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SEHRA BARIŞ

REPUBLIC OF TURKEY

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GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES

EXTRACTION OF PECTIN FROM PISTACHIO HULL AND ITS USE IN EMULSION SYSTEM

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SEHRA BARIŞ

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Supervisor

Assoc. Prof. Dr. Derya KOÇAK YANIK

by

Sehra BARIŞ

August 2020

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

Sehra BARIŞ

ABSTRACT

EXTRACTION OF PECTIN FROM PISTACHIO HULL AND ITS USE IN EMULSION SYSTEM

BARIŞ, Sehra
M.Sc. in Food Engineering
Supervisor: Assoc. Prof. Dr. Derya KOÇAK YANIK
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The aim of this study is to obtain pectin from pistachio hull and to investigate the emulsifying properties of pectin obtained in the oil-in-water emulsion system. Extraction was carried out by using two different methods, conventional extraction (CE) and ultrasound-assisted extraction (UAE). Two different extraction solvents, water and citric acid solutions were used separately as extraction solvents. While the highest yield (32.3±1.44%) was obtained using citric acid solution in CE method, the lowest yield (5.56±0.25%) was obtained using water in UAE method. The physical and chemical properties of the pectin obtained were determined. The results showed that pectin obtained under optimum conditions was low methoxyl (about 19.29% DE) with moisture content of 4.9±0.062%, ash content of 1.49±0,026%, protein content of 1.85±0.14%, total phenolic content of 6.03±0.08 mg GAE/g, total sugar content of 56.84±2.36 mg sugar/g, and acidity of 38.94±0.17%. The galacturonic acid content had been found as 65.81%. Additionally, the emulsifying property of pectin extracted was investigated in an o/w emulsion system. O/W emulsions were prepared at six different pectin concentration (2, 4, 5, 6, 8 and 10% (w/w)) and at fixed oil ratio (20% (w/w)). Emulsion performance was investigated in terms of emulsion stability, morphological characteristics, droplet size and rheological properties were examined. The best emulsion characteristics were obtained at 6% pectin concentration.

Key words: Pistachio Hull, Pectin Extraction, Conventional Extraction, Ultrasound-Assisted Extraction, Oil-in-Water Emulsion

ÖZET

ANTEPFISTIĞI KIRMIZI YUMUŞAK KABUĞUNDAN PEKTİN EKSTRAKSİYONU VE EMÜLSİYON SİSTEMLERİNDE KULLANILMASI

BARIŞ, Sehra
Yüksek Lisans, Gıda Mühendisliği
Danışman: Doç. Dr. Derya KOÇAK YANIK
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Bu çalışmanın amacı, antepfıstığı kırmızı yumuşak kabuktan pektin elde etmek ve elde edilen pektinin yağ/su emülsiyon sisteminde emülsifiye edici özelliklerini araştırmaktır. Konvansiyonel ekstraksiyonu (KE) ve ultrason destekli ekstraksiyonu (UDE) olmak üzere iki farklı yöntem kullanılarak gerçekleştirilmiştir. Ekstraksiyon çözücüleri olarak, su ve sitrik asit çözeltisi ayrı ayrı kullanılmıştır. KE yönteminde sitrik asit çözeltisi kullanılarak en yüksek verim (%32,3±1,44) elde edilirken, UDE yönteminde su kullanılarak en düşük verim (5,56±0,25) elde edilmiştir. Elde edilen pektinin fiziksel ve kimyasal özellikleri belirlenmiştir. Sonuçlar, optimum koşullar altında elde edilen pektinin düşük metoksil (yaklaşık %19,29) olduğunu, %4,9±0,062 nem içeriği, %1,49±0,026 kül içeriği, %1,85±0,14 protein içeriği, 6,03±0,08 mg GAE/g toplam fenolik içeriği, 56,84±2,36 mg şeker/g toplam şeker içeriği, %38,94±0,17 asit içeriği olduğunu gösterdi. Galakturonik asit miktarı %65,81 olarak bulunmuştur. Ek olarak, ekstrakte edilen pektinin emülsifiye edici özellikleri bir yağ/su emülsiyon sisteminde araştırılmıştır. Yağ/su emülsiyonları altı farklı pektin konsantrasyonunda (%2, 4, 5, 6, 8 ve %10 (w/w)) ve sabit yağ oranında (%20 (w/w)) hazırlanmıştır. Emülsiyon performansı, emülsiyon stabilitesi, morfolojik özellikler, damlacık boyutu ve reolojik özellikler açısından incelenmiştir. En iyi emülsiyon karakteristikleri %6 pektin konsantrasyonunda elde edilmiştir.

Anahtar Kelimeler: Antepfıstığı Yumuşak Kabuk, Pektin Ekstraksiyonu, Konvansiyonel Ekstraksiyonu, Ultrason Destekli Ekstraksiyonu, Yağ/Su Emülsiyonu

"dedicated to my beloved mom and dad,

who educated 11 daughters despite all

difficulties"

ZEYNO & ABDULBAKİ BARIŞ

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
BC	Before Christ
CE	Conventional extraction
$CaCI_2$	Calcium Chloride
DE	Degree of Esterification
EAI	Emulsifying Activity Index
ESI	Emulsifying Stability Index
FAO	Food and Agriculture Organization
FW	Food Waste
GalA	Galacturonic Acid Content
HCI	Hydrochloric Acid
NaOH	Sodium Hydroxide
O/W	Oil in Water
PH	Pistachio Hull
SDS	Sodium Dodecyl Sulfate
TPC	Total Phenolic Content
UAE	Ultrasound Assisted Extraction
W/O	Water in Oil

CHAPTER I

INTRODUCTION

One of the issues that are gaining importance both in the world and in our country is food waste. The food and beverage sector is one of the sectors that produced food waste. Many countries, institutions and non-governmental organizations are working on food waste, which has become a global problem. The waste of food brings environmental, economic and ethical problems. The Food and Agriculture Organization (FAO, 2013) reported that around 30% of food produced for human consumption in the world is lost or disposed of every year. Food waste reduction and food security issues are key points identified in the nutrition strategy of the future human population. Prevention or reduction of food waste will ensure the protection of both environment and human health. In addition, economic development will be achieved by preventing financial losses from wastes (Şahin and Bekar, 2018).

Pistachio (Pistacia vera L.) is one of the most valuable agricultural crops not only in Turkey but also in the world. It is a dioecious plant from the family of Anacardiaceae. (Yavuz et al., 2016). There are several species of the genus Pistacia but Pistacia vera (Pistachio) is the only species in this genus that produces commercially acceptable edible nuts (Kashaninejad and Tabil, 2011). Nowadays, the principal manufacturer and exporter of pistachio nut in the world is Iran. (Rafiee et al., 2016). The other principal manufacturers and exporters of pistachio nuts are USA, Turkey, Syria, Italy, Tunisia and Greece after Iran (Bellomo and Fallico, 2006). Pistachio nuts are generally marketed as split for snack food, and non-split ones are utilized for processing. They are used for ice cream, cakes, candies, biscuits, pies and desserts (such as baklava) in food industry (Adal, 2012).

In the food industry, as a result of the processes, large amounts of food wastes are formed. While some of them are destroyed instantly, some of them can cause environmental pollution (Avcı, 2016). Residues which obtained from pistachio processing are; soft hull (epicarp), lesser extent penducles, leaves of the plant, hard shell (endocarp) and remaining kernel (Moghaddam et al., 2009). The pistachio by-products are generally aforethought as agricultural waste. They are oftentimes mixed with soil and less commonly used as a feedstuff for livestock by local farmers and a small portion is utilized in human foods and herbal medicine. If not further processed, this by-product becomes waste and has the potential to be environment contaminant (Al Juhaimi et al., 2016).

Pistachio hull (soft hull, epicarp), which constitutes a significant part of pistachio, accumulates in high amounts during pistachio processing. In addition to economic benefits, the utilization of a pistachio hull (PH) as a food ingredient can provide nutrition and health benefits due to the valuable ingredients present in it (Chaharbaghi et al., 2017).

Pectin is an important as a food additive that generally uses the gel-forming feature. It is a colloidal carbohydrate compound from the group of pectic substances that found in the cell membrane of plants, between the cells or in the middle lamella region (Avcı, 2016). It is a complex hetero-polysaccharide, and mainly containing α-(1→4) linked D-galacturonic acid (at least 65% galacturonic acid) (Endress, 2011). Pectin is commonly considered as thickner, gelling agent, stabilizer and emulsifier in food and pharmaceutical applications (Syeitkhajy, 2017). Pectin is commercially produced from apple, citrus, etc. In the literature, PH has been stated as a good potential for pectin extraction (Chaharbaghi et al., 2017).

The most widely used pectin extraction method is the conventional acid extraction method in which used hot water acidified with a mineral acid. In the acid extraction process the temperature, pH and time changes in the range of 100 °C, 1.3-3.60, and 20-360 min, respectively (Guo et al., 2014). Although the acid extraction method is considered economically advantageous, new methods are being explored in the extraction with the idea that harmful components that may be released during application may cause environmental problems. Currently, new technologies for pectin extraction method are tried to be developed which do not harm both human

health and environment, provide high efficiency and quality pectin, and decrease energy consumption by completing them in a shorter time than conventional acid extraction (Şimşek, 2013). Microwave (Liu et al., 2006), ultra-sonication (Bagherian et al., 2011), ultra high pressure (Guo et al., 2012) and super-high frequency electromagnetic field (Guo et al., 2014) are some of these methods.

Nowadays, there are many studies to investigate the emulsifying properties of pectin in O/W emulsions. Emulsions are heterogeneous systems with a homogeneous appearance that formed by two immiscible liquids, one of which is diffused in another liquid (Güngör et al., 2013). Basically, the emulsion is forming from two phases, one of which is a continuous phase (external phase), and the other is a dispersed phase (internal phase or discontinuous phase) (Chrisman et al., 2012). The emulsion can tend to destabilize by different physicochemical processes, containing flocculation, aggregation, phase separation, coalescence or Ostwald ripening (Guzey at al., 2004). Emulsion applications are common in the food, pharmaceutical, agricultural, cosmetics and petroleum industries (Ichikawa, 2007). Many food products, containing cream, milk, batters, desserts, dressing, beverages, dips, sauces, etc. are examples of water in oil (W/O) or oil in water (O/W) emulsions. One of the most widely used and important methods to increase O/W or W/O emulsions stability is to use emulsifiers which are food hydrocolloids from natural resources. Protein, pectin, and gums are widely used materials for this purpose (Guo et al., 2013).

The main purpose of the thesis is to obtain pectin from PH and to investigate the emulsifying properties of pectin obtained in an O/W emulsion system. In this respect, pectin extraction from PH was carried out by using two different methods, conventional extraction (CE) and ultrasound-assisted extraction (UAE). The physic-chemical properties of the pectin obtained were determined. Additionally, o/w emulsions were prepared at six different pectin concentration (2, 4, 5, 6, 8 and 10% (w/w)) and at a fixed oil (sunflower oil) ratio (20% (w/w)). The stability, droplet size, and rheological properties of prepared emulsions were examined. In addition, morphological characteristics of the samples were observed under a polarized light microscope.

CHAPTER 2

LITERATURE REVIEW

2.1 Food Waste

The world has a scaring future in terms of food, water and other resource shortages. Researches in recent years have shown that waste production is increasing more and more (Luque and Clark, 2013).

One of the most important problems of the world is waste which is experiencing at the present. It will definitely need to be addressed in the coming days. In recent years, food waste has been receiving increasing attention from researchers. Food waste (FW) occurs in all stages of the supply chain, containing harvesting, postharvest, during production, at retailers, within the foodservice industry, and consumption (Kim et. Al., 2020). Almost 40% of foods are lost before they reach the end consumer. This number covers vegetables, fruits, cooked/uncooked raw materials, cereals, and waste food from restaurants and homes (Sindhu et al., 2020). FAO defines FW as “food (containing inedible parts) that lost from the supply chain, not containing food diverted to material uses such as animal feed, bio-based products or sent for redistribution.” According to forecast report of FAO (2016), a global FW of around 1.6 billion tons is pointed out annually, and fruits are said to be one of the highest waste rates (Petkowicz et al., 2017).

The valorization of food waste through value-added products can be an ideal and practical solution for decreasing the global volume of food waste. (Tsang et al., 2019). These value-added products contain functional components such as phenolics, polysaccharides, prebiotics, surfactants, pigments, biofuel, biopolymers and etc.

The extraction of by-products from food wastes can find a great scale of utilization for is not only for the food industry but also for drug and cosmetic industries (Figure 2.1) (Otles and Kartal, 2018).

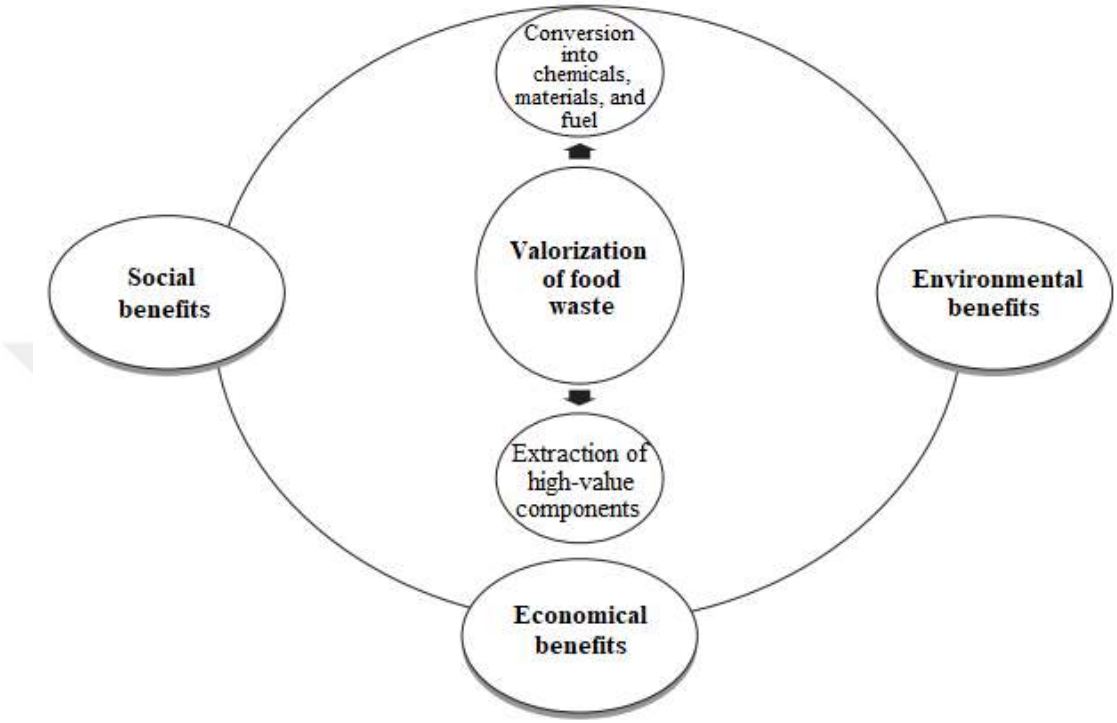

Figure 2.1 Food waste valorization (Otles and Kartal, 2018).

It will fulfill the basics of green chemistry, such as the evaluation of food waste using greener technologies, waste prevention, increasing energy efficiency, design of safer chemical products, use of renewable raw materials and design for degradation (Figure 2.2) (Anastas and Warner, 1998).

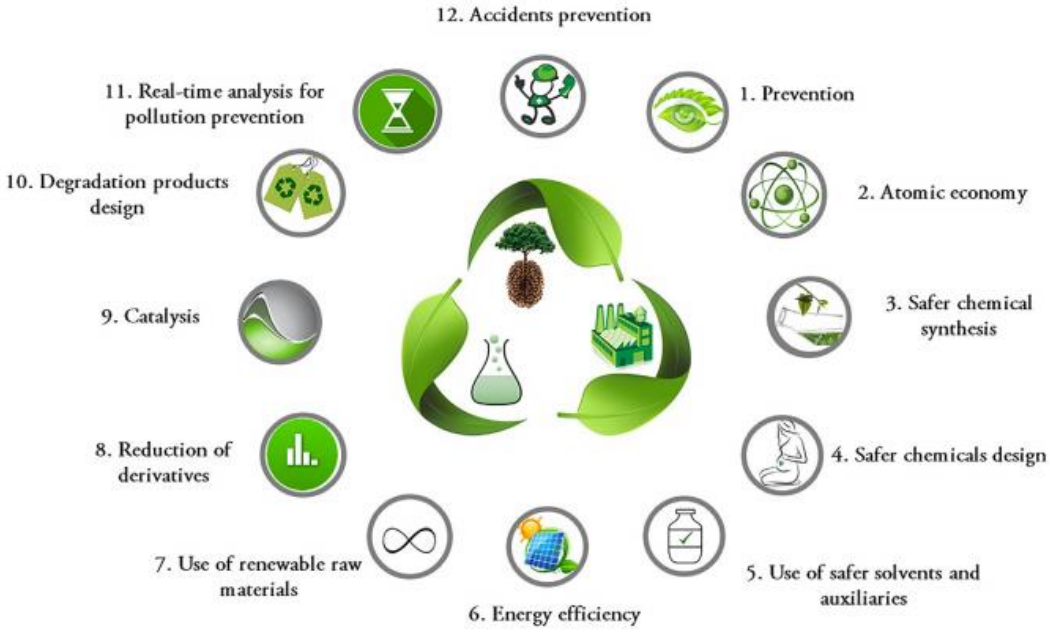

Figure 2.2 Twelve principles of green chemistry (Anastas and Warner, 1998).

2.2 Pistachio Nut (Pistacia vera L.)

Pistachio nut (Pistacia vera L.), is a member of the cashew (Anacardiaceae) family which is the most popular agricultural product in the world. There are a few kinds of the genus Pistacia but Pistacia vera L. is the only kind in this genus that edible nuts**.** Pistachio nut includes a green to yellow-red hull, a lignified shell, a purple seed coat and an edible seed in the center. The pistachio is a unique product in the nut trade, due to the naturally split shell before harvest and can be eaten as fresh, dried and roasted (with or without salt and flavorings). Pistachio kernels can be easily removed from the shell without mechanical cracking, and this advantage provides them to be widely marketed in the shell (Kashaninejad and Tabil, 2011).

2.2.1 Origin and History

The exact place of origin of the pistachio was not known until the beginning of the present century. In archaeological excavations, evidence of pistachio consumption shows that it has long been associated with human activities. Relics of pistachio nuts were found both in southeast Iran and Afghanistan, coming from the sixth millennium BC (Before Christ). Pistachios were widely cultivated in the ancient Persian Empire, and then slowly spread to the west (Alonso et al., 2016). The pistachio history is said to reflect royal characters, pride and endurance. Specifically good pistachios are said to have been a favorite fineness of the Queen of Sheba who

seized all Assyrian consignments. According to legend, the Queen of Sheba raised a restricted amount of pistachios for her special use and guests during her visit to Assyria. Emperor Tiberius, the province's Roman consul, presented pistachios into Italy from Syria at the end of his reign. In time, they had extended into Mediterranean areas in southern Europe and North Africa. Additionally, they were cultivated also in China at around the 10th century and more recently in New Mexico, Australia and California (Kashaninejad and Tabil, 2011).

The pistachio word is a kind of loanwords which come from Zendan Avestan (old Persian language) pista-pistak. This word is the root of the name for the pistachio in most languages. According to Dioskurides, the word pistachio is reproduced from pissa (means resin) and aklomai (means to heal). The general name of pistachio in different languages is Pista/Peste (in Persian), Pistacchio (in Italian), Pistacho (in Spanish), Pistache (in French), Pistazie (in Germany), Fustuq (in Arabic), Fıstık (in Turkish) and Pisutachio (in Japanese) (Joret, 1976). Iran, Asia Minor (now Turkey), Syria, Lebanon, Afghanistan and the Caucasus in Southern Russia are the origins of pistachio (Zohary 1952)**.** Today the pistachios are widely consumed product worldwide, and mostly produced in Iran, the United States, Turkey, Syria, Greece, Italy and Spain. According to the statistics of FAO (2015), in terms of pistachio production, Iran makes a significant contribution to the world and the production is about 480,000 tons per year. The United States is considered to be the second-largest producer worldwide after Iran. Meanwhile, Turkey is the third-largest importer and exporter of pistachio nuts in the world. Turkey has a total of 144,000 tons of pistachio production. The major nuts grown in Turkey are Siirt, Ohadi, Halebi, Kırmızı, Kellekoçi, and Uzun (Şimşek and Gülsoy, 2018).

2.2.2 Varieties

There are many cultivated diversities of pistachio are present in different countries, with important differences in properties of them, especially in terms of color, size, flavor, shape and division. According to the report **of** Maggs (1973), the main pistachio varieties diffused from Iran, Turkey, and Syria to the world. These were achieved through seedling selection in the field (Kashaninejad and Tabil, 2011).

The pistachio of Iran that has a light yellow kernel color are considered as dry. Kerman province is the most important and largest pistachio growing field in Iran.

According to Esmail-pour (2001), more than 70 pistachio varieties have been reported in Kerman. The major varieties are ‘Ohadi (or Fandoghi), Kalle-Ghuchi’, ‘Ahmad-Aghaei’, ‘Badami’, ‘Rezaei’ and ‘Momtaz’ which grow in Kerman (Kashaninejad and Tabil, 2011).

The pistachio of Turkey and Syria that have a green color are said as having high quality. There are about 20 female pistachio varieties in Syria and the main varieties are ‘Ashouri (Red Aleppo)’, ‘Red Oleimy’ and ‘White Batoury’ which grow in Syria and ‘Ashouri’ is the best variety (Kashaninejad and Tabil, 2011). In Turkey, the majority of pistachio culture is located in the dry, barren foothills and lower fields of Southeastern and Western of Turkey. There are many types of pistachios depending on the production areas; the main varieties are; ‘Uzun’, ‘Kırmızı’, ‘Siirt’, ‘Halebi’ and ‘Ohadi’. ‘Kırmızı’ and ‘Uzun’ are two main commonly cultivated varieties in Turkey. Turkish pistachios with a different flavor and uniform green kernels are preferred by many European and USA markets (Satil et al., 2003).

2.2.3 Properties

Pistachio nuts which are largely consumed in many different ways have a very valuable due to their nutritional, sensory and health properties. They are a good sources of fat, protein, vitamins, dietary fibers, minerals and antioxidant phytochemicals depending on the variety and ripeness at harvest time. Nutritionally, they are also better than other varieties of tree nuts and peanut because they have higher in protein, carbohydrate, and potassium and lower in calories and fat content (Kashaninejad and Tabil, 2011). In general, the smaller size has a greener color. Large pistachios with green kernels are very expensive and desirable. Although the taste, aroma, and texture in the kernel of small-sized varieties are superior, the larger size is the more acceptable for the commerce (Ayfer, 1990).

Kamanger and Farsam (1977) studied and reported some chemical composition of Iranian origin pistachio nuts (Table 2.1). Garcia et al (1992) studied and reported some kernel characteristics and fatty acid composition of pistachio nuts grown in Turkey (Table 2.2). Woodroof (1967) reported composition of pistachio nut kernels, shells and hulls of Red Aleppo variety (Table 2.3).

Table 2.1 Chemical composition of pistachio nuts kernel (Kamanger and Farsam, 1977)

Composition (%)					
Moisture	Ash	Oil	Fiber	Protein	Carbohydrate
2.5-4.1	2.2-2.5	55.2-60.5	1.7-2.0	15.0-21.2	14.9-17.7

Mineral Content (%x0.001) of Kernel						
Na	K	Ca	P	Fe	Cu	Mg
4.0-6.7	1048-1120	120-150	494-514	5.8-11	1.0-1.4	157-165

Table 2.2 Kernel fatty acid composition of different pistachio varieties (Garcia et al., 1992)

Variety	**Fatty Acid Compositon (%)**					
	Saturated		Monounsaturated		Polyunsaturated	
	Palmitic	Stearic	Palmitoleic	Oleic	Linoleic	Linolenic
Antep	8.9	2.5	0.7	70.1	17.5	0.6
Bilgen	8.9	1.2	0.9	54.4	33.8	0.6
Kellegouchi	7.3	2.3	0.7	71.8	16.7	0.8
Mümtaz	9.5	1.5	0.9	597	27.3	0.5
Ohadi	7.3	1.3	0.9	54.4	35.3	0.6
Sefidi	7.2	1.9	0.7	65.9	23.3	0.8
Siirt	7.6	2.2	0.6	68.4	19.9	1.1
Vahidi	10.5	0.9	1.4	55.7	30.7	0.4

Table 2.3 Composition of pistachio nut kernels, shells and hulls, % on dry basis (Woodroof, 1967)

	Kernels		**Shells**		**Hulls**	
	Split nuts	Unsplit	Split nuts	Unsplit	Split nuts	Unsplit
Protein	19.41	19.58	0.42	1.06	7.66	9.35
Oil	58.3	54.47	0.56	0.58	7.82	8.27
Crude fiber	1.74	2.19	54.0	53.4	14.1	17.4
Ash	2.95	3.55	0.42	1.6	15.56	13.30
Calcium	0.13	0.13	0.06	0.06	0.07	0.25
Potassium	1.04	1.22	0.22	0.49	6.71	5.88
Phosphorus	0.54	0.64	0.02	0.04	0.12	0.24

2.2.4 Uses

Pistachio which has high nutritional value is one of the most delicious varieties of nuts. It is a unique product in the nuts trade due to the naturally split shell before harvesting.

Pistachio added to many food products is one of the perfect taste enhancer and also improves nutrition, flavor and color of products. The split pistachio form is mainly marketed as a snack food and consumed as fresh, dried, roasted (with or without salt) while non-split pistachio form is mainly utilized in the production of pistachio butter, pistachio oil, pistachio halva and pistachio chocolate (Shakerardekani et al., 2011). In addition, it is utilized as the main ingredient of many Turkish desserts (such as baklava) and utilized in candies, confections, cakes, pastries, ice creams, baked goods, and sausages (Kashaninejad and Tabil, 2011).

The usage of pistachio nuts in producing countries is more diverse than in the importing countries. In Iran, the hull is utilized for manure, feed for ruminants and little amount is made into a flavorful marmalade (Moghaddam et al., 2009). A limited amount of pistachio oil is utilized for production of pistachio butter while a

small amount of pistachio oil is also utilized for eating and utilize in cosmetics (Taghizadeh and Razavi, 2009).

2.2.5 Production Process

The general processing of pistachio as called 'Gold' in Turkey consists of five stages;

1. De-hulling
2. Drying
3. Splitting
4. Salting and Roasting
5. Storage

2.2.5.1 De-hulling

De-hulling is the removal of the hull by separating it from the shell of pistachio. The nuts are usually de-hulled very soon after harvest to decrease the opportunities for microorganisms' growth and to prevent shell staining. De-hulling of pistachio is made using by different types of machines (Woodroof, 1967). If the nuts have been dried in-hull for storage they are softened in water or steam for 1-6 hours to loosen the hulls before the de-hulling in Turkey. After that hulls are removed by rubbing the nuts with stone rollers or fiberglass cylinders. The de-hulling process is completed by the segregation of the nuts from the hulls by the help of vibration sieves. In the United States and Iran, hulls are removed by using abrasive peeling machines, both in the fresh or after drying the hull (Tekin et al., 1995).

2.2.5.2 Drying

The next stage is drying which one of the important stages in the processing of pistachio to prevent spoilage after harvest. During the drying stage, pistachios can be subjected to unwanted reactions that reason deterioration of quality due to the odd colors and flavors formed (Nejad et al., 2003).

The moisture content which can be as high as 45% in the freshly harvested nuts is decreased to 5%. Drying time is depended on many parameters containing temperature, dryer air, ambient relative humidity, initial moisture content of pistachio nuts, variety, drying stage and drying method. In Turkey, Pistachio nuts are generally

dried under the sun for 3 to 4 days (Woodroof, 1967; Yanniotis and Zarmboutis, 1996).

2.2.5.3 Splitting

The next stage is splitting. Even though some of the harvested ones are split naturally, some others could be unsplit. Hence, unsplits ones are splitting by hand or devices in this step.

2.2.5.4 Salting and Roasting

The pistachio nuts are usually eaten as roasted (salted or unsalted) in-shell. Salting and roasting improve the color, texture, flavor and appearance of them. The general procedure is that pistachio nuts are salted by soaking in a salt solution (approximately 15%) and then drying in a rotary-screen drier at 25 °C for 1 hour until the moisture taken up by salting is removed. After the temperature is increased to 65 °C in 30 minutes and kept at this temperature for 20 minutes, and finally is cooled and packaged (Woodroof, 1967; Yonniotis and Zarmboutis, 1996).

2.2.5.5 Storage

The pistachio nuts must be stored under optimum conditions to prevent mold growth, rancidity, off-flavor, discoloration and insect infestation. Since they contain more than 50% oil, temperature, relative humidity, light and gas composition of storage room will affect their quality during storage.

2.2.6 Worldwide Importance and Economical Value

According to FAO (2015) statistics, the world has a total pistachio production of approximately 1,023,000 tons, and the main world pistachio producers are Iran, USA, Turkey, China and Syria produce 480,000, 240,000, 144,000, 80,000 and 57,000 tons yearly, respectively.

Iran is one of the main countries in pistachio nuts production in the world. Pistachio, which has positive effects on the exchange, economy, employment and added value in Iran, is considered as an important agricultural export product. The Kerman province, which has a share of 83 and 80% in total output and total harvest area in 2002, has been accepted as the main center of pistachio production in Iran (Nejada, 2012).

The USA is considered to be the second-largest producer worldwide after Iran. From 1906 to 1976, the USA mainly depended on importing pistachios from Iran. However, due to political relations between the two countries, Iran stopped exporting goods to the USA, and this stimulated the growth of the USA pistachio industry. For the following thirty years, the USA pistachio industry has grown rapidly, and in 1982, the USA entered the world trade market and posing a strong rival against Iran. United States Department of Agriculture (USDA) reported that approximately 98% of USA pistachios are produced in California (Zheng, 2011).

The third-largest producer country of pistachio in the world is Turkey. Especially, the Southeastern Anatolia Region one of the most major gene centers of pistachio nuts in Turkey and has 93.39% of pistachio production. Gaziantep and Şanlıurfa which are the provinces of the Southeastern Anatolia Region in Turkey are the first and second with 53,109 and 47,848 tons of pistachio nuts productions, separately (Şimşek, 2018).

Table 2.4 Pistachio trees' number and production of Southeastern Turkey (Şimşek. 2018)

Provinces	**Production (tons)**	**Number of fruitful trees**	**Number of unfruitful trees**
Gaziantep	53.109	16.412.510	3.575.368
Şanlıurfa	47.848	12.843.690	4.348.102
Adıyaman	15.368	4.209.355	862.476
Siirt	11.221	2.742.800	1.219.000
Kilis	2.271	713.724	237.908
Mardin	1.659	178.157	138.516
Batman	1.654	340.335	255.802
Diyarbakır	1.408	139.980	78.005
Şırnak	43	10.160	61.777
Southeastern Turkey	134.581	37.590.711	10.776.954
TURKEY	144.000	40.597.427	11.632.973

2.3 Pistachio By-Products

Pistachio nut is formed from a kernel (seed), covered by a slim soft and edible skin, encased by a hard inedible shell (endocarp), which is further enclosed by the fleshy inedible hull (epicarp) (Kashaninejad and Tabil, 2011) (Figure2.3). During pistachio nuts processing, a vast quantity of by-product is released and by-product valorization is one of the important issues of the coming years.

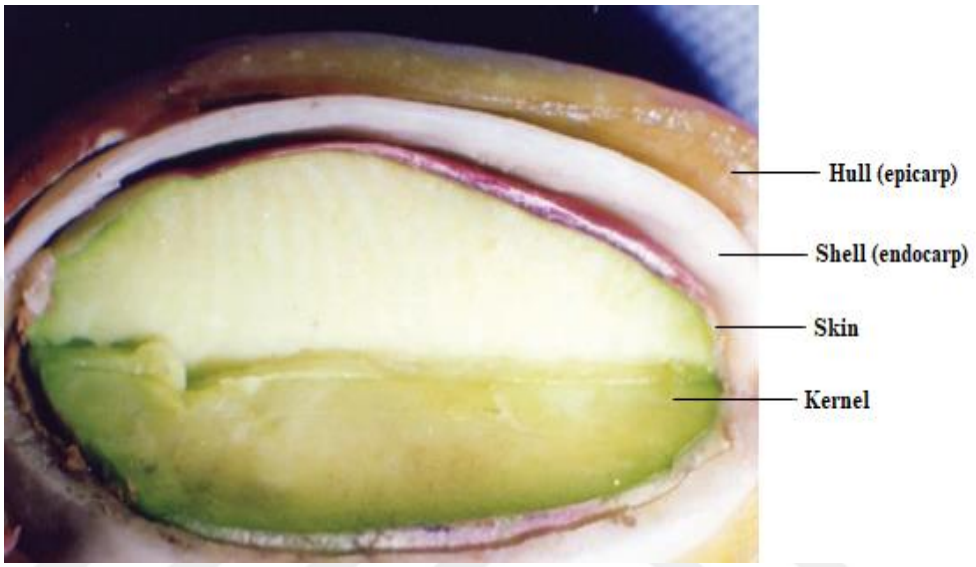

Figure 2.3 Components of pistachio nut (Kashaninejad and Tabil, 2011).

Pistachio by-products consist of the soft hull (epicarp), lesser amount penducles, leaves of the plant during the de-hulling stage of pistachio nuts production while the hard shell (endocarp) and remaining kernel are formed during the other stage of pistachio nuts (Moghaddam et al., 2009). They are used as agricultural waste and are commonly mixed with soil and less commonly utilized as a feedstuff for livestock by local farmers and a small amount are utilized in human foods and herbal medicine. If not further processed, this by-product becomes waste and has the potential to cause environmental contaminant (Al Juhaimi et al., 2016).

PH is one of the by-products accumulated in high volumes during the processing of pistachio nut.

Pistachio Hull

PH is one of the major by-products of pistachio which is surrounding the hard shell that is taken out by de-hulling stage during the post-harvest processing of pistachio (Figure 2.4).

Figure 2.4 Dry pistachio hull

Pistachio contains 18% hull which is a fine source of antioxidants, phenolic compounds, fatty acids, flavonoids, protein, vitamins, minerals and polysaccharides such as pectin (Demiral et al., 2008). The utilization of PH for the production of a value-added ingredient (such as pectin) can both raise the importance of the pistachio production and reduce the ecological contaminant (Kazemi et al., 2019).

2.4 Pectin

Pectin, first isolated and described by Heneri Brancannot in 1825, is a complex mixture of polysaccharides (Srivastava and Malviya, 2011).

2.4.1 Structure

Pectin (water-soluble) is taken place in the cell walls of all living plants. The texture of vegetables and fruits is affected depending on the amount and quality of pectin available during growth, ripening, and storage (Kratchanova et al. 2004). The pectin in plant cell is represented in Figure 2.5.

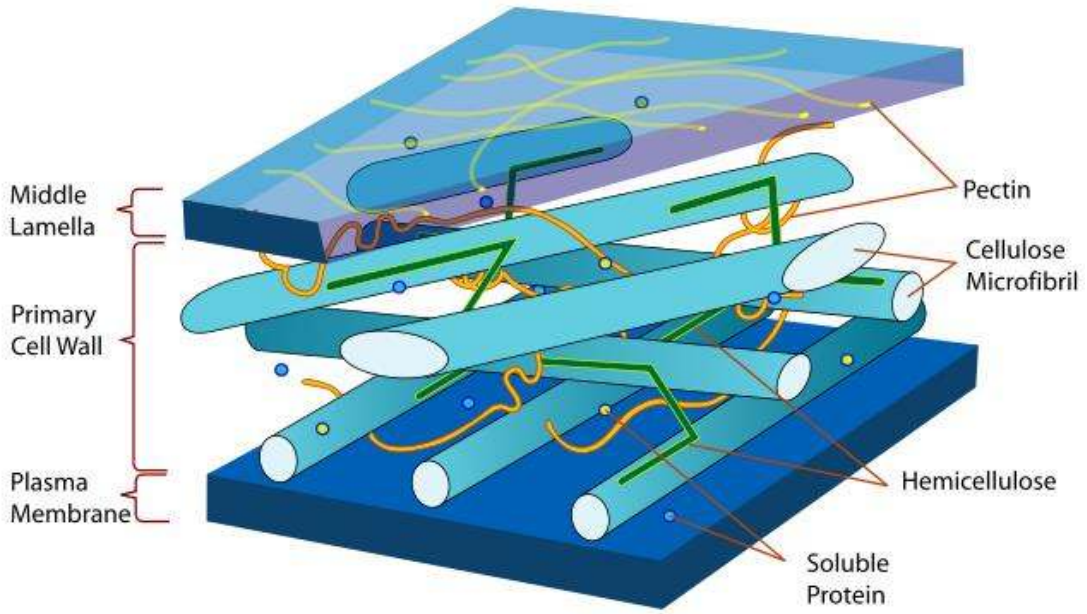

Figure 2.5 Pectin in plant cell (Jain, 2018)

In the early stage of growth, pectin molecules in the plant's cell cannot be extracted with water as they are tightly bound to other molecules of the cell wall. This water-insoluble form is called protopectin. Protopectin is converted into soluble pectin during the maturing of vegetables or fruits (Endress and Rentschler, 1999).

Pectin structure is mainly formed with a backbone of α-(1→4) linked D-galacturonic acid with different degree of methyl esterification. The degree of esterification (DE) is one of the most efficient factors on the pectin application which is the ratio of esterified galacturonic acid groups to total galacturonic acid groups. Based on the factor, the pectin is classified into two groups: low methoxyl which has below 50% DE (Figure 2.6) or high methoxyl pectin which has above 50% DE (Figure 2.7) (Chaharbaghi et al., 2017).

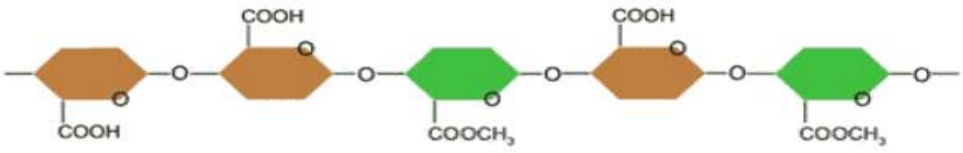

Figure 2.6 The chemical structure of low methoxyl pectin (Ippa, 2017)

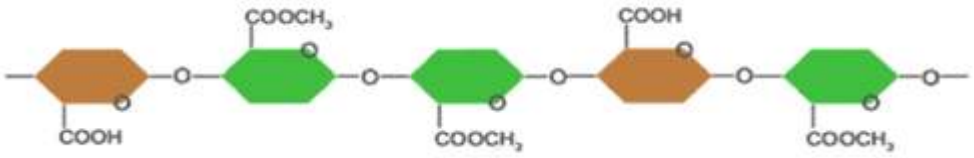

Figure 2.7 The chemical structure of high methoxyl pectin (Ippa, 2017)

2.4.2 Pectin Source

Currently, commercially distributed pectin products are generally extracted from the apple pomace and citrus peel (lemon, orange, grapefruit, etc.). Nonetheless, due to the high request of pectin in the worldwide market (more than of 30,000 tons yearly) (Maran et al., 2015) other new plant sources have been researched for pectin extraction such as watermelon rind (Maran et al., 2014), cocoa husks (Mollea et al., 2008), sisal waste (Santos et al., 2013), passion fruit peel (Kulkarni and Vijayanand, 2010), pomegranate peel (Moorthy et al., 2015), banana peel (Gopi et al., 2014), peach pomace (Pagan and Ibarz, 1999), mango peel (Berardini et al., 2005), papaya peel (Maran and Prakash, 2015), and soy hull (Kalapathy and Proctor, 2001).

2.4.3 Pectin Properties

Pure pectin is light colored and it is soluble in water but it cannot be dissolved in most of the organic solvents. The solubility of pectin in water is related with, degree of esterification, molecular weight and the distribution of methoxyl groups.

The viscosity of the pectin solution is correlated with the concentration of pectin, degree of esterification, molecular weight, the pH, and existence of counter ions in the solution. As the molecular weight of pectin increases, it will exhibit higher viscosity. As concentration increases pectin solutions exert non-Newtonian, pseudolastic characteristics but at low concentration pectin solutions exert Newtonian behavior (Rolin, 2002; Voragen et al., 1995).

The gelation, solubility and viscosity are usually interdependent, i.e, factors that raise gel strength will raise the gel tendency, reduce solubility, and raise viscosity, or vice versa (Srivastava and Malviya, 2011).

Gelation Mechanism of Pectin

One of the most important factors and attraction of pectin is its gel-forming capacity. The mechanism of gelation of pectin mainly depends on degree of esterification and therefore the mechanism of gel-forming differs for both high methoxyl and low methoxyl pectins.

The gel-forming mechanism of high methoxyl pectins is completely different from the gel-forming mechanism of low methoxyl pectins. It can create a gel under the condition pH 2.8-3.6 and more than 55% sucrose concentration due to the hydrogen bond between pectin molecules. The availability of high quantities of soluble sugar necessary for gelling of high methoxyl pectins limits their utilization as a gelling agent in sweetened fruit products such as marmalades, jams, etc. (Zykwinska et al., 2009).

Low methoxyl pectins can create a gel in the existence of divalent cations (usually calcium). The galacturonic acid chain, which is bonded to each other with an ionic bond, forms a three-dimensional network and creates gelling (Syeitkhajy, 2017). Low methoxyl pectins can be used in low-sugar products.

2.4.4 Pectin Extraction

Commercial pectin production consist of different stages (Figure 2.8) and can be extracted from dry or wet plant residues. The most important stage of pectin production is extraction in which insoluble pectin (protopectin) is hydrolyzed and extracted (Garna et al, 2007).

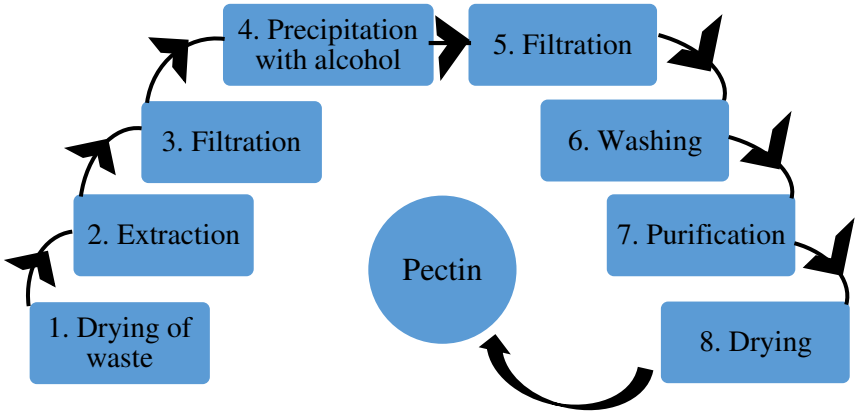

Figure 2.8 General stages in commercial extraction of pectin (Beh and Matharu, 2012).

The literature concerning the most frequently utilized methods for extraction of pectin from sources includes alkaline, enzymatic, and acid methods and their derivatives. The method of alkaline extraction can keep the neutral sugar side chains in pectin; nevertheless, the methyl ester and acetyl groups of pectin are hydrolyzed by the β-elimination reaction. The method of enzymatic extraction is an efficient alternative to reduce the utilization of acid or alkali solutions in pectin extraction. When compared with pectin obtained by acid treatment, it can be said that the pectin obtained from enzymatic treatment has a higher molecular weight and degree of esterification. However, it is a disadvantage of enzymatic treatment that it takes longer time than the acid extraction method. The method of acid extraction, which has convenient and easy operation, is frequently utilized to extract pectin in the food industry (Yang et al, 2018).

Commercial pectin is commonly produced from the conventional acid extraction method which includes hot water acidified with a mineral acid under the pH, temperatures, and process conditions (Koubala et al., 2008). The basic principles in extraction of pectin is acidic hydrolysis of protopectin (Minkov et al., 1996). Pectin extraction is formed from three main stages. The first stage is the formation of the solid phase in the acidic solution as a result of the hydrolysis of protopectin. The second stage is the internal distribution of the pectin that begins to dissolve in the solid phase pores after hydrolysis. At the last stage, the dissolved pectin is distributed outward from the boundary layer (Figure 2.9). That is, the protopectin in the hull turns into a water-soluble pectin and goes into the extraction solution (Minkov et al., 1996).

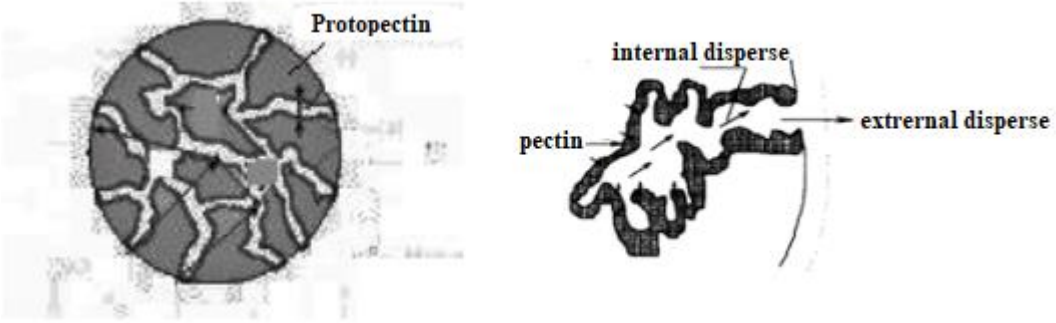

Figure 2.9 The mechanism of transport of pectin from porous particles to the surface as a result of hydrolysis (Minkov et al., 1996).

At the end of extraction, the pectin extract is filtered to remove residues and sediment from the extract solution and then it is precipitated with ethyl alcohol. It is formed the strong gelatinous mass pectin formed after the alcohol is added. After gelatinous mass pectin is separated and washed, it is dried, powdered ad stored (Chaharbaghi et al., 2017).

2.4.5 Pectin Applications

Pectin is a natural food ingredient that is permitted to use in all countries. It is extensively used as gelling agent, thickener, texturizer, emulsifier and stabilizer in food processing industry. There are between 100-500 pectin types on the market. These are generally classified according to their gelling properties (Syeitkhajy, 2017).

Pectin is particularly used in jams, jellies and marmalades as a gelling agent (May, 1990), in fruit juices as a stabilizer, is also used in bakery, confectionary, mayonnaise, ketchup, and beverages industries (Pilnik and Varogen, 1992). Pectin is also used as a stabilizer in the dairy industry that acts as a protective hydrocolloid by forming a complex with casein in low- pH dairy products (Voragen et al., 1995). The fine structure of the pectin profoundly influences its functionality in all application areas.

As functional food products are increased new applications for pectin have emerged. Recently, so many studies have focused on revealing the factors impacting the emulsifying and emulsion-stabilizing properties of pectin (Ngouemazong et al., 2015).

2.5 Ultrasound Assisted Extraction

As technology improves, new extraction methods are being developed. One of them is ultrasound-assisted extraction method. In the early 20^{th} century, while ultrasound was used in the diagnosis of disease, today, it has become a new field of research in its utilizations in the chemical, food, and pharmaceutical industries. Ultrasound is the name given to loud sounds that the human ear cannot hear, and its frequency is over 20000 Hz. In this method, vibrations with a frequency of 20 kHz to 1 GHz are applied to the sample. Sound waves consist of mechanical vibrations that allow the medium to move. The main difference of sound waves from electromagnetic waves

is the need for the environment (air, water, gas, and solid matter) for the propagation of the sound wave and the movement property of the particles (molecules) in the environment. Mechanical vibration is a wave that proceeds with the compression and expansion cycle in the environment. The expansion cycle in the liquid medium creates negative pressure. The molecules separate from each other as they expand, and come together as they get stuck. If the ultrasound is strong enough, the expansion cycle creates bubbles and voids in the liquid. As these bubbles have space limitations to expand, they burst asymmetrically. This event in the ultrasound application is called cavitation, and the event happens in just 400 microseconds (Luque-Garcia and Luque de Castro, 2003). Cavitation increases mass transfer by producing liquid streams between solvent and solid matter. Cavitation on the surface of the solid matter (part of plant) provides particle distribution by the degradation of the cells and increases sample mass transfer to the solvent by increasing the surface area of the solid material (Figure 2.10) (Paniwnyk et al., 2009; Riera et al., 2004).

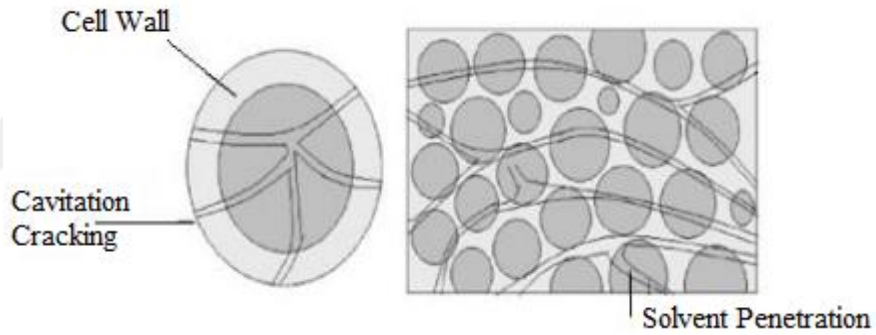

Figure 2.10 As a result of cavitation, the cell wall breaks down and the solvent diffuses into the cell structure (Shirsath et al., 2012).

Ultrasound can have a destructive or constructive effect on cells according to sonication parameters. Thanks to the control unit of the ultrasonic device, time, power, temperature and wave amplitude can be adjusted to the desired level (Figure 2.11.) (Gasmalla et al., 2015).

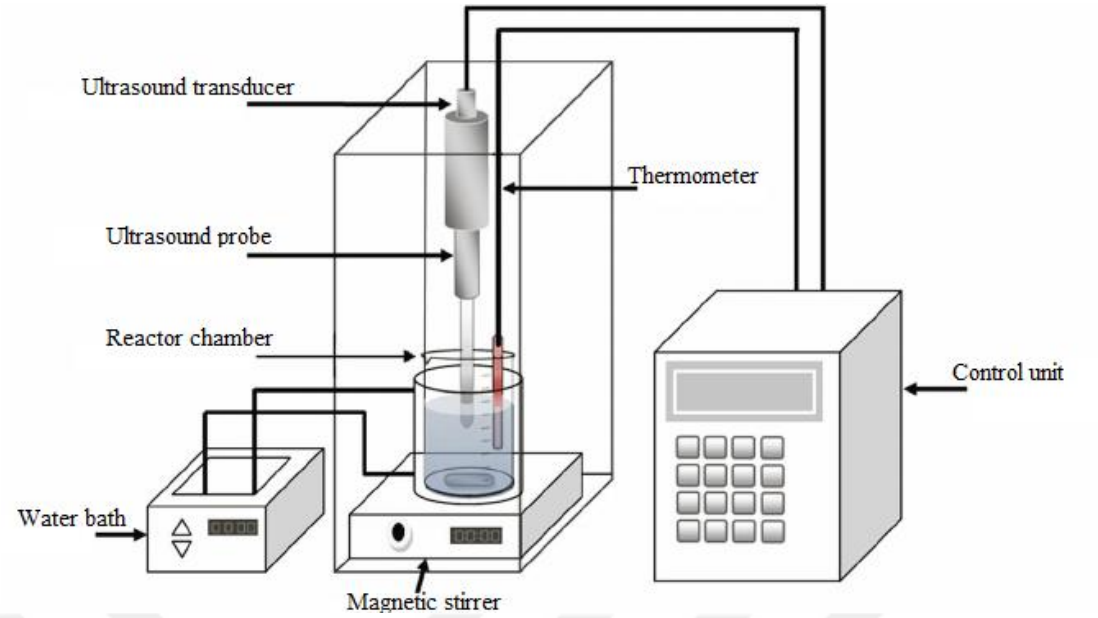

Figure 2.11 Laboratory scale ultrasound-assisted extraction device (Gasmalla et al., 2015).

2.6 Emulsion

The emulsion can be described as a colloidal system in which two immiscible liquids (usually water and oil) are distributed as droplets within each other. An emulsion basically consists of dispersed (internal) and continuous (external) phases and the continuous phase suspends dispersed phase droplets. There are two kinds of emulsions depending on the dispersed phase type: water in oil (W/O) and oil in water (O/W) (Figure 2.12).

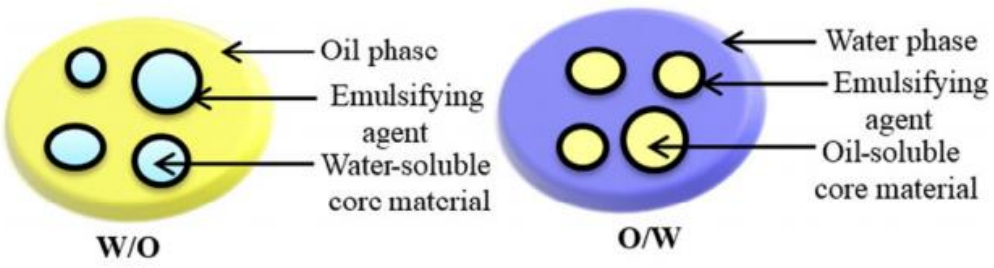

Figure 2.12 Illustration of the two emulsion systems (Amr et al., 2015).

The difference between them is that O/W emulsions have a creamy texture, while W/O emulsions are in oily structure (Zayas, 1997). Food products existing in W/O emulsions contain butter and margarines and existing in O/W emulsions contain mayonnaise, cream, desserts, flavors, soups, salad dressing, sauces, milk, and milk

products. Emulsion food products have various organoleptic and physicochemical properties such as different flavors, texture, taste, and shelf life (McClements, 1999).

In W/O emulsion, water is dispersed in an oil phase, while in O/W emulsions, oil is dispersed in a continuous water phase. The oil and water phase is not always the only component. Each phase can also need a third component for dispersion because their densities are different and the oil droplets tend to merge with each other that cause phase separation in mixtures. This can be avoided by using some natural or synthetic materials called emulsifiers or surfactants. (Guzey and McClements, 2006).

Emulsifiers are one of the most significant main substances as they determine the stability, formation and physicochemical properties of emulsion. They are surface-active substances that naturally collected interface. And they show amphiphilic properties that have both hydrophilic and hydrophobic parts. During the homogenization of emulsion, they form an adsorbed layer that adsorb lipid droplets to the surface. This layer prevents flocculation and coalescence in the emulsion system by reducing interfacial tension that stabilizes the droplet (Robinson and Wilde, 2003).

2.6.1 Pectin as Emulsifier

In 1927, pectin was submitted to be a good potential emulsifying agent for food applications (Rooker, 1927). In recent few decades, pectin has been discerned as an emulsion-stabilizing agent that primarily controls the shelf-life of the emulsions by raising the viscosity of the continuous phase. Lately, it has been observed that some pectins exhibiting some surface activity at the oil-water interface facilitate the formation and stabilization of fine oil droplets during and after emulsifications (Ngouemazong et al., 2015). In general, as with hydrocolloids, the emulsifying activity of pectin is related to its protein portion (Akhtar et al., 2002; Leroux et al., 2003), while the emulsion stabilizing potential is predominantly attributed to structural properties of and conformation of the carbohydrate portion. In addition, pectin has been partially based on the hydrophobic character of acetyl and feruloyl asters due to its ability to ease the formation and retention of fine droplets during emulsification (Ngouemazong et al., 2015).

2.6.2 Homogenization of Emulsion

Homogenization is a good distribution stage by which the dispersed phase is comminuted into small droplets by using mechanical movements. According to Robinson and Wilde (2003), there are three methods of homogenization. The first method is mechanical methods, which induce high shear fields to break up droplets, such as rotor-stator systems. The second method is high pressure homogenizers, they are the most common using in food industry and simply force the premix at high pressures (generally 10-100 MPa or 1500-15000 lb in^{-2}) through a narrow orifice or valve. The third method is breaking up the droplets by forcing the emulsion at high pressure through a valve, creating turbulence and a very high shear force (Robinson and Wilde, 2003).

2.6.3 Mechanism of Emulsion Stability

Emulsion stability can be defined as the resistance of the physicochemical properties of the emulsion against changes that occur over time (Figure 2.13). The stability of the emulsions may deteriorate due to both physical and chemical varied processes. While the chemical instability occurs from modifications in the chemical structure of molecules; the physical instability occurs from modifications in the structural organization of the molecules or their mechanical distribution. While creaming, phase separation, flocculation, partial coalescence and Ostwald ripening are instances of the physical instability (Dickinson and Stainsby, 1982; Dickinson, 1992; Swaisgood, 1996; Walstra, 1996; Walstra, 1996), hydrolysis and oxidation are instances of chemical instability of emulsions (Fennema, 1996).

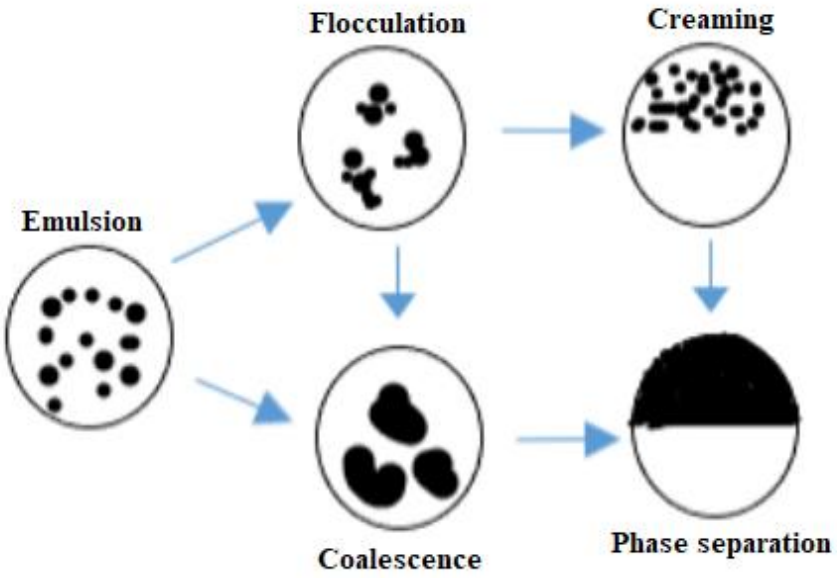

Figure 2.13 Breakdown mechanism of an emulsion (McClements, 2015)

2.6.3.1 Creaming

Creaming of emulsion which is inversely proportional to the viscosity is related to the Stokes equation. In the Stoke equation, if the density of the dispersed phase is lesser than density of the continuous phase, droplets move upward and accumulate on the top of the emulsion (Figure 2.14).

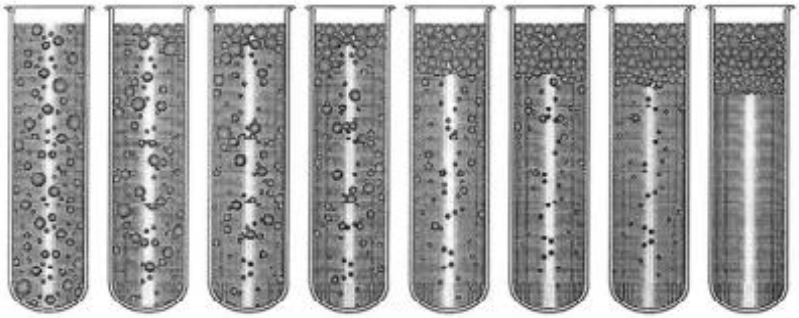

Figure 2.14 Change of creaming formation in emulsion over time (McClements, 1999).

At the same time, creaming is accepted to be the movements of droplets that are not the same size and these movements affect each other and the droplets are deformed thus the creaming occurs. The droplet diameter of the dispersed phase and the density of the continuous phase are factors that directly influence the creaming rate

to assign the stability of the emulsion. Large droplets in the dispersed phase undergo creaming faster than small ones. By decreasing the droplet diameter and the density of the continuous phase, the creaming rate may be minimized (Martin et al., 1993). The creaming and sedimentation mechanism is similar due to density difference but in sedimentation, droplets move to the bottom of the emulsion (Tadros, 2013).

2.6.3.2 Flocculation

The flocculation is process that can occur before, during, or after creaming. It is defined as the reversible aggregation of internal phase droplets which occurs if the attractive forces between the particles are stronger than the driving forces (Figure 2.15) (Walstra and Vliet, 2003).

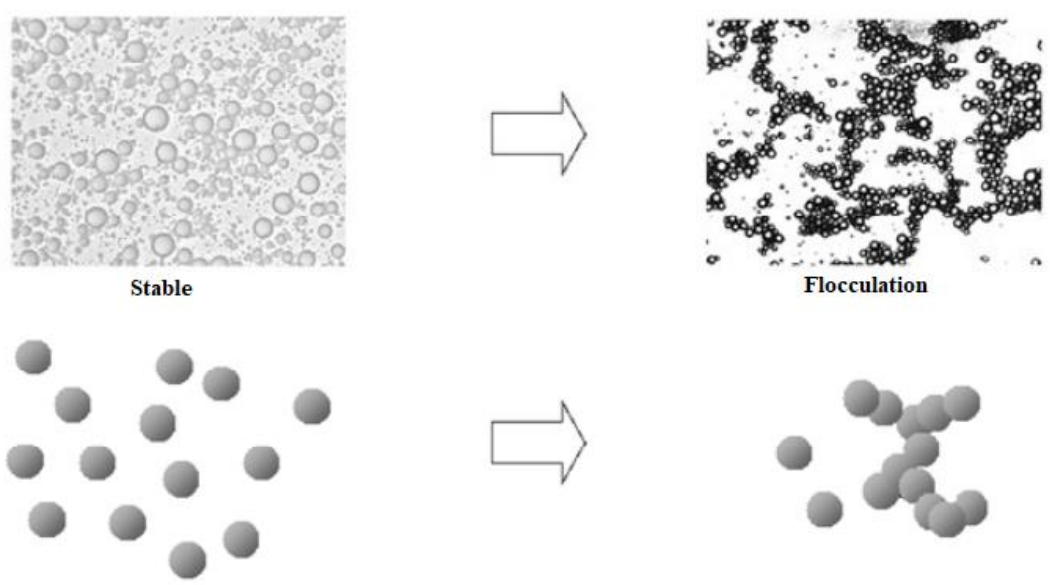

Figure 2.15 Difference between stable emulsion and flocculation emulsion (McClements, 1999).

Flocculation increases the gravitational decomposition rate in diluted emulsions, which is unwanted because it decreases the shelf-life of the product (Tan et al., 2004). Instability resulting from flocculation is closely related to the viscosity and shear stress of the emulsion. High viscosity prevents movement of droplets and causes flocculation (Im-Emsap and Siepmann, 2002). In addition, flocculation may increase the emulsion viscosity and cause gel formation. Flocculation has a direct impact on the properties of the emulsion such as texture, taste, appearance, and shelf life (McClements, 2004).

2.6.3.3 Coalescence

Coalescence is a stage in which two or more droplets combine to create larger droplets in the emulsion system. It causes average droplet diameter growth and ultimately decomposition of the water and oil phase (Figure 2.16).

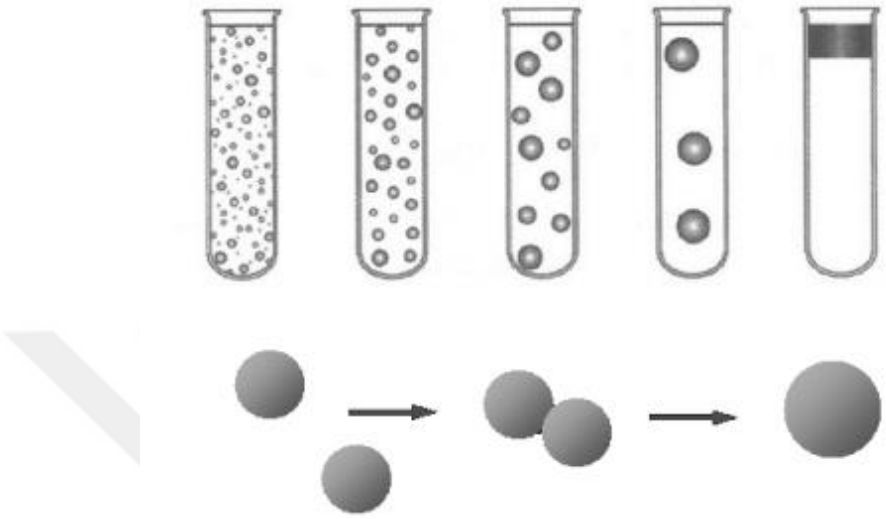

Figure 2.16 The coalescence of the droplet leads to complete division of the oil and water phase (McClements, 1999).

The basic principle of coalescence is the desire of the emulsion system to be thermodynamically most stable because in this case the contact area between the water and oil phase is reduced. As the diameter of the emulsion droplets increases with coalescence, sensitivity to creaming and sediment increases. While coalescence causes the creation of an oil layer on the surface of the product in O/W emulsion system, in the W/O emulsion system, it causes the water phase to accumulate at the lower part of the emulsion (McClementts, 2004).

2.6.3.4 Phase Separation

Phase separation can be defined as the exchange between continuous and disperse phase (Figure 2.17). It can usually form with the variation of proportional and environmental factors such as mechanical agitation, volumetric fraction of the dispersed phase, emulsifier type and concentration and temperature.

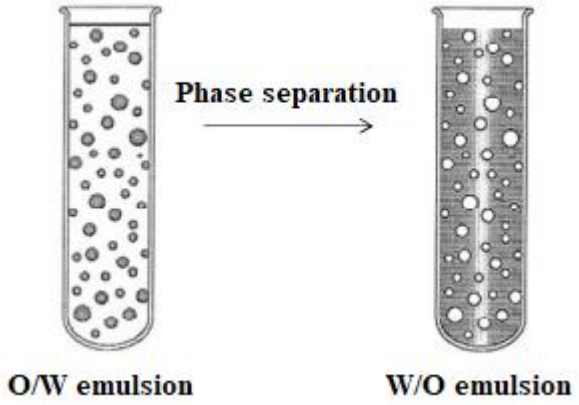

Figure 2.17 Phase separation results from the displacement of the continuous and disperse phase (McClements, 1999).

2.6.3.5 Ostwald Ripening

In simple terms, Ostwald ripening is the diffusion of the dispersed phase over the continuous phase due to mass transfer. It concludes from the solubility differences between smaller and larger droplets (Figure 2.18) (Aslan, 2015).

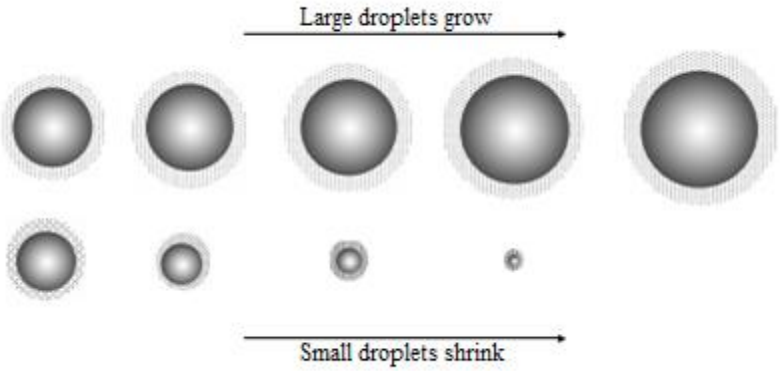

Figure 2.18 Schematic representation of Ostwald ripening in emulsions systems (Weer, 1998).

Ostwald ripening is negligible in many food emulsion systems due to the low mass transfer rate of the multiple solubilities of triacylglycerol and water (Dickinson and Stainsby, 1982).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The PH waste from de-hulling processing of pistachio nuts was obtained from a company in Gaziantep, Turkey and held at 4 °C until used to prevent biodegradation. The collected hulls were dried in the oven at 40 °C for 24 hours. The dried PH waste was ground. Then, the obtained powder was packed in dark bags and held it in dry environment prior to the experimental analyses.

Sunflower oil utilized for preparation emulsion was supplied from Zer Group Food, Gaziantep, Turkey.

Ethanol, citric acid, sodium azide (purity ≥99.5%), $CaCI_2$ (assay: 99-103%), SDS (Sodium Dodecyl Sulfate) pellets (≥99%), and all other chemicals used in this study were of analytical or chromatographic grade purchased from Sigma – Aldrich.

3.2 Extraction of Pectin from Pistachio Hull

In this study, the pectin was extracted from PH utilizing two different extraction methods, conventional extraction (CE) and ultrasound assisted extraction (UAE) methods.

3.2.1 Conventional Extraction of Pectin

In conventional extraction, the extraction was performed according to the method proposed by Jafari et al. (2017) with some modifications. The utilized conditions for the conventional extraction method of pectin were 0.5, 90°C, 30 min and 50 v/w for pH, temperature, time and liquid/solid ratio, respectively. Two different extraction solvent, distilled water, and citric acid solutions (≥99.7%) were tried separately as extraction solvents. 250 ml solvent (distilled water or citric acid solution, separately)

was mixed with 5 g of dried powder of PH. Then, the pH of the solution was set to 0.5 with 0.1 N HCI. The extraction was performed by stirring by magnetic stirrer at 90 °C for 30 min. At the end of the extraction time, the mixture was cooled to room temperature and then filtrated to separate the insoluble material. Then, an equal volume of ethanol 98% (v/v) was added to the solution to precipitated pectin and the solution was stored for 16 h at refrigerator temperature (about 5 °C). In the following step, the supernatant was separated by centrifugation (10,000 rpm for 15 min) (Centrifuge 5810 R, Eppendorf AG, Germany), washed two times with ethanol 98% (v/v). Finally, it was dried in a vacuum oven at 70 °C. The pectin yield of PH was calculated as follows;

$$Extraction\ yield\ (\%) = \frac{m_1\ (g)}{m_2(g)} x100 \quad \textbf{Eq 3.1}$$

where m_1 is weight of dried pectin (g) and m_2 is weight of dried pistachio hull (g).

3.2.2 Ultrasound-Assisted Extraction of Pectin

In ultrasound-assisted extraction, the extraction was applied by using a Bandelin SONEREX DIGIPLUS DL-255 H (Bandelin Corporation, Deutschland). The used conditions for the ultrasound-assisted extraction method of pectin were; 35 kHz, 100%, 0.5, 90°C, 30 min and 50 v/w for ultrasonic frequency, power setting, pH, temperature, time and liquid/solid ratio, respectively. Two different extraction solvent, distilled water, and citric acid solutions (≥99.7%) were tried separately as extraction solvents. 250 ml solvent (distilled water and citric acid solution, separately) was mixed with 5 g of dried powder of PH. Then, the pH of the solution was set to 0.5 with 0.1 N HCI. At the end of the extraction, the mixtures were processed in further steps as explained in the conventional extraction method of pectin. The pectin yield of PH was calculated by using Eq 3.1.

The results (yield and pectin quality) of those two extraction methods were compared to decide the pectin extraction method that will be used for the rest of the study.

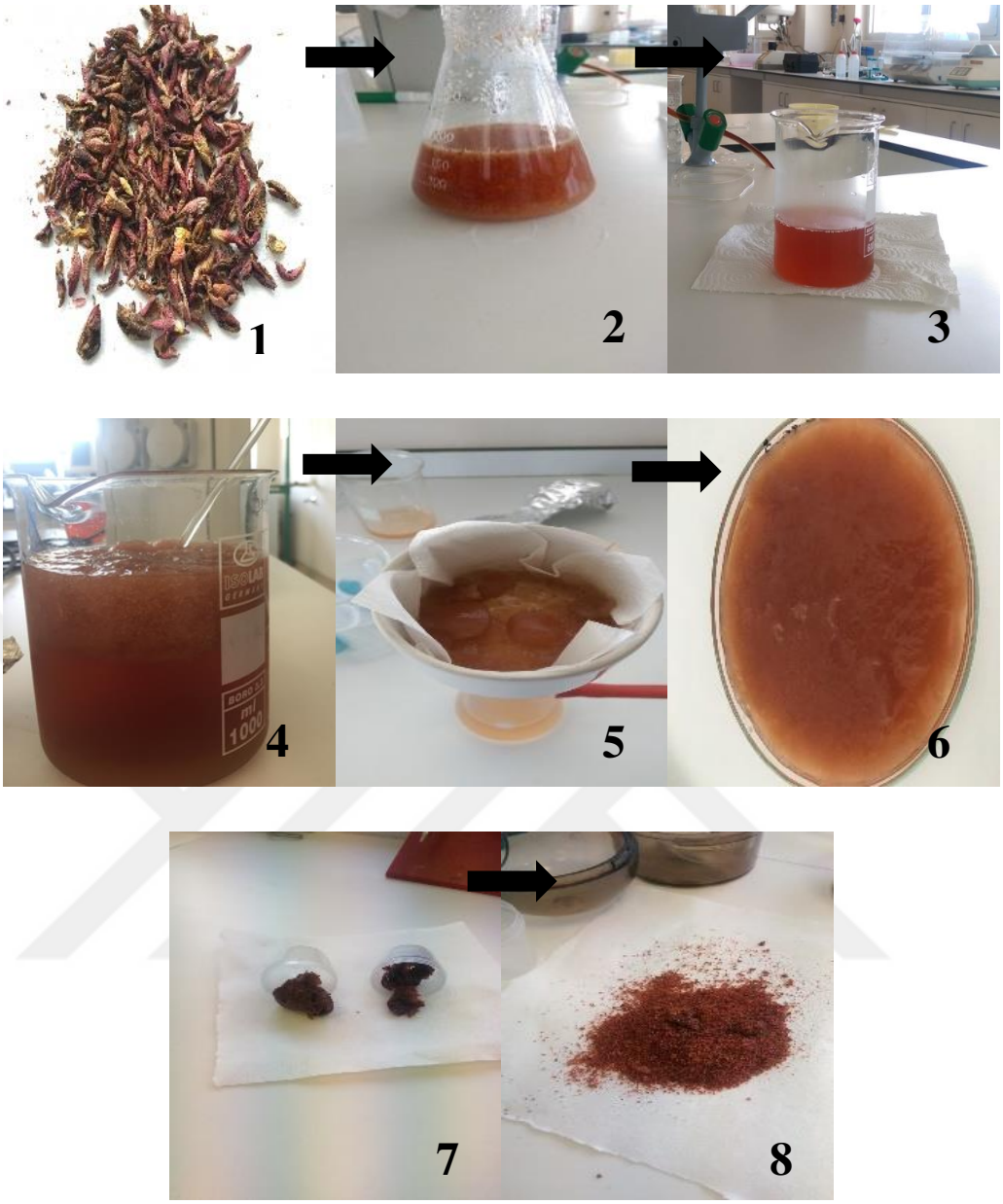

Figure 3.1 Schematic representation of pectin extraction from PH by CE method; 1: dried PH, 2: extraction, 3: filtrated extraction solution to separate insoluble materials, 4: precipitating with ethanol, 5: filtrated pectin after precipitating, 6: accumulating pectin in a petri dish for drying, 7: dried pectin, 8: powder pectin

3.3 Analytical Techniques

3.3.1 Determination of Moisture Content

The moisture content of PH pectin was estimated by the oven method according to Official method of analysis (AOAC, 1995). First, petri dishes were kept at 105 °C in

the oven (JSOF-100, Korea) until constant weight attain. Then 1 g of pectin sample was weighed into the petri dish and dried at 105 °C until constant weight. The analyses were done in triplicate.

3.3.2 Determination of Ash Content

The ash content of PH pectin was determined according to AOAC (1995). 1 g of pectin sample was weighted and placed in a furnace at 550 °C for 6 h. Then, the final weight was measured after cooling in a desiccator. Ash content was calculated from the mass of the residue after this procedure. The analyses were done in triplicate.

3.3.3 Determination of Protein Content

The protein content of PH pectin was estimated according to Kjeldahl Method. 1 ± x g of pectin sample was weighted into digestion flasks which boiling chips are present. Then, 7 g potassium sulphate and 0.01 g copper sulphate were added. Next, 12.5 ml of sulphuric acid was added and then the digestion flasks were placed into the digestion unit (400 °C for 40 min.). After the completion of the digestion, the flasks were placed into the distillation apparatus (Kjeltec 2200 Auto Distillation Unit, Salford, United Kingdom) to complete the distillation stage. Then the obtained distillate was titrated with 0.1 N HCI and recorded the used volume. Finally, the protein content of the pectin sample was calculated (factor 6.25). The analyses were done in triplicate.

3.3.4 Degree of Esterification and Galacturonic Acid Content

The degree of esterification (DE) and galacturonic acid (GalA) content of pectin samples were determined by the titrimetric method mentioned in Food Chemical Codex (FCC, 1981) with some modifications. First, pectin powder (250 mg) was soaked with 2 ml ethyl alcohol and then dissolved in 50 ml distilled water at 40 °C for 2 h. Then completely dissolving the samples, five drops of reagent (phenolphthalein) were added and the solution was titrated with 0.1 N NaOH. The volume of NaOH consumed to reach to end-point was recorded as the first titer or V_1. Next, 10 ml of 0.1 N NaOH was added for hydrolysis, the solution was stirred for 15 min. Then, 10 ml of 0.1 N HCI was added and the solution was shaken quickly until the pink color fully disappeared. After adding five drops of phenolphthalein reagent, the solution was titrated again with 0.1 N NaOH until a pale pink color was

obtained. The volume of NaOH used for this second titration was recorded as the second titer or V_2. The DE and GalA content of pectin was calculated according to Equation 3.2 and 3.3, respectively. The analyses were done in triplicate.

$$Degree\ of\ Esterification\ (\%) = \frac{V_1}{V1 + V_2} x100 \quad \boldsymbol{Eq.3.2}$$

$$Galacturonic\ acid\ content\ (mg) = 194.14\ mg\ mol^{-1} x(V_1 + V_2) ml\ x\ 0.1\ N \quad \boldsymbol{Eq.3.3}$$

3.3.5 Total Phenolic Content by Follin-Ciocalteu's Assay

The Folin-Ciocalteu method was used to determine total phenolic content (TPC) in the pectin sample, according to the method used by Singleton et al. (1999) with some modifications. Extracts (450 µL) were mixed with 2.25 mL of Folin-Ciocalteu reagent, already diluted with distilled water (1:9 v/v). After 3 minutes of shaking at room temperature, 1.8 mL of sodium carbonate solution (75 g/L) was added to samples. Then samples were left in dark for 2 hours at room temperature to react. Phenolic compounds were detected spectrophotometrically (Optima SP 3000 nano, Japan) at a wavelength of 760 nm. Gallic acid solutions (450 µl) in the concentration range of 10-90 µg/ml were prepared and subjected to the above reactions to the plot calibration curve (Figure 3.2). TPC values were stated as gallic acid equivalents (GAE) in mg per g of the dry matter. The analyses were done in triplicate.

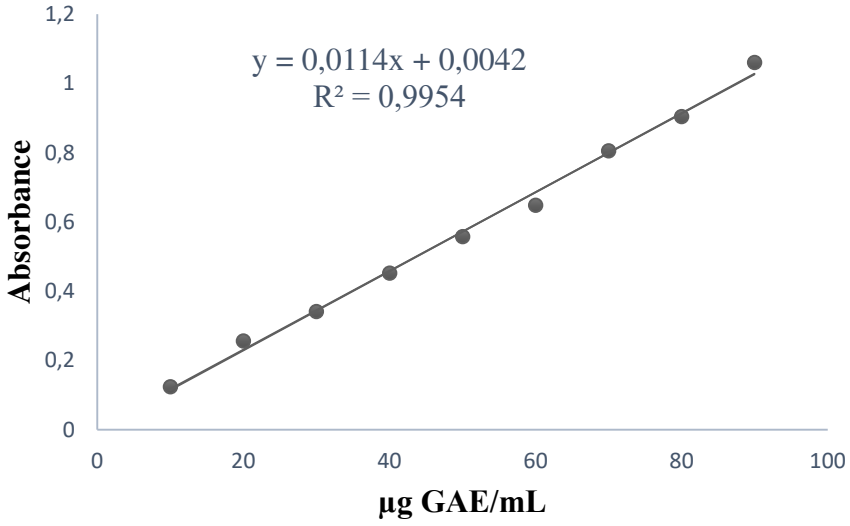

Figure 3.2 Gallic acid solution calibration curve

3.3.6 Total Sugar Content by DNS Method

Preparing DNS reagent; the 0.5 g of DNS was taken and dissolved in 25 mL of distilled water. Then, 15 g of Na-K was added into the solution, slowly. After 10 mL of 2 N NaOH was added and the solution was completed to 50 mL with distilled water. Then, the DNS reagent was stored at 4°C.

Preparing the pectin solution; 1 g sample was taken and diluted in 50 mL distilled water. Then, 1 ml of solution was taken to the test tube and mixed with 3 mL of DNS reagent. After that, 0.50 mL of potassium tartrate (5 N KOH) solution was added into test tube. It was warmed in a water bath at 90 °C for 10 minutes. The tube was put in a cold water bath at 4 °C and waited until cooled to room temperature. The absorbance measurements at 540 nm were taken with a spectrophotometer (Optima SP 3000 Nano, Tokyo, Japan).

Glucose solutions at various concentrations (0.2 – 3 mg/mL) were prepared to form a calibration curve and the results were given as mg glu/g dry matter. The analyses were done in triplicate.

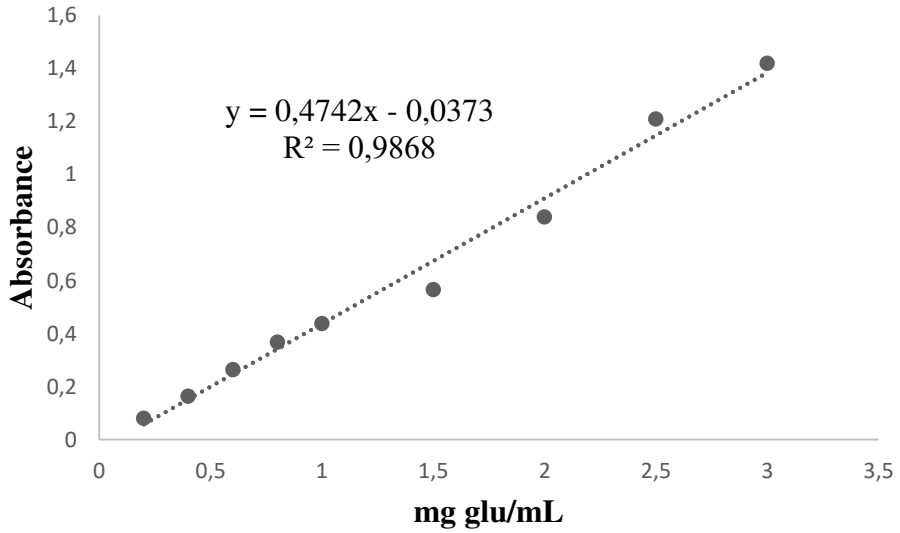

Figure 3.3 Glucose solution calibration curve

3.3.7 Determination of Acidity

The titratable acidity of samples was determined according to the standard method of AOAC (1990). Firstly, 2.5 g of sample was taken and dissolved in 25 ml of distilled

water. After, 2 or 3 drops of phenolphthalein indicator were added and titrated with 0.1 N NaOH. The analyses were done in triplicate.

3.4 Preparation of Oil-in-Water (O/W) Emulsions

Oil-in-water (o/w) emulsions were made by 20.0% (w/w) sunflower oil and 80.0% (w/w) of continuous aqueous phase. Pectin solution were prepared separately at six different pectin concentrations as 2, 4, 5, 6, 8 and 10% w/w in final volume (Figure 3.4). PH pectin was weighed as 2 g, 4 g, 5 g, 6 g, 8 g, and 10 g respectively and dissolved in distilled water. Sodium azide was added to all emulsions at 0.01% to prevent microbial growth and $CaCI_2$ was added to all emulsions at 30 mg $CaCI_2$ / 1 g pectin to create gelation. They were stirred at 300 rpm for 30 min at 80 °C. The temperatures were monitored using a thermometer. After the pectin melted completely, 20 g of sunflower oil was added to the pectin solutions. Then the samples were homogenized with a high shear homogenizer (model IKA, T18 Digital Ultra-Turrax) at 25,000 rpm for 5 min. The composition of emulsions is given in Table 3.1 respectively. Emulsions were prepared in duplicate.

Table 3.1 The composition of pectin, sunflower oil emulsions (w/w)

SAMPLE	PECTIN (%)	WATER (%)	SUNFLOWER OIL (%)
S1	2	78	20
S2	4	76	20
S3	5	75	20
S4	6	74	20
S5	8	72	20
S6	10	70	20

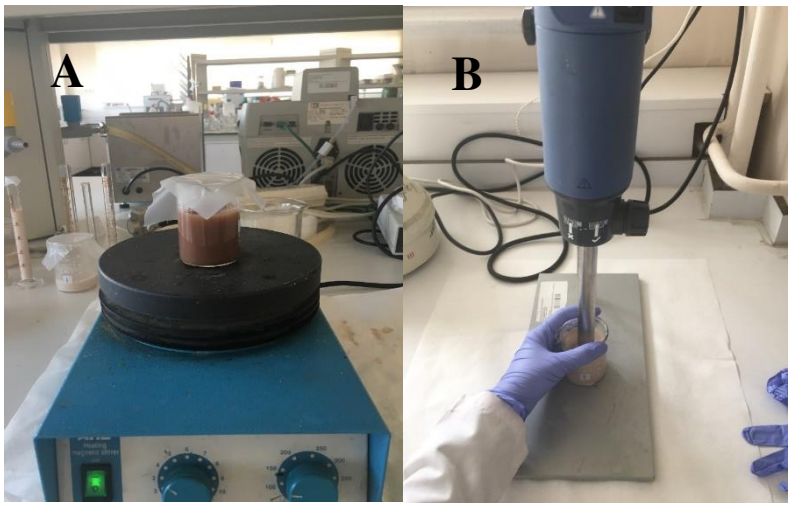

Figure 3.4 Preparation of O/W emulsion; A: dissolved of pectin, B: homogenization

3.5 Emulsifying Properties of Pistachio Hull Pectin

3.5.1 Emulsifiying Activity Index and Emulsion Stability Index

The emulsifying activity index (EAI) and the emulsion stability index (ESI) of the emulsion were determined by the following the Pearce and Kinsella (1978) method with some modifications. 50 µl of the freshly made emulsion was taken by a pipette from the bottom of the beaker at intervals of 0 and 10 minutes. Then, they were diluted with 50 ml of 0.1% (w/v) SDS solution. The absorbance measurements of the diluted emulsions were taken by using a spectrophotometer at 500 nm (Pharmacia Biotech, Novaspec II, Cambridge, UK) in cuvettes. After emulsion preparation, the EAI was determined from the absorbance at 500 nm.

Further, the ESI was calculated from the measurement of absorbance of the emulsion as a time-dependent. The results of the experiment were stated as surface area (m^2) per unit weight (g) of pectin used in the emulsions. The experiments were done in duplicate.

$$EAI = \frac{2T}{\emptyset C}$$

where C is the weight of pectin per unit volume of the aqueous phase before emulsion formed and $\emptyset$ is the volumetric fraction of oil. T is turbidity and $T = 2.303\ x\ A/l$, A is the observed absorbance, l is the path length of the cuvette in cm.

$$ESI(min) = \frac{A_0}{A_0 + A_{10}} x10$$

where A_0 and A_{10} are the absorbance of the diluted emulsion at 0 and 10 min, respectively (Wang et al., 2008).

3.5.2 Creaming Stability

The creaming stability of emulsions was determined by following the method proposed by Anton et al. (2000). 10 ml of the emulsion was taken from prepared emulsion samples to the graduated cylinder separately. After the graduated cylinders were labeled and sealed they were stored at room temperature for a week. The cream separation was expressed as a percentage of the volume of the creaming part over the total volume of the emulsion in the cylinder. The experiments were replicated in duplicate.

$$Creaming\ (\%) = \frac{V_c}{V_t} x100$$

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

3.5.3 Optical Microscopy

The emulsions were observed by using polarized light microscopy (PLM) (Olymous BX 51 model, Tokyo, Japan) and the microscopic images of emulsions were taken with a camera (Pixera PVC 100C, USA). One drop of the freshly made emulsion sample was put on a microscopic slide and enclosed with a coverslip, carefully. Then it was replaced onto the microscope. After a few minutes, images of emulsion were taken with a 20X magnification objective. All emulsion images of different pectin ratio emulsions were recorded.

3.5.4 Droplet Size Measurement

The mean particle size of emulsion was determined using a Mastersizer 3000 laser light scattering instrument (Mastersizer 3000 Malvern, Worcestershire, UK). This instrument uses a laser diffraction technique to measure the mean particle size distribution. The refractive index value of sunflower oil was taken as 1.4. The

average droplet sizes were characterized in terms of volume mean diameter d_{43} defined by;

$$DeBroukere\ Mean: D[4,3] = \sum N_i D_{i4} / N_i D_{i3}$$

where D_i value is the geometric mean (the square root of upper*lower diameters) of diameters, N_i is the number of particles in emulsion with diameter D_i.

3.5.5 Rheological Measurement

Dynamic rheological behavior of the emulsion samples was analysed in the shear rate range of 1-200/s at 25 ± 0.01°C by using Haake Rheostress RS-1 (Karlsruhe, Germany) equipped with TCP/P Peltier temperature controller unit. Measurements were carried out in a cone-plate configuration (cone diameter 35 mm, angle 2°). 2 ml of the emulsion was taken and placed onto the plateau of the rheometer for each rheological measurements, carefully. The consistency coefficient and flow behavior index values of the all emulsion samples were determined. All data analysis were made by software (Reowin Pro Data Manager Version 2.64).

3.5.6 Statistical Analysis

Results were compared by one-way analysis of variance (ANOVA) to test for significant differences. Duncan multiple range test was used to determine that the data is significantly different from each other (Version 25). The results were evaluated at $p \leq 0.05$ significance level.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Comparison of Extraction Efficiencies

In one of the recently published study, Chaharbaghi et al., (2017) studied pectin extraction from PH and consequently determined the optimal conditions to extract pectin from the hull. In this study, the optimal extraction conditions were reported as 0.5, 90°C, 30 min and 50 v/w for pH, temperature, time, and liquid/solid ratio, respectively. Hence, in the present study, pectin extraction from PH was tried under these optimal conditions using two different methods, conventional and ultrasound-assisted. Conventional and ultrasound-assisted extraction of pectin was carried out utilizing distilled water and citric acid solution, separately. The pectin yields obtained are given in Table 4.1 according to extraction methods and used solvents.

Table 4.1 The pectin yield according to extraction methods and solvent type.

Extraction Method	Solvent (ml)	Yield (%)
Conventional	Citric acid (250)	32.3±1.44
	Distilled water (250)	5.96±0,92
Ultrasound Assisted	Citric acid (250)	23.64±1.28
	Distilled water (250)	5.56±0.25

The highest pectin yield (32.3%) was obtained using the citric acid solution in the conventional extraction method, while the lowest yield (5.56%) was obtained using distilled water in the ultrasound-assisted extraction method. According to the results, it has been observed that the conventional extraction method using the citric acid solution is more efficient. In one of the studies, Chaharbaghi et al., (2017) extracted pectin from PH using by conventional extraction method and they pectin obtained yield as 22.01±0.5%. In the other study, Kazemi et al., (2019) extracted pectin from

PH using by microwave-assisted extraction method and they pectin obtained yield as 18.13%. The possible reasons for differences may be parameters such as variety and maturity of raw material, harvesting time, climate, soil conditions or etc.

4.2 Characteristics of Pectin Extracted by Conventional Method

Some physical, chemical and emulsifying properties of pectin obtained by conventional extraction at optimal conditions were discussed in this section.

4.2.1 Moisture content

The moisture content of pectin extract powder at optimum conditions was calculated as 4.9±0.062 %. Generally, when the moisture content is less than 6 % in food systems, the microbial and biochemical reactions could be prevented and, also the degradation of the product can be inhibited (Tan et al., 2015). Pectin with low moisture content is preferable as it prevents the growth of microorganisms that can influence the pectin quality due to the production of pectinase enzymes and is suitable for storage (Ismail et al., 2012). Therefore, pectin obtained powders in this study can be considered resistant to microbial growth and thus to degradation of pectin quality as a result of its low moisture content. In another study extracted pectin from PH, the moisture content was performed as 12.2% (Kazemi et al., 2019). In the literature, it was seen that the moisture content of the pectin varies according to the source from which they were obtained. Some reported moisture content values are as follows 7.88% for white grapefruit peel pectin, 8.96% for red grapefruit peel pectin (Mohamed, 2015), 10.05±0.10 for citric waste (Gama et al., 2015), 8.9% for quince fruits (Açıkgöz and Poyraz, 2006), 8.5-11% for apple pomace (Sharma et al., 2014).

4.2.2 Ash Content

Ash content is an indicator of mineral substances in food (Acquistucci et al., 1991) and low ash content is an important criterion for the purity of the pectin (Miyamoto and Chang, 1992). The ash content of pectin extract powder in this study was obtained as 1.49±0,026 %. The ash content of pectin varies depending on the pectin source and the type of the extraction solvent. According to the study of Ismail et al., (2012) the low ash content (below < 10) in pectin is good for gel formation. Therefore, it can be concluded that the amount of 1.4% ash for the pectin obtained in

this study is very suitable for high quality gel formation. In another study, the ash content in PH pectin was determined as 5.16 % (Kazemi et al., 2019).

4.2.3 Protein Content

The presence of the protein in the pectin plays a major functional role in enhancing the emulsification properties (McKenna et al., 2006). The PH pectin in this study had 1.85±0.14 % protein content. However, in another study, protein content in PH pectin was determined as 6.3 % (Kazemi et al., 2019).

The pectin source, differences in extraction methods, extraction conditions and extraction solvent are some of the factors that affect the protein content of pectin extracted. Some reported protein content values are as follows 6.97% for potato pulp pectin (Yang et al., 2018), 9% for sugar beet pectin (Karnık, 2008), 3.6% for cacao pod husks pectin (Vriesmann et al., 2011), 6% for apple pomace pectin (Marcon et al., 2005), 1.10% for citrus pectin (Mesbahi et al., 2005).

4.2.4 Degree of Esterification and Galacturonic Acid Content

The functional properties of pectin are based on the degree of esterification (Walter, 1991). The presence of methoxyl groups in the pectin molecule gives the methoxyl degree of the pectin. Based on the degree esterification, there are two types of pectin; high metoxyl (higher than 50%) and low methoxyl pectin (lower than 50%) (Morris et al., 2000). The results of the current study showed that DE of PH pectin is 19.29±0.41% and thus, it was under the low methoxy pectin group. In addition, the two studies performed so far reported that pectins obtained from PH are low methoxyl pectin (Chaharbaghi et al., 2017; Kazemi et al., 2019).

High galacturonic acid content is an important criterion indicating pectin purity (Hwang et al., 1992). According to FAO, the galacturonic acid content of good quality pectin should not be less than 65 % (Chaharbaghi et al., 2017). In the current study, the galacturonic acid content of pectin extracted was determined and results indicated that the amount was 65.81±1.51%. Thus, it can be considered that PH pectin has a suitable purity. In studies of Kazemi et al., (2019) and Chaharbaghi et al., (2017) reported that the content of galacturonic acid in PH pectin to approximately 65 % and 66 %, respectively.

4.2.5 Total Phenolic Content

Natural antioxidants are plant polyphenolic compounds which can be obtained from different parts of the plant (Rajaei et al., 2010). PH is a good source of natural phenolics and antioxidants (Al-Juhaimi et al., 2017). According to Bohluli et al., (2008), the phenolic contents and antioxidant activity of PH are more than those of the pistachio nut and skin. In the present study, the total phenolic content of PH pectin was examined and the total phenolic content was found as 6.03±0.08 mg gallic acid equivalents per g of PH pectin (mg GAE/g). In another research, the total phenolic content of PH pectin was analysed and found as 18.18 mg GAE/g pectin (Kazemi et al., 2019). The pistachios used for the study of Kazemi et al., (2019) were obtained from Alborz (Iran), while the PH used in this study was from Gaziantep (Turkey). Hence, the possible reasons for differences may be parameters such as variety and maturity of raw material, harvesting time, climate, soil conditions or etc.

Also, the literature reviews have shown that little research has been done on the total phenolic content of pectin. In one of the studies, Sharma et al., (2015) examined the total phenolic content of tamarind pectin from Tamarindus indica L. pulp and they found as 79.66 ±4.71 mg GAE/g pectin. In another study, Wang et al., (2015) examined the total phenolic content of grapefruit peel pectin and they determined as between 4.21-7.06 mg GAE/g pectin.

4.2.6 Total Sugar Content

The total sugar content of PH pectin was determined by the DNS method. As far we know, there is no specific search on the total sugar content of pectin extracted from PH. In this study, the total sugar content of PH pectin extracted was examined and found as 56.84±2.36 mg sugar/g pectin. In the literature, the total sugar content in pectin obtained from different sources was determined. For example, Yang et al., (2018) stated that the total sugar content in potato pulp pectin was found as 23.18-46.03 mg sugar/g pectin.

4.2.7 Acidity

Citric acid as an extraction solvent, has the advances of very low toxicity and low environmental effect, compared with the mineral acids (Xu et al., 2018). In the current study, pectin was extracted from PH using citric acid. As far we know, there

is no specific search on the acidity of pectin from PH. In the present study, the acidity of PH pectin extracted was determined as 38.94±0.17%. The high acidity content in pectin extracted can be associated with not exactly removed citric acid in pectin during the washing with ethanol.

4.3 Emulsifying Properties of Pectin

4.3.1 Emulsifying Activity Index and Emulsion Stability Index

Emulsifying activity index (EAI) and emulsion stability index (ESI) may mirror the capability of emulsions to adsorb at the interface and resistance against the o/w emulsions instability (Li and Xiang, 2019). In order to examine the activities of the emulsions, six emulsions were prepared using different concentrations of pectin (2, 4, 5, 6, 8 and 10%). As soon as emulsions are prepared, the EAI of emulsions was determined. In emulsions prepared using 2 and 4% pectin concentration, EAI value was not determined since phase separation was observed. EAI values of the emulsion samples were shown in Figure 4.1.

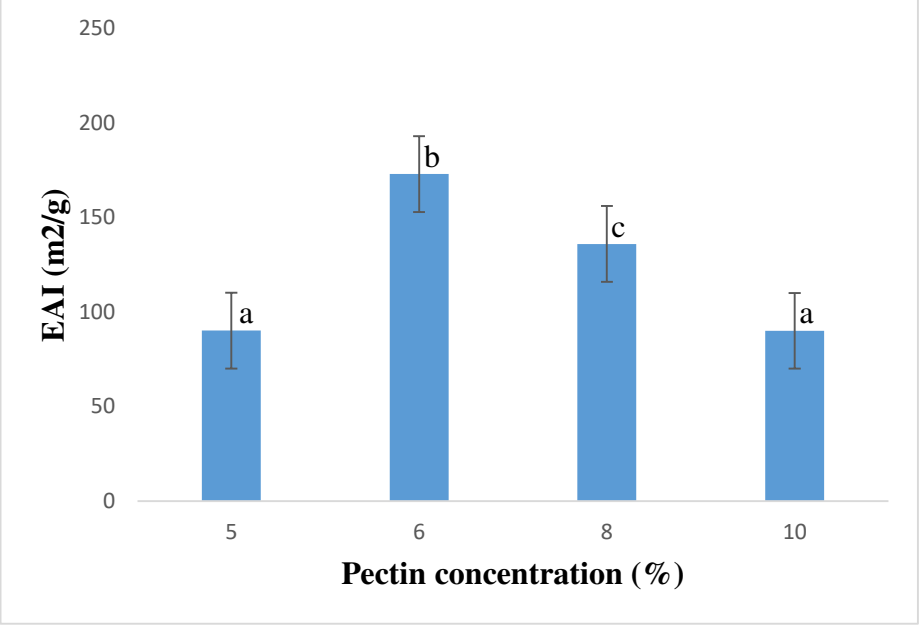

Figure 4.1 EAI values of emulsions at different pectin concentrations. (Different letters as a, b, c indicate statistically significant differences between pectin amounts ($p<0.05$))

The EAI of emulsions containing 5, 6, 8 and 10 % pectin were determined as 90.12, 172.85, 135.88 and 89.97 m^2/g, respectively. As seen in Figure 4.1, an emulsion

containing 6% pectin has the highest EAI value while emulsions containing 5% and 10% pectin have the lowest EAI value. Emulsifying properties of emulsifiers are associated with the surface hydrophobicity which is linked to the emulsifier's capability to adsorb at the oil/water interface. (Lam and Nickerson, 2013). Therefore, in the current study, the o/w emulsion prepared by 6 % pectin concentration can indicate more hydrophobic bonds and improve the hydrophobic properties. The results show that while there are no significant ($p>0.05$) differences between the emulsions containing 5 and 10 % pectin, there are significant ($p<0.05$) differences between other emulsions (Figure 4.1). Compared to 6 % pectin emulsion, the EAI value of emulsions including 5, 8, and 10 % pectin exhibited a significant decrease. Hence it can be concluded that the further addition of pectin can significantly reduce the emulsifying activity of the o/w emulsion and that the oil droplets had good dispersibility at certain concentrations of pectin.

The ESI of an emulsion determines the resistance of the change in the emulsion structure during the specific time period (Aryee et al., 2018). In the present study, the ESI values were determined using a spectrophotometer. The absorbance of the emulsions was measured rapidly at 0 and 10 min. In emulsions prepared using 2 and 4% pectin concentration, ESI value was not determined since phase separation was observed. The trends in ESI were similar to those of EAI. ESI values of the emulsions were shown in Figure 4.2.

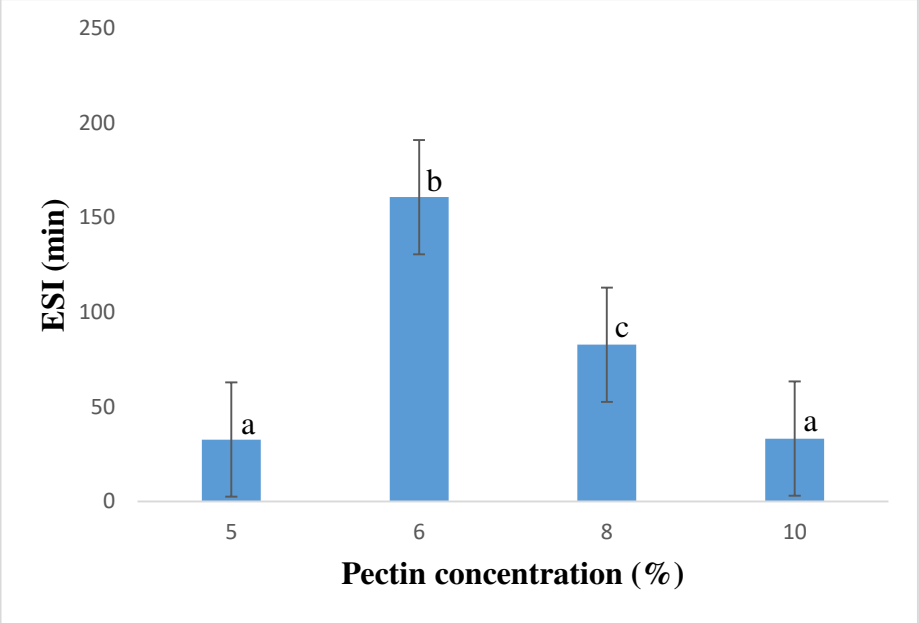

Figure 4.2 ESI values of emulsions at different pectin concentrations. (Different letters as a, b, c indicate statistically significant differences between pectin amounts ($p<0.05$))

The ESI of emulsions containing 5, 6, 8 and 10% pectin were determined as 32.67, 160.83, 82.82 and 33.12, respectively. As seen in Figure 4.2, while the highest ESI was observed in the emulsion containing 6% pectin, the lowest ESI value was observed in the emulsions containing 5 and 10% pectin. As it was seen in Figure 4.2, while no significant ($p>0.05$) difference was found in ESI value between emulsions prepared by 5 and 10 % pectin, a significant ($p<0.05$) difference was found between other emulsions. Emulsions prepared by 5, 8, and 10 % pectin have had lower ESI value than that of prepared by 6 % pectin.

The results indicate that, although the addition amount of pectin up to a certain value (6 %) can improve emulsion stability, more pectin addition after that certain value significantly reduce the emulsifying stability of the o/w emulsion. Consequently, the pectin concentration must be kept at an optimum level to prepare a stable emulsion. The ESI value of o/w emulsion is also a measure of resist against the coalescence (Aslan and Dogan, 2018). The emulsions especially containing 5 and 10 % pectin are more susceptible to coalescence. To wrap up, the most stable emulsion was prepared by 6% pectin addition.

4.3.2 Creaming Stability

Creaming stability is one of the important characteristics of emulsions. Creaming stability gives the volumetric percentage of the separated serum layer. Figure 4.3 shows the image of the emulsions at the end of 7-day storage. The creaming values of emulsion were illustrated in Figure 4.4.

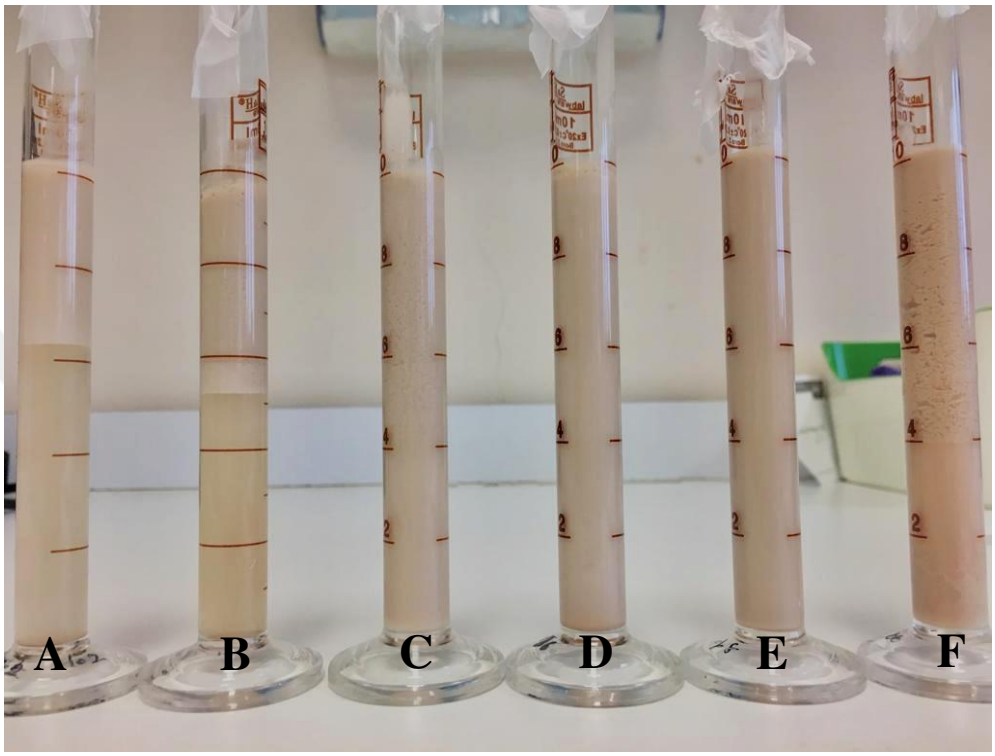

Figure 4.3 Image of emulsions at different pectin concentrations; A) 2%, B) 4%, C) 5%, D) 6%, E) 8% and F) 10% pectin

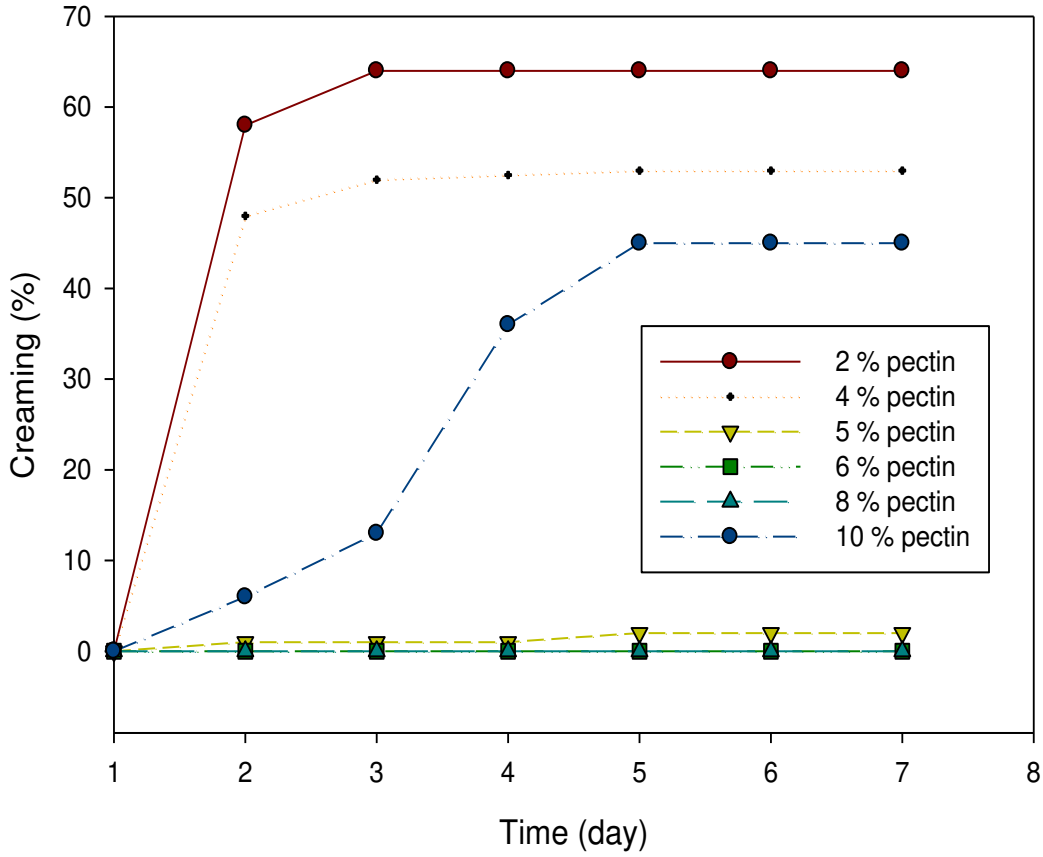

Figure 4.4 Creaming percentages of emulsions at different pectin concentrations.

The highest creaming value (64%) was obtained for emulsion containing 2% pectin, while the lowest one (0%) was observed for emulsions containing 6% and 8% pectin. As seen in Figure 4.4, both the increase and decrease in the amount of pectin in the emulsion have negatively affected the stability of the emulsions. While only serum layer was observed in the emulsion containing 2, 4 and 5 % pectin, both serum layer and flocculation were observed in the emulsion containing 10 % pectin. According to Blijdenstein et al. (2004), when polysaccharides were added to oil/water emulsions, they can cause depletion flocculation or bridging flocculation. While the depletion flocculation can occur in excess polysaccharide, the bridging flocculation can occur in less polysaccharide than optimum. Excess polysaccharides cannot be attached to the oil droplet and thus they start to deplete from the droplet surface. This depletion of polysaccharides from the surface of the oil droplets leads to their attractiveness between them and causes flocculation (Blijendenstein et al .2004). Based on this information, the reason for flocculation seen in the emulsion containing 10% pectin (Figure 4.3) can be explained by the use of high amounts of pectin. Finally, it can be

concluded that the emulsions containing 6 and 8 % pectin have better stability and the pectin concentration of them are at the optimum level.

4.3.3 Microscopic Images

The optical microscopy is the most commonly used kind of microscope to characterize droplet flocculation (McClements, 1999). The microscopic images of prepared emulsion samples were taken by a polarized-light microscope with 20X magnification and recorded to confirm the information obtained from the results of particle size measurements. The light microscope images were shown in Figure 4.5.

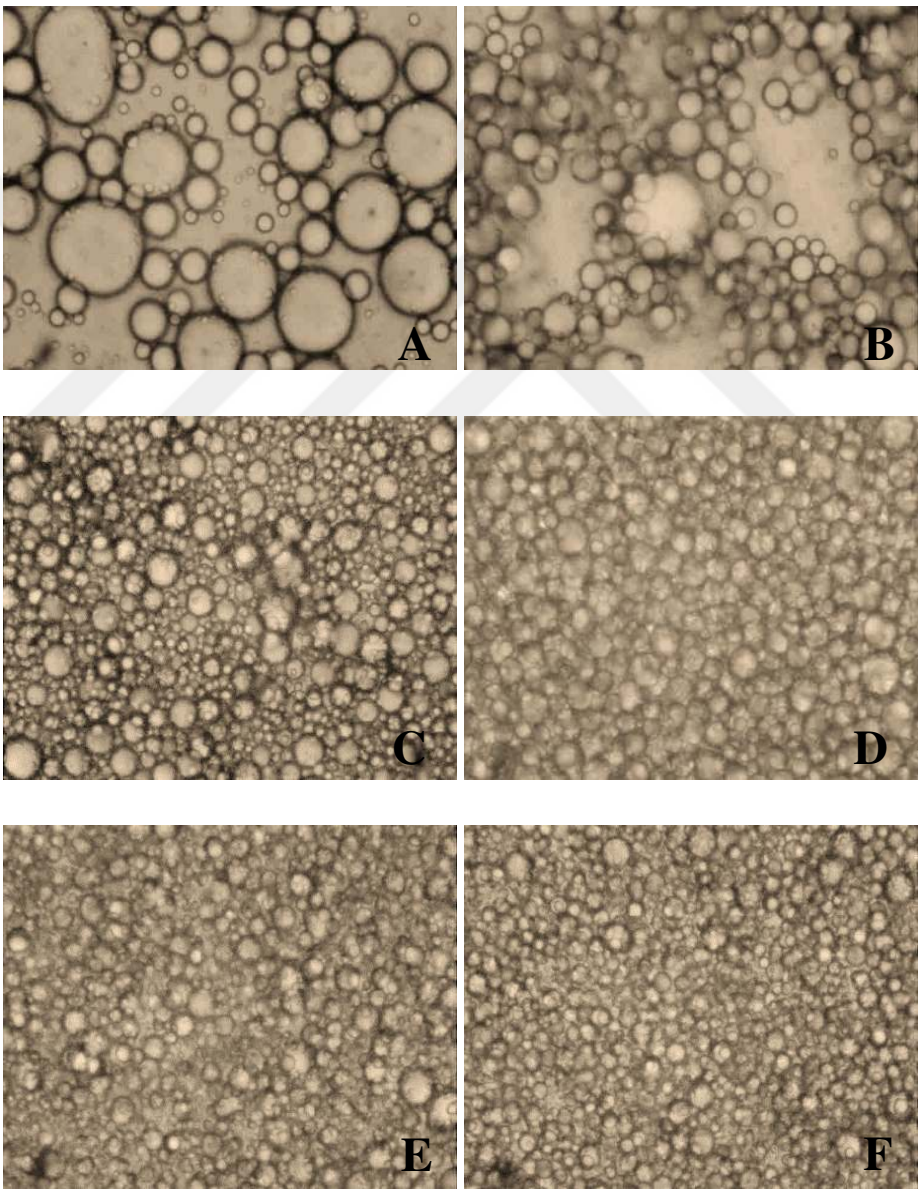

Figure 4.5 Microscopic images of emulsions obtained at 20X magnification: A) 2 %, B) 4 %, C) 5 %, D) 6 %, E) 8 % and E) 10 % pectin

As can be seen in Figure 4.5, A and B, emulsions prepared with 2 and 4 % pectin had larger droplet size due to insufficient saturation of oil with pectin. Additionally, the droplets were not in uniform size and they were not evenly dispersed. Moreover, agglomeration was observed, consistent with particle size. The emulsions prepared by using 5, 6, 8 and 10 % pectin have had more uniform droplet size compared to the emulsions prepared with 2 and 4 % pectin (Figure 4.5). Small droplet size is an important criterion for more stable emulsion. The smaller particle size the more stable emulsion. The microscopic images results of emulsions were found consistent with the stability results. The smallest droplet size was observed in emulsion prepared with 6 and 8 % pectin which were more stable ones.

4.3.4 Droplet Size Properties

Changing in droplet size of emulsions is one of the widely examined features to determine the physical instability of emulsions. Changes in droplet size are undesirable as they change the emulsion microstructure, which often negatively affects emulsion quality and product properties (Schmidt, 2016). According to Schmidt (2016), the higher amount of covalently bound protein to pectin has, the smaller the characteristic droplet size of the emulsions. In the current study, the droplet size of emulsion samples was measured using laser diffraction. The DeBrouckere Mean Diameter (Volume Moment Mean or D [4,3]) values and the volume distribution of droplets in emulsions were measured without storage and presented in Figure 4.6 and Figure 4.7, respectively.

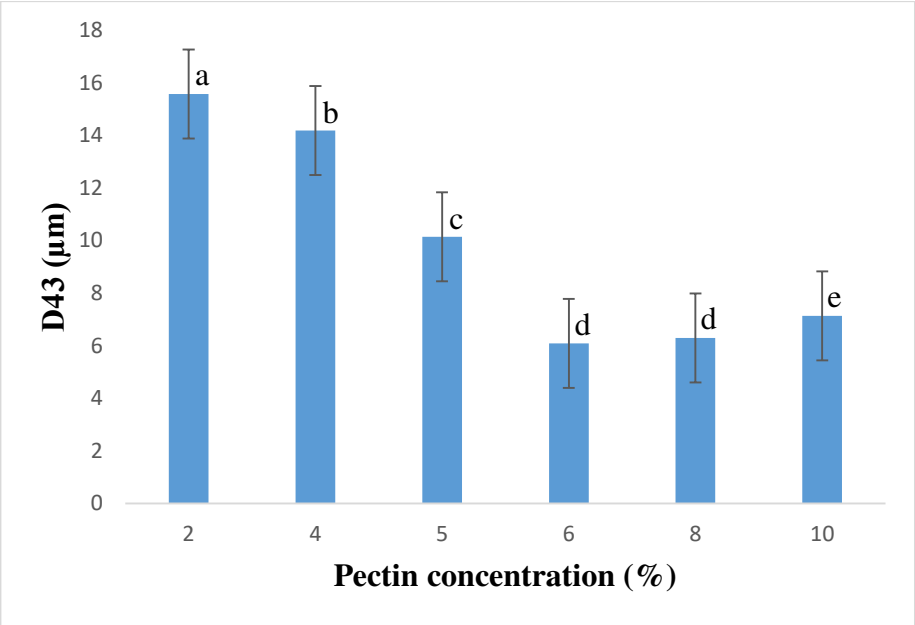

Figure 4.6 The DeBrouckere Mean (mean particle) size measurement results of emulsions at different pectin concentrations. (Different letters as a, b, c, d, e indicate statistically significant differences between pectin amounts ($p<0.05$))

As shown in Figure 4.6, while no significant ($p>0.05$) difference was found between 6 and 8 % pectin emulsion, a significant ($p<0.05$) difference was found between other emulsions. Thus, it can be said that pectin concentrations had a significant effect on the droplet size of emulsions. The particle sizes of emulsions were ranged between 6 and 15 µm. It is obvious that the emulsions with low pectin concentration have had larger droplet sizes than that of emulsions with high pectin concentration (Figure 4.6). Similarly, Akkaya (2019) reported that increase the pectin concentration resulted in a decrease in the mean particle size. In this study, increasing the pectin concentration up to 6 % in emulsions significantly reduced the droplet size, and the smallest droplet size was 6.08±0.04 µm at that concentration. However, it was seen that the addition of extra pectin after 6% caused droplet size growth.

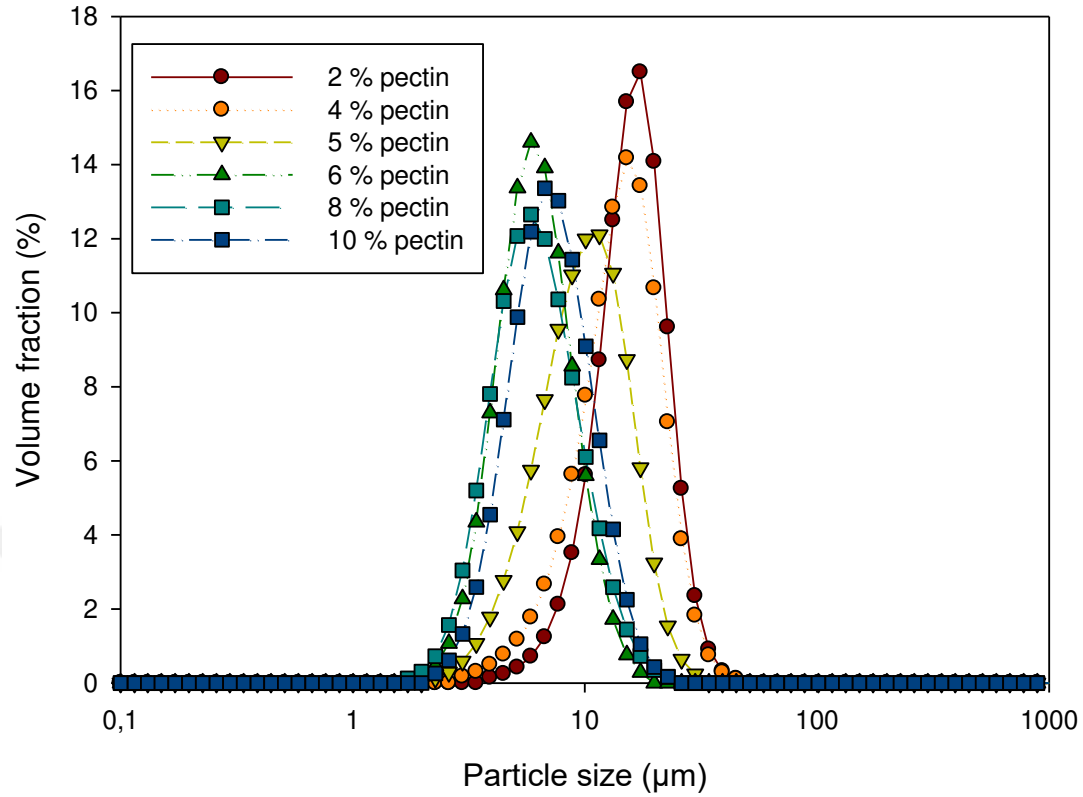

Figure 4.7 Particle size distribution graph of emulsions at different pectin concentrations

As can be seen in Figure 4.7, the particle size distribution of all emulsions prepared in this study indicated a monomodal distribution with a sharp peak in the presence of the pectin, and thus this result can indicate that they have good emulsifying properties. The distribution curves of emulsions including a lower pectin concentration shifted to the bigger particle sizes, by that resulting in a larger average particle size.

4.3.5 Rheological Properties

Rheology is an important science that studies the flow and deformation properties of matter. The science of rheology examines the change and deformation that occurs in the flow of the emulsion with a certain force applied the substances. Therefore, it is important to know the rheological behaviors of emulsions. There are two main categories of the flow behavior of fluid; Newtonian and Non-Newtonian. Newtonian fluids have the most simple flow features which their viscosity is independent to the

shear rate and the shear rate is directly proportional to the shear stress. However, the viscosity of Non-Newtonian fluids is affected and generally it decreases with increasing shear stress resulting in a shear-thinning behavior (Subramanian, 2002).

Pectin can play an important role in emulsification and emulsion stabilization and thusly can impact the rheological behavior of product (Ngouemazong et al., 2015). In the present study, the rheological behaviors of six different emulsion samples that had different pectin concentration were observed. According to the results obtained by examining the rheological properties of the prepared emulsions, the shear stress values against the shear rate of the samples were plotted and shown in Figure 4.8.

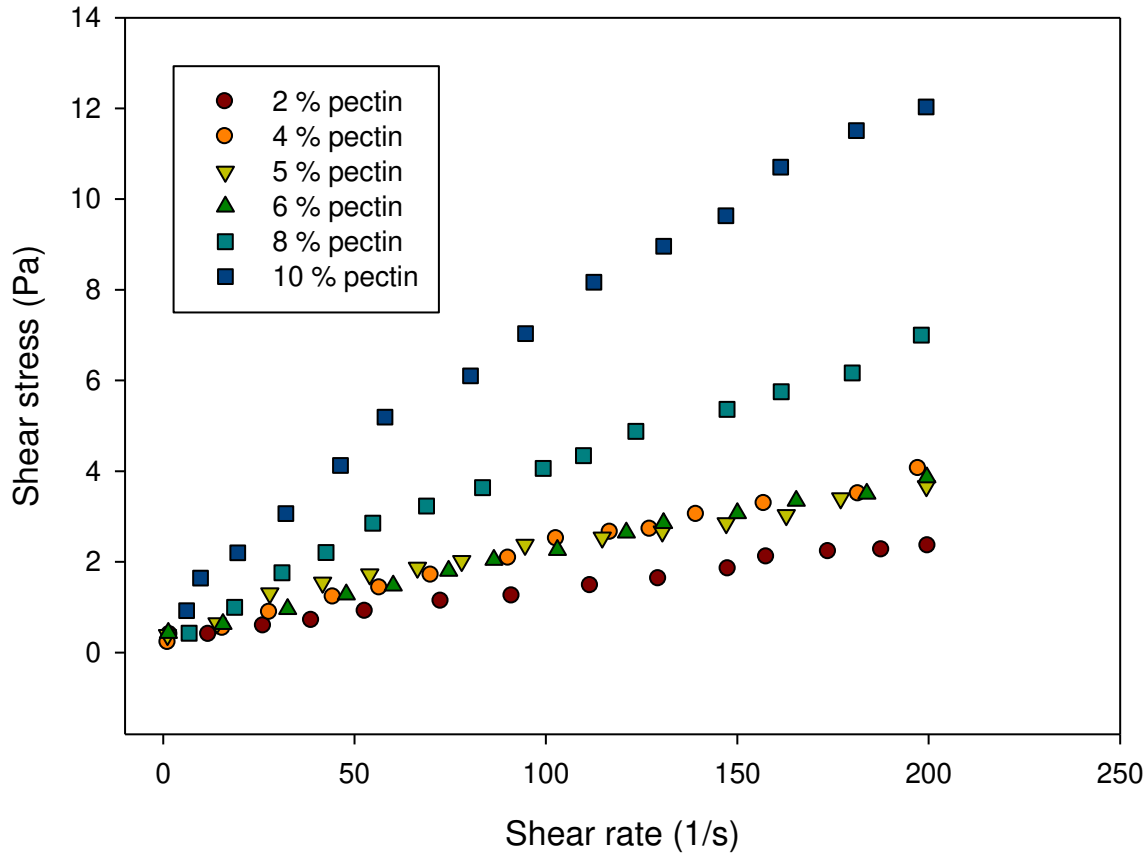

Figure 4.8 Shear stress as a function of shear rate and curves modeled by power law for all emulsions at different pectin concentrations

As a result of the measurements, it was detected that the viscosity of all prepared emulsion samples reduced with increasing shear rate. Emulsions exhibited as Non-Newtonian flow behavior and the data was modeled by Power Law model;

$$\tau = K.\gamma^{n}$$

where k is the consistency index, n is the flow behavior index. The k and n values of modelled flow curves are listed in Table 4.2.

Table 4.2 Rheological parameters of emulsions at different pectin concentrations

Pectin concentration (wt %)	**Oil concentration (wt %)**	**n**	**K**	**R^2**
2	20	0.732±0.029	0.054±0.001	0,9756
4	20	0,776±0.002	0.066±0.002	0,9937
5	20	0.769±0.012	0.067±0.002	0,9863
6	20	0.752±0.005	0.072±0.001	0,9906
8	20	0.742±0.011	0.138±0.003	0,9957
10	20	0.750±0.031	0.232±0.037	0,9981

The flow behavior n<1 shows a shear-thinning fluid, n=1 indicates a Newtonian fluid and n>1 shows shear-thickening. (Erçelebi and Ibanoglu, 2009). Power law can be generally applied to all Non-Newtonian fluids (Karnık, 2008). The consistency index (k) is a parameter that also indicates the viscous behavior of a fluid. As expected the consistency indexes increased with the addition of pectin (Table 4.2). It can be seen that the shear-thinning attitude was more prominent with increasing pectin concentration. When n values were considered, it can be said that all emulsion samples had shear thinning behavior (n<1). Similarly, McClements (2007) reported that food emulsions generally illustrate shear thinning behavior. It can be concluded that the rheology results obtained for emulsions in this study consistent with those mentioned in the literature. The reason for shear-thinning explains by the separation of flocculated droplets at high shear rates or getting droplets aligned to the flow field (McClements, 2007). In the current study, it was seen that n values were significantly affected by pectin concentration.

CHAPTER 5

CONCLUSION

The highest pectin yield (32.3±1.44%) was obtained using the citric acid solution in the conventional extraction method, while the lowest yield (5.56±0.25%) was obtained using distilled water in the ultrasound-assisted extraction method.

The PH pectin obtained by using the conventional extraction method was characterized. According to the results, the degree of esterification of PH pectin was detected to be less than 50% and thus, PH pectin was classified into low methoxyl pectin.

The high amount of galacturonic acid (65.81±1.51%) and low ash content (1.49±0.028%) in PH pectin are two important criteria showing the quality and purity of the pectin obtained.

The emulsifying properties of PH pectin were observed in an oil-in-water emulsion system. Emulsions were characterized by measuring their stabilities, particle sizes, rheological behaviors and morphological properties. The results indicated that emulsifying activities and stabilities were significantly affected by the different pectin concentrations.

The most stable emulsion was obtained at 6% pectin concentration.

It has been observed that the emulsion samples have a flow behavior index of less than 1 and therefore have shear-thinning flow behavior which is one of the Non-Newtonian flow types.

Consequently, the overall results showed that the PH which is the waste or by-products of pistachio nut was a good source of pectin which is classified in low methoxyl pectin. Also, overall results indicated that PH pectin could act as a good

surfactant or emulsifier in emulsions between the water and oil phases. PH can be utilized as a good potential source of pectin, as a way to remediate the environmental and ecological problems and add value to them.

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