INVESTIGATION OF AMINO ACID MODIFICATIONS DERIVED FROM LIPID OXIDATION IN FOODS

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YEŞİM KARADEMİR

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submitted by YEŞİM KARADEMİR in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Food Engineering Department, Middle East Technical University** by,

Prof. Dr. Halil Kalıpçılar
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Serpil Şahin
Head of Department, **Food Engineering, METU** _____

Assoc. Prof. Dr. Halil Mecit Öztop
Supervisor, **Food Engineering, METU** _____

Prof. Dr. Vural Gökmen
Co-Supervisor, **Food Engineering, Hacettepe University** _____

Examining Committee Members:

Prof. Dr. Alev Bayındırlı
Food Engineering, METU _____

Assoc. Prof. Dr. Halil Mecit Öztop
Food Engineering, METU _____

Assist. Prof. Dr. Elif Turabi Yolaçaner
Food Engineering, Hacettepe University _____

Assist. Prof. Dr. Tolgahan Kocadağlı
Food Engineering, Hacettepe University _____

Assist. Prof. Dr. Süreyya Özcan Kabasakal
Chemistry, METU _____

Date: 19.12.2019

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Surname: Yeşim Karademir

Signature:

ABSTRACT

INVESTIGATION OF AMINO ACID MODIFICATIONS DERIVED FROM LIPID OXIDATION IN FOODS

Karademir, Yeşim

Doctor of Philosophy, Food Engineering

Supervisor: Assoc. Prof. Dr. Halil Mecit Öztop

Co-Supervisor: Prof. Dr. Vural Gökmen

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This study aimed to investigate lipid derived amino acid modifications in foods. When the revealed carbonyl pool resulted from the repeated use of frying oils and high absorption rates of the oil during frying are taken into consideration, deep-fat fried foods have a significant potential to investigate this type of reactions. In the first part, effect of heating time of the frying oil on amino acid modifications in potato chips and model doughs was investigated. 2,4-decadienal, total free amino acid, decadien-1-amine, thiobarbituric acid reactive substances (TBARs), 2-pentylpyridine, acrylamide analyses were carried out for this purpose. The results presented here revealed that 2,4-decadienal, one of the lipid oxidation products accumulated in repeatedly used oil, actively involved in Maillard type carbonyl-amine reactions occurring in potatoes during frying. To the best of our knowledge, 2-pentylpyridine and decadien-1-amine in potato chips were reported for the first time in this study as products of the reactions occurred between 2,4-decadienal and amino acids. Frying oil was found to have the highest concentration of 2,4-decadienal after thermal oxidation at 180 ^{o}C for 6 h. Expectedly, potato chips fried in

this oil had the highest concentration of 2,4-decadienal (29 mg/kg). There was a positive correlation (r^2=0.73) between the concentrations of 2,4-decadienal and decadien-1-amine (relative concentration as peak area) formed in potato chips fried in repeatedly used sunflower oil. No 2-pentylpyridine was detected in potato chips fried in unoxidized oil, whereas its concentration ranged between 91 and 154 µg/kg in potato chips fried in oxidized oil. According to the model system studies, addition of glucose and fructose to model doughs consisted of only whey protein and water resulted in lesser amounts of decadien-1-amine, most probably because of the competition of the reducing sugars with lipid carbonyls on the reaction with amino acids. Moreover, the highest yields of decadien-1-amine were observed in the doughs prepared at 4 h heated oil. Model doughs containing potato flour appeared to have similar trends of 2,4-decadienal and decadien-1-amine with potato chips while the decline of total free amino acids in the first 4 h of the heating was more apparent in model doughs (M4) compared to real food systems. Second part of the study included the investigation of the impact of different oil types on amino acid modifications in potato chips. Safflower, corn, canola, hazelnut and olive oils were used in this context and p-anisidine, 2,4-decadienal, total free amino acids, decadien-1-amine, carboxymethyllysine (CML), carboxyethyllysine (CEL) analyses were performed. This impact was observed in decadien-1-amine formation in the order of the safflower>hazelnut>canola>olive>corn oils. CML concentrations of the poato chips were detected to be increased significantly ($p<0.05$) within the first 4 hours of the heating. Mean CML concentrations were determined to be 1063, 958, 815, 680 and 522 ng/g in the chips prepared within the hazelnut, canola, safflower, corn and olive oil respectively. In case of using the oils with very high amounts of linoleic acid such as safflower oil, increase in CML and CEL levels of the potato chips within the first 4 hours of the heating was very obvious in spite of less likely gradual accumulation of the glyoxal (GO) and methylglyoxal (MGO) in the oil during the heating of the oil. For the first 4 hours of the heating, the decrease ($p<0.05$) in the levels of CML and CEL of the chips prepared in the oils (hazelnut, olive, canola) with high oleic acid content was remarkable while the reverse of this behavior was

observed in the high linoleic acid oils (safflower, canola). Finally, in the last part, the effect of different treatments (antioxidant addition to the oil, coating, pan frying) on the amino acid modifications in potato chips and fried chickens was investigated. Similarly, p-anisidine, 2,4-decadienal, total free amino acids, decadien-1-amine, CML, CEL were measured in this part. 1% tocopherol mix which was added to the frying oil in order to increase oxidation stability triggered the oxidation of the oil. When decadien-1-amine, CML and CEL formation was monitored in the chips prepared within the more oxidized oil, the ocurrence of much more modification of the free amino acids was demonstrated. Although no significant difference ($p>0.05$) was detected between p-anisidine values of 0.1% BHT (T2) or 0.1% tocopherol mix (T3) added oils with the control (T1), the highest increment of 2,4-decadienal in chicken meatballs was observed in T1 group while the lowest rises were in T2 and T3 within the first 4 h of the heating the oil. In addition, T1 was found to have the lowest levels of free amino acids. However, while T3 led to the highest decadien-1-amine yields, T2 was determined to form the highest CML levels. Due to the reduced oil absorption, the protective effect of the coating treatment (T4) and pan frying (P) on lipid derived amino acid modifications was obviously observed by the lower levels of decadien-1-amine and CML formation.

Keywords: Lipid browning, decadien-1-amine, 2,4-decadienal, fried foods, CML

ÖZ

GIDALARDA LİPİT OKSİDASYONUNDAN KAYNAKLANAN AMİNO ASİT MODİFİKASYONLARININ ARAŞTIRILMASI

Karademir, Yeşim
Doktora, Gıda Mühendisliği
Tez Danışmanı: Doç. Dr. Halil Mecit Öztop
Ortak Tez Danışmanı: Prof. Dr. Vural Gökmen

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Bu çalışma gıdalarda lipit kaynaklı amino asit modifikasyonlarının araştırılmasını hedeflemiştir. Kızartma yağlarının tekrar tekrar kullanılması sonucu oluşan karbonil havuzu ve kızartma sırasında yağın yüksek oranda absorpsiyonu göz önüne alındığında, derin yağda kızartılmış gıdalar, bu tip reaksiyonları araştırmak için önemli bir potansiyele sahiptir. Birinci bölümde, kızartma yağının ısıtma süresinin patates cipsi ve model hamurlardaki amino asit modifikasyonları üzerine etkisi incelenmiştir. Bu amaçla, 2,4-dekadienal, toplam serbest amino asit, dekadien-1-amin, tiyobarbitürik asit reaktif maddeler, 2-pentilpiridin, akrilamid analizleri gerçekleştirilmiştir. Burada sunulan sonuçlar, art arda kullanılmış yağda biriken lipit oksidasyon ürünlerinden 2,4-dekadienalin, kızartma sırasında patateslerde meydana gelen Maillard tipi karbonil-amin reaksiyonlarında aktif olarak rol oynadığını ortaya koymuştur. Bildiğimiz kadarıyla, 2,4-dekadienal ve amino asitler arasında gerçekleşen reaksiyon ürünleri olarak 2-pentilpiridin ve dekadien-1-amin patates cipslerinde ilk kez bu çalışmada rapor edilmiştir. Kızartma yağının 180 °C' de 6 saat boyunca termal oksidasyonundan sonra en yüksek 2,4-dekadienal konsantrasyonuna

sahip olduğu bulunmuştur. Beklenildiği gibi, bu yağda kızartılmış patates cipsleri de, en yüksek 2,4-dekadienal (29 mg/kg) konsantrasyonuna sahip olmuştur. Tekrar tekrar kullanılan ayçiçek yağında kızartılmış patates cipslerinde oluşan dekadien-1-amin (pik alanı olarak, bağıl konsantrasyon) ve 2,4-dekadienal arasında pozitif bir korelasyon (r^2 = 0.73) tespit edilmiştir. Okside olmuş yağda kızartılan patates cipslerinde 91-154 µg/kg konsantrasyonları arasında 2-pentilpiridin tespit edilirken, okside olmamış yağda kızartılan patates cipslerinde 2-pentilpiridin saptanmamıştır. Model sistem çalışmalarına göre, sadece peynir altı suyu proteini ve su içeren model hamurlara glukoz ve fruktoz ilavesi, büyük olasılıkla, indirgen şekerlerin amino asitlerle olan reaksiyonlarında lipit karbonilleriyle olan rekabetinden dolayı daha az miktarlarda dekadien-1-amin oluşumu ile sonuçlanmıştır. Ayrıca, 4 saat ısıtılmış yağda hazırlanan hamurlarda en yüksek dekadien-1-amin verimi tespit edilmiştir. Patates unu içeren model hamurların (M4), patates cipsi ile benzer 2,4-dekadienal ve dekadien-1-amin trendine sahip olduğu görünürken, ısıtmanın ilk 4 saatindeki toplam serbest amino asitlerin düşüşü gerçek gıda sistemlerine kıyasla model hamurlarda daha belirgin olmuştur. Çalışmanın ikinci kısmı, farklı yağ türlerinin patates cipsindeki amino asit modifikasyonları üzerindeki etkisinin araştırılmasını içermektedir. Bu kapsamda aspir, mısır, kanola, fındık ve zeytinyağı kullanılmış ve p-anisidin, 2,4-dekadienal, toplam serbest amino asit, dekadien-1-amin, karboksimetillizin, karboksietillizin analizleri yapılmıştır. Bu etki, dekadien-1-amin oluşumunda aspir>fındık>kanola>zeytin>mısır yağları sırasına göre gözlemlenmiştir. Patates cipslerindeki CML konsantrasyonlarının, ısıtmanın ilk 4 saati içinde önemli ölçüde arttığı tespit edilmiştir. Ortalama CML miktarları, fındık, kanola, aspir, mısır ve zeytinyağında hazırlanan cipslerde sırasıyla 1063, 958, 815, 680 ve 522 ng/g olarak belirlenmiştir. Aspir yağı gibi çok yüksek miktarda linoleik asit içeren yağların kullanılması durumunda, yağın ısıtılması sırasında gliokzal ve metilgliokzalın yağda kademeli olarak birikmesi düşük olasılıkta olmasına rağmen, ısıtmanın ilk 4 saati içerisinde patates cipslerindeki CML ve CEL seviyeleri çok belirgin bir şekilde artmıştır. Isıtma işleminin ilk 4 saati için, yüksek oleik asit içeriğine sahip yağlarda (fındık, zeytin, kanola) hazırlanan cipslerin CML ve CEL

seviyelerinde azalma dikkat çekerken, bu davranışın tersi yüksek linoleik asit içerikli yağlarda (aspir, kanola) gözlemlenmiştir. Son olarak, son bölümde, farklı işlemlerin (yağa, antioksidan ilaveye, tavada kızartmaya) patates cipsi ve kızarmış tavuklardaki amino asit modifikasyonları üzerindeki etkisi incelenmiştir. Benzer şekilde, bu kısımda p-anisidin, 2,4-dekadienal, toplam serbest amino asit, dekadien-1-amin, CML, CEL ölçülmüştür. Oksidasyon stabilitesini arttırmak için kızartma yağına ilave edilen %1 tokoferol karışımı yağın oksidasyonunu tetiklemiştir. Daha fazla okside olmuş yağ içerisinde hazırlanan patates cipslerinde, dekadien-1-amin, CML ve CEL oluşumu takip edildiğinde çok daha fazla serbest amino asit modifikasyonu olduğu gösterilmiştir. %0,1 BHT (T2) veya %0,1 tokoferol karışımı (T3) ilave edilen yağların p-anisidin değerleri ile kontrol grubu (T1) arasında önemli bir fark görülmemesine rağmen, yağın ilk dört saatlik ısıtılması sırasında, tavuk etinden hazırlanan köftelerde en yüksek 2,4-dekadienal artışı T1 grubunda görülürken, en düşük artış T2 ve T3 grubunda görülmüştür. Ek olarak, T1'in en düşük serbest amino asit seviyelerine sahip olduğu bulunmuştur. Bununla birlikte, T3 en yüksek dekadien-1-amin oluşumuna neden olurken, T2' nin en yüksek CML seviyelerini oluşturduğu belirlenmiştir. Yağ absorpsiyonunun azalmasından dolayı, kaplama (T4) ve tavada kızartma (P) işleminin lipit kaynaklı amino asit modifikasyonları üzerindeki koruyucu etkisi, daha düşük miktarlarda dekadien-1-amin ve CML oluşumu ile açık bir şekilde gözlemlenmiştir.

Anahtar Kelimeler: Lipit esmerleşmesi, dekadien-1-amin, 2,4-dekadienal, kızartılmış gıdalar, karboksimetillizin

To all honorable scientists of all times in the world, who went and will go after the science since he just wants to find answers of his questions in spite of all drawbacks…

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LIST OF ABBREVIATIONS

L·: Alkyl radicals

LOO·: Peroxyl radicals

S: Total Spin

NEB: Non enzymatic browning

CML: N(ε)-carboxymethyllysine

CEL: N(ε)-carboxyethyllysine

MDA: Malondialdehyde

BHA: Butylated hydroxyanisole

BHT: Butylated hydroxytoluene

TBHQ: Tert-butylhydroquinone

PG: Propyl gallate

TBARs: Thiobarbituric acid reactive substances

LYS: Lysine

PTFE: Polytetrafluoroethylene

LC-MS/MS: Liquid chromatography with tandem mass spectrometry

UPLC-HRMS: Ultra performance liquid chromatography high resolution mass spectrometry

FTIR: Fourier transform infrared spectroscopy

GO: Glyoxal

MGO: Methylglyoxal

SD: Standard deviation

SEM: Standard error of the mean

M1: Model dough consisted of 25 g of whey protein and 15.9 mL of pure water

M2: Model dough consisted of 0.5% glucose and 0.5% fructose in addition to 25 g of whey protein and 15.9 mL of pure water

M4: Model dough consisted of 25 g of potato flour and 30 mL of pure water

SO: Sunflower oil

SO+A: Sunflower oil that contains 1% (v/v) tocopherol mix (α-β-δ-γ)

T1: Chicken meatballs prepared in frying oil without any treatment

T2: Chicken meatballs prepared in frying oil with 0.1% (v/v) BHT

T3: Chicken meatballs prepared in frying oil with 0.1% (v/v) tocopherol mix (α-β-δ-γ)

T4: Coated chicken meatballs

P: Pan fried chicken meatballs

LOD: Limit of detection

LOQ: Limit of quantification

LIST OF SYMBOLS

k: reaction rate constant

Ea: Activation energy

CHAPTER 1

INTRODUCTION

1.1. Lipid oxidation

1.1.1. Lipid oxidation and its importance in foods

When its effects on food and complex reaction structure are considered, lipid oxidation is one of the important reactions in food science. While unpleasant odor, flavor and taste formation affect food quality negatively, on the other hand toxic reaction products pose a health risk (Frankel, 1980). As a result of oxidation, the formation of rancid taste and odor adversely affects food consumability, however in some cases, such as ripened cheese and fried foods, a limited level of lipid oxidation is needed to produce the desired taste and odor compounds (Nawar, 1996).

Lipid oxidation is a free radical chain reaction and consists of 4 stages: initiation, propagation, branching and termination. It is thermodynamically difficult for fatty acid to react with oxygen to form free radicals (activation energy: 35 kcal). Therefore, the catalyst is required for initial free radical formation. Free radicals can be formed by metal catalysts, light or direct thermal decomposition of hydroperoxides. The reaction mechanism changes according to the catalysts that play role in the reaction thereof oxidation is pronounced by different names such as peroxidation, photooxidation (Schaich, 2005; Belitz et al., 2009).

In the initial stage, the alkyl radical ($L^{\cdot}$) is formed by the cleavage of H from the C atom adjacent to the double bond on the fatty acid chain, and the exposed single electron delocalizes causing the double bond to be displaced on the chain. In the propagation step, O_2 is added to the free radical chain from the same region to form peroxyl radicals ($LOO^{\cdot}$). Hydroperoxide and new free radicals are formed by the separation of H from fatty acids and other molecules (LH) by peroxyl radicals. The

arising free radicals react with oxygen. The series of reactions repeated in this way allow the reaction to proceed autocatalytically. There are many findings supporting the tendency of oxidation reactions to proceed on the same molecule rather than a new acyl chain (Schaich, 2005). The reaction continues until no hydrogen source is left or the chain reaction is stopped.

Because of their partial stability, hydroperoxides, the first lipid oxidation products discovered, play role in starting the complex reaction series. After decomposition to peroxy and alkoxy radicals, secondary oxidation products such as aldehyde, ketone, alcohol, acid and lactones are produced (Benzie, 1996; Lundberg & Chipault, 1947; Schaich, 2005). Secondary reaction products are responsible for impaired taste, flavor and texture in foods, and reactions which have negative effects on the human body (Eldin et al., 2003). In the termination phase, the alkyl, alkoxy and peroxy radicals formed after the decomposition reactions, and combine to generate more stable dimer and dimer-like products.

Model system studies have shown that autoxidation rate is affected by fatty acid composition, degree of unsaturation, presence and activity of prooxidant and antioxidants, partial pressure of oxygen, surface area that contact with oxygen and storage conditions (temperature, light, moisture). Apart from these factors, the lipid structure and the position of the unsaturated fatty acid in the triacylglycerol molecule also affect the rate of autoxidation. Miyashita and Takagi, showed that free oleic, linoleic and linolenic acid undergoe faster autoxidation compared to methyl esters and it was suggested that the catalytic activity of carboxyl groups on free fatty acids in the decomposition of hydroperoxides might play role in the occurence of this condition (Miyashita and Takagi, 1986; Eldin et al., 2003). In addition, unsaturated fatty acids at the positions of 1 and 3 in triacylglycerol molecule are oxidized more rapidly than those at the position 2 (Belitz et al., 2009).

Propagation

(1) $L\bullet + O_2 \longrightarrow LOO\bullet$

(2) $LOO\bullet + LH \longrightarrow LOOH + L\bullet$

(3) $LO\bullet + LH \longrightarrow LOH + L\bullet$

Branching

(4) $LOOH \longrightarrow LO\bullet + \bullet OH$

(5) $2\ LOOH \longrightarrow LOO\bullet + LO\bullet + H_2O$

Termination

(6) $2\ L\bullet \longrightarrow$

(7) $L\bullet + LOO\bullet \longrightarrow$ } Stable products

(8) $2\ LOO\bullet \longrightarrow$

Figure 1-1. Steps of Lipid Oxidation (Belitz et al., 2009)

1.1.2. Autooxidation phases

1.1.2.1. Initiation

Oxidation of unsaturated fatty acid usually begins with the initial phase, known as the lag phase or more commonly the induction period. Length of induction period and rate of oxygen use depend on fatty acid composition, the presence of prooxidant-antioxidant and catalyst in the medium. In the presence of more allyl groups, induction period is shortened and oxidation rate increases (Belitz et al., 2009).

The mechanism for the formation of the first hydroperoxides causing the initiation of chain reactions is still unknown. Thermodynamically, oxygen can not react directly

with double bonds since spin states are different. The total angular momentum of electrons in an atom is expressed by 2S + 1 (S: Total spin). If the atom has unpaired electrons in its outermost orbitals, such as oxygen, there are two different states depending on whether the spin is parallel or antiparallel. For oxygen, these states are called triplet, $^3O_2$ [2 (½ + ½) + 1 = 3] and singlet, $^1O_2$ [2 (½ - ½) + 1 = 1]. Oxygen in the singlet state is more electrophilic than the oxygen in the triplet state and in the case of high electron density, it reacts 1500 times faster than the triplet oxygen. Singlet oxygen is thought to be effective in the initiation of photooxidation (Nawar, 1996).

Oxygen in the ground state is in the triplet state (two free electrons in different orbitals, with the same spin direction and net positive angular momentum) while the double bond is in the singlet state (no unpaired electrons, the paired electrons in the same orbital and in different spin directions, no net angular momentum). According to quantum mechanics, the angular momentum of the spin must be maintained during the reaction, and the double bond must be excited to be excited to a triplet state. This requires a high amount of activation energy (Ea= 35-65 kcal/mole) (Schaich, 2005). Therefore, reaction can not occur directly. The spin barrier could be overcame and the lipid oxidation is started by electron removal of the reaction initiator or catalyst from the lipid or oxygen or by changing the electron spin of the oxygen. Because only a trace amount of catalyst is needed, in many cases where it is thought as spontaneous and without catalyst, contaminants and undetermined or ignored conditions are effective. Some of the substances that initiate or catalyze the reaction include metal, light, temperature, lipoxygenase, heme proteins and porphyrins, ozone and free radicals (Schaich, 2005). The spin states of oxygen and double bond in the ground states are shown in Figure 1.2.

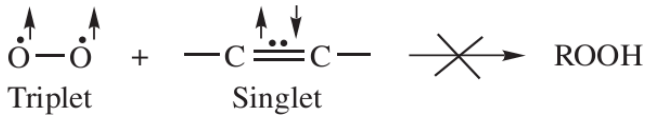

Figure 1.2. Triplet and singlet states of oxygen and double bond (Schaich, 2005)

Hydrogen breakdown by free radicals is generally quite specific and preferably occurs in allylic hydrogen where the C-H bond energy is the lowest. H bond energy according to the position is shown in Table 1.1.

Table 1.1. Bond energies of hydrogens at different positions in the acyl chain (Schaich, 2005)

	E (kJ/mol)	E (kcal/mol)
H–CH=CH_2	431	105
H–CH_2–CH_2–CH_3	419	99
H–CH_2–CH=CH_2	356	85
R–**H**CH–CH=CH–CH_2–CH_3	322	77
R(CH_2=CH)–HC**H**–CH_2–	310	74
R–CH=CH–HC**H**–CH=CH–	272	65
ROO**H**	377	90

1.1.2.2. Propagation

The electron released by the removal of allylic hydrogens is distributed along the double bond which is stabilized by resonance. The electron density at the center is high, while the electron density at the outer positions is partially lower. Therefore, oxygen tends to form a peroxyl radical (LOO·) by bonding to the outermost points (Schaich, 2005).

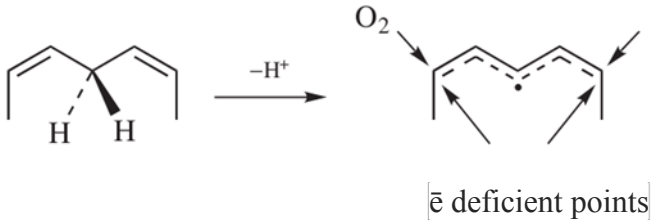

Figure 1.3. Addition of oxygen to the double bond (Schaich, 2005)

1.1.2.2.1. H transfer by peroxyl radicals (LOO•)

After abstraction of H from the 8th and 11th C atoms on the oleic acid chain, the addition of oxygen and hydrogen to the allylic radicals results in a mixture of isomeric hydroperoxide (8-, 9-, 10-, 11-). These 4 isomers can be found in 8 different forms with regards to cis and trans isomers (Neff et al., 1978; Haslbeck & Grosch, 1983; Frankel et al., 1984; Porter et al., 1994; Eldin et al., 2003). Figure 1.4 shows the formation of monohydroperoxides from oleic acid.

Due to its 1,4-pentadiene structure, linoleic acid is more sensitive to oxidation than propene oleic acid. The presence of the double bond in the fatty acid chain weakens the C-H bond at the carbon atom adjacent to the double bond and facilitates the abstraction of H. The dissociation energy for the bis allylic C-H bond is ~ 85 ± 3 kcal/mol, while the dissociation energy for the mono allylic C-H bond is ~ 10 kcal/mol higher (Reich & Stivala, 1969; Wu et al., 1978; Porter, 1986; Gardner, 1989; Eldin et al., 2003).

The separation of H from the 11th C atom adjacent to the two double bonds yields the pentadienyl radical as an intermediate followed by a mixture of 9- and 13-diene hydroperoxide. Released hydroperoxides can exist in both cis-trans and trans-trans forms. The formation of monohydroperoxides from linoleic acid was depicted in Figure 1.5. While the reaction of the alkyl radical (L·) with oxygen to form a peroxyl radical (LOO·) is rapid, subsequent transfer of H from another hydrogen source

(such as unsaturated fatty acids, antioxidants) occurs slowly leading to limitation on the radical formation rate (Knothe, 2007; Belitz et al., 2009).

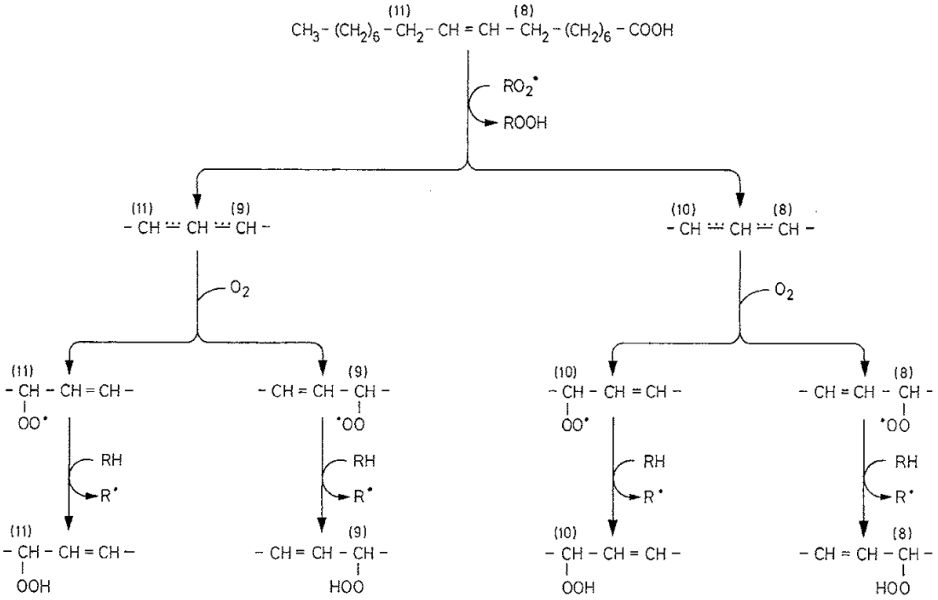

Figure 1.4. Autooxidation of oleic acid and primary oxidation products (Belitz et al., 2009)

Three factors are important for H-cleavage by peroxyl radicals: 1- Available H source in lipid and solvent 2- Viscosity of the medium 3- Temperature (Walling & Padwa, 1963; Schaich, 2005).

For proton-free solvents containing only lipid allyl groups as a source of H, it is easier to break off H in presence of polyunsaturated fatty acids having bisallylic H and in the high lipid concentration where the interaction of fatty acid chains is increased. In low viscosity environments where chain lengths are substantially shortened, H breakage is also favored (Factor et al., 1965; Porter et al., 1995; Schaich, 2005). On the other hand, if the solvent or other compounds in the system has H, H is cleaved off competitively from non-lipid regions. In this case, the

radicals are scavenged and the chain reactions end instead of proceeding. At high temperatures, the thermal energy decreases the bond dissociation energy, making it easier to break H.

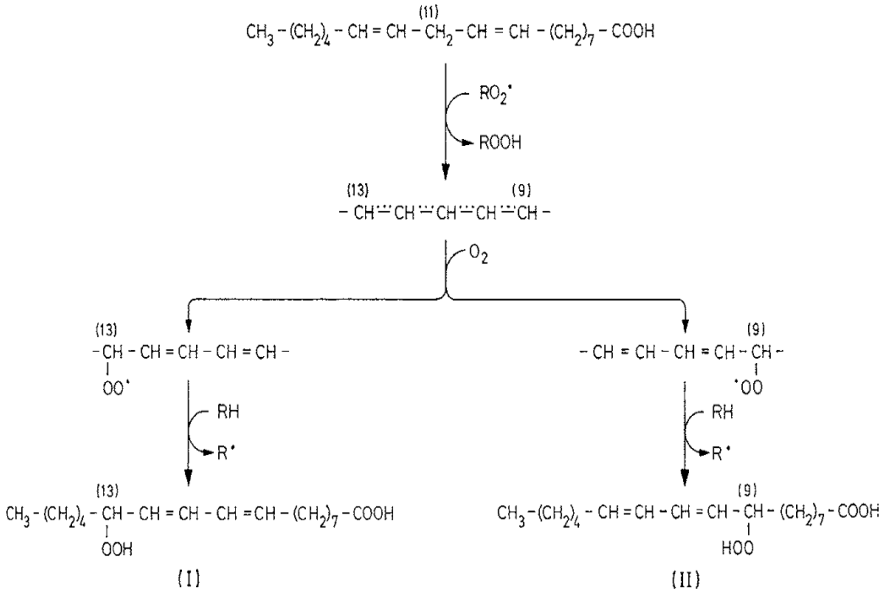

Figure 1.5. Autooxidation of linoleic acid and primary oxidation products

(Belitz et al., 2009)

1.1.2.2.2. Rearrangement and cyclization of peroxyl radicals (LOO•)

Cyclization is observed especially in polyunsaturated fatty acids having 3 or 4 double bonds and radicals with peroxyl group in internal positions. When H is not present in the medium, the peroxyl radicals are attached to the intramolecular double bonds to find electron pairs and form cyclic compounds. The peroxyl groups of 25% of linolenic acid peroxyl radicals and 33% of arachidonic acid peroxyl radicals are in internal position. Therefore, linolenic and arachidonic acid tend to form cyclic

peroxide. The cyclization process of linolenic acid peroxyl radicals is shown in Figure 1.6. .

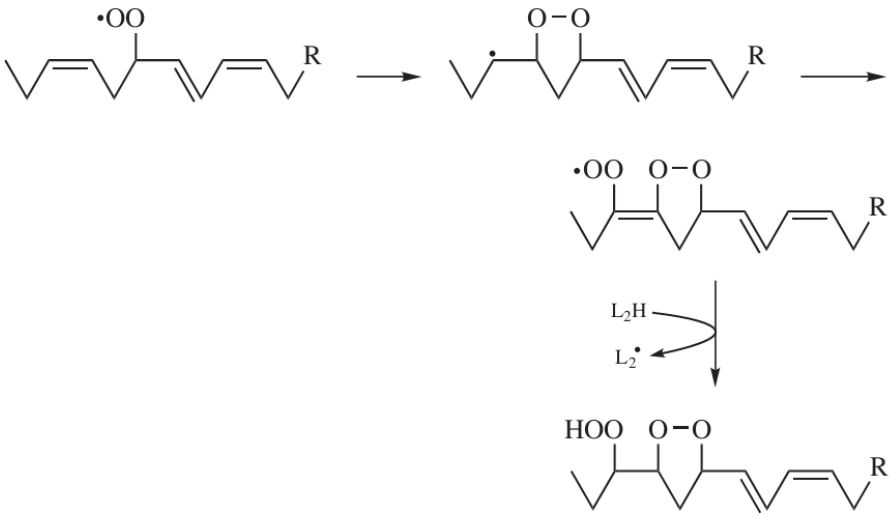

Figure 1.6. Cyclization of linolenic acid peroxyl radicals (Schaich, 2005)

Secondary peroxyl radicals are formed by the addition of oxygen after the formation of the ring structure. The resulting radicals break off H from an H source to form hydroperoxy epidioxides. In this way chain reactions are continued.

Since there is no cis-double bond - hydroperoxide structure (homoallicity) required for the reaction, linoleic acid can not form epidioxide by cyclization during autoxidation. However, as a result of photooxidation of linoleic acid, the number of hydroperoxides in the inner positions (10-, 12-) is close to the number of hydroperoxides in the outer positions (9-, 13-). The hydroperoxides which have hydroperoxide group in the internal position with cis-double bond structure necessary for cyclization. Peroxyl radicals formed by the removal of H from these hydroperoxides undergo cyclization to form high amounts of hydroperoxy epidioxide and epidioxy-hydroperoxides (Frankel et al., 1979; 1982; Mihelich, 1980; Schaich, 2005). This formation is presented in Figure 1.7:

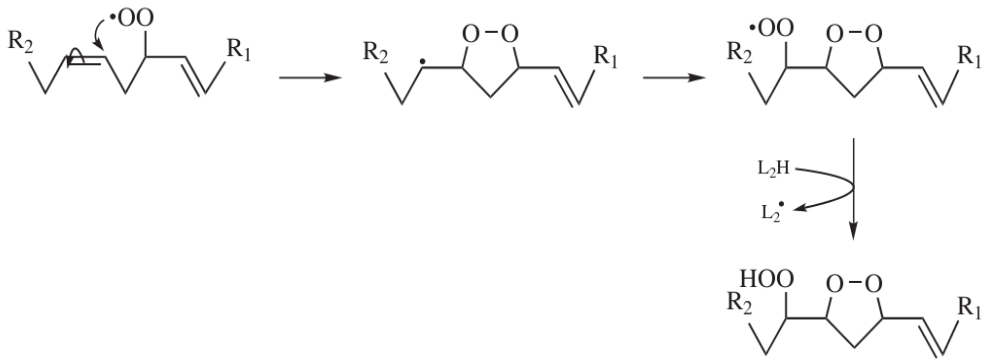

Figure 1.7. Hydroperoxy epidioxide and epidioxy-hydroperoxide formation (Schaich, 2005)

Because epidioxide-OO· radicals are very reactive, they tend to dimerize even at very high temperatures (Neff et al., 1988; Schaich, 2005). This situation makes their analyses difficult.

1.1.2.2.3. Addition of peroxyl radical (LOO•) to double bond

When the available amount of H is limited (in proton free solvents, at low temperature), addition reactions of LOO· occur competitively in the presence of 1,1 disubstituted double bonds or in conjugated, terminal position. (Hiatt & McCarrick, 1975; Schaich, 2005). According to Gardner, LOO· forms the same type of reaction products by adding to conjugated double bonds of oxidation products as well as double bonds of unoxidized linoleic acid (Gardner 1989; Schaich, 2005).

The addition of peroxyl radicals to the double bond yields monomers (epoxides and epidioxides), dimers and polymer reaction products. Peroxyl and alkoxy radicals, which are formed in new positions by subsequent reactions, allow the chain reactions to progress (Schaich, 2005).

When the peroxyl radicals are attached to the double bond, as a result of 1,3 cyclization, epoxides and allylic radicals, and then new peroxyl radicals are formed by the addition of oxygen to the allylic radicals (Figure 1.8.). Polymerization may

occur as a result of addition of hydroperoxide to the released peroxyl radicals, or radical chain reactions proceed with the formation of stable dihydroperoxide dimers by H transfer (Schaich, 2005).

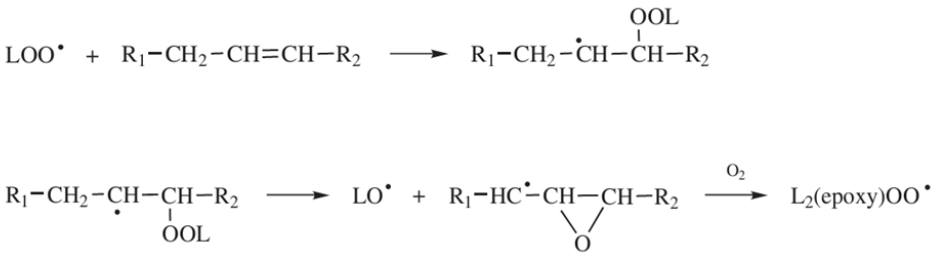

Figure 1.8. Addition of the peroxyl radicals to double bond (Schaich, 2005)

Epoxides derived from the addition of LOO· to double bond and cyclization of LO· are among the major lipid oxidation products. The rate of this reaction increases with increasing temperature, extent of the oxidation and solvent polarity. (Mounts et al., 1970; Frankel et al., 1988; Haynes & Vonwiller, 1990; Schaich, 2005)

1.1.2.3. Branching

The intramolecular rearrangement and addition reactions take place before the formation of hydroperoxide from peroxyl radical by cleaving H. However, during oxidation, the alkoxy radical is formed only by the decomposition of hydroperoxides. Therefore reactions of the alkoxy radical are referred as secondary reactions.

The rate of hydroperoxide formation in the propagation phase is greater than the rate of decomposition. When the amount of hydroperoxide reaches a certain critical value, the rate of decomposition of hydroperoxide in the branching phase is greater (Schaich, 2005).

Each hydroperoxide forms specific degradation products depending on the position of the peroxide group in the molecule (Waters, 1971; Schaich, 2005). Subsequent oxidation and decomposition reactions result in volatile, non-volatile, polymeric secondary oxidation products.

In the first step of the hydroperoxide decomposition, the O-O bond in the hydroperoxide group breaks down to form alkoxy and hydroxy radicals. The alkoxy radical (LO·) is more reactive than the peroxyl radical (LOO·) but it is predominant in the environment as long as hydroperoxide degradation continues. The hydroxyl radical (HO·) is excessively reactive and does not show selective properties and may break H from anywhere along the acyl chain. It can also be easily attached to the double bond (Schaich, 2005).

1.1.2.3.1. Disproportionation of peroxyl radical (LOO·)

Since the recombination of peroxyl radicals produces stable reaction products, this reaction is generally considered during the termination step. However, considering the second reaction (Figure 1.9.), where new alkoxy radicals are formed rather than stable reaction products, it is also considered within the scope of the propagation phase.

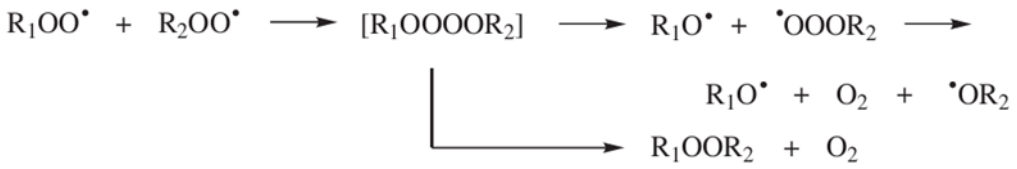

Figure 1.9. Recombination and disproportionation of peroxyl radicals (Schaich, 2005)

The first reaction takes place in oxidized lipids, in aprotic solvents. The β-cleavage reaction is preferred in polar solvents or aqueous solutions and LOO· decomposition increases significantly. In this case, the reaction stands out as a termination reaction rather than propagation (Walling et al., 1970; Heijman et al., 1985; Schaich, 2005).

1.1.2.3.2. β-cleavage reaction of peroxyl radical (LOO·)

β-cleavage reaction breaks the C-O bond in the peroxyl radical and releases O_2. For linoleic acid it competes with the transfer of H from allylic positions (Porter et al., 1981; Schaich, 2005). Another important case in which β-cleavage reaction is important that it is effective in the distribution of isomers at high temperatures and accordingly in the variation of end products. During heating, isomerization occur in the direction of from 13-OOH to 9-OOH (Smith and Waters, 1969; Schaich, 2005).

1.1.2.3.3. H abstraction by alkoxy radicals (LO·)

H transfer of LO· takes place very fast compared to LOO· radical (k ~ 107-108 L M-1 s-1). In addition while LOO· acts on more bis allylic hydrogens, LO· shows less selective feature by acting on the both allylic and bisallicity hydrogen (Bors et al., 1987; Michael et al., 1987; Schaich, 2005). Allylic hydrogens are proned to be removed by secondary alkoxy radicals. Therefore, the classical radical chain reaction presented in Figure 1.10 is preferred for lipid oxidation (Kochi, 1973; Schaich, 2005).

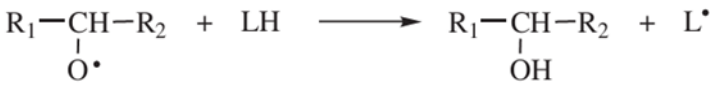

Figure 1.10. H abstraction from the lipid chain by alkoxy radicals (Schaich, 2005)

The removal of H from other lipid chains by LO·, is the most effective in the case where only the lipid allylic group is present as the H source. If the lipid concentration is moderate, H abstraction must compete with internal rearrangements and scission reactions on the other hand if there is low amount of lipid, it becomes insignificant (Walling & Padwa, 1963; Kulik et al., 1998; Schaich, 2005).

LO· is effective on hydroperoxide decomposition by abstraction of H from the lipid hydroperoxides concluding with the initiation of branching reactions. Although the bond dissociation energy for hydroperoxide hydrogen was higher than allylic hydrogen, the H bond between LO· and LOOH greatly reduces the activation energy required for the abstraction (Avila et al., 1995; Schaich, 2005).

Figure 1.11. H abstraction from hydroperoxides by alkoxy radicals (Schaich, 2005)

H abstraction by LO· occurs faster in the case of solvent containing abundant H sources but the effect of the reaction on the development of chain reactions is reduced. Because high temperatures (T > 100 ºC) increase scission reactions rather than abstraction, secondary stages of oxidation become dominant at these temperatures.

1.1.2.3.4. Rearrangement and cyclization of alkoxy radicals (LO·)

Cyclization takes place by the addition of oxygen in the alkoxy group to the adjacent double bond and epoxide forming epoxyallicity radicals. The reaction is shown in Figure 1.12.

Figure 1.12. The cyclization of alkoxy radicals (Schaich, 2005)

Formation of epoxide by LO· cyclization is the predominant reaction when the lipid amount is low (Van Sickle et al., 1967) or it is concentrated at the surface (Wu et al.,

1977; 1978), at room temperature (Bors et al., 1984), at low oxygen pressure (Van Sickle et al., 1967; Mayo, 1958) for aprotic solvents (Schaich, 2005).

While cyclization reactions are dominant at room temperature, H abstraction and scission reactions become important as the temperature increases. Rearrangement reactions at temperatures higher than 100 ^{o}C have a minor effect. As a result of photooxidation, high concentrations of cyclic reaction products are formed (Neff & Frankel, 1980). The level and positional distribution of these products may be identifier in the distinction of autoxidation and photooxidation.

1.1.2.3.5. Addition of alkoxy radicals (LO$^{\cdot}$) to double bond

The addition of an alkoxy radical to the double bond (Figure 1.13.) is not as easy as the addition of a peroxy radical. Since alkoxy radicals are inclined to prefer allylic attack reactions, H abstraction and internal cyclization reactions are dominant as long as in the presence of allylic hydrogens.

Figure 1.13. Addition of alkoxy radicals to the double bond (Schaich, 2005)

The addition reaction occurs substantially in the neat lipid chain and in organic solvents. Compared to scission and rearrangement reactions, a small amount of these reactions also takes place in aqueous solutions.

1.1.2.3.6. β-scission reaction of alkoxy radicals (LO$^{\cdot}$)

C-C bond on the either side of the alkoxy group is broken by the β-scission reaction. The reaction results in a mixture of products containing carbonyl compounds, free radicals, aldehydes, alkanes and oxo-esters (Chan et al., 1976; Schaich, 2005). The

β-scission of alkoxy radicals is the main source of oxidation products in the aldehyde group.

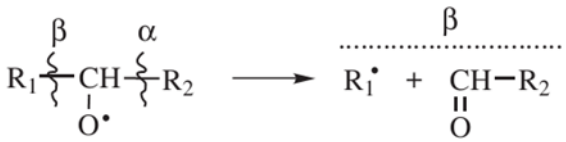

Figure 1.14. β-scission reaction of alkoxy radicals (Schaich, 2005)

Some radicals formed as a result of scission reactions are rearranged into non-radical products, but the most of them continue to radical chain reactions by H abstracting. Unsaturated decomposition products, especially those with conjugated diene structure, tend to oxidize, thus contribute to chain branching by their reactions. H abstraction of radicals from the solvent is the driving force for the reaction, and the scission reactions occur in polar solvents 10-100 times faster than nonpolar organic solvents (Baignee et al., 1983; Baciocchi et al., 2002). β-scission of alkoxy radicals at high temperatures highly contributes to the progression of oxidation (Kochi, 1973; Schaich, 2005).

1.1.2.3.7. Decomposition of peroxide (LOOH)

At high peroxide concentrations, the peroxide molecules combine to form a dimer structures via H bonds. Subsequently, oxidation is further accelerated by bimolecular decomposition (Hiatt & McCarrick, 1975; Sliwiok et al., 1974; Schaich, 2005). Figure 1.15 shows the dimerization and fragmentation reactions.

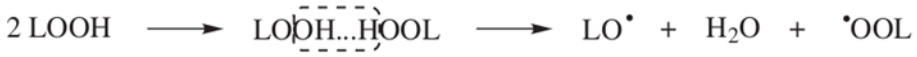

Figure 1.15. Decomposition of peroxides (Schaich, 2005)

1.1.2.4. Termination

If H abstractions or radical quenching reactions occur faster than the formation of new radicals, oxidation begins to slow down, but radical chain reactions are difficult to stop completely. The term termination, therefore, is used for a single radical rather than the entire reaction. Stable reaction products are formed by radical recombination and quenching of unstable reaction products with H.

Temperature and oxygen pressure have key importance on the determination of radical recombination pathway. Alkyl radical (L·) reactions are dominant at low oxygen content and high temperature (oxygen solubility decreases), however peroxyl radical (LOO·) reactions are increasing at high oxygen pressures (Bolland, 1949; Schaich, 2005).

Secondary peroxyl radicals quickly recombine (2k= 108-109 M^{-1} s^{-1}) to form many different products such as alcohol, ketone, alkane, acyl peroxide and peroxyl radicals (Schieberle et al., 1979; Grosch & Megele, 1984; Schaich, 2005). The proposed mechanism for the formation of radical and non-radical products from peroxyl radicals is given in the figure 1.16.

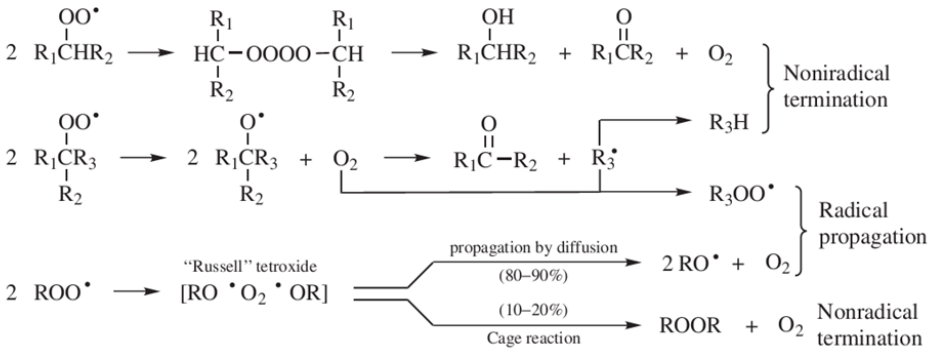

Figure 1.16. Formation of radicals and non-radicals from peroxyl radicals (Schaich, 2005)

LOO· recombination is the basic termination reaction for oleic acid only. The higher the degree of unsaturation, the greater the tendency for internal rearrangement reactions (formation of epoxide etc.).

Unless the oxygen pressure is limiting, LOO· recombination is qualified as a propagation reaction and consequently the formation of non-radical products is low. When the temperature rises, the removal of oxygen from the peroxyl radical by the β-scission reaction becomes dominant. Some suggested mechanisms for the recombination of alkoxy and alkyl radicals are depicted in Figure 1.17:

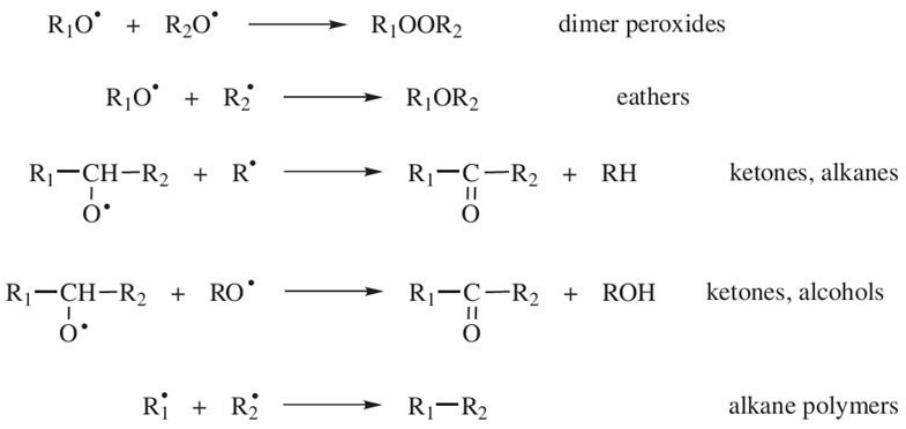

Figure 1.17. Recombination of alkoxy and alkyl radicals (Schaich, 2005)

If lipid molecules are present in polar or dilute in nonpolar solvents, recombination reactions lose their importance. Peroxyl and alkoxy radicals can also cleave H from any suitable source, including non-lipid molecules (amino acids, nucleic acids, antioxidants, carotenoids, carbohydrates) (Gardner et al., 1972; 1976; Yang & Schaich, 1996). In this case, the lipid radical is quenched and radical chain reactions are stopped. However, since there is radical transfer to non-lipid molecules such as proteins or carbohydrates, the oxidation process similar to lipids also occurs in these

molecules. When it is considered from this point of view, lipids cause extensive oxidative damage in foods and biological systems (Karel et al., 1975; Pryor, 1978).

1.2. Non enzymatic browning reactions

Browning reactions could be listed among the most important chemical phenomenas taking place during processing and storage of food. They are mainly classified as enzymatic and non-enzymatic. (Manzocco et al., 2001; Hui, 2006). Non enzymatic browning (NEB) reactions occur as a result of complex chemical reactions of different food components. Although, this type of browning is generally thought to be the result of carbohydrate-protein reactions (Maillard reaction), participation of other food components in NEB reactions via different mechanisms also have been shown (Hidalgo and Zamora, 2000). Maillard reaction, caramelization, ascorbic acid degradation and the reactions between lipid oxidation products and amino acids, which is called also lipid browning, are involved in the NEB reactions.

Maillard reaction and lipid oxidation have a complex reaction network. As a result of both reactions, there are positive or negative changes in the sensory properties and nutritional value of the food with a complex product mixture consisting of very different amounts of compounds (Hidalgo & Zamora, 2005). The oxidation products of lipids generate brown oxypolymers by polymerization (Khayat & Schwall, 1983; Hui, 2006) however in presence of amino acids, peptides or proteins, formation of the products different from those produced by the lipid oxidation was observed. (Gillatt & Rossell, 1992; Hui, 2006). In this case, lipid oxidation and oxidized lipid-amino acid reactions take place simultaneously.

Particularly in foods such as fish, meat and meat products, there is evidence of reaction between oxidized fatty acid and amino groups during storage and processing (Hidalgo et al., 1992; Nawar, 1996). In addition, browning in humans and animals with increasing accumulation of lipofuscin (age-related yellow-brown pigments) has been found to be associated with this reaction (Yin, 1996; Hui, 2006).

Decomposition of the hydroperoxides formed during the initial stage of lipid oxidation generates the reaction products (aldehydes, ketones…) with carbonyl groups. Resulting carbonyl compounds react with nucleophilic amino groups such as carbohydrate-derived carbonyls to cause browning with a series of reactions similar to the Maillard reaction (Zamora & Hidalgo, 2011). The first secondary lipid oxidation product identified to cause NEB is malondialdehyde. Chio and Tappel found that Schiff base and N, N'-disubstituted 1-amino-3-iminopropene are formed by cross-linking between malondialdehyde and two amino groups (Chio & Tappel, 1969). It is also known that this reaction occurs between long chain oxidized lipids and amino acids, proteins (Gardner et al., 1976; 1977; Hidalgo & Zamora, 2000).

Decarboxylation and deamination reactions of amino acids with dicarbonyl and hydroxycarbonyls, which are Maillard reaction intermediates, are known as Strecker degradation. Similarly, lipid oxidation products react with amino acids to form Strecker aldehydes (Figure 1.18).

The reaction follows the pathway in Figure 1.18. In the first step, water is released through the imine formation from lipid carbonyl and amino acid. Then, by the carbon dioxide scission and electronic arrangements, a new imine which is the precursor of Strecker aldehyde is formed (Figure 2.23.). In the next step, after hydrolytic decomposition, Strecker aldehyde is formed.

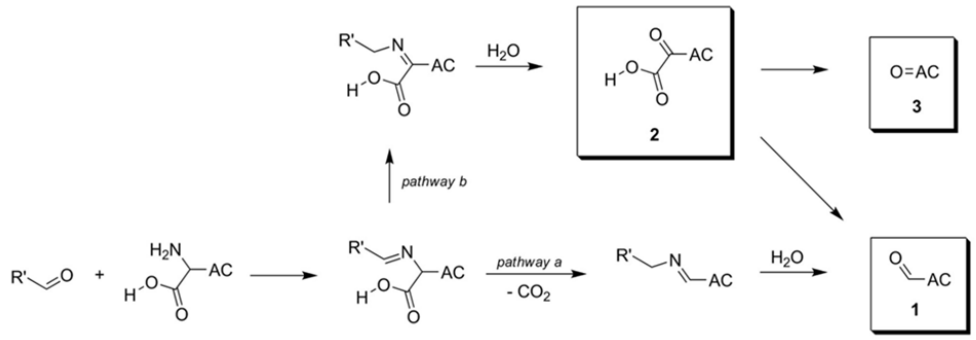

Figure 1.18. Reaction of lipid oxidation products and amino acids (Zamora & Hidalgo, 2011)

It is stated that pyridine and pyrrole derivatives are generated during these reactions and these compounds are known to cause changes in the sensory properties of the food. A similar degradation without decarboxylation takes place via pathway b. As a result of this pathway, α-keto acid with low thermal stability is formed. Therefore, under mild conditions, new flavor molecules are formed.

The first mechanism proposed for the formation of brown polymers is repeated aldol condensation (Figure 1.19.). According to this mechanism, lipid carbonyls form Schiff bases condensing with free amino groups of proteins. Schiff bases also polymerize by aldol condensation to form dimers and high molecular weight brown macromolecules.

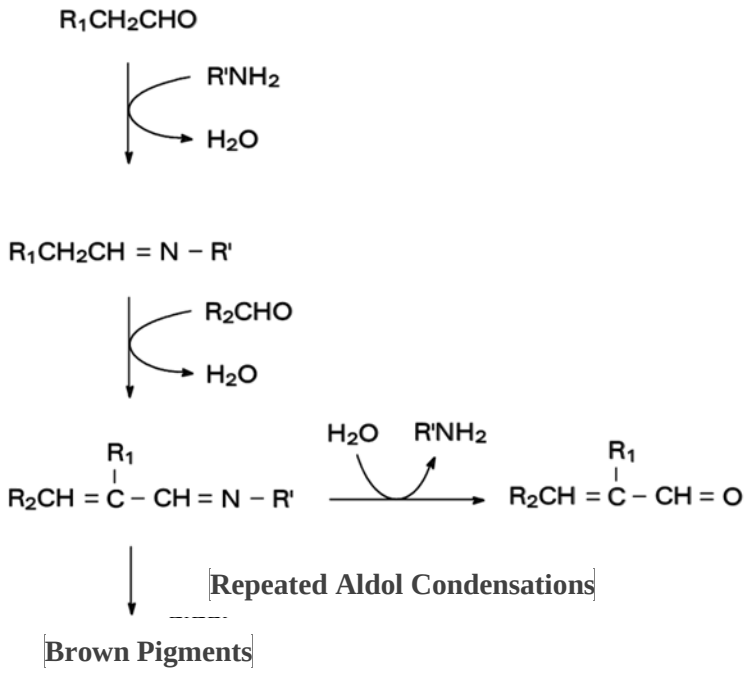

Figure 1.19. Formation of brown pigments by aldol condensations (Hidalgo & Zamora, 2000)

The other proposed mechanism is the spontaneous polymerization of N-substituted hydroxyalkyl pyrrole (2-1-hydroxyalkyl pyrrole) (Figure 1.20.). The reaction of epoxyalkenal (such as 4,5-epoxy-2-alkenal) and unsaturated epoxyketo fatty acids

with amine, amino acid, proteins produces N-substituted hydroxyalkyl pyrrole and more stable N-substituted pyrrole. N-substituted hydroxyalkyl pyrroles can readily polymerize to form melanoidine-like brown macromolecules. Unstable brown polymers are converted to novel volatile compounds which affect the flavor properties of food by decomposition or dehydration during processing or cooking of the food.

The pyrrole formation and subsequent polymerization is the final step before the maximum color and fluorescence formation. Apart from the polymeric structures, low molecular weight monomers are also reported to be effective in color formation (Hidalgo et al., 1993; 1994; 1995; 2000). Examples of these monomers are short chain aldehydes.

4,5-epoxy-2-alkenal reacts with proteins according to the pathway given in Figure 1.20. to form pyrroles and Michael addition products (Hidalgo & Zamora, 1994; Zamora et al., 1999). 4,5-epoxy-2-alkenals are formed as a result of the decomposition of epoxyhydroperoxy fatty acids, which are the lipid oxidation intermediates (Figure 1.20.). Some other lipid oxidation products studied in this context are: 2-alkenals, 4-hydroxyalkenes, 2,4-decadienal, 3-(2-ethyl-5-hydroxy-3-oxo-cyclopentyl)-2-propenal, hexanal.

Food matrices, as complex mixtures of reactive components, reveal nontrivial profiles of reaction products which makes identification of low concentrated compounds derived from lipid and protein interactions in real foods challenging. Wide range of carbonyls formed after oxidation of various fatty acids and presence of different reactive protein residues can constitute various reactant combinations which may lead to complex reaction series. Furthermore, both lipid and carbohydrate derived carbonyls give similar non-enzymatic browning reactions and might result in common products such as N(ε)-carboxymethyllysine (CML), N-substituted hydroxyalkyl pyrroles (Zamora & Hidalgo, 2005; Baynes, 2007).

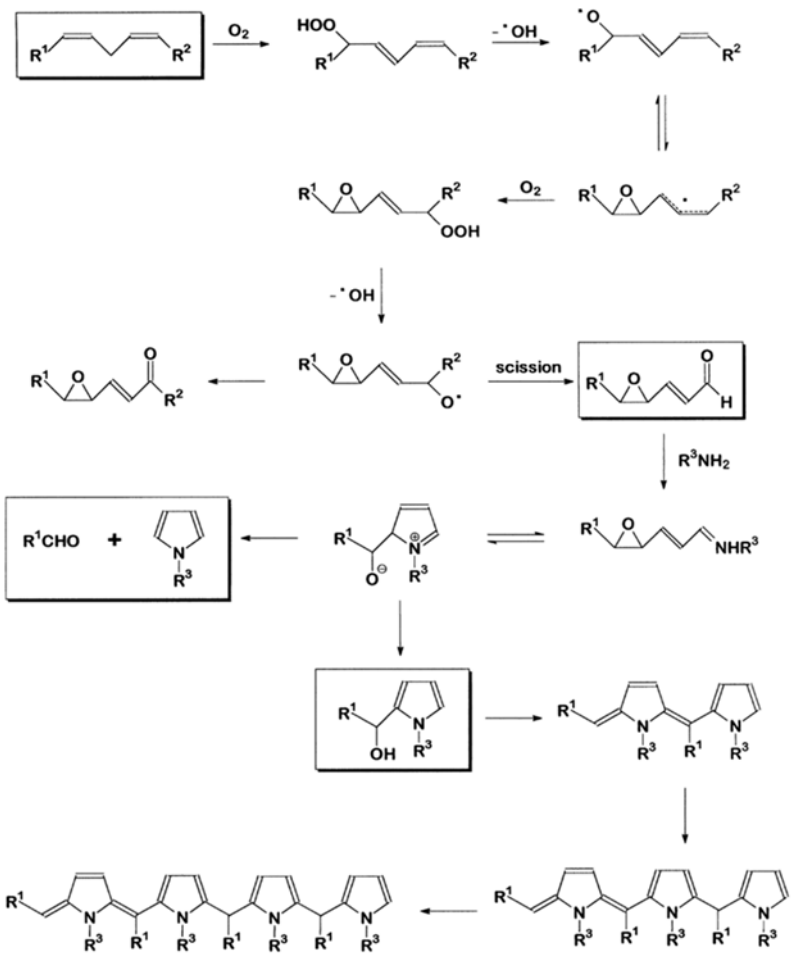

Figure 1.20. Polymerization of N-substituted hydroxyalkylpyrroles (Zamora & Hidalgo, 2005)

Although there are some studies in literature investigating this type of reactions in food (Karademir et al., 2013; Globisch et al., 2015), most of them were implemented in model systems (Adams et al., 2009; Hidalgo et al., 2016).

The studies focused on volatile and flavour compounds rather than reaction intermediates, constitute a considerable amount of this literature (Pokorny 1980, Josephson & Lindsay, 1987, Whitfield, 1992, Kim et al., 1996, Adams et al., 2011). In this context, N-substituted amides and nitriles were reported as the major reaction products of amino acids with triglycerides (Lien & Nawar, 1974, Breitbart & Nawar, 1981, Adams et al. 2011) while 2-pentylpyridine was major product of reaction between valine and linoleic acid or its esters (Henderson and Nawar, 1981). Macku and Shibamoto heated corn oil with glycine and indicated the formation of nitriles, pyridines and pyrroles (Macku & Shibamoto, 1991). In another study, Adams et al. investigated model mixtures consisting of lipid oxidation products - amino acids with and without glucose and identified carbonyl compounds as main products and 2-pentylpyridine from 2,4-decadienal mixtures, 5-butyl-2-propylpyridine from 2-hexenal model systems (Adams et al., 2011).

The discolouration was noted in fish meats prepared in used frying oil because of polyunsaturated fatty acid content of the fish (Pokorny, 1981).

The extent of protein cross linking was found to be higher levels in fried tortilla chips compared to baked ones with lower oil content. Nevertheless, Schiff base or Michael adducts were stated to be present in lower amounts associated with the formation of low levels of fluorescent products (Schaich, 2014; Hu & Jacobsen, 2016).

1.3. Chemistry of frying

Deep fat frying is one of the most sophisticated processes in the food industry when the chemical reactions occurred within the system and complex composition of fried food are considered. Simultaneous heat and mass transfer between food and frying medium are dominating transport phenomenas for the process. As a result of mass

transfer, transition of food components (water, oil, carbohydrate, protein etc.) to frying oil and oil uptake of food material from frying oil are observed (Krokida et al., 2000; Sosa-Morales et al., 2006). Hence, both fried food and frying oil jointly promote the formation of complex chemical reactions: Maillard reaction, thermal oxidation, hydrolysis, cyclization, isomerization and polymerization. These reactions are the main contributors to the texture and flavour of fried foods as well as its nutritional value by producing a broad variety of compounds: Free fatty acids, alcohols, aldehydes, ketones, acids, lactones, hydrocarbons mono and diglycerides, cyclic and epoxy compounds, trans isomers. (Zhang et al., 2012)**.** Free fatty acids, mono- and diacylglycerols and glycerols are resulted from hydrolysis while hydroperoxides, low molecular weighted volatiles such as aldehydes, ketones, carboxylic acids, and short-chain alkanes and alkenes are from oxidation reactions. However, in the course of frying, oxidation rate is higher than hydrolysis rate. Dimers and polymers are formed as a consequence of Diels-Alder reactions. (Choe & Min, 2007)

By heat transfer from the oil, temperature of the fried food increases slowly. When surface temperature reaches to 100 ^{o}C, internal water is transported to outer layers of the food and then the surface is covered with steam which interrupts oil penetration. Consequently two specific zones are formed: 1- Dehydrated surface in which primary changes occur 2- Core with temperature which does not exceed 100 ^{o}C.

During frying, water loss and oil absorption of food material continue interrelatedly due to suppression of internal pressure over the pressure at outside of the food. Water and oil exchange mainly influenced by temperature, time, food, frying oil (Dobarganes et al., 2000)**.** Factors related the food are composition of food (water and lipid content), surface structure, shape of food, surface to weight ratio, porosity, pre-frying treatments (Fillon & Henry, 1998; Saguy & Dana, 2003). Oil uptake in foods may vary from 6% (e.g. in roasted nuts) to approximately 40% (e.g. in potato crisps) (Morton & Chidley, 1988). Oil absorption might occur continuously through the interchange with the evaporated water and after frying. In the first mechanism,

most of the oil is absorbed in the first 20 seconds of frying. According to second mechanism the oil is drawn into the food during cooling by vacuum effect as a result of the drop in internal vapour pressure due to condensation **(**Saguy & Dana, 2003**).**

Oil uptake of the fried food can be reduced by vacuum frying, pretreatments like immersing of the fried material to NaCl solution, blanching, air dehydration and coating (Arslan et al., 2018). In addition, the quality of frying oil also plays role in oil absorption. Through the accumulation of the degradation compounds which has been associated with the decreasing oil quality, the polarity of the fying medium increases with the viscosity of the oil. Increased viscosity induce to higher amounts of the oil around the food surface which contributes to oil absorption by reducing the interfacial tension between the food and oil (Dobarganes et al. 2000). Therefore oil amount on the surface and oil absorption increases during repeated frying (Pinthus & Saguy, 1994).

Because of the compositional similarities between frying oil and the oil absorbed by the food (Dobarganes et al. 2000), the oxidation level of frying oil have significant importance. Nevertheless, to slow down the oxidation reactions during frying has not only importance from the point of the quality of fried food but also to prolong the stability of frying oil.

In order to enhance oxidative stability and thereby lifetime of the frying oils, a wide range of natural and synthetic antioxidants have been studied up to date. Tocopherols, tocotrienols, carotenoids, polyphenols, phenolic acids, flavonoids, phospholipids, ascorbic acids, citric acids, sesame lignans, rosemary and sage extracts would be added as natural antioxidants while butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) have been investigated as synthetic antioxidants in this scope (Choe & Min, 2007;Boskou, 2010; Aladedunye, 2014; Márquez-Ruiz et al., 2014).

However the effectiveness of the antioxidants particularly at frying conditions depends on various factors including thermal stability, volatility, reactivity with the oil molecules and radical scavenging ability (Hu & Jacobsen, 2016).

1.4. A brief overwiew of the study

When the interactions between frying oil and food taken into consideration, carbonyl compounds formed in oxidized oil could modify amino groups in fried foods by means of the absorption of the oil to the fried food. In this respect, deep-fat fried foods may have significant potential to investigate the role of lipid oxidation products on the formation of carbonyl-amine reaction products. In the light of this hypothesis, lipid derived amino acid modifications were investigated mainly in the fried foods within the scope of this dissertation. Briefly, the effect of heating time of the frying oil on amino acid modifications in potato chips and model doughs was investigated in the first part. 2,4-decadienal, total free amino acid, decadien-1-amine, thiobarbituric acid reactive substances (TBARs), 2-pentylpyridine, acrylamide analyses were carried out. Second part of the study included the investigation of the impact of different oil types on amino acid modifications in potato chips. Safflower, corn, canola, hazelnut and olive oils were used for this purpose and p-anisidine, 2,4-decadienal, total free amino acids, decadien-1-amine, carboxymethyllysine (CML), carboxyethyllysine (CEL) analyses were performed in this part. Finally in the last part, the effect of different treatments (antioxidant addition to the oil, coating, pan frying) on the amino acid modifications in potato chips and fried chickens was investigated. Similarly, p-anisidine, 2,4-decadienal, total free amino acids, decadien-1-amine, CML, CEL were measured in this part.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2,4-decadienal, 2-pentylpyridine, acrylamide, lysine (Lys), ammonium formate, potassium hexacyanoferrate ($K_4Fe(CN)_6$), zinc sulfate ($ZnSO_4$), butylated hydroxytoluene (BHT), 2-thiobarbutiric acid, malondialdehyde tetrabutylammonium salt, nonane, ethanol, methanol, 2-propanol, formic acid, hexane, acetonitrile, glacial acetic acid, isooctane, p-anisidine, methanolic potassium hydroxide, carboxymethyllysine (CML), carboxyethyllysine (CEL), butylatedhydroxytoluene (BHT), sodium hydroxide, hydrochloric acid, boric acid, sodium borohydride, the mixture of fatty acids methyl esters (C14-C22), tocopherol mix (α-β-δ-γ) were purchased from Sigma-Aldrich (Germany). Silica gel was obtained from Merck (Germany). XB-C18 column (150 mm x 4.6 mm i.d., 3 μm), AQ column (150 mm x 4.6 mm i.d., 3 μm), Accucore HILIC (150 mm x 2.1 mm i.d., 2,6 μm) were from Welch (USA), ACE (Scotland) and Thermo Scientific (USA) respectively. HP5-MS capillary column (30 m x 0.25 mm i.d.; coating thickness 0.25 μm), CP-Sil 88 capillary column (50 m x 0.25 mm ID. x 0.20 μm film) were purcased from Agilent Technologies (USA) and Chrompak (the Netherlands) respectively. Potatoes (*Solanum tuberosum*), sunflower oil, safflower oil, corn oil, canola oil, hazelnut oil, olive oil, chicken breast meat, coating material for the chicken, commercial potato chips, potato flour were purchased from a local market. Ultra pure water was used throughout the experiments (MilliQ system, Millipore, USA). 15 g of potassium hexacyanoferrate ($K_4Fe(CN)_6$) was dissolved in 100 mL of water for Carrez I solution and 30 g of zinc sulfate ($ZnSO_4$) was dissolved in 100 mL of water for Carrez II solution.

2.2. Methods

2.2.1. Preparation of the model system

Decadien-1-amine, reaction intermediate of 2,4-decadienal with an any amino acid, does not have reference material. In an attempt to determine its possible chromatographic and mass spectrum behavior, a model system was prepared. For this purpose, commercially available sunflower oil (10 mL) was thermally oxidized at 180 ^{o}C for 3 h in closed glass tubes within an oil bath (Wisd, Germany). Afterward, 50 mg silica gel, 100 μL of 10 μmol aqueous lysine solution and 1 mL of oxidized sunflower oil were transferred into a glass tube and mixed with the vortex (Wisd, Germany) for 2 min. Finally 300 mg silica gel was added to cover reaction medium. Tightly closed tubes were heated at 180 ^{o}C for 10 min.

It was also studied on the model dough to understand the effect of food matrice and eliminate the compositional differences coming from the matrice. For this purpose 3 different models (M1, M2 and M4) were prepared. M1 was consisted of 25 g of whey protein and 15.9 mL of pure water while M2 contained the same amounts of protein and water in addition to 0.5% glucose and 0.5% fructose. M4 was prepared from 25 g of potato flour and 30 mL of pure water. Same sized round shaped doughs were fried for 1.5 min (for M1 and M2) and 2 min (for M4) within the 2 L of sunlower oil in a domestic type fryer heated at 180 ^{o}C along 12 hours (at every 4 hours).

2.2.2. Preparation of fried foods

In the first part, roundly sliced nine potato discs (1.5 mm thickness and 5 cm diameter) were fried at 180 ^{o}C for 1.5 min in 2 L of sunflower oil. The same oil at 180 ^{o}C was used repeatedly for 24 h to fry potato discs at different times (0, 6, 12, 18, and 24 h). In the second part, the same procedure was repeated for different type of the oils (safflower, canola, corn, hazelnut, olive) until 12 h (0, 3, 6, 9, 12 h). In the third part, the effect of different process conditions (antioxidant addition, batter-coating, pan frying) on different fried foods (chips, fried chickens) was investigated.

To understand the effect of antioxidant addition to oil, chips were prepared within the sunflower oil (SO+A) that contains 1% (v/v) tocopherol mix (α-β-δ-γ) and the same frying conditions (180 ºC - 0, 4, 8, 12 h) were applied against the control (SO), which does not contain tocopherol mix. With respect to chickens, meatballs were prepared from minced chicken breast meat and except for P group, similar to chips, chickens were deep fried for 2 minutes along the heating of the sunflower oil (2 l) at 180 ºC after certain hours (0, 4, 8, 12 h). Five different treatments (T1, T2, T3, T4, P) were investigated. T1 represents control without any treatment. T2 was prepared in the oil with 0.1% (v/v) BHT and T3 in the oil with 0.1% (v/v) tocopherol mix (α-β-δ-γ). Breaded chickens were used for T4. Finally, P represents pan-frying with 50 mL of pre-oxidized (180 ºC - 0, 4, 8, 12 h) sunflower oil. The oil temperature was monitored by a digital thermometer probe for the whole operations. Frying experiments were replicated. Prepared and commercial potato chips, fried chickens were ground with a domestic type grinder. Ground samples and frying oils samples were stored at -18 ºC until chemical analyses.

2.2.3. Analysis of Decadien-1-amine

2.2.3.1. Extraction of model system

The model reaction mixture was extracted with 5 mL of methanol-water mixture (70:30, v/v) by vortexing for 3 min. Afterward, samples were centrifuged at 7168 x g for 5 min and supernatants were filtered over nylon syringe filter (0.45 µm) into a vial.

2.2.3.2. Extraction of potato chips

1 g of ground sample was extracted with 9.5 mL of methanol-water mixture (70:30, v/v) in three step (4.5; 2.5; 2.5 mL). Only in first step, 0,25 mL of Carrez I and 0.25 mL of Carrez II were added to methanol-water mixture to precipitate the co-extracted colloids. 3 min-vortexing was followed by centrifugation (7168 x g for 5 min) at each stage. Lastly, collected supernatants were passed through the nylon

syringe filter (0.45 μm) into a vial prior to analysis by ultra performance liquid chromatography coupled to high-resolution mass spectrometry (UPLC-HRMS).

2.2.3.3. UPLC-HRMS analysis

An Exactive Plus Orbitrap model UPLC-HRMS system (Thermo Fisher Scientific, San Jose, CA, USA) was used to analyze decadien-1-amine in model system and potato chips. The chromatographic separations were performed on a Welch XB-C18 column (150 mm 4.6 mm i.d., 3 μm). A gradient mixture of formic acid (0.1%, v/v) and ammonium formate (5 mM) solutions in water (A) and methanol (B) was used as a mobile phase at a flow rate of 0.5 mL/min. The injection volume was set as 10 μl. The following elution programme was applied: 0% B was held for first 1 min, increased to 40% B within 3 min, then to 95% B within 5.5 min, isocratic elution with 95% B for 3.5 min followed by 0% B within 3 min. Positive heated electrospray ionization (H-ESI) mode was operated. The automatic gain control target and maximum injection time were set to 1 × 106 and 100 ms respectively. Ionization source parameters were as follows: sheath gas flow rate 45 (arbitrary units), auxiliary gas flow rate 20 (arbitrary units), spray voltage 4 kV, capillary voltage 20 V, tube lens voltage 30 V, skimmer voltage 15 V, capillary temperature 300 ^{o}C, vaporizer temperature 300 ^{o}C. Ions with 50 to 200 m/z were scanned in Full MS mode with high resolution (70000). Relative abundances of decadien-1-amine (m/z: 154.1590) were expressed as the peak area. Peaks with noise levels higher than 10^4 were regarded in the calculations.

2.2.4. Analyses of 2,4-Decadienal and 2-Pentylpyridine

2.2.4.1. Extraction of potato chips

2-propanol (4 mL), Carrez I (0.25 mL) and Carrez II (0.25 mL) solutions were added to ground potato chips (2 g). After 3 min vortexing and 5 min centrifugation (7168 x g), extraction was repeated with 3.5 mL of 2-propanol in the second step. Combined supernatants were cooled to 4 ^{o}C for clarification before centrifuged. Afterwards, the supernatant was transferred to a vial through a PTFE syringe filter (0.45 μm).

2.2.4.2. Extraction of oils

1 g of oil sample was extracted with 6 mL of 2-propanol in two steps. 3 min vortexing and 5 min centrifugation (7168 x g) were performed in each step. Combined supernatants were transferred to a vial through a PTFE syringe filter (0.45 μm) prior to gas chromatography mass spectrometry (GC-MS) analyses.

2.2.4.3. GC-MS analysis

A GC-MS system consisting of Agilent 6890 model gas chromatograph (Böblingen, Germany) and Agilent 5973 model mass selective detector (Böblingen, Germany) was used to analyze 2,4-decadienal and 2-pentylpyridine in potato chips. Chromatographic separations were performed on a HP5-MS capillary column (30 m x 0.25 mm i.d.; coating thickness 0.25 μm). Helium was the carrier gas with 1 mL/min flow rate. 2 μL of the sample was injected at 260 ^{o}C with splitless mode. The initial oven temperature was set to 60 ^{o}C for 2 min then raised to 200 ^{o}C at 6 ^{o}C/min and held for 10 min. The system was worked in electron impact mode at 70 eV. The detector and auxiliary temperature were set to 280 ^{o}C. SIM (Selected Ion Monitoring) mode was used with ions of 81 m/z and 152 m/z for *trans,cis*- and *trans,trans*-2,4-decadienal, 93 m/z and 120 m/z for 2-pentylpyridine. Quantifications were performed through the calibration curves prepared by external standards. LOD and LOQ values for 2,4-decadienal were determined to be 94 and 313 ng/g while for 2-pentylpyridine, 1.49 and 5.00 were determined, respectively.

2.2.5. Analysis of Acrylamide

2.2.5.1. Extraction of potato chips

9.5 mL of water and 0.25 mL of Carrez I and 0.25 mL of Carrez II solutions were added to 1 g of ground potato chips in a tube. After vortexing for 3 min and centrifugation (4032 x g for 5 min), the supernatant was transferred to a tube and the solid residue was extracted with 5 mL of water once more. The collected supernatants were defatted by 5 mL of hexane. Following vortexing (5 min) and

centrifugation (4032 x g for 5 min), separated hexane phase was removed. Finally cooled (4 ^{o}C) extracts were centrifuged again before transferred to a vial over a nylon syringe filter (0.45 μm) prior to liquid chromatography tandem mass spectrometry (LC-MS/MS) analyses.

2.2.5.2. LC-MS/MS analysis

An Agilent 6470 model tandem LC-MS/MS (Böblingen, Germany) system was used to analyze acrylamide in potato chips. An ACE 3 AQ column AQ column (150 mm x 4.6 mm i.d., 3 μm) was used for chromatographic separations. An isocratic mixture of (97:3, v/v) formic acid solution of water (0.1%) and methanol was used as the mobile phase at a flow rate of 0.6 mL/min at 35 ^{o}C. The system was worked in positive electrospray ionization and multiple reaction monitoring mode. Operation parameters were as follows: drying gas temperature, 300 ^{o}C; drying gas flow rate, 11 L/min, nebulizer pressure, 45 psi; sheath gas temperature, 400 ^{o}C; sheath gas flow rate, 11 L/min.; capillary voltage, 2000 V; nozzle voltage, 500 V. Mass transitions from 72 to 55 m/z and 44 m/z were carried out by setting collision energy and fragmentor voltage as 6 (for m/z: 55), 16 (for m/z: 44) and 60 respectively with dwell time of 100 ms. Quantification was performed through the calibration curve prepared by external standards with the concentrations between 1 to 50 μg/L.

2.2.6. Analysis of Free Amino Acids

2.2.6.1. Extraction of potato samples

One gram of ground potato samples was extracted with 20 mL of water in two steps (9.5-10). Carrez I (0.25 mL) and Carrez II (0.25 mL) solutions were added in the first step for clarification. Vortexing (3 min) and centrifugation (7168 x g for 5 min) were performed in each step. Collected supernatants were transferred to vial through nylon syringe filters (0.45 μm) prior to UPLC-HRMS analyses.

2.2.6.2. UPLC-HRMS analysis

Free amino acids were analyzed by using a Q-Exactive Orbitrap model UPLC-HRMS system (Thermo Fisher Scientific-San Jose, CA, USA) according to the method described elsewehere (Gökmen et al., 2012) with some modifications. Chromatographic separations were performed on Accucore HILIC column (150 mm x 2.1 mm i.d., 2,6 μm) (Thermo Fisher Scientific-San Jose, CA, USA) using a gradient mixture of acetonitrile (A) and 0.1% formic acid and in water (B) consisting 10 mM of ammonium formate was used as the mobile phase at a flow rate of 400 μl/min at 30 ºC. The injection volume was set as 5 μL. The same gradient program used by Gokmen et al. was applied. Full MS/dd MS_2 mode was operated with positive heated electrospray ionization mode. Full MS settings were as follows: Resolution 70000, automatic gain control target 3×10^6, maximum injection time 120 ms, mass scan range 60 to 220 m/z. Data Dependent-MS_2 settings were as follows: resolution 35000, automatic gain control target 1×10^5, maximum injection time 50 ms, collision energy 35. Quantification was performed through the calibration curve prepared by external standards of amino acids with the concentrations between 0.1 to 10 mg/L.

2.2.7. Measurement of TBARs value

The TBARS value of sunflower oil repeatedly used for frying was measured by a method described elsewhere (Papastergiadis, Mubiru, Langenhove, & Meulenaer, 2012). 500 mg of oil sample was extracted with 10 mL of 0.01% butylated hydroxytoluene in ethanol. The mixture was vortexed for 4 min and centrifuged at 4032 x g for 3 min. After separation of the supernatant to another tube, the procedure was repeated one more time. 2.5 mL of collected supernatants were mixed with 2.5 mL of 46 mM TBA solution in glacial acetic acid and heated in water bath at 95 ºC for 45 min. For quantification, similarly 2.5 mL of 1-10 μmole of malondialdehyde (MDA) solutions were reacted with 2.5 mL of 46 mM TBA solution in glacial acetic acid. Reaction tubes were cooled and after passing through PTFE (0.45 μm) syringe

filter, absorbance was measured at 532 nm using UV-visible spectrophotometer (Optizen POP, KLAB, Republic of Korea).

2.2.8. Analyses of CML and CEL

20 mg of sample was weighted into glass test tube. Sodium borate buffer was prepared by adjusting 0.2 M of boric acid to pH 9.2 with 0.2 M of sodium hydroxide. 1 M sodium borohydride solution was prepared in 0.1 M sodium hydroxide. 100 µL of deionized water, 450 µL of sodium borate buffer and 500 µL of sodium borohydrate were added to the test tube. After mixing by vortex, it was waited for 4 hours at room temperature. At the end of this period, 2 mL of 8 N hydrochloric acid was added to the tubes then they were tightly closed and hydrolyzed at 110 ^{o}C for 24 h. 50 µL of the hydrolyzate was transferred to another test tube and dried under nitrogen gas. Afterward, 500 µL of deionized water and 500 µL of water-acetonitrile mixture (80:20, v/v) were added and it was passed through a preconditioned OASIS HLB cartridges to vials. The instrumental part of the analysis was performed according to the method described by Akillioglu and Gokmen (Akillioglu & Gokmen, 2014. LOD and LOQ values of CML measurements in potato chips were 0.7 and 2.2 ng/g while for CEL, LOD and LOQ were determined to be 0.5 and 1.7 ng/g, respectively. In fried chickens, LOD and LOQ for CML were 1.3 and 4.4 ng/g while for CEL, 1.4 and 4.7 ng/g were determined.

2.2.9. Analysis of fatty acids

800 µL of isooctane was added to 80 mg of the oil sample in test tube. Then 500 µL of methanolic potassium hydroxide (2 moles/L) was added and the mixture was shaken for 6 minutes in a capped test tube. After 10 min duration for phase separation, upper phase was injected to gas chromatograph (Thermo Scientific, USA). 0.5 µL of the sample was injected with 1:100 split ratio to the system. Injector and FID (Flame Ionization Dedector) temperature was 250 ^{o}C while CP-Sil 88 capillary column (50 m x 0.25 mm ID. x 0.20 µm film, Chrompak, the Netherlands) was 177 ^{o}C at isothermal programme. Fatty acids were detected

according to the retention times of the reference materials. Results were indicated as relative percentage of the peak areas.

2.2.10. Analysis of p-Anisidine value

p-Anisidine value was determined according to the AOCS Official Method-Cd 18-90 (AOCS, 2009).

2.2.11. Oil content analysis

5 g of weighted samples (potato chips and fried chickens) was extracted with 250-300 mL of hexane by the Soxhlet apparatus (Behr Labor-Technik, Germany). After extraction, hexane was evaporated and the percentage of oil in the sample was calculated.

2.2.12. Moisture content analysis

Moisture amount of the samples was detected by infrared moisture analyzer (Radwag, Poland).

2.2.13. Statistical analyses

Results are expressed as mean values ± standard deviations of two separate measurements (Excel 2010; Microsoft, Redmond, WA). Analysis of Variance (One-way ANOVA) with post hoc multiple comparison of means (Tukey) and correlation analysis were performed by Minitab Statistical Software v.16 (Minitab Inc., State College, Pa., USA). The differences with the p value lower than 0.05, were indicated to be significant.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Investigation of heating time of the oil on amino acid modifications in fried foods and model systems

Repeatedly used sunflower oils was used to investigate the role of lipid oxidation products on the formations of decadien-1-amine, 2-pentylpyridine, and acrylamide in potato chips. The TBARS value of sunflower oil, a measure of the formation of secondary oxidation products, was found to increase from 9 to 18 mmoles MDA/kg within 24 h of repeated use for frying at 180 ^{o}C.

3.1.1. Formation of 2,4-Decadienal in frying oil and potato chips

Occurrence of 2,4-decadienal was monitored as a targeted reactive carbonyl compound in sunflower oil and potato chips. As shown in Figure 3.1, concentrations of 2,4-decadienal were found to range from 31 to 97 µg/g for oil, and from 10 to 29 µg/g for potato chips during 24 h repeated frying at 180 ^{o}C. The highest concentration of 2,4-decadienal was determined in oil and potato chips at 6 h. After that, a declining trend of 2,4-decadienal was observed as a result of suppressing the rate of decomposition reactions against the rate of its formation. It has been reported that breakage of the two double bonds results in hexanal and 2-octenal formation which might be responsible for the degradation of 2,4-decadienal on the course of heating (Zamora et al., 2015). In a different study conducted by Boskou et al. (2006) effect of successive frying on 2,4-decadienal formation was investigated and concentration ranges were found to be between 16 and 129 µg/g for oil and 5-11 µg/g for french fries while the samples from sixth frying cycle contained the highest amounts. Similar to above findings, 2,4-decadienal was stated to decrease after reaching a maximum point (Boskou et al., 2006). Determination of higher quantities

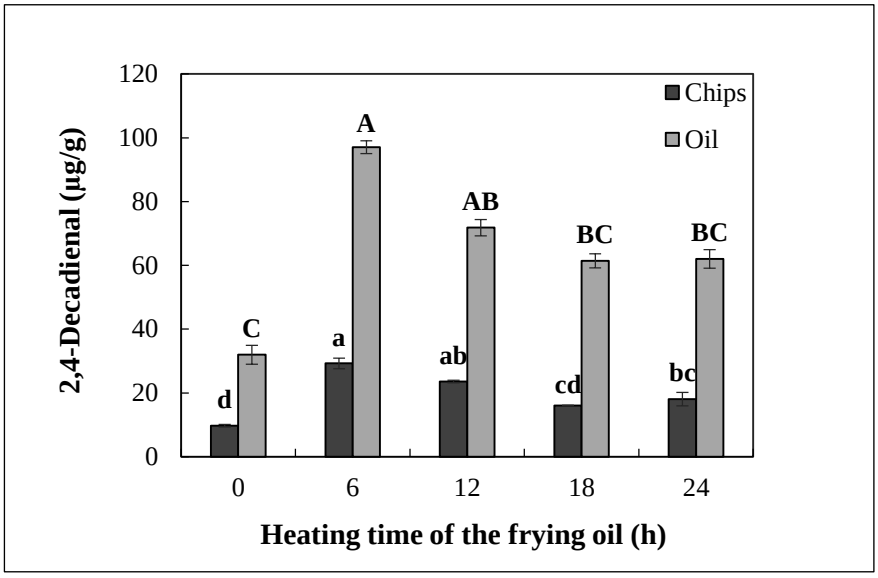

Figure 3.1. Amount of 2,4-decadienal formed in potato chips and oil fried at 180 °C in sunflower oil repeatedly used for 24 h to fry potato discs at different times (0, 6, 12, 18, and 24 h)

in potato chips compared to french fries could be explained by larger surface area of potato chips that reacted with the oxidized oil during frying.

3.1.2. Formation of Decadien-1-amine

Decadien-1-amine stands out with the possibility of much more abundancy in reaction medium via the formation from any amino acid and relatively higher concentrations of 2,4-decadienal among lipid carbonyls in linoleic acid containing systems. Therefore, focus point of this research was to confirm the formation of decadien-1-amine in potato chips fried in thermoxidized oil. The samples of model system composed of 2,4-decadienal and lysine (LYS- 01), potato chips fried at 180 °C in repeatedly used sunflower oil at 6h (6h-01) and at 12 h (12h-01), and commercial potato chips (crisp 01) were analyzed by using an UPLC-HRMS system. Figure 3.2. illustrates the extracted ion chromatograms of decadien-1-amine confirmed in the model system, experimental potato chips and commercial potato chips.

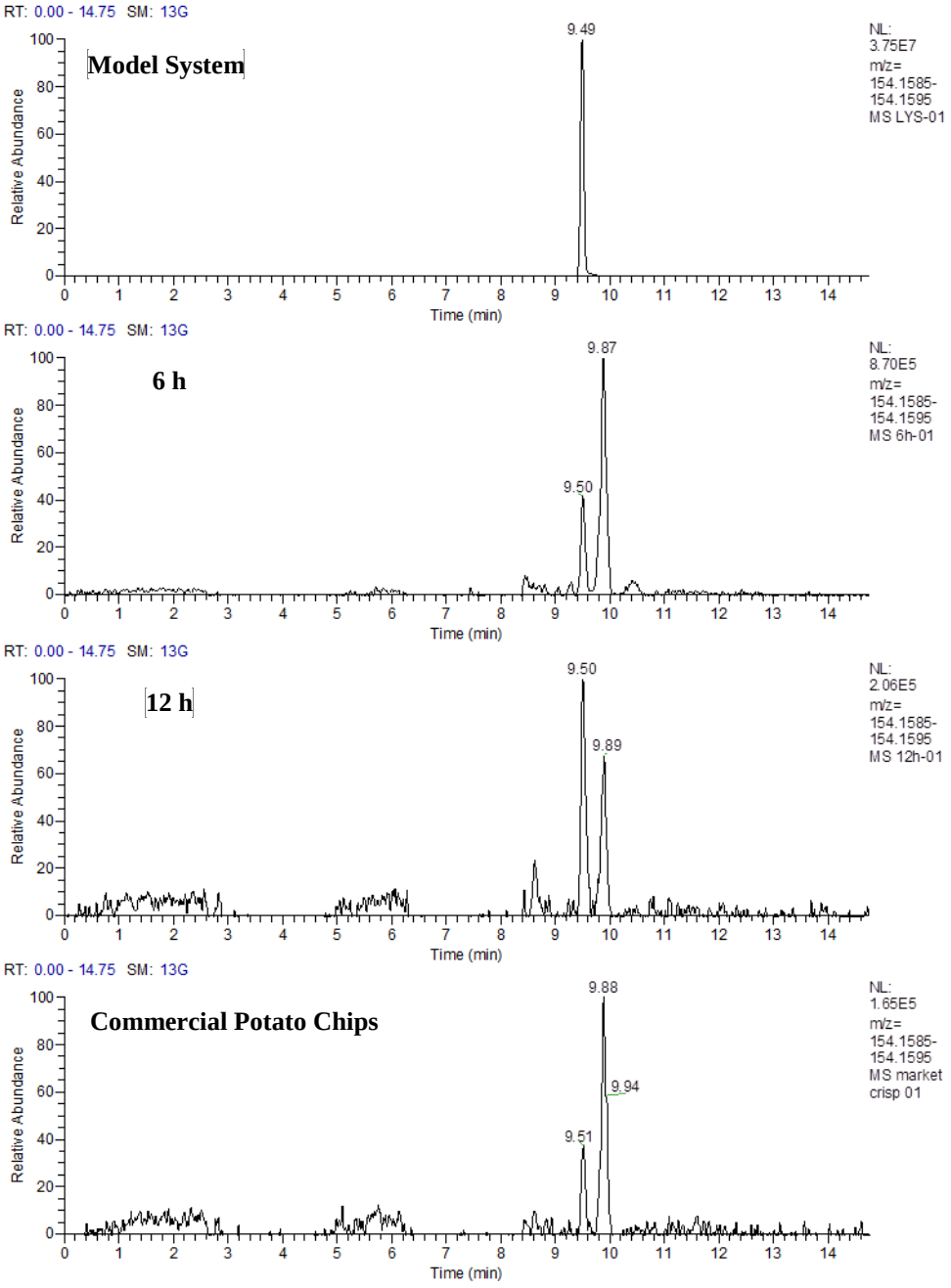

Figure 3.2. Extracted ion chromatograms of decadien-1-amine in the model system (LYS- 01), potato chips fried at 180 ºC in repeatedly used sunflower oil at 6 h (6h-01) and at 12 h (12h-01), and commercial potato chips (crisp 01)

Presence of decadien-1-amine in these samples was confirmed with ultra high accuracy (Δ ppm < 1) ppm by means of the Thermo Exactive Plus Orbitrap mass spectrometry. Although retention time of the molecule was detected as 9.49 min for the model system, two separate peaks were observed at 9.49 and 9.88 min for prepared and commercial potato chips. This might be the result of favored formation of stereoisomers [trans-cis (E,Z) and trans-trans (E,E)] of 2,4-decadienal or substitution of double bonds as (2,4) and (1,3) on the carbon chain.

Figure 3.3. shows the amounts of decadien-1-amine formed in potato chips (reported as peak area) fried at 180 ºC in repeatedly used sunflower oil. Similar to 2,4-decadienal, relative amount of decadien-1-amine increased approximately 4 times at 6 h of repeated frying. There was a positive linear correlation (r^2=0.73) between the concentrations of 2,4-decadienal and decadien-1-amine (relative concentration as peak area).

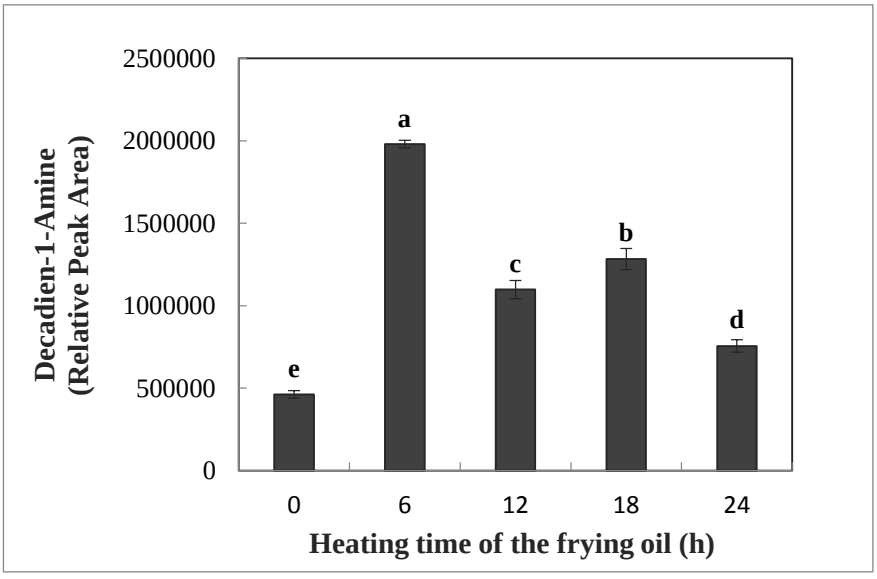

Figure 3.3. Relative amounts of decadien-1-amine in potato chips fried at 180 ºC in repeatedly used sunflower oil for 24 h to fry potato discs at different times (0, 6, 12, 18, and 24 h)

Although it was not statistically significant, total free amino acid concentration of potato chips were found to decrease slightly within the first 6 h (Figure 3.4.).

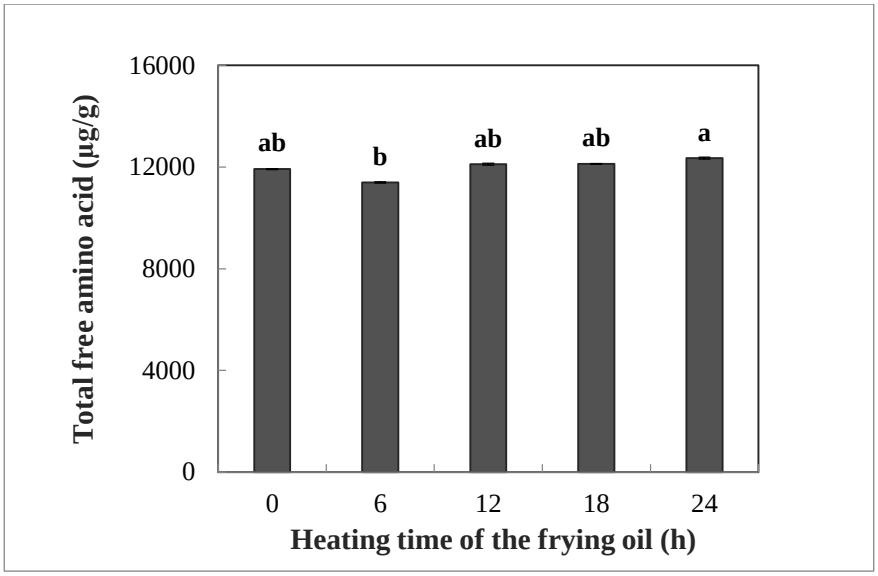

Figure 3.4. Amount of total free amino acid formed in potato chips fried at 180 ºC in repeatedly used sunflower oil for 24 h to fry potato discs at different times (0, 6, 12, 18, and 24 h)

One possible route was adapted for 2,4-decadienal in our previous study pointing out the formation decadien-1-amine in hazelnut during roasting (Karademir et al., 2013). It is typically Strecker degradation of an any amino acid by 2,4-decadienal. This type of degradations contribute considerably to flavour of heat processed foods (Zamora & Hidalgo, 2011). According to this route (Figure 3.5.), initial reaction of 2,4-decadienal (I) with any amino acid (II) at high temperature results in imine (III) formation by dehydration which then proceeds with electronic rearrangement, decarboxylation and hydrolytic cleavage steps. At the end of the route, isomers of decadien-1-amine (V,VI) and corresponding Strecker type aldehyde (VI) are formed with the structures that are only specific to initial lipid carbonyl and amino acid.

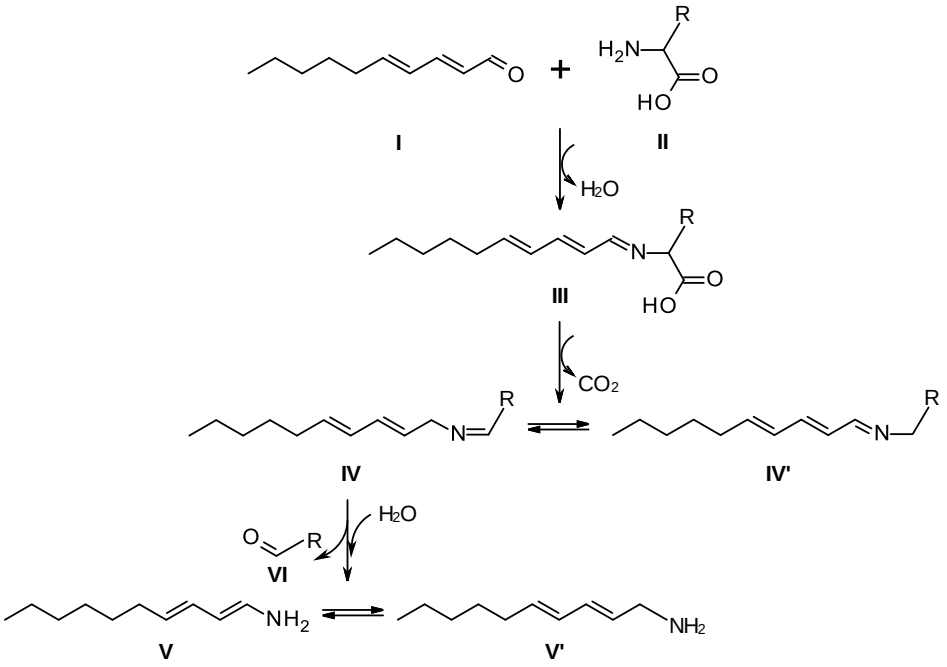

Figure 3.5. Proposed reaction pathway between 2,4-decadienal and an amino acid (adapted from Zamora & Hidalgo, 2011); I: 2,4-decadienal, II: amino acid, III: Schiff base of 2,4-decadienal, IV, IV[I]: decarboxylated isomer forms of Schiff base, V: Deca-1,3-dien-1-amine, VI: Deca-2,4-dien-1-amine VI: Strecker type aldehyde

3.1.3. Formation of 2-Pentylpyridine

As shown in Figure 3.6., 2-pentylpyridine was not detected in potato chips fried in unoxidized oil (0 h) while its concentration increased up to 154 µg/kg in potato chips at 24 h as a result of thermoxidation. Formation of 2-pentylpyridine as a flavor compound originating from 2,4-decadienal was reported in different model systems (amino acid-linoleic acid and amino acid-2,4-decadienal), and in thermally processed meats (lamb, fried chicken and beef) (Henderson & Nawar, 1981; Tang et al., 1983; Kim et al., 1996; Zhang et al., 2018). Its formation was supposed to be through the reaction of 2,4-decadienal with either free ammonia released from amino acids or α-amino groups of amino acids (Kim et al., 1996). In the case of amino acid

initiating route, condensation of 2,4-decadienal carbonyl with amino group of an amino acid was followed by electrocyclic and aromatic rearrangements (Zhang & Ho, 1989; Zhang et al., 2012).

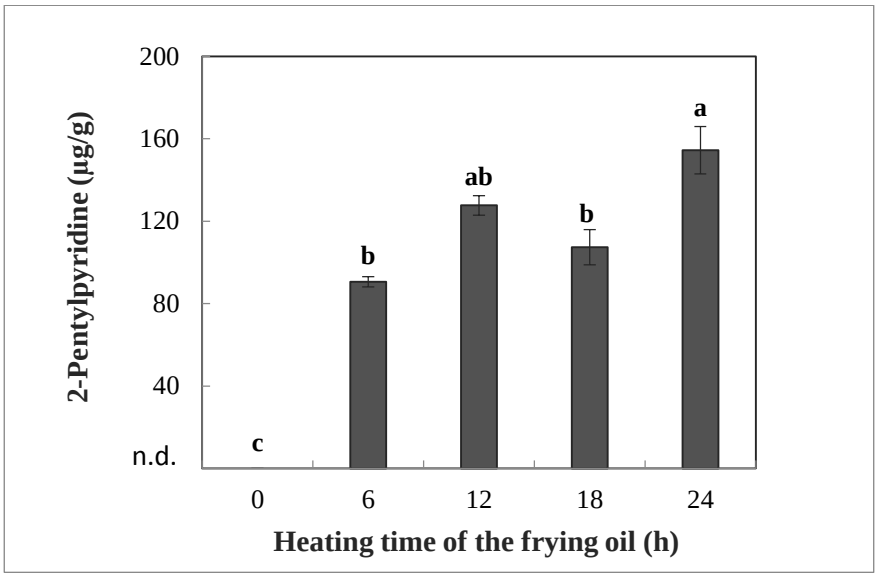

Figure 3.6. Amount of 2-pentylpyridine formed in potato chips fried at 180 ºC in repeatedly used sunflower oil for 24 h to fry potato discs at different times (0, 6, 12, 18, and 24 h)

3.1.4. Formation of Acrylamide

Although acrylamide is mainly formed from the amino acid asparagine and reducing sugars during heating as a result of the Maillard reaction, lipid-derived reactive carbonyls were also shown very active in Strecker type degradation of asparagine to acrylamide in different model studies (Zamora et al., 2008; Hidalgo et al., 2009; Hidalgo et al., 2010).

Due to its proven reactivity, 2,4-decadienal as a response to oil oxidation on acrylamide formation in potato chips was also investigated. Figure 3.7. shows the

concentrations of acrylamide formed in potato chips fried at 180 °C in repeatedly used sunflower oil.

The highest concentration of acrylamide (721 µg/kg) was found in potato chips fried in unoxidized oil (0 h) while the lowest concentration (524 µg/kg) was in potato chips fried in oil thermally oxidized for 12 h.

According to the results shown in Figure 3.6., there were no statistically significant differences ($p>0.05$) in the concentrations of acrylamide as influenced by thermal oxidation level of frying oil. However interestingly, there was a negative linear correlation between the concentrations of 2,4-decadienal and acrylamide formed in potato chips as shown in Figure 3.8..

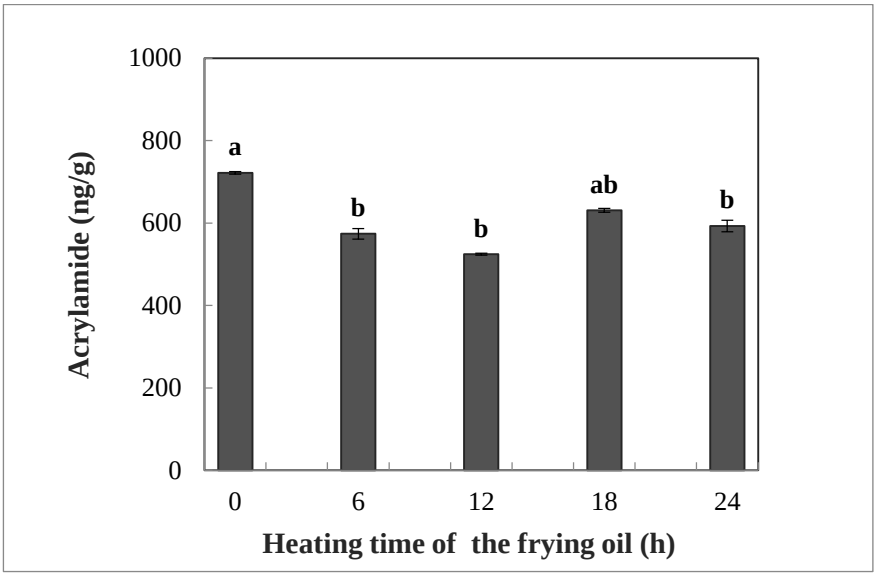

Figure 3.7. Amount of acrylamide formed in potato chips fried at 180 °C in repeatedly used sunflower oil for 24 h to fry potato discs at different times (0, 6, 12, 18, and 24 h)

When the amino acid involved is asparagine, decarboxylated Schiff base in the reaction scheme shown in Figure 3.5. leads to a Strecker aldehyde that can react further with the corresponding alcohol and upon hydrolysis generate acrylamide. It is important to note that the starting carbonyl structure may impact the yields of acrylamide, especially the functional group in the beta position to the nitrogen (Stadler et al., 2004). It is thought that lipid-derived reactive carbonyls like 2,4-decadienal may compete with reducing sugars naturally present in potato on the conversion of asparagine into acrylamide during frying. Slightly lower concentrations of acrylamide attained in potato chips fried in thermally oxidized sunflower oil might be due to lower efficiency of 2,4-decadienal on converting asparagine to acrylamide in comparison to reducing sugars.

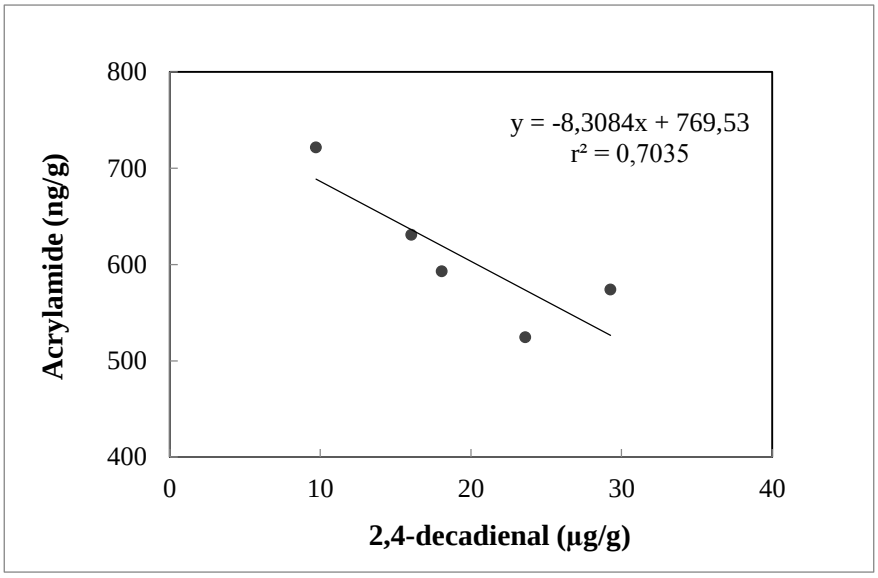

Figure 3.8. Correlation between the concentrations of 2,4-decadienal and acrylamide in potato chips

It was previously reported by different researchers that neither repeated use nor oil type affects significantly acrylamide concentration in French fries (Williams, 2005;

Matthäus et al., 2004). In contrast, frying conducted with 4 different types of vegetable oils in 10 consecutive cycles was found to have significant effects on acrylamide concentrations of sweet potato (Lim et al., 2014). The average acrylamide concentrations of fried sweet potato were reported at the highest (2019 μg/kg) for soy bean oil and the lowest (1443 μg/kg) for palm olein which were proportional to the degree of unsaturation (Lim et al., 2014). Differences in acrylamide concentrations of potato chips fried in different oils were linked with polyunsaturated fatty acid content of the oils (Thürer & Granvogl, 2016).

3.1.5. Investigation of the model systems

Sunflower oil is one of the most common used oils in frying operations both at homes and restaurants. For this reason, it was used in the preparation of model systems and fried foods to research the impact of heating of oil on amino acid modifications in foods. Model systems were used to understand complex matrice effect through eliminating deviations in the results derived from the compositional differences in the fried foods. For this purpose three groups of model doughs (M1, M2 and M4) were prepared. Briefly, M1 was consisted of whey protein and water while M2 contained 0.5% glucose and 0.5% fructose in addition to M1's content. On the other hand, M4 was prepared from potato flour and water to simulate the behavior of potato matrice during frying. Model doughs were fried at 180 ^{o}C in repeatedly used sunflower oil for 12 h at different times (0, 4, 8, 12 h).

Considering secondary oxidation products of linoleic acid which is the major fatty acid of sunflower oil and primary source of desired frying flavor, 2,4-decadienal stands out not only with its higher concentrations but also its boiling point (114-116 °C/10 mmHg(lit.)) which is relatively higher compared to other carbonyls having less C atoms. Therefore 2,4-decadienal was selected as target carbonyl compound. Thermal oxidation degrees of the oils were determined by p-Anisidine value. Formation of decadien-1-amine, revealed from the reaction between 2,4-decadienal

and amino acids was monitored as reaction intermediate. The change in reactant amounts (2,4-decadienal, total free amino acids) were also detected.

p-anisidine values of 0, 4, 8, 12 hours heated oils at 180 ^{o}C were given in Figure 3.9. Along the 12 hours-heating, as time increased, p-anisidine value of the sunflower oil also increased simultaneously. After 12 h heating, it was increased from 15 to 184 and from 15 to 210 for M1 and M2 treatments, respectively. Since p-anisidine values indicate the quantity of aldehydes (primarily 2-alkenals and 2,4-alkadienals) in animal fats and vegetable oils, (Akoh, 2017), the results showed the continuous increase in the amounts of 2-alkenals and 2,4-alkadienals along the 12 hours heating of the frying medium of two models: M1 and M2.

When the oils of two models are compared in terms of oxidation level, except for 0 th hour, M2 oil was found to be more oxidized. Glucose and fructose in the M2-model doughs appeared to favor oxidation of the frying oil and resulted with higher p-anisidine values.

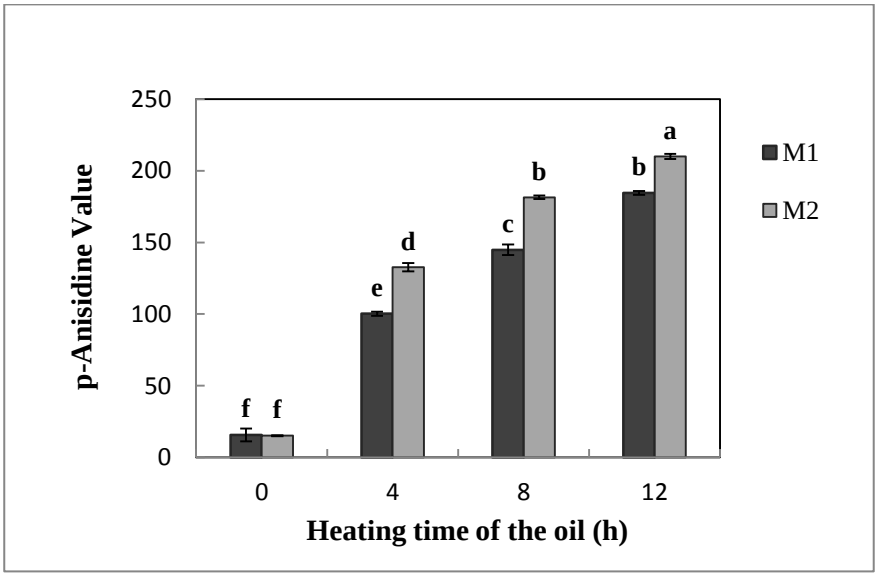

Figure 3.9. p-Anisidine values of the oils used for frying of the model systems without (M1) and with sugar (M2)

Relative amounts of 2,4-decadienal in M1 and M2 samples were detected by UPLC-HRMS system. According to the results, when the oil was heated up to 12 h, 2,4-decadienal amount rised accordingly, similar to p-anisidine values of the oil however there was not significant difference ($p>0.05$) between in M1 and M2 samples in terms of 2,4-decadienal amounts on the contrary of p-anisidine values. In potato chips there was significant increase in 2,4-decadienal amount with the first 6 h heating of the oil while it didn't differ significantly ($p>0.05$) between 6 and 12 h (Figure 3.1.).

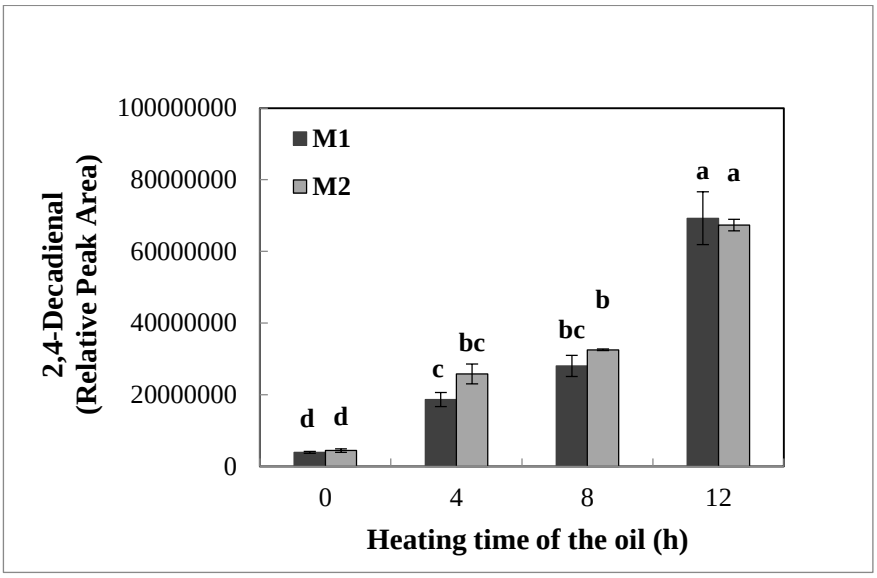

Figure 3.10. Relative amounts of 2,4-decadienal formed in the model systems without (M1) and with sugar (M2) fried at 180 ^{o}C in repeatedly used sunflower oil for 12 h at different times (0, 4, 8, 12 h)

In a study conducted by Zhang et al., soybean oil (SBO) was heated with or without frying of wheat dough model (WD) and chicken breast meat model (CBM) at 180 °C for 8 h/day over a period of 7 successive days. The formation of volatile aldehydes were investigated in these processes. Briefly WD was consisted of 39.00% water, 9.11% protein, 51.52% carbohydrates and 0.03% lipid whereas CBM composed of

72.20% water, 19.80% protein, 2.31% carbohydrates and 4.59% lipid. Because of the composition of the soybean oil (21% of C18:1-55.3% of C18:2) (Hammond, 2003)**,** trans,trans-2,4-decadienal was reported to be generated in the highest yields among the detected volatile aldehydes produced in SBO samples. In the first day of the experiments, the relative contents of trans-trans-2,4-decadienal in SBO, WD and CBM were determined to be 46.13±4.15, 35.66±3.11 and 15.76±1.98, respectively at 2 h while they increased to 78.77±2.32, 46.27±1.66 and 36.46±1.56 at 8 h of the heating. It is notable that frying of the model systems (WD, CBM) within the soybean oil (SBO) was reported to result in the lower amounts of 2,4-decadienal levels of the frying oil. It was explained by both the absorption of the 2,4-decadienal from the oil to the fried materials and the reaction of 2,4-decadienal with the amino compounds within there. On the other hand, WD was found to contain more 2,4-decadienal compared to CBM which was interpreted to be originated from the higher protein contents of CBM indicating the possible reaction between 2,4-decadienal and amino groups in the CBM (Zhang et al., 2015).

Relative amounts of decadien-1-amine formed in the model doughs M1 and M2 were presented in the Figure 3.11. As expected, decadien-1-amine was found significantly higher ($p<0.05$) amounts in M1 group. The presence of glucose and fructose in M2, affected reversely lipid-amino acid reactions by competing with lipid carbonyls transferred by frying oil to the model dough. During the heating of the oil, the most decadien-1-amine was formed in the model doughs fried at 4th hour which was followed by 8 and 12 hours. While there was not significant difference ($p>0.05$) between 8 and 12th hours, 0 hour was found to be the least effective time on decadien-1-amine formation.

Changes in the total free amino acid contents of the M1 and M2 with heating time of the oil were given as dry weight basis in the Figure 3.12.. For M1, after the first 4 h heating of the oil, the free amino acid content decreased from 427 to 299 μg/g, which also explains the remarkable rise ($p<0.05$) of decadien-1-amine in M1 within

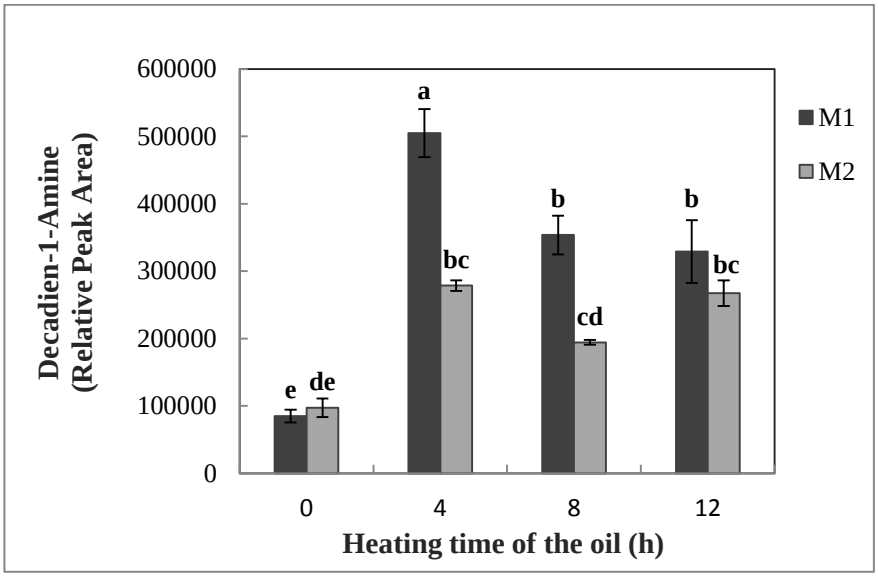

Figure 3.11. Relative amounts of decadien-1-amine formed in the model doughs (M1) and (M2) fried at 180 ºC in repeatedly used sunflower oil for 12 h at different times (0, 4, 8, 12 h)

the first 4 h. After 4 h, the amino acid concentration of M1 did not significantly differ ($p>0.05$). On the other hand, heating time of the oil did not considerably change ($p>0.05$) amino acid content of M2. The possible explanation for this would be that in presence of reducing sugars, depending on the ratio of sugar to lipid carbonyls, dominating compounds in the degradation of free amino acids might be reducing sugars due to Maillard reaction. When all the data, both of M1 and M2 were analyzed together, the total free amino acid concentration of M1 was found to be higher than M2 which was possibly resulted from the further degradation of amino acids by sugar carbonyls.

In Figure 3.13., the change in the relative amounts of 2,4-decadienal in the model dough-M4 prepared from potato flour as a response of heating time of the sunflower oil at 180 ºC was given. 2,4-decadienal reached to the its maximum concentration after 8 h thermo-oxidation of the oil and afterward it decreased slightly. Similarly, the most 2,4-decadienal was observed in potato chips fried at the 6 h heated oil, while there was not significant change ($p>0.05$) between 6 and 12 hours.

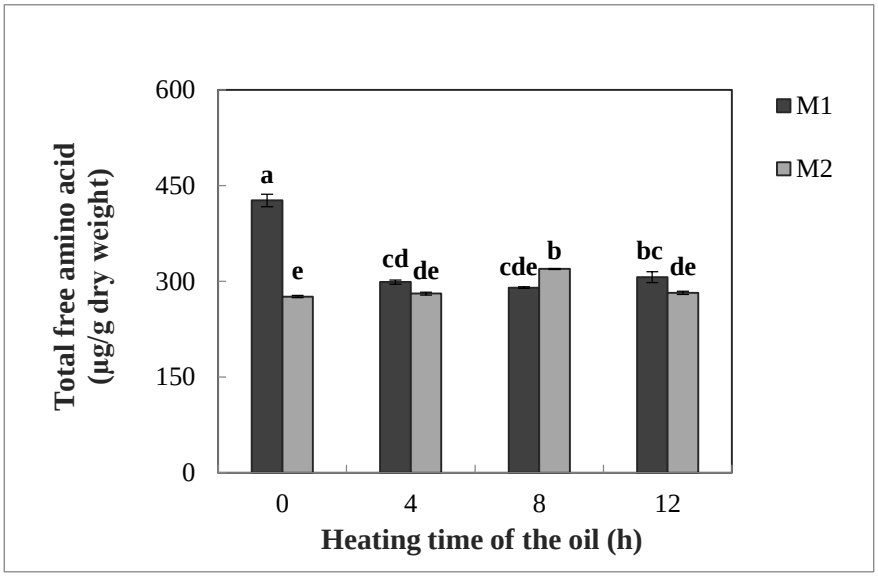

Figure 3.12. Amount of total free amino acid formed in the model systems without (M1) and with sugar (M2) fried at 180 ºC in repeatedly used sunflower oil for 12 h at different times (0, 4, 8, 12 h)

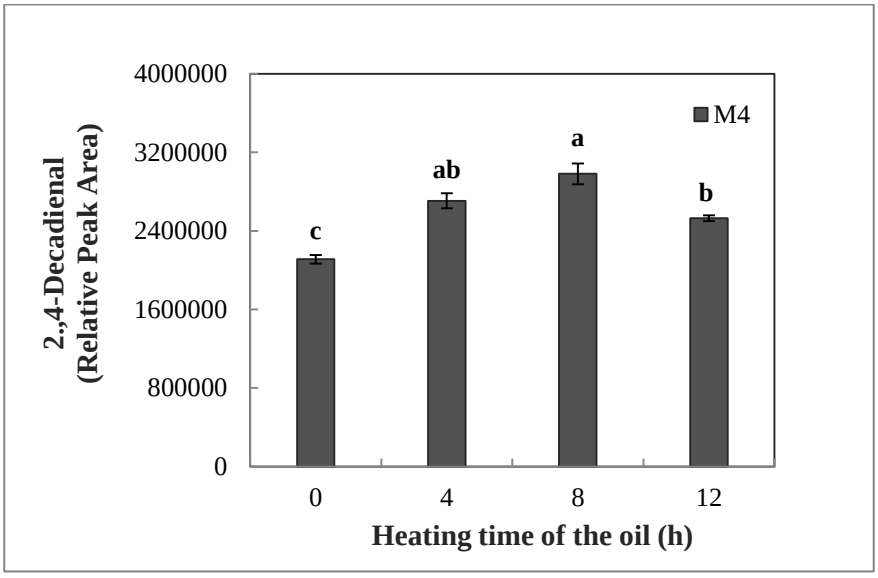

Figure 3.13. Relative amounts of 2,4-decadienal in the model system prepared with potato flour (M4) fried at 180 ºC in repeatedly used sunflower oil for 12 h at different times (0, 4, 8, 12 h)

The relative quantities of decadien-1-amine belongs to M4 were presented in Figure 3.14.. In accordance with the trend observed in potato chips along the 12 hours heating, decadien-1-amine began to decline after a dramatic increase within the first 4 h. This decline could be resulted from higher decomposition rates of 2,4-decadienal compared to production rates due to the aforementioned reasons. 0 or 12 h heating of the oil was not significantly differ ($p>0.05$) to relative concentrations of decadien-1-amine in M4.

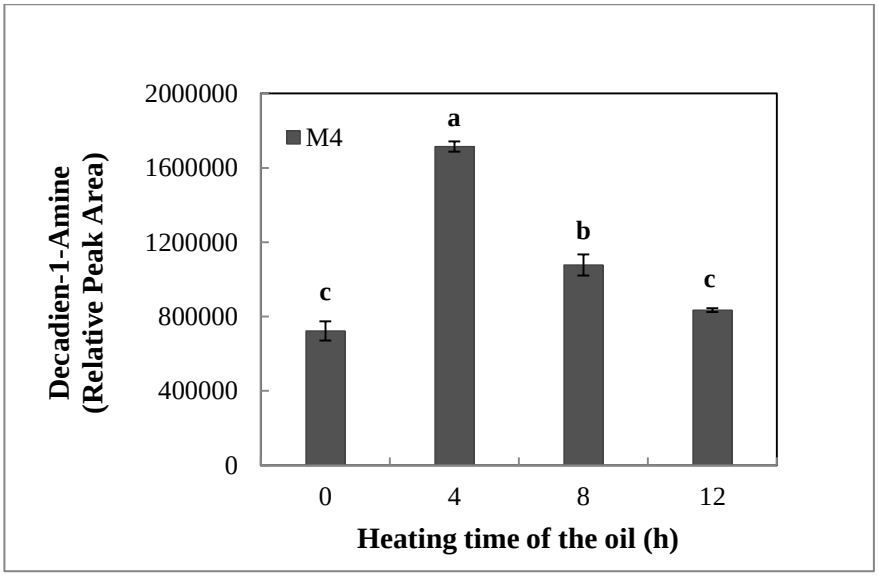

Figure 3.14. Relative amounts of decadien-1-amine in the model system prepared with potato flour (M4) fried at 180 °C in repeatedly used sunflower oil for 12 h at different times (0, 4, 8, 12 h)

Total free amino acid concentration was decreased from 9307 to 7657 µg/kg dry weight within the first 4 hours of the heating which was in aggrement with the results obtained from the potato chips. It also clarified the remarkable increase ($p<0.05$) in decadien-1-amine within this time range. However after 4 h, no significant change ($p>0.05$) was observed in total free amino acid amounts in M4.

3.2. Investigation of the impact of the oil types on amino acid modifications in potato chips

In this part, in order to understand the effect of different type of oils on amino acid modifications, corn, canola, safflower, hazelnut and olive oils were used. Frying experiments were carried out with the home type fryer and potato chips were fried in the oils were heated at 180 ^{o}C for 0, 3, 6, 9, 12 h. The same oil was used along the 12 h-heating to investigate the effect of one type of the oil.

Fatty acid profiles of the used oils have significance from the point of its direct correlation with the distribution of different type of oxidation products. For example, heptane, octane, heptanal, octanal, nonanal, 2-decenal, 2-undecenal are originated from the oxidation of oleic acid (Neff et al., 2000; Nawar, 1998) while pentanal, hexanal, 2-heptenal, 2-octenal, 2,4-octadienal, 2,4-nonadienal, 2,4-decadienal are revealed from the autoxidation of linoleic acid by contributing desired deep-fried flavor (Warner et al., 2001; Nawar, 1998; Min & Smouse, 1985). Similarly, 2,4-heptadienal is associated with the linolenic acid oxidation (Nawar, 1998). Therefore as a source of precursor compound: 2,4-decadienal, the amounts of linoleic acid in the oils have more importance for this research.

Fatty acid compositions of the oils are presented in Table 3.1.. Safflower oil was determined as the most linoleic acid containing oil with the ratio of 74.48% which was followed with a descending order by corn (53.81%), canola (20.16%), hazelnut (14.61%) and olive (9.75%) oils. While linoleic acid was predominant fatty acid in the safflower and corn oils, oleic acid was the major fatty acid of canola, hazelnut and olive oils with the ratios of 61.72%, 76.97% and 71.94%, respectively. Oleic and linoleic acid were the major fatty acids for all types of the analyzed oils. In aggrement with these results, corn, canola, safflower, hazelnut and olive oils were reported by different researchers to contain oleic and linoleic acids with the approximate percentages of 30.5%-52.0%, 60%-20%, 16%-75%, 73.6%-16.6%, 68.7

%-14.8%, respectively (Othón, 2019; Barthet, 2016; Velasco & Fernández-Martínez, 2001; Benitez-Sanchez et al., 2003).

Table 3.1. Fatty acid profile of the oils (Canola, Corn, Hazelnut, Olive, Safflower) used for frying experiments

%	Corn	Canola	Safflower	Hazelnut	Olive
C14:0	0.03	0.02	0.1	-	0.04
C16:0	11.43	4.57	6.84	5.3	12.33
C16:1	0.21	0.3	0.13	0.24	1.07
C18:0	2.00	1.78	3.28	2.37	3.01
C18:1	30.72	61.72	14.57	76.97	71.94
C18:2	53.81	20.16	74.48	14.61	9.75
C18:3	0.13	10.64	0.02	0.06	0.71
C20:0	1.24	0.44	0.31	0.37	0.99
C20:1	-	-	0.26	0.02	0.08
C20:2		0.31	0.15	-	-
C22:0	0.28	-	-	0.06	0.04
C22:1	-	-	-	-	-

The oxidation degrees of different types of used oils during the heating at 180 ^{o}C were assessed by p-anisidine value (Figure 3.15.). When the overall data statistically analyzed, as a representative of aldehydes specifically 2-alkenals and dienals, p-anisidine value was found to be the highest in the safflower oil and it was followed by corn, olive, canola and hazelnut oil, respectively. Furthermore, the oxidation degrees of the oils were increased over the heating of the oil up to 12 h. The composition and concentration of the fatty acids, antioxidants and other minor components in vegetable oils are known to play role in the characteristic oxidation stability of the oil (Tabee et al., 2008; Nogala-Kalucka et al., 2005; Przybylski & Eskin, 2006).

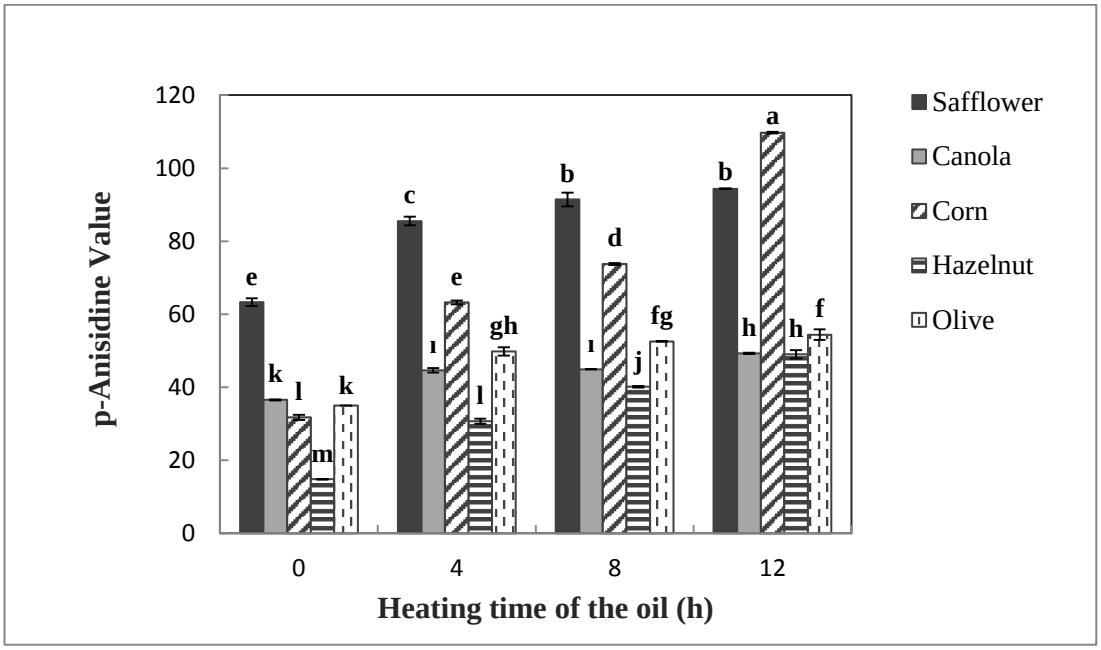

Figure 3.15. p-Anisidine values of different type of the oils (Canola, Corn, Hazelnut, Olive, Safflower) repeatedly used for 12 h at 180 ºC to fry potato discs at different times (0, 4, 8, 12 h)

In the oils with high linoleic acid content, such as safflower and corn oil, due to the higher formation rates of 2-alkenals and dienals, relatively higher values of p-anisidine caught the attention. Among the all heating times, only at 12 h, p-anisidine value of the corn oil was detected to be higher than that of the safflower oil while there was not significant change ($p>0.05$) between 8 and 12 hours of the safflower oil in terms of oxidation degree. p-anisidine values of the oils heated for 0 and 12 h were ranged between 63±1.04 - 94±0.06, 31±0.72 - 109±0.20, 34±0.01 - 54±1.44, 36±0.14 - 49±0.17, 14±0.02 - 49±1.14 for the safflower, corn, olive, canola and hazelnut oils, respectively. Moreover, the highest increment in the amounts of aldehydes was observed between 0 and 4 h for all types of the oils except for the corn oil which oxidized the most through the heating from 8 to 12 h.

Houhoula et al. ran successive fryings of the potatoes within the heated cotton seed oil with 55.5% linoleic acid and 15.9% oleic acid content at 175 ºC for 12 h. They reported the linear increase in p-anisidine values with heating time of the oil which

was associated with continuous accumulation of the aldehydes in the oil in the course of frying. Despite of the fact that the most of the aldehydes generated by the decomposition of primary oxidation products are known to have volatile feature, a partial quantity of them remains in the frying oil (Krishnamurthy et al., 1965). At the end of 12 h, p-anisidine value of the cotton seed oil was determined to reach to almost 140 (Houhoula et al., 2002). Among the oils used in this study, corn oil, showing the most compositional similarity to cotton seed oil was detected to have p-anisidine value of 109 after 12 h heating at 180 ^{o}C. In another study, p-anisidine value of the refined olive oil heated at 180 ^{o}C for 12 hours was found to increase with time and determined to be approximately 90 after 12 h heating (Tabee et al., 2008). However, Ryan et al. reported p-anisidine values of the olive and corn oil heated at 170 ^{o}C for 12 h to be 85 and 170, respectively (Ryan et al., 2007). The differences in the results most probably be derived from both the differences in the experimental conditions and the exact compositions of the used oils. Additionally the reliability of the results are strictly affected by some of the method requirements such as reaction time, mixing of the solutions (B. Matthäus, 2010).

Changes in the 2,4-decadienal amounts of the chips over the 12 h heating were presented in Figure 3.16.. Overall results revealed that the chips fried in the safflower oil, contained the highest amount of 2,4-decadienal, which was quantified with decreasing order in the chips prepared within the corn, olive, canola and hazelnut oils with no significant difference ($p>0.05$) between the chips of canola and hazelnut oils. It is noteworthy that the oil types were observed to have similar impacts on 2,4-decadienal accumulation in the chips and p-anisidine values of the oils. p-anisidine value was also indicated to be well correlated with the significant part of the aldehydes identified in frying oil and overall odor intensity of the fried oil as a result of headspace analyses (Tompkins & Perkins, 1999). According to a method developed by Dubois et al., hexanal, trans-2-hexenal and trans,trans-2,4-decadienal in canola oil, which were thought to be the best representative of the aldehydes were used as standard compounds in the calibration to determine p-

anisidine value by Fourier transform infrared (FTIR) spectroscopy (Dubois et al., 1996).

With respect to heating time of the oils, 2,4-decadienal concentrations of the chips increased in considerable amounts ($p<0.05$) within the first three hours of heating while no significant change ($p>0.05$) was observed up to 9 h which was followed by a decline. In general, after a certain heating time of the oil, the rise of 2,4-decadienal was followed by a declining trend which was most probably due to the lower production rates than that of the decomposition giving rise to formation of 2-octenal, acetaldehyde, 2-octene and glyoxal as a result of oxidation and scission reactions (Nawar, 1984).

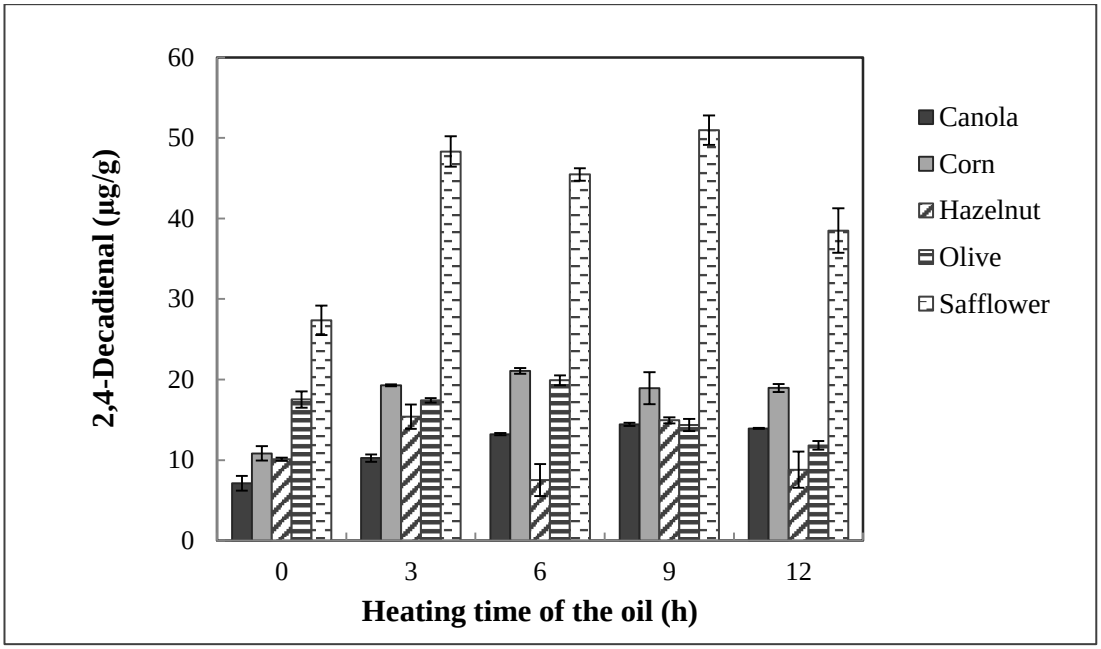

Figure 3.16. Amount of 2,4-decadienal formed in chips fried within the different type of oils (Canola, Corn, Hazelnut, Olive, Safflower) repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 3, 6, 9, 12 h)

At the beginning of the oxidation, the quantity of 2,4-decadienal in the chips prepared within the safflower oil was 27±0.06 μg/g, and it reached to the maximum of 50±0.03 μg/g after 9 h heating of the oil. The highest amounts of 2,4-decadienal in the chips prepared within the corn, olive, hazelnut and canola oils were found to be 21±0.3, 19±0.6, 15±0.2 and 14±0.4 μg/g, respectively.

The concentrations of (E,E)-2,4-Decadienal and (E,Z)-2,4-Decadienal were reported to be 6.34±0.3 and 1.53±0.2 μg/g in the potato chips prepared within the palm oil in a previous study (Wagner & Grosch, 1998). Similarly, in this study, 7.11±0.9 μg/g of 2,4-decadienal was detected in the chips fried in the canola oil which was the closest oil to palm oil among the studied oil types in terms of fatty acid composition (C18:1-38.5%, C18:2-10.5%) (Hammond, 2003).

The quantity of (E,E)-2,4-decadienal both in different oils and the French fries prepared in these oils during the eight successive frying operations at 170 ^{o}C were investigated by Boskou et al.. 2,4-decadienal concentrations were found to be ranged between 5-11, 1-9, 3-6, 2-3 μg/g for the French fries prepared in sunflower, cottonseed, palm and olive oil, respectively which also demonstrates the effectiveness of the oil types in decreasing order. This effect was reported to be associated with the unsaturation degree of the oil. When comparing the results obtained from 0 h, the reason for the determination of higher amounts in this study would be possibly derived from the higher surface to volume ratio of potato chips compared to French fries and accordingly, higher absorption rates of oil. It should be also noted that while all the oil types showed an increase in the 2,4-decadienal amounts of French fries through successive fryings, olive oil didn’t increase after 2nd frying which indicated the lesser impact of oil deterioration in olive oil most probably because of natural antioxidants (Boskou et al., 2006). Similar behavior of the olive oil was also observed in this study with the slight change of 2,4-decadienal concentrations over the heating of the olive oil.

Moumtaz et al. investigated the amounts of (*E,E*)-alka-2,4-dienal in fried potato chips obtained from 12 different fast-food restaurants. 520 μmole/kg, corresponding nearly to 80.83 μg/g of (*E,E*)-alka-2,4-dienal was detected as the highest concentration in the chips prepared within the oil consisted of 50% monounsaturated and 41% polyunsatured fatty acids whereas the estimated *trans,trans*-deca-2-4-dienal content for a typical fried potato chip was indicated to be 9 μg/g (Moumtaz et al., 2019). However, in another study, the estimated concentration of (*E,E*)-deca-2,4-dienal was reported to be 19±2.8 μg/g (Mean±SEM) for the investigated 44 samples of typical fried potato chips purchased from different fast food restaurants (Grootveld et al., 2018).

The levels of 2,4-decadienal in fried foods have importance not only for the amino acid modifications but also for the health related concerns due to its own genotoxic and cytotoxic effects (Loureiro et al., 2000; Nappez et al., 1996) although its major contribution to the deep fried flavor at the same time.

Decadien-1-amine amounts of the chips as functions of type and heating time of the oil were illustrated in Figure 3.17. Heating of the oil increased the content of decadien-1-amine significantly ($p<0.05$) for the first 3 hours according to statistical analysis of all data obtained from different type of the oils heated for different times. While the amounts of decadien-1-amine in the chips didn't change significantly ($p>0.05$) prepared within the 3 - 9 hours heated oils, it reached to the maximum level when the 12 h heated oils were used.

Safflower oil was found to be the most effective oil on the formation of decadien-1-amine in the fried chips which was in accordance with the 2,4-decadienal concentrations of the chips pointing out the highest abundance when the safflower oil was used as a result of its high linoleic acid content (74.48%). The impact of the oil type on the relative amounts of decadien-1-amine in the chips followed the order of the safflower>hazelnut>canola>olive>corn oils. The mean value of the relative amounts of decadien-1-amine resulted from the safflower oil was detected 1.5 times

higher than the hazelnut oil and 2.5 times higher than the canola oil. Although it had the second most linoleic acid content, the corn oil was found to be the least effective oil on decadien-1-amine formation. On the other hand, in spite of its lower linoleic acid content, the hazelnut oil was determined as the second effective oil.

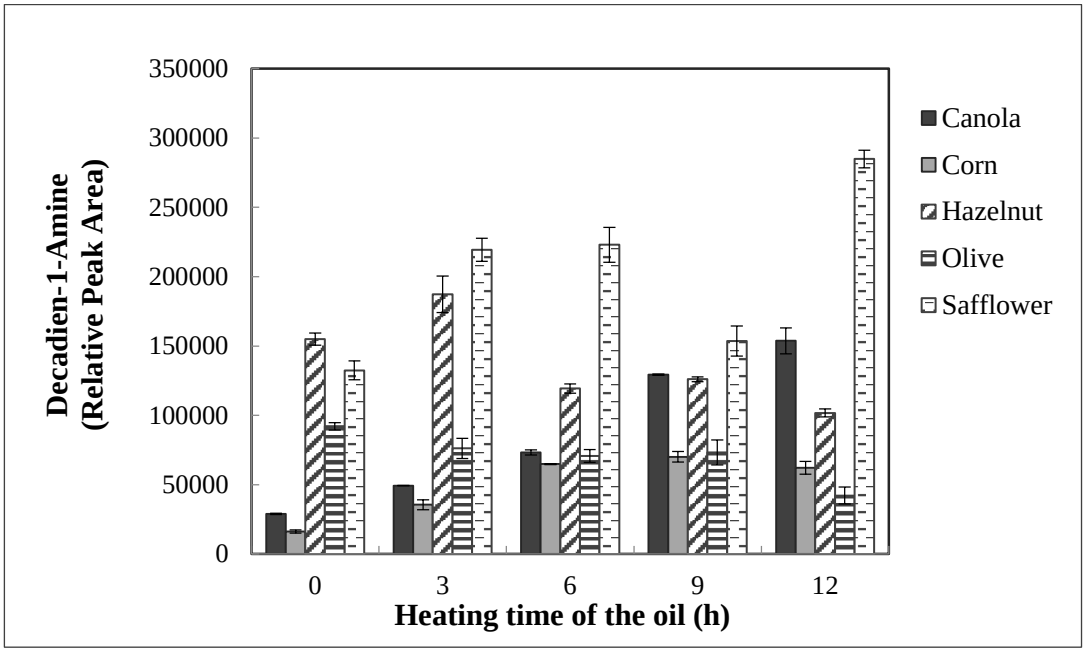

Figure 3.17. Relative amounts of decadien-1-amine in potato chips fried within the different type of oils (Canola, Corn, Hazelnut, Olive, Safflower) repeatedly used for 12 h at 180 ºC to fry potato discs at different times (0, 3, 6, 9, 12 h)

It might be resulted from the differences in the composition of potato tubers. Considering 0 h of decadien-1-amine results whereas the effect of oxidation level was less observed compared to the other hours, the difference between hazelnut and corn oils has already noticed. Although the chips fried in corn and hazelnut oils at 0 h oxidized oils had similar concentrations of 2,4-decadienal, decadien-1-amine level of the chips within the hazelnut oil was detected to be almost 10 times higher than

those within the corn oil. It could be explained by the differences in the free amino acid or sugar contents of the potato tubers.

Total free amino acid concentrations of the chips fried within the specified oils with different oxidized levels were given in the Figure 3.18.. The results were expressed on dry basis. A slight increase was observed in free amino acid levels of the chips, only for the first three hours while there was not significant ($p>0.05$) change when the heating of the oil was prolonged. However, the findings obtained from the model systems in the first part provided the evidence for that total free amino acid contents of the model doughs decreased significantly ($p<0.05$) in the first 4 h-heating of the oil. Therefore, this slight increase might be attributed to the compositional differences of the food matrice.

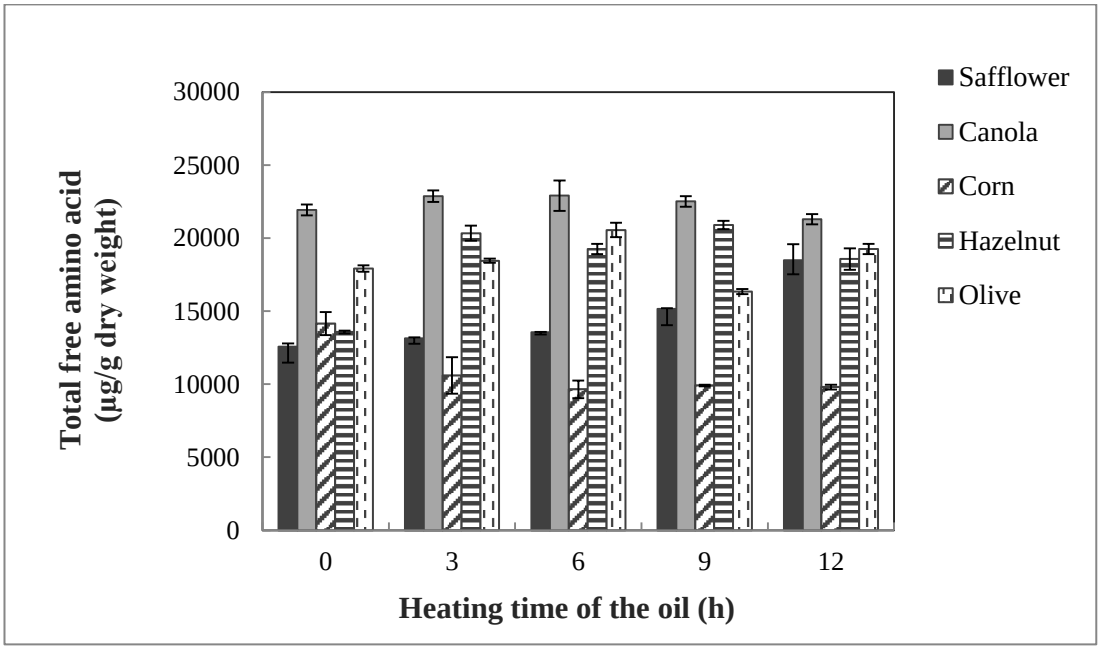

Figure 3.18. Amount of total free amino acid in potato chips fried within the different type of oils (Canola, Corn, Hazelnut, Olive, Safflower) repeatedly used for 12 h at 180 ^{o}C to fry potato discs at different times (0, 3, 6, 9, 12 h)

Among all the oil types, the chips of canola oil had the highest concentration of free amino acids with the means of 22306 μg/g dry weight for the all degrees of oxidation which was followed by hazelnut (18527 μg/g dry weight) and olive (18507 μg/g dry weight), safflower (14584 μg/g dry weight) and corn (10825 μg/g dry weight) oils. When the overall results were analyzed, free amino acid levels of the chips fried in the corn oil was found to be far less than that of the hazelnut oil although the hazelnut oil was determined to be the second most effective oil type on decadien-1-amine formation which might be derived from less concentration of free amino acids in the potato tuber used for corn oil.

Carboxymethyllysine (CML) and carboxyethyllysine (CEL) formed as a result of the reaction of lysine with glyoxal (GO) and methylglyoxal (MGO) respectively which could be generated in both lipid or glucose oxidation (Fu et al., 1996). Frying process was also reported to be a rich source of glyoxal (Degen et al., 2012). Goldberg et al. reported the highest amounts of CML in oily foods among the analyzed 250 food items classified by their protein, oil and carbohydrate content (Goldberg et al., 2004; Han et al., 2013b). Therefore CML and CEL concentrations of the chips were monitored in the chips prepared along the heating of different type of the oils. CML results were given in Figure 3.19..

According to the overall assessment of all type of the oils, within the first 4 hours of the heating, CML concentrations of the chips increased significantly ($p<0.05$), however between 4 and 8 hours no significant change ($p>0.05$) was observed which was followed then by a slight decrease at 12 h.

Regarding the impact of the oil types, hazelnut oil was detected to be the most effective oil on the formation of CML with the means of 1063 ng/g and it was followed by canola (958 ng/g), safflower (815 ng/g), corn (680 ng/g) and olive (522 ng/g) oil, respectively.

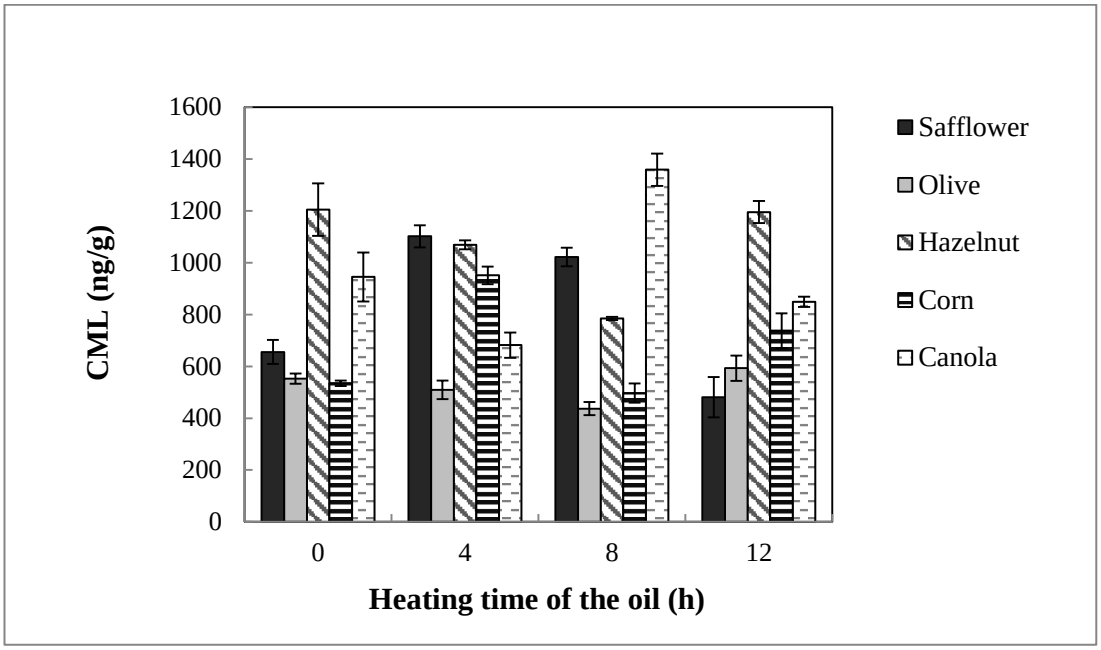

Figure 3.19. Amount of CML formed in potato chips fried within the different type of oils (Canola, Corn, Hazelnut, Olive, Safflower) repeatedly used for 12 h at 180 ^{o}C to fry potato discs at different times (0, 4, 8, 12 h)

CML levels of the chips fried in the hazelnut oil were ranged between 784±6 - 1204± 101 ng/g while it was between 681±48 - 1358±62, 655±46 - 1101±36, 496±37 - 950±33, 437±25-592±48 ng/g in canola, safflower, corn and olive oils, respectively. Zhou et al. reported the CML levels of commercial potato crisps in China to range from 4690±290 to 20000±300 ng/g which were extremely higher than our findings even if the differences derived from the composition of the potatoes and used oils were considered (Zhou et al., 2015). However our results were in agreement with those of Scheijen et al. who noted 1400 ng/g of CML in commercial potato chips (Scheijen et al., 2016).

GO and MGO, precursors of CML and CEL could be generated from the Amodori products during the Maillard reaction or sugar fragmentation (Davídek et al., 2006) Cämmerer et al., 1999; Namiki & Hayeshi, 1983). GO was also suggested to form through the decomposition of the hydroperoxides and as a result of the autooxidation

of linoleic and linolenic acids (Yin & Porter, 2005; Loidl-Stahlhofen & Spitelier, 1994, Miakar & Spiteller, 1994). Positive effects of unsaturated fatty acids (UFA) on CML formation was stated by Han et al. (Han et al., 2013a). Additionally, in a previous study, formation of GO and MGO was pronounced to be higher levels in fish oils compared to vegetable oils after heating at 200 ^{o}C for 1 h because of higher contents of polyunsaturated fatty acids in fish oils (Fujioka & Shibamoto, 2004). Therefore positive correlation might be speculated between the oxidation degree of the frying oil and CML concentrations of the potato chips. On the other hand, no significant difference ($p>0.05$) was reported between the effect of triolein and trilinolein on CML and CEL formation in the model systems consisted of lysine, glucose and one of those triglycerides. It was most probably resulted from the low extent of thermal treatment which was applied at 180 ^{o}C just for 30 min. Nevertheless, the volatility and lower stabilities of GO and MGO, food matrice and the reactions taken place during frying are quite complicated to think so simple. Glyoxylic acid, CO and CO_2 were stated to be secondary products of glyoxal (Moree-Testa & Saint-Jalm, 1981). High concentrations of reducing sugars in the potato matrice and the substantial rates of Maillard reaction as sources of GO and MGO should be taken into consideration simultaneously.

Although the behaviour of CML with respect to heating time of the oil is similar to that of 2,4-decadienal, the impact of the oil types were different. However it should be noted that at 0th hour, the highest amount of CML formation had been already observed in the chips prepared with hazelnut oil rather than following a gradual increase with the heating time of the oil. It might be resulted from the matrice effect in other words because of higher concentrations of free amino acids in the potatoes used in the hazelnut oil.

In Figure 3.20, the change in CEL amounts of the chips with type and heating time of the oil was shown. When canola oil was selected as the frying oil, CEL concentrations of the chips was determined to be the highest (Mean value: 1923 ng/g) and it was followed by safflower oil (Mean value: 1445 ng/g). While no

significant difference ($p>0.05$) was observed between safflower and hazelnut oil (Mean value: 1384 ng/g), corn (Mean value: 1294 ng/g) and olive (Mean value: 1176 ng/g) oils were determined to form lower amounts of CEL.

CEL levels of the chips were quantified between 1509±106 - 2435±86, 1181±83 - 1640±58, 1244±35 - 1673±57, 1191±84 - 1365±38, 1010±68 - 1366±38 ng/g in the oils of canola, safflower, hazelnut, corn and olive, respectively in accordance with

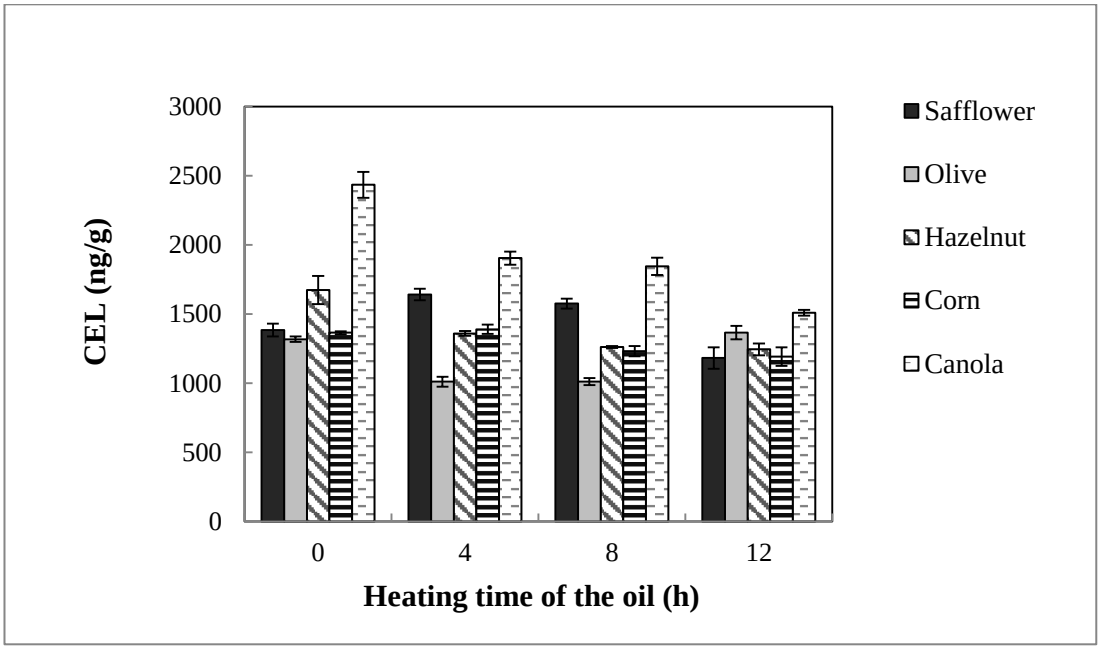

Figure 3.20. Amount of CEL formed in potato chips fried within the different type of oils (Canola, Corn, Hazelnut, Olive, Safflower) repeatedly used for 12 h at 180 ºC to fry potato discs at different times (0, 4, 8, 12 h)

the results of the former studies. Commercial potato crisps in China were reported to contain CEL levels changed between 1970±100 - 4010±340 ng/g by Zhou et al. (Zhou et al., 2015). In another study, CEL quantity of the commercial potato chips was found to be 900 ng/g (Scheijen et al., 2016).

According to the statistical analysis of the all data, heating time of the oil was not found to be correlated with the amount of CEL in potato chips. However in the case of using the oils with very high amounts of linoleic acid such as safflower oil, it is very obvious that CML and CEL concentrations of the chips significantly increased ($p<0.05$) within the first 4 hours in spite of low stability and notably low boiling points of GO (50.4 ºC) and MGO (72.0 ºC) which might also adversely affect the gradual accumulation of the GO and MGO in the oil during the heating of the oil (Figure 3.21.) (Shibamoto, 2006).

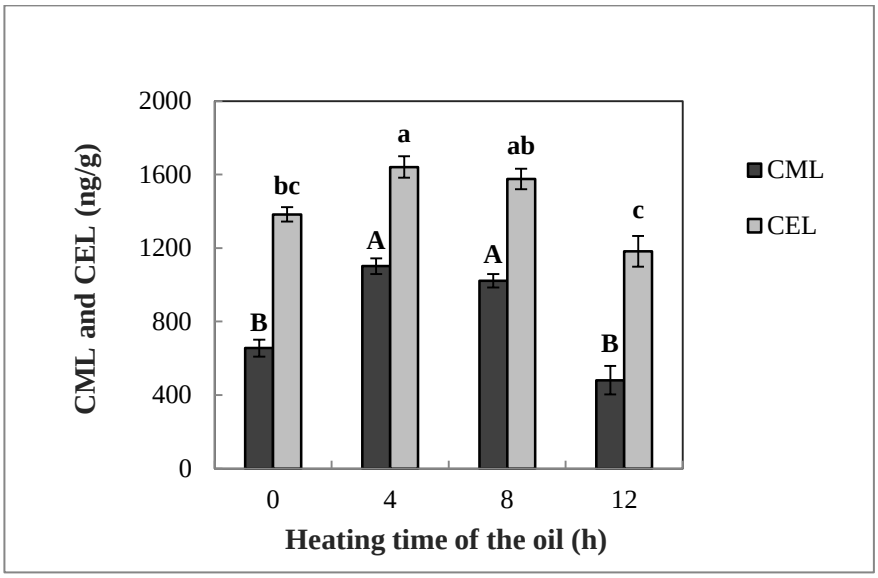

Figure 3.21. Amount of CML and CEL formed in potato chips fried within the safflower oil repeatedly used for 12 h at 180 ºC to fry potato discs at different times (0, 4, 8, 12 h)

CEL concentrations of the chips were found to be higher than CML concentrations for all oil types which might be due to the relatively higher levels of MGO compared to GO. Related with that observation, high temperatures indicated to accelerate CEL generation while mild temperatures promote CML formation (Yu et al., 2016). Fujioka et al. also reported higher rates of CEL formation in comparison with CML

in soybean, corn and olive oil after heating at 200 ^{o}C for 1 h (Fujioka & Shibamoto, 2004).

Additionally, it is important to note that for the first 4 hours of the heating, decrease in the levels of CML and CEL of the chips prepared in the oils (hazelnut, olive, canola) with high oleic acid content was remarkable while the reverse of this behavior was observed in the high linoleic acid oils (safflower, canola). Therefore the suppression rates of the polyunsaturated fatty acids against the other sources of CML and CEL in the food matrice might be determinative in the impact of different type of the oils on the formation of CML and CEL.

3.3. Investigation of the effect of different treatments on the amino acid modifications in potato chips and fried chickens

3.3.1. Addition of antioxidant to the oil during the frying of the potato chips

Oxidation is known to play a significant role in financial and health related considerations in the frying operations by declining stability of the frying oil, organoleptic properties of the food and producing toxic compounds (Pokorny, 1998). For this reason, antioxidant addition to the oil is one of the most common practices which has been tried to reduce oxidation during frying. In this context, some natural and synthetic antioxidants might be used. Among the natural antioxidants, tocopherols have been characterized as the most well recognized and effective ones which could be used at frying temperatures (Warner & Moser, 2009). Therefore, the tocopherol mix (α-β-δ-γ) was used in the experiments as natural antioxidants.

In this part of the study, to understand the antioxidant effect on amino acid modifications in the potato chips, 1 % tocopherol mix (α-β-δ-γ) was added to sunflower oil (SO+A) and the frying experiments were carried out at every 4 hours in the both SO+A and control oil without antioxidant SO which were heated at 180 ^{o}C along the 12 hours.

The oxidation degrees of the oils upon the heating were monitored by p-anisidine values (Figure 3.22.). The addition of 1 % tocopherol mix to the sunflower oil triggered the oxidation reactions by resulting with the significantly higher ($p<0.05$) numbers of p-anisidine (Mean value: 71) in comparison with the control oil (Mean value: 34). On the other hand, heating time of the oil gradually raised the p-anisidine value up to the 8 h at where it culminated and then declined by the 12 h.

p-anisidine values of the SO and SO+A were found to range between 10±0.2 - 54±0.05 and 18±1.44 - 111±1.24, respectively. In a different study, French fries were prepared within different type of oils after 3, 6, 8 and 12 hours of heating of the oil at 170 ^{o}C for each day of 3 consecutive days. p-anisidine value in the sunflower oil increased from 26.6±4.9 to 100.8±6.3 while the heating was continued from 3 h

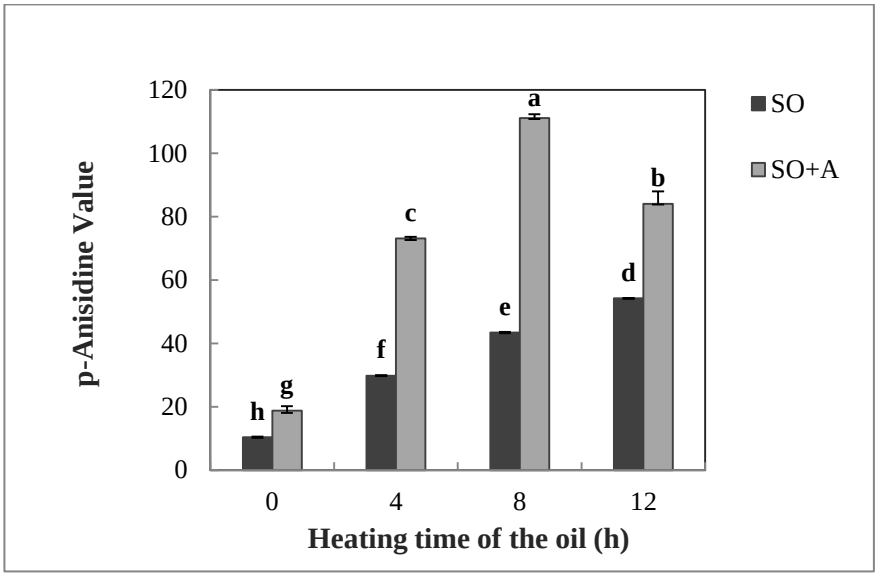

Figure 3.22. p-Anisidine values of the oils with (SO+A) and without 1% tocopherol mix (SO) repeatedly used for 12 h at 180 ^{o}C to fry potato discs at different times (0, 4, 8, 12 h)

to 8 h in the first day of the experiments (Petersen et al., 2013). Although similar results (29.78±0.15) were observed for 4 h heating of the sunflower oil, lower p-anisidine value (43.39±0.19) was observed at 8 h in our study.

The effectiveness of antioxidants was known to drop at frying temperatures in comparison with the room temperatures as a result of losses derived from volatility and decomposition of the molecule (Boskou, 1988; Choe & Min, 2007).

The tendency of antioxidants in the acceleration of the decomposition of hydroperoxides was also reported in the former studies (Hill et. al, 1969; Privett & Quackenbush, 1954). However in another study, the sunflower oils with and without their natural tocopherols were heated at 180 °C for 10 hours and for any heating time, higher amounts of polar compounds were detected in the stripped oils (Barrera-Arellano et al., 2002; Dobarganes et al., 2010).

The antioxidant behavior of α- and γ-tocopherols with the concentrations between 0.0005 - 0.2% in purified triacylglycerols derived from the sunflower oil was investigated at 100 ^{o}C by Yanishlieva et al.. Below to the levels of 0.07%, α-tocopherol was determined to be more active than γ-tocopherol wheras the vice versa was reported for γ-tocopherol at higher concentrations. The loss of antioxidant activity at higher concentrations was stated to be associated with their consumption in side reactions rather than their participation in chain initiation reactions. α- and γ-tocopherol increase the rate of lipid oxidation by reducing lipid hydroperoxides and thereof generating alkoxyl radicals (Yanishlieva et. al, 2002).

In a different study, stripped corn oil was oxidized at 60 ^{o}C for 7 days and the optimum quantities to prevent hydroperoxide formation were reported to be 0.01% for α-tocopherol and 0.025-0.050% for γ-tocopherol. According to this study, while α-tocopherol was found to exhibit prooxidant effect at the concentrations of 0.025% or higher, no prooxidant activity was observed in γ-tocopherol at 0.1% or below. However, hexanal as a decomposition product of linoleate hydroperoxides was

reported to be inhibited further by increasing tocopherol concentration (Huang et al., 1994).

In another study, α-tocopherol and TBHQ were determined to be the most effective antioxidants in accelerating hydroperoxide decomposition (Saaidia, 1985). In this regard, the higher p-anisidine values of the SO+A, probably would be resulted from accelerated decomposition of hydroperoxides which might yield higher numbers of aldehyde oxidation products.

The formation of γ-tocopherolquinone type structures by the rapid degradation of tocopherols at frying temperatures has been demonstrated by Verleyen et al. (Verleyen et al., 2001).

According to the results of different relevant studies in the literature, it is quite difficult to understand and adequately evaluate the antioxidant activity of tocopherols because this activity is frequently affected by their concentration, types of oils and evaluation method to measure oxidation (Yoshida, Kajimoto, & Emura, 1993). Furthermore, as a result of simultaneous oxidative and thermal reactions, evaluation of antioxidant efficacy is much more challenging under frying conditions compared to the moderate temperatures (Márquez-Ruiz et al., 2014).

The precursor of decadien-1-amine, 2,4-decadienal was monitored in the chips fried at 0, 4, 8 and 12 h along the 12 h-heating of the SO and SO+A. In consistent with the results of p-anisidine values, SO+A caused higher 2,4-decadienal accumulation in the chips than the SO did. In a previous study TBHQ addition to the oil was reported also to result in higher yields of 2,4-decadienal (Saaidia, 1985). Considering the overall results, heating time of the oil was determined to rise 2,4-decadienal levels up to 8 h while no significant change ($p>0.05$) was observed between 8 and 12 h.

The relative amounts of decadien-1-amine were given in the Figure 3.23. In accordance with the results obtained from p-anisidine values and 2,4-decadienals, SO+A was found to be more effective than SO on the formation of the decadien-1-

amine which was the most apparent at 4 h. Decadien-1-amine levels significantly raised ($p<0.05$) within the first 4 h of heating and it started to decrease by the 8 h.

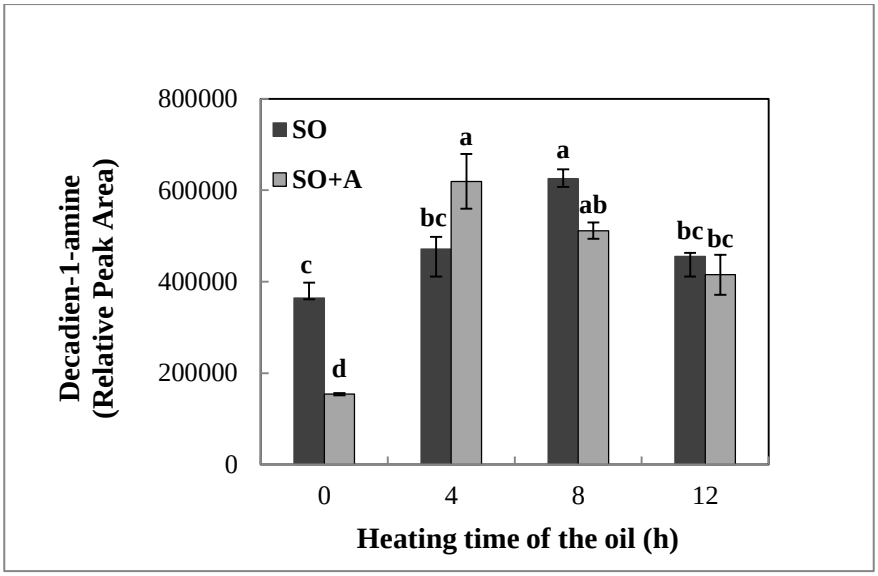

Figure 3.23. Relative amounts of decadien-1-amine in potato chips fried in the oils with (SO+A) and without 1% tocopherol mix (SO) repeatedly used for 12 h at 180 ºC to fry potato discs at different times (0, 4, 8, 12 h)

Concentrations of the total free amino acids determined in the chips were presented in the Figure 3.24. . Total free amino acid levels of the chips fried in the SO decreased from 12738±109 to 8483±345 µg/g within the first 4 hours of the heating because of most likely the highest extent of amino acid modifications by the lipid derived carbonyls while no significant change ($p>0.05$) was observed in the chips prepared in SO+A.

In order to investigate the impact of antioxidant addition to the oil on amino acid modifications of the chips, CML and CEL amounts were also detected and shown in the Figure 3.25. and Figure 3.26., respectively.

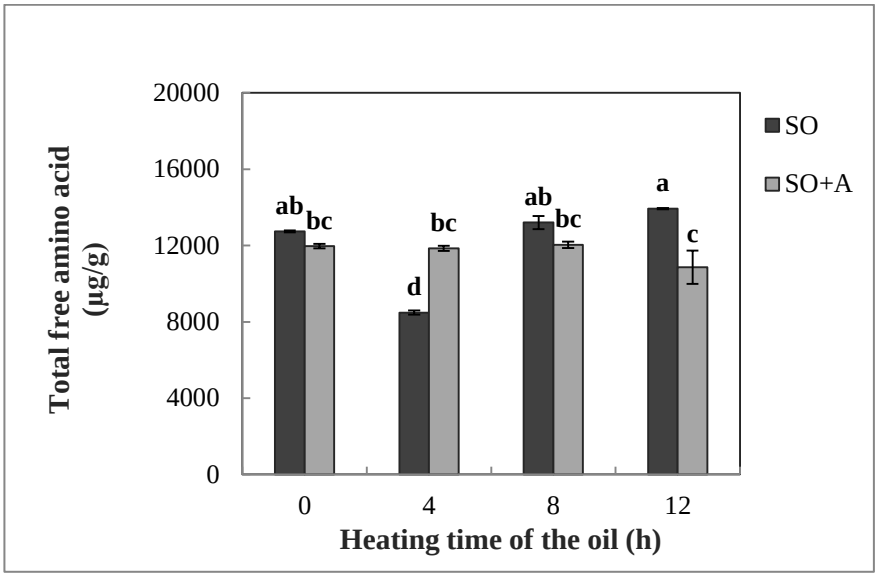

Figure 3.24. Amounts of total free amino acid in potato chips fried in the oils with (SO+A) and without 1% tocopherol mix (SO) repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 4, 8, 12 h)

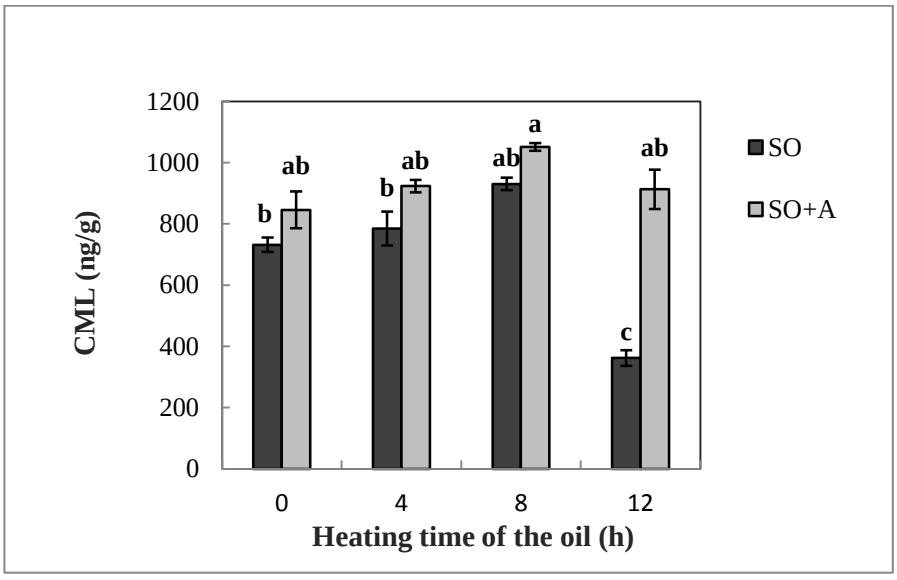

Figure 3.25. Amounts of CML in potato chips fried in the oils with (SO+A) and without 1% tocopherol mix (SO) repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 4, 8, 12 h)

The overall results showed that when 1% tocopherol mix was added to sunflower oil, CML and CEL concentrations of the chips increased significantly ($p<0.05$). It would probably due to the increasing affect of the tocopherol mix on the aldehydes formed during the thermal oxidation of the oil as a result of reported higher decomposition rates of the hydroperoxides.

CML and CEL concentrations of the chips prepared in the SO and SO+A were ranged between 362±25 - 930±20 ng/g (CML), 1135±128 - 2662±94 ng/g (CEL) for SO and 845±59 - 1050±12 ng/g (CML), 1997±141 - 2814±99 ng/g (CEL) for SO+A.

When all the data analyzed, in terms of heating time of the oil, 8 h was found to be the most efficient time on the formation of CML while CEL concentrations of the chips didn't have any correlation with the heating time similar to the other types of the oils investigated in previous section except for the safflower oil with very high linoleic acid content (% 74.48).

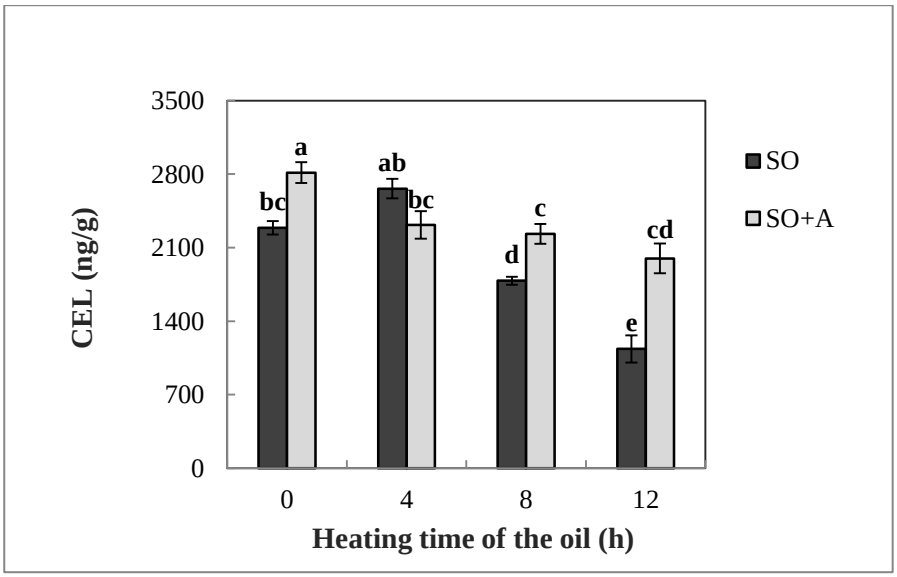

Figure 3.26. Amounts of CEL in potato chips fried in the oils with (SO+A) and without 1% tocopherol mix (SO) repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 4, 8, 12 h)

In conclusion, although it was aimed that to increase oxidation stability of the frying oil by the addition of the 1% tocopherol mix, this treatment triggered the oxidation of the oil. Overall results revealed much more modification of the free amino acids in the chips prepared within the more oxidized oil.

3.3.2. Investigation of the effect of different treatments on the amino acid modifications in fried chickens

Chicken meat, being one of the most prevalent food materials used in frying practices was investigated in this part. For this purpose, five different treatments: which were coded as T1, T2, T3, T4, P were studied. Except for P group, chicken meatballs were deep fried with different treatments in the sunflower oil at 0, 4, 8, 12th hours of the heating of the oil at 180 ^{o}C. On the other hand, chicken meatballs were pan fried within the pre-oxidized (180 ^{o}C-0, 4, 8, 12 h) sunflower oil for the P group only. Briefly, T1 represents control without any treatments. T2 was prepared in the oil with 0.1% (v/v) BHT and T3 in the oil with 0.1% (v/v) tocopherol mix. Finally, coated chickens were used for T4 group.

Because of possible toxic effects, the allowed quantity of the synthetic antioxidants in foods, fats and oils have been limited to 0.02% (w/w) by the U.S. Food and Drug Administration (FDA), the European Food Safety Authority (EFSA), and the World Food and Agricultural Association (FAO). However in order to provide more stability against the oxidation, natural antioxidants could be added to frying oil with the levels more than 0.02% (Hu & Jacobsen, 2016). Therefore in spite of being higher than the allowed limit for the uses of synthetic antioxidants in foods, BHT was used in the rates of 0.1% to be able to compare its effect with tocopherols.

In order to monitor changes in the oxidation degrees of the oils, p-anisidine value is measured. The results were given in the Figure 3.27. .

Considered overall results, no significant changes ($p>0.05$) were observed between T3 (Mean value: 102), T1 (Mean value: 101) and T2 (Mean value: 98) whereas T4 (Mean value: 73) was found to have the least oxidized levels of the oil. Because the

moisture of the fried chickens (40-60%) was transferred to the oil during frying, free fatty acid concentrations of the oil increased which favored the oxidation reactions accordingly. As a result of the coating treatment in T4 group, lesser amounts of food components might be transferred to the oil leading lower p-anisidine values.

In consistent with the obtained results in the previous parts of this study, heating time of the oil was found to be correlated with p-anisidine value (r^2=0.909) of the frying oil. But the most considerable change ($p<0.05$) was observed within the first 4 hours.

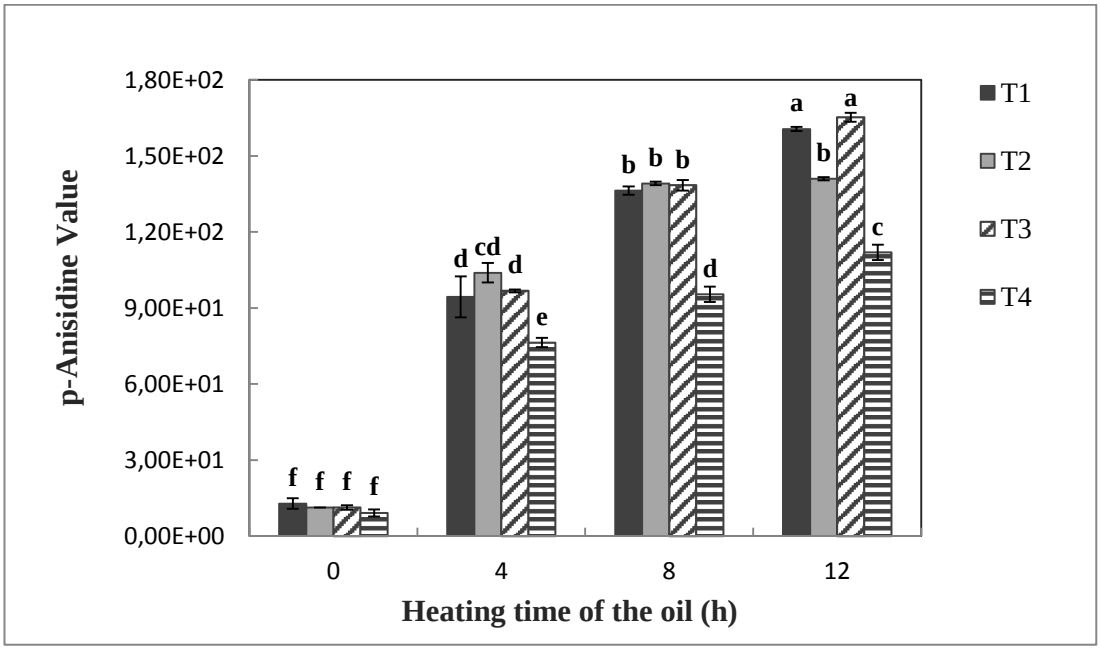

Figure 3.27. p-Anisidine values of the oils repeatedly used for 12 h at 180 ºC to fry chicken meatballs at different times (0, 4, 8, 12 h) for different processes (T1:Control, T2: Oil with 0.1% BHT, T3: Oil with 0.1% tocopherol mix, T4: Breaded)

Thermal decomposition of some antioxidants were investigated by Nawar et al. and approximately one-fourth of the initial amounts of BHT were detected to be lost by the evaporation after just 1 h of heating at 185 ºC (Hamama & Nawar, 1991).

Augustin and Berry reported the loss of 70 % of BHT after 8 h heating and found very few differences between palm olein and palm olein containing 0.02 % BHT (Augustin & Berry, 1983). In addition, no protective effect of the 0.02 % BHT was reported in the heated sunflower oil whereas silicone was determined to have protective role against the oxidation (Freeman, et al., 1973).

In another study, refined olive oil with and without 0.2% of α-tocopherol were heated at 180 ^{o}C along 12 h. In accordance with the our results, p-anisidine values were found to increase during the 12 h heating of the oil at 180 ^{o}C and 0.2% α-tocopherol was detected to induce higher p-anisidine values after 6 h of heat treatment (Tabe et al., 2008) Similarly, 0.01 and 0.1% of α-tocopherol additions to the mixture of rapeseed and palm oil was reported to found to cause higher rates of secondary oxidation products (Nogala-Kalucka, Korczak et al., 2005).

However in a different research conducted by Rossi et al., sunflower oil was heated at 175 ^{o}C, and used for 12 h to fry potato chips and 90% of α-tocopherol was reported to be able to remain in the oil even after 12 h (Rossi et al., 2007). Despite of the fact that radical scavenging mechanism is accepted to be mainly responsible for the antioxidant activity under ambient conditions, it becomes less significant when the frying conditions were considered (Aladedunye, 2014). Oxygen supply is quite restricted in a typical frying operation as a result of both decreasing solubility and steam blanketing at the oil-air interface. Therefore non-radical polymerization of unsaturated fatty acids has been proposed to dominate which might be the one explanation for the poor activity of lipid antioxidants at frying temperatures in spite of their excellent radical scavenging activity under moderate conditions. (Aladedunye, 2014; Gertz et al., 2000; Gertz, 2004; Parkash-Kochhar & Gertz, 2004).

Nevertheless, chemical degradation of antioxidants is considered to be the primary reason of antioxidant loss at frying temperatures even though the volatilization and

steam distillation resulted from the high temperature and steam evaporated from the food have been thought (Dobarganes et al., 2010).

2,4-decadienal levels of chickens fried with different treatments were presented in the Figure 3.28.. The results were expressed as relative peak areas in dry weight basis. Similar to p-anisidine value, 2,4-decadienal levels of the fried chickens showed an increase with the prolonged heating times of the oil by reaching the maximum level at 12 h for all treatments.

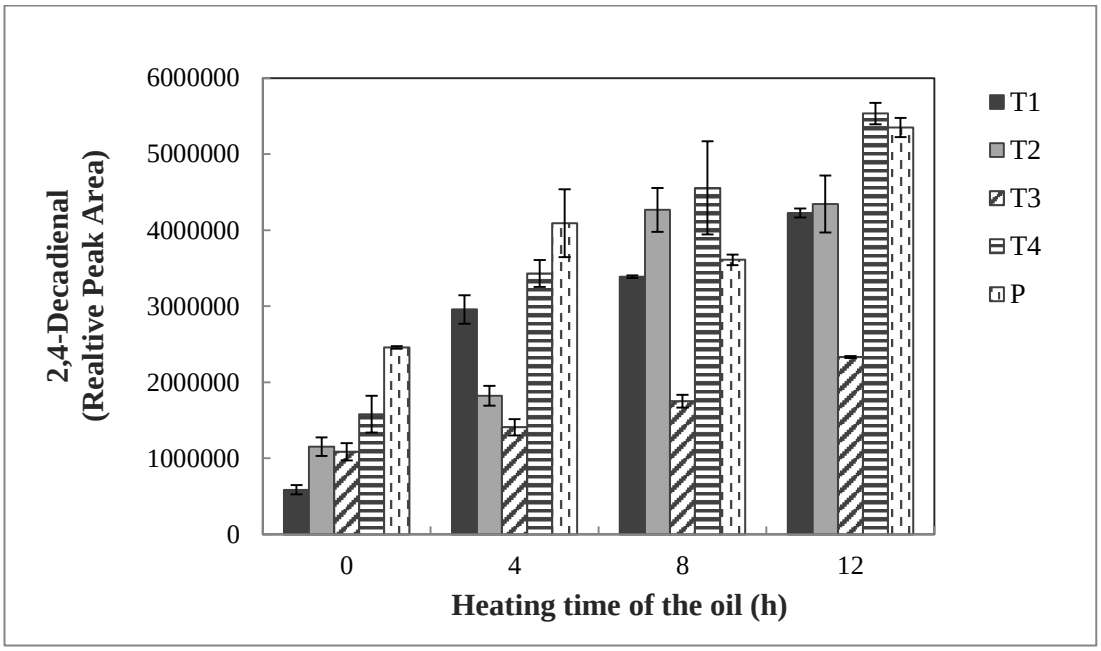

Figure 3.28. Relative amounts of 2,4-decadienal in chicken meat balls (T1:Control, T2: Oil with 0.1% BHT, T3: Oil with 0.1% tocopherol mix, T4: Breaded P: Pan-fried) fried in the oils repeatedly used for 12 h at 180 ^{o}C to fry chicken meatballs at different times (0, 4, 8, 12 h)

The first 4 hours of the heating was determined to be the most effective time range in the accumulation of 2,4-decadienal in the fried chicken meatballs. Among the all treatments, the highest increment (5.00 times) in 2,4-decadienal was observed in T1, control group for this time range whereas T4 showed the second highest rise (2.17

times) which was followed by P (1.66 times). However there was not significant change ($p>0.05$) between 0 and 4 hours of T2 and T3 groups. Although no significant difference ($p>0.05$) was observed between p-anisidine values of T1, T2 and T3 oils, lesser amounts of 2,4-decadienal were determined in the chicken meatballs prepared within 4 hours heated oils with 0.1% BHT or 0.1% tocopherol mix.

According to the overall data, while T3 was found to contain the lowest amounts of 2,4-decadienal, T4 and P groups were determined to possess the highest quantities which were followed by T1 and T2.

The oil contents of T1, T2, T3 and T4 groups were ranged between 30-32% on dry matter whereas it was determined to be 17% (on dry matter) for P group. In terms of the oil content, except for P, T4 did not significantly differ ($p>0.05$) from other treatments, possibly because of the fact that the crust layers of the coated chicken meatballs were not disregarded for the sampling of the oil analysis in order to provide it to represent the consumed form of it. Expectedly, pan fried chickens were detected to have less amounts of oil compared to deep fried ones. When 0.1% BHT was added to frying oil, chicken meatballs were found to have lesser amounts of 2,4-decadienal regardless of the similar p-anisidine values of the oils. Despite of its lower amount of the oil, pan fried group was found to contain more 2,4-decadieanal than T1, T2 and T3 treatments. It was probably originated from the higher 2,4-decadienal amounts of the oil used in pan-frying because of the pre-oxidation of the oil without the food and higher temperatures of the oil in the pan compared to deep fryer. The relative contents of 2,4-decadienals were reported to be 15.76±1.98 and 36.46±1.56 in chicken breast meat fried in soybean oil (C18:2-50.15%, C18:1-22.49 %, C18:3-10.54%) heated at 180 ^{o}C for 2 and 8 h, respectively (Zhang et al., 2015).

In the Figure 3.29, decadien-1-amine amounts of the chicken meatballs prepared by different treatments were presented. When all results were analyzed statistically,

decadien-1-amine levels of the chicken meatballs increased by the highest rate within the first 4 h afterward it started to decrease up to 12 h.

In terms of the effect of the treatments in the decadien-1-amine formation, the following order was determined: T3>T2=T1>P>T4. Expectedly, the coating treatment in T4 group led to lesser amounts of amino acid modifications by lipid oxidation products because of both lower oxidation levels of the T4 oil and retaining of the oil at the coating rather than penetration to inner parts of the foods which might cause non-homogenous distribution of the oil within the food. On the other hand, the reason behind the lower decadien-1-amine levels in the pan-fried chickens was most probably due to the lower amounts of the oil absorption.

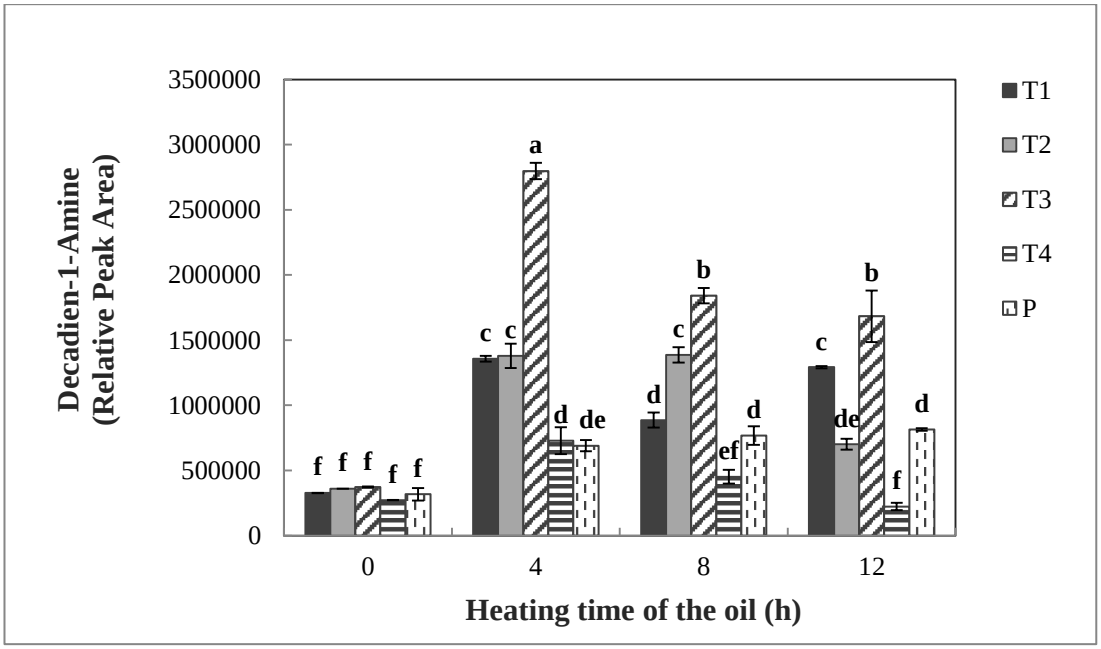

Figure 3.29. Relative amounts of decadien-1-amine formed in chicken meat balls (T1:Control, T2: Oil with 0.1% BHT, T3: Oil with 0.1% tocopherol mix, T4: Breaded P: Pan-fried) fried in the oils repeatedly used for 12 h at 180 ºC to fry chicken meat balls at different times (0, 4, 8, 12 h)

In comparison with the control, 0.1% BHT (T2) did not change significantly ($p>0.05$) lipid derived amino acid modifications in the fried chickens via decadien-1-amine. Although chicken meatballs fried in the oil with 0.1% tocopherol mix (T3) were found to have the lowest amounts of 2,4-decadienal, the opposite case was observed for decadien-1-amine formation which induced the highest rates of modifications, particularly with the outstanding increase ($p<0.05$) after the first 4 h heating of the oil. Contrary to potato chips, the inverse relation was observed between the orders of 2,4-decadienal and decadien-amine levels in the fried chickens. Therefore the explanation for the lower levels of 2,4-decadienal in T3, might be its reaction with the amino acids and thereby decadien-1-amine formation rather than antioxidant activity of BHT.

The concentrations of total free amino acids along the heating of the oil in the fried chickens were given in Figure 3.30. .

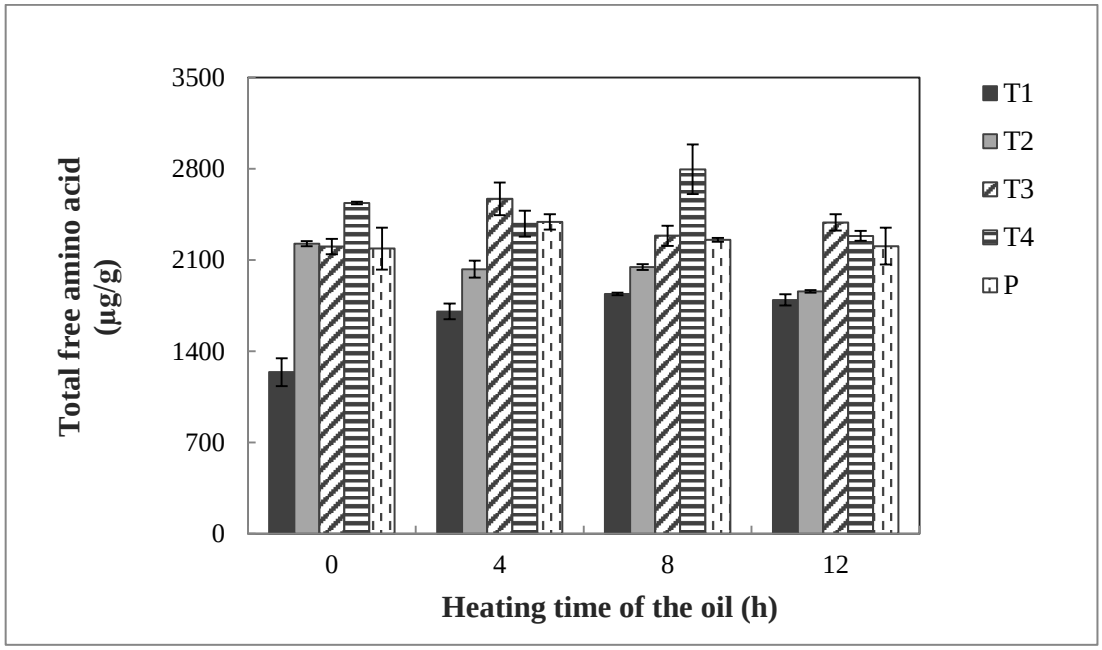

Figure 3.30. Amounts of total free amino acid in chicken meat balls (T1:Control, T2: Oil with 0.1% BHT, T3: Oil with 0.1% tocopherol mix, T4: Breaded P: Pan-fried) fried in the oils repeatedly used for 12 h at 180 ºC to fry chicken meat balls at different times (0, 4, 8, 12 h)

Total free amino acids of fried chickens were between 1238±106-1839±8, 1858±9-2224±20, 2202±58-2567±125, 2284±37-2795±191 and 2186±161-2391±58 μg/g for T1, T2, T3, T4 and P, respectively.

Similarly, 2700 μg/g of total free amino acids was reported in the chicken cordonbleu purchased from German market (Hermanussen et al., 2009). Overall results revealed that the highest levels of free amino acids were determined in the coated chicken meatballs (T4) which supported also the findings belong to decadien-1-amine formation indicating effective inhibition of lipid derived amino acid modifications in fried foods by the coating treatment. Oxidation products of the oil were reported to promote the decomposition of free or bound amino acids in another study (Ribarova et al., 1993). In addition, amino acid transfer from foodstuffs to oil was demonstrated to play role in the browning of frying oil (Totani et al., 2006).

Although there was not significant difference ($p>0.05$) in the oxidation degree of the oils used for T1, T2 and T3 through p-anisidine value, T1 was found to have the lowest levels of free amino acids which might be resulted from higher rates of amino acid modifications as well as lower initial content of free amino acids in T1 group as a result of matrice effect. However no general trend was observed for all treatments in terms of the effect of heating time of the oils on free amino acid concentrations.

To best of our knowledge, there has not been any study related to the effect of heating time and antioxidant level of frying oil on the free amino content of fried chickens in literature. However, the effect of frying on amino acids has been widely investigated in fish fillets and conflictive results have been obtained. Lin Li et al. reported the general increase in the content of free amino acids except for lysine, cysteine, alanine and threonine after frying of grass carp fillets at 180 ºC for 2 min. When the frying time was held for 4 min, while some amino acids reported to show an increase, others decreased as a result of generation and degradation reactions (Li et al., 2016). In another study, higher amounts of proteins were also reported in different type of fried fish fillets on dry weight basis in consistent with the different

previous studies (Erkan et al., 2010; Gokoglu et al., 2004; Steiner-Asiedu et al., 1991). Nevertheless, no significant change or decreases ($p>0.05$) of amino acids were also observed with frying of fish fillets by different researchers (Ismail & Ikram, 2004; GALL et al., 1983; Weber et al., 2008). Oluwaniyi et al. found reduced amounts of total amino acids in fried fish fillets with the lowest levels in the samples prepared in palm oil which was attributed to the reactions of lysine amino groups with carbonyl compounds in the oils (Oluwaniyi et al., 2010). Similarly, Tooley et al. also reported to 17% and 25% of available lysine loss in fish fillets as a result of repeated use of the oil heated for 48 h due to lipid protein interactions (Tooley, 1972). To conclude, while frying originated decreases of free amino acids were ascribed to Maillard reaction, protein-protein interactions, oxidation and heat induced degradation, the increases were thought to be related to dehydration, hydrolysis of the protein (Burger & Walters, 1973; Bordin et al., 2013; Li et al., 2016; Ginger et al., 1954). Simultaneous water loss and oil uptake during the frying process might be also responsible for the arising of this conflict.

CML and CEL results of the fried chickens were depicted in the Figure 3.31. and Figure 3.32., respectively. CML concetrations were determined to be ranged between 2504±70.8 - 2720±57.7, 2315±65.5 - 2936±58.6, 2312±65.4 - 2983±84.4, 1924±44.1 - 2922±62.0 ng/g for T1, T2, T3 and T4 respectively.

T1 and T3 were detected to induce similar levels of CML while T2 was determined to be the most and T4 was the least effective treatment. It can be noted that GO and MGO produced during the oxidation reactions could be transferred from the frying oil to the food and impact amino acid modifications in the fried chickens which was the least observable in the coated chickens through the effect of reduced oil absorption. On the other hand, 0.1% tocopherol did not differ ($p>0.05$) CML levels of the fried chickens while 0.1% BHT mix was found to increase ($p<0.05$) CML concentrations although p-anisidine values of the two oils did not show any considerable difference ($p>0.05$). Zhu et al. noted the promotion of CML and CEL formation by lipid oxidation and reported to concentrations of CML to change

between 1214±12.5 - 1369±3.32 ng/g in chicken breasts pan-fried in soybean oil at 160 ºC for 1-6 min which was in aggrement with our results (Zhu et al., 2019). In another study, 3400 and 1300 ng/g of CML was detected in chicken fillets fried for 10 and 30 min, respectively (Scheijen et al., 2016).

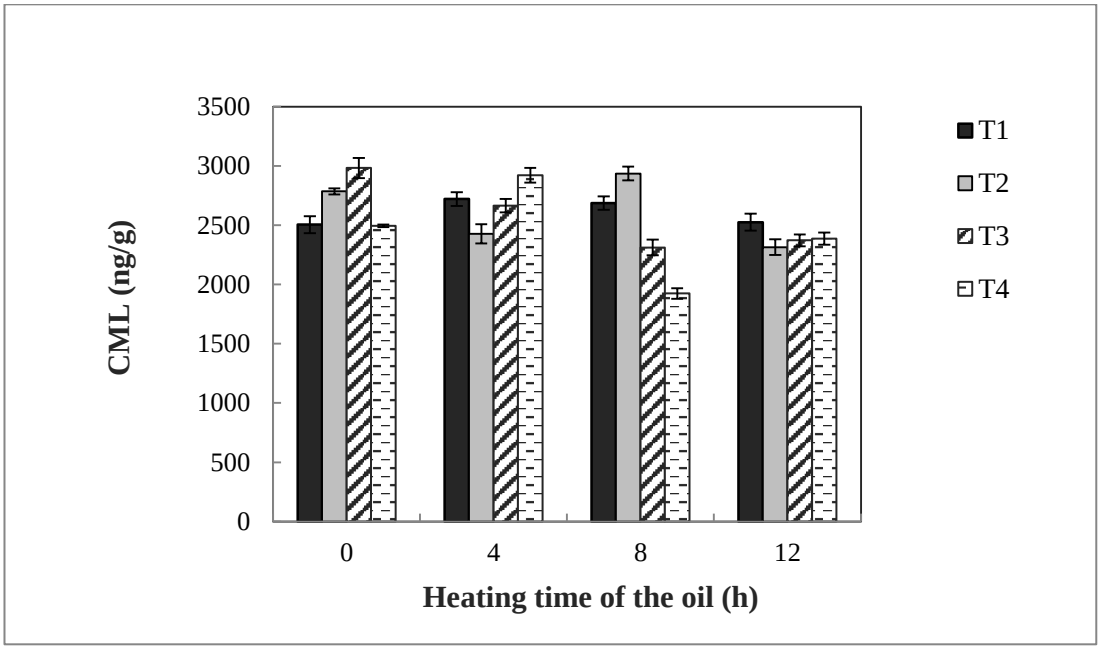

Figure 3.31. Amounts of CML formed in chicken meat balls (T1:Control, T2: Oil with 0.1% BHT, T3: Oil with 0.1% tocopherol mix, T4: Breaded) fried in the oils repeatedly used for 12 h at 180 ºC to fry chicken meat balls at different times (0, 4, 8, 12 h)

CEL amounts of the samples were found to range 2750±220.2 - 3581±141.2, 2058± 72.7-3365±47.5, 2286±83.1 - 3435±97.1, 2117±149.7-3519 ± 124.4 ng/g for T1, T2, T3 and T4, respectively (Figure 3.31.). In accordance with these results, Scheijen et al. found 2600 and 2700 ng/g of CEL in 10 and 30 min fried chicken fillets, respectively (Scheijen et al., 2016).

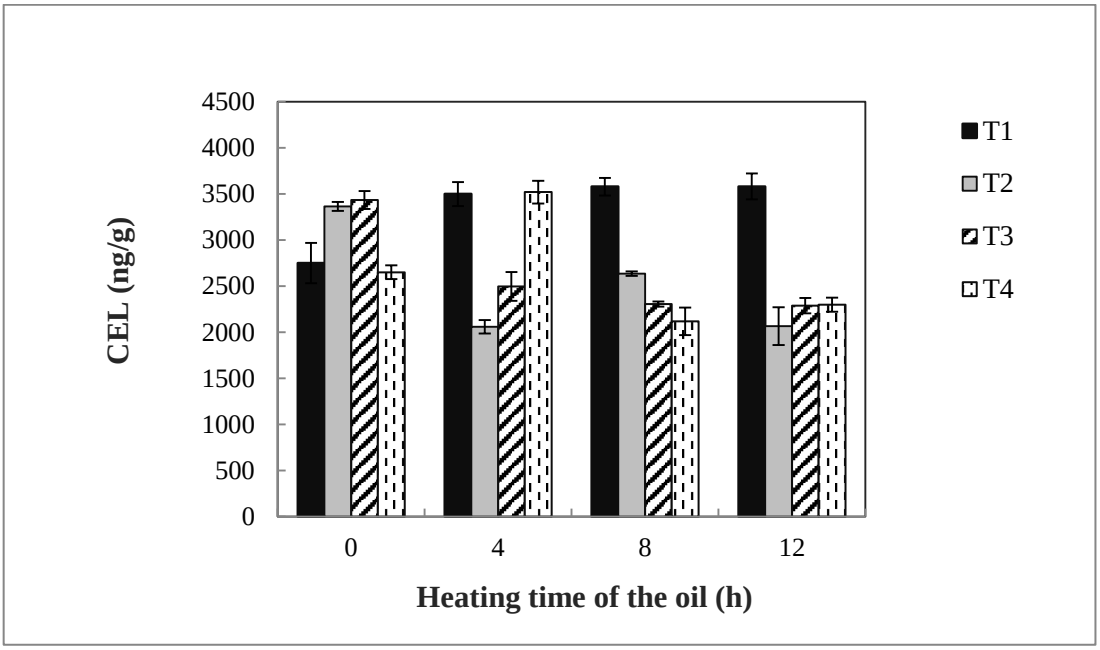

Figure 3.32. Amounts of CEL formed in chicken meat balls (T1:Control, T2: Oil with 0.1% BHT, T3: Oil with 0.1% tocopherol mix, T4: Breaded) fried in the oils repeatedly used for 12 h at 180 ºC to fry chicken meat balls at different times (0, 4, 8, 12 h)

While no correlation was observed between CEL and heating time of the oil, T2, T3 and T4 were determined to induce lesser amounts of CEL compared to the control group, T1 indicating no significant difference ($p>0.05$) of tocopherol, BHT addition or coating treatment.

CHAPTER 4

CONCLUSION

Repeated use of frying oil is a common practice in restaurants, mass consumption places and industrial applications. It results with the accumulation of certain lipid oxidation products which may directly or indirectly affect quality and safety of fried products. The results presented here revealed that 2,4-decadienal, one of the lipid oxidation products accumulated in repeatedly used oil, actively involved in Maillard type carbonyl-amine reactions occurring in potatoes during frying. To the best of our knowledge, 2-pentylpyridine and decadien-1-amine in potato chips were reported for the first time as products of the reaction between 2,4-decadienal and amino acids. However, furher studies should be conducted on the confirmation of decadien-1-amine in addition to high resolution mass spectrometry. Frying oil was found to have the highest concentration of 2,4-decadienal after thermal oxidation at 180 ^{o}C for 6 h. There was a positive correlation (r^2=0.73) between the concentrations of 2,4-decadienal and decadien-1-amine (relative concentration as peak area) formed in potato chips fried in repeatedly used sunflower oil.

According to the model system studies, addition of glucose and fructose to model doughs consisted of only whey protein and water resulted in lesser amounts of decadien-1-amine, most probably because of the competition of the reducing sugars with lipid carbonyls on the reaction with amino acids. The highest yields of decadien-1-amine were observed in the doughs prepared at 4 h heated oil. Model doughs containing potato flour appeared to have similar trends of 2,4-decadienal and decadien-1-amine with potato chips while the decline of total free amino acids in the first 4 h of the heating was more apparent in model doughs (M4) compared to real food systems.

2,4-decadienal was quantified with decreasing order in the chips prepared within the safflower, corn, olive, canola and hazelnut oils in accordance with p-anisidine values of the oils. While heating of the oil up to 12 h, led to increasing levels of p-anisidine, 2,4-decadienal accumulation in the chips reached to the maximum levels at 3 h.

The impact of the oil type on the relative amounts of decadien-1-amine in the chips followed the order of the safflower>hazelnut>canola>olive>corn oil. The differences between the orders of 2,4-decadienal and decadien-1-amine quantities might be derived from compositional differences in the potato tubers. Free amino acid levels of the chips fried in the corn oil was found to be far less than that of the hazelnut oil although the hazelnut oil was determined to be the second most effective oil type on decadien-1-amine formation which might be derived from less concentration of free amino acids in the potato tuber used for corn oil.

CML concentrations of the chips were detected to be increased significantly ($p<0.05$) within the first 4 hours of the heating. In case of using the oils with very high amounts of linoleic acid such as safflower oil, it is very obvious that CML and CEL quantities of the chips significantly increased ($p<0.05$) within the first 4 hours in spite of less likely gradual accumulation of the GO and MGO in the oil during the heating of the oil. CEL concentrations of the chips were found to be higher ($p>0.05$) than CML concentrations for all oil types which might be due to the relatively higher levels of MGO compared to GO. Additionally, it is important to note that for the first 4 hours of the heating, decrease in the levels of CML and CEL of the chips prepared in the oils (hazelnut, olive, canola) with high oleic acid content was remarkable while the reverse of this behavior was observed in the high linoleic acid oils (safflower, canola). Therefore the suppression rates of the polyunsaturated fatty acids against the other sources of CML and CEL in the food matrice might be determinative in the impact of different type of the oils on the formation of CML and CEL.

1% tocopherol mix was added to the frying oil in order to increase oxidation stability however this treatment triggered the oxidation of the oil. The chips prepared within

the more oxidized oil revealed much more modification of amino acids by means of decadien-1-amine, CML and CEL formation.

Although no significant difference ($p>0.05$) was detected between p-anisidine values of 0.1% BHT (T2) or 0.1% tocopherol mix (T3) added oils with control (T1), the highest increment of 2,4-decadienal in chicken meatballs was observed in T1 group while the lowest rises were in T2 and T3 within the first 4 h of the heating the oil. In addition, T1 was found to have the lowest contents of free amino acids. However, while T3 led to the highest decadien-1-amine yields, T2 was determined to form the highest CML levels.

On the other hand because of the reduced oil absorption, the protective effect of coating treatment (T4) and pan frying (P) on lipid derived amino acid modifications in fried chickens is quite observable with the lower amounts of decadien-1-amine and CML formation.

It could be concluded that lipid oxidation might have far more important role in the browning reactions in fried foods from a food safety point of view. Elucidating the complex mechanisms of these reactions would help us to deeper understand their consequences, and to be able to measure potential reaction products. More work is required to evaluate comprehensively the effects of simultaneous lipid oxidation and Maillard reactions on food quality and safety.

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APPENDICES

A. STATISTICAL ANALYSES

Figure 3.1. Amount of 2,4-decadienal formed in potato chips and oil fried at 180 °C in sunflower oil repeatedly used for 24 h to fry potato discs at different times (0, 6, 12, 18, and 24 h)

One-way ANOVA: 2,4-decadienal Chips versus Time

```
Source  DF      SS      MS      F      P
TIME     4  443.57  110.89  36.11  0.001
Error    5   15.35    3.07
Total    9  458.93

S = 1.752   R-Sq = 96.65%   R-Sq(adj) = 93.98%

                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev   -+---------+---------+---------+--------
0H     2   9.719  0.584   (----*---)
12H    2  23.608  0.583                      (----*---)
18H    2  16.051  0.163            (----*---)
24H    2  18.077  3.038               (----*---)
6H     2  29.270  2.327                           (----*---)
                           -+---------+---------+---------+--------
                          7.0      14.0      21.0      28.0

Pooled StDev = 1.752


Grouping Information Using Tukey Method

TIME  N    Mean  Grouping
6H    2  29.270  A
12H   2  23.608  A B
24H   2  18.077    B C
18H   2  16.051      C D
0H    2   9.719        D

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of TIME

Individual confidence level = 98.98%


TIME = 0H subtracted from:

TIME   Lower  Center   Upper  -------+---------+---------+---------+--
12H    6.863  13.889  20.915                        (-----*----)
```

```
18H   -0.694   6.332  13.358                  (-----*-----)
24H    1.332   8.358  15.383                    (-----*-----)
6H    12.525  19.550  26.576                             (-----*-----)
                             -------+---------+---------+---------+--
                                  -12         0        12        24
```

```
TIME = 12H subtracted from:

TIME    Lower  Center   Upper  -------+---------+---------+---------+--
18H   -14.583  -7.557  -0.531       (-----*-----)
24H   -12.557  -5.531   1.494         (----*-----)
6H     -1.364   5.661  12.687                  (-----*-----)
                             -------+---------+---------+---------+--
                                  -12         0        12        24
```

```
TIME = 18H subtracted from:

TIME   Lower  Center   Upper  -------+---------+---------+---------+--
24H   -5.000   2.026   9.051              (-----*-----)
6H     6.193  13.218  20.244                       (-----*-----)
                             -------+---------+---------+---------+--
                                  -12         0        12        24
```

```
TIME = 24H subtracted from:

TIME  Lower  Center   Upper  -------+---------+---------+---------+--
6H    4.167  11.193  18.218                     (-----*-----)
                             -------+---------+---------+---------+--
                                  -12         0        12        24
```

One-way ANOVA: 2,4-Decadienal Oil versus Time

```
Source  DF      SS     MS      F      P
h        4  3033.2  758.3  19.41  0.003
Error    5   195.3   39.1
Total    9  3228.5

S = 6.250   R-Sq = 93.95%   R-Sq(adj) = 89.11%
```

```
                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean   StDev  --+---------+---------+---------+-------
0H     2  26.646   7.316  (----*-----)
12H    2  59.857   3.040                (-----*-----)
18H    2  51.194   2.615             (-----*----)
24H    2  51.697   3.462             (-----*-----)
6H     2  80.850  10.664                           (----*-----)
                        --+---------+---------+---------+-------
                         20        40        60        80

Pooled StDev = 6.250
```

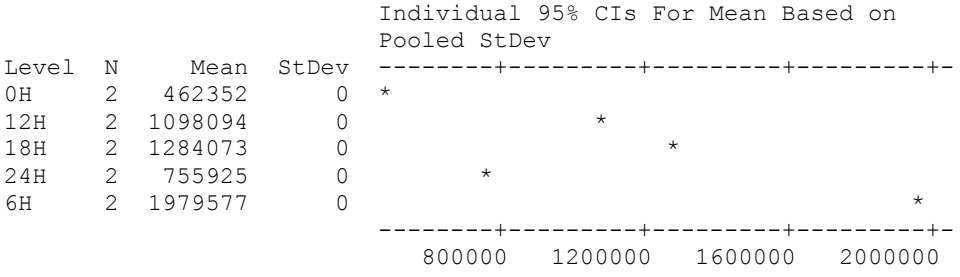
```
Grouping Information Using Tukey Method
```

```
h    N    Mean  Grouping
6H   2  80.850  A
12H  2  59.857  A B
24H  2  51.697    B C
18H  2  51.194    B C
0H   2  26.646      C

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of h

Individual confidence level = 98.98%


h = 0H subtracted from:

h     Lower  Center   Upper  ------+---------+---------+---------+---
12H   8.154  33.211  58.268                   (------*-------)
18H  -0.509  24.548  49.605                 (------*------)
24H  -0.006  25.051  50.109                 (------*------)
6H   29.147  54.204  79.261                         (------*-------)
                            ------+---------+---------+---------+---
                                -35         0        35        70


h = 12H subtracted from:

h      Lower  Center   Upper  ------+---------+---------+---------+---
18H  -33.720  -8.663  16.394        (-------*------)
24H  -33.216  -8.159  16.898         (------*------)
6H    -4.064  20.993  46.050                 (------*------)
                            ------+---------+---------+---------+---
                                -35         0        35        70


h = 18H subtracted from:

h      Lower  Center   Upper  ------+---------+---------+---------+---
24H  -24.554   0.503  25.561           (------*------)
6H     4.599  29.656  54.713                   (------*-------)
                            ------+---------+---------+---------+---
                                -35         0        35        70


h = 24H subtracted from:

h   Lower  Center   Upper  ------+---------+---------+---------+---
6H  4.095  29.152  54.209                  (------*------)
                           ------+---------+---------+---------+---
                               -35         0        35        70
```

Figure 3.3. Relative amounts of decadien-1-amine in potato chips fried at 180 °C in repeatedly used sunflower oil for 24 h to fry potato discs at different times (0, 6, 12, 18, and 24 h)

One-way ANOVA: Decadien-1-amine versus Time

```
Source  DF           SS           MS  F  P
TIME_1   4  2,66249E+12  6,65622E+11  *  *
Error    5            0            0
Total    9  2,66249E+12

S = 0   R-Sq = 100,00%   R-Sq(adj) = 100,00%
```




```
Pooled StDev = 0
```

```
Grouping Information Using Tukey Method

TIME_1  N     Mean  Grouping
6H      2  1979577  A
18H     2  1284073     B
12H     2  1098094       C
24H     2   755925         D
0H      2   462352           E

Means that do not share a letter are significantly different.
```

```
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of TIME_1

Individual confidence level = 98,98%

TIME_1 = 0H subtracted from:

TIME_1    Lower   Center    Upper  -------+---------+---------+---------+--
12H      635742   635742   635742                            *
18H      821721   821721   821721                              *
24H      293573   293573   293573                        *
6H      1517225  1517225  1517225                                       *
                                  -------+---------+---------+---------+--
                                   -700000         0    700000   1400000
```

```
TIME_1 = 12H subtracted from:

TIME_1    Lower   Center    Upper  -------+---------+---------+---------+--
18H      185979   185979   185979                    *
24H     -342169  -342169  -342169            *
6H       881483   881483   881483                              *
                                  -------+---------+---------+---------+--
                                   -700000         0    700000   1400000


TIME_1 = 18H subtracted from:

TIME_1    Lower   Center    Upper  -------+---------+---------+---------+--
24H     -528148  -528148  -528148          *
6H       695504   695504   695504                        *
                                  -------+---------+---------+---------+--
                                   -700000         0    700000   1400000


TIME_1 = 24H subtracted from:

TIME_1    Lower   Center    Upper  -------+---------+---------+---------+--
6H      1223652  1223652  1223652                                   *
                                  -------+---------+---------+---------+--
                                   -700000         0    700000   1400000
```

Figure 3.4. Amount of total free amino acid formed in potato chips fried at 180 °C in repeatedly used sunflower oil for 24 h to fry potato discs at different times (0, 6, 12, 18, and 24 h)

One-way ANOVA: Amino acid versus Time

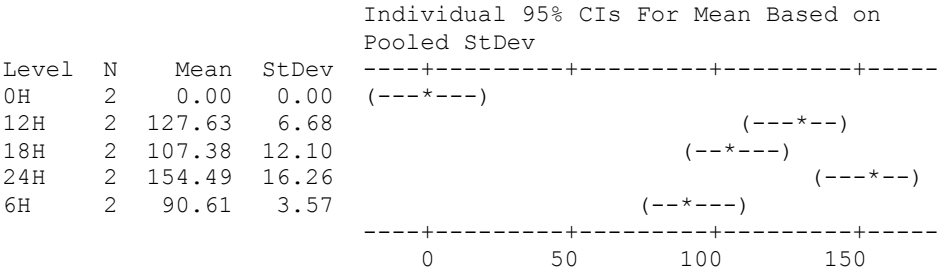
```
Source  DF       SS      MS     F      P
TIME     4  1052400  263100  5,71  0,042
Error    5   230482   46096
Total    9  1282882

S = 214,7   R-Sq = 82,03%   R-Sq(adj) = 67,66%


                        Individual 95% CIs For Mean Based on Pooled StDev
Level  N   Mean  StDev      +---------+---------+---------+---------
0H     2  11918      0                (------*-------)
12H    2  12111    276                   (-------*-------)
18H    2  12124    334                    (------*-------)
24H    2  12348    204                        (-------*-------)
6H     2  11389     36      (-------*-------)
                            +---------+---------+---------+---------
                          11000     11500     12000     12500

Pooled StDev = 215


Grouping Information Using Tukey Method

TIME  N     Mean  Grouping
24H   2  12348,2  A
```

```
18H   2  12123,8  A B
12H   2  12111,2  A B
0H    2  11918,4  A B
6H    2  11389,3    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of TIME

Individual confidence level = 98,98%


TIME = 0H subtracted from:

TIME    Lower  Center   Upper  --------+---------+---------+---------+-
12H    -668,0   192,8  1053,6             (--------*--------)
18H    -655,4   205,4  1066,2             (--------*--------)
24H    -431,0   429,8  1290,6                (-------*--------)
6H    -1389,9  -529,1   331,7      (--------*-------)
                              --------+---------+---------+---------+-
                                  -1000         0      1000      2000


TIME = 12H subtracted from:

TIME    Lower  Center   Upper  --------+---------+---------+---------+-
18H    -848,2    12,6   873,4            (-------*--------)
24H    -623,8   237,0  1097,8              (-------*--------)
6H    -1582,8  -722,0   138,8    (--------*-------)
                              --------+---------+---------+---------+-
                                  -1000         0      1000      2000


TIME = 18H subtracted from:

TIME    Lower  Center   Upper  --------+---------+---------+---------+-
24H    -636,4   224,4  1085,2              (-------*--------)
6H    -1595,3  -734,5   126,3    (--------*-------)
                              --------+---------+---------+---------+-
                                  -1000         0      1000      2000


TIME = 24H subtracted from:

TIME    Lower  Center  Upper  --------+---------+---------+---------+-
6H    -1819,7  -958,9  -98,1  (-------*--------)
                              --------+---------+---------+---------+-
                                  -1000         0      1000      2000
```

Figure 3.6. Amount of 2-pentylpyridine formed in potato chips fried at 180 °C in repeatedly used sunflower oil for 24 h to fry potato discs at different times (0, 6, 12, 18, and 24 h)

One-way ANOVA: 2-Pentylpyridine versus hour

```
Source  DF       SS      MS      F      P
hour     4  27591.7  6897.9  73.66  0.000
Error    5    468.2    93.6
Total    9  28060.0

S = 9.677   R-Sq = 98.33%   R-Sq(adj) = 97.00%
```




```
Pooled StDev = 9.68


Grouping Information Using Tukey Method

hour  N    Mean  Grouping
24H   2  154.49  A
12H   2  127.63  A B
18H   2  107.38    B
6H    2   90.61    B
0H    2    0.00      C

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of hour

Individual confidence level = 98.98%


hour = 0H subtracted from:

hour   Lower  Center   Upper
12H    88.83  127.63  166.43
18H    68.58  107.38  146.18
24H   115.69  154.49  193.29
6H     51.81   90.61  129.41
-80
0
80
160


hour = 12H subtracted from:
```

```
hour   Lower  Center  Upper  ---+---------+---------+---------+------
18H   -59.05  -20.25  18.54        (---*----)
24H   -11.94   26.85  65.65              (---*----)
6H    -75.82  -37.02   1.77      (---*----)
                              ---+---------+---------+---------+------
                               -80         0        80       160


hour = 18H subtracted from:

hour   Lower  Center  Upper  ---+---------+---------+---------+------
24H     8.31   47.11  85.91               (----*----)
6H    -55.57  -16.77  22.03        (----*----)
                              ---+---------+---------+---------+------
                               -80         0        80       160


hour = 24H subtracted from:

hour    Lower  Center   Upper  ---+---------+---------+---------+------
6H    -102.68  -63.88  -25.08  (----*----)
                               ---+---------+---------+---------+------
                                -80         0        80       160
```

Figure 3.7. Amount of acrylamide formed in potato chips fried at 180 °C in repeatedly used sunflower oil for 24 h to fry potato discs at different times (0, 6, 12, 18, and 24 h)

One-way ANOVA: Acrylamide versus Time

```
Source  DF     SS     MS      F      P
TIME_1   4  43523  10881  12,77  0,008
Error    5   4259    852
Total    9  47782

S = 29,19   R-Sq = 91,09%   R-Sq(adj) = 83,96%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev   -+---------+---------+---------+--------
0H     2  721,50   3,54                          (-----*------)
12H    2  524,50  23,33   (------*-----)
18H    2  631,00  15,56               (------*------)
24H    2  593,00   9,90          (------*------)
6H     2  574,00  57,98        (------*-----)
                         -+---------+---------+---------+--------
                        480       560       640       720

Pooled StDev = 29,19


Grouping Information Using Tukey Method

TIME_1  N    Mean  Grouping
0H      2  721,50  A
```

```
18H     2  631,00  A B
24H     2  593,00    B
6H      2  574,00    B
12H     2  524,50    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of TIME_1

Individual confidence level = 98,98%


TIME_1 = 0H subtracted from:

TIME_1    Lower   Center   Upper    -+---------+---------+---------+-------
-
12H     -314,01  -197,00  -79,99    (-------*-------)
18H     -207,51   -90,50   26,51           (-------*-------)
24H     -245,51  -128,50  -11,49         (------*-------)
6H      -264,51  -147,50  -30,49       (-------*-------)
                                   -+---------+---------+---------+-------
-
                                  -300      -150         0       150


TIME_1 = 12H subtracted from:

TIME_1   Lower  Center   Upper    -+---------+---------+---------+--------
18H     -10,51  106,50  223,51                      (-------*-------)
24H     -48,51   68,50  185,51                    (-------*------)
6H      -67,51   49,50  166,51                  (-------*-------)
                                -+---------+---------+---------+--------
                               -300      -150         0       150


TIME_1 = 18H subtracted from:

TIME_1    Lower  Center  Upper    -+---------+---------+---------+--------
24H     -155,01  -38,00  79,01              (------*-------)
6H      -174,01  -57,00  60,01            (-------*-------)
                                -+---------+---------+---------+--------
                               -300      -150         0       150


TIME_1 = 24H subtracted from:

TIME_1    Lower  Center  Upper    -+---------+---------+---------+--------
6H      -136,01  -19,00  98,01               (-------*-------)
                                -+---------+---------+---------+--------
                               -300      -150         0       150
```

Figure 3.9. p-Anisidine values of the oils used for frying of the model systems without (M1) and with sugar (M2)

General Linear Model: p-anisidine_2 versus Time_2; Model

```
Factor  Type   Levels  Values
Time_2  fixed       4  0; 4; 8; 12
Model   fixed       2  M1; M2


Analysis of Variance for p-anisidine_2, using Adjusted SS for Tests

Source        DF  Seq SS  Adj SS  Adj MS        F      P
Time_2         3   74942   74942   24981  3803,25  0,000
Model          1    2205    2205    2205   335,64  0,000
Time_2*Model   3     837     837     279    42,50  0,000
Error          8      53      53       7
Total         15   78037


S = 2,56286   R-Sq = 99,93%   R-Sq(adj) = 99,87%


Grouping Information Using Tukey Method and 95,0% Confidence

Time_2  N   Mean  Grouping
12      4  197,3  A
 8      4  163,2    B
 4      4  116,5      C
 0      4   15,5        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Model  N   Mean  Grouping
M2     8  134,8  A
M1     8  111,4    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time_2  Model  N   Mean  Grouping
12      M2     2  210,0  A
12      M1     2  184,5    B
 8      M2     2  181,5    B
 8      M1     2  144,9      C
 4      M2     2  132,7        D
 4      M1     2  100,2          E
 0      M1     2   15,8            F
 0      M2     2   15,2            F

Means that do not share a letter are significantly different.
```

Figure 3.10. Relative amounts of 2,4-decadienal formed in the model systems without (M1) and with sugar (M2) fried at 180 °C in repeatedly used sunflower oil for 12 h at different times (0, 4, 8, 12 h

General Linear Model: 2,4-DEC-KM-3 versus TIME-KM-3; OIL-KM-3

Factor	Type	Levels	Values
TIME-KM-3	fixed	4	0; 4; 8; 12
OIL-KM-3	fixed	2	M1; M2

Analysis of Variance for 2,4-DEC-KM-3, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME-KM-3	3	8,75388E+15	8,75388E+15	2,91796E+15	302,45	0,000
OIL-KM-3	1	2,63071E+13	2,63071E+13	2,63071E+13	2,73	0,137
TIME-KM-3*OIL-KM-3	3	4,88150E+13	4,88150E+13	1,62717E+13	1,69	0,246
Error	8	7,71809E+13	7,71809E+13	9,64761E+12		
Total	15	8,90618E+15				

S = 3106061 R-Sq = 99,13% R-Sq(adj) = 98,38%

Unusual Observations for 2,4-DEC-KM-3

Obs	2,4-DEC-KM-3	Fit	SE Fit	Residual	St Resid
7	74456171	69261555	2196316	5194617	2,37 R
8	64066938	69261555	2196316	-5194617	-2,37 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME-KM-3	N	Mean	Grouping
12	4	68323281,1	A
8	4	30288064,9	B
4	4	22260646,1	C
0	4	4188799,2	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

OIL-KM-3	N	Mean	Grouping
M2	8	32547460,0	A
M1	8	29982935,6	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME-KM-3	OIL-KM-3	N	Mean	Grouping
12	M1	2	69261554,6	A
12	M2	2	67385007,5	A
8	M2	2	32529076,5	B
8	M1	2	28047053,4	B C
4	M2	2	25840791,0	B C
4	M1	2	18680501,2	C
0	M2	2	4434965,1	D
0	M1	2	3942633,2	D

Means that do not share a letter are significantly different.

Figure 3.11. Relative amounts of decadien-1-amine formed in the model doughs (M1) and (M2) fried at 180 °C in repeatedly used sunflower oil for 12 h at different times (0, 4, 8, 12 h)

General Linear Model: D-1-A-3 versus TIME-3; OIL-3

Factor	Type	Levels	Values
TIME-3	fixed	4	0; 4; 8; 12
OIL-3	fixed	2	M1; M2

Analysis of Variance for D-1-A-3, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME-3	3	1,90179E+11	1,90179E+11	63393054907	101,97	0,000
OIL-3	1	47410163252	47410163252	47410163252	76,26	0,000
TIME-3*OIL-3	3	33234021917	33234021917	11078007306	17,82	0,001
Error	8	4973460619	4973460619	621682577		
Total	15	2,75797E+11				

S = 24933,6 R-Sq = 98,20% R-Sq(adj) = 96,62%

Grouping Information Using Tukey Method and 95,0% Confidence

TIME-3	N	Mean	Grouping
4	4	391794,2	A
12	4	298251,3	B
8	4	273903,3	B
0	4	90961,0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

OIL-3	N	Mean	Grouping
M1	8	318162,1	A
M2	8	209292,8	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME-3	OIL-3	N	Mean	Grouping
4	M1	2	505005,5	A
8	M1	2	353564,5	B
12	M1	2	329247,5	B
4	M2	2	278583,0	B C
12	M2	2	267255,0	B C
8	M2	2	194242,0	C D
0	M2	2	97091,0	D E
0	M1	2	84831,0	E

Means that do not share a letter are significantly different.

Figure 3.12. Amount of total free amino acid formed in the model systems without (M1) and with sugar (M2) fried at 180 °C in repeatedly used sunflower oil for 12 h at different times (0, 4, 8, 12 h)

General Linear Model: AA-2 versus Time-2; Models-2

Factor	Type	Levels	Values
Time-2	fixed	4	0; 4; 8; 12
Models-2	fixed	2	M1; M2

Analysis of Variance for AA-2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time-2	3	9647,6	9647,6	3215,9	127,07	0,000
Models-2	1	6686,3	6686,3	6686,3	264,20	0,000
Time-2*Models-2	3	17748,1	17748,1	5916,0	233,76	0,000
Error	8	202,5	202,5	25,3		
Total	15	34284,4				

S = 5,03068 R-Sq = 99,41% R-Sq(adj) = 98,89%

Grouping Information Using Tukey Method and 95,0% Confidence

Time-2	N	Mean	Grouping
0	4	351,5	A
8	4	304,9	B
12	4	294,0	B C
4	4	289,9	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Models-2	N	Mean	Grouping
M1	8	330,5	A
M2	8	289,6	B

Means that do not share a letter are significantly different.
Grouping Information Using Tukey Method and 95,0% Confidence

```
Time-2  Models-2  N   Mean  Grouping
 0      M1        2  426,8  A
 8      M2        2  319,4    B
12      M1        2  306,0    B C
 4      M1        2  298,9      C D
 8      M1        2  290,3      C D E
12      M2        2  282,0        D E
 4      M2        2  280,8        D E
 0      M2        2  276,2          E

Means that do not share a letter are significantly different.
```

Figure 3.13. Relative amounts of 2,4-decadienal in the model system prepared with potato flour (M4) fried at 180 °C in repeatedly used sunflower oil for 12 h at different times (0, 4, 8, 12 h)

One-way ANOVA: M4-2,4-DEC versus Time

```
Source  DF           SS           MS      F      P
Time     3  8,01074E+11  2,67025E+11  53,70  0,001
Error    4  19891317149   4972829287
Total    7  8,20966E+11

S = 70518   R-Sq = 97,58%   R-Sq(adj) = 95,76%


                          Individual 95% CIs For Mean Based on Pooled
StDev
Level  N     Mean   StDev    ----+---------+---------+---------+-----
 0     2  2110744   44776    (---*----)
 4     2  2706929   76564                       (---*----)
 8     2  2982150  105435                                (---*----)
12     2  2529769   30132                 (---*----)
                             ----+---------+---------+---------+-----
                          2100000   2400000   2700000   3000000

Pooled StDev = 70518


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
 8    2  2982150  A
 4    2  2706929  A B
12    2  2529769    B
 0    2  2110744      C

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 98,48%


Time =  0 subtracted from:
```

```
Time   Lower  Center    Upper   -----+---------+---------+---------+----
 4    308969  596186   883402                      (-----*-----)
 8    584190  871406  1158622                            (----*-----)
12    131808  419025   706241                   (----*-----)
                                -----+---------+---------+---------+----
                              -500000         0    500000   1000000


Time =  4 subtracted from:

Time    Lower   Center   Upper   -----+---------+---------+---------+----
 8     -11996   275220  562437                (-----*----)
12    -464377  -177161  110056         (----*-----)
                                -----+---------+---------+---------+----
                              -500000         0    500000   1000000


Time =  8 subtracted from:

Time    Lower   Center    Upper   -----+---------+---------+---------+----
12    -739598  -452381  -165165   (-----*-----)
                                -----+---------+---------+---------+----
                              -500000         0    500000   1000000
```

Figure 3.14. Relative amounts of decadien-1-amine in the model system prepared with potato flour (M4) fried at 180 °C in repeatedly used sunflower oil for 12 h at different times (0, 4, 8, 12 h)

One-way ANOVA: M4-D-1-A versus TIME

```
Source  DF           SS           MS       F      P
TIME     3  1,17938E+12  3,93125E+11  235,32  0,000
Error    4   6682424109   1670606027
Total    7  1,18606E+12

S = 40873   R-Sq = 99,44%   R-Sq(adj) = 99,01%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N     Mean  StDev  ---------+---------+---------+---------+
 0     2   722708  51103  (--*--)
 4     2  1714174  27378                                  (--*--)
 8     2  1077756  56675             (--*--)
12     2   834914  10455      (--*--)
                        ---------+---------+---------+---------+
                            900000   1200000   1500000   1800000

Pooled StDev = 40873


Grouping Information Using Tukey Method

TIME  N     Mean  Grouping
 4    2  1714174  A
 8    2  1077756     B
12    2   834914       C
```

```
 0    2   722708      C
```

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of TIME

Individual confidence level = 98,48%

TIME = 0 subtracted from:

```
TIME   Lower  Center    Upper  -------+---------+---------+---------+--
 4    824992  991466  1157939                                  (--*-)
 8    188575  355048   521521                       (--*--)
12    -54268  112206   278679                  (--*--)
                           -------+---------+---------+---------+--
                            -600000         0    600000   1200000
```

TIME = 4 subtracted from:

```
TIME     Lower   Center    Upper  -------+---------+---------+---------+--
 8     -802891  -636418  -469944       (-*--)
12    -1045733  -879260  -712787  (-*--)
                           -------+---------+---------+---------+--
                            -600000         0    600000   1200000
```

TIME = 8 subtracted from:

```
TIME    Lower   Center   Upper  -------+---------+---------+---------+--
12    -409316  -242843  -76369            (--*--)
                           -------+---------+---------+---------+--
                            -600000         0    600000   1200000
```

Figure 3.15. p-Anisidine values of different type of the oils (Canola, Corn, Hazelnut, Olive, Safflower) repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 4, 8, 12 h)

General Linear Model: p-Anisidine versus TIME; OIL

```
Factor  Type   Levels  Values
TIME    fixed       4  0; 4; 8; 12
OIL     fixed       5  CA; CO; HA; OL; SA
```

Analysis of Variance for p-Anisidine, using Adjusted SS for Tests

```
Source    DF   Seq SS   Adj SS  Adj MS        F      P
TIME       3   6472,9   6472,9  2157,6  3373,46  0,000
OIL        4  13283,2  13283,2  3320,8  5192,09  0,000
TIME*OIL  12   2841,2   2841,2   236,8   370,18  0,000
Error     20     12,8     12,8     0,6
Total     39  22610,1
```

S = 0,799743   R-Sq = 99,94%   R-Sq(adj) = 99,89%

Unusual Observations for p-Anisidine

Obs	p-Anisidine	Fit	SE Fit	Residual	St Resid
5	92,776	91,423	0,566	1,353	2,39 R
6	90,070	91,423	0,566	-1,353	-2,39 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME	N	Mean	Grouping
12	10	71,4	A
8	10	60,6	B
4	10	54,8	C
0	10	36,3	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

OIL	N	Mean	Grouping
SA	8	83,6	A
CO	8	69,6	B
OL	8	47,9	C
CA	8	43,8	D
HA	8	33,7	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME	OIL	N	Mean	Grouping
12	CO	2	109,7	A
12	SA	2	94,4	B
8	SA	2	91,4	B
4	SA	2	85,5	C
8	CO	2	73,8	D
0	SA	2	63,3	E
4	CO	2	63,2	E
12	OL	2	54,4	F
8	OL	2	52,6	F G
4	OL	2	49,8	G H
12	CA	2	49,3	H
12	HA	2	49,0	H
8	CA	2	44,9	I
4	CA	2	44,6	I
8	HA	2	40,2	J
0	CA	2	36,5	K
0	OL	2	35,0	K
0	CO	2	31,7	L
4	HA	2	30,7	L
0	HA	2	14,8	M

Means that do not share a letter are significantly different.

Figure 3.16. Amount of 2,4-decadienal formed in chips fried within the different type of oils (Canola, Corn, Hazelnut, Olive, Safflower) repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 3, 6, 9, 12 h)

General Linear Model: 2,4-DEC-Chips versus HeatingTime; OilType

Factor	Type	Levels	Values
HeatingTime	fixed	5	0; 3; 6; 9; 12
OilType	fixed	5	Canola; Corn; Hazelnut; Olive; Safflower

Analysis of Variance for 2,4-DEC-Chips, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
HeatingTime	4	458,20	458,20	114,55	93,63	0,000
OilType	4	6507,50	6507,50	1626,88	1329,73	0,000
HeatingTime*OilType	16	647,45	647,45	40,47	33,07	0,000
Error	25	30,59	30,59	1,22		
Total	49	7643,74				

S = 1,10610 R-Sq = 99,60% R-Sq(adj) = 99,22%

Unusual Observations for 2,4-DEC-Chips

Obs	2,4-DEC-Chips	Fit	SE Fit	Residual	St Resid
19	36,5540	38,4982	0,7821	-1,9442	-2,49 R
20	40,4425	38,4982	0,7821	1,9443	2,49 R
39	7,2070	8,8032	0,7821	-1,5962	-2,04 R
40	10,3994	8,8032	0,7821	1,5962	2,04 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

HeatingTime	N	Mean	Grouping
9	10	22,7	A
3	10	22,1	A
6	10	21,4	A
12	10	18,4	B
0	10	14,6	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

OilType	N	Mean	Grouping
Safflower	10	42,1	A
Corn	10	17,8	B
Olive	10	16,2	C
Canola	10	11,8	D
Hazelnut	10	11,4	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HeatingTime	OilType	N	Mean	Grouping
9	Safflower	2	51,0	A
3	Safflower	2	48,3	A B
6	Safflower	2	45,5	B
12	Safflower	2	38,5	C
0	Safflower	2	27,4	D
6	Corn	2	21,1	E
6	Olive	2	19,9	E
3	Corn	2	19,3	E F
12	Corn	2	18,9	E F G
9	Corn	2	18,9	E F G
0	Olive	2	17,5	E F G H
3	Olive	2	17,4	E F G H
3	Hazelnut	2	15,4	F G H I
9	Hazelnut	2	14,9	F G H I J
9	Canola	2	14,4	G H I J K
9	Olive	2	14,3	H I J K
12	Canola	2	13,9	H I J K
6	Canola	2	13,2	H I J K L
12	Olive	2	11,8	I J K L M
0	Corn	2	10,8	J K L M N
3	Canola	2	10,2	K L M N
0	Hazelnut	2	10,1	K L M N
12	Hazelnut	2	8,8	L M N
6	Hazelnut	2	7,5	M N
0	Canola	2	7,1	N

Means that do not share a letter are significantly different.

Figure 3.17. Relative amounts of decadien-1-amine in potato chips fried within the different type of oils (Canola, Corn, Hazelnut, Olive, Safflower) repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 3, 6, 9, 12 h)

General Linear Model: D-1-A versus HT; OT

Factor	Type	Levels	Values
HT	fixed	5	0; 3; 6; 9; 12
OT	fixed	5	Canola; Corn; Hazelnut; Olive; Safflower

Analysis of Variance for D-1-A, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
HT	4	10001088679	10001088679	2500272170	41,70	0,000
OT	4	1,50570E+11	1,50570E+11	37642395162	627,80	0,000
HT*OT	16	58026761293	58026761293	3626672581	60,49	0,000
Error	25	1498991203	1498991203	59959648		
Total	49	2,20096E+11				

S = 7743,36 R-Sq = 99,32% R-Sq(adj) = 98,67%

Grouping Information Using Tukey Method and 95,0% Confidence

HT	N	Mean	Grouping
12	10	128947,5	A
3	10	113558,9	B
9	10	110463,9	B
6	10	110234,8	B
0	10	84922,8	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

OT	N	Mean	Grouping
Safflower	10	202681,3	A
Hazelnut	10	137880,5	B
Canola	10	86912,4	C
Olive	10	70877,5	D
Corn	10	49776,2	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HT	OT	N	Mean	Grouping
12	Safflower	2	284912,5	A
6	Safflower	2	223054,0	B
3	Safflower	2	219433,5	B
3	Hazelnut	2	187330,0	C
0	Hazelnut	2	155056,0	D
12	Canola	2	153767,5	D
9	Safflower	2	153612,0	D
0	Safflower	2	132394,5	D E
9	Canola	2	129374,5	D E
9	Hazelnut	2	126036,0	D E
6	Hazelnut	2	119322,5	E F
12	Hazelnut	2	101658,0	E F G
0	Olive	2	92067,5	F G H
3	Olive	2	76188,0	G H I
9	Olive	2	73243,5	G H I J
6	Canola	2	73235,0	G H I J
6	Olive	2	70723,0	G H I J
9	Corn	2	70053,5	G H I J
6	Corn	2	64839,5	H I J K
12	Corn	2	62234,0	H I J K
3	Canola	2	49280,0	I J K L
12	Olive	2	42165,5	J K L M
3	Corn	2	35563,0	K L M
0	Canola	2	28905,0	L M
0	Corn	2	16191,0	M

Means that do not share a letter are significantly different.

Figure 3.18. Amount of total free amino acid in potato chips fried within the different type of oils (Canola, Corn, Hazelnut, Olive, Safflower)

repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 3, 6, 9, 12 h)

General Linear Model: AA versus TIME; OIL

Factor	Type	Levels	Values
TIME	fixed	5	0; 3; 6; 9; 12
OIL	fixed	5	CA; CO; HA; OL; SA

Analysis of Variance for AA, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME	4	62900924	62900924	15725231	20,01	0,000
OIL	4	1081796789	1081796789	270449197	344,18	0,000
TIME*OIL	16	258827191	258827191	16176699	20,59	0,000
Error	25	19644461	19644461	785778		
Total	49	1423169365				

S = 886,441 R-Sq = 98,62% R-Sq(adj) = 97,29%

Unusual Observations for AA

Obs	AA	Fit	SE Fit	Residual	St Resid
45	23412,8	21859,1	626,8	1553,7	2,48 R
46	20305,3	21859,1	626,8	-1553,7	-2,48 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME	N	Mean	Grouping
12	10	21287,9	A
9	10	19320,7	B
6	10	19316,1	B
3	10	19049,8	B
0	10	17787,1	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

OIL	N	Mean	Grouping
CA	10	24688,3	A
HA	10	22350,0	B
OL	10	21512,1	B
SA	10	16451,0	C
CO	10	11760,3	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME	OIL	N	Mean	Grouping

```
 9    CA   2  25688,8  A
 3    CA   2  25583,3  A
 6    HA   2  25101,3  A B
 9    HA   2  25013,6  A B C
 6    CA   2  24484,2  A B C
12    CA   2  24219,7  A B C
12    HA   2  24174,4  A B C
12    OL   2  23688,6  A B C
 0    CA   2  23465,5  A B C
12    SA   2  23292,0  A B C
 3    HA   2  22243,6  A B C D
 6    OL   2  21859,1    B C D
 3    OL   2  21765,2    B C D
 0    OL   2  21391,6      C D
 9    OL   2  18856,0        D E
 9    SA   2  16424,4          E F
 0    HA   2  15217,0            F G
 0    CO   2  14926,1            F G
 6    SA   2  14742,5            F G
 0    SA   2  13935,5            F G H
 3    SA   2  13860,7            F G H
 3    CO   2  11796,5              G H
12    CO   2  11064,8                H
 9    CO   2  10620,8                H
 6    CO   2  10393,3                H
```

Means that do not share a letter are significantly different.

Figure 3.19. Amount of CML formed in potato chips fried within the different type of oils (Canola, Corn, Hazelnut, Olive, Safflower) repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 4, 8, 12 h)

General Linear Model: CML-CIPS versus OIL; TIME

```
Factor  Type   Levels  Values
OIL     fixed       5  CA; CO; HA; OL; SA
TIME    fixed       4  0; 4; 8; 12


Analysis of Variance for CML-CIPS, using Adjusted SS for Tests

Source    DF   Seq SS   Adj SS  Adj MS       F      P
OIL        4  1483904  1483904  370976  121,71  0,000
TIME       3    53364    53364   17788    5,84  0,005
OIL*TIME  12  1487805  1487805  123984   40,68  0,000
Error     20    60962    60962    3048
Total     39  3086035

S = 55,2096   R-Sq = 98,02%   R-Sq(adj) = 96,15%


Grouping Information Using Tukey Method and 95,0% Confidence

OIL  N    Mean  Grouping
HA   8  1063,4  A
CA   8   958,3    B
```

```
SA   8   815,0      C
CO   8   680,0        D
OL   8   522,9          E
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

```
TIME   N   Mean  Grouping
 4    10  862,4  A
 8    10  819,8  A B
 0    10  778,5    B
12    10  771,0    B
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

```
OIL  TIME  N    Mean  Grouping
CA    8    2  1358,4  A
HA    0    2  1204,9  A B
HA   12    2  1195,0  A B
SA    4    2  1101,8    B C
HA    4    2  1068,9    B C D
SA    8    2  1021,9    B C D
CO    4    2   950,8      C D E
CA    0    2   944,6      C D E
CA   12    2   848,7        D E F
HA    8    2   784,7          E F G
CO   12    2   737,4          E F G H
CA    4    2   681,6            F G H I
SA    0    2   655,3            F G H I J
OL   12    2   592,9              G H I J
OL    0    2   552,8                H I J
CO    0    2   535,1                H I J
OL    4    2   509,0                  I J
CO    8    2   496,9                  I J
SA   12    2   480,9                  I J
OL    8    2   437,0                    J
```

Means that do not share a letter are significantly different.

Figure 3.20. Amount of CEL formed in potato chips fried within the different type of oils (Canola, Corn, Hazelnut, Olive, Safflower) repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 4, 8, 12 h)

General Linear Model: CEL-CIPS-2 versus OIL; TIME

```
Factor  Type   Levels  Values
OIL     fixed       5  CA; CO; HA; OL; SA
TIME    fixed       4  0; 4; 8; 12
```

Analysis of Variance for CEL-CIPS-2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
OIL	4	2619681	2619681	654920	122,02	0,000
TIME	3	613573	613573	204524	38,10	0,000
OIL*TIME	12	1040306	1040306	86692	16,15	0,000
Error	20	107350	107350	5368		
Total	39	4380911				

S = 73,2633 R-Sq = 97,55% R-Sq(adj) = 95,22%

Unusual Observations for CEL-CIPS-2

Obs	CEL-CIPS-2	Fit	SE Fit	Residual	St Resid
9	2542,80	2435,08	51,81	107,72	2,08 R
10	2327,36	2435,08	51,81	-107,72	-2,08 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

OIL	N	Mean	Grouping
CA	8	1923,4	A
SA	8	1445,5	B
HA	8	1384,5	B C
CO	8	1294,2	C
OL	8	1176,4	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME	N	Mean	Grouping
0	10	1635,0	A
4	10	1460,7	B
8	10	1385,0	B C
12	10	1298,6	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

OIL	TIME	N	Mean	Grouping
CA	0	2	2435,1	A
CA	4	2	1904,0	B
CA	8	2	1845,4	B C
HA	0	2	1673,3	B C D
SA	4	2	1640,9	B C D E
SA	8	2	1576,1	C D E F
CA	12	2	1509,3	D E F G
CO	4	2	1388,9	D E F G H
SA	0	2	1383,3	D E F G H
OL	12	2	1366,4	E F G H
CO	0	2	1365,1	E F G H
HA	4	2	1359,3	E F G H
OL	0	2	1318,0	F G H
HA	8	2	1261,1	G H I

```
HA   12    2  1244,4             G H I
CO    8    2  1231,7             G H I
CO   12    2  1191,1               H I
SA   12    2  1181,8               H I
OL    8    2  1010,8                 I
OL    4    2  1010,3                 I
```

Means that do not share a letter are significantly different.

Figure 3.21. Amount of CML and CEL formed in potato chips fried within the safflower oil repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 4, 8, 12 h)

One-way ANOVA: SA-CML versus HeatingTime_1

```
Source          DF      SS      MS      F      P
HeatingTime_1    3  524368  174789  89,66  0,000
Error            4    7798    1949
Total            7  532166

S = 44,15   R-Sq = 98,53%   R-Sq(adj) = 97,44%


                          Individual 95% CIs For Mean Based on Pooled StDev
Level  N    Mean  StDev    +---------+---------+---------+---------
 0     2   655,3    0,0           (----*---)
 4     2  1101,8   42,4                                  (---*---)
 8     2  1021,9    0,0                               (---*---)
12     2   480,9   77,4    (---*---)
                           +---------+---------+---------+---------
                          400       600       800      1000

Pooled StDev = 44,2


Grouping Information Using Tukey Method

HeatingTime_1  N     Mean  Grouping
 4             2  1101,77  A
 8             2  1021,87  A
 0             2   655,33    B
12             2   480,85    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of HeatingTime_1

Individual confidence level = 98,48%


HeatingTime_1 =  0 subtracted from:

HeatingTime_1    Lower   Center   Upper
 4              266,61   446,44  626,28
 8              186,71   366,54  546,38
```

```
12             -354,31  -174,48    5,36

HeatingTime_1  ------+---------+---------+---------+---
 4                                (---*---)
 8                               (--*---)
12                       (---*--)
               ------+---------+---------+---------+---
                  -500         0       500      1000


HeatingTime_1 =  4 subtracted from:

HeatingTime_1    Lower   Center    Upper
 8             -259,73   -79,90    99,93
12             -800,75  -620,92  -441,09

HeatingTime_1  ------+---------+---------+---------+---
 8                        (--*---)
12             (---*--)
               ------+---------+---------+---------+---
                  -500         0       500      1000


HeatingTime_1 =  8 subtracted from:

HeatingTime_1    Lower   Center    Upper
12             -720,85  -541,02  -361,19

HeatingTime_1  ------+---------+---------+---------+---
12               (--*---)
               ------+---------+---------+---------+---
                  -500         0       500      1000
```

One-way ANOVA: CEL-SA versus HeatingTime

```
Source       DF      SS     MS      F      P
HeatingTime   3  257250  85750  22,89  0,006
Error         4   14985   3746
Total         7  272235

S = 61,21   R-Sq = 94,50%   R-Sq(adj) = 90,37%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
 0     2  1383,3   39,1            (-----*-----)
 4     2  1640,9   58,0                         (-----*-----)
 8     2  1576,1   55,7                      (-----*-----)
12     2  1181,8   83,6  (-----*-----)
                         -------+---------+---------+---------+--
                             1200      1400      1600      1800

Pooled StDev = 61,2


Grouping Information Using Tukey Method

HeatingTime  N     Mean  Grouping
```

```
 4           2  1640,87  A
 8           2  1576,12  A B
 0           2  1383,31    B C
12           2  1181,82      C

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of HeatingTime

Individual confidence level = 98,48%


HeatingTime =  0 subtracted from:

HeatingTime    Lower   Center   Upper  --------+---------+---------+-------
--+-
 4              8,27   257,57  506,86                    (-----*------)
 8            -56,48   192,81  442,10                  (-----*-----)
12           -450,78  -201,48   47,81         (-----*-----)
                                        --------+---------+---------+-------
--+-
                                             -400         0       400
800


HeatingTime =  4 subtracted from:

HeatingTime    Lower   Center    Upper
 8           -314,05   -64,76   184,53
12           -708,34  -459,05  -209,76

HeatingTime  --------+---------+---------+---------+-
 8                    (-----*------)
12           (------*-----)
             --------+---------+---------+---------+-
                  -400         0       400       800


HeatingTime =  8 subtracted from:

HeatingTime    Lower   Center    Upper
12           -643,58  -394,29  -145,00

HeatingTime  --------+---------+---------+---------+-
12             (-----*-----)
             --------+---------+---------+---------+-
                  -400         0       400       800
```

Figure 3.22. p-Anisidine values of the oils with (SO+A) and without 1% tocopherol mix (SO) repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 4, 8, 12 h)

General Linear Model: p-Anisidine-2 versus TIME-2; OIL-2

```
Factor  Type   Levels  Values
```

```
TIME-2  fixed       4  0; 4; 8; 12
OIL-2   fixed       2  SU; SU-A


Analysis of Variance for p-Anisidine-2, using Adjusted SS for Tests

Source        DF   Seq SS  Adj SS  Adj MS        F      P
TIME-2         3   9303,8  9303,8  3101,3  1267,14  0,000
OIL-2          1   5562,0  5562,0  5562,0  2272,58  0,000
TIME-2*OIL-2   3   1853,1  1853,1   617,7   252,38  0,000
Error          8     19,6    19,6     2,4
Total         15  16738,5


S = 1,56443   R-Sq = 99,88%   R-Sq(adj) = 99,78%


Unusual Observations for p-Anisidine-2

Obs  p-Anisidine-2     Fit  SE Fit  Residual  St Resid
 15         86,777  83,982   1,106     2,794      2,53 R
 16         81,188  83,982   1,106    -2,794     -2,53 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

TIME-2  N  Mean  Grouping
 8      4  77,2  A
12      4  69,1    B
 4      4  51,5      C
 0      4  14,6        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

OIL-2  N  Mean  Grouping
SU-A   8  71,7  A
SU     8  34,4    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

TIME-2  OIL-2  N   Mean  Grouping
 8      SU-A   2  111,1  A
12      SU-A   2   84,0    B
 4      SU-A   2   73,1      C
12      SU     2   54,2        D
 8      SU     2   43,4          E
 4      SU     2   29,8            F
 0      SU-A   2   18,7              G
 0      SU     2   10,4                H

Means that do not share a letter are significantly different.
```

General Linear Model: 2,4-Decadienal in SO and SO+A

Factor	Type	Levels	Values
TIME-3	fixed	4	0; 4; 8; 12
OIL-3	fixed	2	SU; SU-A

Analysis of Variance for 2,4-DEC-3, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME-3	3	2,14600E+13	2,14600E+13	7,15333E+12	72,75	0,000
OIL-3	1	1,74185E+13	1,74185E+13	1,74185E+13	177,15	0,000
TIME-3*OIL-3	3	2,33423E+12	2,33423E+12	7,78076E+11	7,91	0,009
Error	8	7,86613E+11	7,86613E+11	98326624829		
Total	15	4,19993E+13				

S = 313571 R-Sq = 98,13% R-Sq(adj) = 96,49%

Grouping Information Using Tukey Method and 95,0% Confidence

TIME-3	N	Mean	Grouping
12	4	4752063,8	A
8	4	4667660,8	A
4	4	3746314,7	B
0	4	1874060,2	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

OIL-3	N	Mean	Grouping
SU-A	8	4803410,9	A
SU	8	2716638,9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME-3	OIL-3	N	Mean	Grouping
12	SU-A	2	5931952,5	A
8	SU-A	2	5576851,0	A
4	SU-A	2	5311478,0	A
8	SU	2	3758470,5	B
12	SU	2	3572175,0	B C
0	SU-A	2	2393362,0	C D
4	SU	2	2181151,5	D
0	SU	2	1354758,5	D

Means that do not share a letter are significantly different.

Figure 3.23. Relative amounts of decadien-1-amine in potato chips fried in the oils with (SO+A) and without 1% tocopherol mix (SO) repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 4, 8, 12 h)

General Linear Model: D-1-A versus TIME; OIL

Factor	Type	Levels	Values
TIME	fixed	4	0; 4; 8; 12
OIL	fixed	2	SU; SU-A

Analysis of Variance for D-1-A, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME	3	2,37839E+11	2,37839E+11	79279664679	77,31	0,000
OIL	1	10687114262	10687114262	10687114262	10,42	0,012
TIME*OIL	3	68988473423	68988473423	22996157808	22,43	0,000
Error	8	8203443661	8203443661	1025430458		
Total	15	3,25718E+11				

S = 32022,3 R-Sq = 97,48% R-Sq(adj) = 95,28%

Grouping Information Using Tukey Method and 95,0% Confidence

TIME	N	Mean	Grouping
8	4	591766,3	A
4	4	566314,0	A
12	4	450515,5	B
0	4	282891,3	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

OIL	N	Mean	Grouping
SU	8	498716,4	A
SU-A	8	447027,1	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME	OIL	N	Mean	Grouping
8	SU	2	643149,5	A
4	SU-A	2	640941,5	A
8	SU-A	2	540383,0	A B
4	SU	2	491686,5	B C
12	SU	2	470857,5	B C
12	SU-A	2	430173,5	B C
0	SU	2	389172,0	C
0	SU-A	2	176610,5	D

Means that do not share a letter are significantly different.

Figure 3.24. Amounts of total free amino acid in potato chips fried in the oils with (SO+A) and without 1% tocopherol mix (SO) repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 4, 8, 12 h)

General Linear Model: AA versus TIME-3; OIL

Factor	Type	Levels	Values
TIME-3	fixed	4	0; 4; 8; 12
OIL	fixed	2	SU; SU-A

Analysis of Variance for AA, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME-3	3	15828136	15828136	5276045	31,35	0,000
OIL	1	666694	666694	666694	3,96	0,082
TIME-3*OIL	3	22120433	22120433	7373478	43,81	0,000
Error	8	1346298	1346298	168287		
Total	15	39961561				

S = 410,228 R-Sq = 96,63% R-Sq(adj) = 93,68%

Unusual Observations for AA

Obs	AA	Fit	SE Fit	Residual	St Resid
13	11417,5	12037,1	290,1	-619,6	-2,14 R
14	12656,7	12037,1	290,1	619,6	2,14 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME-3	N	Mean	Grouping
8	4	12621,2	A
12	4	12393,8	A
0	4	12353,2	A
4	4	10171,3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

OIL	N	Mean	Grouping
SU	8	12089,0	A
SU-A	8	11680,7	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME-3	OIL	N	Mean	Grouping
12	SU	2	13929,4	A
8	SU	2	13205,4	A B
0	SU	2	12738,1	A B
8	SU-A	2	12037,1	B C
0	SU-A	2	11968,4	B C
4	SU-A	2	11859,3	B C
12	SU-A	2	10858,2	C
4	SU	2	8483,2	D

Means that do not share a letter are significantly different.

Figure 3.25. Amounts of CML in potato chips fried in the oils with (SO+A) and without 1% tocopherol mix (SO) repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 4, 8, 12 h)

General Linear Model: CML-2 versus OilType; HeatingTime-2

Factor	Type	Levels	Values
OilType	fixed	2	SU; SU-A
HeatingTime-2	fixed	4	0; 4; 8; 12

Analysis of Variance for CML-2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
OilType	1	213569	213569	213569	57,79	0,000
HeatingTime-2	3	257987	257987	85996	23,27	0,000
OilType*HeatingTime-2	3	136459	136459	45486	12,31	0,002
Error	8	29565	29565	3696		
Total	15	637579				

S = 60,7912 R-Sq = 95,36% R-Sq(adj) = 91,31%

Unusual Observations for CML-2

Obs	CML-2	Fit	SE Fit	Residual	St Resid
11	1015,24	923,19	42,99	92,06	2,14 R
12	831,13	923,19	42,99	-92,06	-2,14 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

OilType	N	Mean	Grouping
SU-A	8	933,2	A
SU	8	702,1	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HeatingTime-2	N	Mean	Grouping

8	4	990,6	A
4	4	853,7	A B
0	4	788,8	B
12	4	637,5	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

OilType	HeatingTime-2	N	Mean	Grouping
SU-A	8	2	1051,0	A
SU	8	2	930,1	A B
SU-A	4	2	923,2	A B
SU-A	12	2	912,8	A B
SU-A	0	2	845,8	A B
SU	4	2	784,3	B
SU	0	2	731,8	B
SU	12	2	362,2	C

Means that do not share a letter are significantly different.

Figure 3.26. Amounts of CEL in potato chips fried in the oils with (SO+A) and without 1% tocopherol mix (SO) repeatedly used for 12 h at 180 °C to fry potato discs at different times (0, 4, 8, 12 h)

General Linear Model: CEL_2 versus HeatingTime_2; OilType

Factor	Type	Levels	Values
HeatingTime_2	fixed	4	0; 4; 8; 12
OilType	fixed	2	SU; SU-A

Analysis of Variance for CML_2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
HeatingTime_2	3	2547739	2547739	849246	78,13	0,000
OilType	1	551521	551521	551521	50,74	0,000
HeatingTime_2*OilType	3	785830	785830	261943	24,10	0,000
Error	8	86955	86955	10869		
Total	15	3972045				

S = 104,257 R-Sq = 97,81% R-Sq(adj) = 95,90%

Grouping Information Using Tukey Method and 95,0% Confidence

OilType	N	Mean	Grouping
SU-A	8	2339,5	A
SU	8	1968,2	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HeatingTime_2	N	Mean	Grouping

```
 0             4  2551,7  A
 4             4  2489,1  A
 8             4  2008,3    B
12             4  1566,4      C
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

```
HeatingTime_2  OilType  N    Mean  Grouping
 0             SU-A     2  2814,4  A
 4             SU       2  2662,6  A B
 4             SU-A     2  2315,6    B C
 0             SU       2  2289,0    B C
 8             SU-A     2  2230,7      C
12             SU-A     2  1997,4      C D
 8             SU       2  1785,8        D
12             SU       2  1135,5          E
```

Means that do not share a letter are significantly different.

Figure 3.27. p-Anisidine values of the oils repeatedly used for 12 h at 180 °C to fry chicken meatballs at different times (0, 4, 8, 12 h) for different processes (T1:Control, T2: Oil with 0.1% BHT, T3: Oil with 0.1% tocopherol mix, T4: Breaded)

General Linear Model: p-Anisidine-4 versus TIME-4; OIL-4

```
Factor  Type   Levels  Values
TIME-4  fixed       4  0; 4; 8; 12
OIL-4   fixed       4  T1; T2; T3; T4
```

Analysis of Variance for p-Anisidine-4, using Adjusted SS for Tests

```
Source        DF   Seq SS   Adj SS   Adj MS        F      P
TIME-4         3  84411,1  84411,1  28137,0  3746,99  0,000
OIL-4          3   4676,6   4676,6   1558,9   207,59  0,000
TIME-4*OIL-4   9   2409,6   2409,6    267,7    35,65  0,000
Error         16    120,1    120,1      7,5
Total         31  91617,5
```

S = 2,74030   R-Sq = 99,87%   R-Sq(adj) = 99,75%

Unusual Observations for p-Anisidine-4

```
Obs  p-Anisidine-4     Fit  SE Fit  Residual  St Resid
  3         88,739  94,428   1,938    -5,689     -2,94 R
  4        100,117  94,428   1,938     5,689      2,94 R
```

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

```
TIME-4  N   Mean  Grouping
12      8  144,7  A
 8      8  127,4    B
 4      8   92,9      C
 0      8   11,2        D
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

```
OIL-4  N   Mean  Grouping
T3     8  102,9  A
T1     8  101,1  A B
T2     8   98,9    B
T4     8   73,2      C
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

```
TIME-4  OIL-4  N   Mean  Grouping
12      T3     2  165,3  A
12      T1     2  160,6  A
12      T2     2  141,1    B
 8      T2     2  139,2    B
 8      T3     2  138,4    B
 8      T1     2  136,4    B
12      T4     2  112,0      C
 4      T2     2  103,9      C D
 4      T3     2   96,8        D
 8      T4     2   95,4        D
 4      T1     2   94,4        D
 4      T4     2   76,4          E
 0      T1     2   12,9            F
 0      T3     2   11,3            F
 0      T2     2   11,3            F
 0      T4     2    9,2            F
```

Means that do not share a letter are significantly different.

Figure 3.28. Relative amounts of 2,4-decadienal in chicken meat balls (T1:Control, T2: Oil with 0.1% BHT, T3: Oil with 0.1% tocopherol mix, T4: Breaded P: Pan-fried) fried in the oils repeatedly used for 12 h at 180 °C to fry chicken meatballs at different times (0, 4, 8, 12 h)

General Linear Model: 2,4-DEC-2 versus TIME-2; OIL-2

```
Factor  Type   Levels  Values
TIME-2  fixed       4  0; 4; 8; 12
OIL-2   fixed       5  P; T1; T2; T3; T4
```

Analysis of Variance for 2,4-DEC-2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME-2	3	4,82298E+13	4,82298E+13	1,60766E+13	311,31	0,000
OIL-2	4	2,61270E+13	2,61270E+13	6,53174E+12	126,48	0,000
TIME-2*OIL-2	12	1,03188E+13	1,03188E+13	8,59897E+11	16,65	0,000
Error	20	1,03284E+12	1,03284E+12	51641885943		
Total	39	8,57083E+13				

S = 227249 R-Sq = 98,79% R-Sq(adj) = 97,65%

Unusual Observations for 2,4-DEC-2

Obs	2,4-DEC-2	Fit	SE Fit	Residual	St Resid
29	4123665	4555996	160689	-432331	-2,69 R
30	4988327	4555996	160689	432331	2,69 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME-2	N	Mean	Grouping
12	10	4357401,3	A
8	10	3514250,1	B
4	10	2742481,2	C
0	10	1372205,2	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

OIL-2	N	Mean	Grouping
P	8	3878113,6	A
T4	8	3775000,1	A
T2	8	2896131,8	B
T1	8	2789877,6	B
T3	8	1643799,1	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME-2	OIL-2	N	Mean	Grouping
12	T4	2	5533833,5	A
12	P	2	5350758,0	A B
8	T4	2	4555996,0	B C
12	T2	2	4344303,0	C D
8	T2	2	4266832,0	C D E
12	T1	2	4225900,5	C D E
4	P	2	4092234,0	C D E
8	P	2	3610947,5	D E F
4	T4	2	3430557,0	D E F
8	T1	2	3388720,5	E F
4	T1	2	2958643,5	F G
0	P	2	2458515,0	G H
12	T3	2	2332211,5	G H
4	T2	2	1822155,5	H I

8	T3	2	1748754,5	H I
0	T4	2	1579614,0	H I
4	T3	2	1408816,0	I J
0	T2	2	1151236,5	I J
0	T3	2	1085414,5	I J
0	T1	2	586246,0	J

Means that do not share a letter are significantly different.

Figure 3.29. Relative amounts of decadien-1-amine formed in chicken meat balls (T1:Control, T2: Oil with 0.1% BHT, T3: Oil with 0.1% tocopherol mix, T4: Breaded P: Pan-fried) fried in the oils repeatedly used for 12 h at 180 °C to fry chicken meat balls at different times (0, 4, 8, 12 h)

General Linear Model: D-1-A versus TIME-2; OIL

Factor	Type	Levels	Values
TIME-2	fixed	4	0; 4; 8; 12
OIL	fixed	5	P; T1; T2; T3; T4

Analysis of Variance for D-1-A, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME-2	3	5,90627E+12	5,90627E+12	1,96876E+12	448,35	0,000
OIL	4	7,16318E+12	7,16318E+12	1,79080E+12	407,83	0,000
TIME-2*OIL	12	3,59568E+12	3,59568E+12	2,99640E+11	68,24	0,000
Error	20	87821353787	87821353787	4391067689		
Total	39	1,67529E+13				

S = 66265,1 R-Sq = 99,48% R-Sq(adj) = 98,98%

Unusual Observations for D-1-A

Obs	D-1-A	Fit	SE Fit	Residual	St Resid
23	1823178	1683023	46857	140155	2,99 R
24	1542868	1683023	46857	-140155	-2,99 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME-2	N	Mean	Grouping
4	10	1390253,6	A
8	10	1066451,6	B
12	10	942647,6	C
0	10	329969,9	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

OIL	N	Mean	Grouping

T3	8	1673177,3	A	
T1	8	965638,9		B
T2	8	956639,6		B
P	8	647273,5			C
T4	8	418924,1				D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME-2	OIL	N	Mean	Grouping				
4	T3	2	2797181,0	A				
8	T3	2	1839914,5		B			
12	T3	2	1683023,0		B			
8	T2	2	1386582,0			C		
4	T2	2	1379314,0			C		
4	T1	2	1356930,5			C		
12	T1	2	1292125,0			C		
8	T1	2	886235,0				D	
12	P	2	814240,5				D	
8	P	2	768094,0				D	
4	T4	2	728393,0				D	
12	T2	2	700624,5				D	E
4	P	2	689449,5				D	E
8	T4	2	451432,5					E F
0	T3	2	372590,5					F
0	T2	2	360038,0					F
0	T1	2	327265,0					F
0	P	2	317310,0					F
0	T4	2	272646,0					F
12	T4	2	223225,0					F

Means that do not share a letter are significantly different.

Figure 3.30. Amounts of total free amino acid in chicken meat balls (T1:Control, T2: Oil with 0.1% BHT, T3: Oil with 0.1% tocopherol mix, T4: Breaded P: Pan-fried) fried in the oils repeatedly used for 12 h at 180 °C to fry chicken meat balls at different times (0, 4, 8, 12 h)

General Linear Model: AA versus TIME-2; PROCESS

Factor	Type	Levels	Values
TIME-2	fixed	4	0; 4; 8; 12
PROCESS	fixed	5	PAN; T1; T2; T3; T4

Analysis of Variance for AA, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME-2	3	195956	195956	65319	8,77	0,001
PROCESS	4	3564701	3564701	891175	119,68	0,000
TIME-2*PROCESS	12	894846	894846	74570	10,01	0,000
Error	20	148926	148926	7446		
Total	39	4804428				

S = 86,2919   R-Sq = 96,90%   R-Sq(adj) = 93,96%
Unusual Observations for AA

Obs	AA	Fit	SE Fit	Residual	St Resid
37	2660,18	2795,26	61,02	-135,08	-2,21 R
38	2930,34	2795,26	61,02	135,08	2,21 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME-2	N	Mean	Grouping
8	10	2243,5	A
4	10	2214,2	A
12	10	2105,9	B
0	10	2077,8	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

PROCESS	N	Mean	Grouping
T4	8	2498,3	A
T3	8	2360,8	B
PAN	8	2259,4	B
T2	8	2039,1	C
T1	8	1644,0	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

TIME-2	PROCESS	N	Mean	Grouping
8	T4	2	2795,3	A
4	T3	2	2568,0	A B
0	T4	2	2536,6	A B C
4	PAN	2	2391,4	B C D
12	T3	2	2388,0	B C D
4	T4	2	2377,3	B C D E
8	T3	2	2284,9	B C D E
12	T4	2	2284,2	B C D E
8	PAN	2	2253,9	B C D E
0	T2	2	2224,4	B C D E
12	PAN	2	2205,4	C D E F
0	T3	2	2202,4	C D E F
0	PAN	2	2186,8	D E F G
8	T2	2	2044,3	D E F G H
4	T2	2	2029,0	E F G H
12	T2	2	1858,8	F G H
8	T1	2	1839,0	G H
12	T1	2	1792,9	H
4	T1	2	1705,2	H
0	T1	2	1238,8	I

Means that do not share a letter are significantly different.

Figure 3.31. Amounts of CML formed in chicken meat balls (T1:Control, T2: Oil with 0.1% BHT, T3: Oil with 0.1% tocopherol mix, T4: Breaded) fried in the oils repeatedly used for 12 h at 180 °C to fry chicken meat balls at different times (0, 4, 8, 12 h)

General Linear Model: CML_1 versus HeatingTime_1; Process

Factor	Type	Levels	Values
HeatingTime_1	fixed	4	0; 4; 8; 12
Process	fixed	4	T1; T2; T3; T4

Analysis of Variance for CML_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
HeatingTime_1	3	852998	852998	284333	79,26	0,000
Process	3	365491	365491	121830	33,96	0,000
HeatingTime_1*Process	9	1285559	1285559	142840	39,82	0,000
Error	16	57399	57399	3587		
Total	31	2561447				

S = 59,8950 R-Sq = 97,76% R-Sq(adj) = 95,66%

Grouping Information Using Tukey Method and 95,0% Confidence

HeatingTime_1	N	Mean	Grouping
4	8	2800,6	A
0	8	2692,0	B
8	8	2464,6	C
12	8	2399,9	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Process	N	Mean	Grouping
T2	8	2732,6	A
T1	8	2609,0	B
T3	8	2583,6	B
T4	8	2432,0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HeatingTime_1	Process	N	Mean	Grouping
0	T3	2	2983,0	A
8	T2	2	2936,3	A B
4	T4	2	2922,5	A B C
4	T2	2	2893,4	A B C D
0	T2	2	2785,5	A B C D
4	T1	2	2720,6	B C D E

8	T1	2	2685,6		C	D	E	
4	T3	2	2666,0			D	E	
12	T1	2	2525,0				E	F
0	T1	2	2504,6				E	F
0	T4	2	2494,9				E	F
12	T4	2	2386,3					F
12	T3	2	2372,9					F
12	T2	2	2315,2					F
8	T3	2	2312,4					F
8	T4	2	1924,4					G

Means that do not share a letter are significantly different.

Figure 3.32. Amounts of CEL formed in chicken meat balls (T1:Control, T2: Oil with 0.1% BHT, T3: Oil with 0.1% tocopherol mix, T4: Breaded) fried in the oils repeatedly used for 12 h at 180 °C to fry chicken meat balls at different times (0, 4, 8, 12 h)

General Linear Model: CEL versus HeatingTime_2; Process

Factor	Type	Levels	Values
HeatingTime_2	fixed	4	0; 4; 8; 12
Process	fixed	4	T1; T2; T3; T4

Analysis of Variance for CEL, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
HeatingTime_2	3	1194229	1194229	398076	30,55	0,000
Process	3	3433435	3433435	1144478	87,83	0,000
HeatingTime_2*Process	9	6180661	6180661	686740	52,70	0,000
Error	16	208485	208485	13030		
Total	31	11016810				

S = 114,150 R-Sq = 98,11% R-Sq(adj) = 96,33%

Grouping Information Using Tukey Method and 95,0% Confidence

HeatingTime_2	N	Mean	Grouping
0	8	3050,4	A
4	8	2893,5	A
8	8	2659,2	B
12	8	2558,3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Process	N	Mean	Grouping
T1	8	3352,4	A
T4	8	2646,7	B
T3	8	2631,4	B
T2	8	2530,8	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HeatingTime_2	Process	N	Mean	Grouping
12	T1	2	3581,1	A
8	T1	2	3578,8	A
4	T4	2	3519,8	A
4	T1	2	3499,4	A
0	T3	2	3436,0	A
0	T2	2	3365,2	A
0	T1	2	2750,4	B
0	T4	2	2649,9	B C
8	T2	2	2634,6	B C
4	T3	2	2496,8	B C D
8	T3	2	2306,3	B C D
12	T4	2	2300,1	B C D
12	T3	2	2286,8	C D
8	T4	2	2117,1	D
12	T2	2	2065,4	D
4	T2	2	2058,1	D

Means that do not share a letter are significantly different.

CURRICULUM VITAE

PERSONAL INFORMATION

Surname, Name	: Karademir, Yeşim
Nationality	: Turkish (TC)
Date and Place of Birth	: 26 December 1988, Rize
Phone	: +903124806678
E-mail	: yesim.karademir@metu.edu.tr

EDUCATION

Degree	Institution	Year of Graduation
MS	Hacettepe University Food Engineering	2013
BS	Ankara University Food Engineering	2010

FOREIGN LANGUAGES

English, Advanced

PUBLICATIONS

1. Karademir, Y., Gökmen, V., and Öztop, H. M. (2019). Investigation of lipid-derived formation of decadien-1-amine, 2-pentylpyridine, and acrylamide in potato chips fried in repeatedly used sunflower oil. *Food Research International, 121*, 919–925.

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