbonyl compounds with amino acids during roasting at 170°C. Symbols indicate experimental data; straight line (predicted 1), model fit according to Figure 4.1; dashed line (predicted 2), the model fit that involves the reaction of dicarbonyl compounds with amino acids in Figure 4.3.

Therefore, reactions of α-dicarbonyl compounds with amino acids were excluded from the comprehensive model (Figure 4.3) and only their degradation reactions (k_{22}, k_{23}, k_{24}, k_{25}, k_{26}) were considered in the proposed model (Figure 4.1). After all, the model fits of amino acids were fitted well with the experimental data and the degradation rate constants of each α-dicarbonyl compound were estimated. The reactivity of 1-DG could not be well estimated in the 95% HPD interval. Degradations of 3-DG and 3,4-DG were also not included in the proposed model (Figure 4.1) because their fits were better in that case. The degradation rate constants of α-dicarbonyl compounds were followed the order of methylglyoxal (k_{23}) > dimethylglyoxal (k_{24}) > glyoxal (k_{25}) > HMF (k_{26}) and they all increased with roasting temperature. It could be speculated that the degradation rate constants of α-dicarbonyl compounds might enlighten their reactivity in formation of newly formed compounds such as carbohydrate based melanoidins. However, further research is necessary to thoroughly understand their role in melanoidin formation.

4.4 Conclusion

The proposed kinetic model provided an insight to the Maillard reaction and caramelization of a real food, hazelnut, during thermal treatment. The model discriminations and the reaction rate constants unraveled the quantitatively most important reaction steps together with rate-determining steps during hazelnut roasting. Isomerization of glucose and fructose via 1,2-enolization, formation of HMF through fructofuranosyl cation rather than 3-DG pathway, Amadori product formation through glucose-amine reaction, glyoxal formation through degradation of glucose, methylglyoxal and dimethylglyoxal formation through degradation of 1-DG was the reaction steps whose kinetically importance was stressed during roasting of hazelnuts at high temperatures. Additionally, 3,4-DG formation from dehydration of 3-DG was found to be a rate-determining step in the HMF formation. The temperature dependence of the reactions was found to be more complicated than defined by Arrhenius equation. Furthermore, it could also be stated that advance and final stages of the Maillard reaction together with caramelization play crucial role during hazelnut roasting as indicated by mass balance of the reactants and products.

GENERAL CONCLUSION AND DISCUSSION

From the scientific point of view, chemical reactions occurring in hazelnuts during roasting are not known thoroughly. Unaware of the formation of undesirable compounds during roasting, hazelnuts are traditionally roasted until to obtain a desirable color, texture or aroma. However, it is reasonable to unravel the chemistry behind roasting to be able to understand and control the chemical reactions by balancing the formation of both desirable and undesirable compounds or by identifying neo formed compounds that could be tracked during roasting. In this thesis, chemical reactions, especially Maillard reaction and caramelization, induced by roasting in hazelnuts were explained entirely and a multiresponse kinetic model was performed in order to understand the important reaction steps.

Proximate composition of fourteen hazelnut varieties belong to two consecutive harvest years were evaluated to enlighten the reactants of the chemical reactions in hazelnuts. The data obtained herein provided in depth understanding of the content of both lipid and non-lipid fraction of hazelnuts. The lipid fraction of hazelnuts was rich in monounsaturated fatty acids, especially oleic acid, followed by linoleic, palmitic and stearic acids. The triacylglycerol profile supported the distribution of fatty acids in hazelnuts as oleic acid containing triacylglycerols, OOO and OOL, were predominant while the triacylglycerols containing stearic acid were respectively in lower amounts. Hazelnut varieties were found to contain α-, β-, and γ-tocopherol and rich in especially α-tocopherol. In the water soluble fraction of hazelnut varieties, the predominant amino acids were glutamic acid, arginine and aspartic acid. Sucrose was in highest concentrations in all varieties. The other sugars were fructose and glucose, followed by stachyose, raffinose, myo-inositol in decreasing order. Hazelnuts were also rich in vitamins and minerals. Pantothenic and nicotinic acids, pyridoxal, biotin, thiamine and nicotinamide were the vitamins found in hazelnuts. The most predominant mineral was potassium and it was followed by magnesium while the iron and copper were the least abundant ones.

Roasting of hazelnuts resulted in the loss of sugars and amino acids at all roasting temperatures studied. The decreases in their concentrations were obvious even after 15 min of roasting. Sucrose and total amino acid contents of hazelnuts were almost depleted with 90% and 85% of losses at the end of roasting at 170°C for 120 min, respectively. The decreases in the concentration of sugars were related to not only sugar degradation but also

their reaction with amino compounds in Maillard reaction. Lysine was the only amino acid in proteins which was modified by carbonyl compounds originating from sugar degradation, lipid oxidation, Maillard reaction or either their common products during roasting. α-Dicarbonyl compounds were formed during roasting of hazelnuts. 3-DG gradually increased during roasting and reached to a maximum concentration of 6.7±0.1 mg/kg dw after roasting at 150°C for 120 min. The concentration of 3,4-DG was almost one fifth of the concentration of 3-DG and a lag phase was observed in the formation of 3,4-DG due to its formation from 3-DG. The least abundant deoxyhexulose was 1-DG whose concentration was reached to a maximum of 0.31±0.03 mg/kg dw after roasting at 160°C for 90 min. Dimethylglyoxal was also quantified in hazelnuts during roasting. Glyoxal was found in raw hazelnuts and its concentration increased up to four times after 15 min of roasting at all roasting temperatures. Methylglyoxal reached to a maximum concentration of 6.6±0.5 mg/kg dw after roasting at 160°C for 90 min. When all the measured α-dicarbonyl compounds was considered, the most abundant ones were 3-DG, glyoxal and methylglyoxal. Furosine, as a marker of early glycation, increased at all roasting temperatures after roasting for 15 and 30 min and followed a decreasing trend during prolonged roasting of hazelnuts. The reason for this degradation could be explained with the degradation or oxidation of fructosyllysine which could result in the formation of CML, an advanced glycation end product. Concentration of furosine was found to be four times more than the concentration of CML in roasted hazelnuts. Maillard reaction proceeded to the formation of colored compounds. L^* values of hazelnuts decreased with roasting, a^* value showed an increase and then decreased during prolonged roasting as well as b^* value. HMF, acrylamide and furan were the process contaminants analyzed in roasted hazelnuts. HMF did not create a health risk considering the dietary intake and daily consumption. Acrylamide could not be found in roasted hazelnuts as the asparagine was very limited in hazelnuts. Furan concentration was very low probably due to the processing conditions of hazelnuts and/or volatility of furan.

Effect of oil in hazelnuts to the formation of Maillard reaction advanced stage products was evaluated by heating hazelnut resembling oil:non-fat hazelnut mixtures. Changing the amount of oil in the mixtures did not cause any difference in the concentration of fructosyllysine, CML, formyline, maltosine, and pyrraline although their concentrations increased with roasting time. MP-lysine was the advanced lipation product whose

concentration in heated mixtures increased with roasting time and dependent on the amount of oil. The modifications of lysine were predominated by pyrraline (maximum 392 mg/kg protein), CML (maximum 305 mg/kg protein), formyline (maximum 46.5 mg/kg protein), MP-lysine (maximum 22 mg/kg protein) and maltosine (maximum 3.70 mg/kg protein).

A kinetic model was proposed enlightening the Maillard reaction and caramelization reactions during roasting of hazelnuts. A series of models from more simples to the comprehensive one had been also tested until to obtain the most appropriate model. The important reaction steps in the most appropriate model of the hazelnut roasting were interconversion of glucose to fructose via 1,2-enolization, HMF formation from fructofuranosyl cation formation, Amadori product degradation, formation of glyoxal from glucose, formation of dimethylglyoxal and methylglyoxal through degradation of 1-DG. In addition, HMF was formed mainly from fructofuranosyl cation which occurs degradation of sucrose. In the formation of HMF, 3,4-DG formation from 3-DG was the rate determining step. Moreover, the mass balance showed that advanced and final stages of the Maillard reaction was also important.

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Publications

(Publications within this PhD thesis are indicated with an asterisk.)

[1]* Taş, N.G., Gökmen, V., Profiling of the contents of amino acids, water-soluble vitamins, minerals, sugars and organic acids in Turkish hazelnut varieties, *Polish Journal of Food and Nutrition Sciences*, **2017**. (under review)

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Book Chapters

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Oral and Poster Presentations

(Presentations within this PhD thesis are indicated with an asterisk.)

Oral Presentations

(The presenter author underlined.)

[1]* Taş, N.G., Gökmen, V., Multiresponse kinetic modelling of Maillard reaction and caramelisation, *12th International Symposium on the Maillard Reaction*, 1-4 September, Tokyo, Japan, **2015**.

[2] Göncüoğlu, N., Gökmen, V., Targeted screening of sugar dehydration products in frying oils by high resolution mass spectrometry, *CEFood 2012: 6th Central European Congress on Food*, 23-26 May, Novi Sad, Serbia, **2012**.

[3] Göncüoğlu, N., Mogol, B.A., Durmaz, G., Gökmen, V., HMF formation in dried apricots, *CEFood 2010: 5th Central European Congress on Food*, 19-22 May, Bratislava, Slovakia, **2010**.

Poster Presentations

(The presenter author underlined.)

[1] Taş, N.G., Gökmen, V., Effect of alkalization on Maillard reaction products during cocoa roasting, *3rd International Congress on Cocoa Coffee and Tea*, 22-24 June, Aveiro, Portugal, **2015**. (Best Poster Award)

[2]* Taş, N.G., Gökmen, V., Bioactive compounds in skins of different hazelnut varieties, *7th International Conference and Exhibition on Nutraceuticals and Functional Foods*, 14-17 October, Istanbul, Turkey, **2014**.

[3] Göncüoğlu, N., Gökmen, V., Lipid oxidation and sugar dehydration products creating health risks in repeatedly used frying oils, *EuroFoodChem XVII*, 7-10 May, Istanbul, Turkey, **2013**. (National Food Technology Platform- Special Award)

[4] Göncüoğlu, N., Gökmen, V., High resolution mass spectrometry analysis of the Maillard reaction products formed in 2,4-decadienal-amino acid model systems during heating. *11th International Symposium on the Maillard Reaction "Centenary of the Maillard Reaction Discovery (1912-2012)"*, 16-20 September, Nancy, France, **2012**.

[5] Kocadağlı, T., Göncüoğlu, N., Hamzalıoğlu, A., Gökmen, V., In depth study of acrylamide formation in coffee during roasting, *11th International Symposium on the Maillard Reaction "Centenary of the Maillard Reaction Discovery (1912-2012)"*, 16-20 September, Nancy, France, **2012**.

[6] Mogol, B.A., Göncüoğlu, N., Kocadağlı, T., Gökmen, V., High resolution mass spectrometry analysis of reaction products and intermediates formed in carbonyl-asparagine model system during heating, *11th Recent Advances in Food Analysis*, 1-4 November, Prague, Czech Republic, **2011**.

[7] Göncüoğlu, N., Mogol, B.A., Gökmen, V., Regeneration of frying oils by using adsorbent resins, *11th International Congress on Engineering and Food*, 22-26 May Athens, Greece, **2011**.

[8] Göncüoğlu, N., Mogol, B.A., Gökmen, V., Removal of hydroxymethylfurfural from frying oil by using adsorbent resin, *2nd Food Safety Congress*, 9-10 December, Istanbul, Turkey, **2010**. (Second Best Poster Award)

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GRADUATE SCHOOL OF SCIENCE AND ENGINEERING
THESIS/DISSERTATION ORIGINALITY REPORT

HACETTEPE UNIVERSITY
GRADUATE SCHOOL OF SCIENCE AND ENGINEERING
TO THE DEPARTMENT OF FOOD ENGINEERING

Date: 15/06/2017

Thesis Title / Topic: INVESTIGATION OF CHEMICAL REACTIONS IN HAZELNUT INDUCED BY ROASTING

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