EXTRACTION OF PHENOLIC COMPOUNDS FROM HAZELNUT SHELL WASTE

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ABSTRACT

EXTRACTION OF PHENOLIC COMPOUNDS FROM HAZELNUT SHELL WASTE

The main objective of this study is to obtain phenolic compounds from hazelnut shell waste by extraction and to add value to hazelnut shell wastes. Soxhlet extraction, ultrasonic extraction and combined extraction (soxhlet followed by ultrasonic extraction) methods were used for the extraction of hazelnut shell to obtain phenolic and antioxidant compounds. The effect of extracting solvent (ethanol, methanol, n-hexane, acetone and chloroform), extraction time (8h, 2 cycle and 3 cycle) (1 cycle = 20 min for hexane, 25 min for chloroform, 40 min for ethanol, 45 min for methanol and 35 min for acetone), solid-liquid ratio (4, 8 and 12 g / 250 ml) and size of hazelnut shell (1 mm and 2 mm) were investigated on the phenolic content and antioxidant capacity.

Gas Chromatography equipped with a Mass Spectrometry (GC-MS) was used for the analysis of liquid products obtained from the extraction of hazelnut shell. Palmitic acid and oleic acid variations were detected at high ratios. The combined extraction method, which was composed of soxhlet and ultrasonic extractions, resulted in a significant increase in the yield of extraction. Also, higher yield was obtained from methanol and ethanol extraction because of the higher polarity of the solvents. On the other hand, it was observed that there was no significant effect of the extraction time on the extraction yield. The highest phenolic content was 0.166 mg gallic acid equivalent/ml and this value was obtained with methanol by combined extraction using 4 g hazelnut shell and 250 ml solvent.

ÖZET

FENOLİK BİLEŞENLERİN FINDIK KABUĞU ATIKLARINDAN EKSTRAKSİYONU

Bu çalışmanın esas amacı fındık kabuğu atığından ekstraksiyon yöntemi ile fenolik bileşenler elde etmek ve fındık kabuğu atıklarının değerlendirilmesini sağlamaktır. Fenolik ve antioksidan bileşenlerin eldesinde sokslet ekstraksiyonu, ultrasonik ekstraksiyon ve bu iki ekstraksiyon yönteminin birleşmesinden oluşan kombine ekstraksiyon yöntemi kullanılmaktadır. Çeşitli çözgen tiplerinin (n-hekzan, kloroform, etanol, metanol ve aseton), ekstraksiyon süresinin (2 döngü (1 döngü=hekzan için 20 dk, kloroform için 25 dk, etanol için 40 dk, metanol için 45 dk ve aseton için 35 dk), 3 döngü ve 8 saat), katı sıvı oranın (4, 8 ve 12 g fındık kabuğu/ 250 ml çözgen) ve fındık kabuğu boyutunun (1 mm ve 2 mm) fenolik ve antioksidan bileşenler üzerindeki etkisi incelenmektedir.

Fındık kabuğu ekstraksiyonundan elde edilen sıvı ürün analizinde Gaz Kromatogram-Kütle Spektrometri (GC-MS) kullanılmış, yüksek oranda palmitik asit ve oleik asit varyasyonları tespit edilmiştir. Sokslet ve ultrasonik ekstraksiyonların birleşiminden oluşan kombine ekstraksiyon yöntemi, ekstraksiyon veriminde önemli bir artışa sebep olmuştur. Ayrıca metanol ve etanol çözgenlerinin polaritesinin yüksek olması fındık kabuğundan daha fazla verim elde edilmesini sağlamıştır. Öte yandan, ekstraksiyon süresinin verime ciddi bir etkisinin olmadığı gözlemlenmiştir. En yüksek fenolik bileşen değeri metanol kombine ekstraksiyonunda 4 g fındık kabuğu ve 250 ml çözgen kullanılarak 0.166 mgGAE/ml elde edilmiştir.

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CHAPTER 1

INTRODUCTION

A considerable amount of plant and animal waste is being produced in the world and it is observed that these wastes have been disposed instead of being used. Taking advantage of these wastes has become very important in recent years and the value of the contents of these wastes has been understood. Animal wastes are mostly used in fertilizer and agricultural activities and also the vegetable wastes are evaluated in terms of phenolic components and antioxidant activities. These phenolic components and antioxidants are very beneficial for human health and the pharmaceutical, medical and cosmetic sectors also benefit from these components (Luque-Garcia and Luque De Castro, 2003; Tavman et al., 2009). These antioxidants and phenolic compounds are the natural origin and have begun to be used to prevent many diseases. Also, phenolic compounds reduce oxidative activity and clear free radicals (Naczk and Shahidi, 2004). Antioxidants are anti-tumor (Kumar et al., 2013), antimutagenic and anticarcinogenic (Surh, 2003). Phenolic compounds and antioxidants provide protection against cancer, heart and neurological diseases when taken at a sufficient level in the body (Naczk and Shahidi, 2004; Surh, 2003; Geybels et al., 2013; Cavuldak et al., 2016). For this reason, the acquisition of these components has recently become important.

Many methods have been developed for obtaining these components from vegetable wastes. One of the most commonly applied methods is the extraction. Extraction briefly means to obtain desired products in a separation process by using selective solvents. A number of different extraction methods have been used to isolate important components of plant wastes. Ultrasonic assisted, microwave assisted, and supercritical extraction methods are examples of modern methods. In new methods, extraction times are shortened and solvent usage rates are reduced (Perez-Serradilla et al., 2007; Wang et al., 2006; Tavman et el., 2009). Soxhlet, a traditionally used solid liquid extraction method, is one of the most widely used methods despite the high level of solvent use and duration of time.

Polyphenols and antioxidants have been obtained from very different plant wastes. Various extraction methods have been studied to isolate these phenolic compounds and antioxidants. For obtaining these components, peanut skin (Yu et al., 2005), coconut

(Rodrigues et. al, 2008), almond hulls and pine sawdust (Pinelo et al., 2004), chest nut tree wood (Gironi and Piemonte, 2011), pecan nut shell (do Prado et al., 2014), cashew nut shell (Yuliana et al., 2012), almond shells (Moure et al., 2007) were used as a waste source.

In this study, phenolic components were obtained from hazelnut shell using three different extraction methods; soxhlet extraction, ultrasonic extraction and combined extraction (soxhlet extraction followed by ultrasonic extraction). The parameters of the the study were extraction time (2, 3 cycles and 8h), solid liquid ratio of hazelnut shell and solvent (4, 8 and 12 g / 250 ml), size of hazelnut shell (1 and 2 mm) and type of extracting solvents (ethanol, methanol, n-hexane, acetone and chloroform). At the end of each experiment, compositions of liquid product and solid residue were analysed. The solid residue was analyzed by using Fourier Transform-Infrared Spectroscopy (FTIR). The liquid product was analyzed via Gas Chromatography equipped with a Mass Spectroscopy (GC-MS). Total phenolic content and antioxidant activity of the final liquid products were determined by Folin Ciocalteu and ABTS methods, respectively.

1.1. Description of Hazelnut and Hazelnut Shell

Hazelnut (*Corylus avellena)* is the genus *Corylus* of the *Corylear* subfamily of the *Betulaceae* family of the *Fagales* team (Gümrük ve Ticaret Bakanlığı Kooperatifçilik Genel Müdürlüğü, 2017). Hazelnut, which is a high nutrient source with its healthy oils, is an important food source in terms of nutritional value (Shahidi et al., 2007).

The main producers of hazelnuts are Turkey, Italy, USA and Spain in the world. In Turkey the annual production of hazelnut is approximately 400.000-500.000 tons. In Italy, yearly production has decreased to 110.000 tons. The hazelnuts have been produced in Campania, Sicily and Latium region in Italy. While the production of hazelnut is 25.000-30.000 tons in the USA. 18. 000 tons of hazelnut have been harvested hazelnut in Spain, Catalonia region (Koksal, 2000). The leading country in the world in terms of exportation of hazelnut is Turkey. Besides, Italy, USA and Spain are affected by the other importer countries from the point of hazelnut production (Kılıç and Alkan, 2006).

Turkey has been producing hazelnuts in the north of Turkey for 2300 years. It is grown in almost all coasts of the Black Sea, especially Giresun, Ordu, Trabzon and Rize.

Turkey has a %70 share in hazelnut production in the world (Gümrük ve Ticaret Bakanlığı Kooperatifçilik Genel Müdürlüğü, 2017). Moreover, these hazelnuts are an important economical source of export for our country. %70-75 of the world's export belongs to Turkey. There are three main varieties of hazelnut separated by length, width an tickness.

Hazelnut is mainly used in bakery, chocolate and candy industry. Also, it can be used that development of the products such as; cosmetics, hand and house tools applications, fertilizer and pharmaceutical applications. (Fındık Tanıtım Grubu, 2012). Although the hazelnut shell can be used in many applications such as; oil refinery, resin production, plastic production, termosettings, isolation in buildings and heating of houses, they are usually considered as a waste because of the lack of evaluating potential value. However, there are many antioxidants and phenolic components in them. After the cracking process, the hazelnut shell can be obtained. The density of the hazelnut shell is approximately 0.23 g/cm^3 (Çöpür et al., 2007). While carbon content of hazelnut shell is 51.6%, oxygen and hydrogen contents of hazelnut shell are 40.2% and 5.2%, respectively (Demirbaş, 2002). The hazelnut shell includes approximately 43.1% lignin, 27.5% hemicellulose and %24.7 cellulose (Çöpür et al., 2007).

Hazelnut grows in humic and humid soil in mild and rainy climates. Also, it requires rainfall of 1000-2000 mm per year. Frost conditions and summer drought reduce the yield. Therefore, it is easier to grow hazelnuts on the Black Sea coastline (Fındık Tanıtım Grubu, 2012). Moreover hazelnut is important in the struggle against erosion when it is thought that the regions where the nuts are grown are slippery and rainy lands (Gümrük ve Ticaret Bakanlığı Kooperatifçilik Genel Müdürlüğü, 2017). Hazelnut is grown in Turkey would be ready to harvest towards the middle of August. Figure 1.1 demonstrates the by-products of hazelnut and hazelnut. The byproducts of the hazelnut are hazelnut leaves, hazelnut shell and hazelnut skin. Hazelnut kernel covers with skin. Skin, hazelnut shell and leaves protect the hazelnut kernel. The matured hazelnuts are collected and dried in the sun and seperated from their leaves. The obtained hazelnuts are again dried with the sun. First of all, the hazelnut leaves are separated from the kernel of hazelnut. Hazelnut shells are mechanically broken and hazelnut fruit is obtained. This kernel can be used up raw or roasted (removed skin). (Shahidi et al., 2007). Although the hazelnut shell is not a commercial value, it is valorized as a source of heating, ethanol production. In addition, due to the phenolic components and antioxidants in the content of the hazelnut shell has gained value.

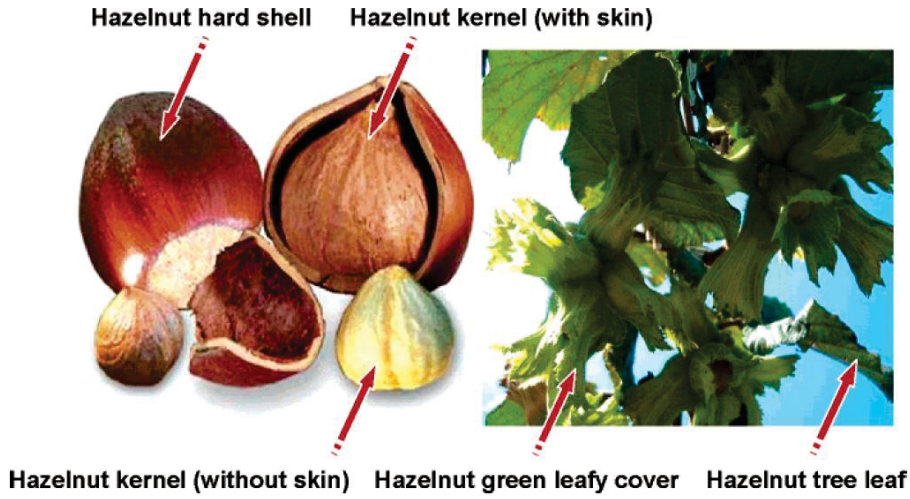


Figure 1.1. Hazelnut and hazelnut by-products
(Source: Shahidi et al., 2007)

1.2. Valorization of Hazelnut Shell Waste

1.2.1. Ethanol Production

The necessity of new alternative energy sources has increased due to the depleted energy resources in the world. Because of the depletion of energy resources such as coal, natural gas and oil, renewable energy sources have become an important place (Kim and Holtzapple, 2005; Arslan and Saraçoğlu, 2010). In particular, ethanol has become an important source of energy in terms of being renewable and harmless to nature (Kumar et al., 2009; Arslan and Saraçoğlu, 2010). It also has an advantage in terms of performance with its high octane content in ethanol use (Von Blottnitz and Curran, 2007; Hoşgün et al., 2017).

Lignocellulosic biomass is used in ethanol synthesis. Lignocellulosic biomass consists of cellulose hemicellulose and lignin. For this reason, biomass-synthesized ethanol is renewable and harmless to the environment. Ethanol is synthesized from plantal wastes because they are contained in lignocellulosic components (Balat et al., 2008; Arslan and Saraçoğlu, 2010).

Hazelnut shell is also used in ethanol production due to the lignocellulosic components it contains. Hazelnut shell contains approximately 43.1% lignin, 27.5% hemicellulose and 24.7% cellulose (Çöpür et al., 2007; Arslan and Saraçoğlu, 2010).

400.000 - 500.000 tons of hazelnuts are produced annually in Turkey (Koksal, 2000). For this reason, a significant amount of hazelnut shell in Turkey. Although hazelnut shell has no commercial value, it has become a significant role in terms of renewable energy production.

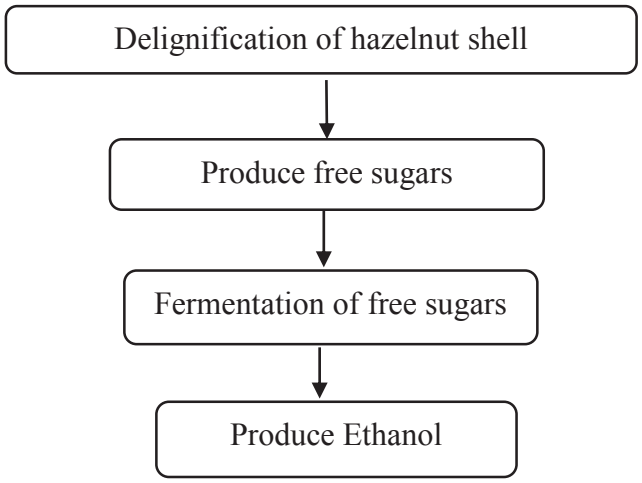


Figure 1.2. Production of ethanol from hazelnut shell

Figure 1.2 shows that production of ethanol from lignocellulosic material. In the production of ethanol from lignocellulosic materials. Firstly, lignin, cellulose and hemicellulose are separated from each other by the help of acid and enzyme. Hemicellulose and cellulose are released. Free sugar is obtained by depolymerization of hemicellulose and cellulose and ethanol is obtained by fermentation of these sugars (Laopaiboon et al., 2010; Arslan and Saraçoğlu, 2010).

1.2.2. Antioxidants and Phenolic Compounds

Hazelnuts are an important nutrient source for the human health. The proteins, carbohydrates, vitamins, minerals, beta sitosterol, antioxidants and phenolic compounds are beneficial to human health. Around 60% of the oleic acid, linoleic acid, palmitic acid and steraic acid properties of oil ratio in the positive effects are observed for human health (Ciemniewska-Zytkiewicz et al., 2015). Hazelnut is a very rich source of nutrients in terms of minerals such as, magnesium, calcium, potassium and phosphorus. Moreover, hazelnuts consist of vitamin E and B. (Fındık Tanıtım Grubu, 2012). Hazelnut consists of many phytochemical compounds, antioxidants and phenolic compounds. These

compounds help to decrease many diseases such as; many types of cancer, inflammation disease, neurodegenerative disease, cholestrerol, paralysis, heart and other diseases. etc. They also reduce the harmful effects of free radicals (Shahidi et al., 2007; Surh, 2003; Watson, 2003). The phenolic acids of hazelnut are gallic, caffeic, p-courmaric, ferulic, sinapic caffeolytartaric and caffeoylquinic acids (Amaral et al., 2005; Oliveira et al., 2007; Peev et al., 2007; Shahidi et al., 2007).

In the literature, there are many studies about antioxidant activity and phenolic content. One of these is the hazelnut kernel and hazelnut by products which were hazelnut skin, hazelnut hard shell, hazelnut green leafy cover and tree leaf to understand the yield phenolic contents and antioxidant activity. One of these studies, in the extraction, 80:20 (v/v) ethanol/water mixture was used for 6 g of sample/100 mL solvent at 80 ^{o}C in a thermostated bath. In order to determine the phenolic content Folin Ciocalteu method was used and for the antioxidant activity $ABTS^{+}$ solution was prepared. According to Table 1.1, the highest yield and phenolic content were obtained from hazelnut skin. The yield and phenolic contents were 10.28 and 577.7 mg of CE per gram of extract respectively. The highest antioxidant activity was obtained from hazelnut tree leaf which was found 148 micromoles of Trolox equivaltent per gram of extract. On the other hand, the antioxidant activity of hazelnut kernel was the lower than antioxidant activity of hazelnut byproducts. (Shahidi et al., 2007)

Table 1.1. The yield phenolic content and antioxidant activities of hazelnut kernel and hazelnut byproducts (Source: Shahidi et al., 2007)

Extract	Yield	Phenolic Content	Antioxidant activity
Hazelnut kernel	2.26	13.7	29.0
Hazelnut skin	10.28	577.7	132.0
Hazelnut hard shell	2.53	214.1	120.0
Hazelnut green leafy cover	3.59	127.3	117.0
Hazelnut tree leaf	1.64	134.7	148.0

1.2.3. Heating Source

Non-renewable energy sources (coal, natural gas, fuel and oil etc.) supply a large part of our energy needs. However, the deplementation of these resources has increased the demand for renewable energy sources. For this reason, biomass energy has come to

an important point in renewable energy sources. Biomass energy is used to produce fuel for electricity, chemicals and vehicles (Şen, 2002; Özçimen and Meriçboyu, 2009). Energy is produced by thermochemical destruction of biomass and this energy is used in many industries.

Hazelnut shell is also used as a source of heat in Turkey. %60-70 of the world hazelnut production is being met from Turkey (Koksal, 2000). The contents of the hazelnut shell are %51.6 carbon, %6.2 hydrogen, %40.2 oxygen, %1.6 nitrogen, %0.04 sulfur and %1.4 ash (Demirbaş, 2002). For this reason, heating of the house is provided with a nut shell in Turkey. Moreover, the cost of hazelnut shell is very low compared to other non-renewable energy sources and it is friendly to the environment.

1.3. Description of Extraction

Extraction is a separation process. It is a process of separating one or more components from a mixture of different or the same phase with the extraction agent. Extraction is used in gold, medicine, petroleum, cosmetics, food and many other industries.

There are two main extraction methods based on nature of phases:

- Liquid - liquid extraction
- Solid - liquid extraction (Perry, 1985).

The modern techniques are developed for reducing solvent use and saving time.

- Ultrasonic Extraction
- Supercritical Fluid Extraction
- Pressurized Fluid Extraction
- Microwave Assisted Extraction

1.3.1. Liquid - Liquid Extraction

Liquid - liquid extraction is known as solvent extraction. When a homogeneous liquid mixture or solution contains multiple components and one of these components is desired to be separated from the solution, a suitable solvent is used, usually water and an

organic solvent. The separation in this process called liquid - liquid extraction. A liquid in a solution which is important in liquid-liquid extraction needs to be dissolved in the solvent. This method based on their relative solubilities in two different liquids (Alpay, 2012).

This process consists of a feed stream, a solubilising component and a carrier solvent. The solvent stream dissolves the solubilising component in the feed stream, which stream can also be formed from a pure solvent or mixture. At the end of the extraction, from the process extracted and raffinated streams exit. The extract stream contains the solvent and the solubilising component, while the raffinate stream contains the carrier solvent and solubilizing component (Alpay, 2012).

This process is used in the production of fine organic compounds, nuclear processing, ore processing, processing of perfumes and other industries. Selection of the solvent plays an important role in this process. Selectivity, capacity, miscibility, density, surface tension, viscosity, vapor pressure, chemical and thermal stability, recovery, flammability, corrosivity, toxicity and cost are all important criteria for the selection of the extraction solvent.

1.3.2. Solid - Liquid Extraction

Soluble components in the solid liquid extraction are separated from the solid material using the solvent. In the daily activity, making tea or coffee is an extraction process. Water is used as a solvent and coffee or tea particles are extraction materials. The zest of the coffee or tea is a transition component.

The size of the solid in the extraction is important in terms of extraction rate. Table 1.2 shows that the boiling point and polarities of solvents used in the study. As the surface area of the particle increases, the interaction with the solvent increases, so using smaller size particles can shorten the extraction period. Temperature is also an important parameter for extraction. It should be taken to avoid degradation of the feedstock in spite of increased yields at high temperatures (Mccabe et al., 2001; Wingard and Philips, 1951). The solvent chosen for the extraction processes must dissolve the desired substance. Examples of using solvents in extraction are hexane, chloroform, acetone, methanol, ethanol, petroleum ether, isopropanol, benzene, toluene, etc. (Ramluckan et al., 2014) The polarity of the solvents used in extraction affects the extraction efficiency.

Table 1.2. The boiling points and polarities of the solvents used in extraction (Chen et al., 2009; Ramluckan et al., 2014)

Solvent	Boiling Point (°C)	Polarity Index
Hexane	69.0	0.1
Chloroform	60.5-61.5	4.1
Acetone	56.0	5.1
Methanol	64.7	5.1
Ethanol	78.0	5.2

There are many solid liquid extraction methods. However the main extraction method was soxhlet since the 1890s. Soxhlet extraction is still used in many laboratories. However, in this method, the duration of extraction is very long and the amount of solvent used is high. By modern extraction techniques developed the duration of extraction has been shortened and the amount of solvent usage is reduced. These modern techniques are ultrasonic extraction, supercritical extraction, micro-waved assisted extraction and pressurized liquid extraction (Wan and Wong, 1996; Eskilsson and Bjorklund, 2000).

1.3.2.1. Soxhlet Extraction

Soxhlet extraction was invented in history by Franz Ritter von Soxhlet in 1879 to obtaine milk fat (Soxhlet, 1879). Soxhlet apparatus is used for solid-liquid extraction. Soxhlet extraction generally used to make teas and perfumes in history (Levey, 1959). Soxhlet extraction has many uses in the daytime, although it is designed to separate lipids from solid matter. In the Soxhlet extraction, the solid component must be dissolved in a certain amount in the liquid solvent. Soxhlet extraction is not possible if the solids are not soluble in the solvent. Pure organic solvents or mixtures thereof are used in extraction. The solid components must be thermally stable at the boiling temperature of the solvent.

Figure 1.3 shows the soxhlet extraction unit. The soxhlet consists of a solvent bottle, a medium-flow liquid flow pipe (siphon), a cooled condenser and a heating system. In soxhlet extraction, the solid material is placed in a filter paper and the filter paper is put to the thimble flask. The solvent is poured into the solvent flask according to the volume of a thimble. The solvent flask is placed on the heating system and the system temperature is adjusted according to the boiling point of the solvent. When the

temperature reaches the boiling point of the solvent, the first evaporation occurs. The condenser locates on the thimble flask, which cools the solvent vapors and the solvent starts to condense into thimble flask. When the solvent level in the thimble flask reaches the siphon level, the first cycle is completed and the solid is washed with solvent.

Soxhlet extraction has some attractive advantages. It is constantly in contact with the fresh solvent. Thus, the products extracted from the solid becomes easier to handle. Also it is a low-cost equipment (Luque Garcia and Luque De Castro., 2004; Büyüktuncel, 2009). Besides, in soxhlet extraction method, large amount material can be extracted and no need to use filtration after the process.

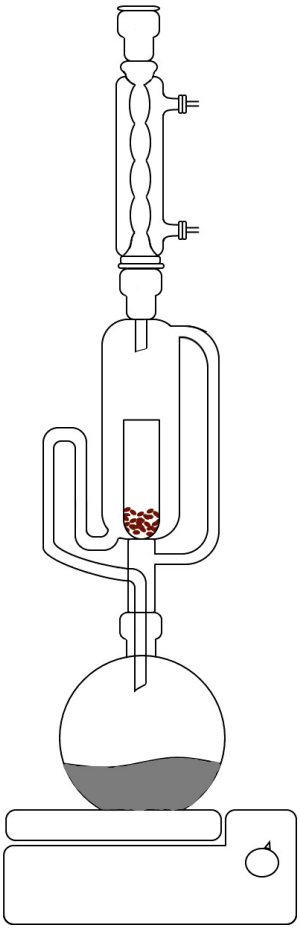


Figure 1.3. The picture of soxhlet extraction unit
(Source: Büyüktuncel, 2012)

Unfortunately, this method has some diasadvatages. A considerable amount of solvent is used in the soxhlet extraction, and the duration of this extraction is long. If some examples of long extraction time are given that 12 h, 22 h and 24 h (Subramanian et al., 2016; Contini et al., 2008). The amount of solvent used generally 200 and 250 ml in this

extraction method (Jadhav et al., 2009; Subramanian et al., 2016). This method has been used for years, despite the loss of time and is harmful to the environment.

Soxhlet extraction is used in the evaluation of waste. It provides isolation of valuable components in the contents of wastes. There are many studies in the literature about soxthlet extraction. In one of these, antioxidant activity and phenolic content of the hazelnut byproducts, which were hazelnut shell waste, skin waste whole roasted hazelnuts and skin waste of chopped hazelnuts using the soxthlet extraction was investigated. The effect of different solvents on extraction was studied. 1:10 (w/v) solid extracted with using 80% ethanol, methanol and acetone solutions. After extraction part, the rotary evaporator was used to remove the solvents at 40 ºC. Table 1.3 indicates the yield and phenolic contents of hazelnut byproducts with different solvent types. The highest yield and phenolic content of extraction were found roasted skin hazelnuts. When the solvents compared in terms of total soluble phenolic content in hazelnut by products, the highest content was sorted as acetone ethanol and methanol. Also there was no difference between methanol and ethanol in terms of yield and phenolic content values.(Contini, 2008)

Table 1.3. The yield and phenolic contents of hazelnut byproducts with different solvent types (Source: Contini et al., 2008)

Sample	Solvents	Yield (g/100g)	Total Soluble Phenolic Content (mg GAE/g)
Hazelnut shell	Methanol	2.7	1.5
	Ethanol	2.7	1.6
	Acetone	2.8	2.1
Roasted skin waste of hazelnut	Methanol	28.9	123.4
	Ethanol	27.8	139.6
	Acetone	32.6	152.2
Chopped skin waste of hazelnut	Methanol	20.8	20.3
	Ethanol	20.0	34.9
	Acetone	23.5	48.5

1.3.2.2. Ultrasonic Extraction

Ultrasonic extraction is based on sound waves. In this method, it is possible to extrude solids or liquids with different vibrations at different frequencies. Small bubbles formed in the liquid medium mechanically shake to solid in a solvent and that causes the bond to break in solid. This is called cavitation (Capelo et al., 2005). The ultrasonic extraction runs generally in an ultrasonic water bath or ultrasonic probe (Santos et al., 2007). An ultrasonic bath is cheaper than the ultrasonic probe. Ultrasonic probes provide more homogenity but are costly and have a short lifespan. However, in solid-liquid extraction ultrasonic probe can be offered because the duration of extraction in an ultrasonic probe is shorter than an ultrasonic bath (Taedo, 2010).

Figure 1.4 demonstrates ultrasonic solid bath. In ultrasonic extraction the solid material and solvent are placed in a flask and this flask is put in the ultrasonic bath. The temperature and frequency of the ultrasonic bath are regulated according to the boiling point of the solvents and extraction occurs.

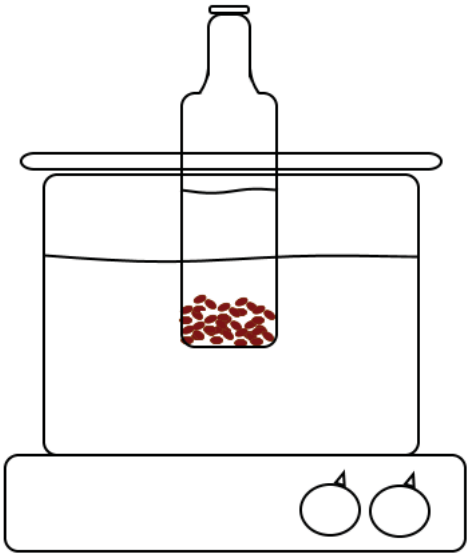


Figure 1.4. The picture of ultrasonic bath
(Source: Büyüktuncel, 2009)

The ultrasonic device, solvent, temperature and frequency are important parameters for increasing the efficiency in the ultrasonic extraction. Besides, the size and amount of solid material are affected by the yield.

Ultrasonic extraction has advantages as well as disadvantages. The advantages of this method; it is a low cost and rapid method. The extraction takes generally 2-20 mins.

In addition, large amount of material can be extracted. However, at the end of the extraction, the substance to be extracted must be filtered and this method also uses a large amount of solvent like soxhlet extraction.

Ultrasonic extraction is used in organic or inorganic extraction in solid or liquid phase. There are some studies in the literature about this. One of these is the extraction of vanillin from the vanillin pods using two different extraction methods which were soxhlet and ultrasound assisted extraction. In the study the type of solvent which was ethanol, methanol, acetonitrile, acetone, chloroform and hexane and the amount of vanillin (1-3 g) were investigated for the soxhlet extraction. The effect of different solvents was researched for the ultrasound assisted extraction method. 2 g vanilla beans were used for 200 mL with the different type of solvents at 95 ^{o}C. In the soxhlet extraction method the vanillin concentration is higher in the extraction of ethanol and methanol than the other solvents of extraction. The concentration of vanillin was 150 ppm in 6 hours ethanol extraction. However, this concentration remains at 50 ppm in the work done with hexane. The most effective solvent in this study was ethanol and then methanol.

The effect of the initial amount of vanilla beans (1-3 g) was investigated with ethanol extraction. The maximum vanillin concentration per unit vanilla beans was detected in 1 g of vanillin beans because 1 g vanillin beans had higher related with the proportion of ethanol than other amounts. At the end of the 8 h soxhlet extraction, the highest amount of vanillin concentration per unit vanilla beans was approximately 85 mL/mg for 1 g vanilla beans.

The parameters of the ultrasound assisted extraction was the different solvents which were ethanol, methanol, acetonitrile, acetone, chloroform and hexane. When the the polarity of the solvent increases, the vanillin concentration increased. The highest solid extracted was obtained in ethanol ultrasound assisted extraction. The vanillin concentration was 110 ppm approximately in ethanol ultrasound extraction. The concentration of vanillin showed a decrease in these solvents: Ethanol > Methanol > Acetone > Acetonitrile > Chloroform (Jadhav et al., 2009).

Ultrasound assisted extraction is more rapid than the soxhlet extraction (Fu et al., 2006). The operating time of soxhlet extraction and ultrasound assisted extraction was 8h and 1h, respectively. When the two types of extraction method were compared, the vanillin concentration of the soxhlet extraction was higher than the ultrasound extraction method in Figure 1.5. However, the ultrasonic extraction can be more profitable in the

short time of the extraction in terms of trade (Hromádková et al., 2008 and Jadhav et al., 2009).

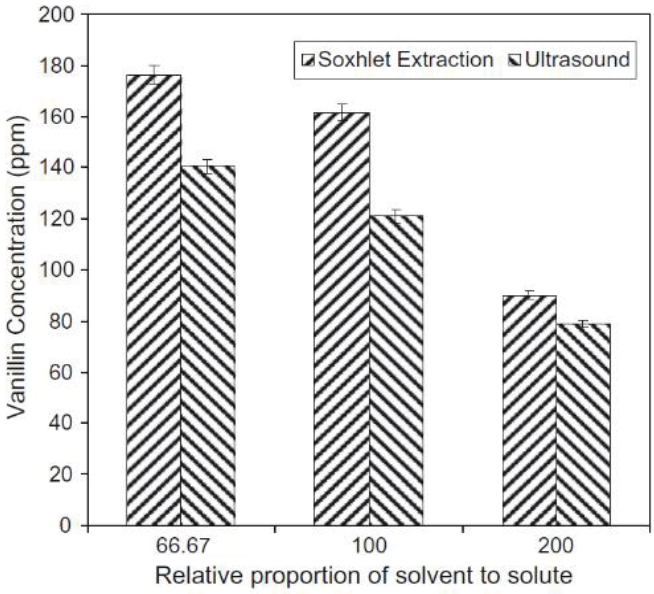


Figure 1.5. To compare soxhlet extraction and ultrasound assisted extraction method (Source: Jadhav et al., 2009)

1.3.2.3. Other Important Solid-Liquid Extraction Methods

Ideally, a separation should be rapid, simple and cheap, should give high recovery ratios without any loss or degradation, and should yield a solution of the analyte that is sufficiently concentrated to perform analytical measurements, and should generate little or no laboratory wastes that have to be disposed of. Therefore, new extraction methods have been developed for reducing solvent use and saving time. These modern methods are supercritical extraction, pressurized liquid extraction and microwave-assited extraction.

1.3.2.3.1. Supercritical Fluid Extraction

Supercritical fluid extraction is a process of separating one component in a solution with using supercritical fluids as a solvent. In supercritical fluid extraction, the

extraction solvent is at the critical point. The solvent is above its own critical temperature and pressure. This substance is neither liquid nor solid. These materials are high density and high solvent power like liquids, but also they have low viscosity, low surface tension and high diffusivity as gases. Dissolution and spreading power are more than liquid. For this reason, extraction is faster than solvent extractions (Zougagh et al., 2004 & Mira et al., 1999). The supercritical fluids are used in dying, foaming and foaming films and extraction cleaning areas.

The typical supercritical liquids are CO_2, H_2O, methane, ethane, propane, methanol, ethanol, acetone, N_2, Ar and NH_3. While SC-CO_2 are non-polar, subcritical H_2O is polar. The most common supercritical fluid is SC-CO_2. It is cheap, it has chemical stability and non- flammability so that it can be used in radioactive applications. Also, it is non-toxic and friendly with environment. The carbon dioxide critical temperature is at 304.1 K and critical pressure is at 7.38 mPa.

The density is an important thermodynamic property in supercritical fluids. The density changes rapidly at around the critical pressure. When the temperature increases, the change of density is less than changing with pressure. It is difficult to control the density close to the critical temperature, and since many effects are correlated with the density, control of experiments and processes can be difficult. Other properties, such as enthalpy also show dramatic changes close to the critical temperature (Sengers and Kiran, 1994).

The advantages of the supercritical extraction; it is more rapid than the other traditional extraction methods. The supercritical fluids are easier to remove from the device. This extraction method is safer than others and the extraction is more efficient to provide power generation. Besides, the method is less polluting and friendly to the environment (Johnston and Penninger, 1988).

In the literature, there are many studies about this method. One of them is the effect of extraction methods on characteristic and composition of Indonesian cashew nut shell liquid. In this study, four different extraction methods were compared. These were soxhlet extraction, supercritical water extraction, supercritical carbon dioxide and two step extraction (soxhlet extraction followed by supercritical water extraction). In soxhlet extraction n-Hexane and methanol were used. The amounts of gum, wax and CNSL obtained with different extraction methods are shown in Table 1.4. According to the results, the amount of CNSL was obtained by soxhlet extraction was higher than that is isolated SCW extraction and SC-CO_2 extraction. It is probably caused by the much longer

extraction time in soxhlet. The amount of CNSL obtained in soxhlet extraction by using methanol is higher than hexane. SCW and soxthlet extraction of CNS yield is 27.31% CNSL and 32.01% respectively (Yulina et al., 2012).

Table 1.4 Gum, wax and CNSL amount in the crude extract obtained by different extraction methods (Source: Yulina et al., 2012) .

	Soxhlet Extraction		**SCW Extraction**	**SC-CO_2 Extraction**	**Two step extraction**	
	Hexane	Methanol	SCW	SC-CO_2	Hexane-SCW	Methanol-SCW
Gum	1.31	3.48	3.27	1.18	1.16	3.48
Wax	8.65	6.5	0.17	1.34	7.75	6.39
CNSL	32.01	38.36	27.31	15.895	58.56	62.48

1.3.2.3.2. Micro-Wave Assisted Extraction

The microwave is high frequency electromagnetic waves. The frequency of the microwave is between 300 and 300000 MHz. Microwave energy is generated by heating the molecules with the conduction of ions and the dipole moment. Thanks to this energy, the solution heats up. The extraction occurs by heating the molecules (Camel, 2001 and Eskilsson and Björklund, 2000). Therefore, solvent selection is very important. This solvent must interact with the substance to be extracted and the solvent can make microwave radiation. Non-polar solvents are not easily heated by this method while polar solvents are more easily heated (Lopez-Avilla, 1999). A closed system is usually used for extraction. The solvent in the system can be heated from the boiling point by applying pressure (Renoe, 1994).

1.3.2.3.3. Pressurized Liquid Extraction

Pressurized liquid extraction is a fairly new and modern method. Solvents can reach higher temperatures using high pressure in this extraction. At this high pressure and temperature, the solvents are in liquid form. Van der Walls and hydrogen bonds involved in extracts are ruptured at high temperatures, thereby increasing extraction yield (Richter

et al., 1996). The surface tension of the extract is decreased at high temperature so that it is more soluble in the solvent.

When the amount of solvent used is reduced in this method, the extraction time is shortened. Due to the high temperature, the kinetics of extraction is also faster. However, at this high pressure and temperature the extract may be deteriorated. For this reason, the most important factors that will affect the efficiency are extraction time, solvent and amount of extract (Bjorklung et al., 1999). In this method, there is no need for filtration.

In the literature, one of the studies, low-lewel polycyclic aromatic hydrocarbons in sediment revealed are extracted by using soxhlet, microwave-assisted extraction and pressurized liquid extraction. In soxhlet extraction, n-hexane/acetone (1:1 volume) dichloromethane, ethyl acetate and dichloromethane/ethyl acetate (1:1 volume) were used as solvent. In soxhlet extraction 150 mL solvents were extracted for 4 h. After choosing of the solvents, Hex/Ace was extracted for 8, 16, 24 h. In microwave assisted extraction three different solvents were used. They were dichloromethane, ethyl acetate and dichloromethane/ethyl acetate (1:1 volume). The extraction run at 150 ^{o}C. Toluene was extraction solvent in pressurized liquid extraction. The pressurized liquid extraction run at 150 ^{o}C under 15 mPa. According to the results, the efficieny was decreased from pressurized liquid extraction, microwave- assisted extraction and soxhlet extraction. It can be said that the pressurized liquid extraction is more efficient than other methods (Itoh et al., 2008).

In another study, the phenolic components antioxidant activity and antimicrobial activity of the pecan nut shell was extracted with using diffrent methods. The using extraction methods used in the study were supercritical extraction, ethanol extraction, infusion and infusion followed by spray drying. Infusion part is followed by using the atomization spray dryer. According to the results, the total phenolic contents, condensed tannis and antioxidant activity data are greater than the only infusion, ethanol extraction and supercritical extraction data. In other words, the infusion by atomization spray extraction was more efficient than the other extraction methods. In infusion followed by the spray dryer, the total pheolic content was 590.78 mg GAE/g and antioxidant activity in Trolox was 4124.83 μmol TEAC/g (Prado et al., 2014).

1.4. Aim and Importance Of The Study

In the study, the main objective was to obtain phenolic compounds from hazelnut shell waste by using various extraction methods. Hazelnut production in Turkey is in first place in the world. Therefore high amount hazelnut shells are produced in our country as a waste. The purpose of this study is to benefit from the content of hazelnut shells. The extracted oil from hazelnut shell contains phenolic and antioxidant components.

Soxhlet extraction, ultrasonic extraction and combined extraction of soxhlet and ultrasonic were preferred for the extraction of hazelnut shell for obtaining the beneficial oil, phenolic content and antioxidant activity. In soxhlet extraction, extraction time (2 cycles (1 cycle = 20 min for hexane, 40 min for ethanol, 45 min for methanol and 35 min for acetone), 3 cycles and 8h), solid-liquid ratio of hazelnut shell and solvent (4 g hazelnut shell/250 ml solvent, 8 g hazelnut shell/250 ml solvent,12 g hazelnut shell/250 ml solvent), size of hazelnut shell (1 mm and 2 mm) and type of extracting solvents (ethanol, methanol, n-hexane, acetone and chloroform) were investigated. In ultrasonic extraction, the solid-liquid ratio (4 g hazelnut shell/250 ml solvent, 8 g hazelnut shell/250 ml solvent) and the type of extracting solvents (ethanol, methanol, n-hexane and acetone) experimented. Besides in combined extraction method, the solid-liquid ratio (4 g hazelnut shell/250 ml solvent, 8 g hazelnut shell/250 ml solvent), size of hazelnut shell (1 mm, 2 mm) and type of extracting solvents (ethanol, methanol and acetone) were studied.

The solid product was investigated with the help of FTIR. The chemical structure and chemical bonds of the shell were analyzed by using of FTIR. Besides, the yield of the extraction was calculated.

The liquid product was examined with the Folin Ciocalteu method, gas chromatography-mass spectrophotometer (GC-MS) and ABTS method. Folin Ciocalteu method provided to understand the total phenolic content. GC-MS was used to find out the content of hazelnut shell oil. The antioxidant activity was calculated by using the ABTS method.

In the literature, there are many extraction methods in different shells and nuts. The phenolic content and antioxidant activity of the hazelnut shell were obtained by the help of different extraction methods have not been studied. For this reason, in this study it was decided to extract hazelnut shell with soxhlet extraction, ultrasonic extraction and soxhlet extraction followed by ultrasonic extraction.

CHAPTER 2

EXPERIMENTAL AND PRODUCT ANALYSIS

2.1. Materials

2.1.1. Chemicals

The raw material of this study is hazelnut shell, which was obtained from Gürsoy, Ordu. Hazelnut shells were ground in different sizes (1 mm and 2 mm) for the extraction experiments. Ethanol (99.5% purity), methanol (99.5% purity), acetone (99.9% purity) and hexane (96% purity), were bought from Merck and chloroform (99% purity) was purchased from Sigma-Aldrich. Folin Ciocalteu reagent (Merck), gallic acid (Merck) and sodium carbonate (Sigma-Aldrich) were used to determine the total phenolic content of the extract. Potassium persulfate was provided from Fluka and $ABTS^{+}$ solution was purchased from Sigma-Aldrich.

2.2. Experimental Apparatus and Procedure

In the study, three different extraction methods were used: Soxhlet extraction, ultrasonic extraction and combined extraction of soxhlet and ultrasonic. For all extraction processes 4 g, 8 g and 12g hazelnut shells were used as raw material.

2.2.1. Soxhlet Extraction

In Soxhlet extraction, the system consists of 250 ml thimble flask, 500 ml solvent flask, a condenser and a heating system (Wisd, DH.WHM 12295).

In this extraction method, the effects of extraction time (2 cycles, 3 cycles and 8h), solid liquid ratio (4 g hazelnut shell/250 ml solvent, 8 g hazelnut shell/250 ml solvent 12 g hazelnut shell/250 ml solvent), size of hazelnut shell (1 mm and 2 mm) and type of extracting solvents (ethanol, methanol, n-hexane, acetone and chloroform) were investigated.

The soxhlet unit used for the soxhlet extraction is given in Fig 2.1. Firstly, the hazelnut shell was weighted by assay balance (ATX224, Shimadzu) and then transferred to the filter paper. This filter paper was placed in the thimble flask in order to prevent the solvent flow. 250 ml extracting solvent was poured into the solvent flask. The temperature of the heating system was regulated according to the boiling point of the solvents. When the solvent started to evaporate at the boiling point, the vapor condensed with the help of the condenser and began to fill the thimble flask. The thimble flask was filled with solvent. When the level of solvent passed to the siphon level, the extracted product and solvent were discharged into the solvent bottle. In this way, one cycle was completed.

Figure 2.1. The picture of soxhlet extraction unit

At the end of the extraction, the extracted oil was separated from the solvent with the help of the rotary evaporator (Laborota 4001, Heidolph) that is given in Figure 2.2. The temperature of the water bath in the rotary evaporator was set at the boiling point of the solvent by keeping the frequency of the rotary at 60 rpm. The vacuum valve was provided to lower pressure in the condenser in order to evacuate solvent more rapidly. The liquid product was heated to the boiling point of the solvent. The evaporated solvent was condensed with the help of condenser and the condensing solvent was collected in

the collecting flask at the other end of the condenser. The oil was obtained and became ready to be analyzed.

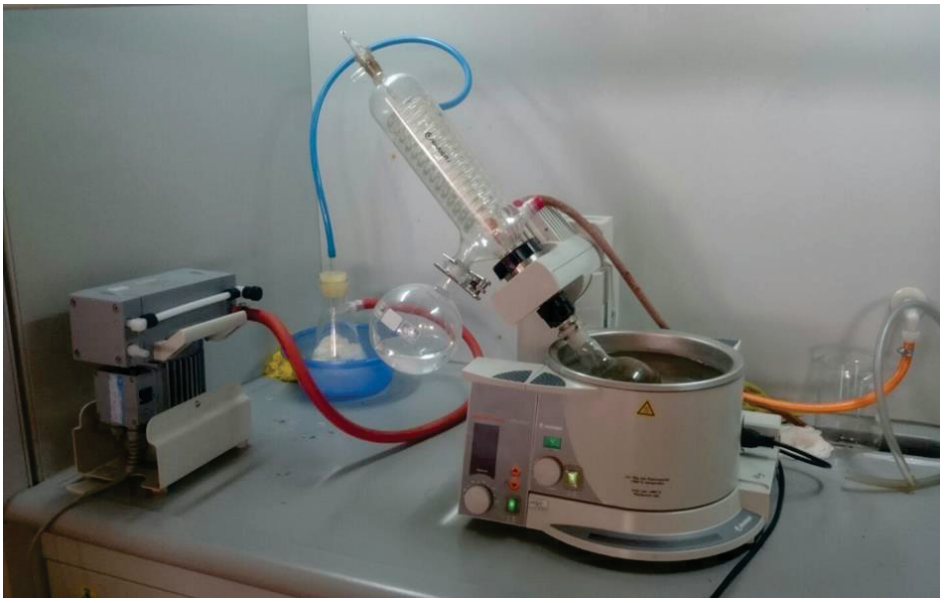


Figure 2.2. The picture of rotary evaporation

The remained solid product was placed in the vacuum oven (Jsr jsvo-60T) at 50 ^{o}C for overnight to remove the remained solvent. After the solid product was dried, it was weighted to calculate the extraction yield from the given equation below:

$$\text{Yield}(\%) = \frac{\text{mass of initial hazelnut shell} - \text{mass of solid residue}}{\text{mass of initial hazelnut shell}} \times 100$$

2.2.2. Ultrasonic Extraction

In ultrasonic extraction, the ultrasonic bath (WUC-D06H, WiseClean) was used. The capacity of the bath was 6 liters and the frequency was up to 40 kHz.

The solid liquid ratio (4 g hazelnut shell /250 ml solvent, 8 g hazelnut shell /250 ml solvent) and the type of extracting solvents (ethanol, methanol, n-hexane and acetone) were the parameters of ultrasonic extraction method.

The ultrasonic bath used for ultrasonic extraction is given in Fig 3.3. Firstly, the hazelnut shells were weighted by assay balance (ATX224, Shimadzu). The weighted hazelnut shells and 250 mL solvent were transferred to 500 mL flask. This flask was placed in the ultrasonic bath and the extraction took around 8 h. The temperature of the

water in the ultrasonic bath was regulated according to the boiling point of the solvents. Besides the frequency of the bath was adjusted 50% (approximately 20 kHz). At the end of the extraction, the hazelnut shells were separated from the solvent by using filter paper. The solid product was placed in the vacuum oven at 50 ^{o}C for overnight to remove remained solvent like soxhlet extraction method. The dried solid product was weighted for the calculation of the extraction yield. The rotary evaporator was used to separate the extracted oil from the solvent.

Figure 2.3. The picture of ultrasonic extraction bath

2.2.3. Combined Extraction

The combined extraction was performed with soxhlet extraction followed by ultrasonic extraction under the same conditions. In this extraction method, the parameters of the process are the solid liquid ratio (4 g hazelnut shell/250 ml solvent and 8 g hazelnut shell/250 ml solvent), size of hazelnut shells (1 mm, 2 mm) and type of extracting solvents (ethanol, methanol and acetone).

Table 2.1. Experimental study of hazelnut shell extraction

Extraction Method	Experiments	Solvent Types	Solid/Liquid Ratio (g/ml)	Extraction Time	Particle Size (mm)
SOXHLET	**HEX 1**	Hexane	4/250	2 cycles	1
	HEX 2	Hexane	4/250	2 cycles	1
	HEX 3	Hexane	4/250	2 cycles	1
	HEX 4	Hexane	4/250	2 cycles	1
	HEX 5	Hexane	4/250	2 cycles	1
	CHL 6	Chloroform	4/250	3 cycles	1
	CHL 7	Chloroform	4/250	2 cycles	1
	HEX 8	Hexane	4/250	3 cycles	1
	EtOH 9	Ethanol	4/250	8 h	1
	EtOH 10	Ethanol	4/250	2 cycles	1
	EtOH 11	Ethanol	4/250	3 cycles	1
	MetOH 12	Methanol	4/250	2 cycles	1
	MetOH 13	Methanol	4/250	3 cycles	1
	Ace 14	Acetone	4/250	2 cycles	1
	Ace 15	Acetone	4/250	3 cycles	1
	MetOH 16	Methanol	4/250	8 h	1
	Ace 17	Acetone	4/250	8 h	1
	EtOH 18	Ethanol	8/250	3 cycles	1
	MetOH 19	Methanol	8/250	8 h	1
	EtOH 20	Ethanol	8/250	2 cycles	1
	MetOH 21	Methanol	8/250	2 cycles	1
	EtOH 22	Ethanol	8/250	8 h	1
	MetOH 23	Methanol	8/250	8 h	1
	EtOH 24	Ethanol	12/250	3 cycles	1
	MetOH 25	Methanol	12/250	3 cycles	1
	Ace 26	Acetone	12/250	3 cycles	1
	MetOH 27	Methanol	12/250	8 h	1
	Ace 28	Acetone	8/250	3 cycles	1
	Ace 29	Acetone	8/250	2 cycles	1
	Ace 30	Acetone	8/250	8 h	1
	Hex 38	Hexane	8/250	8 h	1
ULTRASONIC	**MetOH 31**	Methanol	4/250	8 h	1
	EtOH 32	Ethanol	4/250	8 h	1
	Ace 33	Acetone	4/250	8 h	1
	Hex 34	Hexane	4/250	8 h	1
	MetOH 35	Methanol	4/250	8 h	2
	EtOH 36	Ethanol	4/250	8 h	2
	MetOH 37	Methanol	8/250	8 h	1
	EtOH 39	Ethanol	8/250	8 h	1
	Ace 40	Acetone	8/250	8 h	1
	Ace 42	Acetone	4/250	8 h	2
	Hex 43	Hexane	4/250	8 h	2
COMBINED	**MetOH 44**	Methanol	4/250	8 h & 8 h	1
	EtOH 45	Ethanol	4/250	8 h & 8 h	1

(Cont. on next page)

Table 2.1 (cont.)

Ace 46	Acetone	4/250	8 h & 8h	1
MetOH 47	Methanol	8/250	8 h & 8h	1
MetOH 48	Methanol	8/250	8 h & 8h	2

Firstly, the soxhlet extraction was performed according to the procedure previously described. The soxhlet extraction took 8 h. At the end of the first extraction, the remained solid product was placed in a vacuum oven at 50 °C for overnight to remove the remained solvent. The following day, ultrasonic extraction was applied to the solid product, which was taken from the vacuum oven in the same procedure that was mentioned before. The same solvent used in the soxhlet extraction, was also used in the ultrasonic extraction. After 8 hours of ultrasonic extraction, the hazelnut shells were placed into the vacuum oven under the same conditions again. Then it was weighted to calculate the extraction yield. The extracted oil was obtained with the help of the rotary evaporator. The experimental study of hazelnut shell extraction is shown in Table 2.1.

Briefly, three different extraction methods were studied after the preparation. Extracted oil was obtained by using rotary evaporator. Also, the solid residue was procured from the vacuum oven and they were ready for the analyses. The general diagram of the experimental process of this study is given in Figure 2.4.

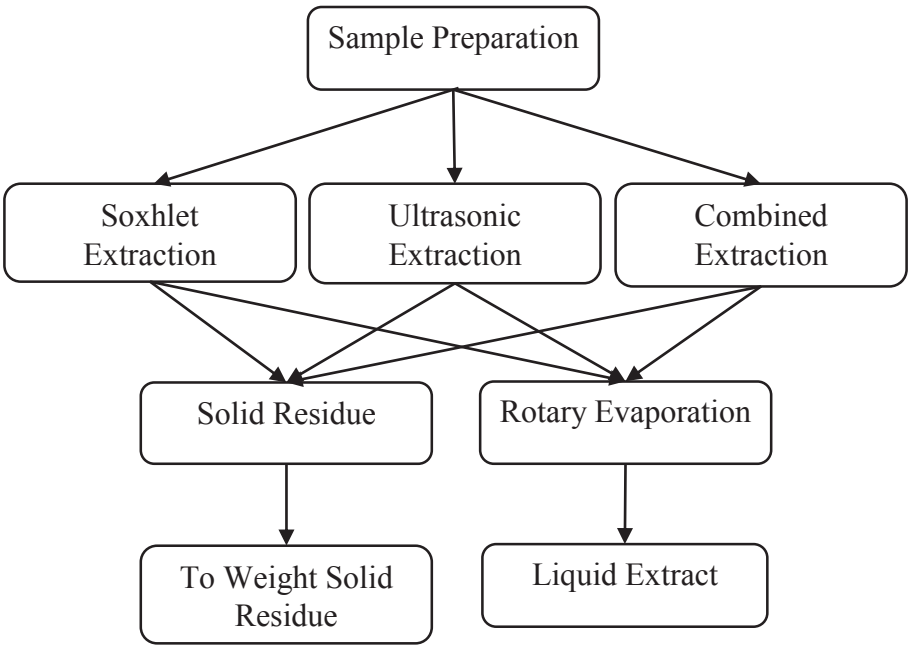


Figure 2.4. The general diagram of an experimental process of this study

2.3. Characterization Method

Solid residue and liquid product were obtained in all three processes. The solid residue was analyzed by Fourier Transform-Infrared Spectroscopy (FTIR). Gas Chromatography equipped with a Mass Spectroscopy (GC-MS) was used for the identification of liquid products. Total phenolic content and antioxidant activity of the final liquid products were also determined. The product analyses were grouped as given in Figure 2.5.

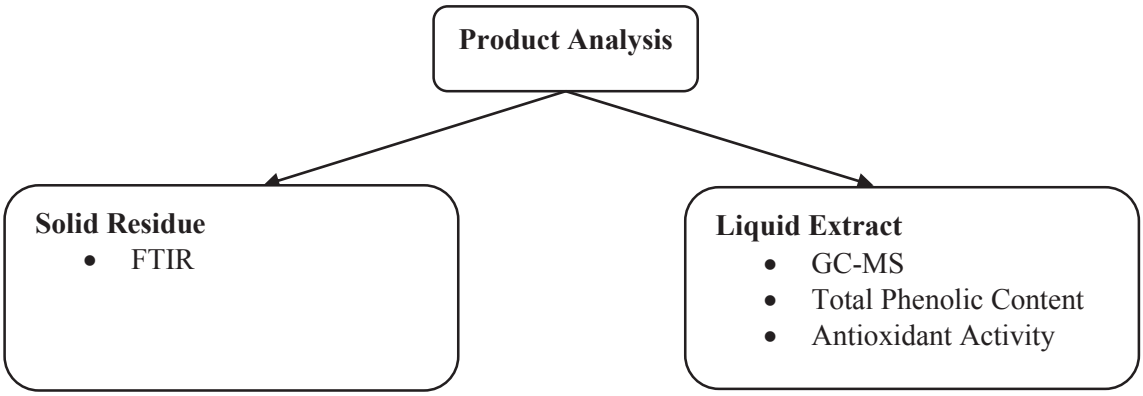


Figure 2.5. The general diagram of an experimental analysis

2.3.1. Solid Product Analysis

The solid product was similarly obtained from the vacuumed oven in all three processes. The solid product was analyzed by FTIR. The yield was calculated according to the mass difference of initial value and the residue, which was formulated before in Eq 1. In addition, the solid residue was kept in the desiccator to avoid moisture adsorption.

In Fourier Transform-Infrared Spectroscopy chemical structure and chemical bonds of the remained hazelnut shells after the extraction process have been analysed. The analysis was performed at wavelengths ranging from 4000 to 400 cm^{-1} by using the instrument of Perkin Elmer Spectra at Biotechnology and Bioengineering Research and Application Center of IZTEC.

2.3.2. Liquid Product Analysis

The liquid product was obtained from rotary evaporation in all methods that were mentioned previously. The liquid product was analyzed by Folin Ciocalteu method for the determination of total phenolic content and by ABTS Method for the evaluation of antioxidant activity, respectively. GC-MS was used for the identification of the products in the liquid solution.

The total phenolic content of the liquid extract was determined by using of Folin Ciocalteu method. For this method, the stock solution was prepared. After that, 50 mg gallic acid was diluted with 100 ml distilled water. The calibration curve was plotted using samples with different concentrations. The total phenolic content was determined by using the equation of the calibration curve that is given in Figure 2.6.

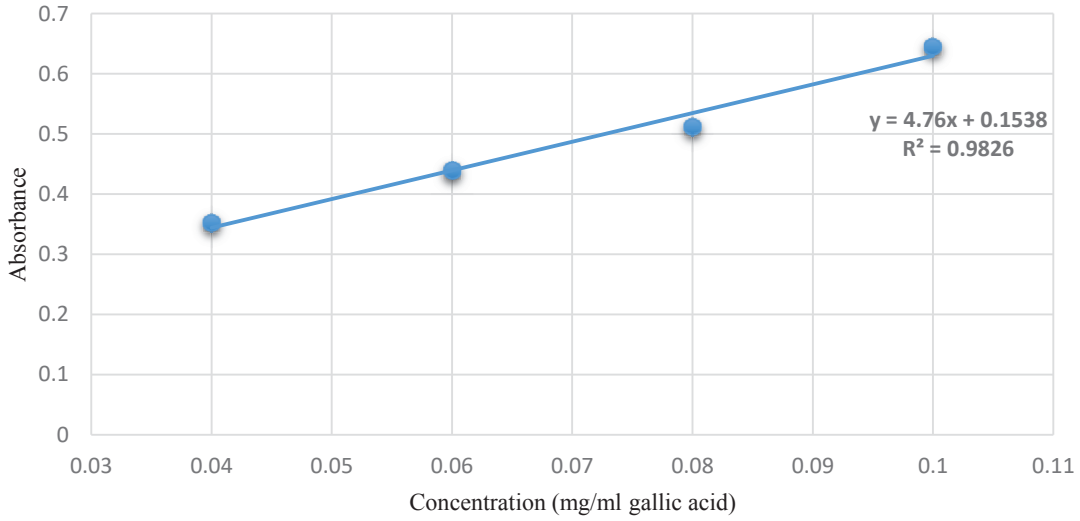


Figure 2.6. The calibration curve for the total phenolic content of the liquid extract

Firstly, 1:9 (v/v) Folin solution was prepared with Folin reagent and distilled water. Secondly, 7.5% (v/v) Na_2CO_3 solution was made with sodium carbonate and distilled water. Subsequently, 0.5 ml Folin solution, 0.5 ml of liquid extract, 1 ml of Na_2CO_3 solution and 8 ml of distilled water were mixed and covered the bottles with the aluminum foil and the mixture was kept in the dark for 45 mins at 25 ^oC. The prepared solution was analyzed by UV at 725 nm by keeping the distilled water as the blank solution.

Antioxidant activity of liquid was evaluated by ABTS method. For this method, 0.014 M $ABTS^+$ solution and 0.0049 mM potassium persulfate solution was prepared

with distilled water. Afterwards, both of them were mixed with the volume ratio of 1:1 and the solution was kept for 16 h in the dark room. The $ABTS^+$ solution was diluted with ethanol (1:50 volume-ratio). 4 ml of $ABTS^+$ solution and 1 ml of the liquid product solution were mixed. The absorbance of the solution was determined with UV analyzer at 734 nm. Distilled water was used as a reference solution. The antioxidant activity was evaluated by the equation of the calibration curve given that in Figure 2.7.

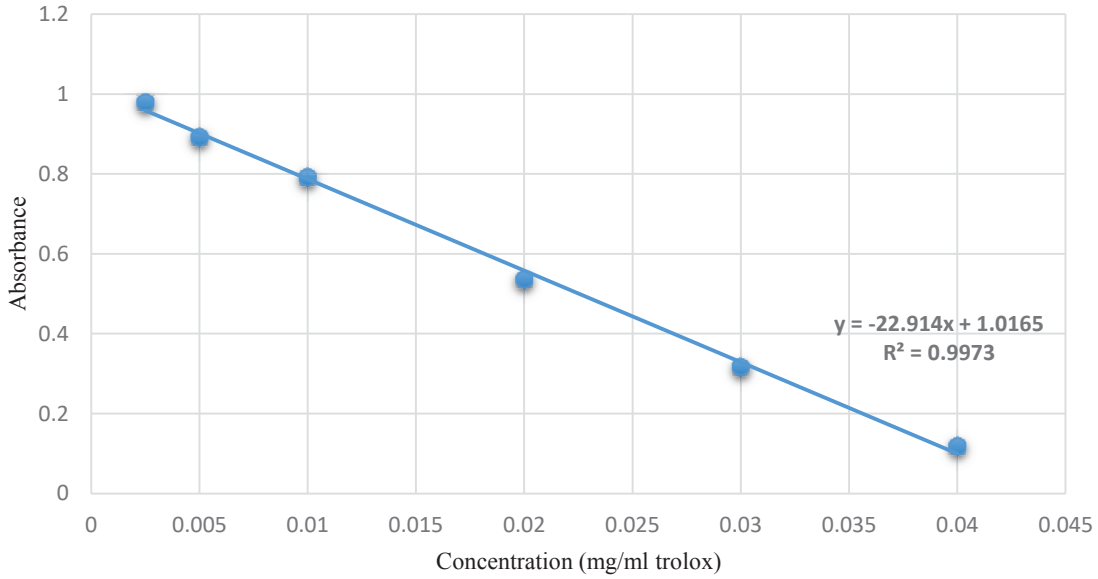


Figure 2.7. The calibration curve for antioxidant activity of the liquid extract

The oil content obtained after extraction was determined using GC-MS (Agilent Technologies 6890 N - 5973 N Network). In the analysis, two capillary columns (Agilent 19091S-433 and Agilent 19091-316) were used. Injection volume was 1.0 microliters. Hellium gas was used as an eluent gas in this method.

CHAPTER 3

RESULTS AND DISCUSSION

In this study, hazelnut shell was extracted by using various extraction solvents (hexane, chloroform, ethanol, methanol and acetone), different extraction time (2 cycle, 3 cycle and 8 h), solid-liquid ratio (4 g hazelnut shell/250 ml solvent, 8 g hazelnut shell/250 ml solvent, 12 g hazelnut shell/250 ml solvent), different extraction methods (soxhlet extraction, ultrasonic extraction and combined extraction) and particle size (1 mm, 2 mm).

3.1. Effect of Extracting Solvent

The extraction solvent type is the most important parameter in this study. The solubilities of these solvents in different natural substances are different due to having polarity difference. Polar substances dissolve better in polar solvents. Similarly, nonpolar substances dissolve better in nonpolar solvents. As the polarity of the solvents increases, the dissolution also increases in natural products (Jadhav et al., 2009). Hexane used in the study has a very low polarity, while the polarity of ethanol and methanol is very high (Chen et al., 2009; Ramluckan et al., 2014).

Figure 3.1. Pictures of hazelnut shell extracts (Amount of hazelnut shell: 4 grams; extraction time: 3 cycles by soxhlet extraction; solvent volume: 250 ml (hexane, chloroform, ethanol, methanol and acetone).

In this study, 4 grams of hazelnut shell were extracted at 3 cycles by soxhlet extraction using hexane, chloroform, ethanol, methanol and acetone. As can be seen in Figure 3.1, the liquid product obtained by extracting hazelnut shell with hexane was quite

lighter than the sample extracted with ethanol. The color of the liquid product obtained from the extraction with acetone and chloroform was not as dark as the extraction performed with methanol and ethanol. However, it was not as light as the extraction with hexane.

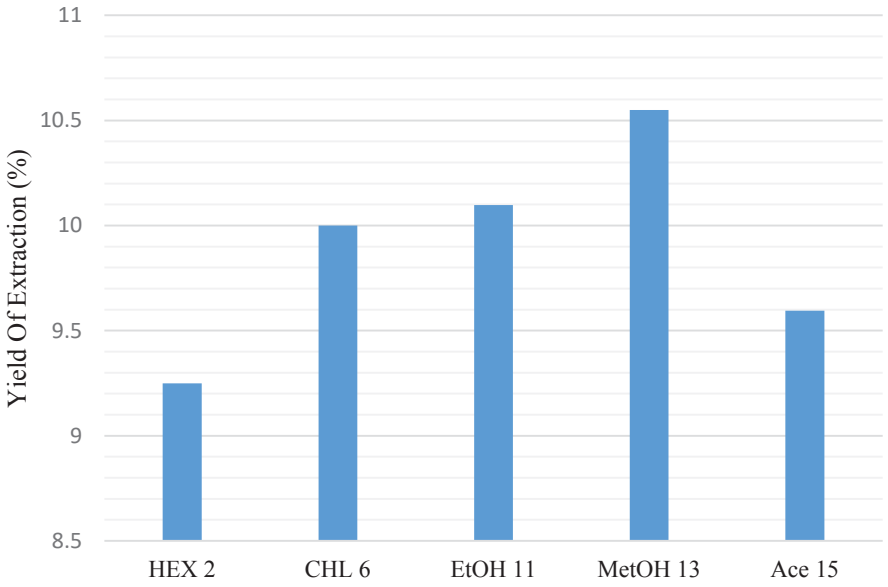


Figure 3.2. Effect of extracting solvent on yield (Amount of hazelnut shell: 4 grams; extraction time: 3 cycles by soxhlet extraction; solvent volume: 250 ml (hexane, chloroform, ethanol, methanol and acetone)

As shown in Figure 3.2, the yield with methanol extraction was 10.55%. However, extraction with hexane as a non-polar solvent, the yield was very low. As the yield with ethanol was found 10.1%, acetone extraction yield decreased to 9.6%. Extraction with ethanol and methanol reached maximum yield and it was suggested as the optimum solvent in this study.

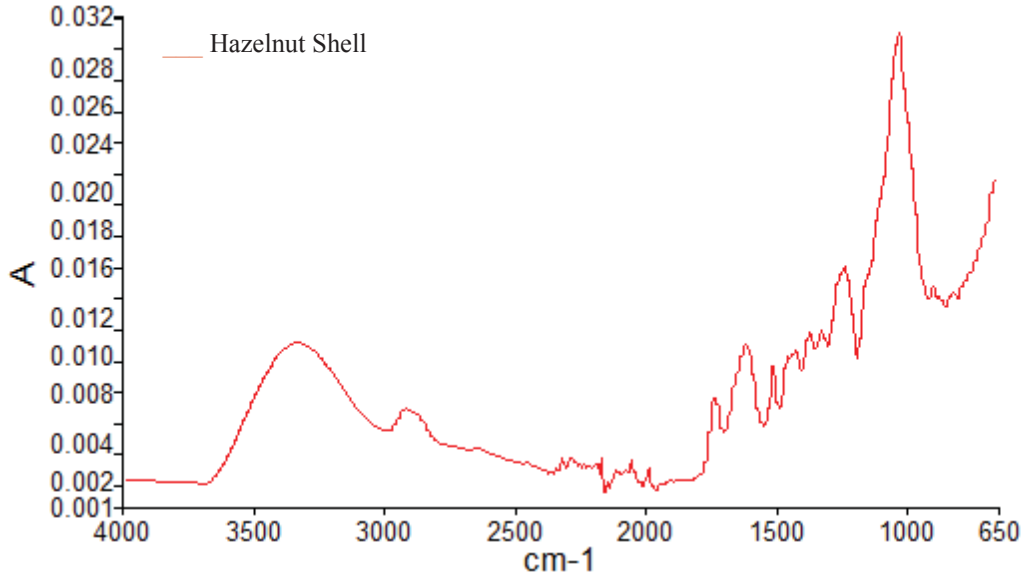


Figure 3.3. FTIR analysis of hazelnut shell which was used in the study

The FTIR analysis of hazelnut shells was given in Figure 3.3. According to this analysis, the structures in the peaks and the bond structures are given in Table 3.1.

Table 3.1. FTIR analysis of the peak banding and structure of using hazelnut shell in the study (Cheng et al. 2009, Pavlovic et al. 2013, Gözaydın, 2016).

Peak	Banding	Structure
1030 cm^{-1}	C-O stretching of alcohols	Cellulose and hemicellulose
1215- 1275 cm^{-1}	Aliphatic C=C stretching	Lignin
1375 cm^{-1}	C-O stretching carboxylic acids	Cellulose and hemicellulose
1600 cm^{-1}	C-C	Lignin
2980 cm^{-1}	C-O stretching esters Aliphatic C-H stretching	Cellulose and hemicellulose
3300 cm^{-1}	O-H bonded	Lignin

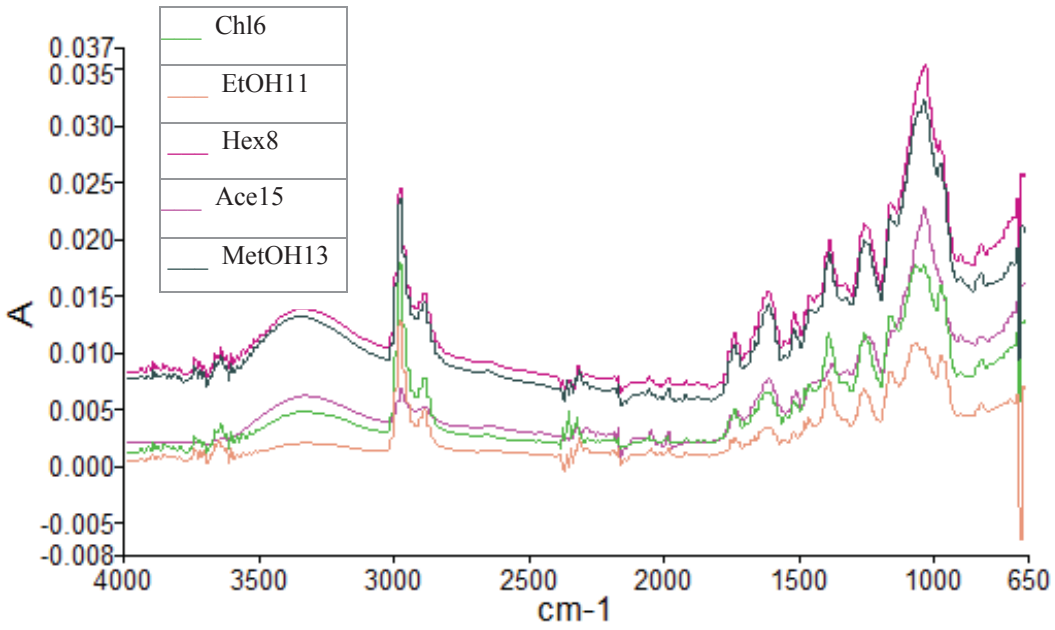


Figure 3.4. FTIR analysis of hazelnut shell with different types extracting solvent (Amount of hazelnut shell: 4 grams; extraction time: 3 cycles by soxhlet extraction; solvent volume: 250 ml (hexane, chloroform, ethanol, methanol and acetone).

The FTIR analysis performed with 4 grams of hazelnut shells which were extracted with different types of solvents (hexane, methanol, ethanol, acetone and chloroform) is depicted in Figure 3.4. This graph shows the structure of O-H bonded lignin at 3300 cm^{-1}. The highest level of lignin in the hazelnut shell was extracted with hexane, whereas the lowest level of lignin was extracted with ethanol. Peak C-O stretching at 2980 cm^{-1} exhibited esters where the cellulosic and hemicellulosic components were found higher in extraction with hexane. The peak at 1600 cm^{-1} indicates the lignin components in the hazelnut shell and accordingly the highest lignin component is found with methanol and hexane extraction. Peaks at 1030 cm^{-1} and 1375 cm^{-1} show C-O stretching of alcohol and carboxylic acid bonds. The cellulosic and hemisellulosic components were found to be higher in the experiment with hexane. According to the results, the high amount of lignin, hemicellulose and cellulosic components which are released as a result of the decomposition of the hazelnut shell, can be ordered as hexane > methanol > acetone > chloroform > ethanol (Cheng et al. 2009, Pavlovic et al. 2013, Gözaydın, 2016).

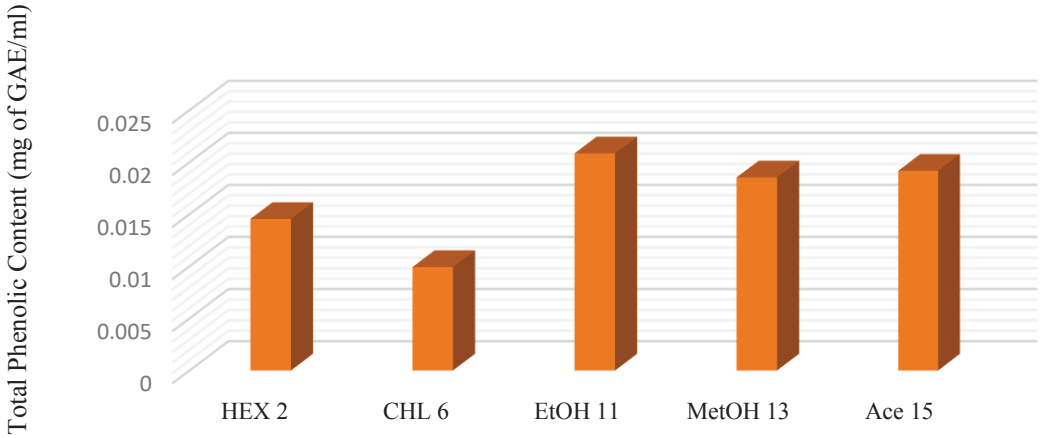


Figure 3.5. Effect of extracting solvent on total phenolic content (Amount of hazelnut shell: 4 grams; extraction time: 3 cycles by soxhlet extraction; solvent volume: 250 ml (hexane, chloroform, ethanol, methanol and acetone)

Shahidi et al. (2007) worked on antioxidant phytochemicals in hazelnut kernel and hazelnut byproducts. Results showed that, gallic acid, caffecic acid, p-courmaric acid, ferulic acid and sinapic acid were detected in phenolic content of hazelnut shell. Besides, the phenolic content of hazelnut shell was found 214.1 mg of CE/g extract defatted samples. Yu et al. (2005) studied to obtain phenolics with extracted peanut skin with using water, 80% (v/v) ethanol and methanol. The maximum total phenolic content was obtained in ethanol extraction.

According to Figure 3.5, the total phenolic content was found 0.015, 0.0010, 0.02, 0.0185 and 0.02 mg GAE/ml for hexane, chloroform, ethanol, methanol and acetone extraction, respectively. It has been determined that the amount of phenolic content obtained from the ethanol extraction is greater than the amount of phenolic content obtained with other solvent extractions.

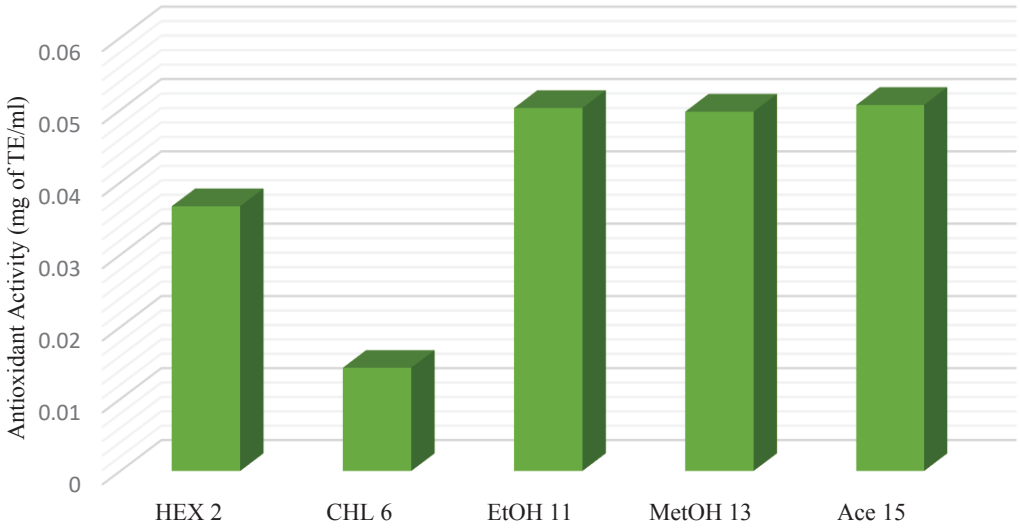


Figure 3.6. Effect of extracting solvent on antioxidant activity (Amount of hazelnut shell: 4 grams; extraction time: 3 cycles by soxhlet extraction; solvent volume: 250 ml (hexane, chloroform, ethanol, methanol and acetone)

According to the Figure 3.6, the antioxidant activities of different types of solvent were 0.037, 0.0144, 0.0503, 0.05 and 0.0507 mg TE/ml for hexane, chloroform, ethanol, methanol and acetone extraction, respectively. The maximum antioxidant activity achieved by ethanol extraction was found to be similar with studies in literature. Shahidi et al. (2007) studied antioxidant acitivity of hazelnut shell extraction with 80:20 (v/v) ethanol/water mixture mixture at 80 ^{o}C. The antioxidant activity of hazelnut shell was found 120 μmol of TE/g of ethanol extract. Also, Alasalvar et al. (2006) indicated that the antioxidant activity obtained with 80% (v/v) ethanol extraction was found to be lower than the antioxidant activity with 80% (v/v) acetone extraction.

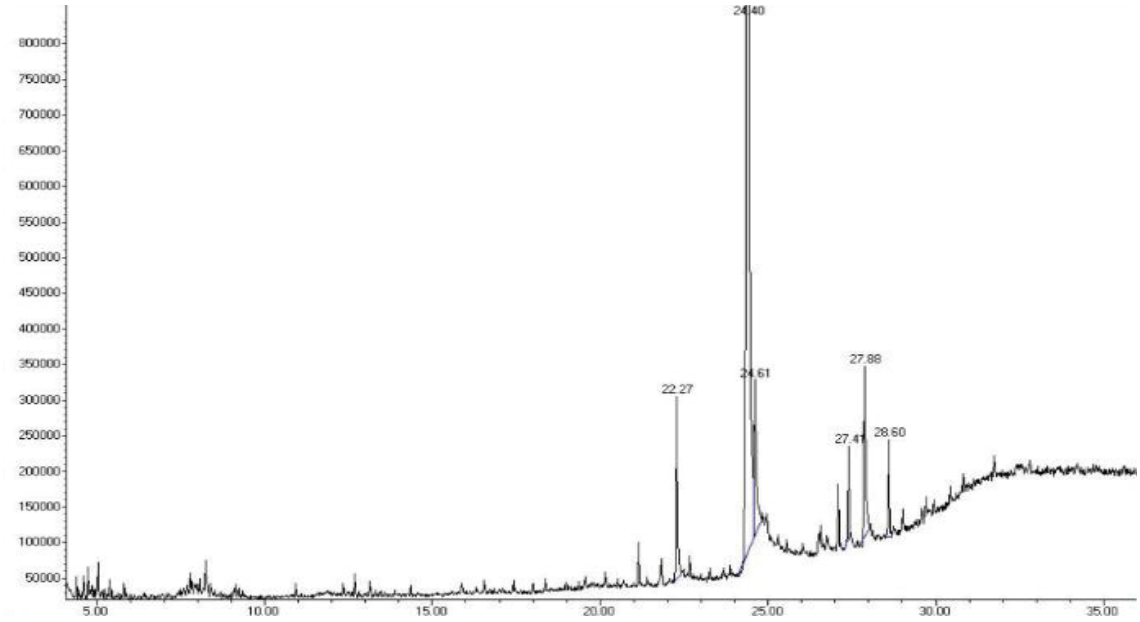


Figure 3.7. GC-MS chromotogram of hazelnut shell extracts (Amount of hazelnut shell: 4 grams; extraction time: 3 cycles by soxhlet extraction; solvent volume: 250 ml hexane)

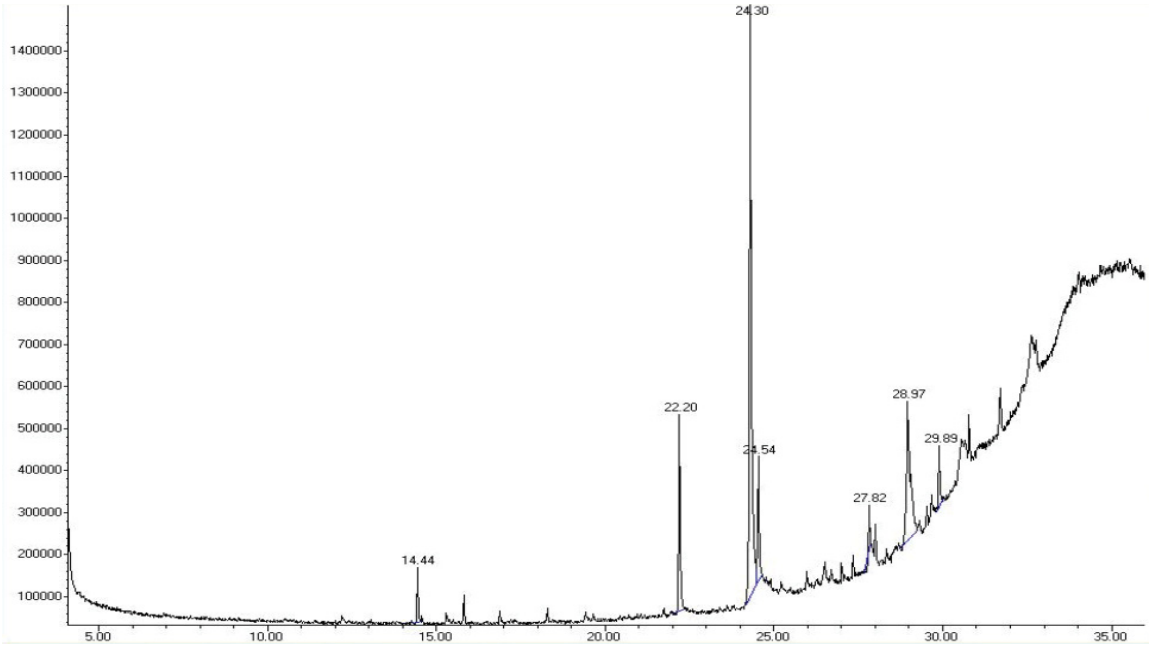


Figure 3.8. GC-MS chromotogram of hazelnut shell extracts (Amount of hazelnut shell: 4 grams; extraction time: 3 cycles by soxhlet extraction; solvent volume: 250 ml chloroform)

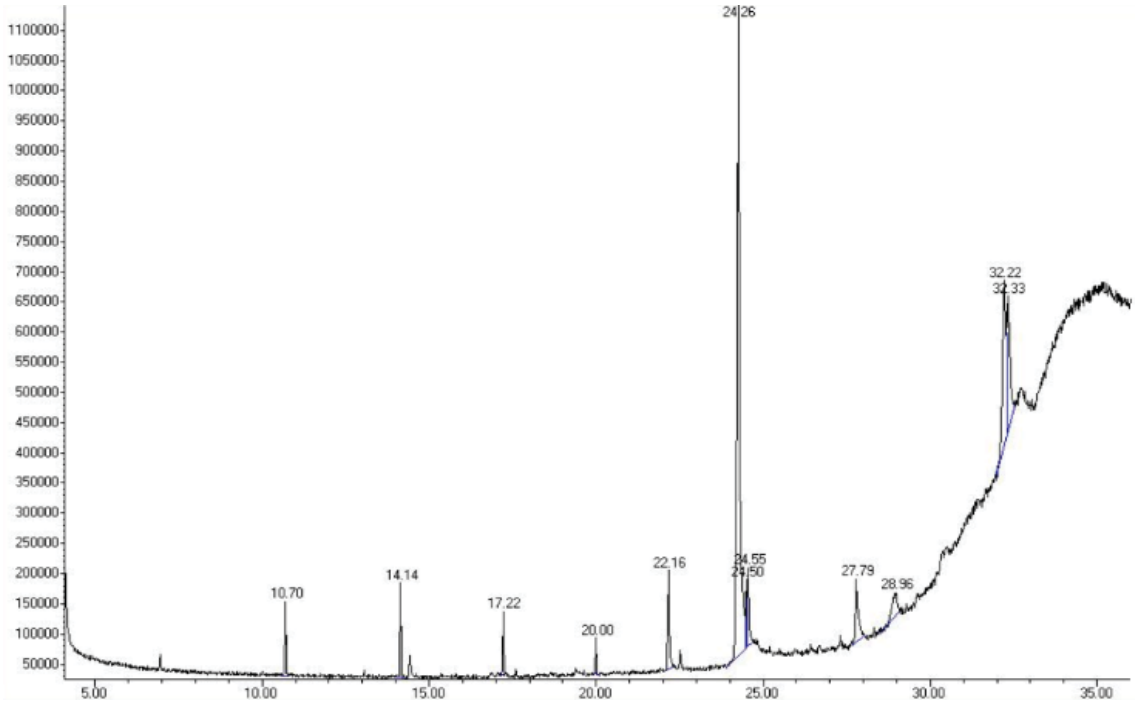


Figure 3.9. GC-MS chromotogram of hazelnut shell extracts (Amount of hazelnut shell: 4 grams; extraction time: 3 cycles by soxhlet extraction; solvent volume: 250 ml ethanol)

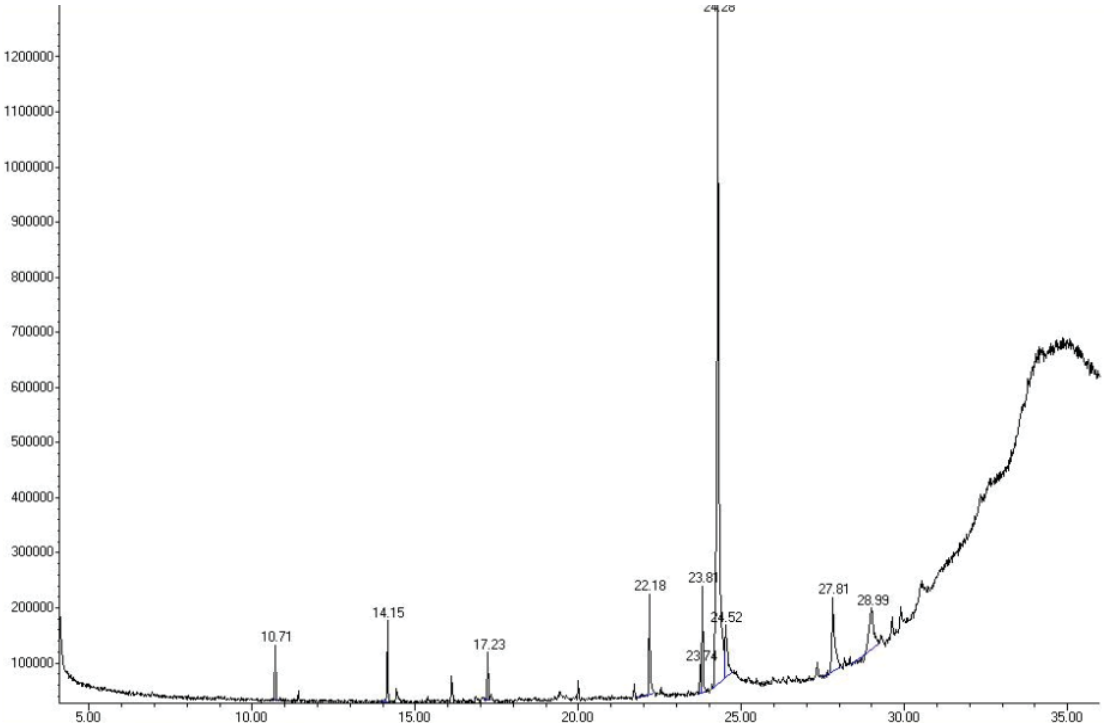


Figure 3.10. GC-MS chromotogram of hazelnut shell extracts (Amount of hazelnut shell: 4 grams; extraction time: 3 cycles by soxhlet extraction; solvent volume: 250 ml methanol)

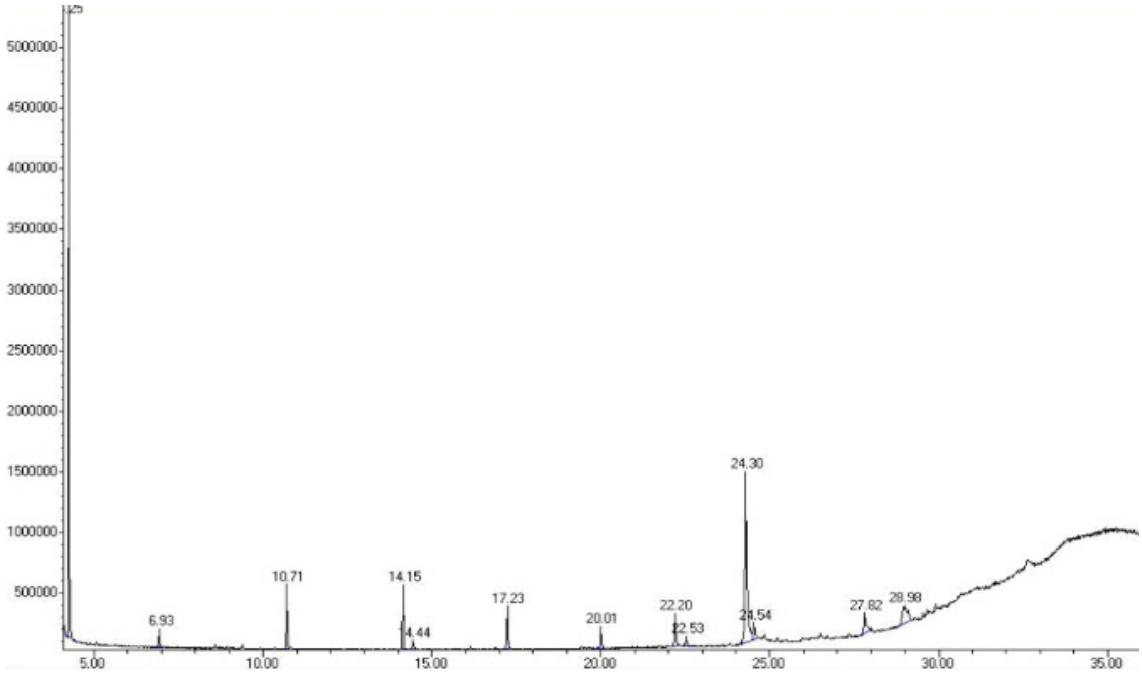


Figure 3.11. GC-MS chromotogram of hazelnut shell extracts (Amount of hazelnut shell: 4 grams; extraction time: 3 cycles by soxhlet extraction; solvent volume: 250 ml acetone)

Demirbaş (2008) studied the soxhlet and supercritical extraction using n-hexane and methanol from hazelnut kernel husk and hazelnut shell for biodiesel production. According to the results of Demirbaş and oil analysis obtained from this study, hazelnut shell consists of palmitic acid, stearic acid, palmioleic acid, oleic acid and linoleic acid. It was detected in Figure 3.7, Figure 3.8, Figure 3.9, Figure 3.10 and Figure 3.11. According to GC-MS results that is given in Table 3.2, palmitic acid, oleic acid and octyl phthalate was determined as a result of 4 g of hazelnut shell with 3 cycles of hexane soxhlet extraction.

Table 3.2. The retention time, area and components of GC-MS chromatograms hazelnut shell extracts (Amount of hazelnut shell: 4 grams; extraction time: 3 cycles by soxhlet extraction; solvent volume: 250 ml (hexane, chloroform, ethanol, methanol and acetone)

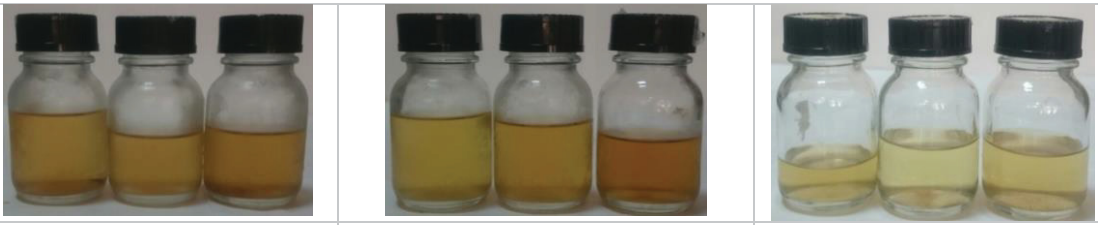
Solvent Types	Retention Time	Area (%)	Component Name	Common Name
Hexane	22.27	5.56	n-Hexadecanoic acid	Palmic acid
	24.40	77.71	9- Octadecenoic acid	Oleic acid
	24.62	6.86	3Octadec-9-enoic acid	Oleic acid
	27.41	2.07	9-Octadecenoic acid	Oleic acid
	28.60	2.26	Bis(2-ethylhexyl) phthalate	Octyl phthalate
Chloroform	14.44	2.80	Vanillin	Vanillin
	22.20	10.99	n-Hexadecanoic acid	Palmitic acid
	24.30	51.20	9-Octadecenoic acid	Oleic acid
	24.54	7.45	Octadecanoic acid	Oleic acid
	29.89	3.78	Heptacosane	Heptacosane
Ethanol	10.70	2.01	1-Dodecene	Alpha-olefin
	14.14	2.62	1-Tetradecene	Acyclic olefin
	17.22	1.69	1-Hexadecene	Cetene
	19.99	1.00	5-Octadecene	Alpha-olefin
	22.16	4.28	n-Hexadecanoic acid	Palmitic acid
	24.26	47.83	9-Octadecenoic acid	Oleic acid
	24.50	2.80	9,17-Octadecadienal	Oleic acid
	24.55	3.32	9-Octadecenoic acid	Oleic acid
	27.79	4.98	9-Octadecenal	Oleic acid
	32.33	11.47	Milbemycin b	Milbemycin b
Methanol	10.71	2.12	1-Dodecene	Alpha olefin
	14.14	3.12	Cyclododecane	Acyclic olefin
	17.23	1.82	1-Hexadecene	Cetene
	22.18	5.50	n-Hexadecanoic acid	Palmitic acid
	23.74	1.22	9,12-Octadecadienoic acid	Oleic acid
	23.80	4.95	10-Octadecenoic acid	Oleic acid
	24.31	60.00	9-Octadecenoic acid	Oleic acid
	24.52	5.18	Octadecanoic acid	Oleic acid
	29.00	7.68	.gamma.-Sitosterol	Clionasterol
Acetone	10.72	4.18	1-Dodecene	Alpha olefin
	14.15	4.50	1-Tetradecene	Acyclic olefin
	14.44	0.94	Vanillin	Vanillin
	17.24	3.04	1-Hexadecene	Cetene
	22.20	3.49	n-Hexadecanoic acid	Palmitic acid
	24.29	25.38	9-Octadecenoic acid	Oleic acid
	24.54	2.17	9-Octadecenoic acid	Oleic acid
	27.82	2.74	9-Octadecenal	Oleic acid
	28.98	6.92	gamma.-Sitosterol	Clionasterol

In the study with liquid product obtained from chloroform extraction according to Table 3.2, vanillin and heptacosane were found differently from hexane. Oleic acid is palmitic acid revealed according to analysis of the liquid product obtained from ethanol soxhlet extraction, as well as alpha olefin, acyclic olefin, retinoid and milbemycin b. The content of the liquid product of methanol extraction is close to the content of liquid product detected in ethanol extraction, only the clionasterol occurred. The liquid product obtained from the acetone extraction, vanillin and cetene unlike the liquid product obtained from methanol and ethanol extraction as to. The highest peak for all types of solvent was obtained between retention times of 24.30 and 24.40. The retention time area at 24.40 was 77.71% in hexane extraction. In chloroform, the retention time area at 24.30 is 51.20%, while the area in ethanol extraction is 47.83%. In the experiment with methanol, the area was 60%, while in acetone this value decreased to 25.38%. As the GC-MS analysis area increases, the proportion of the component formed increases. Results showed that, the highest amount of oleic acid was obtained from hexane and methanol extraction.

3.2. Effect of Extraction Time

In this study, the second important parameter is the extraction time. 4 grams of hazelnut shell was extracted at 2 cycles (1 cycle = 20 min for hexane, 40 min for ethanol, 45 min for methanol and 35 min for acetone), 3 cycles and 8 h by soxhlet extraction using hexane, ethanol, methanol and acetone.

Chan and Choo (2013) studied on different extraction conditions with using cocoa husks. Cocoa husk was extracted substrate-water 1:25 (w/v) at 95 ^{o}C with different extraction time. Extraction time was found as 1.5 hours and 3 hours. Although the yield was higher in the 3 hour extraction, there was not a significant difference compared to 1.5 hour extraction. The colors of liquid products of ethanol, methanol and acetone for 8 hour extractions observed were quite dark compared to 2 cycles and 3 cycles extractions. The darkest liquid product color was observed in the methanol extraction for 8 hours (Figure 3.12).



(a) EtOH10-EtOH11-EtOH9 **(b)** MetOH12-MetOH13-MetOH16 **(c)** Ace14-Ace15-Ace17

Figure 3.12. Pictures of hazelnut shell extracts (Amount of hazelnut shell: 4 grams; extraction time: 2 cycles, 3 cycles and 8 h by soxhlet extraction; solvent volume: 250 ml (ethanol, methanol and acetone))

Figure 3.13 shows the yields of 2 cycle of 3 cycle and 8 h of extraction using hexane methanol ethanol and acetone. The lowest yield was found 7% in hexane extraction for 2 cycle. In the extraction with using ethanol, the yield for 2-cycle experiment is 9.7% while the yield for 3-cycle is 10.09% and the yield for 8-h experiment it is 10%. In methanol extraction, the values of yield were found 9.85%, 10.55%, 10.5% for 2 cycle, 3 cycle and 8 h respectively. In acetone extraction, while the yield for 3 cycle is 9.59%, this value decreased to 9.47% for 8 h. According to the results, the maximum yield was seen in the extraction of 3 cycles. However, there is no significant difference in yield of 8 hour extraction.

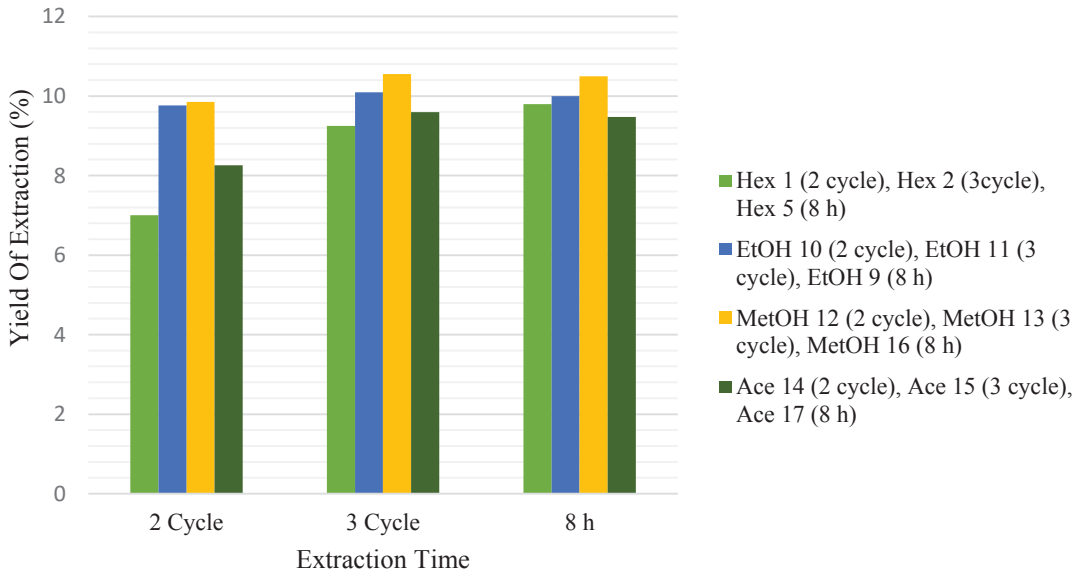


Figure 3.13. Effect of extraction time on yield (Amount of hazelnut shell: 4 grams; extraction time: 2 cycles, 3 cycles and 8 h by soxhlet extraction; solvent volume: 250 ml (hexane, ethanol, methanol and acetone))

Vuong et al. (2013) worked on the effect of extraction time on total phenolic contents and antioxidant activities of papaya leaf. According to this study, total phenolic content increased with increasing extraction time. Figure 3.14 shows that extraction time affected to the total phenolic content during the extraction of hazelnut shell. The total phenolic content values of the 2 cycle and 3 cycle extractions were very close to each other. However, total phenolic contents were found higher for 8 hour extraction. The maximum phenolic content value was observed in the extraction with methanol for 8 hours and it was 0.15 mg of GAE/ml.

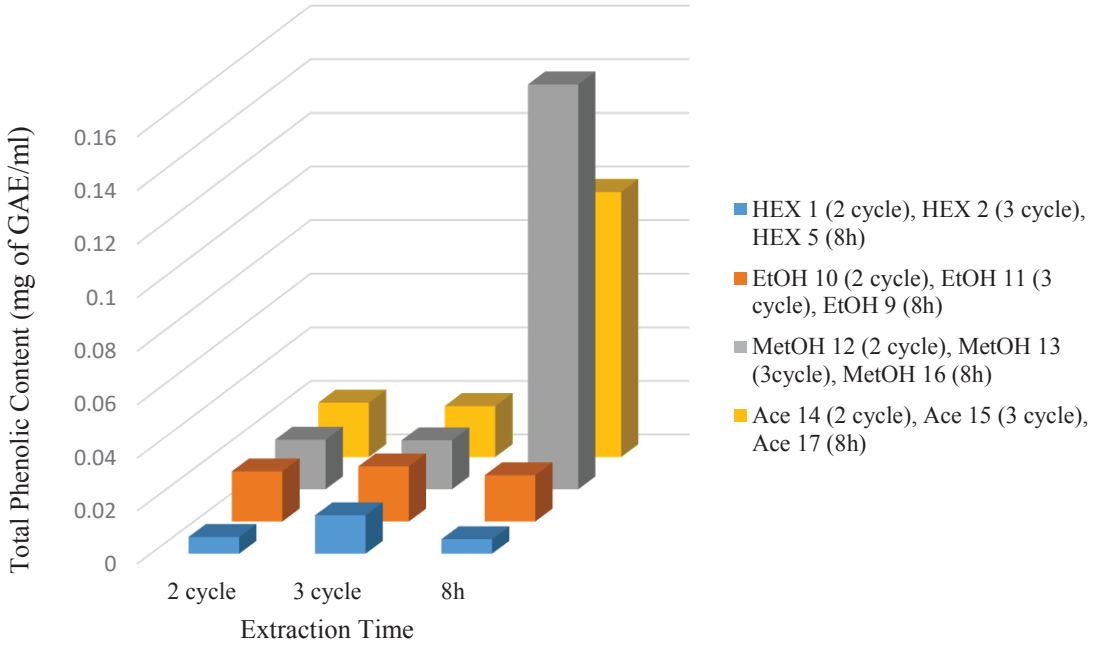


Figure 3.14. Effect of extraction time on total phenolic content (Amount of hazelnut shell: 4 grams; extraction time: 2 cycles, 3 cycles and 8 h by soxhlet extraction; solvent volume: 250 ml (hexane, ethanol, methanol and acetone))

Rusak et al., (2008) studied the effects of green and white tea extract on the antioxidant and phenolic contents were investigated under different extraction conditions. The duration of extraction in this study is 5, 10 and 15 min. The solvents used in the study are water, water + lemon juice, 10% ethanol, 30% ethanol, 70% ethanol. Antioxidant capacity is expected to increase as time increases. However, this study does not show a great change in solvents other than hexane in the antioxidant capacity at different times. The antioxidant activity of ethanol, methanol and acetone has 0.05 mg TE/ml at different extraction times in Figure 3.15. However, antioxidant activity is lower in experiments

with hexane. The antioxidant activity with 2 cycles of hexane is around 0.016 mg TE/ml, whereas with 8h of hexane it is approximately 0.0082 mg TE/ml. In the 3-cycle experiment with hexane, the antioxidant activity was higher than that of the other hexane and 0.036 mg TE/ml.

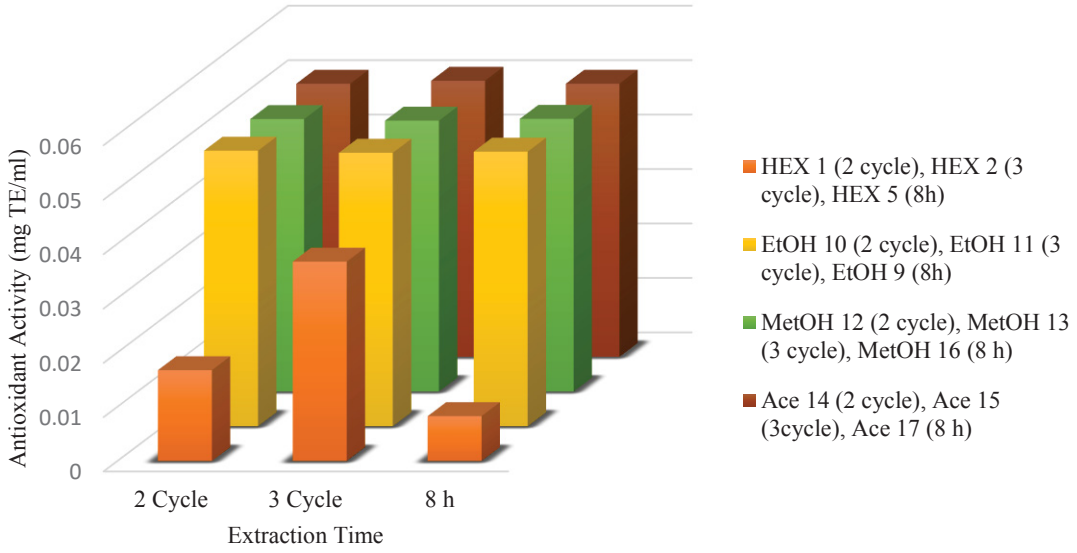


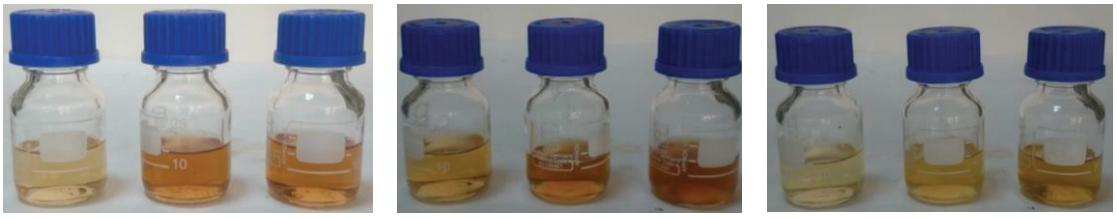
Figure 3.15. Effect of extraction time on antioxidant activity (Amount of hazelnut shell: 4 grams; extraction time: 2 cycles, 3 cycles and 8 h by soxhlet extraction; solvent volume: 250 ml (hexane, ethanol, methanol and acetone))

3.3. Effect of Solid Liquid Ratio

Concerning the effect of different solvent types, ethanol, methanol and acetone yields were higher than the yields obtained by chloroform and hexane, so the experiments were continued using ethanol, methanol and acetone. In this section, 3 cycle soxhlet extraction with different solid liquid ratios (4/250, 8/250 and 12/250 g hazelnut shell/ml solvent) were examined.

Figure 3.16 (a) shows the liquid product obtained from the rotary using 4/250, 8/250 and 12/250 g hazelnut shell/ml of ethanol extraction. Figure 3.16 (b) shows that 4/250, 8/250 and 12/250 g hazelnut shell/ml of methanol extraction. In Figure 3.16 (c), the color of liquid products is given with different solid liquid ratio using acetone as solvent (4/250, 8/250 and 12/250 g hazelnut shell/ml acetone). According to the results, as the solid-liquid ratio increased, the intense color was observed in the liquid product.

Ethanol and methanol liquid product colors were observed dark brown compared to acetone.



(a)EtOH11- EtOH 18- EtOH 24 (b)MetOH13- MetOH 19- MetOH 25 (c)Ace15- Ace28- Ace26

Figure 3.16. Pictures of hazelnut shell extracts (Solid-liquid ratio: 4/250 g hazelnut shell/ml solvent, 8/250 g hazelnut shell/ml solvent, 12/250 g hazelnut shell/ml solvent (ethanol, methanol and acetone); extraction time: 3 cycles by soxhlet extraction)

Jadhav et al., (2009) studied with soxhlet and ultrasonic extraction using different amounts (1g-3g) of vanilla pods. As a result of this study, extraction yield of 1 g was higher than that of 2 g and 3 g. The reason is that, as the amount of the extract decreases, the effect of the solvent on the extract increases. For this reason, the yield of 3 g had the lowest value. While the minimum vanilla concentration was 66.67 ml/g for initial quantity as 3 g, the maximum vanilla concentration was obtained approximately 85 ml/g for initial quantity as 1 g. The results of experiments using hazelnut shells in different solid liquid ratios (4/250, 8/250 and 12/250 g hazelnut shell/ml solvent) were depicted in Figure 3.17 with ethanol, methanol and acetone extraction. In the study, as the solid liquid ratio was reduced, the effect of the solvent on the extract was increased, and the yield is higher in the extraction of 4/250 g hazelnut shell/ml solvent. The yields of 4/250 g hazelnut shell/ml solvent extraction with different types of solvent are the highest, while the yields of the 12/250 g hazelnut shell/ml solvent with different types of solvent extraction were found as the lowest. At 4/250 g hazelnut shell/ml methanol extraction, the yield was found 10.55%. This yield is 9.63% in the extraction with 12/250 g hazelnut shell/ml methanol. The lowest yield was found as 7.62% in the experiment using 12/250 g/ml of solid liquid ratio in acetone extraction and the yield of in acetone extraction using 4/250 g/ml solid liquid ratio was 9.55%.

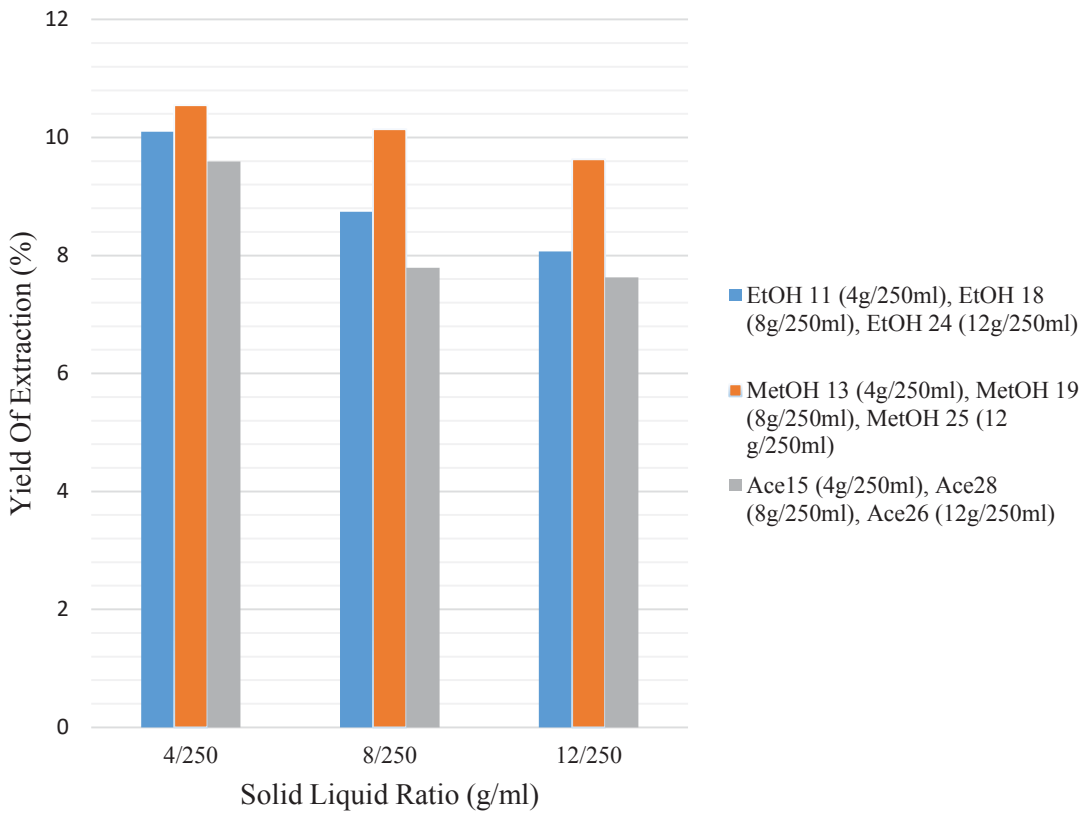


Figure 3.17. Effect of solid liquid ratio on yield (Solid-liquid ratio: 4/250 g hazelnut shell/ml solvent, 8/250 g hazelnut shell/ml solvent, 12/250 g hazelnut shell/ml solvent (ethanol, methanol and acetone); extraction time: 3 cycles by soxhlet extraction)

Figure 3.18 shows the total phenolic content versus 3 cycle soxhlet extraction with 4/250, 8/250 and 12/250 g hazelnut shell/ml solvent. According to the results, the highest total phenolic content value was obtained from 8/250 g hazelnut shell/ ml methanol by 3-cycle. The total phenolic content of this experiment was found 0.1588 mg GAE/ml. The next highest value was obtained by extraction of 8/250 g of hazelnut shell/ml ethanol and this value was 0.1519 mg GAE/ml. The total phenolic values obtained from extraction of ethanol, methanol and acetone from 4/250 g hazelnut shell/ ml solvent were quite low and these values were found as 0.021, 0.018 and 0.019 mg GAE/ml, respectively. In the same way, the values which were obtained from methanol, acetone and ethanol extraction from 12/250 g hazelnut shell/ml solvent, were slightly higher than the results of 4/250 g hazelnut shell/ml solvent, and these results are 0.024, 0.025 and 0.023 mg GAE/ml, respectively.

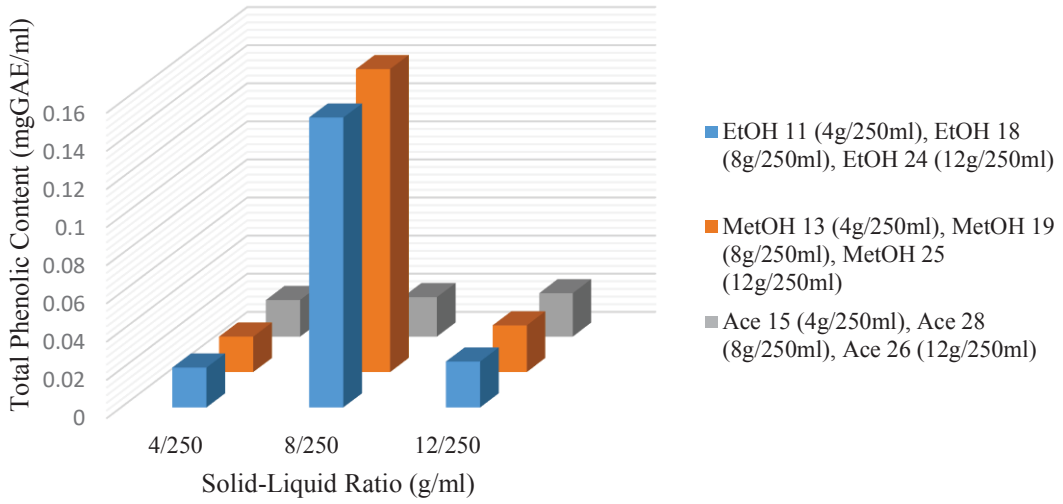


Figure 3.18. Effect of solid liquid ratio on total phenolic content (Solid-liquid ratio: 4/250 g hazelnut shell/ml solvent, 8/250 g hazelnut shell/ml solvent, 12/250 g hazelnut shell/ml solvent (ethanol, methanol and acetone); extraction time: 3 cycles by soxhlet extraction)

In Figure 3.19, antioxidant capacity of different solid liquid ratios of ethanol, methanol and acetone for 3 cycles of extraction were given. According to the results, the highest antioxidant capacity was obtained in extraction of 4/250 g hazelnut shell/ml acetone and this value was found 0.00317 TE. This value decreased with extraction of 8/250 g hazelnut shell/ml acetone and 12/250 g hazelnut shell/ ml acetone for 3 cycle extraction and found 0.00157 TE and 0.00104 TE, respectively. In the methanol extraction, the highest antioxidant capacity was found 4/250 g hazelnut shell/ml methanol and this value was 3.11 TE. Antioxidant capacity was reduced by methanol extraction with 8/250 and 12/250 g hazelnut shell/ml methanol and antioxidant capacity were found 0.00156 TE and 0.00104 TE, respectively. Also, the highest antioxidant capacity was found in extraction of 4/250 g hazelnut shell/ml ethanol. When the solid liquid ratio increased, antioxidant capacity decreased.

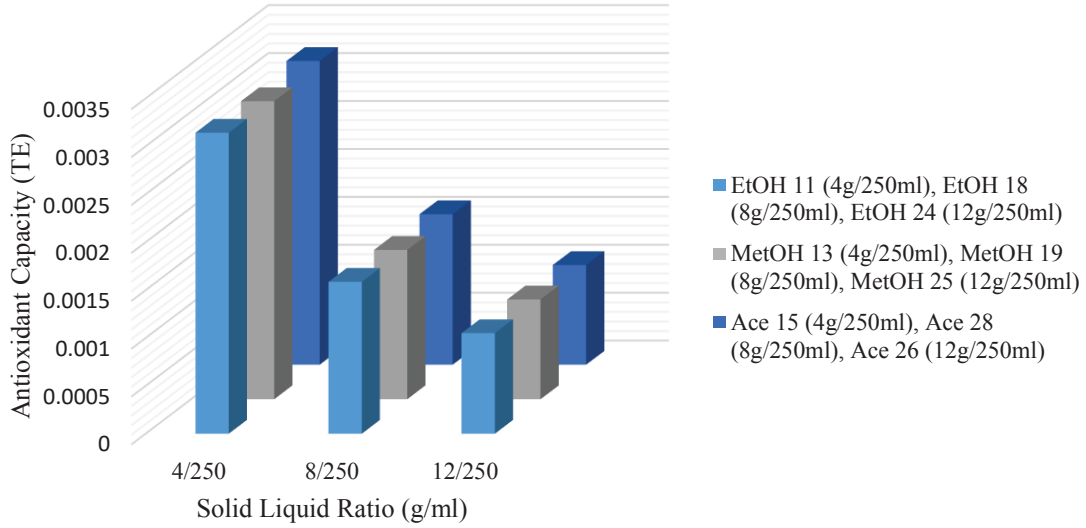


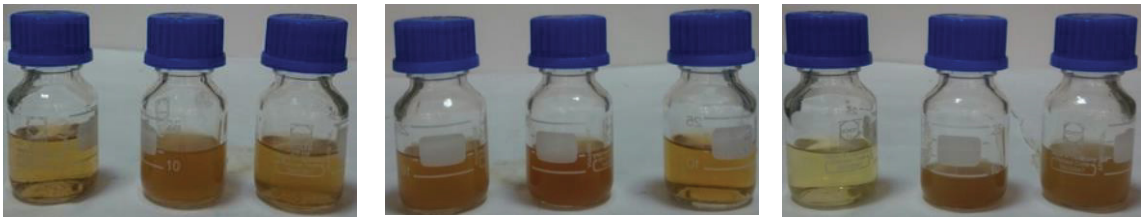
Figure 3.19. Effect of solid liquid ratio on antioxidant capacity (Solid-liquid ratio: 4/250 g hazelnut shell/ml solvent, 8/250 g hazelnut shell/ml solvent, 12/250 g hazelnut shell/ml solvent (ethanol, methanol and acetone); extraction time: 3 cycles by soxhlet extraction)

3.4. Effect of Extraction Methods

In this part of the study, the effect of extraction methods was investigated. 4 grams of hazelnut shells were extracted for 8 hours with 250 ml ethanol methanol and acetone using different methods (soxhlet extraction, ultrasonic extraction and combined extraction which means soxhlet extraction (8 h) followed by ultrasonic extraction (8 h) and the effect of these different extractions was investigated.

Figure 3.20 shows the liquid products obtained as a result of 8 hours soxhlet, ultrasonic and combined extractions using 4 g hazelnut shells with different solvents. Figure 3.20 (a) shows the liquid products obtained by ethanol extraction, and Figure 3.20 (b) and Figure 3.20 (c) shows the products by methanol and acetone extractions, respectively. The first liquid product for each picture was obtained by soxhlet extraction, the second product was obtained by ultrasonic extraction and the third product was obtained by combination of these extraction methods. As mentioned in the effect of the solvent type, the liquid product color was the darkest in the extraction with methanol. The liquid product which was obtained from ethanol extraction was darker in color than the liquid extraction which was obtained from acetone extraction. However, the color of liquid products which were obtained by soxhlet extraction were clear and lighter. The

liquid products which were obtained by ultrasonic extraction were darker in color and liquid products were not clear. Therefore, hazelnut shell was put directly into the solvent at the ultrasonic extraction and the solid products were filtered at the end of the extraction. As a result, liquid products obtained from ultrasonic extraction were blurred. When liquid products obtained from combined extraction were examined, the color of the products was not as dark as the color of the liquid products obtained from the ultrasonic extraction, but it was not as light as the liquid products obtained from the soxhlet extraction. Also, the liquid product color obtained from the combined extraction was clearer than the other extraction methods.



(a)EtOH9- EtOH32-EtOH45 (b)MetOH16-MetOH31-MetOH 44 (c)Ace17-Ace33-Ace46

Figure 3.20. Pictures of hazelnut shell extracts (Amount of hazelnut shell: 4 grams; extraction time: 8 h; extraction method: soxhlet extraction, ultrasonic extraction and combined extraction; solvent volume: 250 ml (ethanol, methanol and acetone))

Jadhav et. al, (2009) compared the ultrasonic extraction and soxhlet extraction with using vanilla pods. In soxhlet extraction 180 ppm vanillin concenration was obtained for 8h at 95 ^{o}C. However, in ultrasonic extraction just for 1 h 140 ppm vanillin concentration was obtained. It can ben understood from these vanillin concentrations ultrasonic extraction is more effective than soxhlet extraction. The cavitation of the ultrasonic extraction increases the diffusion rate of the extruded material. When the diffusion rate increases, mass transfer is getting easier between solvent and extracted material. For this reason, higher yields were obtained in experiments with ultrasonic extraction. Figure 3.21 shows the yields of 4 g hazelnut shell for 8 h extraction with ethanol, methanol and acetone. The yield obtained by ultrasonic extraction was found higher than that of soxhlet extraction in different solvents. The maximum yield was obtained in soxhlet extraction and it is 9.19% in the extraction with ethanol. This yield was up to 12.80% in the ultrasonic extraction with ethanol. Combined extraction consists of 8 hours of soxhlet extraction and 8 hours of ultrasonic extraction. Therefore, the yield

obtained by combined extraction was found higher than ultrasonic extraction. The highest yield in the combined extract was obtained by methanol. The yield of soxhlet extraction with methanol is 7.8%, while ultrasonic extraction increased to 11.19%. The maximum yield is obtained in combined extraction with methanol 15.41%.

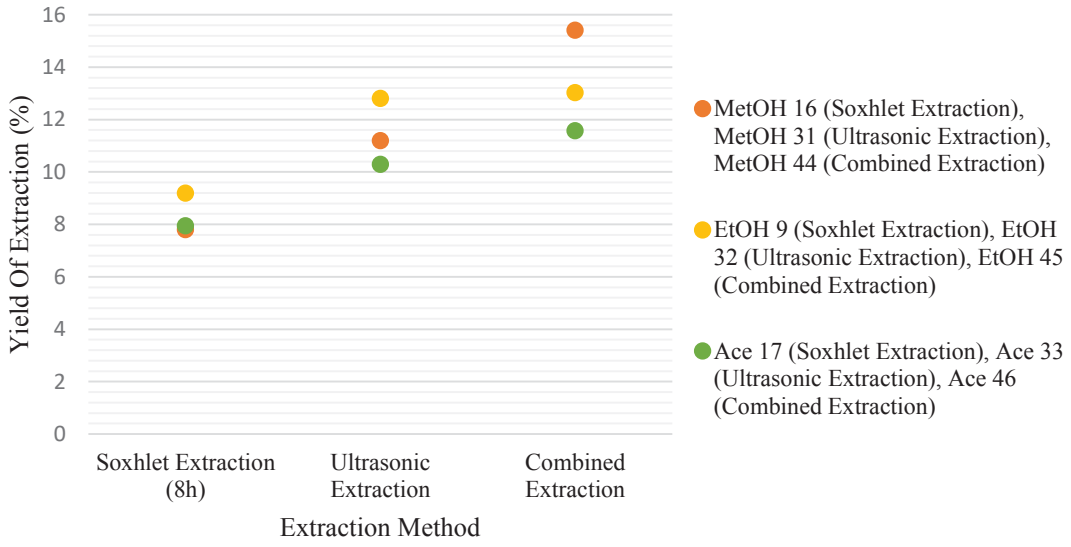


Figure 3.21. Effect of extraction methods on yield (Amount of hazelnut shell: 4 grams; extraction time: 8 h; extraction method: soxhlet extraction, ultrasonic extraction and combined extraction; solvent volume: 250 ml (ethanol, methanol and acetone))

In the ethanol soxhlet extraction using 4 g hazelnut shells, alpha olefin, oleic acid, palmitic acid varieties were determined. In the ultrasonic extraction, in the experiment with ethanol, alpha olefin, acyclic olefin, palmitic acid, oleic acid, eicosane, tetracosane, pentacosane and heptacosane were obtained. The reason for the detection of the different components in the ultrasonic extraction is that increasing diffusion rate resulting from the cavitation helps to obtain the different components by causing higher mass transfer but the area under the peaks in ultrasonic extraction is lower than the other extraction methods. GC-MS chromotogram of 4 g hazelnut shell extracts for 8 h combined ethanol extraction was given in Figure 3.22. In the combined extraction, the area under the peak obtained at 24.48 reached the highest level (90.37%) and oleic acid formation was higher in this method than other methods (Table 3.3).

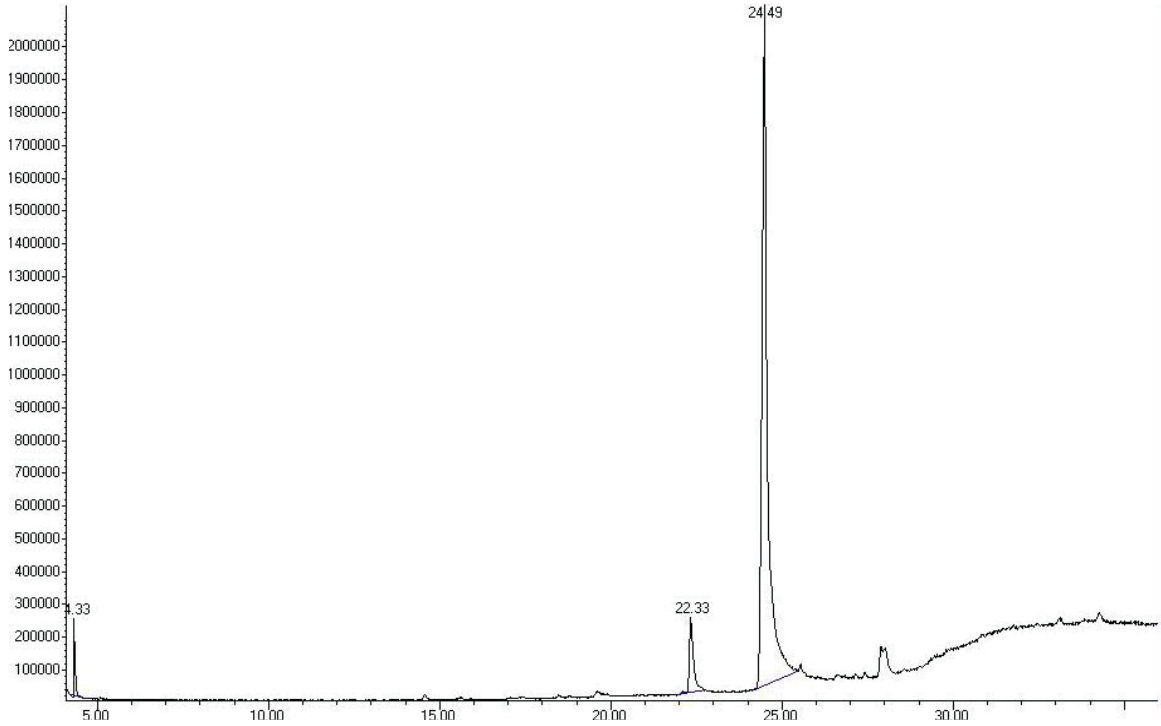


Figure 3.22. GC-MS chromotogram of hazelnut shell extracts (Amount of hazelnut shell: 4 grams; extraction time: 8 h by combined extraction; solvent volume: 250 ml ethanol)

Table 3.3. The retention time, area and components of GC-MS chromotogram of hazelnut shell extracts (Amount of hazelnut shell: 4 grams; extraction time: 8 h by combined extraction; solvent volume: 250 ml ethanol)

Retention Time	**Area (%)**	**Component Name**	**Common Name**
4.33	2.01	2-Pentanone	Methyl propyl ketone
22.34	7.62	n-Hexadecanoic acid	Palmitic acid
24.48	90.37	9-Octadecenoic acid	Oleic acid

Figure 3.23 demonstrastes the total phenolic contents obtained by ethanol methanol and acetone in different extraction types. According to this figure, the lowest total phenolic content is determined in the soxhlet extraction with using ethanol. This value increased in the combined extraction and reached 0.15 mg GAE/ml in the ultrasonic extraction. The highest total phenolic content is found combined extraction with using methanol this phenolic content is 0.16 mg GAE/ml. In these different types of extractions with using methanol, the total phenolic content did not show any significant change and were found to be 0.15 mg GAE/ml in soxhlet extraction and 0.14 mg GAE/ml in ultrasonic extraction. In the ultrasonic extraction with acetone, the total phenolic content reached the highest value and this value close to 0.17 mg GAE/ml. This value has

decreased to 0.10 mg GAE/ml in combined extraction and 0.09 mg GAE/ml in soxhlet extraction. It is understood from this in the experiments done by ultrasonic extraction, the total phenolic content was found to be higher than the total phenolic content obtained by combined extraction. The lowest total phenolic contents are obtained in the soxhlet extract.

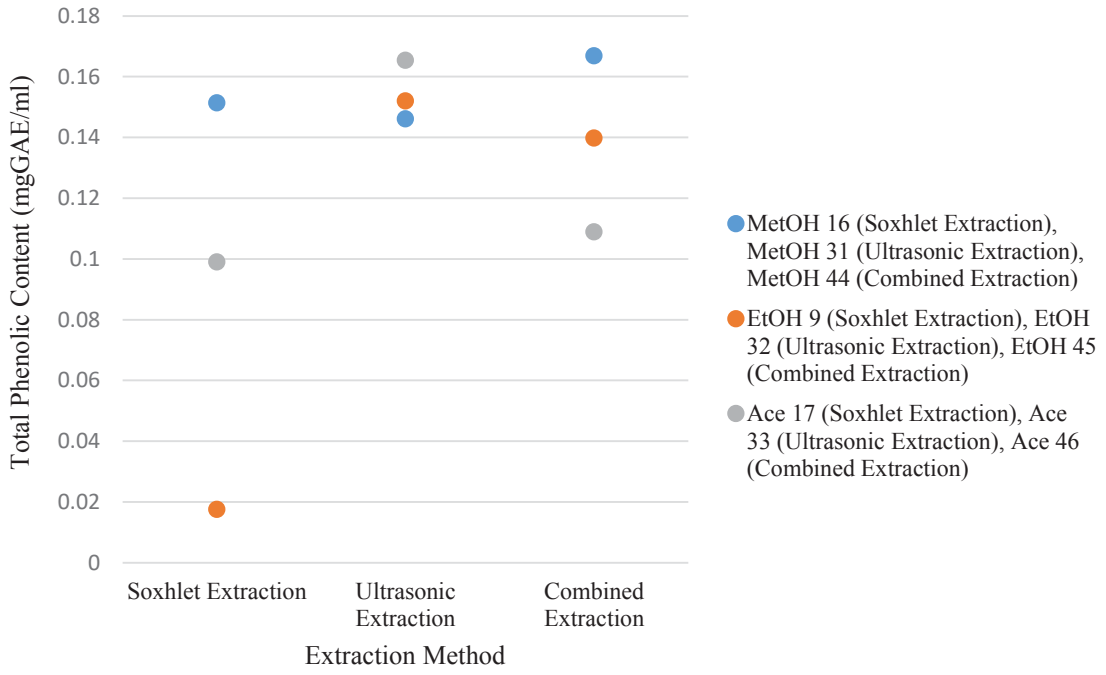


Figure 3.23. Effect of extraction methods on total phenolic content (Amount of hazelnut shell: 4 grams; extraction time: 8 h; extraction method: soxhlet extraction, ultrasonic extraction and combined extraction; solvent volume: 250 ml (ethanol, methanol and acetone))

Figure 3.24 shows the antioxidant activity of experiments using different extraction methods with methanol ethanol and acetone. Antioxidant activities were very close in the three different extraction methods with methanol. Antioxidant activities with using methanol, soxhlet, ultrasonic and combined extraction were found to be 0.0501 mg TE/ml, 0.0504 mg TE/ml and 0.046 mg TE/ml, respectively. The highest antioxidant activity is in ultrasonic extraction with methanol. When antioxidant activities of soxhlet, ultrasonic and combined extraction with using ethanol were investigated, the results were found to be 0.0505 mg TE/ml, 0.0475 mg TE/ml and 0.0462 mg TE/ml respectively. The highest level of antioxidant activity is in soxhlet extraction with using ethanol. Antioxidant activities of aceton extraction were 0.0501 mg TE/ml in soxhlet extraction, 0.035 mg TE/ml in ultrasonic extraction and 0.0429 mg TE/ml in combined extraction.

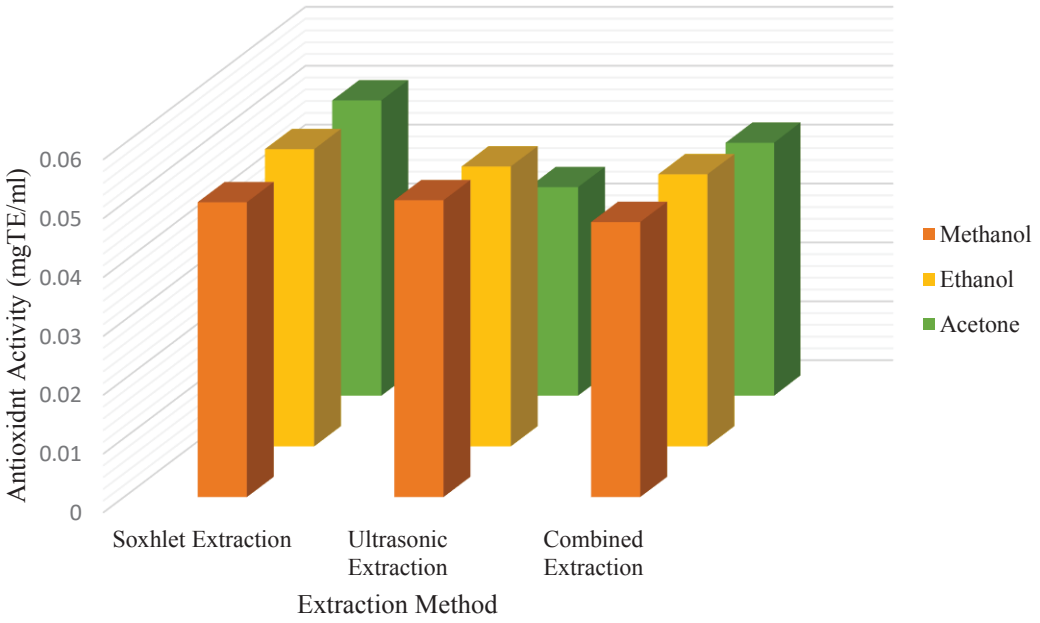


Figure 3.24. Effect of extraction methods on antioxidant activity (Amount of hazelnut shell: 4 grams; extraction time: 8 h; extraction method: soxhlet extraction, ultrasonic extraction and combined extraction; solvent volume: 250 ml (ethanol, methanol and acetone))

3.5. Effect Of Particle Size

In this part of the study, 4 g hazelnut shell with a different particle size (1mm, 2mm) was extracted with using 250 ml of solvent (ethanol, methanol, acetone and hexane) for 8 hours using an ultrasonic bath.

In Figure 25, ultrasonic extraction of 4 g hazelnut shells at different sizes of 1 mm and 2 mm using ethanol, methanol and acetone were given respectively. The first liquid product in each picture shows the extraction of a 2 mm hazelnut shell and the second image shows the extraction of a hazelnut shell of 1 mm. Accordingly, the liquid product color obtained as a result of ultrasonic extraction of 2 mm hazelnut shell is darker.

(a)EtOH36-EtOH32 (b)MetOH35-MetOH31 (c)Ace42-Ace33

Figure 3.25. Pictures of hazelnut shell extracts (Amount of hazelnut shell: 4 grams; extraction time: 8 h by ultrasonic extraction; particle size: 1mm and 2 mm; solvent volume: 250 ml (ethanol, methanol and acetone))

Coats and Wingard (1950) investigated the effect of particle size with using soybeans. In this study using soybean and the amount of residual oil were examined by increasing particle size. According to the results as the particle size increases, the amount of residue oil obtained increases. %0.85/dry basis residue oil was obtained in the extraction using 0.0085 inc soybean for 5 min extraction and %2.48/dry basis oil was obtained in the experiment using 0.0135 inc soybean for 5 min extraction. Table 3.4 shows the yields of ultrasonic extraction from 1 mm and 2 mm hazelnut shells using different solvents (methanol, ethanol, acetone and hexane) for 8 hours. According to these results, the yield obtained from the extraction of the 2 mm hazelnut shell is higher than the yield obtained from the extraction of the 1 mm hazelnut shell. The highest yield was obtained from methanol extraction of 2 mm hazelnut shell and this value was 13.76%.

Table 3.4. Effect of particle size on yield (Amount of hazelnut shell: 4 grams; extraction time: 8 h by ultrasonic extraction; particle size: 1mm and 2 mm; solvent volume: 250 ml (ethanol, methanol and acetone))

	Yield Of Extraction (%)		
Solvent Types	**Methanol**	**Ethanol**	**Acetone**
1 mm	11.19	12.80	10.28
2 mm	13.76	12.85	11.97

Figure 3.26 demonstrates total phenolic contents obtained from ultrasonic extraction of hexane, ethanol, acetone and methanol of 4 g hazelnut shell with different particle size (1 mm, 2 mm) are given. According to the graph, the total phenolic content

of 1 mm hazelnut shell is higher than the total phenolic content of 2 mm hazelnut shell. While the phenolic content obtained from the 1 mm hazelnut shell was 0.022 mg GAE/ml, the total phenolic content obtained from the hazelnut shell of 2 mm was 0.016 mg GAE/ml. The phenolic content obtained in the experiment with the hexane is lower than the phenolic content value obtained from other solvents because hexane is a non-polar solvent and the strength of dissolving hazelnut shell is low. In the extraction with acetone, the total phenolic content of the 1 mm hazelnut shell was 0.163 mg GAE/ml and the highest phenolic content was obtained here. For acetone extraction with 2 mm hazelnut shell, this value decreased to 0.13 mg GAE/ml. In the ultrasonic extraction with ethanol, the phenolic content obtained from the 1 mm hazelnut shell was 0.15 mg GAE/ml while the phenolic content obtained from the 2 mm hazelnut shell was 0.10 mg GAE/ml. It is clear from the graph that the total phenolic content value obtained from the 1 mm hazelnut shell is higher than the total phenolic content 2 mm hazelnut shell. The reason of this is that, as the surface area gets smaller, the diffusion rate increases.

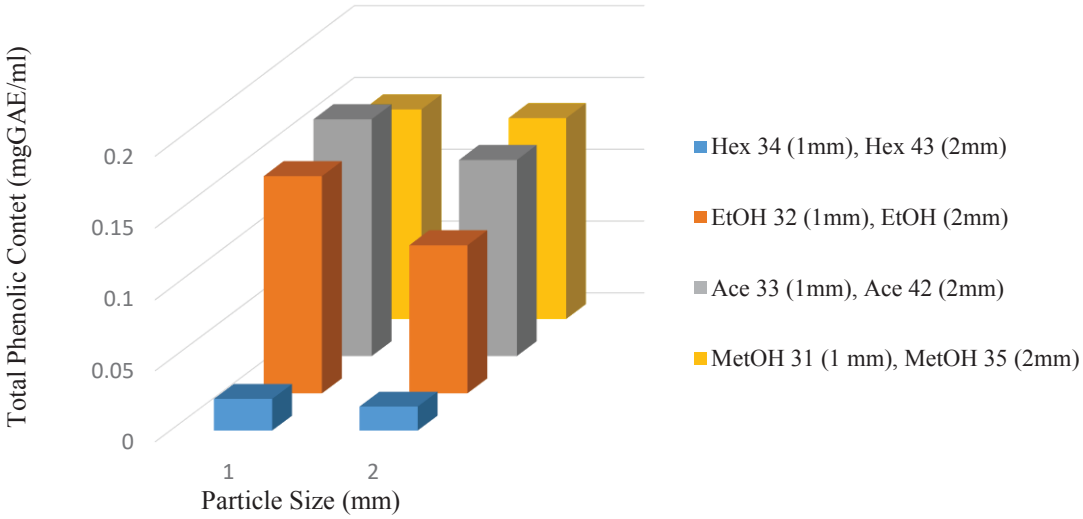


Figure 3.26. Effect of particle size on total phenolic content (Amount of hazelnut shell:4 grams; extraction time: 8 h by ultrasonic extraction; particle size: 1mm and 2 mm; solvent volume: 250 ml (ethanol, methanol and acetone))

The antioxidant activities of 1 mm and 2 mm hazelnut shells obtained from ultrasonic extraction of ethanol, methanol, acetone and hexane for 8 hours were shown in Figure 3.27. The antioxidant activity obtained from 1 mm hazelnut shell by hexane extraction was 0.040 mg TE/ml while the antioxidant activity obtained from hexane extraction from 2 mm hazelnut shell was 0.048 mg TE/ml. The antioxidant activity obtained from extraction of 1 mm and 2 mm hazelnut shell by ethanol is very close to

each other. In the extraction with methanol, the antioxidant activity obtained from the 1 mm hazelnut shell is 0.050 mg TE/ml and the antioxidant activity from the 2 mm hazelnut shell is 0.051 mg TE/ml. In the extraction with acetone, the antioxidant activity was significantly lower than the antioxidant activities of other solvents. As consequences, it can be said that the antioxidant activity obtained from the 2 mm hazelnut shell was higher than the one with 1 mm hazelnut shell.

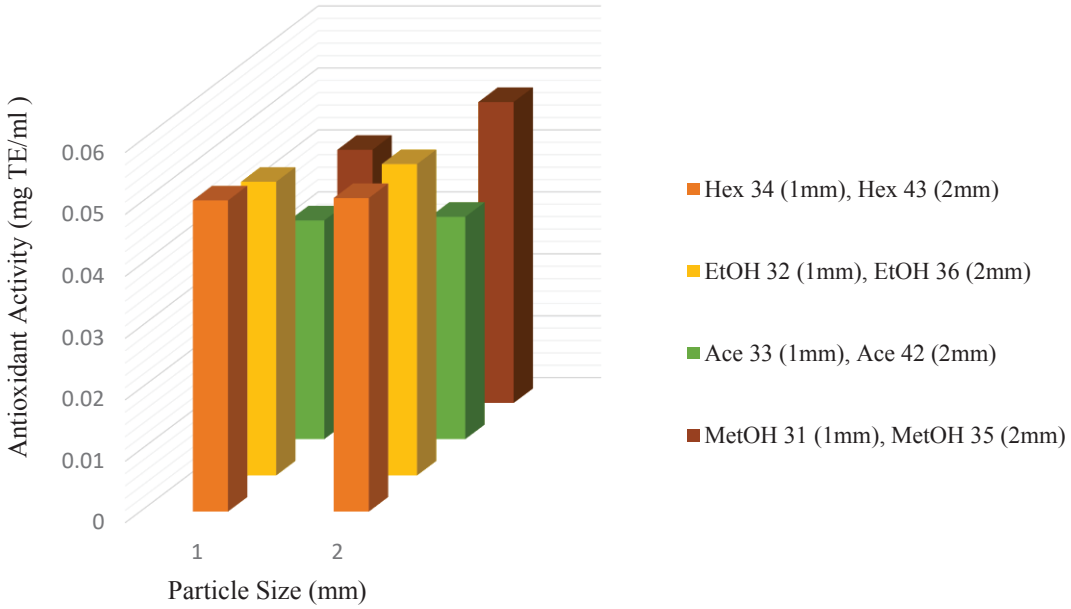


Figure 3.27. Effect of particle size on antioxidant activity (Amount of hazelnut shell: 4 grams; extraction time: 8 h by ultrasonic extraction; particle size: 1mm and 2 mm; solvent volume: 250 ml (ethanol, methanol and acetone))

CHAPTER 4

CONCLUSION

Extraction of hazelnut shell was performed using soxhlet extraction, ultrasonic extraction and combined extraction. The effects of type of extracting solvents (ethanol, methanol, n-hexane, acetone and chloroform), extraction time (2 cycles, 3 cycles and 8h), solid liquid ratio (4, 8 and 12 g / 250 ml) and size of hazelnut shell (1 mm and 2 mm) were investigated on capacity of phenolic and antioxidant components, extraction yield, contents of liquid and solid product which were obtained extraction.

The highest yields were detected in ethanol and methanol extracts. The darkest color of liquid product was also found obtained extraction of solvents. Combined extraction showed the highest yield extraction among all extraction methods. On the other hand, there is no significant effect of the extraction time on yield, antioxidant activity and phenolic content. It was also found that extraction yield decreased when the solid - liquid ratio increased. The yield obtained from extraction of 2 mm hazelnut shell is higher than that obtained from 1 mm hazelnut shell extraction. As a result of the GC-MS analyzes, high content of oleic acid and palmitic acid were found in the liquid product obtained from extraction. In FTIR analysis of the solid product, the lignin, hemicellulose and cellulose structures was determined to be rich in hazelnut shell.

According to the results obtained at the end of the study, optimum conditions were detectioned by using 4 g hazelnut shell in methanol combined extraction. The phenolic content and antioxidant activity which were obtained from this extraction was 0.166 mg GAE/ml and 0.046 mg TE/ml, respectively. Besides, the maximum yield was found as 15.40% from methanol combined extraction.

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