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M.Sc. in Food Engineering

EZGİ SOĞANCI

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EXTRACTION AND FOOD APPLICATION OF MUCILAGE FROM OKRA

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EXTRACTION AND FOOD APPLICATION OF MUCILAGE FROM OKRA

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in

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Supervisor

Prof. Dr. Medeni MASKAN

by

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June 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.

Ezgi SOĞANCI

ABSTRACT

EXTRACTION AND FOOD APPLICATION OF MUCILAGE FROM OKRA

SOĞANCI, Ezgi

M.Sc. in Food Engineering

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The main objective of this study is to investigate the extraction of mucilage from okra (*Abelmoschus esculentus)* thereby evaluate and compare the functional properties of okra. Experimental design was performed by using Response Surface Methedology (RSM). The independent variables used in this study were extraction temperature (25–80ºC), extraction time (1-7 hr) and water to raw material ratio (4:1–10:1). The dependent variables were extraction yield, swelling index, emulsion capacity, emulsion stability, antioxidant activity and phenolics content. By numerical optimization of independent process variables, it was found that the optimum variables for extraction time, water to okra ratio and extraction temperature were 1.52 hr, 7.2 and 50.51ºC, respectively. At these optimum conditions; extraction yield, swelling capacity, emulsion capacity, emulsion stability, antioxidant capacity and total phenolics content were predicted to be 23.24 %, 44.2 (mL/g), 47.05 %, 50.18 %, 38.95% and 36.75, respectively. Oiling off was decreased with addition of mucilage and stability of the emulsion was enhanced. Organoleptic evaluation of okra mucilage found to be acceptable and satisfactory. The okra mucilage was used in pistachionuts paste as a stabilizer. It was found that as the percentages of okra mucilage in pistachionuts paste increased, ability of holding oil increased and amount of oil accumulated at the top decreased. Results showed that okra mucilage can be used as a promising additive in food formulations.

Key Words: Mucilage, Okra, Extraction, Optimization, Emulsion Stability

ÖZET

BAMYADAN MÜSİLAJ EKSTRAKSİYONU VE GIDA UYGULAMASI

SOĞANCI, Ezgi

Yüksek Lisans Tezi, Gıda Mühendisliği

Danışman: Prof. Dr. Medeni MASKAN

Haziran 2020

72 sayfa

Bu çalışmanın temel amacı bamyadan (*Abelmoschus esculentus)* müsilaj ekstraksiyonunu araştırmak böylece bamyanın fonksiyonel özelliklerini değerlendirmek ve karşılaştırmaktır. Deney tasarımı, Yanıt Yüzey Metodolojisi (RSM) kullanılarak yapılmıştır. Bu çalışmada kullanılan bağımsız değişkenler ekstraksiyon sıcaklığı (25–80ºC), ekstraksiyon süresi (1-7 saat) ve su: hammadde oranı (4: 1–10: 1) dır. Bağımlı değişkenler ekstraksiyon verimi, şişme indeksi, emülsiyon kapasitesi, emülsiyon stabilitesi, antioksidan aktivite ve fenolik içeriğidir. Bağımsız proses değişkenlerinin sayısal optimizasyonu ile ekstraksiyon süresi, su-bamya oranı ve ekstraksiyon sıcaklığı için optimum değişkenler sırasıyla 1.52 saat, 7.2 ve 50.51ºC olarak bulunmuştur. Bu optimum koşullarda; ekstraksiyon verimi, şişme kapasitesi, emülsiyon kapasitesi, emülsiyon stabilitesi, antioksidan kapasite ve toplam fenolik içeriğinin sırasıyla % 23.24, 44.2 (mL/g), % 47.05, % 50.18, % 38.95 ve 36.75 olduğu tahmin edilmektedir. Müsilaj ilavesiyle yağ ayrılması azaltılmıştır ve emulsiyon stabilitesi korunmuştur. Bamya zamkının organoleptik olarak değerlendirilmesi kabul edilebilir ve tatmin edici bulunmuştur. Bamya müsilajı antep fıstığı ezmesinde stabilize edici olarak kullanılmıştır. Antep fıstığı ezmesindeki bamya müsilajı yüzdeleri arttıkça, yağ tutma kabiliyetinin arttığı ve üstte biriken yağ miktarının azaldığı bulunmuştur. Sonuçlar bamya müsilajının gıda formülasyonlarında umut verici bir katkı maddesi olarak kullanılabileceğini göstermiştir.

Anahtar Kelimeler: Müsilaj, Bamya, Ekstraksiyon, Optimizasyon, Emülsiyon Stabilitesi

To my family

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
TPC	Total Phenolics Content
DPPH	1,1-Diphenyl -2-picrylhydrazyl radical
GAE	Gallic Acid Equivalent
RSM	Response Surface Methodology
BBD	Box–Behnken design
SC	Swelling Capacity
EC	Emulsion Capacity
ES	Emulsion Stability
EY	Extraction Yield

CHAPTER 1

INTRODUCTION

1.1 Okra

1.1.1 Origin and Geographic Distribution

Okra *(Abelmoschus esculentus* L.) is one of the oldest cultivated plants, which is suited to be raised in warm climatic zones and one of the most economically valuable plants of the Malvaceae family. Although okra is a very susceptible to cold climate and grown mostly in tropical and sub-tropical regions, its distribution and utilization is quite common. The homeland of okra is considered to be Ethiopia and northern parts of Africa, but the origin of the plant is still uncertain. A picture of the okra is shown in Figure 1.1 (Lamont, 1999).

Figure 1.1 A picture of Okra (Abelmoschus esculentus)

However, okra by plant explorers has been proven to be grown in 2000 BC as one of the oldest crops planted in Egypt. Nevertheless, some are thought to be the original home of India. While it is certain that Okra's origins date back centuries, it is now

widely cultivated in India, the Mediterranean region and North Africa, Arabia and the United States of America (Gemede et al., 2014; Mir et al., 2017).

1.1.2 Cytogenetic Relationships

Table.1.1 demostrated the chromosome numbers of various *Abelmoschus esculentus* species (Benchasri, 2012). The plant, spreading in different parts of the world, is a cultigen and shows this diversity feature in the number of chromosomes.

Table 1.1 Chromosome numbers in *Abelmoschus* spp.

Species	Chromosome numbers(2n)	References
Abelmoschus esculentus	62	IBPGR (1991)
Abelmoschus esculentus	66	Ford (1938)
Abelmoschus esculentus	72	Ugale et al (1976), Datta & Naug (1968), kamalova (1977) and Qhureshi (2007)
Abelmoschus esculentus	108	Datta & Naug (1968)
Abelmoschus esculentus	118	Tischler (1931)
Abelmoschus esculentus	120	Purewal & Randhawa (1947) and Datta & Naug (1968)
Abelmoschus esculentus	122	Tischler (1931)
Abelmoschus esculentus	124	Kuwada (1964, 1966)
Abelmoschus esculentus	126-134	Chizaki (1934)
Abelmoschus esculentus	130	Joshi & Hardas (1976), Singh & Bhatnagar (1975) and Kumar et al. (2010)
Abelmoschus esculentus	131-143	Datta & Naug (1968) and Siemonsma (1982)
Abelmoschus esculentus	132	Ford (1938) and Datta & Naug (1968)
Abelmoschus esculentus	144	Datta & Naug (1968)

Genetic studies have shown that *Abelmoschus esculentus,* a hybridized specie, is contributed of *Abelmoschus tuberculatus* Pal and H. B. Singh (2n = 58) and

Abelmoschus ficulneus L. Wight and Arn (2n = 72). The chromosome number of okra is 2n=130, ranging from 66 to 144.

This feature is manifested by plant characteristics such as plant shape, environmental tolerance, productivity, and consumption depending on these differences (Benchasri, 2012). The okra plant presents a feature of annual in temperate climates, perennial in tropic climates. Okra plant, known to belong to the Malvaceae or mallow family of *Abelmoschus esculentus* L. Moench was first included in the genus Hibiscus, then separated and finalized the name as Abelmochus. Figure 1.2 showed scientific classification of *Abelmoschus esculentus* (Badrie, 2016).

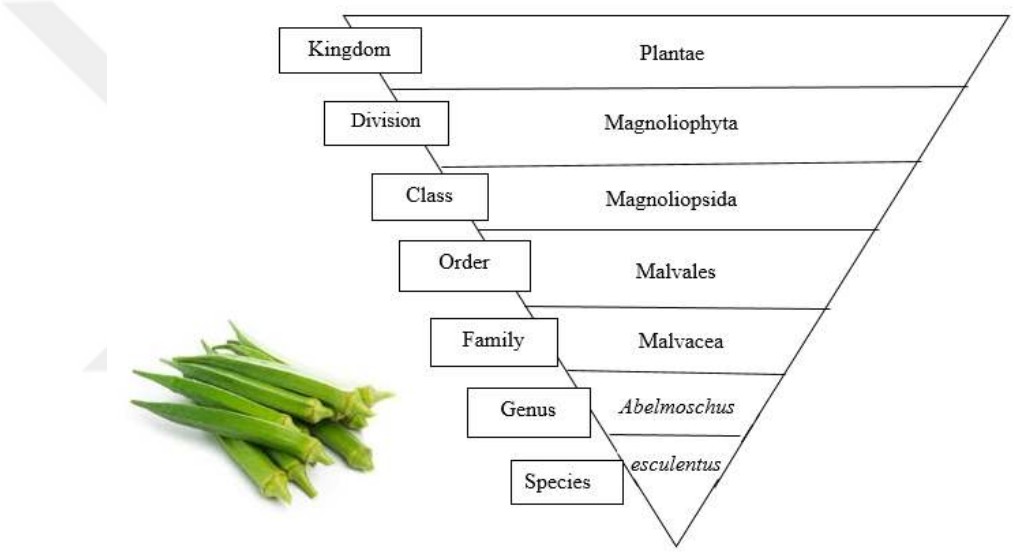


Figure 1.2 Scientific Classification of okra (Abelmoschus esculentus)

About 50 species are characterized under the title of genus *Abelmoschus*. The most detailed study of the genus *Abelmoschus* to date has been initiated by van Borssum Waalkes (1966) and continued by Bates (1968). Two hypotheses have been proposed regarding the geographical origin of *Abelmochus esculentus*. Some scientists claim that the species *Abelmoschus tuberculatus,* native to the Uttar Pradesh region, is a hypothetical ancestor, and that the species comes from North India.

Others claimed that Ethiopia or Northern Egypt was a domestication area, based on signs of ancient cultures and presumed ancestors, although there was no conclusive evidence (Pravin et al., 2018).

Abelmochus esculentus is a globally known plant that can be grown in various regions of the world and in different climates. Spreading areas of the plant is shown in Figure 1.3. It was demonstrated (Figure 1.3) that Southeast Asia is the intersection point of cultivated and wild okra species, also the richness of the region is remarkable (Hamon and Van Sloten, 1995).

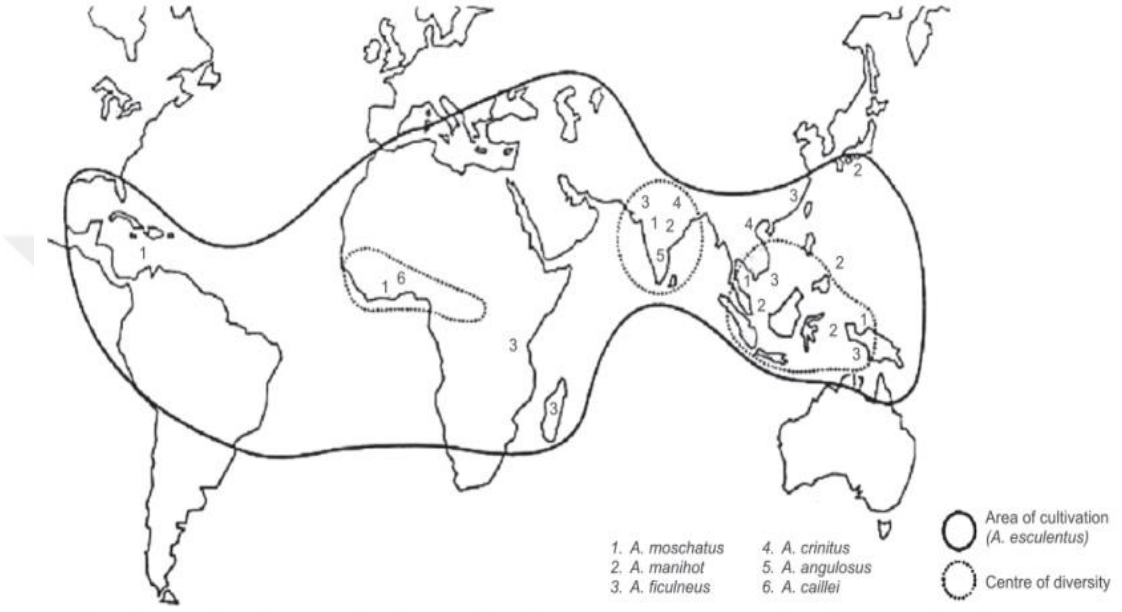


Figure 1.3 Geographically spread areas of Abelmoschus species

1.1.2.1 Okra Names

Okra plant's local names in abundance in the world. In motherland Ethiopia, Kenkase (Berta), Andeha (Gumuz), or Bamia (Oromica/Amharic), bhindi in India, gombó or quingombó in Porteguese and Spain, mbamia in Sweden, okura in Japan, qiu kui in Taiwan, bamiah in Arabia, bamia, gombo, lady's finger or okra in England. Lastly, in Turkey and most of the West Asia, it is called Bamya.

There are many other names such as Kacang Bendi, qiu kui, Quiabos, Ochro, Quiabo, Bendi, Kopi Arab (Badrie, 2016).

1.1.3 Biochemical Composition and Uses

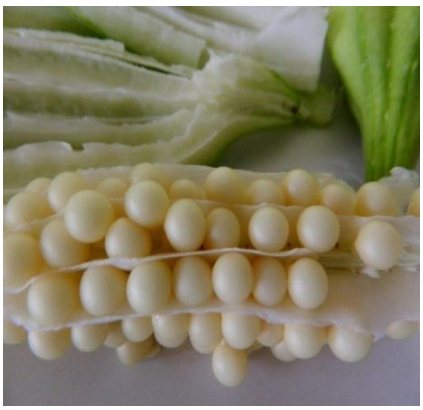
Okra cultivation as a commercially valuable herbal product initiated in the African continent and spread all over the world. This plant is used as horticulture or in large

agricultural land. Okra plant is an essential nutrient in human nutrition with rich protein, carbohydrate, vitamin, fiber and potassium content. The composition, nutritional and functional properties of this plant have been investigated for many years and it is suggested that it should be present in nutrition in developed countries. Okra is an important dietary vegetable among the cultivated vegetables (Xu et al., 2017).

Production of okra in Turkey is concentrated in Aegean and Marmara regions but is done in all regions of the country. Okra is consumed as fresh, dried, frozen, canned and in brine. Okra is one of the integral and recommended part in human diet owing to considerable nutritional elements. It is produced in a several countries for its green non-fibrous fruits or pods containing round seeds. Okra fruit, leaf, seed and roots and flower are consumed. Height of okra reaches 3 to 6 ft (0.9 to 1.8 m). Parts of the okra fruit are illustrated in Figure 1.4 (Lamont, 1999).

Flower, Leaves and Root

Fruit



Seed

Figure 1.4 Name of the okra parts

1.1.3.1 Root

Okra root structure of the plant can go down to 100-120 cm, a main root wrapped around the secondary pile root and a small amount of fringes on the sides consists of roots (Lamont, 1999).

1.1.3.2 Leaves

Okra leaves resemble cotton and vine leaves morphologically. Leaves vary according to their varieties, but their colors may be light green, dark green and red, and their shapes may be one-piece or piece. The top of the leaf is bright and the bottom contains a large amount of hispid. It is known to be consumed as a vegetable in some African countries. Okra leaves can be consumed raw or cooked.

Amount of water is the highest among other components as 81.5 gr. It is followed by amount of carbohydrate and protein with 11.30 gr and 4.40 gr respectively. There are 0.60 gr of fat, 2.10 gr of fibre, 532.00 mg of Ca, 70.00 mg of P, 0.70 mg of Fe, 59.00 mg of ascorbic acid, 385.00 μ of β-carotene, 0.25 mg of thiamin, 2.80 mg of riboflavin and 0.20 mg of niacin are the other nutritional elements that is found in 100 gr of okra leaves. Lastly, amount of energy gain is 235.00 kJ (56.00 kcal).

Leaf stem length and leaf size are the factors that should not be overlooked in the production of okra and the collection of fruits. Okra species with small leaves and long leaf stems are preferred for easy harvesting of fruits (Benchasri, 2012).

1.1.3.3 Fruit

According to the varieties, okra fruits are of different shapes, colors and sizes and the number of seed houses in the fruits varies between 5 and 8. The fruit shape can be long, pyramid-shaped or close to the round. Fruits are pentagonal or hexagonal. Fruits color varies light green to wine red color. Depending on the fruit stalk and fruit varieties may be abundant hairy, less hairy or glabrous. Fruit grows very quickly. During harvesting, it is observed to reach 8-10 cm fruit lenght. Ripe fruits can take up to 30 cm height.

In Turkey, okra with small fruit length is preferred, while larger fruit varieties are grown and consumed in USA, Africa and Australia. Okra fruit drying and storage method also varies from region to region. In some regions, it can be collected and consumed as a fruit before it matures, or dried and consumed. In Turkey, okra fruits

are strung to be dried and used in the winter, in African countries, sliced, dried under the sun, stored and pulverized.

Okra is a versatile plant that can be cooked and evaluated in many different ways. Depending on consumer demand, boiled, steamed, sautéed, sliced, fried or added to soups are available. Egypt, Iran, Iraq, Greece and the eastern Mediterranean countries such as Turkey, okra cooked meal of meat consumed together. Indian cuisine is popular with sauce. In South America, it is consumed by frying in bread with oil (Archana et al., 2013).

The aqueous solution of okra has a thick and sticky property and is known to result from the acidic polysaccharides in its structure. Mucilage represents a slimy-looking and high viscosity solution of up to 30 % in aqueous solution. Okra mucilage is an advantageous structure that can be evaluated in food, non-food and medical purposes. It provides an affordable source for industrial and medical applications (Samavati, 2013; Ahiakpa et al., 2013).

When examining the nutritional elements of okra pods and its energy values, it has been found that amount of water is the highest with 88.6 gr among other components. Energy intake is 144.00 kJ (36 kcal) with 100 gr of okra pods. It is followed by amount of carbohydrate and protein with 8.20 gr and 2.10 gr respectively. There are 0.20 gr of fat, 1.70 gr of fibre, 84.00 mg of Ca, 90.00 mg of P, 1.20 mg of Fe, 47.00 mg of ascorbic acid, 185.00 μg of β-carotene, 0.04 mg of thiamin, 0.08 mg of riboflavin and 0.60 mg of niacin are the other nutritional elements that is found 100 gr of okra leaves. 81 % of purchased okra pods are ends trimmed.

Okra fruits are composed of long chain molecules, mostly sugar and amino acids. Essential components of the sugars are consisting of galacturonic acid, galactose, rhamnose and amino acids with 27%, 25%, 22% and 11%, respectively. Also, Okra is known for having a large quantity of phenolics compounds in all parts, making it a preferable source of natural antioxidants (Benchasri, 2012).

1.1.3.4 Seeds

Okra seed is considered a substantial source of protein and fat, with protein content ranging from 20 to 27% and fat content ranging from 20 to 40%. The oil of okra seed consists of polyunsaturated fatty acid as lineoleic and oleic acid (about 70%) which is

accepted as essential for human body. It is explained that a 9-inch-long okra fruit contains nearly 100 seeds. Okra seed color varies from white to yellow depending on factors such as soil type and okra genus.

Even though okra seeds are partly candidates for oil production due to their rich oil content, it is common in Central America, Africa and South East Asian countries to add dried okra seeds as roasting or grinding to coffee.

The grain weight of 1000 gum seeds varies between 30-80 grams. Okra, because it has a thick seed shell, requires pre-treatment before planting. In the study conducted in Egypt, okra seeds were kept in distilled water followed by growth regulators for 12 consecutive hours. Gibberellic acid, indole-3-acetic acid, and naphthalene acetic acid were chosen to be plant growth regulators.

1.1.4 Growth and Planting

Okra is a self-pollinating plant, but its effectiveness in cross-pollination with honey bees and wasp's increases. To maintain purity, the insulation distance of 400 meters was required according to India's minimum seed certification standard.

Okra is a tropical vegetable that grows directly from seeds and lasts for 90-100 days. Soil moisture and temperature during planting, soil and seed structure are effective factors on germination. It is recommended to keep the old seeds in wet cloth or water for 1 day before planting. During planting soil temperature is above 20℃ and soil moisture is the most appropriate value is advantageous. Excess moisture can cause seed decay. In addition, the cream layer on the soil surface can prevent germination.

Okra is an annual herbaceous plant that shows irregular growth but grows in a positive correlation with day length. The shortest critical day length is reported 12.30 hours. There are factors that affect its growth, such as season type, soil type and fertilization (Lamont, 1999; Benchasri, 2012).

1.1.4.1 Season Type

Although cultivated areas are spread over a large area in the world, it is more common in tropic and sub-tropic climatic regions because it is a hot climate vegetable.

While the normal growth temperature of okra plant is 20°C, air temperature during seed sowing is 15-20°C and soil temperature is minimum 15°C are factors that increase productivity. The production of okra can be done even at 5-6°C when accustomed. When taken into other elements in account which are effective on germination percentage and rate of emergence, temperature is became the most prominent factor. Optimum temperature is desired to be 30-35°C. However, the effect of temperature becomes negative after 40-42°C and causes drying.

Besides temperature, it is an effective factor on yield in irrigation. Drip irrigation has been successful and it is recommended to give 1 to 1.5 inch (25 to 38 mm) of water per week to increase the moisture and nutritional value to prevent disease formation. It was observed that drought weakened the pods and leaves and eventually caused the plant to lose its viability. Okra needs a moist and warm environment with about 80-100 cm of rainfall for better flowering and fruiting during the growth phase (Lamont, 1999).

1.1.4.2 Soils

Okra is a plant that can grow in all kinds of soil including poor and clay soils but provides the best growth and yield in sandy soils rich in organic matter. During seed sowing, it is essential to grind the soil in all soil types and moisten it in sufficient amounts. Okra is susceptible to excessive moisture and calcareous soil during seed sowing. Therefore, especially in nitrogen-rich soils, yield and the number of fruits decreases. If calcium and magnesium is needed, dolomitic calcification can be performed for proper lime. Okra is a very resistant to environmental conditions after germination and the pH of the soil is ranked from 6.0 to 7.0 (Lamont, 1999; Badrie, 2016).

1.1.4.3 Fertilization

Since the production period of okra has a longer period compared to other vegetables, fertilization process is of great importance in terms of preventing overgrowth of plant height and plant productivity.

If no soil test is available, N is 30 lb / acre (34 kg/ha), P is 26 - 34 lb / acre (29 - 38 kg/ha) and K is between 49 and 66 lb / acre (55 to 74 kg/ha) general application is recommended. If the area is very rainy, it may be necessary to neutralize the previously applied fertilization and enrich the soil with N (Lamont, 1999; Badrie, 2016).

1.2. Mucilage

The use of natural polymers of vegetable origin in the food industry and pharmacy facilitates human life. It can be used not only in these areas, but also in agriculture, food packaging and in reducing environmental pollution. The most common natural polymers to be used are aloe mucilage, guar pentose gum, black gum, bhara gum, sodium alginate, carob gum gum and flaxseed gum (Deogade et al., 2012; Malviya et al., 2011).

Mucilages are physiological metebolism products of complex polysaccharide structure. It has a high molecular weight about 170,000 mainly consisting of sugar units and aminoacids (Badrie, 2016). It can be found in all parts of the plant including roots, seeds, leaves, flowers. It is naturally occurring in the cell or deposited in the layers, although they are generally dense in small areas of the surface area of the plants. Mucilage forms a slippery colloidal dispersion that is optically active, hydrolysable and fermentable with water. Also, mucilage can be synthesized by some microorganisms and mucilages are metebolized products (Ahiakpa et al., 2013).

Mucilage, a plant hydrocollloid, has a transparent fluid and amorphous structure and acts as a protective agent during plants injury, supporting water storage and food storage. Mucilages in complex polysaccharide structure provide water retention capacity due to hydroxyl groups. Although the physical function of mucilage in almost every part of the plant is still unclear, but act to be a role of food storage.

It has been reported that the mucilage in the leaves can be assumed to be secondary plant metebolite, providing water transport, frost tolerance and response to injury (Malviya et al., 2011; Assi et al., 2017). Mainly, plant parts provide the main source of the mucilage, then, animals, seaweeds and fungi are other sources. In human body, mucilage is indigestible in the small intestine and passes directly into the large intestine (Sangwan et al., 2011; Ahiakpa et al., 2013).

Mucilages are classified into three groups: ıntra cell mucilage, cell membrane mucilage and secreting hairs. For instance, *Orchids* sp., *Urginea maritime* L., Baker (Squill) and *Allium* sp. (onion, garlic) are the species are the members of intra cell mucilage. *Aloe*, *Althaea officinalis* L. and *Cinnamomum* sp. are the species whose mucilages area kind of cell membrane mucilage and found in the bark of the plant. Thirdly, *Cydonia vulgaris* L. *Viola tricolor* L. are the examples of species which have secreting hairs of the mucilages on their leaves.

The term mucilage is generally used with the term gum alternately. Although there are significant differences in mucilage with gum and pectin physically, it is chemically closely related. Mucilage, gum and pectins exhibit different behaviors in their contact with water. Gums swell in contact with water to form adhesive and colloidal dispersions. Pectin gelatinizes in water. It has been found that the viscosity of the aqueous phase increases when different parts of many fruits are kept in water for a short or long time. This thickening and slimy effect increases in parallel with certain factors such as increasing temperature to a certain degree and increasing surface area of the fruit (Deogade et al., 2012; Jani et al., 2009).

When gums and mucilages are analyzed, gums are a pathological product that occurs under harsh conditions such as cell damage, disintegration or drought, mucilages are physical products, normal metebolism products that occur within the cell. The gums show a good solubility behavior in water, while the mucilage forms a sticky slimy form (Prajapati et al., 2013; Bhosale et al., 2014). Gum and mucilages are translucent, amorphous substances produced by plants. The gum was obtained from the hydrocolloids of the plant and is of two types. These are anionic and nonionic polysaccharides.

Gum and mucilage can be obtained from the middle coverslips, from the cell wall - endodermia, and from some secretarial cells. These are found for example in algae, in the seed epidermis and in schizogenous sacs, respectively (Jones and Smith, 1949).

1.2.1 Mucilage from Okra

Almost all parts of the okra plant, *Abelmoschus esculentus* L., can be subjected to water-based extraction. The structure formed after dissolving into the solution is called

mucilage. Okra mucilage is the thick and spongy sticky colloid liquid formed by the extraction of the okra plant in water. The carbohydrate in the okra is present in the form of mucilage (Farooq et al., 2013). Mucilage of okra and its slimy appearance are shown in Figure 1.5.

Figure 1.5 Mucilage of okra

1.2.1.1 Chemical Composition of Okra Mucilage

Okra mucilage, a hydrophilic polymer, is reported to be acidic polysaccharide. It is consisted of mostly galacturonic acid, secondly galactose and then rhamnose with 27%, 25% and 22%. Aminoacids are of 11% among other components. The okra mucilage is an amorphous polysaccharide. It is composed of D-galactose, L-rhamnose and L-galactouronic acid. Chemical structure of the okra mucilage is illustrated in Figure1.6.

The acetyl content of okra mucilage was determined to be 5% (w / w) and hemicellulose and pectic (xylan, xyloglucan) structures were revealed (Archana et al., 2013). Zaharuddin et al. (2014), tested the pH value of the okra mucilage as 6.59; and moisture content of 14.83%. Specific gravity of okra mucilage is 0.9975 gm/ml in 0.5% (w/v) dispersion of okra mucilage.

Also, microbial count was declared to be 200 CFU/gm of the mucilage. It is reported that role of rhamnogalacturonan in the structure is to support cell division (Zaharuddin et al., 2014).

The characterization of okra mucilage turned out to be highly soluble in water, but insoluble in organic solvents. The reason for showing high viscosity when dissolved in water is due to the dissolution of macromolecules in the structure (Sangwan et al., 2011; Zaharuddin et al., 2014).

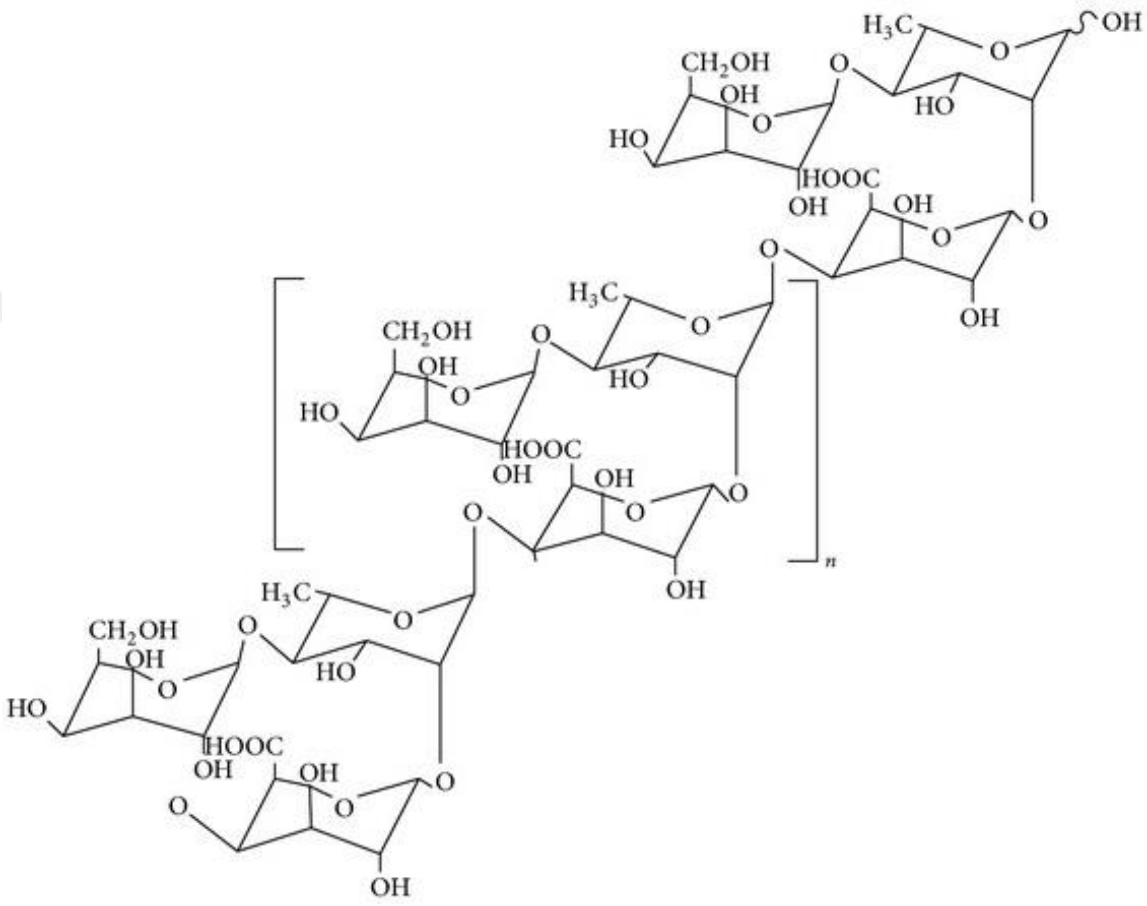


Figure 1.6 Chemical structure of okra mucilage polysaccharide (Zaharuddin et al., 2014)

1.2.1.2 Application Area of Okra Mucilage

Okra is a very economical plant that should be used for multipurpose due to its widespread cultivation in the world, quik growing, easy cultivation in many soil types and resistant to hot and dry weather.

Okra in itself is a valuable nutrient widely used in the world cuisine, as well as okra mucilage is considered as an important source of natural polymer, hydrocollid, in industrial and pharmaceuticals. Mucilage has a high amount of polysaccharide and

protein, neutral sugar, and mineral. Okra mucilage is a valuable complex polysaccharide whose nutritional and functional properties have been the research issue of many investigations and have a wide range of uses mainly in food and medical applications also cosmetics, textiles, and paper-making (Ahiakpa et al., 2013; Archana et al., 2013).

1.2.1.2.1 Application of Okra Mucilage in Foods

Okra mucilage is a hydrophilic polymer whose aqueous solution reaches upper limit of the viscosity at neutral pH and lean to rupture bonds at high temperature. Okra mucilage is known to be used as a stability-promoting agent in local foods, such as soups, in some parts of West Africa (Ahiakpa et al., 2013). In the food industry, it is used as a food additive, thickener, stabilizer, water retention agent, suspending agent, binder, emulsion stabilizer.

Functional properties of the aqueous solution of mucilage, for example, viscosity change, swelling index, stability and capacity of emulsion systems, are worthy of consideration in order to shed light on new applications in the food industry and to improve the quality of foods. Additionally, mucilage is a high molecular weight, highly hydrophilic complex polysaccharide. The transfer and dissolution of macromolecules into the aqueous phase affects the rheological character of the liquid and changes the mechanical properties of the water.

Due to its high water solubility and water holding capacity, it is used as a food additive by increasing the viscosity of the dissolved liquid, acting as a thickener, gelling agent, stabilizing agent and improving food quality appertaining to texture and appearance properties. One of the peculiar capability of okra mucilage is that it is used instead of egg white in the food formulations such as cakes. Because mucilage solution of 25% shows more qualified than egg white in terms of ropiness and stringiness. It is also used as a stabilizer in ice cream, sauces and salads and as a clarifying agent in the sugar cane juice (Ndjouenkeu et al., 1996; Mir et al., 2017).

Furthermore, okra mucilage has a rich antioxidant and phenolics content that supports human health against free radicals and has a protective effect against diseases (Mir et al., 2017). Free radicals are unstable molecules. They can react with other molecules

quickly and damage them. These free radicals can destroy our body very quickly without antioxidants. The main weapon of the human body that can prevent damage caused by free radicals is antioxidants. It is important to take antioxidants from foods. The best antioxidant sources; plant-based foods, especially fruits and vegetables. Especially as the age increases, fruits and vegetables containing antioxidants should be consumed to protect against free radicals. If the required amount cannot be taken, the probability of seeing diseases such as cancer, heart disease, vascular occlusion and high blood pressure increases (Xia et al., 2015; Karabulut and Gülay, 2016). In previous studies, nutrient profile, functional properties and antioxidant activity of different parts of okra plant was investigated. It was shown that okra mucilage showed antioxidant and anti-glucosidase activity (Graham et al., 2017; Xia et al., 2015; Cahyana and Kam, 2016). Additionaly, Adetuyi and Dada (2014) was researched the mucilage from okra, water leaf and Jews mallow. They were dwelled on the healing effect of okra mucilage with regarding high antioxidant capacity.

The food products which have oil in water emulsion system are great of numbers in marketplace. Food stability and texture is one of the key factor that attracts people. Okra mucilage has a pivotal role as natural emulsifiers and improve the stability of products in terms of emulsifying properties. Emulsifying stability and capacity are associated with maintaining the substantiality of the system under heat and force. Okra mucilage is found to be favorable as an emulsion stabilizer in coconout milk emulsion system (Noorlaila et al., 2015).

Another example is that okra mucilage can be using as a fat replacer. The research was applied on chocolate bar cookies and results are found to be satisfactorial in the way of color, smell and flavor (Romanchik-Cerpovicz et al., 2002). Okra mucilage are being used in food products that has emulsion system such as mayonessie, salad dressing, sauces and frozen desserts because of good thickening and stabilizing ability (Mir et al., 2017).

1.2.1.2.2 Application of Okra Mucilage in Medicine

Okra, which has been accepted as a medicinal plant since ancient times, comes to mind for curing various diseases with the increasing trend towards traditional methods of medicine. In case of stomach irritations and stomach ulcers, it is recommended to eat

okra in folk medicine because of its therapeutic effect of high mucilage content. Studies reported the advantegeous effects of okra mucilage over anti-diabetes, reducing blood lipid and neuroprotection and activity of antioxidation in vitro (Gürbüz et al., 2005; Ahiakpa et al., 2013).

Mucilage is widely used as a binder and disintegrant agent in tablet preparation thanks to ability of adhesiveness. This feature is supported by high water absorption and swelling capacity, subsequently swollen particules disperse and lead to improve dissolution rate. Also, mucilages can be used by themselves or in other combinations as a gelling agent due to their non-toxic, emollient and non-irritating properties (Prajapati et al., 2013). Okra mucilage, a hydrophilic polymer has an advantageous over synthetic excipitients due to it high adhesive and cohesive properties.

Besides, it has a various role in pharmacy as emulsifiers, suspending agents, stabilizing agents, thickening agents. Additionally, mucilage is successfully applied as a film forming agent and coating agents in periodontal films and microcapsules respectively. (Deogade et al., 2012; Ahiakpa et al., 2013).

1.2.2 Mucilage from Other Plants

Plant derived polymers are attracted attention recently due to their advantages of biodegradability, biocompatibility, low in cost, nontoxicity and enviromental friendly processsing against semi synthetic and syntetic polymers.

Natural gums and mucilages are plant derived polymers whose polysaccharides contain significant amounts of hydroxyl groups. Hydroxyl groups are responsible of water absorbing and binding capacity of the mucilage. The well known, mucilage-rich plants are cacti (and other succulents), flaxseed, carrageen (*gigartina mammilosa*), *Malva slyvestris*, *Althea officinalis*, *Tilia* sp.(ıhlamur), isabgol (pisilyum plant), fenugreek, cinnamon, algae (agar, chondrus) and fungus (*Magnaporthe grisea conidia*).

Quince plant, rich antioxidant, phenolics and flavanoid content with high nutritional value, is a fruit used in the treatment of various diseases. 75% of its production in the world is met by Iran and is recommended in Iranian traditional medicine. The leaves are used as a soothing agent against diseases, the fruit is used in the treatment of

dysentery disease, and quince seed mucilage is used as a fixing and emulsifying agent in hair lotions and has been the subject of research in edible film applications. Quince seed gum content (about 20% of the seed by weight) is the most important criteria follwed by color of the solution after water based extraction and freeness from foreign materials. As the quince seed mucilage has a tasteless and cohesive colloidal structure, it is favarouble to use as stabilizing agent on chocolate milk, chocolate-flavored drinks, and ice cream (Jouki et al., 2014).

Much of current literature focused on benefits of chia gum whose mucilage content %5-6 for industrial applications. Chia gum is revelaed itself by water extraction and have a slimy, mucilageous characterics that can be used for food formulations (Bemiller et al., 1993).

Customer demands have an ascending tendency of healthy foods this respect leads to industrial applications make a research for new healthy source. Frozen desserts, for example ice cream, have a complex emulsion system. Using this approach, chia mucilage, basil gum and flaxseed gum are undertaken to improve mechanical properties of frozen desserts due to high water retention ability of mucilage content (Chaves et al., 2018).

1.3 Extraction

Extraction is defined as a transition of compounds from one location to another which can be either solvent or phase in the form of liquid or solid. It is known that the concentration of water increases by dispersing the dissolved macromolecules in the aqueous phase as a result of the immersion or retention of plant-derived structures in water. High viscosity liquid is called mucilage formed by the extraction of macromolecules such as polysaccharides proteins or glycoproteins found in parts of plants such as roots (e.g., orchids), fruit (e.g., okra), tree skins (e.g., mamaku), leaves (e.g., baobab) or seeds (e.g., quince).

In this binary system formed by the plant parts and the water phase, it is seen that the rheological and textural properties of water have changed during the process of converting them to high viscosity mucilage form. The use of hydrocolloids in food systems have mainly two basic tasks, that is flow behaviour (viscosity) and mechanical

solid property (texture). Okra mucilage, one of the plant hydrocolloid, is used as food additive and have an impact on food formulations due to its functional properties. The transformation of low-viscosity water into a viscous colloidal dispersion comprises of three distinct stages.

Molecular transition from high to the low density occurs during diffusion. When the extraction of okra fruit in an aqueous environment is examined, it is seen that it occurs in three distinguishable stages.

Stage.1, okra fruit is soaked into aqueous solvent. Thus leads the hydration and swollen of the solid material. In Stage.2, the hydrated macromolecules pass from the solid material to the aqueous phase, thereby increasing the concentration of the aqueous phase. Macromolecules can be assign in Stage.3, thickening role or connection between oil and water molecules. This is resulted in changing of the rheological characteristics of water (Ritzoulis, 2017). Schematic represantation of water based okra mucilage extraction is shown in Figure 1.7.

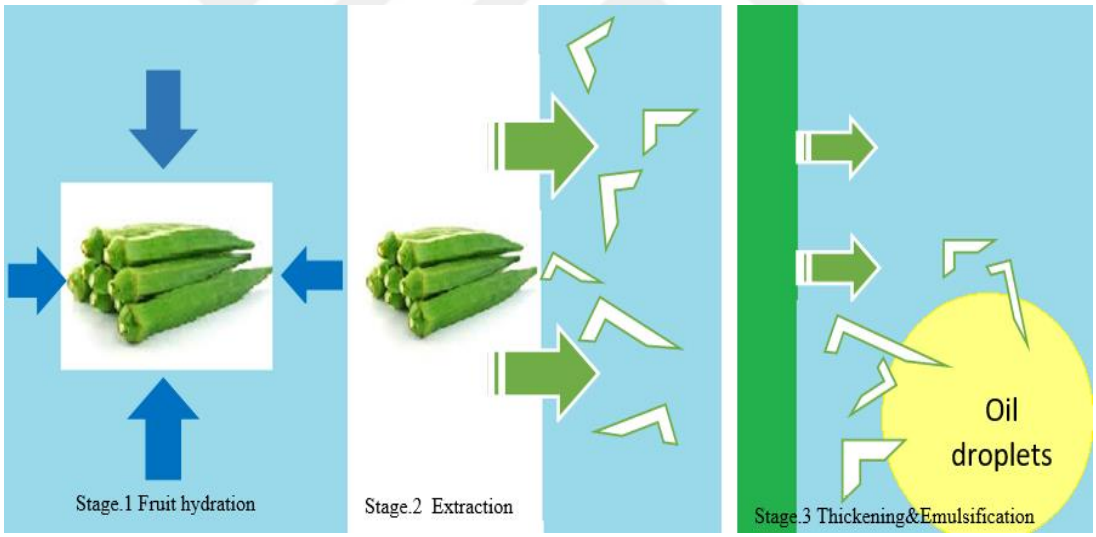


Figure 1.7 Schematic represantation of water based okra mucilage extraction

Wetting of the fruit body of the okra is the first and obligatory step. It is followed by absorbing water molecules in the structure of fruit and migration of molecules from fruit to the water. As the molecules relocate to the water phase, it is expected fruit body decomposes. The key factor of better swelling is claimed to be the larger surface area of the solid material. Larger areas were permitted to molecules penetrate into water

more efficiently. As in any aqueous extraction, the presence of hydrogen bonds between the water molecules and the solid molecules in dissolution state is considered in the okra-water binary system. Fundemental principle of mucilage formation is increasing the concentration of macromolecules in the water phase that is adequate to change rheology of the water (Milani and Maleki, 2012; Ritzoulis, 2017).

1.4 Freeze –Drying Method

Drying is the removal of water or other liquids contained in the substance without disturbing the structure of the substance. Drying process is a method used for many years to make foodstuffs more durable and long lasting. Freeze-drying is used interchangeably with the term lyopilization. It means suspending the vitality of the product by protecting rancy characteristics. The elementary intend of freeze drying within the food industry is to lenghten the shelf-life of the food while retaining the quality (Nireesha et al., 2013; Stawczyk et al., 2006).

The freeze-drying method differs from the conventional drying method in terms of its application and properties of the final product. Freeze drying method, compared to other high temperature drying methods, less change in the nutritional value of the dried product and sensory properties such as color, smell and taste. Therefore, the best quality final product is obtained by freeze drying method when compared with other methods. It has become popular in the food industry to achieve successful results in foodstuffs such as coffee, soups and fruits which are considered very difficult to dry by conventional drying methods and temperature sensitive substances, such as some biologic substances and drugs. Through freeze drying method, besides the whole nutritional profile of the product is increased, the durability period is improved as well by the help of decreasing final product moisture concent to considerably low decrease.

Freeze-drying is a method of food preservation and does not usually cause the dried material to toughen or shrinkage. When water was contributed to the freeze-dried material again, it achieves its structure of integrity very quickly before drying on account of its non-shrunken porous structure. One of the previous study, Kırmacı et al. (2008) have worked on the freeze drying system and made the manufacturing, design and performance experiments of this system. In his study on drying strawberries, he observed that the amount of moisture decreased from 91.2 % to 9.1 %.

Freeze drying is a water removal process, but since the basic principle of this process is sublimation, the water molecules or other frozen solvents are removed from the ice form to the gas form under vacuum. Thus, the water or aqueous solution in the freeze-dried substance must be below the triple point. Freeze drying is a process that constituted of three significant steps.

These are freezing phase, first drying phase and second drying phase. During the freezing stage, the material is cooled below its triple point. This is the last-place at three phases of the material can concur (Sadıkoğlu and Özdemir, 2003). A phase diagram of water is illustrated in Figure 1.8.

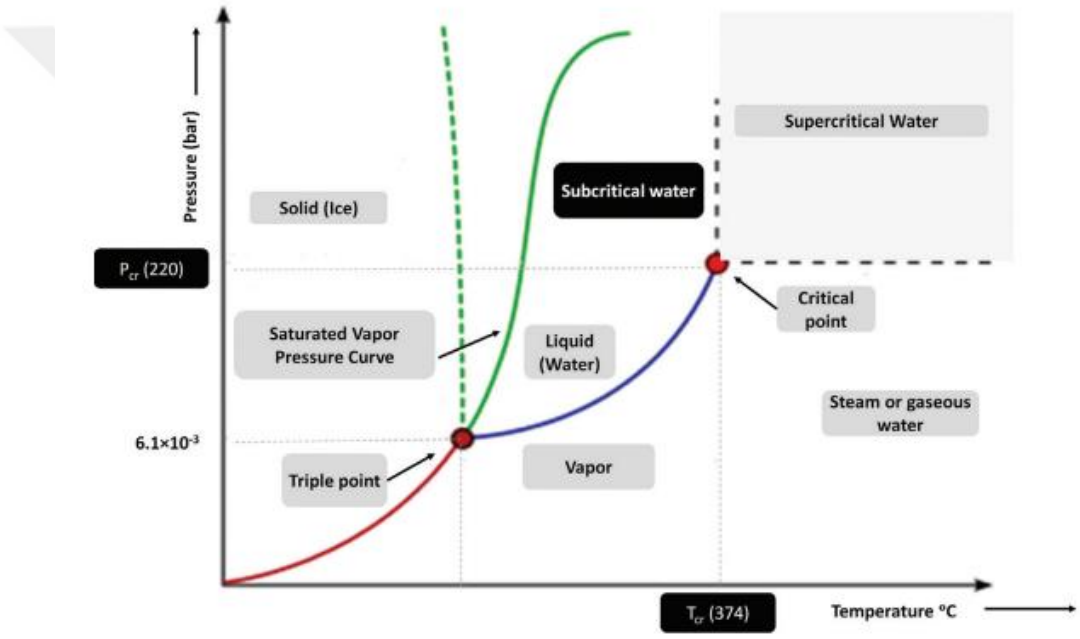


Figure 1.8 Phase diagram of water (Gbashi et al., 2017)

In the first drying phase, frozen water (ice) is removed from the substance by sublimation, the sublimation interface moves down and the dried layer remains in the porous structure. All frozen water in the substance to be dried is accepted to be the end of the first drying period. Up to 95% of the water is sublimated in the first drying phase. The second drying phase targeted to remove unfrozen water. 10-35% of the total water is bound water. Removing of bound water impacts the drying speed and total drying time. This part of the freeze-drying process is conducted by the material's adsorption isotherms.

To summarize, compared to the traditional method, it has been observed that the possibility of an enzymatic or non-enzymatic reaction is reduced and protein denaturation is prevented due to the freeze drying method operating at low temperature and low relative humidity, and the loss of water occurs very quickly locally (Franks, 1997; Sadıkoğlu and Özdemir, 2003).

1.5 Aim of the Study

Nature is a bottomless source which is equipped with great variety of components to increase the welfare of human and sustainability of healthiness. In recent years, righteously, seeking of natural source are attracted great interest by the understanding of substances of natural origin may have contribute tremendous benefits. Utilizing plant hydrocolloids are a good example in the scope of diversifying and supporting food products by reason of mainly that it can be obtainable from many parts of the plants.

Okra has been a subject of many researches on nutritional and functional properties over the years. Mucilage, as a plant derived hydrocolloid, is obtained from okra in this research. Some advantages as biodegradability, low-cost and biocompability are reasons of preferring okra mucilage in this study. Okra mucilage has a strong potential of using as a diluent, binder, disintegrant, thickener and gelling agents in pharmaceutical industry additionaly, thickener, stabilizer, emulsifier in food industry.

The aims of this study are; 1) to pore over the extraction of mucilage from okra under the optimized conditions and acquired to powder form of mucilage with least effect of temperature by freeze-drying method. 2) to use the mucilage in pistachionuts paste in order to improve its quality characteristic and long-term stability.

CHAPTER 2

LITERATURE REVIEW

2.1 Summaries of the Recent Studies

Archana et al. (2013) examined the mucilage extraction and characterization obtained from seaweed and the head of the okra which is considering as biowaste. The precipitation of the mucilage obtained from these two different plants was done by using acetone and methanol and further compared the percentage yield, emulsifying capacity and swelling index. The percentage yield of mucilage was 8.6% from acetone precipitation, but 0.28% from methanol precipitation. Emulsifying capacity was obtained 54.75% from acetone precipitation, but 50% from methanol precipitation. It gives similar result with swelling index too. Swelling index was 93.9% with acetone precipitation but, 80.31% with methanol precipitation. It was reported that besides okra mucilage has a large application area as a thickener, binder and stabilizer in pharmaceutical and food industry, it has a potential for tissue engineering as a biopolymer due to their eco-friendly characteristic.

In another study, Nair and Fahsa (2013) concerned with mucilage characterization and extraction of 5 different *Abelmoschus* species according to their maturation period. It was determined that the mucilage yield of the *Abelmoschus esculentus* species collected during the period of flowering was highest among the other species and the yield decreased as the ripening time increased. It was also found that the swelling index shown to be strongly related to the mucilage concentration is higher in distilled water than HCl and phosphate buffer. This study once again demonstrated the sufficiency of the use of *Abelmoschus esculentus* mucilage as a suspending agent for the pharmaceutical formulation and tablet preparation.

Optimization of extraction from quince seed mucilage was performed by Jouki et al. (2014). The effect of extraction conditions such as extraction time, temperature and water / seed ratio on yield, antioxidant activity, emulsion stability were investigated.

It has been found that the extraction conditions have a considerable effect on these analyzes and the optimum extraction condition was investigated as being temperature 65°C, time 5 minutes and water / raw material ratio 25: 1.

Sangeethapriya et al. (2014) examined the total phenolics content and antioxidant activity of *Zizyphus mauritiana* mucilage (ZZM) along with functional properties such as swelling capacity, emulsion capacity and stability. It was observed that swelling capacity and absorption of water which resulted in increasing cell weight is strongly linked with the dietary fiber (mucilage) content of ZZM. Also, it was revealed that ZZM possess good emulsification property which is effective in providing stability of oil water phases. Emulsion capacity and stability of ZZM was observed to be 54.2% and 42.14% respectively. In addition, ZZM was described to be a favourable source of antioxidant capacity and must have exploited beneficially against various diseases.

Similarly, Thanatchaand and Pranee (2011) studied the extraction of mucilage from *Ziziphus mauritiana*. They optimized the conditions for highest yield and investigated the emulsion capacity comparing with other gums. It was revealed that the optimum extraction conditions for highest yield were as follows: extraction temperature of water of 60°C, and the ratio of water to *Ziziphus mauritiana* pulp is 7:1 and the ratio of ethenol to precipitating the mucilage solution is 3:1. Also, it was observed that emulsion capacity of mucilage give a proportional result with guar gum, but lower values than xantgam gum.

Samavati (2013) shed light on the extraction process of the mucilage from *Abelmoschus esculentus* and optimized the extraction conditions including the extraction time, extraction temperature, number of extraction and the ratio of water to raw material factors using the Response surface methodology. It was determined that the extraction efficiency was strongly dependent on the extraction conditions and was reached the optimum value of 16.8% when the conditions were set to 94.97°C extraction temperature, 4.94 hours' extraction time, 4 number of extraction and 21.74 raw material to water ratio. Additionally, it was once again indicated that, okra mucilage can be a good candidate for being a source of hydrocolloid.

Mir et al. (2017) were performed mucilage extraction from Okra (*Hibiscus esculentus*) to investigate the functional properties of mucilage such as emulsion capacity and to obtain mucilage yield.

Regarding the emulsion capacity mucilage from okra, it was found that emulsion capacity of okra mucilage is 46.87 %. Also, Results showed that the mucilage yield extracted from okra, which was highly related to the extraction procedure, was 1.2% and revealed the suitability of okra mucilage as emulsion stabilizer for different food formulations.

In another study, extraction and characterization of mucilage from okra was evaluated by Farooq et al. (2013) in order to use as solid oral dosage form of supplement for pharmaceutics. Additionaly, okra mucilage was showed to be highly soluble characteristics in water while insoluble in organic solvents. Regarding extraction yield of okra mucilage, it was obtained to be 11.44%. Also, the sensory evaulation of okra mucilage was observed to be fairly agreeable for dosage form.

Noorlaila, et al. (2015) were performed the extraction of okra (*Abelmoschus esculentus*) mucilage and revealed the highest yield and emulsifying properties of mucilage with respect to different maturity stages of okra. In addition, stabilizing behavior of different concentrations of okra mucilage solutions on coconut milk was investigated and okra mucilage was found to be highly acceptable for different food formulations. The highest emulsion stability and capacity values of coconut milk was obtained from the maturity index 2 by 1% concentration.

Assi et al. (2017) studied on mucilage extraction from different plants including *Abelmoschus esculentus*. It was found that the mucilage yield of okra was 34.86% and mucilage could be used against various diseases.

Ndjouenkeu et al. (1997) examined the emulsifying, thickening and stabilizing properties of 3 food hydrocolloids from okra (*Hibiscus esculentus*), dika nut (*Irvingia gabonensis*), and khan (*Belschmiedia* sp.). Based on the findings, it was reported that khan gum provided a stability of emulsion system more efficient than okra gum and dika nut in terms of food emulsion system. It was observed that the behavior of okra gum was showed thickening effect more than a stabilizing.

Cahyana and Kam (2016) examined the extraction of mucilage from okra *(Abelmoschus esculentus)* in order to optimize the extraction conditions and investigated the antioksidant activities of mucilage to use its therapeutic role against diabetic disorders.

The extraction conditions were as follows: water-based extraction time (1, 4, 8 and 12 hours), 2 different temperatures (room and refrigerator temperature) and the ratio of fruit to water (1: 3 and 1: 6). The results revealed that the antioxidant activity was imposed by the time of extraction, the extraction temperature and the ratio of the fruit to water, and the highest antioxidant activity was achieved in the refrigerator temperature at 1: 6 ratios for 12 hours.

Zaharuddin et al. (2014) studied the extraction of mucilage in order to evaluate the usefulness of okra gum as a binding agent for tablet preparation. It was reported that solubility of okra powder was observed to be high in water, but less in organic solvents. When the retarding effect of okra powder was compared with HPMC and sodium alginate, longer term sustainability was observed, it was inferred that the use of okra powder as a binder could be a good candidate for controlling the release.

Biswal et al. (2014) studied the extraction of mucilage from *Abelmoschus esculentus* using ethanol as precipitating agent and it was found that the use of okra as one of the natural binders was effective as an adjuvant in tablet formulations.

Ahiakpa et al. (2014) performed the mucilage extraction with the participitation of 21 okras *(Abelmoschus* spp. L.) from 8 regions of Ghana in order to investigate the high mucilage yield for the raising of the latest okra species with high mucilage content. Although results showed that there was a considerable difference between the mucilage levels of the 21 participants. It was concluded that the level of mucilage from the West African Region (*Abelmoschus callei)* was higher than that of the Asian region *(Abelmoschus esculentus).*

The extraction of mucilages from the okra fruits (*Hibiscus esculentus*) and Baobab leaves (*Adansonia digitata*) was studied by Woolfe et al. (1977). The aim was to increase the potential of its use in culinary system by characterizing the mucilage which was already used in local kitchen applications. According to the results obtained, although the viscosity behavior of the okra mucosa was decreased with temperature, it was revealed that mucilage could be included as a food additive in food formulations and could be used in cooking and boiling procedures.

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CHAPTER 3

MATERIALS & METHODS

3.1 Materials

All the chemicals used in this study were of analytical grade. All reagents and solvents which were used in analysis are; Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid, 1,1 – Diphenyl – 2 – Picrylhydrazyl (DPPH), sodium bicarbonate ($NaHCO_3$). All these were analytically reagent grade and obtained from Merck (Merck, Darmstadt, Germany) and Sigma (Sigma-Aldrich, Steinheim, Germany). Pistachionuts paste, sunflower oil and 99.9 % pure acetone were also purchased.

3.2 Sample Preparation

Okra (*Abelmoschus esculentus*) fruits were obtained from the marketplace in Gaziantep, Turkey. Okra fruits have been stored in the freezer at -20°C in order to protect against harmful modificatins caused by natural oxidation and browning reactions over time. Samples were taken out of the freezer by making sure that okra fruits were green, fresh and small, for thawing at room temperature the day before the experiment. Samples were transferred to the laboratory in sterile rigid plastic container. In the laboratory, samples were washed thoroughly with running water to remove any dirt and debris. Then, with a sharp knife cut into two, and the seeds were cleaned. Seeds were extracted from the fruit because they do not contain mucilage. The cleaned okra was homogenized by laboratory blender for about one minute until they became slurry.

3.3 Extraction Procedure

The extraction of mucilage from okra was carried out using a methods altered from Ameena et al. (2010). The extraction procedure of okra mucilage is shown in Figure 3.1 in details.

Cleaning of okra fruit

Extracting from seeds and Slicing

Homogenizing of pods

Extraction

Precipitation

Freeze drying

Okra mucilage

Figure 3.1 Extraction procedure of okra mucilage

Firstly, 80 g of sample was taken from the homogenized okra pods and weighed. The ratio of water to okra was ranged from 4:1-10:1. Then, a known amount of distilled water was added to the glass beaker and glass beaker was placed on a magnetic stirrer to ensure complete dispersion.

The temperature of the aqueous system graded from 25 to 80ºC and was checked within ±2.0ºC using a manual laboratory thermometer. Weighed okra samples were added to the beaker and water based extraction was started. The speed of stirrer was set constant at 400 rpm. The okra-water slurry was dispersed and extracted from the beginning to the end of the extraction period of 1 to 7 hr.

The solution was separated from residue using white muslin cloth. Then same volume of pure acetone was poured into the solution which caused precipitation of mucilage. Okra mucilage was directly precipitated by adding acetone in cream and light green color and slimy viscous form. Precipitated mucilage was poured into boiling flask and dried for 4 days in the freeze-dryer (CHRIST Alpha 1-4 LDPlus, Martin Christ, Germany). The moisture content of powdered okra mucilage was determined and it was stored in a refrigerator at 4℃ for further analysis.

3.4 Data Analysis and Optimization of Okra Mucilage Extraction

In this study, Box–Behnken (BBD) statistical experiment design and the Response Surface Methedology (RSM) were utilized. The effects of the three independent variables (extraction temperature of 25-80°C, time of 1-7 hr and water to okra ratio of 4:1–10:1 (mL/gr) on the responses were seeked for and settled on the extraction of mucilage polysaccaride from okra fruit. Experiments were demostrated on the basis of Box–Behnken design with three factors at three levels.

Remarkable features of Box-Behnken design can be explained as follows: independence, rotatability and quadratic design. There is no imbedded factorial or fractional factorial points where the variable combinations are at the midpoints of the edges of the variable space and at the center (Maran et al., 2013; Ferreira et al., 2017). Optimization strategy is a complex multi-variable process which has two kinds of variables: responses and factors.

In the present study, the independent variables were extraction time in hr (A), water to okra ratio (B), extraction temperature in °C (C). The independent variables and variation levels are shown in Table 3.1.

Table 3.1 Process variables used in box–behnken design design for three independent variables

Independent variables	Variable Level Codes			
	Code	- 1	0	1
Extraction time	A	1	4	7
Water to okra ratio	B	4	7	10
Extraction temperature	C	25	52.5	80

The responses are the dependent variables and every single independent variable was coded at three levels between −1, 0 and +1. Dependent variables were extraction yield, emulsion capacity, emulsion stability, antioxidant activity, phenolics content and swelling index. The experimental design established of 17 experiments with five center points.

Design-Expert version 7.0.0 (Minneapolis, USA) was used. Experiments were randomized so as to lessen the effects of unperceived variability in the observed responses because of extraneous factors. The results were compared by one-way analysis of variance (one-way ANOVA) to test for significant differences. The goodness of fit of the model was detected by Fisher test (F-test) value. Differences among sample means were reported to be significant when $p< 0.05$. The array of an experiment runs with coded and actual levels are demonstrated in Table 3.2.

Table 3.2 Experimental design for extraction of okra mucilage experiment with coded and actual variable levels

	Coded levels			Actual levels		
Number of Runs	A	B	C	Extraction time (hr)	Water to okra ratio	Extraction temperature (°C)
1	0	0	0	4	7	52.5
2	0	1	-1	4	10	25
3	1	1	0	7	10	52.5
4	0	-1	1	4	4	80
5	0	-1	-1	4	4	25
6	-1	0	1	1	7	80
7	0	0	0	4	7	52.5
8	-1	1	0	1	10	52.5
9	0	0	0	4	7	52.5
10	1	-1	0	7	4	52.5
11	0	1	1	4	10	80
12	1	0	1	7	7	80
13	1	0	-1	7	7	25
14	-1	-1	0	1	4	52.5
15	-1	0	-1	1	7	25
16	0	0	0	4	7	52.5
17	0	0	0	4	7	52.5

Additionally, numerical optimization was implemented for independent variables of okra mucilage powder. To achieve that goal, desirability function of Response Surface Methodology was used. The desirability formula is shown in Equation 3.1.

$$N = K^2 + K + C_p \tag{3.1}$$

where, K is the factor number and Cp is the replicate number of the central point (Edrissi et al., 2008).

The desirability approach consists of the following steps:

(1) Conducting the experiments and fitting response models (yi) for all m responses.

(2) Defining individual desirability functions for each response (di).

(3) Maximising the overall desirability with respect to the controllable factors

The general approach is to first convert each response yi into an individual desirability function di that varies over the range $0 \leq di \leq 1$ where if response yi is at its target value, then di = 1, and if it is outside an acceptable region, di = 0. Then the design variables were chosen to maximize the overall desirability. Desirability formula is given in Equation 3.2.

$$D_o = (d1d2 \dots dm)^{\frac{1}{m}} \quad (3.2)$$

where m is the number of responses and Do is the overall desirability (Myers and Montgomery, 2002). It was aimed to achieve the independent variables of extraction yield, emulsion capacity, emulsion stability, antioxidant activity, phenolics content and swelling index to their maximum level for parameter optimization.

RSM is a remarkably effective method used in optimization of multivariable processes. This strategy helps the compherension of mathematical and statistical relationships between one or more dependent and independent variables. Many researchers have used RSM in order to optimize extraction procedures (Karazhiyan et al., 2011; Jouki et al., 2014). Regression analysis and ANOVA were used in order to settle the model and view the statistical significance of the terms.

3.5 Methods

3.5.1 Moisture Content Analysis

Moisture determination was carried out by moisture analyzer at the laboratory of Gaziantep University. Sufficient amount of homogenized okra sample was taken and analysed (Khodabux et al., 2007). Dried okra mucilage was analysed as well.

3.5.2 Percentage Yield

The percentage yield was determined from the ratio of weight (w) of homogenized okra pods, as a starting material and the dried okra mucilage powder, as a final material both are on dry basis, times 100% (Samavati, 2013) as shown in Equation 3.3.

$$\%\text{Extraction Yield} = \frac{Dried\ okra\ mucilage\ powder(g)}{Homogenized\ okra\ pods\ (g)} \text{ x } 100 \qquad (3.3)$$

3.5.3 Swelling Capacity

Swelling capacity (SC) of the okra mucilage is formulated as milliliter per gram of mucilage (mL/g). The method was derived from Sangeethapriya and Siddhuraju (2014), outlined in Figure 3.2 and calculated from Equation 3.4.

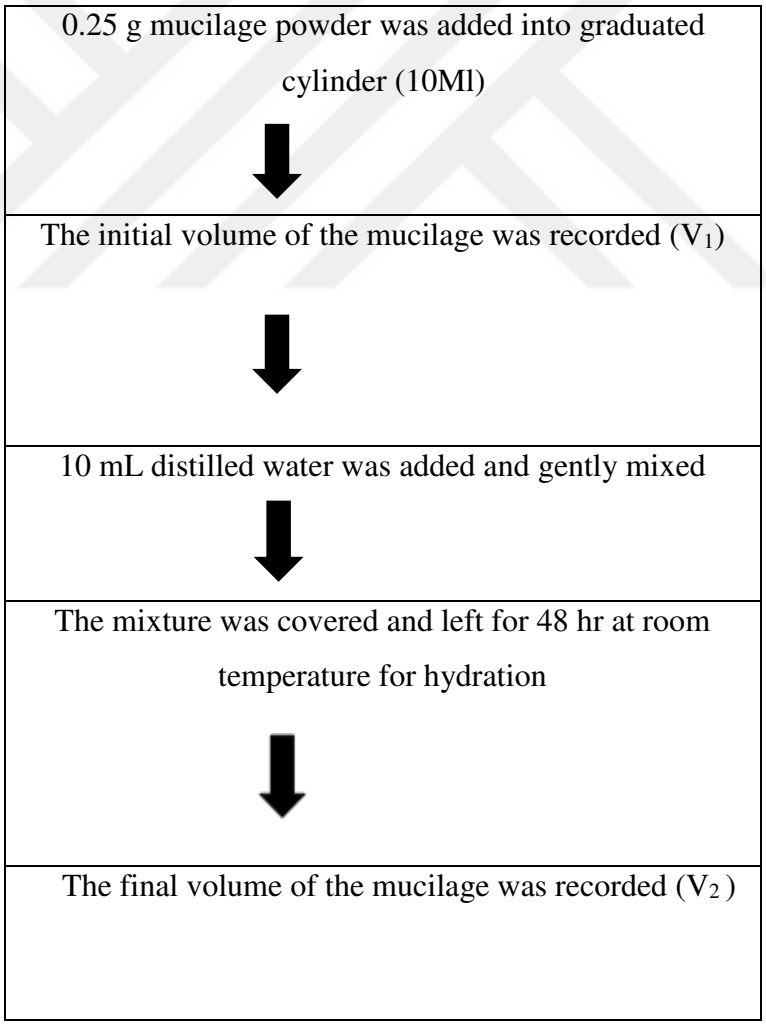

Figure 3.2 Schematic representation of swelling capacity procedure

$$Swelling\ Capacity\ (mL/g) = \frac{(V_2 - V_1)}{0.25} \quad (3.4)$$

3.5.4 Emulsifying Properties

3.5.4.1 Emulsion Capacity

Okra mucilage samples were precisely weighed (0.25 g). Then, it was dissolved in 20 ml distilled water, and 20 ml refined sunflower oil was added to mucilageous solution. Then, by homogenizing for 2-3 min with a homogenizer (Art-Miccra D-8, Germany) emulsion was prepared. It was centrifuged at 8000 rpm for 5 min. Finally, the height of emulsified layer was measured and compared with the height of whole layer (Thanatcha and Pranee, 2011). Emulsion capacity (EC) was calculated by the Equation 3.5.

$$Emulsion\ Capacity\ \% = \frac{Height\ of\ emulsified\ layer\ x\ 100}{Height\ of\ whole\ layer} \quad (3.5)$$

3.5.4.2 Emulsion Stability

The same procedure was applied with emulsion capacity method and emulsion was prepared. It was heated at 80ºC for 30 min. then centrifuged at 8000 rpm for 5 min (Noorlaila et al., 2015; Thanatcha and Pranee, 2011). Emulsion stability (ES) evaulated by the Equation 3.6.

$$\text{Emulsion Stability \%} = \frac{\text{Height of emulsified layer after heating x100}}{\text{Height of whole layer}} \quad (3.6)$$

3.5.5 Radical-Scavenging Activity (RSA) of Okra mucilage

By the previously reported method used by Jouki et al. (2014), antioxidant activity of okra mucilage was measured relying on the scavenging ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical test. Firstly, the powder of okra mucilage was dissolved in distilled water at 1 mg/ml. For preparing at a concentration of 0.1 mM of DPPH ethanol solution, 1.97 mg DPPH powder was dissolved into ethanol in 50 ml of volumetric flask. Then, 2 ml extract solution was mixed with 4 ml DPPH solution into test tubes for sample. Also, 2 ml of extract solution was mixed with 4 ml of DPPH solution into test tubes for control tubes. Ethanol was used as a reference.

After vigorous stirring of all test tubes it was counted 30 min dark incubation at room temperature. Then, absorbance was measured at 517 nm by using spectrophotometre. The experiment was employed triplicate and averaged. The scavenging activity of DPPH radicals was calculated for using the Equation 3.7.

$$Scavenging\ ability\ \% = A_0 - \left(\frac{A_1}{A_0}\right) x100 \quad (3.7)$$

where, A_0 and A_1 are the absorbance of control (without sample) and sample, respectively. The scavenging activity of okra mucilage solutions was determined instantly after preparation.

3.5.6 Determination of Total Phenolics Content (TPC)

Total phenolics content (TPC) of okra mucilage was calculated on the basis of Folin-Ciocalteau method used by Ahiakpa et al. (2013). As a first step, 20% Na_2CO_3 solution was prepared by 5 g of Na_2CO_3 was dissolved into 25 ml of volumetric flask with distilled water and stirred until to get clear solution. Second step, 0.005 mg of okra mucilage powder was dissolved in 50 ml distilled water for extract solution (1:1, v/v). Third step was the Folin-Ciocalteau reagents (FCR) solution. 400 microliter extract solution and 1000 microliter Folin-Ciocalteau reagents was mixed and waited for 5 min. Then, 3000 microliters of Na_2CO_3 was added to all test tubes. They were stored at room temperature for 30 min in darkness. Absorbance values were measured spectrophotometrically at 760 nm using a Spectrophotometer (UV/Vis – SP3000nano, Optima, Tokio, Japan). All determinations were employed in triplicate and averaged.

A calibration curve was derived using the following dilution regimes; 0.01mg/ml, 0.02mg/ml, 0.04 mg/ml, 0.06mg/ml, 0.08mg/ml from a stock solution of 10 mg/ml Gallic acid by dissolving 0.5 gr gallic acid into 50 ml distilled water.

TPC was calculated from a calibration curve using gallic acid as standard and the results were expressed as mg G.A.E /mL (The calibration curve equation was Conc.= 0.0786.Abs – 0.0002, R^2= 0.9886).

3.5.7 Oiling Off

Oiling off was determined by using the method used by Maskan and Göğüş (2000). Emulsions were prepared by dissolving 0.2 g okra mucilage in 20 ml distilled water and then adding 20 ml sunflower oil.

The mixture was homogenized (Art-Miccra D-8, Germany) at 8000 rpm for 2-3 min. Homogenized mixtures were poured seperately into measuring cylinders (10 ml) of the same size and kept at the constant temperature of 30ºC. The height of the clear liquid (oil) accumulated at the top was measured after 24 hr.

3.5.8 Organoleptic Evaluation of Mucilage from Abelmoschus esculentus

Okra mucilage was characterized for organoleptic properties such as colour, odour, taste, touch and texture. Orgonoleptic evaluation is carried out by 10 trained panellists. 5 randomly choosen okra mucilage sample were placed on white plates and given to the panelist. Samples were scored from 1 to 5. Organoleptic evaluation is given in Table 3.3. On the scales for colour 5= honey, on the odor scale 1= odourless, on the taste scale 1= tasteless, on the touch and texture scale 5= too rough (Baker and Darfler, 1977).

Table 3.3 Organoleptic evaluation of okra mucilage sample

Name of the panelist	Colour	Odor	Taste	Touch and texture
Sample 1				
Sample 2				
Sample 3				
Sample 4				
Sample 5				

3.5.9 Solubility Test

In order to determine the solubility of the okra mucilage, 10 mg of okra powder was added to 10 mL water, acetone and ethanol solutions (1% dispersion) (Zaharuddin et al., 2014). Solubility was ascertained by visual observation and noted into the paper. 10 trained panellists observed and noted their consideration as ‘’Soluble’’ or ‘’Insoluble’. Solubility test is given in Table 3.4 (Alalor et al., 2014).

Table 3.4 Solubility test for okra mucilage sample

Solvent type	Solubility
Water	
Acetone	
Ethanol	

3.5.10 Effect of Okra Mucilage on Oiling Off of Pistachionuts Paste

Okra mucilage powders were dissolved in distilled water by adjusting the percentage of mucilage powder in the test samples to 0.8 %, 1% and 1.2 %. Control sample has no mucilage powder. Subsequently, the mixtures with mucilage additives were prepared by taking 5 grams of pistachionuts paste and were centrifuged. Each experiment was repeated 3 times and averaged. The oiling off of pistachionuts paste was found by subtracting the initial mass of fat from the supernatant oil after centrifugation and dividing the result by the initial fat amount and multiplying by 100. Oiling off formula is given in Equation 3.8.

$$Oiling\ off = \frac{Initial\ mass\ of\ fat - supernatant\ oil\ mass}{Initial\ mass\ of\ fat} x100 \quad (3.8)$$

CHAPTER 4

RESULTS AND DISCUSSION

4.1 General

In the present study, for optimization of the experiment, factors and their upper and lower levels were determined based on prelimary studies and previous works. The most critical factors affecting extraction conditions were settled on extraction time (hr), water to okra ratio and extraction temperature (ºC). The upper and lower levels of extraction time ranged between 1-7 hr, water to okra ratio 4:1–10:1 (mL/gr) and extraction temperature 25-80ºC. Stability of emulsions increased with addition of okra mucilage because oiling off results supported as previously thought. Additionally, pistachionuts paste was chosen as a case study with the awareness that the appearance of foods is important and pistachionuts paste is a preferable food for the consumer which has an unstable emulsion system.

The mucilage sample used in this case study has both high extraction efficiency and high emulsion properties. Thus, it was aimed to be economical and efficient. By adding mucilage, amount of oil accumulated at the top decreased. Product stability of pistachionuts paste was increased to 97.1% by adding 1.2% mucilage.

4.2 Experimental Design and Model Fitting

Response surface methodology was used for experimental design. ANOVA was applied to evaluate the significant effects of independent variables on the responses. The significance of coefficients of fitted models was assesed by using F-test, p-value, lack of fit and coefficient of determination. Coefficient of determination (R^2) varied from 0.62 to 0.93. Results of responses received from three-factor, three-level Box–Behnken design. It is shown in Table 4.1.

Table 4.1 Results of responses obtained from three-factor, three-level box–behnken design

Run	Time (hr)	Ratio (water to okra)	Temperature (°C)	Yield (%)	EC (%)	ES (%)	RSA (%)	TPC	SC (mL/g)
1	4	7	52.5	22.76	49.75	50	54.74	26.68	50.24
2	4	10	25	16.92	57	60	15.77	18.97	32.3
3	7	10	52.5	18.51	18.95	27	29.82	21.41	37.9
4	4	4	80	18.9	8.75	17	20.38	14.73	44
5	4	4	25	20.09	47.5	53,5	18.52	32.73	40.4
6	1	7	80	32.19	28	24,5	22.86	42.63	41.2
7	4	7	52.5	21.39	53.75	57,5	56.76	26.99	51.8
8	1	10	52.5	21.37	53.25	54,5	7.17	14.49	49.2
9	4	7	52.5	23.95	50.5	51,5	55.34	26.36	47.8
10	7	4	52.5	24.52	34	56,5	29.82	15.44	55.44
11	4	10	80	19.91	17.75	23,5	13.07	10.64	50.8
12	7	7	80	21.93	27.05	24	19.76	54.34	34.4
13	7	7	25	27.09	52.5	56	11.35	9.54	46
14	1	4	52.5	17.1	43	56	11.25	26.83	38.2
15	1	7	25	20.47	53.25	58	16.82	60.47	30.5
16	4	7	52.5	20.89	53.5	57	51.75	27.23	46.6
17	4	7	52.5	20.4	50.5	56	53.73	25.1	45.5

The effects of independent variables (extraction time, water to okra ratio, extraction temperature) on responses were evaluated to determine optimum conditions. Six responses (extraction yield, swelling capacity, emulsion capacity, emulsion stability, DPPH, TPC) were determined for better investigation of okra mucilage. The influences of process variables were illustrated by 3D response surface plots. The regression model graphs described the effects of process variables by linear and quadratic equations.

4.3 Percantage Yield

The first response was the percentage yield. The effects of extraction temperature, extraction time and water to okra ratio on percentage yield were investigated.

Range of extraction temperature was selected 25 to 80°C, extraction time 1 to 7 hr while ratio of water to okra 4:1-10:1.

Table 4.2 shows quadratic effect of process variables on yield. The model is significant ($p<0.05$). For quadratic effect, AB, AC, A^2 and B^2 are significant model terms ($p<0.05$).

However, from Table 4.2 it can be seen that influences of extraction temperature are not significant on this response ($p>0.05$). There were many previous studies investigated hydrocolloids extractions with modificated methods and different solvents. In this study, water based extraction method was used for the extraction of mucilage from okra fruit. The percentage yield was calculated on dry basis %. The yield of okra mucilage was obtained between 16.92 to 32.19 %.

Table 4.2 ANOVA results for response surface quadratic model analysis for yield

Source	Sum of squares	df	Mean square	F value	p-value
Model	205.4239	9	22.82487444	8.10	0.0058*
A-Time	0.11	1.	0.11	0.04	0.8519
B-Ratio	1.90	1	1.90	0.67	0.4385
C-Temperature	8.74	1	8.74	3.10	0.1217
AB	26.42	1	26.42	9.37	0.0183
AC	71.23	1	71.23	25.27	0.0015
BC	4.37	1	4.37	1.55	0.2532
A^2	25.92	1	25.92	9,20	0.0190
B^2	66.83	1	66.83	23.71	0.0018
C^2	4.74	1	4.74	1.68	0.2358
Residual	19.73	7	2.82		
Lack of Fit	11.26	3	3.75	1.77	0.2912**

*Significant at p-value < 0.05, **Not significant at p-value>0.05, R^2=0.91

The relationship between extraction yield and independent variables of extraction time, water to okra ratio and extraction temperature in terms of coded variables are given in Equation 4.1. In this equation, EY is the extraction yield, A is the extraction time, B is the water to okra ratio and C is the extraction temperature.

Regression analysis revealed that the coefficient of determination (R^2) of quadratic model was 0.91.

$$EY\ \% = 21.88 + 0.12A - 0.4B + 1.04C - 2.57AB - 4.22AC + 1.04BC + 2.48A^2 - 3.98B^2 + 1.06C^2 \quad (4.1)$$

It is seen that variables have a quadratic effect on the responses. In Figure 4.1, the effect of extraction temperature and extraction time on extraction yield, in Figure 4.2, the effect of water to okra ratio and extraction time on yield are shown. Figure 4.1 shows the variation of extraction yield depending on the extraction temperature and time at constant water to okra ratio. ANOVA results and Figure 4.1 revealed that interaction effects of extraction time and water to okra ratio have significant effect on yield ($p<0.05$). Also, interaction effects of extraction time and extraction temperature have significant effect on yield ($p<0.05$). As shown, extraction efficiency increased with temperature. In addition, extraction time is another effective influence on extraction efficiency. Extraction efficiency appeared to have two different increases in constant water okra ratio. One of these increased efficiency with increasing temperature in low extraction time, and the second, efficiency increased with increasing extraction time in low temperature. Maximum yield was reached at 80°C and 1-hour extraction time.

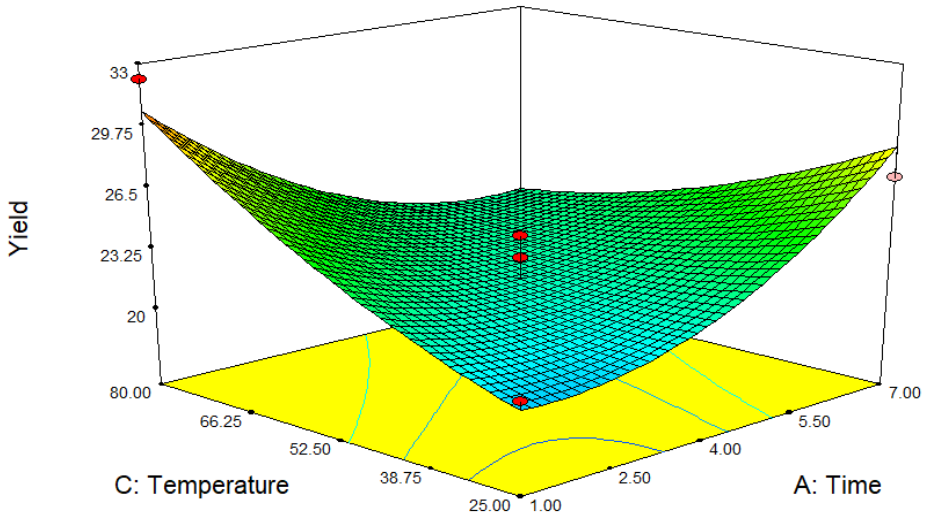

Figure 4.1 3D response surface of the effects of extraction temperature and extraction time on extraction yield

In Figure 4.2, the results point out that quadratic effect of water to okra ratio and extraction time had significant effects on extraction yield ($p<0.05$). It was illustrated that increasing water to okra ratio increased extraction yield about ratio of 8.50 then, slight decrease was observed on yield. On the other hand, increasing extraction time had positively affected with extraction yield. The graph has increasingly continued tendency.

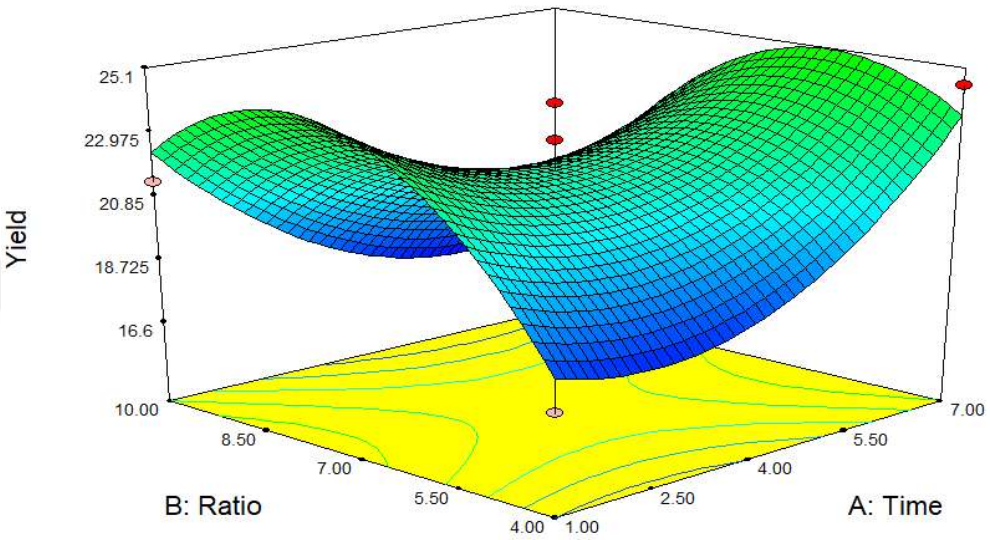

Figure 4.2 3D response surface of the effects of water to okra ratio and extraction time on extraction yield

4.4 Swelling Capacity

Second response was the swelling capacity. Table 4.3 shows quadratic effect of extraction time, water to okra ratio and extraction temperature on swelling capacity. The model is significant ($p<0.05$). The effects of extraction time and extraction temperature on swelling capacity was shown in Figure 4.3 and the effects of water to okra ratio and extraction time on swelling capacity was shown in Figure 4.4. ANOVA results revealed that AB, AC, C^2 are significant model terms ($p<0.05$). As Table 4.3 indicated that interactive effect between extraction time and extraction temperature on swelling capacity were significant ($p<0.05$). Also interactions of extraction time and water to okra ratio was significant ($p<0.05$). In this present study, range of swelling capacity was between 30.5 to 55.44 mL/g.

The term of swelling is defining 'the volume seized over a known weight of fibre under the conditions used' and used to explain hydration properties of natural polymers especially classified as hydrocolloids from plant origins (Sangeethapriya and Siddhuraju, 2014).

Sangeethapriya and Siddhuraju (2014) determined the swelling capacity of *Zizyphus mauritiana* fruits mucilage as 19.34 mL/g. They reported that mucilage content of *Zizyphus mauritiana* fruits is correspond to coconut and mango fiber with 18.3 mL/g, 18.7 mL/g, respectively.

Nevertheless, it is lower than pitaya and carrot in order of 35.95 mL/g, 60.2 mL/g. Controversely, Archana et al. (2013) investigated the swelling capacity of *Gracilaria corticata* and *Abelmoschus esculentus* polysaccharide with methanol and acetone. Methanol and acetone extract of okra polysaccarides were almost identical. Also, high swelling capacity is attributed to higher water holding capacities.

Table 4.3 ANOVA results for response surface quadratic model analysis for swelling capacity

Source	Sum of squares	Df	Mean square	F value	p-value
Model	734.9423	9	81.66026	5.864936	0.0147*
A-Time	26.7912	1	26.7912	1.924176	0.2080
B-Ratio	7.6832	1	7.6832	0.551817	0.4818
C-Temperature	56.18	1	56.18	4.034914	0.0845
AB	203.6329	1	203.6329	14.62516	0.0065
AC	124.3225	1	124.3225	8.928989	0.0203
BC	55.5025	1	55.5025	3.986255	0.0861
A^2	52.36296	1	52.36296	3.76077	0.0936
B^2	0.440641	1	0.440641	0.031647	0.8638
C^2	196.7905	1	196.7905	14.13372	0.0071
Residual	97.46428	7	13.92347		
Lack of Fit	70.5094	3	23.50313	3.487774	0.1294**

*Significant at p-value < 0.05, **Not significant at p-value>0.05, R^2=0.88

The relationship between swelling capacity and independent variables of extraction time, water to okra ratio and extraction temperature in terms of coded variables is given in Equation 4.2 where, A is the extraction time, B is the water to okra ratio and C is the extraction temperature. Regression analysis revealed that the coefficient of determination (R^2) of quadratic model was 0.88.

$$\text{Swelling Capacity} = 48.39 + 1.83A - 0.98B + 2.65C - 7.14AB - 5.58AC + 3.72BC - 3.53A2 + 0.32B2 - 6.84C2 \quad (4.2)$$

Figure 4.3 shows that increasing extraction temperature and extraction time has increasing effect on swelling capacity. The minimum swelling capacity found to be 30.5 mL/g at the lowest extraction temperature and extraction time. Figure 4.4 explains the effect of water to okra ratio and extraction time on swelling capacity. Decreasing extraction time causes to rapid decline on the swelling capacity also, decreasing water to okra ratio decreases the swelling capacity.

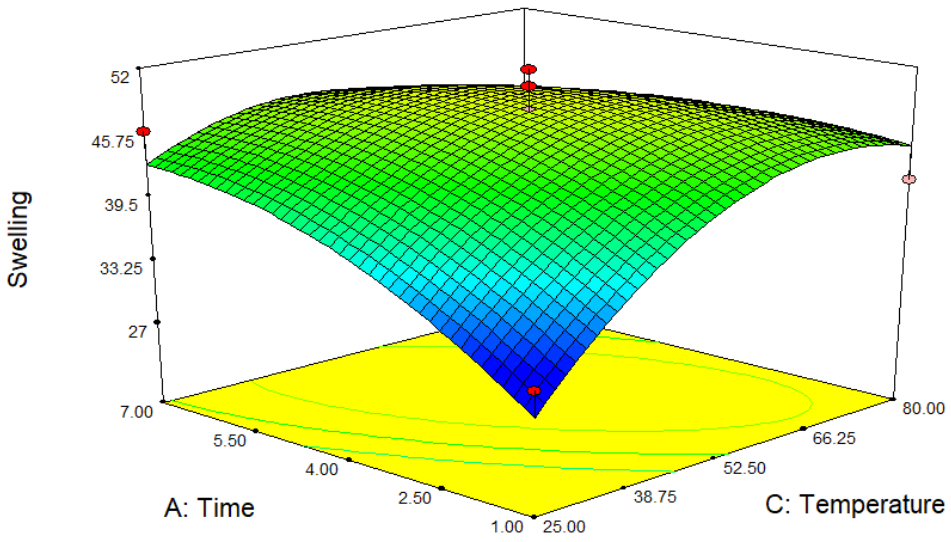

Figure 4.3 3D response surface of the effects of extraction time and extraction temperature on swelling capacity

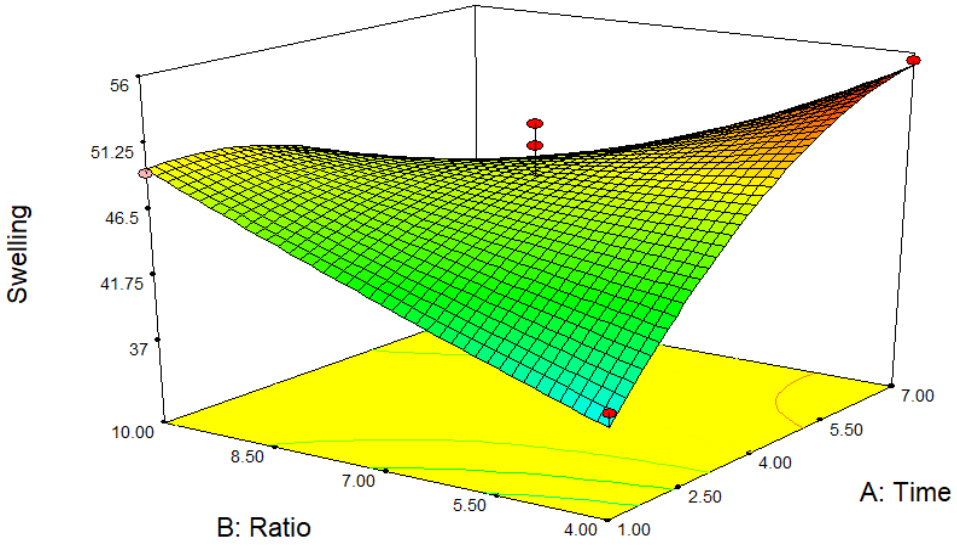

Figure 4.4 3D response surface of the effects of water to okra ratio and extraction time on swelling capacity

After the duration of 48 hr, volume occupied by okra mucilage is clearly shown in Figure 4.5. Initial weight of the samples was constant, and 0.25 g. The average value of initial volume of the samples is 2.14 ±057 ml. After 48 hour of duration, final volume of the samples is recorded and averaged as 13.03±1.47 ml. Mucilage powder into that sample was produced under extraction conditions of 4 hr of extraction time, 4:1 of water to okra ratio and 25°C of extraction temperature. As it is anticipated, swelling capacity of okra mucilage was satisfactory.

This result shows that okra mucilage is the potential candidate using as thickening agent for nutraceutical and food systems. Under favour of high water absorbing ability, okra mucilage has a strong potential on food and pharmaceutical industry. Thanks to its swelling capacity, it takes place in these areas. Because of controlled release dosage in diffusion, swelling is a fundemental mechanism. Previous researches demonstrated that okra mucilage is useful excipient in tablets about the issue of sustained release drug delivery system thanks to strong matrix polymeric structure and it forms viscous, swellable dispersion in water (Archana et al., 2013).

Figure 4.5 A picture of swelling capacity of okra mucilage on test tube

4.5 Emulsifying Properties

Emulsion systems are comprised of two liquids which are not dissolved in each other. These two immiscible liquids are mostly liquid and water. By time, two distinctive phases occur that can be noticeable by visual observation in food emulsion systems. Definitions such as emulsion capacity, stability and activity are used for the emulsion properties of proteins.

Emulsion capacity is the ability of the protein solution or suspension to emulsify the oil and is shown by the amount of fat emulsified by 1 gram of protein under specific conditions. Emulsion stability is the capacity of the emulsion droplets to stay soluble without forming the turbidity forming the layer formation and the combination of the drops (Zayas, 2012).

The next response is the emulsifying properties of okra mucilage. The influences of extraction time, water to okra ratio and extraction temperature on emulsion capacity and emulsion stability were shown in Table 4.4 and Table 4.5, respectively. Figure 4.6 and Figure 4.7 demonstrated the effect of independent variables on emulsion capacity.

On the other hand, the effect of independent variables on emulsion stability is demonstrated on Figure 4.8 and Figure 4.9. ANOVA results shows that extraction temperature is the only significant model term for emulsion capacity and stability ($p<0.05$). Linear model was significant on both responses ($p<0.05$).

Table 4.4 ANOVA results for response surface linear model analysis for emulsion capacity

Source	Sum of squares	Df	Mean squares	F value	p-value
Model	2347.05	3	782.35	7.05	0.0047*
A-Time	253.12	1	253.12	2.28	0.1549
B-Ratio	23.46	1	23.46	0.21	0.6533
C-Temperature	2070.46	1	2070.46	18.66	0.0008
Residual	1442.65	13	110.97		
Lack of Fit	1428.57	9	158.73	45.11	0.0012*

*Significant at p-value < 0.05, **Not significant at p-value>0.05, R^2=0.62

The relationship between emulsion capacity and independent variables extraction time, water to okra ratio and extraction temperature in terms of coded variables is given in Equation 4.3 where, A is the extraction time, B is the water to okra ratio and C is the extraction temperature. Regression analysis revealed that the coefficient of determination (R^2) of linear model was 0.62.

$$Emulsion\ Capacity = 41.12 - 5.63A + 1.71B - 16.09C \quad (4.3)$$

From the Figure 4.6 and Figure 4.7, it is examined the linear effect of interactions of parameters on emulsion capacity. Figure 4.6 showed that the emulsion capacity was negatively analogical to the main effects of extraction temperature. Also, increases in extraction time decreased the emulsion capacity slightly. Extraction temperature is the

only significant term and influences of water to okra ratio and extraction time is not significant ($p>0.05$) on response. It is clear from Figure 4.7 that decreasing extraction time incerased the emulsion capacity gradually.

The maximum and minimum emulsion capacity was found to be 57% and 8.75% respectively. Silva et al. (2019) extracted mucilage from a cactus known as *Pereskia aculeata* Miller. They reported that increasing the mucilage concentration is boosting the emulsion capacity. Similarly, Jideani and Bello (2009) claimed that there is a strong relation between emulsifying properties and mucilage. They pointed out that importance of the existence of the mucilage in the emulsion system and its stabilizing effect. Their study indicated that samples of high mucilage protein concentrate (HMPC) and whole okra flour (WOF) showed a great emulsion activity, nearly 100%. In this present study, the highest emulsion capacity was found as 57% which is higher than the results of Archana et al. (2013) (54.75%), and Mir et al. (2017). They reported the emulsion capacity of okra mucilage as 46.87 %. The emulsion capacity of okra mucilage powder was analogous to that of Locust bean gum (52%) but less than xanthan gum (about 94%) and *Ocimum canum* seeds (about 74.41%).

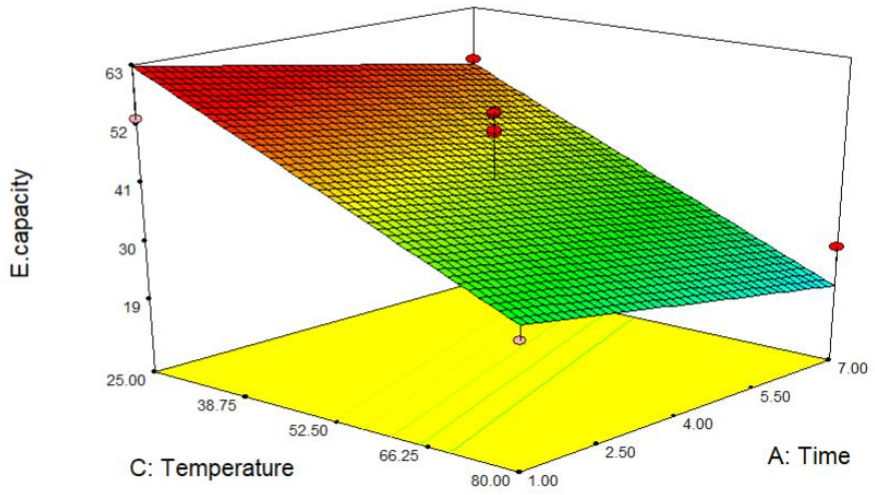

Figure 4.6 3D response surface of the effects of extraction temperature an extraction time on emulsion capacity

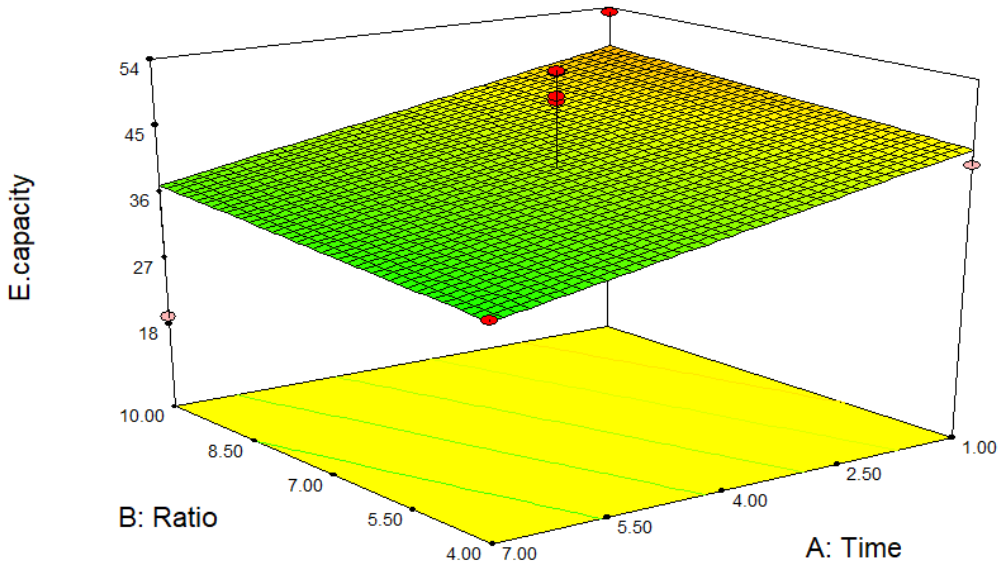

Figure 4.7 3D response surface of the effects of water to okra ratio and extraction time on emulsion capacity

Table 4.5 ANOVA results for response surface linear model analysis for emulsion stability

Source	Sum of squares	Df	Mean square	F value	p-value
Model	2547.06	3	849.02	8.58	0.0021*
A-Time	108.78	1	108.78	1.10	0.3134
B-Ratio	40.50	1	40.50	0.41	0.5333
C-Temperature	2397.78	1	2397.78	24.25	0.0003
Residual	1285.67	13	98.90		
Lack of Fit	1238.97	9	137.66	11.79	0.0150*

*Significant at p-value < 0.05, **Not significant at p-value>0.05, R^2=0.66

The relationship between emulsion stability and independent variables extraction time, water to okra ratio and extraction temperature in terms of coded variables is given in Equation 4.4 where, A is the extraction time, B is the water to okra ratio and C is the

extraction temperature. Regression analysis revealed that the coefficient of determination (R^2) of linear model was 0.66.

$$Emulsion\ Stability = 46.03 - 3.69A - 2.25B - 17.31C \quad (4.4)$$

Emulsion stability of okra mucilage was ranged from 17 to 60% in the present study. From Figure 4.8, it was demonstrated that increasing temperature was negatively effective on emulsion stability. However, Figure 4.9 shows that there is no significant interaction between extraction time and water to okra ratio ($p>0.05$). Results were contrary with Jouki et al. (2014) and Koocheki et al. (2009). Jouki et al. (2014) explained the relationship between emulsion stability of quince seeed mucilage and independent variables such as water to seed ratio, extraction temperature and extraction time. Controversely, they demonstrated that water to seed ratio, extraction temperature and extraction time are significant terms ($p < 0.001$) on linear effect. Therefore, they claimed that increasing temperature had increasing effect up to 45°C. If the temperature continues to be raised, the emulsion has a negative effect on stability thus, emulsion stability decreases. Similar explanations reported by Koocheki et al. (2013) that increasing temperature has decreasing effect on emulsion stability.

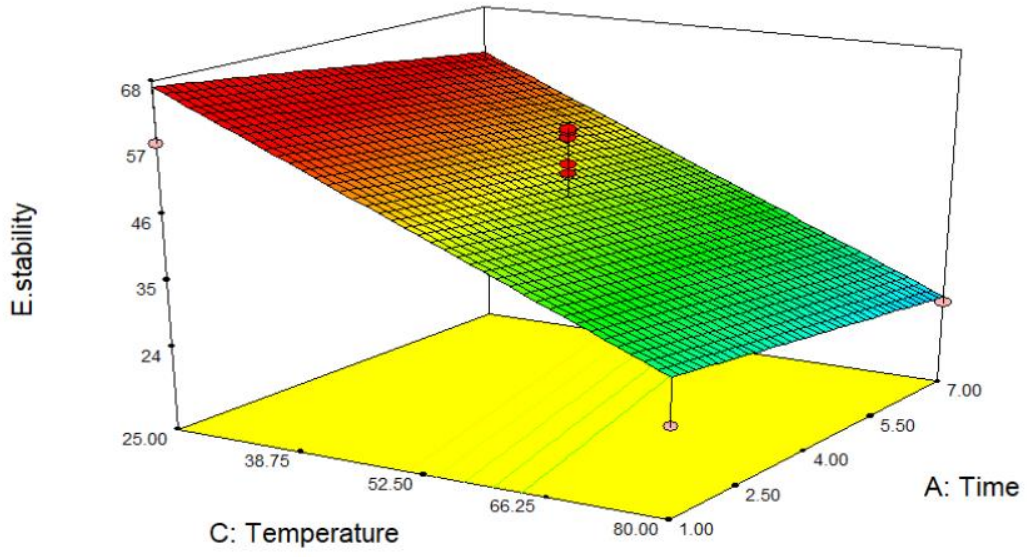

Figure 4.8 3D response surface of the effects of extraction temperature and extraction time on emulsion stability

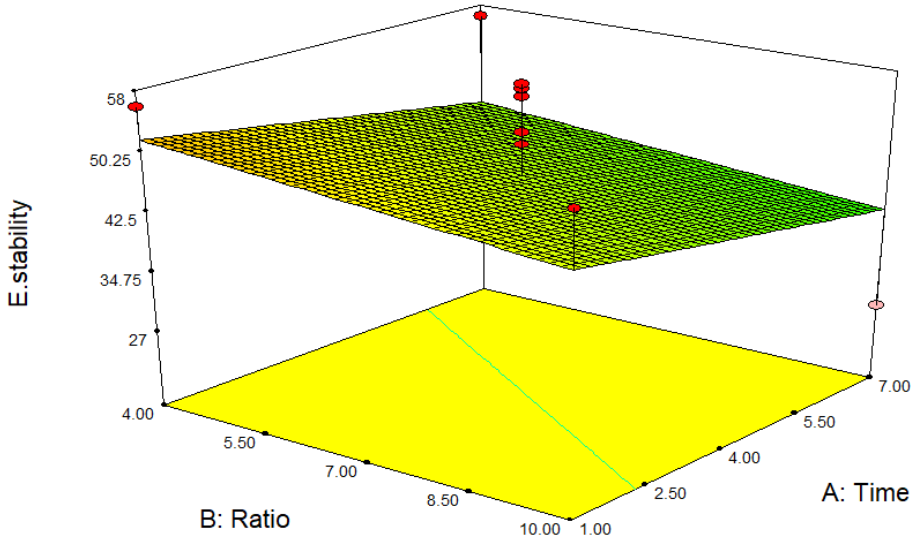

Figure 4.9 3D response surface of the effects of water to okra ratio and extraction time on emulsion stability

It is clearly seen from the Figure 4.10 that from top to bottom, liquid layer, stabilized layer and water, respectively. Okra mucilage was added into the sample which was produced under the conditions of 4 hr of extraction time, 7 of water to okra ratio and 52°C of extraction temperature. In the present study, result demonstrated that okra mucilage is a strong promising potential to be used as an emulsifier and stabilizer on food products consisted of oil and water. Similarly, emulsifying properties of okra mucilage was investigated on coconut milk emulsion and found to be succesfull natural emulsifier for food emulsion systems (Noorlaila, 2015).

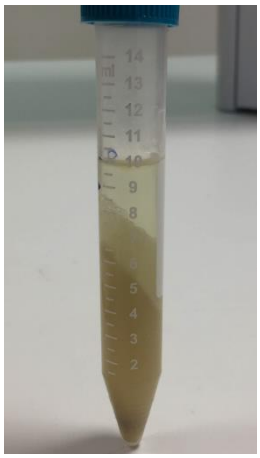

Figure 4.10 A picture of emulsifying capacity of okra mucilage on test tube after centrifugation

4.6 Radical-Scavenging Activity of Okra Mucilage

Another important response is the antioxidant capacity. Antioxidant is a substance that prevents or slows down the hazard brought on by unbalanced molecules and free radicals. These harmful molecules are produced by the body as a reaction to environmental and other pressures.

Okra is an advantageous participant to antioxidant activity along with a hopeful chemo-preventive agent. Pharmacological studies have shown that okra has a possession of antioxidant, neuroprotective, antidiabetic, antihyperlipidemic, and anti-fatigue activities.

Quadratic effects of extraction temperature, extraction time and water to okra ratio on antioxidant activity were demonstrated on Table 4.6. The model is significant ($p<0.05$). From the quadratic model, A^2, B^2, C^2 are significant ($p<0.05$) model terms. The ANOVA results showed that extraction time, extraction temperature and water to okra ratio were the crucial factors on antioxidant activity of mucilage from okra. Antioxidant activity of okra mucilage was determined on the basis of scavenging ability (%). It was ranged between 56.76% to 7.16%. Figure 4.11 and Figure 4.12 show effects of extraction temperature and extraction time on DPPH radical-scavenging activity, effects of water to okra ratio and extraction temperature on DPPH radical-scavenging activity, respectively. It was observed that there are strong interactions between process variables as shown on the 3D model graphs. From the Figure 4.11, it was seen that extraction time less than 4 hr causes decrease in antioxidant activity. This may be due to the insufficient time for the antioxidant substances in okra mucilage to pass into the water.

Similarly, excessive extraction time seems to have a reducing effect on antioxidant activity as in low extraction time. It was interpreted that compounds tend to deteriorate when exposed to long time extraction duration (Jouki et al., 2014). Moreover, according to Cahyana and Kam (2016), extended extraction time caused decrease in antioxidant activity. It was claimed that antioxidant activity was getting higher in the first 8 hr of duration, then showed decreasing in prolong hours. With the same mechanism, high extraction temperature causes a reduction in antioxidant activity as similar with low extraction temperature. Decrease in antioxidant activity in exceded temperatures may led loosing the ability of reactants against free radicals. This may be

an indication that phenolics substances are sensitive to temperature. Jouki et al. (2014) stated that an increase in extraction temperature caused a decrease in the antioxidant activity of quince seed mucilage. Similarly, Miranda (2009) claimed that extraction temperature had a negative effect on antioxidant capacity of of aloe vera gel.

Table 4.6 ANOVA results for response surface quadratic model analysis for DPPH radical scavenging activity of okra mucilage

Source	Sum of squares	Df	Mean square	F value	p-value
Model	4886.36	9	542.9289	10.628	0.0026*
A-Time	133.2528	1	133.2528	2.608465	0.1503
B-Ratio	24.99245	1	24.99245	0.489235	0.5068
C-Temperature	23.15401	1	23.15401	0.453247	0.5224
AB	4.1616	1	4.1616	0.081465	0.7836
AC	1.404225	1	1.404225	0.027488	0.8730
BC	5.1984	1	5.1984	0.10176	0.7590
A^2	1230.228	1	1230.228	24.0821	0.0017
B^2	1342.433	1	1342.433	26.27854	0.0014
C^2	1629.628	1	1629.628	31.90048	0.0008
Residual	357.5933	7	51.08476		
Lack of Fit	343.5736	3	114.5245	32.67527	0.0028*

*Significant at p-value < 0.05, **Not significant at p-value>0.05, R^2=0.93

The relationship between antioxidant activity and independent variables extraction time, water to okra ratio and extraction temperature in terms of coded variables is given in Equation 4.5 where, A is the extraction time, B is the water to okra ratio and C is the extraction temperature. Regression analysis revealed that the coefficient of determination (R^2) of qudaratic model was 0.93.

$$DPPH\ Radical - Scavenging\ Activity = 54.46 + 4.08A - 1.77B + 1.70C + 1.02AB + 0.59AC - 1.14BC - 17.09A^2 - 17.86B^2 - 19.67C^2 \quad (4.5)$$

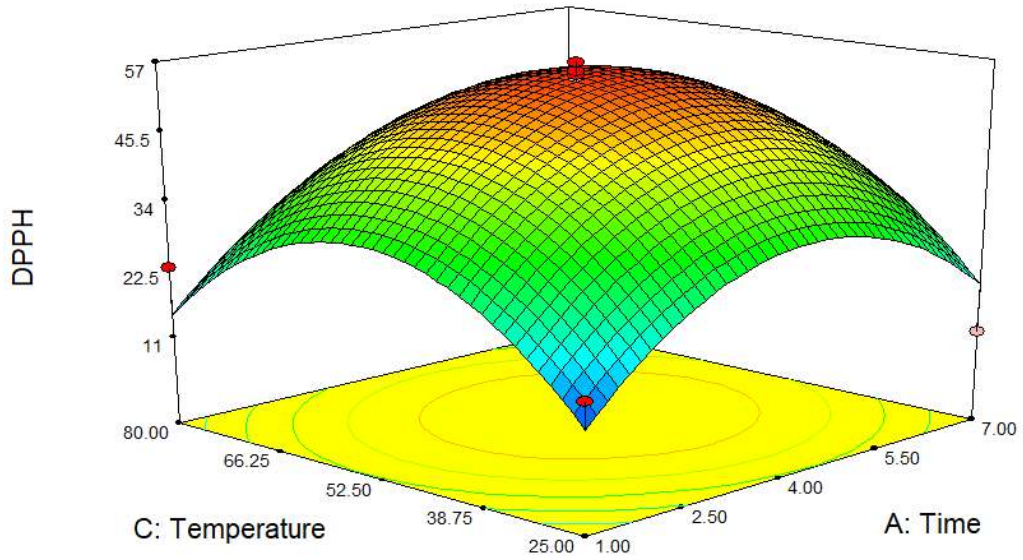

Figure 4.11 3D response surface of the effects of extraction temperature and extraction time on DPPH radical scavenging activity

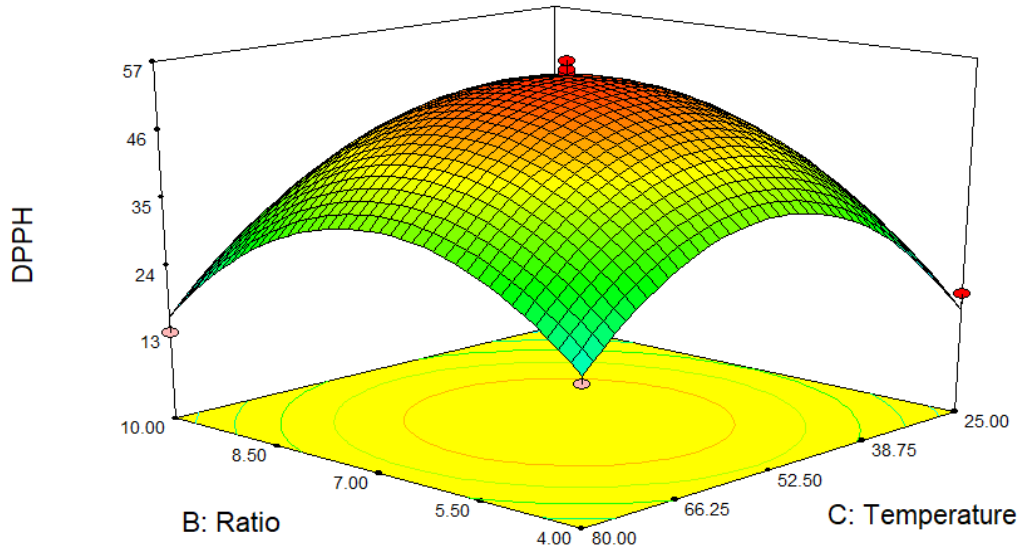

Figure 4.12 3D response surface of the effects of water to okra ratio and extraction temperature on DPPH radical scavenging activity

Figure 4.12 demonstrated that another main effect, water to okra ratio, was positively proportional to the antioxidant activity of mucilage until it reaches to the ratio of 7:1. Antioxidant activity of okra mucilage increased when the water to okra ratio increased up to 7:1. However it decreased at higher water to okra ratios.

This behaviour has been observed by Wong et al. (2013). They reported that solid to solvent ratio was a vital factor on antioxidant activity. The antioxidant activity increased with increasing solid to solvent ratio until it reaches the optimum level. This can be expounded by the higher water to solid ratio, as the transition from the dissolved material to the solution during diffusion occurs with a greater concentration gradient. Meanwhile, it resulted in higher efficiency of extraction (Wong et al., 2013). Additionally, Antioxidant activity of quince seed mucilage showed a similar trend as it was reported by Jouki et al. (2014). Moreover, Sangeethapriya and Siddhuraju (2014) isolated the crude mucilage from *Ziziphus mauritiana* fruit and found that antioxidant activity of mucilage is strong to defeat and to control Alzheimer's disease, skin diseases and food spoilage.

4.7 Total Phenolics Content (TPC)

It is supported by many years of studies that the antioxidant effects of plant foods have its source in phenolics compounds such as ascorbic acid, carotenoids, anthocyanins, phytosterols. These phenolics compounds protect the cells from the invasion of free radicals, thus helping prevent many serious diseases such as cancer, diabetes and heart diseases.

In Table 4.7, it was seen that the process variables have quadratic effect on total phenolics content and the model was significant ($p<0.05$). The results were expressed as mg G.A.E/mL. Gallic acid is a common phenolics standard mostly employed to determine the total phenolics content in plant-derived foods (Dalar et al., 2012; Adetuyi and Dada, 2014). From the quadratic model, the terms AC, B^2 are significant model terms ($p<0.05$). In this study, Figure 4.13 clearly shows the interaction between extraction temperature and extraction time on total phenolics content of okra mucilage. The interactive effect of extraction temperature and extraction time on total phenolics content was significant ($p<0.05$). Total phenolics content decreased as the extraction time decreased and extraction temperature increased.

From the plot, it can be observed that when the water to okra ratio decreased from 10:1 to 7:1, the phenolics content increased (Figure 4.14).

Thus, it can be concluded that phenolics content of okra mucilage increased with decreasing okra to water ratio until it reaches the optimum level around 7:1. After that point, reduction of the ratio causes to decrease the phenolics content. In other respect, it was claimed by Wong et al. (2013) that high solid-to-solvent ratio was positively proportional to total phenolics content of Dukung Anak (*Phyllanthus niruri*).

Table 4.7 ANOVA results for response surface quadratic model analysis for total phenolics content of okra mucilage

Source	Sum of squares	df	Mean square	F value	p-value
Model	2731.64	9	303.5155	4.0455	0.0394*
A-Time	238.602	1	238.602	3.180281	0.1177
B-Ratio	73.32605	1	73.32605	0.977349	0.3558
C-Temperature	0.049612	1	0.049612	0.000661	0.9802
AB	83.81403	1	83.81403	1.117141	0.3256
AC	980.9424	1	980.9424	13.07479	0.0086
BC	23.37723	1	23.37723	0.311591	0.5941
A^2	254.4635	1	254.4635	3.391695	0.1081
B^2	910.2859	1	910.2859	12.13303	0.0102
C^2	236.779	1	236.779	3.155981	0.1189
Residual	525.1782	7	75.02546		
Lack of Fit	522.3971	3	174.1324	250.4529	< 0.0001*

*Significant at p-value < 0.05, **Not significant at p-value>0.05, R^2=0.84

The relationship between total phenolics content and independent variables extraction time, water to okra ratio and extraction temperature in terms of coded variables is given in Equation 4.6 where, A is the extraction time, B is the water to okra ratio and C is the extraction temperature. Regression analysis revealed that the coefficient of determination (R^2) of quadratic model was 0.84.

$$Total\ phenolic\ content = 26.47 - 5.56A - 3.03B + 0.079C + 4.58AB + 15.66AC + 2.42BC + 7.77A^2 + 14.70B^2 + 7.50C^2 \quad (4.6)$$

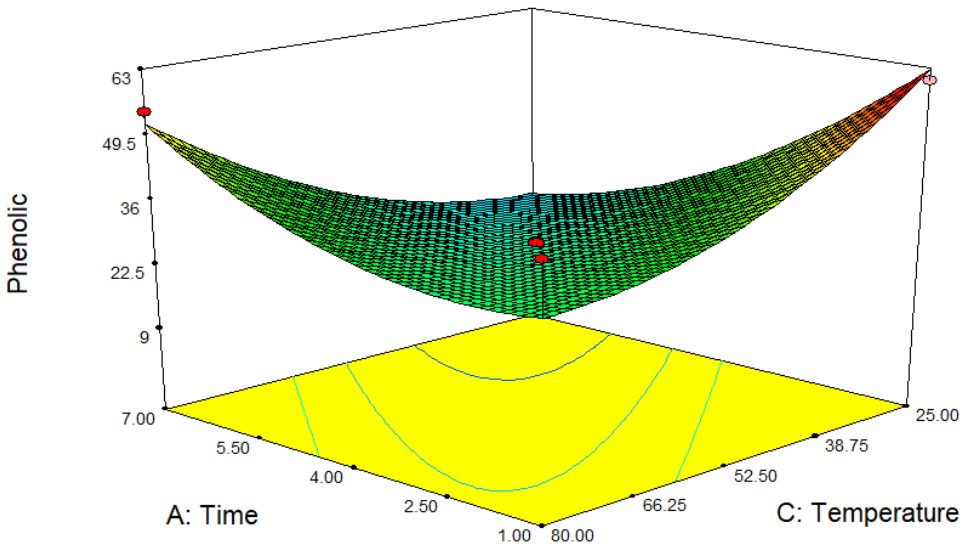

Figure 4.13 3D response surface of the effects of extraction time and extraction temperature on total phenolics content

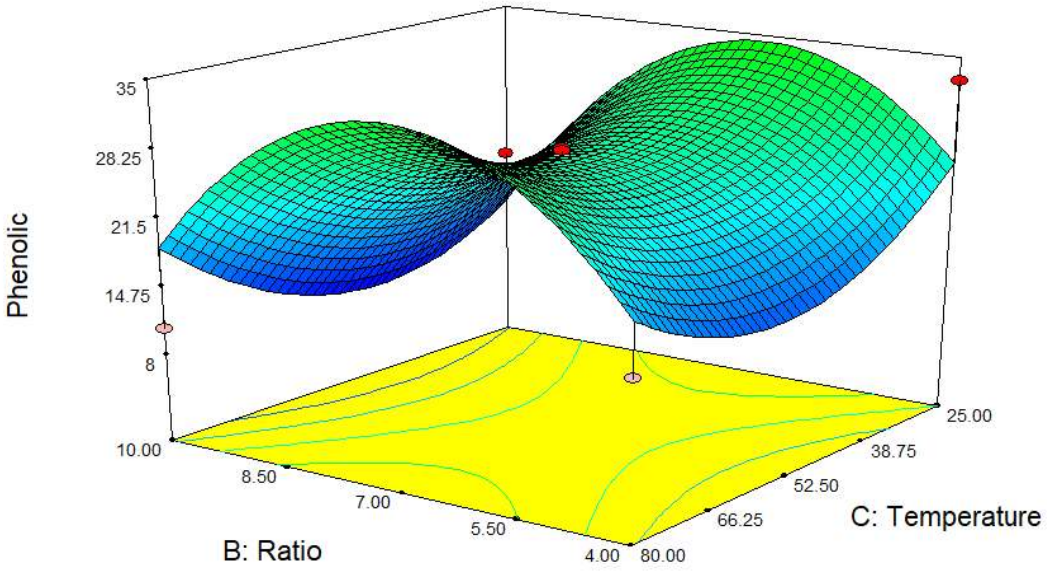

Figure 4.14 3D response surface of the effects of water to okra ratio and extraction temperature on total phenolics content

4.8 Oiling Off

The principle of the method is migration of oil droplets from the food system to the top during the storage time due to the density difference. According to the previous researches, okra mucilage as a natural food hydrocolloid, may have role in the use of emulsifier, thickener and stabilizer in food formulations to support the food quality in terms of textural and consistency characteristics.

In progress of time, continious oil droplets combine into larger droplets and exhibit a tendency of accumulating on upper part of the emulsion due to the difference of density. From the point of consumer view, food external appearance and consistency are important for purchasing. This makes emulsion properties regarding with oiling off more important. Oiling off method measures the amount of oil remaining on the top, after a specified time and at constant temperature. This method explains the unstable emulsion system thermodynamically.

As it is seen from Figure 4.15, there are 2 distinct layers. The oil (at the top) and mucilage-water mixture. Stability effect of okra mucilage can clearly be seen from these results that the consistency of the solution is almost completely stand stable on the example sample after the duration of 24 hr. Thus, it can be resulted from the picture that, addition of the mucilage decreased the oiling off and instability of the emulsion. The easily seperable two liquids, such as water and liquid, can retain integrity by the help of mucilage. Okra mucilage which was used in that experiment produced at the 1 hr of extraction time, 4:1 of water to okra ratio and 52°C of extraction temperature.

Figure 4.15 A picture of okra mucilage water emulsion after 24 hr of duration

4.9 Organoleptic Evaluation of Okra Mucilage

Okra mucilage has undergone orgonoleptic evaluation by the organs of sense and investigated the characterictics of okra mucilage powder regarding the parameters of colour, odor, taste, touch and texture as shown in Table 4.8. These parameters are important for the selection and valuation of the material. According to the results obtained from panellists, the orgonoleptic evaluation of okra mucilage powder was found to be acceptable in all cases. Okra mucilage powder is honey in colour, odorless, tasteless and rough in touch and texture. Okra mucilage that is seen on Figure 4.16 was produced under the experimental conditions of 4 hr of extraction time, 4:1 of water to okra ratio and 25°C of extraction temperature and named as Sample 3.

Table 4.8 Organoleptic evaluation of okra mucilage

Sample	Colour	Odor	Taste	Touch and texture
Sample 1 extraction time: 7hr extraction temp: 52.5 water to okra ratio: 10:1	4.3	1.5	1.3	4.6
Sample 2 extraction time: 7hr extraction temp: 80 water to okra ratio: 7:1	4.2	1.4	1.4	4.5
Sample 3 extraction time: 4hr extraction temp: 25 water to okra ratio: 4:1	4.8	1.1	1.1	4.1
Sample 4 extraction time: 4hr extraction temp: 80 water to okra ratio: 4:1	4.4	1.3	1	4.3
sample 5 extraction time: 1hr extraction temp: 52.5 water to okra ratio: 4:1	4.2	1.2	1.1	3.9

Figure 4.16 A picture of powder form of okra mucilage

4.10 Solubility

According to the observations of the panellist, okra mucilage is found to be greatly soluble in water, but insoluble in acetone and ethanol. Results from solubility test are given in Table 4.9. From the previous works, okra mucilage powder was observed to dissolve well in hot water and cold water as reported by Malviya (2011). Viscous and slimy form was observed while okra mucilage powder dissolved in water.

Nonetheless, with acetone and ethanol okra mucilage powder was observed to be clumped and precipitated. This demonstrated that acetone is comparatively better precipitating agent. As previous results confirmed that acetone gives better extraction yield than methanol (Archana et al., 2013).

Table 4.9 Solubility test for okra mucilage sample

Solvent type	Solubility
Water	Soluble
Acetone	Insoluble
Ethanol	Insoluble

4.11 Model Optimization

Response surface methedology is a very well-received technique for optimization of the processes in latest years. It has been a crucial point to develop the performance of the system and to reform the process in order to take best efficiency without increasing the cost.

The term of optimization refers to the improving of the process while maximizing the quantities of desired ones and minimizing undesired ones. Model optimization is executed by Box–Behnken design. The optimization of okra mucilage was performed by maximizing all of the six process variables which are extraction yield, swelling capacity, emulsion capacity, emulsion stability, DPPH and TPC. For optimizing the extraction conditions, desirability function of numerical optimization was used (Bezerra et al., 2008). Table 4.10 shows the optimized conditions for okra mucilage.

According to numerical optimization, the optimum extraction time, water to okra ratio and extraction temperature were 1.52 hr, 7:2 and 50.51°C, respectively.

By applying these optimum conditions, it was predicted to get 23.24 % of extraction yield, 47.05 % of emulsion capacity, 50.18 % of emulsion stability, 38.95 % DPPH radical scavenging activity, 36.75 of total phenolic content and 44.20 (mL/g) of swelling capacity.

In these circumstances, desirability was 0.6. The following 3D model graphs (Figure 4.17, Figure 4.18 and Figure 4.19) shows the desirability functions at optimum process conditions. Figure 4.17 illustrated at 1.52 hr of extraction time and 50.51°C of extraction temperature while water to okra ratio was constant. It is shown that desirability function decreases with decreasing temperature and time. At constant water to okra ratio, decreasing of time and temperature moves away the model from ideality. Figure 4.18 illustrated at 7:2 ratio of water to okra and 1.52 hr of extraction time while constant extraction temperature. In this conditions, desirabilitiy function of the model was not affected from the extraction time considerably. However, water to okra ratio around 7:1 increases the model compatability. Apart from this point, increasing or decreasing the ratio of water to okra has a reducing effect on the desirability function. Lastly, Figure 4.19 illustrated at 50.51°C of extraction temperature, 7:2 ratio of water to okra and constant extraction time.

It can be interpreted that 52.5℃ of extraction temperature increases the model adequacy. Increasing the temperature towards 80℃ or decreasing to around 25℃ causes a sharp decrease in model compatibility.

Also, water to okra ratio except from 7:1, desirability function doesn't seem suitable. The desirability value is between 0 and 1. As the nearness of desirability approaches to 1, reasonableness of the model increases and response gets more suitable.

Table 4.10 Optimum process conditions for okra mucilage

	Time (hr)	Ratio (Water to okra)	Temperature (°C)
Okra mucilage	1.52	7.2	50.51

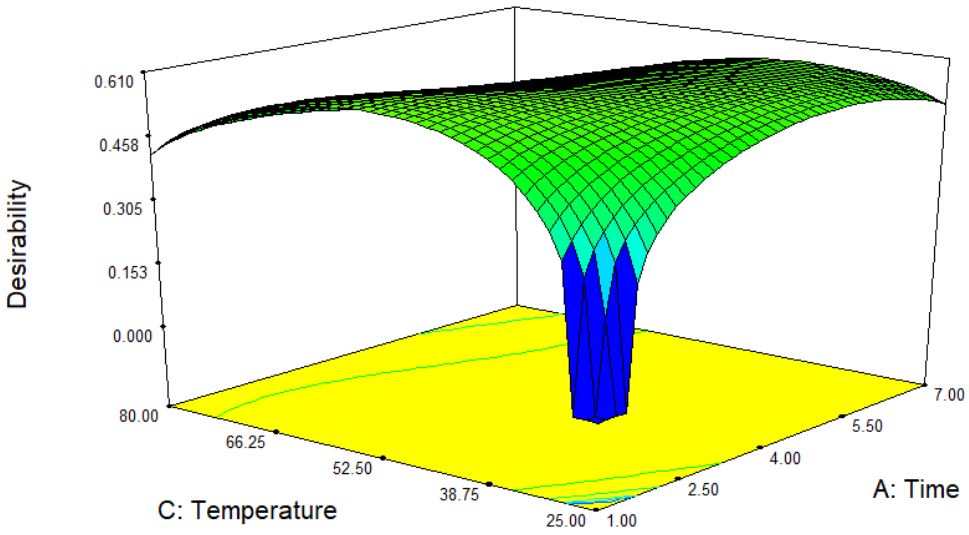

Figure 4.17 3D response surface of the effect of extraction temperature and extraction time on desirability function

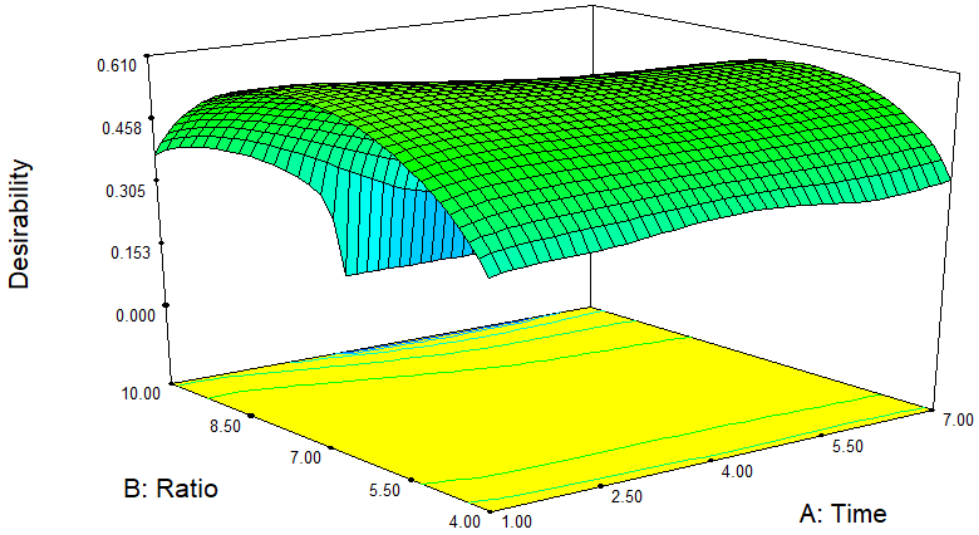

Figure 4.18 3D response surface of the effect of water to okra ratio and extraction time on desirability function

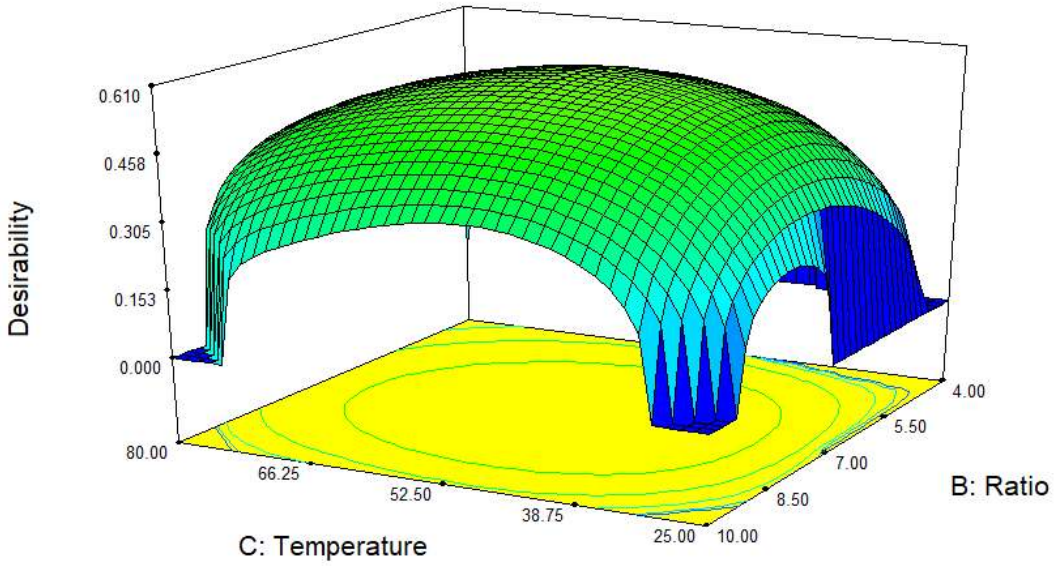

Figure 4.19 3D response surface of the effect of extraction temperature and water to okra ratio on desirability function

4.12 Effect of Okra Mucilage on Oiling Off of Pistachionuts Paste

Pistachionuts paste was chosen in the case study since it has an unstable form because of its high unsaturated oil content. In such products, oil phase is seperated with time and this instability of the product may decrease the consumer perception. In this regard, okra mucilage was used as an emulsifying agent for improving the stability of the pistachionuts paste.

The mucilage sample obtained at the conditions of 4 hr of extraction time, 52.5°C of extraction temperature and 7:1 of water to okra ratio was used in this application. This sample was preferred because it gives comparatively better results both emulsion properties and extraction yield (higher than 50% and 20% respectively). Then, it was added to pistachionuts paste. From the Figure 4.20 it can be clearly seen that adding okra mucilage increased consistency of the emulsion system and slowed the movability of oil phase to top.

As the percentages of okra mucilage in pistachio paste increased, ability of holding oil increased and amount of oil accumulated at the top decreased. The pistachinuts paste sample that did not contain any mucilage (control sample) had a product stability of 80.79%. Increasing mucilage percentages from 0.8 %, 1 %, 1.2 %, increased product stability about 93%, 94.68 % and 97.19 %, respectively. This was shown in Figure 4.21 that oil content of pistachionuts paste containing mucilage was less than that of the control sample.

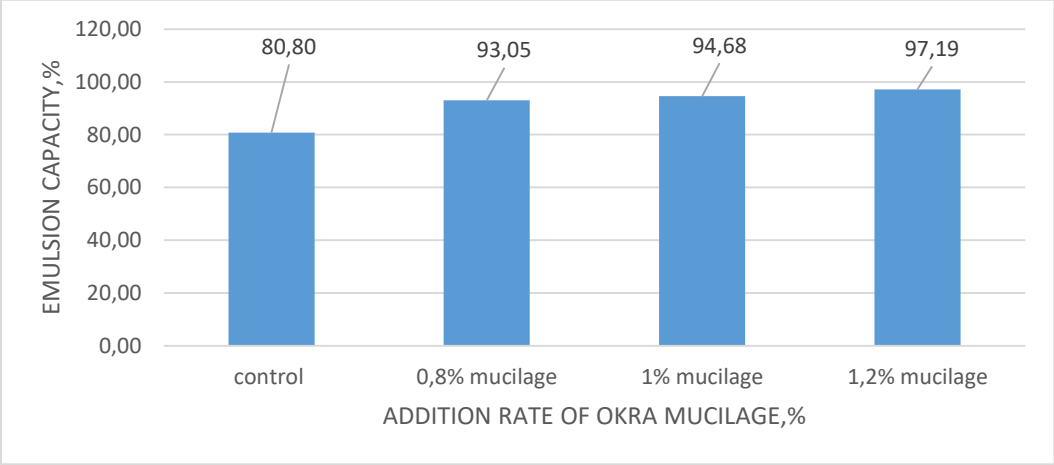

Figure 4.20 Oiling off of pistachionuts paste containing different percentages of okra mucilage

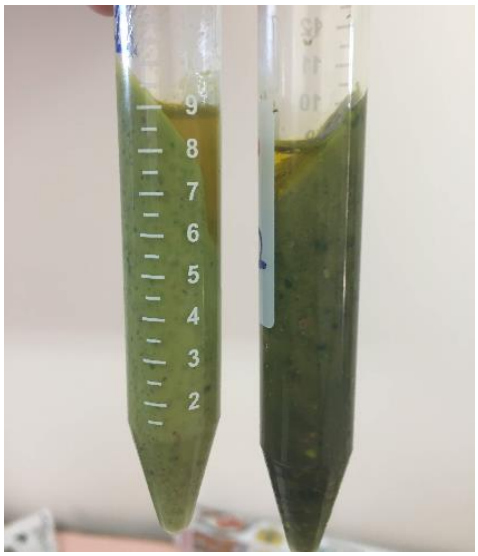

Figure 4.21 A picture of pistachionuts paste after centrifugation without mucilage (on the left) and added mucilage (on the right)

CHAPTER 5

CONCLUSION

5.1 Conclusion

In this study, the first part was to obtain okra mucilage in powder form by extracting from the plant using suitable solvents and then drying. The study showed that extraction conditions have significant effect on extraction yield, swelling capacity, emulsion capacity & stability, antioxidant capacity, total phenolics content and oiling off. Results revealed that characteristics of okra mucilage are suitable to be used as thickener, stabilizer, emulsifier on food formulations.

At the second part, okra mucilage was used as a food additive into pistachionuts paste with which is not implemented yet. Role of mucilage as emulsifier was developed the stability of pistachionuts paste up to 97.19 % with 1.2 % mucilage.

5.2 Future Work

Results investigated that okra mucilage can be a good candidate for different food formulations and possible to its adjustment to food industry.

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