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Ph.D. in Food Engineering

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NATURAL & APPLIED SCIENCES

EFFECT OF OZONE TREATMENT ON QUALITY PARAMETERS AND STRUCTURE OF VEGETABLE OILS

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Effect of Ozone Treatment on Quality Parameters and Structure of Vegetable Oils

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Supervisor

Prof. Dr. Esra İBANOĞLU

by

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This is to certify that we have read this thesis and that in our consensus opinion it is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

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Hicran UZUN KARKA

ABSTRACT

EFFECT OF OZONE TREATMENT ON QUALITY PARAMETERS AND STRUCTURE OF VEGETABLE OILS

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Ph.D. in Food Engineering
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The effects of ozone treatment on the chemical and structural properties of hazelnut oil (HO) were investigated. The HO treated with ozone gas for 1, 5, 30, 60, 180 and 360 min. The ozone reactivity with the HO were analysed by ^{1}H, ^{13}C NMR, FTIR and GC. Iodine, peroxide and free fatty acid value, viscosity, colour variables were measured. Reaction products were identified according to Criegee mechanism. New signals were assigned to the rings proton of 1,2,4 trioxolane in ozonated oils in ^{1}H and ^{13}C NMR. The viscosity increased, whereas iodine value decreased with extended ozone treatment time. The oxidation kinetics of HO was studied by DSC at isothermal temperatures. E_a and $\Delta H^{\ddagger}$ of the ozone treated oils showed a reducing trend and reflected an increased oxidation sensitivity after ozone treatment. Also, the variations in melting and cooling profile of HO with ozone treatment were examined. The melting and crystallization peaks shifted towards lower temperature and became broader. Further, emulsion activity index (*EAI*) and emulsion stability decreased as ozone treatment time extended. Extensive droplet flocculation occurred in the emulsions containing ozone treated oils and the emulsions exhibited more solid like behaviour when ozone treatment time increased.

Key Words: Ozone, hazelnut oil, oxidation, DSC, GC, NMR

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ÖZET

OZON MUAMELESİNİN BİTKİSEL YAĞLARIN KALİTE PARAMETRELERİ VE YAPISAL ÖZELLİKLERİ ÜZERİNE ETKİLERİ

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Bu çalışmada ozon uygulamasının fındık yağının kimyasal ve yapısal özellikleri üzerine etkileri incelenmiştir. Fındık yağı ozon ile 1, 5, 30, 60, 180 ve 360 dakika muamele edilmiştir. Ozonun fındık yağı ile reaksiyonu ^{1}H, ^{13}C NMR, FTIR ve GC ile analiz edilmiştir. Reaksiyon ürünleri Criegee mekanizmasına göre belirlenmiştir. Ozonlanmış yağda, 1,2,4 trioxolane´e ait olan sinyaller ^{1}H, ^{13}C NMR´da tespit edilmiştir. İyot, peroksit ve serbest yağ asidi değerleri ile viskozite ve renk değerleri ölçülmüştür. Artan ozon muamele süresi ile iyot değeri azalırken, viskozite değerinin arttığı tespit edilmiştir. Fındık yağının oksidasyon kinetiği izotermal sıcaklıklarda sıcaklık taramalı kalorimetre ile çalışılmıştır. Ozon ile muamele edilen fındık yağlarının aktivasyon enerjisi (E_a) ve entalpi ($\Delta H^{\ddagger}$) değerleri azalan bir eğilim sergilemiştir ve ozonlama sonrası yağların oksidasyon duyarlılığının arttığını yansıtmıştır. Ozonlama ile erime ve kristalizasyon pikleri daha düşük sıcaklıklara kaymış ve daha da genişlemiştir. Ayrıca, ozon ile muamele edilmiş fındık yağı içeren emülsiyonların emülsiyon aktivitesi ve stabilizesinin de ozonlama süresi ile azaldığı tespit edilmiştir. Ozon ile muamele edilmiş fındık yağı içeren emülsiyonlarda büyük damlacık flokülasyonları oluşmuştur ve emülsiyonlar ozonlama süresi arttıkça daha fazla katıya benzer davranış sergilemiştir.

Anahtar Kelimeler: Ozon, fındık yağı, oksidasyon, DSC, GC, NMR.

TO MY HUSBAND ABDULLAH KARKA

AND MY SWEET ANGEL ASYA

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

LIST OF ABBREVIATIONS

WPI	Whey Protein Isolate
ANOVA	Analysis Of Variance
DSC	Differential Scanning Calorimetry
HPLC	High Pressure Liquid Chromatography
T_0	Oxidative Induction Time
$\Delta H^{\ddagger}$	Activation Enthalpy
$\Delta S^{\ddagger}$	Activation Entropy
E_a	Activation Energy
EAI	Emulsion Activity Index
ESI	Emulsion Stability Index
k	Rate Constant
G'	Elastic (Storage) Modulus
G"	Viscous (Loss) Modulus
SPSS	Statistical Package for Social Sciences
NMR	Nuclear Magnetic Resonance
FTIR	Fourier Transforms Infrared Radiation
IV	Iodine Value
FFA	Free Fatty Acid
PV	Peroxide Value
HO	Hazelnut Oil
OHO	Ozone Treated Hazelnut Oil
OHO1	Ozone Treated Hazelnut Oil for 1 Min
OHO5	Ozone Treated Hazelnut Oil for 5 Min
OHO30	Ozone Treated Hazelnut Oil for 30 Min
OHO60	Ozone Treated Hazelnut Oil for 60 Min
OHO180	Ozone Treated Hazelnut Oil for 180 Min
OHO360	Ozone Treated Hazelnut Oil for 360 Min
PLM	Polarized Light Microscope
R^2	Correlation Coefficient

σ	Shear Stress
γ	Shear Rate
μ	Viscosity
L*	Lightness
a*	Redness or Greenness
b*	Yellowness or Blueness
Y1	Yellowness Index
T_{on}	Initial Temperature of Crystallization or Melting
T_{off}	Final Temperature of Crystallization or Melting
TPC	Total Plate Count

CHAPTER 1

INTRODUCTION

Ozone has several potential applications as a sanitizing and disinfecting agent in the food industry because of its advantages over other traditional food preservation methods. The utilization of the ozone in food industry has been accepted as safe chemical agent by Food Drug Administration in 1997 and then ozone have been used for many applications in the food industry such as increasing shelf life of vegetables and fruits, preventing of decontamination of perishable foods, the reduction microbial load in storage atmosphere of nuts and cereals and the disinfecting of food plant equipment, water and packaging materials (Gusel-Seydim et al., 2004).

The ozone which could be used as gas or as dissolved in water, chemically react with food materials, as well as disinfecting them. Food materials have complex structures which are composed of various components and oxidation of these components during ozone treatment is likely to occur and this manifests itself as a change in the physical, chemical properties of food and quality loss. However, in literature, there has been a limited knowledge about deleterious effects of ozone on these components. The reactivity of ozone with the unsaturated bonds in lipid fraction of nuts can form several compounds such as ozonides, peroxides and aldehydes. In this study, our aims are to investigate the influence of ozone treatment on the structure and quality parameters of hazelnut oil (HO). The unsaturated triglycerides in vegetable and nut oils give the oil several suitable properties. However, the ozone reacts with the double bonds present in unsaturated fatty acids of the oils. Several oxygenated compounds such as hydroperoxides, ozonides, aldehydes, peroxides, diperoxides and polyperoxides can be produced during reaction of ozone with unsaturated oils. HO is composed of triacylglycerol and phospholipids. The HO contains unsaturated fatty acids which are about ~ 83% monounsaturated (MUFA) and ~9% polyunsaturated (PUFA) and saturated fatty acids (~8%). Therefore, determining chemical and structural variations in the oil upon ozone treatment might be useful to predict the effect of ozone treatment on the

lipid component of hazelnut kernel when they are exposed to ozone for hygiene in their storage atmosphere.

The effects of ozone treatment on structural and chemical properties of the HO were determined by following some chemical and instrumental analysis. Firstly, HO treated with ozone gas for 1, 5, 15, 30, 60, 180 and 360 min. Ozone treated and untreated oils were stored in a hermetically sealed glass bottle kept in a dark place at room temperature up to 150 days and analysed at specific time intervals (3, 7, 10, 20, 30, 40, 55, 70, 85, 100, 125 and 150 days). After ozone application, peroxide value (PV), free fatty acid (FFA) value and iodine value (IV) of ozone treated hazelnut oil were measured as a function of storage time and ozone treatment time. The PV is an indication of oxidation while FFA value of the oxidized oils increases due to cleavage of double bonds. Iodine value (IV) is often used to determine the amount of unsaturation in fatty acids. In order to determine the effects of ozone treatment on the unsaturated bonds of the oil samples, IV of samples was analysed by using chemical analysis method. The ozone reactivity with the HO during ozone treatment was analysed by ^{1}H, ^{13}C NMR (Nuclear Magnetic Resonance) spectroscopy, FTIR (Fourier Transform Infrared Radiation) spectroscopy and GC. The products formed from ozone treatment was determined and identified according to Criegee mechanism. Viscosity and colour variables (L*, a* and b*) of untreated and ozone treated oils were determined. Viscosity of the oil samples was measured by Rheometer (Bohlin C-VOR) to compare variations in viscosity of the untreated and ozone treated oil samples. The ozone reacts with the carotenoids and lead to partial oxidation of these components. The variations in colour values (L*, a*and b*) were measured by Hunter Colorimeter. The oxidation kinetic mechanism of untreated and ozone treated HO was studied. Rate constant (k) of oxidation reaction was determined from oxidation thermogram obtained from Differential Scanning Calorimeter (DSC). Kinetic parameters (activation energy (E_a), enthalpy ($\Delta H^{\ddagger}$) and entropy ($\Delta S^{\ddagger}$) of untreated and ozonated oils was calculated based on Arrhenius equation. The slope of plots ln k versus 1/T gives the activation energy divided by the molar gas constant, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values of ozonated oils were determined by ln (k/T) versus 1/T plot via Eyring equation. Also, heating and melting profile of untreated and ozone treated HO samples were obtained from DSC thermograms. Emulsifying properties of emulsions prepared with untreated and ozone treated HO

were determined. After emulsion formation, the emulsifying activity (*EAI*) was measured from absorbance immediately and the emulsion stability index (*ESI*) was estimated from the change in the following absorbance readings as dependent the time. Also, flow behaviour of the emulsions prepared with untreated and ozone treated oil samples according to elastic modulus (G') and viscous modulus (G'') values was obtained from dynamic rheological measurement. Further, polarized light microscopy was used to measure droplet sizes of emulsions prepared with untreated and ozone treated oil samples. Polarized light microscopy was used to observe the morphology of emulsions. In order to investigate the influence of ozone treated hazelnut oil on microbial growth, total plate count, yeast and mould count of the oil samples were analysed.

CHAPTER 2

LITERATURE SUMMARY

2.1 OZONE

2.1.1 Chemical and Physical Properties of Ozone

Ozone molecule contains three oxygen atoms with the chemical formula O_3. It is more unstable molecule than that of diatomic allotrope (Cuthbertson and Cuthbertson, 1914). Ozone molecule dissociates rapidly oxygen atoms at room temperature and, thus, ozone gas does not accumulate substantially in atmosphere. At room temperature, ozone gas has pale blue colour (Miller et al., 1978; Peleg, 1976). Ozone is an inorganic molecule which has a pungent, characteristic odour (Coke, 1993). Detectable limit of the ozone gas is at 0.01–0.05 ppm level (Mehlman and Borek, 1987; Miller et al., 1978; Mustafa, 1990). It is occurred in stratosphere (Earth's atmosphere) photochemically, but exits in low concentrations at ground. In gaseous phase, it has a longer half-life than that of dissolved phase in aqueous solution. Ozone gas has a solubility in water is about 13 times higher than oxygen at 0–30 ^{0}C (Rice et al., 1981). Ozone is progressively more soluble in cold water and the solubility altered with respect to the temperature of water (Rice, 1986). It decomposes back to oxygen, rapidly in aqueous solution which includes impurities; however, the decomposition of ozone is more slowly in untreated water or in the gaseous state (Hill and Rice, 1982).

Table 2.1 Physical properties of ozone (pure) (Manley and Niegowski, 1967).

Boiling point (^{0}C)	-111.9
Melting point (^{0}C)	-192.5
Critical temperature (^{0}C)	-12.1
Critical pressure (atm)	54.6
Critical volume (cm^3/mol)	111

Ozone is the second strong oxidizing agent; the first one is fluorine which has highest oxidation potential value. The oxidation potential value of fluorine and ozone are 3.06 mV and 2.07 mV, respectively. The other oxidizing agents are permanganate, chlorine dioxide, hypochlorous acid and chlorine gas and their oxidation potential values are lower than that of ozone (Manley and Niegowski, 1967).

Ozone decomposes to oxygen atoms too fast at higher water temperatures (Rice et al., 1981). Explosion in liquid ozone occur easily while ozone to oxygen mixtures is greater than 20%. Electrical sparks or sudden variations in temperature or pressure can cause these explosions. However, in practical applications, ozone explosion can occur, rarely (Guzel-Seydim et al., 2004). The structure of ozone molecule is arranged as a resonance hybrid of the four canonical forms; the included angle is approximately $116^{0}490'$ and the bond length is 1.278 Å. Figure 2.1 shows the different structure of ozone molecule (Oehlschlaeger, 1978).

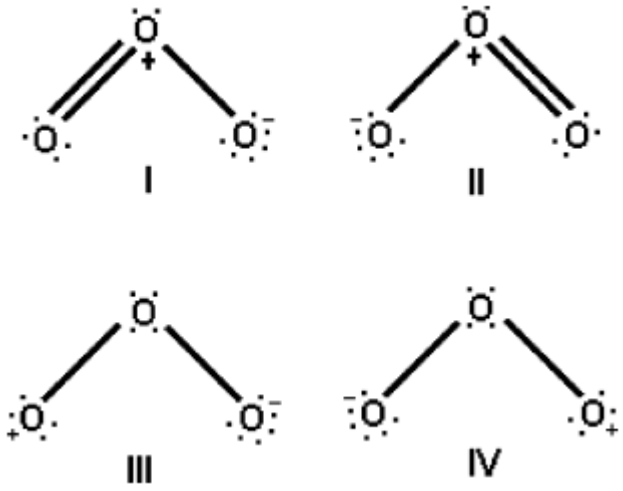


Figure 2.1 Resonance Structures of Ozone Molecule (Oehlschlaeger, 1978).

FDA announces that maximum ozone level as 50 ppb for protection of human health and reducing the occurrence of potentially adverse health effects. Although ozone is known as extremely safe gas in low concentrations, it may be fatal to humans at high concentration (Schwartz et al., 1976). The high concentration of

ozone (>0.2 ppm) can cause severe damages to the respiratory tract (Schwartz et al., 1976).

2.1.2 Earl history of ozone

C.F. Schönbein in 1839 discovered ozone molecule (Guzel-Seydim et al., 2004). He had observed special odour during electrolysis and sparking experiments. He also noticed that the odour was the same with odour observed after a flash of lightning. Schönbein concluded that this odour was due to a new substance. The word of “ozone" derived from "ozein" which is a Greek word, meaning "to smell" (Schönbein, 1840). He concluded that ozone could be formed by passing moist air over stick phosphorus. Further, he said that ozone was produced by plunging a heated glass rod into a mixture of air and vapours of ether. After his experiments; he found that ozone occurs naturally in the ambient atmosphere (Schönbein, 1868).

In 1848, Hunt (1848) announced that ozone is triatomic oxygen. In 1860, Andrew and Tait (1860) reported that ozone could be converted to oxygen by the action of heat or otherwise. Also, they found that small amounts of mercury or metallic silver had the power to destroy ozone (Andrew and Tait, 1860).

Ozone technology was advanced greatly by the development of ozone generation. Ozone generating tubes was introduced by von Siemens (1857). This type of ozone generator has served as a prototype for majority presently used electric discharge generators. The generator consisted of two glass tubes which were coated with tin foil. The metallic surfaces of the tubes were connected to the terminals of an induction coil or electrical machine. When air feed gas was passed through the annular space, 3-8% of dry oxygen could be converted to ozone (Siemens, 1857).

While decomposition of gaseous ozone occurs spontaneously over a period of hours at ambient temperatures, dissolved ozone in aqueous phase decomposes at a faster rate, usually measurable in minutes. In 1894 Mailfert (1894) determined that solubility of ozone in water as 0.64, 0.45, 0.11, 0 liter/liter water at 0, 15, 40, and 60 ^{0}C, respectively (Mailfert, 1894).

In order to investigate ozone generation process under the low temperature conditions, Hautefeuille and Chappuis (1882) passed an electric discharge through oxygen gas at very low temperatures. They obtained concentrations of 14.9 % by weight of ozone at 0 ^{0}C and 21.4 % at -23 ^{0}C. Further, these researchers succeeded in producing liquid ozone by applying a pressure at 125 atm and -100 ^{0}C. Liquid ozone was observed to have a dark indigo-blue colour (Hautefeuille and Chappuis, 1882; Encylopedia Britannica, 1885).

FDA (United States Food and Drug Administration) announced that ozone was generally safe for use in food industry (USDA, 1997). In food industry, ozone was first used in disinfection of urban water in 1907 in Nice and in 1910 in St. Petersburg (Kogelschatz, 1988). FDA stated that maximum treatment level was 0.4 mg of ozone per liter of bottled water. Nowadays, ozone has been used commonly as gaseous phase or an aqueous solution for many applications in food industry such as for decontaminating surfaces of plant equipment, recycling wastewater, and reducing amount of pesticide on fresh produce (Rice et al., 1982; Guzel-Seydim, 1996; Majchrowicz, 1998; Dosti, 1998).

Several foods for example vegetables, fruits, nuts, meats etc., can be treated with ozone directly or indirectly for improving microbial and physical quality and increasing their shelf-life (Norton, et al., 1968). The gaseous ozone can be used to reduce microbial load in the air and on the surface of food products (Yang and Chen, 1979; Sheldon and Brown, 1986).

2.1.3 Ozone Generation

In order to generate ozone, there are ultraviolet radiation (188 nm wavelength) and corona discharge methods. In these methods, firstly, free radical oxygen can be formed from air to generate ozone. Generally, the corona discharge method is usually used for production of commercial level ozone (Fig. 2.2) (Rice et al., 1981).

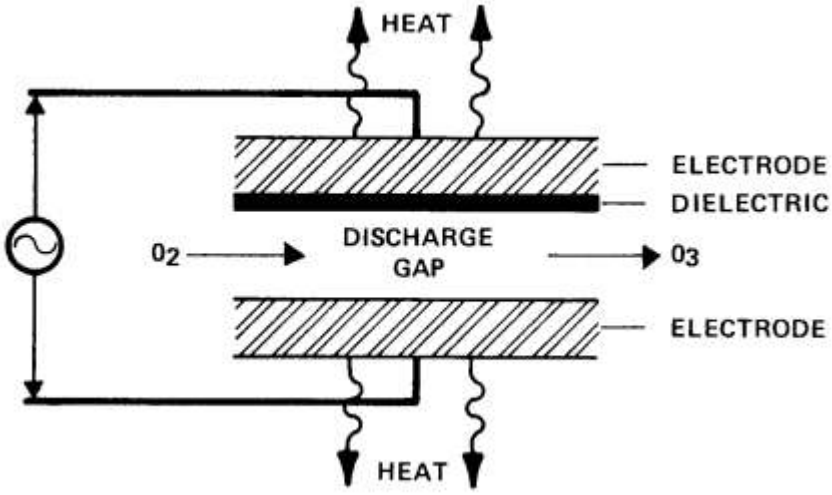


Figure 2.2 Typical corona cell configuration.

Two electrodes are present in the corona discharge method. One of them is high tension electrode and the other is the low tension electrode. These electrodes can be separated from a dielectric medium and there is a discharge gap in the corona discharge (Fig. 2.2). While a suitable kinetic energy which is about 6–7 eV generates between electrons for dissociation of the oxygen molecule, the collisions occur in a certain fraction and then a molecule of ozone can be generated from dissociated oxygen atom. Generally air or pure oxygen was used as a feed gas. Ozone can be produced from air however, 6% ozone yields can be obtained from pure oxygen (Rice et al., 1981). The generated ozone gas cannot accumulate in the atmosphere since it dissociates back to oxygen atoms spontaneously (Coke, 1993; Kogelschatz, 1988; Wickramanayaka, 1991).

2.1.4 Application of Ozone in Food Industry

Ozone treatment has many recommended applications in the food industry for example; disinfection of food manufacturing equipment, waste water (Rice et al., 1982), surface of food material, carcasses of poultry and chilling water in the poultry industry (Yang and Chen, 1979; Sheldon and Brown, 1986), increasing shelf-life of fruit and vegetables (Norton et al., 1968), etc. In poultry industry, ozone has been commonly used in sanitizing of recycled chilling water used in poultry and disinfecting of poultry carcasses (Sheldon and Brown, 1986). The Code of Federal Regulations declared that the reduction in total microorganisms, coliforms, *Escherichia coli*, and *Salmonella spp* must be at least 60% (USDA, 1997). Waldroup

et al., (1993) determined that viable *Escherichia coli* or coliforms cannot present in recycled chill water after ozone treatment and there was low count of the total aerobic bacteria. After ozone treatment to poultry carcasses directly, the all carcass microorganisms were destroyed more than 2 log-units. There were no lipid oxidations, off-flavour development or variation in carcass skin colour (Sheldon and Brown 1986). In order to preserve fruits and vegetables against microbial contamination, ozone treatment has been used in food industry (Norton et al., 1968; Rice et al., 1982). Treatment with ozone resulted in the reduction in microbial decontamination of blackberries and grapes (Beuchat, 1992), the decreased mould and bacterial counts in onions without any variation in chemical and physical attributes and the decreased bacterial content in shredded lettuce in water (Kim et al., 1999). Ozone has been used experimentally as a safety disinfection agent in whole black peppercorns and milled black pepper. Although ozone treatment of milled black pepper causes an oxidation of volatile oils, slightly, ozone treatment had no important influence on the volatile oils of whole peppercorns. After ozone treatment, new components were not detected (Zhao and Cranston, 1995). However, the ozone treatment successfully reduced microbial loads. In order to decrease mould on surfaces of Cheddar cheese and in the cheese storage rooms, ozone inhibit the moulds present at high ozone concentrations (Gibson et al., 1960).

Chemical sanitizers have been commonly preferred for sanitation in the food industry because of being cheaper than other sanitizing methods. The chemical agents which contain chlorinate are used throughout the world for disinfection of water, waste water and for sanitization of food processing plant equipment and foods. Although chlorinated sanitizers have hazardous affects such as causing to form carcinogenic compounds and causing toxicity affects to the environment, causing harmful and irritating at high concentrations, these compounds are cheaper than other bactericides which inhibit vegetative forms of bacteria (Guzel-Seydim et al., 2004). In food processing industry, the alternative sanitizing and disinfecting agents which is no damage to human health and safe for environment and also effective against microbial spoilage and pathogens are researched. Furthermore, these agents must also be non-reacted to food plant equipment (Bellar et al., 1974; Trussell and Umphres, 1978).

Ozone is an alternative sanitizing agent to chlorine for utilize in disinfection purposes in the food industry and use of water treated with ozone has been suggested as sanitizing agent for dairy and food plants (Greene et al., 1993). In previous research, the sanitation effects of the ozone treated water and chlorinated sanitizer on stainless steel surfaces were compared by incubating the equipment with UHT milk inoculated with either *Pseudomonas fluorescens* or *Alcaligenes faecalis* at 32 ^{0}C for 4–24 h. It has been found that ozone treated water and chlorinated sanitizer exhibited almost similar effectiveness against bacteria present on dairy surfaces, both treatments decreased bacterial load by 99%. The disinfection effects of ozone, chlorine and heat were compared with each other against food spoilage bacteria in synthetic broth (Dosti, 1998). The ozone treatment for 10 min caused the highest reduction in the microbial load with a mean reduction over all species of 7.3 log units followed by heat (5.4 log reduction) and chlorine (3.07 log reduction) (Dosti, 1998).

2.1.5 Reaction mechanism of ozone with oils

Ozone treatment of unsaturated triglycerides present in vegetables and nuts oil has been suggested recently due to usage of ozone treated oils in several applications (Soriano et al., 2003). The oils treated with ozone have been used in food, cosmetics and pharmaceutical industry as antibacterial and fungicidal agent (Zanardi et al., 2008). The chemical reactions of ozone with unsaturated fatty acids present in oils are very complex mechanism. The structural and chemical analyses related with these oxidation reactions give some useful information to determine functional group formed from ozone treatment as well as the identification of the reaction products, according to Criegee mechanism (Criegee, 1975). In the mechanism, the reaction products which are known as ozonides can be formed from alkenes and ozone. The reaction of ozone with the unsaturated bonds present in the oils produces several compounds such as ozonides, peroxides and aldehydes according to the mechanism described by Criegee. The initial stage is the formation of the primary ozonide which is called as 1,2,3 trioxolane. And then, a diradical intermediate is produced from the primary trioxolane by O-O bond scission. The diradical can decompose into an aldehyde and a carbonyl oxide. In aqueous environment, the carbonyl oxide can form a hydroxyhydroperoxide, which breaks down to an aldehyde and hydrogen peroxide. In the relatively anhydrous environment, the primary ozonide rearranges to form the

secondary ozonide (1,2,4 trioxolane), which can decompose to a hydroperoxide and an aldehyde. The hydroperoxide can initiate lipid peroxidation. Several oxygenated compounds such as hydroperoxides, ozonides, aldehydes, peroxides, polyperoxides are produced by Criegee mechanism (Figure 2.3), (Pryor, (1994).

In our study, hazelnut oil was treated with ozone in a glass gas washing bottle, directly. Ozone was bubbled into the hazelnut oil without using any chemical solvent. In literature, many studies have not sufficiently examined reaction mechanism of ozone which to be contacted to oil directly. Generally, the studies have been related with reaction mechanism of typical ozonation procedure where ozone gas is bubbled through a solution of oil in a suitable solvent. Based on the results of the mentioned studies, reaction products formed from ozonation in this research were analysed and evaluated by ^{1}H and ^{13}C NMR (Nuclear Magnetic Resonance) and FTIR (Fourier Transform Infrared Radiation).

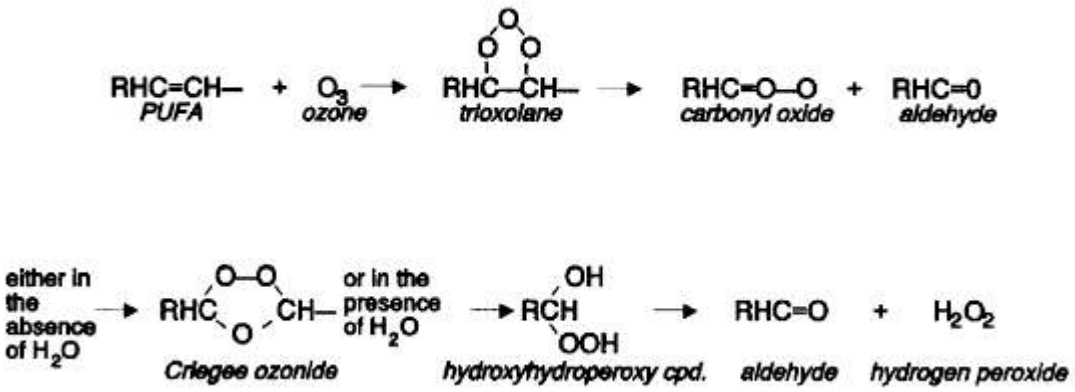


Figure 2.3 Reaction of the ozone with double bond of unsaturated fatty acids.

2.2 OXIDATION MECHANISMS OF FATTY ACIDS

2.2.1 Fatty acids

Fatty acids are carboxylic acids and they have aliphatic carbon chain. They have been in saturated or unsaturated forms. If the aliphatic carbon chain is composed of single bonds between carbon atoms, it is called as saturated fatty acids; if there is one or more of double bonds in the carbon chain, it is called as unsaturated fatty acids. Most of the natural fatty acids have been consist of even number of carbon atoms with hydrogen atoms along the length of the in their carbon chain. The

most common fatty acids have 12-22 carbon atoms in their structure. While the carboxyl group (−COOH) is at the one end of the chain and there is H atom at the other end (IUPAC, Compendium of Chemical Terminology, 2007). The free fatty acids are the fatty acids which are not bonded to other molecules. The carboxyl group and double bonds of the fatty acids is reactive sites, methylene group attached to them are also increased their reactivity. Fatty acids are commonly found that in triglyceride structure in nature. Triglycerides is composed of fatty acids and glycerol (an alcohol) (Scrimgeour, 2005).

There are over 1000 fatty acids which are known in nature, however, only about 20 fatty acids are found in significant amounts in the oils and fats (Table 2.2). They are classified as short, medium or long chain fatty acids with respect to length of their carbon chain. Short chain fatty acids consist from 2 to 6 carbon atoms, while, the length of the carbon chain from 8 to 10 is medium chain fatty acids. Additionally, long chain fatty acids have from 12 to 24 carbon atom in their chains (Akoh and Min, 2002).

Table 2.2 Significant sources of common fatty acids (Akoh and Min, 2002).

Fatty acid	Significant source
4:0 butyric	Butter, milk fat
6:0 caproic	Coconut oil, palm kernel oil
8:0 caprylic	Coconut oil, palm kernel oil
10:0 capric	Coconut oil, palm kernel oil
12:0 lauric	Coconut oil, palm kernel oil
14:0 myristic	Coconut oil, palm kernel oil
16:0 palmitic	Cotton seed oil, palm kernel oil
18:0 stearic	Cocoa butter, tallow
18:1 oleic	Cottonseed, olive, palm, hazelnut oil
18:2 linoleic	Corn, sesame, soybean, sunflower oil
18:3 α-linolenic	Linseed oil
20:1 erucic	High euric rare
20:5 eicosapentaenoic	Fish and animal fats
22:6 docosahexaenoic acid	Fish and animal fats

Table 2.3 Composition of fatty acid present in common oils (wt. %) (Gunstone et al., 1995).

Common oils	16:0	18:1	18:2	18:3	Other
Butter	28	14	1	1	56
Coconut	9	6	2		83
Corn	13	31	52		4
Cottonseed	24	19	53		4
Peanut	13	37	41		9
Olive	10	78	7		5
Palm	44	40	10		6
Rape	4	56	26	10	4
Sesame	9	38	45		8
Soybean	11	22	53	8	6
Sunflower	6	18	69		7
Hazelnut	5	77	12	0.05	6

Hazelnut oil is one of the most commonly used nuts oils which is produced cold pressed method from hazelnut kernels. Hazelnut oil contains oleic acid as monounsaturated fatty acid (MUFA), while linoleic acid and linolenic acid are found as the polyunsaturated fatty acids (PUFA). While major fatty acid of hazelnut oil is oleic acid (74.2–82.8%), small quantity of linolenic acid is found in the hazelnut oil (0.03-0.08%). Additionally, linoleic acid is the PUFA and its range was between 9.82% and 18.7%. Percentages of palmitic, stearic and palmitoleic acids are 5.87%, 2.49%, and 0.48%, respectively (Köksal et al., 2006).

Oleic acid is the major monounsaturated fatty acid in the hazelnut oil. It is odourless, colourless oil. Oleic acid is named as a monounsaturated omega-9 fatty acid, chemically, and abbreviated with a lipid number of 18:1 cis-9. It's chemical formula is $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$ (Thomas, 2002).

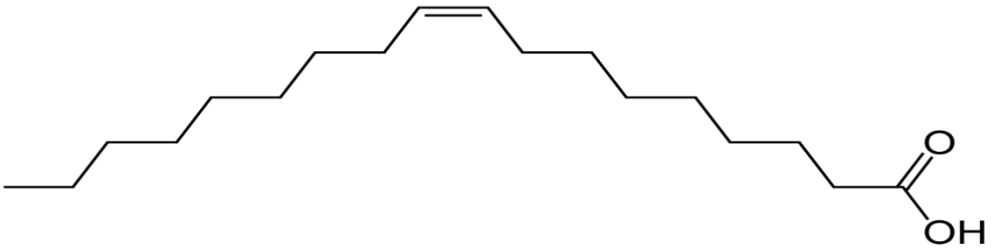


Figure 2.4 Chemical structure of oleic acid (C_{18}:1) (Kaneko et al., 1997).

Linoleic acid is a polyunsaturated omega-6 fatty acid. Hazelnut oil contains high amount of linoleic acid as unsaturated fatty acids after that oleic acid. Linoleic acid is known as polyunsaturated fatty acid which has two cis double bond in its carbon chain; the first double bond located at the sixth carbon and second double bond at the nine carbon atom from the methyl end (Anneken et al., 2006). The double bonds present in hazelnut oil are susceptible to oxidation. Linoleic acid also is named as essential fatty acids, which means that it is not synthesized by human body. Therefore, human can take on linoleic acid from food materials, especially, vegetable and nut oils.

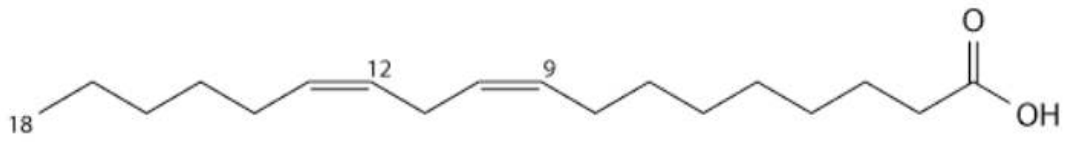


Figure 2.5 Chemical structure of linoleic acid (Anneken et al., 2006).

2.2.2 Oxidation

Oxidation reactions in fatty acid alkyl chain can occur both at double bonds and adjacent allylic carbon atoms. Unsaturated oils and fats can be deteriorated because of photooxidation and formation of free radicals at allylic carbons in the chain due to oxidation reaction. As a result of oxidation, rancidity increases and rancid flavour occur in oils and nutritional value of the oil reduced. There are two common encountered oxidation in food materials containing high amount of lipids; autoxidation and photooxidation. However, autoxidation can occur commonly in food stuff (Scrimgeour, 2005).

2.2.2.1 Autoxidation

Autoxidation include a series of complex oxidation reactions which are initiate, propagate, and terminate the chain in the presence of oxygen. It is also known as auto-catalytic production of free radicals.

In the initiation step, hydrogen atom is abstracted to give lipid radical in the presence of initiators such as light, heat, metals, oxygen. The lipid radical is formed by dissociation of hydroperoxides present or already generated by photoxidation (Scrimgeour, 2005). Then, the free lipid radical reacts with oxygen molecule resulting in generated lipid peroxide radicals during propagation step. This step is much faster than the following step. In this step, another hydrogen atom is abstracted by the lipid peroxide radical, resulting in generation of hydroperoxide and a new free radical (Frankel, 1998). The chain reaction is terminated by several reactions which produce more hydroperoxide radicals by hydrogen abstraction. These reactions is that two hydroperoxide radicals combine with each other for causing to nonradical products and molecular oxygen or it react with an antioxidant for producing a more stable radical (Akoh and Min, 2002).

2.2.2.2 Photooxidation

Photooxidation is a chemical oxidative reaction of unsaturated fatty acids in the presence of light. The several chemical oxygenated compounds such as hydroperoxides, peroxides, and carbonyl and other oxygen containing compounds, and free radicals can be generated by the oxidation reactions (Frankel, 1998). These compounds can be decomposed by Ultraviolet radiation. Natural colour pigments such as chlorophyll and riboflavin exhibit a sensitizing behaviour. Light stimulate these sensitizing agents to the triplet state that promotes oxidation. Unlike autoxidation, there is no induction period (Doleiden, et al., 1974).

There are two different type photooxidation mechanisms which are type I and II. In the type I oxidation, electron or hydrogen is abstracted from the structure of unsaturated bond present in the oil by the triplet state sensitizer, and then produced radicals. Additionally, in type II oxidation mechanism, molecular oxygen are converted to excited singlet oxygen by triplet sensitizer due to transfer of energy from sensitizer to oxygen molecule. Singlet oxygen which highly electrophilic

produces hydroperoxides resulting from its oxidation reaction with olefins. The singlet oxygen attaches to carbons in the double bond and then causes to shift double bond and converts cis form to trans (Rawls and Santen, 1970; Min and Akoh, 1998).

2.2.2.3 Oxidative cleavage

Several oxidizing agents or named as oxidants such as oxygen, ozone, hydrogen peroxide, fluorine, chlorine etc. react with double bonds and produce carboxylic acids, aldehydes, or alcohols (Scrimgeour, 2005).

One of these oxidizing agents is ozone. The ozone molecule reacts directly with one or more double bonds present in olefins and cause oxidation of them (Christie, 2002). Results of the oxidation reactions, firstly molozonide (1,2,3 trioxolane) is formed by the addition of ozone on the carbon–carbon double bonds. The molozonide which is very unstable is rapidly dissociated to an aldehyde and a zwitterion. The next step of the reaction is related to reaction medium which is protic or aprotic (Nishikawa et al., 1995). In an aprotic media, zwitterions and aldehydes reacts with each other to form Criegee ozonide (1,2,4 trioxolane) and peroxidic compounds, while in a protic medium, the zwitterions react with water to generate carboxylic acids and hydro-peroxides, oligomeric peroxides, etc. (Murray, 1968).

2.3 HAZELNUT OIL

2.3.1 Hazelnut production and oil extraction

Hazelnut is one of the most popular nuts belonging to *Betulaceae* family. Hazelnut is derived from species of the genus Corylus and its species is known as *Corylus avellana L.* (Alasalvar et al., 2009). It is mainly grown in the Black Sea region of Turkey (Ordu and Giresun), the south part of Europe and in some location of the US. Furthermore, hazelnut can also be grown in some other countries such as New Zealand, China, Azerbaijan, Iran, and Georgia among others (Alasalvar et al., 2009). The best quality of hazelnuts are grown in Turkey due to having suitable climatic condition for cultivation of high quality varieties hazelnuts. Turkey is the main high quality hazelnut producing country in the world and provide 65% of the total hazelnut production of the world (Ayfer et al., 1997; Köksal, 2002).

Hazelnuts usually begin to mature in the end of summer and they are harvested after maturation completed in autumn. In autumn, over matured nuts fall into the ground while the others is still on the hazelnut tree. Therefore, the hazelnuts are harvested either by manual or by special machine mechanically. Hazelnut is found in nature as clusters of one or more hazelnuts together and the hazelnuts in the clusters are covered by a husk. The hazelnut has oval or round shape depending on the varieties. While unripe hazelnuts have a green colour husk, the colour of the husk turns from green to brown during maturation. The hazelnut is 12-25 mm long and 12-20mm broad. After pollination, the hazelnut reaches to maturity about 7-8 months (Hazelnuts in Ontario – Growing, Harvesting and Food Safety, 2012).

Turkey is the main hazelnut producer in the world. Hazelnut production of Turkey is 625000 tons annually. Turkey is the top exporter of the hazelnut, 75% of world is hazelnut production is supplied from Turkey (USDA, 2004). There are several varieties of hazelnut such as Tombul, Cakıldak, Sivri, Yassi Badem, Çakildak Badem, etc. cultivated in Turkey. Furthermore, a small amount of Ham which is known as wild is also harvested. Among these species, Tombul (round) hazelnut which is grown especially in Giresun is named as first quality due to its high flavor and nutritional value. The other species are called as second grade quality. They contain lower oil contents, flavors and nutritional values than first quality ones. Among other nut species, hazelnut has an important role in terms of its positive influence on nutrition and health (Köksal et al., 2006; Alasalvar et al., 2006). Hazelnut contains mainly valuable lipids which include about 60% of its composition. Hazelnut is composed of high amount of monounsaturated fatty acids (oleic acid), and then linoleic, palmitic, and stearic acids, respectively. Also, Hazelnut kernels have high content of protein about as between 10-24%. The carbohydrate contents of hazelnut kernels vary between about 10% and 22% (Alasalvar et al., 2006; Maguire et al., 2004; Shahidi et al., 2007). Furthermore, hazelnut kernels include valuable vitamins, such as B1, B2, B6, niacin and α-tocopherol. Also, they are sources of several of minerals. Hazelnut commonly utilizes as an ingredient due to its unique and different flavor in food industry such as confectionery, bakery, ice-cream and dairy, candy and chocolate products (Alasalvar et al., 2004; Alasalvar et al., 2003).

The hazelnut oil is obtained from the hazelnut kernels by generally cold-pressed method (Figure 2.6). Hazelnut oil is clear, yellow to yellow-brown oil that is rich in monounsaturated fatty acids (oleic acid) and has an extremely nutty smell and taste. Hazelnut kernel contain high amount of oil about between 58-68%. Refined hazelnut oil is obtained by refinement of cold pressed oil (Figure 2.7). Hazelnut oil has been commonly used for cooking, deep frying, salad dressing, and flavouring ingredients in Turkey. Additionally, hazelnuts play a significant in human nutrition and health. The hazelnuts have a special fatty acid composition which contains high percentages of oleic acid and then linoleic acids as unsaturated fatty acids. Also, hazelnuts include a small amount of palmitic and stearic acid. Furthermore, some health-promoting components such as tocopherols, phytosterols, polynenols and squalene are present in hazelnuts. Therefore, Hazelnut oil is stable against lipid oxidation due to its antioxidant content, especially tocopherols content. Generally, about 100,000-150,000 tons of hazelnut kernel is used annually as raw material for production of refined hazelnut oil (Alasalvar, 2003).

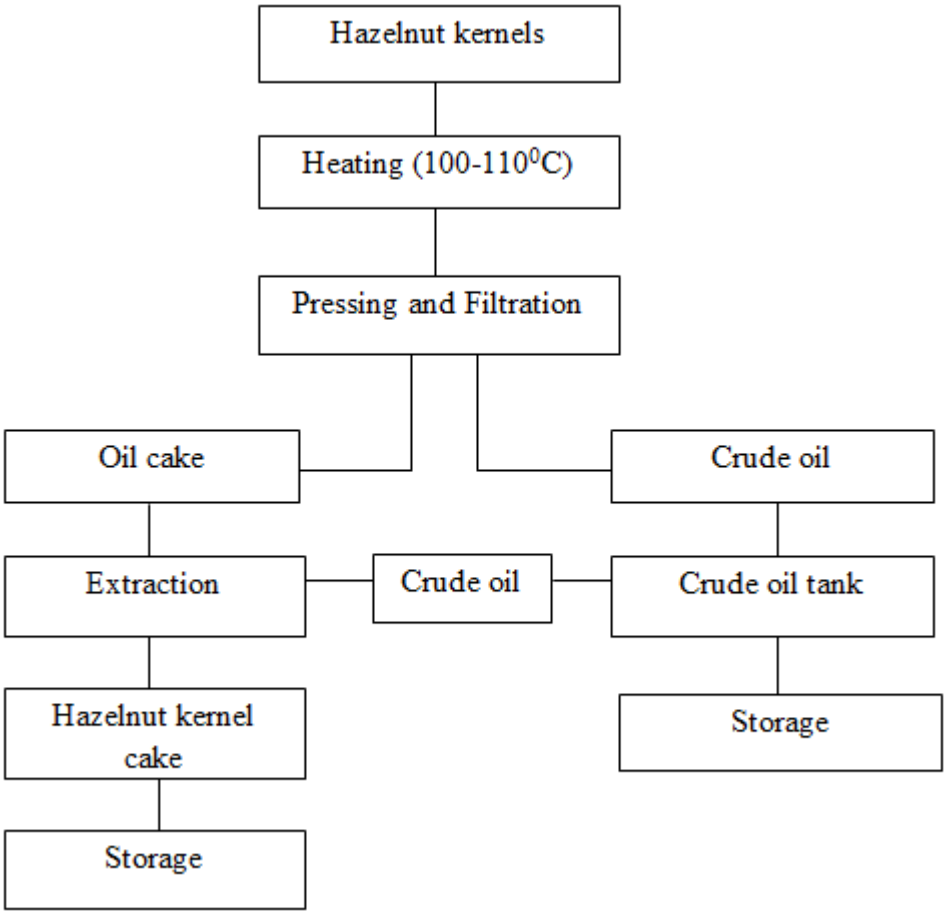


Figure 2.6 Production of crude or extra virgin hazelnut oil (Altaş Company).

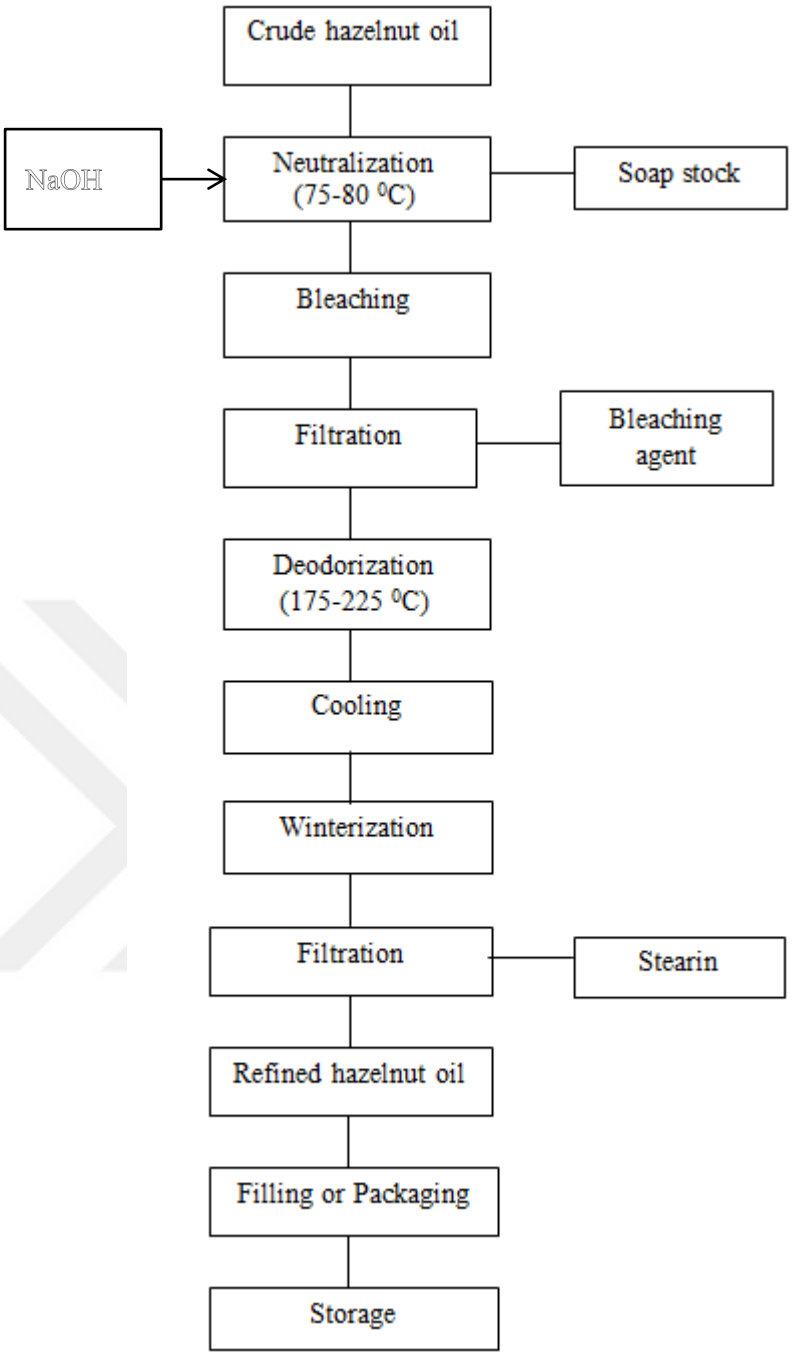


Figure 2.7 Production of refined hazelnut oil (Altaş Company).

2.3.2 Composition of hazelnut kernel oil

Hazelnut kernel composed of valuable nutritional constituents such as proteins, vitamin, minerals and lipids (Alphan et al., 1997; Durak et al., 1999; Mercanlıgil et al., 2007). Especially, it contains some valuable fatty acids such as oleic acid, linoleic acid which are known as unsaturated fatty acids for human diet. Fat content of the hazelnut kernel is about 60-65% and it include high amount of oleic acid as monounsaturated fatty acid and then linoleic acid as polyunsaturated fatty acid.

Small amount of tocopherols, phytosterols, polyphenols, squalene, minor components, and phytochemicals are also present in the hazelnut kernel (Alasalvar, et al., 2006; Maguire et al., 2004; Shahidi et al., 2007). The hazelnut oil is composed of triglycerides which is an ester formed from the binding of three fatty acids to glycerol. Table 2.4 showed that the composition of different Turkish hazelnut varieties which are grown in the Black Sea Region of Turkey. According to the table, hazelnut kernel contains about 20.59% protein, 10% carbohydrates and 61.66% oil. The oil contents of hazelnut kernel varieties are mostly composed of oleic acid (74.2-82.8%) and then linoleic acid while it contains small amount of linoleic acid (0.03-0.08%). Furthermore, hazelnut kernel gives energy about between 27.17-28.46 MJ/kg. The hazelnut varieties have low moisture (2.49-5.25%) and ash content (2.22-2.36%). The hazelnut kernel oil also contains saturated fatty acids, such as palmitic and stearic (Alasalvar, et al., 2003; Bada et al., 2004; Özdemir et al., 1998). The ratio of unsaturated/saturated fatty acid is about 13.1 and the ratio of polyunsaturated/ saturated fatty acids is about between 1.23 and 2.87 in the hazelnut kernel varieties (Alasalvar, et al., 2003; Parcerisa et al., 1998; Özdemir et al., 1998). The unsaturated fatty acid content of the hazelnut oil gives insight about its nutritional value for human consumption. However, high amount of unsaturated fatty acid increases its sensitivity to oxidation. The rates of oxidation of fatty acids are approximately 1:10:100:200 for stearic acid, oleic acid, linoleic acid, and linolenic acid, respectively. The amounts of these fatty acids in hazelnuts widely affect the rates of oxidation, loss in nutritional value, and shelf life of hazelnut (Ragnarsson, and Labuza, 1977).

Table 2.4 Composition of hazelnut kernel varieties

Moisture (%)	**Total oil (%)**	**Protein (%)**	**Carbohydrate (%)**	**Ash (%)**	**References**
4.73	62.21	15.4	15.53	2.09	(Baş et al., 1986).
3.39	63.60	16.38	---	2.04	(Özdemir et al., 1998).
7.72	69.06	20.59	---	---	(Özdemir et al., 1998).
3.90	61.21	15.35	17.3	2.24	(Alasalvar et al., 2003).
6.74	56.10	15.72	10.0	2.29	(Bada et al., 2004).

2.3.3 Chemical and physical characteristics of hazelnut oil

2.3.3.1 General

Hazelnut oil is produced from hazelnut kernel which is named as *Corylus avellana L.* botanically by using cold-pressed method. Usually, the pressed hazelnut oil is passed through refinement process to produce refined hazelnut oil. Characteristic properties of hazelnut oil are shown in Table 2.5 (Alasalvar, et al., 2003; Parcerisa et al., 1998; Özdemir et al., 1998). Hazelnut oil appear clear, yellow to yellow-brown colour and has strong nutty smell and taste.

Table 2.5 Fatty acid composition of hazelnut oil (%).

Palmitic (C16:0)	Palmitoleic (C16:1)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	References
6.38	0.51	1.68	76.78	14.75	---	(Baş et al., 1986).
4.52	---	1.99	71.37	7.77	---	(Özdemir et al., 1998).
5.58	0.2	2.7	75.27	16.15	0.12	(Parcerisa et al., 1998).
4.85	0.16	2.73	82.72	8.89	0.1	(Alasalvar et al., 2003).
5.26	0.19	4.3	81.37	10.28	0.13	(Bada et al., 2004).

2.3.3.2 Colour

In food industry, colour of food materials is described by different colour scales. Usually, the most common used methods for colour measurement are Hunter colour values (L, a, b), CIE systems and the Munsell colour solids (Giese, 2000). Colours of food materials has been determined by colour parameters including L*, a* and b* value accepted by the Commission Internationale d'Eclairage (CIE) (Papadakis et al., 2000). These parameters are accepted as an international standard and "L" demonstrates lightness (0 = black, 100 = white), "b" demonstrates chromaticity on a blue (-) to yellow (+) axis and "a" demonstrates chromaticity on a green (-) to red (+) axis (Rocha and Morais, 2003). Among other nut species, hazelnuts have a rich composition in terms of Vitamin E and magnesium. Also, hazelnut contains olate, β-carotene, vitamin K, lutein+zeaxanthin, phosphorus, copper, selenium, potassium, and zinc (King et al., 2008). Furthermore, carotenoid

pigments which give the yellow colour to the hazelnut oil such as β-carotene, lutein, and zeaxanthin are found in the hazelnut.

2.3.3.3 Percentage of free fatty acid (FFA)

Hazelnut oils generally contain small amount of FFA. FFA content of hazelnut oil raise with an extension in storage time due to inappropriate storage conditions. After harvesting, FFA content of the hazelnut oil is about 0.5%, while FFA content increases to about 1.4% as a function of storage period (TS 6581, Turkish Standard, 2003). During storage, unsuitable handling, moisture, microbial contamination and other factors stimulate to dissociation of triacylglycerol that result in a dramatic rise in FFA (Sanders, 1979). Neutralization is applied to the refined hazelnut oil to remove FFA. However, crude oil deteriorates immediately due to its high FFA content. Therefore, the losing in nutritional quality, flavour and stability of crude oil occur in a short storage period (Sanders et al., 1982).

2.3.3.4 Iodine value (IV)

In analytical chemistry, iodine value indicates the amount of unsaturation of the oil. IV is an important measurement related to some chemical properties of the oil such as melting point, oxidative stability (Sanders et al., 1982). The sensitivity of the oil against oxidation and degree of unsaturation of the oil increases when the oil has higher IV. Among vegetable oils, hazelnut oil (IV; 81-92 g/100g) has higher degree of unsaturation than coconut oil (IV; 7.7-10.5 g/100g), palm oil (IV; 44-54 g/100g), or butter (IV; 25-42 g/100g), however, it is greatly more saturated than corn oil (IV; 103-128 g/100g), cottonseed oil (IV; 99-113 g/100g) or linseed oil (IV; 155-205 g/100g) (TS 6581, Turkish Standard, 2003).

2.3.3.5 Peroxide value (PV)

Peroxide value is the important quality parameters of the oils related to lipid oxidation. During improper storage, oils can be oxidized under the influence of light, moisture, oxygen, metals, etc. (Warner and Mounts, 1993). The concentration of primary oxidation products namely hydroperoxides increase as a result of reaction of oxygen with unsaturated bonds. And then, PV of the oil rise rapidly with oxidative reactions. Therefore, PV is an indication of the oxidative degradation of the oils. It means that amount of reactive oxygen per 1000 g fat (meq O_2/1000g oil or fat). PV can be maximum 15 meq O_2/ kg oil for cold pressed vegetable oils and 10 meq O_2/ kg oil for refined vegetable oils (TS 6581, Turkish Standard, 2003).

2.3.3.6 Antioxidant activity

Hazelnut oil contains various vitamins and minerals. Among the vitamins, vitamin E especially is an important role to delay the oxidation reactions. Vitamin E which shows an antioxidant property is soluble in lipids. Tocopherol which is related to Vitamin E is organic compounds indicated antioxidant effectivity (Packer 1991). They work as an antioxidant agents and preserve the oils and lipid membrane against oxidation by reacting with lipid radicals which generate lipid peroxides. In prior studies, researchers studied the tocopherol content of oil produced from different nuts and found that highest vitamin E content is present in hazelnut oil (33.1 mg/100 g oil) (Le Poole, 1995). And then, high amount of vitamin E are present in almond, peanut, pistachio, pine nut, walnut, Brazil nut, pecan in following order, respectively. It has been reported that hazelnut oil has the highest amount of α-tocopherol content than other tree nuts (Wefers and Sies, 1988; Herrera and Barbas, 2001; Kornsteiner et al., 2006).

Table 2.6 Tocopherol content of hazelnut (mg/100g).

α-Tocopherol	β-Tocopherol	γ-Tocopherol	References
38.60	0.89	0.24	(Kornsteiner et al., 2006)
31.01	---	6.12	(Maguire et al., 1998)
38.23	1.15	3.89	(Alasalvar et al., 2003)
40.45	1.96	4.19	(Bada et al., 2004)

Table 2.7 Chemical and physical properties of hazelnut oil (TS 6581, Turkish Standard, 2003).

Characteristics	**Value**
Flavour	Bland
Colour (visual)	Yellow, yellow brown
FFA (as oleic acid, maximum)	0.3%
IV (g/100g)	81-92
PV (maximum) (meq O_2/ kg oil)	10
Refractive index (n_D, 20^0C)	1.4681-1.4735
Dynamic viscosity (μ, Pas, 0^0C)	66-76
Saponification value (mg KOH/g oil)	188-198

2.4 METHODS FOR MEASURING LIPID OXIDATION

2.4.1 Peroxide Value (PV)

PV is a measurement of the hyroperoxides which is formed by initial stage of lipid oxidation (Riuz et al., 2001). Therefore, it plays an important role to determine oxidative alterations during production and storage of fats and oils (Antolovich et al., 2002; Riuz et al., 2001). In order to measure PV, international standard iodometric titration method is performed. In this method, saturated potassium iodide solution is added to the oil sample. The iodide reacts with hydroperoxides which is produced by oxidation reactions. The released iodine (unreacted) is then titrated with sodium thiosulfate solution which has a certain concentration (Shahidi et al., 2002; Antolovich et al., 2002). The PV is calculated from the used volume of the sodium thiosulfate solution and reported as milliequivalents of oxygen per kilogram of oil or fat (meq O_2/ kg oil) (Kiritsakis et al., 2002).

2.4.2 Free Fatty Acid Value (FFA)

FFA value is an indication of deterioration of the oils resulting from hydrolytic rancidity. The triglycerides in the oil are decomposed during storage in inappropriate conditions, and then free fatty acids from triglyceride structure are released. FFA of the oil is determined from used volume of sodium hydroxide required to neutralize the free acids in 1.0 g of the substance (British Pharmacopoeia, 2000). FFA value is expressed as % oleic acid which is major fatty acid found in the oils (Sadowska et al., 2008).

2.4.3 Iodine Value (IV)

Iodine value or iodine number is a chemical analysis to measure the degree of unsaturation of the oil. The oils which are composed of more double bonds have higher iodine value (Thomas, 2002).

The principle of the analysis of iodine value is based on treatment of fatty acids in the oil with iodine monobromide (IBr) present in the Hanus solution or iodine monochloride (ICl) present in the Wijs solutions. And then, unreacted iodine monobromide (or monochloride) react with potassium iodide, transforming it to iodine. The concentration of the iodine is calculated from used volume of sodium thiosulfate during titration (Firestone, 1993).

2.4.4 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis is an instrumental analysis method which has used for quality and control in the various industrial areas (Dobarganes and Velasco, 2002; Kiritsakis et al., 2002). The FTIR method is a suitable method to investigate oxidation of the oils based on the variations in the functional groups found in oil after oxidation reaction. In the FTIR spectrum, an absorption band at about 2.93 μm demonstrates the formation of hydroperoxides. After oxidation, the absorption band at about 3.20 μm disappears because of replacing of the hydrogen atom on a double bond or occurring of polymerization. An increase at 5.72 μm observes due to the formation of aldehydes, ketones, or acids at the end of oxidative reactions. Furthermore, cis, trans isomerization and formation of conjugated dienes can be assigned with the variations in the absorption band between the ranges of 10 μm to 11 μm (Shahidi et al., 2002).

2.4.5 Differential Scanning Calorimetry (DSC)

The differential scanning calorimetry (DSC) technique has been commonly used instrumental method for evaluating oxidative stability of fats and oils and detecting the onset of oxidation (Tan and Che Man, 2002). DSC gives unique information about thermal energy profile of oxidation and determines the temperature and heat flows related with lipid oxidation as a function of time and temperature (Tan and Che Man, 1999a). At isothermal or non-isothermal conditions, DSC measures the thermal alterations which are occurred from lipid oxidation, such as heat flow into (endothermic) or out of (exothermic) oil sample (Velasco et al., 2004; Tan and Che Man, 2002). The various heating time is used to determine the oxidation curves of the sample. A strong exothermic curve is seen in thermogram at the induction of oxidation. The oxidative induction time is a time where exothermic reaction occurs rapidly between oil and oxygen. Induction period (IP) is obtained

from the intersection of extrapolated baseline and tangent line (leading edge) of the exotherm (Figure 2.8) (Velasco et al., 2004; Tan and Che Man, 2002).

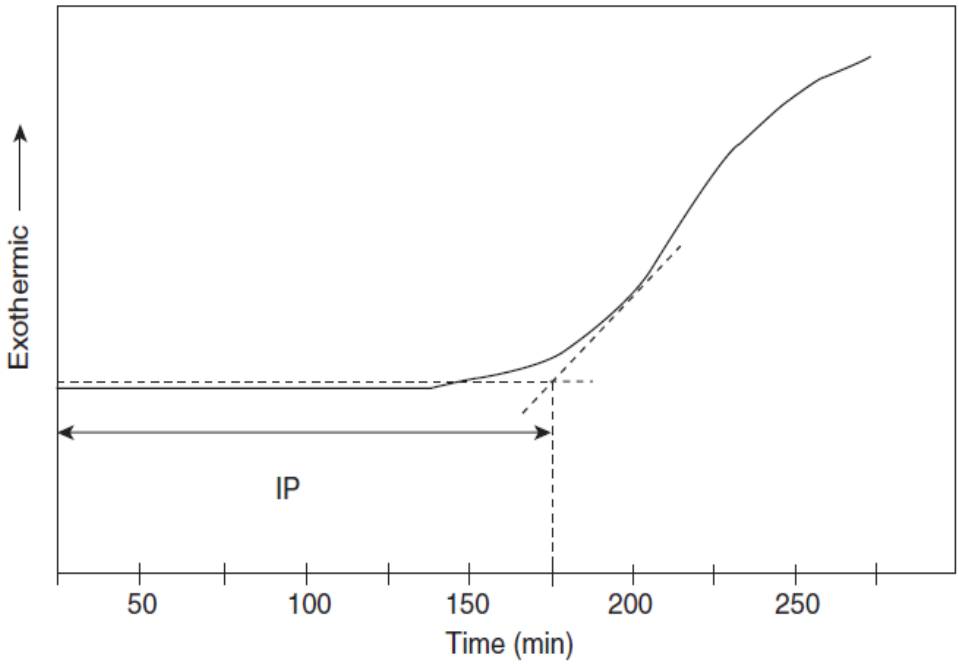


Figure 2.8 Determination of induction period (IP) by DSC (Tan and Che Man, 2002).

2.4.6 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy which is a safe, rapid and nonchemical technique has been used to evaluate lipid oxidation. The NMR spectroscopy gives reliable measurements associated with oxidative variations in oil samples. Therefore, it is said that NMR spectroscopy is more convenient method for estimating lipid oxidation than chemical methods (Shahidi et al., 2002).

The oxidative deterioration of fats and oils is detected by ^{1}H NMR spectroscopy. It determines hydrogen atoms found in various locations in the triacylglycerol molecules. The hydrogen atoms which are found in strong magnetic field absorb energy in the radiofrequency range. The variations in absorbed energy by hydrogen atoms occur during the oxidation process. These variations may be monitored by NMR spectroscopy depending on oxidation level of oils (Shahidi et al., 2002).

In addition to ^{1}H NMR, ^{13}C NMR and ^{31}P NMR are also strong technique to estimate oxidative stability of oils (Medina et al., 1998; Diehl and Hamilton, 1998).

^{13}C NMR provides observation of carbon atoms, directly. ^{13}C NMR evaluates lipid oxidation by monitoring the variations of carbon chains in triacylglycerol molecules, displaying the specific sites that oxidative degradation occurs (Medina et al., 1998).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The crude hazelnut oil was supplied from FİSKOBİRLİK EFİT Inc., Giresun). The chemicals required for acidity, peroxide value and iodine value (Sodium hydroxide (NaOH, diethyl ether, ethyl alcohol, phenolphthalein, potassium bifitalate, sodium thiosulfate ($Na_2S_2O_3$), starch, potassium iodide (KI), chloroform, acetic acid, boric acid, potassium iodate (KIO_3), hydrochloric acid (HCI), Wijs solution, cyclohexane,) were purchased from INTERLAB Chemical Company. Buffer salts, potassium dihydrogen orthophosphate, disodium hydrogen orthophosphate and sodium dodecyl sulfate (SDS) were purchased from Analar analytical reagent; BDH Chemical Ltd. Sodium chloride (JT Baker), sodium hydroxide (Riedel-de-Haen), were used. KI was obtained from Merck Company. All chemicals used were of analytical grade.

3.2 Ozone Treatment of Hazelnut oil

Ozone gas was generated by ozone generator (Ozone Marine, OMS Model, Izmir, Turkey) operating according to the corona-discharge method at a constant flow rate of 1L/min and ozone concentration was 40 mg/L. Oxygen required for ozone generation was provided from the air.

A 100 ml of oil sample was placed into a glass bottle. Ozone gas was directed into the bottle and bubbled through the oil sample for different periods of time (1, 5, 30, 60,180 and 360 min). Ozone treated samples and untreated sample were stored in a hermetically sealed glass bottle kept in a dark place at room temperature up to 120 days and analysed at specific time intervals (5, 10, 15, 20, 30, 40, 50, 60, 75, 90 and 120 days).

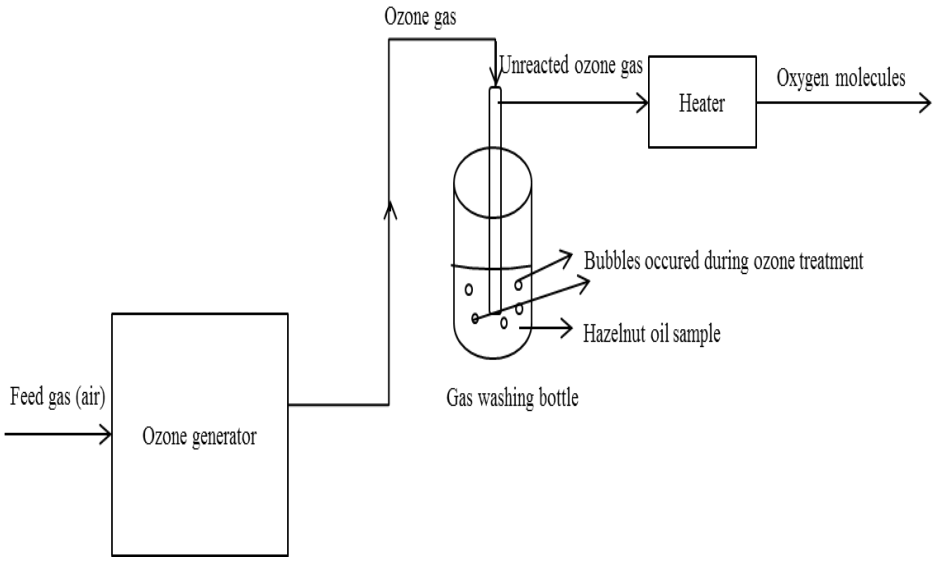


Figure 3.1 Scheme of ozone treatment of hazelnut oil

3.3 Peroxide value (PV)

After ozone treatment, peroxide value of untreated and ozone treated hazelnut oil samples were determined by applying the American Oil Chemists' Society (AOCS) official method. According to the AOCS official method, the following chemical reactions occur as shown in below:

$$2KI + 2CH_3COOH \rightarrow 2HI + 2CH_3COO^- K^+ \quad (3.1)$$

$$R•OO•H + 2HI \rightarrow ROH + H_2O + I_2 \quad (3.2)$$

Firstly, iodine is liberated from the reaction of peroxides (R•OO•H) with potassium iodide in the presence of acetic acid. The liberated iodine is titrated with sodium thiosulfate solution. PV is calculated from following formula given in below (AOCS, 1998):

$$PV = \frac{(S-B)xNx1000}{w} \quad (3.3)$$

where PV is the peroxide value (meq/kg fat), S is the volume $Na_2S_2O_3$ used, B is the volume $Na_2S_2O_3$ for blank, N is the normality of $Na_2S_2O_3$, w is the mass of fat.

3.4 Free fatty acid value (FFA)

Free fatty acid value (FFA) is a measurement of percentage of free fatty acids resulting from the breakdown of triglycerides present in the vegetable oils (British Pharmacopoeia, 2000). FFA of the oil samples was determined by dissolving 5 g of each oil in 40 ml of neutral alcohol (mixture of diethyl ether and ethanol 1:1) in a measuring flask to obtain a solution which was titrated with sodium hydroxide in triplicate, using phenolphthalein as indicator. FFA was calculated as oleic acid from the formula shown in below;

$$FFA\ \% = 2.8\frac{V}{w} \tag{3.4}$$

where w is the mass of oil sample, v is the volume of used NaOH.

3.5 Iodine value (IV)

The total number of double bonds present in the hazelnut oil samples was measured by using chemical methods. Iodine value (IV) is a measurement of the amount of iodine (in grams) which will react with the unsaturated bonds in 100 g of sample. IV of untreated and ozone treated hazelnut oils were measured according to the European Pharmacopoeia, 2004. The IV was calculated by the following formula:

$$IV = \frac{1.269x(A-B)}{m} \tag{3.5}$$

where A is the volume of thiosulphate solution (ml) used for applied a blank test, B is the volume of thiosulphate (ml) solution used for the titration, and m the amount of substance (g).

3.6 Viscosity Measurements

The viscosity of untreated and ozone treated oil samples were measured by using a controlled stress rheometer (Bohlin CVO-R, Malvern Instruments Limited, UK) with cone and plates (2^0/ 20 mm). Shear rates in the range of 0-300 s^{-1} under steady shear conditions were performed to oil samples and shear stress was determined at 25 ^{0}C.

3.7 Hunter colorimeter

Colour of untreated and ozone treated hazelnut oil samples were measured using a Hunter Lab colorimeter (Colour Flex, Hunter Associates Laboratory Inc., Reston, Virginia, USA). The instrument (65^0/ 0^0 geometry, D25 optical sensor, 10^0 observers) was calibrated by white and black reference tiles. The colour values were expressed as L* related with whiteness or brightness/darkness, a* related with redness/greenness and b* related with yellowness/ blueness. L*, a* and b* were given as mean of triplicate measurements of each sample.

3.8 Differential Scanning Calorimetry (DSC)

3.8.1 The measurement of oxidative stability

Differential scanning calorimetry (DSC) (Perkin-Elmer DSC 6 equipped with a Pyris software, Perkin-Elmer Inc., Wellesley USA) analysis was performed to determine oxidative stability of untreated and ozone treated hazelnut oil. The DSC instrument firstly was calibrated by using pure indium and the baseline of the thermogram was obtained by using empty open aluminium pan (Anonymous, 1995). About 5 mg of oil samples were weighed into the aluminium pans and then placed in the sample chamber of the instrument. An empty open aluminium pan which was used as reference. DSC was run at different isothermal temperatures (100, 110, 120, 130, and 140 °C) and purified oxygen (99.8%) was passed through the sample chamber at a flow rate of 50 ml/min. The T_0 (oxidative induction time) of the oxidative reaction corresponded closely to the intersection of the extra-polated baseline and the tangent line (leading edge) of the exotherm. The onset time (T_0) of the oxidation reaction (so called oxidative induction time) was determined from the resulting curve as the intersection of the extrapolated baseline and the tangent line (leading edge) of the exotherm. All DSC experiments were performed in triplicate and the average value of T_0 was used in calculations.

The lipid oxidation in DSC was conducted with a large excess of oxygen generated by a constant flow rate which allows the formation of oxidation products independent of the oxygen concentration (Tan et al., 2001). This allows the assumption of a first order oxidation reaction of oil in DSC at isothermal mode in many works (Tan et al., 2001; Tan and Che Man, 1999b; Micic et al., 2015). The

value of heat evolved at time t is proportional to the amount of reacted substrate (Thurgood et al., 2007). The same extent of conversion reached is observed as oxidation induction period at different temperatures. Therefore the induction time which reflects the effect of temperature is proportional to the rate constant. Kinetic parameters including activation energy (E_a), the rate constant (k), activation enthalpy ($\Delta H^{\ddagger}$) activation entropy ($\Delta S^{\ddagger}$) were calculated by methods modified from Tan et al. (2001). The relationship between isothermal temperatures and the kinetic rate constant of lipid oxidation was explained by the Arrhenius equation:

$$\ln(k) = \ln A - E_a \ /RT \qquad (3.6)$$

where k is the rate constant and determined as reciprocal T_0. A (h^{-1}) is the pre-exponential factor or frequency factor. E_a (kJ mol^{-1}) is the activation energy. R (8.314510 J K^{-1} mol^{-1}) is the molar gas constant and T (K) is the absolute temperature. A linear least squares regression of ln (k) vs. $1/T$ exhibits activation energy and frequency factor from the slope and intercept, respectively. The regression of ln (k) vs. $1/T$ in the equation derived from activated complex theory gives enthalpy ($\Delta H^{\ddagger}$) and entropy ($\Delta S^{\ddagger}$) of activation from the slope and interface, respectively,

$$\ln\left(\frac{k}{T}\right) = (\ln k_B \ /h) + (S^{\ddagger}/R) - (\Delta H^{\ddagger}/R)(1/T) \qquad (3.7)$$

where k_B is the Boltzmann constant (1.380658×10^{-23} J K^{-1}) and h is Planck's constant ($6.6260755 \times 10^{-34}$ J s).

3.8.2 Heating and cooling thermogram analysis

The melting and cooling profile of untreated and ozone treated oils were analysed by differential scanning calorimetry (DSC) (Perkin-Elmer DSC 6 equipped with a Pyris software, Perkin-Elmer Inc., Wellesley USA). The instrument was calibrated with pure indium and baseline was obtained with an empty open aluminium pan (Anonymous, 1995). Nitrogen gas which was used as the purge gas was passed through the sample chamber at a flow rate of 40 ml/min. About 5 mg of sample was weighed into aluminium pans and sealed. An empty aluminium pan which sealed hermetically was used as reference. The previous thermal history of the sample was deleted by heating the sample to 80^0C in the DSC instrument and

holding it for 10 min. The sample was then cooled to -60^{0}C at a rate of 5^{0}C/min and held at -60^{0}C for 10 min. After, the sample was heated at 5^{0}C/min to 80^{0}C. The onset *(T_{on})* and offset *(T_{off})* temperature and enthalpy *(ΔH)* of melting and cooling were determined from DSC thermogram.

3.9 Gas Chromatography (GC) Analysis

The fatty acid composition of hazelnut oil was determined by using Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) with a split/ splitless injector, flame ionisation detector and HP-88 capillary column (88% cianopropylaryl; 100 m×0.250 mm, 0.20 μm i.d.). The injector and detector temperatures were programmed as 250 and 260 °C, respectively. The oven temperature was scheduled as follows: 1 min at 120 °C, from 120 to 175 °C at 10 °C/min, 10 min at 175 °C, from 175 to 210 °C at 5 °C/min, 5 min at 210 °C, from 210 to 230 °C at 5 °C/min and 5 min at 230 °C. Helium was the carrier gas with a flow rate of 1.5 mL/min.

For fatty acid methyl esters (FAME) preparation, 2 mL n-hexane and 2 mL KOH (in methanol) were added onto 1 drop of oil and mixture was shaken vigorously. The supernatant (FAME) was taken and a 1μL of FAME mixture was injected into GC system.

3.10 Fourier transforms infrared radiation (FT-IR) Analysis

FT-IR analyses were applied to investigate the variations in the functional groups found in hazelnut oil after ozone treatment by FT-IR spectrometer (FT-IR 100, Perkin Elmer Incorporation, USA). About 2 μl of sample were deposited between two disks of KBr, without any air bubble formation. The IR spectra of samples were measured and recorded in the 4000–650 cm^{-1} region at room temperature.

3.11 Nuclear magnetic resonance (NMR) Analysis

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Minispec spectrometer 400 MHz at 25 $^{\circ}$C in $CDCl_3$. All the experiments were performed under the same experimental conditions (about 100 μL of oil sample in 750 μL $CDCl_3$). The reaction

of ozone with HO was studied by ^{1}H and ^{13}C NMR at certain time interval (1, 60,180 and 360 min).

3.12 Emulsifying Properties

3.12.1 Preparation of Emulsions

The emulsifying properties of the emulsions which is stabilized by whey protein isolate (WPI) and prepared by using untreated and ozone treated hazelnut oil were determined by the modified Pearce and Kinsella (1978) method. To prepare emulsions, 50.0% (v/v) hazelnut oil and 50.0% (v/v) of protein solution was homogenized using Soniprep 150 Ultrasonic Disintegrator at 72 kHz for 30 s. The final protein concentration of 0.25% (w/w) in emulsions. Emulsion stability and activity of the emulsions were measured and compared depending on containing ozone concentrations. Emulsions were prepared in duplicate.

3.12.2 Emulsifying Activity and Emulsion Stability

An about of 0.05 ml aliquot of the emulsion was pipetted from the bottom of the beaker at different time intervals and then diluted with 50 ml of 0.1% (w/v) SDS solution. The absorbance of the diluted emulsion was measured at 500 nm by using spectrophotometer (Pharmacia Biotech, Novaspec II, UK). After emulsion preparation, the emulsifying activity index (*EAI*) was determined immediately from the absorbance at 500 nm. Further, the emulsion stability index (*ESI*) was calculated from measurement of absorbance of the emulsion as a time dependent. The results of experiment were expressed as surface area (m^2) per unit weight of protein used in the emulsions. The experiments were replicated in two times.

$$EAI = 2T/\emptyset C \tag{3.8}$$

where *C* is the weight of protein per unit volume of aqueous phase before the emulsion is formed. *T* is turbidity and *T= 2.303 A/l.* A is the observed absorbance *l* is the path length of the cuvette. Ø is the volume fraction of the dispersed phase.

$$ESI = \Delta T/\Delta t \tag{3.9}$$

where ΔT is the change in turbidity, occurring during the time interval Δt.

3.12.3 Creaming Analysis

The stability of the emulsions prepared with untreated and ozone treated hazelnut oil against creaming behaviour was determined. Also, emulsions were prepared from ozone treated oils to observe the effect of ozone gas on creaming. Firstly, a 10 ml of freshly prepared emulsion was placed into a graduated tube and sealed. Then, the emulsions were stored at ambient temperature (~22–24 ^{0}C). The variations in the length of serum layer of the emulsion were measured regularly as a time dependent at a certain time intervals. The volume of the creaming part of the emulsion and the initial volume of the emulsion were measured and then % creaming was calculated following formula;

$$\%creaming = 100 - \left[\left(\frac{Vc}{Vt}\right)\right] x100 \qquad (3.10)$$

where V_c is the volume of the creaming part of the emulsion and V_t is the total volume of emulsion (Anton, Beaumal, and Gandemer, 2000).

3.13 Optical Microscopy

In order to measure the droplet images of the emulsions, polarized light microscopy (PLM) was used at room temperature. A volume of 100 µL of emulsion was diluted in 1 mL of 0.1% (w/v) SDS solution. Then a small drop of diluted emulsion was placed onto the microscope slide and a cover slip was used to cover emulsion droplet, carefully. After a few minutes, photomicrographs (40× magnification) of emulsion were taken by using Olympus BX 51 microscope (Tokyo, Japan) equipped with a camera (Pixera PVC 100C, USA).

3.14 Rheological measurement

Dynamic rheological measurements were applied at 25±0.01^{0}C, by a Bohlin CVO-R controlled stress rheometer (Bohlin Instruments, Gloucestershire, UK), using a plate plate geometry (40mm diameter, 1mm gap). For each rheological measurement, 2.0 ml of emulsion were carefully placed onto the pleateau of the rheometer. After the emulsion on the pleateau has been contacted to the plate , the exposed surface of sample was covered a thin layer of silicone oil with for keeping away from the evaporation during the measurement. Frequency sweep

tests (1 Pa stress value at 25^0C) were performed in the linear viscoelastic region (0.01 to 10 frequency (Hz)). The elastic (G') and viscous (G'') moduli were measured against frequency. Data analysis software (Reowin Pro Data Manager Version 2.64) was used to analyse rheological parameters (elastic, viscous modulus, phase angle etc.).

3.15 Microbial Analysis

Total plate count (ISO 4833-1, 2013), yeast and mold count (ISO 21527-2, 2008) were applied to ozonated hazelnut oil and untreated oil as control. In order to investigate the influence of ozonated hazelnut oil on microbial growth, the ozonated hazelnut oils and untreated hazelnut oil as control were added to inoculated plates and then cultivated. Applying different ozone treatment time (15 and 60 min) to the hazelnut oil. The microbial analyses were performed triplicate.

3.16 Statistical Analysis

The SPSS Statics 16.0, version 2.0 (2006), (SPSS Inc., Chicago) was used for all statistical analysis. Experimental results were evaluated by using analysis of variance (ANOVA). Duncan multiple range test method was performed to determine that the data is significantly different from each other. The results were evaluated as significant when means of compared parameters differed at $p<0.05$ significance level.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Peroxide value (PV) and free fatty acid value (FFA)

Peroxide value (PV) and free fatty acid (FFA) value for the untreated and ozone treated hazelnut oils are shown in Table 4.1. PV indicates that the oil deterioration in the presence of oxygen. High level of PV in the oils forms when the oil exposed to oxygen molecules due to oxidative rancidity. Peroxides which are oxygenated compounds are produced during oxidation reactions between the oxygen and the double bond present in the oil structure. Further, FFA in the oils released resulting from hydrolytic rancidity of the oils. Hydrolytic rancidity can occur in the oils while the oil is stored in unfavorable conditions such as excessive moisture, high temperature, light, oxygen, metal contamination, etc. During hydrolytic rancidity, triglycerides in the oils can break down to diglycerides, monoglycerides, and unbound fatty acids, respectively. Thus, the FFA value increases when the oil is exposed the inadequate conditions. As seen in the Table 4.1, the first row represents the variation in PV and FFA of untreated and ozone treated oil samples immediately after ozone treatment. The others indicate the PV and FFA of oil samples measured after being stored for predetermined periods. All PV and FFA values in Table 4.1 were statistically analysed and they showed significant differences ($p<0.05$). Statistically that there was no interaction between ozone treatment period and storage time ($p>0.05$). PV increased with respect to the ozone treatment time, dramatically, while a slight increase in PV was observed as a function of storage time.

Table 4.1 Peroxide and free fatty acid values of untreated and ozone treated hazelnut oils.

Time	FFA (% oleic acid)					PV (meq/kg oil)				
day	HO	OHO1	OHO5	OHO60	OHO180	HO	OHO1	OHO5	OHO60	OHO180
0	0.0145±0.0001a,a	0.0155±0.0001a,b	0.0157±0.0001a,b	0.0174±0.0001a,c	0.0201±0.0000a,d	12.17±0.00a,a	15.91±0.02a,b	33.72±0.03a,c	120.63±0.03a,d	207.25±0.05a,e
3	0.0150±0.0000b,a	0.0161±0.0000b,b	0.0164±0.0001b,c	0.0180±0.0000b,d	0.0201±0.0000a,e	13.24±0.02b,a	18.35±0.05b,b	41.53±0.05b,c	125.87±0.05b,d	211.32±0.02b,e
7	0.0150±0.0001b,a	0.0165±0.0000c,b	0.0167±0.0000c,b	0.0180±0.0000b,c	0.0201±0.0000a,d	14.25±0.00c,a	23.19±0.02c,b	44.43±0.09c,c	130.45±0.00c,d	213.64±0.01c,e
10	0.0150±0.0002b,a	0.0168±0.0000d,b	0.0167±0.0002c,b	0.0181±0.0001b,c	0.0207±0.0001b,d	15.12±0.03d,a	26.19±0.03d,b	44.59±0.08c,c	133.62±0.02d,d	218.90±0.00d,e
20	0.0150±0.0001b,a	0.0168±0.0001d,b	0.0174±0.0001d,c	0.0185±0.0000c,d	0.0211±0.0001c,e	16.51±0.00e,a	28.55±0.03e,b	47.67±0.07d,c	137.89±0.06e,d	221.32±0.00e,e
30	0.0154±0.0000c,a	0.0174±0.0001e,b	0.0177±0.0000e,c	0.0188±0.0001d,d	0.0214±0.0001d,e	17.23±0.02f,a	30.95±0.05f,b	56.61±0.06e,c	143.61±0.04f,d	236.73±0.08f,e
40	0.0157±0.0002d,a	0.0178±0.0001f,b	0.0180±0.0000f,b	0.0192±0.0002e,c	0.0220±0.0000e,d	20.13±0.01g,a	32.53±0.02g,b	65.98±0.05f,c	152.55±0.02g,d	237.51±0.03g,e
55	0.0158±0.0000d,a	0.0177±0.0001f,b	0.0180±0.0002f,b	0.0202±0.0003f,c	0.0290±0.0005f,d	25.03±0.01h,a	35.77±0.01h,b	66.67±0.01g,c	153.69±0.11h,d	248.04±0.08h,e
70	0.0157±0.0000d,a	0.0177±0.0000f,b	0.0185±0.0001g,c	0.0218±0.0000g,d	0.0334±0.0001g,e	26.24±0.00i,a	49.09±0.03i,b	68.58±0.01h,c	153.81±0.08h,d	269.13±0.08i,e
85	0.0158±0.0001d,a	0.0184±0.0002g,b	0.0188±0.0003g,c	0.0234±0.0001h,d	0.0364±0.0005h,e	28.69±0.03j,a	54.96±0.02j,b	69.53±0.03i,c	163.81±0.03i,d	280.52±0.07j,e
100	0.0161±0.0000e,a	0.0190±0.0002h,b	0.0212±0.0003h,c	0.0268±0.0001i,d	0.0387±0.0008i,e	30.17±0.02k,a	60.94±0.08k,b	74.18±0.02j,c	177.88±0.05j,d	292.14±0.02k,e
125	0.0164±0.0001f,a	0.0195±0.0001i,b	0.0220±0.0001i,c	0.0281±0.0002j,d	0.0422±0.0003j,e	32.23±0.00l,a	69.01±0.03l,b	76.91±0.08k,c	189.21±0.05k,d	300.05±0.05l,e
150	0.0180±0.0007g,a	0.0211±0.009j,b	0.0222±0.0008i,c	0.0295±0.0003k,d	0.0468±0.0002k,e	38.19±0.05m,a	78.43±0.09m,b	110.60±0.03l,c	193.56±0.07l,d	315.42±0.07m,e

Each value in the table represents the mean ±standart deviation of triplicate analyses. Abreviations: FFA, free fatty acid; PV, peroxide value. Values with different letters in each column and rows are significantly different ($p < 0.05$). First letter shows that comparison of changes in PV and FFA values of the untreated and ozone treated samples as a function of storage time (day), second letters shows that comparison of changes in PV and FFA values of the untreated and ozone treated samples with ozone treatment time (min), separately.

In the study, PV of untreated and ozone treated hazelnut oils were measured as a function of storage time and also with different ozone treatment time. Ozone treated and untreated oils were stored in a dark place at 20°C up to 150 days. This period was assumed to be long enough to observe the oxidative changes (Seydim and Ertekin, 2006). It was observed that the PV of ozone treated oil samples raised with increase in storage period. The increase in PV in 150 days was observed as to be 3.13 times higher in untreated oil. The raise in PV in ozone treated oil samples were found as 4.92, 3.27, 1.60, and 1.52 times for 1, 5, 60 and 180 min ozone treatments, throughout the same storage period, respectively. The increase in PV was observed to slow down in 60 and 180 min ozone treatment, since the oxidation of oil was expected to accomplish during ozone treatment. A reduction in the reaction rate was occurred because of disappearance of the double bond with increasing in ozone treatment time (Zanardi et al., 2008).

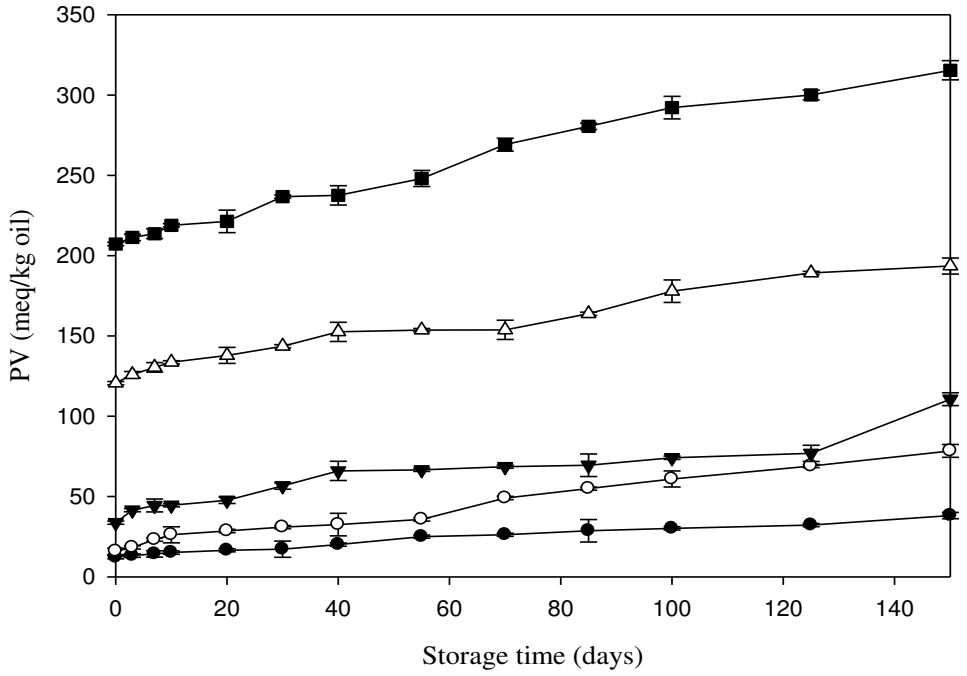


Figure 4.1 Changes in PV of untreated and ozone treated hazelnut oil as a function of storage time.(●; HO, ○; OHO1; ▼; OHO5, Δ; OHO60; ■ ; OHO180).

As shown in Figure 4.2, an exponential increase in the PV of oil samples with the increase in ozone treatment time was observed. While PV of untreated oil was measured as 12.1 meq/kg oil, PV were 15.91, 33.72, 120.63, and 207.25 meq/kg oil for 1, 5, 60, and 180 min ozone treated samples, respectively. The increase in PV with respect to untreated sample is 17 times after 180 min ozone treatment. PV of oil is used as a measure of the extent to which oxidative rancidity reactions have occurred during ozone treatment. As the treatment time is extended, more ozone molecules contact the sample of oil, whose volume was kept constant in each ozone treatment, causing an extensive oxidation. PV of ozone treated oil samples correlate well with data in previous studies on different vegetable fats and oils in relation to the ozone treatment time (Sadowska et al., 2008). The reaction of ozone with vegetable oils occurs in double bonds present in unsaturated fatty acids. After oxidation reactions, the several compounds such as hydroperoxides, ozonides, aldehydes, peroxides, diperoxides and polyperoxides are generated (Bailey, 1982). Hence, the chemical and structural properties of the oils can change. Researchers stated a higher PV in ozone treated soybean oil as compared to olive oil which could be explained by the presence of high proportion of unsaturated fatty acid chains in soybean oil than olive oil (Sadowska et al., 2008).

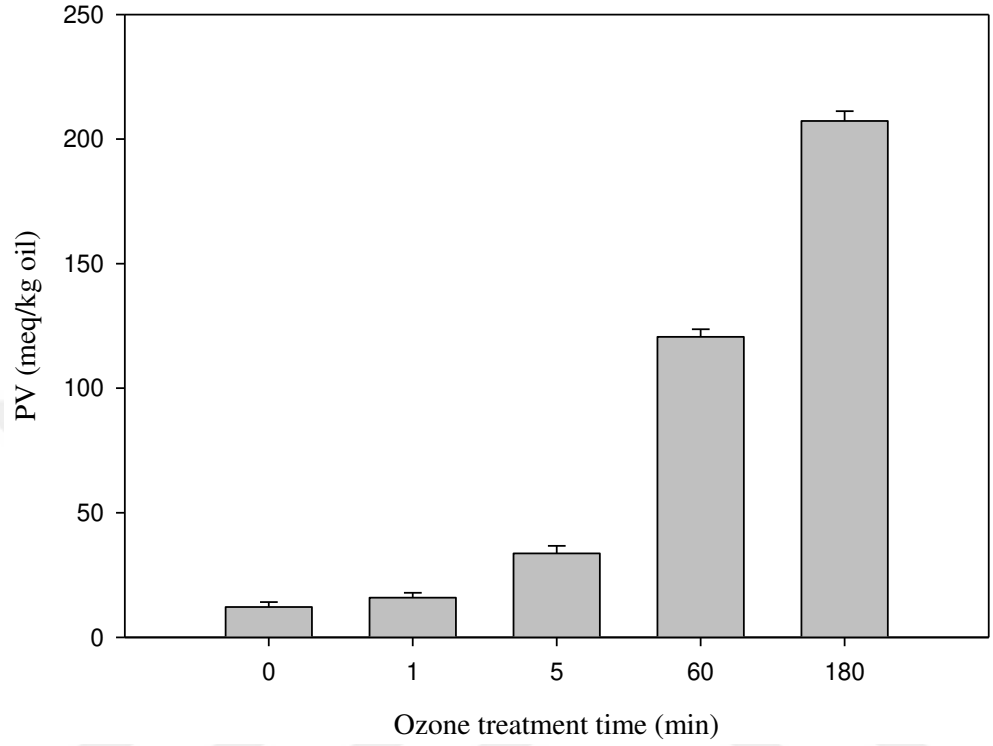


Figure 4.2 Changes in peroxide values of hazelnut oil samples with ozone treatment time.

A rise in FFA of the oil samples was observed when they were measured throughout the storage period (Figure 4.3). FFA of untreated oil increased 1.24 times in 150 days, whereas FFA of ozonated oil samples increased 1.36, 1.41, 1.69, and 2.32 times for 1, 5, 60, and 180 min ozone treatments, respectively. As expected, the highest FFA value was seen in oil sample ozonated for 180 min and stored for 150 days (Table 4.1), resulting from a high amount of ozone exposure. Recently, it has been reported that partial hydrolysis takes place during the ozonation process gives a rise unbound fatty acids which gave fats an acidic feature (Skalska et al., 2009). Additionally, all oils and fats, depending on minor components including moisture and other impurities together with storage temperature, air (oxygen) concentration, and light can exhibit oxidation during storage which is likely to release free fatty acids (Syed, 2016; Fu et al., 2016). In a recent study, it has been revealed that monounsaturated fatty acid content of soybean, canola, and corn oils were slightly increased during storage exhibiting a common trend which was an increase in the monounsaturated fatty acid content and a decrease in that

of polyunsaturated fatty acids (Syed, 2016). Considering that, fat molecules which were already exposed to oxidation with ozone, might be more sensitive to oxidation during storage resulting from structural changes. This may cause an inevitable increase of FFA values after storage period.

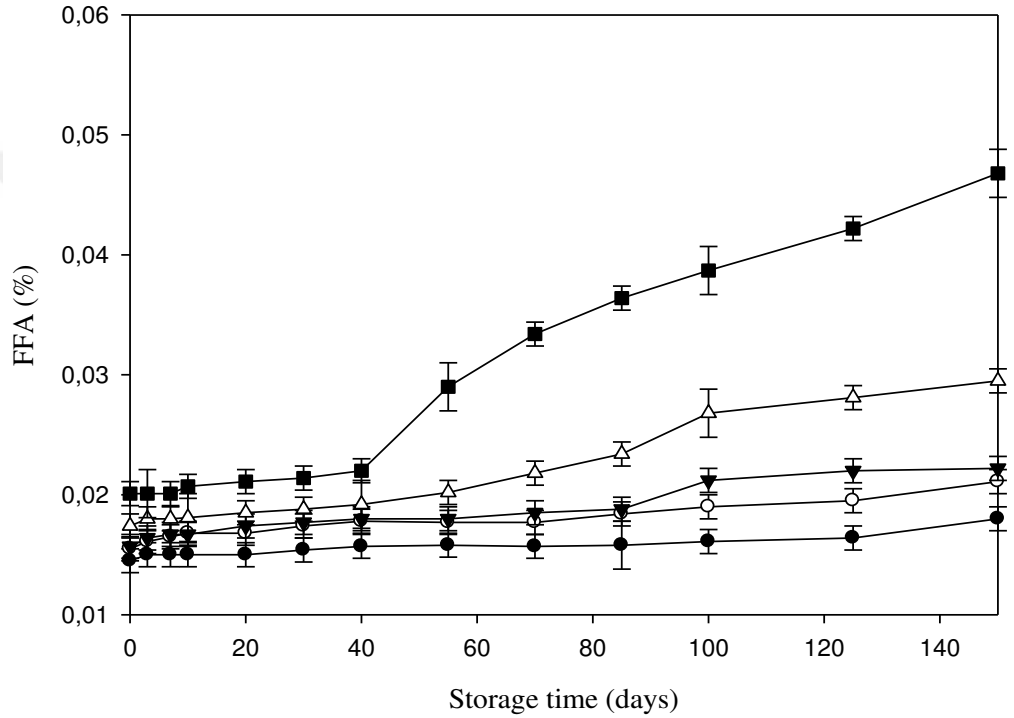


Figure 4.3 Changes in free fatty acid values of untreated and ozone treated hazelnut oil as a function of storage time.(●; HO, ○; OHO1; ▼; OHO5, Δ; OHO60; ■ ; OHO180).

As well as PV, FFA of oils is very important in terms of quality measurements in the food industry. In the present study, FFA of oil samples was measured as a function of ozone treatment period and also, as a function of storage time (Table 4.1, Figure 4.3 and 4.4). As seen in Figure 4.4, while FFA value of untreated oil sample was measured as 0.0145 % oleic acid, the FFA of ozone treated oils were 0.0155, 0.0157, 0.0174 and 0.0201 % oleic acid for 1, 5, 60, and 180 min ozone treatment, respectively. FFA of oil samples were observed to increase as much as 1.06, 1.08, 1.20, and 1.38 times in ozone treated samples for 1, 5, 60, and 180 min ozone treatment, respectively. An increase in the FFA value correlates well with the increase in PV in the ozone treatment process. In a previous study (Skalska et al., 2009), it was noticed that acidity number of sunflower

oil increase with increase of ozone dosage. Also, it was reported that the fatty acid was oxidised rapidly and the acid value and peroxide value would increase when peanuts were treated with high concentration ozone for a long time (Chen et al., 2014).

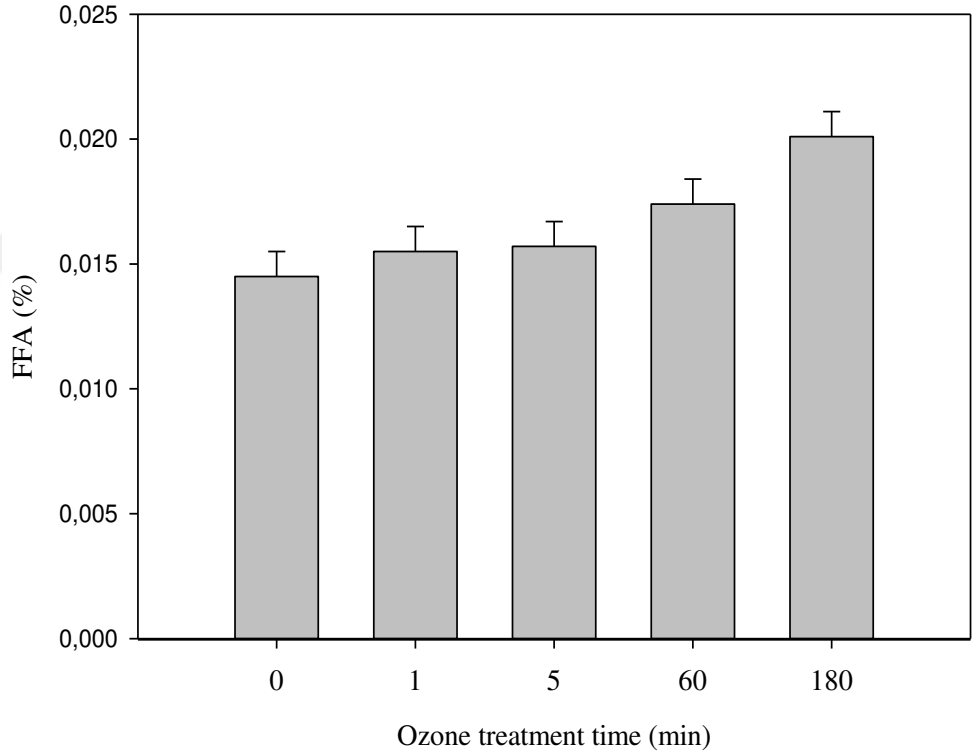


Figure 4.4 Changes in free fatty acid values of hazelnut oil samples with ozone treatmenttime.

4.2 Iodine value (IV)

Table 4.2 Changes in iodine value (g/100g oil) of untreated and ozone treated hazelnut oil.

Storage time (days)	Iodine value (IV) (g/100g)						
	HO	**OHO1**	**OHO5**	**OHO30**	**OHO60**	**OHO180**	**OHO360**
0	95.47±0.1a,a	72.08±0.3b,a	65.09±0.5c,a	32.13±0.1d,a	22.30±0.4e,a	18.27±0.1f,a	13.46±0.3g,a
5	92.54±0.2a,a	70.95±0.2b,a	64.74±0.4c,a	32.32±0.5d,a	20.90±0.6e,a	18.09±0.4f,a	13.67±0.5g,a
10	93.36±0.2a,a	72.08±0.1b,a	64.56±0.5c,a	31.95±0.7d,a	22.23±0.2e,a	17.44±0.5f,a	13.96±0.3g,a
15	92.45±0.1a,a	71.66±0.1b,a	63.95±0.7c,a	31.95±0.3d,a	21.97±0.7e,a	18.25±0.2f,a	13.54±0.2g,a
20	95.36±0.3a,a	71.85±0.5b,a	64.11±0.3c,a	32.08±0.3d,a	21.76±0.5e,a	18.40±0.3f,a	13.51±0.1g,a
30	92.34±0.1a,a	72.02±0.2b,a	63.85±0.1c,a	32.17±0.4d,a	21.76±0.5e,a	18.02±0.7f,a	13.79±0.5g,a
40	95.23±0.4a,a	72.05±0.4b,a	64.19±0.5c,a	31.38±0.8d,a	21.54±0.2e,a	17.93±0.7f,a	13.48±0.6g,a
50	95.01±0.8a,a	72.18±0.9b,a	65.24±0.4c,a	31.89±0.2d,a	21.24±0.3e,a	17.42±0.1f,a	13.22±0.4g,a
60	92.43±0.2a,a	72.34±0.6b,a	63.39±0.9c,a	32.43±0.5d,a	21.93±0.7e,a	18.35±0.3f,a	13.59±0.5g,a
75	95.27±0.2a,a	71.98±0.3b,a	64.78±0.1c,a	32.03±0.3d,a	22.14±0.1e,a	18.17±0.3f,a	13.64±0.2g,a
90	94.33±0.5a,a	72.52±0.1b,a	64.24±0.4c,a	32.38±0.2d,a	22.28±0.5e,a	18.08±0.7f,a	13.23±0.2g,a
120	95.11±0.3a,a	72.10±0.1b,a	65.32±0.6c,a	32.42±0.5d,a	22.20±0.3e,a	18.24±0.9f,a	13.88±0.7g,a

Each value in the table represents the mean ±standard deviation of double analyses.

Iodine numbers give information about the amount of double bonds in unsaturated fatty acids which react with iodine compounds. The higher the iodine number shows the more double bonds are present in the lipids (Thomas, 2002). Hazelnut oil, was composed of a high amount of monounsaturated fatty acids, particularly oleic acid (79.15%), and also polyunsaturated fatty acids, especially linoleic acid (11.43%) and small amount of gandoic acid (0.181%) (Table 4.12). As illustrated in Table 4.2, the IV of hazelnut oil was 95, approximately. In previous work, the iodine value of the hazelnut oil ranges from 90.6 to 97.4 (Xu et al., 2007).In present work, IV of the untreated and ozone treated oil samples were measured by iodimetric titration method, chemically. It was determined that IV of untreated and ozone treated hazelnut oil samples was significantly different ($p<0.05$).

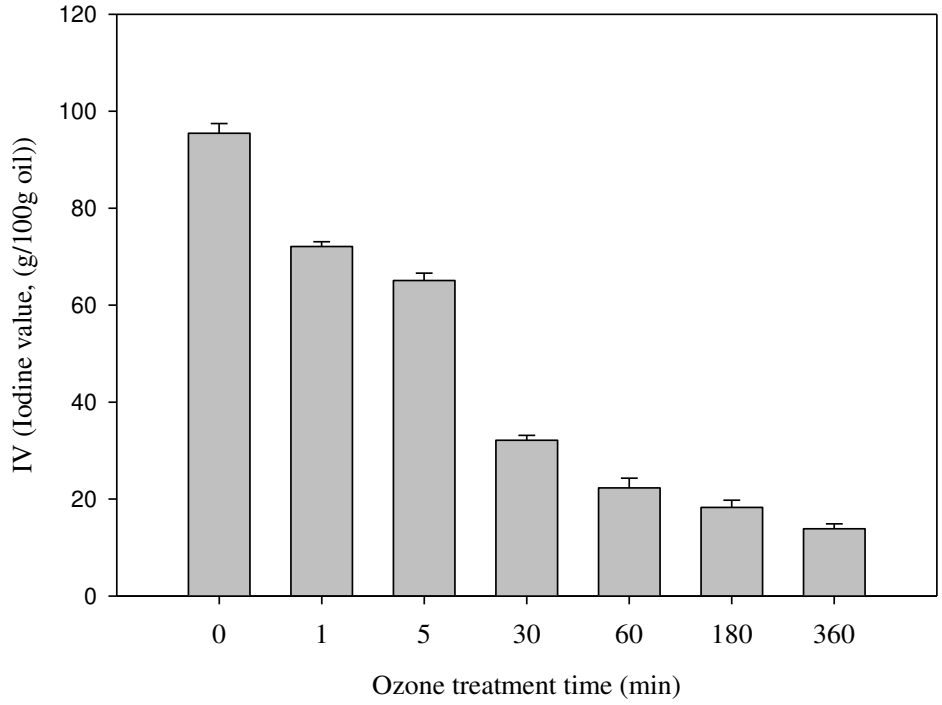


Figure 4.5 Changes in iodine value (g/100g oil) of hazelnut oil with ozone treatment time.

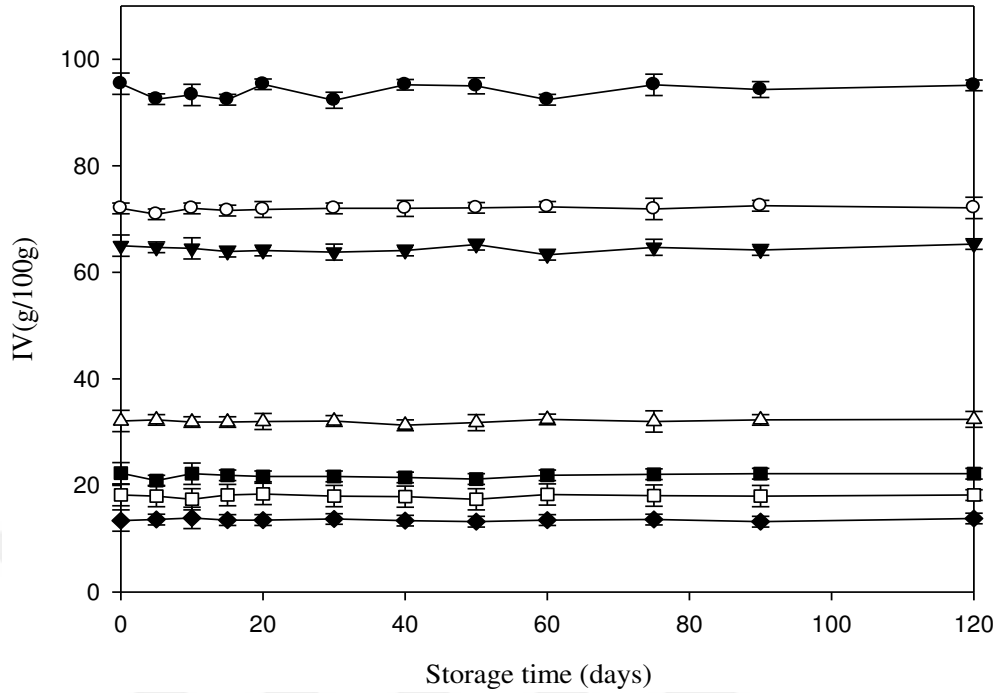


Figure 4.6 Changes in iodine value (g/100g oil) of untreated and ozone treated hazelnut oil as a function of storage time (●; HO; ○; OHO1, ▼; OHO5, Δ; OHO30, ■; OHO60, □; OHO180, ♦; OHO360).

The IV of samples dramatically reduced with ozone treatment time (Figure 4.5). A sharp reduction in iodine values of samples were observed after ozone treatment, whereas the iodine values of ozone treated samples slightly changed as a function of storage time. Statistical results showed that the change in IV as a function of storage was not significantly different ($p>0.05$) (Figure 4.6). During ozonation reaction, ozone reacted with double bonds found in the unsaturated fatty acid of hazelnut oil. This view was verified by the decrease in IV with ozone treatment time, indicating a measure of unreacted double bonds. The result was correlated with a change in unsaturated fatty acid composition of hazelnut oil after ozone treatment (Table 4.12). Zanardi et al., (2008) reported that IV of sesame oil samples decreased with ozone treatment. The IV is indicative that double bonds in the oil samples reacted with ozone result from double bond disappearance. Tan and Man (1999a) reported that IV of oils reduced with heating process and this showed deterioration in oils during heating. Skalska et al., (2009) stated that almost all unsaturated bonds in the oils were saturated in the oxidation reaction with ozone.

4.3 Viscosity measurement

Figure 4.7 shows that flow behaviours of control and hazelnut oils treated with ozone for 1, 5, 30, 60, 180 and 360 min. The flow behaviours of untreated and ozone treated hazelnut oil samples were described by steady shear measurements at 25 ^{0}C. All of the samples exhibited the similar flow behaviour which could be described by straight lines as illustrated in the Figure 4.7. The figure showed that the shear stress linearly increased with shear rate. Therefore, this type of flow behaviour was characterised by the formula shown in below

$$\sigma = \mu.\gamma \qquad (4.1)$$

where σ is shear stress, γ is shear rate, and μ is viscosity. The viscosity of the untreated and ozone treated oils was calculated from the slope of the curve. In previous studies, it was stated that fresh vegetable oils exhibited Newtonian flow behaviours (Santos et al., 2004). However the viscosity values of the oil samples varied distinctly depending on the ozone treatment time. The highest viscosity was observed at 360 min ozone treated oil samples followed by 180, 60 and 30 min, respectively. However, the viscosities of 1 and 5 min ozone treated samples were observed as close to the viscosity of crude oil. The increase in viscosity of HO with respect to ozone treatment time could be explained with the decrease in unsaturation (Abramovic and Klofutar, 1998, Sega et al., (2010). Adhvaryu et al., (2000) reported that as the oxidation reactions proceed, products formed lead to oil thickening. This was explained that van der Waals interactions in the oil molecules increased because of the disappearance of the double bonds (Zanardi et al., 2008). The alteration of the unsaturated bond influences the mobility and the reactivity of the molecules in the reaction. The reaction of ozone with unsaturated fatty acids generates different types of peroxidic compounds as explained by the Criegee mechanism. Van der Waals interactions of these peroxidic compounds increase to form species having high molar masses. Therefore, ozonation of double bond in oils increase the viscosity, due to those high molar mass species. However, the viscosities of untreated and ozone treated HO was not observed to change with respect to storage time. Sega et al., (2010) reported that double bonds of sesame oil decreased and peroxide value and viscosity of sesame oil increased with ozone treatment.

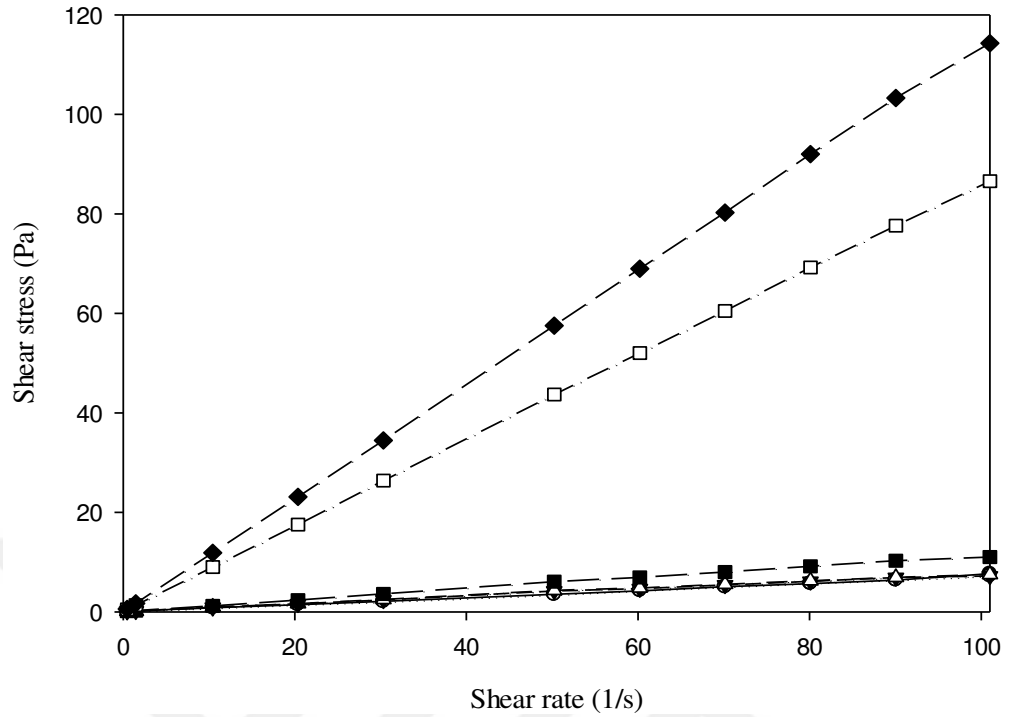


Figure 4.7 Curves of shear stress vs. shear rate of untreated and ozone treated hazelnut oils.(●; HO; ○; OHO1, ▼; OHO5, Δ; OHO30, ■; OHO60, □; OHO180, ♦; OHO360.

Figure 4.8 shows that changes in viscosities of untreated and ozone treated hazelnut oil samples as a function of storage time up to 120 days. As shown in the figure, the viscosity values of untreated and ozone treated hazelnut oil samples cannot change as a function of storage time, dramatically. It could be evaluated that the change in viscosity of ozone treated hazelnut oil was related with amount of unsaturated fatty chain. Therefore, it might be said that the amount of double bonds of ozone treated oils could not change as a function of storage time. However, the change in viscosity as a function of storage time did not show a correlation with the change in PV and FFA. As mentioned in previous section, PV and FFA values of ozone treated oils increased as a function of storage time. As a result, it can be evaluated that ozone chemically reacted with the unsaturated double bond in the hazelnut oil during ozone treatment process. The oxidation reactions can proceed with storage time, mostly leading to form several oxygenated compounds. Hydroperoxides known as primary oxidation products can be produced firstly and then they decompose to secondary oxidation products (aldehydes, ketones, alcohol, and hydrocarbons). Adhvaryu et al., (2000) reported that as the oxidation reactions normally progress, oxidation products to form viscous materials, leading to oil

thickening. In addition, during oxidation reactions, oleic acid which is predominant unsaturated fatty acid in hazelnut oil was broken; results in the hazelnut oil become saturated (Souza et al., 2004).

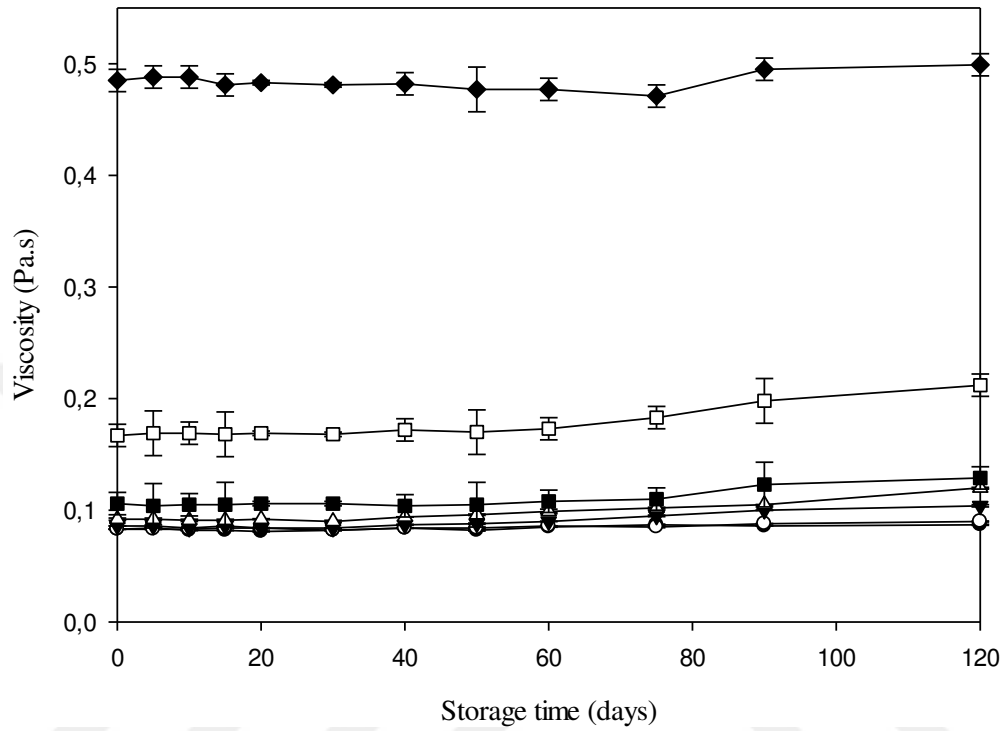


Figure 4.8 Changes in viscosity values of untreated and ozone treated hazelnut oils as a function of storage time (●; HO; ○; OHO1, ▼; OHO5, Δ; OHO30, ■; OHO60, □; OHO180, ♦; OHO360).

The fatty acid composition of untreated and ozone treated hazelnut oil were analysed by gas chromatography. Hazelnut oil, as expected, was composed of a high amount of monounsaturated fatty acids especially oleic acid (79.15%), and also polyunsaturated fatty acids, particularly linoleic acid (11.43%) and small amount of gandoic acid (0.181%) (Table 4.12). As shown in this table, the major unsaturated fatty acid of hazelnut oil was oleic acid ($C_{18:1}$). According to chromatograms, it could be determined that composition of unsaturated fatty acid of the hazelnut oil decreased with ozone treatment time. Figure 4.9 shows relationship between viscosity and oleic acid composition. The viscosity values of control and ozone treated oils were plotted against percentages of oleic acid found that in oils. It was obtained that there was a highly positive correlation between them (R^2=*0.951*). The result demonstrated that oleic acid composition of hazelnut oil had more effect on the flow behaviour characteristic of oil. Especially, an increase in the viscosity values

was observed when oleic acid composition of hazelnut oil reduced with ozone treatment time. The increases in viscosity values were 0.085, 0.106, 0.169, and 0.485 Pa.s and the changes in fatty acid composition of oils were 79.15, 72.90, 45.80, and 10.81 % for control and ozone treated oils for 60, 180 and 360 min, respectively. Therefore, it could be said that viscosity values of hazelnut oil increased 5.7 times with ozone treatment for 360 min. In previous studies, it was reported that viscosity of oils depends on the unsaturated fatty acid composition of them. They reported that viscosity of oils decreased with a rise in unsaturated fatty acid content of oils (Kim et al., 2010). Since, unsaturated fatty acid contains one or more double bond in its fatty acid chain. Double bonds may be in either a cis or a trans form related with the geometry of the double bond. This bonds which form a twist in the fatty acid chain do not allow fatty acids to come together. Consequently, fatty acids which contain double bonds have a lower melting point. Skalska et al., (2009) stated that viscosity of ozone treated sunflower oil increased with ozone treatment process. Furthermore, the viscosity could be related with dimensions and orientation of oil molecules. The decrease in unsaturated bonds present in the oil contributed to an increase in viscosity of ozone treated oils (Sadowska et al., 2008).

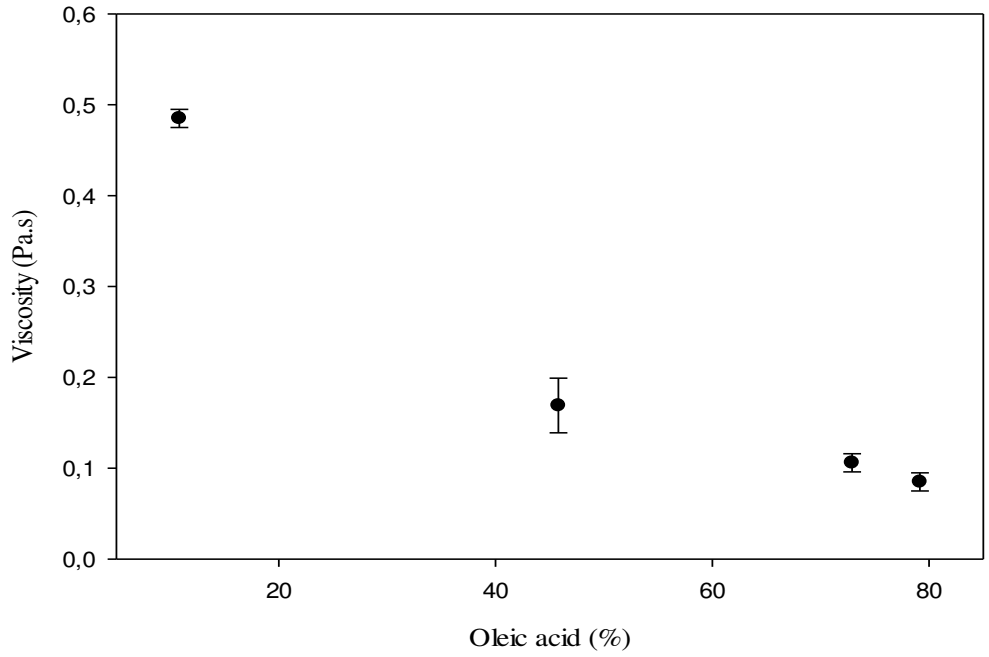


Figure 4.9 Correlation of oleic acid (C18:1) composition of untreated and ozone treated oils with viscosity (R^2=0.951)

4.4 Hunter colorimeter

Hazelnut oil is a natural material which includes fatty acids, pigments, vitamins, water soluble and volatile compounds. Hazelnut oil contains high amount of oleic acid and a small amount of linoleic compared to other nut oils. It contains a unique amount of vitamin E (α-tocopherols) and sterols (Alasalvar et al., 2003). Carotenoids in nut oils are β-carotene, lutein+zeaxanthin (King et al., 2008). In previous studies, it was reported that the main pigments of olives are the chlorophylls and the carotenoids and refined palm oil contains β -carotene and vitamin E (Cloo et al., 1993; Poku, 2002). Carotenoid pigments which give red or yellow colour to the oils contain one or more double bonds. Carotenoids are composed of a polyenic chain which contains double bonds from 3 to 15(Melendez-Martinez et al., 2007). The carotenoids can be influenced reactions of oxidation and hydrolysis because of including unsaturated polyenic chain. The trace amounts of several compounds which have a low molecular weight are formed resulting from the oxidation of carotenoids. Depending on the concentration of ozone, the percentage decay of the β-carotene increases due to possible Criegee mechanism.

Table 4.3 L*, a*, b*and Y1 values of untreated and ozone treated hazelnut oils.

Ozone treatment time (min)	Hazelnut oil			
	L*	a*	b*	Y_1
0	63.37±2.2a	-3.18±0.2a	57.16±1.5a	93.23±2.4a
1	61.54±2.5a	-3.25±0.5a	28.52±1.8b	56.83±0.5b
5	58.75±3.7a	-3.71±0.5a	15.22±0.8c	32.74±3.21c
30	57.56±2.3a	-3.28±0.8a	13.13±0.7d	29.05±1.54d
60	58.34±3.2a	-2.61±0.5a	9.06±1.3e	20.06±2.21e
180	57.99±2.7a	-2.33±0.8a	7.37±0.9f	16.26±1.56f
360	61.44±3.2a	-2.61±0.7a	2.63±1.3 g	13.32±1.32g

Each value in the table represents the mean ±standart deviation of triple analyses. Letters show that comparison of changes in colour parameters with ozone treatment time.

Table 4.3 and Figure 4.10 showed that changes in colour parameters (L*, a*, b*and Y1 values) of untreated and ozone treated hazelnut oils. As illustrated in table 4.3 and figure 4.10, it was observed that b* (b+ yellowness and b- blueness) and Y_1 (yellowness index) values of oil samples reduced with ozone treatment time, dramatically when a* (a+ redness and a- greenness) values increased with ozone treatment time, slightly. L* (lightness) values changed with ozone treatment time slightly. The statistical results showed that changes in b* and Y_1 values was significantly different ($p<0.05$) with ozone treatment time, but, it was determined that there were no significant ($p>0.05$) changes in a* and L* values with ozone treatment time. Therefore, It may arise from the pronounced effect of ozone treatment on carotenoid pigments which give yellow colour of the oils. The colour of

hazelnut oil and turned pale as ozone treatment time increased. The loss in the colour of oils was more evident after 360 min ozone treatment. This was correlated well with previously reported studies. Carotenoids in nut oils are β-carotene, lutein, zeaxanthin (King et al., 2008).

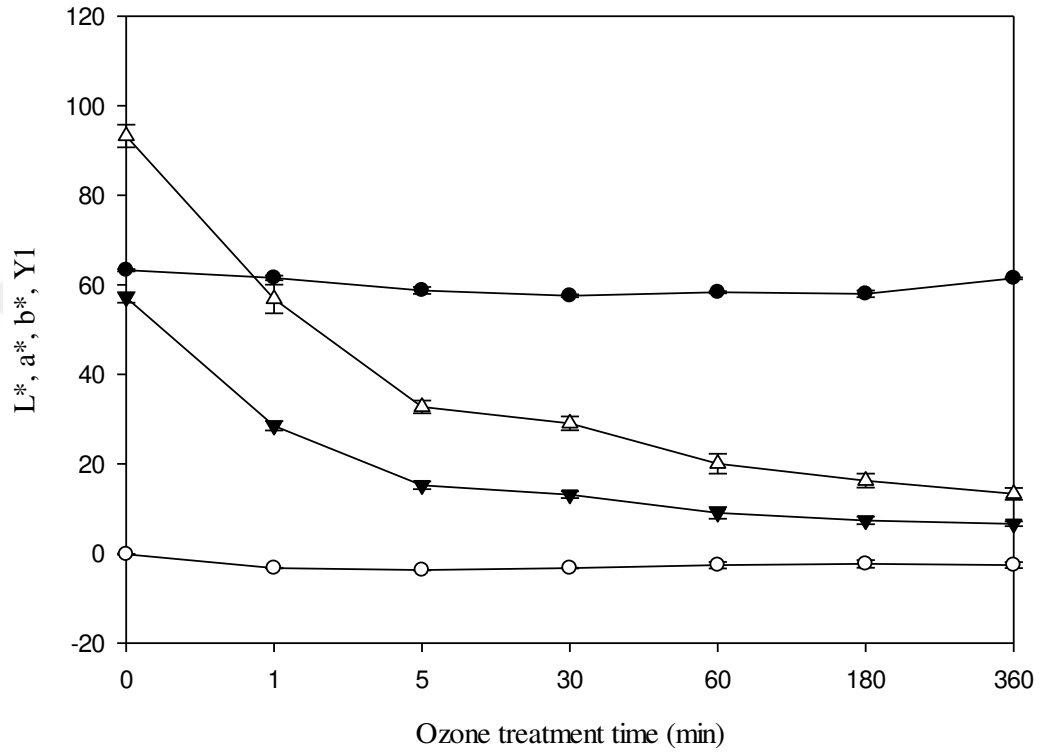


Figure 4.10 Changes in color parameters of untreated and ozone treated hazelnut oils with ozone treatment time (●; L*, ○; a*,▼; b*, Δ;Y1).

4.5 DSC kinetics

Oxidative stability of ozone treated hazelnut oil was evaluated using kinetic parameters, the rate constant (k), the activation energy (E_a), activation enthalpy ($\Delta H^{\ddagger}$), activation entropy ($\Delta S^{\ddagger}$). The ozone treatment process was carried out at 20°C, because the solubility of oxygen decreases by almost 25% for each 10°C rise in temperature (Tan et al., 2001). Oleic acid is the main unsaturated component in the hazelnut oil. The ozone treatment time for complete consumption of double bonds has been reported to be 2.26 h for oleic acid, and up to 20 h for oils (Sadowska et al., 2008). For the ozone oxidation to be accomplished, the samples were kept in a dark environment for 20 hours at 20°C, before the analyses were conducted. The stability of ozone oxidized hazelnut oil to thermal oxidative decomposition was carried out at

high isothermal temperatures in DSC. The oxygen was kept in large excess providing that the concentration of it was very high compared to the amount of oil. The substrate concentration remains nearly constant for the reaction allowing the assumption that the reaction is a first order reaction which is an essential assumption for the calculation of kinetic parameters (Micic et al., 2015). DSC method allows the detection of energy necessary for the transfer of oxygen molecule to an unsaturated fatty acid which was observed as an exothemic change. The oxidative stability of the oils was estimated on the basis of the oxidation induction time (T_0) (Tan and Che Man, 1999a) detected from the resulting thermogram at different isothermal temperatures (373, 383, 393, and 403K). Figure 2.8 shows the curves of oxidation at which the induction period was observed as the intersection of the extrapolated baseline and the tangent line to the leading edge. Thermal oxidation rate constant (k) of untreated and ozone treated hazelnut oil samples were determined as the reciprocal of induction time, considering that T_0 is proportional to the effect of temperature on the rate of thermal lipid oxidation as the concentration is assumed to be constant.

Table 4.4 The reaction rate constant (k) of untreated and ozone treated oils different temperatures.

	Reaction rate constant at different isothermal temperatures, **k ($x10^3$ min^{-1})**			
	373 K	383 K	393 K	403 K
HO	1.80±0.05a,a	3.70±0.2b,a	7.80±0.3c,a	16.90±2.1d,a
OHO1	2.07±0.7a,b	4.12±0.9b,b	8.28±1.2c,b	16.94±1.5d,b
OHO5	7.02±0.8a,c	14.11±2.3b,c	28.20±0.8c,c	56.49±2.7d,c
OHO60	12.80±1.1a,d	25.68±3.2b,d	51.65±0.9c,d	103,62±4.2d,d
OHO180	17.52±0.6a,e	35.10±2.1b,e	68.77±0.8c,e	137.36±3.9d,e

Each value in the table represents the mean ± standard deviation of triplicate analyses. First letter shows that comparison of k values of untreated and ozone treated samples individually, for each temperature, second letters shows that comparison of k values of the untreated and ozone treated samples with each other's for each temperature.

Table 4.4 shows the change in *k* values of untreated and ozone treated hazelnut oils at isothermal temperatures applied in DSC. Those *k* values of oil samples were analysed statistically with respect to isothermal temperature and with respect to ozone treatment time separately, and found to be significantly different ($p<0.05$). Also, there was statistically no interaction between isothermal temperature and ozonation period ($p<0.05$). As seen in Table 4.4, a dramatic increase in rate constant was observed as the isothermal temperature was increased for each ozonated sample. *k* values also showed an exponential rise with the increase of ozone treatment time. At 373 K, *k* value increased almost 10 times with ozone treatment of 180 minutes. Consequently, it was determined that rate of oxidation reaction can be increased with ozone treatment time. Ozone reacts with double bonds of the oils and causes oxidation of unsaturated fatty acids. Cataldo (2003) stated that ozone attacks the unsaturated fatty acids present in oils with the possible consequent lipid peroxidation. It has been detected that unsaturated fatty acid composition of the oils decreases with ozone treatment (Thurgood et al., 2007). Generally, edible oils including high amount of unsaturation exhibits more sensitivity to oxidation. The range of oleic acid (C18:1) content of hazelnut oil has been found ranging from 73.48% to 81.57% (Balta et al., 2006). However, untreated hazelnut oil showed that higher oxidative stability than ozone treated oils, even though it is composed of a high quantity of oleic acid and it is commonly known for its resistance to oxidative degradation. This resistance is likely related to it's the large quantity of α-tocopherol composition. The α-tocopherol which is a natural antioxidant is found in nuts oils (Parcerisa et al., 2000). Although it has been known that a higher level of unsaturation favours oxidation process, presence of antioxidants plays an important role in the rate of lipid oxidation. Tocopherols are very efficient antioxidant agents and give hydrogen on the hydroxyl group to lipid peroxyl radical. α-Tocopherol hinders the propagation step of lipid oxidation and retard oxidation rate. Researchers investigated the tocopherol content of oil which is extracted from different nuts and reported that hazelnut oil has highest quantity of tocopherol content (33.1 mg a-tocopherol equivalents/100 g oil) (Kornsteiner et al., 2006). Therefore, the chemical composition of the oil influences its oxidative stability. The influence of α-tocopherol as an antioxidant is associated with the concentration of tocopherol in the food material. At low concentrations, it exhibits antioxidant behaviour, but at high concentrations an act as a prooxidant (Jung and Min, 1990) which is clearly

demonstrated by the increased oxidation rate during the induction period, but this situation may or may not be related with the length of the induction period. The behaviour was suggested to be evaluated as the ratio of induction period in the presence and absence of the antioxidant, which is more critical, and the ratio of the rate of oxidation in the presence and absence of the antioxidant (Kamal-Eldin and Budilarto, 2015). The proposed mechanisms for the prooxidant behaviour of tocopherol at high concentration are chain transfer reaction by the tocopheroxyl radical to abstract a hydrogen atom from fatty acid or hydroperoxide, autoinitiation by the decomposition of hydroperoxide, formation of intermediate radicals, and reactions of the oxidized products of tocopherol (Chapman et al., 2009). The loss of antioxidant efficacy and the reduction in the rate of oxidation reaction may make the evaluation of lipid oxidation by kinetic approach difficult. However, in the interpretation of the oil oxidation stability through kinetic parameters few assumptions have been taken into consideration. The threshold concentration of tocopherol for the prooxidant behaviour is 1 mmole/L, which is more than the tocopherol content of the hazelnut oil (Naumov and Vasil'ev, 2003). The reaction rate between α-tocopherol and a radical is 10000 times greater than that between the radical and another lipid molecule and heating at high temperature caused rapid tocopherol degradation (Chapman et al., 2009), which increases the probability of tocopherol degradation mainly in the ozonolysis of oil rather than in the thermal oxidation by DSC. Therefore, the results presented exhibits a good approximation for the prediction of the oxidative stability of the ozone treated hazelnut oil. It has been detected that unsaturated fatty acid composition of the oils decreases with ozone treatment (Thurgood et al., 2007). This has been confirmed by the measurement of IV in our study and it was found that IV of untreated oil and 1,5,60 and 180 min ozone treated oil were 95.47, 72.08, 65.09, 22.30 and 18.27 (g/100g oil), respectively. However, a discrepancy was observed between the IV and the oxidation reaction rate constant obtained by thermal analysis. Although IV of untreated hazelnut oil was high, it showed almost 10 times slower oxidation rate as compared to 180 min ozone treated hazelnut oil (Table 4.4). This may be explained by the presence of a high FFA content which was exposed due to partial hydrolysis taking place during the ozonation process (Skalska et al., 2009). This was also evidenced by increased FFA in ozonated samples (Table 4.1). It has been (Tan et al., 2001) found that free fatty acids are more prone to oxidation compared to fatty acids bound to

glycerol backbone. Furthermore, Kang and Dasgupta (1999), informed that oxidation is a very complex process at which generation of several oxidation products including several intermediates. The oxidation rate constant of these compounds are different from each other. Thurgood et al., (2007) stated that the relationship between oxidation rate in lipids and degree of unsaturation of fatty acids present in the oil was not straightforward. They reported that there might be several interactions between different fatty acids responsible for the lack of linearity observed in the oxidation kinetics of complex lipid mixtures. Therefore, it is not true to measure the extent of oxidative degradation in terms of a single parameter, i.e. unsaturated fatty acids. We have noticed that chemical standard analyses have important effect on the oxidation reactions.

Table 4.5 Changes in kinetic constants for ozone treated (1 min) hazelnut oil with storage period.

Time (day)	OHO1						
	k x 10^{-3} (min^{-1})				E_a (kJ mol^{-1})	$\Delta H^{\ddagger}$ (kJ mol^{-1})	$\Delta S^{\ddagger}$ (J $K^{-1}mol^{-1}$)
	373 K	383 K	393 K	403 K			
0	2.07±0.05	4.15±0.5	8.28±0.07	16.68±0.31	86.72±0.25a	83.51±0.20a	-74.41±0.91a
3	2.15±0.08	4.31±0.5	8.64±0.13	17.42±0.27	86.91±0.33a	83.70±0.37a	-73.56±0.55a
14	2.28±0.07	4.57±0.5	9.16±0.09	18.28±0.55	86.58±0.41a	83.36±0.42a	-73.98±1.32a
30	3.99±0.1	7.95±0.5	16.05±0.24	31.54±0.62	86.25±0.37a	83.02±0.35a	-72.87±0.75a
60	4.75±0.05	9.48±0.5	18.97±0.23	37.97±0.36	86.52±0.35a	83.30±0.18a	-73.13±0.34a
90	4.84±0.09	9.71±0.5	19.49±0.12	38.81±0.47	86.68±0.45a	83.46±0.23a	-73.21±0.15a

Each value in the table represents the mean ± standard deviation of double analyses. Abbreviations: k, reaction rate constant; E_a, activation energy; $\Delta H^{\ddagger}$, enthalpy of formation of the activated complex; $\Delta S^{\ddagger}$, entropy of formation of the activated complex. The letters show that comparison of each kinetic parameter as a function of storage time.

Table 4.5 shows the change in k, E_a, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ of ozonated hazelnut oil as a function of storage time. The kinetic parameters were calculated for oil samples ozonated for 1 min and stored up to 90 days. Untreated and treated oil samples were stored in hermetically sealed glass bottle and dark place at room temperature. Samples were analysed by DSC at specific time intervals (0, 3, 14, 30, 60, and 90) at different isothermal temperatures (373, 383, 393, and 403K). Based on Arrhenius equation, the slope of plots ln k versus 1/T gives the activation energy divided by the molar gas constant, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values of ozonated oils were determined by regressing ln (k/T) versus 1/T plot via Eyring equation. As seen in table 4.5, the statistical analysis of kinetic parameters didn't show a significant difference (p>0.05). The change in E_a, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ during storage for 90 days were in the range of experimental error. Contrary to observations in PV and FFA which were observed to increase during storage (Table 4.1), kinetic parameters indicated that the storage period did not affect the temperature sensitivity, rate of oxidation reaction, and hence the quality change or deterioration of ozone treated oil samples. This may be related with the composition of oil sample that the oxidation that was detected by DSC might reflect more the extent of unsaturation rather than the formation of oxidation products.

Table 4.6 Kinetic constants for untreated and ozone treated hazelnut oil at different ozone treatment times.

Ozonation time (min)	E_a (kJ mol^{-1})	$\Delta H^{\ddagger}$ (kJ mol^{-1})	$\Delta S^{\ddagger}$ (J K^{-1} mol^{-1})
0	91.44±0.21a	88.33±0.34a	-75.03±0.30a
1	87.72±0.10b	84.51±0.35b	-74.41±0.27b
5	86.83±0.14c	83.42±0.45c	-64.15±0.15c
60	85.03±0,20d	81.88±0.25d	-63.72±0.33d
180	84.56±0.15e	80.34±0.18e	-62.34±0.52e

Each value in the table represents the mean ± standard deviation of triplicate analyses. Abbreviations: Ea, activation energy; $\Delta H^{\ddagger}$, enthalpy of formation of the activated complex; $\Delta S^{\ddagger}$, entropy of formation of the activated complex. The letters show that comparison of each kinetic parameter with ozone treatment time.

Table 4.6 shows change in kinetic constants (E_a, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$) for untreated and ozonated hazelnut oils with ozone treatment time (1, 5, 60 and 180 min) for studied DSC thermogram at different temperatures (373, 383, 393 and 403 K). Fitted data has a high correlation for determination ($R^2 > 0.96$) indicating that temperature dependent oxidation of ozonated hazelnut oil could be characterized by activation complex theory. Statistically, kinetic parameters were significantly different ($p < 0.05$) with respect to ozone treatment time. E_a of ozone treated oils showed a reducing trend as the ozone exposure period increased. E_a value represents the minimum energy required to start oxidation reaction (Laidler, 1987). This result indicates the increased oxidation sensitivity of oils after ozone treatment either due to partial hydrolysis of triglycerides or by destruction of compounds having high antioxidant activity. In a prior work, it was determined that the activation energy for oxidation of sunflower oil was higher as the amount of artificial antioxidants were increased (Souza et al., 2004). The lower activation energy means less energy required for oxidation products to form. $\Delta H^{\ddagger}$ of hazelnut oil were observed to decrease with increased ozone exposure. $\Delta H^{\ddagger}$ represents the difference in energy between ground state and the transition state in a chemical reaction. The higher activation enthalpy means the more energy required for the products to form in activated state (Atkins and De Paua, 2006). In general, a reaction will take place faster, if the value of $\Delta H^{\ddagger}$ and E_a are low. $\Delta S^{\ddagger}$ is the difference between entropy of transition state and the sum of entropies of the reactants. In bimolecular reactions, when a complex is formed by association of two individual molecules, there may be a loss of transitional and rotational freedom. More often, there is a decrease in entropy in passing to activated state leading to a negative $\Delta S^{\ddagger}$ (Moore, 1972). However, an increase in $\Delta S^{\ddagger}$ was observed as the ozone exposure was increased indicating an increase in disorderness in activated complex state. This can be evaluated that activated complex state for lipid oxidation in ozone treated hazelnut oil is more probable and oxidation reaction rate is faster. These results were correlated well with change in k values. It could be concluded that increasing of k and $\Delta S^{\ddagger}$ and decreasing of E_a and $\Delta H^{\ddagger}$ indicated a faster oxidation reaction when ozone exposure time of hazelnut oil was extended.

Table 4.7 Predicted T_0 values at room temperature (298 K).

ln k vs. 1/T (temperature)			
Ozonation time (min)	Regression equation [a]	Correlation determination, R^2	Predicted T_0 at room temperature (298 K) (day)
0	ln$k$=-11000(1/T)+23.150	0.9995	659
1	ln$k$=-10431(1/T)+21.769	0.9989	388
5	ln$k$=-10436(1/T)+23.004	0.9995	115
60	ln$k$=-10227(1/T)+23.052	0.9995	54
180	ln$k$=-10171(1/T)+23.221	0.9995	38

T_0: oxidation induction time.

Table 4.7 provides the regression parameter for relationships between reaction rate constant (k) and temperature for oil samples treated with ozone at different times. As shown in the Table 4.5., ln k values exhibit linear relationship with isothermal temperatures. From the regression equations, firstly k values were calculated for a given temperatures. Furthermore, the regression equations could be used for predicting T_0 values of untreated and ozone treated hazelnut oils at room temperature (298 K). This parameter may be of interest for determining effects of ozone treatment on the oxidative stability of oils without effects of isothermal temperature. The T_0 values of untreated and ozone treated hazelnut oils at room temperature (298 K) were predicted from the regression equations are also given in Table 4.5. As shown in Table 4.5, the predicted T_0 values for untreated oil were 659 days and these values decreased with ozone treatment time. It was found that the predicted T_0 values for untreated oil were almost conformed to shelf life mentioned in Turkish Standard for hazelnut oil. According to Turkish Standard, it was stated that shelf life of hazelnut oil was maximum 2 years (Turkish Standard 6581, 2003). The predicted T_0 values for hazelnut oils treated with ozone for 1,5, 60 and 180 minutes were 388, 115, 54 and 38 days, respectively. From Table 4.7, it was said that ozone is more powerful oxidizing agent. The oxidation effects of ozone on the oil samples increases when ozone treatment time increased. Gusel-Seydim et al., (2004) reported that ozone is the strong oxidizing agent. The oxidation potentials of fluorine, ozone, permanganate, chlorine dioxide, hypochlorous acid and chlorine gas are 3.06, 2.07, 1.67, 1.50, 1.49 and 1.36 respectively. Predicted induction times of 10 different oils have been estimated through regression equations found by fitting the experimental data in accelerated shelf-life testing (Tan et al., 2001) in a previous work and it has been stated that the estimated values at low temperatures can be

considered as approximate values. Different reaction pathways that are followed by the lipid oxidation at high or low temperatures may be the reason together with the effects of oxygen solubility, metal catalysts and antioxidants (Thurgood et al., 2007). Moreover, the ambient conditions in storage would have a limited oxygen concentration in comparison to the oxidation stability tests conducted with 99.5% untreated oxygen. Thus, predicted T_o may reflect uncertainties which are not included in the regression equation.

Table 4.8 Pearson correlation coefficient between chemical methods and oxidation reaction rate constant[a].

	Reaction rate constant	Peroxide value	Free fatty acid value
Reaction rate constant	1.000	0.941	0.951
Peroxide value	0.941	1.000	0.977
Free fatty acid value	0.951	0.977	1.000

[a] Significance at 0.05 level (P<0.05).

Table 4.9 Relationship between oxidation reaction rate and indicator of oil oxidation[a].

Indicator (Y)	Regression equation Reaction rate constant (X)
Peroxide value	Y= 0.941X+3.588
Free fatty acid value	Y= 0.951X+0.098

[a] Significance at 0.05 level (P<0.05).

As seen in Table 4.1 and 4.4, FFA and PV and reaction rate constants showed an increase with ozone treatment time. However, FFA indicated a slight and gradual increase when a dramatic increase in PV and reaction rate constant was observed. Berger (1984) reported that the measurement of FFA in the oil could not determine suitability of heated oils for further use. The PV was increased dramatically when oxidation reaction could be continued with ozone treatment time (Table 4.1). Since peroxidic compounds are the main products that are formed from oxidation reactions of the oils (Guillen and Cabo, 2002). It has been suggested that a high correlation existed between each of DSC parameters (peak temperature and enthalpy) and standard chemical methods including FFA, PV which are used to estimate the deterioration of edible oils (Tan and Che Man, 1999b).The correlation coefficients between reaction rate constant calculated from DSC thermogram and standard

chemical methods were shown in Table 4.8. The rate constant of oxidation reaction showed excellent correlations with the standard chemical methods. The coefficients of correlation for each comparison were also highly significant ($p<0.05$). As seen in Table 4.9, we noticed a considerable correlation between FFA, PV and *k*. The experimental results showed that FFA increased with the increase in reaction rate constant as the ozone treatment time was increased. Similarly, PV showed a dramatic increase with *k* as the ozone treatment time was extended. Regression equations and the respective correlations between FFA and *k* (Y= 0.951X+0.098 with R^2=0.961) and PV and *k* (Y= 0.941X+3.588 with R^2=0.952) were verified by establishing linear regression equations at isothermal temperature of 373 K. A high correlation between *k* and FFA and PV reveals that DSC is an appropriate method for determining the extent of oxidation in oils. It can be used as a reliable, rapid method for detecting the extent of lipid oxidation.

4.6 DSC heating and cooling thermogram

DSC heating thermograms of untreated and ozone treated hazelnut oils are shown in Figure 4.11. DSC heating thermograms of vegetable oil which overlapping melting peaks of various polymorphic forms of crystals are usually more complicated than cooling thermogram (Tan and Che Man, 2000). As seen in Figure 4.11, untreated hazelnut oil heating thermograms showed a well-defined endothermic peaking at -5.6^{0}C. Previous DSC reports indicated that hazelnut oil had melting peak at -6.1^{0}C (Xu et al., 2007). Previous researches informed that there is either single melting peak (Tan and Che Man, 2000) in DSC heating thermogram of hazelnut oil, or a wide endothermic event consisting of four overlapped peaks. The overlapped peaks are related to the melting of triacylglycerol (TAG) (Tand Che Man, 2002). Differences in melting profile of hazelnut oil can be observed because of different experimental conditions and /or DSC instrumentation and / or nature of the sample. The heating thermograms of hazelnut oil showed some variations which were more pronounced from 30 min ozone treatment onwards. The endothermic peaks slightly shifted towards lower temperature. The melting curve of hazelnut oil samples became broader and less evident from 30 min ozone treatment time onwards. After 180 and 360 min ozone treatment, it was observed that the melting curves became less evident or disappeared. The result have been explained in previous researches that an important rise in lipid oxidation products and free fatty acids may have

hindered crystallization of TAG, leading to the formation of distinct and unstable crystal polymorphic forms as compared to those of the untreated hazelnut oils (Che Man and Swe, 1995). These unstable crystals and molecules produced from oxidative reactions (monoacylglycerols, diacylglycerols, free fatty acids, primary and secondary oxidation products) may have probably melted at lower temperature because of their lower stability.

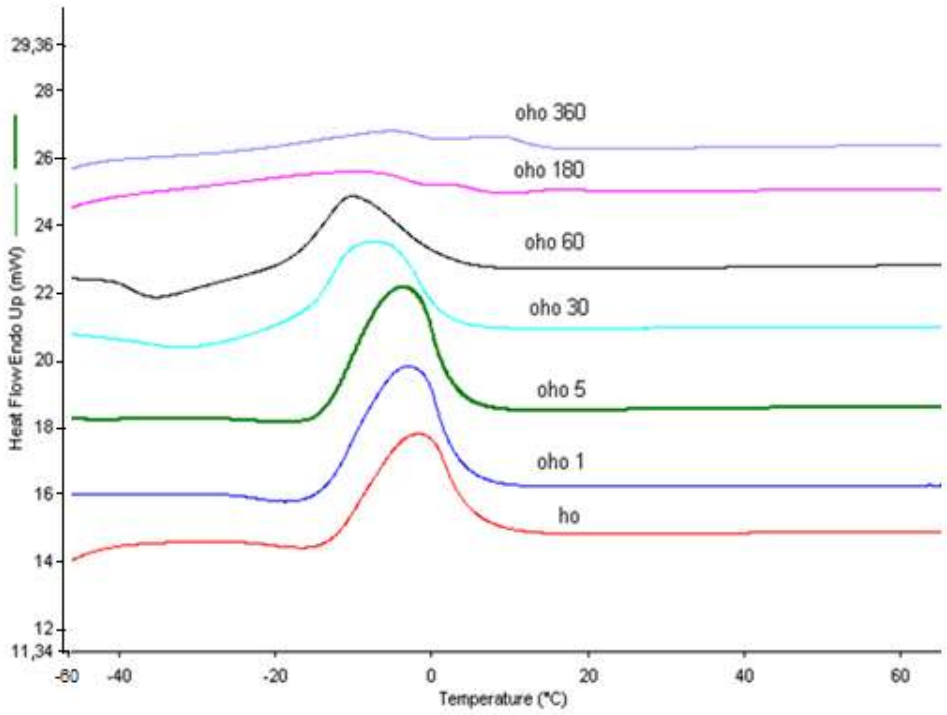


Figure 4.11 DSC heating thermogram of untreated and ozone treated hazelnut oils.

Table 4.10 DSC data obtained from the heating thermograms of untreated and ozone treated oils[a].

Ozone treatment time (min)	T_{on} (°C)	T_{off} (°C)	ΔH (J/g)	Peak height (mW)	Range (°C)
0	-12.45± 0.3a	5.14±0.5a	58.28±0.1a	3.16±0.3a	17.59±0.2a
1	-13.22±0.4b	3.60±0.4b	57.79±0.3b	3.68±0.2b	16.82±0.4b
5	-13.51±0.3b	2.91±0.4c	56.83±0.8c	3.79±0.2b	16.42±0.5b
30	-16.67±0.1c	1.94±0.3d	50.31±1.2d	2.74±0.4c	18.61±0.9c
60	-18.72±0.5d	2.02±0.4d	42.49±0.7e	2.34±0.5c	20.74±0.7d
180	-20.82±0.2e	0.90±0.2e	42.90±0.5e	1.03±0.7d	31.72±0.5e
360	-24.16±0.1f	0.65±0.1f	33.48±0.7f	0.66±0.3e	37.81±0.8f

[a] Same letters within each column do not significantly different ($p<0.05$).

Table 4.10 showed that thermal parameters obtained from DSC heating thermograms of untreated and ozone treated hazelnut oil samples. Phase transition of hazelnut oil showed a significant decrease of overall enthalpy. The enthalpy of heating transition in hazelnut oils decreased as ozone treatment time increased. The lowest enthalpy of transition in hazelnut oil was found at 360 min ozone treatment when major peak (Fig. 4.11) became smaller. This situation probably arised from formation of heterogeneous structure of TAG crystals due to significant increase of oxidation products with ozone treatment process. And also, T_{on} and T_{off} values of transition altered with ozone treatment time. T_{on} and T_{off} shifted towards lower temperatures with ozone treatment time. After ozone treatment, formation of the oxidation products which were observed from 30 min of treatment onwards may affect dramatically the production of weak TAG crystals so lower energy value required to melt (Che Man and Swe, 1995). The range of transition temperature was observed to be expanded at the 180 and 360 min of ozone treatment as the major endothermic peak became broader. In previous studies, similar results were obtained. Chiavaro et al., (2010), studied that effect of microwave heating on vegetable oils. They were determined that enthalpy of vegetable oils decreased with microwave heating time. And also, they were reported that T_{on} and T_{off} shifted towards lower temperatures at different times of microwave heating of vegetable oils.

Samples undergoing cooling exhibited an exothermic phase transition due to crystallization of hazelnut oil such as that shown in Figure 4.12 for untreated and ozone treated hazelnut oils. Cooling thermogram of untreated hazelnut oil was similar to that previously reported in researches for this type of nut oils (Tan and Che Man, 2000). The hazelnut oil cooling profile showed a major exothermic peak at about -37^0C related to the crystallization of triunsaturated TAG, and a minor exothermic peak at about -15^0C attributed to the crystallization of disaturated TAG (Tan and Che Man, 2002; Tan and Che Man, 2000). Ozone treatment process changed cooling profile of both exothermic peaks, especially the major one that shifted towards lower temperature with ozone treatment times, became also broadening over a larger temperature range and decreasing in peak height. The minor peak became less clear as ozone treatment time increased. In previous work related with cooling profiles of vegetable oils after deep frying, conventional and microwave heating treatments, it was observed that a decrease in the height of crystallization

peak as well as broadening and shifting towards lower temperature (Chiavaro et al., 2008; Tan and Che Man, 1999a; Tan et al., 2002, Vittadini et al., 2003). All these situations were related to the rise of lipid oxidation products and molecules which are free fatty acids, monoacylglycerols, diacylglycerols that may have prevented to form crystals from TAG molecules (Chiavaro et al., 2008; Tan and Che Man, 1999a; Tan et al., 2002, Vittadini et al., 2003).

As seen in Figure 4.12, sharp exothermic peak of untreated hazelnut oil was observed at -37^0C while a less defined shoulder peak was showed at -22^0C. The sharp exothermic crystallization shifted towards lower temperature with ozone treatment process and becoming broader and shorter as treatment time increased. The minor peak became less evident and apparently disappeared when ozone treatment proceeded. All these variations in cooling profile may be associated with oxidative products (i.e. free fatty acids, peroxide value, and oxidative induction time decrease (Chiavaro et al., 2008; Tan and Che Man, 1999a; Tan et al., 2002).

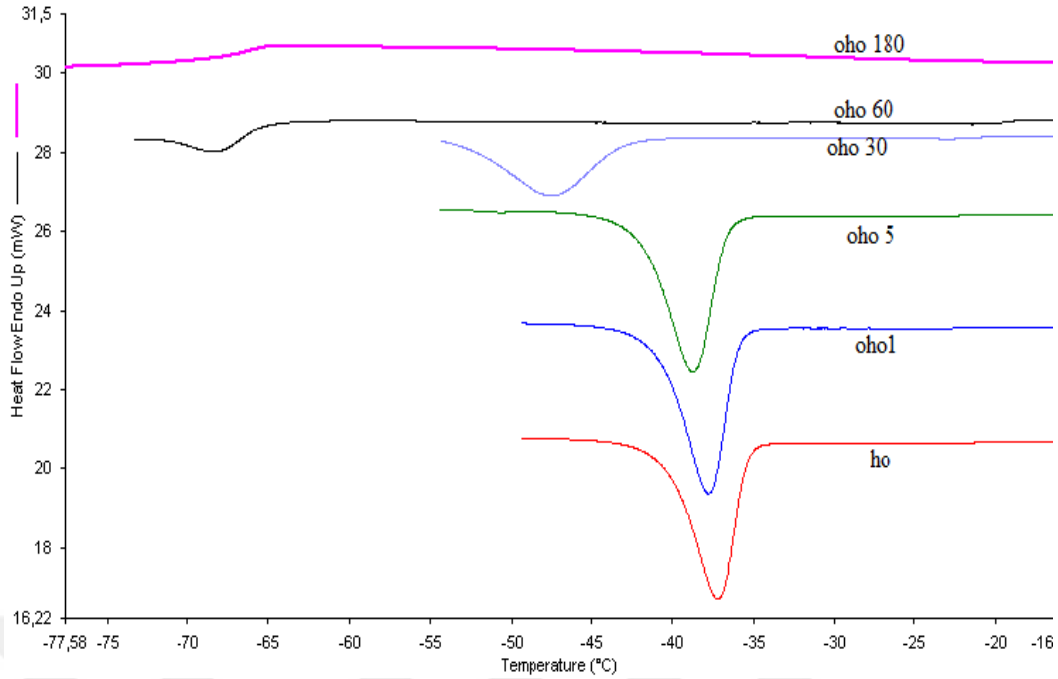


Figure 4.12 DSC cooling thermogram of untreated and ozone treated hazelnut oils.

Table 4.11 DSC data obtained from the cooling thermograms of untreated and ozone treated oils.

Ozone treatment time (min)	T_{on} (°C)	T_{off} (°C)	ΔH (J/g)	Peak height (mW)	Range (°C)
0	-35.40± 0.3a	-40.14±0.5a	-48.28±0.1a	-3.91±0.3a	4.74±0.2a
1	-35.95±0.4b	-40.74±0.4b	-47.43±0.3b	-4.16±0.2b	4.79±0.4b
5	-36.68±0.3b	-41.83±0.4c	-42.98±0.8c	-3.88±0.2b	5.15±0.5b
30	-43.06±0.1c	-52.64±0.3d	-25.99±1.2d	-1.42±0.4c	9.58±0.9c
60	--65.65±0.5d	-71.34±0.4d	-11.18±0.7e	-0.59±0.5c	5.69±0.7d
180	-----	-----	-----	-----	-----

Same letters within each column do not significantly different ($p<0.05$).

Table 4.11 showed that thermal properties of untreated and ozone treated hazelnut oils including peak enthalpy, initial and final temperature of crystallization, range of transition, peak height obtained by cooling thermogram. With ozone treatment, the oxidation products such as dimers, polymers, hydroperoxides and aldehydes formed in the hazelnut oil. As mentioned in previous works, the free fatty acids and oxidation products present in the oil caused shifting of the melting range to a lower temperature (Che Man and Swe, 1995). The similar

alterations are expected for DSC crystallization parameters. The crystallization enthalpy of hazelnut oil decreased with increasing time of ozone treatment. Samples treated for 30 and 60 min exhibited a marked decrease in peak enthalpy due to higher ozone treatment time. Also, the crystallization peaks could not be detected for 180 and 360 min ozone treated samples and the crystallization enthalpy could not be calculated. On the other hand, samples ozone treated at 1 and 5 min showed a low reduction in DSC crystallization enthalpy that indicates shorter ozone treatment of the oil cannot affect crystallization event. A decrease in crystallization enthalpy was previously observed for cooling thermogram of thermooxidised oils (Chiavaro et al., 2008; Tan and Che Man, 1999a; Vittadini et al., 2003). This situation may relate to the partial lysis of TAG and the formation of their degradation products (i.e. free fatty acids, monoacylglycerols, diacylglycerols, oxidised TAG) (Gloria and Aguilera, 1998; Tan and Che Man, 1999a). Crystallization T_{on} and T_{off} significantly shifted towards to lower temperature from 1 min ozone treatment onwards and the shift in the crystallization temperatures became more evident after 30 min ozone treatment. The shift of crystallization peak to lower temperatures and the decrease in crystallization enthalpy could be due to the depletion of TAG, the increase of the free fatty acid content, and the increase in viscosity during ozone treatment.

4.7 Gas chromatography analysis

Table 4.12 Fatty acid composition of untreated and ozone treated HO.

	Fatty acid composition (%)							
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:1
HO	-	5.31	-	2.75	79.15	11.43	-	0.18
OHO60	-	8.44	-	5.11	72.90	5.84	-	-
OHO180	-	16.07	-	8.70	45.80	1.74	-	-
OHO360	-	24.81	-	14.74	10.81	-	-	-

HO; hazelnut oil, (C16:0; palmitic acid, C18:0; stearic acid, C18:1; oleic acid, C18:2; linoleic acid, C18:3; linolenic acid, C20:1, gondoic acid)

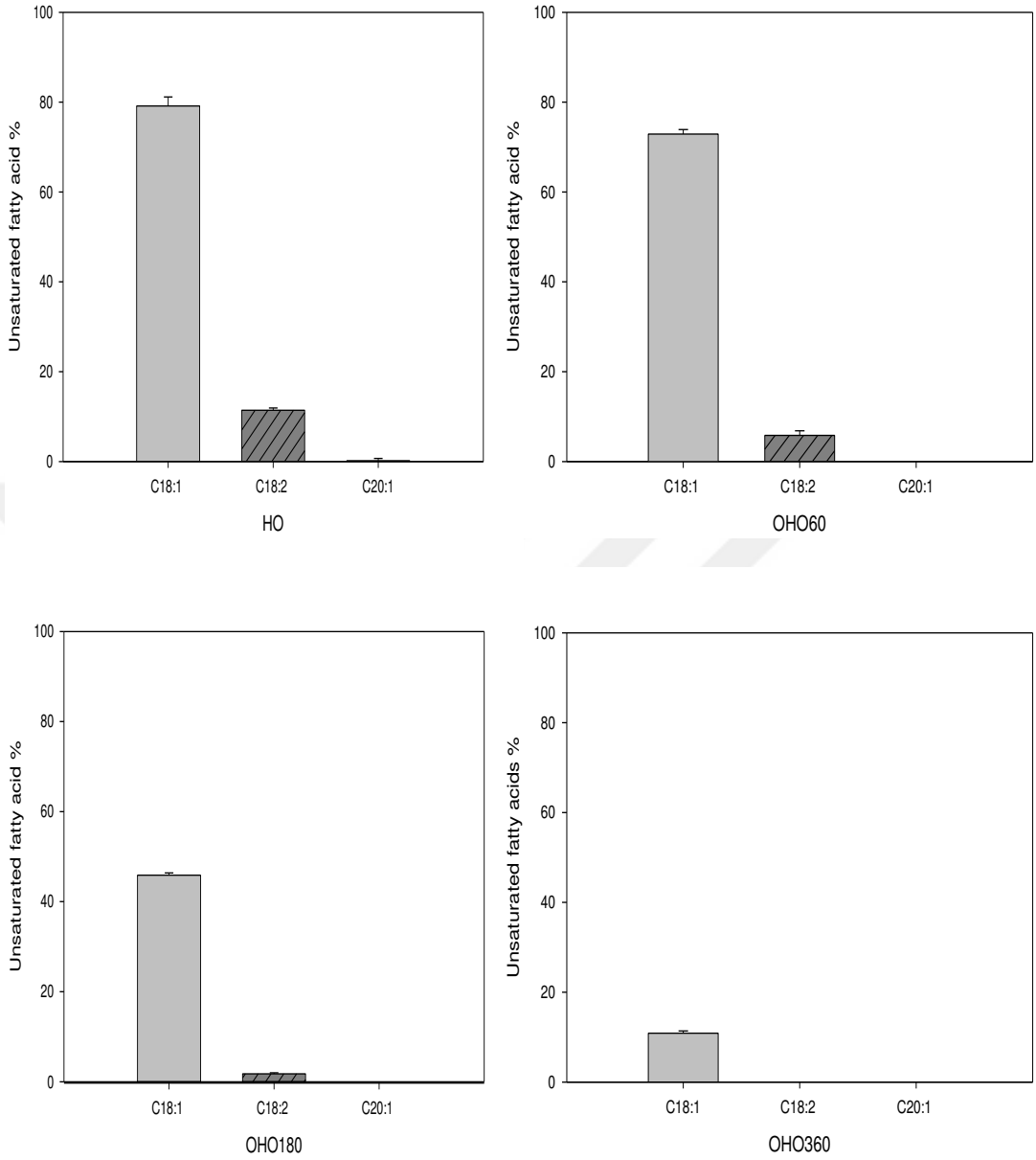


Figure 4.13 Chromatogram of fatty acids of untreated and ozone treated hazelnut oil.

Table 4.12 shows the composition of fatty acid present in untreated hazelnut oil. According to this figure, we determined that hazelnut oil was composed of 79.15 % oleic acid (C18:1), 11.43 % linoleic acid (C18:2), 5.31 % palmitic acid (C16:0), 2.74 % stearic acid (C18:0) and a small amount of gondoic acid (0.181%). Gondoic acid (11-eicosenoic acid) is a monounsaturated fatty acid found in a several types of plant oils and nuts (Miwa, 1971). From the chromatogram, it could be calculated that unsaturated fatty acid composition of untreated hazelnut oil was about 90.76 %. Similar proportions of fatty acid compositions for hazelnut oil were

stated in the previous studies. Chiavaro et al., (2008) reported that hazelnut oil contained high proportion of unsaturated fatty acids (77.23 % of oleic acid, 11.39% of linoleic acid) and small proportion of saturated ones (5.61% of palmitic acid and 3.44 % of stearic acid). Gustone et al., (1995) determined that hazelnut oil was composed of palmitic (4.7%), stearic (1.6%), oleic (76.4%), linoleic acid (16.3%). Also, it was found that palmitic, stearic, oleic, linoleic acid proportions of hazelnut oil which was extracted from hazelnuts grown in Turkey were between 4.39-8.85%, 1.67-3.18%, 73.48-81.57%, and 10.46-14.95%, respectively (Balta et al., 2006). From literature, we know that ozone reacts with unsaturated fatty acids in the oils and affects the double bonds in the unsaturated fatty acids. Cataldo (2003) stated that attack of the ozone molecules to the unsaturated fatty acids present in oils result in lipid peroxidation occur. In order to determine effects of ozone on the fatty acids, we performed gas chromatography analysis after ozone treatment process. Firstly, oil samples were treated with the ozone gas for 60, 180, 360 min. Then, untreated and ozone treated oils were converted to fatty acid methyl esters by using methanol as solvent. The percentages of fatty acid composition of untreated and ozone treated oils were compared to each other's.

Figure 4.13 showed that variations in unsaturated fatty acids present in hazelnut oil with ozone treatment time (60, 180 and 360 min). From this figure, it was determined that percentage of oleic acid and linoleic acid decreased result from oxidation reaction with ozone. The reductions in percentages of linoleic acid an oleic acid of OHO60 were about 49% and 7.9%, respectively. A decrease in the percentage of linoleic acid was higher than oleic acids. Because linoleic acid which has two double bonds in its structure is more prone to oxidation than oleic acid. However, it was observed that the percentages of saturated fatty acids (palmitic acid and stearic acid) increased after ozone treatment for 60 min. The increases in percentages of palmitic and stearic acid were found about 59% and 86% respectively. As a result, it was determined that the portions of unsaturated fatty acids in hazelnut oil reduced while the portions of saturated increased with ozone treatment. In previous study, it was concluded that oxidation causes a reduction in the unsaturated fatty acids content and an increase in the saturated fatty acid content in the oil (Liu and White, 1992).

As seen in Figure 4.13, after 180 and 360 min ozone treatment, a dramatic reduction was observed after 180 and 360 min ozone treatment. The decrease in the percentage of oleic acid was highest (~86%) and linoleic acid was destroyed completely after ozone treatment for 360 min. Furthermore, an increase in the percentages of the saturated fatty acid was reached to highest value with 360 min ozone treatment. The percentages of palmitic acid and stearic acid increased to 4.6 and 5.36 times during 360 min ozone reaction.

Table 4.13 New peaks assigned in chromatogram after ozone treatment process.

	Ozone treatment time (min)			
Number of carbon atoms belonging to new peaks (%)	HO	OHO60	OHO180	OHO360
C 4-6	-	-	0.68	1.41
C 8-10	-	4.05	16.65	33.84
C 17-18	-	-	0.60	-
C 18	-	1.67	6.47	14.36
C 24-22	-	0.87	1.28	-
C 24-22	-	-	1.16	-

Also, new peaks were observed in the chromatograms of ozone treated hazelnut oils for 60, 180 and 360 min as seen in Table 4.13. As mentioned before, oleic acid is a predominant unsaturated fatty acid present in hazelnut oil. The hazelnut contains a small amount of linoleic acid. Therefore, we suggested that the new peaks formed mainly from the reaction of ozone with oleic acid and secondly with linoleic acid. The oxidation reaction of oleic acids with ozone involves the decomposition of double bond at position nine of carbon chain. The cleaving of unsaturation has been caused to form reactive medium. In general, the oxidation reactions of unsaturated fatty acids with ozone are known as Criegee mechanism (Criegee, 1975). The reaction products are named as Criegee intermediates. The intermediates are highly reactive and some reactions including ester and acid formation and hydroperoxide formation occur. In the mechanism, 1,2,3 trioxolane which is known as primary ozonide are formed from reaction of ozone with unsaturated fatty acids. 1,2,3 trioxolane is an unstable intermediate and then can

easily decompose to 1,2,4 trioxolane (secondary ozonide) (Criegee, 1975; Atkinson and Carter, 1984). It was evaluated that the unknown peaks seen in the chromatograms might be related with these products which are formed from decomposition of oleic acid and linoleic acid present in hazelnut oil after ozone treatment.

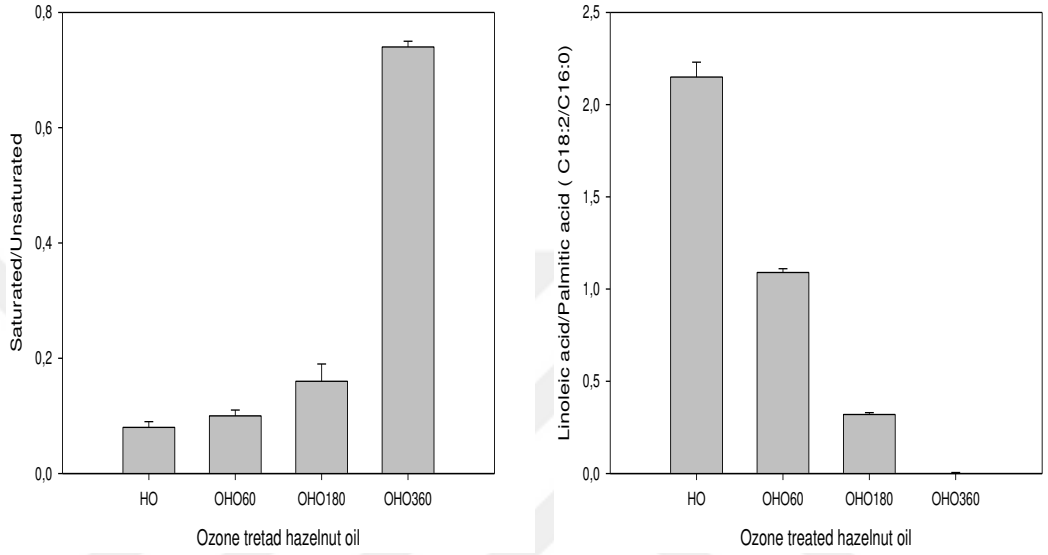


Figure 4.14 Changes in saturated/unsaturated fatty acid ratio with ozone treatment for different time (60, 180 and 360 min).

As seen in Figure 4.14, the ratio of $C_{18:2}/C_{16:0}$ decreased from 2.15 to 0.10 as ozone treatment time increased. During ozone treatment for 360 min, the ratio could not be calculated due to disappearance of linoleic acid completely. The ratio demonstrated that changes in ratio of linoleic acid to palmitic acid. Linoleic acid and palmitic acid can be used to determine extent of oil deterioration. Linoleic acid is more sensitive to oxidation while palmitic acid is more stable to oxidation. Generally, oils which are composed of higher percentages of unsaturated fatty acids are more susceptible to oxidation than those containing lesser amounts (Tan et al., 1999a). Furthermore, the ratios of saturated to unsaturated fatty acids were calculated to determine changes in nutritional value of oils with ozone treatment. From the table, it could be said that nutritional value of oils decreased as ozone treatment progressed. With ozone treatment, an increase in percentages of the saturated fatty acid occurs while decreasing of the percentages of unsaturated fatty acids. The untreated hazelnut oil had the highest nutritional value while the

nutritional value of ozone treated sample for 360 min was minimum value. Tsanev et al., (1998) reported that smaller ratio of saturated to unsaturated fatty acids considered as positive from the viewpoint of nutritional content of oils.

4.8 FTIR (Fourier transform infrared radiation) analysis

FT-IR spectroscopy measurements were performed to investigate the changes in the functional groups present in hazelnut oils during ozone treatment. As expected, intensities of all bands related with double bonds decreased with ozone treatment time. Since ozone reacts with the double bonds of unsaturated fatty acids. This reaction produces some compounds such as hydroperoxides, ozonides, aldehydes, peroxides, diperoxides and polyperoxides (Bailey, 1978; Criegee, 1975). Summary of characteristic bands found in hazelnut oil were shown in Table 4.14 and figure 4.15 showed the FTIR spectra of untreated and ozone treated hazelnut oil. As seen in the figure, there were several major peaks in the spectrum which were belonging to functional groups of triglycerides. These peaks were assigned around 2922 cm^{-1}(C-H stretching (asymmetry)), 2853 cm^{-1}(C-H stretching (symmetry)), 1743 cm^{-1} (C=O stretching),1463 cm^{-1} (C-H bending), 1377 cm^{-1} (C-H bending), 1237 cm^{-1} (C-O stretching), 1160cm^{-1} (C-O stretching), 722 cm^{-1} (C-H bending (rocking. Also, a very weak peak was observed around 1654 cm^{-1} which was corresponding to functional group of (C=C) double bond of olefins (cis).

Figure 4.15 and 4.16 showed that FTIR spectra of untreated and ozone treated HO. As seen in these figures, the weak bands corresponded to the C=C-H stretch at 3006 cm^{-1} reduced when ozone treatment time increased. In an earlier study, the higher frequency of the band (=CH stretching vibration assigned around 3006 cm^{-1}) for large proportion of polyunsaturated fatty acid content of the oil were observed than those of large proportion of monounsaturated fatty acid of the oil (Guillen and Cabo, 2000). We observed that the frequency of the band of HO reduced, sharply as ozone treatment proceeded. A dramatic reduction in the intensity of band at around 722 cm^{-1} corresponding to overlapping methylene ($-CH_2$) rocking vibration of olefins was observed as ozone treatment continued (Silverstein et al., 1974).

The band at 2922 and 2853 cm^{-1} corresponded to asymmetrical and symmetrical stretching of methylene ($-CH_2$) group of mainly unsaturated bonds in

the lipids (Dogan et al., 2007). It was observed that intensities of the bands decreased slightly during ozone treatment.

The untreated HO showed a relatively sharp band due to carbonyl stretch of triglyceride ester group at 1743 cm^{-1} (Figure 4.15 and 4.16). It could be observed that the broadening of the band and decreasing of the frequency occurred due to formation of new carbonyl compounds as ozone treatment extended (Soriano et al., 2003).

A small band assigned at 1463 cm^{-1} corresponds to the bending vibrations of CH_2 and CH_3 groups (Che Man and Setiowaty, 1999). It was detected that absorbance value of the band decreased as ozone treatment time increased (Figure 4.15 and 4.16). The bands at 1237 and 1160cm^{-1} related(=CH stretching vibration) was assigned around 3006 cm^{-1} with stretching vibrations of C-O group in esters were observed in the spectrum of HO(Guillen and Cabo, 1997).

The band at 1105 cm^{-1}, getting broader and stronger in FTIR spectrum of HO as the ozonation period increases was assigned corresponding to C-O stretch of the secondary ozonide as consistent with a previous research (Wu et al., 1992).

The bands at 1237, 1160, 1119, 1095, 1031 and cm^{-1} related with stretching vibrations of C-O group in esters were observed in all samples. Some researchers assigned the bands at 1238 cm^{-1} to bending vibration out-of-plane of methylene group (Safar et al., 1994). However, absorbance values of the bands decreased as ozone treatment proceeded. Especially, it was detected that the band at 1160 cm^{-1} shifted to 1146 cm^{-1} in spectrum of ozone treated sample for 360 min. With increasing duration of ozone treatment, the bands at 1119 and 1095 cm^{-1} became broader and relatively broad and strong band was detected at 1104 cm^{-1} in spectrum of ozone treatment for 180 and 360 min. It might be concluded that the cleavage of final ozonide was minimum. Prior studies reported that trans isomer of ozonide absorbed at around 1300 cm-1 while its cis isomer at around 800cm^{-1} (Bailey, 1978; Murray et al., 1967). The band around 1031 cm^{-1} shifted toward lower freguency values while ozone treatment time increased. After 360 min ozone treatment, the band was detected at near 966 cm^{-1}. A reduction in the intensity of band at around 722 cm^{-1} was observed as ozone treatment process continued. The band

corresponding to overlapping methylene (-CH2) rocking vibration and to the out of plane vibration of cis –disubtituted olefins (Silverstein et al., 1974).

Therefore, the variations were observed in frequency and absorbance of FTIR spectra bands after ozone treatment. It was reported that oxidation of oils leads to changes in the frequency of the same bands (Guillen and Cabo, 2000). The intensities of the bands related to double bonds in unsaturated oil decreased as ozone treatment extended. Double bonds finally disappeared during ozone treatment for 360 min. The broad and relatively strong bands corresponding to primary or secondary ozonide were observed as ozone treatment proceeded.

Table 4.14 FTIR band assignment for functional groups commonly found in an oil spectrum[a].

Frequency (cm^{-1})	Functional group assignment
3006	(=C-H) Cis double bond stretching
2922 and 2853	Asymmetrical and symmetrical stretching of methylene (-CH_2) group
1743	(C=O) Ester carbonyl functional group of triglycerides
1654	(C=C) cis double bond of acyl groups of olefins
1463	Bending vibrations of the CH_2 and CH_3 aliphatic groups
1417	Rocking vibrations of CH bonds of cis –disubtituted olefins
1402	=C-H bending vibration
1377	Bending vibrations of the CH_2 groups
1237 and 1160	C-O stretching
1119 and 1095	Stretching vibration of the C-O ester group
1031	C-O stretching
856	=CH_2 wagging
722	Overlapping methylene (-CH_2) rocking vibration and to the out of plane vibration of cis –disubtituted olefins

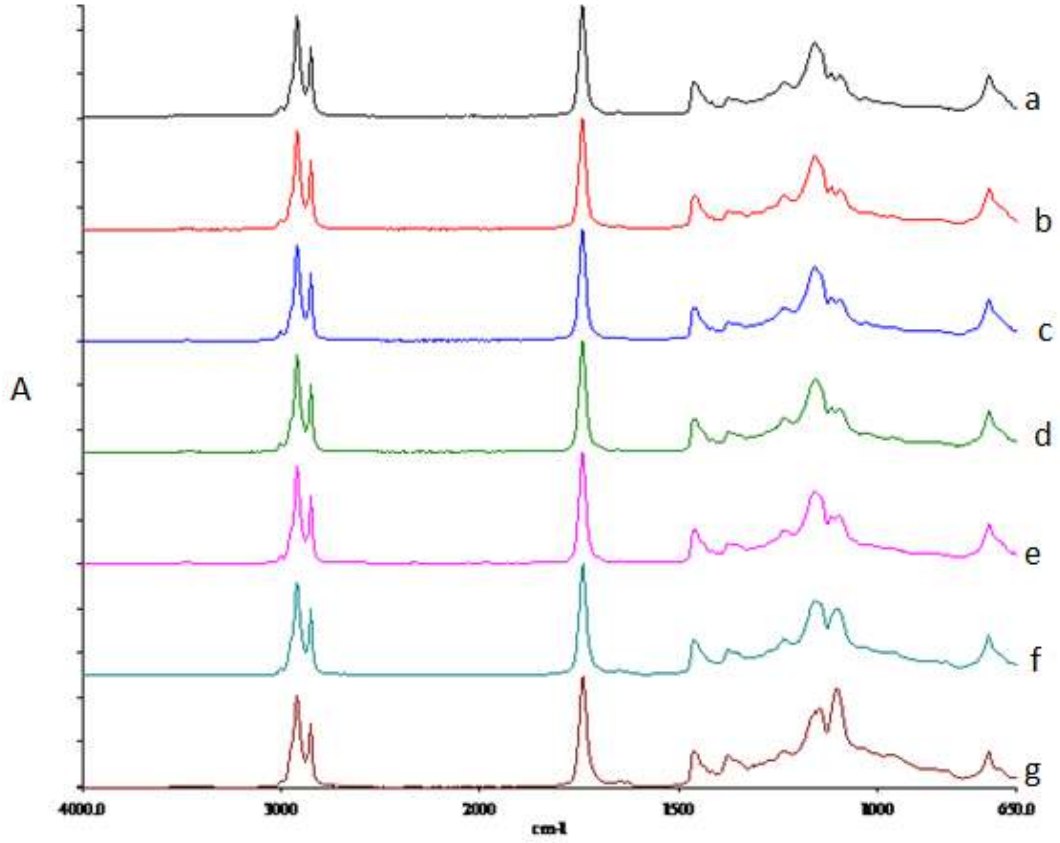


Figure 4.15 FT-IR spectra of untreated and ozone treated hazelnut oils (a: HO, b: OHO1, c: OHO5, d: OHO30, e: OHO60, f: OHO180, g: OHO360).

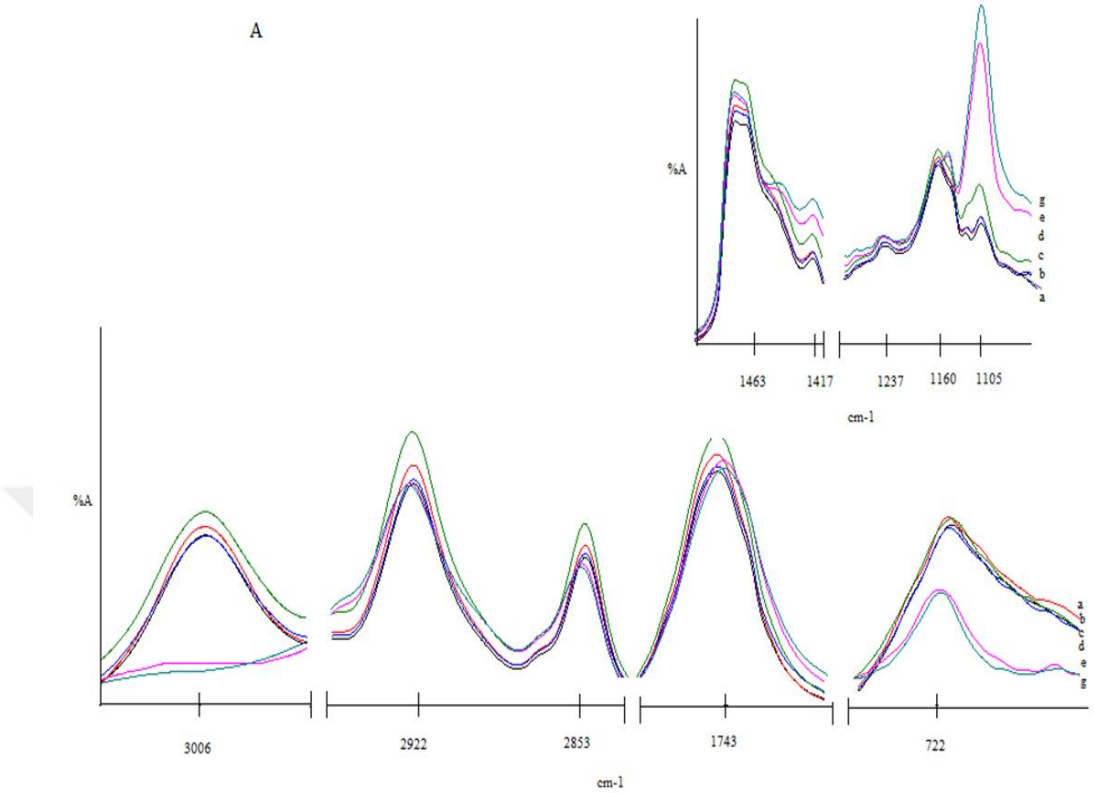


Figure 4.16 Major bands in FTIR spectra. (a: HO, b: OHO1, c: OHO5, d: OHO30, e: OHO60, f: OHO180, g: OHO360).

4.9 NMR (Nuclear magnetic resonance) analysis

Nuclear Magnetic Resonance (NMR) spectroscopy analysis for untreated and ozone treated oils were performed in ^{1}H and ^{13}C NMR spectra on a Bruker Avance 500 MHz spectrometer at $25^{0}C$ in $CDCl_3$ to identify changes in the structure of hazelnut oil as a function of ozone treatment time. The ^{1}H and ^{13}C NMR assignments to principal peaks of untreated hazelnut oil are summarized in Table 4.15. The ^{1}H NMR and ^{13}C NMR resonances were assigned according to data in the literature determined for similar vegetables oils (Sega et al., 2010, Sadowska et al., 2008, Soriano et al., 2003).

Table 4.15 ^{1}H NMR intensities for main groups in ozone reaction with HO.

Ozone treatment time		HO	OHO60	OHO180	OHO360
Groups	δ ^{1}H				
α-metyhlene group	1.39	---	2.35	4.77	5.04
CH_2-CH_2COOH (All acyl chains)	1.65	---	3.19	3.49	8.12
CH_2CH=CH (All unsaturated acyl chains)	2.00	5.37	3.47	0.74	0.41
CH_2-COOH(All acyl chains)	2.30	2.93	3.06	3.04	3.05
α-metyhlene group	2.43	---	0.34	0.28	0.21
CH=CHCH$_2$CH=CH (Linolenyl and linolenyl chains)	2.75	0.36	0.15	---	---
CH_2-OCOR (Triglycerides)	4.30	0.97	1.00	0.97	0.91
1,2,4-trioxolane	5.15	---	0.93	1.09	1.07
CH=CH (All unsaturated fatty acids)	5.30	3.03	1.87	1.09	0.90
Aldehyde	9.75	---	0.04	0.07	0.07

δ ^{1}H (ppm): Chemical shifts

Table 4.16 ^{1}H NMR and ^{13}C NMR new signals of ozone treated samples.

^{13}C Shift (ppm)	^{1}H Shift (ppm)	Functional group
104.35	5.15	1,2,4-trioxolane
-	1.39	α-metyhlene group
-	1.65	α-metyhlene group
43.9	2.43	A-Methylene group
202.58	9.75	Aldehyde

NMR analysis confirmed that the structural changes in the hazelnut oil occurred during ozone treatment (Figure 4.17, 4.18, and 4.19). The new signals found in ozone treated hazelnut oil were summarized in Table 4.16. In ^{1}H NMR spectra of ozone treated hazelnut oil, new signal at 5.15 ppm was found, the new signal was assigned to the ring proton of 1,2,4 trioxolane (Sadowska et al., 2008). The result was confirmed by the appearance of the signal in ^{13}C NMR spectra at 104.35 ppm assigned by Wu et al. (1992), as ring carbon in the same structure. Area of the new peaks increased with ozone treatment time as shown in the figures. Ozone treated oil samples also exhibited new peaks belonging to aldehydic proton and α-methylene group at 9.75 and 2.43 ppm in ^{1}H NMR and at 202.58 and 43.9

ppm in ^{13}C NMR, respectively. The new signals formed in ozone treatment process gradually increased until 360 min of ozone treatment. The signals of unsaturated bonds at 2.00 and 5.30 ppm in ^{1}H NMR spectra and 27.1 and 128-130 ppm in ^{13}C NMR spectra dramatically decreased with ozone treatment time and finally disappeared after ozone treatment for 360 min. As illustrated in the Figures, signal of linolenyl chains at 0.85 ppm in ^{1}H NMR spectra and at 33.8 ppm in ^{13}C NMR spectra decreased as ozone treatment time increased. In addition, after ozone treatments for 180 and 360 min, signal of linolenyl chains which contain two double bond at 2.75 in ^{1}H NMR and at 24.8 ppm in ^{13}C NMR completely disappeared. As seen in NMR spectra for ozone treated oil for 360 min, the double bond of unsaturated fatty acids had been consumed, thoroughly. Furthermore, the protons of the methylene group α to the sp^2 hybridized carbons which assigned at 2.00 and 2.75 ppm in the spectra of untreated hazelnut oil shifted to 1.39 and 1.65 ppm after ozone treatment due to formation of 1,2,4 trioxolane.

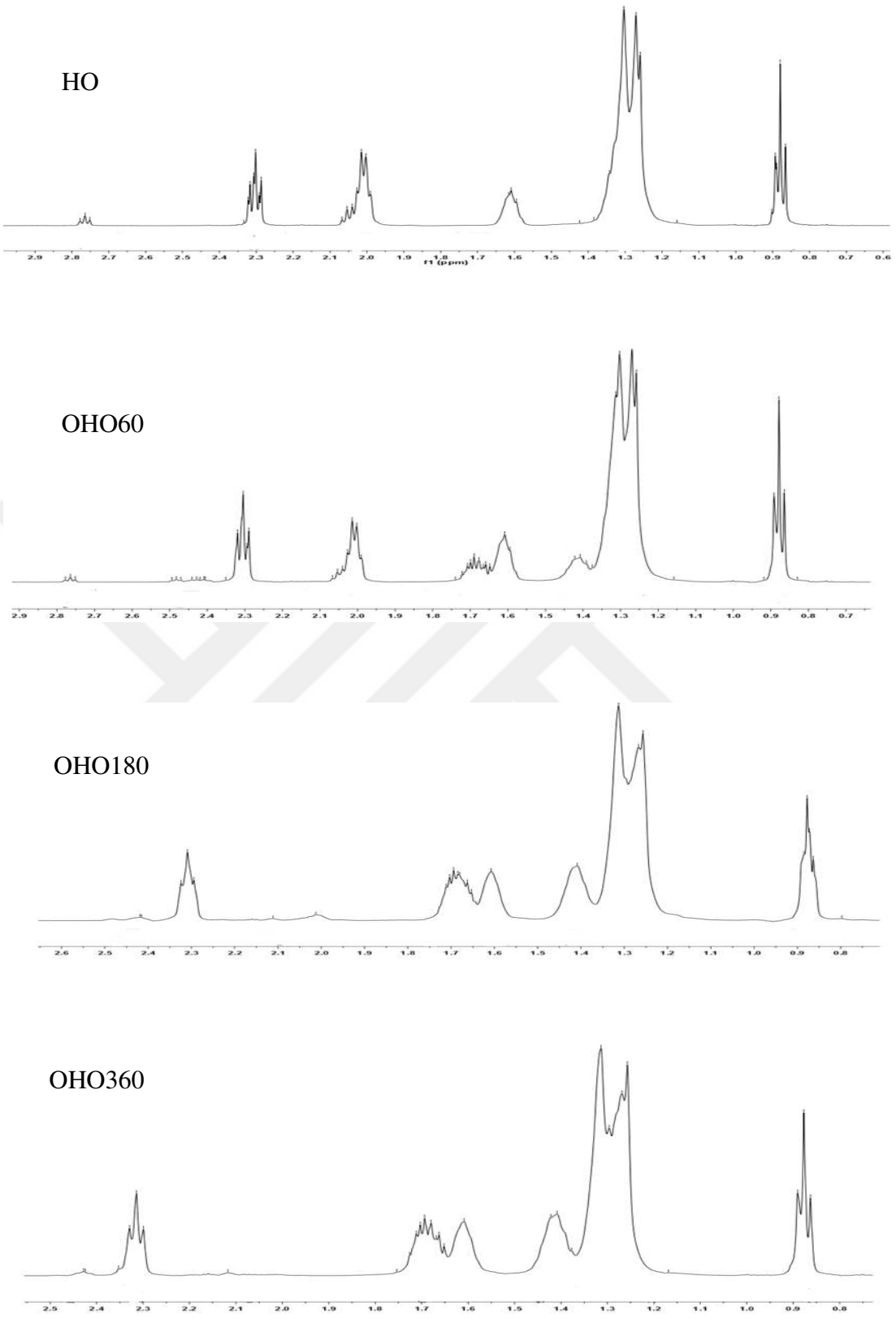


Figure 4.17 ^{1}H NMR spectra of untreated and ozone treated hazelnut oils. (0-3 ppm)

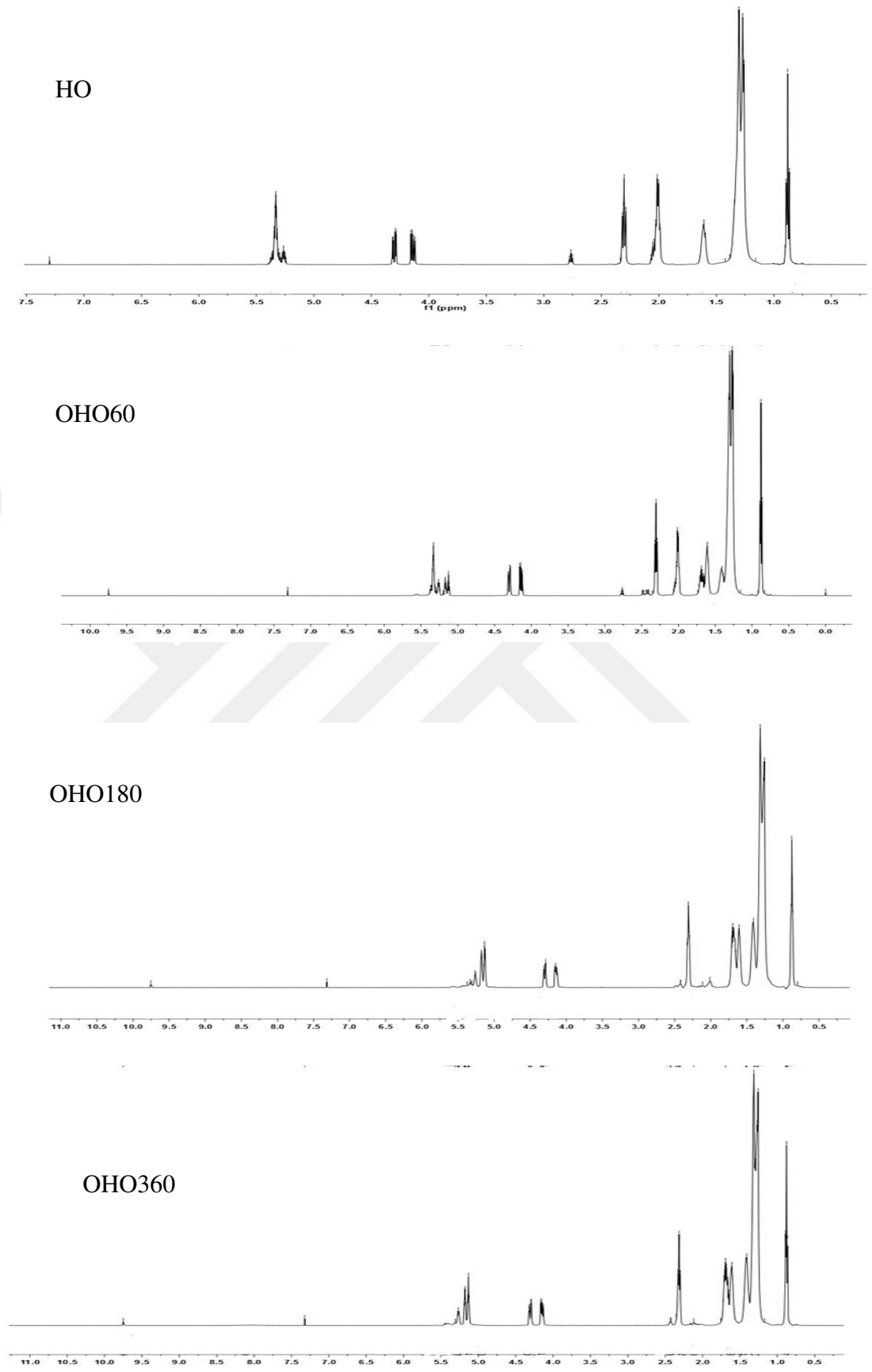


Figure 4.18 ^{1}H NMR spectra of untreated and ozone treated hazelnut oils.(0-11 ppm)

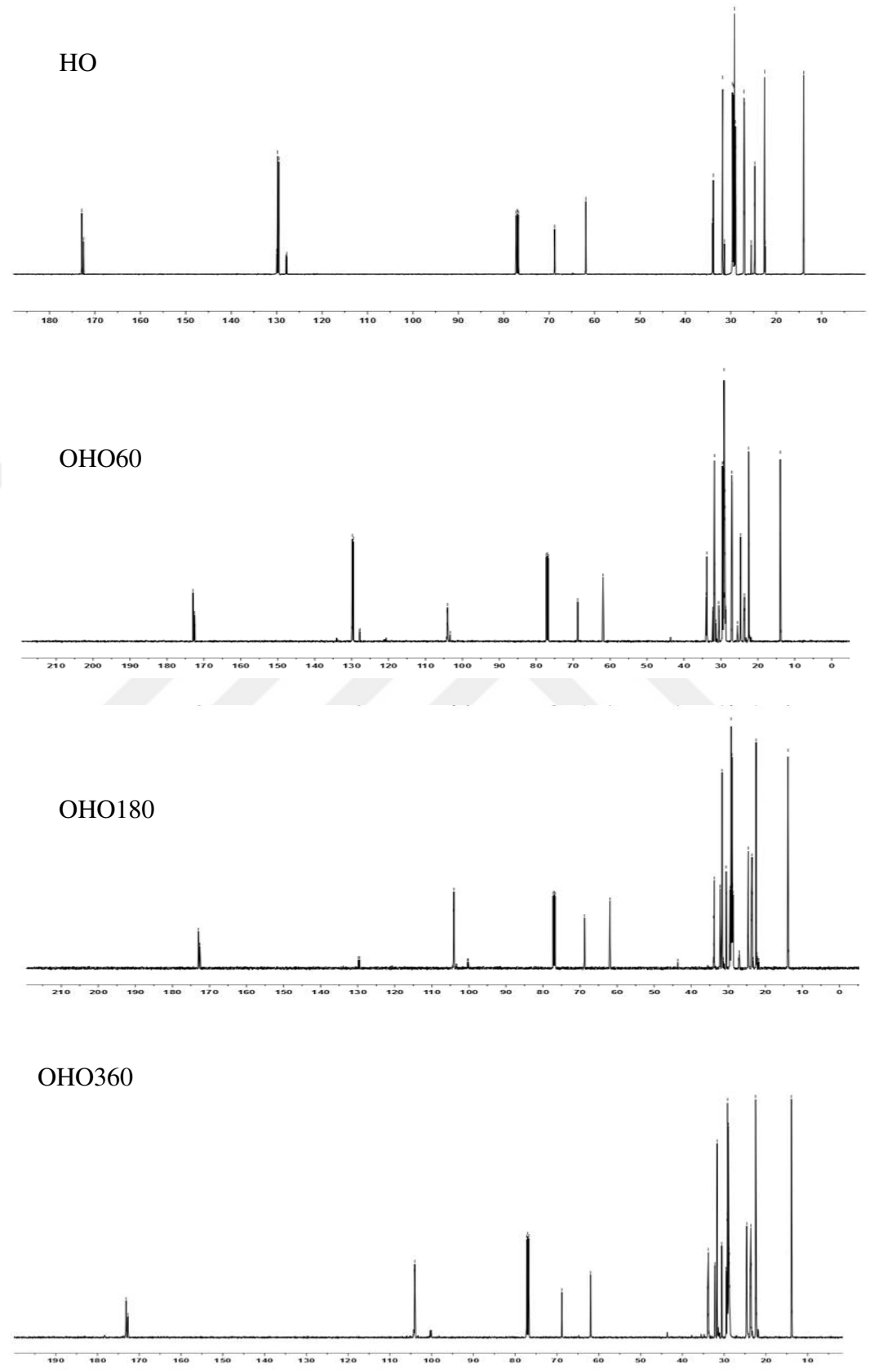


Figure 4.19 ^{13}C NMR spectra of untreated and ozone treated hazelnut oils.

In literature, the products formed from cycloaddition of a Criegee intermediate with double bond of fatty acids has been detected from the reaction of ozone with oleic acid in a aqueous medium (Zahardis et al., 2006). The reaction mechanism of ozone with unsaturated fatty acids and formed oxidative products changes related to the reaction conditions (Nishikawa et al., 1995). In an anhydrous medium, secondary ozonide (1,2,4 trioxolane) and peroxide oligomers are formed by the reaction of zwitterions and aldehydes whereas in a aqueous medium, the zwitterions react with water to produce different peroxidic species (hydro-peroxides, oligomeric peroxides, etc.) and carboxylic acids (Murray, 1968). The NMR spectra of HO were correlated with gas chromatograph results and IV (Table 4.12). In gas chromatography, unsaturated fatty acid composition of the hazelnut oil decreased with ozone treatment time. Also, The IV was decreased as a function of ozone treatment time due to oxidation of double bonds present in unsaturated fatty acids.

4.10 Emulsifying properties

In this section, emulsifying properties of emulsions which is stabilised with whey protein and prepared with untreated / ozone treated oils were examined at % 0.25 w/w protein concentration. Emulsions were prepared by using an ultrasound disintegrator (15 amplitudes for 30 s). Guzey (2001) reported that ultrasonic processing in high intensity improves emulsifying properties of the emulsions. In previous studies (Pongsawatmanit et al., 2006; Gulseren et al., 2007) the emulsions had been treated with high intensity ultrasonic waves to disrupt any flocculated droplets.

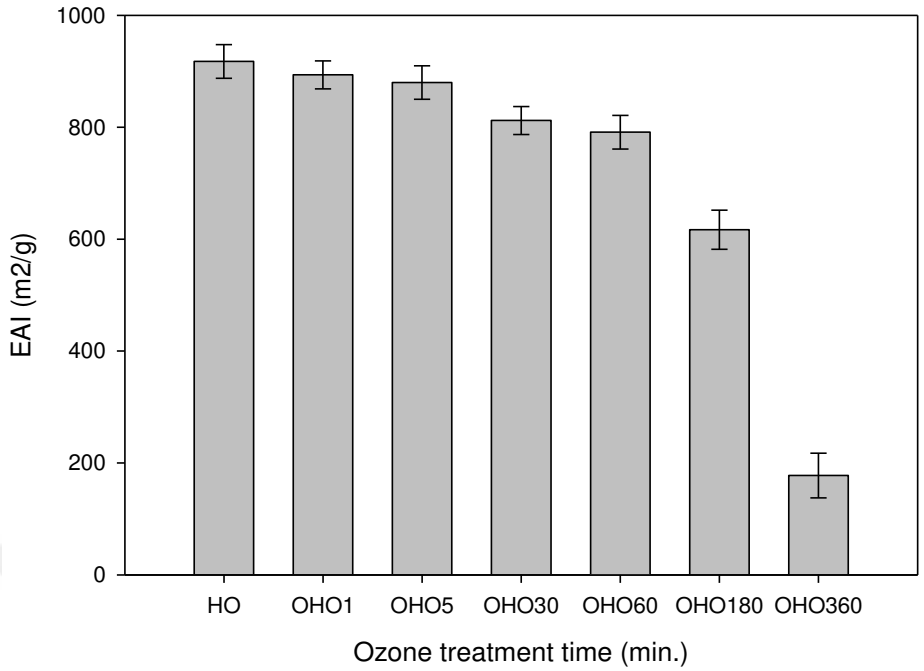


Figure 4.20 EAI of emulsions prepared with untreated and ozone treated hazelnut oils.

Emulsifying properties of whey protein stabilised emulsions were expressed in terms of emulsifying activity index (EAI) and emulsion stability index (ESI) and were determined by the modified Pearce and Kinsella (1978) method. In this study, whey protein isolate (WPI) was used to stabilize the emulsions. Whey proteins are composed of beta lactoglobulin (β-Lg), alpha lactalbumin (α-La), bovine serum albumin (BSA) and immunoglobulins (Ig) (Verheul et al., 1998). They exist as compact, globular proteins. Whey proteins have been commonly used as foaming and emulsifying agents in food industry. They mainly form viscoelastic films by polymerization with disulphide bonds and hydrophobic interactions (Monahan et al., 1993; Lee et al., 1992). The EAI and ESI of the emulsions were measured for long period (about 10 days) to observe behaviour of droplets in the emulsion as a function of storage time. Figure 4.20 showed that EAI of emulsions prepared untreated and ozone treated oil samples. EAI was measured as 917 m^2/mg for emulsion containing untreated hazelnut oil and 893, 880, 812, 791, 616 and 177 m^2/mg for emulsion containing ozone treated hazelnut oils for 1, 5, 30, 60, 180 and 360 min, respectively. As seen in the figure, EAI of the emulsion containing untreated hazelnut oil was the higher value than emulsions containing ozone treated oils. It was observed that ozone treatment significantly affected emulsion activity

negatively ($p<0.05$). And also, as mentioned previous part (4.1), FFA content of the hazelnut oil increased with ozone treatment time. A dramatic rise in FFA causes increasing acidity in emulsion medium. Therefore, the emulsions were prepared by using buffer solutions (at pH: 7.0 and 0.5M ionic strength) to minimize effects of acidity on the emulsifying properties. Also, pH value of the emulsions was measured immediately. It was determined that pH value of emulsions prepared with ozone treated oil reduced as a function of ozone treatment time. pH value of the emulsion prepared with untreated hazelnut oil was 6.88 when pH values of emulsions containing ozone treated oil for 1, 5, 30, 60, 180, and 360 min were 6.71, 6.35, 5.01, 4.08, 3.35, and 2.56, respectively. Unfortunately, we observed that buffer solution was not sufficient to prevent increasing acidity of the emulsions. The ozone treatment has a negative effect on emulsion activity. It was observed that the negative effect of ozone on emulsion activity was more pronounced at the emulsion containing ozone treated oils for 360 min. In previous studies, it was stated that the adsorption of whey proteins to the interface, and the adsorption of whey protein components with each other was depending on the pH of the medium (Pearce and Kinsella, 1978). At pH 5, whey proteins have the highest adsorption at the interface (Hunt and Dalgleish, 1994; Shimizu et al., 1986). Shimizu et al., (1986) suggested that at low pH, the adsorption of β-lactoglobulin decreases by structural alterations. These alterations cause a reduction in flexibility and thereby emulsifying properties of β-lactoglobulin. Also, the net charge of the proteins is influenced by the pH of the solution. Generally, the proteins have high solubility in acidic or alkaline pH values because of the excess of charges of the same sign. The same signed charges generate repulse force between molecules, consequently, proteins exhibit highest solubility (Pelegrine and Gasparetto, 2005). The solubility of the proteins is a considerable functional property which influence on the other functional properties. Proteins demonstrate strong functional properties including emulsifying, foaming, gelling, etc. when they have high solubility (Nakai and Chan, 1985; Wit, 1989). At isoelectric point, solubility of the proteins is minimum because of the increasing in protein-protein interaction. The electrostatic forces of the molecules are very low and interaction of water with the protein molecules is at a minimum. Proteins form precipitate in solution because of aggregation of protein molecules at the isoelectric point (Kakalis and Regenstein, 1986; Wit, 1989; Vojdani, 1996).

Oxidative reaction of the proteins with ozone influences their structural properties. Enzyme activity, charge, nutritive value (loss of essential amino acids) and conformational structure of the protein (exposure of hydrophobic groups, changes in secondary structure and disulphide groups) unfavourably are affected result from oxidation reaction (Howell et al., 2001). In addition, as well as structural properties, functional properties of the proteins may be affected by ozone reaction (Howell et al., 2001). It was also informed that at isoelectric point of β-lactoglobulin, it does not exhibit minimum solubility because of the product is not a pure protein. The whey proteins are found in milk as a mixture and precipitation occurs at the isoelectric point of their proteins, and not at the isolectic point of β-lactoglobulin.

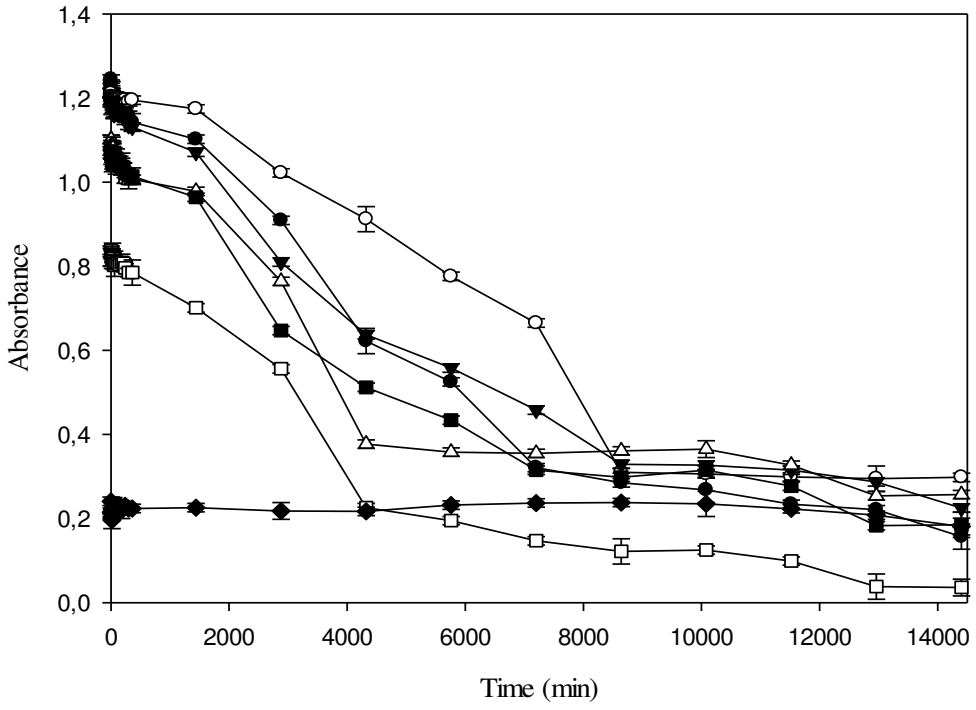


Figure 4.21 ESI of emulsions prepared with untreated and ozone treated hazelnut oil (●; HO; ○; OHO1, ▼; OHO5, Δ; OHO30, ■; OHO60, □; OHO180, ♦; OHO360).

Emulsion stability index (ESI) of emulsions were determined by following the time-dependent change in the absorbance of emulsions preparing with untreated and ozone treated hazelnut oils. As seen in the Figure 4.21, ESI of ozone treated samples for 1, 5, 30 and 60 min initially reduces fast for approximately 4000 -5760 min, and then gradually slows down and reaches to almost a plateau value. From

the figure, it could be evaluated that emulsion stability were significantly affected by ozone treatment process ($p<0.05$). It could be determined that emulsion stability had been more pronounced affected negatively as ozone treatment time increased. Especially a dramatic reduction in the emulsion stability was observed with ozone treatment for 30, 60 and 180 min, respectively. As mentioned above, pH value of the emulsions prepared with ozone treated oils decreased related with ozone treatment time. The emulsions exhibited minimum stability approaching isoelectric point of whey proteins. The isoelectric point of whey protein is about pH~5 (Fox and McSweeney, 1998). This behaviour has explained that at approaching the isoelectric point net charge is very low and a dramatic reduction happens in electrostatic and steric repulsions. Thus, flocculation and creaming are observed extensively in emulsion droplets and consequently, accelerating destabilization of emulsions (Klemaszewski and Kinsella, 1992). Stability of the emulsion is associated with the consistency of the interphase which does not altered with time. Emulsions at which suitable pH and high net negative charge blocking interactions of droplets with each other retard the rate of coalescence, and then, more stable emulsions are formed (Klemaszewski and Kinsella, 1992). The stability of emulsion containing ozone treated oils for 360 min was changed with time, slowly. As mentioned previous section about viscosity, the viscosity of the oil samples increased with ozone treatment time (Abramovic and Klofutar, 1998). The increase in viscosity with ozone treatment time could be explained that the unsaturated fatty acids in the oil can be oxidized by ozone and then the percentages of unsaturated fatty acids in the oil decreased when the percentages of saturated fatty acids in the oil increase. Result from an increase in ratio of saturated fatty acid to unsaturated fatty acid in the oil, the viscosity of ozonated oils increased. An increase in viscosity was highest value for 360 min ozone treatment. Therefore, it might be said that the motion of oil droplets in the emulsion slowed down when the viscosity of oil increased. Thus, better stability was observed in the emulsion prepared with ozone treated oil for 360 min. In previous studies, it was stated that viscosity of the continuous phase could be increased for retarding the gravitational separation of the emulsions (Guzey and McClements, 2006).

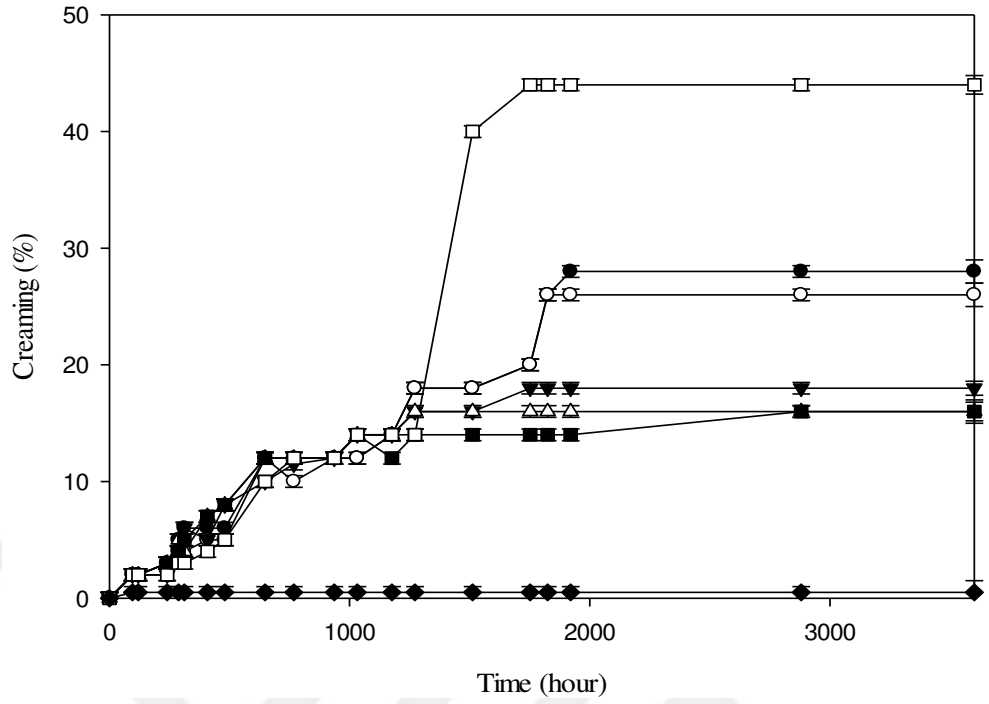


Figure 4.22 Creaming behaviour of emulsions prepared with untreated and ozone treated hazelnut oil.(●; HO; ○; OHO1, ▼; OHO5, Δ; OHO30, ■; OHO60, □; OHO180, ♦; OHO360).

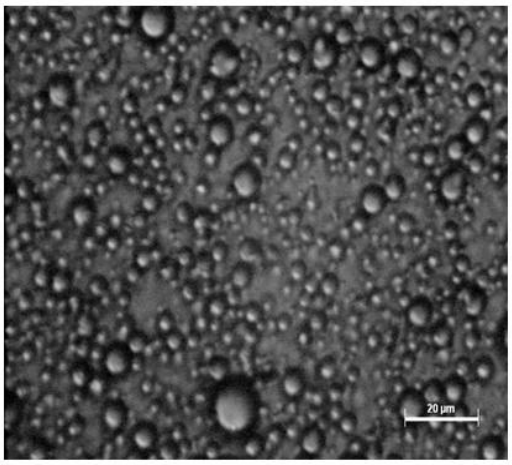
Creaming arises from the destabilizing mechanism of the food emulsions and may cause from gravitational separation associated with the differences in densities of the dispersed and continuous phases. Creaming is formed from the upward motion at which the density of the droplets is lower than that of the surrounding liquid (McClements et al., 2007). Creaming behaviour of whey protein stabilized emulsions prepared with untreated and ozone treated oils was illustrated in Figure 4.22. Creaming behaviour of samples were significantly affected with ozone treatment time ($p<0.05$). Creaming proceeded for about 75 days dramatically, and then, slowed down and reached to plateau value for all emulsions except emulsion prepared with ozone treated oil for 360 min. The creaming behaviour of emulsions containing 5, 30 and 60 min ozone treated oil was similar to each other's. Also, a similar creaming behaviour was observed in emulsions prepared with untreated and 1min ozone treated oil. While 23% creaming was observed after 75 days storage of the emulsion containing untreated oil, creaming rate of emulsions prepared with ozone treated oil for 1, 5, 30, 60 and 180 min was determined as 24%, 18%, 16% and 44%. Therefore, it can be determined that creaming rate of the emulsion

prepared with short time ozone treated oils retarded compared to creaming behaviour of the emulsion containing untreated oil. However, a dramatic increase was observed in the creaming rate of the emulsion prepared with 180 min ozone treated oil. This may result from the change in pH values of emulsions containing ozone treated oils. After ozone treatment, acidity of the hazelnut oil increased and result in a reduction in pH of emulsions was observed depending on ozone treatment time. pH values of emulsion containing ozone treated oil for 180 min were 3.35. Previous researches showed that pH is influenced physical stability of emulsions (Demetriades et al., 1997; McClements, 2012). Generally, a dramatic increase in formation of the flocculation is observed at isoelectric point of protein emulsifier which is 4.8 for whey protein (McClements, 2012). At the isoelectric point, the emulsions stability is affected negatively because of elimination of the electrostatic repulsion between droplets (Demetriades et al., 1997). The stability of the emulsions containing either untreated and ozone treated oil were highly dependent on the pH. At low pH around 3, there were rapid creaming and droplet aggregations, as evidenced by the emulsion stability measurements. The creaming was not detected in emulsions containing ozone treated oils for 360 min. Creaming is a gravitational separation in which less dense particles than the continuous liquid tend to rise top of the sample and thus, forming cream layer (Alben and Ibanoglu, 2007). It can be said that there was no sufficient interaction in the emulsion system between the oil droplets and WPI solution depending on rising in the viscosity and acidity of the oil after ozone treatment for 360 min. It might be concluded that the creaming could not be observed because of a dramatic increase in viscosity of oil after ozone treatment for 360 min. While the viscosity increases in the emulsion system, the movement of oil droplets slowed down and thus, increases stability (Dickinson et al., 1998; Huang et al., 2001).

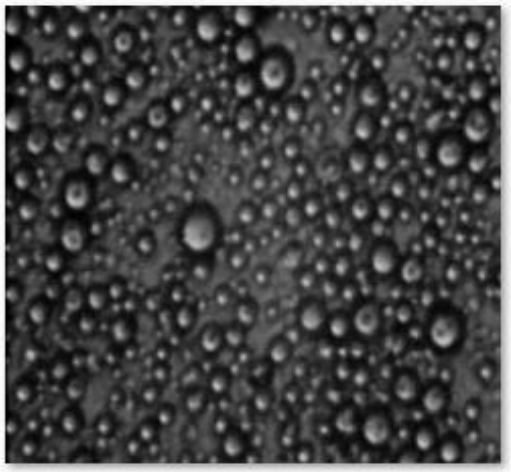
4.11 Polarised light microscope (PLM)

Images of emulsions prepared with either untreated or ozone treated hazelnut oils have been monitored by polarized light microscopy with 40x magnification to define any microstructural variations between the emulsions. Therefore, as shown in Figure 4.23A, for whey protein stabilized emulsion prepared with untreated hazelnut oil, the image indicated that small and isolated spherical oil droplets. In such a system, at the interface, the electrostatic repulsion between protein

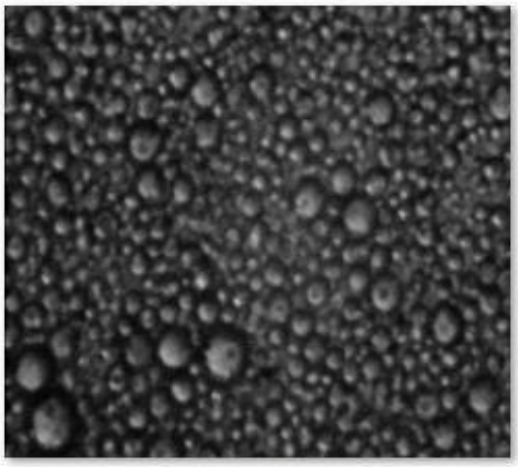
molecules hindered the formation of flocculate, result in occurred a well-stabilised emulsion which is in a liquid form, due to the small particle size of emulsion droplets. Also, the optical microscopy image obviously demonstrated that large droplet flocculation formed in the emulsions containing long time ozone treated oil. The oil droplet size of emulsions enlarged when the ozone treatment time increased. Especially, the optical microscopy measurements of emulsion microstructure confirmed that large droplet flocculation formed in the emulsions containing ozone treated oils for 60, 180 and 360 min (Figure 4.23E, F, G). These measurements indicated that the emulsions containing ozone treated oils was unstable to droplet flocculation due to the whey protein coated droplets have a low net charge close to the isoelectric point. Thus, there is a weak electrostatic repulsion between the droplets to eliminate their aggregation (McClements, 2004). As mentioned in previous section, pH value of the emulsions prepared with ozone treated oil decreased when the ozone treatment time increased. The emulsifying properties of the proteins can be affected from environmental conditions including pH, ionic strength and temperature (Das and Kinsella, 1989; McClements, 2004). As seen in figure 4.23, the droplet size distributions of the emulsions shifted towards large sizes with ozone treatment time because of a decrease in pH of emulsions. At near isoelectric point, aggregation of droplets was further promoted by a decrease in the electrostatic repulsion between oil droplets. Finally, the emulsion prepared with 360 min ozone treated oil had a fairly coarse microstructure (figure 4.23G) As mentioned previous creaming section, although, coarse droplets were observed in this emulsion, the creaming rate of the emulsion was determined to be minimum level due to an increase in viscosity of the oil result from ozone treatment for 360 min. An increase in viscosity slows down the upward movement of the oil droplets. While the viscosity increases in the emulsion system, the movement of oil droplets slowed down and thus, increases stability (Dickinson et al., 1998; Huang et al., 2001).



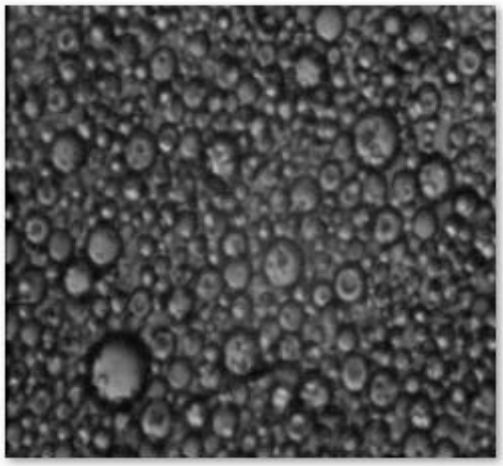
A



B



C



D

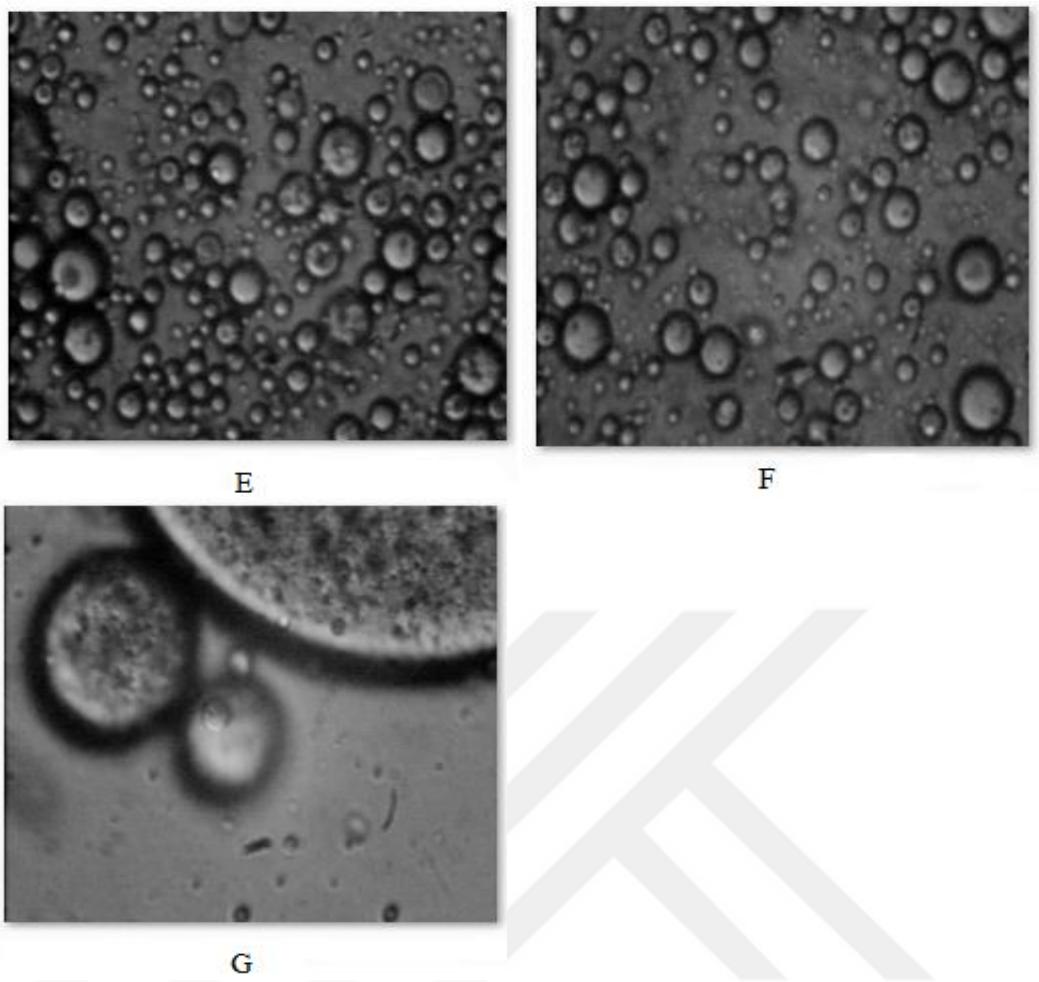
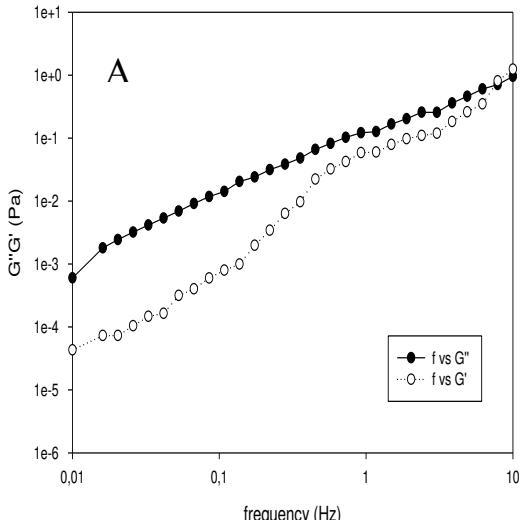


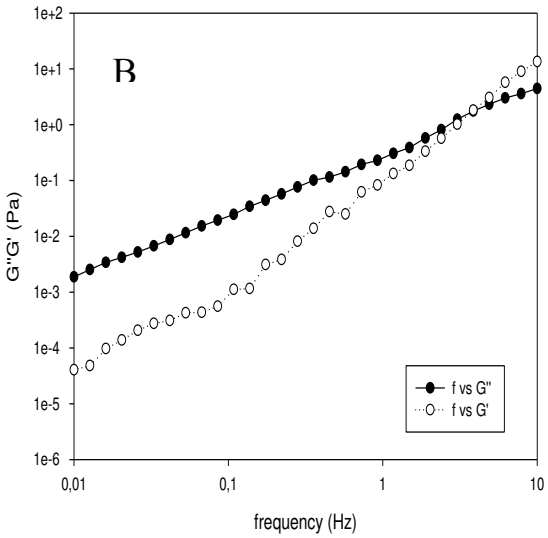
Figure 4.23 Polarized light micrographs of emulsions prepared with untreated and ozone treated hazelnut oils. (A: HO; B: OHO1; C: OHO5; D: OHO30; E: OHO60; F: OHO180; and G: OHO360; Scale bar = 20μm).

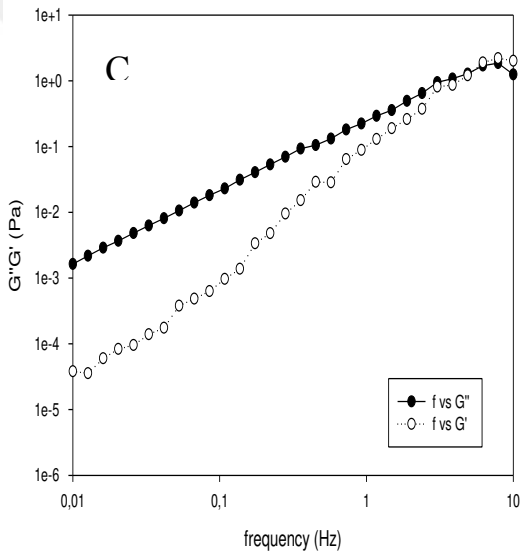
4.12 Rheological properties of emulsions

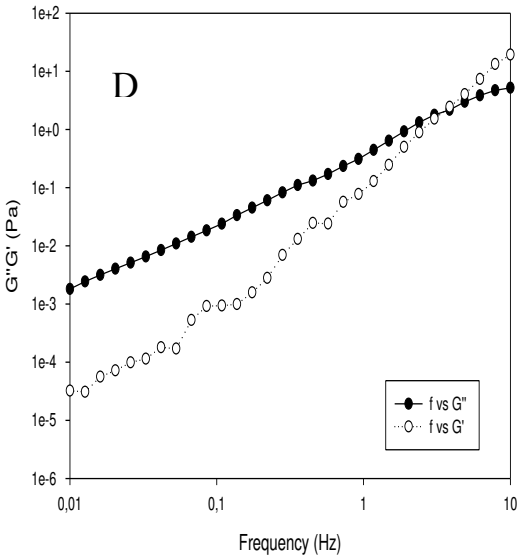
In oscillatory instruments, samples are exposed to various stresses or strain harmonically. The dynamic method has been commonly used to investigate the viscoelastic behaviour of foods. The suggested applications such as estimation of gel strength, investigation of starch gelatinization, examination of the glass transition, observation of protein denaturation and coagulation, evaluation curd formation in dairy products, shelf life testing, etc. are studied by this method (Steffe, 1996). The effects of ozone on rheological behaviour of whey protein isolate stabilized emulsions (0.25% w/w) were examined in this study. In order to examine viscoelastic behaviour of whey protein isolate stabilised emulsions, untreated and ozone treated oils at different times (1, 5, 30, 60, 180 and 360 min) were used. Dynamic frequency sweep tests were applied in the linear

viscoelastic range for determination of the frequency depend on the elastic (G') and viscous moduli (G''). Figure 4.24 showed the viscoelastic behaviour of whey protein isolate stabilized emulsions prepared with untreated and ozone treated hazelnut oils. G' is a measure of the energy stored in a cycle of oscillation and G'' is a measure of the energy dissipated as a viscous flow in a cycle of oscillation (Tadros, 2004). As shown in the figures, a more fluid-like behaviour was determined at low frequencies, where the loss modulus, G'', was higher than the storage modulus, G'. The crossover between G'' and G' curves was observed at the different frequency ranges depending on ozone treatment time of hazelnut oils. For example, the crossover between G'' and G' curves of emulsion prepared with untreated hazelnut oil was observed at 7.88 Hz, while the crossover between G'' and G' curves of emulsion prepared with ozone treated hazelnut oil for 360 min was observed at 2.39 Hz. The crossover between G'' and G' curves were changed as 3.85, 4.89, 3.85, 3.03 and 2.39 Hz for emulsions containing ozone treated hazelnut oil for 1, 5, 30, 60, 180 and 360 min, respectively. It was said that the crossover between G'' and G' curves shifted towards to low frequencies when ozone treatment time increased. Therefore, it was determined that emulsions containing ozone treated oils exhibited more solid like behaviour when ozone treatment time increased. At frequencies bigger than the crossover frequency, a solid-like behaviour was observed where G' was higher than G''. According to Steffe (1992), this description of the mechanical spectra is characteristic of the viscoelastic fluids.


A
G''G' (Pa)
1e+1
1e+0
1e-1
1e-2
1e-3
1e-4
1e-5
1e-6
0,01
0,1
1
10
frequency (Hz)
f vs G''
f vs G'


B
G''G' (Pa)
1e+2
1e+1
1e+0
1e-1
1e-2
1e-3
1e-4
1e-5
1e-6
0,01
0,1
1
10
frequency (Hz)
f vs G''
f vs G'


C
G''G' (Pa)
1e+1
1e+0
1e-1
1e-2
1e-3
1e-4
1e-5
1e-6
0,01
0,1
1
10
frequency (Hz)
f vs G''
f vs G'


D
G''G' (Pa)
1e+2
1e+1
1e+0
1e-1
1e-2
1e-3
1e-4
1e-5
1e-6
0,01
0,1
1
10
Frequency (Hz)
f vs G''
f vs G'

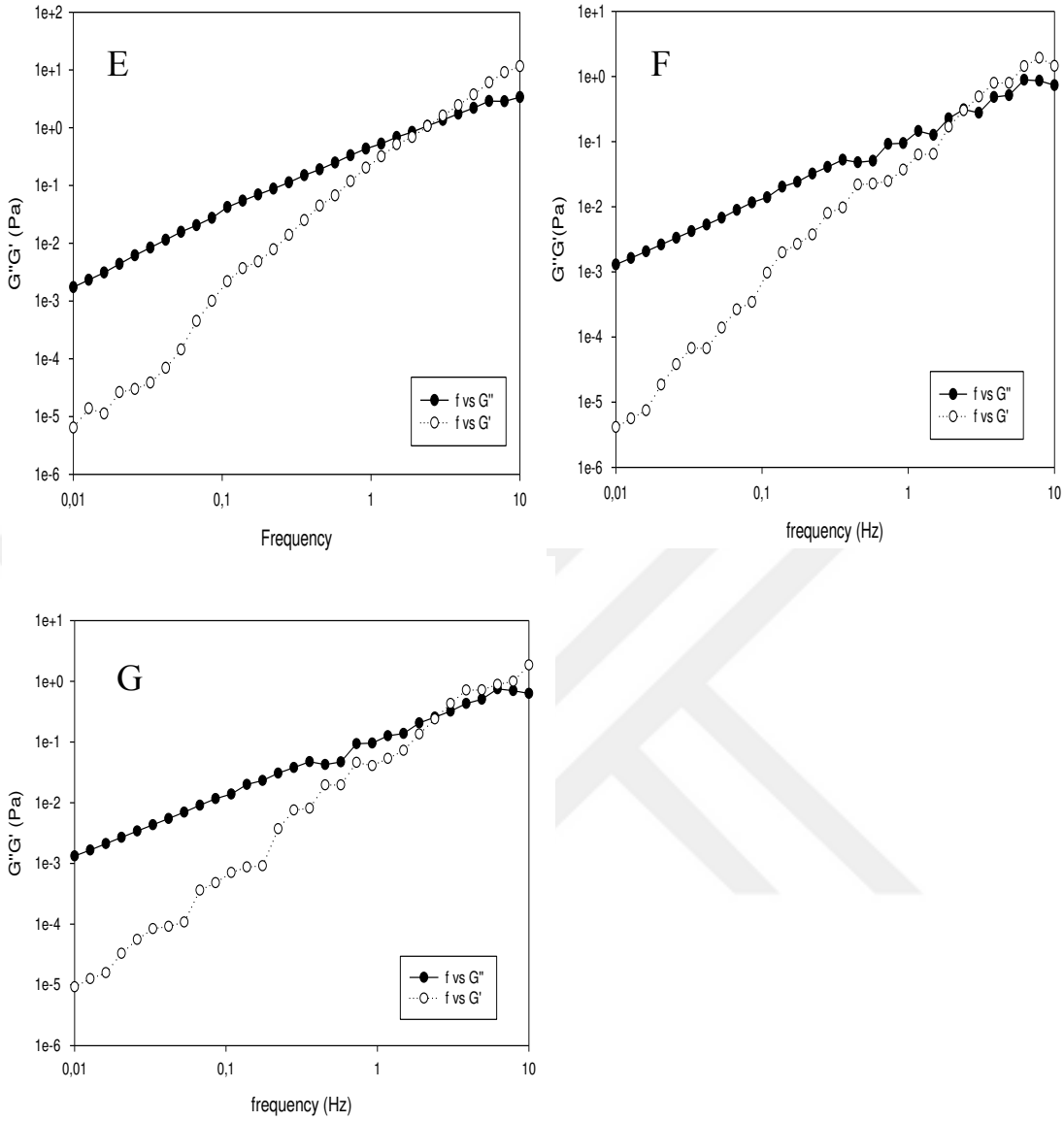


Figure 4.24 Viscoelastic moduli for emulsions prepared with untreated and ozone treated hazelnut oils (A: HO; B: OHO1; C: OHO5; D: OHO30; E: OHO60; F: OHO180; and G: OHO360).

Loss tangent (defined as tan δ= *G″/G′*) is a rheological parameter which a measurement of the relative magnitudes of the viscous and elastic components, i.e., the smaller the value of tan δ indicates the more elasticity of the sample (Tadros, 2004). The behaviour of phase angle (*tanδ*) with time is also characteristic of a viscoelastic liquid at short times (around 90^0) and reaches a value characteristic of an elastic solid (near 0^0) at larger times (Valdez et al., 2006). According to our results, phase angle of all emulsions were around 90^0 at shorter times. Thus, it was

evaluated that all emulsions prepared with untreated and ozone treated oil exhibits viscoelastic liquid behaviour.

4.13 Microbiological analysis

Microbiological studies for ozone treated hazelnut oils were performed at different treatment times for 15 and 60 min to investigate lethal effects of ozone on microorganism found in hazelnut oil. Ozone which known as strong oxidizing agent has been used commonly in food industry. Ozone treatment is a non-thermal process however; it destroys microorganisms by reacting with some components in the microbial cell, especially the components including double bonds, sulfhydryl groups, and phenolic rings. Therefore, phospholipids, intracellular enzymes, and genomic material are targeted by ozone; as a result of these reactions microbial cell is damaged and microorganisms are killed by ozone treatment.

Table 4.17 Changes TPC and mould and yeast in hazelnut oil exposed to different times in ozone treatment.

	Ozone treatment time (min)		
Microorganism (cfu/g)	HO	OHO15	OHO60
Total plate count (TPC)	$2x10^2$	<10	<10
Mold and yeast	<10	<10	<10

Table 4.17 showed that total plate count (TPC) and mold and yeast changes in untreated and ozone treated hazelnut oil. With ozone treatment process, TPC in the hazelnut oil decreased rapidly. As seen in the table, TPC of ozone treated hazelnut oil totally destroyed, while TPC of untreated hazelnut oil was 200 cfu/g. Mold and yeast in untreated hazelnut oil could not be detected and so, any change in mould and yeast could not be observed after ozone treatment. These results demonstrated that ozone treatment causes immediately killing effect on the microorganisms in hazelnut oil. In previous studies, it was reported that TPC in wheat flour decreased significantly, as the increase of ozone treatment time (Li et al., 2013). They investigated that fungicidal effectiveness of ozone treated sunflower oil. They

suggested that ozone treated sunflower oil inhibited expansion and growth of microorganism. In this study, it could be detected that microorganism in the hazelnut oil sample could be destroyed totally for 15 min ozone treatment time. However, they reported that TPC of 15 min ozone treated wheat flour sample was reduced at 17% percentages. The differences in microbial lethal effectiveness might be due to the differences in physical and chemical properties between wheat flour and hazelnut oil.

CHAPTER 5

CONCLUSIONS

The present work presents the influences of ozone treatment on chemical and structural properties of hazelnut oil were investigated and the following results were obtained.

Peroxide value (PV) and free fatty acid value (FFA);

PV of ozone treated oil samples raised with increase in storage period. The increase in PV was observed to slow down in 60 and 180 min ozone treatment, since the oxidation of oil was expected to accomplish during ozone treatment. A reduction in the reaction rate was occurred because of disappearance of the double bond with increasing in ozone treatment time. An exponential increase in the PV of oil samples with the increase in ozone treatment time was observed. As the treatment time is extended, more ozone molecules contact the sample of oil, whose volume was kept constant in each ozone treatment, causing an extensive oxidation.

FFA of oil samples were observed to increase as a function of ozone treatment time. An increase in the FFA value correlates well with the increase in PV in the ozone treatment process. The fat molecules which were already exposed to oxidation with ozone might be more sensitive to oxidation during storage resulting from structural changes. This may cause an inevitable increase of FFA values after storage period.

Iodine value (IV);

The iodine value (IV) of untreated and ozone treated hazelnut oil samples was significantly different ($p<0.05$). The IV of samples reduced with ozone treatment time, dramatically. A sharp reduction in iodine values of samples were observed after ozone treatment, whereas the iodine values of ozone treated samples slightly changed as a function of storage time. Statistical result showed that the change in IV as a function of storage was not significantly different ($p>0.05$).

Viscosity measurement;

The viscosity values of the oil samples varied distinctly depending on the ozone treatment time. The highest viscosity was observed at 360 min ozone treated oil samples followed by 180, 60 and 30 min, respectively. However, the viscosities of 1 and 5 min ozone treated samples were observed as close to the viscosity of crude oil. The increase in viscosity of HO with respect to ozone treatment time could be explained with the decrease in unsaturation.

The viscosity values of untreated and ozone treated hazelnut oil samples cannot change as a function of storage time, dramatically. The change in viscosity of ozone treated hazelnut oil was related with amount of unsaturated fatty chain. Therefore, the amount of double bonds of ozone treated oils could not change as a function of storage time.

Hunter calorimeter;

b* (b+ yellowness and b- blueness) and Y_1 (yellowness index) values of oil samples reduced with ozone treatment time, dramatically when a* (a+ redness and a- greenness) values increased with ozone treatment time, slightly. L* (lightness) values changed with ozone treatment time slightly. The statistical results showed that changes in b* and Y_1 values was significantly different ($p<0.05$) with ozone treatment time, but, there were no significant ($p>0.05$) changes in a* and L* values with ozone treatment time.

DSC kinetics;

Oxidative stability of ozone treated hazelnut oil was evaluated using kinetic parameters, the rate constant (k), the activation energy (E_a), activation enthalpy ($\Delta H^{\neq}$), activation entropy ($\Delta S^{\neq}$). k values showed an exponential rise with the increase of ozone treatment time. Consequently, the rate of oxidation reaction can be increased with ozone treatment time.

The kinetic parameters were calculated for oil samples ozonated for 1 min and stored up to 90 days. The kinetic parameters indicated that the storage period did not affect the temperature sensitivity, rate of oxidation reaction, and hence the quality change or deterioration of ozone treated oil samples. This may be related

with the composition of oil sample that the oxidation that was detected by DSC might reflect more the extent of unsaturation rather than the formation of oxidation products.

Statistically, kinetic parameters were significantly different ($p<0.05$) with respect to ozone treatment time. E_a of ozone treated oils showed a reducing trend as the ozone exposure period increased. Increasing of k and $\Delta S^{\ddagger}$ and decreasing of E_a and $\Delta H^{\ddagger}$ indicated a faster oxidation reaction when ozone exposure time of hazelnut oil extended. The rate constant of oxidation reaction showed excellent correlations with the standard chemical methods. The experimental results showed that FFA increased with the increase in reaction rate constant as the ozone treatment time was increased. Similarly, PV showed a dramatic increase with k as the ozone treatment time was extended.

DSC heating and cooling thermogram;

The endothermic melting peaks slightly shifted towards lower temperature when ozone treatment time is extended. The melting curve of hazelnut oil samples became broader and less evident from 30 min ozone treatment time onwards. After 180 and 360 min ozone treatment, the melting curves became less evident or disappeared.

The sharp exothermic crystallization shifted towards lower temperature with ozone treatment process and becoming broader and shorter as treatment time increased. The minor peak became less evident and apparently disappeared when ozone treatment proceeded. All these variations in cooling profile may be associated with oxidative products (i.e. free fatty acids, peroxide value, and oxidative induction time decrease.

Gas chromatography analysis;

A dramatic reduction in the unsaturated fatty acids content and an increase in the saturated fatty acid content in the oil was observed when ozone treatment proceeded. The decrease in the percentage of oleic acid was highest and linoleic acid was destroyed completely after ozone treatment for 360 min. Furthermore, an increase in the percentages of the saturated fatty acid was reached to highest value

with 360 min ozonation. Also, new peaks which being suggested formed mainly from the reaction of ozone with oleic acid and secondly with linoleic acid, were observed in the chromatograms.

FTIR (Fourier transform infrared radiation) analysis;

The variations were observed in frequency and absorbance of FTIR spectra bands after ozone treatment. Oxidation of oils leads to changes in the frequency of the same bands. The intensities of the bands related to double bonds in unsaturated oil decreased as ozone treatment extended. Double bonds finally disappeared during ozone treatment for 360 min. The broad and relatively strong bands corresponding to primary or secondary ozonide were observed as ozone treatment proceeded.

NMR (Nuclear magnetic resonance) analysis;

NMR analysis confirmed that the structural changes in the hazelnut oil occurred during ozone treatment. The new signals found in ozone treated hazelnut oil. In ^{1}H NMR spectra of ozone treated hazelnut oil, new signal at 5.15 ppm was found, the new signal was assigned to the ring proton of 1,2,4 trioxolane (secondary ozonide or Criegee ozonide). The result was confirmed by the appearance of the signal in ^{13}C NMR spectra at 104.35 ppm assigned by them, as ring carbon in the same structure. Area of the new peaks increased with ozone treatment time. The signals of unsaturated bonds dramatically decreased with ozone treatment time and finally disappeared after ozone treatment for 360 min.

Emulsifying properties;

Ozone treatment significantly affected emulsion activity negatively ($p<0.05$). Emulsion stability had been more pronounced affected negatively as ozone treatment time increased. The better stability was observed in the emulsion prepared with ozone treated oil for 360 min because of indicating highest viscosity value of oil at 360 min ozone treatment. Therefore, the motion of oil droplets in the emulsion slowed down when the viscosity of oil increased.

Creaming rate of the emulsion prepared with short time ozone treated oils retarded compared to creaming behavior of the emulsion containing untreated oil.

However, a dramatic increase was observed in the creaming rate of the emulsion prepared with 180 min ozone treated oil. Creaming could not be observed because of a dramatic increase in viscosity of oil after ozone treatment for 360 min.

Polarised light microscope (PLM);

The image related with whey protein stabilized emulsion prepared with untreated hazelnut oil was indicated that small and isolated spherical oil droplets. The oil droplet size of emulsions enlarged when the ozone treatment time increased. Especially, the optical microscopy measurements of emulsion microstructure confirmed that large droplet flocculation formed in the emulsions containing ozone treated oils for 60, 180 and 360 min. The emulsion prepared with 360 min ozone treated oil had a fairly coarse microstructure.

Rheological properties of emulsions;

The crossover between G'' and G' curves shifted towards to low frequencies when ozone treatment time increased. Therefore, the emulsions containing ozone treated oils exhibited more solid like behaviour when ozone treatment time increased.

Microbiological analysis;

With ozone treatment process, TPC in the hazelnut oil decreased rapidly. TPC of ozone treated hazelnut oil totally destroyed, while TPC of untreated hazelnut oil was 200 CFU/g. Mould and yeast in untreated hazelnut oil could not be detected and so, any change in mould and yeast could not be observed after ozone treatment.

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APPENDICES

PV

Table A.1 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of different ozone treatment time on PV of hazelnut oil for first day.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	86097,160	4	21524,290	217431,712	,000
Within Groups	,990	10	,099		
Total	86098,150	14			

Duncan

	N	Subset for alpha = .05				
Time	1	2	3	4	5	1
,00	3	12,1500				
1,00	3		15,7767			
5,00	3			33,6000		
60,00	3				120,5333	
180,00	3					207,2700
Sig.		1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

PV

Table A.2 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of different ozone treatment time on PV of hazelnut oil for tenth day.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	90348,984	4	22587,246	205625,230	,000
Within Groups	1,098	10	,110		
Total	90350,083	14			

Duncan

	N	Subset for alpha = .05				
time	1	2	3	4	5	1
,00	3	15,1333				
1,00	3		26,4700			
5,00	3			44,5567		
60,00	3				133,4867	
180,00	3					218,6333
Sig.		1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3,000.

Table A.3 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of different ozone treatment time on PV of hazelnut oil for thirty days.

PV

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	102266,864	4	25566,716	181564,596	,000
Within Groups	1,408	10	,141		
Total	102268,272	14			

Duncan

	N	Subset for alpha = .05				
Time	1	2	3	4	5	1
,00	3	17,3667				
1,00	3		30,6567			
5,00	3			56,5367		
60,00	3				143,6367	
180,00	3					236,7633
Sig.		1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.4 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of different ozone treatment time on PV of hazelnut oil for hundredth days.

PV

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	138885,513	4	34721,378	85446,274	,000
Within Groups	4,064	10	,406		
Total	138889,576	14			

Duncan

	N	Subset for alpha = .05				
Time	1	2	3	4	5	1
,00	3	30,3800				
1,00	3		60,7200			
5,00	3			74,5500		
60,00	3				177,4867	
180,00	3					292,1267
Sig.		1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.5 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of different ozone treatment time on PV of hazelnut oil for 150 days.

PV

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	145161,122	4	36290,281	267259,529	,000
Within Groups	1,358	10	,136		
Total	145162,480	14			

Duncan

	N	Subset for alpha = .05				
Time	1	2	3	4	5	1
,00	3	38,1967				
1,00	3		78,3833			
5,00	3			110,5233		
60,00	3				193,3133	
180,00	3					315,4200
Sig.		1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.6 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of 1 min ozone treatment on PV of hazelnut oil.

PV

ANOVA for 1min ozone treatment

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14614,850	12	1217,904	3958188,625	,000
Within Groups	,008	26	,000		
Total	14614,858	38			

Duncan

days	N	Subset for alpha = .05											
	1	2	3	4	5	6	7	8	9	10	11	12	13	1
0	3	15,92												
3	3		18,35											
7	3			23,19										
10	3				26,18									
20	3					28,55								
30	3						30,95							
40	3							32,53						
55	3								35,77					
70	3									49,09				
85	3										54,96			
100	3											60,94		
125	3												69,02	
145	3													78,43
Sig.		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.7 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of 5 min ozone treatment on PV of hazelnut oil.

PV

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7001,539	11	636,504	1878207,299	,000
Within Groups	,008	24	,000		
Total	7001,548	35			

Duncan

days	N	Subset for alpha = .05											
	1	2	3	4	5	6	7	8	9	10	11	12	1
0	3	33,72											
3	3		41,53										
7	3			44,43									
10	3				44,58								
20	3					47,67							
30	3						56,62						
40	3							65,96					
55	3								66,67				
70	3									68,58			
85	3										69,53		
100	3											74,16	
125	3												76,92
Sig.		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.8 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of 60 min ozone treatment on PV of hazelnut oil.

PV

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8999,468	10	899,947	2516800 ,425	,000
Within Groups	,008	22	,000		
Total	8999,476	32			

Duncan

days	N	Subset for alpha = .05										
	1	2	3	4	5	6	7	8	9	10	11	1
0	3	120,63										
3	3		125,87									
7	3			130,45								
10	3				133,62							
20	3					137,87						
30	3						143,60					
40	3							152,55				
55	3								153,67			
70	3									153,82		
85	3										163,85	
1000	3											177,87
Sig.		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.9 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of 180 min ozone treatment on PV of hazelnut oil.

PV

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4489,390	7	641,341	78893,868	,000
Within Groups	,130	16	,008		
Total	4489,520	23			

Duncan

days	N	Subset for alpha = .05							
	1	2	3	4	5	6	7	8	1
0	3	207,5							
3	3		211,32						
7	3			213,65					
10	3				218,90				
20	3					221,33			
30	3						236,75		
40	3							237,53	
55	3								248,05
Sig.		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.10 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of storage time on PV of untreated hazelnut oil.

PV

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2359,666	11	214,515	617803,505	,000
Within Groups	,008	24	,000		
Total	2359,675	35			

Duncan

days	N	Subset for alpha = .05											
	1	2	3	4	5	6	7	8	9	10	11	12	1
0	3	12,17											
3	3		13,24										
10	3			14,25									
20	3				16,52								
30	3					17,23							
40	3						20,13						
55	3							25,03					
70	3								26,24				
85	3									28,69			
100	3										30,17		
125	3											32,23	
145	3												38,19
Sig.		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.11 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of storage time on FFA of untreated hazelnut oil.

FFA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,001	12	,000	188,648	,000
Within Groups	,000	26	,000		
Total	,001	38			

Duncan

	N	Subset for alpha = .05						
days	1	2	3	4	5	6	7	1
0	3	,102267						
3	3		,106100					
7	3		,106167					
20	3			,108133				
10	3			,109200				
30	3			,109267				
55	3				,111000			
70	3				,111000			
40	3				,111667	,111667		
85	3				,112000	,112000		
100	3					,112600		
125	3						,116267	
145	3							,126000
Sig.		1,000	,911	,080	,133	,147	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.12 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of 1 min ozone treatment on FFA of hazelnut oil.

FFA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,004	12	,000	5201,010	,000
Within Groups	,000	26	,000		
Total	,004	38			

Duncan

days	N	Subset for alpha = .05										
	1	2	3	4	5	6	7	8	9	10	11	1
0	3	,1091										
3	3		,1133									
7	3			,1162								
10	3				,1181							
20	3				,1181							
30	3					,1223						
40	3						,1241					
55	3							,1262				
70	3							,1262				
85	3								,1292			
100	3									,1331		
125	3										,1375	
145	3											,1481
Sig.		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.13 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of 5 min ozone treatment on FFA of hazelnut oil.

FFA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,008	12	,001	250,093	,000
Within Groups	,000	26	,000		
Total	,008	38			

Duncan

Days	N	Subset for alpha = .05						
	1	2	3	4	5	6	7	1
,00	3	,113333						
3,00	3	,115033	,115033					
7,00	3		,117000					
10,00	3		,117000					
20,00	3			,122333				
30,00	3			,124233	,124233			
40,00	3				,126200			
55,00	3				,126200			
70,00	3					,130200		
85,00	3					,132300		
100,00	3						,149233	
125,00	3							,154233
150,00	3							,156300
Sig.		,209	,170	,162	,170	,124	1,000	,129

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.14 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of 60 min ozone treatment on FFA of hazelnut oil.

FFA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,032	12	,003	21255,885	,000
Within Groups	,000	26	,000		
Total	,032	38			

Duncan

Days	N	Subset for alpha = .05											
	1	2	3	4	5	6	7	8	9	10	11	12	1
0	3	,1221											
3	3		,1262										
7	3		,1262										
10	3			,1271									
20	3				,1301								
30	3					,1313							
40	3						,1351						
55	3							,1421					
70	3								,1531				
85	3									,1641			
100	3										,1880		
125	3											,1971	
150	3												,2071
Sig.		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3,000.

Table A.15 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of 180 min ozone treatment on FFA of hazelnut oil.

FFA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,167	12	,014	1390380 ,769	,000
Within Groups	,000	26	,000		
Total	,167	38			

Duncan

Days	N	Subset for alpha = .05										
	1	2	3	4	5	6	7	8	9	10	11	1
0	3	,14110										
3	3	,14110										
7	3	,14110										
10	3		,14510									
20	3			,14810								
30	3				,15010							
40	3					,15410						
55	3						,20310					
70	3							,23410				
85	3								,25510			
100	3									,27110		
125	3										,29610	
150	3											,32810
Sig.		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3,000.

Table A.16 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of different ozone treatment time on FFA of hazelnut oil for first days.

FFA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,003	4	,001	12309,680	,000
Within Groups	,000	10	,000		
Total	,003	14			

Duncan

	N	Subset for alpha = .05				
time	1	2	3	4	5	1
,0000	3	,102200				
1,0000	3		,109200			
5,0000	3			,110233		
60,0000	3				,122300	
180,0000	3					,141233
Sig.		1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.17 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of different ozone treatment time on FFA of hazelnut oil for tenth days.

FFA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,002	4	,001	182,464	,000
Within Groups	,000	10	,000		
Total	,002	14			

Duncan

	N	Subset for alpha = .05			
time	1	2	3	4	1
,000	3	,10600			
1,000	3		,11700		
5,000	3		,11700		
60,000	3			,12600	
180,000	3				,14100
Sig.		1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.18 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of different ozone treatment time on FFA of hazelnut oil for thirty days.

FFA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,002	4	,001	216,214	,000
Within Groups	,000	10	,000		
Total	,002	14			

Duncan

	N	Subset for alpha = .05			
time	1	2	3	4	1
,000	3	,10900			
1,000	3		,12200		
5,000	3		,12400		
60,000	3			,13000	
180,000	3				,14800
Sig.		1,000	,174	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.19 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of different ozone treatment time on FFA of hazelnut oil for hundredth days.

FFA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,047	4	,012	922091,263	,000
Within Groups	,000	10	,000		
Total	,047	14			

Duncan

	N	Subset for alpha = .05				
Time	1	2	3	4	5	1
,0000	3	,113167				
1,0000	3		,133100			
5,0000	3			,149100		
60,0000	3				,188100	
180,0000	3					,271100
Sig.		1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.20 ANOVA and Multiple Comparison Test (Duncan Test) table for the effect of different ozone treatment time on FFA of hazelnut oil for 150 days.

FFA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,079	4	,020	1096539,074	,000
Within Groups	,000	10	,000		
Total	,079	14			

Duncan

	N	Subset for alpha = .05				
Time	1	2	3	4	5	1
,0000	3	,126100				
1,0000	3		,148167			
5,0000	3			,156100		
60,0000	3				,207133	
180,0000	3					,328167
Sig.		1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.21 ANOVA and Multiple Comparison Test (Duncan Test) table for the reaction rate constant of untreated hazelnut oil at different temperatures.

Rate for HO

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	407,056	3	135,685	626239,321	,000
Within Groups	,002	8	,000		
Total	407,057	11			

Duncan[a]

		Subset for alpha = 0.05			
Temperature	N	1	2	3	4
373,00	3	1,8100			
383,00	3		3,7100		
393,00	3			7,8167	
403,00	3				16,9267
Sig.		1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3,000.

Table A.22 ANOVA and Multiple Comparison Test (Duncan Test) table for the reaction rate constant of OHO1 at different temperatures.

Rate for OHO1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	389,856	3	129,952	222775,100	,000
Within Groups	,005	8	,001		
Total	389,861	11			

Temperature	N	Subset for alpha = 0.05			
		1	2	3	4
373,00	3	2,0500			
383,00	3		4,1233		
393,00	3			8,2500	
403,00	3				16,9200
Sig.		1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3,000.

Table A.23 ANOVA and Multiple Comparison Test (Duncan Test) table for the reaction rate constant of OHO5 at different temperatures.

Rate for OHO5

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4299,400	3	1433,133	2605697,106	,000
Within Groups	,004	8	,001		
Total	4299,405	11			

Duncan[a]

Temperature	N	Subset for alpha = 0.05			
		1	2	3	4
373,00	3	7,0267			
383,00	3		14,1167		
393,00	3			28,2267	
403,00	3				56,4600
Sig.		1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

Table A.24 ANOVA and Multiple Comparison Test (Duncan Test) table for the reaction rate constant of OHO60 at different temperatures.

Rate for OHO60

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14529,401	3	4843,134	5588231,295	,000
Within Groups	,007	8	,001		
Total	14529,408	11			

Duncan[a]

Temperature	N	Subset for alpha = 0.05			
		1	2	3	4
373,00	3	12,8267			
383,00	3		25,6500		
393,00	3			51,6400	
403,00	3				103,6233
Sig.		1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3,000.

Table A.25 ANOVA and Multiple Comparison Test (Duncan Test) table for the reaction rate constant of OHO180 at different temperatures.

Rate for OHO180

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25169,827	3	8389,942	8532144,610	,000
Within Groups	,008	8	,001		
Total	25169,834	11			

Duncan[a]

Temperature	N	Subset for alpha = 0.05			
		1	2	3	4
373,00	3	17,5500			
383,00	3		35,1400		
393,00	3			68,7567	
403,00	3				137,3400
Sig.		1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3,000.

Table A.26 ANOVA and Multiple Comparison Test (Duncan Test) table for the variations in the activation energy (Ea) of OHO1 with storage time.

Ea

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,059	5	,012	,286	,912
Within Groups	,498	12	,041		
Total	,557	17			

Duncan[a]

Time	N	Subset for alpha = 0.05
		1
30,00	3	86,6633
3,00	3	86,7100
60,00	3	86,7133
14,00	3	86,7367
90,00	3	86,7533
,00	3	86,8500
Sig.		,328

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3,000.

Table A.27 ANOVA and Multiple Comparison Test (Duncan Test) table for the variations in the activation enthalpy (ΔH) of OHO1 with storage time.

Enthalpy

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,101	5	,020	,265	,924
Within Groups	,914	12	,076		
Total	1,015	17			

Duncan[a]

Time	N	Subset for alpha = 0.05
		1
14,00	3	83,3000
90,00	3	83,3300
3,00	3	83,4533
60,00	3	83,4567
30,00	3	83,4733
,00	3	83,4967
Sig.		,442

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3,000.

Table A.28 ANOVA and Multiple Comparison Test (Duncan Test) table for the variations in the activation entropy (ΔS) of OHO1 with storage time.

Entropy

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,156	5	,031	,164	,971
Within Groups	2,283	12	,190		
Total	2,439	17			

Duncan[a]

Time	N	Subset for alpha = 0.05
		1
60,00	3	73,4333
30,00	3	73,4967
90,00	3	73,5133
14,00	3	73,5300
,00	3	73,6467
3,00	3	73,7067
Sig.		,498

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3,000.

Table A.29 ANOVA and Multiple Comparison Test (Duncan Test) table for the variations in the activation energy (Ea) with ozone treatment time.

Ea

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	90,520	4	22,630	2259,997	,000
Within Groups	,100	10	,010		
Total	90,621	14			

Duncan[a]

ozonationtime	N	Subset for alpha = 0.05				
		1	2	3	4	5
180,00	3	84,5400				
60,00	3		85,0300			
5,00	3			86,8133		
1,00	3				87,7467	
,00	3					91,4400
Sig.		1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3,000.

Table A.30 ANOVA and Multiple Comparison Test (Duncan Test) table for the variations in the activation enthalpy (ΔH) with ozone treatment time.

Enthalpy

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	110,369	4	27,592	275922,600	,000
Within Groups	,001	10	,000		
Total	110,370	14			

Duncan[a]

ozonationtime	N	Subset for alpha = 0.05				
		1	2	3	4	5
180,00	3	80,3300				
60,00	3		81,8700			
5,00	3			83,4100		
1,00	3				84,5100	
,00	3					88,3200
Sig.		1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3,000.

Table A.31 ANOVA and Multiple Comparison Test (Duncan Test) table for the variations in the activation entropy (ΔS) with ozone treatment time

Entropy

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	466,711	4	116,678	380471,011	,000
Within Groups	,003	10	,000		
Total	466,714	14			

Duncan[a]

ozonationtime	N	Subset for alpha = 0.05				
		1	2	3	4	5
180,00	3	62,3333				
60,00	3		63,7167			
5,00	3			64,1500		
1,00	3				74,4133	
,00	3					75,0133
Sig.		1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3,000.

Table A.32 ANOVA and Multiple Comparison Test (Duncan Test) table for the variations in the colour parameters (a*) with ozone treatment time.

a*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,498	6	,083	,483	,810
Within Groups	2,406	14	,172		
Total	2,904	20			

Duncan[a]

ozonation	N	Subset for alpha = 0.05
		1
5,0000	3	-3,360000
30,0000	3	-3,280000
1,0000	3	-3,270000
,0000	3	-3,253333
360,0000	3	-3,126667
180,0000	3	-2,966667
60,0000	3	-2,930000
Sig.		,275

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3,000.

Table A.33 ANOVA and Multiple Comparison Test (Duncan Test) table for the variations in the colour parameters (b*, L*) with ozone treatment time.

b*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5953,959	6	992,326	42975,574	,000
Within Groups	,323	14	,023		
Total	5954,282	20			

Duncan

ozonation	N	Subset for alpha = 0.05						
		1	2	3	4	5	6	7
360,0000	3	6,55500						
180,0000	3		7,35667					
60,0000	3			9,05333				
30,0000	3				13,12000			
5,0000	3					15,22000		
1,0000	3						28,54667	
,0000	3							57,17667
Sig.		1,000	1,000	1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

Table A.34 ANOVA and Multiple Comparison Test (Duncan Test) table for the variations in the colour parameter (L*) with ozone treatment time.

L*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17,641	6	2,940	,891	,527
Within Groups	46,203	14	3,300		
Total	63,843	20			

Duncan[a]

ozonation	N	Subset for alpha = 0.05
		1
180,0000	3	59,396667
30,0000	3	59,720000
360,0000	3	61,046667
1,0000	3	61,046667
5,0000	3	61,183333
60,0000	3	61,213333
,0000	3	62,323333
Sig.		,100

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

Table A.35 ANOVA and Multiple Comparison Test (Duncan Test) table for the variations in the colour parameter (Y1) with ozone treatment time.

Y_1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14705,998	6	2451,000	9561,945	,000
Within Groups	3,589	14	,256		
Total	14709,587	20			

		Subset for alpha = 0.05						
ozonation	N	1	2	3	4	5	6	7
360,0000	3	13,30333						
180,0000	3		16,35000					
60,0000	3			20,31000				
30,0000	3				29,11666			
5,0000	3					32,83666		
1,0000	3						56,83000	
,0000	3							93,25000
Sig.		1,000	1,000	1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3,000.

Table A.36 ANOVA and Multiple Comparison Test (Duncan Test) table for the variations in the iodine value (IV) with ozone treatment time.

IV

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18233,068	6	3038,845	61544,738	,000
Within Groups	,691	14	,049		
Total	18233,760	20			

Duncan[a]

		Subset for alpha = 0.05						
Ozonation time	N	1	2	3	4	5	6	7
360,0000	3	13,576667						
180,0000	3		18,253333					
60,0000	3			22,366667				
30,0000	3				32,233333			
5,0000	3					65,293333		
1,0000	3						72,203333	
,0000	3							95,503333
Sig.		1,000	1,000	1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3,000.

Table A.37 ANOVA and Multiple Comparison Test (Duncan Test) table for the variations in the iodine value (IV) with storage time.

IV

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,023	10	,102	,048	1,000
Within Groups	46,553	22	2,116		
Total	47,576	32			

Duncan[a]

Storage time	N	Subset for alpha = 0.05
		1
90,0000	3	93,110000
10,0000	3	93,536667
,0000	3	93,566667
40,0000	3	93,580000
120,0000	3	93,603333
30,0000	3	93,663333
5,0000	3	93,706667
15,0000	3	93,706667
60,0000	3	93,733333
50,0000	3	93,736667
75,0000	3	93,783333
Sig.		,626

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3,000.

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Degree	Instution	Graduation year	GPA
Ph.D.	University of Gaziantep		3.79/4.00
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B.Sc.	University of Gaziantep	2006	2.71/4.00

WORK EXPERIENCE

2010-……	Lecturer	University of Gaziantep
2007-2010	Food Engineer	Kahraman Tarım Inc.

PROJECTS

2009-2013	FP7 project Marine algae as biomass for biofuels (MABFUEL)	European Commission Research and Technological Project
2007-2009	Effects of ozone on functional properties of proteins	TUBITAK

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