

**Optimization of Microwave-Assisted Extraction of
Pomegranate (*Punica granatum L.*) Seed Oil and Evaluation
of Its Physicochemical and Bioactive Properties**

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**Supervisor
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**by
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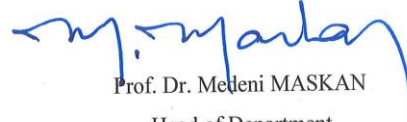
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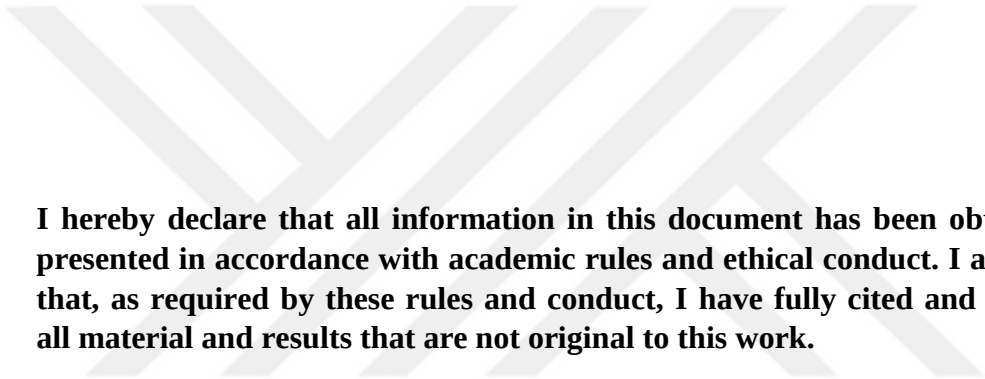
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ABSTRACT

OPTIMIZATION OF MICROWAVE-ASSISTED EXTRACTION OF POMEGRANATE (*PUNICA GRANATUM L.*) SEED OIL AND EVALUATION OF ITS PHYSICOCHEMICAL AND BIOACTIVE PROPERTIES

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Pomegranate seed oil was extracted in a closed-vessel high-pressure microwave system. The characteristics of the obtained oil such as fatty acid composition, free fatty acidity, total phenolic content, antioxidant activity and color, were compared to those of the oil obtained by cold solvent extraction.

Response surface methodology was applied to optimize extraction conditions: power (176-300 W), time (5-20 min), particle size ($d = 0.125\text{-}0.800$ mm), solvent/sample ratio (2:1, 6:1 and 10:1, by mass). The predicted highest extraction yield (35.19 %) was obtained using microwave power of 220 W, particle size in the range of 0.125-0.450 mm and solvent/sample ratio of 10:1 (by mass) in 5 min extraction time.

Microwave-assisted solvent extraction (MASE) resulted in higher extraction yield than that of Soxhlet (34.70 % in 8 h) or cold extraction (17.50 % in 8 h). The dominant fatty acid of pomegranate seed oil was punicalic acid (86 %) irrespective of the extraction method. Oil obtained by MASE had better physicochemical properties, total phenolic content and antioxidant activity than the cold solvent extracted oil.

Key words: pomegranate seed oil, microwave-assisted solvent extraction, extraction optimization, bioactive properties

ÖZET

NAR ÇEKİRDEĞİ YAĞININ MİKRODALGA DESTEKLİ EKSTRAKSİYONUNUN OPTİMİZASYONU VE FİZİKOKİMYASAL VE BİYOAKTİF ÖZELLİKLERİNİN DEĞERLENDİRİLMESİ

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Nar çekirdeği yağı, yüksek basınçlı kapalı bir mikrodalga sisteminde ekstrakt edildi. Elde edilen yağın yağ asidi kompozisyonu, serbest yağ asitliği, toplam fenolik içeriği, antioksidant aktivitesi ve renk özellikleri soğuk çözücü ekstraksiyonundan elde edilen yağ ile karşılaştırıldı.

Ekstraksiyon şartlarını optimize etmek için yüzey tepki metodolojisi uygulandı: güç (176-300 W), zaman (5-20 dk), numune boyutu ($d = 0,125-0,800$ mm), çözücü numune oranı (kütlece 2:1, 6:1 ve 10:1). Tahmin edilen en yüksek verim (% 35.19) 220 W mikrodalga gücü, 0,125-0,400 mm aralığındaki numune boyutu ve kütlece 10:1 oranlı çözücü numune oranı ile elde edildi.

Mikrodalga destekli çözücü ekstraksiyonu (MASE) hem Soxhlet ekstraksiyonu (8 saat, % 34,70 verim) hem de soğuk ekstraksiyondan (8 saat, % 17,50 verim) daha yüksek ekstraksiyon verimi ile sonuçlandı. Nar çekirdeği yağındaki baskın yağ asidi ekstraksiyon metoduna bakılmaksızın punikik asitti. MASE ile elde edilen yağ, toplam fenolik içerik ve antioksidant aktivite yönünden soğuk çözücü ekstraksiyonun ile elde edilen yağa göre daha iyi fizikokimyasal özelliklere sahipti.

Anahtar Kelimeler: nar çekirdeği yağı, mikrodalga yardımcılı çözücü ekstraksiyonu, ekstraksiyon optimizasyonu, biyoaktif özellikler



TO MY FAMILY...

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LIST OF SYMBOLS/ABBREVIATIONS

CLA	Conjugated linolenic acid
CSE	Cold solvent extraction
CSEO	Cold solvent extraction oil
DPPH	2,2-diphenyl-1-picrylhydrazyl
EHF	Extra high frequency
ELF	Extra low frequency
EO	Essential oil
FFA	Free fatty acid
GAE	Gallic acid equivalent
HF	High frequency
HUFA	Highly unsaturated fatty acid
IC ₅₀	Concentration of antioxidant that causes 50 % inhibition of DPPH
LDL	Low density lipoprotein
LF	Low frequency
LLE	Liquid-liquid extraction
MAE	Microwave assisted extraction
MASE	Microwave assisted solvent extraction
MASEO	Microwave assisted solvent extraction oil
MF	Medium frequency
PAH	Polycyclic aromatic hydrocarbon
PMAE	Pressurized microwave assisted extraction
PTFE	Polytetrafluoroethylene
PUFA	Poly unsaturated fatty acid
RSM	Response surface methodology
SEM	Scanning electron microscope
SFMAE	Solvent free microwave assisted extraction

SHF	Super high frequency
UHF	Ultra high frequency
ULF	Ultra low frequency
VHF	Very high frequency
VLf	Very low frequency
PCB	Polychlorinated biphenyls



CHAPTER 1

INTRODUCTION

Microwave-assisted solvent extraction (MASE) is a process that has emerged as an attractive alternative oil extraction method recently. A more effective extraction in a shorter time compared to the conventional processes is provided by the rapid heating and destruction of biological cell structure in a microwave. Furthermore, a small amount of solvent is needed for extraction in MASE and it produces high-quality oil with respect to chemical and physical properties. The lower energy requirement resulting in a considerable decrease in environmental impact and financial costs is another important advantage of this technique (Abbasi et al., 2008 a; Azadmard-Damirchi et al., 2010; Amarni and Kadi, 2010; Camel, 2000; Taghvaei et al., 2014; Clodoveo and Hbaieb, 2013; Tsukui et al., 2014).

Researchers have started to focus on the microwave-assisted extraction instead of the conventional methods to obtain natural substances such as pectin, essential oil and phenolics in the last decade. MASE has been extensively studied to investigate its effect on the extraction of high-value components with high yield and quality from various plant food materials, industrial food wastes and by-products. It has been widely used for the extraction of lycopene from tomato peel (Ho et al., 2015), polyphenols from red grape pomace, grape seed and potato peel (Drosou et al., 2015; Li et al., 2011; Wu et al., 2012), or essential oil and pectin from orange peel (Balı et al., 2012).

Pomegranate (*Punica granatum L.*) is an edible fruit cultivated in the Mediterranean area and Near and Far East countries (Abbasi et al., 2008 a). The edible part of whole fruit is composed of 75-85 % juice and 15-25 % seeds. The edible part of the pomegranate is called aril and constitutes about 52% w/w of the total fruit and comprises of 78% w/w juice and 22% w/w seeds (Ahangari and Sargolzaei, 2012). Pomegranate seed which is basically obtained from pomegranate juice industry is one of the most valuable food wastes (Carvalho, 2014). Pomegranate seed has 12-25

% crude oil which is rich in bioactive lipids (Orak et al., 2012; Özgül and Yücel, 2012)

It contains tocopherols, phytosterols and punicic acid, which have several potential health benefits (Liu et al., 2009). 74–85 % of the total fatty acid content of pomegranate seed oil is punicic acid. Punicic acid is known for its antioxidant, antitumor, immunoregulatory, anti-atherosclerotic and serum lipid-lowering activities (Verardo et al., 2014; Carvalho et al., 2010). Eikani et al. (2012) compared the efficiency of Soxhlet, cold pressing and superheated hexane extraction methods in the extraction of pomegranate seed oil.

Tian et al. (2013) optimized the conditions for ultrasonic-assisted extraction of pomegranate seed oil. Fadavi et al. (2006) looked into the total lipid content and fatty acid composition of pomegranate seed oil extracted from 25 different varieties grown in Iran. Sadeghi (2010) evaluated the physical and chemical characteristics of four pomegranate cultivars. However, pomegranate seed oil extraction using a closed vessel high-pressure microwave system has not been studied before. To investigate the effects of extraction time, solvent/solid ratio (by mass), particle size and microwave power on oil extraction yield in a microwave system using response surface methodology and to compare the yield and the quality parameters such as physical, chemical and bioactive properties of the oil produced by MASE and cold solvent extraction are main objectives of this study (Çavdar et al., 2017).

CHAPTER 2

LITERATURE REVIEW

2.1 Pomegranate

The pomegranate (*Punica granatum L.*) is one of the oldest fruits usually grows in tropical and subtropical regions such as Near and Far Eastern countries and the Mediterranean area, which includes Turkey and Iran (Kiralán et al., 2009; Abbasi et al., 2008 b). Because of its pleasant taste and excellent health benefiting properties, it is better known as the fruit of Eden (Al-Quran) in some countries (Akhtar et al., 2015).

In the medieval age it is named after “Pomuni granatum” that means seedy apple (LaRue, 1980; Oğuz et al., 2011).

Hicaz pomegranate, Fellahyemez, Ernar, Eksilik, Katırbasili, Eksi Goknar, Lefan, Erdemli Asinar and Silifke Grafting are some of the major pomegranate types that has become popular all over the world these days. On the other hand, some domestic varieties have become synonymous with the field because they have special importance in pomegranate cultivation. Dislinar-“Toothed pomegranate” (Zivzik pomegranate) which has large grains and is grown in Dislinar Village of Sirvan province in Siirt/Turkey is the most typical example to this (Kurt and Şahin, 2013).

Basically, the pomegranate consists of three parts, the outer skin (peel), mesocarp (inside peel) and arils (pulp and seeds), which also include the interior network of membranes. The arils of pomegranate comprise approximately 55–60% of the total fruit weight and contain 80% juice and 20% seeds. The fresh juice contains 85% water and 15% sugars, pectins, ascorbic acid, polyphenols, flavonoids, anthocyanins and amino acids (Roy and Vaskar, 1997; Heber et al., 2006).

The edible fractions of pomegranate contain sugars, polysaccharides, polyphenols, vitamins and minerals. However, the content of these ingredients may change

depending on many factors, such as cultivars, growth factors, maturation period, postharvest processing and storage conditions. These factors can influence both the fruit quality and how beneficial it is for health (Schwartz et al., 2009). Approximately half of the total fruit mass is the peel, which is distinguished by the existence of high molecular weight ellagitannins, proanthocyanidins, phenolics, complex polysaccharides, flavonoids and significant amount of microelements all of which display strong anti-mutagenic, antioxidant, antimicrobial and apoptotic features (Ismail et al., 2012). The fruit comprises various flavonoids, corresponding to approximately 0.2–1.0% of the fruit weight. Nearly 30% of all fruit anthocyanidins are accumulated in the peel part. Concentration of these anthocyanidins varies according to the type of cultivar and different phases of fruit development, and it causes changes in the peel color (Fischer et al., 2011; Kumari et al., 2012; Zhao et al., 2013).

2.2 Pomegranate Process

Arils are commonly consumed fresh or used for beverages (Figure 2.1), canned fruit, jams and jelly. Arils are also employed in the extract development to be utilized as ingredient in herbal medicine and food supplements (Goula and Adamopoulos, 2012). This edible part is rich in potassium (259 mg potassium/100 g pomegranate) (Ebcioğlu, 2003).

Pomegranate not freshly consumed is mostly processed into the juice in industry and Table 2.1 shows that the amount of pomegranate processed into the juice increases by year which results from the increasing production of the pomegranate as seen on Table 2.3. By looking at the Table 2.1, 2.2, and 2.3 various calculations can be made about pomegranate wastes from consumption and percentage productions.

Table 2.1 Pomegranate processing datas by years (Anonymous a, 2008)

Year	Amount of pomegranate processed into juice (thousand tonnes)	Amount of pomegranate processed into concentrate-65 brix (thousand tonnes)
2000	-	-
2001	-	-
2002	-	-
2003	-	-
2004	-	-
2005	17.6	2.8
2006	46.6	6.9
2007	57.5	5.6
2008	49.5	5.2

Amount of Pomegranate production in the worldwide is about three million tonnes by year 2014.

Table 2.2 Pomegranate production and exportation of some countries (2008 – 2010) (Anonymous b, 2012)

Order	Country	Production (tonnes)	Exportation (tonnes)
1	India	1.140.000	35.000
2	Iran	705.000	60.000
3	Chine	700.000	-
4	Turkey	217.572	86.271
5	A.B.D.	120.000	17.000
6	Iraq	100.000	-
7	Spain	80.000	40 000
8	Syria	70.000	-
9	Azerbaijan	60.000	15 000
10	Afghanistan	60.000	1.000
11	Egypt	43.000	-
12	Uzbekistan	35.000	10.000
13	Pakistan	30.000	4.500

Table 2.3 Pomegranate production in Turkey on the basis of years (Anonymous c, 2018)

Year	Number of Trees (Thousand)		Production (Tonnes)
	Bearing	Nonbearing	
1988	1 989	509	45 000
1989	2 028	500	48 000
1990	2 110	456	50 000
1991	2 194	504	53 000
1992	2 200	485	50 000
1993	2 245	484	55 000
1994	2 258	476	58 000
1995	2 304	495	53 000
1996	2 350	520	56 000
1997	2 420	570	56 000
1998	2 320	500	55 000
1999	2 410	730	58 000
2000	2 485	809	59 000
2001	2 530	840	60 000
2002	2 670	855	60 000
2003	3 190	1 100	80 000
2004	3 200	1 220	73 000
2005	3 220	1 409	80 000
2006	3 136	1 502	90 737
2007	3 611	3 367	106 560
2008	4 017	5 929	127 760
2009	5 092	5 794	170 963
2010	6 431	5 679	208 502
2011	7 881	6 432	217 572
2012	10 011	5 789	315 150
2013	11 087	5 089	383 085
2014	11 756	6 034	397 335
2015	13 310	4 072	445 750
2016	13 859	3 482	465 200
2017	13 662	3 123	502 606

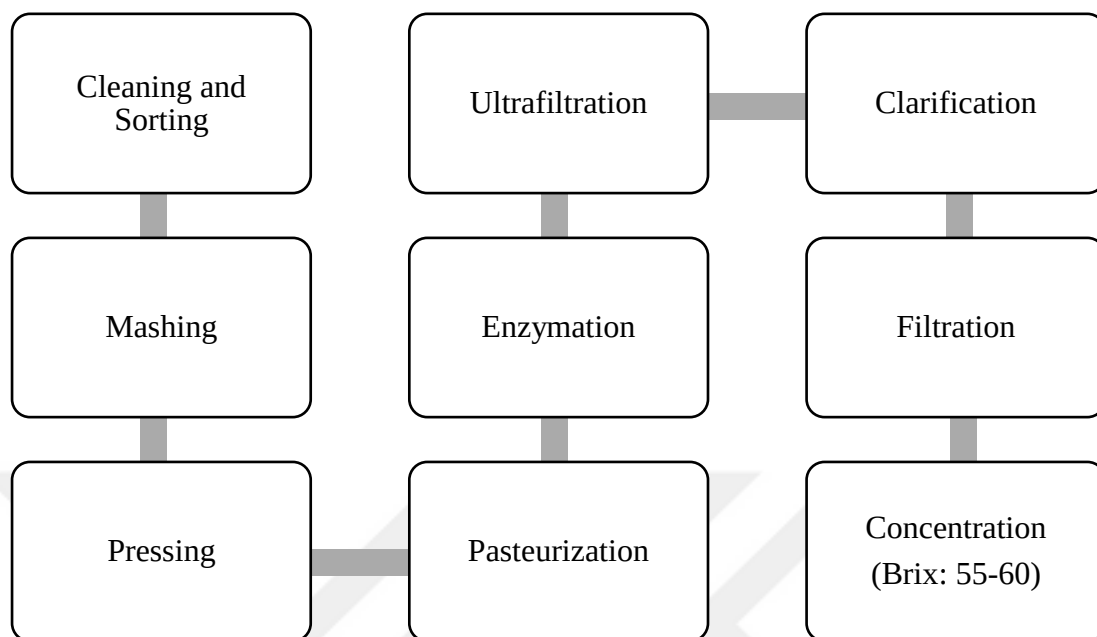


Figure 2.1 Flow diagram of pomegranate juice

2.3 Evaluation of Pomegranate Waste

Pomegranate (*Punica granatum L.*) is among the oldest known fruits and it has highest concentration of total polyphenols compared to other fruits tested (Fazaeli et al., 2013). Huge amounts of waste composed of peels and seeds is produced from the industrial handling of pomegranate. This waste possesses a problem for the environment. It has been stated that the bioactivity of the peels and seeds of some fruits is higher than bioactivities of the pulps (Goula and Lazarides, 2015).

Peel is the major waste product of the pomegranate and it has been extensively studied since it has a wide range of biologically active compounds. These include antioxidants such as phenolic acids and flavonoids (Singh et al., 2002; Li et al., 2006). Phenolic compounds have become increasingly popular as they have high antioxidant activity and healthful effects in some illnesses (Rice-Evans et al., 1996; Lodovici et al., 2001; Kim and Lee, 2004). Latest studies have revealed that the peel has higher antioxidant capacity compared to the aril juice (Li et al., 2006; Tzulker et al., 2007). Consequently, the peel fraction of pomegranate is very appealing due to its obvious woundhealing characteristics (Chidambara et al., 2004),

immunoregulatory activity (Gracious et al., 2001), antimicrobial activity (Navarro et al., 1996), antiatherosclerotic and antioxidative activities (Tzulker et al., 2007).

Oil percentage of seeds changes between 12 and 20 on a dry basis (Al-Maiman and Ahmad, 2002). It was recorded that pomegranate seed oil has biological features (Eikani et al., 2012), such as antioxidant and eicosanoid enzyme inhibition properties (Qu et al., 2010), immune function and lipid metabolism (Yamasaki et al., 2006), estrogen content (Tong et al., 2006), skin photoaging inhibition effect (Park et al., 2010), lipoperoxidation and activity of antioxidant enzymes (Melo et al., 2010), toxicological evaluation (Meerts et al., 2009) and protective effect against gentamicin induced nephrotoxicity (Asadpour et al., 2010).

Hence, due to health beneficial effects of pomegranate peels and seeds and their large annual production as industrial waste, they deserve more valuable practices in the food industry than just being animal feed or part of the cosmetics. In Figure 2.2, there is an example of pomegranate waste utilization processes.

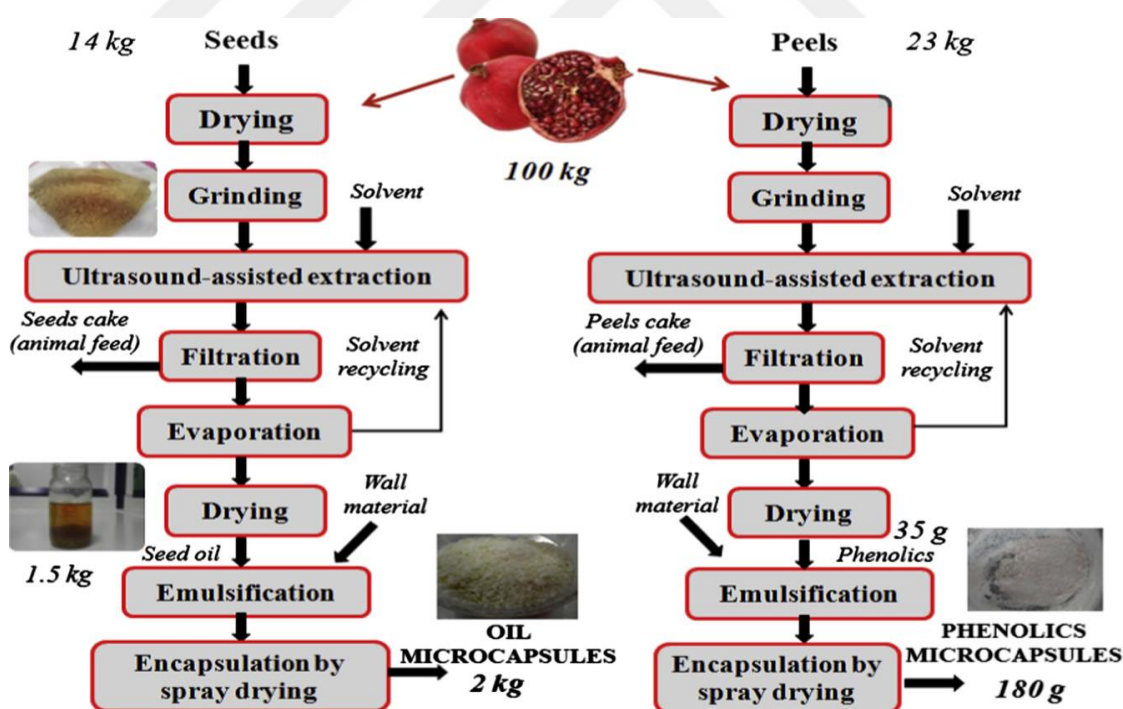


Figure 2.2 An example of pomegranate waste utilization process.

2.4 Pomegranate Seed and Seed Oil

Pomegranate seed is a residue obtained from juice and it contains vitamin E, sterols and punicic acid. Seeds can constitute up to nearly 20% of the fruit weight, varying between 9.3% and 57.5% (Fernandes et al., 2015) related to the variety, growth conditions, maturity level, etc. Pomegranate seeds contain mostly fiber and lipid (Eikani et al., 2012; Hernandez et al., 2011). Their oil percentage varies between 12 and 20 on dry weight basis (Lansky and Newman, 2007). Seed exhibit antioxidant behavior (Jing et al., 2012; Pande and Akoh, 2009). Various reports have demonstrated that pomegranate seed oils are rich in polyunsaturated fatty acids, particularly punicic and linoleic acids (Eikani et al., 2012; Jing et al., 2012; Liu et al., 2012), and tocopherols (Jing et al., 2012). They are a by-product of the pomegranate industry, but their potential use as a source of seed oil with favourable health effects has been accentuated by the recent reports. The seed content of the pomegranate shows average contents of about 37–143 g/kg of fruit. The seeds are rich in lipids, which vary between 140 and 270 g/kg dry matter (Al-Maiman and Ahmad, 2002; Melgarejo et al., 1995).

Significant amounts of polyunsaturated fatty acid (PUFA) are present in pomegranate seed oil, including conjugated linolenic acid (CLA) isomers as well as a low saturated fatty acid content (Özgül and Yücel, 2005). CLA isomers may protect against diseases such as cancer (Yasui et al., 2005). Furthermore, consumption of oils, containing rich amounts of highly unsaturated fatty acids (HUFA), prevents diseases such as colorectal, breast, colon and prostate cancers (Gerber et al., 2005). Essential fatty acids such as omega3-, and 6-fatty acids, decrease plasma triacylglycerols and LDL cholesterol, which are risk factors for cardiovascular disease (Dubois et al.; 2007). Moreover, pomegranate is extensively utilized in remedial formulations, cosmetics and food seasonings (Viuda et al., 2010).

2.5 Oil Extraction Methods

2.5.1 Soxhlet Extraction

Soxhlet extraction is a well-known and commonly used extraction method (De Castro et al., 2010). Franz Ritter von Soxhlet invented the Soxhlet extractor in 1879 (Chen and Urban, 2015). The classical of Soxhlet extraction was originally

performed for milk fat analysis (Soxhlet, 1879). Here, the material to be extracted is put in a thimble holder. Then, the condensed solvent coming from a distillation vessel is slowly filled into the thimble holder. When the solute-solvent mixture gets to siphon level on the thimble-holder, it is poured into the distillation flask. This process is continued until extraction is finished (De Castro et al., 2010).

At present, Soxhlet extraction has numerous applications. Unlike other techniques, in Soxhlet extraction the compounds fixed in matrices are leached thoroughly with freshly distilled extractant. Here are the basic steps of the procedure: (De Castro et al., 2010) (1) the extractant is vaporized, (2) the solvent vaporized is condensed, (3) the solutes in the sample are leached by the condensed solvent, (4) The extract is collected in the thimble-holder, (5) the collected extract is poured into the solvent flask from the siphon, (6) these steps are repeated until the extraction is completed (1)–(5).

Soxhlet extraction can be considered as a semi-batch operation. The solvent is gradually given to the system, so the operation is a batch system. Still, it is also a continuous system due to the recirculation of the solvent by means of the siphon.

Soxhlet extraction has been commonly used for a long time and today the majority of the studies making use of this technique include collecting the extracts for other processes (Prados et al., 2003; Sukhija and Palmquist, 1988) or comparing it with other sample preparation methods such as microwave-assisted extraction (Egizabal et al., 1998; Frost et al., 1997), super fluidic extraction (Snyder et al., 1992; Erstfeld and Chen, 1998), and accelerated extraction (Bandh et al., 2000; Abdullah et al., 2006). While Soxhlet extraction is generally preferred for studying complex materials (Akinlua and Smith, 2009; Chung et al, 2010), it requires too much time for the process.

There are some agreeable advantages of conventional Soxhlet extraction. The sample is consistently brought into contact with fresh portions of extractant, which eases shifting of the transfer equilibrium. Besides, the heat applied to the distillation flask which reaches the extraction cavity to some extent keeps the system at a relatively high temperature. Moreover, no filtration is needed after leaching and amount of sample to be analyzed may be increased by carrying out multiple consecutive

extractions, thanks to the affordable equipment. Furthermore, Soxhlet extraction is an easy technique that calls for little education, more sample mass can be extracted compared to the many of the modern techniques such as microwave-assisted extraction or supercritical fluid extraction, and apparently it is not exposed to the matrix effects. However, this claim may not always be true, as in the case of comparing Soxhlet extraction with supercritical fluid extraction for the analytes firmly bound to their matrix (De Castro et al., 2012). There is a wide variety of official methods involving a sample preparation step based on Soxhlet extraction (De Castro et al., 2010).

The consumption of excess amount of solvent, which is too valuable to throw away and also cause of environmental risks, and long extraction time are the most important disadvantages of Soxhlet extraction in comparison with other methods for solid sample preparation. Thermolabile target species may be thermally decomposed because the samples are generally extracted at the boiling point of the solvent for long periods. Besides, there is no agitation to accelerate the process in a conventional Soxhlet system. Also, an evaporation–concentration step is needed after the extraction for large amounts of extractant used. Lastly, Soxhlet extraction is dependent on the solvent.

2.5.2 Solvent Extraction

Bucholz's extraction in 1805 was possibly the first reported solvent extraction study, where he obtained uranium from a nitric acid solution into ether. He then extracted uranium into pure water.

Solvent extraction method has been established before that, but in a different field of chemistry. Andres Marggraf noticed formation of crystals after the extraction of beetroot with brandy in 1747.

A new extraction method for uranium (VI) with ethyl acetate or diethyl ether was discovered by Professor Eugène-Melchior Péligot from Institute National Agronomique in 1842.

Walther Hermann Nernst (1864-1941) built the solvent extraction methods on a solid ground by formulating an equation satisfying thermodynamic consideration of

concentration as well as the activity coefficient of solute both in aqueous and the organic phase. The origin of solvent extraction is not certain, but it might have been performed already by the Persian alchemist as-Razi who is believed to have used extraction to separate metals (Vértes et al., 2010).

Solvent extraction, liquid-liquid extraction (LLE) or partitioning is a method to separate compounds in two different immiscible liquids due to their relative solubilities (i.e. ones that cannot get mixed up together and separate into layers when shaken together). These liquids are generally water and an organic solvent. In LLE, a substance is extracted from one liquid into another liquid phase. Extraction of substances by solvents which are often employed both in a laboratory and in large scale manufacturing is the most common use of the distribution principle. Organic compounds are generally more easily dissolved in organic solvents such as benzene, chloroform, ether, etc. than in water, and these solvents are immiscible with water. Organic compounds are then quite easily separated from the mixture with inorganic compounds in aqueous medium by adding benzene, chloroform etc. These are separated into two layers by shaking. More organic compounds would pass into a non-aqueous layer because their distribution ratio largely in favor of benzene phase. Finally, the purified compound is obtained by removing and distilling this non-aqueous layer (Rydberg, 2004).

Solvent extraction has become a popular separation technique in analytical chemistry recently because it is simple, rapid, and applicable to both large and small amounts of metal ions. The fundamentals of solvent extraction are well understood (Fifield and Kealey, 1975; De et al., 1970).

2.5.3 Microwave Assisted Solvent Extraction

Microwave-assisted extraction (MAE) or simply microwave extraction which unites microwave and traditional solvent extraction is a comparatively new method. In microwave-assisted extraction microwaves are applied to heat the solvents and solutes, and this raises extraction kinetic. Shorter extraction time, less solvent, higher extraction rate and lower cost are some advantages of MAE over traditional method. MAE in extraction of natural products have come into use in the late 1980s and it has now become one of the popular and cost-effective extraction methods available today by means of the technological developments. A number of advanced MAE

instrumentations and methodologies such as pressurized microwave-assisted extraction (PMAE) and solvent-free microwave-assisted extraction (SFMAE) have become available (Delazar et al., 2012).

In MAE, electromagnetic waves cause changes in the cell structure and the extraction occurs. It has been proposed that the heat and mass transfer gradients working in the same direction may be the cause of the extraction acceleration observed in MAE. Kinetics of extraction are increased by using microwaves for heating the solvents and plant tissues, thus various advantages over traditional solvent extraction, including shorter extraction times, higher extraction rates, lower costs, and less solvent use are obtained. MAE has been utilized in the extraction of various compounds from natural sources, including essential oils (EOs) from leaves, terpenes from must, and gossypol from cottonseeds (Beoletto et al., 2016).

In microwave-assisted extraction the solvent together with the sample is heated with microwaves. The separation of the desired solutes from the sample depends on the temperature and solvent characteristics. It must be noted that, unlike classical heating, microwaves heat the entire sample simultaneously without heating the vessel, so extraction occurs in a very short time since the solution approaches its boiling point quickly (Camel, 2000).

Although compounds are related from plants have an important place in medicine, researchers have still been dealing with safety and quality problems which are related with the lack of high performance and reliable extraction technique (Huie, 2002). Since it is the beginning for the isolation and purification procedures, extraction is an important step for obtaining new bioactive compounds. Several active phytoconstituents may abundantly exist in an individual plant together with certain constituents of low activity profile. Therefore, development of high performance extraction techniques is essential (Smith, 2003). In order to decrease the extraction time and the cost of solvent consumption there has been a need for better and newer extraction techniques in the herbal drug industry (Nyiredy, 2004). Soxhlet extraction has been the most commonly used among other traditional and conventional extraction techniques. Soxhlet extraction serves as both an extraction technique for phytoconstituents and a reference to compare newer extraction techniques. Having longer extraction period and requiring significant amount of heat energy are the

downsides of Soxhlet extraction. The time consuming procedure causes lowering the number of samples to be analyzed (Sanghi and Kannamkumarath, 2004; Luque-Garcia and De Castro, 2004). In addition to aforesaid disadvantages, excessive amounts of organic solvent are used in these kind of extractions and this usage is a potential hazard to the environment (Zuloaga and Madariaga, 1999; Luque and Garcia-Ayuso, 1998; Tatke and Jaiswal, 2011).

2.5.4 Microwave Theory

2.5.4.1 Microwave Principle

Microwave is a radio wave, and radio wave is an electromagnetic wave.

The electromagnetic wave can be spread in a vacuum since it is spread by the interaction of electric field and magnetic field.

Electromagnetic wave has two elements; wavelength and frequency. The wave length is about the length of the wave from top to top, and the frequency is the number of waves that appear in a second.

The velocity of electromagnetic wave in a free space, such as vacuum and air, is constantly 300000 km per second regardless of frequency. Wavelength equals to this divided by frequency.

As shown in Figure 2.3, depending on the frequency, electromagnetic waves are called differently. According to their characteristics, they are used in several applications. Light is also a kind of electromagnetic wave. Radio waves are electromagnetic waves that are less than 3000 GHz and microwaves are radio waves that have frequency of 300 MHz to 300 GHz (wavelength of 1m to 1mm).

Microwaves have been used in communication, radio telescope for astronomy, radar surveillance system, and also in GPS positioning system known as car navigation system. Heating is another application of microwave (Finkenzeller, 1999).

Frequency	Wave length	Designation			Use	
100 —	—10pm	*1	Gamma ray		Medical, Material Inspection	
10 —	—100		X ray		Medical, Material Inspection	
1EHz —	—1nm		Ultraviolet ray		Bactericidal lamp*Electronic device Manufacturing equipment	
100 —	—10	*2	Visible ray		Infrared heating appliance	
10 —	—100		Infrared ray			
1PHz —	—1 μ m		Far infrared ray			
100 —	—10	*3	Decimillimetric wave		Radar system	
1THz —	—1mm		EHF	Millimetric wave		Microwave
100 —	—10		SHF	Centimetric wave		
10 —	—100		UHF	Decimetric wave		
1GHz —	—1m		VHF Meter wave		FM radio, Television	
100 —	—10		HF Decametric wave		Shortwave radio	
1MHz —	—100		MF Hectometric wave		Middle-wave radio	
100 —	—1km		LF Kilometric wave		Ship, Aircraft Communication, Induction heating cooking device	
10 —	—10		VLF Myriametric wave		50/60HzHigh-voltage transmission line Electric home appliances	
1kHz —	—10 ³ km		ELF			
100 —	—10 ⁴ km	*4	ULF			Biological electric phenomena, such as brain wave
10 —	—10 ⁵ km				Radio wave, radiation around earthquake source	
1Hz —	—10 ⁶ km					

*1 Ionizing radiation

*2 Light

*3 Nonionizing radiation

*4 Radio wave

*1 Ionizing radiation *2 Light *3 Nonionizing radiation *4 Radio wave

Figure 2.3 Application and classification of electromagnetic wave

Microwave energy is a non-ionizing radiation with a frequency of 300-300 000 MHz. It leads to the migration of ions and rotation of dipoles resulting in molecular motion. Dipole rotation refers to the alignment of molecules in the solvent and samples that have dipole moments thanks to the electric field (either permanent or induced by the electric field). As the field decreases, thermal disorder is restored and thermal energy

is released. At 2450 MHz (which is the frequency used in commercial systems), the alignment of the molecules followed by their return to disorder occurs 4.9×10^9 times per second and this leads to rapid heating. A dielectric compound is necessary for the heat generation in the sample in the microwave field. When dielectric constant is higher, more thermal energy is liberated and heating will be faster. Actually, the heat formation in a material depends partially on the dissipation factor. It is the ratio of the material's dielectric loss to its dielectric constant. The dielectric constant is a measure of how much microwave energy can be absorbed by the material, and the loss factor is the ability of the material to dissipate the absorbed energy. Therefore, both the solvent and the matrix are very important in terms of the effect of microwave energy. Usually, a solvent with a high dielectric constant is chosen, so that it strongly absorbs the microwave energy (Pare et al., 1994).

On the other hand, sometimes while extracting heat sensitive compounds the sample alone can be heated by using a solvent with a low dielectric constant. It is proven that MAE allows the migration of the compounds out of the matrix during the extraction of essential oils from plant materials. Indeed, the microwave selectively interact with the free water molecules in the system. This provides a localized heating leading to raise of the temperature to the boiling point of water (Pare et al., 1994). These systems experience an apparent expansion, and their walls are broken. So the essential oil flows into the solvent. Classical solvent extraction in which the solvent diffuses into the matrix and extracts the components by solubilization is quite different from this process. Moreover, since the technique should be less dependent on a high solvent affinity, a wider range of solvents could be used in MAE (Camel, 2000).

Molecules are heated by microwaves through dual mechanism of ionic conduction and dipole rotation (Tatke and Jaiswal, 2011). Microwaves are non-ionizing electromagnetic waves situated between the X-ray and infrared rays in the electromagnetic spectrum with frequency between 300 MHz to 300 GHz. The electric field and magnetic field are the two types of oscillating perpendicular fields that form microwaves. Substances are heated by the ionic conduction and/or dipole rotation when the microwaves interact with polar solvents. Moving of ions due to the changing electric field is known as ionic conduction (Anastassiades et al., 1992).

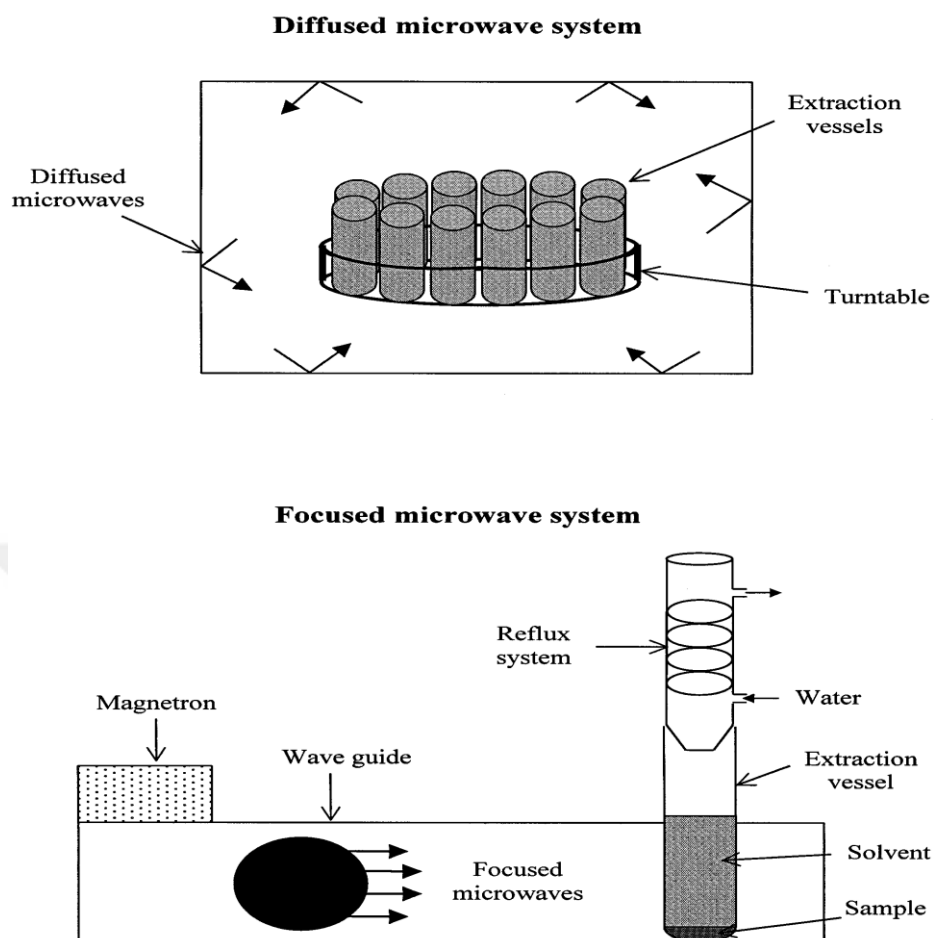


Figure 2.4 Diffused microwave system and focused microwave system

If the solution resists this motion, the solution is heated by means of the generated friction. Dipole rotation is the realignment of the dipoles of the molecule with the rapidly changing electric field (Burfoot, 1967). Heating process occurs at a frequency of 2450 MHz. The electric field is changed by microwaves at a high speed (4.9×10^4 times/second). As a result of this, the solvent molecules move according to the changing electric field. Thus, the heat is generated through frictional force (Burkert et al., 1993). Heating does not happen when the frequency is higher or lower than 2450 MHz (Carroll, 1970; Tatke and Jaiswal, 2011). Only dielectric materials having permanent dipoles are not heated by microwave (Tatke and Jaiswal, 2011).

2.5.4.2 Microwave Extraction Principle

The basics of the microwave extraction are not similar to the conventional extractions because in MAE, electromagnetic waves altering the cell structure enable the extraction.

The reason of the process acceleration and high extraction performance in MAE, may be the effect of a synergistic combination of two transport phenomena: heat and mass gradients working in the same direction (Chemat et al., 2009). Conversely, in conventional extractions the direction of the mass transfer is outwards, while the direction of the heat transfer is inwards (Figure 2.5). Besides, in MAE, the medium is volumetrically heated, whereas the heat is transferred from the heating medium to the interior of the sample in conventional extraction.

In the extraction process, there is not a linear relation between the extract recovery and time: an unsteady condition occurs since the solute concentration in the solid changes. It is considered that the following steps are taking place during the separation process in extraction. Some important steps must take place during the interaction period between the solid and the solvent, involving (1) the solvent permeates into the solid parts; (2) the components are solubilized; (3) the solute moves out of the matrix; (4) the extracted solute migrates from the solid surface into the solution; (5) the extract moves according to the solid; and (6) the extract and the solid are separated (Aguilera, 2003).

Hence, the solvent passes through the sample matrix by diffusion, and dissolves the solute. The solution containing the solute diffuses to the surface by effective diffusion. Finally, the solution is transferred from the surface to the bulk solution by natural or forced convection (Figure 2.5).

During the extraction, different forces may be exposed such as dispersion and driving forces. Diffusion, chemical interactions, the persistence and strength of these events may be strongly dependent on the solvent characteristics (Table 2.4) such as solubilization power, purity, polarity, etc. (Majors, 2008).

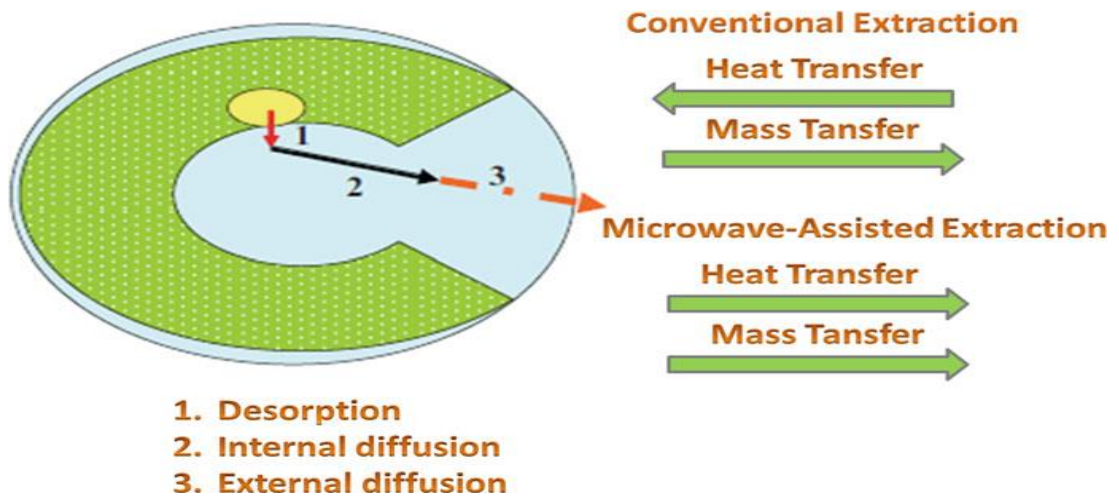


Figure 2.5 Basic transfer mechanisms in microwave-assisted extraction and conventional extraction (Veggi et al., 2012).

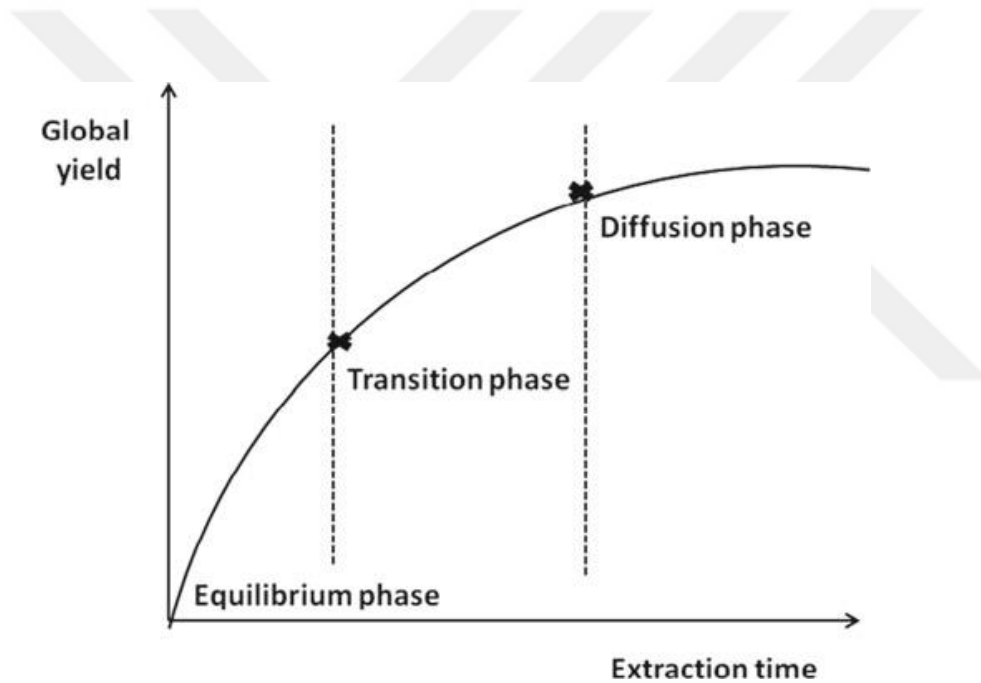


Figure 2.6 Global yield versus time during the extraction (Veggi et al., 2012).

Table 2.4 Physical constants and dissipation factors for commonly used solvents of microwave assisted extraction (Kingston and Haswell, 1997; Zlotorzynski, 1995).

Solvent	Dielectric constant, ^a (ϵ')	Dissipation factor $\tan \delta$ ($\times 10^{-4}$)	Boiling point, ^b ($^{\circ}\text{C}$)	Viscosity, ^c (cP)
Acetone	20.7	5.555	56	0.30
Acetonitrile	37.5		82	
Ethanol	24.3	2.500	78	0.69
Hexane	1.89		69	0.30
Methanol	32.6	6.400	65	0.54
2-Propanol	19.9	6.700	82	0.30
Water	78.3	1.570	100	0.89
Ethyl acetate	6.02	5.316	77	0.43
Hexane-ethanol (1:1)			52	

^aDetermined at 20 $^{\circ}\text{C}$, ^bDetermined at 101.4 kPa, ^cDetermined at 25 $^{\circ}\text{C}$

Using domestic microwave ovens were the first applications of MAE (Ganzler et al., 1986). However, it is vitally important to use only the equipment approved for MAE because the application of microwave energy to flammable organic compounds such as solvents may induce serious hazards. There are many studies explaining the operation of the common commercial extractors in detail (Renoe, 1994; Lopez-Avilla et al., 1994). Only the frequency level of 2450 MHz is applied in all of them. Microwave energy can be applied in two ways: using open vessels or closed vessels (Letellier and Budzinski, 1999; Demesmay and Olle, 1993). They are often called as pressurized MAE or focused MAE. Both applications are shown in Figure 2.4. Since the microwave systems are considered simple, it must be noted that the level of hazard and exposure to accidents will increase if proper safety measures are not practiced or if the experiments are not properly performed. Therefore, it is strongly recommended to use only approved equipments and specifically sound procedures (Camel, 2000).

2.5.4.3 Microwave Extraction Systems (Closed and Open Vessel)

In closed vessels, the solvent can be heated above its boiling point at atmospheric pressure, thus both extraction speed and efficiency are enhanced. Such systems allow controlling the extraction process. Additionally, using several vessels to perform parallel extractions may usually increase sample throughput. A turntable is used since the electric field is not homogeneous in the cavity (Camel, 2000).

The CEMMES 1000 is the most commonly used closed system (Renoe, 1994). This system provides extracting 12 samples at a time, so the extraction process is shortened. In addition, existence of a detector monitoring the presence of solvent in the cavity, availability of a pressure-burst membrane in each vessel, and temperature and pressure control systems contribute to the safety of the operation. Yet, because of the pressure build-up in the vessels, safety measures should be followed. Therefore, the rupture membrane in all vessels should be changed before every extraction to prevent leaking of the solvent vapor in the cavity. Besides, the pressure is controlled in only one vessel, so the samples to be extracted in parallel should be very similar; particularly in terms of water level and organic content (Lopez-Avila et al., 1995). Because solvent blanks get heated more rapidly than the samples, they should not be heated together.

The MARS-X enables to extract up to 14 samples at a time. The design of its vessels ensures enhanced safety and fast cooling.

In these systems if the temperature of the extraction vessel increases quickly, solutes that are more volatile break up into the headspace and these compounds may be lost. This is the main disadvantage of such systems. Furthermore, when the extraction is finished, it is very important to cool the vessels to room temperature before they can be opened in order to avoid losses of volatile solutes. However, this step significantly increases the overall extraction time (Camel, 2000).

In the open systems, the highest temperature the extraction can reach depends on the boiling point of the extractant at atmospheric pressure. Focused microwaves are used in such systems providing homogeneous and very efficient heating of the sample. Prolabo Soxwave 100 is the most commonly used one. Here, there is a reflux system on top of the extraction vessel to prevent losses of vapor. Very recently, an improved version of this system, called Soxwave 3.6, has been introduced to perform multiple extractions. Sample handling safety of these systems is higher than the closed vessel systems. Moreover, extraction of larger materials can be performed in these systems compared to the closed vessels (Camel, 2000).

Open vessel extraction systems are generally chosen for the extraction of organometallic compounds. This is because the destruction of the bonds between

carbon and metal elements is prevented since the energy transferred to the sample is precisely controlled (Szpunar, 1996).

Increased pressure inside the vessel raises the boiling point of the solvents used, so higher temperatures can be reached in these systems. Loss of volatile compounds is not considered in a closed system vessel. Also, little amount of solvent is needed. Addition of solvents repeatedly is not necessary; this reduces the risk of air-borne contamination (Tatke and Jaiswal, 2011).

A closed vessel system has two main disadvantages. Only small amount of sample can be analyzed and use of high pressure can be dangerous. Besides, the vessel should remain closed until it cools down to the room temperature; otherwise, loss of extracted volatile constituents will occur (Tatke and Jaiswal, 2011).

Safety of open vessel systems is higher than closed vessel systems where high pressures are used and open vessel systems allows addition of reagent at any time of the process. Polytetrafluoroethylene (PTFE), glass or quartz materials can be used for vessels of the oven and excess of solvents can be removed easily. A large amount of sample can be analyzed in open these systems and this is the main advantage of the instrument. Also, the equipment is affordable (Tatke and Jaiswal, 2011).

Open vessel method is generally less accurate compared to the method of closed vessel systems. Longer extraction times are needed in open vessel systems to get extraction yield as high as yield of closed vessel systems (Tatke and Jaiswal, 2011).

2.5.4.4 Factors Affecting Microwave Assisted Solvent Extraction

2.5.4.4.1 Particle Size of Sample

Besides the above-mentioned parameters, sample properties also affect the MAE technique. The extraction yield increases with increasing surface area and a better microwave penetration is obtained with finer particles (Huie, 2002). However, problems may occur when the particles are too small; so, centrifugation or filtration may be performed for matrix preparation (Mandal et al., 2007; Tatke and Jaiswal, 2011). Grinding and homogenization are applied to the sample in the preparation step for higher contact surface area. The particle size is mostly between 100 μm and 2 mm (Eskilsson and Björklund, 2000). Sometimes, an improved yield is obtained by

immersing the material in the solvent before MAE. This technique is known as pre-leaching extraction (Mandal et al., 2007).

Like particle size and the nature of the material the matrix characteristics will influence the compound recovery significantly. When the particle size of the sample is smaller, the surface area increases and the effect of microwave will be better (Huie, 2002). However, small particles may cause difficulty during the extraction. This difficulty can be overcome with an extra process such as filtration.

Sometimes the samples can be pretreated with solvents which have microwave absorption ability. Thus, the cells take up the solvent and dual heating of the solvent will occur upon microwave application (Talebi et al., 2004). Immersing the matrix with solvent before microwave application (preleaching extraction) is applied by some researchers to get higher efficiency. With preleaching, the yield of tashinones was observed to be increased (Pan et al., 2001). In closed vessel systems, microwave power and temperature affect each other directly, so these two parameters should be taken into consideration together. When the temperature increases, extraction efficiency is improved because removal of the materials to be extracted from the matrix is facilitated (Tatke and Jaiswal, 2011).

2.5.4.4.2 Microwave Power

Increasing the microwave power may raise the temperature and increase the efficiency of the extraction to a certain extent (Hu et al., 2008; Xiao et al., 2008; Chemat et al., 2005). The microwave power determines how much energy is given to the matrix. This energy turns into heat in the dielectric material, which in turn controls.

Efficacy of the solvent is better at higher temperatures because the viscosity and surface tension are decreased. Hence, the solubilization is facilitated and sample wetting penetration are improved (Mandal et al., 2007; Li et al., 2010; Khajeh et al., 2010). Moreover, the temperature may exceed the boiling point of the solvent in closed vessel systems in MAE. Thus, extraction efficiency is improved by the solutes released from the matrix (Eskilsson and Björklund, 2000). On the other hand, Routray and Orsat (2012) indicate that the increase in yield with increasing temperature lasts until an ideal point and then further increase in temperature causes

the efficiency to decrease. The reason for this is that the selection of ideal extraction temperature is directly related to the stability and, therefore, to the yield of the target compound.

The quantity of sample and the extraction time needed is directly linked with the microwave power. The localized heating in the sample provided by the microwave power serves as a driving force to disrupt matrix, which enables the solute to be separated from the matrix and diffuse into the solvent. Hence, the extraction yield will be better and the extraction time will be shorter when the power is increased (Hu et al., 2008; Chan et al., 2011). However, when high microwave power is applied, a poor extraction yield may be observed as a result of the degradation of thermolabile substances. Also, when higher power is used, the cell walls break down rapidly at a higher temperature. Therefore, impurities may accompany the solute in the solvent (Mandal et al., 2007). So, proper selection of the MAE power is essential to reduce the period required to achieve the desired temperature (Eskilsson and Björklund, 2000). Furthermore, it was found that the extraction yield is decreased due to the excess exposure to microwaves, even if the working conditions such as temperature or power are kept low, because the chemical constitution of the compounds is disrupted.

Since only the level of microwave power is not enough to understand the microwave energy taken into the system. Therefore, the energy density term was created by Alfaro et al. (2003) to examine the effect of power on MAE. It is the microwave irradiation energy per unit of solvent volume for a given unit of time (W/mL). As stated by Li et al. (2010) the energy density should be taken rather than microwave power level. Also, he obtained different extraction rates of anthocyanin from grape peel when the microwave power, extraction time, and solvent/feed ratio values were the same because of different energy density levels.

Raner et al. (1993) noted that the yield of flavonoids was not significantly affected by the power variation between 500 to 1000 W. At temperatures higher than 110°C, the extraction yield was found to be decreased due to the thermal degradation of flavonoids (Xiao et al., 2008). In another study, thermal degradation of phenols was observed at a higher power level of 350 W (Song et al., 2011). Also, Yan et al.

(2010) found that temperature rise from 50°C to 70°C increased the yield remarkably; however, above 70°C, it increased slowly and even decreased.

Microwave power and irradiation times affect each other considerably. Generally, low or moderate power is combined with longer exposure in order to optimize the MAE procedure. Although the application of high power is associated with thermal degradation, there are studies indicating that increasing the microwave power did not significantly influence the flavonoid yield (Raner et al., 1993).

2.5.4.4.3 Temperature

Temperature is very important in guaranteeing efficient extraction. Elevated temperatures generally improve the extraction because the solvent better diffuses into the matrix at high temperatures. Besides, release of the analytes from the matrix is enhanced. Pressure is a crucial factor in closed systems, yet it is directly affected by the temperature. So, the temperature may be controlled to avoid degradation (Camel, 2000).

2.5.4.4.4 Time

Time is also a parameter to take into consideration as in other extraction techniques. As an example, higher yields of Polycyclic aromatic hydrocarbons (PAHs) from a certified marine sediment was obtained by increasing the extraction time for 5-10min (at 30W in open vessels) (Budzinski et al., 1996). However, longer extraction times had no further effect, as was also observed for Polychlorinated biphenyls (PCBs) from sludge samples (Dupont et al., 1999). Likewise, better recoveries were obtained with 20-min extractions than 5-min extractions in closed vessels for PAHs in soils and sediments (Lopez et al., 1994). Besides, extraction time was not a significant factor for the PMAE of methylmercury from sediments (Vazquez et al., 1997).

Prolonged extraction periods may result in degradation of heat sensitive compounds, as in the case of organotins (Donard et al., 1995).

Extraction times in MAE are mostly between a couple of minutes and half an hour, which is very short compared to conventional techniques. This provides avoiding from potential thermal degradation and oxidation (Al-Harabsheh and Kingman, 2004; Chan et al., 2011). When the solvent dielectric properties are high, as in

ethanol and methanol overheating happens (Routray and Orsat, 2012). Longer extraction time mostly shows a tendency to increase the efficiency of extraction. However, as the time increases, the increase in efficiency declines (Wang et al., 2008).

In some cases, if an extended time of extraction is needed, the extraction may be performed in several steps implementing serial extraction rounds (Chan et al., 2011). Here, the new solvent is given to the sample and the procedure is done repeatedly to ensure the consumption of the matrix. The extraction efficiency is improved and overheating is avoided with this procedure (Routray and Orsat, 2012; Chan et al., 2011). The number of runs is based on the type of sample and the solute. As reported by Li et al. (2010), for MAE of triterpene saponins from yellow horn, three cycles of 7 min were suitable, while cycles of 5 min each were advised in the extraction from *Ganoderma atrum* (Chen et al., 2008). Yan et al. (2010) reported that three round of extraction each lasting 5 min were ideal to extract astragalosides from *Radix astragali*. According to their results, the extraction yield rapidly increases by raising the application time from 1 to 5 min. The efficiency raises to the highest level at 5 min, and after that, it decreases with further increasing of the time. In the extraction of flavonoids from *Radix astragali*, the yield was increased by prolonging the exposure up to 25 min and after that, it began to decline (Xiao et al., 2008). In the study of Chen et al. (2007) in the extraction of triterpenoid saponins from *Ganoderma atrum*, the yield rose to the highest level at 20 min; then, the decomposition of the desired compounds took place as a result of long exposure to high temperature. Song et al. (2011) observed the same behaviour in their work.

2.5.4.4.5 Solvent/Sample Ratio

The optimization of the solvent/solid (feed or sample) ratio is important in extraction. The volume of solvent must be sufficient to ensure that the sample is entirely soaked in the solvent during whole microwave application process. This is particularly important while using a swellable matrix (Eskilsson and Björklund, 2000; Mandal et al., 2007; Tatke and Jaiswal, 2011).

The extraction recovery is increased by the use of large volumes of solvent in conventional extractions. According to studies the extraction solution should be less than 34% (w/v) (Eskilsson and Björklund, 2000). In many applications the ideal

ratios were between 10:1 (v/w) to 20:1 (v/w) (Talebi et al., 2004; Pan et al., 2003). Additionally, the volume of solvent is a significant factor to be taken into account because more energy and time is necessary to condense the extraction solution in the later step and for purification process when too much solvent is used (Eskilsson et al., 1999).

Sometimes, small volumes of solvent are enough to obtain the desired compounds. In the extraction of some phenolic compounds from oils ideal conditions were obtained when solvent/feed ratio reached 2 (Llompart et al., 1997). On the contrary, a better extraction rate was obtained by using more solvent in the MAE of artemisinin from *Aretimisia annua L.* (Lu et al., 2008). The recovery of triterpenoid saponins from *Ganoderma atrum* was improved by increasing the volume of solvent until the S/F (solvent/feed) rose to 25, and after that the yield was reduced quickly (Chen et al., 2007).

There must be enough solvent volume to soak the matrix entirely in the solvent from beginning to end of the operation. Better extraction yields are obtained by using higher ratio of solvent volume to solid matrix in conventional extractions, whereas in MAE it may not give better efficiency because the microwaves may not reach the matrix homogeneously (Tatke and Jaiswal, 2011).

2.5.4.4.6 Solvent Type

Proper selection of the solvent is the main factor affecting the process of extraction. The solvent is chosen according to the interactive relation of solvent and matrix, the solubility of the analyte of interest, and solvent's ability to take in microwaves in an experimental setup (Chen et al., 2008). The solvent should exhibit a higher affinity to the desired analyte and lower affinity to the other components of the matrix. Also it should adapt well to further chromatographic analytical steps. If the solvent does not absorb microwaves, it will not get heated. In the reverse situation, the extraction efficiency will be improved. For example, hexane is a solvent transparent to microwaves while ethanol is an excellent microwave absorbing solvent. Sometimes high and low microwave absorbing solvents are mixed to get optimum extraction yields (Tatke and Jaiswal, 2011).

There is some moisture at inner parts of plant tissues. When the water molecules are heated the cell walls are destroyed and the phyto-components in cell walls pass to the plant environment. For extractions, which use only the moisture in plant tissues without using any solvent in order to obtain oils especially from aromatic plant tissues, method has been improved that is called solvent free microwave assisted extraction (Tatke and Jaiswal, 2011).

The most outstanding factor influencing MAE is the choice of solvent. A more efficient extraction is obtained with the choice of the appropriate solvent. Solvent selection is made based on the solubility of the desired compounds, solvent penetration, its interaction with the sample matrix, its dielectric constant (Chen et al., 2008), and the mass transfer kinetics of the process (Spigno and De Faveri, 2009). A solvent should ideally have a high affinity for desired solutes and low affinity for undesired matrix components. It is also important to know that the solvents used in conventional extractions cannot be directly selected for the MAE process. The solvent is chosen according to solvent's capacity of microwave absorption (Chan et al., 2011; Eskilsson and Björklund, 2000; Mandal et al., 2007; Routray and Orsat, 2012).

Generally, when the solvent has high dielectric constant and dielectric loss, the capacity of the solvent to absorb microwave energy is high (Spigno and De Faveri, 2009). Not only polar but also nonpolar solvents may be employed in MAE. Solvents that are polar enough to absorb microwaves such as ethanol and water are heated by microwave irradiation (Brachet et al., 2002). In this regard, solvent characteristics may be adjusted by mixing different solvents. This allows diversification of the solvent selectiveness (Brachet et al., 2002). The heating rate may also be increased by the addition of salts to the mixture, because the ion conductivity is the one of the main reasons of polarization (Spigno and De Faveri, 2009). According to the studies, the presence of water in the extracting solvent in small amounts enables the diffusion of water into the matrix, which leads to an improved heating and favoring the transfer of compounds into the solvent more rapidly.

While extracting volatile compounds, solvents that have low dielectric constants can be used to prevent overheating of (Routray and Orsat, 2012). Hexane is commonly preferred for the extraction of volatile oils (Mandal et al., 2007).

Studies have showed that poor microwave absorbers; such as hexane can be used together with ethanol or water to enhance the extraction yield. Hexane-acetone is a very common solvent mixture (Eskilsson and Björklund, 2000). To accelerate the heating, some water should be used in nonpolar solvents like hexane and toluene (Eskilsson and Björklund, 2000). Different mixtures of ethanol and hexane were evaluated by Zhou and Liu (2006) in solanesol extraction from tobacco leaves; the best yield was obtained with a ratio of 1:3. In rice bran oil extraction, approximately 40% more oil was extracted with hexane at 40°C compared to the isopropanol. However, rice bran oil extraction yield of hexane was not significantly high when the temperature increased, while 25% more yield was obtained with isopropanol at 120°C (Zigoneanu et al., 2008).

The use of solvent mixtures in MAE has been studied by some authors. For microwave extraction of gymnemagenin from *Gymnema sylvestre* R. Br a combination of methanol and water (85:15) found to be an effective solvent. The extraction yield reduced with higher water concentration since it increases the mixture polarity more than adequate. Talebi et al. (2004) observed the same condition when extracting paclitaxel from *Taxus baccata*: the best combination was a methanol–water (90:10) mixture. In the extraction of sweet potato leaves, Song et al. (2011) reported that 60–80% (v/v) ethanol concentration in water was ideal.

2.5.4.4.7 Mixing Speed

The mass transfer process in the solvent phase is directly affected by stirring, which triggers convection in the headspace. Hence, the balance between the liquid and vapor phases may be obtained more quickly. In MAE, stirring speeds up the extraction by improving desorption of compounds in the matrix (Ruan and Li, 2007). The disadvantages of using of low solvent/solid ratio may be reduced by stirring (Chan et al., 2011). In the study of Kovacs et al. (1998) the difference between stirred solutions and non-stirred solutions is observable. The researchers reported agitation with magnetic stirrer provided to reach the desired temperature more rapidly and there wasn't a noticeable temperature differentiation between the vessels.

2.5.4.5 Applications of Microwave Assisted Solvent Extraction

Lately, extraction phenolic compounds from tea leaves has been studied (Pan et al., 2003). Furthermore, microwave radiation has been utilized to extract other biological substances, such as essential oil extraction from rosemary and peppermint leaves (Chen and Spiro, 1994), glycyrrhizic acid extraction from licorice root (Pan et al., 2000), extraction of taxanes from *Taxus* biomass (Mattina et al., 1997) and extraction of ergosterol and total fatty acids from fungal hyphae and spores, mushrooms, filtered air, artificially contaminated corn, naturally contaminated grain dust, and soil (Young, 1995).

MAE process is a new way to extract constituents in plants. This way has high efficiency and low cost. Extraction of catechin by MAE was made by using the leaves of *Anacardium occidentale*. Phytoconstituent yields obtained by using MAE were higher than the yields in conventional extraction methods (Tatke and Jaiswal, 2011).

Studies have been reported about the MAE of pesticides, persistent organic pollutants, phenols, metals, polymers, pharmaceuticals, natural products and miscellaneous substances (Tatke and Jaiswal, 2011).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Pomegranate (*Punica granatum*) seeds, Hicaznar cultivar variety were purchased from a fruit juice company in Turkey. Folin-Ciocalteu phenol reagent and gallic acid were provided by Merck (Darmstadt, Germany). The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), sodium carbonate, hexane, methanol, and other solvents were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All reagents and solvents were of analytical or chromatographic grade.

3.1.1 Sample Preparation

Pomegranate seeds were cleaned from foreign materials, pomegranate shell, and dirt. The seeds were dried to moisture content of 3.55 % in a vacuum oven (model VD 23; Binder Inc., Bohemia, NY, USA) at 35 °C for 3 h. Dried seeds were ground in a particle grinder (model PRG 259; Premier, Istanbul, Turkey). Ground particles were sieved into different particle sizes d_1 (fine particles) = 0.125-0.450 mm (1), d_2 (moderately coarse particles) = 0.450-0.530 mm (2) and d_3 (coarse particles) = 0.530-0.800 mm (3) through meshes, collected and used in the experiments. Particles bigger than 800 mm sizes were not used in experiments because they had low extraction yield. Dried and ground seed samples were stored in refrigeration conditions at 4°C until processes had started. In addition, moisture content changes of dried seeds were controlled by infrared moisture analyzer.

3.2 Preliminary Experiments

Before main experiments some preliminary experiments and works such as choosing the extraction solvent (hexane-water ratio), the system type (closed or open), power, time, seed particle size etc. were occurred to determine main experiment conditions. There are four options for stirring speed: off, low (440 rpm), medium (570 rpm), and

high (800 rpm). Stirring speed is high and stirring time is 2 min. In closed system works, solvent amounts were used flask volume by completing to 20 mL. In open system works, solvent amounts were used flask volume by completing to 50 mL.

Oil yields were calculated according to equation (3.1):

$$Yield (\%) = \frac{m(\text{g extracted oil})}{m(\text{g dry seeds})} \cdot 100 \quad (3.1)$$

3.3 Soxhlet Extraction

Conventional Soxhlet extraction was performed using 10 g of fine powdered seeds ($d_1 = 0.125\text{--}0.450$ mm) and 220 mL of *n*-hexane in a classical Soxhlet apparatus at 110 °C for 8 h.

3.4 Solvent Extraction (Cold-Hexane Extraction)

For cold solvent extraction, 40 g of ground fine particle (0.125–0.400 mm) seeds and 400 mL of *n*-hexane were put into a glass beaker and stirred by magnetic stirrer (613.01.001 model, Isolab, Wertheim, Germany) with extraction time of 8 h at 25 °C. Finally, the solid residue was precipitated by centrifugation (EBA 20, Hettich, Tuttlingen, Germany 6000 rpm, 10 min). The supernatant was separated by decantation. Hexane was removed at 40°C using rotary vacuum evaporator (Hei-VAP Advantage HL/G1, Heidolph Instrument GmbH & Co. KG., Schwabach, Germany) in both extraction methods. The extracted oil was kept at -20 °C until the analyses were carried out.

3.5 Microwave Assisted Solvent Extraction

Microwave assisted solvent extraction (MASE) was applied for all sized particles in time-pressure-temperature-mixing speed controlled CEM Discover SP-D microwave instrument (Figure 3.1) at 2450 (CEM Corporation, Matthews, NC, USA) according to the RSM Methodology. The extraction was checked and monitored by computer. Microwave instrument has maximum 300 watts output power.

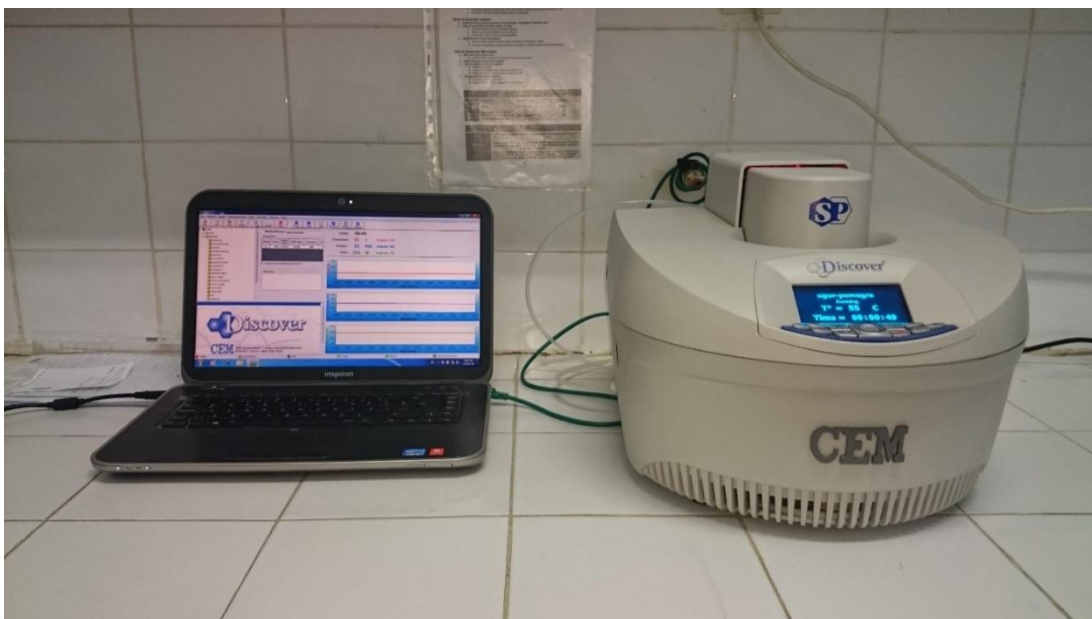


Figure 3.1 Microwave instrument

Ground pomegranate seeds and *n*-hexane at different solvent/sample ratios (Table 3.1) were put into 35-mL microwave quartz vessel closed with snap-on caps. The values of power and time were adjusted as given in the experimental central composite design (Table 3.1) using CEM Synergy. Stirring was set at high level. The extractions were performed in dynamic mode and PowerMax function, which blows air to eliminate additional heating was on. The required time for heating up and cooling down were not included in total extraction time. After extraction, aqueous solution was centrifuged for 10 minutes at 6000 rpm for achieving clarification and supernatant was collected. Hexane was removed at 40 °C along 20 minutes using rotary vacuum evaporator (Hei-VAP Advantage HL/G1, Heidolph Instrument GmbH & Co. KG., Schwabach, Germany). So, pure oil was obtained. The yield was calculated using equation (3.1). The extracted oil was kept at -20 °C for using analysis.

3.6 Experimental Design and Optimization by Response Surface Methodology (RSM)

A three-level, four-factorial face-centered central composite design was applied to evaluate the effects of extraction parameters on oil extraction yield and to determine optimum extraction conditions according to the lowest time, in the range of power, and in the range of solvent/sample ratio for obtaining highest extraction yield. The design consisted of 30 experimental runs with six replications at the central point

(Table 3.1). The extraction variables were time (5–20 min), power (176–300 W), solvent/sample ratio (2:1, 6:1 and 10:1) by mass and particle size ($d = 0.125$ – 0.800 mm). The response was the oil extraction yield (Y). The data were analyzed as reported by Keskin et al. (2016). The predicted values given by the model fitting technique in Design Expert.7.0 (Stat Ease, Inc., Minneapolis, MN, USA) were closely correlated to the experimental results.

Table 3.1 A three-level, four factorial face-centered central composite design generated for microwave-assisted extraction of pomegranate seed oil

Run	Factors			
	Power (W)	Time (min)	Solvent/sample ratio (m/m)	Particle size (d)
1	176	5	2:1	1
2	300	5	2:1	1
3	176	20	2:1	1
4	300	20	2:1	1
5	176	5	10:1	1
6	300	5	10:1	1
7	176	20	10:1	1
8	300	20	10:1	1
9	176	5	2:1	3
10	300	5	2:1	3
11	176	20	2:1	3
12	300	20	2:1	3
13	176	5	10:1	3
14	300	5	10:1	3
15	176	20	10:1	3
16	300	20	10:1	3
17	176	12.5	6:1	2
18	300	12.5	6:1	2
19	238	5	6:1	2
20	238	20	6:1	2
21	238	12.5	2:1	2
22	238	12.5	10:1	2
23	238	12.5	6:1	1
24	238	12.5	6:1	3
25	238	12.5	6:1	2
26	238	12.5	6:1	2
27	238	12.5	6:1	2
28	238	12.5	6:1	2
29	238	12.5	6:1	2
30	238	12.5	6:1	2

Particle size: $d_1 = 0.125$ – 0.450 mm (1), 2: $d_2 = 0.450$ – 0.530 mm (2) and $d_3 = 0.530$ – 0.800 mm (3). mass/mass=m/m.

3.7 Chemical Analysis

3.7.1 Fatty Acid Composition

The fatty acid composition of the extracted oil was determined by using Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) with a split/splitless injector, flame ionization detector and HP-88 capillary column (88 % cyanopropylaryl; 100 m×0.250 mm, 0.20 µm i.d.). The method proposed by Çiftçi et al. (2009) was used with some modifications. The injector and detector temperatures were 250 and 260 °C, respectively. The oven temperature was scheduled as follows: 1 min at 120 °C, from 120 to 175 °C at 10 °C/min, 10 min at 175 °C, from 175 to 210 °C at 5 °C/min, 5 min at 210 °C, from 210 to 230 °C at 5 °C/min and 5 min at 230 °C. Helium was the carrier gas with a flow rate of 1.5 mL/min.

3.7.2 Percent Free Fatty Acid (Titration Method)

The determination of the free fatty acid content was carried out according to AOCS Ca 5a-40 (AOCS, 1998). 10 mL of diethyl ether was added to 10 mL ethanol. Then, 2-3 drop of phenolphthalein was added. Solution was titrated with 0.1 N NaOH until pink color was observed (Volume₁). 2.5 g of oil was taken and 10 mL ethanol was added. Then, 10 mL of diethyl ether was added and mixed. After that, 2-3 drop of phenolphthalein was added to the solution. Solution was titrated with 0.1 N of NaOH until pink color was observed (Volume₂). Finally, FFA% was calculated by the equation (3.2). Results were given by puniceic acid. Analysis were made using MASE oil which was obtained at optimum conditions according to RSM and CSE oil.

$$FFA = \frac{N \times \frac{MW}{10} \times V}{m} \quad (3.2)$$

Here, N is normality of titrate solution (NaOH), MW is molecular weight of puniceic acid (g/mol), V is consumed volume (volume₂ – volume₁) (mL), m is used sample amount (g).

3.7.3 Total Phenolic Content

The total phenolic content of the extracted oil was determined using the Folin-Ciocalteu colorimetric method proposed by Gutfinger (1981) with some modifications. The oil obtained by microwave-assisted or cold solvent extraction (2.5 g) was dissolved in *n*-hexane (5 mL). Oil-in-hexane solution together with 5 mL of methanol and water (60:40, by volume) mixture was vortexed to extract the phenolic compounds. Centrifugation (EBA 20, Hettich, Tuttlingen, Germany 6000 rpm, 10 min) was used to separate the two phases. This extraction procedure was done in triplicate. The extracts in methanol were mixed and 0.2 mL of the methanolic phase was diluted to 5 mL with distilled water. Folin-Ciocalteu reagent (0.5 mL) was added to this mixture. After 3 min, 1 mL of Na₂CO₃ (20 %, by mass per volume) was added to the reaction mixture, which was diluted to a final volume of 10 mL with distilled water and held for 1 h in the dark. The absorbance values were measured against a blank sample at 765 nm using Lambda 25 UV/Vis spectrophotometer (PerkinElmer, Shelton, CT, USA).

3.7.3.1 Preparation of Calibration Curve

Stock solution was prepared by using gallic acid and methanol-water (60:40) solution. The stock solution was diluted with methanol-water solution at the range of 0-60 mg/mL to obtain calibration curve. 0.2 mL calibration solution was taken and 4.8 mL water was added. Then, 0.5 mL Folin-C reagent was added and kept for 3 minutes. 1 mL of 20 % (w/v) Na₂CO₃ was added and mixed. Solution was diluted to 10 mL with water. After that, it was put into the dark zone for 1 hour. Finally, absorbance was measured by spectrophotometric method at 765 nm. For each diluted solution, this procedure was applied. Then, calibration curve was formed according to absorbance. For blank measurements the same procedure was applied by using 0.2 mL of methanol-water (60:40, by volume) solutions instead of calibration solution or methanolic phase solution. The results were defined as mg of gallic acid equivalents per g of sample dry mass (mg GA/g oil).

3.7.4 Peroxide Value of Seed Oil

Cd 8-53 method (AOCS, 1997) was used to determine peroxide value of seed oil. 5 g of seed oil was taken. 1 mL of saturated KI was added into oil and oil – KI mix was shaken for 5 minutes. 25 mL of acetic acid – chloroform (3:2 v/v) solution was added

into the oil – KI mix. The solution bottle was sealed and stored at dark for 5 minutes. After that, 75 mL of distilled water was added into this solution. Prepared solution was titrated with 0.01 N of Na₂SO₃ solution. Peroxide values were calculated by equation (3.3). Results were given mmol of O₂ per kg of oil.

$$PV = \frac{(A-B) \times N \times 1000}{m} \quad (3.3)$$

Here, A is amount of sodium thiosulfate used for sample (oil) in titration (mL), B is amount of sodium thiosulfate used for blank in titration (mL), N is normality of sodium thiosulfate (N), and m is amount of sample used (g).

3.7.5 Antioxidant Activity of Seed Oil

The antioxidant activity of the oil was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical with the method proposed by Kalantzakis et al. (2006). To obtain 0.1 mmol of DPPH, 0.00197 g DPPH was added into ethyl acetate by total volume of 50 mL. 4 mL of freshly prepared DPPH solution (0.1 mmol) were added to 1 mL of oil and ethyl acetate solution at different concentrations (0.05-25 mg/mL). Each of solution at different concentrations was sealed immediately and vortexed for 2 minutes. After 30 min of incubation at 25 °C in the dark, the absorbance of the final solution was measured at 515 nm with Lambda 25 UV/Vis spectrophotometer (Perkin Elmer). The percentage of inhibition was calculated using equation (3.4):

$$\text{Inhibition (\%)} = \frac{A_c - A_s}{A_c} \cdot 100 \quad (3.4)$$

Here, A_c is absorbance of control solution (without using sample) at 515 nm and A_s is absorbance of sample solution at 515 nm.

DPPH scavenging activity was expressed as IC₅₀, which indicated the effective sample concentration needed to scavenge 50 % of DPPH radicals and was calculated by a linear regression analysis between the oil concentration and percent inhibition.

3.8 Physical Analysis

3.8.1 Color Analysis of Seed Oil

Colour measurements were performed using a HunterLab ColorFlex model A60-1010-615 colourimeter (Reston, VA, USA). Colour values were reported as L* (lightness), a* (redness), b* (yellowness) according to the Hunter colour scale.

3.8.2 SEM Analysis

Untreated pomegranate seed and solid residues after conventional and microwave-assisted extraction were examined using scanning electron microscopy (SEM) to analyse the effect of extraction methods on the surface morphology of the seeds. Gold/palladium was used for the coating of the samples in an SC7620 sputter coater (Emitech, Kent, UK). Images of samples were taken JSM-6390LV (JEOL Ltd., Tokyo, Japan) scanning electron microscope. The SEM images were created at 12.5 kV under high vacuum condition and 1000× magnification.

3.8.3 Size Distribution

Seeds were ground in a coffee grinder (model PRG 259; Premier, Istanbul, Turkey) for 3 minutes at room conditions (25 °C) after they were dried to moisture content of 3.5 % in a vacuum oven (model VD 23; Binder Inc., Bohemia, NY, USA) at 35 °C for 3 h. Ground seeds were sieved by using different size sieves. Four different sizes of sieve which is 0.125 mm, 0.450 mm, 0.530 mm, and 0.800 mm were used for this purpose and dried-sieved seeds were categorized according to their size ranges at four types by 0.125 – 0.450 mm, 0.450 – 0.530 mm, 0.530 – 0.800 mm, and >800 mm (Figure 3.2). Size distribution of pomegranate seed sample were calculated by percentage.



0.530 – 0.800 mm

0.450 – 0.530 mm

0.125 – 0.400 mm

Figure 3.2 Appearance of pomegranate seeds in different sizes



CHAPTER 4

RESULTS AND DISCUSSION

4.1 Preliminary Experiments

Preliminary works were performed using two methods: Microwave assisted solvent extraction and Soxhlet extraction. In application of microwave assisted solvent extraction, a basis for the optimization of extraction conditions was provided by using different values of some parameters which are considered to have an effect on extraction. These parameters were the type of system: open-closed, power level, extraction time, solvent type and particle size of the sample. All results of preliminary works were given at Table 4.1.

Percent efficiencies obtained by using ground samples in open and closed systems were very close to each other at Table 4.1. However, a condensation unit for solvent control was needed in the open system. Moreover, the positive effect of extra pressure formation on extraction efficiency in closed system was not present in open extraction system. Therefore, since it is also simpler to operate, the closed system was preferred.

Three different power levels were applied in the preliminary works: 250, 275 and 300 W. The effects of these levels on oil yield were found similar at Table 4.1. There was a decrease in the yield at high power level. Based on the preliminary works, we can say that the increase of power contributes to the extraction efficiency to a certain level, and then this positive effect disappears and the efficiency starts decreasing.

It was observed in the preliminary works that hexane alone had a much better effect than the hexane-water mixture in terms of oil recovery (oil yields; at the same conditions by hexane-water mixture= 13.820 % and by only hexane= 16.049 %). This is because water absorbs microwave energy more than hexane does, since water has a high dielectric constant.

Another parameter analyzed for its effect on extraction efficiency was the extraction time. 15, 20 and 25 minutes of extractions were applied and a significant difference among these times in terms of extraction yield was not observed.

The last parameter analyzed for extraction efficiency in preliminary works was the particle size of sample. The particle size and oil yield seemed to be inversely proportional. As the particle size decreased, oil yield increased. The increase of microwave penetration and unit area affected by microwave was considered as the reason of this effect. Ground samples mean no size separation particles (Table 4.1).

Five experiments were performed in the Soxhlet extraction part of the preliminary works. The highest yield was obtained from the sample with the smallest particle size as in microwave assisted solvent extraction. The oil yield for the particle size of 0.125-0.400 mm was between 34-35 % while it was between 19-21 % for the ground sample.

Operation temperature range was between 51-105 °C in all microwave assisted solvent extraction experiments. Although we did not perform an interaction study between temperature and oil yield, temperature changes depended on microwave power and particle size. Generally, when microwave power or particle size increased, the extraction temperature was higher.

4.2 Comparison of Extraction Yields on Various Extraction Methods

Three methods were used for comparison of extraction yields: Microwave assisted solvent extraction, Soxhlet extraction, and cold solvent extraction. A higher extraction yield of 35.10 % in 5 min was obtained with microwave-assisted solvent extraction than those of Soxhlet extraction (34.70 % in 8 h) and cold solvent extraction (17.50 % in 8 h). Orak et al. (2012) and Özgül-Yücel (2012) found that the oil content of seeds from different genotypes of *Punica granatum L.* was between 17.84 and 24.96 % (dry mass basis) which is lower than our results. Ahangari and Sargolzaei (2012) found that the oil content of pomegranate seed was 22.31 %. The differences in the yields might be a result of the genetic backgrounds and the growth conditions of the pomegranate or the applied extraction methods. Taghvaei et al. (2014) reported that the efficacy of microwaves on fast extraction of oil from oilseeds was related to the destruction of oil cell structures within the plant tissues

through denaturation of cell proteins by heat generated from the movement of polar molecules including water.

Table 4.1 Preliminary works results

Sample Amount (g)	Solvent Used	Sample Size	Solvent Amount (mL)	System Type	Power Used (Watt)	Hold Time (min)	Yield (%)
2.5020	Hexane	Ground	17	Closed	250	15	16.598
2.5049	Hexane	Ground	17	Closed	275	15	16.049
2.5034	Hexane	Ground	17	Closed	275	20	17.149
2.5051	Hex-water	Ground	15.3-1.7	Closed	275	15	13.820
2.5071	Hexane	Ground	17	Closed	275	25	16.138
2.5089	Hexane	Ground	17	Closed	300	25	15.437
2.5064	Hex-water	Ground	16.15-0.85	Closed	275	15	13.370
2.5035	Hexane	0.125-0.450 mm	17	Closed	275	20	31.024
2.5014	Hexane	>800mm	17	Closed	275	20	6.340
2.5036	Hexane	0.450-0.530mm	17	Closed	275	20	26.002
2.5064	Hexane	0.530-0.800mm	17	Closed	275	20	9.670
2.5012	Hexane	0.125-0.450 (ground from 0.800mm)	17	Closed	275	20	20.640
1.0046	Hexane	Ground	19	Closed	275	20	17.520
2.5042	Hexane	0.125-0.450 mm	17	Closed (repeat)	275	20	30.824
1.0002	Hexane	Ground	49	Open	275	20	13.840
5.0020	Hexane	Ground	44	Open	275	20	17.323
10.003	Hexane	Ground	39	Open	275	20	14.396
1.0011	Hexane	Ground	49	Open (repeat)	275	20	18.110
11.5227	Hexane	Ground	-----	Soxhlet	-----	-----	20.734
10.0044	Hexane	Ground	-----	Soxhlet	-----	-----	21.588
10.0903	Hexane	Ground	-----	Soxhlet	-----	-----	19.192
12.3928	Hexane	0.125-0.450mm	-----	Soxhlet	-----	-----	34.700
9.2936	Hexane	0.125-0.450mm	-----	Soxhlet	-----	-----	34.630

4.3 Pomegranate Oil Yield by Microwave Assisted Solvent Extraction

The effects of different parameters on the MASE of oil from pomegranate seeds were analyzed. The experimental and predicted values of the oil extraction yield at each of the 30 runs given by response surface methodology (RSM) are shown in Table 4.2.

Firstly, according to the preliminary works, range for each extraction parameters was determined. After that, extraction conditions were optimized for the highest oil extraction yield using Design Expert software 7.0 (Stat-Ease, Inc.). The predicted oil extraction yield was 35.19 % under the optimum conditions of 220 W, 5 min, 10:1 solvent/sample ratio (by mass) and $d_1 = 0.125\text{--}0.450$ mm particle size 1 (fine particles). Three experiments were carried out at these optimum conditions to validate the predicted result, and average oil extraction recovery was 34.91 % (Figure 4.1). The predicted and actual (experiment) values were so close and in good agreement.



Figure 4.1 Pomegranate seed oil obtained by microwave assisted solvent extraction

4.3.1 Effect of Particle Size on Extraction Yield

Particle size was the most important factor that affected the oil yield in MASE (Table 4.3). Negative coefficient of the term stated that decreasing the particle size increased the oil extraction yield. The smaller particle size might increase the penetration of irradiation into the interior cell walls and the mass transfer surface area by association of the seed matrix and hexane enhancing the oil extraction. Kwon et al. (2003) and Singh et al. (2014) also documented that there was an inverse relationship between the particle size and microwave extraction yield of different compounds. The effect of interaction between particle size and time on oil extraction yield was also statistically important (Table 4.3). Figure 4.2 shows the interaction effect of the particle size and time on extraction yield at a constant solvent/sample ratio of 6:1 (by mass) and power of 238 W. The extraction yield when using fine particles was significantly higher (32 %) than that when coarse particle size (11 %) were used at the end of 5 min extraction under the same extraction conditions. The

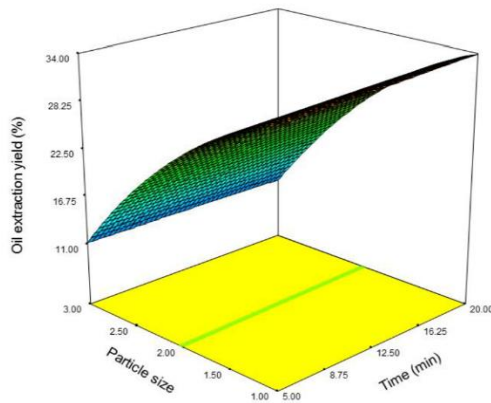
extraction yields were 34 and 11 % at the end of 20 min of extraction when fine and coarse particles were used respectively. This inverse effect might be explained with limited penetration of microwave irradiation into the coarse seeds. It is basically because of zero dielectric constant of cellulose, which is the main component of the seed. Thus, extraction yield could not be improved when using larger particles even though the time increased. Quadratic term of particle size was negative, indicating the presence of maximum value for this variable (Table 4.3).

Table 4.2 A three-level, four factorial face-centered central composite design generated for microwave-assisted extraction of pomegranate seed oil

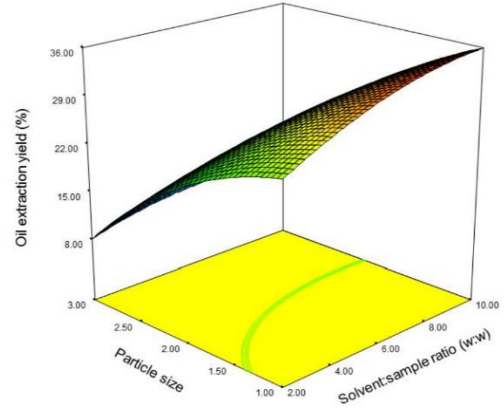
Run	Factors				Y/%	
	P (W)	t (min)	Solvent/sample ratio (m:m)	Particle size (d)	Experimental	Predicted
1	176	5	2:1	1	25.32±0.01	25.96
2	300	5	2:1	1	26.03±0.03	25.96
3	176	20	2:1	1	27.33±0.11	27.41
4	300	20	2:1	1	27.56±0.12	27.41
5	176	5	10:1	1	35.14±0.07	35.19
6	300	5	10:1	1	35.29±0.08	35.19
7	176	20	10:1	1	36.21±0.16	36.64
8	300	20	10:1	1	36.51±0.07	36.64
9	176	5	2:1	3	7.88±0.09	8.07
10	300	5	2:1	3	9.67±0.12	8.07
11	176	20	2:1	3	7.65±0.13	7.97
12	300	20	2:1	3	7.60±0.05	7.97
13	176	5	10:1	3	10.19±0.06	10.48
14	300	5	10:1	3	10.06±0.08	10.48
15	176	20	10:1	3	10.66±0.16	10.38
16	300	20	10:1	3	11.52±0.08	10.38
17	176	12.5	6:1	2	25.16±0.19	25.43
18	300	12.5	6:1	2	25.38±0.06	25.43
19	238	5	6:1	2	24.80±0.08	25.09
20	238	20	6:1	2	25.42±0.06	25.77
21	238	12.5	2:1	2	20.44±0.05	20.62
22	238	12.5	10:1	2	26.27±0.08	26.44
23	238	12.5	6:1	1	34.26±0.17	33.20
24	238	12.5	6:1	3	9.71±0.25	11.13
25	238	12.5	6:1	2	24.95±0.31	25.43
26	238	12.5	6:1	2	26.32±0.12	25.43
27	238	12.5	6:1	2	25.67±0.18	25.43
28	238	12.5	6:1	2	25.01±0.25	25.43
29	238	12.5	6:1	2	26.20±0.15	25.43
30	238	12.5	6:1	2	25.82±0.16	25.43

All values are expressed as mean ± standard deviation of three replicates.

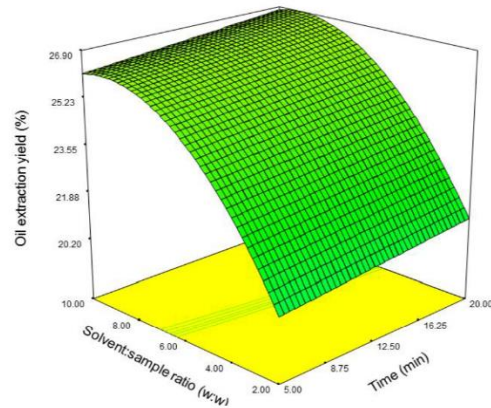
Particle size: $d_1 = 0.125\text{--}0.450$ mm (1), 2: $d_2 = 0.450\text{--}0.530$ mm (2) and $d_3 = 0.530\text{--}0.800$ mm (3)



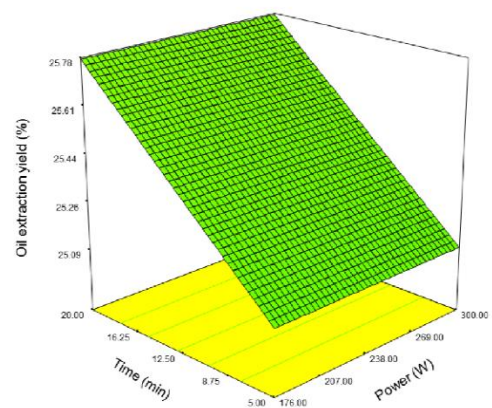
a) Power: 238 (W); solvent:sample ratio (w:w): 6:1



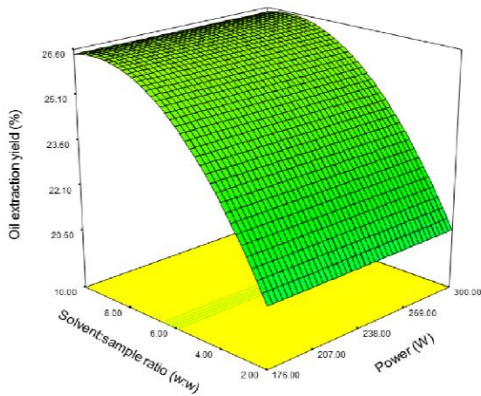
b) Power: 238 W; time: 12.5 min



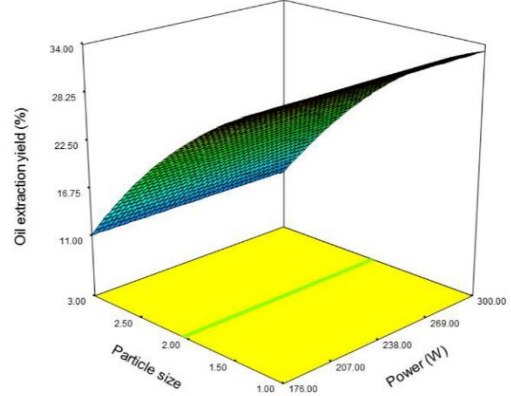
c) Power 238 W; particle size^a: 2



d) Solvent:sample ratio (w:w): 6:1; particle size^a: 2



e) Time: 12.5 min; particle size^a: 2



f) Solvent:sample ratio (w:w): 6:1; time: 12.5 min

Figure 4.2 Response surface plots for oil extraction yield as a function of: a) particle size and time; b) particle size and solvent/sample ratio; c) solvent/sample ratio and time; d) time and power; e) solvent/sample ratio and power; and f) particle size and power. Particle size: 1 ($d_1 = 0.125\text{--}0.450$ mm), 2 ($d_2 = 0.450\text{--}0.530$ mm) and 3 ($d_3 = 0.530\text{--}0.800$ mm)

Table 4.3 ANOVA and model equation for response surface quadratic model of microwave assisted oil extraction

Source	Coefficient	F value	p-value Prob > F
Model*		762.29	< 0.0001
Intercept	25.44		
Linear			
Time	0.34	4.27	0.0507 ^a
Solvent/sample ratio	2.91	317.05	< 0.0001 ^b
d (particle)	-11.04	4564.10	< 0.0001 ^b
Interactive			
t×d (particle)	-0.39	5.00	0.0358 ^b
Solvent/sample ratio×d (particle)	-1.71	96.78	< 0.0001 ^b
Quadratic			
Solvent/sample ratio×solvent/sample ratio	-1.90	25.95	< 0.0001 ^b
d (particle)×d (particle)	-3.27	76.54	< 0.0001 ^b
Lack of fit		1.56	0.3290

*The coefficient of determination (R^2) of the model was 0.99; ^a not significant at 'Prob>F'>0.05, ^b significant at 'Prob>F'<0.05

4.3.2 Effect of Solvent/Sample Ratio on Extraction Yield

Statistical results showed that the solvent/sample ratio affected extraction yield significantly (Table 4.3). When fine particles of pomegranate seed were used in the microwave extraction, increasing solvent/sample ratio from 2:1 to 10:1 (by mass) caused a rise in the extraction yield from 26 to 36 %. The solvent/sample ratio had statistically significant interaction effect with particle size. However, when the extraction was done with coarse particles of pomegranate seed, increasing solvent/sample ratio from 2:1 to 10:1 (by mass) increased the extraction yield from 8 to 12 % (Figure 4.2b). This might be explained again by the limited penetration of microwaves into the interior of the coarse seeds. Because of the limited penetration, all of the oil could not be released from the seeds. Hence, there was no extra oil to be dissolved in the medium even though the amount of solvent increased. Quadratic term of solvent/sample ratio was found to be statistically significant (Table 4.3).

4.3.3 Effect of Time on Extraction Yield

The linear term of extraction time was statistically ineffective on oil extraction yield (Table 4.3). Nde et al. (2015) also reported that time was statistically inefficient on the microwave extraction of neem oil. However, its effect can be understood more

obviously by considering the principle of closed-vessel high-pressure microwave extraction. In the present study, closed system microwave application resulted in high pressure extraction of the oil. The combination of the microwave and high pressure resulted in higher extraction yields at short extraction times than when using domestic or focused microwave ovens. Optimum extraction times found by Gai et al. (2013) and Jiao et al. (2014) were 83 and 66 min, respectively, which was longer than the value found in this study. In Figures 4.1c and d, it was also seen that increasing the extraction time increased the oil extraction yield. However, these improvements were not statistically significant (Table 4.3). Similar to this result, Wang et al. (2008) also found that the increase in the extraction yield was very small as a function of time in microwave extraction (Figure 2.6). The reason for the insignificant increase could be explained by the fact that MASE of different compounds is usually completed within a few minutes to half an hour, depending on the material extracted as mentioned in literature (Nde et al., 2015; Spigno and De Faveri, 2009; Sumi and Horikoshi, 2013; Veggi et al., 2012).

4.3.4 Effect of Microwave Power on Extraction Yield

The linear term of power was omitted by backward elimination to conserve the hierarchy of the model since it had statistically insignificant effect on the oil extraction yield. Figures 4.1d, e and f showed that raising the power from 176 to 300 W did not improve the oil extraction yield. The reason for this result might be related to the limited increase in extraction temperature with increasing power. It is well known that higher temperatures increase the oil extraction efficiency due to the higher solubility of oil at higher temperatures. Hexane, which is a microwave-transparent solvent and has low dielectric constant, cannot be heated up to temperature higher than 55 °C during extraction. Thus, hexane might limit the capacity of power to increase extraction efficiency.

4.3.5 Effect of Solvent Type on Extraction Yield

In literature, there are studies in which it was noted that poor microwave absorbers like hexane can be mixed with ethanol or water to enhance the extraction performance (Veggi et al., 2012) because water has high dielectric constant so it is good microwave absorber. However, in this study use of different water contents

(water:hexane ratio; 0:100, 5:95 and 10:90, v:v) in the extraction medium decreased the extraction yield. The oil extraction yield from ground seeds with no size separation was 16.04 when the microwave assisted extraction was performed at power of 275 W, time of 15 min and solvent/sample ratio of 10:1 (v:v). However, the extraction yield decreased to 13.82 and 13.37% when water:hexane mixtures of 5:95 and 10:90 (v:v) were used respectively at the same extraction conditions. The reason of this can be related to compound (oil), desired to be extracted, which has a non-polar property.

4.4 Comparison of Characteristic of Pomegranate Oil Extracted by Various Methods

4.4.1 Comparison of Fatty Acid Composition

The pomegranate seed oil samples obtained from microwave-assisted and cold solvent extraction had almost mostly identical fatty acid profiles (Table 4.4). The total unsaturated fatty acid content of pomegranate seed oil obtained by MASE was 95.57 %. The predominant fatty acid was punicic acid (86.53 %) in the oil extracted using microwaves (Table 4.4). Oleic, linoleic, palmitic and stearic acids were present in minor amounts at 4.10, 3.84, 2.04 and 1.71%, respectively. The punicic acid and total unsaturated fatty acid content of the investigated pomegranate seed oil were higher than in the papers of Melo et al. (2014), Fadavi et al. (2006) and Khoddami et al. (2014). The dissimilarity might be related to the differences in pomegranate cultivars and climatic conditions during growth. In addition, molecular weight of oil was calculated by using percentages of fatty acid compositions. Molecular weight of pomegranate seed oil was 909.52 g/mol.

Table 4.4 Fatty acid composition of pomegranate seed oil extracted by cold and microwave-assisted extraction

Fatty acid	w%	
	MASEO	CSEO
C16:0	2.04±0.01	2.34±0.01
C17:0	0.04±0.01	0.04±0.01
C18:0	1.71±0.02	1.69±0.01
C18:1	4.10±0.01	4.07±0.01
C18:2	3.84±0.02	3.85±0.02
C18:3	86.53±0.01	86.42±0.02
C20:0	0.37±0.02	0.37±0.01
C20:1	0.69±0.01	0.70±0.01
C20:3	0.09±0.01	0.09±0.01
C20:4	0.32±0.01	0.31±0.01
C24:0	0.27±0.01	0.12±0.02
Total polyunsaturated fatty acids	90.78	90.67

Total monounsaturated fatty acids	4.79	4.77
Total saturated fatty acids	4.43	4.56

All values are expressed as mean±standard deviation of three replicates.

4.4.2 Comparison of Peroxide Value of Seed Oil

Peroxide value and acidity are the indices for the determination of the oxidative degradation of products in oil. The peroxide value of pomegranate seed oil obtained by cold solvent extraction was 4 mmol of O₂ per kg, while that of the oil extracted by microwave-assisted extraction was 0 mmol of O₂ per kg (Table 4.5). The long extraction time (8 h) and an extraction process under atmospheric pressure could be the reasons for higher peroxide value of the oil extracted by cold solvent extraction. On the other hand, performing the extraction in a short time and usage of closed system in microwave extraction can be the reasons for no peroxide formation. In addition, usage of solvents in the extraction may provide lower peroxide values.

4.4.3 Comparison of Percent Free Fatty Acid (Titration Method)

Percentages of free fatty acid were determined for two extraction methods. In the oil extracted by cold solvent and microwave-assisted solvent extractions, there was 0.42% and 0.44% free fatty acids expressed as punicic acid, respectively. One can say that microwave assisted solvent extraction method did not affect free fatty acid percentage.

Table 4.5 Physical, chemical and bioactive properties of pomegranate seed oil extracted by microwave-assisted and cold extraction

Property	MASEO	CSEO
Peroxide value (mmol of O ₂ per kg of oil)	0.00±0.05	4.00±0.12
FFA as punicic acid (%)	0.42±0.03	0.44±0.02
Total phenols as GAE (mg/g)	7.42±0.12	1.73±0.05
IC ₅₀ /(mg/mL)	5.12±0.22	17.00±0.21
L*	56.03±0.01	58.91±0.02
a*	-5.64±0.03	-2.45±0.03
b*	22.06±0.02	14.44±0.57

All comparisons of MASEO and CSEO in the all analysis were significant, with p<0.05.

All values are expressed as mean±standard deviation of three replicates.

L*=lightness, a*=redness, b*=yellowness

4.4.4 Comparison of Total Phenolic Contents

The total phenolic content expressed as gallic acid equivalents of the pomegranate seed oil obtained by microwave-assisted and cold solvent extraction were 7.42 and 1.73 mg/g, respectively (Table 4.5). The higher total phenolic content of pomegranate seed oil obtained by MASE can be related to higher pressure during the extraction. The presented extraction system combines the advantage of microwave-assisted and pressurized solvent extraction. Bayramoglu et al. (2008) also reported that higher internal pressure of the solid media and hence enhancement of the extraction may be the reason for higher phenolic content of the oil obtained by MASE. Similarly, Haddadi-Guemghar et al. (2014) also found that the phenolic content of the oil extracted by conventional method was significantly lower than that of the oil obtained by MASE.

4.4.5 Comparison of Antioxidant Activity of Seed Oil (DPPH Method)

IC₅₀ value of the oil extracted by microwave-assisted extraction (5.12 mg/mL) was significantly lower than that of the oil extracted by cold extraction (17.00 mg/mL extract concentrations) (Table 4.5). This significant difference in the IC₅₀ values might be related to the differences in total phenolic content of the extracted oil depending on the extraction method. He et al. (2011) demonstrated a significant relationship between DPPH activities and total phenolics ($R^2=0.751$) in pomegranate seed residues. Gil et al. (2000) also reported that pomegranate fruit is a rich source of two types of polyphenolic compounds: anthocyanins and hydrolyzable tannins, which account for 92 % of the antioxidant activity of the whole fruit. This showed that higher total phenolic content in the oil obtained by microwave-assisted extraction significantly increased the antioxidant activity of the oil when compared to that of the oil obtained by cold solvent extraction.

4.4.6 Comparison of Color Analysis of Seed Oil

The color values of the oil extracted by two different methods were significantly different (Table 4.5). The lightness (L*) of pomegranate seed oil extracted by cold extraction method (58.91) was higher than that of the oil extracted by MASE (56.03). The a* value measures redness (+) and greenness (-) and the b* value indicates yellowness (+) and blueness (-). The b* value of the oil extracted by MASE (22.06) was higher than that of the oil extracted by cold extraction (14.44), while a* value of

the oil extracted by MASE (-5.64) was smaller than that of the oil extracted by cold solvent extraction (-2.45). These results showed that MASE was more efficient in extracting the chlorophyll and carotene present in the pomegranate seeds.

4.4.7 Structural Changes After Extraction (SEM)

SEM analyses were performed to observe the microscopic changes in pomegranate seed before and after extraction to compare the influence of conventional and microwave-assisted solvent extractions on the pomegranate seed structure. The fat globules were dispersed uniformly in the tissues of pomegranate seed before the extraction (Figure 4.3a). On the SEM images of the residues after Soxhlet and cold solvent extraction, the fat globules disappeared while the cell structure was preserved and the cells were still entirely unbroken (Figures 4.3b and c). However, MASE caused some structural changes in the seed tissues (Figure 4.3d). Most of the cell walls and membranes of the pomegranate seeds were ruptured and broken down completely after MASE (Figure 4.3d). It is clearly seen that the oil can be released from the cell structure and extracted efficiently by cell wall rupture in a short time. There was a good correspondence between these results and the findings of Jiao et al. (2014), who studied the Soxhlet and MASE of pumpkin seeds.

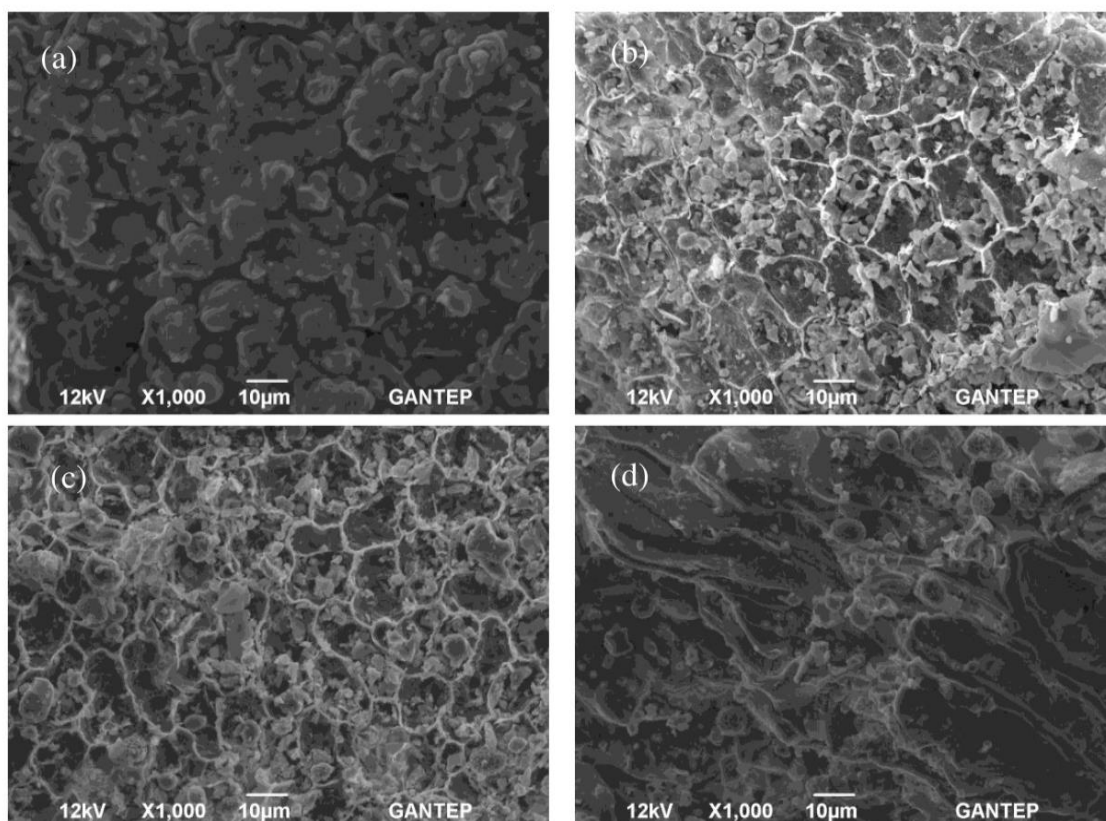


Figure 4.3 SEM images of pomegranate seed samples: (a) untreated pomegranate seed; (b) solid residue after Soxhlet extraction; (c) solid residue after cold extraction; (d) solid residue after MASE. MASE was performed at optimum conditions.

4.4.8 Size Distribution

To determine size distribution of dried pomegranate seed particles, approximately 15 gram of seeds were used. Dried seeds were ground in a particle grinder (model PRG 259; Premier, Istanbul, Turkey) for 3 minutes and sized by using sieves that have different pore sizes (0.125 mm, 0.450 mm, 0.530 mm, and 0.800 mm). According to this analysis, size distributions were given at the Table 4.6.

Table 4.6 Size distribution of dried pomegranate seed samples

Sieve Range (mm)	Weight Percentage of seed (%)
0.125 – 0.450	34.7533
0.450 – 0.530	29.3688
0.530 – 0.800	29.4153
>0.800	6.4626

CHAPTER 5

CONCLUSION

Closed-vessel high-pressure microwave system was optimized to obtain high quality pomegranate seed oil and the highest performance in extraction of pomegranate seed oil which is process waste. Verification experiments resulted in the extraction yield of 34.91 % under the optimum extraction conditions and are in good agreement with the predicted value of 35.19 %. The oil extraction yield obtained by MASE was higher than those obtained by conventional extractions. The fatty acid compositions of the oil extracted by cold and microwave-assisted solvent extractions were statistically similar to each other ($p < 0.05$). Oil extracted by MASE had significantly lower peroxide value (0 mmol of O_2 per kg of oil), free fatty acidity (0.42 %) and higher total phenolic content expressed as gallic acid equivalents (7.42 mg/g) and antioxidant activity (5 mg/mL) than those of the oil obtained by cold extraction. SEM results demonstrated that microwave method broke down cell walls and membranes efficiently thus increasing the efficient release of oil from the seed in shorter time compared to conventional extraction. The results indicated that higher quality oil could be obtained using MASE than when using cold extraction. The application of high-efficiency short-time MASE can be a valuable alternative to conventional oil extraction methods, especially for healthcare, pharmaceutical and healthy food industries.

REFERENCES

- Abbasi, H., Rezaei, K., Rashidi, L. (2008 a). Extraction of essential oils from the seeds of pomegranate using organic solvents and supercritical CO₂. *Journal of the American Oil Chemists' Society*, **85**(1), 83-89.
- Abbasi, H., Rezaei, K., Emamdjomeh, Z., Mousavi, S. M. E. (2008 b). Effect of various extraction conditions on the phenolic contents of pomegranate seed oil. *European Journal of Lipid Science and Technology*, **110**(5), 435-440.
- Abdullah, H. A., Hauser, A., Ali, F. A., Al-Adwani, A. (2006). Optimal conditions for coke extraction of spent catalyst by accelerated solvent extraction compared to Soxhlet. *Energy & Fuels*, **20**(1), 320-323.
- Aguilera, J. M. (2003). Solid-liquid extraction. In *Extraction Optimization in Food Engineering* (pp. 51-70). CRC Press.
- Ahangari, B., Sargolzaei, J. (2012). Extraction of pomegranate seed oil using subcritical propane and supercritical carbon dioxide. *Theoretical Foundations of Chemical Engineering*, **46**(3), 258-265.
- Akhtar, S., Ismail, T., Fraternali, D., Sestili, P. (2015). Pomegranate peel and peel extracts: Chemistry and food features. *Food Chemistry*, **174**, 417-425.
- Akinlua, A., Smith, R. M. (2009). High temperature steam extraction for the determination of aliphatic hydrocarbons in petroleum source rock. *Chromatographia*, **69**(11-12), 1333-1339.
- Alfaro, M. J., Bélanger, J. M., Padilla, F. C., Paré, J. J. (2003). Influence of solvent, matrix dielectric properties, and applied power on the liquid-phase microwave-assisted processes (MAP™) 1 extraction of ginger (*Zingiber officinale*). *Food Research International*, **36**(5), 499-504.
- Al-Harashsheh, M., Kingman, S. W. (2004). Microwave-assisted leaching—a review. *Hydrometallurgy*, **73**(3-4), 189-203.

Al-Maiman, S. A., Ahmad, D. (2002). Changes in physical and chemical properties during pomegranate (*Punica granatum L.*) fruit maturation. *Food Chemistry*, **76(4)**, 437-441.

Amarni, F., Kadi, H. (2010). Kinetics study of microwave-assisted solvent extraction of oil from olive cake using hexane: comparison with the conventional extraction. *Innovative Food Science & Emerging Technologies*, **11(2)**, 322-327.

Anastassiades, J., Perea, E., Pozevara, A., Kaminsky, D. (1992). *Solid-State Microwave Generation* (Vol. 4). Springer.

Anonymous a (2008). www.meyed.org.tr.

Anonymous b (2012). BATEM; TÜİK.

Anonymous c (2018). Ministry of Food, Agriculture, and Livestock

AOCS Official Method Ca 5a-40. Free fatty acids. In: Firestone D, editor. Official methods and recommended practices of the AOCS. Champaign, IL, USA: American Oil Chemist's Society (AOCS); 1998.

AOCS Official Method Cd 8-53. Peroxide value. In: Firestone D, editor. Official methods and recommended practices of the AOCS. Champaign, IL, USA: American Oil Chemist's Society (AOCS); 1997.

Asadpour, E., Boroushaki, M. T., Sadeghnia, H. (2010). Protective effect of pomegranate seed oil against gentamicin induced nephrotoxicity in rat. *Toxicology Letters*, **196**, S232.

Azadmard-Damirchi, S., Habibi-Nodeh, F., Hesari, J., Nemati, M., Achachlouei, B. F. (2010). Effect of pretreatment with microwaves on oxidative stability and nutraceuticals content of oil from rapeseed. *Food Chemistry*, **121(4)**, 1211-1215.

Balu, A. M., Budarin, V., Shuttleworth, P. S., Pfaltzgraff, L. A., Waldron, K., Luque, R., Clark, J. H. (2012). Valorisation of orange peel residues: waste to biochemicals and nanoporous materials. *ChemSusChem*, **5(9)**, 1694-1697.

- Bandh, C., Björklund, E., Mathiasson, L., Näf, C., Zebühr, Y. (2000). Comparison of accelerated solvent extraction and Soxhlet extraction for the determination of PCBs in Baltic Sea sediments. *Environmental Science & Technology*, **34**(23), 4995-5000.
- Bayramoglu, B., Sahin, S., Sumnu, G. (2008). Solvent-free microwave extraction of essential oil from oregano. *Journal of Food Engineering*, **88**(4), 535-540.
- Beoletto, V. G., de las Mercedes Oliva, M., Marioli, J. M., Carezzano, M. E., Demo, M. S. (2016). Antimicrobial Natural Products Against Bacterial Biofilms. *Antibiotic Resistance*, 291.
- Brachet, A., Christen, P., Veuthey, J. L. (2002). Focused microwave-assisted extraction of cocaine and benzoylecgonine from coca leaves. *Phytochemical Analysis*, **13**(3), 162-169.
- Burfoot, J. C. (1967). *Ferroelectrics: An Introduction to the Physical Principles*. Van Nostrand.
- Burkert, R., Helberg, H. W., Von Schütz, J. U. (1993). Longitudinal and transverse conductivity in (2, 5-Me₂-DCNQI) 2 Cu fibres. *Synthetic Metals*, **56**(1), 2519-2524.
- Budzinski, H., Baumard, P., Papineau, A., Wise, S., Garrigues, P. (1996). Focused microwave assisted extraction of polycyclic aromatic compounds from standard reference materials, sediments and biological tissues. *Polycyclic Aromatic Compounds*, **9**(1-4), 225-232.
- Camel, V. (2000). Microwave-assisted solvent extraction of environmental samples. *TrAC Trends in Analytical Chemistry*, **19**(4), 229-248.
- Carroll, J. E. (1970). *Hot Electron Microwave Generators*. American Elsevier Pub. Co.
- Carvalho Filho, J. M. (2014). Pomegranate seed oil (*Punica granatum* L.): A source of punicic acid (conjugated α -linolenic acid). *J Human Nutri Food Sci*, **2**(1), 1-11.
- Carvalho, E. B. T. D., Melo, I. L. P. D., Mancini-Filho, J. (2010). Chemical and physiological aspects of isomers of conjugated fatty acids. *Food Science and Technology*, **30**(2), 295-307.

Chan, C. H., Yusoff, R., Ngoh, G. C., Kung, F. W. L. (2011). Microwave-assisted extractions of active ingredients from plants. *Journal of Chromatography A*, **1218**(37), 6213-6225.

Chemat, F., Abert-Vian, M., Huma, Z. (2009). Microwave-assisted separations: green chemistry in action. In *Green Chemistry Research Trends* (pp. 33-62). Nova Science Publishers, New York, NY.

Chemat, S., Aït-Amar, H., Lagha, A., Esveld, D. C. (2005). Microwave-assisted extraction kinetics of terpenes from caraway seeds. *Chemical Engineering and Processing: Process Intensification*, **44**(12), 1320-1326.

Chen, L., Song, D., Tian, Y., Ding, L., Yu, A., Zhang, H. (2008). Application of on-line microwave sample-preparation techniques. *TrAC Trends in Analytical Chemistry*, **27**(2), 151-159.

Chen, S. Y., Urban, P. L. (2015). On-line monitoring of Soxhlet extraction by chromatography and mass spectrometry to reveal temporal extract profiles. *Analytica Chimica Acta*, **881**, 74-81.

Chen, S. S., Spiro, M. (1994). Study of microwave extraction of essential oil constituents from plant materials. *Journal of Microwave Power and Electromagnetic Energy*, **29**(4), 231-241.

Chen, Y., Xie, M. Y., Gong, X. F. (2007). Microwave-assisted extraction used for the isolation of total triterpenoid saponins from *Ganoderma atrum*. *Journal of Food Engineering*, **81**(1), 162-170.

Chidambara Murthy, K. N., Reddy, V. K., Veigas, J. M., Murthy, U. D. (2004). Study on wound healing activity of *Punica granatum* peel. *Journal of Medicinal Food*, **7**(2), 256-259.

Clodoveo, M. L., Hbaieb, R. H. (2013). Beyond the traditional virgin olive oil extraction systems: Searching innovative and sustainable plant engineering solutions. *Food Research International*, **54**(2), 1926-1933.

Çavdar, H. K., Yanık, D. K., Gök, U., Göğüş, F. (2017). Optimisation of Microwave-Assisted Extraction of Pomegranate (*Punica granatum L.*) Seed Oil and Evaluation of Its Physicochemical and Bioactive Properties. *Food Technology and Biotechnology*, **55**(1), 86.

Çiftçi, O. N., Fadıloğlu, S., Göğüş, F. (2009). Conversion of olive pomace oil to cocoa butter-like fat in a packed-bed enzyme reactor. *Bioresource Technology*, **100**(1), 324-329.

De A. K., Khopkar S. M., Chalmers R. A. (1970). “*Solvent Extractions of Metals*”, Van Nostrand, Reinhold Co., London.

De Castro, M. L., Priego-Capote, F. (2010). Soxhlet extraction: Past and present panacea. *Journal of Chromatography A*, **1217**(16), 2383-2389.

F. Soxhlet, (1879). Dingers’ Polyt. **232**, 461.

De Castro, M. D. L., Valcarcel, M., Tena, M. T. (2012). *Analytical Supercritical Fluid Extraction*. Springer Science & Business Media.

Delazar, A., Nahar, L., Hamedeyazdan, S., Sarker, S. D. (2012). Microwave-assisted extraction in natural products isolation. In *Natural Products Isolation* (pp. 89-115). Humana Press.

De Melo, I. L. P., de Carvalho, E. B. T., e Silva, A. M. D. O., Mancini-Filho, J. (2010). Effects of pomegranate seed oil on lipoperoxidation and activity of antioxidant enzymes in liver and brain of rats. *Free Radical Biology and Medicine*, **49**, S189.

Demesmay, C., Olle, M. (1993). Utilisation des micro-ondes dans les laboratoires d'analyse: Préparation d'échantillons. *Spectra 2000 Analyse*, **22**(175), 27-32.

Donard, O., Lalere, B., Martin, F., Lobinski, R. (1995). Microwave-assisted leaching of organotin compounds from sediments for speciation analysis. *Analytical Chemistry*, **67**(23), 4250-4254.

Drosou, C., Kyriakopoulou, K., Bimpilas, A., Tsimogiannis, D., Krokida, M. (2015). A comparative study on different extraction techniques to recover red grape pomace

polyphenols from vinification byproducts. *Industrial Crops and Products*, **75**, 141-149.

Dubois, V., Breton, S., Linder, M., Fanni, J., Parmentier, M. (2007). Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. *European Journal of Lipid Science and Technology*, **109(7)**, 710-732.

Dupont, G., Delteil, C., Camel, V., Bermond, A. (1999). The determination of polychlorinated biphenyls in municipal sewage sludges using microwave-assisted extraction and gas chromatography-mass spectrometry. *Analyst*, **124(4)**, 453-458.

Ebcioğlu, N. (2003). Sağlığımızın Yapıtaşları Sebze ve Meyveler. *Remzi Kitabevi, İstanbul*.

Egizabal, A., Zuloaga, O., Etxebarria, N., Fernandez, L. A., Madariaga, J. M. (1998). Comparison of microwave-assisted extraction and Soxhlet extraction for phenols in soil samples using experimental designs. *Analyst*, **123(8)**, 1679-1684.

Eikani, M. H., Golmohammad, F., Homami, S. S. (2012). Extraction of pomegranate (*Punica granatum L.*) seed oil using superheated hexane. *Food and Bioproducts Processing*, **90(1)**, 32-36.

Erstfeld, K. M., Chen, C. Y. (1998). Comparison of Supercritical fluid and Soxhlet extraction methods for the determination of chlorothalonil from cranberry bog soils. *Journal of Agricultural and Food Chemistry*, **46(2)**, 499-503.

Eskilsson, C. S., Björklund, E. (2000). Analytical-scale microwave-assisted extraction. *Journal of Chromatography A*, **902(1)**, 227-250.

Eskilsson, C. S., Björklund, E., Mathiasson, L., Karlsson, L., Torstensson, A. (1999). Microwave-assisted extraction of felodipine tablets. *Journal of Chromatography A*, **840(1)**, 59-70.

Fadavi, A., Barzegar, M., Azizi, M. H. (2006). Determination of fatty acids and total lipid content in oilseed of 25 pomegranates varieties grown in Iran. *Journal of Food Composition and Analysis*, **19(6-7)**, 676-680.

Fazaeli, M., Yousefi, S., Emam-Djomeh, Z. (2013). Investigation on the effects of microwave and conventional heating methods on the phytochemicals of pomegranate (*Punica granatum L.*) and black mulberry juices. *Food Research International*, **50(2)**, 568-573.

Fernandes, L., Pereira, J. A., López-Cortés, I., Salazar, D. M., Ramalhosa, E., Casal, S. (2015). Fatty acid, vitamin E and sterols composition of seed oils from nine different pomegranate (*Punica granatum L.*) cultivars grown in Spain. *Journal of Food Composition and Analysis*, **39**, 13-22.

Fifield F. W., Kealey D. (1975). "*Principals and Practice of Analytical Chemistry*", International textbook Company limited, 450 Edgware Rd., London W2 IEG.

Finkenzeller, K. (1999). *RFID Handbook: Radio-frequency Identification Fundamentals and Applications* (p. 131). New York: Wiley.

Fischer, U. A., Carle, R., Kammerer, D. R. (2011). Identification and quantification of phenolic compounds from pomegranate (*Punica granatum L.*) peel, mesocarp, aril and differently produced juices by HPLC-DAD–ESI/MSn. *Food Chemistry*, **127(2)**, 807-821.

Frost, S.P., Dean, J.R., Evans, K.P., Harradine, K., Cary C., Comber, M.H.I. (1997). Extraction of hexaconazole from weathered soils: A comparison between Soxhlet extraction, microwave-assisted extraction, supercritical fluid extraction and accelerated solvent extraction. *Analyst*, **122(9)**, 895-898.

Gai, Q. Y., Jiao, J., Mu, P. S., Wang, W., Luo, M., Li, C. Y., ...Fu, Y. J. (2013). Microwave-assisted aqueous enzymatic extraction of oil from *Isatis indigotica* seeds and its evaluation of physicochemical properties, fatty acid compositions and antioxidant activities. *Industrial Crops and Products*, **45**, 303-311.

Ganzler, K., Salgó, A., Valkó, K. (1986). Microwave extraction: A novel sample preparation method for chromatography. *Journal of Chromatography A*, **371**, 299-306.

- Garcia-Ayuso, L. E., Sánchez, M., Fernández de Alba, A., Luque de Castro, M. D. (1998). Focused microwave-assisted soxhlet: an advantageous tool for sample extraction. *Analytical Chemistry*, **70(11)**, 2426-2431.
- Gerber, M., Thiébaud, A., Astorg, P., Clavel-Chapelon, F., Combe, N. (2005). Dietary fat, fatty acid composition and risk of cancer. *European Journal of Lipid Science and Technology*, **107(7-8)**, 540-559.
- Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M., Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry*, **48(10)**, 4581-4589.
- Goula, A. M., Adamopoulos, K. G. (2012). A method for pomegranate seed application in food industries: seed oil encapsulation. *Food and Bioproducts Processing*, **90(4)**, 639-652.
- 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.
- Gutfinger, T. (1981). Polyphenols in olive oils. *Journal of the American Oil Chemists Society*, **58(11)**, 966-968.
- Haddadi-Guemghar, H., Janel, N., Dairou, J., Remini, H., Madani, K. (2014). Optimisation of microwave-assisted extraction of prune (*Prunus domestica*) antioxidants by response surface methodology. *International Journal of Food Science & Technology*, **49(10)**, 2158-2166.
- Heber, D., Schulman, R. N., Seeram, N. P. (Eds.). (2006). *Pomegranates: Ancient Roots to Modern Medicine*. Boca Raton, FL. CRC press.
- He, L., Xu, H., Liu, X., He, W., Yuan, F., Hou, Z., Gao, Y. (2011). Identification of phenolic compounds from pomegranate (*Punica granatum L.*) seed residues and investigation into their antioxidant capacities by HPLC–ABTS+ assay. *Food Research International*, **44(5)**, 1161-1167.

Hernandez, F., Melgarejo, P., Martínez, J. J., Martínez, R., Legua, P. (2011). Fatty acid composition of seed oils from important spanish pomegranate cultivars. *Italian Journal of Food Science*, **23(2)**, 188-193.

Ho, K. K. H. Y., Ferruzzi, M. G., Liceaga, A. M., San Martín-González, M. F. (2015). Microwave-assisted extraction of lycopene in tomato peels: effect of extraction conditions on all-trans and cis-isomer yields. *LWT-Food Science and Technology*, **62(1)**, 160-168.

Huie, C. W. (2002). A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. *Analytical and Bioanalytical Chemistry*, **373(1-2)**, 23-30.

Hu, Z., Cai, M., Liang, H. H. (2008). Desirability function approach for the optimization of microwave-assisted extraction of saikosaponins from Radix Bupleuri. *Separation and Purification Technology*, **61(3)**, 266-275.

Ismail, T., Sestili, P., Akhtar, S. (2012). Pomegranate peel and fruit extracts: a review of potential anti-inflammatory and anti-infective effects. *Journal of Ethnopharmacology*, **143(2)**, 397-405.

Jiao, J., Li, Z. G., Gai, Q. Y., Li, X. J., Wei, F. Y., Fu, Y. J., Ma, W. (2014). Microwave-assisted aqueous enzymatic extraction of oil from pumpkin seeds and evaluation of its physicochemical properties, fatty acid compositions and antioxidant activities. *Food Chemistry*, **147**, 17-24.

Jing, P. U., Ye, T., Shi, H., Sheng, Y., Slavin, M., Gao, B., ... Yu, L. L. (2012). Antioxidant properties and phytochemical composition of China-grown pomegranate seeds. *Food Chemistry*, **132(3)**, 1457-1464.

Keskin, H., Koçak Yanık, D., Mucuk, H. N., Göğüş, F., Fadıloğlu, S. (2016). Valorization of Olive Pomace Oil with Enzymatic Synthesis of 2-Monoacylglycerol. *Journal of Food Science*, **81(4)**.

Khajeh, M., Moghaddam, A. R. A., Sanchooli, E. (2010). Application of Doehlert design in the optimization of microwave-assisted extraction for determination of zinc and copper in cereal samples using FAAS. *Food Analytical Methods*, **3(3)**, 133-137.

Khoddami, A., Man, Y. B. C., Roberts, T. H. (2014). Physico-chemical properties and fatty acid profile of seed oils from pomegranate (*Punica granatum L.*) extracted by cold pressing. *European Journal of Lipid Science and Technology*, **116**(5), 553-562.

Kim, D. O., Lee, C. Y. (2004). Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. *Critical Reviews in Food Science and Nutrition*, **44**(4), 253-273.

Kingston, H. M., Haswell, S. J. (1997). *Microwave-Enhanced Chemistry*. American Chemical Society.

Kiralan, M., Golukcu, M., Tokgoz, H. (2009). Oil and conjugated linolenic acid contents of seeds from important pomegranate cultivars (*Punica granatum L.*) grown in Turkey. *Journal of the American Oil Chemists' Society*.

Kovacs, A., Ganzler, K., Simon-Sarkadi, L. (1998). Microwave-assisted extraction of free amino acids from foods. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A*, **207**(1), 26-30.

Kumari, A., Dora, J., Kumar, A., Kumar, A. (2012). Pomegranate (*Punica granatum*)—Overview. *Int. J. Pharm. Chem. Sci*, **1**, 1218-1222.

Kurt, H., Şahin, G. (2013). Study of agricultural geography: Pomegranate (*Punica granatum L.*) cultivation in Turkey. *Marmara Geogr Rev*, **27**, 551-574.

Kwon, J. H., Lee, G. D., Bélanger, J. M., Jocelyn Paré, J. R. (2003). Effect of ethanol concentration on the efficiency of extraction of ginseng saponins when using a microwave-assisted process (MAP™). *International Journal of Food Science & Technology*, **38**(5), 615-622.

Lansky, E. P., Newman, R. A. (2007). *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology*, **109**(2), 177-206.

LaRue, J. H. (1980). Growing pomegranates in California. UC Fruit & Nut Research Information Center.

Letellier, M., Budzinski, H. (1999). Microwave assisted extraction of organic compounds. *Analisis*, **27(3)**, 259-270.

Li, J., Zu, Y. G., Fu, Y. J., Yang, Y. C., Li, S. M., Li, Z. N., Wink, M. (2010). Optimization of microwave-assisted extraction of triterpene saponins from defatted residue of yellow horn (*Xanthoceras sorbifolia* Bunge.) kernel and evaluation of its antioxidant activity. *Innovative Food Science & Emerging Technologies*, **11(4)**, 637-643.

Liu, G., Xu, X., Gong, Y., He, L., Gao, Y. (2012). Effects of supercritical CO₂ extraction parameters on chemical composition and free radical-scavenging activity of pomegranate (*Punica granatum* L.) seed oil. *Food and Bioproducts Processing*, **90(3)**, 573-578.

Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., Cheng, S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry*, **96(2)**, 254-260.

Li, Y., Skouroumounis, G. K., Elsey, G. M., Taylor, D. K. (2011). Microwave-assistance provides very rapid and efficient extraction of grape seed polyphenols. *Food Chemistry*, **129(2)**, 570-576.

Llompart, M. P., Lorenzo, R. A., Cela, R., Pare, J. J., Belanger, J. M., Li, K. (1997). Phenol and methylphenol isomers determination in soils by in-situ microwave-assisted extraction and derivatisation. *Journal of Chromatography A*, **757(1-2)**, 153-164.

Lodovici, M., Guglielmi, F., Meoni, M., Dolara, P. (2001). Effect of natural phenolic acids on DNA oxidation in vitro. *Food and Chemical Toxicology*, **39(12)**, 1205-1210.

Lopez-Avila, V., Young, R., Beckert, W. F. (1994). Microwave-assisted extraction of organic compounds from standard reference soils and sediments. *Analytical Chemistry*, **66(7)**, 1097-1106.

Lopez-Avila, V., Young, R., Benedicto, J., Ho, P., Kim, R., Beckert, W. F. (1995). Extraction of organic pollutants from solid samples using microwave energy. *Analytical Chemistry*, **67**(13), 2096-2102.

Luque-Garcia, J. L., De Castro, M. L. (2004). Focused microwave-assisted Soxhlet extraction: devices and applications. *Talanta*, **64**(3), 571-577.

Lu, Y., Ma, W., Hu, R., Dai, X., Pan, Y. (2008). Ionic liquid-based microwave-assisted extraction of phenolic alkaloids from the medicinal plant *Nelumbo nucifera* Gaertn. *Journal of Chromatography A*, **1208**(1-2), 42-46.

Majors, R. E. (2008). Practical Aspects of Solvent Extraction. *LC GC North America*, **26**(12).

Mandal, V., Mohan, Y., Hemalatha, S. (2007). Microwave assisted extraction—an innovative and promising extraction tool for medicinal plant research. *Pharmacognosy Reviews*, **1**(1), 7-18.

Mattina, M. I., Berger, W. I., Denson, C. L. (1997). Microwave-assisted extraction of taxanes from *Taxus* biomass. *Journal of Agricultural and Food Chemistry*, **45**(12), 4691-4696.

Meerts, I. A. T. M., Verspeek-Rip, C. M., Buskens, C. A. F., Keizer, H. G., Bassaganya-Riera, J., Jouni, Z. E., ... Van de Waart, E. J. (2009). Toxicological evaluation of pomegranate seed oil. *Food and Chemical Toxicology*, **47**(6), 1085-1092.

Melgarejo, P., Salazar, D. M., Amoros, A., Artes, F. (1995). Total lipids content and fatty acid composition of seed oils from six pomegranate cultivars. *Journal of the Science of Food and Agriculture*, **69**(2), 253-256.

Melo ILP, Carvalho EBT, Mancini-Filho J (2014) Pomegranate Seed Oil (*Punica Granatum* L.): A Source of Punicic Acid (Conjugated α -Linolenic Acid). *J Hum Nutr Food Sci* 2(1): 1024.

Navarro, V., Villarreal, M. L., Rojas, G., Lozoya, X. (1996). Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. *Journal of Ethnopharmacology*, **53(3)**, 143-147.

Nde, D. B., Boldor, D., Astete, C. (2015). Optimization of microwave assisted extraction parameters of neem (*Azadirachta indica* A. Juss) oil using the Doehlert's experimental design. *Industrial Crops and Products*, **65**, 233-240.

Nyiredy, S. (2004). Separation strategies of plant constituents—current status. *Journal of Chromatography B*, **812(1-2)**, 35-51.

Oğuz, H. İ., Ukav, İ., Eroğlu, D. (2011). Pomegranate Production and Marketing in Southeastern Anatolia, *GAP VI. Agriculture Congress*, 09 –12 May 2011, p. 108 – 112.

Orak, H. H., Yagar, H., Isbilir, S. S. (2012). Comparison of antioxidant activities of juice, peel, and seed of pomegranate (*Punica granatum* L.) and inter-relationships with total phenolic, tannin, anthocyanin, and flavonoid contents. *Food Science and Biotechnology*, **21(2)**, 373-387.

Özgül-Yücel, S. (2005). Determination of conjugated linolenic acid content of selected oil seeds grown in Turkey. *Journal of the American Oil Chemists' Society*, **82(12)**, 893-897.

Pande, G., Akoh, C. C. (2009). Antioxidant capacity and lipid characterization of six Georgia-grown pomegranate cultivars. *Journal of agricultural and food chemistry*, **57(20)**, 9427-9436.

Pan, X., Liu, H., Jia, G., Shu, Y. Y. (2000). Microwave-assisted extraction of glycyrrhizic acid from licorice root. *Biochemical Engineering Journal*, **5(3)**, 173-177.

Pan, X., Niu, G., Liu, H. (2001). Microwave-assisted extraction of tanshinones from *Salvia miltiorrhiza* bunge with analysis by high-performance liquid chromatography. *Journal of Chromatography A*, **922(1-2)**, 371-375.

- Pan, X., Niu, G., Liu, H. (2003). Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chemical Engineering and Processing: Process Intensification*, **42**(2), 129-133.
- Pare, J. J., Belanger, J. M., Stafford, S. S. (1994). Microwave-assisted process (MAP™): a new tool for the analytical laboratory. *TrAC Trends in Analytical Chemistry*, **13**(4), 176-184.
- Park, H. M., Moon, E., Kim, A. J., Kim, M. H., Lee, S., Lee, J. B., ... Kim, S. Y. (2010). Extract of *Punica granatum* inhibits skin photoaging induced by UVB irradiation. *International Journal of Dermatology*, **49**(3), 276-282.
- Prados-Rosales, R. C., García, J. L., De Castro, M. L. (2003). Rapid analytical method for the determination of pesticide residues in sunflower seeds based on focused microwave-assisted Soxhlet extraction prior to gas chromatography–tandem mass spectrometry. *Journal of Chromatography A*, **993**(1-2), 121-129.
- Qu, W., Pan, Z., Ma, H. (2010). Extraction modeling and activities of antioxidants from pomegranate marc. *Journal of Food Engineering*, **99**(1), 16-23.
- Raner, K. D., Strauss, C. R., Vyskoc, F., Mokbel, L. (1993). A comparison of reaction kinetics observed under microwave irradiation and conventional heating. *The Journal of Organic Chemistry*, **58**(4), 950-953.
- Renoe, B. W. (1994). Microwave assisted extraction. *American Laboratory*, **26**(12), 34-40.
- Rice-Evans, C. A., Miller, N. J., Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, **20**(7), 933-956.
- Routray, W., Orsat, V. (2012). Microwave-assisted extraction of flavonoids: a review. *Food and Bioprocess Technology*, **5**(2), 409-424.
- Roy, S. K., Waskar, D. P. (1997). Pomegranate. *Postharvest physiology and storage of tropical and subtropical fruits*. Cab International, Wallingford, UK, 365-374.

- Ruan, G. H., Li, G. K. (2007). The study on the chromatographic fingerprint of *Fructus xanthii* by microwave assisted extraction coupled with GC–MS. *Journal of Chromatography B*, **850**(1-2), 241-248.
- Rydberg, J. (Ed.). (2004). *Solvent Extraction Principles and Practice, Revised and Expanded*. CRC Press.
- Sadeghi, H. (2010). Physical and chemical characteristics of four native pomegranate cultivars in Mazandaran province of Iran. *Journal of Food, Agriculture & Environment*, **8**(2), 570-572.
- Sanghi, R., Kannamkumarath, S. S. (2004). Comparison of extraction methods by soxhlet, sonicator, and microwave in the screening of pesticide residues from solid matrices. *Journal of Analytical Chemistry*, **59**(11), 1032-1036.
- Schwartz, E., Tzulker, R., Glazer, I., Bar-Ya'akov, I., Wiesman, Z., Tripler, E., ... Amir, R. (2009). Environmental conditions affect the color, taste, and antioxidant capacity of 11 pomegranate accessions' fruits. *Journal of Agricultural and Food Chemistry*, **57**(19), 9197-9209.
- Smith, R. M. (2003). Before the injection—modern methods of sample preparation for separation techniques. *Journal of Chromatography A*, **1000**(1-2), 3-27.
- Singh, N., Shrivastava, P., Shah, M. (2014). Microwave-assisted extraction of lemongrass essential oil: study of the influence of extraction method and process parameters on extraction process. *J Chem Pharm Res*, **6**, 385-9.
- Singh, R. P., Chidambara Murthy, K. N., Jayaprakasha, G. K. (2002). Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *Journal of Agricultural and Food Chemistry*, **50**(1), 81-86.
- Snyder, J. L., Grob, R. L., McNally, M. E., Oostdyk, T. S. (1992). Comparison of supercritical fluid extraction with classical sonication and soxhlet extractions for selected pesticides. *Analytical Chemistry*, **64**(17), 1940-1946.
- Song, J., Li, D., Liu, C., Zhang, Y. (2011). Optimized microwave-assisted extraction of total phenolics (TP) from *Ipomoea batatas* leaves and its antioxidant activity. *Innovative Food Science & Emerging Technologies*, **12**(3), 282-287.

- Spigno, G., De Faveri, D. M. (2009). Microwave-assisted extraction of tea phenols: a phenomenological study. *Journal of Food Engineering*, **93**(2), 210-217.
- Sukhija, P. S., Palmquist, D. L. (1988). Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *Journal of Agricultural and Food Chemistry*, **36**(6), 1202-1206.
- Sumi, T., Horikoshi, S. (2013). Microwave synthesis, extraction, improvement and degradation in oil chemistry. *Journal of Oleo Science*, **62**(7), 443-451.
- Szpunar, J., Schmitt, V. O., Donard, O. F., Lobin, R. (1996). Low-power focused microwave technology as a new tool for rapid preparation of solid samples for speciation analysis. *TrAC Trends in Analytical Chemistry*, **15**(4), 181-187.
- Taghvaei, M., Jafari, S. M., Assadpoor, E., Nowrouzieh, S., Alishah, O. (2014). Optimization of microwave-assisted extraction of cottonseed oil and evaluation of its oxidative stability and physicochemical properties. *Food Chemistry*, **160**, 90-97.
- Talebi, M., Ghassempour, A., Talebpour, Z., Rassouli, A., Dolatyari, L. (2004). Optimization of the extraction of paclitaxel from *Taxus baccata* L. by the use of microwave energy. *Journal of Separation Science*, **27**(13), 1130-1136.
- Tatke, P., Jaiswal, Y. (2011). An overview of microwave assisted extraction and its applications in herbal drug research. *Research Journal of Medicinal Plant*, **5**(1), 21-31.
- Tian, Y., Xu, Z., Zheng, B., Lo, Y. M. (2013). Optimization of ultrasonic-assisted extraction of pomegranate (*Punica granatum* L.) seed oil. *Ultrasonics Sonochemistry*, **20**(1), 202-208.
- Tong, P., Kasuga, Y., Khoo, C. S. (2006). Liquid chromatographic-mass spectrometric method for detection of estrogen in commercial oils and in fruit seed oils. *Journal of Food Composition and Analysis*, **19**(2-3), 150-156.
- Tsukui, A., Júnior, H. S., Oigman, S. S., De Souza, R. O. M. A., Bizzo, H. R., Rezende, C. M. (2014). Microwave-assisted extraction of green coffee oil and quantification of diterpenes by HPLC. *Food Chemistry*, **164**, 266-271.

Tzulker, R., Glazer, I., Bar-Ilan, I., Holland, D., Aviram, M., Amir, R. (2007). Antioxidant activity, polyphenol content, and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. *Journal of Agricultural and Food Chemistry*, **55**(23), 9559-9570.

Vazquez, M. J., Carro, A. M., Lorenzo, R. A., Cela, R. (1997). Optimization of methylmercury microwave-assisted extraction from aquatic sediments. *Analytical Chemistry*, **69**(2), 221-225.

Veggi, P. C., Martinez, J., Meireles, M. A. A. (2012). Fundamentals of microwave extraction. In *Microwave-Assisted Extraction for Bioactive Compounds* (pp. 15-52). Springer US.

Verardo, V., Garcia-Salas, P., Baldi, E., Segura-Carretero, A., Fernandez-Gutierrez, A., Caboni, M. F. (2014). Pomegranate seeds as a source of nutraceutical oil naturally rich in bioactive lipids. *Food Research International*, **65**, 445-452.

Vértes, A., Nagy, S., Klencsár, Z., Lovas, R. G., Rösch, F. (Eds.). (2010). *Handbook of Nuclear Chemistry: Vol. 2: Elements and Isotopes: Formation, Transformation, Distribution*. Springer Science & Business Media.

Viuda-Martos, M., Fernández-López, J., Pérez-Álvarez, J. A. (2010). Pomegranate and its many functional components as related to human health: a review. *Comprehensive Reviews in Food Science and Food Safety*, **9**(6), 635-654.

Wang, Y., You, J., Yu, Y., Qu, C., Zhang, H., Ding, L., ... Li, X. (2008). Analysis of ginsenosides in *Panax ginseng* in high pressure microwave-assisted extraction. *Food Chemistry*, **110**(1), 161-167.

Wu, T., Yan, J., Liu, R., Marcone, M. F., Aisa, H. A., Tsao, R. (2012). Optimization of microwave-assisted extraction of phenolics from potato and its downstream waste using orthogonal array design. *Food Chemistry*, **133**(4), 1292-1298.

Xiao, W., Han, L., Shi, B. (2008). Microwave-assisted extraction of flavonoids from *Radix Astragali*. *Separation and Purification Technology*, **62**(3), 614-618.

Yamasaki, M., Kitagawa, T., Koyanagi, N., Chujo, H., Maeda, H., Kohno-Murase, J., ... Yamada, K. (2006). Dietary effect of pomegranate seed oil on immune function and lipid metabolism in mice. *Nutrition*, **22**(1), 54-59.

Yan, M. M., Liu, W., Fu, Y. J., Zu, Y. G., Chen, C. Y., Luo, M. (2010). Optimisation of the microwave-assisted extraction process for four main astragalosides in *Radix Astragali*. *Food Chemistry*, **119**(4), 1663-1670.

Yasui, Y., Hosokawa, M., Sahara, T., Suzuki, R., Ohgiya, S., Kohno, H., ... Miyashita, K. (2005). Bitter gourd seed fatty acid rich in 9c, 11t, 13t-conjugated linolenic acid induces apoptosis and up-regulates the GADD45, p53 and PPAR γ in human colon cancer Caco-2 cells. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, **73**(2), 113-119.

Young, J. C. (1995). Microwave-assisted extraction of the fungal metabolite ergosterol and total fatty acids. *Journal of Agricultural and Food Chemistry*, **43**(11), 2904-2910.

Zhao, X., Yuan, Z., Fang, Y., Yin, Y., Feng, L. (2013). Characterization and evaluation of major anthocyanins in pomegranate (*Punica granatum L.*) peel of different cultivars and their development phases. *European Food Research and Technology*, **236**(1), 109-117.

Zhou, H. Y., Liu, C. Z. (2006). Microwave-assisted extraction of solanesol from tobacco leaves. *Journal of Chromatography A*, **1129**(1), 135-139.

Zigoneanu, I. G., Williams, L., Xu, Z., Sabliov, C. M. (2008). Determination of antioxidant components in rice bran oil extracted by microwave-assisted method. *Bioresource Technology*, **99**(11), 4910-4918.

Zlotorzynski, A. (1995). The application of microwave radiation to analytical and environmental chemistry. *Critical Reviews in Analytical Chemistry*, **25**(1), 43-76.

Zuloaga, O., Etxebarria, N., Fernández, L. A., Madariaga, J. M. (1999). Optimisation and comparison of microwave-assisted extraction and Soxhlet extraction for the determination of polychlorinated biphenyls in soil samples using an experimental design approach. *Talanta*, **50**(2), 345-357.