PRODUCTION AND CHARACTERIZATION OF MICROFLUIDIZED OLIVE POWDER

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

SİNEM ARĞÜN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
FOOD ENGINEERING

JANUARY 2022

Approval of the thesis:

THESIS TITLE

submitted by **SİNEM ARĞÜN** in partial fulfillment of the requirements for the degree of **Master of Science** i**n Food Engineering, Middle East Technical University** by,

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

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

Prof. Dr. Behiç Mert
Supervisor, **Food Engineering, METU** _____

Examining Committee Members:

Prof. Dr. Aziz Tekin
Food Engineering, Ankara University _____

Prof. Dr. Behiç Mert
Food Engineering, METU _____

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

Assoc. Prof. Dr. İlkay Şensoy
Food Engineering, METU _____

Assist. Prof. Dr. Leyla Nesrin Kahyaoğlu
Food Engineering, METU _____

Date: 26.01.2022

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

Name, Last name : Sinem Arğün

Signature :

ABSTRACT

PRODUCTION AND CHARACTERIZATION OF MICROFLUIDIZED OLIVE POWDER

Arğün, Sinem
Master of Science, Food Engineering
Supervisor: Prof. Dr. Behiç Mert

January 2022, 102 pages

Microfluidization has been gaining popularity as a size reduction process in recent years. Its application in food research has shown that reducing the particle size of food materials not only alters their structure but also develops their functional properties. Therefore, microfluidization technique was used in this study for olives to change the cell wall structure and decrease the size of oil droplets. The aim of this study is to develop a new value-added product *(an olive powder)* from olive with enhanced functionality and to characterize physical and stability properties of olive powder in water suspensions and to compare it with regular olive powders produced conventionally freeze drying. In the study, green, black and raw olive powders were produced with freeze drying following microfluidization at 1200 bar. Control samples were prepared without microfluidization. Peroxide values, antioxidant activity (DPPH assay), total phenolic content, lipolysis (free fatty acid content), color, moisture content and microscopic analysis (Scanning Electron Microscopy) were conducted for the powder samples. Stability measurements through *Turbi Scan* and rheological characterization were performed for the suspensions prepared from the powders at different concentrations. When the effect of microfluidization is investigated, it can be concluded that antioxidant activity was not affected

significantly for all olive powders while total phenolic content decreased for green and raw olive powders. Among all olive powders, raw olive gave the highest antioxidant activity and phenolic content. In addition, raw olive gave the best results in peroxide values and free acidity experiments. In the microscopic analysis of olive powders, SEM images revealed that microfluidization results in finer particles which are self-encapsulated by trapping the oil droplets inside of the olive. Stability analysis and rheological measurements of olive powder suspensions showed that microfluidization process is a good way to obtain more stable suspensions having higher water holding capacity. Herschel-Bulkley model was found for the flow behaviors of olive powder suspensions. Microfluidized olive powder suspensions had higher elastic (G') and viscous (G") moduli than unmicrofluidized ones because microfluidization produces more hydrophilic moieties and results in larger moduli values. To conclude, microfluidization technique helped to make the olive powder more stable compared to conventional olive powders.

Keywords: Olive Powder, Microfluidization, Freeze Drying, Oxidation, Stability

ÖZ

MİKROFLUDİZASYON YÖNTEMİ İLE ZEYTİN TOZU ÜRETİMİ VE KARAKTERİZASYONU

Arğün, Sinem
Yüksek Lisans, Gıda Mühendisliği
Tez Yöneticisi: Prof. Dr. Behiç Mert

Ocak 2022, 102 sayfa

Mikrofludizasyon, son yıllarda boyut küçültme işlemi olarak popülerlik kazanmaktadır. Gıda araştırmalarındaki uygulaması, gıda maddelerinin parçacık boyutunun küçültülmesinin sadece yapılarını değil aynı zamanda fonksiyonel özelliklerini de iyileştirdiğini göstermiştir. Bu nedenle bu çalışmada, zeytinlerin hücre duvar yapısını değiştirmek ve yağ damlacıklarının boyutunu azaltmak için mikrofludizasyon yöntemi kullanılmıştır. Bu çalışmanın amacı, zeytinden gelişmiş işlevselliği olan katma değeri yüksek bir ürün *(zeytin tozu)* geliştirmek, zeytin tozunun su süspansiyonlarındaki fiziksel ve stabilite özelliklerini karakterize etmek ve geleneksel olarak dondurarak kurutma ile üretilen normal zeytin tozları ile karşılaştırmaktır. Çalışmada 1200 bar'da mikrofludizasyonun ardından dondurarak kurutma ile yeşil, siyah ve ham zeytin tozları üretilmiştir. Kontrol numuneleri mikrofludizasyon olmadan hazırlanmıştır. Toz numuneler için Peroksit değeri tayini, antioksidan aktivite (DPPH testi), toplam fenolik içerik, lipoliz (serbest yağ asidi içeriği), renk, nem içeriği ve mikroskobik analiz (Taramalı Elektron Mikroskobu) yapılmıştır. Farklı konsantrasyonlardaki tozlardan hazırlanan süspansiyonlar için *Turbi Scan* ile stabilite ölçümleri ve reolojik karakterizasyon yapılmıştır. Mikrofludizasyonun etkisi incelendiğinde, antioksidan aktivitenin tüm zeytin tozları

için önemli ölçüde etkilenmediği, yeşil ve ham zeytin tozları için toplam fenolik içeriğin azaldığı görülmüştür. Tüm zeytin tozları arasında en yüksek antioksidan aktiviteyi ve fenolik içeriği ham zeytin vermiştir. Ayrıca peroksit değer ve toplam serbest asitlik deneylerinde de en iyi sonucu ham zeytin vermiştir. Zeytin tozlarının mikroskobik analizinde, SEM görüntüleri, mikrofludizasyonun, zeytinin içindeki yağ damlacıklarını yakalayıp kendi kendine kapsülleyerek daha ince partiküller oluşturduğunu ortaya koymuştur. Zeytin tozu süspansiyonlarının stabilite analizi ve reolojik ölçümleri, mikrofludizasyon işleminin, daha yüksek su tutma kapasitesine sahip daha kararlı süspansiyonlar elde etmek için iyi bir yol olduğunu göstermiştir. Zeytin tozu süspansiyonlarının akış davranışları için Herschel-Bulkley modeli bulunmuştur. Mikrofludize zeytin tozu süspansiyonları, mikrofludize olmayan zeytin tozu süspansiyonlarından daha yüksek elastik (G') ve viskoz (G'') modüllere sahip olduğu ortaya çıkmıştır çünkü mikrofludizasyon daha fazla hidrofilik kısımlar üretir ve daha büyük modül değerleri ile sonuçlanır. Sonuç olarak, mikrofludizasyon yöntemi zeytin tozunun geleneksel zeytin tozlarına kıyasla daha stabil olmasına yardımcı olmuştur.

Anahtar Kelimeler: Zeytin Tozu, Mikrofludizasyon, Dondurarak Kurutma, Oksidasyon, Stabilite

To my beloved family and my grandmother…

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my advisor Prof. Dr. Behiç Mert for his valuable support, guidance, understanding, immense knowledge, patience and encouragement throughout this study. He always helped me and provided quick solutions when I had difficulties in writing my thesis even if I was working in İstanbul.

I would like to express my special thanks to Assoc. Prof. Dr. Mecit Halil Öztop for his continuous support, knowledge, energy and motivation. It wouldn't be possible to complete this thesis without his motivation and support. He always tried to make everything easy for me and encouraged me in my stressful days.

I would like to thank 'Öztop Lab' members and research assistants at our department, Ülkü Ertuğrul, Eda Ceren Kaya, Şirvan Uğuz, Gökhan Uğur Atıl, Seren Oğuz and Selen Güner for their continuous help during my experiments. They enlightened me with their scientific knowledge whenever I faced with a problem during my study.

I am so grateful to my best friends Merve Eşin, Bayram Salihoğlu, Emine Değerli and Nurbanu Taştepe for their endless support and encouragement. They always supported me when I faced difficulties in my life. I am also so thankful to my close friends from the department Kübra Koyuncu and Feyza Nur Şimşek for their continuous support and motivation. I am incredibly grateful to have you all in my life.

I am so thankful to my colleague Res. Asst. Yağmur Küçükduman and Assist. Prof. Dr. Özge Taştan for their continuous support, motivation, and not leaving me alone in my difficult times. Also, I would like to thank Assist. Prof. Dr. Emrah Kırtıl for his valuable support, encouragement and helps in my thesis.

Last but not the least, I would like to express my deepest appreciation to my grandmother, Kesra Arğün for her supports and prayers, my parents, Zinet and

Abdullah Arğün, for their love and being with me in every decision I make, and my sisters Leyla, Tuğba, Şükran, Melike and Yağmur Arğün for their endless love. This accomplishment would not have been possible without their valuable supports. Therefore, I would like to thank every each of you for always being there for me.

Finally, I would like to thank all the people who have passed through my life.

TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

LIST OF ABBREVIATIONS

ABBREVIATIONS

MF : Microfluidized

UnMF : Unmicrofuidized

MF_Green : Microfluidized green olive powder

MF_Black : Microfluidized black olive powder

MF_Raw : Microfluidized raw olive powder

UnMF_Green : Unmicrofuidized green olive powder

UnMF_Black : Unmicrofuidized black olive powder

UnMF_Raw : Unmicrofuidized raw olive powder

DPPH : 2,2-diphenyl-1-picryl-hydrazyl-hydrate

TPC : Total phenolic content

FFA : Free fatty acid

GAE : Gallic acid equivalent

ANOVA : Analysis of variances

EVOO : Extra virgin olive oil

SFA : Saturated fatty acid

MUFA : Monounsaturated fatty acids

PUFA : Polyunsaturated fatty acids

LIST OF SYMBOLS

SYMBOLS

τ	: Shear stress
γ	: Shear rate
τ_0	: Yield stress
k	: Consistency coefficient
n	: Flow behavior index
G'	: Elastic modulus
G"	: Viscous modulus

CHAPTER 1

INTRODUCTION

1.1 Olive Fruit Description

The olive fruit is an oval-shaped fruit obtained from the olive tree (*Olea europaea*). Olive is an agricultural product of which its fruit can be processed as oil or consumed as table olive and the by-products can further be utilized (Çelik, et al., 2016). The olive tree is grown in the temperate climate of the Mediterranean basin and its history lasts back to 6000 years. Besides surrounding all the Mediterranean countries, it grows beyond Mediterranean countries such as Jordan, Portugal, Iran, Iraq and Turkmenistan. Olive is an important crop in edible oil production by ranking 6th place in all over the world. It is also one of the most significant crops in Mediterranean countries, particularly Spain, Italy, Tunisia, Greece, Morocco and Turkey (Kiritsakis & Shahidi, 2017). Turkey has been one of the major producers of olives among the Mediterranean countries having the longest coastline on the Mediterranean Sea (Republic of Turkey Ministry of Economy, 2018).

Olive oil production has tripled in all around the world, reaching about 3 million tons in the last 60 years. In Turkey, olive oil production is 225,000 tons in 2019/20 (International Olive Council, 2021a). Table olive consumption in Turkey has increased from 110,000 tons in 1990/91 to 340,000 tons in 2019/20 as increasing production triggered consumption (International Olive Council Newsletter, 2021). Recently, the interest in extra virgin olive oil has risen significantly because of its nutritional and health properties and because it has been one of the essential components of Mediterranean diet (Kiritsakis & Shahidi, 2017).

1.2 Composition of Olive

Olive fruit is rich source of oil and phenolic compounds. Ripe olives consist of oil, water, proteins, sugars, and cellulose. Other essential components are phenolic compounds; pectin; tannins; organic acids such as malonic, fumaric, citric, tartaric, acetic, oxalic and triterpenic acids; and inorganic salts. Olive pulp comprises some minerals such as calcium, iron, magnesium, potassium, manganese, copper and phosphorus (Kiritsakis & Shahidi, 2017).

The composition of olive fruit differs with respect to the cultivar, the degree of ripeness and the environment (Kiritsakis & Markakis, 1988; Garcia et al., 2012). These parameters affect the final quality of olive as well as the corresponding olive oil. In addition, olives' health state (whether infested by pests or not) affects the final quality of the oil (Suarez, 1975).

The raw olive flesh consists of 60 – 68 % water, 12 – 28 % oil, 8 – 12 % carbohydrate, 0.7 – 2 % protein and 0.4 – 1.1 % ash minerals (Kailis & Harris, 2007). Also, the olive flesh contains approximately 1-3% of phenolic compounds (Yorulmaz & Tekin, 2008). Olive fruit comprises of mainly two parts: *pericarp and endocarp*. The pericarp corresponds 66 to 85% of the total weight of the fruit and consists of the mesocarp (or pulp) and the epicarp (or skin). The endocarp (or kernel) contains seed which corresponds to lower than 3% of the total fruit weight.

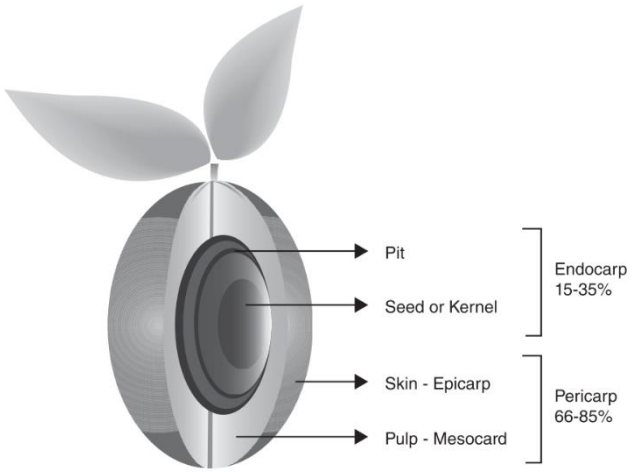

Figure 1.1 Parts of olive fruit (Kiritsakis & Shahidi, 2017)

While the pericarp accounts for 96–98% of the total oil content, the remaining oil is found in the endocarp. Raw olives' oil fraction is composed of 98% triglycerides, 1.1% diglycerides and 0.3% free fatty acids. In addition, phospholipids and galactolipids are also found in olive fruit cell membranes (Kiritsakis & Shahidi, 2017). Oil droplets of olive in cell structure is shown in Figure 1.2. Big cellular structures show olive oil droplets under Scanning Electron Microscopy.

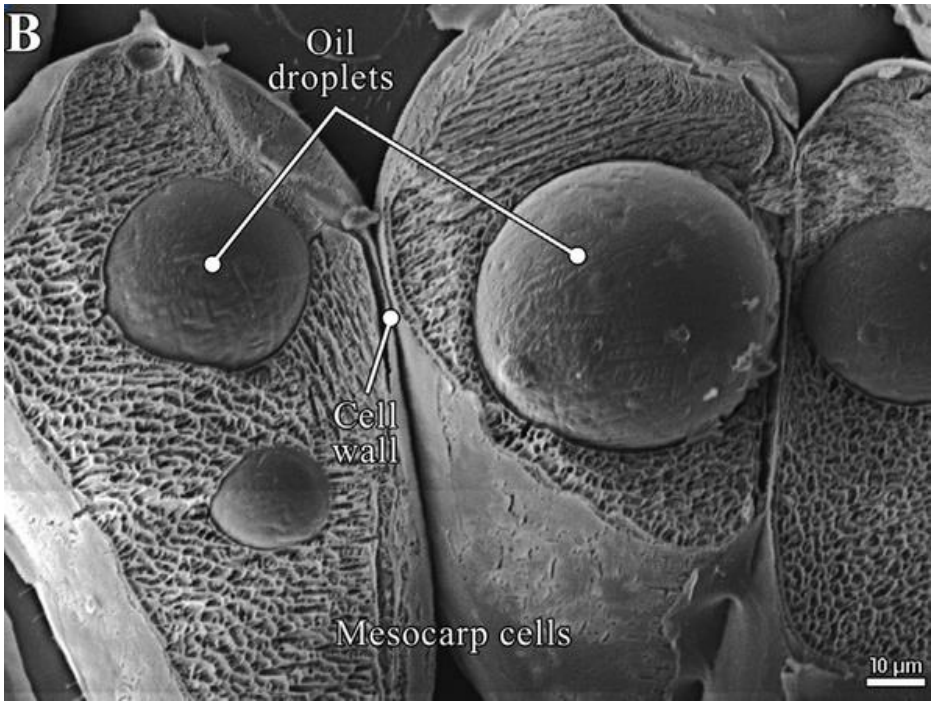

Figure 1.2 Oil droplets of olive in cell structure under Scanning Electron Microscopy (SEM) (Moretti et al., 2018)

Olive fruit contains some oil soluble compounds which are sterols, tocopherols (Vitamin E) and triterpenic acids. Oil composition in olive fruit changes between varieties, however; it is comparatively consistent to individual varieties cultivated under the same conditions. Higher oleic acids are found in oil fraction of olive if olive trees are cultivated in cool regions when compared with hot regions (Kailis & Harris, 2007).

Oil fraction of raw olive fruit flesh has different fatty acids; 70-80% oleic acid (MUFA), 5-10% linoleic acid (PUFA), <1 % linolenic acid (PUFA), 10-15% palmitic acid (SFA) and 2-3% stearic acid (SFA) (Hernández, 2021). The carbohydrate values of raw olive flesh changes between 8-12% (w/w). It consists of soluble sugars including glucose, sucrose, fructose and mannitol, and sugar polymers including pectin, cellulose, hemicellulose and lignin. The structural components of the cell wall comprise of cellulose and hemicellulose which also contribute to flesh

texture. During ripening or processing, these polysaccharides can be change or reduce which affects the organoleptic characteristics of olive (Kailis & Harris, 2007).

According to many researches, the cultivar might affect olive's fatty acid composition, polyphenol and tocopherol contents significantly. However, some researchers argue that it is the geographical location that affects those parameters notably (Kiritsakis & Shahidi, 2017).

Phenolic compounds in olive and olive oil consist of mainly tyrosols such as hydroxytyrosols and its derivatives named as secoiridoids. Oleuropein which is the major secoiridoid of olive gives to the olive fruit a bitter taste. During maturation of the fruit, its concentration declines while hydroxytyrosol concentration rises (Yorulmaz et al., 2013). Both β-glucosidase and esterase is found naturally in olive flesh. They help the production of hydroxytyrosols by oleuropein hydrolysis (Segovia-Bravo et al., 2009). Apart from that, phenolics found in olive and olive oil behave as antioxidants and also radical scavengers. They have anti-inflammatory and antitumor properties. Particularly, their benefits on oxidative damage and plasma lipid levels leads to a positive health declaration which is approved by EFSA which is European Food Safety Authority (Kiritsakis & Shahidi, 2017). Furthermore, phenolic compounds are responsible for the many of the sensory characteristics of plant based foods which are mainly astringency, bitterness and oxidative stability (Pandey & Rizvi, 2009; Li et al., 2014).

Phenolic compounds of olives are influenced by several factors. These consist of the geographical region, cultivar, age of the tree, maturity stage, agronomical practices, intensity of light, preservation conditions of fruit after harvesting and processing conditions which are the mainly temperature and water quantity (Kiritsakis, 1998; Benevente-Garcia et al., 2000; Salvador et al., 2001). The composition of fatty acid of olive oil is also considerably affected by the ripeness, cultivar, climatic condition and irrigation (Demirag & Konuskan, 2021).

1.3 Types of olives

During maturity stage, as ripening progresses, some metabolic processes might take place. The oil content of fruit rises, while color of the fruit changes from green to yellowish green and then finally blackish purple color as seen in Figure 1.3 (Gallardo-Guerrero et al., 2012). Green olives are obtained during ripening period before the color change. Black olives are harvested when they are black, before the olives overripe or being shriveled by frost (International Olive Council, 2021b).

Figure 1.3 Color evaluation of olive during maturation (Escuela Superior del Aceite de Oliva, n.d.)

1.4 Quality Changes of Olive and Olive Oil

The quality of olive oil is affected by some factors depending on its origin and alterations in composition which arise from tree to table. These changes considerably influence the functional components of oil. The most important quality deteriorations of olive oil result from hydrolysis and oxidation. Hydrolysis, also called as lipolysis, generally starts when the oil inside in the fruit, however; oxidation commences primarily after the oil is extracted from the fruit and as well as during storage (Kiritsakis & Shahidi, 2017). Hydrolysis leads to release of fatty acids from

triglyceride and thus acidity, known as free fatty acids (FFAs), increases and it causes changes in flavor.

Hydrolysis is affected by many factors including enzymes, microorganisms, temperature and moisture. Microorganisms found on the olive fruit releases lipases which leads to hydrolysis of triglycerides (Kiritsakis & Markakis, 1984). Enzymatic lipolysis occurs by microbial and endogenous enzymes such as lipases that are present the olive fruit. Lipase activity starts after the fruit begins turning purple. If the olive fruit is kept under improper conditions before processing, lipases may cause an increase in acidity. Since olive fruit as a breathing organism makes transpiration which liberates heat, therefore; increase in temperature causes the rise in the activity of lipolytic enzymes. In addition, the presence of water eases lipolysis by dissolving the enzymes and triggers microbial growth (Kiritsakis & Shahidi, 2017).

Oxidative rancidity can be described as the basic deteriorative change of olive oil. Oxidation occurs because of the oxidation of unsaturated fatty acids and following formation of certain compounds. These compounds cause unpleasant flavor and odor thus it might negatively affect the functional and nutritional values of the oil. Olive oil oxidation might take place in dark (*autoxidation*), or in light (*photooxidation*) (Dugan, 1961). Autoxidation occurs with free-radical mechanism and proceeds in three stages; initiation, propagation and termination. Firstly, in the initiation step, abstraction of hydrogen occurs as seen in below. Two free radicals are formed. Since polyunsaturated fatty acids contributes higher numbers of double bonds, oxidation takes place more in oils containing higher PUFAs.

$$RH \rightarrow R\bullet + H\bullet$$

In the propagation stage, alkyl radical reacts with the ground stage oxygen ($^1O_2$) to form alkyl peroxyl radical. Then, hydrogen can be abstracted from another

unsaturated molecule to form a hydroperoxides (ROOH) and another free radical as seen in below.

$$R\bullet + {}^1O_2 \rightarrow ROO\bullet$$
$$ROO\bullet + RH \rightarrow ROOH + R\bullet$$

These reactions proceed until unsaturated compounds are depleted or free radicals inactivate each other. The mutual destruction of free radicals is named as termination. Since hydroperoxides, primary oxidation products, are very unstable, they decompose to the secondary oxidation products such as aldehydes, secondary alcohols and ketones which several of them could possess toxic properties. Radical induced chain reactions might be terminated with several ways and one of them is shown below.

$$R\bullet + R\bullet \rightarrow RR$$
$$R\bullet + ROO\bullet \rightarrow ROOR$$
$$ROO\bullet + ROO\bullet \rightarrow ROOR + O_2$$

These compounds cause rancid flavor of oil with unpleasant taste and odor. However, antioxidants (AH) might prevent lipid oxidation by reacting with free radicals as seen in below (Kiritsakis, & Markakis, 1984).

$$R\bullet + AH \rightarrow RH + A\bullet$$
$$A\bullet + A\bullet \rightarrow AA$$

Olive oil resists to autoxidation as it has low content of PUFAs and it has natural antioxidants. Nevertheless, it is quite vulnerable to photooxidation due to its chlorophyll content which acts as a photosensitizer. Chlorophylls may act as photosensitizers because they are able to convert energy from light to triplet oxygen and gives off singlet oxygen which reacting with unsaturated fatty acids, resulting in

the formation of hydroperoxides. However, chlorophylls protect the oil from oxidation acting as antioxidants in the dark (Kiritsakis & Dugan, 1985). Since olive oil has high levels of MUFAs, it is more stable and less susceptible to oxidation when comparing to oils containing PUFAs.

The enzymatic lipid oxidation also can be occurred by the enzyme lipoxygenase found in lots of plant and animal tissues. Fatty acids can be oxidized by the lipoxygenase enzyme present in olive fruit thus autoxidation reactions may proceed and hydroperoxides are formed. These hydroperoxides are the precursors of further conversion by enzymatic reaction as lipoxygenase initiates the producing of hydroperoxides from substrate of single fatty acids (Ahmed et al., 2016).

The browning reaction is a widespread phenomenon that occurs due to mechanical injury during processing or post-harvest storage of fruit. Polyphenoloxidase (PPO) is the responsible enzyme causing browning by catalyzing oxidation of *o*-dihydroxyphenols to *o*-quinones. Then, quinones cause the formation of dark-colored pigments (Martinez & Whitaker, 1995).

When maturation of the olive fruit increases, from green olive to ripe olive, some metabolic processes might proceed. That is, polyphenolic compounds declines and oxidative stability of the olive reduces (Gutierez et al., 1999; Salvador et al., 2001). According to the Salvador et al. (2001) and Fuentes et al. (2013), there existed a positive correlation between total polyphenols and oxidative stability during maturation. The oxidation gives olive oil an unpleasant flavor and odor which affects the nutritional and functional value of oil.

1.5 Production of Olive and Olive Oil

1.5.1 Table Olive Production

For table olive production, olives need to undergo a series of processes which considerably depends on the growth region and variety. Since oleuropein found in olive fruit gives a strong bitter taste to olive, it needs to be removed from the fruit after harvesting. The olive fruit is usually treated in brine, sodium or potassium hydroxide or rinsed in water to obtain table olives. After green olives are picked, they are transported to the processing plant, then immersed in brine at 2.5 - 10% concentrations in reverse relationship with fruit size and protected from air. Black olives need to be transported to the factory facility as soon as possible. Then, they are sorted, rinsed and placed in 8 to 10% brine solution. The brine solution triggers the microbial activity for the fermentation process and decreases the oleuropein levels. At the beginning of fermentation, the tanks are sealed tightly in order not to expose to air (International Olive Council, 2021b).

1.5.2 Olive Oil Production

Since olive oil extraction is not in the scope of this thesis, extraction methods will not be discussed comprehensively. However; in traditional olive oil production there are three main processes for the production of olive oil; *pressing, two phase and three phase systems* as seen in Figure 1.4 – 1.5. The pressing is the most traditional process and still used even though it is not widespread as it has high labor costs and low production efficiency (Domingues et al., 2021). Olive oil extraction process consists of three main processes; *crushing, malaxation and centrifugation*. Firstly, olive fruits are washed and they are crushed by using stone-mill, de-stoning machines, disc crushers or blades. Crushing step is made for facilitating the release of oil droplets from olive flesh. Then, at the malaxation step, obtained olive paste is

stirred constantly and slowly in order to promote the coalescence of small oil droplets into larger ones, therefore; this process eases the separation of oil and water. In pressing extraction, the paste is pressed in order to release oily must which consists oil and water. Then phase separation by decanting or centrifugation is applied to separate oil from waste water (Yüksel Aydar, 2019). Separation of olive oil in two and three phase systems occurred by centrifugation process and olive oil is separated from olive pomace and water. In two phase system, water is not used. After malaxation, centrifugation and filtration steps, the olive paste is directly separated into oil and semi-solid waste, named as two phase olive-mill waste (TPOMW). In three phase system, at the malaxation step crushed olives are mixed with water in order to rise extraction yield and processing capacity (Souilem et al., 2017). Then it passes to the centrifugation and filtration steps. This continuous system is the most used in many countries of the Mediterranean region. It enables the separation of three products; oil, pomace and olive-mill wastewater (OMWW) so it is called three phase system (Morillo et al., 2009). Malaxation and crushing are the important steps of the extraction because it affects the final olive oil quality. Obtaining good quality of olive oil depends some important factors, which are maturity of fruit, harvest time, mode of harvesting (i.e. hand picking), storage of fruit before processing, mode of crushing and the extraction system (Kiritsakis & Markakis, 1988).

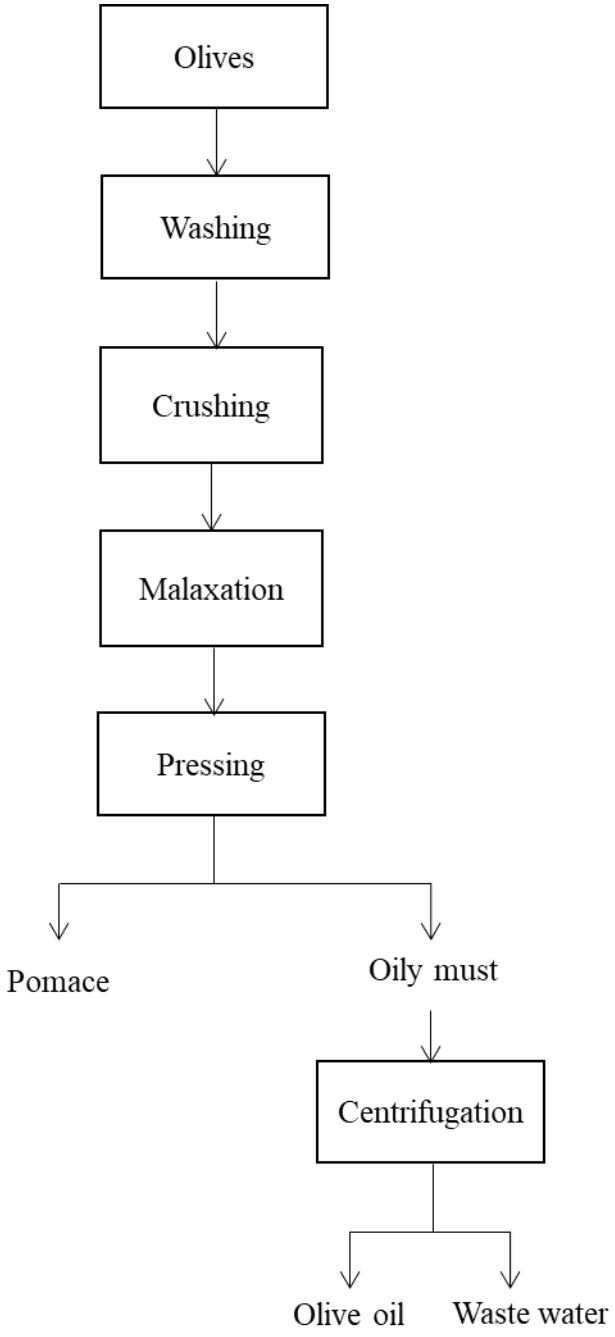

Figure 1.4 Flowchart of pressing process for olive oil extraction

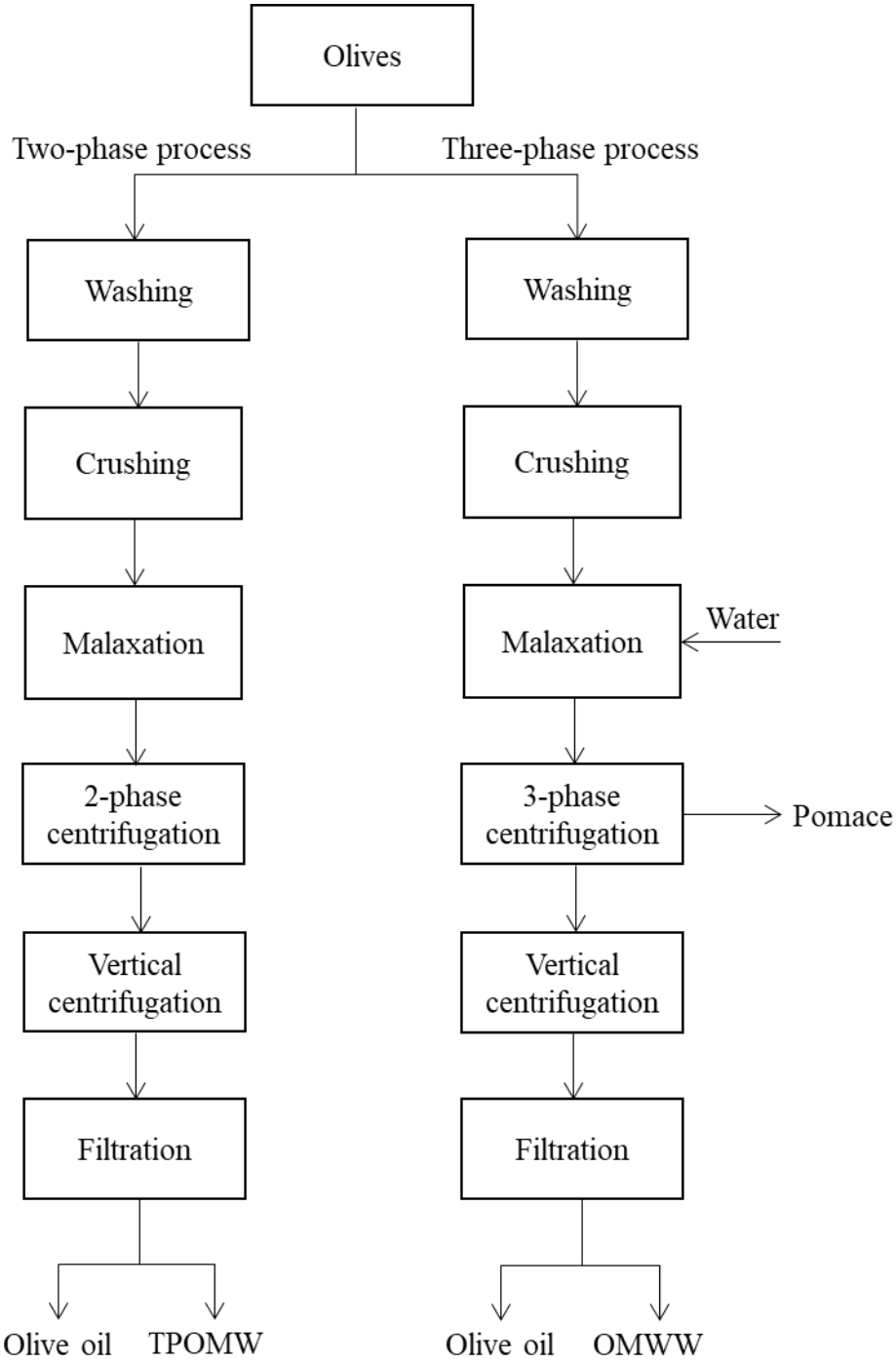

Figure 1.5 Flowchart of two-phase and three-phase process for olive oil extraction

1.6 Olive Powder

Olive leaf powders, olive stone powders, and by-products of olive oil extraction, which are olive pomace, olive mill wastewater are also utilized to obtain beneficial powder forms. Since the processing of olive oil results in high amounts of solid waste, the idea of using the leftovers into a beneficial value-added product has become important (Sarmin et al., 2021). Spain is the leading producer of olive oil all around the world and during processing too much organic waste is acquired. Therefore, olive powder produced by by-product of two phase extraction process of olive oil is being used as a food ingredient such as in pasta, bread and soups. As it is rich in bioactive compounds such as antioxidants, consisting polyphenols, tocopherols, oleic acid, oleuropein and chlorophylls, olive powder used as a food ingredient (Marco et al., 2011).

Olive powder is produced by conventional drying, spray drying or freeze-drying technique. In a study, olive powder was used as an additive to increase the antioxidant and quality characteristics of yoghurt products. First olives were dried by using freeze dryer and then dried olives were grinded. By using this technique, olive powder is obtained in the form of an olive paste, therefore; it is susceptible to oxidation if it is not stored properly (Cho et al., 2017). In addition, Chasekioglou et al. (2017) used olive mill waste water by spray drying method and a value-added food by-product was produced. Since three phase extraction process is the most widely used among olive oil extraction processes, use of by-products of this process, which are olive cake and olive mill wastewater, is important to decrease the amount of waste. In another study, olive by products were produced by drying with a forced draft oven at 55 °C for 24 h and then grinded in a mill (de Moraes Crizel et al., 2016). Olive powder had also been reported to have bacteriostatic effects against *Bacillus cereus* spores (Marco et al., 2011). Also, there exists a freeze dried olive powder product, named as Oliva Negra Sosa, obtained from mixing black olives, black olive flour, salt and stabilizer (Gourmet Versand, 2021). There is a patented olive powder,

which is produced by freeze drying of olive paste obtained after extraction (Escoda, 2016).

Olive droplets in olive fruits are captured in cell membranes. When olives are grinded to get a powder form, the cell membranes of olive are disrupted, thus oil droplets are released and grinded dry olives become an oily paste. Therefore, this makes impossible to get dry powder form of olive from the paste. In a patented study, powdered oil absorbing substances which contains ground cereals, starch, extruded flours, resistant starch, pre-gelatinized starch and protein are used to obtain powder form as an invention. When olive paste is mixed immediately with these substances during grinding, the released oil is absorbed, thus oil cannot be accumulated and cake is not formed in some parts of the mixture (Borcaklı et al., 2014).

By using microfluidization technique, the releasing of oil problem can be solved. During microfluidization, bigger oil droplets becomes smaller due to the high pressure. These small oil droplets probably absorbed by protein and sugars found in olive fruit such as glucose, fructose, mannitol, pectin, cellulose and hemicellulose and cause self-encapsulation of olive oils. Therefore, dry olive powder form can be obtained by microfluidization technique.

1.7 Microfluidization

Microfluidization is an emerging size reduction technique and it is also known as ‘high pressure microfluidization’ or ‘dynamic high pressure microfluidization’. It is a high-pressure homogenization method where the fluid is pressurized and changed as a consequence of high shear rate and impact forces (Mert, 2012). The fluid is forced to separate into two microstreams causing to the collision of the streams at

ultra-high pressures up to 200 MPa within less than 5 seconds. Thus, this operation distributes large particles homogenously into more uniform particles by causing deformation and breakage of structures of particles (Liu et al., 2009; Mert et al., 2014). The schematic representation of microfluidizer is shown in Figure 1.6.

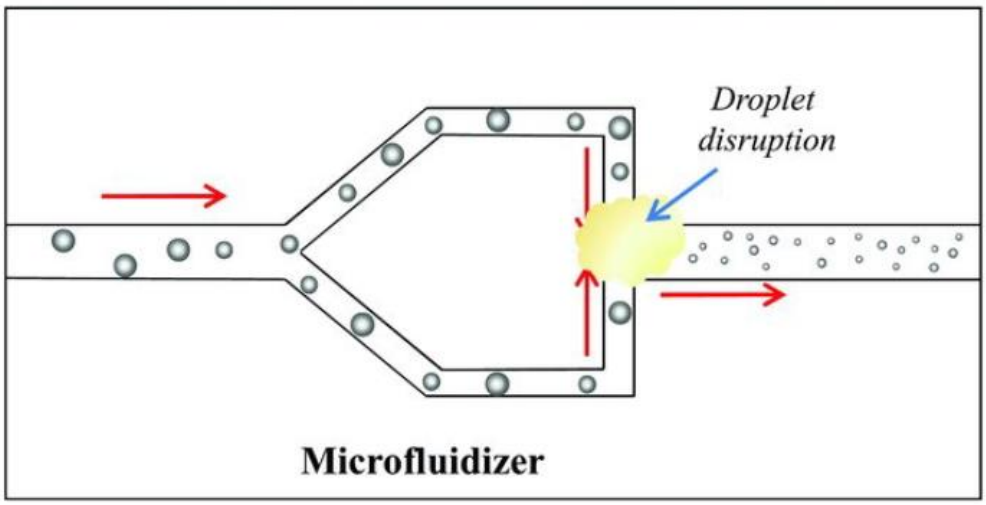

Figure 1.6 Schematic representation of microfluidizer (McClements & Rao, 2011)

Microfluidization allows for continuous operation with less loss of nutritious substances, low temperature treatment, and a quick process time as compared to other modification methods (Hu et al., 2011). By reducing particle size and generating more homogenous food particles, it enhances the texture, stability, color, and taste of food samples. The microfluidization process alters macromolecules' functional and physical properties, such as particle size, solubility, antioxidant activity, and emulsifying abilities (Bitik et al., 2019; Liu et al., 2016; Liu et al., 2017; Wang et al., 2013). According to Mert et al. (2014), microfluidization resulted in more fibrous wheat bran structures with increasing water holding capacity. The existence of high amount of water-binding macromolecules like fibers, emulsifiers and hydrocolloids is regarded to increase the mixing characteristics and shelf life of

baked food products. In addition, starch molecules are quite hydrophilic and when they are modified properly, they include hydrophilic and hydrophobic groups being attracted to the oil micelles' surface. Thus, modification of starch molecules with microfluidization may also increase water holding capacity of samples (Varavinit et al., 2001). Furthermore, microfluidization process has an impact on the rheological and sensory qualities of food (Ciron et al., 2011).

This approach has been employed in a variety of fields, including cosmetics and pharmaceutics. It's been utilized in milk homogenization (Hardham et al., 2000; Kumar et al., 2019), yoghurt (Demirkesen et al., 2018), cream liquers (Paquin & Giasson, 1989), cheese (Vuillemard, 1991), wheat bran (Koo et al., 2018), high methoxyl pectin (Chen et al., 2016), and microfluidization increased the quality of the ketchup samples, according to Mert (2012). Furthermore, according to Wang et al. (2012), the microfluidization technique reduced the bulk density of wheat bran and increased the porosity of the sample. The water holding capacity of the samples was also enhanced as a result of the smaller size and increased surface area. As a result, the wheat bran samples' water holding capacity was improved using the microfluidization approach (Mert et al., 2014).

1.8 Objective of the study

Olive and olive oil represent an essential part of the Mediterranean diet. The olive fruit is an important crop in edible oil production by ranking 6th place in all over the world and Turkey has been one of the major producers of olives among the Mediterranean countries. Since it is not possible to produce olive powder without discarding the oil in the olive or without adding any additives, %100 natural olive powders are not available in markets. The main objective of this study is to develop

a new value added product from olive with increased functionality and to characterize its properties. Specific objectives of the study can be listed as;

- *Investigate the effect of olive type on the physicochemical properties of green/black/raw olives*
- *Investigate the physicochemical properties of the powders such as antioxidant activity, phenolic content, peroxide value, color and moisture content*
- *Investigate the physical properties of the olive powder suspensions through Turbiscan and rheology experiments*
- *Showing that microfluidization helped to make the powder more stable compared to conventional olive powders.*

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Green, black and raw olives were obtained from a local market. Green and black olives are table olives which are consumed in daily life while raw olive is unprocessed olive obtained after harvesting. Distilled water were used for microfluidization process. Ethanol, methanol, acetic acid, DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) reagent, Folin reagent, sodium carbonate, chloroform, sodium thiosulfate solution, potassium iodide, potato starch, phenolphthalein and hexane were supplied from by Sigma Aldrich Chemical Co. (St. Louis, USA).

2.2 Methods

2.2.1 Olive Powder Production

2.2.1.1 Microfluidized Olive Powder Production

For microfluidization process, aqueous solution is required; therefore, olives are needed to be mixed with water before microfluidization. Firstly, olive seeds were discarded from olives and remaining olive pulp was crushed by using a laboratory blender (Retsch knife mill Grindomix GM 300, Germany) for 30 seconds. Then, crushed olives and distilled water were mixed at a ratio of 2:5 (w/w) with blender for 2 minutes. Olive-water solution was passed through a colloid mill (Magic Lab, IKA, Staufen, Germany) in order to decrease size of the solid particles previous to microfluidization process. This process was repeated twice. Then, microfluidization was carried out to the final solution by using microfluidizer (M-110Y, Microfluidics,

USA). The solution was passed two times through the microfluidizer at 1200 bar. Then, the solution was first frozen, later freeze dried in freeze dryer (Christ, Alpha 2–4 LD plus, Germany) for about 48 hours. These processes were applied for each type of olive; green, black and raw. Production scheme of microfluidized olive powder is shown in Figure 2.1.

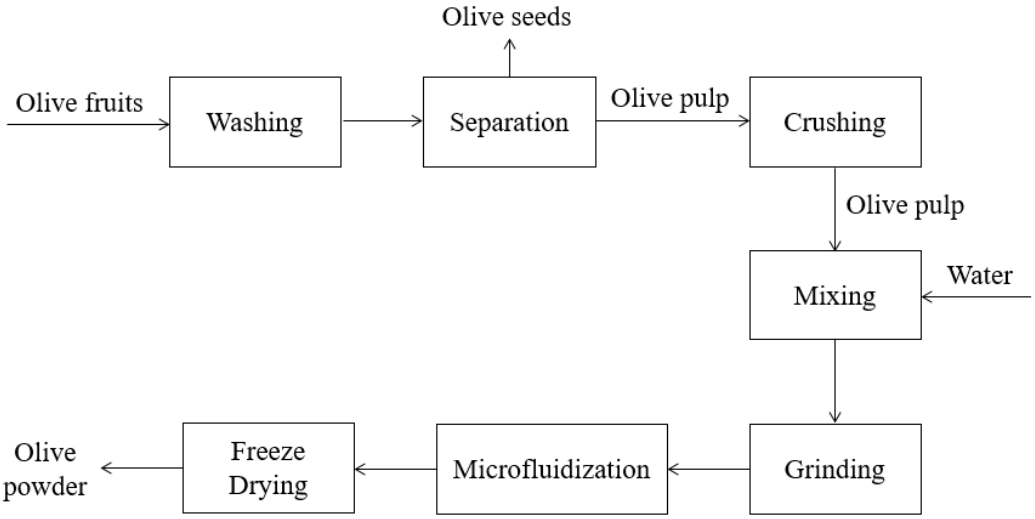

Figure 2.1 Flow-chart of microfluidized olive powder production

2.2.1.2 Unmicrofluidized Olive Powder Production

Unmicrofluidized olive powder was obtained only by freeze drying. After discarding the seeds of the olives, olive pulp was frozen before putting freeze dryer. The sample was dried about 48 hours in freeze dryer. Then, freeze dried olives were crushed with blender at 2000 rpm for 1.5 minutes to get powder form. These processes were applied for each type of olive; green, black and raw. Flow-chart of unmicrofluidized olive powder production is shown in Figure 2.2.

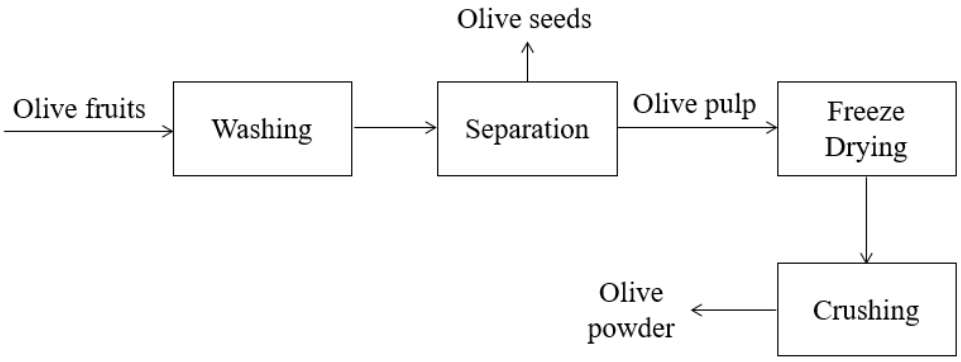

Figure 2.2 Flow-chart of unmicrofluidized olive powder production

2.2.2 Physical and Chemical Characterization of Olive Powder

2.2.2.1 Moisture Content

The moisture content of the UnMF and MF green, black and raw olive powder samples was measured with an infrared moisture analyzer (Radwag, MAC 50). Three replicates were done for each olive type.

2.2.2.2 Antioxidant Activity

DPPH assay was used to determine the antioxidant activity of UnMF and MF initial green, black and raw olive powders and for powders stored for 1.5 and 3 months (Brand-Williams et.al., 1995) and kept in glass jars under refrigerator conditions. First, extraction solvent was prepared by mixing 42 ml water, 8 ml acetic acid and 50 ml ethanol. In order to prepare the DPPH solution, 2.5 mg of DPPH reagent was

added to 100 ml methanol and the solution was mixed. Then, 0.1 g of sample was mixed with the solvent and DPPH solution. Final solution was hold for 24 hours in dark at refrigerator conditions. The absorbance values of the powder samples were read and recorded at 517 nm by using UV Spectrophotometer (Optizen Pop Nano Bio, Korea). Finally, radical scavenging activities of samples (mg Trolox/ g powder) were calculated (Zhang et al., 2018).

2.2.2.3 Characterization of Total Phenolic Content

Folin Ciocalteu total phenolic content method was used to determine the amount of total phenolic content of initial UnMF and MF green, black and raw olive powders and powders stored for 1.5 and 3 months. Modified version of the Folin Ciocalteu method reported by Scapin et al. (2016) was used. First 2 gr of sample was mixed with the 50 ml methanol: water solution (80:20) in a stirrer for a short period of time. This is important to liberate all phenolic compounds from the sample. Samples were held in the dark for 24 hours at refrigerator conditions. Then, mixtures were filtered using 0.45μm plastic micro-filters. After that, Folin Ciocalteu reagent was mixed with the samples and the mixture was kept in dark for a short time. For the next step, 7.5 % (w/v) ratio of Na_2CO_3 was added to the mixture. Final mixture was kept in dark for 1 hour. Then, absorbance was read by a UV Spectrophotometer (Optizen Pop Nano Bio, Korea) at 765 nm. All measurements were made in triplicate. For the calibration curve, gallic acid was used as a standard. Values were reported as gallic acid equivalent divided by amount of sample (mg GAE/g) (Scapin et al., 2016).

2.2.2.4 Peroxide Values

Peroxide values were determined on the oil extracted from samples. For the extraction, classical extraction method (maceration) was used. Hexane was used as the solvent. Olive powder and hexane were mixed at a ratio of 1:10 (w/v). Then, mixture was stirred by using magnetic stirrer at 45℃ for 1 hour. Then, the mixture was cooled to room temperature and it was centrifuged at 4,000 rpm for 15 minutes. Finally, liquid part was separated and hexane was evaporated.

Oxidative stability of extracted oil was determined by Peroxide Value – AOCS Method according to the Lubrizol Standard Test Procedure (2006). Peroxide values of UnMF and MF green, black and raw olive powders were determined. Five (±0.05) grams of olive oil sample was weighed into an Erlenmeyer flask. 30 ml of acetic acid-chloroform mixture was added to the flask and it was swirled until it was totally dissolved. Then, 0.5 ml of saturated potassium iodide solution was added and mixed exactly for one minute. 30 ml distilled water was added directly and total mixture was shaken vigorously to release the iodine from the chloroform layer. The initial color of the solutions was deep red orange. Titration was done with 0.1 N sodium thiosulfate slowly until the color lightens. The following equation was used for the calculation of peroxide value.

$$\text{Peroxide value}=\frac{(S-B)\ x\ N\ thiosulfate\ x\ 1000}{weight\ of\ sample} \quad \text{(Eq. 2.1)}$$

S: volume of titration of sample

B: volume of titration of blank

N thiosulfate: Normality of thiosulfate solution

2.2.2.5 Free Fatty Acid Content

Oil was extracted as described before. Free fatty acid (FFA) contents of UnMF and MF green, black and raw olive powders were determined using the AOCS standard method Ca 5a-40 (2004). Results of the % FFA is expressed as the percentage of oleic acid. The weight of the oil samples and the properties of the chemicals used in the experiment were made based on the estimated %FFA values they contain. For olive oil samples, 0.25 N NaOH was used for the range of 1-30 % FFA. Firstly, 1 g of olive oil sample which is extracted from olive powder was weighed carefully and mixed with 15 ml ethanol and shaken vigorously. 2-3 drops of phenolphthalein indicator was added to the mixture and then solution was titrated with 0.25 N NaOH solution slowly until the pink color appears. Measurements were conducted in triplicate for each oil sample (Ayyıldız & Hüseyin, 2015).

2.2.2.6 Rheological Properties of Olive Powder Suspensions

Rheological measurements of green and raw olive powder suspensions at different powder concentrations (15%, 40% and 60%) were conducted using a rheometer (Kinexus Dynamic Rheometer, Malvern, Worcestershire, UK). Because the rheological characteristics of green and raw olive powder solutions are comparable, the black olive powder solution was not investigated. At a temperature of 25°C, all measurements were performed using parallel plate geometry with a 40 mm diameter and a 2 mm gap thickness. Shear stress was measured as a function of shear rate, which was varied between 0.1 and 100 s^{-1} throughout the flow measurements. In oscillatory trials, each sample was subjected to a frequency sweep test ranging from 0.1 to 100 Hz at a strain rate of 0.5 %. The elastic (G') and viscous (G") moduli were then measured. All measurements were made in triplicate, and mean values were used to create the graphs for each frequency.

2.2.2.7 Stability Measurements

Stability of the olive powder suspensions was determined using Turbiscan Lab Expert type stability analyzer (Formulaction, Toulouse, France) at concentrations of 10, 40 and 60%. Sample solutions with volumes of 20 ml were placed in a Turbiscan cylindrical glass tube. This instrument applies a pulsed near-infrared light source (λ = 880 nm). The sample was scanned every 30 minutes for 60 hours at 25 °C. Each scan measures two signals; transmittance (T) and backscattering (BS). The emitted light partly passes through the sample tube while most of it are scattered by the particles in the sample. Turbiscan detects the intensity of these lights over the height of the whole tube (Formulaction, 2021).

The changes in size of droplets and phase separation in olive powder solutions were monitored by measuring the backscattering (Delta BS (ΔBS), %) at different times and ΔBS and Turbiscan Stability Index (TSI) values were investigated. TSI values were calculated using Eq. 2.2 with the help of Turbiscan Easy Soft where *n* is the time of scan, x_i is the light intensity of backscattering at scanning time of *i*, and x_{bs} is the average light intensity of backscattering (Yang et al., 2017). Since green and raw olive powders gave similar results, black olive powder was not analyzed.

$$\text{TSI} = \sqrt{\frac{\sum_{i-1}^{n}(x_i - x_{bs})^2}{n-1}} \qquad \text{(Eq. 2.2)}$$

2.2.2.8 Scanning Electron Microscopy

Microstructural analysis of both MF and UnMF green olive powders were examined using a Scanning Electron Microscope (SEM) (QUANTA 400F Field Emission SEM, Holland). Black and raw olive powders were not investigated because the aim of this analysis is to see the effect of microfluidization process on microstructure of

olive powders, thus one olive type was enough. Samples were coated with gold (3 nm) to make samples electrically conductive using a sputter coater (Au/Pd). Images were analyzed at the magnification levels of 100×, 250×, 500× 1000× and 2000× with 30 kV of accelerating voltage. Analyses were performed at METU Central Laboratory (Ankara, Turkey).

2.2.2.9 Color

Color of the UnMF and MF green, black and raw olive powders were measured using a spectrophotometer (Konica Minolta Spectrophotometer, CM-5, Japan). CIELAB method was used. L*, a* and b* values were recorded for each olive powder samples. ΔE values of the results were calculated using Eq. 2.3 in order to examine differences between samples (Sant'Anna, 2013).

$$\Delta E=\sqrt{(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2} \quad \text{(Eq. 2.3)}$$

2.2.2.10 Statistical Analysis

Analysis of Variance (ANOVA) was performed to analyze whether there is significant difference between olive powder samples using MINITAB (Version 19.1.0.0, Minitab Inc., Coventry, UK). The effect of microfluidization on antioxidant activity, total phenolic content, free fatty acid content, peroxide value and color were examined for each type of olive powder. Tukey's comparison test was used at a confidence level of 95% (p = 0.05). All experiments were performed with duplicates and/or triplicates.

2.3 Experimental Design

Table 2.1 Parameters of the experimental design

Factors	Levels	Responses
Olive type	Green Black Raw	● Moisture Content ● Antioxidant Activity by DPPH method ● Total Phenolic Content ● Peroxide Values ● Free Fatty Acid ● Rheological Properties of suspensions ● Stability Measurements ● Scanning Electron Microscopy (SEM) ● Color
Processing	Microfluidization @ 1200 bar (MF)/ Unmicrofluidized (UnMF)	
Storage Time	0, 1.5 and 3 months	

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Moisture Content

Moisture content of the olive powders were found as between 1-4%. This result shows that food powders are dried enough because the moisture content of food products depending on the different type of food must be below around 10% to prevent microbial growth (Vera Zambrano et al., 2019).

3.2 Antioxidant Activity by DPPH Radical Scavenging Method

Antioxidants have significant effect on prevention or slowing down the oxidation of fats, oils or foods which contains fatty acids. Therefore, it is important to know antioxidant activity of food samples especially containing higher amount of fat/oil content in order to predict shelf life of foods (Kiritsakis & Shahidi, 2017).

Antioxidant activity of olive powder samples were analyzed by scavenging the DPPH radical. The analysis was performed for t=0, 1.5 and 3 months for each type of olive powder. Results for green olive powders are given in Fig. 3.1. For UnMF green olive powder, the results showed that there was not a significant decrease in antioxidant activity with time ($p>0.05$). For MF green olive powder, there was significant reduction in antioxidant activity of olive powder at the end of 3 months ($p<0.05$).

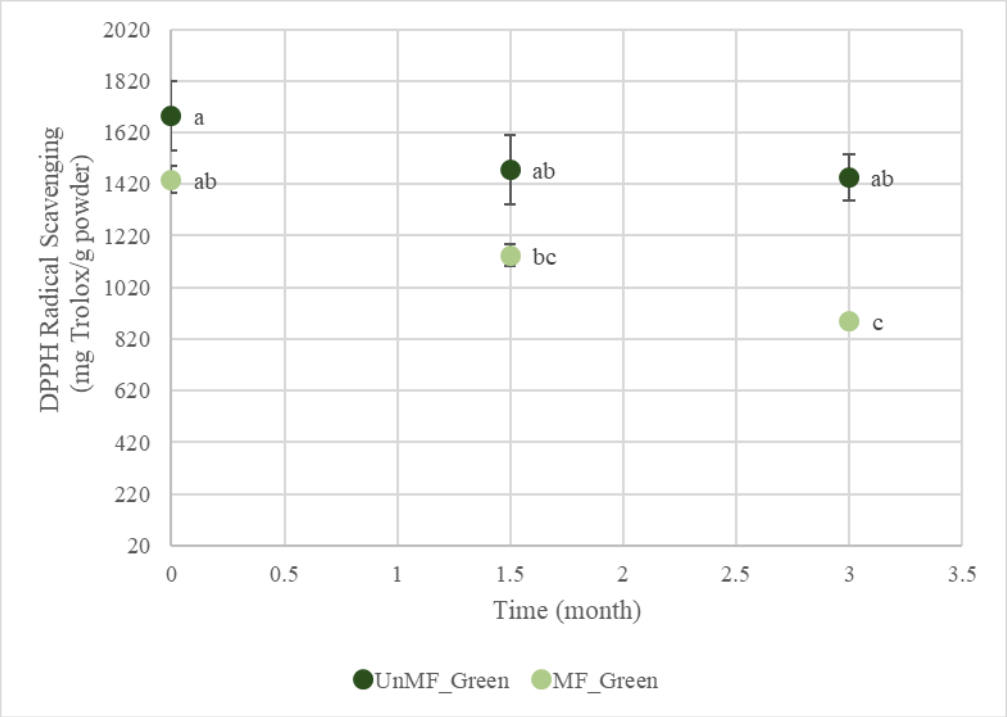

Figure 3.1 DPPH Radical Scavenging (mg Trolox / g powder) of green olive powders at t=0, 1.5 and 3 month

For black olive powder, antioxidant activity decreased significantly after 1.5 months for both UnMF and MF powders, and remained same for 3 months as seen in Figure 3.2.

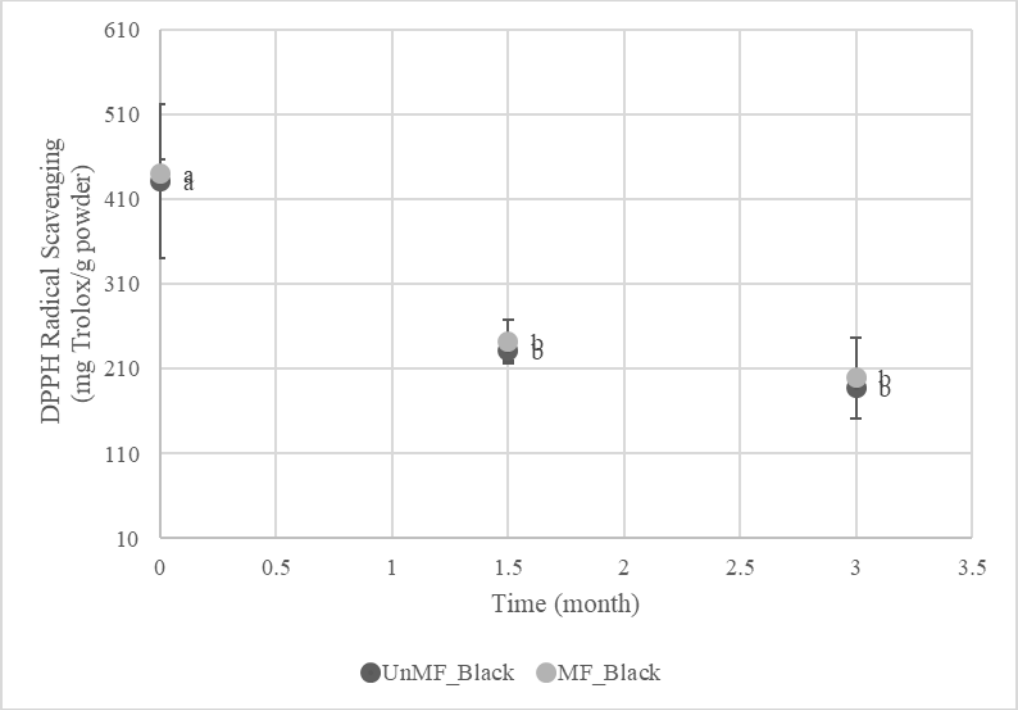

Figure 3.2 DPPH Radical Scavenging (mg Trolox / g powder) of black olive powders at t=0, 1.5 and 3 month

For UnMF raw olive powder, antioxidant activity was not affected significantly with time and process type as shown in Figure 3.3 ($p>0.05$).

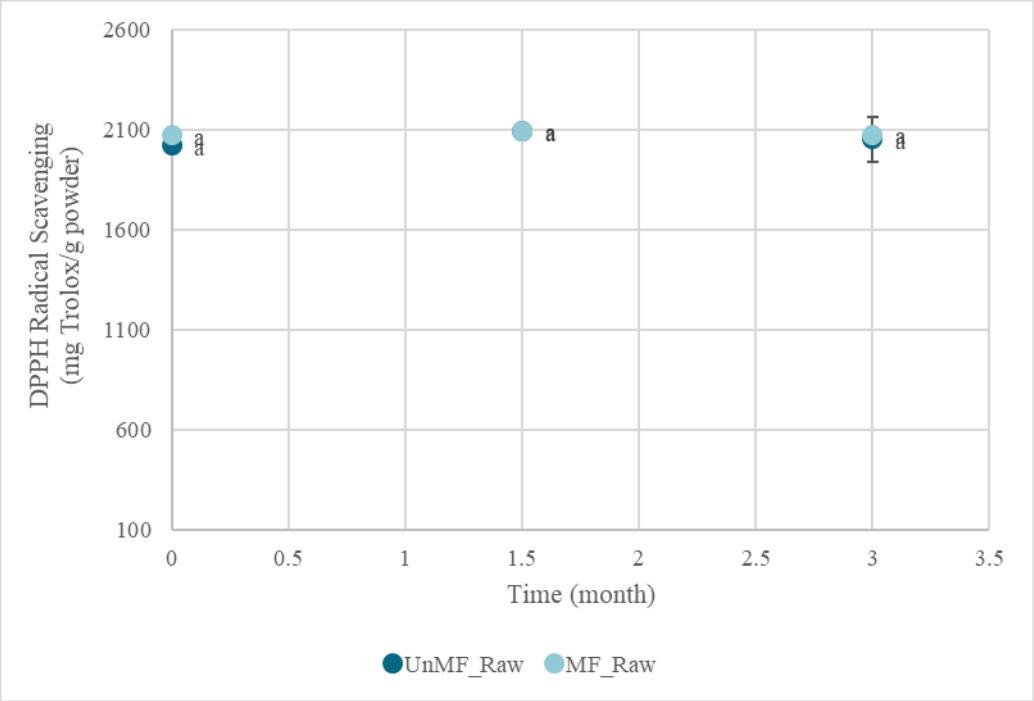

Figure 3.3 DPPH Radical Scavenging (mg Trolox / g powder) of raw olive powders at t=0, 1.5 and 3 month

According to Wang et al. (2019), high pressure microfluidization had no significant effect on DPPH scavenging when compared to conventional homogenization for peach juice samples. Likewise, Tarafdar et al. (2021) reported that no substantial variation in DPPH radical scavenging of sugarcane juice was examined.

Antioxidant activities of MF green and both UnMF and MF black powders were decreased with time ($p<0.05$). During storage of fruit, antioxidant activity changes due to the oxidation reactions because antioxidants may participate lipid oxidation reactions by reacting with free radicals (Kiritsakis, & Markakis, 1984; Pokorný & Schmidt, 2001).

When processes are compared, for green olive powder, there was not significant change in antioxidant activity in the application of microfluidization process initially. Black and raw olives followed the same pattern.

When the antioxidant activities of green, black and raw olive powder are compared, it is seen that raw olive has the highest antioxidant activity followed by green olive and black olive, respectively ($p<0.05$). The reason is the fact that during fermentation stage of table olive production, water-soluble compounds in olive such as polyphenols, fermentable substrates and other nutrients pass from olives to brine. That is, about 60-80% of total polyphenols, sugars and flavonoids are lost from olive fruit at the end of the fermentation. Therefore, the result that black and green olive have lower antioxidant capacity than raw olive fruit is consistent with the previous studies (Kiai & Hafedi, 2014). In addition, when maturation of the olive fruit increases, from green olive to ripe olive, polyphenolic compounds decreases (Gutierez et al., 1999; Salvador et al., 2001). Thus, the results of polyphenolic compounds showing antioxidant activity of black olive is the lowest which is also consistent with the literature.

3.3 Total Phenolic Content

Total phenolic content of olive powder samples was determined for 0, 1.5 and 3 months. For UnMF green olive powder, the results showed that there was a significant decrease in total phenolic content at the end of 1.5 months ($p<0.05$) and remained same at the end of 3 months. For MF green olive powder, no significant change was observed after 1.5 and 3 months. When the effect of process is considered, it is seen in Fig. 3.4 that microfluidization process caused a decrease in the total phenolic content of green olive powder at t=0 month.

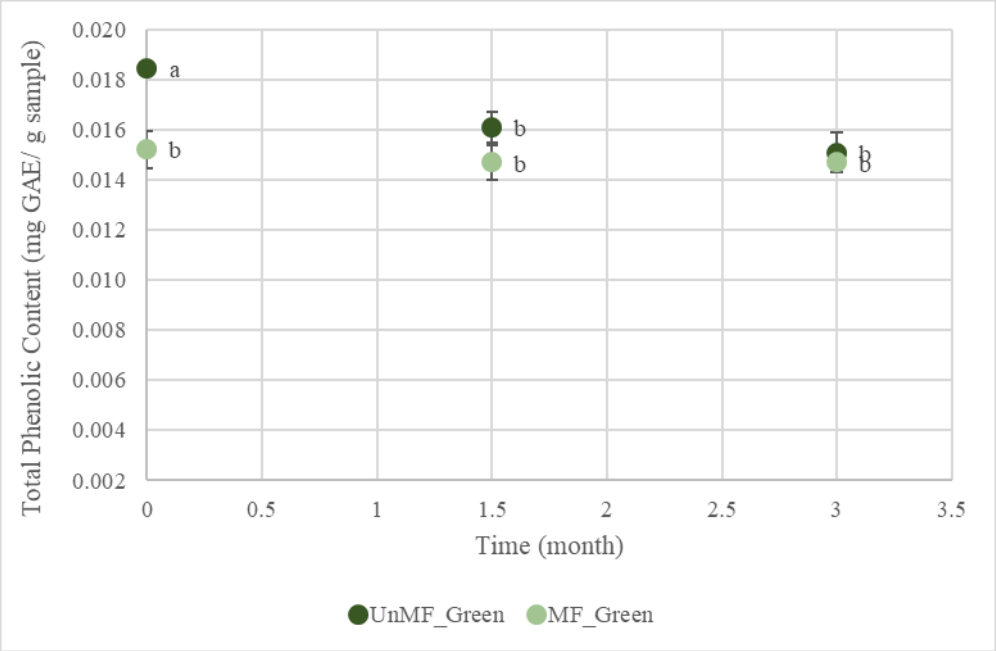

Figure 3.4 Total phenolic content (mg GAE/g sample) of green olive powders at t=0, 1.5 and 3 month

For both UnMF and MF black olive powder, the total phenolic content did not change notably ($p>0.05$) after 1.5 and 3 months as shown in Figure 3.5. Microfluidization also did not have significant effect.

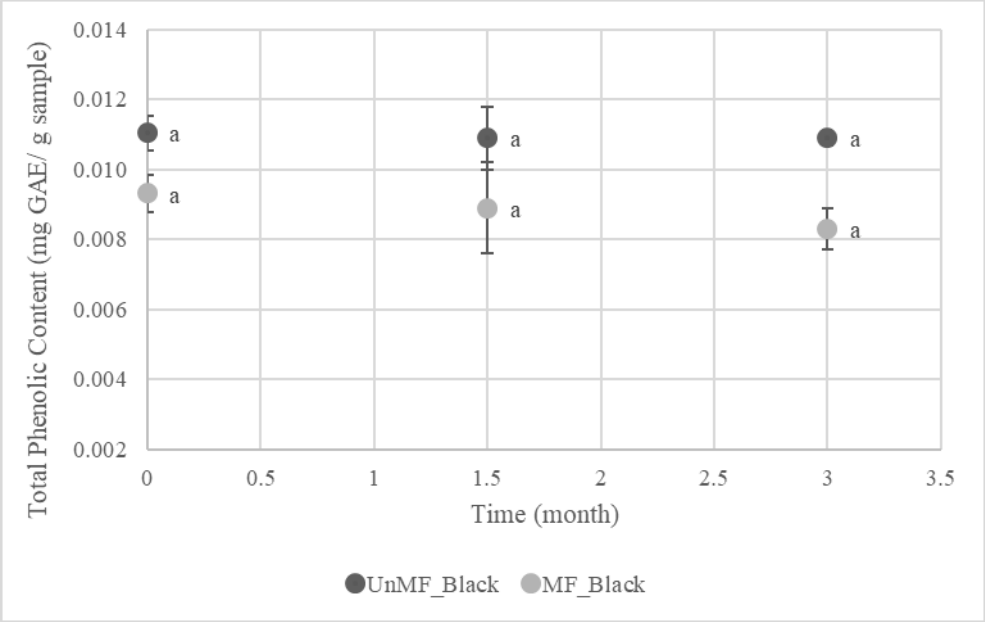

Figure 3.5 Total phenolic content (mg GAE/g sample) of black olive powders at t=0, 1.5 and 3 month

For UnMF raw olive powder, total phenolic content decreased significantly after 1.5 months ($p<0.05$) and remained same at the end of 3 months. However, for MF raw olive powder, total phenolic content declined significantly with time as shown in Figure 3.6 ($p<0.05$). When the effect of process is considered, it is seen in Fig. 3.6 that microfluidization process resulted in a reduction in the total phenolic content of raw olive powder at t=0 month.

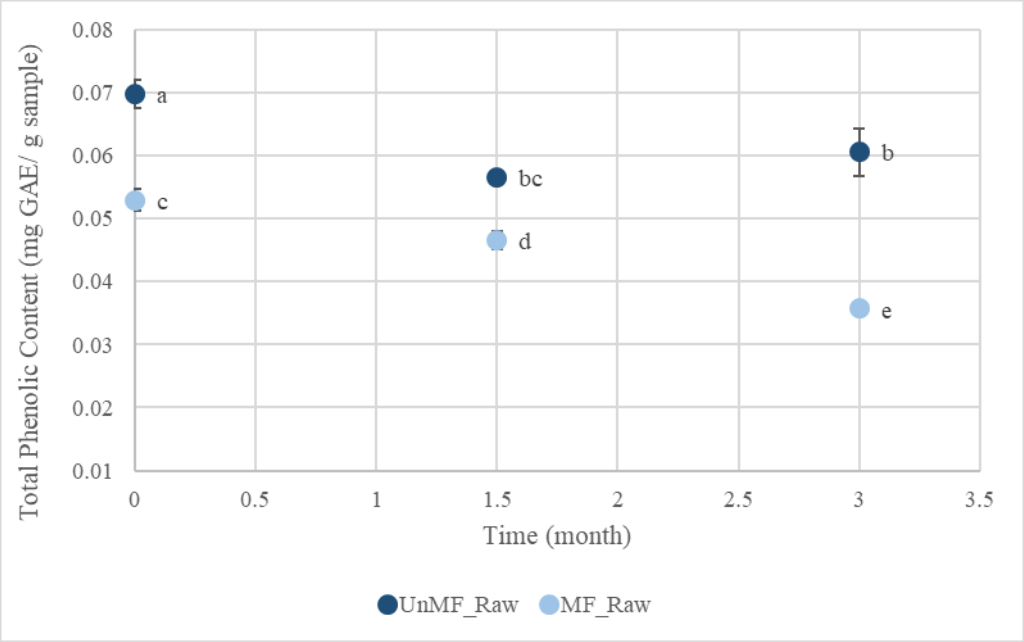

Figure 3.6 Total phenolic content (mg GAE/g sample) of raw olive powders at t=0, 1.5 and 3 month

Total polyphenol content of olive is affected by several factors such as maturity stage, cultivar, age of tree and processing conditions which are amount of water and temperature. Depending on these factors, total phenol content of olive oils changes from 50 to 1000 mg caffeic/kg oil (Aguilera et al., 2005). According to the Garcia et al. (2003), for commercial olive oil it is about 400 mg/kg in terms of caffeic acid equivalent. Furthermore, according to the Tanilgan et al. (2007), total phenol contents of some olive varieties in Turkey which are Gemlik, Kilis, Uslu, Tirilye and Ayvalık are between 22.5 and 97.1 mg gallic acid equivalent/kg oil. These values are close to the experimental results which are shown in Figures 3.4-3.6 (*for the initial samples*). In addition, according to the Bayram et al. (2012), total phenolics of 55 extra virgin olive oil varieties ranged from 40 to 530 mg GAE/kg oil. Also, there is a study about total phenolic content of different monovarietal and commercial Portuguese olive oils. In this research, same method which is Folin–Ciocalteu method is used. Phenolic content of these olive oils changed from 62.77 to 219.7 mg GAE/kg (Gouvinhas et al., 2014). When the results are compared, it is seen that total

phenolic content of raw olive powder used in this study is in the similar range. The results of the raw olive powders are consistent with literature while results of the green and black olive powders were lower than the literature. The reason could probably due to the effect of drying, crushing or microfluidization processes applied to olives. Also, the reason might be due to the fact that the founded olive varieties in the literature are not the same type of the used olives. As it is mentioned before, total phenolics of oils are affected by cultivar, maturation, geographical origin and processing conditions (Vinha et al., 2005).

Total polyphenol content of raw olive is higher than the table olives which are green and black olives seen in Figure 3.4 – 3.6 ($p<0.05$). When maturation of the olive fruit increases, from green olive to ripe olive, polyphenolic compounds decreases and oxidative stability of the olive declines (Gutierez et al., 1999; Salvador et al., 2001). The results of phenolic content of black olive is the lowest which is also consistent with the literature.

Phenolic compounds decreased significantly after microfluidization process in green and raw olive powders while in black olive it didn't show notable reduction. The reason of the decrease could be that phenolic compounds might be destroyed during crushing of olive pulp with water, during grinding or microfluidization process. According to Inarejos-Garcia et al. (2011), crushing stage in olive oil production have a huge impact on concentration of phenolic compounds that affects the final quality of olive oil by changing the volatile compounds and phenolic compounds of the oil. In addition, in the microfluidization process, there is a slight increase in temperature of the tubes and flowing liquid product because of high pressure. Temperature has an important effect on antioxidant activity as well as corresponding phenolic compounds since most phenolic compounds show also antioxidant activity. Volatile constituents and phenolic compounds may have been lost due to the high temperature. It is also possible that water-soluble substances having phenolic

compounds could have diffused from olive to the water during grinding and microfluidization steps (Kiritsakis & Shahidi, 2017).

Many authors have stated that the polyphenols in the virgin olive oil are the most effective antioxidants (Allouche et al., 2007). The contribution of phenolic compounds to the oxidative stability is about 50%. These antioxidants react with lipid radicals in order to form more stable compounds by interrupting the initiation and propagation stages of the oxidative reactions. Folin-Ciocalteu method which is used to measure the total phenolic content of product by colorimetric assay may be insufficient because it doesn't show the compounds really involved in antioxidant activity of the fraction of total phenolics (Aparicio et al., 1999; Gutierrez, 2001).

DPPH & TPC Correlation

Previous studies report that antioxidant activity of plants is well-correlated with their polyphenol contents. According to the Gouvinhas et al. (2014), there is a significant correlation between antioxidant activity and phenolic compounds of Portuguese olive oil samples. In this study, there is a positive correlation between antioxidant activity and total phenolic contents of olive powders ($r=0.799$; $p<0.05$).

3.4 Peroxide Value

Peroxide value for olive powder samples was determined for each types of olives shown in Figure 3.7. For UnMF green olive powder, peroxide value was found as 20.46 ± 2.11 meq O_2 / kg of oil while the result is 27.45 ± 2.12 meq O_2 / kg of oil for MF one ($p>0.05$). The result is 60.17 ± 0.12 meq O_2 / kg of oil for UnMF black olive powder and it is 22.39 ± 0.71 meq O_2 / kg of oil for MF one. In spite of the green olive powder results, there is a significant decrease with microfluidization in black

olive powder ($p<0.05$). Lastly, for UnMF raw olive powder, the result is 5.00 ± 0.02 meq O_2 / kg of oil, for MF one the result is 9.53 ± 0.68 meq O_2 / kg of oil which shows there is significant rise after microfluidization ($p<0.05$).

The International Olive Oil Council (IOC) and European Union (EU) standard for the virgin olive oils is $\leq$20 meq O_2/kg oil, for the olive pomace oils and olive oils it is $\leq$15 meq O_2/kg oil and for the refined olive oils it is $\leq$5 meq O_2/kg oil (International Olive Council, 2019). For the IOC and EU standards, green olive powders are close to the limit of 20 meq O_2/kg oil but exceeds the limit a little. UnMF black olive powders result exceeds the limit too much while MF one exceeds a little. Since olive powder has not been studied in literature yet, comparisons were made with olive oil results in literature.

As it is mentioned before, peroxide value is an indicator for the primary oxidation status of olive oil determined by the presence of hydroperoxides. Raw olive powders' oils are below the limits. According to Halvorsen & Blomhoff (2011), oxidation of oil generally rises when oil is exposed to heat. As peroxides are primary oxidation products, it is expected that peroxide value is higher in MF olive powder because temperature of the olive suspension passing through the microfluidizer's tubes increases during microfluidization process. Also, as water was used for microfluidization process and hydrolysis reactions could take place, resulting in release of free fatty acids from triacylglycerol, subsequently causing oxidation (Ahmed et al., 2016). These reasons could explain the increase in peroxide value after microfluidization process. In the same research, however; 2 out of 4 olive oils the peroxide value is decreased after heating. Because of instability of hydroperoxides to heat, they can decompose to the more stable secondary oxidation products; aldehydes and ketones. In other words, the decomposition of hydroperoxides could be possibly faster than the formation in this case. Furthermore, thanks to the microfluidization process, the oil in the olive powders is expected to be

more preserved due to self-encapsulation. This could explain the decrease in peroxide value in black powders with microfluidization.

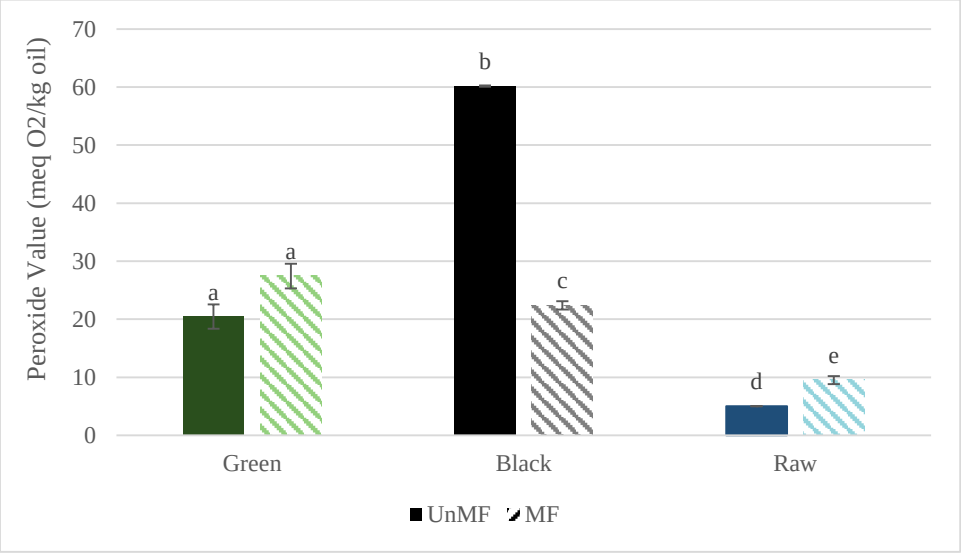

Figure 3.7 Peroxide value (meq O_2/kg oil) of green, black and raw olive powders

When olive types are compared, it is seen that peroxide value of raw, green and black olive are different from each other and raw olive has the lowest peroxide value. Lower peroxide value may be interpreted as a good quality of olive oil. Also, since raw olives have the highest antioxidants among olive types, it can be said that antioxidants prevented the oxidation of lipids in raw olives. Furthermore, according to the study of Yorulmaz et al. (2013), peroxide value has been found either rise, fall or remains constant for two common Turkish olive varieties (Memecik and Edremit) during ripening, and there is slight change between different maturation levels. Peroxide value changes from 2.88 to 4.17 meq O_2/kg oil for Memecik and 2.87 to 5.16 meq O_2/kg oil for Edremit olive. The peroxide value result of UnMF raw olive is closer to those values. According to Demirag & Konuskan (2021), peroxide value of different olives which harvested from Mersin, Osmaniye Adana and Hatay in the

Eastern Mediterranean region of Turkey were ranged from 8.87 to 18.87 meq O_2/kg oil. Also, Yıldırım (2009) found that peroxide values of oils obtained from Erkence and Ayvalık olives was 23.57 and 10.07 meq O_2/kg oil, respectively. Peroxide value results of green, raw and MF black olive powder are close to these values. However, the result of UnMF black olive powder is very high when compared to other results. In another study, where olive fruits at two different maturity stages (purple and black) were analyzed, peroxide value rises with higher temperature except for oils produced from black stage where the rise in temperature ends up with a rise in peroxide value up to a certain point. Further increase in temperature leads to decrease in this quality parameter (Cevik et al., 2017). Since temperature increase occurs due to microfluidization process, it is not surprising to see a drop in peroxide value of black olive powder.

3.5 Free Fatty Acid Content

Free fatty acid content (FFA) of olive powder samples was examined for each types of olives. The free acidity of UnMF and MF green olive powder was found as 32.40 ± 0.43 % and 36.86 ± 0.26 %, respectively. The result is 3.02 ± 0.18 % for UnMF black olive powder while it is 37.21 ± 0.14 % for MF one. There is significant increase in both green and black olive powders after microfluidization is applied ($p<0.05$) as can be seen in Figure 3.8. Finally, it is 0.68 ± 0.04 % and 0.73 ± 0.04 % for UnMF and MF raw olive powder. For raw olive powder, there is not a notable increase in FFA.

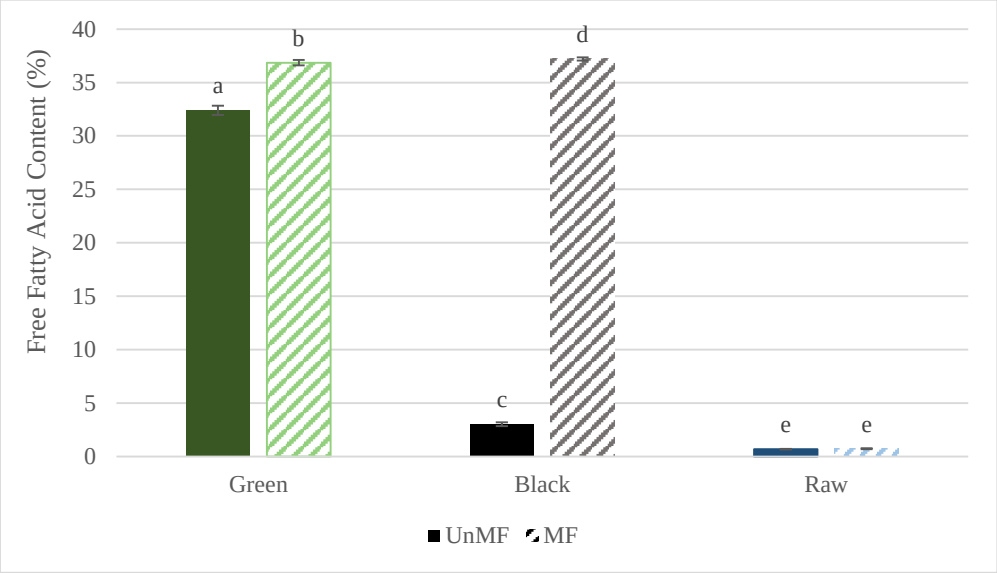

Figure 3.8 Free Fatty Acid Content (%) of green, black and raw olive powders

The fatty acid composition of olive oil is importantly affected by the cultivar, ripeness, climatic condition and irrigation (Demirag & Konuskan, 2021). Free fatty acids result from the hydrolysis of fatty acids from triglycerides in olive. Hydrolysis occurs due to the action of enzymes (lipases) and it is further stimulated by heat, light and water. The effect of crushing and inadequate storage of olive also could cause the hydrolysis of triglycerides (Gharbi et al., 2015).

Free fatty acids are less stable when compared to triglycerides and they are prone to oxidize and cause rancidity (Wei et al., 2013). Hence, FFA content is a quality parameter in olives and it points out how fresh and well handled the olives before milling process (Tena et al., 2015). According to the Konuskan & Mungan (2016), free acidity of oils showed statistically significant differences allied with the varieties, growing area and ripening ($p < 0.05$).

According to a research, free acidity values of extra virgin olive oil samples growing different regions are between 0.28 and 12.69% in terms of oleic acid (Turk, 2016). In the other research, it was found that the free acidity levels ranged from 0.39% to 2.23% for virgin olive oils extracted from Hatay and Mersin Gemlik olives, respectively (Bozdogan Konuskan & Mungan, 2016). According to the Turkish Food Codex, free acidity of extra virgin olive oil (EVOO) must be below the 0.8% oleic acid. Furthermore, FFA of raw olive oil, which is not suitable for consumption, must be below 2% (Türk Gıda Kodeksi Zeytinyağı Ve Pirina Yağı Tebliği, 2017). Since raw olives are not treated with heat or water like table olives, their free acidity is below the limit and this shows its quality. Apart from the both green olive powders and MF black olive powder results, the founded results for UnMF black olive powder and both raw olive powders are close with the literature values. However, UnMF black olive powder results exceeded the limits in the Turkish Food Codex. The increase in free acidity after microfluidization could be because of the water used, heat and crushing steps during processing.

When peroxide value and free fatty acid content of olive powders are compared, it is observed that peroxide value of UnMF black olive powder gave high results. On the other hand, free acidity of UnMF black olive powder result was low. Since free fatty acids are less stable when compared to triglycerides and they are prone to oxidize, the free fatty acids found in UnMF black olive powder could had been participated oxidation reactions resulting in decrease in FFA content and as well as increase in peroxide value (Wei et al., 2013).

3.6 Rheological Properties of Olive Powder Suspensions

The shear stress (τ) versus shear rate (γ) data for all MF and UnMF olive powder suspensions were fitted well to Herschel-Bulkley model at 25 ℃ (Eq. 3.1) (Sahin & Sumnu, 2006).

$$\tau = \tau_0 + k\,(\gamma)^n \qquad (3.1)$$

In the equation, τ (Pa) represents shear rate, τ_0 (Pa) represents yield stress, γ (s^{-1}) is shear rate, k ($Pa.s^n$) is the consistency coefficient, and n is flow behavior index.

Table 3.1 Herschel-Bulkley parameters of olive powder suspensions at 25 ℃.

Sample	**τ_0 (Pa)**	**k ($Pa.s^n$)**	**n**
UnMF_Green 15%	2.31	0.88	0.84
UnMF_Green 40%	161.94	15.28	0.64
UnMF_Green 60%	412.35	25.11	0.49
MF_Green 15%	86.48	23.02	0.69
MF_Green 40%	457.21	138.31	0.53
MF_Green 60%	1337.50	125.87	0.56
UnMF_Raw 15%	1.44	0.53	0.99
UnMF_Raw 40%	148.98	13.43	0.70
UnMF_Raw 60%	383.21	13.86	0.56
MF_Raw 15%	108.45	20.15	0.64
MF_Raw 40%	1173	148.26	0.53
MF_Raw 60%	1954.40	224.19	0.49

Herschel-Bulkley model parameters' results for green and raw olive samples are shown in Table 3.1. Shear thinning (pseudoplastic) behavior was seen for all samples as flow behavior index is lower than 1 (n<1). Fruit and vegetable products such as banana puree or concentrated fruit juices are the examples of pseudoplastic fluids in food systems. Olive powder solutions also can be categorized as vegetable products. Generally, in fruit and vegetable products, the consistency coefficient (k) rises exponentially while n declines slightly with concentration (Krokida et al., 2001). As can be seen in Table 3.1, nearly for all samples k values increases exponentially while n decreases slightly with the increase in concentration indicating that results are compatible with literature. The flow behavior index changes between 0.49 to 0.99. UnMF raw olive powder at concentration of 15% showed the highest n value as 0.99 meaning that it behaves like Newtonian fluid (n=1) (Sahin & Sumnu, 2006).

A certain amount of yield stress was observed in olive powders suspensions. Yield stress can be explained as minimum stress needed to apply to start the flow. Thus, rises in yield stress with microfluidization and concentration shows that more forces are needed to deform olive suspensions (Mert et al., 2014). MF suspensions showed much higher yield stress and consistency index values than UnMF ones. Microfluidization enables higher water holding capacity by forming finer particles with branched structure and larger surface area. More water could be bound because of the hydroxyl groups present in fiber structure, therefore; it causes to lower available water in product. According to Mert et al. (2014), the presence of high amount of water-binding macromolecules like fibers, emulsifiers and hydrocolloids is regarded to increase the mixing characteristics and shelf life of baked food products. The reason of the highest yield stress and consistency index values could be the higher water holding capacity of MF powder suspensions (Demirkesen et al., 2010).

The flow curves in Figures 3.9-3.12 show shear stress values at different shear rates for raw and green olive powder suspensions at 15%, 40% and 60% concentrations. Since physically there was no big difference between green and raw olive powder suspensions, black olive powder suspension was not analyzed.

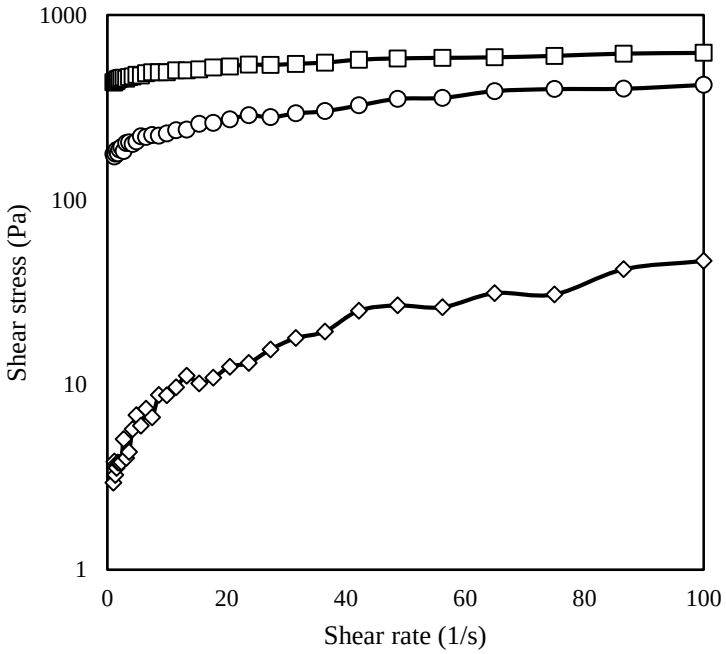

Figure 3.9 Flow curves obtained for UnMF green olive powder solutions at different concentrations. (□): 60%, (○): 40%, (◊): 15%.

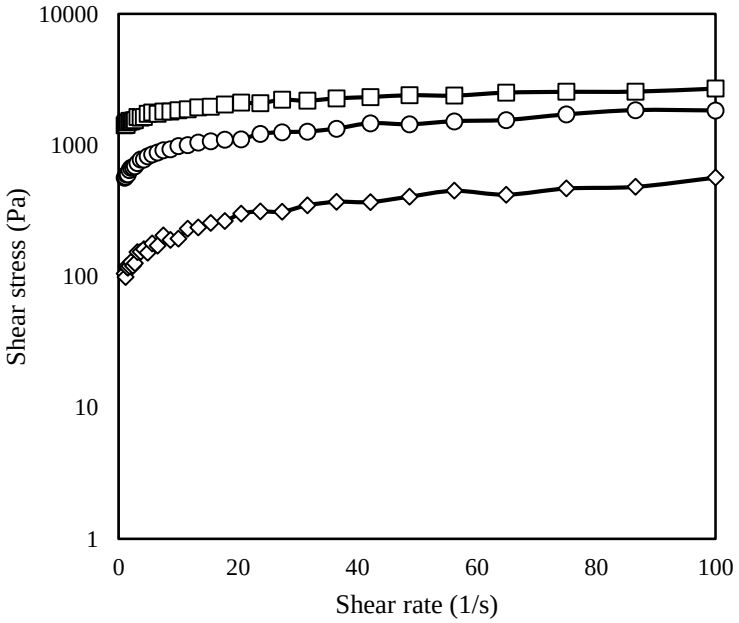

Figure 3.10 Flow curves obtained for MF green olive powder solutions at different concentrations. (□): 60%, (○): 40%, (◊): 15%.

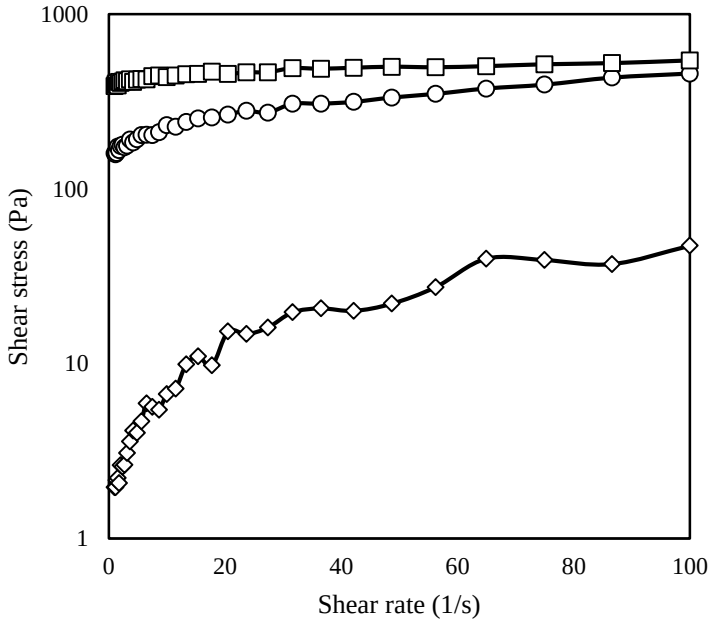

Figure 3.11 Flow curves obtained for UnMF raw olive powder solutions at different concentrations. (□): 60%, (○): 40%, (◊): 15%.

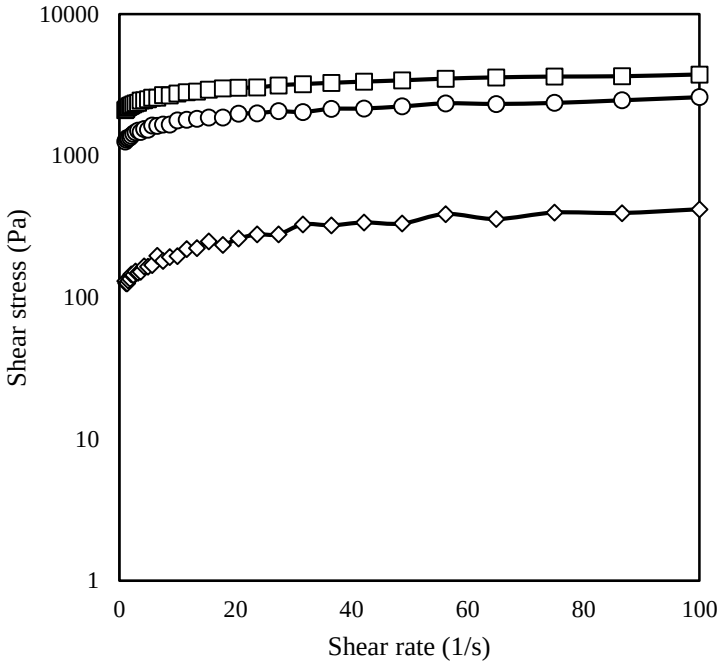

Figure 3.12 Flow curves obtained for MF raw olive powder solutions at different concentrations. (□): 60%, (○): 40%, (◊): 15%.

As can be seen in Figures 3.9-3.12, shear stress versus shear rate data shows that higher viscosity values were achieved at higher concentrations. That is, when powder concentration increases, viscosity rises which is an expected result. Furthermore, both green and raw MF olive powder solutions for each concentration resulted higher viscosity than UnMF ones. Since microfluidization creates finer particles and larger surface area, it causes increase in water absorption by powder particles. As a result of this, viscosity rises in MF powder suspensions (Demirkesen et al., 2010). In addition, for the concentration of 60% in all suspensions, shear stress values remained relatively constant with the increase in frequency (0.01 – 100 Hz) when compared to other concentrations. Since its concentration is very high, it acts like solid and doesn't show much increase in shear stress at higher frequencies.

Figures 3.13-3.16 illustrate the elastic (storage) modulus (G') and viscous (loss) modulus (G") of olive powder solutions. G' and G" values rose with frequency in all samples, according to the results. These powder suspensions have viscoelastic behavior, which means they are viscous and elastic at the same time. The elastic moduli values in all samples were found to be greater than the viscous moduli values, indicating that the olive powder suspensions behave solidly. Furthermore, MF olive powder suspensions had larger elastic and viscous moduli than UnMF olive powder suspensions. Because microfluidization produces greater shear rates for longer periods of time, more hydrophilic moieties are formed, resulting in larger moduli values. Microfluidizers, on the other hand, provide a more uniform particle dispersion, as previously stated. Microfluidization produces finer particles by maintaining a constant pressure throughout the process (Mert, 2012). It may be stated that when the concentration of powders decreases, the difference between G' and G" values decreases. Figures 3.13–3.16 indicate that all samples followed the same pattern. The difference between G' and G" values reduces as the water content rises. This is to be anticipated since water dilutes the dispersions, making the sample act like a viscous liquid rather than an elastic solid. There were crossover frequencies for G' and G" values in the green olive powder suspensions shown in Figures 3.13–3.14 at a concentration of 15%. The loss modulus rose more at first, indicating that it acts like a liquid product. However, this crossover was seen at some sites, indicating that this is the position of the viscous-to-elastic transition. In other words, the suspension behaved similarly viscous and elastically at that frequency. After this threshold, elastic modulus takes precedence over viscosity (Masalova et al., 2011). The crossover was observed at lower frequencies in the MF powder suspension when compared to the UnMF one. Since microfluidization results in fibrous structure and larger surface area, it behaves like solid at lower frequencies which was an expected result (Yildiz et al., 2016).

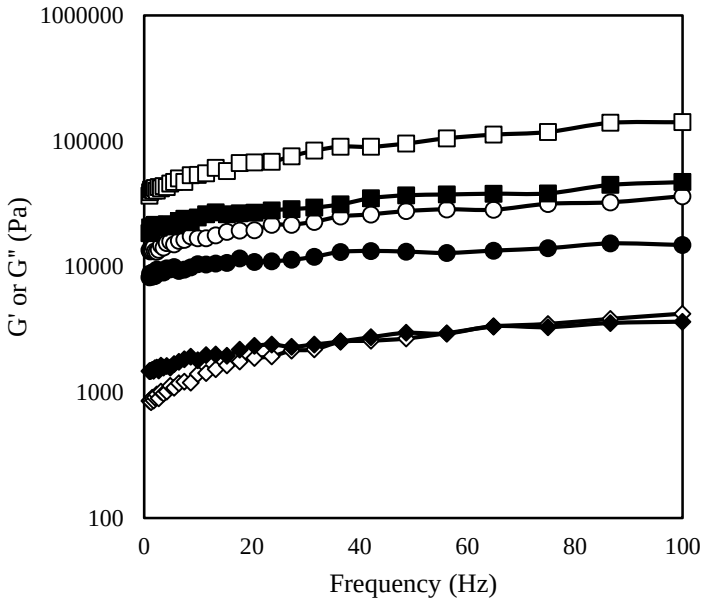

Figure 3.13 Elastic and viscous modulus obtained for UnMF green olive powder solutions at different concentrations. (□): G' at 60%, (○): G' at 40%, (◊): G' at 15%, (■) G" at 60%, (●): G" at 40%, (♦): G" at 15%.

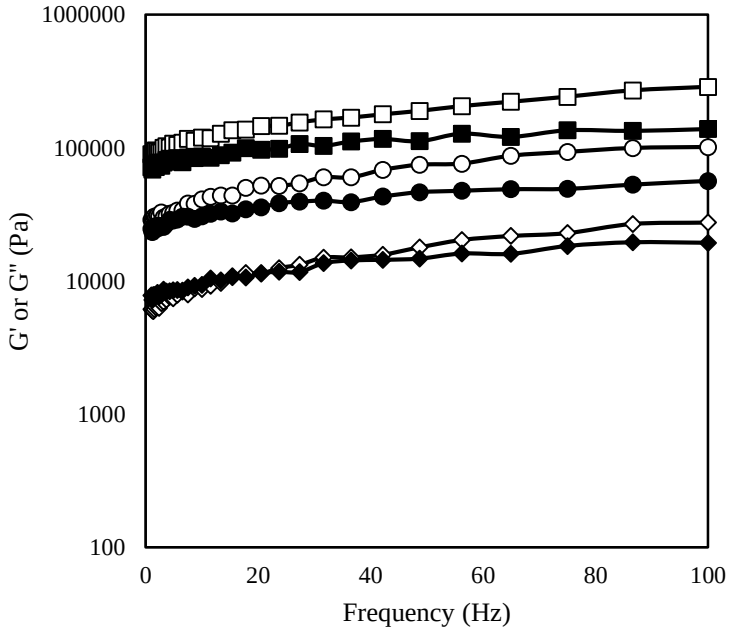

Figure 3.14 Elastic and viscous modulus obtained for MF green olive powder solutions at different concentrations. (□): G' at 60%, (○): G' at 40%, (◊): G' at 15%, (■) G" at 60%, (●): G" at 40%, (♦): G" at 15%.

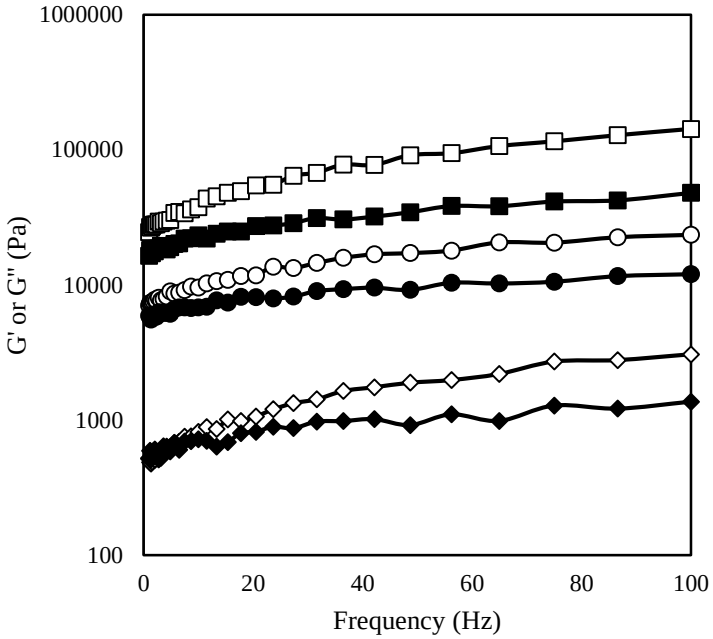

Figure 3.15 Elastic and viscous modulus obtained for UnMF raw olive powder solutions at different concentrations. (□): G' at 60%, (○): G' at 40%, (◊): G' at 15%, (■) G" at 60%, (●): G" at 40%, (♦): G" at 15%.

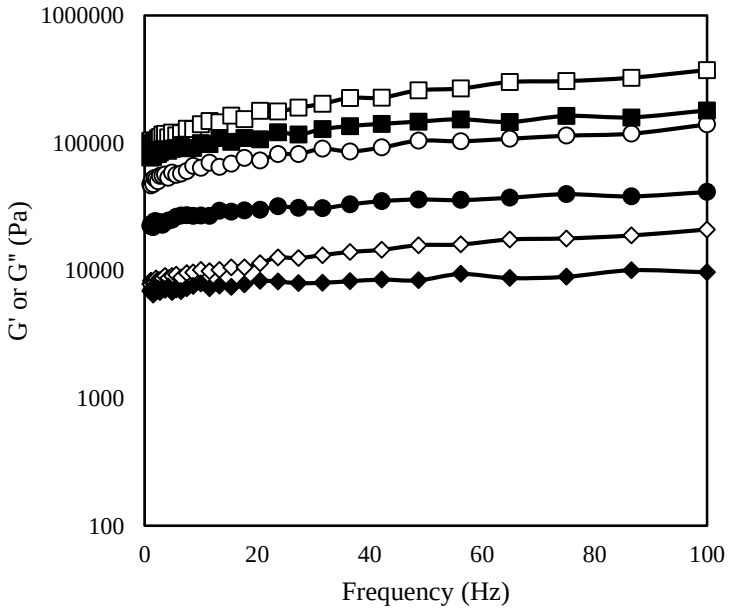

Figure 3.16 Elastic and viscous modulus obtained for MF raw olive powder solutions at different concentrations. (□): G' at 60%, (○): G' at 40%, (◊): G' at 15%, (■) G" at 60%, (●): G" at 40%, (♦): G" at 15%.

3.7 Stability Analysis

The stability of green and raw olive powder solutions at 60%, 40%, and 10% was investigated. MF olive powder solutions were like solid dough at a concentration of 60% because MF olive powders have a granular shape that allows for a large surface area when combined with water, as seen in Figures 3.18 and 3.20 therefore it couldn't be analyzed. In addition, the microfluidization procedure, according to Mert et al. (2014), enhances the water holding capacity of samples, resulting in more stable product forms. That is, this process results in long intertwined fibrous structure thus it can hold more water in its structure. In the same study, less water separation was observed in microfluidized wheat bran samples. Backscattering (ΔBS, %) is measured at different periods to track changes in droplet size and phase separation in olive powder solutions. Figure 3.21 - 3.24 shows that olive suspensions prepared with MF olive powders are more stable than those prepared with UnMF olive powders when the impact of the microfluidization process is taken into account. That example, at a 40% concentration of UnMF suspensions, the backscatter light is bigger and the curve varies, but at the same concentration of MF suspensions, there is no variation on the baseline. When the influence of powder concentration is examined, it is clear that a drop in concentration in UnMF suspensions leads in increased backscatter light and curve variation, indicating that the suspensions are not particularly stable at low concentrations within 60 hours. At 40% concentration, there is no variation, while slight variations are detected at 10% concentration for MF ones. Finally, owing to the enhanced water holding capacity of powders, more stable olive powder solutions may be created using the microfluidization approach.

Also, TSI graphs of suspensions are shown in Figures 3.25 – 3.28. In all samples, TSI values increases with decrease in concentration. According to Polowczyk et al. (2015), high TSI value indicates a high variation in particle size or concentration of the system, presenting unstable system. It can be concluded that at higher concentrations, more stable suspensions were obtained. Also, Du et al. (2022) states

that higher TSI values represents less stable samples. When the effect of microfluidization is taken into account, that example at 10% concentration of UnMF green olive powder suspensions, TSI value is around 20, while it is 2 for MF one after reaching the plateau. Therefore, lower TSI value indicates that microfluidization results in more stable suspensions showing that the results are compatible with literature. When raw olive powder suspensions were analyzed, it can be said that they had slightly higher TSI results when compared to green olive powder suspensions. Thereby, less stable suspensions were obtained in raw olive powders when compared to green one.

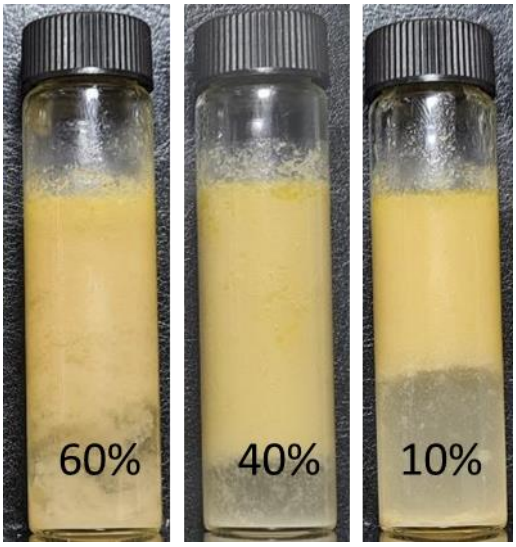

Figure 3.17 UnMF green olive powder emulsions at different concentrations; 60%, 40% and 10%

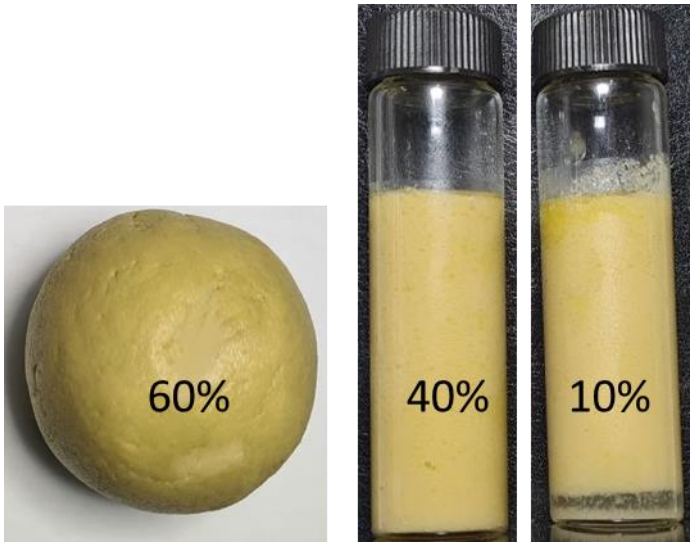

Figure 3.18 MF green olive powder emulsions at different concentrations; 60%, 40% and 10%

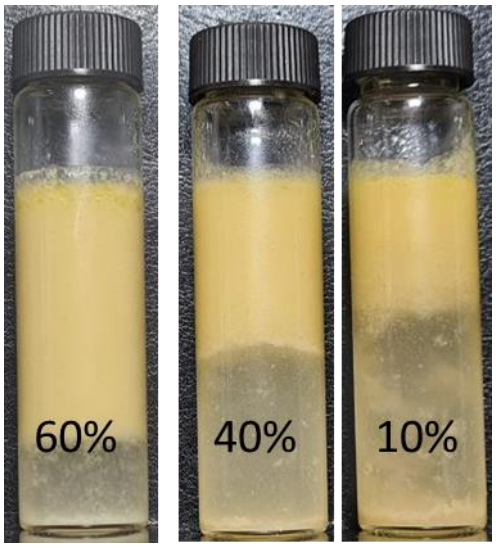

Figure 3.19 UnMF raw olive powder emulsions at different concentrations; 60%, 40% and 10%

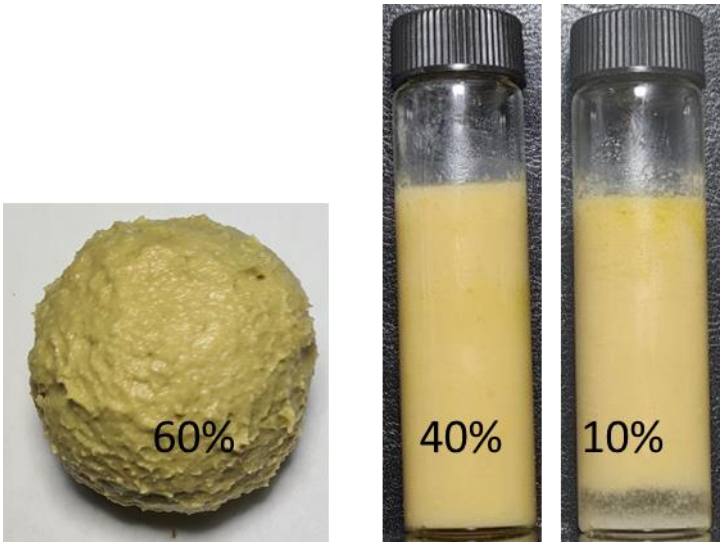

Figure 3.20 MF raw olive powder emulsions at different concentrations; 60%, 40% and 10%

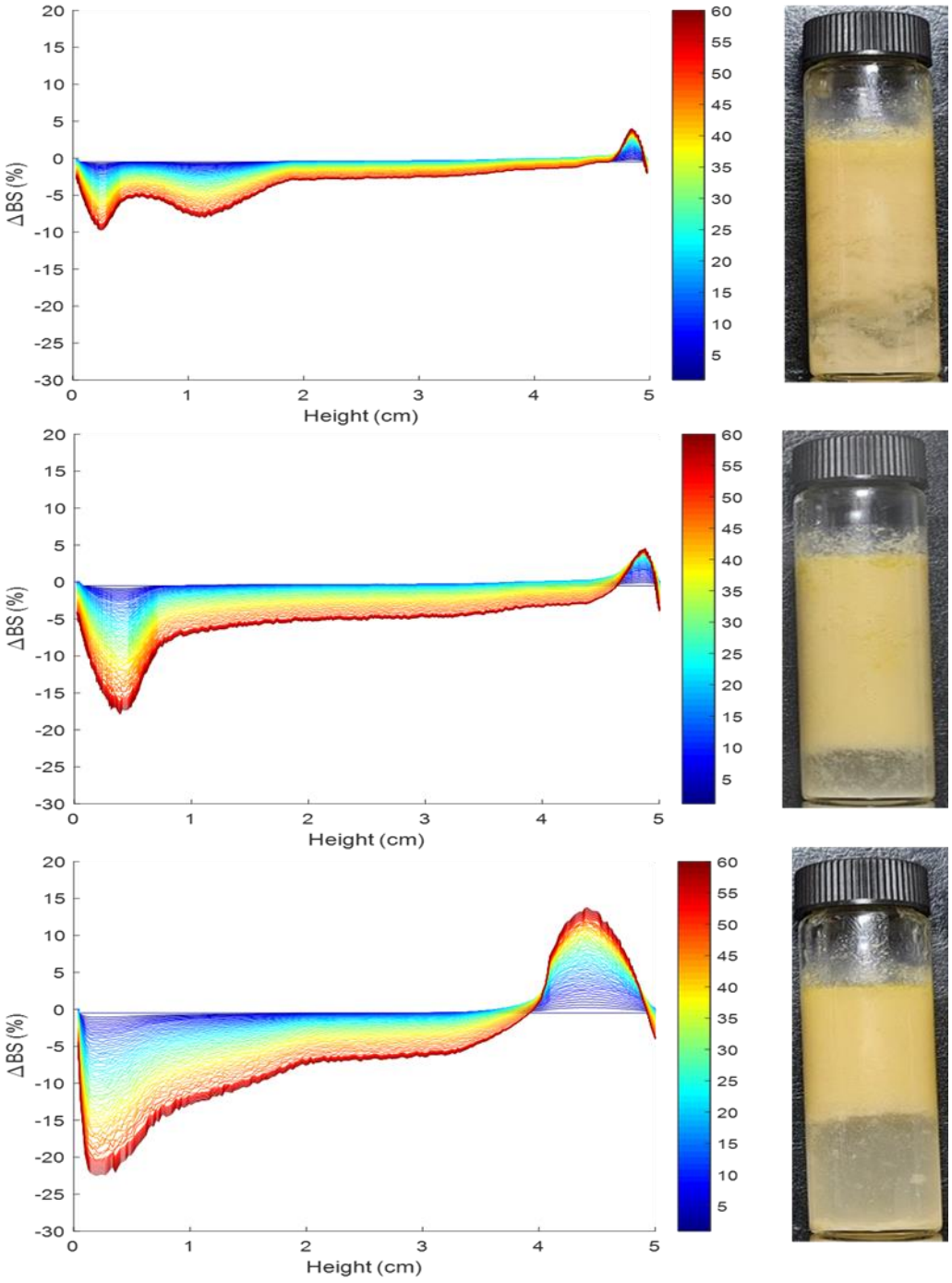

Figure 3.21 Delta backscattering (ΔBS) profiles of UnMF green olive powder solutions at concentrations of 60%, 40% and 10% (from top to bottom).

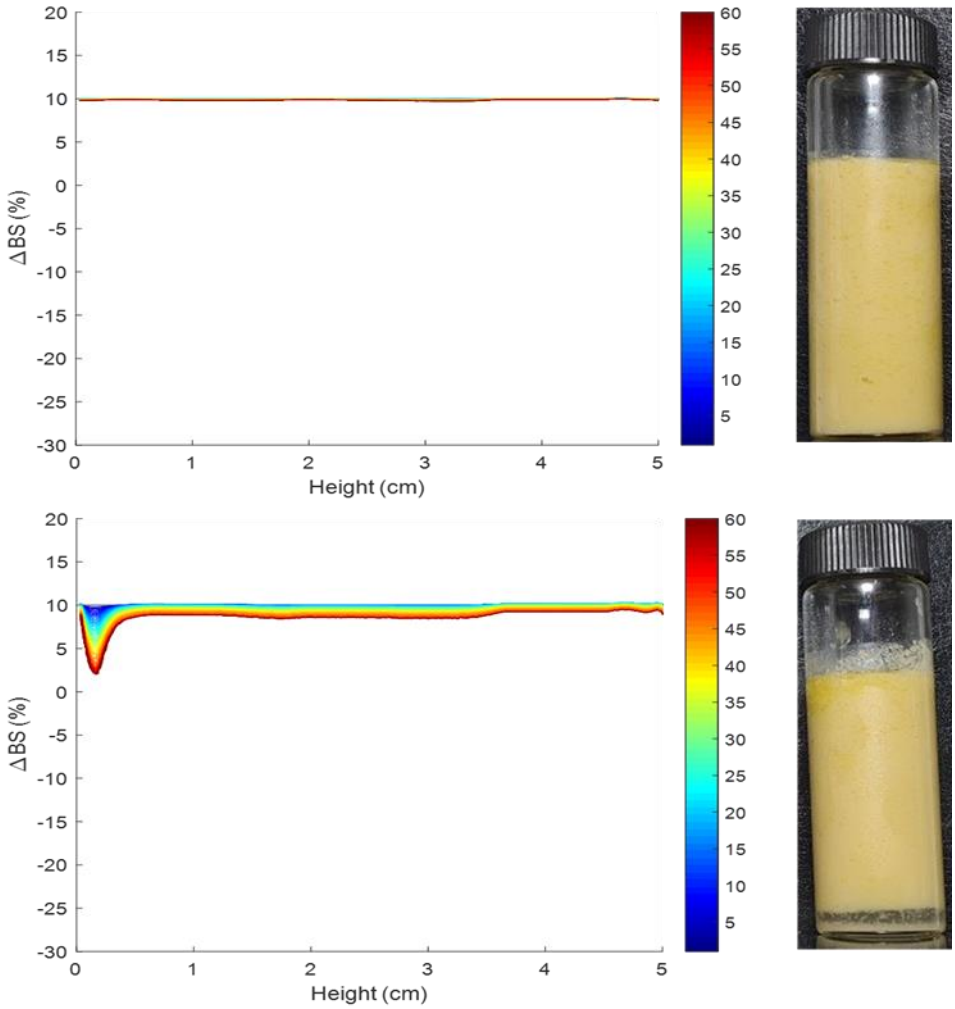

Figure 3.22 Delta backscattering (ΔBS) profiles of MF green olive powder solutions at concentrations of 40% and 10% (from top to bottom).

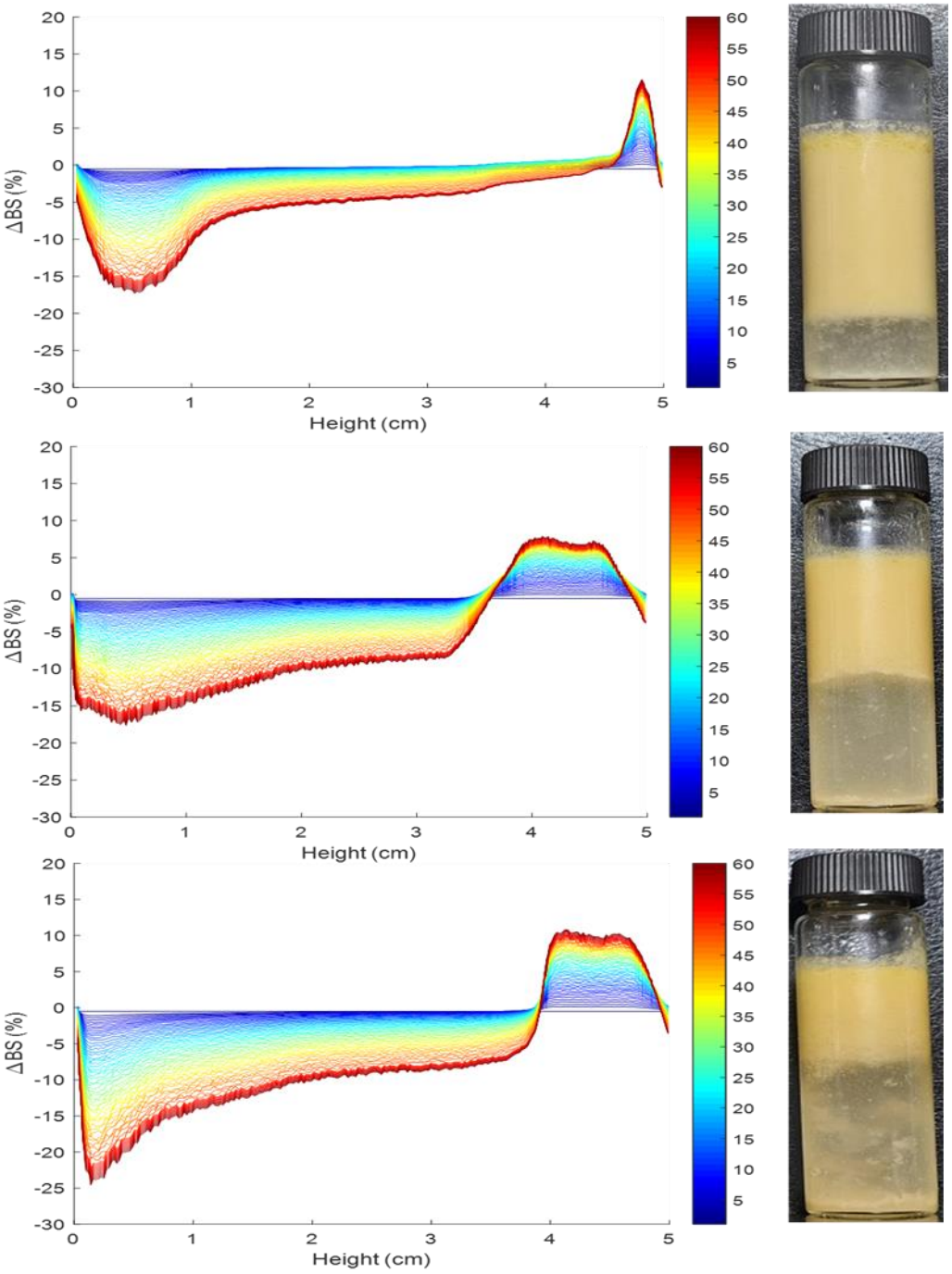

Figure 3.23 Delta backscattering (ΔBS) profiles of UnMF raw olive powder solutions at concentrations of 60%, 40% and 10% (from top to bottom).

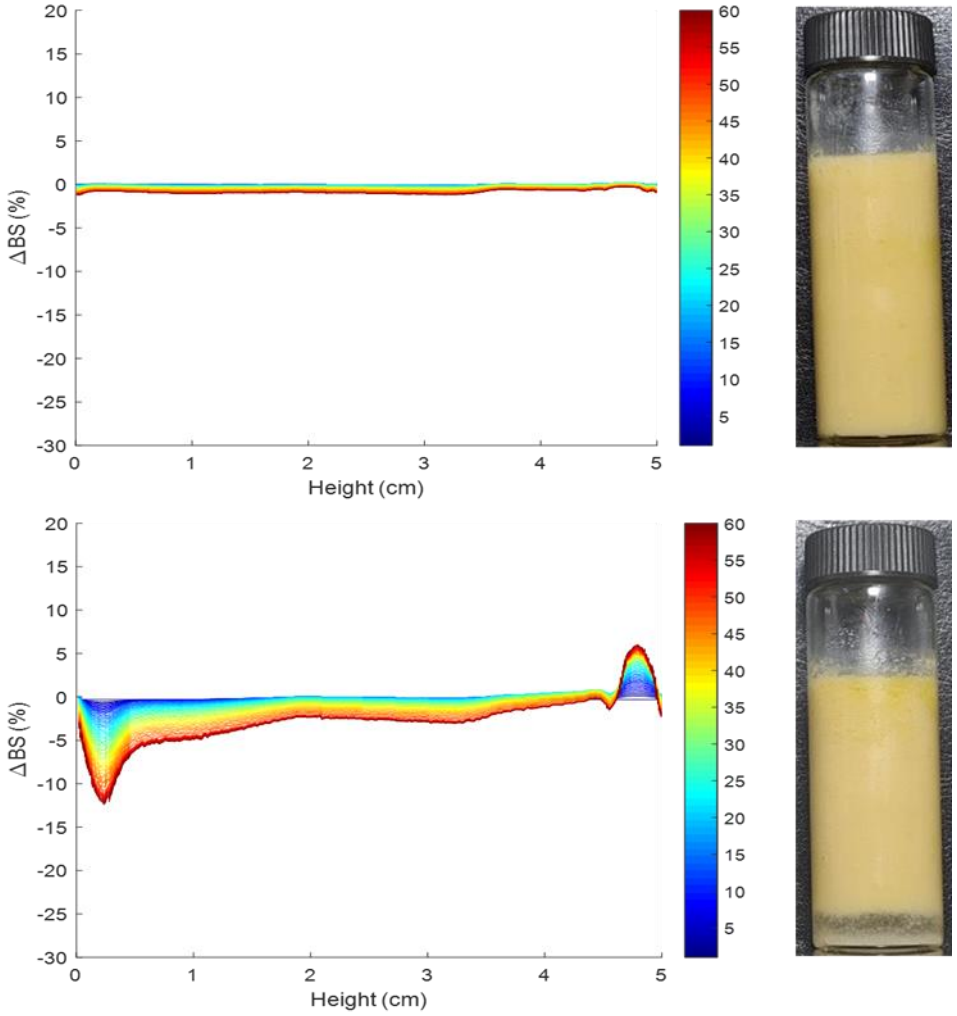

Figure 3.24 Delta backscattering (ΔBS) profiles of MF raw olive powder solutions at concentrations of 40% and 10% (from top to bottom).

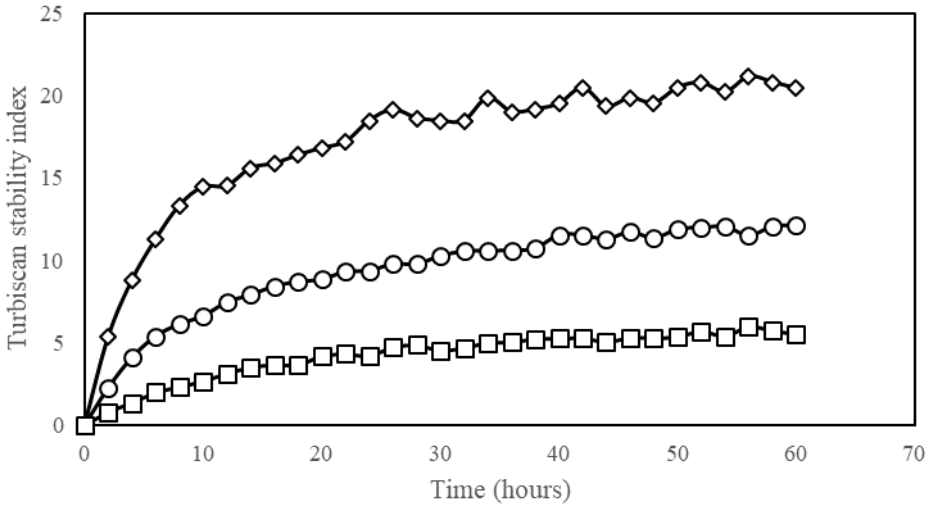

Figure 3.25 Turbiscan Stability Index values for UnMF green olive powder suspensions at different concentrations. (□): 60%, (○): 40%, (◊): 10%.

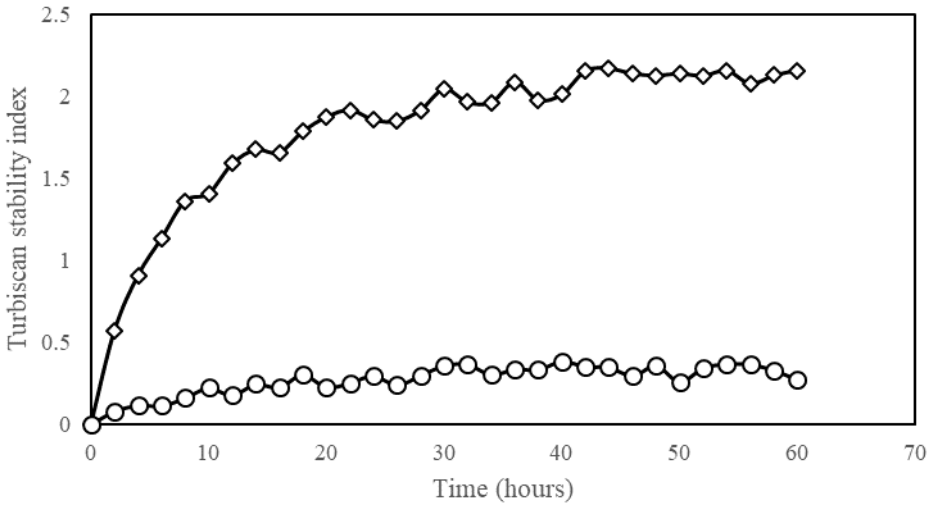

Figure 3.26 Turbiscan Stability Index values for MF green olive powder suspensions at different concentrations. (○): 40%, (◊): 10%.

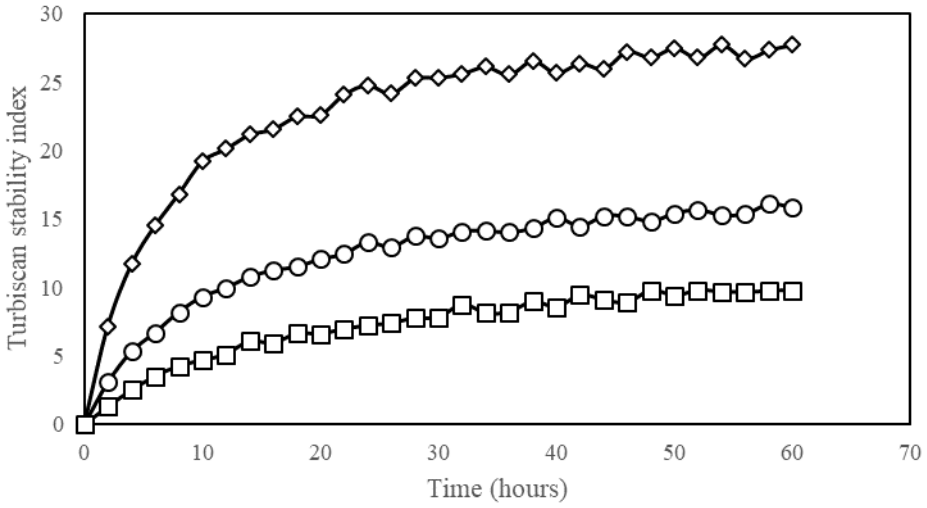

Figure 3.27 Turbiscan Stability Index values for UnMF raw olive powder suspensions at different concentrations. (□): 60%, (○): 40%, (◊): 10%.

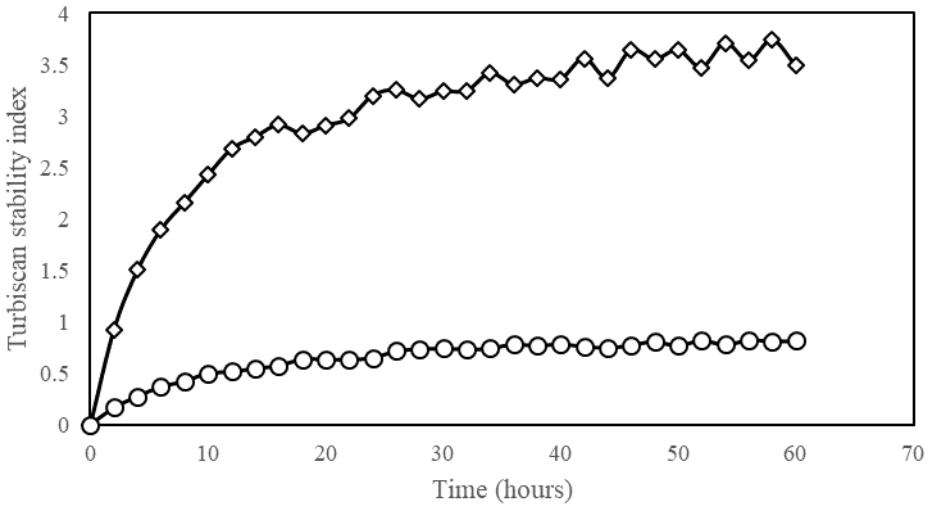

Figure 3.28 Turbiscan Stability Index values for MF raw olive powder suspensions at different concentrations. (○): 40%, (◊): 10%.

3.8 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) analysis is significant to observe morphological differences. In this study, SEM analysis were conducted for UnMF and MF green olive powder in order to see the effect of microfluidization process. Black and raw olive powders were not investigated because the aim of this analysis was to see the effect of microfluidization process on microstructure of olive powders, thus one olive type was enough for the analysis. SEM images showed that microfluidization process could cause encapsulation by trapping the oil droplets inside of the olive. In this study, after microfluidization of olives, bigger oil droplets could become smaller and encapsulated with the substances in the olive itself. This observation in MF olive powder resulted in smaller oil droplets outside of the powder when compared to UnMF one as seen in Figure 3.29 and Figure 3.30. In Fig. 3.29, a more oily surface was observed, however; in Fig. 3.30, less oil is seen outside of the particles.

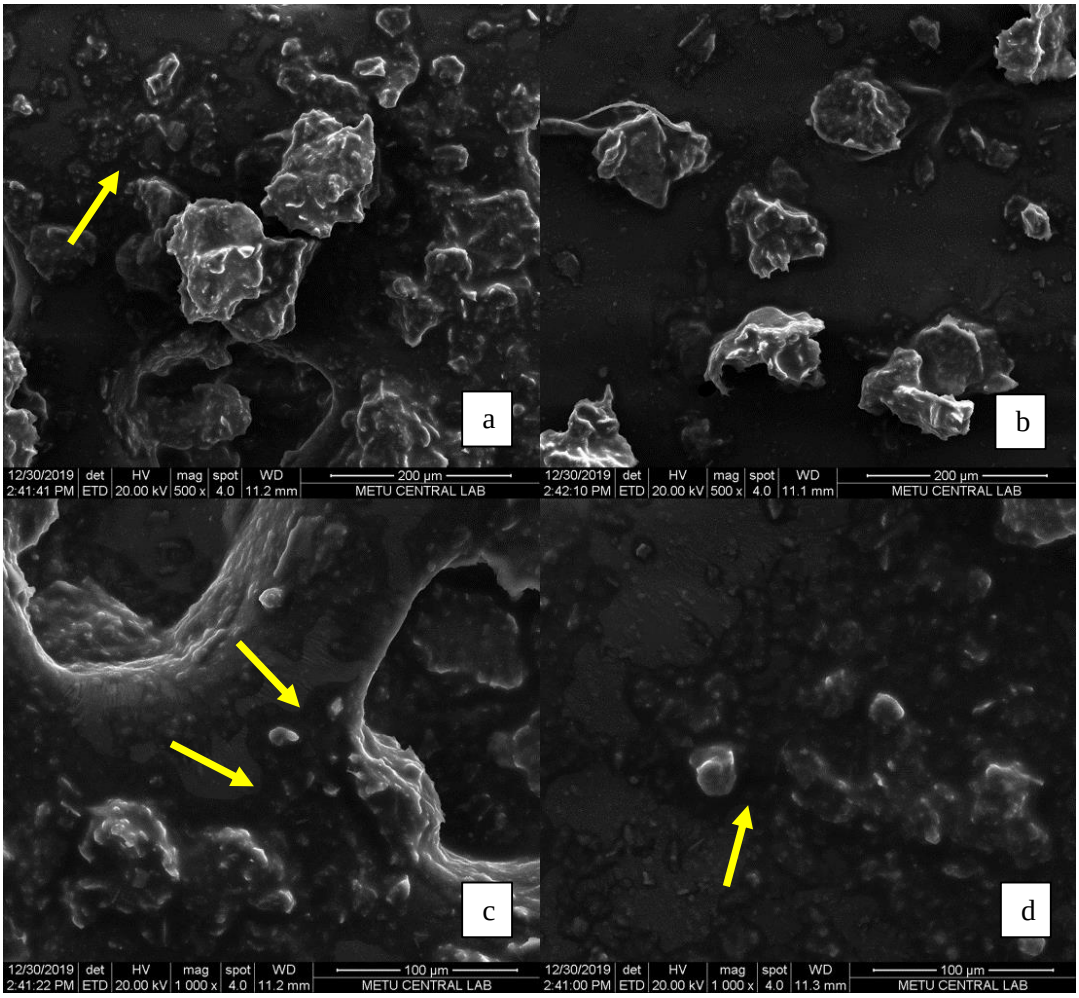

Figure 3.29 Scanning electron microscope images of UnMF green olive powder Magnification: 500× (a), (b) and 1000× (c), (d)

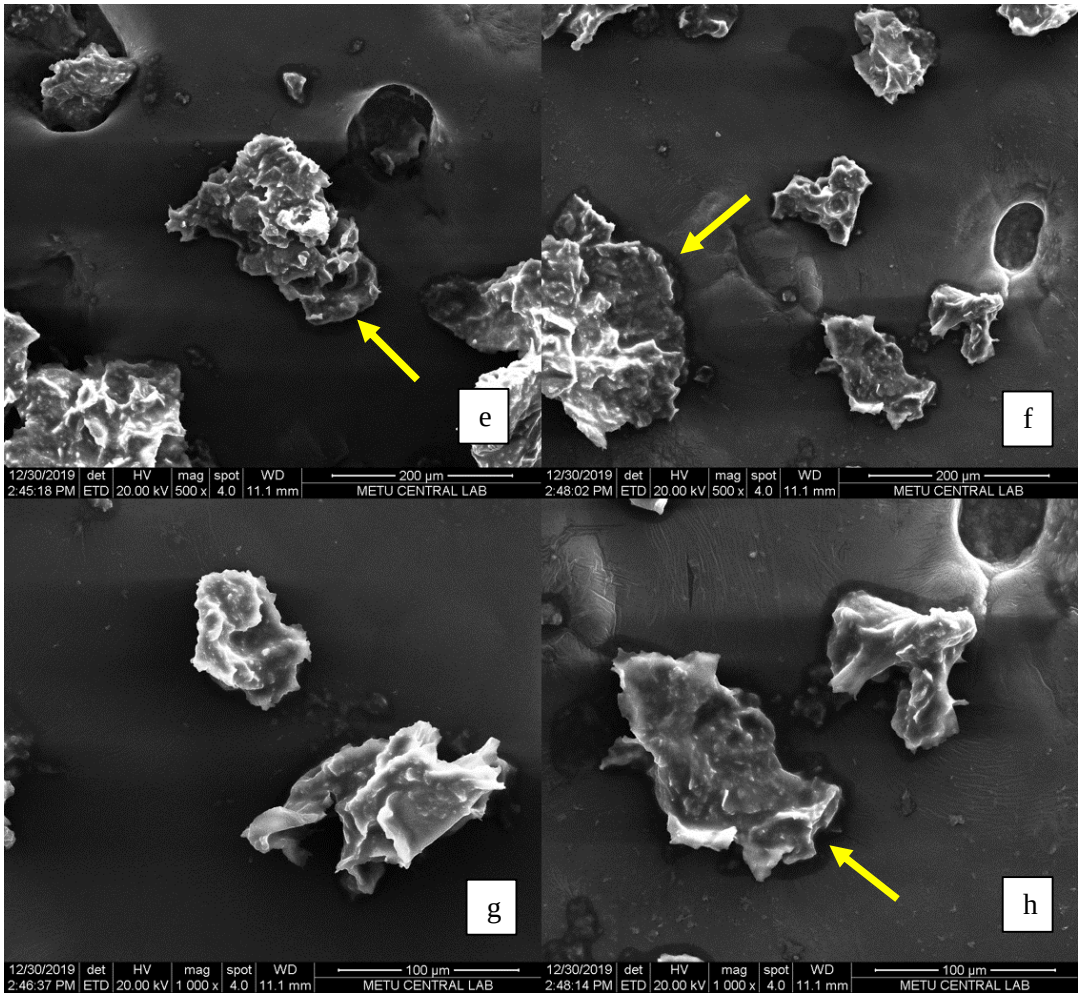

Figure 3.30 Scanning electron microscope images of MF green olive powder Magnification: 500× (e), (f) and 1000× (g), (h)

3.9 Color

Color of a food product is an important parameter to comprehend the product quality and may influence consumers' perception. Color changes of food products could be associated with denaturation, gelatinization or browning reactions that take place during food processing. In this study, CIELAB model was used to evaluate color changes and ΔE values were calculated by using L*, a*, b* color components to see differences between olive powders (Özcan, 2008). The results are given in Table 3.2.

Table 3.2 CIELAB constants of the olive powders at the first day of storage

Sample	L^*	a^*	b^*	ΔE
UnMF_Green	44.64 ± 0.28^{c}	5.45 ± 0.12^{a}	21.91 ± 0.45^{b}	89.42 ± 0.17^{b}
MF_Green	60.00 ± 3.46^{a}	3.86 ± 0.34^{b}	26.93 ± 0.08^{a}	36.34 ± 0.14^{d}
UnMF_Black	25.88 ± 0.17^{e}	0.08 ± 0.01^{d}	0.25 ± 0.02^{f}	30.89 ± 0.18^{e}
MF_Black	38.31 ± 0.16^{d}	1.40 ± 0.04^{c}	3.05 ± 0.05^{e}	5.22 ± 0.62^{f}
UnMF_Raw	34.84 ± 0.04^{d}	-0.68 ± 0.05^{e}	12.28 ± 0.07^{d}	102.52 ± 0.07^{a}
MF_Raw	51.70 ± 0.06^{b}	1.47 ± 0.01^{c}	14.28 ± 0.07^{c}	42.53 ± 0.08^{c}

Small letters (a-f) represents that they differ significantly for each sample in the same column ($p<0.05$).

Results clearly shows that MF olive powders had higher lightness values (L*) than UnMF ones ($p<0.05$). MF green olive powder had higher L* (lightness/darkness) value than UnMF one as seen easily in Figure 3.31. Lightness value has changed significantly in olive powder types ($p<0.05$). Color of green olive powder turns black because of oxidation reactions. Enzymatic browning reactions is a widespread phenomenon that take place due to the mechanical injury, during processing of fruit or post-harvest storage. Polyphenol oxidase (PPO) is the main enzyme causing browning (Martinez & Whitaker, 1995). As microfluidization process acts like making encapsulation by trapping oils, cell structures are protected more due to less exposure to oxygen in MF olive powder when it is compared with UnMF olive powder. Therefore, MF olive powders are less exposed to oxygen and less oxidation reactions occur. Results of a* (redness/greenness) and b* (yellowness/blueness) indicates that there are significant differences between olive powder samples ($p<0.05$). Yellowness of MF olives were higher than UnMF ones.

ΔE results demonstrates color differences between samples. The results showed that there is significant difference between MF and UnMF olive powders and also among all olive powders ($p<0.05$).

Figure 3.31 UnMF (left) and MF (right) green olive powder

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In this thesis, production and characterization of new value-added powder products from olive were studied. Microfluidization technique was used as a size reduction process to reduce the size of the big oil droplets present in olive fruit. Green, black and raw olive powders were obtained with microfluidization and dried with freeze drying method. For comparison, olive powders were also obtained from conventional freeze-drying method. Peroxide values were performed for the measurement of lipid oxidation products. Moreover, for the physical and chemical characterization of olive powders, antioxidant activity (DPPH assay), total phenolic content and lipolysis (free fatty acid content) were conducted. Furthermore, for the characterization of powders, color, moisture content, stability measurements, rheology and microscopic analysis (Scanning Electron Microscopy) were conducted.

When the effect of microfluidization is investigated, it can be concluded that antioxidant activity was not affected significantly for all olive powders. However, total phenolic content decreased for green and raw olive powders. In addition, when time effect was observed, antioxidant activities decreased with time for all powders except raw olive powder. However, total phenolic contents decreased with time for green and raw olive powder. Among all olive powders, raw olive gave the highest antioxidant activity and phenolic content.

Peroxide values of olive powders were studied for the oxidation stability of powders. Except from the UnMF black olive powder, the other powder results were closer to the literature values. When olive types are compared, it is seen that raw olive has the

lowest peroxide value indicating a good quality of olive oil. Furthermore, free acidity of raw olive powder was below the limit so this shows its quality.

In the microscopic analysis of olive powders, the effect of microfluidization operation was clearly observed. SEM images show that microfluidization process results in finer and dry particles encapsulated by trapping the oil droplets inside of the olive. Stability analysis and rheological measurements of olive powder suspensions revealed that microfluidization technique is a good way to obtain more stable suspensions having higher water holding capacity.

Since there is no literature study about production and characterization of MF olive powders, this study was vital. As future study, bioavailability of olive powders in *vitro* digestion fluids may be investigated.

REFERENCES

Ahmed, M., Pickova, J., Ahmad, T., Liaquat, M., Farid, A., & Jahangir, M. (2016). Oxidation of lipids in foods. *Sarhad Journal of Agriculture, 32*(3), 230-238.

Aguilera, M.P., Beltr´an, G., Ortega, D., Fern´andez, A., Jim´enez, A., & Uceda, M. (2005). Characterisation of virgin olive oil of Italian olive cultivars: 'Frantoio' and 'Leccino', grown in Andalusia. *Food Chemistry, 89,* 387–391.

Allouche, Y., Jiménez, A., Gaforio, J. J., Uceda, M., & Beltrán, G. (2007). How heating affects extra virgin olive oil quality indexes and chemical composition. *Journal of Agricultural and Food Chemistry, 55*(23), 9646–9654. https://doi.org/10.1021/jf070628u

AOCS. (2004). Method Ca 5a-40: Free Fatty Acids. Official methods and recommended practices of the American Oil Chemists' Society, 5th ed., American Oil Chemists' Society. Champaign, IL, USA.

Aparicio, R., Roda, L., Albi, M. A., & Gutiérrez, F. (1999). Effect of various compounds on virgin olive oil stability measured by Rancimat. *Journal of agricultural and food chemistry, 47*(10), 4150-4155.

Ayyıldız, H. F., & Hüseyin, K. (2015). Yemeklik Yağlardaki Serbest Yağ Asidi Düzeyinin Standart Metotlara Alternatif Geliştirilen Akış Enjeksiyon Analiz Metoduyla Belirlenmesi. *Selçuk Üniversitesi Fen Fakültesi Fen Dergisi,* (40), 23-40.

Bayram, B., Esatbeyoglu, T., Schulze, N., Ozcelik, B., Frank, J., & Rimbach, G. (2012). Comprehensive Analysis of Polyphenols in 55 Extra Virgin Olive Oils by HPLC-ECD and Their Correlation with Antioxidant Activities. *Plant Foods for Human Nutrition, 67*(4), 326–336. https://doi.org/10.1007/s11130-012-0315-z

Benavente-Garcia, O., Castillo, J., Lorente, J., Ortuno, A., & Del-Rio, J.A. (2000) Antioxidant activity of phenolics extracted from Olea europaea L. leaves. *Food Chemistry 68*, 457–462.

Bitik, A., Sumnu, G., & Oztop, M. (2019). Physicochemical and structural characterization of microfluidized and sonicated legume starches. *Food and Bioprocess Technology*, *12*(7), 1144-1156.

Borcakli, M., Ozturk, T., Uygun, N., & Gozum, E. (2014). U.S. Patent No. 2014/0178560 A1. United States Patent Application Publication.

Bozdogan Konuskan, D., & Mungan, B. (2016). Effects of Variety, Maturation and Growing Region on Chemical Properties, Fatty Acid and Sterol Compositions of Virgin Olive Oils. *JAOCS, Journal of the American Oil Chemists' Society, 93*(11), 1499–1508. https://doi.org/10.1007/s11746-016-2904-8

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology, 28*(1), 25–30. doi:10.1016/s0023-6438(95)80008-5

Cevik, S., Aydin, S., Sermet, O. S., Ozkan, G., & Karacabey, E. (2017). Optimization of Olive Oil Extraction Process by Response Surface. *Akademik Gıda, 15*(4), 337–343. https://doi.org/10.24323/akademik-gida.370090

Chasekioglou, A. N., Goula, A. M., Adamopoulos, K. G., & Lazarides, H. N. (2017). An approach to turn olive mill wastewater into a valuable food by-product based on spray drying in dehumidified air using drying aids. *Powder Technology, 311*, 376–389. doi:10.1016/j.powtec.2017.02.008

Chen, L., Chen, J., Yu, L., & Wu, K. (2016). Improved emulsifying capabilities of hydrolysates of soy protein isolate pretreated with high pressure microfluidization. *LWT - Food Science and Technology, 69*, 1–8. https://doi.org/10.1016/j.lwt.2016.01.030

Cho, W. Y., Yeon, S. J., Hong, G. E., Kim, J. H., Tsend-Ayush, C., & Lee, C. H. (2017). Antioxidant Activity and Quality Characteristics of Yogurt Added

Green Olive Powder during Storage. *Korean journal for food science of animal resources, 37(*6), 865–872. https://doi.org/10.5851/kosfa.2017.37.6.865

Ciron, C. I. E., Gee, V. L., Kelly, A. L., & Auty, M. A. E. (2011). Effect of microfluidization of heat-treated milk on rheology and sensory properties of reduced fat yoghurt. *Food Hydrocolloids, 25*, 1470-1476.

Çelik, M., Aksoy, M., Özkaya, D. F. (2016). Zeytin Bilimi. *Zeytin Bilimi, 6*(2), 69–75.

de Moraes Crizel, T., Hermes, V. S., de Oliveira Rios, A., & Flôres, S. H. (2016). Evaluation of bioactive compounds, chemical and technological properties of fruits byproducts powder. *Journal of Food Science and Technology, 53*(11), 4067–4075. doi:10.1007/s13197-016-2413-7

Demirag, O., & Konuskan, D. B. (2021). Quality properties, fatty acid and sterol compositions of east mediterranean region olive oils. *Journal of Oleo Science, 70*(1), 51–58. https://doi.org/10.5650/jos.ess20179

Demirkesen, I., Mert, B., Sumnu, G., & Sahin, S. (2010). Rheological properties of gluten-free bread formulations. *Journal of food Engineering*, *96*(2), 295-303.

Demirkesen, I., Vilgis, T. A., & Mert, B. (2018). Effect of microfluidization on the microstructure and physical properties of a novel yoghurt formulation. *Journal of Food Engineering, 237*, 69–77. doi:10.1016/j.jfoodeng.2018.05.02

Domingues, E., Fernandes, E., Gomes, J., Castro-Silva, S., & Martins, R. C. (2021). Olive oil extraction industry wastewater treatment by coagulation and Fenton's process. *Journal of Water Process Engineering, 39,* 101818. doi:10.1016/j.jwpe.2020.101818

Du, F., Qi, Y., Huang, H., Wang, P., Xu, X., & Yang, Z. (2022). Stabilization of O/W emulsions via interfacial protein concentrating induced by thermodynamic incompatibility between sarcoplasmic proteins and xanthan gum. *Food Hydrocolloids*, *124*, 107242.

Dugan, L. R. Jr. (1961). Development and inhibition of oxidative rancidity of foods. *Food Technol. 15*, 10

Escoda, T. C. (2016). Spain Patent No. WO2016024034A2. Organización Mundial de la Propiedad Intelectual Oficina internacional.

Escuela Superior del Aceite de Oliva. (n.d.). When should olives be harvested to obtain top quality evoo? Blog Escuela Superior del Aceite de Oliva. https://blog.esao.es/en/when-should-olives-be-harvested-to-obtain-top-quality-evoo

Formulaction SA. (2011, September 15). Turbiscan - Formulaction dispersion stability. [Video]. YouTube. https://www.youtube.com/watch?v=lmhP8ltrTZI

Formulaction. (2021). Turbiscan: The Reference Technology for Dispersion Stability Analysis. Formulaction. http://www.formulaction.com/en/our-solutions/stability-and-shelf-life

Fuentes, M.M., De Miguel Gordillo, C., Mar´ın Exp´oxito, J., S´anchez Casas, J., Mart´ınez Cano, M., Mart´ın Verte- dor, D., & Franco Baltasar, M.N. (2013) Chemical composition of virgin olive oils according to the ripening in olives. *Food Chemistry 141*, 2575–2581.

Gallardo-Guerrero, L., Gandul-Rojas, B., Mínguez-Mosquera, M. I., & Roca, M. (2012). Olives and Olive Oil. Tropical and Subtropical Fruits, 503–528. doi:10.1002/9781118324097.ch26

Gandul-Rojas, B., & Mınguez-Mosquera, M. I. (2006). Olive processing. Handbook of Fruits and Fruit Processing, 10, 491.

García, A., Brenes, M., García, P., Romero, C., & Garrido, A. (2003). Phenolic content of commercial olive oils. *European Food Research and Technology, 216*(6), 520-525.

Garcia, B., Magalhães, J., Fregapane, G., Salvador, M. D., & Paiva-Martins, F. (2012). Potential of selected Portuguese cultivars for the production of high

quality monovarietal virgin olive oil. *European Journal of Lipid Science and Technology*, *114*(9), 1070–1082. doi:10.1002/ejlt.201200046

Gharbi, I., Issaoui, M., Mehri, S., Cheraief, I., Sifi, S., & Hammami, M. (2015). Agronomic and Technological Factors Affecting Tunisian Olive Oil Quality. *Agricultural Sciences*, *06*(05), 513–526. https://doi.org/10.4236/as.2015.65051

Goula, A. M., & Lazarides, H. N. (2015). Integrated processes can turn industrial food waste into valuable food by-products and/or ingredients: The cases of olive mill and pomegranate wastes. *Journal of Food Engineering, 167*, 45–50. doi:10.1016/j.jfoodeng.2015.01.00

Gourmet Versand. (2021). Powder - black olive, freeze dried Sosa. https://www.gourmet-versand.com/en/article23038/powder-black-olive-freeze-dried-sosa-150-g.html

Gouvinhas, I., Machado, J., Gomes, S., Lopes, J., Martins-Lopes, P., & Barros, A. I. R. N. A. (2014). Phenolic composition and antioxidant activity of monovarietal and commercial portuguese olive oils. *JAOCS, Journal of the American Oil Chemists' Society, 91*(7), 1197–1203. https://doi.org/10.1007/s11746-014-2462-x

Gutierez, F., Jimenez, B., Ruiz, A., & Albi, M.A. (1999) Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and on the different components involved. *Journal of Agricultural and Food Chemistry 47*, 121–127.

Gutierrez, F., Arnaud, T., & Garrido, A. (2001). Contribution of polyphenols to the oxidative stability of virgin olive oil. *Journal of the Science of Food and Agriculture*, *81*(15), 1463-1470.

Hardham, J. F., Imison, B. W.,& French, H. M. (2000). Effect of homogenisation and microfluidisation on the extent of fat separation during storage of UHT milk. *Australian Journal of Dairy Technology , 55* (1), 16-22.

Hernández, M.L, Sicardo, M.D., Belaj, A. & Martínez-Rivas, J.M. (2021) The Oleic/Linoleic Acid Ratio in Olive (*Olea europaea* L.) Fruit Mesocarp Is Mainly Controlled by *OeFAD2-2* and *OeFAD2-5* Genes Together With the Different Specificity of Extraplastidial Acyltransferase Enzymes. *Front. Plant Sci.* 12:653997. doi: 10.3389/fpls.2021.653997

Hu, X., Zhao, M., Sun, W., Zhao, G., & Ren, J. (2011). Effects of microfluidization treatment and transglutaminase cross-linking on physicochemical, functional, and conformational properties of peanut protein isolate. *Journal of agricultural and food chemistry*, *59*(16), 8886-8894.

Halvorsen, B. L., & Blomhoff, R. (2011). Determination of lipid oxidation products in vegetable oils and marine omega-3 supplements. *Food and Nutrition Research*, *55*. https://doi.org/10.3402/fnr.v55i0.5792

Inarejos-Garcia, A.M., Salvador, M.D., & Fregapane, G. (2011) Effect of crushing on olive paste and virgin olive oil minor components. *European Food Research & Technology 232*, 441–451.

International Olive Council. (2019, November). *Trade Standard Applying to Olive Oils and Olive Pomace Oils.* Madrid.

International Olive Council. (2021a, January 4). *World's Olive Oil Production Has Tripled.* Madrid.

International Olive Council. (2021b). *Olive World - Table Olives*. https://www.internationaloliveoil.org/olive-world/table-olives/#about-olives

International Olive Council Newsletter. (2021, January). *Table olives – Provisional 2019/20 crop year and estimated 2020/21 crop year*, 160, 11-12.

Kailis, S. G., & Harris, D. (2007). *Producing table olives*. Collingwood, Austràlia: Landlinks.

Kiai, H., & Hafedi, A. (2014) Chemical composition changes in four green olive cultivars during spontaneous fermentation. *LWT – Food Science and Technology*. DOI:http://dx.doi.org/10.1016/j.lwt.2014.02.011

Kiritsakis, A.K., & Dugan L.R. (1985) Studies in photooxidation of olive oil. *Journal of the American Oils Chemists' Society, 62*, 892–896.

Kiritsakis, A.K., & Markakis, P. (1984) Effect of olive collection regimen on olive oil quality. *Journal of the Science of Food and Agriculture 35*(6), 677–678.

Kiritsakis, A., & Markakis, P. (1988). Olive Oil: A Review. *Advances in Food Research, 31*(C), 453–482. https://doi.org/10.1016/S0065-2628(08)60170-6

Kiritsakis, A., & Shahidi, F. (2017). Olive oil quality and its relation to the functional bioactives and their properties. *Olives and Olive Oil as Functional Foods: Bioactivity, Chemistry and Processing,* 205.

Koo, C. K. W., Chung, C., Ogren, T., Mutilangi, W., & McClements, D. J. (2018). Extending protein functionality: Microfluidization of heat denatured whey protein fibrils. *Journal of Food Engineering, 223,* 189–196. https://doi.org/10.1016/j.jfoodeng.2017.10.020

Krokida, M.K., Maroulis, Z.B., & Saravacos, G.D. (2001). Rheological properties of fluid fruit and vegetable products: Compilation of literature data. *International Journal of Food Properties, 4,* 179–200.

Kumar, A., Badgujar, P. C., Mishra, V., Sehrawat, R., Babar, O. A., & Upadhyay, A. (2019). Effect of microfluidization on cholesterol, thermal properties and protein digestibility (in-vitro and in-vivo) of milk. *LWT,* 108523. doi:10.1016/j.lwt.2019.108523

Li, A.-N., Li, S., Zhang, Y.-J., Xu, X.-R., Chen, Y.-M., & Li, H.-B. (2014) Resources and biological activities of natural polyphenols. *Nutrients, 6,* 6020–6047.

Libretexts. (2020, August 13). Lipids and Triglycerides. Chemistry LibreTexts. https://chem.libretexts.org/@go/page/58857

Liu, C. M., Liang, R. H., Dai, T. T., Ye, J. P., Zeng, Z. C., Luo, S. J., & Chen, J. (2016). Effect of dynamic high pressure microfluidization modified insoluble dietary fiber on gelatinization and rheology of rice starch. *Food Hydrocolloids, 57,* 55-61.

Liu, G. X., Tu, Z. C., Wang, H., Zhang, L., Huang, T., & Ma, D. (2017). Monitoring of the functional properties and unfolding change of Ovalbumin after DHPM treatment by HDX and FTICR MS: Functionality and unfolding of Oval after DHPM by HDX and FTICR MS. *Food chemistry*, *227*, 413-421.

Liu, W., Liu, J., Liu, C., Zhong, Y., Liu, W., & Wan, J. (2009). Activation and conformational changes of mushroom polyphenoloxidase by high pressure microfluidization treatment. *Innovative Food Science & Emerging Technologies, 10*(2), 142–147. doi:10.1016/j.ifset.2008.11.009

Lubrizol Standard Test Procedure. (2006). Peroxide Value - AOCS Method. Noveon Consumer Specialties.

Mackinney, G., (1961). Coloring matters in the orange. In Sinclair, W. B. (Ed.), Its biochemistry and physiology. Univ. of California. http://dx.doi.org/10.2172/4072030.

Malheiro, R., Mendes, P., Fernandes, F., Rodrigues, N., Bento, A., & Pereira, J. A. (2014). Bioactivity and phenolic composition from natural fermented table olives. *Food Funct., 5*(12), 3132–3142. doi:10.1039/c4fo00560k

Marco, A., Ferrer, C., Velasco, L. M., Rodrigo, D., Muguerza, B., & Martínez, A. (2011). Effect of Olive Powder and High Hydrostatic Pressure on the Inactivation of Bacillus cereus Spores in a Reference Medium. *Foodborne Pathogens and Disease, 8*(6), 681–685. doi:10.1089/fpd.2010.0712

Martinez, M. V., & Whitaker, J. R. (1995). The biochemistry and control of enzymatic browning. *Trends in Food Science & Technology, 6*(6), 195-200.

Masalova, I., Foudazi, R., & Malkin, A. Y. (2011). The rheology of highly concentrated emulsions stabilized with different surfactants. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 375(1-3), 76–86. doi:10.1016/j.colsurfa.2010.11.06

McClements, D. J., & Rao, J. (2011). Food-Grade Nanoemulsions: Formulation, Fabrication, Properties, Performance, Biological Fate, and Potential Toxicity.

Critical Reviews in Food Science and Nutrition, 51(4), 285–330. doi:10.1080/10408398.2011.559558

Mert, B. (2012). Using high pressure microfluidization to improve physical properties and lycopene content of ketchup type products. *Journal of Food Engineering, 109*(3), 579–587. https://doi.org/10.1016/j.jfoodeng.2011.10.021

Mert, B., Tekin, A., Demirkesen, I., & Kocak, G. (2014). Production of Microfluidized Wheat Bran Fibers and Evaluation as an Ingredient in Reduced Flour Bakery Product. *Food and Bioprocess Technology, 7*(10), 2889-2901. doi:10.1007/s11947-014-1258-1

Moretti, S., Francini, A., Minnocci, A., & Sebastiani, L. (2018). Does salinity modify anatomy and biochemistry of Olea europaea L. fruit during ripening? *Scientia Horticulturae, 228,* 33-40. doi:10.1016/j.scienta.2017.10.003

Morillo, J. A., Antizar-Ladislao, B., Monteoliva-Sánchez, M., Ramos-Cormenzana, A., & Russell, N. J. (2009). Bioremediation and biovalorisation of olive-mill wastes. *Applied microbiology and biotechnology*, *82*(1), 25-39.

Opaliński, I., Chutkowski, M., & Hassanpour, A. (2016). Rheology of moist food powders as affected by moisture content. *Powder Technology, 294*, 315–322. https://doi.org/10.1016/j.powtec.2016.02.049

Özcan, A. (2008). Kağıt Yüzey Pürüzlülüğünün L*A*B* Değerleri Üzerine Etkisinin Belirlenmesi. *İstanbul Ticaret Üniversitesi Fen Bilimleri Dergisi,* 7, 53–61.

Pandey, K.B. & Rizvi, S.I. (2009) Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity 2,* 270–278.

Paquin, P., & Giasson, J. (1989). Microfluidization as an homogenization process for cream liqueur. *Lait, 69,* 491-498.

Pokorný, J., & Schmidt, S. (2001). Natural antioxidant functionality during food processing. *Antioxidants in food,* 331-350.

Polowczyk, I., Bastrzyk, A., Koźlecki, T., & Sadowski, Z. (2015). Stability of Three-Phase Water-Particle-Oil Systems. *Chemical Engineering & Technology*, *38*(4), 715-720.

Réblová, Z. (2012). Effect of temperature on the antioxidant activity of phenolic acids. *Czech Journal of Food Sciences, 30*(2), 171–177. https://doi.org/10.17221/57/2011-cjfs

Republic of Turkey Ministry of Economy. (2018). *Olive oil and table olives*. Directorate General of Experts.

Sahin, S., & Sumnu, S. G. (2006). *Physical properties of foods*. Springer Science & Business Media.

Salvador, M.D., Aranda F., & Fregapane, G. (2001) Influence of fruit ripening on Cornicabra virgin olive oil quality: a study of tour crop seasons. *Food Chemistry 73*, 45–53.

Sant'Anna, V., Gurak, P. D., Ferreira Marczak, L. D., & Tessaro, I. C. (2013). *Tracking bioactive compounds with colour changes in foods – A review. Dyes and Pigments, 98(3), 601–608.* doi:10.1016/j.dyepig.2013.04.011

Sarmin, S. N., Jawaid, M., Awad, S. A., Saba, N., Fouad, H., Alothman, O. Y., & Sain, M. (2021). Olive fiber reinforced epoxy composites: Dimensional Stability, and mechanical properties. Polymer Composites.

Scapin, G., Schmidt, M. M., Prestes, R. C., & Rosa, C. S. (2016). Phenolics compounds, flavonoids and antioxidant activity of chia seed extracts (Salvia hispanica) obtained by different extraction conditions. *International Food Research Journal, 23*(6), 2341-2346. doi:10.1055/sos-sd-046-00152

Segovia-Bravo, K. A., Jarén-Galán, M., García-García, P., & Garrido-Fernández, A. (2009). Browning reactions in olives: Mechanism and polyphenols involved. *Food Chemistry, 114*(4), 1380–1385. doi:10.1016/j.foodchem.2008.11.01

Simpson, K. L., Lee, T. C., Rodriguez, D. B. & Chichester, C. O. (1976). Metabolism in senescent and stored tissues. In Goodwin, T. W. (Ed.), *Chemistry and Biochemistry of Plant Pigments*. Academic: London, New York, San Francisco.

Souilem, S., El-Abbassi, A., Kiai, H., Hafidi, A., Sayadi, S., & Galanakis, C. M. (2017). Olive oil production sector: environmental effects and sustainability challenges. Olive Mill Waste, 1–28. doi:10.1016/b978-0-12-805314-0.00001-7

Suarez, I. M. M. (1975). Preliminary operations. (In J. M. M. Moreno, Eds.), In *"Olive Oil Technology*". FAO, Rome.

Tanilgan, K., Özcan, M. M., & Ünver, A. (2007). Physical and chemical characteristics of five Turkish olive (Olea europea L.) varieties and their oils. Grasas y Aceites, 58(2), 142–147. https://doi.org/10.3989/gya.2007.v58.i2.78

Tarafdar, A., Kumar, Y., Kaur, B. P., & Badgujar, P. C. (2021). High-pressure microfluidization of sugarcane juice: Effect on total phenols, total flavonoids, antioxidant activity, and microbiological quality. *Journal of Food Processing and Preservation*, *45*(5), 1–10. https://doi.org/10.1111/jfpp.15428

Tena, N., Wang, S. C., Aparicio-Ruiz, R., García-González, D. L., & Aparicio, R. (2015). In-depth assessment of analytical methods for olive oil purity, safety, and quality characterization. *Journal of Agricultural and Food Chemistry*, *63*(18), 4509–4526. https://doi.org/10.1021/jf5062265

Türk Gıda Kodeksi Zeytinyağı Ve Pirina Yağı Tebliği (2017/26) (2017, September 17). *Resmi Gazete*. https://www.resmigazete.gov.tr/eskiler/2017/09/20170917-9.htm

van Dijk, S. J., Feskens, E. J., Bos, M. B., de Groot, L. C., de Vries, J. H., Müller, M., & Afman, L. A. (2012). Consumption of a high monounsaturated fat diet reduces oxidative phosphorylation gene expression in peripheral blood mononuclear cells of abdominally overweight men and women. *The Journal of nutrition*, *142*(7), 1219-1225.

Varavinit, S., Chaokasem, N., & Shobsngob, S. (2001). Studies of flavor encapsulation by agents produced from modified sago and tapioca starches. *Starch-Stärke*, *53*(6), 281-287.

Vinha, A. F., Ferreres, F., Silva, B. M., Valentao, P., Gonçalves, A., Pereira, J. A., ... & Andrade, P. B. (2005). Phenolic profiles of Portuguese olive fruits (Olea europaea L.): Influences of cultivar and geographical origin. *Food chemistry*, *89*(4), 561-568.

Vera Zambrano, M., Dutta, B., Mercer, D. G., MacLean, H. L., & Touchie, M. F. (2019). Assessment of moisture content measurement methods of dried food products in small-scale operations in developing countries: A review. *Trends in Food Science and Technology*, *88*(April), 484–496. https://doi.org/10.1016/j.tifs.2019.04.006

Vuillemard, J.-C. (1991). Recent advances in the large-scale production of lipid vesicles for use in food products: microfluidization. *Journal of Microencapsulation*, *8*(4), 547–562. doi:10.3109/02652049109021878

Wang, T., Raddatz, J., & Chen, G. (2013). Effects of microfluidization on antioxidant properties of wheat bran. *Journal of Cereal Science*, *58*(3), 380-386.

Wang, T., Sun, X., Zhou, Z.,& Chen, G. (2012). Effects of microfluidization process on physicochemical properties of wheat bran. *Food Research International* *48*(2), 742–747.

Wang, X., Wang, S., Wang, W., Ge, Z., Zhang, L., Li, C., … Zong, W. (2019). Comparison of the effects of dynamic high-pressure microfluidization and conventional homogenization on the quality of peach juice. *Journal of the Science of Food and Agriculture.* doi:10.1002/jsfa.9874

Wei, F., Zhao, Q., Lv, X., Dong, X. Y., Feng, Y. Q., & Chen, H. (2013). Rapid magnetic solid-phase extraction based on monodisperse magnetic single-crystal ferrite nanoparticles for the determination of free fatty acid content in

edible oils. *Journal of Agricultural and Food Chemistry, 61*(1), 76–83. https://doi.org/10.1021/jf303840q

Yang, H., Kang, W., Wu, H., Yu, Y., Zhu, Z., Wang, P., … Sarsenbekuly, B. (2017). Stability, rheological property and oil-displacement mechanism of a dispersed low-elastic microsphere system for enhanced oil recovery. *RSC Advances, 7*(14), 8118–8130. doi:10.1039/c6ra26849h

Yıldırım, G. (2009). Effect of Storage Time On Olive Oil Quality, The Graduate School of Engineering and Sciences of Izmir Institute of Technology, İzmir.

Yildiz, E., Demirkesen, I., & Mert, B. (2016). High Pressure Microfluidization of Agro by-product to Functionalized Dietary Fiber and Evaluation as a Novel Bakery Ingredient. *Journal of Food Quality, 39(6), 599–610.* doi:10.1111/jfq.12246

Yorulmaz, A., Erinc, H., & Tekin, A. (2013). Changes in olive and olive oil characteristics during maturation. *JAOCS, Journal of the American Oil Chemists' Society, 90*(5), 647–658. https://doi.org/10.1007/s11746-013-2210-7

Yorulmaz, A., & Tekin, A. (2008). Zeytin ve Zeytinyağı Fenolikleri. I. Ulusal Öğrenci Kongresi. 17-18 Mayıs, Balıkesir, s. 24-31.

Yüksel Aydar, A. (2019). Emerging Extraction Technologies in Olive Oil Production. Technological Innovation in the Olive Oil Production Chain. doi:10.5772/intechopen.81390

Zhang, X., Guo, Y., Guo, L., Jiang, H., & Ji, Q. (2018). In vitro evaluation of antioxidant and antimicrobial activities of melaleuca alternifolia essential oil. *BioMed Research International*, *2018*. https://doi.org/10.1155/2018/2396109

APPENDICES

A. Calibration Curves

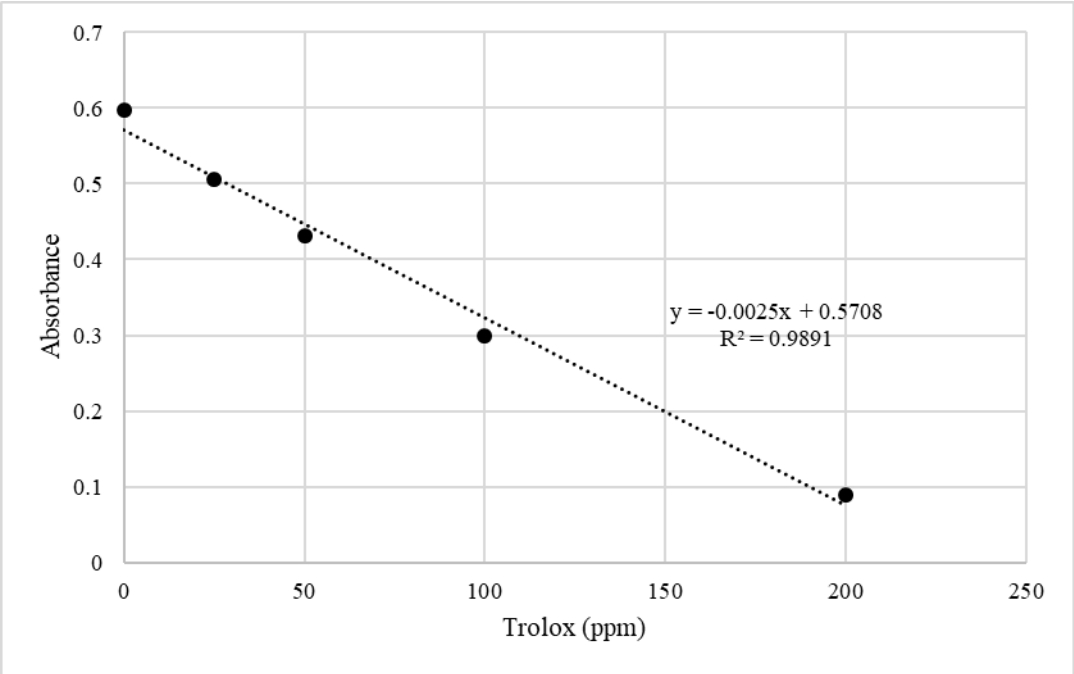

Figure A. 1 Calibration Curve for DPPH

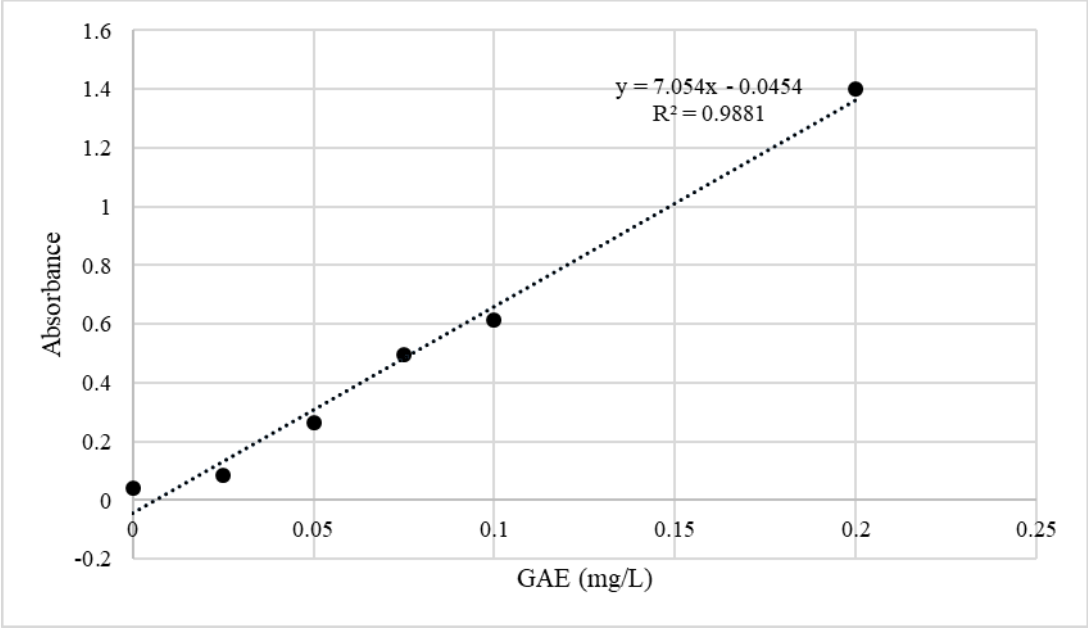

Figure A. 2 Calibration Curve for Gallic Acid Equivalent

B. Statistical Analysis

Table B. 1 ANOVA results of DPPH of green olive powder

General Linear Model: Concentration versus Process; Time (month)

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Process	Fixed	2	MF; UnMF
Time (month)	Fixed	3	0,0; 1,5; 3,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	429578	429578	27,43	0,000
Time (month)	2	445193	222597	14,22	0,001
Process*Time (month)	2	121257	60628	3,87	0,053
Error	11	172241	15658		
Total	16	1044236			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
125,133	83,51%	76,01%	62,89%

Comparisons for Concentration

Tukey Pairwise Comparisons: Process

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
UnMF	9	1501,01	A
MF	8	1179,43	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Time (month)

Grouping Information Using the Tukey Method and 95% Confidence

Time (month)	N	Mean	Grouping
0,0	6	1561,80	A
1,5	6	1287,00	B
3,0	5	1171,86	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Process*Time (month)

Grouping Information Using the Tukey Method and 95% Confidence

Process*Time (month)	N	Mean	Grouping	
UnMF 0,0	3	1685,33	A	
UnMF 3,0	3	1454,51	A	B
MF 0,0	3	1438,26	A	B
UnMF 1,5	3	1363,20	A	B
MF 1,5	3	1210,80	B	C
MF 3,0	2	889,22		C

Means that do not share a letter are significantly different.

Table B. 2 ANOVA results of DPPH of black olive powder

General Linear Model: Concentration versus Process; Time (month)

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Process	Fixed	2	MF; UnMF
Time (month)	Fixed	3	0,0; 1,5; 3,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	316	316,5	0,16	0,701
Time (month)	2	133156	66578,2	34,10	0,001
Process*Time (month)	2	4	2,0	0,00	0,999
Error	6	11716	1952,7		
Total	11	145193			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
44,1890	91,93%	85,21%	67,72%

Comparisons for Concentration

Tukey Pairwise Comparisons: Process

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
MF	6	294,172	A
UnMF	6	283,901	A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Time (month)

Grouping Information Using the Tukey Method and 95% Confidence

Time (month)	N	Mean	Grouping
0,0	4	435,874	A
1,5	4	237,381	B
3,0	4	193,854	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Process*Time (month)

Grouping Information Using the Tukey Method and 95% Confidence

Process*Time (month)	N	Mean	Grouping

MF 0,0	2	440,360 A	
UnMF 0,0	2	431,387 A	
MF 1,5	2	242,410	B
UnMF 1,5	2	232,351	B
MF 3,0	2	199,744	B
UnMF 3,0	2	187,965	B

Means that do not share a letter are significantly different.

Table B. 3 ANOVA results of DPPH of raw olive powder

General Linear Model: Concentration versus Process; Time (month)

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Process	Fixed	2	MF; UnMF
Time (month)	Fixed	3	0,0; 1,5; 3,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	2563	2563	0,95	0,351
Time (month)	2	6440	3220	1,19	0,340
Process*Time (month)	2	2007	1003	0,37	0,698
Error	11	29744	2704		
Total	16	40886			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
51,9998	27,25%	0,00%	0,00%

Comparisons for Concentration

Tukey Pairwise Comparisons: Process

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
MF	8	2081,99	A

UnMF	9	2057,15 A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Time (month)

Grouping Information Using the Tukey Method and 95% Confidence

Time (month)	N	Mean	Grouping
1,5	6	2094,09	A
3,0	5	2066,65	A
0,0	6	2047,99	A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Process*Time (month)

Grouping Information Using the Tukey Method and 95% Confidence

Process*Time (month)	N	Mean	Grouping
UnMF 1,5	3	2094,52	A
MF 1,5	3	2093,65	A
MF 3,0	2	2078,91	A
MF 0,0	3	2073,42	A
UnMF 3,0	3	2054,39	A
UnMF 0,0	3	2022,56	A

Means that do not share a letter are significantly different.

Table B. 4 Comparisons of olive types for DPPH results

Comparisons for DPPH

Tukey Pairwise Comparisons: Olive Type

Grouping Information Using the Tukey Method and 95% Confidence

Olive Type	N	Mean	Grouping	
Raw	17	2058,52	A	
Green	17	1356,24	B	
Black	12	289,04		C

Means that do not share a letter are significantly different.

Table B. 5 ANOVA results of TPC of green olive powder

General Linear Model: Concentration versus Process; Time (month)

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Process	Fixed	2	MF; UnMF
Time (month)	Fixed	3	0,0; 1,5; 3,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	0,000011	0,000011	30,17	0,000
Time (month)	2	0,000011	0,000006	15,11	0,001
Process*Time (month)	2	0,000006	0,000003	7,38	0,011
Error	10	0,000004	0,000000		
Total	15	0,000033			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0006111	88,64%	82,96%	70,96%

Comparisons for Concentration

Tukey Pairwise Comparisons: Process

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
UnMF	9	0,0165800	A
MF	7	0,0148710	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Time (month)

Grouping Information Using the Tukey Method and 95% Confidence

Time (month)	N	Mean	Grouping
0,0	6	0,0168557	A
1,5	5	0,0154262	B
3,0	5	0,0148946	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Process*Time (month)

Grouping Information Using the Tukey Method and 95% Confidence

Process*Time (month)	N	Mean	Grouping
UnMF 0,0	3	0,0184860	A
UnMF 1,5	3	0,0161232	B
MF 0,0	3	0,0152254	B
UnMF 3,0	3	0,0151309	B
MF 1,5	2	0,0147292	B
MF 3,0	2	0,0146583	B

Means that do not share a letter are significantly different.

Table B. 6 ANOVA results of TPC of black olive powder

General Linear Model: Concentration versus Process; Time (month)

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values

Process	Fixed	2 MF; UnMF
Time (month)	Fixed	3 0,0; 1,5; 3,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	0,000010	0,000010	7,87	0,017
Time (month)	2	0,000001	0,000000	0,36	0,709
Process*Time (month)	2	0,000003	0,000001	1,03	0,388
Error	11	0,000014	0,000001		
Total	16	0,000028			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0011270	50,26%	27,65%	0,00%

Comparisons for Concentration

Tukey Pairwise Comparisons: Process

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
UnMF	9	0,0103897	A
MF	8	0,0088382	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Time (month)

Grouping Information Using the Tukey Method and 95% Confidence

Time (month)	N	Mean	Grouping
0,0	6	0,0099093	A
3,0	6	0,0096021	A

1,5 5 0,0093304 A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Process*Time (month)

Grouping Information Using the Tukey Method and 95% Confidence

Process*Time (month)	N	Mean	Grouping
UnMF 3,0	3	0,0109252	A
UnMF 0,0	3	0,0105000	A
UnMF 1,5	3	0,0097439	A
MF 0,0	3	0,0093186	A
MF 1,5	2	0,0089169	A
MF 3,0	3	0,0082790	A

Means that do not share a letter are significantly different.

Table B. 7 ANOVA results of TPC of raw olive powder

General Linear Model: Concentration versus Process; Time (month)

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Process	Fixed	2	MF; UnMF
Time (month)	Fixed	3	0,0; 1,5; 3,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	0,001140	0,001140	315,32	0,000
Time (month)	2	0,000494	0,000247	68,36	0,000
Process*Time (month)	2	0,000135	0,000067	18,66	0,000

Error	10	0,000036	0,000004
Total	15	0,001719	

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0019015	97,90%	96,85%	93,78%

Comparisons for Concentration

Tukey Pairwise Comparisons: Process

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
UnMF	8	0,0623067	A
MF	8	0,0451139	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Time (month)

Grouping Information Using the Tukey Method and 95% Confidence

Time (month)	N	Mean	Grouping	
0,0	6	0,0613694	A	
1,5	6	0,0516114	B	
3,0	4	0,0481500		C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Process*Time (month)

Grouping Information Using the Tukey Method and 95% Confidence

Process*Time (month)	N	Mean	Grouping
UnMF 0,0	3	0,0697571	A
UnMF 3,0	2	0,0605897	B

UnMF 1,5	3	0,0565731	B	C		
MF 0,0	3	0,0529818		C		
MF 1,5	3	0,0466497			D	
MF 3,0	2	0,0357102				E

Means that do not share a letter are significantly different.

Table B. 8 Comparisons of olive types for TPC results

Comparisons for TPC

Tukey Pairwise Comparisons: Olive Type

Grouping Information Using the Tukey Method and 95% Confidence

Olive Type	N	Mean	Grouping	
Raw	16	0,0541656	A	
Green	16	0,0152625	B	
Black	17	0,0094476		C

Means that do not share a letter are significantly different.

Table B. 9 ANOVA results of Peroxide Value of green olive powder

General Linear Model: Peroxide Value versus Process

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels Values
Process	Fixed	2 MF; UnMF

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	48,989	48,989	10,98	0,080
Error	2	8,925	4,462		
Total	3	57,914			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2,11245	84,59%	76,88%	38,36%

Comparisons for Peroxide Value

Tukey Pairwise Comparisons: Process

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
MF	2	27,4547	A
UnMF	2	20,4555	A

Means that do not share a letter are significantly different.

Table B. 10 ANOVA results of Peroxide Value of black olive powder

General Linear Model: Peroxide Value versus Process

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Process	Fixed	2	MF; UnMF

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	1427,67	1427,67	5571,94	0,000
Error	2	0,51	0,26		
Total	3	1428,18			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,506187	99,96%	99,95%	99,86%

Comparisons for Peroxide Value

Tukey Pairwise Comparisons: Process

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
UnMF	2	60,1746	A
MF	2	22,3901	B

Means that do not share a letter are significantly different.

Table B. 11 ANOVA results of Peroxide Value of raw olive powder

General Linear Model: Peroxide Value versus Process

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Process	Fixed	2	MF; UnMF

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	20,5003	20,5003	89,45	0,011
Error	2	0,4584	0,2292		
Total	3	20,9586			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,478737	97,81%	96,72%	91,25%

Comparisons for Peroxide Value

Tukey Pairwise Comparisons: Process

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
MF	2	9,52887	A
UnMF	2	5,00115	B

Means that do not share a letter are significantly different.

Table B. 12 ANOVA results of % FFA content of green olive powder

General Linear Model: %FFA versus Process

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Process	Fixed	2	MF; UnMF

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	29,8539	29,8539	233,55	0,000
Error	4	0,5113	0,1278		
Total	5	30,3652			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,357529	98,32%	97,90%	96,21%

Comparisons for %FFA

Tukey Pairwise Comparisons: Process

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping

MF	3	36,8574 A	
UnMF	3	32,3962	B

Means that do not share a letter are significantly different.

Table B. 13 ANOVA results of % FFA content of black olive powder

General Linear Model: %FFA versus Process

Method

Factor coding	(-1; 0; +1)

Factor Information

Factor	Type	Levels Values
Process	Fixed	2 MF; UnMF

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	1753,09	1753,09	69795,18	0,000
Error	4	0,10	0,03		
Total	5	1753,19			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,158486	99,99%	99,99%	99,99%

Comparisons for %FFA

Tukey Pairwise Comparisons: Process

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
MF	3	37,2054 A	
UnMF	3	3,0187	B

Means that do not share a letter are significantly different.

Table B. 14 ANOVA results of % FFA content of raw olive powder

General Linear Model: %FFA versus Process

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Process	Fixed	2	MF; UnMF

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	0,003642	0,003642	2,35	0,200
Error	4	0,006209	0,001552		
Total	5	0,009851			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0393984	36,97%	21,21%	0,00%

Comparisons for %FFA

Tukey Pairwise Comparisons: Process

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
MF	3	0,725327	A
UnMF	3	0,676052	A

Means that do not share a letter are significantly different.

Table B. 15 Correlation between DPPH and TPC of green olive powder

Correlation: DPPH; TPC

Method

Correlation type	Pearson
Rows used	17

Correlations

	DPPH
TPC	0,646

Table B. 16 Correlation between DPPH and TPC of black olive powder

Correlation: DPPH; TPC

Method

Correlation type	Pearson
Rows used	17

Correlations

	DPPH
TPC	0,245

Table B. 17 Correlation between DPPH and TPC of raw olive powder

Correlation: DPPH; TPC

Method

Correlation type	Pearson
Rows used	17

Correlations

	DPPH
TPC	-0,371

Table B. 18 Correlation between DPPH and TPC of all olive powders

Correlation: DPPH; TPC

Method

Correlation type	Pearson
Rows used	51

Correlations

	DPPH
TPC	0,799