NOVEMBER 2021

Ph.D. in Food Engineering

ŞAKİR SELÇUK SEÇİLMİŞ

REPUBLIC OF TURKEY

GAZİANTEP UNIVERSITY

GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES

MICROWAVE-ASSISTED BLEACHING OF VEGETABLE OILS

Ph.D. THESIS

IN

FOOD ENGINEERING

BY

ŞAKİR SELÇUK SEÇİLMİŞ

NOVEMBER 2021

MICROWAVE-ASSISTED BLEACHING OF VEGETABLE OILS

Ph.D. Thesis

in

Food Engineering

Gaziantep University

Supervisor

Prof. Dr. Sibel FADILOĞLU

by

Şakir Selçuk SEÇİLMİŞ

November 2021

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

Şakir Selçuk SEÇİLMİŞ

ABSTRACT

MICROWAVE-ASSISTED BLEACHING OF VEGETABLE OILS

SEÇİLMİŞ, Şakir Selçuk
Ph.D. in Food Engineering
Supervisor: Prof. Dr. Sibel FADILOĞLU
November 2021
91 pages

In this thesis, sunflower oil and olive pomace oil were bleached by using microwave technique which is a novel bleaching method. In the first part of the thesis, the effect of bleaching parameters on the bleaching quality was investigated. Response surface methodology was employed to optimize the effect of microwave power, bleaching time and amount of clay for both oils. Optimum conditions obtained for microwave-assisted bleaching were 80-102 W, 8-20.07 min and 0.4-4.15% of clay for sunflower oil and pomace oil, respectively. Physical and chemical properties of both oil type investigated were sterol composition, acid value, oxidation parameters (PV, AV, Totox, K_{232} and K_{270}), color parameters (chroma and hue angle values), chlorophyll, carotenoid- tocopherol content and harmful components such as PAHs. In the second part of the thesis, the quality of microwave assisted bleached oils was compared with industrially bleached oils obtained from same batch. In the third part of the thesis, the repetitive use of bleaching clay was examined at the end of the microwave assisted bleaching of sunflower oil, which is important because it reduces the production cost of oil and environmental pollution. According to the findings, microwave bleaching was found advantageous with a 50%-17% and 73%-83% reduction in the amount of clay usage and bleaching time for sunflower oil and olive pomace oil, respectively. The results of this thesis indicated that, promising bleaching quality was obtained by microwave assisted method when compared to the industrial method. So, it has been concluded that microwave bleaching could be an alternative and advantageous method for the bleaching of vegetable oils.

Key Words: Microwave, Bleaching, Sunflower Oil, Olive Pomace Oil

ÖZET

BİTKİSEL YAĞLARIN MİKRODALGA DESTEKLİ AĞARTILMASI

SEÇİLMİŞ, Şakir Selçuk
Doktora Tezi, Gıda Mühendisliği
Danışman: Prof. Dr. Sibel FADILOĞLU
Kasım 2021
91 sayfa

Bu tez çalışmasında, yeni bir ağartma yöntemi olan mikrodalga tekniği kullanılarak ayçiçek yağı ve pirina yağı ağartılmıştır. Tezin ilk bölümünde, ağartma parametrelerinin ağartma kalitesine etkisi araştırılmıştır. Her iki yağ türü için mikrodalga gücü, ağartma süresi ve toprak miktarının etkisini optimize etmek için tepki yüzey metodu kullanılmıştır. Mikrodalga destekli ağartma için elde edilen optimum koşullar ayçiçek yağı ve pirina yağı için sırasıyla 80-102 W, 8-20,07 dakika ve %0,4-4,15 topraktır. İncelenen her iki yağ türünün fiziksel ve kimyasal özellikleri; sterol bileşimi, asit değeri, oksidasyon parametreleri (PV, AV, Totox, K_{232} ve K_{270}) renk parametreleri (renk doygunluğu ve renk açısı değerleri), klorofil, karotenoid ve tokoferol içeriği ve PAHs gibi zararlı bileşen miktarı belirlenmiştir. Tezin ikinci bölümünde, mikrodalga destekli ağartılmış yağların kalitesi, aynı partiden elde edilen endüstriyel ağartılmış yağlarla karşılaştırılmıştır. Tezin üçüncü bölümünde ise ayçiçek yağının mikrodalga destekli ağartılması sonunda, üretim maliyetini ve çevre kirliliğini azalttığı için önem arz eden tekrarlı ağartma toprağı kullanımı incelenmiştir. Bulgulara göre; ayçiçek yağı ve zeytin pirinası yağı için sırasıyla, toprak kullanım miktarı ve ağartma süresindeki %50-%17 ve %73-83 düşüşle, mikrodalga ağartma avantajlı bulunmuştur. Bu tezin sonuçları; mikrodalga destekli yöntemle, endüstriyel yöntem karşılaştırıldığında, umut verici bir ağartma kalitesi elde edildiğini göstermiştir. Bu nedenle, mikrodalga ağartmanın bitkisel yağların ağartılmasında alternatif ve avantajlı bir yöntem olabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Mikrodalga, Ağartma, Ayçiçek Yağı, Pirina Yağı

"Dedicated to my dear parents and beloved my wife and children"

ACKNOWLEDGEMENTS

I would like to express my special sense of respect, gratitude and appreciation to my supervisor Prof. Dr. Sibel FADILOĞLU for her invaluable guidance, patience, advice and constant support during this thesis. You have provided excellent advice and motivation to enable me to progress.

I would also like to express my sincere thanks to Prof. Dr. Fahrettin GÖĞÜŞ and Assoc. Prof. Dr. Derya KOÇAK YANIK for their guidance, patience, criticism and comments during this research. Thank you for your continuous guidance, caring, and enlightening discussions regarding this work. Your guidance will be very important in every time of my life.

I would also thank to Dr. Aysel ELİK, Dr. Hatice Neval MUCUK and Food Engineer Nazan ÇAĞLAR employed in the Department of Food Engineering for their support in the lab, endless helpful characteristics and guidance.

To my wonderful parents, the best I could ever ask for thank you for being there for me at all times, Thank you for your prayers, advice, love and for supporting me during every stage of my life.

I would like to thanks to my beloved my wife Dr. Nisa SEÇİLMİŞ for her patience, help and support. Of course, another thanks goes to my precious sons Alper and Kubilay...

Financial support for this thesis is provided by the Scientific and Technological Research Council of Turkey (TUBITAK) 1002 Project No: 217O348.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AV	P- anisidine Value
BaP	Benzo[a]pyrene
BE	Bleaching Efficiency
CCRD	Central Composite Rotatable Design
HPLC	High pressure liquid chromatography
IBSO	Industrial Bleached Sunflower Oil
IBPO	Industrial Bleached Pomace Oil
MWB	Microwave-assisted Bleaching
MWBSO	Microwave-assisted Bleached Sunflower Oil
MWBPO	Microwave-assisted Bleached Pomace Oil
NPO	Neutralized Pomace Oil
NSO	Neutralized Sunflower Oil
PAHs	Polycyclic Aromatic Hydrocarbons
PO	Pomace Oil
PV	Peroxide Value
RSM	Response Surface Methodology
SEM	Scanning Electron Microscopy
SPE	Solid Phase Extraction
Totox	Total Oxidation Value

CHAPTER I

INTRODUCTION

Fats and oils are important and valuable materials for the processing of many food and non-food products and also for human nutrition. The terms as fat and oil relate to their melting point, degree of saturation and number of carbons in the fatty acids. Fats are also obtained from animals but oils usually obtained from plants. Vegetable oils, subjected in this study, rich in bioactive components such as sterols, carotenes, tocols, phenolic substances etc, have an important place for healthy life. These bioactive components vary according to the region where it grows and its climate. Oil not only provides energy to the body, but also plays a role in maintaining body temperature and tissues, carrying soluble vitamins and performing many other important functions.

Crude vegetable oils also contain some impurities such as phosphatides, color pigments, oxidation products and trace elements that should be removed from the oil as they cause problems in their chemical structure. Vegetable oil consumption is 158 million tons / year in the all around the world; it is observed that this figure is gradually increasing [1, 2]. Therefore, obtaining better oil quality and meeting consumer expectations depend on the good refinery conditions. In this case, the main goal of oil refining is to minimize the loss of oil and valuable components, while removing unwanted impurities as much as possible without occurring harmful components. For this reason, researching of new methods to prevent the nutritional losses of vegetable oils continues, and studies on the prevention of formation harmful components are also recommended [3, 4].

Many food processing depends on the heating which is very crucial for better food quality. It is directly effective on the obtaining the good or bad quality of food product. It serves different kind of aims such as microbial and enzyme inactivation, physical and chemical changes of foods and etc., during processes. In general,

conventional heating sources such as steam and hot air are frequently used for numerous food processes. On the other hand, in recent years, electric heating technologies such as microwave, radio frequency, ohmic and infrared have been used for many food processes. Therefore, the use of microwave is very popular in various industrial applications.

Microwave is used especially in food industry to reduce process time and increase food quality. The unit processes such as baking, blanching, thawing, sterilization and drying can be carried out by using microwave treatment [5]. Therefore, researches on microwaves and its experiments on different products have usually concentrated on these issues. Nutritional quality and some sensorial properties of foods are protected very well in the microwave treatment compared with conventional heating [6]. Some microwave applications related with vegetable oils in the literature include deep frying of foods, oil extraction and activation of bleaching clays supporting to the mineral acids [7-10]. However, microwave treatment has not been found in the vegetable oil refining process such as bleaching in the literature.

There are vast numbers of studies to improve the bleaching process of oils. In all these studies, the type and amount of bleaching agent, mixing time, temperature and pressure are the parameters examined [11-15] by keeping the conventional method as the same. Only in few studies, new technologies such as ultrasound and high voltage electric field were used as alternative techniques to the conventional process [16-18]. In the existing conventional bleaching methods, the desired oil color quality is achieved by increasing the amount of clay that leads to increased oil losses and environmental problems. Therefore, the development of new bleaching methods to overcome these problems is necessary for the vegetable oil industry.

The aim of this thesis was to perform bleaching of sunflower and pomace oils by using microwave treatment. In detail, neutralized sunflower oil (NSO) and pomace oil (NPO) were bleached by using microwave and the bleaching parameters such as microwave power, bleaching time and amount of bleaching clay were optimized by response surface methodology (RSM). First of all, optimized conditions for microwave-assisted bleaching of sunflower oil (MWBSO) and pomace oil (MWBPO) were compared with the conventional bleaching conditions by considering the bleaching time and amount of clay. After that bleached oils quality;

such as color, chlorophyll, carotenoid and tocopherol content, sterol composition and oxidation parameters and harmful components such as PAHs, was evaluated for both methods. At the same time, the repetitive use of bleaching clay at the end of the MWBSO, which is important because it reduces the production cost of oil and environmental pollution, will be examined in this thesis.

CHAPTER II

LITERATURE REVIEW

2.1 Description of Vegetable Oil

Vegetable oils are obtained from plants such as nuts, seeds, fruits and cereal grains. But, plants only having commercial quantities are used for oil production. Even if they have commercial value, some of them might not be suitable for human nutrition in terms of chemical structure and including materials. Hence, the suitable vegetable oils for human consumption are called as edible vegetable oils.

Vegetable oils include more unsaturated fatty acids compared with that of animal originated fats. Therefore, they are generally liquid at room temperature because of including unsaturated fatty acids. But, as an exception, coconut oil is solid at room temperature because of having saturated fatty acids content. Especially, edible vegetable oils are usually used for cooking, in salads, in production of margarines and in baking etc. Their chemical composition depends on the origin of plant and that of growing conditions. These factors cause different quantites of component contents in the oil. But, some nonglyceridic components can be changed depending on the process and storage conditions [19]. A typical vegetable oil consists of three mole fatty acid with one mole glycerol bonded via esterification (Figure 2.1). Vegetable oils having non-polar characteristics are soluble in organic solvents because of their hydrophobic property.

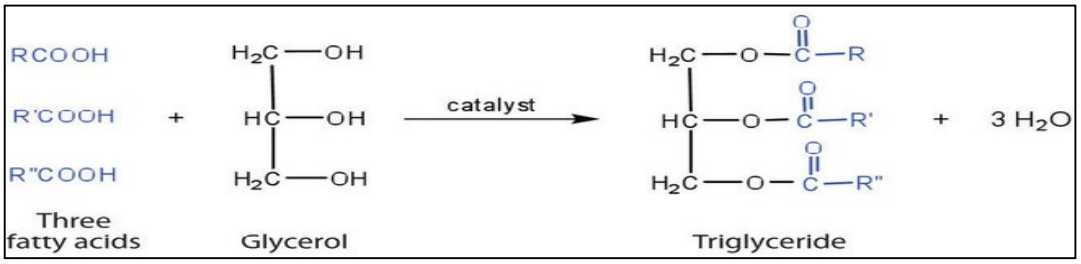

Figure 2.1 Structure of oil [20]

2.2 **Sunflower Oil and Its Properties**

It is the most common vegetable oil obtained from a plant called as sunflower (*Helianthus annuus* L.) in the world. The main source of oil is the sunflower seeds containing high amount oil (40-50%) (Figure 2.2). Mechanical pressing and solvent extraction are used for obtaining of oil from seeds. Sunflower oil (SO) mainly includes polyunsaturated fatty acids and linoleic acid (C-18:2) and oleic acid (C-18:1) are the major components (90%) in a typical sunflower oil. The remaining of palmitic (C-16:0) and stearic (C-18:0) have 8-10% ratio.

The nonglyceridic components such as tocopherols and sterols are the other important components of SO. These components are the effective on the nutritional and technological properties of the oil. However, others such as waxes, free fatty acids, and phospholipids negatively effect on oil stability and quality [21].

There are also different varieties of sunflower seed including different kind of fatty acids. For example, SO including higher oleic acid alternative to olive oil has a greater oxidative stability than traditional SO [22]. Therefore, high stearic (C-18:0) SO can be cheap and healthy alternative to cocoa butter, a source of stearic fatty acids [23]. Hence, SO can be used in different food industries such as cooking, salad dressing and fat spreads. A pale yellow color and mild flavor are the main characteristics for refined SO. The melting point of SO is below 0 °C. This provides an advantage for some products such as processing of sauces and emulsions having to keep cold [24].

Figure 2.2 Sunflower seeds and sunflower plant

2.3 Olive Pomace Oil and Its Properties

High amounts of waste water (100 liters/100 kg olive fruits) and olive pomace (35 kg /100 kg olive fruits) occur during the processing of olive oil [25]. Being a solid material pomace (Figure 2.3) consists of pieces of skin, pulp, stone and olive kernel.

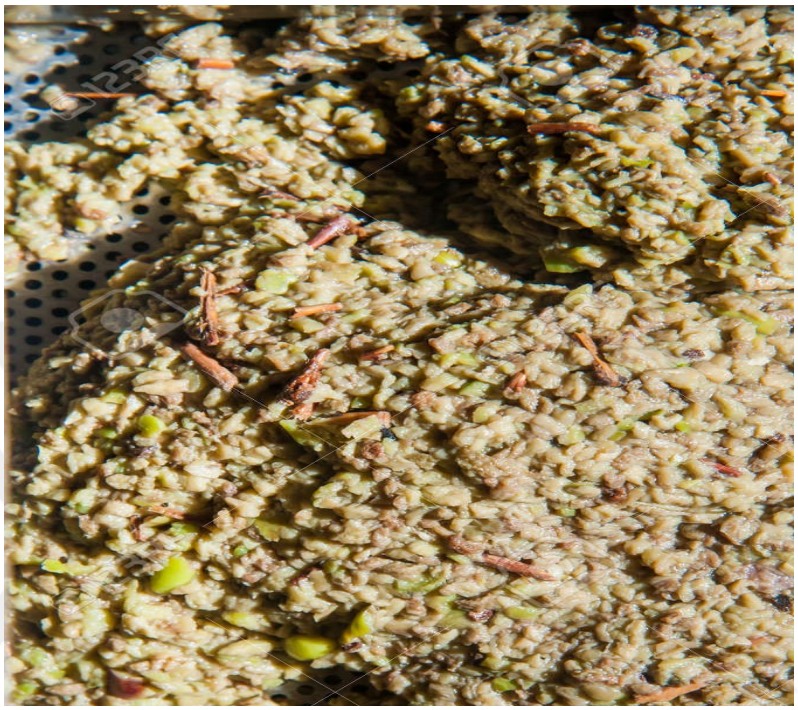

Figure 2.3 Olive pomace after the pressing process

Of the total olive weight, the pulp forms about 70-90%, the stone 9-27% and the seed about 2-3% [26]. This sludge includes a certain amount of oil and water. That of amount of oil and water content changing with olive oil processing conditions (press, two-phase and three-phase decanter), determines its commercial value. The ratios of water in the pomace are 70%, 45% and 25-30% for two, three phase and press, respectively [27].

Olive pomace also includes proteins, carbohydrates, phenols and a number of inorganic compounds (Table 2.1). Therefore, this valuable waste is being evaluated for different applications such as obtaining olive pomace oil (PO), production of bioethanol, biogas, methane and activated carbon [28, 29]. PO contains functional components at least as much as extra virgin olive oil. Hence, in recent years there has been increasing demand in PO [30].

Table 2.1 Characteristics of the olive oil processing solid wastes [31]

Parameters	Press process	Two phases	Three phases
Moisture (%)	27.2	50.23	56.80
Fats and oils (%)	8.72	3.89	4.65
Proteins (%)	4.77	3.43	2.87
Total sugars (%)	1.38	0.99	0.83
Cellulose (%)	24.1	17.37	14.54
Hemicellulose (%)	11.0	7.92	6.63
Ash (%)	2.36	1.70	1.42
Lignin (%)	14.1	10.21	8.54
Total nitrogen	0.71	0.51	0.43
Phosphorous as P_2O_5 (%)	0.07	0.05	0.04
Phenolic compounds (%)	1.14	0.33	2.43
Potassium as K_2O (%)	0.54	0.39	0.32
Calcium as CaO (%)	0.61	0.44	0.37
Total Carbon (%)	42.9	29.03	25.37
C/N ratio	60.7	57.17	59.68
C/P ratio	588.7	552.9	577.2

The crude PO is obtained by using solid-liquid extraction with solvents (especially, hexane) followed by other processes. Besides solvent extraction, it can be also separated by using centrifugation (physical extraction) process (Figure 2.4 and Figure 2.5).

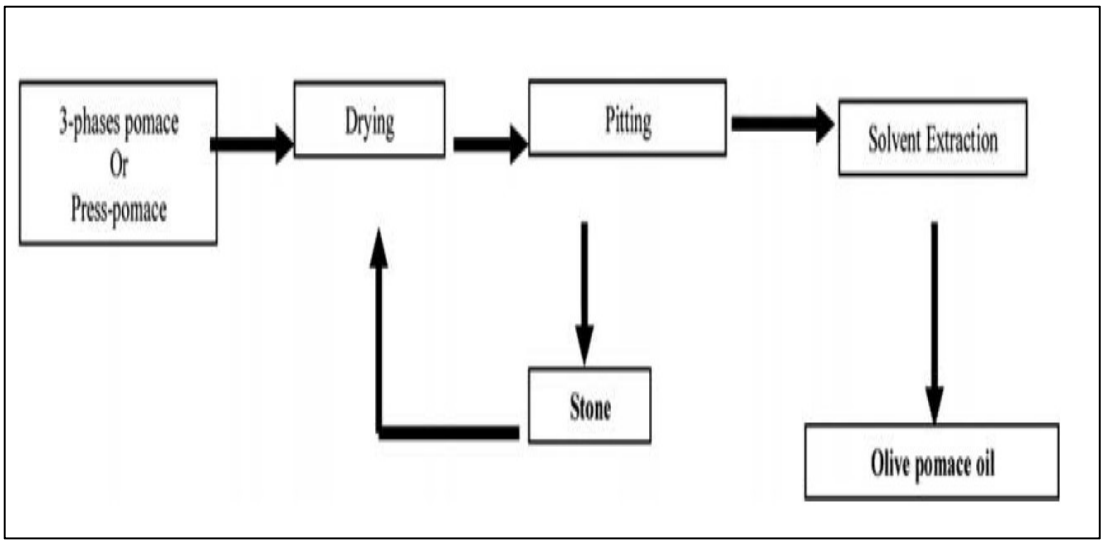

Figure 2.4 The pomace oil production from pressed pomace or 3-phase pomace [26]

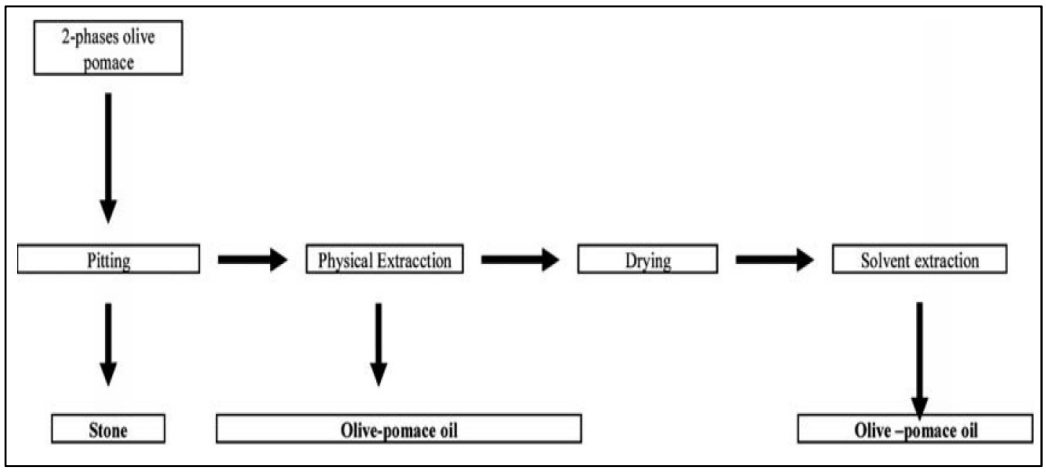

Figure 2.5 The pomace oil production from 2-phase pomace [26]

Pomace obtained from press system contains a higher amount of oil than two and three-phase systems and also that of water content is lower in this system (Table 2.1). Therefore, the more energy consumption and harder drying conditions are needed for two and three-phase methods. The better quality oil is obtained from pomace including the lower water content [25]. Hence drying plays very important role for processing of olive PO. The ideal moisture content is approximately 5% in order to increase yield of the oil extraction.

Drying conditions are very effective on the properties of the PO. The applied higher temperature (min 500 °C) affects negatively both sensorial and chemical properties. It causes the worsening of color and odor and even the formation of harmful components such as polycyclic aromatic hydrocarbons (PAHs). As increasing temperature, the concentration of oxidized compounds also enhances. On the other hand, fermentation and enzyme activity in the pomace are ended by drying.

The other important process for pomace oil is the solvent extraction. The size of dried pomace is reduced to increase extraction yield. Batch and continuous extraction systems are used in the industry. In the batch method, pomace is filled in the first tank after that hexane is added. This mixture is stirred at a certain temperature for a while. After this first extraction, pomace is taken another tank and fresh hexane is added and this situation can be serially maintained for up to 5-8 tanks. At the end of the extraction the crude pomace oil is obtained. But it is not suitable for cooking oil due to higher acidity and including unwanted materials such as oxidation products and PAHs. Refinery is needed to enhance edible PO quality. Chemical or physical

refining can be applied for PO in the industry. However, physical refining is recommended for crude PO with high acidity due to the advantages of physical refining. Refined PO has been added to certain amount of olive oil or offered directly to consumption especially for frying oil.

2.4 Refining of Vegetable Oils

Triacylglycerols are expressed as the most important component of vegetable oils. Vegetable oils also includes minor components such as free fatty acids, monoacylglycerols, diacylglycerols, phospholipids, free and esterified plant sterols, phenolic substances, triterpene alcohols, tocols (tocopherols, tocotrienols), hydrocarbons (squalene, carotenes, etc.), trace metals (iron, sulfur, copper, etc) oxidation products, sticky substances, waxes, pesticide residues, flavor and odor components [32]. Of these components, tocols, phenolic substances, plant sterols, carotenes and squalene are the most important bioactive components in vegetable oil.

Therefore, the main purpose of vegetable crude oil refining is; minimizing the loss of oil and valuable components, to remove as much as possible various unwanted impurities in oil. When considered commercially, vegetable oil refining is necessary to meet market needs. Vegetable oil refining includes basic processes such as degumming, neutralization, bleaching, winterization and deodorization. Figure 2.6 shows the oil refining steps and the function of each process.

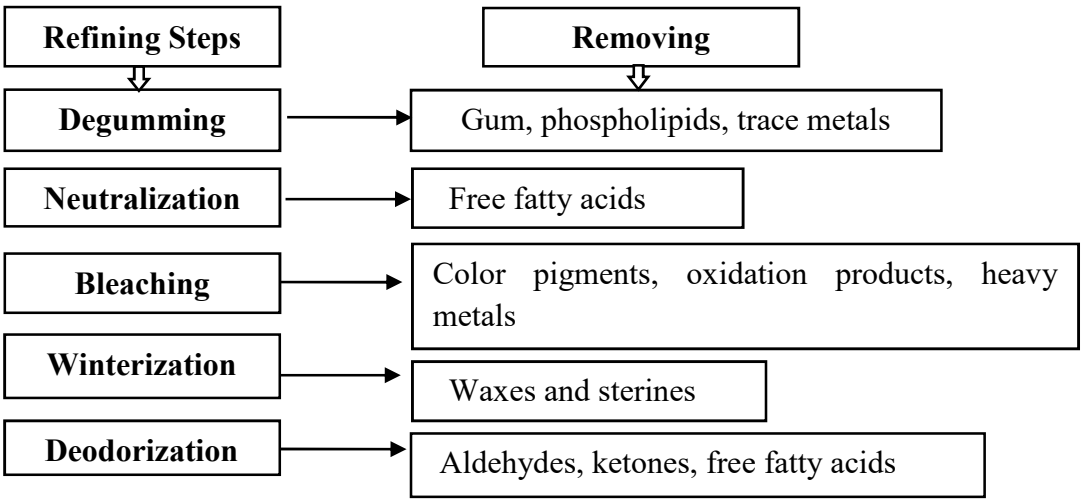

Figure 2.6 Vegetable oil refining steps and functions [33]

The refining process can be carried out with two different processes called chemical and physical refining. The most important difference between the two processes is the way they are implemented. While each process is carried out separately in chemical refining, in physical refining, deacidification (neutralization) and deodorization are carried out in a single step, using water vapor distillation [34]. However, caustic (NaOH) is used for the neutralization process in chemical refining.

The refining process to be applied is determined according to the type and quality of the crude oil (especially the amount of phospholipid). The advantages and disadvantages of physical and chemical refining are given in Table 2.2. Apart from these two refining methods, there is also a method known as cold refining, which is used especially for SO and other wax-containing crude oils [35].

Table 2.2 Advantages and disadvantages of physical and chemical refining [36]

Physical Refining		**Chemical Refining**	
Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
Higher yields	Runs not stable with poor crude oil quality	Less consumption bleaching clay	Lower yields. High oil losses due to emulsification and saponification neutral oil
Less chemical usage	Higher consumption bleaching clay	Stable with poor crude oil quality	Higher chemical usage
Less waste production	Lower concentration of tocopherols in distillate (Mainly consist of FFA)	Concentration of tocopherols in distillate is higher	Generates more waste which is difficult and more expensive to treat

2.5 Bleaching of Vegetable Oils

The bleaching is the most important step in the edible oil refining process and can be described as a process which is based on removing undesirable color pigments and the other undesirable impurities from the neutralized oil by using a certain amount of bleaching earth or active carbon under the determined conditions. Generally, the bleaching process is carried out at temperatures between 90-120 °C, by using clay 0.5-2.0%, in ranging times 20-30 min [37]. However, these values may change according to the physical and chemical properties of the oil, type of oil and market demand.

2.5.1 Mechanism of Bleaching

Bleaching mechanism depends on the adsorption. It can be occurred by involving various physical and chemical interactions. These interactions play an important role in obtaining desirable bleached oil quality. But some of them may affect bleaching quality adversely. Adsorption is described that molecules adhere to a surface of material (Figure 2.7).

Bleaching is carried out in vegetable oil refining by using adsorbent materials which allow the other material to adhere its surface but do not penetrate inside of absorbent, such as bleaching clay and activated carbon. Van der Waal's force provides attraction between adsorbent and impurities [35].

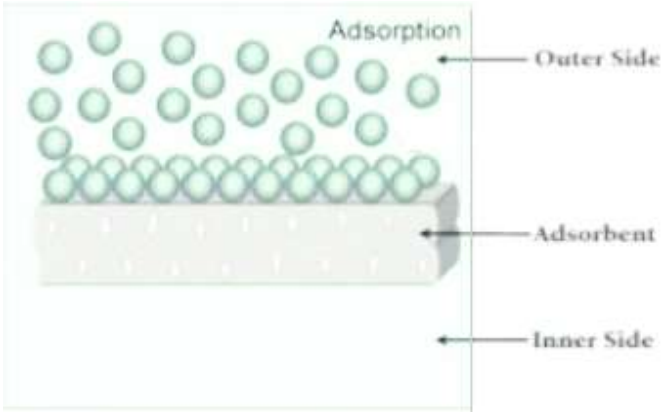

Figure 2.7 Mechanism of adsorption [38]

This interaction continues until a balance occurs between the pigment concentration in the oil and the color pigments deposited on the adsorbent surface. This balance is affected by many parameters such as adsorbent type, temperature, mixing time, amount of adsorbent and color of material [39]. Some equilibrium isotherms have been used to describe adsorption process such as Freundlich, Langmuir and Redlich-Peterson [40].

2.5.2 Factors Affecting the Bleaching Process

Bleaching process depends on temperature, processing time and the amount and type of bleaching clay [41]. Therefore, changing of any these parameters directly affects the bleaching quality.

2.5.3 The Effect of Bleaching Adsorbents

There are many types of bleaching adsorbent in the edible oil refinery. Bentonite, activated clays, activated carbon and others have been usually used for bleaching process. Besides some seed hulls and sawdust, cheap and waste materials can be used as adsorbent material [42].

In the literature, there are many studies on the effects of different adsorbent types on bleaching. Achary et al. [43] used industrial clay, activated bentonite, sepiolite and unactivated bentonite as a bleaching agent to remove chlorophyll in flaxseed oil. The amount used for each adsorbent group was determined as 4%. While the chlorophyll removal rate was 99.4% mostly in industrial clay used; this is followed by activated bentonite with 97.8%, sepiolite with 82.7% and non-activated bentonite with 47.2%.

Chetima et al. [44] carried out a bleaching process by using activated carbons obtained from waste cottonseed and mahogany tree seed hulls. The bleaching efficiency with activated carbon at 5% was compared with the results of commercial bleaching clay used at the same amount, and as a result, they was found that activated carbon was effective on color pigments by about 96-98%, while commercial clay had a performance of 98.4%. Besides, as an adsorbent, it is reported that natural or synthetic adsorbents such as chitosan, activated carbon, carbon produced from soy shell, attapulgite and other clay minerals, magnesol XL, aluminum oxide, magnesium silicate, silicon dioxide can be used [45].

Surface area of adsorbents is very critical feature in the bleaching process. Therefore, clays are activated by mineral acids in order to obtain the better bleaching quality with increasing specific surface area. The clay having 40-60 m^2/g dry clay surface area originally can be increased up to 200 m^2/g dry clay [46].

Figure 2.8 represents activation degree of clay in terms of removing impurities. Clays activated by acid treatment; although it increases the bleaching efficiency, trace amount of acid remains in their structure. Due to higher amount of clay used this trace amount of acid in their body increases the free acidity content in the neutralized oil and adversely affects the product quality [47].

Besides, in the existing conventional methods, the desired oil color quality is achieved by higher the amount of clay but this leads increasing in oil losses because of absorbing approximately 0.36 kg of oil/kg of clay [35] and environmental problems due to removal of spent clay.

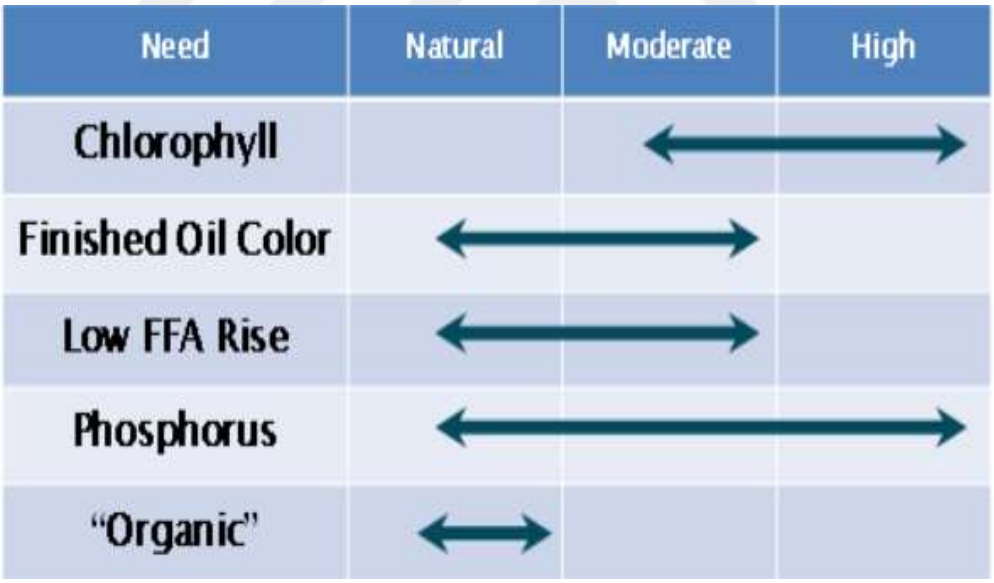

Figure 2.8 Activation degrees of clay for better bleaching [48]

2.5.4 The Effect of Mixing Time

Another important parameter to obtain better quality oil is mixing or contact time. Mixing time starts with adding clay to oil and ended with filtration. Determination of optimum contact time is crucial to avoid color deterioration in oil. Because prolonged bleaching may lead undesirable color of oil [49]. Optimum bleaching time

depends on initial oil properties, type of adsorbent and temperature. Although bleaching time ranges from 15 to 45 min, the most common has been using for 20 to 30 min [48].

2.5.5 The Effect of Temperature

The bleaching temperature used is usually above the 90 °C. Decreasing oil viscosity with increasing temperature provides better dispersion of clay particles. Therefore, interaction of oil/adsorbent can be improved in this way Besides higher temperature values can obtain some benefits such as increasing chlorophyll removal and facilitating filtration but undesirable reactions occur, in which increased oxidation products are formed [48].

2.5.6 The Effect of Vacuum

Vacuum is not only preventing formation of oxidation products but also increase removal of color pigments. As the color removal increases due to the use of vacuum, it contributes to the reduction of the amount of bleaching earth used [50]. Besides vacuum provides drying of oils and clays at low temperatures [51].

2.6 New Bleaching Methods Alternative to Conventional Method

Bleaching is a costly process in the edible oil refining. Therefore, increasing of unit bleached oil capacity is very crucial to decrease unit cost of bleaching. In addition, reducing amount of used of clay and bleaching time can contribute to the reduction of bleaching cost.

On the other hand, obtaining better bleached oil quality is the other important goal. In this respect new bleaching methods have been studied in the literature. Ultrasound-assisted bleaching, high voltage electrical field and membrane filtration are summarized below.

2.6.1 Ultrasound-assisted Bleaching

Thanks to the cavitation forces of ultrasound, it is a promising process in the food industry. These forces have been changing chemical and physical structure of materials [52]. Therefore, some advantages can be obtained with ultrasound such as

shorter processing time and lower temperature. In this respect there are many ultrasound assisted bleaching studies in the literature.

Different kinds of vegetable oils (olive, rapeseed, soybean, sunflower, and canola) have been investigated in these studies [16, 18, 53-56]. As a result, the bleaching time, temperature, and the amount of bleaching clay used were successfully reduced in these studies.

2.6.2 Bleaching with High Voltage Electrical Field

This method was first applied by Abedi et al. [17]. In this study, bleaching process was carried out by using electrode surfaces. Thanks to electrode surface, impurities which are undesirable in oil were polarized under high voltage electric field. Therefore, it was aimed to reduce the bleaching time, amount of clay and temperature in this method.

2.6.3 Bleaching with Membrane Filtration

Membrane filtration is a material separation method, depends on the size exclusion. Dadfar et al. [57] were studied this method for olive oil bleaching. However, bleaching was not carried out alone by using ultrafiltration. At low temperature (36.5 °C) conditions, bleaching clay (0.7%) and activated carbon (0.3%) were added to olive oil and mixed for a while before ultrafiltration separation. The authors emphasized that this method can be an alternative to the conventional method.

2.7 Microwave Application

The use of microwaves has become very popular in various industrial applications in the recent years. Microwave is used especially in food industry to reduce process time and increase food quality. In the literature; microwave heating is described as a continued promising method among the other electric heating technologies such as radio frequency, ohmic, and infrared [58, 59]. Some microwave food applications in the literature are summarized in Table 2.3.

Table 2.3 Some microwave-assisted applications used in food processing [60]

Applications	Objectives	Foods
Vacuum drying	Reduce moisture content	Seed, grains and citrus juices
Freeze drying	Reduce moisture content	Meat, vegetable and fruits
Drying	Reduce moisture content	Macaroni, rice and snacks
Cooking	Modify flavor and texture	Bacon, meat patties, potatoes
Blanching	Inactivate spoilage enzymes	Fruit, corn and potato
Baking	Heat and activate leavening agents	Bread, pastry, donuts
Roasting	Heat and promote heat reactions	Nuts, coffee, cocoa
Pasteurization	Inactivation of vegetative microorganisms	Dairy products and prepared foods
Sterilization	Inactivation of bacterial spores	Dairy products and prepared foods, fruit juices

2.7.1 Microwave Description and Its Properties

Microwaves are electromagnetic waves in the frequency range of 0.3-300 GHz [61]. Figure 2.9 shows the usable microwave area in the electromagnetic spectrum. If the microwave is intended to be used outside of these ranges, it will become uneconomical as it will require extra radiation shielding to prevent interference with other radio systems.

Therefore, while microwave ovens used at home operate at 2450 MHz, industrial systems operate at either 2450 MHz or 915 MHz [62].

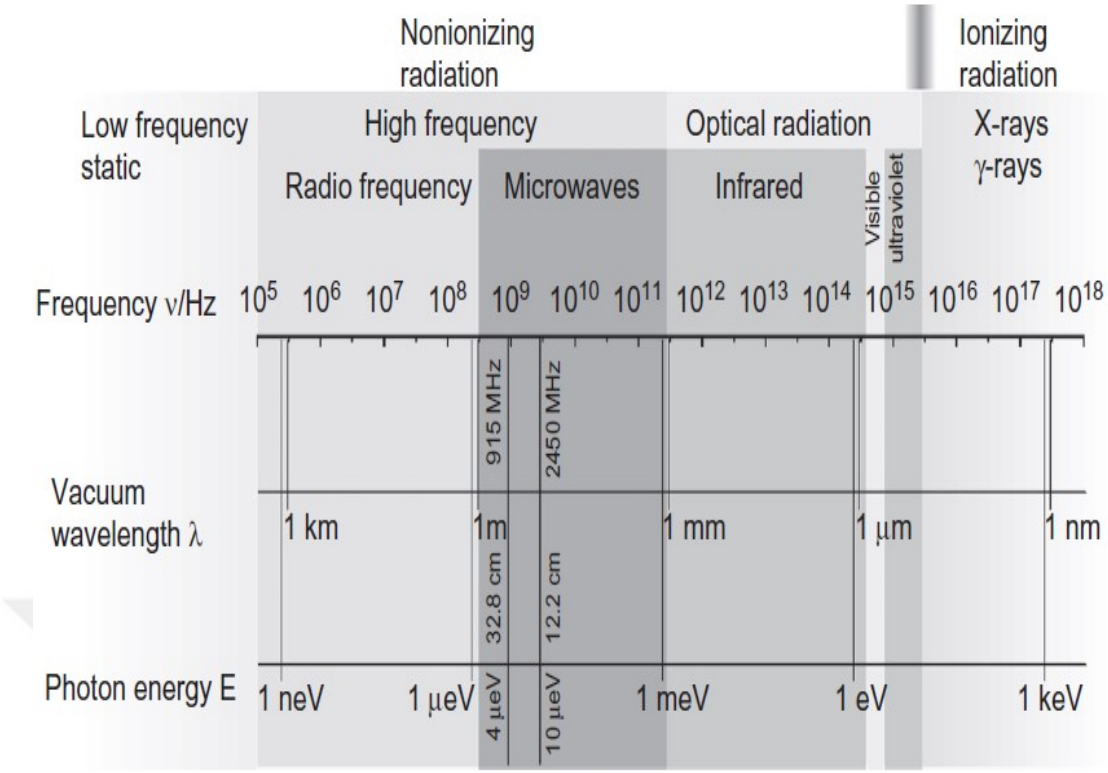

Figure 2.9 Electromagnetic spectrum for microwave [63]

Due to their structure, microwaves have electric and magnetic fields that are perpendicular to each other. These fields cause heating in a short time by moving the molecules very fast in the materials they are transmitted to. Unlike conventional heating, microwave heating does not depend on the temperature gradient for heat transfer [64]. In other words, thermal gradients obtained by microwave heating are the opposite of thermal gradients obtained by conventional heating [65].

Microwave heating is instantaneous and volumetric and high power densities can be also applied [64]. Microwave, which provides a relatively homogeneous temperature distribution over the entire sample, has a penetrating and scattering feature.

On the other hand, conventional heating from the surface to the center; it is called the heat transfer method, which makes the surface temperature very high [66]. This difference between the two methods is shown in Figure 2.10.

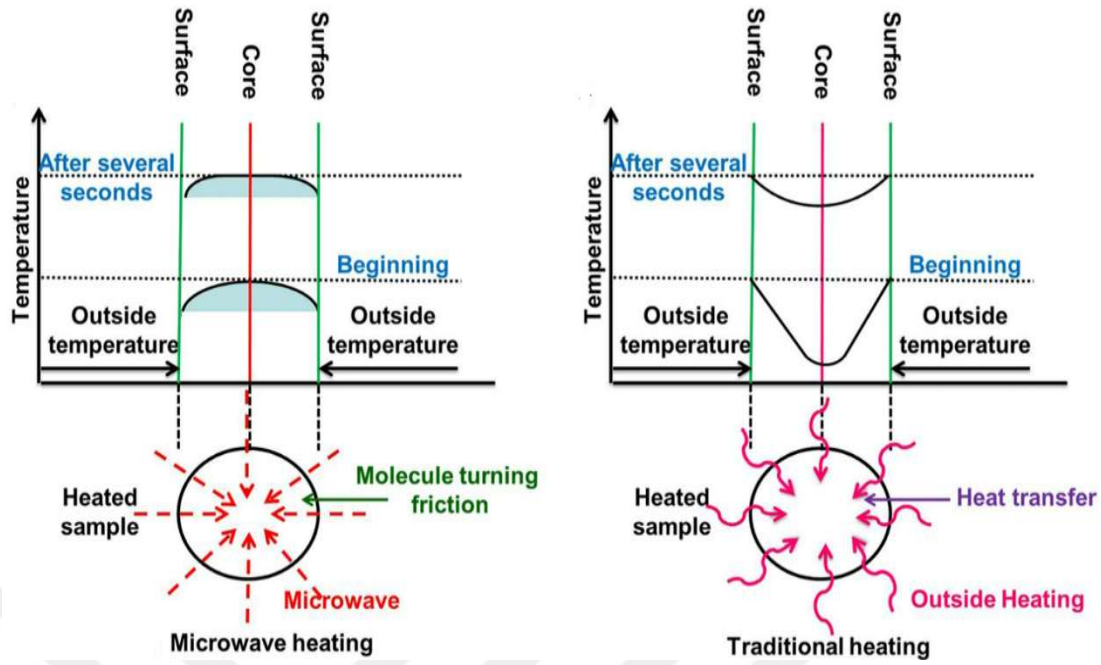

Figure 2.10 Mechanism of microwave and conventional heating [66]

2.7.2 Mechanisms of Microwave Heating

One of the mechanisms that explain the heat generation of microwaves is dipolar rotation [67]. Dipolar rotation occurs due to the clustering of molecules with a dipole moment in the electric field. In this case, the resulting oscillations cause the surrounding molecules to vibrate, causing collisions and releasing heat [68]. Thanks to this feature, microwave has been used in the extraction process in recent years.

With microwave assisted extraction; it is stated that the processing time is shortened, high extraction efficiency is obtained, it has high selectivity, and the quality of the target extract is high [69]. It is also reported that it reduces the amount of solvent used and has a lower energy requirement [70]. Another theory of microwave heat generation is the ionic mechanism. In other words, heat may occur as a result of the oscillation migration of the ions in the food [71].

2.7.3 Factors Affecting Microwave Heating

There are many factors that affect microwave heating and its heat distribution. The most important of these are dielectric properties and penetration depth [72]. The microwave energy absorbed by the material varies depending on the dielectric

properties of that material [73]. The importance of polar molecules in microwave heating is well known. Water has a high dielectric coefficient due to its polar properties and plays a critical role in the microwave heating of food.

On the other hand, in microwave heating, specific heat is usually neglected while focusing on dielectric properties [74]. However, specific heat is very important factor in the microwave heating [75]. Especially, materials having low specific heat can be heated rapidly when subjected to microwave. Vegetable oils have a lower specific heat than water so they may be heated by microwave faster than the water of the same weight [74].

2.7.4 Microwave Heating of Edible Oils

Microwave is preferred for heating of edible oils because of reduction in heating time, providing inner penetration and faster heating [73]. It has been reported that microwave heating increases oxidation reactions in oils. However, it is observed that these increases occur at very high microwave power conditions and during long-term operation. Dostolava et al. [76] treated different vegetable oils with a 500 W (for 25 g oil) microwave power for 3-40 min to evaluate effect of microwave on the heating of oil. As a result of these findings, it is stated that the amount of oxidation increases as the time increases.

In another study, Malheiro et al. [77] examined the changes in the oil by heating olive oil with a microwave power of 1000 W (for 50 ml oil) for 1-15 min. It is expressed that there is no remarkable change in the oil, especially in the first 3 min, but there are changes in the oil quality depending on the increase in the time.

Microwave heating also have been used for deep frying in the literature. Aydınkaptan et al. [78] studied on the heating of SO at frying temperatures. The range of microwave power as 360-600 and 900 W were used in this study. It was observed that highest degradation in oil samples was found in the lower microwave power because of higher heating time.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

Chemicals and solvents used in this thesis were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA.) and Merck (Darmstadt, Germany). All solvents and reagents were analytical or chromatographic grade.

3.1.2 Raw Materials

NSO and NPO samples were taken from the bleaching inlet and outlet of a refinery located in Gaziantep and İzmir, respectively. The non-bleached and industrially bleached sunflower (IBSO) and pomace oil (IBPO) samples obtained from the same batch were placed in dark bottles and stored at –18 °C for microwave-assisted bleaching (MWB) and subsequent analysis. Acid activated (Suprefast M1FF) bleaching clay and activated carbon were also supplied from Gaziantep refinery.

3.2 Methods

3.2.1 Experimental Design

A three-level, five factorial central composite rotatable designs (CCRD) was employed to analyze the effects of microwave assisted factors on the bleaching and to optimize the bleaching conditions to obtain maximum bleached oil quality. The employed CCRD is composed of 20 experiments consisting of 6 center points for both oil types (Table 4.1 and Table 5.1). Preliminary experiments were carried out to obtain some information about the independent variables and their levels before experimental design. In the preliminary studies, the higher microwave power levels were studied but burning of the clay was observed. Higher power values also resulted

in increased totox value. The independent variables were selected as microwave power (A), amount of clay (B) and bleaching time (C). Levels of the independent variables were defined as between A, 70-120 W; B, 0.01-0.5% (w/w); C, 2-10 min and A, 70-110 W; B, 2-6% (w/w); C, 20-40 min for NSO and NPO, respectively. Besides activated carbon is important to reducing polycyclic aromatic hydrocarbons in the PO. But, amount of activated carbon was determined depending of color quality of PO in this thesis.

According to preliminary experiments, the higher amount of activated carbon was not improved desired color of MWBPO. Therefore, the proportion of activated carbon was set at 0.5% during all experiments. At the end of optimization, verification of experiments was carried out under the optimal conditions and the average values of the experiments were compared with the predicted value (Table 4.1 and Table 4.9).

3.2.2 Microwave-assisted Bleaching

MWB treatments were performed by using CEM Discover SP (Figure 3.1) microwave reactor (CEM Corporation, Matthews, USA). Bleaching was carried out in a 35 ml glass vessel (CEM Corporation, Matthews, USA). In all experiments, 20 g of sunflower oil and pomace oil were charged into the vessel and the vessel was not completely filled to prevent the oil from splashing. After the addition specified amount of clay, the tube was sealed by using a rubber lid (CEM Corporation, Matthews, USA) and placed in microwave reactor. In all experiments, clay, activated carbon (it was used just for PO) and oil samples were just stirred inside the reactor.

This microwave system consisted of an infrared sensor for temperature measurement. At the end of microwave treatment, the NSO and clay mixture were separated by EBA 20 centrifugation (Andreas Hettich GmbH & Co KG, Tuttlingen, Germany) at 6000 rpm and 25 ˚C for 10 min.

MWBSO was easily separated due to the clay sticking to the walls of the tube. However, MWBPO was centrifuged (Eppendorf 5810R, Eppendorf AG, Hamburg, Germany) at 9.000 rpm at 25 °C for 10 min. After centrifugation, MWBPO was filtered by using 0.45 µm PTFE filter (ISOLAB, Germany) to separate mix of

clay/activated carbon under vacuum. MWBSO and MWBPO samples were stored in dark amber sample bottles at -18 °C until further analysis.

Figure 3.1 Microwave system used in bleaching experiments

3.2.3 Industrial Bleaching Conditions

According to the information from the refinery company; 8 kg acid activated bleaching clay was mixed with 1000 kg NSO and industrial bleaching (IB) was carried out at 100 °C for 30 min under vacuum. On the other hand; bleaching clay, activated carbon and mixing time were applied as 5%, 1.1% and 120 min, respectively for IBPO according to the information from the refinery company.

3.2.4 Color Measurement

Color of oil samples was determined by using HunterLab Colorflex colorimeter with standard illumination D_{65}, and colorimetric normal observer angle of 10°. Color values were evaluated as L*, a* and b*. Hue angle and chroma were calculated from

a* and b* values according to the following formula given by Eqn. (3.1) and (3.2), respectively.

$$\text{Hue angle} = \tan^{-1}(b^*/a^*) \quad (3.1)$$

$$\text{Chroma} = \sqrt{(a*)\text{^}2 + (b*)\text{^}2} \quad (3.2)$$

3.2.5 Determination of Oxidation Parameters, FFA and Soap Content

Peroxide value (PV) was determined according to AOCS method [79]. The p-anisidine value (AV) in oil samples was determined by the spectrophotometric (Optima, SP-3000nano, Japan) method as described in AOCS method [80]. PV and AV were converted to total oxidation value (Totox) by using Eqn (3.3). Spectrophotometric indices (K_{232} and K_{270}) for PO samples were performed according to AOCS method [81]. Free fatty acids (FFA) were determined by titration methods as described in AOCS method [82]. Soap content of sunflower oil was determined in terms of sodium oleate by titration method according to AOCS [83].

$$\text{Totox value} = 2\,\text{PV} + \text{AV} \quad (3.3)$$

3.2.6 Determination of Bleaching Efficiency

Bleaching efficiency (BE) of sunflower oil samples was determined by using UV-VIS spectrophotometer (Optima, SP-3000nano, Japan), measuring absorbance (A) of bleached oils at 450 nm. The calculation was carried out by the Eqn. (3.4) given below according to Ajemba and Onukwili [84].

$$\text{BE (\%)} = \frac{(A_{unbleache} - A_{bleached})}{A_{unbleached}} \times 100 \quad (3.4)$$

3.2.7 Determination of Total Chlorophyll and Carotenoid Content

Total chlorophyll and carotenoid contents of bleached oils were determined spectrophotometrically according to Minguez-Mosquera et al. [85] by using UV–VIS Spectrophotometer (Optima, SP-3000nano, Japan). 7.5 g SO and 2.5 g PO were dissolved in cyclohexane and the final volume was completed to 25 ml. Then

chlorophyll and carotenoid concentration of oil samples were measured at 670 and 470 nm, respectively, according to Allalout et al. [86] with the Eqn. (3.5) and (3.6).

$$\text{Chlorophyll (mg/kg)} = A_{670} \times 10^6 / 613 \times 100 \times d \quad (3.5)$$

$$\text{Carotenoid (mg/kg)} = A_{470} \times 10^6 / 2000 \times 100 \times d \quad (3.6)$$

A: Absorbance, d: Spectrophotometric cell diameter (1 cm).

3.2.8 Determination of Sterol Compositions

Preparation of unsaponifiable matter from 0.25 g of SO and 5 g of PO and determination of the composition of the sterol fractions were done according to EN ISO 12228-1 [87] and IOC.COI/T.20/Doc [88], respectively. Preparation of unsaponifiable matter from and determination of the composition of the sterol fractions were done according to derivatives of the sterols and analyzed using a Agilent Technologies 7890 A gas chromatography (Agilent Technologies, Santa Clara, USA) equipped with a fused silica capillary column HP-5 (30m-0.25mm ID and 0.25 μm film thickness) (Agilent Technologies, Santa Clara, USA). Betulin and cholesterol (Sigma-Aldrich) were quantified as an internal standard for SO and PO, respectively.

The impurities of the internal standards were taken into account in the calculations. Identifications of individual peaks were carried out using available reference standards and comparing known retention times of the sterols in SO and PO. The split ratio was 30:1 and the carrier gas was nitrogen at 1 ml/min. Injector, column and detector temperatures were 320, 265 and 320 °C, respectively. Three injections of 1 μl were applied for each oil.

3.2.9 Determination of Tocopherol Content in Sunflower Oil

The tocopherol content of SO was determined by Shimadzu Prominence/ LC-20AB HPLC using the method proposed by AOCS method [89]. The sample was prepared by dissolving 1 g of oil in 9 ml of hexane and injected into a normal phase HPLC to analyze tocopherol content. The chromatographic separation was done with a Lichrosorb Si60-5 (250 x 4.6 mm id, particle size S-5μm) (Hichrom, Reading, UK). The column temperature was maintained at 25 °C. Separation was carried out

isocratic elution with n-hexane (99%) and iso-propanol (1%) at 1 ml/min. The eluate was monitored at 295 nm. Tocopherol content was quantified based on peak areas compared with an external standard, α- tocopherol.

3.2.10 Determination of Benzo[a]pyrene Content in Pomace Oil

A solid phase extraction (SPE) method previously described by Moret and Conte [90] was used for extraction and clean-up procedure of benzo[a]pyrene (BaP). Purification was performed using a SPE mini column in a visiprep solid- phase extraction. Oil sample (2.5 g) was diluted with n-hexane in 10 ml volumetric flask. Then, 1.0 ml of the sample solution was transferred onto 5 g silica SPE cartridge (Mega Bond Elute, 20 ml, Varian, Palo Alto, CA, US) previously conditioned with 20 ml of dichloro-methane, dried completely under vacuum and conditioned with 20 ml of n-hexane.

PAHs solution was eluted with mixture of n-hexane and dichloromethane (70:30 v/v). The first 8 ml of elute was discharged, and the following 8 ml elution including PAH fraction was collected in a tube. The flow rate was adjusted to one drop per second.

Elute was evaporated with nitrogen. The concentrate was dissolved in 1 ml of acetonitrile and injected into the HPLC (Agilent 1260 series, Santa Clara, USA) equipped with a fluorescence detector. An Agilent Zorbax Eclipse PAH C_{18} column (3 mm, 250 mm, 5 µm) at 20 °C was used for separation. The BaP and total PAHs were quantified based on peak area compared with that of external standard.

3.2.11 Determination of the Reusability of Bleaching Clay

This study was only carried out at the end of MWBSO. The clay used in the MWBSO was regenerated according to Foletto et al. [91], and Nursulihatimarsyila et al. [92]. The mixture was stirred at 40 °C for 30 min. Then the bleaching activity was tested after the degreased clay was dried for 8 hours at 120 °C. Regeneration was carried out continuously after each MWB until clay efficiency was reduced. Repeated use of bleaching clay was determined according to the color parameters (L*, a*, b*, hue angle and chroma) and bleaching efficiencies (Eqn. 3.4).

3.2.12 Scanning Electron Microscopy Analysis

The effect of repetitive usage of the bleaching clay on its surface morphology was examined by using scanning electron microscopy (SEM). Gold/palladium was used for the coating of the samples in an SC7620 sputter coater (Emitech, Kent, UK). Images of samples were taken with Zeiss GeminiSem300 (Carl Zeiss Ltd. Co., Germany) scanning electron microscope. The SEM images were obtained at 4-5 kV under high vacuum condition and 300×magnification.

3.2.13 Statistical Analysis

Experimental design and statistical analysis were conducted using Design Expert version 7.0 software (State-Ease Inc., Minneapolis, Minn., U.S.A.). Statistical parameters such as lack of fit, the coefficient of determination (R^2) and the F-test value obtained from the analysis of variance (ANOVA) were used to evaluate the best fitting model.

Regression analysis and three-dimensional surface plots were generated to understand the effect of microwave assisted conditions on response variables. SPSS 22 (SPSS Inc. Chicago, IL, USA) statistical package program was used to analyze the results of the experiments and results were evaluated by one-way t-test and Duncan multiple comparison tests. All experiments were performed in three replicates and the results were given as mean ± standard deviation.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Microwave-assisted Sunflower oil Bleaching

NSO samples were separated from the clay by decantation after bleaching and made ready for analysis (Figure 4.1a and b).

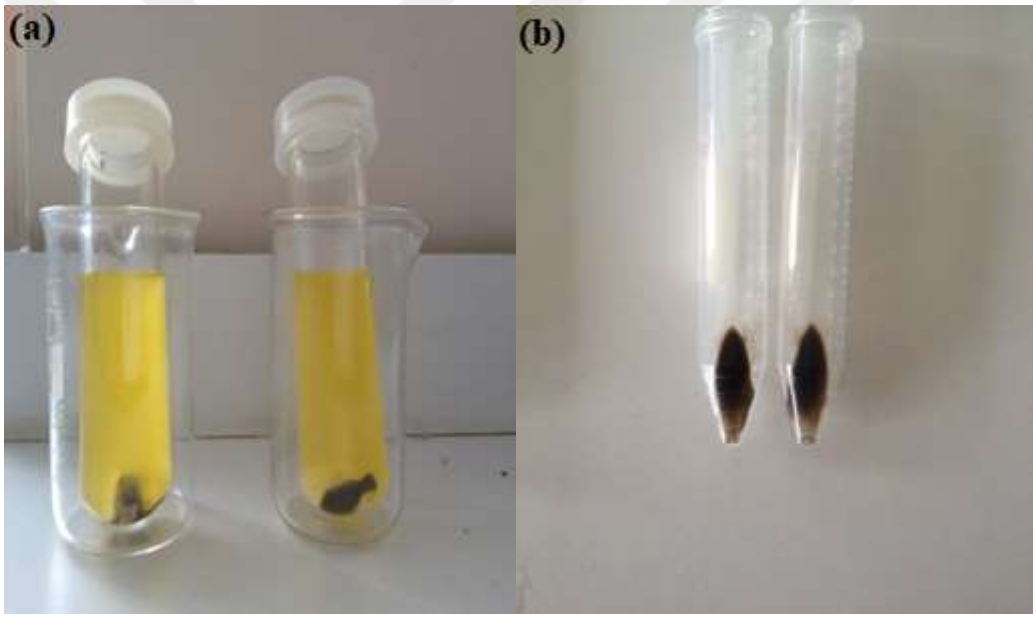

Figure 4.1 Prepared NSO samples for MWB and separated clay with decantation **(a)** NSO samples before bleaching **(b)** Separated clay after bleaching

4.1.1 Optimization of Microwave-assisted Bleaching of Sunflower oil

4.1.1.1 The Central Composite Rotatable Design and Optimization

4.1.1.1.1 Fitting Model

RSM was employed with CCRD to evaluate the effects of microwave power, bleaching time and amount of clay on bleaching efficiency and oil quality in terms of hue angle, chroma and totox value of the MWBSO. The experimental design and the

experimental results for MWBSO have been presented in Table 4.1. In this study, the totox value ranged from 19.27 to 29.91 while the hue angle and chroma ranged from 92.78 to 99.43 and 30.57 to 55.48, respectively.

Table 4.1 Three factor-five level experiment design generated for MWBSO

Run	FACTORS			RESPONSES					
	X	Y	Z	Totox		Hue Angle		Chroma	
	(Watt)	(%)	(s)	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	95	0.26	360	22.02±0.61	22.36	96.24±0.05	96.73	41.21±0.03	39.80
2	110	0.11	241	20.50±0.06	19.49	93.55±0.02	93.41	48.02±0.01	49.86
3	120	0.26	360	22.73±0.08	23.82	96.99±0.01	97.30	39.70±0.01	37.37
4	95	0.26	120	19.27±0.01	19.72	92.78±0.02	92.90	50.19±0.01	49.07
5	80	0.11	241	19.28±0.01	19.76	93.56±0.01	93.52	50.05±0.01	50.41
6	110	0.40	503	29.91±0.90	28.69	99.43±0.01	99.47	30.57±0.02	30.41
7	95	0.50	360	21.73±0.24	22.43	97.48±0.01	97.40	37.47±0.01	38.67
8	95	0.26	360	23.14±0.73	22.36	96.84±0.02	96.73	38.97±0.01	39.80
9	95	0.26	360	22.85±0.73	22.36	96.73±0.01	96.73	39.40±0.01	39.80
10	110	0.40	241	21.26±0.01	21.12	96.36±0.01	96.08	40.34±0.05	41.72
11	95	0.26	360	21.69±1.14	22.36	96.63±0.02	96.73	40.44±0.04	39.80
12	80	0.40	503	23.31±0.14	23.59	97.31±0.01	97.44	37.18±0.04	35.60
13	110	0.11	503	26.17±0.01	26.12	96.06±0.01	95.86	42.75±0.01	44.12
14	95	0.01	360	20.55±0.02	20.90	93.67±0.01	93.76	55.48±0.01	53.92
15	95	0.26	360	22.98±0.03	22.36	97.10±0.01	96.73	38.67±0.01	39.80
16	95	0.26	600	28.58±0.02	29.16	97.76±0.01	97.65	34.80±0.01	35.55
17	70	0.26	360	19.84±0.01	19.80	96.00±0.01	95.70	40.15±0.01	42.12
18	95	0.26	360	21.65±0.74	22.36	96.86±0.01	96.73	40.05±0.04	39.80
19	80	0.40	241	19.70±0.07	19.01	94.05±0.02	94.24	47.06±0.02	45.94
20	80	0.11	503	24.01±1.21	23.41	95.56±0.01	95.78	46.34±0.01	45.60

X: Microwave Power; Y: Bleaching time; Z: Amount of clay. ± Standard Deviation.

The multiple regressions and backward elimination were employed to get the best fitting statistical model for each response. Table 4.2 summarizes the ANOVA and regression analysis' results for the hue angle, chroma and totox value.

Table 4.2 ANOVA results for sunflower oil responses

Source	Sum of squares	Df	Mean Square	F value	p- value
Model (Totox)	146.07	6	24.35	31.60	< 0.0001
A-MW power	19.73	1	19.73	25.61	0.0002
B-Clay content	2.82	1	2.82	3.65	0.0782
C-Time	107.44	1	107.44	139.45	< 0.0001
AB	2.84	1	2.84	3.69	0.0770
AC	4.45	1	4.45	5.77	0.0319
C^2	8.79	1	8.79	11.41	0.0049
Residual	10.02	13	0.77		
Lack of fit	7.72	8	0.96	2.10	0.2154
Pure error	2.30	5	0.46	31.60	< 0.0001
Core total	156.09	19	-	25.61	0.0002
R^2=0.94, Adj. R^2= 0.91, Pred. R^2=0.83, Adeq Precision =18.37					
Model (Hue Angle)	54.30	7	7.76	95.13	< 0.0001
A-MW power	3.11	1	3.11	38.17	< 0.0001
B-Clay amount	15.99	1	15.99	196.12	< 0.0001
C-Time	27.24	1	27.24	334.08	< 0.0001
AB	1.90	1	1.90	23.32	0.0004
BC	0.44	1	0.44	5.45	0.0378
B^2	2.33	1	2.33	28.57	0.0002
C^2	3.77	1	3.77	46.19	< 0.0001
Residual	0.98	12	0.08		
Lack of fit	0.56	7	0.08	0.97	0.5329
Pure error	0.42	5	0.08	95.13	< 0.0001
Core total	55.27	19	-	38.17	< 0.0001
R^2=0.98, Adj. R^2=0.97, Pred. R^2=0.95. Adeq Precision=36.56					
Model (Chroma)	627.77	6	104.63	36.32	< 0.0001
A-MW power	27.47	1	27.47	9.54	0.0086
B-Clay amount	280.68	1	280.68	97.44	< 0.0001
C-Time	220.64	1	220.64	76.59	< 0.0001
BC	15.25	1	15.25	5.29	0.0386
B^2	76.89	1	76.89	26.69	0.0002
C^2	11.54	1	11.54	4.01	0.0667
Residual	37.45	13	2.88		
Lack of fit	32.85	8	4.11	4.47	0.0580
Pure error	4.59	5	0.92	36.32	< 0.0001
Core total	665.22	19		9.54	0.0086
R^2=0.94, Adj.R^2=0.92, Pred. R^2=0.83, Adeq Precision =22.02					

The quadratic model was determined as the best fitting model according to non-significant lack of fit and R^2 values for all responses (Table 4.2). Non-significant lack of fit and high R^2 value indicates that the statistical model fits well with data [18, 92]. Besides adequate precision values obtained for each response were higher than 4, it can be said that there is a good correlation between the estimated values and experimental data [93]. Similarly, Chew et al. [13] and Garcia-Moreno et al. [95]

have also reported the quadratic model for the bleaching studies based on totox and color parameters.

The reduced models created with the terms that are only statistically significant are expressed in Equation (4.1), (4.2), (4.3) for totox (Y1), hue angle (Y2) and chroma values (Y3), respectively.

$$Y1 = 22.03 + 1.20xA + 0.45xB + 2.80xC + 0.59xAxB + 0.74xAxC + 0.77xC2 \quad (4.1)$$

$$Y2 = 99.67 + 0.47xA + 1.08xB + 1.41xC + 0.48xAxB + 0.24xBxC - 0.4xB2 - 0.51xC2 \quad (4.2)$$

$$Y3 = 39.78 - 1.41xA - 4.43xB - 4.02xC - 1.38xBxC + 2.30xB2 + 0.89xC2 \quad (4.3)$$

The magnitude of coefficients for bleaching time and amount of clay are higher than the coefficients for current microwave power and other interactions. On the other hand, the magnitude of coefficients for microwave power and amount of clay are higher than the coefficients for current hue angle, chroma and other interactions, indicating that these parameters have a more significant effect on hue angle, chroma and totox value. The other parameters seem to have a quadratic effect on the hue angle, chroma and totox value.

4.1.1.1.2 The Effect of MWB Parameters on the Oxidation of Oil

Oxidation of fats and oils during the bleaching is one of the important deteriorative reactions. Totox value is one of the important ways to express the total oxidation degree of fats and oils. Hence totox value was followed as oxidation indicator in this study.

Among the independent variables microwave power, time, and their interaction (AC) were found significantly affected ($p < 0.05$) on the totox value. Second-order term of time (C^2) had also significant ($p < 0.05$) effects on the totox value. On the other hand, neither the first-order term of amount of bleaching clay nor its interactions with other responses (AB, BC) had no significant ($p > 0.05$) effect on the totox value of MWBSO.

3D response surface plots for totox value of MWBSO samples as a function of independent variables were shown in Fig 4.2a, b and c. The totox value of SO

increased with rising of microwave power and longer irradiation time. The exposure of material to high microwave power increases the temperature of the material. Therefore the increase in temperature due to high microwave power negatively affects the oxidation stability of oil.

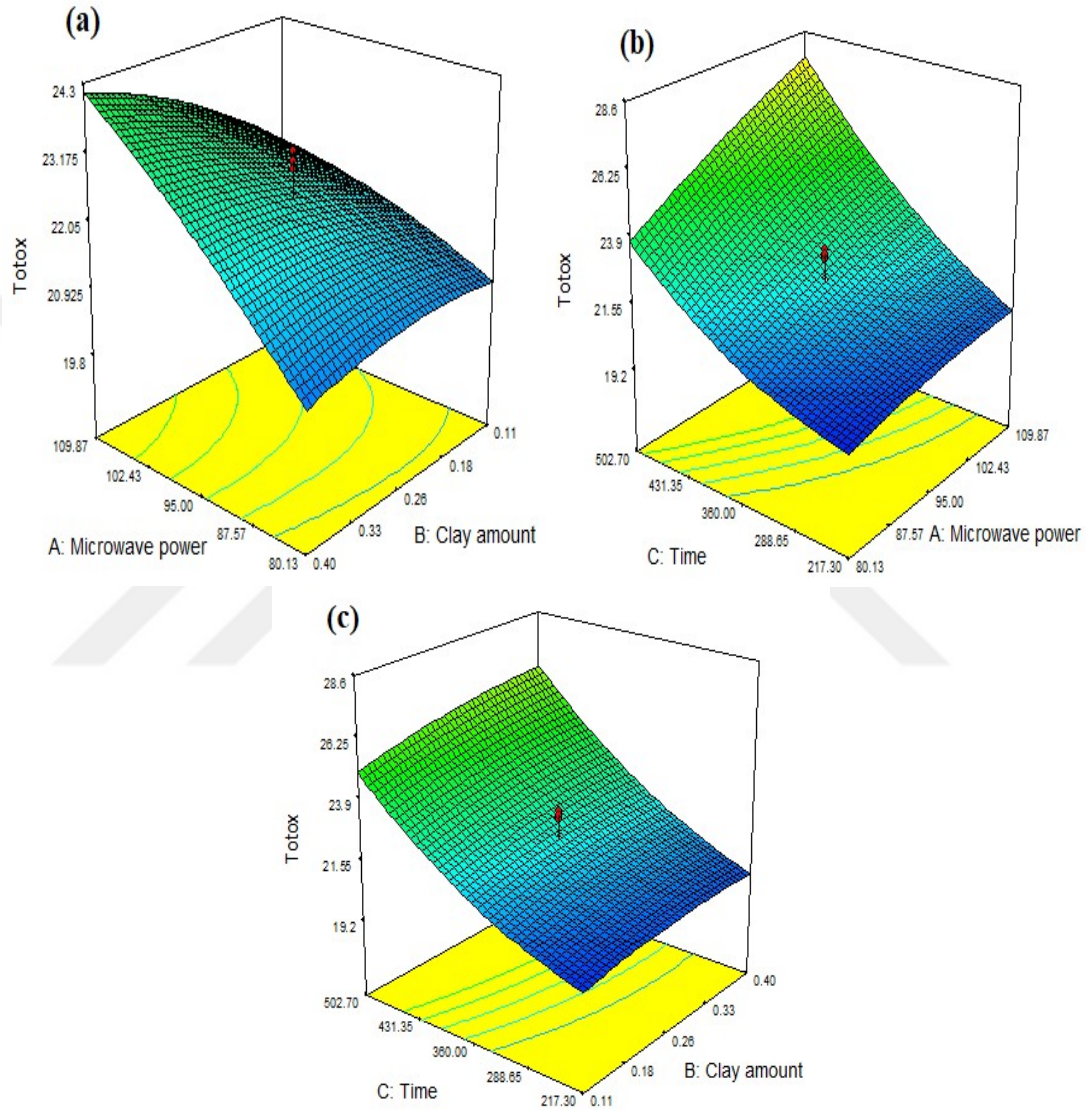

Figure 4.2 3D response plots totox value

Marrakchi et al. [96] stated that the bleaching temperature significantly affects the oxidation stability. In another study in which optimization of IB conditions was studied, it was emphasized that temperature and bleaching earth had significant effect on totox value [13]. Contrary to the findings of Chew et al. [13] the amount of clay in MWB was not found to be effective on the totox value than that of

commercial bleaching process. It may be due to using of low amount of clay and lower bleaching time in MWB conditions.

İçyer and Durak [18] emphasized that especially p-anisidine and totox value increased as increasing bleaching time in both conventional and ultrasonic bleaching method. The maximum totox value (29.91) obtained in MWB was found comparable with totox value (31.07) reported by İçyer and Durak [18] for 30 min conventional bleaching. In general, as increasing the amount of clay, removal of unwanted compounds in the oil during bleaching can be facilitated.

On the other hand the catalytic properties of acid-activated bleaching earth convert hydro peroxides into secondary oxidation products. Consequently, this situation affects the totox value [97, 98]. It is quite clear from our findings that the low microwave power (80 W) and short processing time (8 min) has no effect on oil quality and just little effect on peroxide and p-anisidine value of the oil processed by microwave. Even if totox value increased slightly with increasing peroxide and p-anisidine values, they are similar to the findings obtained by conventional bleaching process [13, 97, 98].

In addition, it should be kept in mind that the following deodorization process will remove the formed oxidation products which are commonly produced also in conventional technology. It is emphasized in the literature in many sources [12, 37, 99].

4.1.1.1.3 The Effect of MWB Parameters on Color Properties

Although Lovibond tintometer is accepted as a standard method in measuring the color of oils, HunterLab has been used in many studies to measure the color of oils [13, 17, 54]. In addition, Abedi et al. [54] have reported a correlation between b * and a * values and carotenoid and chlorophyll concentrations, respectively. Hue angle and chroma have been followed for the prediction of color change during MWBSO. While hue angle represents color appearance (0°: red, 90°: yellow and 180°: blue), chroma is defined as a measure of whiteness and vividness of color [85].

All independent variables (A, B and C) were found significantly ($p < 0.05$) effective on the hue angle and chroma value. Besides some interactions such as microwave

power/amount of clay (AB), amount of clay/time (BC) and second-order term of time (C^2) were found significantly effective ($p<0.05$) on the hue angle. Garcia-Moreno et al. [95] found a similar correlation between hue angle and chroma.

Response surface plots for chroma and hue angle value of bleached sunflower oil samples as a function of independent variables were shown in Fig 4.3a, b and c, respectively. Based on Fig 4.3b, microwave power and amount of clay had significant positive effect on hue angle. Moreover interactions of microwave power and amount of clay had a synergistic effect on hue angle. The interaction of microwave power and amount of clay has caused an increase in hue angle. This means that the redness was removed from the SO.

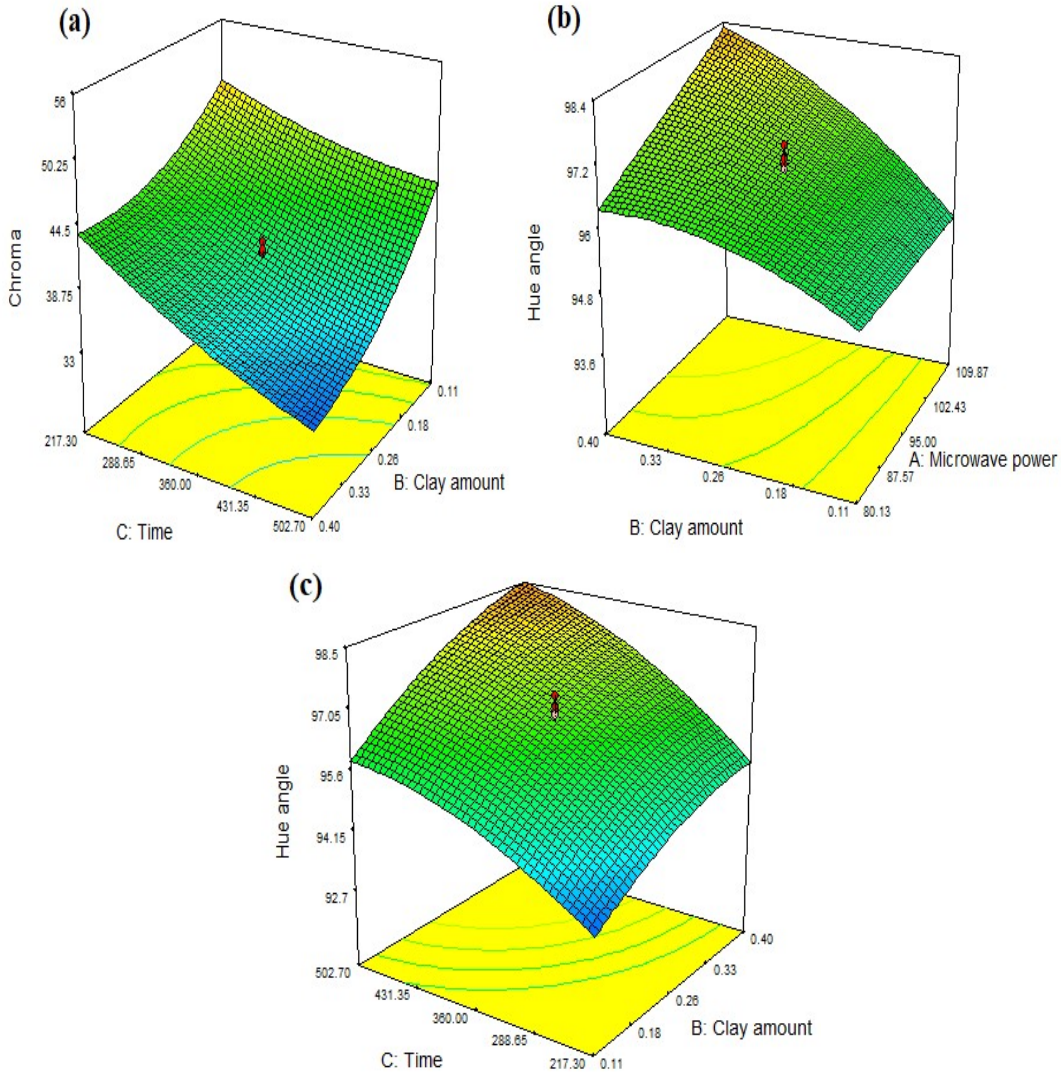

Figure 4.3 3D response plots for chroma and hue angle values

Bleaching time was found as other important parameters for hue angle. Especially, interaction of time and amount of clay caused a positive effect on the hue angle (Figure 4.3c). Furthermore, the second order effects of these two variables on the hue angle had a negative coefficient which means that these variables had reached an optimum value in the studied range. In other words, the increase in time and amount of clay did not cause further rising in hue angle after a certain point. Interactions of BC and second-order term of amount of clay (B^2) were found significant on the chroma. Chroma value decreased with increasing amount of clay and time and vice versa (Figure 4.3a).

A negative correlation was determined between the hue angle and chroma value. Although the hue angle increases, the chroma values decrease. Marrakchi et al. [96] reported a similar trend in their study.

4.1.1.1.4 Optimization and model verification

Optimization of MWBSO was carried out by minimizing totox and chroma and maximizing hue angle in SO samples. In the optimum conditions, the temperature started with ambient temperature and reached to 100 °C at the end of the bleaching. In other words, the temperature increased during the treatment period but remained below 100 °C without applying vacuum. Optimum conditions for MWBSO were found as 80 W, 8.0 min and 0.4% for microwave power, time and amount of clay, respectively.

Compared with the studies in the literature on reducing of clay and bleaching time, it is seen that optimized MWBSO conditions provides more effective decreases. For example, Abedi et al. [54] was reported that bleaching of different two oils were carried out by using 1.22 and 1.35% clay for 28 min. The same researchers in a different study [17] on the bleaching of sunflower and soybean oil by using high voltage electric field found optimum conditions as 1% clay and 20 minutes; 0.5% clay and 20 min for soybean and sunflower oil, respectively. In another study Asgari et al. [56] have studied on bleaching of olive oil and they reported 13 min for bleaching time and 1.21% for the amount of clay.

The bleaching time in none of these studies could not been decreased below 10 min. It was ranged from 15 min to 30 min. Among these studies amount of clay used was minimum 0.8% determined by İçyer and Durak [18].

Temperature is one of the important parameters in order to obtain effective bleaching. The temperature values used in the ultrasonic bleaching process can be as a limiting factor. Because with the rapid increase of the liquid temperature, a dense of bubble formation begins in the liquid and continues increasingly. But the tendency of the bubbles to collapse becomes harder due to the high pressure inside of them acting as a bed [100]. Therefore, in the ultrasound assisted bleaching process, the effect of high temperature bleaching required to achieve the desired oil quality cannot be obtained due to sonication. [54].

In addition, in cases where high temperatures are inevitable, unwanted taste and odors has been observed in ultrasonic method [53]. But due to the having lower specific heat, vegetable oils can be heated to high bleaching temperature easily in a short time by microwave which is important advantage compared to ultrasound method. Abedi et al. [17] used 1.5% bleaching earth and 30 min bleaching time to imitate the industrial process as a control system. The microwave bleaching results have shown that MWB is very advantageous in terms of usage of clay (0.4%) and bleaching time (8 min) than those used in industrial conditions.

For verification of the response values estimated as a result of the optimization, three independent MWB studies were carried out at optimum conditions and experimental response values were determined. Experimental and estimated values for each response under optimum conditions are given in Table 4.3.

Table 4.3 Comparison of experimental and estimated values for sunflower oils

Response	Experimental	Predicted	p value
Totox value	23.31 ± 0.28	22.48	0.13
Hue angle	96.91 ± 0.02	97.28	0.19
Chroma	37.66 ± 0.02	35.67	0.08

± Standard Deviation. Data are means of triplicates.

The optimum values obtained from Design Expert for totox, hue angle and chroma were found as 18.37, 36.56 and 22.02, respectively. Furthermore, one-way t-test showed that there was no statistically significant difference between the experimental data and the predicted values for all three responses ($p > 0.05$).

4.1.2 Comparison of MWBSO with IB

4.1.2.1 Processing Conditions for Bleaching Techniques

Table 4.4 summarizes the real IBSO conditions and optimized conditions of MWBSO. In optimum conditions of MWBSO, the amount of clay and bleaching time were decreased by 50% and 73%, respectively, relative to those used in conventional bleaching.

Table 4.4 Processing parameters for industrial and optimized MWB

Parameters	Industrial Bleaching	Microwave-Assisted Bleaching
Amount of clay (% m/m)	0.8	0.4
Bleaching time (min)	30	8
Process temperature (ºC)	100	<100
Vacuum use	Yes	No

The bleaching was applied to hot fed oil at a temperature of 100 °C for 30 min under the vacuum in conventional process while the oil temperature reached to 100 °C from ambient temperature during the microwave bleaching process in 8 min at a power of 80 W under atmospheric pressure. In other words, microwave treatment caused the heating and bleaching of oil in a short time (8 min) below 100 °C because of its low specific heat capacity and high bleaching efficiency.

Hence, MWB might be advantageous with its short heating time, low process temperature and the absence of vacuum. The used amount of clay and processing time in MWB were also lower compared to those of new bleaching techniques such as ultrasound and high voltage electric field [17, 54, 56].

4.1.2.2 The Effects of Bleaching Processes on Color Properties

Minor components in the oil such as chlorophyll and carotenoids are responsible for the color of crude and refined oils. Especially, green-yellowish, orange and redness occur in the presence of these components [101]. The color and color related values of bleached SO obtained by MWB were given in Table 4.5. Topkafa et al. [39] stated that the amount of chlorophyll in four different crude sunflower oils was between 403-1021 µg/kg. On the other hand, Kreps et al. [101] reported the amount of chlorophyll in standard sunflower oil and sunflower oil with high oleic acid as 4320 and 5620 µg/kg, respectively. Abbasi et al. [55] found that the amount of chlorophyll in neutralized sunflower oil was 8720 µg/kg. These differences are due to different sunflower species, climatic conditions and soil characteristics of cultivated regions. Chlorophyll is desired to be removed from the oils as much as possible, as it transforms into pheophytin, which gives a dark color to the oils, and supports oxidation with the effect of temperature [102, 103].

Chlorophyll destruction was found to be 85.37 and 87.80% in MWBSO and IBSO, respectively. Kreps et al. [101] reported that destruction of chlorophyll was 95% after bleaching.

Table 4.5 Color and color related values of bleached sunflower oils

Parameters	Before Bleaching	Industrial Bleaching	Microwave-Assisted Bleaching
L*	60.26^{a}±0.02	78.28^{b}±0.01	78.59^{b}±0.02
a*	-0.70^{a}±0.01	-3.85^{b}±0.01	-4.60^{c}±0.01
b*	44.20^{a}±0.02	36.89^{b}±0.02	37.52^{b}±0.01
Hue angle	90.91^{a}±0.03	95.96^{b}±0.02	96.99^{c}±0.02
Chroma	44.21^{a}±0.01	36.99^{b}±0.01	37.80^{b}±0.02
Chlorophyll (µg/kg)	3009.78^{a}±0.99	367.05^{b}±0.96	440.45^{c}±0.93
Carotenoid (mg/kg)	1.91^{a}±0.08	0.37^{b}±0.05	0.48^{c}±0.03
Bleaching efficiency (%)	-	85.68^{a}±0.02	83.76^{b}±0.01

Different letters (a. b. etc.) in each line indicate statistical difference ($p < 0.05$). Data are means of triplicates. ±: Standard Deviation (n=3).

The carotenoid content of sunflower oil is generally low, and Gunstone [104] and Abbasi et al. [55] stated the carotenoid content of sunflower oil as 1-1.5 mg/kg and 5.95 mg/kg, respectively. In this study, it was found as 1.91 mg/kg in pre-bleaching. The loss of carotenoid content was found as 80.63% in IBSO and 74.87% in MWBSO. Abbasi et al. [55] determined the carotenoid loss to be at 75.4% and 76.6% in conventional and ultrasonic bleaching, respectively for 30 min. Thus, despite the short bleaching time, MWB is quite effective, and the carotenoid content of the oil bleached with this method was found to be slightly higher than that of the oil in IBSO.

The lower a^* value of MWBSO than that of IBSO indicates that the microwave process reduces redness more and the results confirm each other when looking at the chlorophyll content and a^* values. In both applications, the yellowness value (b^*) of the oil decreases, while the yellowness is slightly higher in MWB. The high b^* value in MWBSO, where the carotenoid content is high, confirms this. While the L* value was increased in both methods compared to the NSO, the brightness values were found very close for MWBSO and IBSO.

Hue angle refers to pure red at 0°, pure yellow at 90° and pure blue at 180° [105]. Chroma is defined as a measure of whiteness and vividness of color [85]. The hue angle value increased in IB and MWB methods compared to unbleached oil. However, slightly higher hue angle and chroma value in MWBSO can be explained by the relatively high b^* value. A negative correlation was determined between hue angle and chroma value. Garcia-Moreno et al. [95] found a similar correlation between them.

The bleaching efficiency can be expressed as a measure of the extent to which unwanted color pigments are removed from the oil. The different bleaching effect is due to the different a^* and b^* values of the oils after both bleaching methods.

4.1.2.3 Effects of Bleaching Processes on Totox, FFA and Soap Residues

Table 4.6 summarizes the oxidation parameters and free acidity content and soap content in MWBSO and IBSO compared to NSO. Although it seems a decrease in

the peroxide value of the oil samples in both bleaching methods compared with NSO, there was no significant change in the PV in MWB.

Table 4.6 Oxidation parameters, FFA and soap content in bleached sunflower oils

Parameters	Before Bleaching	Industrial Bleaching	Microwave-Assisted Bleaching
Peroxide (meq/kg)	$9.90^{a} \pm 0.06$	$7.59^{b} \pm 0.03$	$9.28^{a} \pm 0.05$
P-anisidine	$0.40^{a} \pm 0.02$	$3.68^{b} \pm 0.02$	$3.83^{c} \pm 0.04$
Totox	$20.20^{a} \pm 0.11$	$18.86^{b} \pm 0.15$	$22.39^{c} \pm 0.18$
FFA (%)	$0.27^{a} \pm 0.02$	$0.20^{b} \pm 0.01$	$0.28^{a} \pm 0.01$
Soap content (ppm)	6.06 ± 0.03	N.D	N.D

Different letters (a. b. etc.) in each line indicate statistically difference ($p < 0.05$). Data are means of triplicates. ±: Standard Deviation (n=3). N.D: Not Detected.

Peroxide values showed a decreasing trend in IBSO with a rate of 23.3%. On the other hand, it was observed that PV in MWBSO was not change statistically compared with before bleaching. This is thought to be due to 50% less clay use compared to IBSO and short bleaching time. The fact that the peroxide value does not change in MWB can be explained by the very fast oxidation mechanism. In other words, while existing primary oxidation products are rapidly transformed into secondary oxidation products, new primary oxidation products are likely to form. It has been reported in the literature that peroxides turn into secondary oxidation products during bleaching [106]. Abedi et al. [17] stated that when 1.5% clay was used in high voltage bleaching process, the PV decreased from 5.21 meq O_2/kg to 0.98 meq O_2/kg (81.19%) after 30 min. In the same study, it was found that the peroxide value decreased by 67.56% under IB.

In some studies, it has been stated that as the amount of clay used during bleaching increases, the PV decreases [13, 54, 107, 108]. Ortega-Garcia et al. [109] reported that during bleaching, the amount of clay should be in the range of 1-1.5% to reduce the PV near zero. Silva et al. [107] expressed that the PV in palm oil was reduced to near zero by using more than 1% of the acid activated clay. However, in the same study, when natural (inactivated) clay is used, it is expressed that this value is 2%.

AV increased rapidly in both bleaching methods. Although the PV of MWBSO does not change, the AV is slightly higher than the IBSO. Despite the lower temperature and short duration in MWB, this may be due to the fact that vacuum is not applied (under open conditions to the atmosphere). Although vacuum is not applied, it is quite successful to obtain a value close to IBSO in a short bleaching time. As a result of bleaching carried out under MWBSO conditions, totox values are slightly higher than IBSO. A great deal of oxidation products are removed from the oil during deodorization that is emphasized in the literature [11, 37]. This small difference is not at a level that will adversely affect oil quality.

FFA is the most important determinant of the suitability and edibility of oils. Increase in FFA adversely affects the taste, odor and oxidative stability of oils [110]. The increase in FFA compared to non-bleached oil is interpreted as the result of the leakage of trace amounts of acid in the acid activated clay into the oil [101]. Therefore, decreasing the amount of acid activated clay can prevent the increase of FFA. While FFA decreased in IBSO, there was no significant change in acidity in MWBSO compared to unbleached oil. This is probably due to the reduced amount of clay used and the short bleaching time. A similar result was noted by Jahouach-Rabai et al. [53]. The authors noted that there was no big difference in acidity in unbleached oil and ultrasound bleached oil.

Shelf life and durability of oils depend on soap content and low soap content is expressed as high shelf life [110]. They cause the formation of undesirable destruction products such as long chain or cyclic ketones [37]. The soap content was not detected in both IBSO and MWBSO. Therefore, MWB was found to be very effective in removing soap residues from oil.

While Shah et al. [110] stated the amount of soap in NSO as 121 ppm, Girgis [111] determined it as 40 ppm. The amount of soap in NSO given in the literature is considerably higher than that of the SO used in this study. This difference in soap content is thought to be due to the neutralization conditions applied and the free acidity content of crude SO. Shah et al. [110] reported that the amount of soap in SO after bleaching was reduced from 121 ppm to 60.4 ppm. On the other hand, Girgis [111] emphasized that it was reduced from 40 ppm to 8 ppm.

4.1.2.4 Effect of Industrial and Microwave Bleaching on Sterol Composition and Tocopherol Content

The major sterol components in sunflower oil are reported to be campesterol, stigmasterol, β-sitosterol, Δ5- and Δ7-avenasterols, and Δ7-stigmastenol [112]. Sterol composition and tocopherol content of MWBSO and IBSO are shown in Table 4.7. The dominant component in NSO was found as β-sitosterol. No statistical difference was observed between pre-bleaching and MWBSO and IBSO when compared in terms of sterol composition. Javidipour et al. [113] stated the tocopherol amount in refined SO as 513.01 ppm, while Kreps et al. [101] expressed it as 332 ppm in crude SO.

Table 4.7 Sterol composition and tocopherol content of bleached sunflower oils

Parameters	Before Bleaching	Industrial Bleaching	Microwave Assisted Bleaching
Sterol composition(%)			
Cholesterol	0.55^{a}±0.09	0.27^{b}±0.05	0.21^{b}±0.04
Brassikasterol	0.09^{a}±0.01	0.09^{a}±0.01	0.08^{a}±0.01
Campesterol	8.45^{a}±0.43	8.32^{a}±0.43	8.59^{a}±0.44
Stigmasterol	8.85^{a}±0.67	8.60^{a}±0.65	8.73^{a}±0.44
Beta sitosterol	53.19^{a}±0.39	53.32^{a}±0.4	54.33^{a}±0.41
Δ5-avenasterol	4.74^{a}±0.94	5.02^{a}±0.99	4.45^{a}±0.89
Δ7-stigmastenol	11.13^{a}±0.68	10.65^{a}±0.65	10.85^{a}±0.66
Δ7-avenasterol	5.25^{a}±1.01	4.92^{a}±0.95	5.05^{a}±0.97
Tocopherol (ppm)			
α-Tocopherol	487.50^{a} ±3.00	448.50^{b} ±3.00	449.50^{b} ±3.00

Different letters (a. b. etc.) in each line indicate statistically difference ($p < 0.05$). Data are means of triplicates. ±: Standard Deviation (n=3).

Tocopherol loss in MWBSO was found statistically the same as IBSO. It has been reported that the degradation of the tocopherol is due to the alkalinity or acidity of the bleaching earth used [101]. Javidipour et al. [113] stated in their study that when 10 g of SO was treated at 600 W microwave energy for 9 min, the tocopherol loss was 16.85%. It can be said that this high amount of tocopherol loss is due to the high

microwave energy used. However, it is possible to say that when low microwave energy is used in short bleaching times, MWB preserves tocopherol loss.

4.2 Reusability of bleaching clay used in microwave-assisted bleaching

As shown in Table 4.8, the bleaching quality of sunflower oil gradually decreased after the second use of clay. L* value did not change statistically up to third use, but a* and b* values were found significantly different ($p<0.05$) after the first use.

Especially, yellowness (b*) of the oil dramatically increased with increased number of reuse of the clay for bleaching. This situation can be seen more clearly when the hue angle and chroma values are examined. As the frequency of reuse increases, the hue angle value had presented an approach towards 90°, which is considered as pure yellow.

Table 4.8 The bleaching performance of reused clay

Parameters	First use	Second use	Third use	Fourth use
L*	$78.59^{a}\pm0.02$	$78.25^{a}\pm0.06$	$77.63^{b}\pm0.25$	$74.79^{c}\pm0.54$
a*	$-4.60^{a}\pm0.01$	$-4.25^{b}\pm0.07$	$-3.83^{c}\pm0.02$	$-3.42^{d}\pm0.03$
b*	$37.52^{a}\pm0.01$	$43.36^{b}\pm1.57$	$50.81^{c}\pm0.47$	$54.17^{d}\pm0.33$
Hue Angle	$96.99^{a}\pm0.02$	$95.55^{b}\pm0.23$	$94.30^{c}\pm0.01$	$93.63^{d}\pm0.33$
Chroma	$37.80^{a}\pm0.02$	$43.57^{b}\pm1.56$	$50.95^{c}\pm0.46$	$54.28^{d}\pm0.62$
Bleaching Efficiency (%)	$83.76^{a}\pm0.01$	$75.83^{b}\pm0.53$	$56.25^{c}\pm0.72$	$27.70^{d}\pm0.20$

Different letters (a. b. etc.) in each line indicate statistically difference ($p<0.05$). Data are means of triplicates.±: Standard Deviation (n=3).

The fresh and used clay have been presented in Figure 4.4. Depending on the number of uses, the chroma values increased, in other words, the color in the bleached oil had lost its vividness and whiteness.

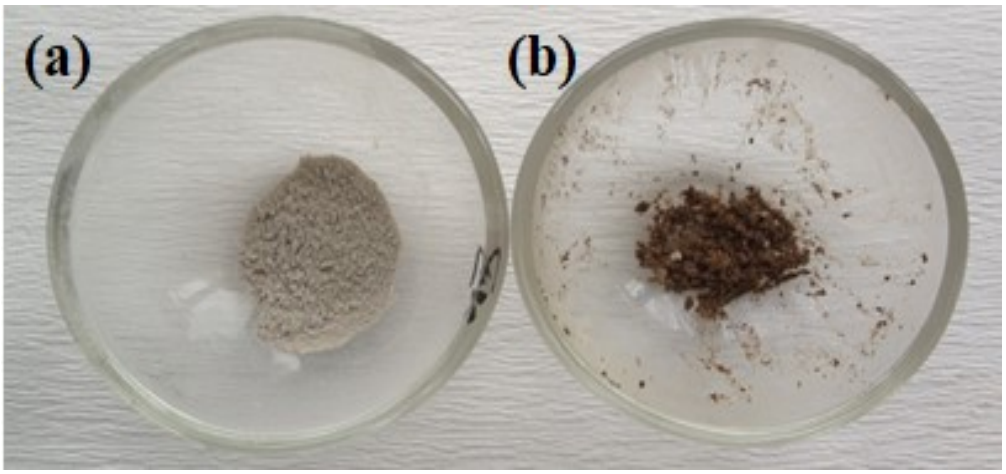

Figure 4.4 Physical situation of bleaching clay
(a) Fresh clay (b) Used clay

It was observed that the physical structure of the bleaching clay changed from powder to the flakes form with repetitive use (Figure 4.5).

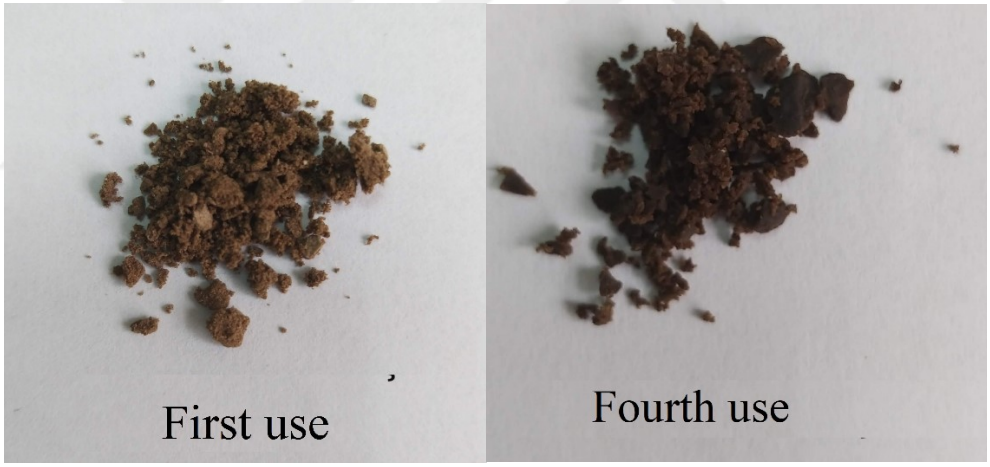

Figure 4.5 Physical structure of bleaching clay during repetitive use

After the third use, the clay was dispersed in the oil as flakes (Figure 4.6) and this situation caused a decrease in the bleaching efficiency due to its lower absorption capacity. Foletto et al. [91] reported that the bleaching efficiency was between 32-67% (with hexane it is 34%) by using 1% spent bleaching clays that were deoiled by using different solvents. Compared to these results, MWB, which uses 60% less clay, has a higher bleaching effect (75.83%) in second use, and it is thought that microwave makes a serious contribution for reuse of the clay.

Figure 4.6 The clay dispersed in the form of flakes in oil after repetitive use

4.2.1 The surface morphology of the fresh and used clay

The surface morphology of the fresh and used clay in MWB were investigated using SEM. The images are given in Fig 4.7.

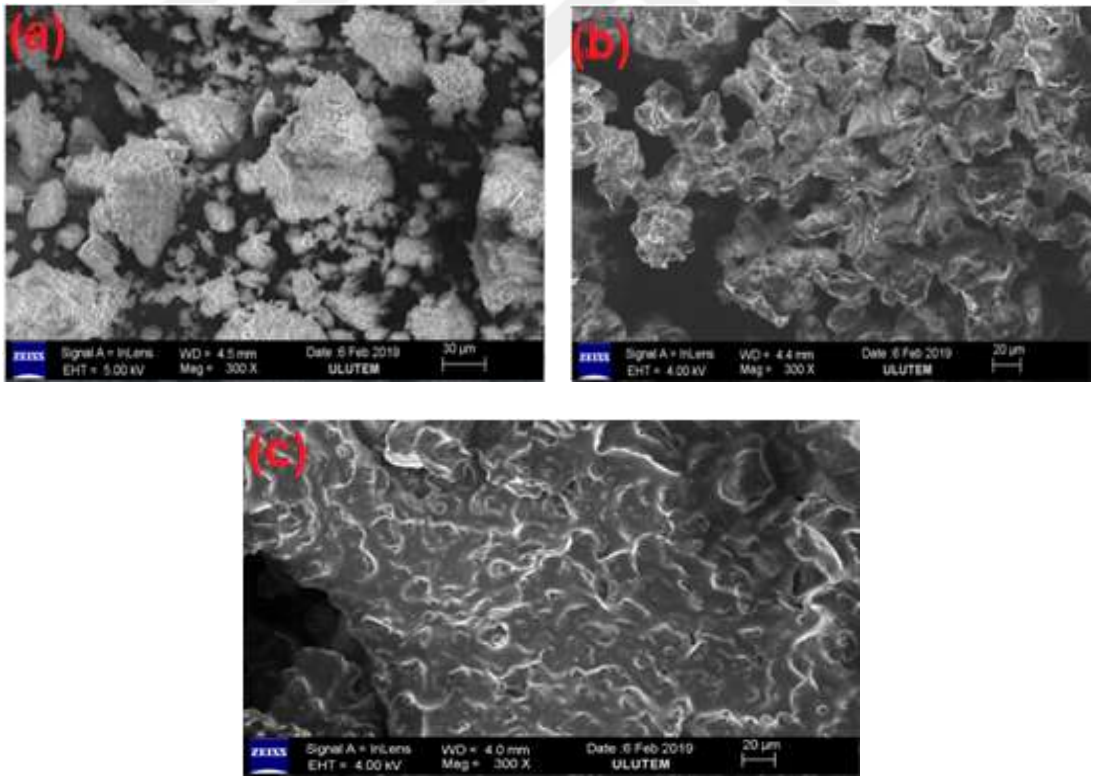

Figure 4.7 SEM images of activated bleaching clays before and after use
(a) Fresh clay (b) After the first use (c) After the fourth use

It was observed that there are important differences between fresh and used clay. As increasing the number of uses it was observed that morphological structure of clay tightens and becomes denser due to closing of the gaps. It can be said that this situation is caused by residual sunflower oil that holds onto the clay and contains impurities such as color pigments, oxidation products, soap residue, etc.

On the other hand, fresh clay had a structure with densely voids between particles. It was also observed that the clay color changed from gray to brown depending on the number of uses and gradually became darker.

4.3 Microwave-assisted Bleaching of Pomace Oil

Bleached PO samples were separated from the clay/activated carbon by centrifugation and filtered under vacuum after bleaching and made ready for analysis (Figure 4.8).

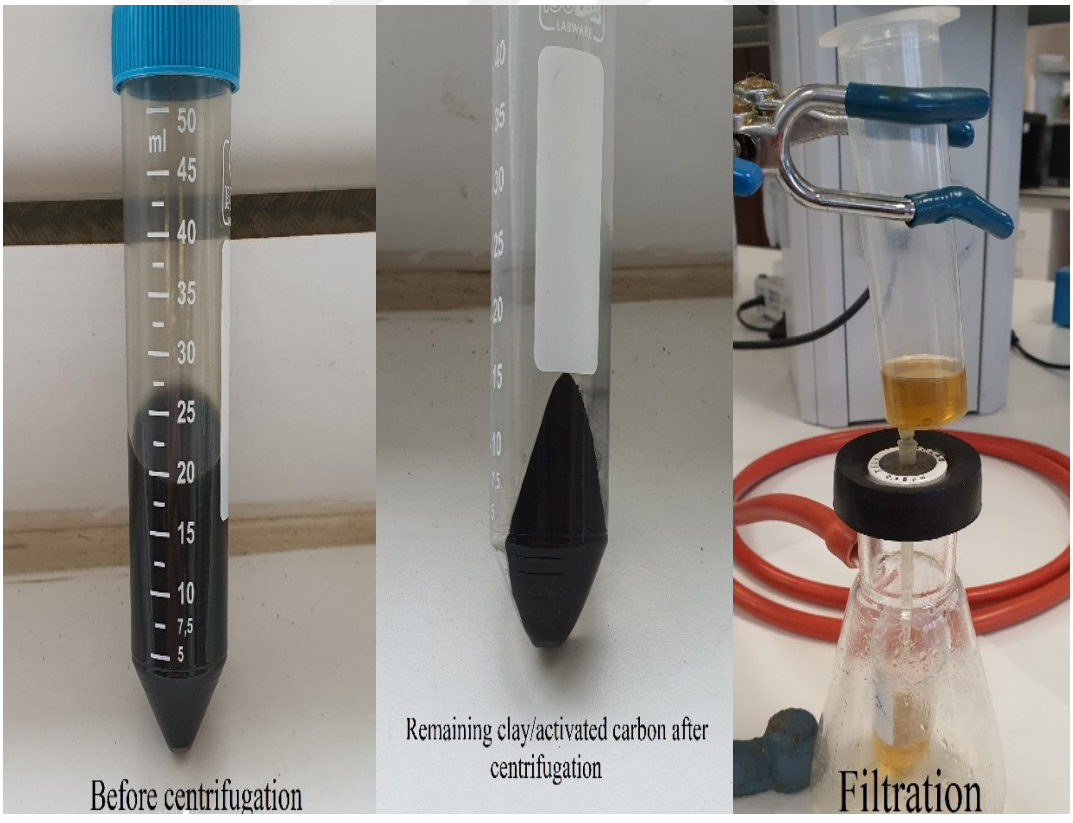

Figure 4.8 Applied physical processes after MWB

4.3.1 Optimization of Microwave-assisted Bleaching of Olive Pomace Oil

4.3.1.1 The Central Composite Rotatable Design and Optimization

4.3.1.1.1 Fitting model

The experimental design and the experimental results for MWBPO are summarized in Table 4.9. The bleaching efficiency and the oil quality have been analyzed in terms of hue angle and chroma values for MWBPO, respectively.

The hue angle and chroma values of MWBPO obtained in this study ranged from 77.47 to 91.50 and 58.59 to 69.24, respectively.

Table 4.9 Three factor-five level experiment design generated for MWBPO

Runs	FACTORS			RESPONSES			
	A	B	C	Hue Angle		Chroma	
	(Watt)	(%)	(min)	Experimental	Predicted	Experimental	Predicted
1	78	2.81	34.93	80.34 ± 0.06	80.22	67.74 ± 0.08	68.42
2	102	5.19	20.07	88.88 ± 0.07	88.20	64.29 ± 0.05	63.24
3	90	4.00	27.50	84.09 ± 0.03	84.21	65.19 ± 0.04	66.71
4	90	2.00	27.50	79.49 ± 0.04	78.19	66.24 ± 0.06	66.68
5	102	5.19	34.93	91.50 ± 0.06	89.62	58.59 ± 0.09	59.32
6	90	4.00	40.00	85.13 ± 0.04	85.40	66.60 ± 0.04	66.37
7	90	4.00	15.00	81.52 ± 0.02	83.02	67.63 ± 0.03	67.05
8	90	4.00	27.50	83.17 ± 0.03	84.21	68.93 ± 0.05	66.71
9	110	4,00	27.50	85.42 ± 0.02	86.10	66.83 ± 0.04	65.93
10	102	2.81	20.07	79.77 ± 0.04	81.04	69.75 ± 0.04	70.39
11	90	4.00	27.50	85.15 ± 0.05	84.21	66.09 ± 0.05	66.71
12	70	4.00	27.50	81.60 ± 0.04	82.33	69.24 ± 0.04	67.50
13	90	4.00	27.50	85.93 ± 0.05	84.21	65.07 ± 0.04	66.71
14	102	2.81	34.93	81.96 ± 0.04	82.46	67.92 ± 0.04	66.47
15	78	5.19	34.93	85.67 ± 0.04	87.38	66.93 ± 0.04	66.30
16	90	4.00	27.50	84.35 ± 0.03	84.21	65.15 ± 0.04	66.71
17	90	4.00	27.50	86.00 ± 0.05	84.21	65.86 ± 0.04	66.71
18	78	5.19	20.07	89.76 ± 0.02	86.00	62.07 ± 0.05	63.14
19	90	6.00	27.50	87.00 ± 0.04	90.23	59.00 ± 0.06	58.85
20	78	2.81	20.07	77.47 ± 0.04	78.80	66.09 ± 0.03	65.31

The multiple regressions and backward elimination were employed to get the best fitting statistical model for each response. Table 4.10 summarizes the ANOVA and regression analysis' results for the hue angle and chroma values. The linear and quadratic models were determined as the best fitting model according to non-significant lack of fit and R^2 values for hue angle and chroma values, respectively.

Table 4.10 ANOVA results for pomace oil responses

Source	Sum of squares	Df	Mean Square	F value	p- value
Model (HueAngle)	199.04	3	66.35	21.86	< 0.0001
A-MW power	17.09	1	17.09	5.63	0.0305
B-Clay amount	175.11	1	175.11	57.69	< 0.0001
C-Time	6.84	1	6.84	2.26	0.1526
Residual	48.56	16	3.04	-	-
Lack of fit	42.38	11	3.85	3.11	0.1100
Pure error	6.18	5	1.24	-	-
Core total	247.60	19	-	-	-
R^2=0.81, Adj. R^2=0.77, Pred. R^2=0.70 Adeq Precision=15.46					
Model (Chroma)	143.12	6	23.85	12.50	< 0.0001
A-MW power	2.94	1	2.94	1.54	0.2363
B-Clay amount	74.04	1	74.04	38.79	< 0.0001
C-Time	0.56	1	0.56	0.29	0.5980
AB	12.38	1	12.38	6.49	0.0243
AC	24.65	1	24.65	12.92	0.0033
B^2	28.55	1	28.55	14.96	0.0019
Residual	24.81	13	1.91		
Lack of fit	13.96	8	1.75	0.80	0.6274
Pure error	10.85	5	2.17		
Core total	167.93	19			
R^2=0.85, Adj.R^2=0.80, Pred. R^2=0.67 Adeq Precision =14.11					

Non-significant lack of fit indicates that the statistical model fits well with data [93, 96]. Adequate precisions for hue angle and chroma were found as 15.46 and 14.11. These values were higher than 4. Therefore, it can be said that there is a good correlation between the estimated values and experimental data [94]. The reduced model created with the terms that are only statistically significant are expressed in Eqn. (6) and Eqn. (7) for hue angle (Y_1) and chroma (Y_2), respectively.

$$Y1 = 84.21 + 1.12xA + 3.58xB \quad (6)$$

$$Y2 = 66.71 - 2.33xA - 1.24xAB - 1.76xAC - 1.39xB\text{^}2 \quad (7)$$

The magnitude of coefficients for amount of clay is higher than the coefficients for current microwave power. Besides the magnitude of coefficients for microwave power is higher than the coefficients for current hue angle, chroma and other interactions, indicating that these parameters have a more significant effect on hue angle and chroma.

The other parameters seem to have a linear effect on the hue angle; on the other hand there is a quadratic effect on the chroma. The microwave power (A) and interactions (AB, AC and B^2) exhibit significant negative effects on the response (Y_2); the increase of these variables results in the decrease of chroma.

4.3.1.1.2 Optimization and model verification

Some independent variables (A and B) were found significantly effective on the hue angle. On the other hand, clay amount was only found significantly effective ($p<0.05$) on the chroma. In addition, some interactions such as microwave power/amount of clay (AB), microwave power/time (AC) and second-order term of amount of clay (B^2) were found significantly effective ($p<0.05$) on chroma.

Response surface plots for chroma and hue angle value of bleached PO samples as a function of independent variables were shown in Fig 4.9. (a, b, c and d). B and interactions of AB, AC and B^2 caused significantly a negative effect on the chroma.

Furthermore, these variables on the chroma had a negative coefficient which means that these variables had reached an optimum value in the studied range. In other words, the increase in these values did not cause further rising in chroma after a certain point.

Optimization of MWBPO was carried out by maximizing hue angle and chroma values. In the optimum condition, the temperature started with ambient temperature and reached to 165 °C at the end of the bleaching without applying vacuum. Optimum conditions for MWBPO were found as 102 W, 20.07 min and 4.15% for microwave power, time and amount of clay, respectively. In these conditions the predicted hue angle and chroma values were 86.11 and 68.06, respectively.

For verification of the response values estimated as a result of the optimization, three independent MWBPO studies were carried out at optimum conditions and the average hue angle and chroma were determined as 85.28 and 67.50, respectively. One-way t-test showed that there was no statistically significant difference between the experimental data and the predicted values for all responses ($p > 0.05$). Experimental and estimated values for each response under optimum conditions are given in Table 4.11.

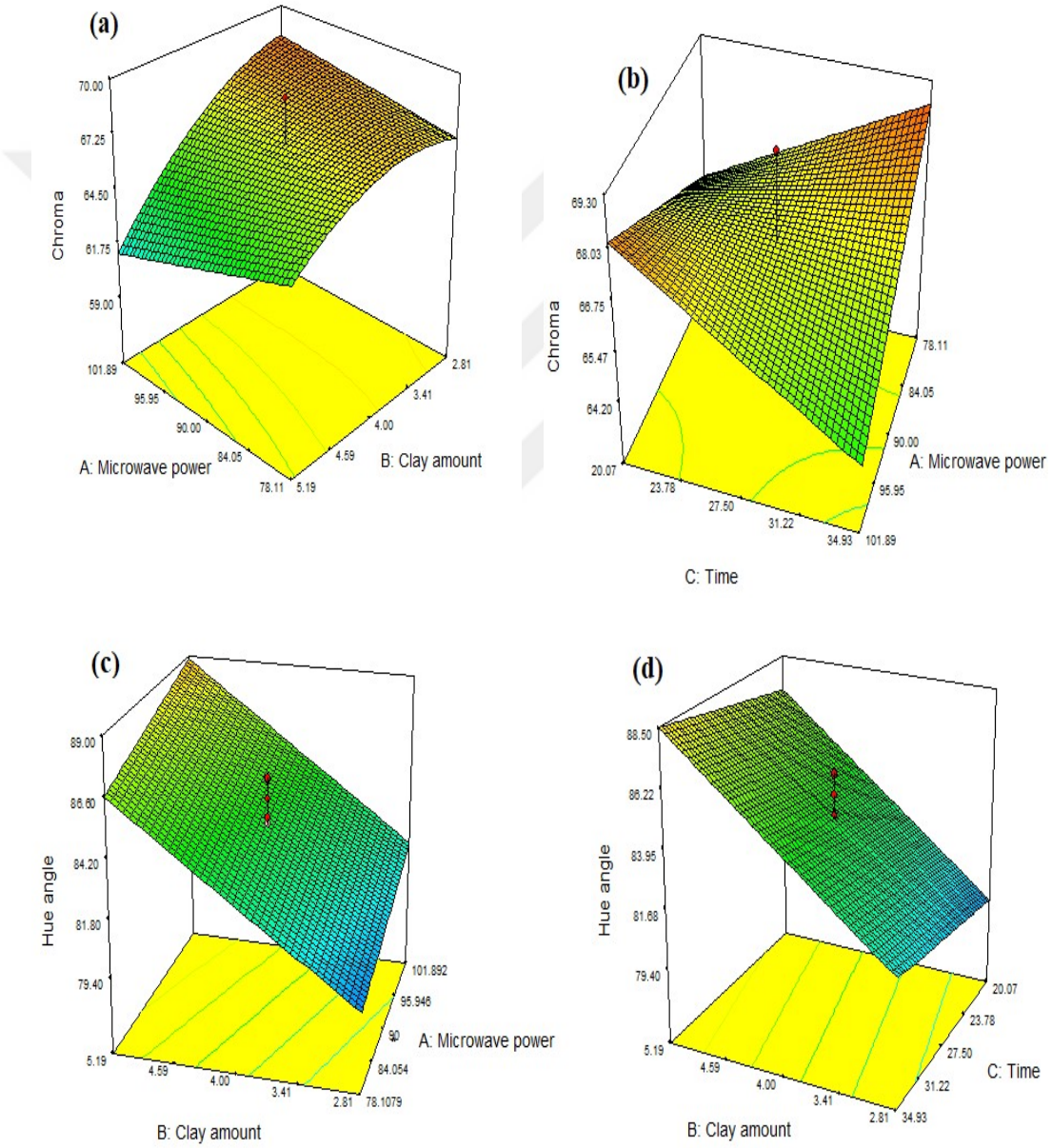

Figure 4.9 3D response plots for chroma and hue angle values

Table 4.11 Comparison of experimental and estimated values for pomace oils

Response	Experimental	Predicted	p value
Hue angle	85.25 ± 0.25	86.11	0.19
Chroma	67.50 ± 0.25	68.06	0.11

± Standard Deviation. Data are means of triplicates.

4.3.2 Comparison of MWBPO with IB

4.3.2.1 Processing Conditions for MWB and IB Techniques

While there was a slight decrease in the clay amount, the processing time and the amount of activated carbon were significantly reduced in MWBPO compared to IBPO.

In that respect the amount of clay, bleaching time and amount of activated carbon were decreased by 17%, 83% and 50%, respectively, under the optimum MWBPO conditions, relative to those used in IBPO. The IBPO was applied to hot fed oil at a temperature of 100 °C for 120 min under the vacuum in industrial process while the oil temperature reached to 165 °C from ambient temperature during the microwave bleaching process in 20.07 min at a power of 102 W without vacuum.

4.3.2.2 The Effects of Bleaching Processes on Color Properties

The appearances of NPO, IBPO and MWBPO were presented in Fig 4.10. The color and color related values of NPO, IBPO and MWBPO were also given in Table 4.12.

Hue angle and chroma have been followed for the prediction of color change during MWBPO. While hue angle represents color appearance (0°: red, 90°: yellow and 180°: blue), chroma is defined as a measure of whiteness and vividness of color [85].

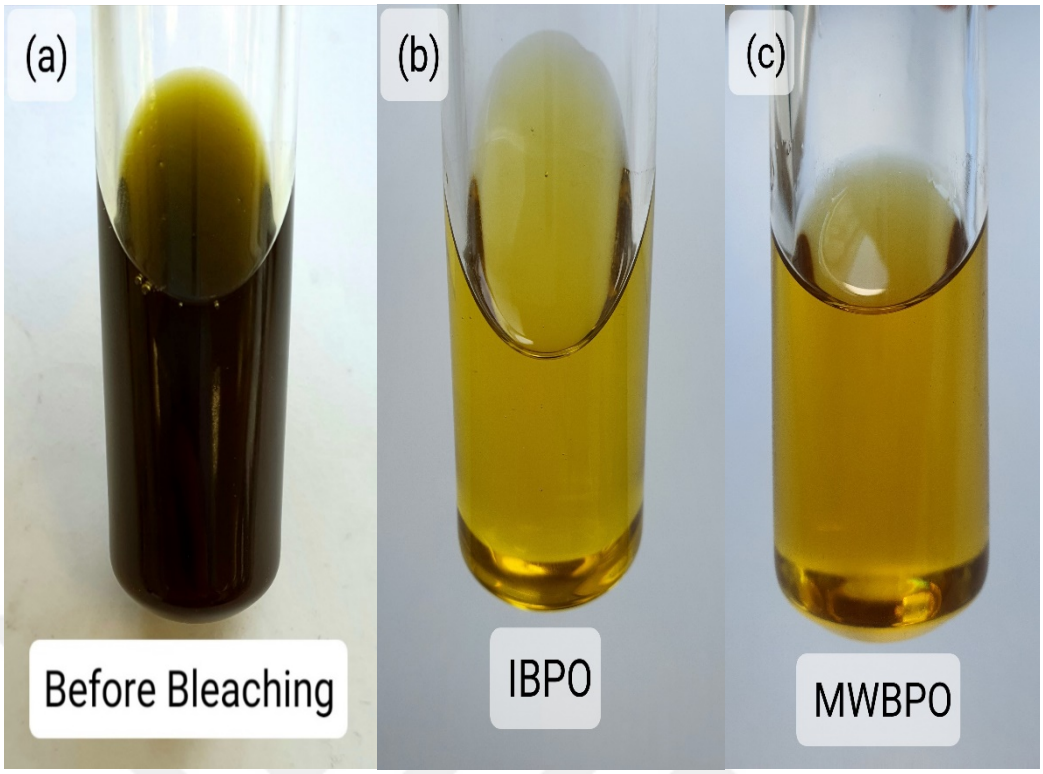

Figure 4.10 The appearances of olive pomace oil
(a) Neutralized unbleached pomace oil, (b) Industrial bleached pomace oil,
(c) microwave- assisted bleached pomace oil

The hue angle and chroma values are increased in both bleaching methods compared to NPO. However, slightly higher hue angle and chroma values in IBPO can be explained by the relatively higher b^* and lower a* value compared with MWBPO. The greenish increased with negative a* value in the IBPO. On the other hand, the redness decreased with MWBPO and IBPO compared with NPO. But the greenish did not increase in MWBPO in terms of positive a* value. Marrakchi et al. [96] were reported that hue angle and chroma values are found as 102 and 51 in the crude lampante olive oil, respectively. Hue angle and chroma values after bleaching were within the limits of 106-110 and 20-39 depending on the bleaching conditions.

Escolar et al. [114] studied on the 107 Spanish olive oil five of which were pomace oil purchased from local outlets. In this study, the average of L*, a* b* and chroma values were found to be 94.40, -5.40, 46 and 46, respectively. Besides, Cho and Lee [115] presented that the average of L*, a* and b* values are 95.54, -10.71 and 35.61 in refined pomace oil including 15-20% virgin olive oil, respectively. Our results

were found very different compared with other findings in the literature. These findings could be because of a wide range of the chlorophyll and carotenoid content in oil samples affected from different olive species, climatic conditions, harvest season, soil characteristics of cultivated regions and processing conditions.

Table 4.12 Color and color related values of bleached pomace oils

Parameters	Before Bleaching	Industrial Bleaching	Microwave-Assisted Bleaching
L*	$13.43^{a} \pm 0.12$	$69.21^{b} \pm 0.49$	$65.21^{c} \pm 0.74$
a*	$9.20^{a} \pm 0.04$	$-2.17^{b} \pm 0.03$	$4.99^{c} \pm 0.76$
b*	$20.71^{a} \pm 0.11$	$72.22^{b} \pm 0.05$	$68.62^{c} \pm 0.14$
Hue angle	$66.06^{a} \pm 0.09$	$91.72^{b} \pm 0.02$	$85.84^{c} \pm 0.62$
Chroma	$22.76^{a} \pm 0.18$	$72.25^{b} \pm 0.05$	$68.80^{c} \pm 0.20$
Chlorophyll (mg/kg)	$5.46^{a} \pm 0.63$	$0.67^{b} \pm 0.94$	$0.26^{c} \pm 0.51$
Carotenoid (mg/kg)	$1.01^{a} \pm 0.05$	$0.15^{b} \pm 0.02$	$0.14^{b} \pm 0.02$

Different letters (a. b. etc.) in each line indicate statistically difference ($p < 0.05$). Data are means of triplicates. ±: Standard Deviation (n=3).

Chlorophyll is desired to be removed from the oils as much as possible, as it transforms into pheophytin, which gives a dark color to the oils, and supports oxidation with the effect of temperature [102, 103]. Chlorophyll destruction was found to be 87.93% and 95.24 in IBPO and MWBPO, respectively. According to these findings microwave was found to be very successful removing chlorophyll from NPO. On the other hand, the loss of carotenoid content was found as 85% in both IBPO and MWBPO. Giuliani et al. [116] was presented that chlorophyll content of different varieties of olive oils obtained from different countries is in a wide range between 2.42 and 64.10 mg/kg. Cho and Lee [115] were reported that the chlorophyll content was found between 5.66-8.48 mg/kg; 1.63-2.67 mg/kg and 1.03-2.66 mg/kg for extra virgin olive oil, pure olive oil and refined pomace oil, respectively. They also were expressed that carotenoid content was between 6.48-10.82 mg/kg; 1.20-2.50 mg/kg and 1.27-2.52 mg/kg in the same oils.

4.3.2.3 Effects of Bleaching Processes on Oxidation Parameters and FFA

Table 4.13 summarizes the oxidation parameters and free acidity content in MWBPO and IBPO compared to NPO. There was a decrease in the PV of the oil in both bleaching methods. However, there was no significantly difference in the PV between MWBPO and IBPO. Besides the rate of decrease in peroxide was between 85-91% in both methods. Jahouach et al. [117] were expressed that the PV of NPO was found to be 6.93 mmol/kg (13.86 meq/kg).

After the bleaching process the peroxide values were found as 6.50 (13.00 meq/kg), 6.80 (13.60 meq/kg) 5.54 (11.08 meq/kg) and 6.60 (13.20 meq/kg) mmol/kg by using 2% different kind of clay (actisyl, tonsil, T1 and T2) for 45 min at 85-90 °C. According to this study the highest decrease rate in PV was obtained as 20% by using T1 type clay. On the other hand the highest decrease in PV could be obtained with the higher clay amount and at the lowest time in MWB. Bouaziz et al. [118] were stated that the peroxide values were found to be 3.33 and 2.68 meq/kg in refined olive and refined pomace oil, respectively. Bulut and Yılmaz [119] were reported that the PV of refined pomace oil was found as 3.49 meq/kg.

Table 4.13 Oxidation parameters and FFA soap in bleached pomace oils

Parameters	Before Bleaching	Industrial Bleaching	Microwave-Assisted Bleaching
Peroxide (meq/kg)	$19.66^{a} \pm 0.83$	$2.61^{b} \pm 0.30$	$1.76^{b} \pm 0.29$
P-anisidine	$11.80^{a} \pm 0.22$	$6.35^{b} \pm 0.09$	$30.87^{c} \pm 0.04$
Totox	$51.13^{a} \pm 1.45$	$11.57^{b} \pm 0.15$	$34.23^{c} \pm 0.33$
FFA (%)	$0.09^{a} \pm 0.01$	$0.13^{a} \pm 0.01$	$0.29^{b} \pm 0.01$
K_{232}	$5.21^{a} \pm 0.03$	$3.83^{b} \pm 0.22$	$4.27^{c} \pm 0.14$
K_{270}	$1.19^{a} \pm 0.07$	$1.36^{b} \pm 0.02$	$2.97^{c} \pm 0.01$

Different letters (a. b. etc.) in each line indicate statistically difference ($p < 0.05$). Data are means of triplicates. ±: Standard Deviation (n=3).

Ortega-Garcia et al. [109] reported that during bleaching, the amount of clay should be in the range of 1-1.5% to reduce the PV near zero. Silva et al. [107] expressed that

the PV in palm oil was reduced to near zero by using more than 1% of the acid activated clay. Despite the use of much higher clay than the amount of required for bleaching other vegetable oils, the PV of PO in both methods could not be brought to a value close to zero. Because crude pomace oil has generally higher PV compared with other vegetable oils. It has been reported in the literature that peroxides turn into secondary oxidation products during bleaching [106, 120]. AV increased rapidly in MWBPO compared with NPO. Although the PV of MWBPO was found the same statistically with that of IBPO, the AV of MWBPO was higher than that of IBPO. Despite the short duration in MWB, this may be due to the fact that vacuum is not applied (under open conditions to the atmosphere) and higher bleaching temperature. Related with this, totox value was also found higher in MWBPO than IBPO.

On the other hand, K_{232} value decreased in both methods compared with NPO. That of MWBPO was slightly higher than IBPO. Besides value increased in both methods. Similarly, K_{270} value of MWBPO was higher than IBPO. However, great deals of oxidation products are removed from the oil during deodorization that is emphasized in the literature [11, 37, 99]. This difference is not at a level that will adversely affect oil quality. K_{232} and K_{270} values in NPO were found as 4.94 and 0.34 by Jahouach et al. [117], respectively. The minimum K_{232} and K_{270} values were reported as 4.40 and 3.52 at the end of the conventional bleaching of PO by using 2% T1 clay for 45 min at 85-90 °C. These values are higher than MWBPO. These differences may be due to initial contents of impurities of NPO and the use of two times higher amount of clay in MWBPO.

FFA is the most important determinant of the suitability and edibility of oils. Increase in FFA adversely affects the taste, odor and oxidative stability of oils [105]. The increase in FFA compared to non-bleached oil is interpreted as the result of the leakage of trace amounts of acid in the acid activated clay into the oil [101]. Therefore, decreasing the amount of acid activated clay can prevent the increase of FFA content. While FFA increased in MWBPO, there was no significant change in acidity in IBPO compared to NPO. This is probably due to the reduced amount of clay and the short bleaching time. In addition, PO could also contain some water just after the neutralization process. Therefore, it is dried under vacuum to remove water in refinery industry prior to bleaching. However, drying process was not applied in

this study before bleaching, so residual water in NPO could be a possible reason of increased FFA.

4.3.2.4 The Effects of Bleaching Processes on sterol composition and benzo[a]pyrene content

Sterol composition and benzo[a]pyrene content of MWBO and IBPO are shown in Table 4.14.

Table 4.14 Sterol composition and BaP content for bleached pomace oils

Parameters	Before Bleaching	Industrial Bleaching	Microwave Assisted Bleaching
Sterol composition			
Cholesterol	$0.35^{a} \pm 0.02$	$0.30^{b} \pm 0.01$	$0.35^{a} \pm 0.02$
Brassikasterol	$0.09^{a} \pm 0.02$	$0.05^{b} \pm 0.01$	$0.06^{b} \pm 0.01$
24-methylene-	$0.03^{a} \pm 0.01$	$0.10^{b} \pm 0.01$	$0,06^{c} \pm 0.01$
Campesterol	$3.19^{a} \pm 0.23$	$4.82^{b} \pm 0.35$	$3.18^{a} \pm 0.23$
Campestanol	$0.31^{a} \pm 0.02$	$0.22^{b} \pm 0.01$	$0.31^{a} \pm 0.02$
Stigmasterol	$1.34^{a} \pm 0.01$	$3.62^{b} \pm 0.28$	$1.29^{a} \pm 0.10$
Δ-7-campesterol	$0.24^{a} \pm 0.02$	$0.40^{a} \pm 0.15$	$0.23^{a} \pm 0.02$
ß-sitosterol	$93.43^{a} \pm 0.56$	$85.17^{b} \pm 0.51$	$93.50^{a} \pm 0.51$
Δ7-stigmastenol	$0.52^{a} \pm 0.04$	$3.54^{b} \pm 0.26$	$0.58^{a} \pm 0.05$
Δ7-avenasterol	$0.50^{a} \pm 0.04$	$1.78^{b} \pm 0.13$	$0.43^{a} \pm 0.04$
Erythrodiol + uvaol	$30.58^{a} \pm 3.47$	$21.39^{b} \pm 2.31$	$25.39^{b} \pm 0.01$
Total sterols (ppm)	$2094^{a} \pm 347.63$	$2132^{a} \pm 353.93$	$1837^{a} \pm 304.88$
Benzo[a]pyrene (ppb)	$7.9^{a} \pm 0.36$	< 2	$3.2^{b} \pm 0.15$
Total PAHs (ppb)	$42.1^{a} \pm 7.37$	< 10	$12.2^{b} \pm 0.31$

Different letters (a. b. etc.) in each line indicate statistically difference ($p < 0.05$). Data are means of triplicates. ±: Standard Deviation (n=3).

PO contains functional components at least as much as extra virgin olive oil. Therefore, in recent years there has been increasing demand in PO [30]. Sterols and terpenic alcohols (erythrodiol and uvaol) are some of them. The major sterol components in PO are reported to be β-sitosterol and campesterol [121-123]. Agreement with these findings, the dominant sterol components in NPO and bleached PO were found as β-sitosterol and campesterol in this study. Except for

brassikasterol and 24-methylene- cholesterol, others were found statistically the same compared with NPO and MWBPO. Despite no statistical difference was observed between unbleached PO, MWBPO and IBPO when compared in terms of total sterol content, sterol composition in IBPO was found different compared with NPO and MWBPO. Boskou [124] reported that total sterol content was more or equal to 2500 mg/kg in PO. β -sitosterol and campesterol were found also as 93% and 5% of the total sterol fraction, respectively. Their findings are very close to those found in this study. Yorulmaz [122] expressed that decreasing in sterol components were determined at the bleaching step for brassicasterol, campesterol, stigmasterol and β-sitosterol. Especially reduction of β-sitosterol in MWB was found lower than conventional bleaching. Antonopoulos et al. [121] stated that process conditions and additives together with high vapor pressure and high vacuum had an impact to sterol removal. Therefore, higher reduction of β-sitosterol in conventional bleaching may be due to applied vacuum and using of higher amount of activated carbon. This might have caused an increase or decrease in other sterol components.

Erythrodiol and uvaol are part of the unsaponifiable fraction of pomace oil. They are usually determined with sterol fractions. And they also have antioxidant effect on the microsomal membranes of rat liver with positive effects on the inflammatory process or in the prevention and treatment of brain tumors and other cancers [125-127]. Reina et al. [128] reported that the level of erythrodiol and uvaol can be used to determine the presence of pomace oil in virgin oil. These components decreased with both bleaching methods. However, there was no difference between MWBPO and IBPO, statistically.

Drira et al. [123] expressed that erythrodiol and uvaol content increased at each refining processes. On the other hand, Sánchez-Gutiérrez et al. [129] reported that percentage of unsaponifiable matter decreased with refining. These difference results from the partial decreasing on the amount of total sterols. Besides these differences may also be due to the removal of varying quantities of squalene in each study.

Although this study did not focus on removing BaP content in PO, it was reduced approximately 60% in a shorter time and using the lower amount of clay and activated carbon by MWB compared with IBPO. On the other hand, the reduction of total PAHs content was found as 71%. The BaP content and total PAHs in MWBPO

were found slightly higher than the upper limit (2 µg/kg, 10 µg/kg for BaP and PAHs, respectively.) of European Union (EU) legislation (EU No 835/2011) for oils and fats intended for direct human consumption.

Kiralan et al. [130] reported that the reduction rate of PAHs was 33% when using 3% bleaching earth during bleaching process. But they expressed that the percent decrease was enhanced to 86%, 87% and 91% by using 0.3, 0.6 and 0.9% activated carbon, respectively. In another study, Kiralan et al. [131] emphasized that 75% reduction of total PAHs content was found in olive pomace oil with pre-microwave application. Their study highlighted the possibility of an alternative innovative strategy to apply in a process to suggest a simple solution for a significant industrial problem. Therefore, our study may be innovative strategy both reducing PAHs content and bleaching process.

CHAPTER V

CONCLUSION AND RECOMMENDATION

In this thesis, it was aimed to perform microwave-assisted bleaching of sunflower and olive pomace oils. Microwave was used for the first time for bleaching in this study. A strategy consisting of three steps was proposed to reach the better microwave-assisted bleaching process. In the first step, important bleaching parameters were determined and optimized for SO and PO. Optimization of bleaching factors (microwave power, mixing time, and amount of clay /activated carbon) was performed by RSM to enhance the bleaching quality. The results indicate that all parameters were important for MWB. Optimum bleaching conditions for SO were found as 0.4% clay, 80 W microwave power and 8 min mixing time to obtain minimum totox and chroma values (23.31 and 37.66, respectively) and maximum hue angle value (96.91). On the other hand, bleaching conditions for PO were found as clay-activated carbon amount of 4.15-0.5 %, 102 W microwave power and 20.07 min to get maximum hue angle and chroma values (86.91 and 68.06), respectively.

Second step was comparison study of MWB with IB. At optimum conditions of MWBSO, the amount of clay and bleaching time were decreased by 50% and 73%, respectively, relative to IBSO. On the other hand, there was a slight decrease in the clay amount; the processing time and the amount of activated carbon were significantly reduced in MWBPO compared to IB. In that respect the amount of clay, bleaching time and amount of activated carbon were decreased by 17%, 83% and 50%, respectively, under the optimum MWBPO conditions, relative to IBPO.

Although the tocopherol and sterol compositions of SO bleached by both methods were found to be similar, the totox value of microwave bleached oil was found slightly higher than the industrially bleached one. Chlorophyll and carotenoid content were also decreased in microwave bleaching for SO. Free fatty acid content of the oil

decreased in the industrial bleaching, but it did not change in MWBSO. Removal of soap residues by microwave bleaching has been as successful as industrial bleaching.

Totox value was also found higher in MWBPO than IBPO. On the other hand K_{232} value decreased in both methods compared with NPO. K_{232} value of MWBPO was slightly higher than IBPO. Besides K_{270} value increased in both methods. Similarly, K_{270} value of MWBPO was higher than that of IBPO. Chlorophyll and carotenoid content were also decreased in microwave bleaching. Free fatty acid content of the PO did not change in the industrial bleaching, but it increased in MWB. Although sterol composition of NPO was not change with MWB except for brassikasterol and 24-methylene- cholesterol, IBPO was found to be different statistically compared with MWBPO. The BaP content and total PAHs in MWBPO were slightly higher than the upper limit of European Union legislation for oils and fats intended for direct human consumption. However, the use of higher amount of activated carbon could be useful to decrease the content of PAH into acceptable limits of regulations.

Third step includes examining the possibility of the repetitive use of bleaching clay because its repeated use will reduce the production cost of the oil as well as the environmental pollution. This step was only carried out in the clay remaining after bleaching of SO because NPO has a very dark color. The results presented that the bleaching capacity of the clay used in the microwave bleaching began to decrease in the second use.

The results of this thesis indicated that, promising bleaching quality was obtained by MWB when compared to the conventional method. So, it can be concluded that microwave bleaching could be an alternative and advantageous method for the bleaching of vegetable oils. This method should be experimented on the different kinds of vegetable oils which have dark color by using variable clays. Further MWB studies also suggested to be carried out to see the performance of the technique at higher capacities on pilot scale.

REFERENCES

[1] Mahlia, T. M. I., Ismail, N., Hossain, N., Silitonga, A. S. (2019). Palm oil and its wastes as bioenergy sources: a comprehensive review. *Environmental Science and Pollution Research*. ***26*(15)**, 14849-14866.

[2] Ostfeld, R., Howarth, D., Reiner, D., Krasny, P. (2019). Peeling back the label exploring sustainable palm oil ecolabelling and consumption in the United Kingdom. *Environmental Research Letters*. **14(1)**, 014001.

[3] Hidalgo, F.J., Zamora, R. (2006). Peptides and proteins in edible oils: Stability, allergenicity, and new processing trends. *Trends in Food Science and Technology*. **17(2)**, 56-63.

[4] Cheng, W.W., Liu, G.Q., Wang, L.Q., Liu, Z.S. (2017). Glycidyl fatty acid esters in refined edible oils: a review on formation, occurrence, analysis, and elimination methods. *Comprehensive Reviews in Food Science and Food Safety*. **16(2)**, 263-281.

[5] Gupta, M., Leong, E. W. W. (2008). *Microwaves and metals*. John Wiley and Sons: New York.

[6] Vadivambal, R., Jayas, D.S. (2010). Non-uniform temperature distribution during microwave heating of food materials – a review. *Food and Bioprocess Technology*. **3,** 161-171.

[7] Yang, D., Wu, G., Li, P., Qi, X., Zhang, H., Wang, X., Jin, Q. (2020). Effect of microwave heating and vacuum oven drying of potato strips on oil uptake during deep-fat frying. *Food Research International*. **137**, 109338.

[8] Boukerroui, A., Ouali, M. S. (2002). Edible oil bleaching with a bentonite activated by micro wave irradiation. *In Annales de Chimie Science des Materiaux.***27(4),**73-81.

[9] Foletto, E. L., Paz, D. S., Gündel, A. (2013). Acid-activation assisted by microwave of a Brazilian bentonite and its activity in the bleaching of soybean oil. *Applied Clay Science*. **83**, 63-67.

[10] Yanık, D. K. (2017). Alternative to traditional olive pomace oil extraction systems: Microwave-assisted solvent extraction of oil from wet olive pomace. *LWT-Food Science and Technology.* **77**, 45-51.

[11] Škevin, D., Domijan, T., Kraljić, K., Gajdoš Kljusurić, J., Neđeral, S., Obranović, M. (2012). Optimization of bleaching parameters for soybean oil. *Food Technology and Biotechnology*. **50(2)**, 199-207.

[12] Salawudeen, T. O., Arinkoola, A. O., Jimoh, M. O., Akinwande, B. A. (2014). Clay characterization and optimisation of bleaching parameters for palm kernel oil using alkaline activated clays. *Journal of Minerals and Materials Characterization and Engineering*. **2(6)**, 586.

[13] Chew, S. C., Tan, C. P., Nyam, K. L. (2017). Optimization of bleaching parameters in refining process of kenaf seed oil with a central composite design model. *Journal of Food Science*. ***82*****(7),** 1622-1630.

[14] Mustafa, A. M., Abusabah, E. K. (2019). Using of Activated Jurdiga for Bleaching of Sunflower Edible Oils. *Gezira Journal of Engineering and Applied Sciences*. **13(2)**.

[15] Boroujeni, L., Ghavami, M., Piravi Vanak, Z., Ghasemi Pirbalouti, A. (2020). Optimization of sunflower oil bleaching parameters: using Response Surface Methodology (RSM). *Food Science and Technology*. **40**, 322-330.

[16] Su, D., Xiao, T., Gu, D., Cao, Y., Jin, Y., Zhang, W., Wu, T. (2013). Ultrasonic bleaching of rapeseed oil: effects of bleaching conditions and underlying mechanisms. *Journal of Food Engineering*. **117**, 8-13.

[17] Abedi, E., Sahari, M. A., Barzegar, M., Azizi, M. H. (2016). Designing of high voltage electric field for soybean and sunflower oil bleaching. *Innovative Food Science & Emerging Technologies*. **36**, 173-180.

[18] Icyer, N. C., Durak, M. Z. (2018). Ultrasound-assisted bleaching of canola oil: Improve the bleaching process by central composite design. *LWT-Food Science and Technology*. **97**, 640-647.

[19] Savva, S. C., Kafatos, A. (2016). *Vegetable oils: Dietary importance*. Academic Press: Oxford.

[20] https://chem.libretexts.org/Bookshelves/Introductory_Chemistry/The_Basics_of_GOB_Chemistry_(Ball_et_al.)/17%3A_Lipids/17.02%3A_Fats_and_Oils, 15.05.2021.

[21] Velasco, L., Ruiz-Méndez, M. V. (2015). *Sunflower oil minor constituents. In Sunflower*. AOCS Press: USA.

[22] Sanchez-Muniz, F. J., Bastida, S. and Benedí, J. (2016). *Sunflower Oil. In Encyclopedia of Food and Health*. Elsevier: Oxford.

[23] Romanić, R. (2020). *Cold pressed sunflower (Helianthus annuus L.) oil. In Cold Pressed Oils*. Academic Press: London.

[24] Salas, J. J., Bootello, M. A., Garcés, R. (2015). *Food uses of sunflower oils. In Sunflower*. AOCS press: USA.

[25] Göğüş, F., Bozkurt, H. (2009). *Zeytinyağı*. Eflatun Yayınevi: Ankara.

[26] Sánchez-Moral, P., Ruiz-Méndez, M. (2006). Production of pomace olive oil. *Grasas y Aceties.* **57(1)**, 47-55.

[27] Skaltsounis, A. L., Argyropoulou, A., Aligiannis, N., Xynos, N. (2015). *Recovery of high added value compounds from olive tree products and olive processing byproducts. In Olive and Olive Oil Bioactive Constituents*. AOCS Press: USA.

[28] Romero-García, J. M., Niño, L., Martínez-Patiño, C., Álvarez, C., Castro, E., Negro, M. J. (2014). Biorefinery Based on Olive Biomass. *State of the Art and Future Trends. Bioresour. Technology*. **159**, 421–432.

[29] Guinda, Á. (2006). Use of Solid Residue from the Olive Industry. *Grasas y Aceites*. **57**, 107-115.

[30] Pérez-Camino, M. C., Cert, A. (1999). Quantitative determination of hydroxy pentacyclic triterpene acids in vegetable oils. *Journal of Agricultural and Food Chemistry*. ***47*(4)**, 1558-1562.

[31] Vlyssides, A. G., Loizides, M., Karlis, P. K. (2004). Integrated strategic approach for reusing olive oil extraction by-products. *Journal of Cleaner production*. **12(6)**, 603-611.

[32] Ghazani, S.M., Marangoni, A.G., (2013). Minor components in canola oil and effects of refining on these constituents: A review. *Journal of the American Oil Chemists' Society*. **90**, 923-932.

[33] Demirkol, E. (2015). Rafine yağ tesislerinde deodorize kolonu seviye ölçümü. https://www.gida2000.com/rafine-yag-tesislerinde-deodorizekolonu-seviye-olcumu.html, 27.04.2021.

[34] Tandy, D. C., McPherson, W. J. (1984). Physical refining of edible oil. *Journal of the American Oil Chemists Society*. ***61*(7)**, 1253-1258.

[35] Gupta, M. (2017). *Practical guide to vegetable oil processing*. Elsevier: Oxford.

[36] Palm Oil Knowledge. Oil Purification Process–An Introduction. https://oilpalmblog.wordpress.com/2015/08/01/oil-purification-process-an-introduction, 08.06.2021.

[37] Zschau, W. (2001). Bleaching of edible fats and oils. *European Journal of Lipid Science and Technology*. **103**, 505-551.

[38] Sharma, R., Saini, P. (2016). *Graphene-based composites and hybrids for water purification applications. In Diamond and Carbon Composites and Nanocomposites*. IntechOpen: London.

[39] Topkafa, M., Ayyildiz, H. F., Arslan, F. N., Kucukkolbasi, S., Durmaz, F., Sen, S., Kara, H. (2013). Role of different bleaching earths for sunflower oil

in a pilot plant bleaching system. *Polish Journal of Food and Nutrition Sciences*. **63**, 147-154.

[40] Aksu, Z. (2002). Determination of the equilibrium, kinetic and thermodynamic parameters of the batch biosorption of nickel (II) ions onto Chlorella vulgaris. *Process biochemistry*. **38(1)**, 89-99.

[41] Seçilmiş, Ş. S., Yanik, D. K., Fadiloğlu, S., & Göğüş, F. (2021). A Comparative Study on Performance of Industrial and Microwave Techniques for Sunflower Oil Bleaching Process. *Food Chemistry*. **365.**

[42] Omar, S., Girgis, B., Taha, F. (2003). Carbonaceous materials from seed hulls for bleaching of vegetable oils. *Food Research International*. **36(1)**, 11-17.

[43] Aachary, A., A., Liang, J., Hydamaka, A., Eskin, N. M., Thiyam-Holländer, U. (2016). A new ultrasound-assisted bleaching technique for impacting chlorophyll content of cold-pressed hempseed oil. *LWT-Food Science and Technology*. **72**, 439-446.

[44] Chetima, A., Wahabou, A., Zomegni, G., Ntieche Rahman, A., Bup Nde, D. (2018). Bleaching of neutral cotton seed oil using organic activated carbon in a batch system: Kinetics and adsorption isotherms. *Processes*. **6**, 22.

[45] Febrianto, N.A., Yang, T.A. (2011). Producing high quality edible oil by using eco-friendly technology: A review. *Advance Journal of Food Science Technology*. **3**, 317-326.

[46] Hymore, F.K. (1996). Effects of some additives on the performance of acid activated clays in the bleaching of palm oil. *Applied Clay Science*. **10**, 379 385.

[47] Taylor, D. R., Ungermann, C. B. (1991). Process for making acid activated bleaching earth using high susceptibility source clay and novel bleaching earth product. https://patents.google.com/patent/US5008226A/en, 22.05.2021.

[48] Brooks, D. D., Berbesi, R., Hodgson, A. S. (2019). Optimization of bleaching process. AOCS lipid library: https://lipidlibrary.aocs.org/edible-oilprocessing /optimization-of-bleaching-process, 22.05.2021.

[49] Mustapha, S. I., Mohammed, A. A., Zakari, A. Y., Mohammed, H. A. (2013). Performance evaluation of local clays from northern Nigeria for the refining of palm oil. *Journal of Chemical Engineering and Materials Science*. **4(5)**, 58-66.

[50] Singleton, W. A., McMichael, C. E. (1955). Continuous countercurrent vacuum bleaching. *Journal of the American Oil Chemists' Society*. **32(1)**, 1-6.

[51] Usman, M. A., Ekwueme, V. I., Alaje, T. O., & Mohammed, A. O. (2012). Characterization, acid activation, and bleaching performance of Ibeshe clay, Lagos, Nigeria. *International Scholarly Research Notices*. **2012**.

[52] Wang, Z. N., Hou, X. F., Sun, J., Li, M., Chen, Z. Y., and Gao, Z. Z. (2018). Comparison of ultrasound-assisted ionic liquid and alkaline pretreatment of Eucalyptus for enhancing enzymatic saccharification. *Bioresource Technology*. **254**, 145-150.

[53] Jahouach-Rabai, W., Trabelsi, M., Van Hoed, V., Adams, A., Verhe, R., De Kimpe, N., Frikha, M. H. (2008). Influence of bleaching by ultrasound on fatty acids and minor compounds of olive oil. Qualitative and quantitative analysis of volatile compounds (by SPME coupled to GC/MS). *Ultrasonics sonochemistry*. **15,** 590-597.

[54] Abedi, E., Sahari, M. A., Barzegar, M., Azizi, M. H. (2015). Optimisation of soya bean oil bleaching by ultrasonic processing and investigate the physico-chemical properties of bleached soya bean oil. *International Journal of Food Science and Technology*. **50**, 857-863.

[55] Abbasi, R., Gharachorloo, M., Ghavami, M., Mahmood-Fashandi, H., Mousavi Khaneghah, A. (2017). The Effect of ultrasonic waves in bleaching of olive and sunflower oils and comparison with conventional bleaching. *Journal of Food Processing and Preservation*. **41**, e13079.

[56] Asgari, S., Sahari, M. A., Barzegar, M. (2017). Practical modeling and optimization of ultrasound assisted bleaching of olive oil using hybrid artificial neural network-genetic algorithm technique. *Computers and Electronics in Agriculture*. **140**, 422-432.

[57] Dadfar, T., Sahari, M. A., Barzegar, M. (2020). Bleaching of Olive Oil by Membrane Filtration. *European Journal of Lipid Science and Technology*. ***122*(4)**, 1900151.

[58] Torrealba-Meléndez, R., Sosa-Morales, M.E., Olvera-Cervantes, J.L., Corona-Chávez, A. (2015). Dielectric properties of cereals at frequencies useful for processes with microwave heating. *Journal of Food Science and Technology*. **52,** 8403-8409.

[59] Zhang, M., Tang, J., Mujumdar, A.S., Wang, S. (2006). Trends in microwave-related drying of fruits and vegetables. *Trends Food Science Technology*. **17**, 524-534.

[60] Fito, P., Chiralt, A., Martı, M. E. (2004). *Current state of microwave applications to food processing. In Novel food processing technologies.* CRC Press: Florida.

[61] Camel, V. (2001). Recent extraction techniques for solid matrices supercritical fluid extraction, pressurized fluid extraction and microwave-assisted extraction: their potential and pitfalls. *Analyst*. **126(7)**, 1182-1193.

[62] Dibben, D. (2001). *Electromagnetics: Fundamental Aspects and Numerical Modeling. Handbook of microwave technology for food application*. Marcel Dekker: New York.

[63] Regier, M., Knoerzer, K., Schuber, H. (2017). *Introducing microwave-assisted processing of food: fundamentals of the technology. The Microwave Processing of Foods.* Woodhead publishing: Cambridge.

[64] Renshaw, R. C., Dimitrakis, G. A., Robinson, J. P., Kingman, S. W. (2019). The relationship of dielectric response and water activity in food. *Journal of Food Engineering*. **244**, 80-90.

[65] Kutbay, I., Kuşkonmaz, N. (2004). Mikrodalga Isıtmanın Seramik Üretiminde Kullanımı. Metalurji, *TMMOB Metalurji Mühendisleri Odası Yayın Organı*. **137**, 52-56.

[66] Zhang, F., Zhou, T., Liu, Y., Leng, J. (2015). Microwave synthesis and actuation of shape memory polycaprolactone foams with high speed. *Scientific- reports*. **5(1)**, 1-12.

[67] Thuery, J. (1992). *Microwaves and matter. Microwaves: industrial, scientific, and medical applications*. Artech House: London.

[68] Kaufmann, B., Christen, P. (2002). Recent extraction techniques for natural products: microwave-assisted extraction and pressurized solvent extraction. *Phytochemical analysis*. **13**, 105-113.

[69] Chen, Y., Xie, M. Y., Gong, X. F. (2007). Microwave-assisted extraction used for the isolation of total triterpenoid saponins from Ganoderma atrum. *Journal of Food Engineering*. **81**, 162-170.

[70] Maeng, J.H., Muhammad Shahbaz, H., Ameer, K., Jo, Y., Kwon, J.H. (2017). Optimization of microwave assisted extraction of bioactive compounds from coriolus versicolor mushroom using response surface methodology. *Journal of Food Process Engineering*, **40(2)**, e12421.

[71] Datta, A. K., Davidson, P. M. (2000). Microwave and radio frequency processing. *Journal of Food Science.* **65**, 32–41.

[72] Chandrasekaran, S., Ramanathan, S., Basak, T. (2013). Microwave food processing: A review. *Food Research International*. **52**, 243-261.

[73] Gjorgjevich, M.P., Velevska. J., Najdoski, M. (2012). Effect of microwave radiation on dielectric behavior of two vegetable oils. *Journal of Physical Sciences*. **2**, 427-433.

[74] Schiffmann, R.F. (2014). *Microwave and dielectric drying*. CRC press: New York.

[75] Meda, V., Orsat, V., Raghavan, V. (2017). *Microwave heating and the dielectric properties of foods. The microwave processing of foods.* Woodhead Publishing: Cambridge.

[76] Dostalova, J. A. N. A., Hanzlik, P. A. V. E. L., Reblova, Z. U. Z. A. N. A., Pokorny, J. (2005). Oxidative changes of vegetable oils during microwave heating. *Czech Journal of Food Sciences*. **23**, 230.

[77] Malheiro, R., Oliveira, I., Vilas-Boas, M., Falcão, S., Bento, A., Pereira, J. A. (2009). Effect of microwave heating with different exposure times on physical and chemical parameters of olive oil. *Food and Chemical Toxicology*. **47**, 92.

[78] Aydınkaptan, E., Mazi, B. G., Barutçu Mazi, I. (2016). Microwave heating of sunflower oil at frying temperatures: Effect of power levels on physicochemical properties. *Journal of Food Process Engineering*. **40(2)**, e12402.

[79] AOCS Official Method Cd 8-53. (2003). *Peroxides in fats and oils. American Oil Chemists Society*. Champaign: Illinois.

[80] AOCS Official Method Cd 18-90. (2011). *P-Anisidine value. American Oil Chemists Society*. Champaign: Illinois.

[81] AOCS Official Method Ch 5-91. (2006). *Specific Extinction of Oils and Fats, Ultraviolet Absorption. American Oil Chemists Society*. Champaign: Illinois.

[82] AOCS Official Method Ca 5a-40. (2017). *Free Fatty Acids in Crude and Refined Fats and Oils. American Oil Chemists Society*, Champaign: Illinois.

[83] AOCS Official Method Cc 17-95. (2017). *Soap in Oil Titrimetric Method. American Oil Chemists Society*, Champaign: Illinois.

[84] Ajemba, R.O., Onukwuli, O.D. (2013). Adsorptive removal of colour pigment from palm oil using acid activated nteje clay. Kinetics, equilibrium and thermodynamics. *Physicochemical Problems of Mineral Processing*. **49**, 369–381.

[85] Minguez-Mosquera, M. I., Rejano-Navarro, L., Gandul-Rojas, B., SanchezGomez, A. H., Garrido-Fernandez, J. (1991). Color-pigment correlation in virgin olive oil. *Journal of the American Oil Chemists Society*. **68**, 332-336.

[86] Allalout, A., Krichène, D., Methenni, K., Taamalli, A., Oueslati, I., Daoud, D., Zarrouk, M. (2009). Characterization of virgin olive oil from super intensive Spanish and Greek varieties grown in northern Tunisia. *Scientia horticulturae*. **120(1)**, 77-83.

[87] EN ISO 12228-1. (2014). Determination of individual and total sterols contents - Gas chromatographic method. Part 1: Animal and vegetable fats and oils. https://www.iso.org/standard/60248.html, 05.02.2020.

[88] IOC. COI/T.20/Doc. No. 30/Rev. 1. (2015). Method for the determination of the composition and content of sterols and triterpene dialcohols by capillary column gas chromatography. https://www.internationaloliveoil.org/what-we-do/chemistry-standardisation-unit/standards-and-methods, 05.02.2020.

[89] AOCS Official Method Ce 8-89. (2017). *Tocopherols and Tocotrienols in Vegetable Oils and Fats by HPLC. American Oil Chemists Society*, Champaign: Illinois.

[90] Moret, S., Conte, L. S. (2002). A rapid method for polycyclic aromatic hydrocarbon determination in vegetable oils. *Journal of Separation Science*. **25(1-2)**, 96-100.

[91] Foletto, E. L., Alves, C. C. A., Sganzerla, L. R., Porto, L. M. (2002). Regeneration and utilization of spent bleaching clay. *Latin American Applied Research*. **32**, 205-208.

[92] Nursulihatimarsyila, A. W., Cheah, K. Y., Chuah, T. G., Siew, W. L., Choong, T. S. Y. (2010). Deoiling and regeneration efficiencies of spent bleaching clay. *American Journal of Applied Sciences*. **7**, 434-437.

[93] Islam, M.A., Tan, Y.L., Islam, M.A., Romić, M., Hameed, B.H. (2018). Chitosan–bleaching earth clay composite as an efficient adsorbent for carbon dioxide adsorption: Process optimization. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. **554**, 9-15.

[94] Gasemloo, S., Khosravi, M., Sohrabi, M. R., Dastmalchi, S., Gharbani, P. (2019). Response surface methodology (RSM) modeling to improve removal of Cr (VI) ions from tannery wastewater using sulfated carboxymethyl cellulose nanofilter. *Journal of Cleaner Production*. **208**, 736-742.

[95] Garcia-Moreno, P. J., Guadix, A., Gómez-Robledo, L., Melgosa, M., Guadix, E. M. (2013). Optimization of bleaching conditions for sardine oil. *Journal of Food Engineering*. **116**, 606-612.

[96] Marrakchi, F., Kriaa, K., Hadrich, B. Kechaou, N. (2015). Experimental investigation of processing parameters and effects of degumming, neutralization and bleaching on lampante virgin olive oil's quality. *Food and Bioproducts Processing*. **94**, 124-135.

[97] Zschau, W. (2000). *Bleaching. Introduction to fats and oils technology.* AOCS Press: USA.

[98] Bonveh, J.S., Torrent, M.S., Coll, F.V. (2001). A laboratory study of the bleaching process in Stigmasta-3,5-diene concentration in olive oils. *Journal of American Oil Chemistry's Society*. **78**, 305–310.

[99] Shahidi, F., Wanasundara, P.K.J.P.D., Wanasundara, U.N. (1997). Changes in edible fats and oils during processing. *Journal of Food Lipids*. **4**, 199–231.

[100] Thompson, L.H., Doraiswamy, L.K. (1999). Sonochemistry. Science and engineering. *Industrial & Engineering Chemistry Research*. **38(4)**, 1215-1249.

[101] Kreps, F., Vrbikova, L., Schmidt, S. (2014). Influence of industrial physical refining on tocopherol, chlorophyll and beta-carotene content in sunflower and rapeseed oil. *European Journal of Lipid Science and Technology*. **116**, 1572-1582.

[102] Strieder, M. M., Pinheiro, C. P., Borba, V. S., Pohndorf, R. S., Cadaval Jr, T. R., Pinto, L. A. (2017). Bleaching optimization and winterization step evaluation in the refinement of rice bran oil. *Separation and Purification Technology*. **175**, 72-78.

[103] Liang, J., Aachary, A. A., Hydamaka, A., Eskin, N. M., Eck, P., Thiyam-Holländer, U. (2018). Reduction of Chlorophyll in Cold-Pressed Hemp (Cannabis sativa) Seed Oil by Ultrasonic Bleaching and Enhancement of Oxidative Stability. *European Journal of Lipid Science and Technology.* **120**, 1700349.

[104] Gunstone, F.D. (2002). *Vegetable oils in food technology–composition, properties and uses*. Whiley-Blackwell Publishing: Oxford.

[105] Tuyen, C. K., Nguyen, M. H., Roach, P. D., Stathopoulos, C. E. (2014). Microencapsulation of Gac oil: Optimization of spray drying conditions using response surface methodology. *Powder Technology*. **264**, 298-309.

[106] Moigradean, D., Poiana, M.A., Gogoasa, I. (2012). Quality charac¬teristics and oxidative stability of coconut oil during stor¬age. *Journal of Agroalimentary Processes and Technologies*. **18**, 272–276.

[107] Silva, S. M., Sampaio, K. A., Ceriani, R., Verhé, R., Stevens, C., De Greyt, W., Meirelles, A. J. (2014). Effect of type of bleaching earth on the final color of refined palm oil. *LWT-Food Science and Technology*. **59**, 1258-1264.

[108] Alpaslan, M., Tepe, S., Simsek, O. (2001). Effect of refining processes on the total and individual tocopherol content in sunflower oil. *International Journal of Food Science and Technology.* **36**, 737-739.

[109] Ortega-García, J., Medina-Juarez, L. A., Gamez-Meza, N., Noriega-Rodriguez, J. A. (2005). Optimization of bleaching conditions for soybean oil using response surface methodology. *Food Science and Technology International*. **11**, 443-449.

[110] Shah, S. N., Mahesar, S. A., Sherazi, S. T. H., Panhwar, M. A., Nizamani, S. M., Kandhro, A. A. (2018). Influence of commercial refining on some quality attributes of sunflower oil. *Ukrainian Food Journal*. **7**, 234-243.

[111] Girgis, A. Y. (2005). Reuse of discarded deactivated bleaching earth in the bleaching of oils. *Grasas y Aceites*. **56**, 34-45.

[112] Broughton, R., Ruíz-Lopez, N., Hassall, K. L., Martínez-Force, E., Garcés, R., Salas, J. J., Beaudoin, F. (2018). New insights in the composition of wax and sterol esters in common and mutant sunflower oils revealed by ESI-MS/MS. *Food Chemistry*. **269**, 70-79.

[113] Javidipour, I., Erinç, H., Baştürk, A., Tekin, A. (2017). Oxidative changes in hazelnut, olive, soybean, and sunflower oils during microwave heating. *International Journal of Food Properties*. **20**, 1582-1592.

[114] Escolar, D., Haro, M. R., Ayuso, J. (2007). The color space of foods: Virgin olive oil. *Journal of Agricultural and Food Chemistry*, **55(6)**, 2085-2093.

[115] Cho, E. A., Lee, Y. S. (2014). A study on the classifying quality standard by comparison with physicochemical characteristics of virgin, pure, pomace olive oil. *The Korean Journal of Food and Nutrition*. **27**, 339-347.

[116] Giuliani, A., Cerretani, L., Cichelli, A. (2011). Chlorophylls in olive and in olive oil: chemistry and occurrences. *Critical Reviews in Food Science and Nutrition*. **51(7)**, 678-690.

[117] Jahouach, W., Essid, K., Trabelsi, M., & Frikha, M. H. (2006). Alteration of chemical composition and the oxidative stability of bleached Pomace olive oil on activated clays. *Journal of Agricultural and Food Chemistry*. **54(19)**, 7137-7143.

[118] Bouaziz, M., Feki, I., Ayadi, M., Jemai, H., & Sayadi, S. (2010). Stability of refined olive oil and olive-pomace oil added by phenolic compounds from olive leaves. *European Journal of Lipid Science and Technology*. **112(8)**, 894-905.

[119] Bulut, E., Yılmaz, E. (2010). Comparison of the frying stability of sunflower and refined olive pomace oils with/without adsorbent treatment. *Journal of the American Oil Chemists' Society*. **87(10)**, 1145-1153.

[120] Marina, A. M., Man, Y. C., & Amin, I. (2009). Virgin coconut oil: emerging functional food oil. *Trends in Food Science & Technology*. **20(10)**, 481-487.

[121] Antonopoulos, K., Valet, N., Spiratos, D., Siragakis, G. (2006). Olive oil and pomace olive oil processing. *Grasas y Aceites*. **57(1)**, 56-67.

[122] Yorulmaz, A. (2018). Influence of industrial refining on some characteristics of olive pomace oil. *Rivista Italiana delle Sostanze Grasse*. **95(3)**, 167-172.

[123] Drira, M., Jabeur, H., Marrakchi, F., & Bouaziz, M. (2018). Delta-7-stigmastenol: quantification and isomeric formation during chemical refining of olive pomace oil and optimization of the neutralization step. *European Food Research and Technology*. **244(12)**, 2231-2241.

[124] Boskou, D. (2009). *Other important minor constituents. Olive oil. Minorconstituents and health.* CRC Press: New York.

[125] Perona, J. S., Arcemis, C., Ruiz-Gutierrez, V., Catalá, A. (2005). Effect of dietary high-oleic-acid oils that are rich in antioxidants on microsomal lipid peroxidation in rats. *Journal of Agricultural and Food Chemistry*. **53(3)**, 730-735.

[126] Marquez-Martin, A., De La Puerta, R., Fernandez-Arche, A., Ruiz-Gutierrez, V., Yaqoob, P. (2006). Modulation of cytokine secretion by pentacyclic triterpenes from olive pomace oil in human mononuclear cells. *Cytokine*. **36(5-6)**, 211-217.

[127] Martín, R., Ibeas, E., Carvalho-Tavares, J., Hernández, M., Ruiz-Gutierrez, V., Nieto, M. L. (2009). Natural triterpenic diols promote apoptosis in astrocytoma cells through ROS-mediated mitochondrial depolarization and JNK activation. *PLoS One*. **4(6)**, e5975.

[128] Reina, R. J., White, K. D., Jahngen, E. G. (1997). Validated method for quantitation and identification of 4, 4-desmethylsterols and triterpene diols in plant oils by thin-layer chromatography–high resolution gas chromatography mass spectrometry. *Journal of AOAC International*. **80(6)**, 1272-1280.

[129] Sánchez-Gutiérrez, C. A., Ruiz-Méndez, M. V., Jiménez-Castellanos, M. R., Lucero, M. J. (2017). Influence of refining processes on content of bioactive compounds, rheology, and texture of olive pomace oil for use in topical formulations. *European Journal of Lipid Science and Technology*. **119(9)**, 1600408.

[130] Kiralan, S. S., Toptancı, İ., Tekin, A. (2019). Further evidence on the removal of polycyclic aromatic hydrocarbons (PAHs) during refining of olive pomace oil. *European Journal of Lipid Science and Technology*. **121(4)**, 1800381.

[131] Kiralan, S. S., Erdogdu, F., Tekin, A. (2017). Reducing polycyclic aromatic hydrocarbons (PAHs) formation in olive pomace oil using microwave pre-heating of olive pomace. *European Journal of Lipid Science and Technology*. **119(1)**, 1600241.

CURRICULUM VITAE

EDUCATION

Degree	Institution	Graduation year
Ph.D.	Gaziantep University, Food Engineering Department	2021
M.Sc.	Gaziantep University, Food Engineering Department	2013
B.Sc.	Atatürk University, Food Engineering Department	2003

ACADEMIC/WORK EXPERIENCES

Year	Place	Enrolment
2004-2005	Sof Süt ve Süt ürünleri Tic. Lim. Şti	Quality Control Engineer
2005-2010	Özkaradeniz Gıda Mad. Tic Aş	Quality and R&D Director
2010-2014	DC Danış. Müh. Tic. Ltd. Şti	Consultant for R&D Projects
2014- Present	Vocational School of Tech. Sci. of GAUN Department of Food Processing	Prelector

PROJECTS

2018-2019 A new method for the bleaching of vegetable oil: Determination of microwave conditions and optimization — TUBITAK-1002

PUBLICATIONS

Papers Published in International indexed Journals

Seçilmiş, Ş.S., Koçak Yanık, D., Fadıloğlu, S., Göğüş, F. (2022). Microwave-assisted Bleaching of Olive Pomace Oil: Optimization of Bleaching Parameters and Comparison with Conventionally Bleached Oil. "under review".

Seçilmiş, Ş.S., Koçak Yanık, D., Fadıloğlu, S., Göğüş, F. (2021). A novel bleaching approach: Microwave-assisted sunflower oil bleaching and optimization. Grasas Aceties, 72, e437. "in-print".

Seçilmiş, Ş.S., Yanik, D. K., Fadiloğlu, S., & Göğüş, F. (2021). A Comparative Study on Performance of Industrial and Microwave Techniques for Sunflower Oil Bleaching Process. Food Chemistry. 365.

Seçilmiş Ş.S, Kocak D., Gogus F. (2015). Processing of a novel powdered herbal coffee (Pistacia Terebinthus L. Fruits Coffee) and its Sensorial Properties. Journal of Food Science and Technology. 52(7).

Papers Published in National indexed Journals

Seçilmiş, Ş.S. (2020). Yağ Rafinasyonu Sırasında İnsan Beslenmesinde Önemli Fonksiyonel Bileşenlerde Değişimler ve Zararlı Rafinasyon Bileşenlerinin Oluşumu. Akademik Gıda, 18(3).

National Conference Papers Published in Proceedings

Ş. Selçuk Seçilmiş, Derya Koçak Yanık, Sibel Fadıloğlu Fahrettin Göğüş. (2019). Ayçiçek Yağının Ağartılmasında Yeni Bir Metot: Mikrodalga Uygulama Koşullarının Belirlenmesi ve Optimizasyonu. Yabited IV. Yağ Kongresi 18-19 Nisan, İstanbul. (Poster).

International Conference Papers Published in Proceedings

Fikret Nafi Çoksöyler, Şennur Özkaya, Can Çoksöyler, Berfin Elmas, **Ş. Selçuk Seçilmiş**, Sümeyye Kara, Fırat Kara. (2019). A newly developed TLC system for the quantitative determination of aflatoxins in foods. 3. International and 26. National Iranian Food Science and Technology Congress 17-19 September, Tehran-Iran.

PATENTS

Faydalı Model No: 2008 00566; Kuru Gıdalarda Ozonlama ile Mikrobiyal Dekontaminasyon için Gerekli Endüstriyel Sistemin Geliştirilmesi. (2008). Başvuru sahipleri: Vista Kuruyemiş Gıda San. Tic. Ltd. Şti. Buluş Sahibi: **Ş. Selçuk Seçilmiş**.

Patent Başvuru No:2017/0992; Nötralize Bitkisel Yağın Mikrodalga Yöntemi ile Ağartılması. Başvuru sahipleri: Gaziantep Üniversitesi. Buluş Sahipleri: Derya Koçak Yanık, Sibel Fadıloğlu, **Ş. Selçuk Seçilmiş**, Fahrettin Göğüş.