

DECEMBER 2019

M.Sc. in Food Engineering

SELMA KULPU

**REPUBLIC OF TURKEY
GAZİANTEP UNIVERSITY
GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES**

MICROWAVE APPLICATION OF PISTACHIO NUT

**M.Sc. THESIS
IN
FOOD ENGINEERING**

**BY
SELMA KULPU
DECEMBER 2019**

MICROWAVE APPLICATION OF PISTACHIO NUTS

**M.Sc. Thesis
in
Food Engineering
Gaziantep University**

**Supervisor
Prof. Dr. Hüseyin BOZKURT
Co- Supervisor
Prof. Dr. Medeni MASKAN**

**by
Selma KULPU
December 2019**



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REPUBLIC OF TURKEY
GAZİANTEP UNIVERSITY
GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES
FOOD ENGINEERING

Name of the Thesis : Microwave Application of Pistachio Nuts

Name of the Student : Selma KULPU

Exam Date :04.12.2019

Approval of the Graduate School of Natural and Applied Sciences

Prof. Dr. A. Necmeddin YAZICI
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of
Master of Science.

Prof. Dr. Medeni MASKAN
Head of Department

This is to certify that we have read this thesis and that in our consensus/majority
opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master
of Science.

Prof. Dr. Medeni MASKAN
Co-Supervisor

Prof. Dr. Hüseyin BOZKURT
Supervisor

Examining Committee Members:

Signature

Prof. Dr. Hüseyin BOZKURT

.....

Assoc. Prof. Dr. Çiğdem SOYSAL

.....

Asst. Prof. Dr. Hidayet SAĞLAM

.....



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Selma KULPU

ABSTRACT
MICROWAVE APPLICATION OF PISTACHIO NUT

KULPU, Selma
M.Sc. in Food Engineering
Supervisor: Prof. Dr. Hüseyin BOZKURT
Co-Supervisor: Prof. Dr. Medeni MASKAN
December 2019
74 pages

In this study, the effects of microwave (MW) heating on physical, chemical and microbial properties of the inoculated (6 log cfu/g) chopped pistachios with *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, from the G (-) and G (+) bacterial groups, respectively were investigated. Response surface methodology was applied for optimizing MW heating conditions. Number of *S.aureus* and *E.coli* microorganisms were the response variables of the model. *S.aureus* showed a minimum reduction of approximately 1.5 log and a maximum reduction of 4.0 log cfu/g after microwave treatment. *E. coli* showed a minimum 2.5 log cfu/g and a maximum decrease of 6 log cfu/g decrease after microwave. The optimum conditions of MW heating were based on the minimum levels of responses. The optimum MW power, MW treatment time for, *S.aureus* count and moisture content were estimated as 323.76 W, 37.90 min for, 2.28 log cfu/g and 1.66 % respectively. The optimum MW power, MW treatment time for, *E.coli*, mold and yeast count and moisture content were estimated as 330.00 W, 40.00 min for, -2.46 log cfu/g, 1.91 log cfu/g and 1.65 % respectively. Based on this study, it has been found that the microwave can increase shelf life and control its quality.

Key Words: Microwave Heating, Pistachio, Quality, Safety, *Escherichia coli*, *Staphylococcus aureus*

ÖZET
ANTEPFISTIĞINA MİKRODALGA UYGULAMASI

KULPU, Selma
Yüksek Lisans Tezi, Gıda Mühendisliği
Danışman: Prof. Dr. Hüseyin BOZKURT
İkinci Danışman: Prof. Dr. Medeni MASKAN
Aralık 2019
74 sayfa

Bu çalışmada, G (-) ve G (+) bakteri gruplarından, *Escherichia coli* ATCC 25922 ve *Staphylococcus aureus* ATCC 25923 ile 6,0 log kob/g aşılınmış pirinç antepfistiğinin mikrodalga ısıtma sisteminin fiziksel, kimyasal ve mikrobiyal özellikleri üzerine etkileri sırasıyla incelenmiştir. Mikrodalga ısıtma koşullarını optimize etmek için yanıt yüzey yöntemi uygulanmıştır. Mikrodalgada uygulama zamanı (0-40 dk) ve uygulama gücü (90-330 W) bağımsız değişken olarak belirlenmiştir. *S.aureus* ve *E.coli* bakterileri sayısı, modelin cevap değişkenleridir. Mikrodalga işleminden sonra *S.aureus*, minimum 1,5 log kob/g, maksimum 4,0 log kob/g düşüş göstermiştir. Mikrodalga işleminden sonra *E. coli*, minimum yaklaşık 2,5 log kob/g ve maksimum 6 log kob/g düşüş göstermiştir. Optimum mikrodalga ısıtma koşulları minimum yanıt seviyesine göre belirlenmiştir. Optimum mikrodalga gücü, mikrodalgada işlem süresi, *S.aureus* sayısı ve nem içeriği sırasıyla 323,76 W, 37,90 dk, 2,28 log kob/g ve % 1,66 olarak hesaplanmıştır. Optimum mikrodalga gücü, mikrodalgada işlem süresi, *E.coli* ve küf ve maya sayısı, nem içeriği sırasıyla 330,00 W, 40,00 dk, -2,46 log kob/g, 1,91 log kob/g ve % 1,65 olarak hesaplanmıştır. Bu çalışmalara dayanarak, mikrodalga'nın gıdaların raf ömrünü artırabildiği ve kalitesini kontrol edebileceği belirlenmiştir.

Anahtar Kelimeler: Mikrodalgada ısıtma, Antepfistiği, Kalite, Güvenirlilik, *Escherichia coli*, *Staphylococcus aureus*



To my mother...

ACKNOWLEDGEMENTS

I would like to express my deepest respect and gratitude to my supervisors Prof. Dr. Hüseyin BOZKURT and Prof. Dr. Medeni MASKAN for their patient supervisions, continuous guidance, invaluable suggestions and continuous encouragements. I am so grateful to them for their advices, understanding and behavior in all situations.

I express appreciation to Chemical Engineer Sevgi KUYUCU, Food Engineer Nazan ÇAĞLAR, Food Technician Zeynep ÖZTÜRK and Chemist Mustafa FİDAN for their helps and advices. I am also very grateful to Res. Assist. Erhan HORUZ for his guidance and encouragement.

Finally, I am very grateful to my family; especially my mother, my cousin Gülbahar DEMİR and my friends Nazmiye KAYA and Nurtaç Rojbin KESKİN for their guidance, encouragement, support and endless love.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
KI	Potassium iodide
MW	Microwave
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
RSM	Response Surface Methodology
VRBA	Violet Red Bile Agar

CHAPTER 1

INTRODUCTION

It is known that pistachio (*Pistacia vera* L.), a hard-shelled fruit called “Green gold”, “King of fruits”, “Golden tree” and “King fruit” was first cultivated by Etiler in Southern Anatolia (Maskan and Karataş, 1999; Tekin et al., 2001; Kashaninejad et al., 2003). It is estimated that the Roman King Vitallis brought the pistachio, which has been cultivated since ancient times in Anatolia, Northern Syria and Iran, from Syria to Italy in the first century. From there it is reported that he was taken to Spain, the Mediterranean islands, South France and North Africa (Özbek, 1978). Pistachio (*Pistacia vera* L.) was classified as in the systematic place; Realm: Plantae (Plant Kingdom), Division: Phanerogamae (Seed Plants), Class: Magnoliopsida (Magnolia plants), Team: Sapindales, Family: Anacardiaceae (Masticaceae), Genus: *Pistacia*, Species: *Pistacia vera* L. (Bilgen, 1973). Among almost all species of *Pistacia* species of hard-shelled, *Pistachio vera* L. is the only species that can be eaten by consumers (Shokraii et al., 1988; Altuntaş and Mutlu, 2007). In the world, pistachio is grown in suitable microclimate areas of 35-40° parallel of North and South hemispheres (Tekin et al., 1995). Pistachio has two gene centers: Near East Gene Center (Anatolia, Iran, Caucasus and Turkmenistan) and Central Asia Gene Center (North of India, Tajikistan, Pakistan and Afghanistan) (Ulusaraç, 1993). Turkey is in Near East Gene Center, located in the northern hemisphere (Atlı et al., 1998). Although pistachio can be grown in poor soils and other irrigated, chalky, rocky, stony areas where other fruit species are difficult to grow, winter is relatively cold; the summer months require special climate conditions that are hot, dry and long (Özbek, 1978; Ayfer, 1990; Bolu, 2002; Anonymous, 2010). Turkey, pistachio (*Pistacia vera* L.) due to favorable ecological conditions of cultivation, are among the countries that can be efficiently product (Kaska, 1995). In addition to being able to grow pistachio in 56 provinces of our country, especially pistachio is cultivated economically in Şanlıurfa, Adıyaman, Gaziantep, Siirt and Kahramanmaraş provinces (TUIK, 2017). About 90% of pistachios cultivation in Turkey takes place in the Southeastern Anatolia Region.

Buttum (*Pistacia khinjuk Stocks.*) and melengiç (*Pistacia terebinthus L.*) trees are used as a rootstock for pistachio that has great importance for the growth of trees as a natural (Anonymous, 1999; Bolu, 2002). According to Food and Agriculture Organization of the United Nations in 2017, 5 countries realize 96.6% of world pistachio production. Among these countries, Iran ranks first with 575 thousand tons of pistachio production. On the other hand, our country ranks 4th with 6.99 % of world production with 78 thousand tons of production (Table 1.1).

Table 1.1Pistachio production in the world and ratio (FAOSTAT, 2017)

Countries	Amount of production (tons)	Ratio (%)
Iran	574 987	51.56
USA	272 291	24.42
China	95 294	14.08
Turkey	78 000	6.99
Syria	56 508	5.07
Other countries	37 986	3.40
Total	1 115 066	100

Pistachio cultivation in the USA started as a result of regular and extensive research. In the 1930s, the selection was made in Iran and the Middle East by breeding new varieties. USA has passed our country with today's production, has become the second largest producer country in the world. This rapid development;

- ✓ to do breeding under water conditions, nematodes and disease resistant rootstocks, efficient and high-quality single varieties,
- ✓ to do good organization of standardization, marketing and promotion,
- ✓ establishment of strong producer association,

can be attributed to such reasons.

Drip irrigation system is mostly used in the USA. Appropriate and sufficient fertilization is done, mechanical pruning system is applied. With the completion of other maintenance measures, an average of 16-18 kg of product can be collected

from a 25-30 year old tree. In our country, the average yield per tree in dry conditions is around 3-4 kg (Arpacı et al., 1997).

The main purpose of this project is to reduce the microbial load, which is one of the main problems of pistachio processing, to a permissible level and also to use a more effective and less energy microwave system for the destruction of pathogenic microorganisms. With this technology, which has not been used in the pistachio processing industry before, it will open up many companies in the sector. Pistachio enterprises have difficulties in supplying quality and reliable raw materials. Farmers dry the harvested pistachios on the soil and outdoors under the sun and stock them under inappropriate storage conditions. In this process, microorganisms can multiply rapidly, especially when the number of mold and yeast reaches very high values, and pathogenic microorganisms easily grow. Raw material differences and the presence of microorganisms from different species can threaten production considerably.

1.1 Aim of the Study

The rationale and objective of the basic launch of this study is to make the pistachio processing more modern. Thus to obtain reliable and quality pistachio products. Microwave power and treatment time should be adjusted according to different forms of pistachios. It is necessary to apply different time and power to the chopped with all grains, otherwise there may be quality losses while the desired levels of microbial load does not decrease. Pistachio is a vegetable product that is identified with our region (Southeastern Anatolia) and takes its name from city of Gaziantep and about 45% of its production is realized in this city. Despite the existence of many pistachio processing plants in the region, companies and methods that produce more efficient and reliable products in the technological sense should be brought into the economy. With microwave application,

- ✓ energy will be saved,
- ✓ microbial load and color losses will be reduced,
- ✓ reliable (reduced pathogenic microorganism) and high quality (unchanged, unburned) pistachio processing will be increased,
- ✓ long time and high temperature application will be improved.

As a result, the objectives of this project were;

- to prevent the quality losses (blackened burned) by adapting this modern method for production,
- to produce reliable pistachio products is possible by reducing the number of pathogenic microorganisms.



CHAPTER 2

LITERATURE REVIEW

2.1 Pistachio

Pistachio has great commercial importance because of its edible seeds. In addition for consumption as a cookie; cake, chocolate, baklava, ice cream and salami, sausage, sausage meat products (Tous and Ferguson, 1996; Yahia, 2011). While Siirt and Ohadi varieties are consumed as snack bars due to their round shape and high cracking ratio; Red, Halebi and Uzun varieties are used as raw materials in confectionery and sweet industries due to their high aroma, taste and dark green color (Tunalıoğlu and Taşkaya, 2003).

Storage conditions should be considered in order not to cause adverse health effects of nuts. Therefore, the relative humidity of storage should not be more than 60 %, moisture content of the seed should not exceed 5 % and should be stored in a cool place. Aflatoxins can be produced by some molds when stored in hot and humid conditions can cause tumors or damage to tissues, especially the liver (Ayaz, 2012). Pistachio, peanut, pine nut, various walnuts, almonds and their products are among the most risky foods seen with aflatoxin (Çeliktaş and Dağlıoğlu, 2008).

Pistachio shelled at 65-70 % relative humidity, 0-7.2 °C temperatures and 4-6 % moisture content for 1 year; It maintains its quality for a maximum of 1 year without bark at 0 °C. It can be stored for 3 years at -18 °C at 65-70 % relative humidity without shells (Ferguson et al., 1995; Perry, 1998).

After harvest and within processing, exporting, storage and marketing of pistachio nuts has problems such a insect infestation occurs by pests. Indianmeal moth (*Plodia interpunctella*) can be given an example to cause mould growth and also causes the reduction of quality of pistachio (Johnson et al., 2002; Johnson et al., 2003). In developing countries, chemical fumigation is used. For example, methyl bromide

(MeBr) and phosphine are used to control insects in tree nuts and dried fruits. However, use of them have negative impacts on health of people and the environment (USEPA, United States Environmental Protection Agency, 2001). In addition, post-harvest technologies are important for controlling target insects and maintaining the nutritional quality of pistachios and environments. There are a lot of unknown or could be said new developing without chemical alternatives for fumigation, such as cold storage, ionizing radiation, thermal treatments, controlled atmospheres (Whiting et al., 1999; Hoa et al., 2006; Mexis and Kontominas, 2009; Jiao et al., 2013). There are also conventional-thermal treatments, such as using steam or hot air. They are quite easily applied and give no chemical residues. However, with these methods, product quality is damaged because of long-term exposure to heat. Thus, it is suitable to develop for being of practical, effective, economical and environmentally friendly method to disinfest the pistachio nuts.

2.2 Production of Pistachio in Turkey

The pistachio species are mainly originated in Turkey (Kaska, 1995). Annual production of pistachio in Turkey is as follows (Table 2.1).

Table 2.1 Annual production of pistachio in Turkey (TUIK, 2017)

Year	Quantity (tons)
2011	112 000
2012	150 000
2013	88 600
2014	80 000
2015	144 000
2016	170 000
2017	78 000
2018	240 000

Although pistachio cultivation has been carried out since ancient times in our country, production has not increased at the desired level. The reason for this is that the cultivation is carried out in completely dry conditions and mostly in arid, stony

and sloping lands. Except Iran and United States manufacturers, growing conditions in other countries are similar to Turkey. All of the cultivation in Iran and the United States is carried out in irrigated conditions and fertile ground areas. In Turkey, the edible species of *Pistacia vera* L. is grown in different regions (Balta, 2002) but with Southeast Anatolia Regional Project (GAP), Turkey's annual pistachio production will increase by irrigation in the following years (Kaska, 1995). Material (rootstock and variety), technology and mechanization should be planned. Pistachio Research Institute is carrying out studies on variety and rootstock selection. As a result, it is aimed to reach advanced producer countries in terms of efficiency and quality (Arpacı et al., 1997).

2.3 The Importance of Pistachio Consumption in Health

Hard-shell fruits are very important for human nutrition and health thanks to the saturated, unsaturated fatty acids, proteins, carbohydrates, vitamins, minerals, dietary fiber and vegetable sterols they contain. In addition to contain vitamin E which has antioxidant properties, monounsaturated fatty acids and non-lipid components in its structure have a lowering effect on cholesterol and lipoproteins. Hard-shell fruits are included in the group of foods that reduce the risk of cancer (Özer and Güven, 2008).

According to Michael et al. (2006), pistachio is very rich in terms of protein and dietary fibers. 28 g of roasted pistachios meet 13 % of the recommended amount of protein and 12 % of dietary fiber. The main components of pistachio are as follows (Table 2.2).

Table 2.2 Composition of pistachio (Özcan, 2008)

Pistachio Nutrition Information (100 g)	
Total fat (%)	53.7
Carbonhydrate (%)	19.0
Protein (%)	19.3
Ca (mg)	131.0
P (mg)	500.0
Fe (mg)	7.30
K (mg)	972.0
Vitamin A (iu)	230.0
Vitamin B ₁ (mg)	0.67
Vitamin B ₆ (mg)	1.40
Energy (cal)	597

Compared with walnuts, almonds, peanuts, pine nuts and hazelnuts, pistachio has the lowest energy with 557 kcal and the highest protein content (20.6 g/100 g) after almond. Pistachio, walnut (67.8 %), hazelnut (61 %) and almonds (54 %), respectively, in terms of fat content (44.4 %) is the last. Pine nuts contain 68 % fat and peanuts 50 % fat (Shi et al., 2010).

As seen in Table 2.2, it is possible to describe the pistachio fruit as a condensed energy pill. The plant needs adequate nutrition, water and maintenance for the formation of this source. It has emerged in many studies that the yield will increase by meeting these, the quality will increase and the periodicity will decrease significantly.

2.4 Microwave Heating Process

Microwave technology was discovered during the Second World War in the production and design of military equipment. Areas where microwave technology is used; food processing (heating, dissolving, quality control), drying of industrial products (paper, wood), acceleration of chemical reactions (microreaction control), melting of industrial products (glass, rubber, slurry), sintering (ceramic, mineral powder), plasma production, mineral processes (crushing, crushing), waste treatment and recycling processes (Gwarek and Celuch-Marcysiak, 2004). The food industry is the area where microwave energy is most used. This energy is used in cooking,

defrosting, tempering, drying, freeze-drying, pasteurization, sterilization, baking and heating (Oliveira and Franca, 2002).

Vacuum tubes are sources of microwaves at high power and frequency. Vacuum tubes for microwave heating; magnetron, moving wave tubes (TWT) and klystron (Thostenson and Chou, 1999). Magnetron tube is the most widely used microwave source in industry and at home. At the center of the magnetron is an electron-emitting cathode surrounded by the anode (Regier and Schubert, 2005).

Microwaves are waves of electromagnetic at frequencies changes between 300 MHz to 300 GHz (Chandrasekaran et al., 2013). Domestic microwave and industrial microwave frequencies are 2.45 GHz, 915 MHz or 2.45 GHz respectively. Volumetric heating in microwave occurs from the inside to outside of the food thus food is not damaged and processing time is minimized by this way (Vadivambal and Jayas, 2010). One of the newest applications applied to food is microwave heating process is used as a heating source to inactivate or reduce the number of microorganisms to obtain reliable and quality food products (Ohlsson, 1991; Salazar-González et al., 2012). Microwave heating system is used in many fields in the food industry such as heating, drying, pasteurization and preservation of foods and other areas (Chandrasekaran et al., 2013) because of these applications has many advantages so recently microwave heating techniques have been commonly used in the food industry.

The presence of water causes dielectric heating because of dipolar structure of water. When an electric field occurs on the water molecules, polarized dipolar molecules try to arrange in the direction of the electric field. This arrangement occurs at a million times per second because of internal friction of molecules causes volumetric heating of the material at high frequencies of electric field (Datta and Davidson, 2000). Friction occurs because of rotation of molecules with surrounding medium as a result, heat is generated. Apart from materials containig of water, microwave heating of a material is affected from several factors, such as size, shape, form of state (e.g., water or ice), properties of the material and the processing equipment.

2.5 Dielectric Properties

Microwave heating occurs in food materials because of having dielectric properties are expressed as dielectric constant and dielectric loss. The real part of dielectric property is a dielectric constant. It means that imaginary part of dielectric property and ability to store electric energy are dielectric loss, means that the ability to convert electric energy into heat,

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \quad (2.1)$$

where ε' signifies dielectric constant and ε'' signifies dielectric loss, $j = \sqrt{-1}$. Loss tangent is the ratio of dielectric loss to dielectric constant and it is expressed as,

$$\tan \delta = \frac{\kappa''}{\kappa'} = \frac{\varepsilon''}{\varepsilon'} \quad (2.2)$$

where κ' is relative dielectric constant and κ'' is relative dielectric loss, these are given as $\kappa' = \varepsilon' / \varepsilon_0$ and $\kappa'' = \varepsilon'' / \varepsilon_0$. Here, ε^0 expresses the permittivity of free space ($\varepsilon_0 = 8.854 \times 10^{-12}$ F/m). Used operating temperature and the microwave frequency significantly affect dielectric properties. Materials are categorized according to their ability of microwave absorption. These are, (i) absorbers also are described as high dielectric loss materials those are strong microwaves absorbers, (ii) transparent also is described as low dielectric loss materials which materials absorb very little microwave energy and finally, (iii) opaque also is described as conductors which reflect the microwaves. It is important that information about dielectric properties is necessary because of classify the materials into the above three categories. The penetration depth (D_p) is described as the depth into a material where the microwave power has dropped to $1/e$ of its transmitted value.

$$D_p = \frac{c}{\sqrt{2\pi f \left[\kappa' \left\{ \sqrt{1 + \left(\kappa'' / \kappa' \right)^2} - 1 \right\} \right]^{1/2}}} \quad (2.3)$$

where c signifies velocity of light given as $c = (\mu_0 \varepsilon_0)^{-1/2}$, ω signifies angular frequency and μ_0 expresses permeability of free space ($\mu_0 = 4\pi \times 10^{-7}$ H/m).

Equation (3) is convenient for non-magnetic food materials ($\mu_r = 1$). The power changes with the square of the electric field is given as,

$$q = \frac{1}{2} \omega \varepsilon_0 K'' |E|^2 \quad (2.4)$$

E signifies electric field. Except the dielectric properties and penetration depth, microwave food processing is affected from several various factors such as design and frequency of microwave, food material placement inside microwave, water activity, composition, density, shape and size of food materials (Icier and Baysal, 2004a). As a general, dielectric properties are estimated as a result of knowing water activity in a food material. Because water is a good absorber of microwave energy. For designing of microwave pasteurization and sterilization processes, it is necessary known the dielectric properties of food materials (Curet et al., 2014; Franco et al., 2015).

2.6 Factors Affecting Food Heating with Microwave

The dielectric properties are determined with several factors. For example, in hygroscopic materials, water activity in agricultural foods is a very important factor for estimating the dielectric characteristic of them. The dielectric properties of materials change because of some reasons. Some of them are:

2.6.1 Frequency

Since the penetration of electromagnetic fields into food is proportional to $\sim 1/f$, the microwave source used frequency (f) affects the treatment time. As the frequency of the source used increases, food penetration the amount decreases. Therefore, frequency selection according to the size of the food to be heated is important (Schiffmann, 1986).

2.6.2 Microwave Power and Heating Speed

Many microwave systems used in the industry operate at microwave power ranging from 5-100 kW. As the power of the system increases, the speed of heating the same amount of mass increases as well (Schiffmann, 1986). In this case, depending on the food warming time reduces (George and Burnett, 1991).

2.6.3 Temperature

Temperature affects the dielectric properties of foods. Dielectric loss may increase or decrease with temperature depending on the material (Giese, 1992). The initial temperature of microwave-heated foodstuffs must be checked or known. Thus, the power of the microwave can be adjusted to obtain a homogeneous final temperature (Schiffmann, 1986). The higher the initial temperature of foods, the faster the microwave is heated (Harrison, 1980).

2.6.4 Mass of Food

There is a direct relationship between the mass of the food and the microwave power required to achieve the desired heating (Schiffmann, 1986). Large objects generally absorb more microwave power than small objects. However, heating of large objects in a microwave oven takes longer time (Heddleson and Doores, 1993). If the total mass is small, a batch system is more suitable for processing. As the mass increases, the use of banded systems is more convenient (Schiffmann, 1986).

2.6.5 Water Content

The dielectric properties of foods are determined based on their chemical composition and mainly on the constant dipole moments related with water. Also, the dielectric properties of food materials such as flour, bread, fruits and vegetables are determined by their water content.

Dipole or ionic polarization structure of materials affects the ability of products for absorbing microwaves. Microwave heating is highly affected especially from

presence of water in food materials (Von Hippel, 1954; Mudgett, 1985; Nelson and Kraszewski, 1990). Microwave energy is absorbed if there is water in foods and as a result of this

reason better heating occurs. The dielectric properties of water change based on free or not. Pure form of water is a classic example of a polar structure.

2.6.6 Density

In particulate or granular materials, the bulk density of the air particle mixture is another factor which effects the dielectric properties. The density of the food affects the dielectric constant of the food. The dielectric constant of the air is one and is completely permeable to the frequencies used for heating in the industry. Therefore, as the amount of air in the food structure increases, the dielectric constant of that food decreases. However; as the density of the material increases, the dielectric constant generally increases linearly (Schiffmann, 1986).

2.6.7 Physical Geometry

If the size of the food to be heated by microwave is higher than the applied electromagnetic wavelength or penetration depth, homogeneous heating cannot be done. Since sharp corners and edges will become more heated, it is necessary to avoid overheating (Schiffmann, 1986). Since microwaves penetrate food from all sides, the shape of the food has a significant effect on microwave heating. The ideal shape for microwave heating is the sphere. The best shape after the sphere is the cylinder (Giese, 1992).

2.6.8 Thermal Properties

Specific heat and thermal conductivity values of materials in heating processes are important parameters (George and Burnett, 1991). The specific heat is used to calculate the amount of energy required to perform the heating operation.

Since the specific temperature value is closely related to the moisture content of the food, this value has significant effects on the microwave heating process of foods

(Giese, 1992). Thermal conductivity is an important factor in cases where the penetration depth is not high enough to heat the product homogeneously to the core during heating of foods with a high mass or if the microwave heating time is long (Schiffmann, 1986). Knowing the thermal conductivity values of each food component ensures that the food is heated optimally (George and Burnett, 1991).

2.6.9 Electrical Conductivity

Electrical conductivity is the event of the transfer of ions and electrons by the electric current in the material. While the dipolar rotation mechanism is generally effective for producing heat in microwave systems, it is thought that ionic conduction plays an important role especially in the heating of foodstuffs. Adding salt to the microwave heated product increases the heating rate of the product. Because the addition of salt directly affects the depth of penetration and may cause overheating on the surface (Schiffmann, 1986).

Dielectric properties were studied for solutions containing sugar, salt, and carboxymethylcellulose (CMC) at several different temperatures and microwave frequencies (Coronel et al., 2005). According to the results, CMC doesn't affect dielectric properties. On the other hand, with increasing temperature and the sugar concentration, an increase in dielectric constant of sugar solutions occurs. However, when dielectric loss factor decreases with sugar concentration but increases with temperature because of non-polar structure of sugar. Thus, salt, sugar, and CMC can be used to simulation the dielectric properties of the food product to be processed (Coronel et al., 2005).

2.6.10 Dielectric Properties

Dielectric properties of foods are very important factors during microwave heating. Dielectric constant (ϵ') and dielectric loss factor (ϵ'') determine these important properties. The dielectric constant shows how much food can store energy, while the dielectric loss factor shows how much of food energy can be converted into heat. Apart from these, another feature is the missing tangent ($\tan \delta$). The missing tangent

is the ratio of the dielectric loss factor of the material to the dielectric constant(ϵ''/ϵ') and refers to the amount of heat consumed by the microwave energy permeating the material in the electromagnetic field (George and Burnett, 1991). The dielectric properties of most materials vary depending on the water content, frequency of the electric field applied, temperature, density, chemical composition and physical structure of the material (Venkatesh and Raghavan, 2004). Water, fat, carbohydrates and proteins, all of the essential components of foods, are directly effective on microwave heating (George and Burnett, 1991).

Boldor et al. (2004) studied on the dielectric properties of both in-shell and shelled peanuts at different densities, temperature and moisture content above the frequency of between 300 and 3000 MHz. For low moisture content of food products, the dielectric properties were estimated based on temperature at frequencies between 915 and 2450 MHz but temperature has less significant importance on determination of the dielectric properties of higher moisture contents (Boldor et al., 2004).

2.7 Measurement of Dielectric Properties

The dielectric properties are evaluated at different frequencies by using some different techniques. These are as follows:

- first one is lumped circuit method is used for frequencies below 100 MHz but unsuitable for having low dielectric loss materials,
- the other one is cavity resonator technique is suitable for frequencies between 50 MHz and 100 GHz. This technique is desirable both high or low temperatures and also used for very low loss materials,
- the other one is transmission line method is usually suitable for liquid and solid materials used for frequencies range of 30 MHz to 100 GHz, and,
- the last one is free space method is used for measuring samples in different forms can be large, flat, thin and parallel-faced applied for high frequencies range of 3 GHz to 100 GHz and at high temperatures (Icier and Baysal, 2004b; Ryyanen, 1995).

Dielectric constant and loss factors based on frequency and moisture content for nuts are reported at frequency range of 1 to 50 MHz (Nelson, 1973). Nelson (1991) obtained information about the dielectric properties of chopped pecans in detail at frequency between 50 kHz to 12 GHz, also moisture content between 3-9 %, at 22°C. The dielectric constant and loss factors were estimated at frequency range of 100 kHz to 110 MHz and moisture contents based on temperature between 0-40°C (Lawrence et al., 1992; Lawrence et., 1998). Dielectric constant and loss factors increased with moisture content. In contrast, when frequency increases, dielectric properties decreases. The effect of temperature has a little importance on dielectric loss and constant factors for containing low moisture contents whereas for containing high moisture contents, dielectric constant and loss factors increased with temperature.

2.8 Microwave Applications in Food Processing

The use of microwaves in food processing has been investigated in many laboratory studies and a few of these processes have been successfully applied on an industrial scale (Venkatesh and Raghavan, 2004). The processes in which the microwave is applied in the food industry are indicated in Table 2.3(Giese, 1991).

Table 2.3 Microwave applications in food processes

Application	Frequency (MHz)	Power (kW)	Food product
Drying	915-2450	30-50	Pasta, cookies etc.
Tempering (with vacuum, freezing)	915	30-70	Meat, fish, poultry etc.
Preheating	915	50-240	Meatball, poultry, etc.
Pasteurization/ sterilization	2450	10-30	Packaged foods, ready to eat foods, etc.
Cooking	915	2-10	Bread, pastry etc.

2.9 Sterilization with Microwave Energy

The ways in which microorganisms are affected by microwave energy have not yet been determined. Some researchers (Hume and Makinson, 1975) suggest that heat can cause lethal effects, while others (Rohrer, 1986; Martin, 1985) cannot reach the level required to kill microorganisms, and may result in some cell alterations, perhaps due to cell explosion. They stated that there could be death. Rohrer and Bulard (1985) state that oxygen metabolism of microorganisms may be affected.

In their study, Hume and Makinson (1975) reported that *Staphylococcus aureus* and *Herpes simplex*, which were infected on various hand tools used in dentistry, were kept in microwave irradiation for up to 12 minutes but did not die.

Rohrer and Bulard (1985) conducted research on aerobic and anaerobic microorganisms, including fungal, virus, spore and non-spore forms. They reported that all microorganisms had died in the modified microwave oven for 5-10 minutes. They argued that microwave power can be used to sterilize metal tools, turbines and handpieces, burs and acrylic dentures in a short time. They reported that it was necessary to use RAM (absorbent material) to make it 3-plane by making some changes in the furnace and not to damage the magnetron from the heat reflected from the metals.

In a joint study with Young and Graves (1985), the same researchers used microwave energy to sterilize the nasal part used in anesthesia with nitrous oxide. They sterilized the parts infected with *Rhinovirus*, *Parainfluenza virus*, *Adenovirus* and *Herpes simplex* virus in a 3-plane microwave oven and stated that 95% of the microorganisms were inactivated at the end of 1 minute irradiation and completely at the end of 4 minutes of irradiation.

Davis et al. (1989) observed that *Serratia marcescens* died in a very short time, although *Bacillus Subtilis*, which was infected with plaster models, did not die in a study using a kitchen microwave oven. However, the researchers stated that the

dehydration effect of the plaster or chemical toxicity may have an effect on this result and these effects should be investigated.

Akşit et al. (1993) in order to evaluate the sterilization efficiency of microwave energy, 3 types of non-spore bacteria (*S.aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) and a spore bacteria (*Bacillus subtilis*) strains of bacteria after a study of *Bacillus subtilis* but died in 1 minute. *Bacillus subtilis* required a period of 20 minutes.

Again, Akşit et al. (1993) reported that all vegetative bacteria had died at the end of 1 min, but this time was 20 min for *Bacillus subtilis*.

Tuncer et al. (1990) 3 types of non-spore bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*) on gypsum models to investigate the effectiveness of microwave sterilization in preventing cross contamination with gypsum models, a spore bacteria (*Bacillus subtilis*) and a fungal genus (*Candida albicans*). They then irradiated in a kitchen-type microwave oven. They found that although *Bacillus subtilis* was kept in the microwave for 20 minutes, it did not die, but all other microorganisms died in as little as 1 minute. As a result of these findings, although microwave energy is not recommended as a sterilization method, they reported that it may have an effect on microorganisms.

Microwave sterilization can heat the product 3 to 5 times faster than conventional sterilization. During this short time, the temperature of the product does not extremely increase it has been reported that microwave treated products have better taste, texture and appearance than traditionally treated products (Lau, 2000). Packed food products are sterilized using several different technologies containing UV light, microwave irradiation, ozone and cold plasma (Guillard et al., 2010). Some of the components of packaging materials such as plasticizers and benzene have significant toxic effect and disappear in higher quantities by using some techniques. Ozone plays a significant role as a disinfectant for processing foods and food packaging materials is a strong oxidant in the form of gaseous or aqueous. Similarly, if wavelength range between 250-280 nm, it is efficient for inactivating

microorganisms. However, ozone and UV light can cause damage polymers of food packages. On the other hand, the irradiation and microwave heating treatments have advantages for saving on cost and time and improving the quality. Glass, paper and ceramics were used for microwave packaging (Guillard et al., 2010) when UV, laser and microwave heat treatments were applied respectively. Studies reported that the microorganisms were efficiently inactivated by using laser, microwave and UV treatment respectively (Maktabi et al., 2011). U.S. Food and Drug Administration (2000) reported that, inactivation effect of microwave processing on microorganisms is not enough.

Microwave pasteurization and sterilization applications have some disadvantages as well as advantages. At the beginning of these; short duration of these operations applied to products microorganisms in the products due to the survival. Four strategies are recommended for the control of viable bacteria in food. These; food wrapping with plastic film, reducing the size of food as much as possible, minimizing microwave power and extending processing time (Lin and Sawyer, 1988).

Listeria monocytogenes, *Staphylococcus aureus* and *Salmonella spp.* food-borne pathogens, such as those that are ubiquitous and cause they have been the focus of many studies on food safety (Heddleson and Doores, 1993). In a study by Pucciarelli and Benassi (2005), *Salmonella enteritidis* was used in fresh chicken meat inoculated. It was investigated to what extent the microwave power at different 2 levels (high and medium) applied, destroyed this microorganism. As a result of this study, it was determined that this microorganism did not survive in 100 g samples exposed to high microwave power for 110 seconds.

2.10 The Effects of Microwave Processing on Health of People

Electromagnetic waves are divided into two main groups: ionizing and non-ionizing radiation. X-rays and gamma rays in the ionizing radiation group form radioactive compounds such as uranium and radium. Non-ionizing microwaves with low frequency and energy have no harmful and cumulative effects. Therefore, heat can be

produced safely with microwaves and foods do not become radioactive (Bih, 2003). However, health problems have been reported in people exposed to microwaves. As the tissues and organs in the human body contain high amounts of water. The temperature of these structures absorbing the microwave is increasing. In addition, since the heat cannot dissipate quickly in organs with slow blood circulation. These organs are more damaged than microwaves. The eye is one of the organs sensitive to temperature increase. Cataracts occur as a result of the thermal denaturation of lens proteins in the eyes due to the increased temperature with microwave radiation. As a result of studies on experimental animals; microwave radiation applied at a given power, frequency and time to the central nervous system.

In addition, chromosomal abnormalities have been identified in human lymphocytes exposed to microwave radiation (Lambert, 1980). In order to prevent these harmful effects of microwaves for human health, legal regulations have been made regarding the use of microwave ovens. According to these regulations; 5 cm from the furnace surface in factories distance should not exceed 1 MW/cm^2 , 5 MW/cm^2 if the consumer uses the microwave oven for the first time at home and 10 MW/cm^2 during the lifetime of the oven (Knutson et al., 1987).

2.11 Advantages of Microwave Processing

In conventional heating methods, heat is transmitted from the surface of the food to the interior. Compared to these methods, the heating rate is due to the fact that heat is produced in the material during microwave heating and the processing time is short. It is stated that vitamin and mineral losses are less in foods processed with microwave. Other advantages are that the equipment takes up little space, easy to clean and saves energy during the process. The energy savings are related to the conversion efficiency of microwaves to heat. Heat efficiency ranges from 7 to 14% in conventional ovens and up to 40% in microwave ovens (Knutson et al., 1987; Oliveira and Franca, 2002). In addition, since microwaves heat materials internally, temperature distributions are more homogeneous, surface overheating can be prevented and product quality can be improved by preventing certain events such as surface hardening. In addition, the selective absorption of microwaves by the

materials that absorb these waves better results in selective heating of foods, particularly during drying (Schiffmann, 1986).

2.12 Disadvantages of Microwave Processing

The vessel to be used for heating purposes must be made of microwave-permeable material; the shape and size of the container also have a significant effect on the reaction. These can be considered as difficulties in using microwave. In addition, magnetrons that function as generators in microwave ovens are more expensive than conventional heating elements. Therefore, their use in industry is developing slowly. Prevention of radiation leakage from microwave oven is very important for human health because the human body is constantly and excessively exposure to microwaves is inconvenient. 100 watt in microwave energy increases the body temperature by 5°C in 5 minutes and is extremely dangerous. The most sensitive organs to microwaves are the especially eye and brain. The temperature rise in both organs, especially in the eye with poor blood circulation, becomes dangerous in a short time since it cannot be carried by the blood. The maximum microwave radiation that the eye can withstand is 0.08 watt/cm² at 2.4 GHz. This is why the use of microwave ovens requires different safety measures than conventional ovens. Microwave producing and using devices must always be a sealed type closed system (Gümüşderelioğlu and Kaynak, 2012).

2.13 Antimicrobial Effects of Microwave

Microwave heating inactivates or reduce the pathogenic microorganisms in food products. Bacterial inactivation by microwave heating reported in the literature for following microorganisms: *Bacillus cereus*, *Clostridium perfringens*, pathogenic *Escherichia coli*, *Enterococcus*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella*. Foodborne pathogens are inactivated by microwave heating in poultry, fish, milk and eggs. Bacteria are more resistant to thermal inactivation than yeasts and molds and bacterial spores are more resistant than vegetative cells by microwave heating (Erkmen,2008).

In a different study for pasteurization of milk, 27 MHz was used and it was shown that the microwave only had a thermal effect on food. In parallel with microwave and conventional heating methods, no significant difference was found between the two methods and it was determined that both methods were not sufficient to eliminate *Bacillus cereus* spores. In a study on *Bacillus subtilis* and *E.coli* at 2450 MHz, it was found that the microwave had a thermal effect on both bacteria (Goldblith and Wang, 1967).

Four mechanisms are reported for inactivation of microorganisms by microwaves. These are selective heating, electroporation, cell membrane rupture and magnetic field coupling. Selective heating refers that solid microorganisms are heated more effectively by microwaves than the surrounding medium. On the other hand, microwave heating includes inactivation of microorganisms by heat, such as denaturation of enzymes and proteins, vital other components, besides the disruption of membranes. The energy absorption from microwaves can increase the temperature of the food high enough to inactivate microorganisms for effective pasteurization and sterilization. For inactivation of microorganisms and also degradation of vitamin B₁ and thiamin, for these reasons the thermal effect is necessary. For example, *E.coli* and *B.subtilis* are inactivated at 2450 MHz, but not effective on *B.subtilis* spores. Because of the nonuniform heating effects microwave heating seems less effective. Microwave heating has some problems during operating such as injury of microorganisms and increases loss of ultraviolet-absorbing cellular material and also elongates time because of production of enterotoxin. Microwaves facilitate oxidative reactions, maybe in membrane lipids, and decreases recovery of exposed cells. In combination of conventional and microwave heating also destroy the 16S subunit of RNA of sublethally-heated cells, only microwave heating also effects structure of the 23S subunit. Moreover, when cells are allowed to recover injury, it took longer for the microwave treated cells to restore their 23S RNA (Erkmen, 2008).

The original point of this project is; in order to reduce the microbial load to the desired level in the process of processing pistachios, the application of this method has not been tried before and the application of this method to obtain reliable and

high quality products as well as to prevent economic losses, especially to provide energy savings.

Pistachio is produced in traditional ways in production facilities. The first stage of this production process begins with wetting dry pistachios and removing the red outer shell and the final process is roasting and packaging. The roasting process is carried out both to give aroma and to reduce the microbial load to a safe level. Existing microorganisms grow rapidly as water activity increases as a result of the contact of pistachios with water during processing process. Significant contamination occurs in the processing process, especially in the production of rice pistachios. This leads to health and safety problems in pistachios. Microwave application was used for drying pistachios in 3 or 4 studies in laboratories in research centers abroad. In our country, no study was performed to reduce microbial load either in laboratory conditions or in pistachio processing process.

Standard and high quality pistachio production method was developed by applying microwave system to pistachio. With the realization of the study;

- Bringing a new method to the pistachio processing industry,
- Improving the relations between academia and society with the university-industry cooperation,
- Reduction of combustion (color change) caused by high temperature and long time in conventional ovens by microwave application,

One of the contributions of this project is the development and application of new techniques for quality improvement by testing a modern method or technology that has never been applied in the field of pistachio production.

2.14 Microbiological Methods Used in Experiments

2.14.1 Spread Plate Method

In spread plate method, colonies are produced on the surface of agar plates (PCA or other nonselective media). Some advantages of this method are:

- ✓ the use of translucent media is not necessary with a surface of plate method,
- ✓ the morphology of surface colonies can be better investigated,
- ✓ different colony types can be distinguished easily,
- ✓ organisms are not exposed to the heat of the melted agar medium,
- ✓ colonies are easily removed for further study,
- ✓ the use of a spread plate method may result in higher counts.

Disadvantage of spread plate method is the use of relatively small volumes (0.1 to 0.5 ml) of sample (Erkmen, 2011).

2.14.2 Plate Count Agar

Plate Count Agar (PCA), also called a standard plate count medium is a microbiological growth medium commonly used to assess "total" or viable bacterial growth of a sample. The composition of PCA is; tyryptone 5.0 g/L; yeast extract 2.5 g/L; D(+) glucose 1.0 g/L; agar-agar 14.0 g/L.

Ingredients except agar in 1.0 L of distilled water were dissolved by steaming. pH was adjusted to 7.0. Agar was added, and brought to boil and continued to heat until dissolving the agar. After that, sterilized at 121 °C for 15 min in autoclave. Then, cooled slowly about to 45 °C and poured into sterile petri dishes. All sterile petri dishes used were labeled with the dilution rate, date, name of media, and other desired information on a minimal area prior to preparation of dilutions (Erkmen, 2011).

2.14.3 Potato Dextrose Agar

It is used as a selective solid medium for yeasts and molds in standard in vitro microbiological analysis. The composition of PDA is; potato, infusion from 200.0 g/L; D(+) glucose 20.0 g/L; agar-agar 15.0 g/L.

Infusion of carbohydrates and potatoes in the composition promotes yeast and mold growth. Low pH suppresses bacterial growth. It is recommended to reduce the pH to 3.5 if only mold count is to be made in the material. The companion flora in the sample to be analyzed may also be used without acidification if the bacterial load is not excessive or not important. Mold develops on this medium by showing their typical morphology.

200 g of peeled potatoes was boiled for 1 hour in 1.0 L of distilled water. It was filtered and made up the filtrate to one liter. Glucose was added and mixed. Agar was added and heated to dissolve agar. Then, sterilized at 121 °C for 15 min. After that, cooled to about 50 °C. Finally, poured into sterile petri dishes. All sterile petri dishes used were labeled with the dilution rate, date, name of media, and other desired information on a minimal area prior to preparation of dilutions (Erkmen, 2011).

2.14.4 *Escherichia coli*

E. coli is the genus of the *Enterobacteriaceae* family. It is catalase positive, oxidase negative, fermentative, and Gram-negative. *E. coli* can be differentiated from other members of the *Enterobacteriaceae* on the basis of biochemical and serological tests.

E. coli must confirm to the indicate and fecal coliform group definitions. *E. coli* is used to indicate recent fecal contamination or unsanitary processing. Its natural habitat is the lower part of the intestines of humans and animals. The strains that colonize in the human intestine are harmless commensal. Although most strains of *E. coli* are not regarded as pathogens, they can be opportunistic pathogens that can cause infections in immunocompromised hosts. *E. coli* can be used as an indicator of fecal and nonfecal contamination. There are pathogenic strains that cause distinct syndromes of diarrheal disease and that have been associated with the foodborne illnesses. Hence, the presence of *E. coli* in food or water became accepted as indicative of recent fecal contamination and the possible presence of pathogens (Erkmen, 2011).

2.14.5 *Staphylococcus aureus*

Staphylococcus aureus is a nonmotile, nancapsulated, Gram-positive coccus, and forming spherical to ovoid cells. It is a member of *Micrococcaceae*, catalase positive, oxidase negative, facultative anaerobic, fermenting glucose and mannitol anaerobically and mesophilic. The natural habitats of *S.aureus* are nose, throat, hair, skin, and the mucous membranes of healthy humans and animals. *S. aureus* associates with the nasal tract in 20 to 50 % of healthy individuals. The bacteria are also present in air, dust and sewage and on the surface of food processing equipments. Food contamination generally occurs from these sources especially by food handlers. The ingestion of 100 ng of enterotoxins is sufficient to intoxication for the susceptible people. *S. aureus* produce enterotoxins if the numbers are higher than 10^4 per g of food. Staphylococcal food poisoning results from the ingestion of enterotoxin produce mainly by toxic strains of *S.aureus*. *S. aureus* has the ability to grow in food with very little available water.

CHAPTER 3

MATERIALS AND METHODS

Materials: *Pistacia vera* L. (pistachio) was obtained from Gaziantep (Turkey). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, from the G (-) and G (+) bacterial groups, respectively and also all other microbiological materials; incubators (at 25 °C and 37 °C), autoclave, oven, inoculating loop (3 mm diameter), test tubes, microscope, microscope slide, were obtained from microbiological laboratory in Gaziantep University. Petri dishes (90 x 17 mm), sterile pipettes (disposable-1 ml), automatic pipette (0.2-2 µl), yellow pipette tip (200 µl) were obtained from ISOLAB. The other equipments; dessicator, mechanical grinder, erlenmeyer flask, balloon, balloon joje, burette, coarse filter paper were obtained from laboratories in university.

Reagents: Sterile 0.1 % buffered peptone water (Merck) was obtained from microbiological laboratory, distilled water, acetic acid (CH_3COOH), isooctane solution ($\text{CH}_3\text{C}(\text{CH}_3)_2\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_3$), was obtained from chemical laboratory of school. Anhydrous potassium iodide (KI), anhydrous 98 % sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5 \text{H}_2\text{O}$), starch soluble, ($\text{C}_{27}\text{H}_{48}\text{O}_{20}$) were obtained from Sigma-Aldrich.

Media: Nutrient broth, Plate Count Agar (PCA), and Potato Dextrose Agar (PDA).

3.1 Sampling and Preparation for Analysis

In this study, pistachios in the form of chopped were used. The 10 g of pasteurized pistachios at 80 °C in an oven were transferred to the microbiology laboratory for inoculation of *Escherichia coli* and *Staphylococcus aureus* bacteria ($6.0 \log \text{cfu/g}$). Their transportation to the microbiology laboratory, and the preparation for analysis was applied according to the literature (Erkmen, 2011). Sampling and transporting were applied carefully at aseptic conditions. Meanwhile, be careful about microbial contamination of sample from environment, air, sample containers, and sampling devices. The package of food product was opened only in the laboratory near the fire

for preventing microbial contamination. All equipments used for sampling previously was wrapped and sterilized.

Sampling apparatus was sterilized in an oven because after dipping the instrument in alcohol and igniting could be inadequate for sterilizing equipments (Erkmen, 2011).

3.2 Experimental Design and Data Analysis for Microwave Heating of Inoculated Pistachio

The Hexagonal design for two independent variables was applied for microwave heating of inoculated pistachios. The independent variables were microwave power (90-330 watt), and microwave treatment time (0-40 min). The independent variables and variation levels are shown in Table 3.1. The complete design was composed of 20 runs. Half of them was done for *E. coli* inoculation, the others were for *S. aureus* inoculation, respectively. The outline of experimental design with actual levels is presented in Tables 3.2 and 3.3. Response surface methodology (Stat-Ease, Design-Expert software, version 6.0.1) was used for experimental data, for generation of response surface mathematical models and plots. It was used for statistical analysis of experimental data and optimization of microwave heating conditions.

Optimization process is used to make process designs efficient, to improve production and quality and to minimize costs. With the application of mathematics, numerical analysis and engineering in computer software, there has been much progress in optimization theories and techniques in the last two decades (Saguy, 1984). The response surface method is used not only for optimization purposes but also for determining the effects of individual and interactions of process variables on responses (Koç and Ertekin, 2009).

Table 3.1 Process variables used in the hexagonal design for the two independent variables

Independent variables	Levels	
Mw power (watt)	90	330
Mw treatment time (min)	0	40

The fitness of the model was determined by evaluating the coefficient of determination (R^2) and Fisher test value (F -Value) as obtained from an analysis of variance (ANOVA). The level of significance for all tests was set at 95% confidence level.

Table 3.2 Experimental design of microwave heating of inoculated pistachios with *S.aureus* experiment with actual variable levels

Run	Microwave power(watt)	Microwave treatment time(min)
1	210	20
2	210	20
3	330	20
4	150	3
5	270	38
6	210	20
7	150	38
8	210	20
9	90	20
10	270	3

Table 3.3 Experimental design of microwave heating of inoculated pistachios with *E.coli* experiment with actual variable levels

Run	Microwave power (watt)	Microwave treatment time(min)
1	210	20
2	150	3
3	210	20
4	330	20
5	270	3
6	270	38
7	90	20
8	210	20
9	150	38
10	210	20

3.3 Microbiological Analysis

3.3.1 Preparation of Dilutions

The purpose of the dilution is to reduce the number of excess microorganisms using various liquids and make them countable. Dilution fluids should be selected and used according to the sample examined or the characteristics of the microorganism culture. The dilution used must not cause any damage (such as osmotic shock) to the microorganisms. Sterile distilled water is therefore unsuitable for dilution. Commonly used diluents are 0.1 % peptone water, phosphate buffer solution, quarter-strength Ringer's solution and 0.85 % NaCl solution. Peptone water (0.1 %) has a greater protective effect than the others (Erkmen, 2011).

Peptone from Meat Pancreatic (Merck 1.07214) was dissolved in distilled water to a concentration of 0.1 % and appropriately dispersed into tubes and flasks and then, sterilized in an autoclave at 121 °C for 15 minutes. In flasks with 90 ml for sample preparation, and into tubes with 9 ml for dilution of samples.

Pistachio was aseptically weighed 10 g into a containing 90 ml of steril diluent (buffered peptone water) to make a 1:10 dilution. Then, pistachio in diluent was shaken for several minutes for mixing homogeneously. 1 ml of mixed pistachio was withdrawn with pipette (disposable-1 ml) and transferred to the tube containing 9 ml diluent labeled as 10^{-1} with the tip touching the side of the tube 2-3 cm above the level of the diluent. The pipette must not contact with the diluting fluid. This pipette used was discarded. The pistachio in diluent was mixed by rotating the tube back and forth vigorously between the hands. 1 ml of the diluent was withdrawn with a fresh sterile pipette and transferred to a second tube of sterile 9 ml diluent labeled as 10^{-2} . This pipette used was discarded. A fresh sterile pipette was taken and prepared further dilution in the same manner as described above. An appropriate dilution range was determined between 10^{-1} - 10^{-6} for the most accurate colony count that is the total number of colonies should be formed in a plate between 30 and 300 (Erkmen, 2011).

3.3.2 Inoculation

About 0.2 ml of diluted sample was placed into sterile petri dishes in duplicate by using a sterile pipette. It was poured 15 to 20 ml of melted medium (at 45 °C, PCA, PDA) into each of petri dish containing 0.2 ml inoculum by gently lifting the cover of the petri dish just high enough to pour the medium. It was avoided spilling the medium on the outside of the container or on the inside of the plate lid when pouring. As each plate was poured, thoroughly mixed the medium with the test portions in the petri dish by rotating the plates in the route drawing 8 while it was lying on the tabletop. The mixture was not splashed over the edge and there should be any bubbles on the surface. Plates were allowed to solidify on a flat surface area. After solidification, the plates were inverted to prevent spreaders, and promptly placed them into an incubator.

3.3.3 Incubation

Plates were incubated depending on the nature of the food and the type of microbial flora to be enumerated. For count of total bacteria, PCAs were incubated at 37 °C for 24 to 48 hr, and for count of mold and yeast, PDAs were incubated at 25 °C for 2 to 5 days (Erkmen, 2011).

3.3.4 Colonies Selecting and Counting on Plates

All colonies on selected plates were counted after incubation. Plates were counted with 30 to 300 colonies and averaged the counts for using duplicate plates and obtained the cfu per g.

3.3.5 Calculation and Reporting Counts

The number of colonies counted or estimated on each plate was recorded and dilutions were used. When colonies on duplicate plates and consecutive dilutions are counted, the results were averaged and rounded to two significant figures (such as

58.6372 = 58.64). Number per plate by the reciprocal of the dilution used was averaged and divided by inoculation amount.

3.3.5.1 Enumeration of *E.coli* Inoculated Pistachios on Solid Medium

About 10 g of inoculated pistachio was added into a flask containing sterile 90 ml of 0.1 % buffered peptone water and shaken for several minutes. Dilutions were prepared with sterile buffered peptone water diluent. Level of dilutions to be prepared depends on expected bacteria number. Sample preparation, dilution and microbial preparation were explained in details in 3.1 and 3.3.1 sections.

About 1 ml of diluted sample into each tubes from each dilution 10^{-1} to 10^{-6} was transferred. Pipette was held at angle so that its lower edge rests against the tube. Pipette drain was let for 2-3 sec. Pistachio with diluent was shaken until all dilutions were inoculated in duplicate form into PCA by using spread plate method. Details of methods were explained in Section 2.14.1. After that, 0.2 ml of sample was inoculated each plates numbered from 10^{-1} to 10^{-6} . They were waited for about 10 min until drying. Then, incubated at 37 °C for 24-48 hr. Plates containing 30-300 colonies that were least 0.5 mm in diameter counted. Number of *E.coli* was calculated from the following equation (3.1).

$$\text{No of } E. coli \text{ (cfu/g)} = \frac{\text{formed colonies each plates in totally (1 and 2)}}{\text{volume of sample inoculated (g)}} \quad (3.1)$$

3.3.5.2 Enumeration of *S. aureus* Inoculated Pistachios on Solid Medium

About 10 g of *S.aureus* inoculated pistachio was added into a flask containing sterile 90 ml of 0.1 % buffered peptone water and shaken for several minutes. Dilutions were prepared with sterile buffered peptone water diluent. Level of dilutions to be prepared depends on expected bacteria number. Sample preparation and dilution were explained in details in 3.1 and 3.3.1 respectively.

About 1 ml of diluted sample into each tubes from each dilution 10^{-1} to 10^{-6} was transferred. Pipette was held at angle so that its lower edge rests against the tube.

Pipette drain was let for 2-3 sec. Pistachio with diluent was shaken until all dilutions were inoculated in duplicate form into PDA by using spread plate method. Details of methods were explained in Section 2.14.1. After that, 0.2 ml of sample was inoculated each plates numbered from 10^{-1} to 10^{-6} . They were waited for about 10 min until drying. Then, incubated at 25 °C for 2-5 days. Plates containing 30-300 colonies that were least 0.5 mm in diameter counted. Number of *S. aureus* was calculated from the following equation (3.2).

$$\text{No of } S. aureus \left(\frac{\text{cfu}}{\text{g}} \right) = \frac{\text{formed colonies each plates in totally (1 and 2)}}{\text{volume of sample inoculated (g)}} \quad (3.2)$$

3.4 Microbial Preparation and Microwave Treatment Of Pistachio

In order to reveal the effect of microwave application and to reduce the effect of microorganism in the samples, chopped prepared had been pasteurized. Pasteurized pistachio groups were used to inoculate a certain number of G (-) bacteria group of *Escherichia coli* and G (+) bacteria group of *Staphylococcus aureus*. The dose inoculated here; 6 log. Different application time and microwave power intervals were tried with the help of the design created by the surface method to respond to pistachios. Firstly, bacteria used for inoculation of pistachio (*E.coli* and *S.aureus*) were multiplied into nutrient broth. After, procedure for enumerating of formed bacteria in previous sections was applied. Amount of used bacteria for inoculation to pistachio was determined after counting of forming bacteria. The result of inoculated bacteria to pistachio must be 6 log. After inoculation, for each microwave treatment, 10 g of chopped pistachio were weighed and were placed on the center of the turntable plate into microwave oven according to design of the experiments by adjusting RSM. Experimental design for *S.aureus* and *E.coli* bacteria already showed above at tables 3.2 and 3.3 respectively. Shortly after, for microwaving samples were homogenized in peptone water for determining mold and yeast count by surface plating on potato dextrose agar (PDA) after incubation 25 °C for 2-5 days and for determining total bacteria count by surface plating on plate count agar (PCA) after incubation at 37 °C for 24-48 hr. Effectiveness of different microwave treatment time and power intervals on 6-log bacteria inoculated pistachio was discussed in the

results and discussion section. All operations to pistachio are shown in the flow chart below (Figure 3.1).

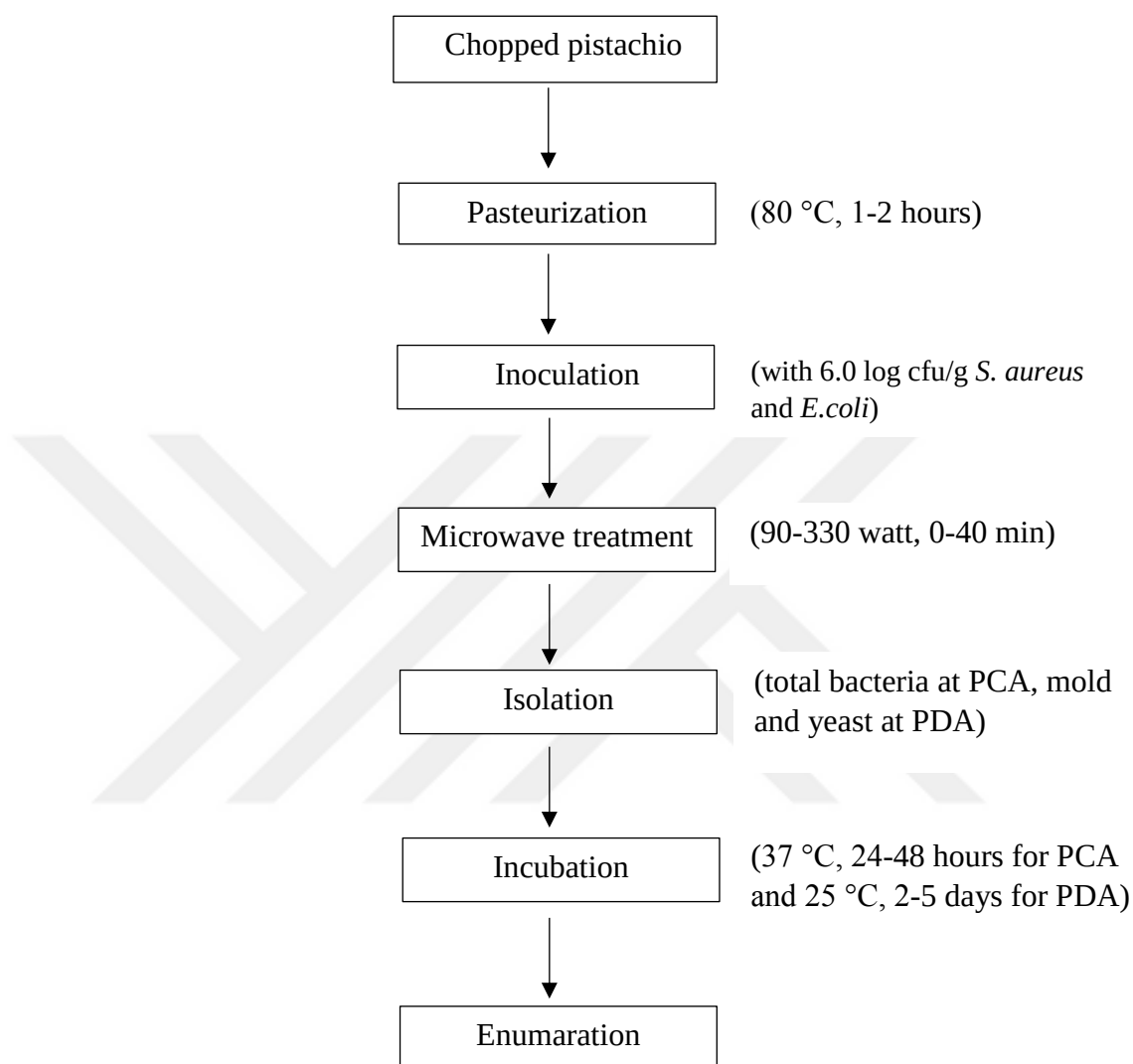


Figure 3.1 Flow chart of microbial preparation and microwave treatment of pistachio

In these processes, microbiological changes in pistachio were examined. The experiments were carried out for the most suitable time for the chopped pistachios and the re-validation attempts at the microwave power and the following physical, microbial and chemical contents of the product were compared with the conventional application.

3.5 Chemical Analysis

3.5.1 Determination of Moisture Content

The sample was previously ground in a thoroughly cleaned mechanical grinder. Weighing container and lid were placed separately in the desiccator and weighed for at least 30 minutes in desiccator. 2 g of the ground sample was placed in the weighing dish, spreading uniformly to the base of the container and weighed again. The vessel containing the test specimen was removed from the oven and placed in the desiccator by placing the container in an oven at a temperature of 105 °C and 2 hours after the temperature reached 105 °C. It was weighed after cooling to laboratory temperature. The vessel was re-incubated for 1 hour, removed again, cooled to laboratory temperature and weighed. If the difference between the two scales was less than 0.005 g or 0.005 g, the process would be considered as finished. If not equal, the process was repeated for 1 hour until the difference between two consecutive weighings was less than 0.005 g or equal to 0.005 g. Two parallel determinations were made from the same prepared sample. The amount of moisture was calculated from the following formula (3.3) as weight percent of the product (Turkish Standard 972, 1971).

$$Moisture = (M_1 - M^2) * \frac{100}{M_1 - M^0} \quad (3.3)$$

in percent by weight,

M_0 = Weight of empty weighing container, g

M_1 = The weight of the container in which sample is placed before drying, g

M_2 = The weight of the container after the drying process, g

3.5.2 Determination of Peroxide Value in Oil of Pistachio

About 50 ml acetic acid / isooctane solution was placed and the lid was closed. The sample was shaken and rotated until dissolved. With a suitable pipette, add 0.5 ml of saturated potassium iodide solution and re-cover the flask. The solution was allowed to stand at room temperature for 1 min with agitation at least three times during this period. Then, immediately add 30 ml of distilled water. Until the yellow iodine

discolouration was lost, it was titrated continuously with vigorous stirring while adding sodium thiosulphate solution. 0.5 ml starch solution was added. Titration was continued by the dropwise addition of sodium thiosulphate solution until the blue color disappeared. The amount of peroxide was calculated from the following formula (3.4 and 3.5)(TS 4964 ISO 3960, 2004).

Peroxide value per milligram of active oxygen per kilogram,

$$P = 1000 * (V - V_0) * C/m \quad (3.4)$$

V = Volume of sodium thiosulfate solution used for determination, ml,

V₀ = The volume of the sodium thiosulfate solution used in the assay, ml,

c = Concentration of sodium thiosulfate solution, mol/L

m = Mass of sample, g

Peroxide value per millimole per kilogram,

$$P = (V - V_0) * c/2m \quad (3.5)$$

3.5.3 Determination of Color

The first judgment in food quality is based on the color of the product. Therefore, it is important for businesses to accurately measure color. In the analysis of quality changes as a result of factors such as processing and storage of foods, color measurements are used to determine the conformity of food quality with standards.

Color analysis by Hunter method is based on the principle of reading L, a, b values of different food samples using Hunter Colorimeter. Photoelectric colorimeter which is cheaper, simpler and faster than spectrophotometric system. The Hunter colorimeter has three color values: In this system, the abbreviations L*, a*, b* are used. The L value indicates brightness and L:0 indicates black, L:100 white, + a indicates redness (-a green), + b indicates jaundice (-b blue).

In colorimetric measurement, the color of the sample is evaluated by comparing it to the color of the standard. Color measurements are used to determine quality in many foods such as freshly processed fruits and vegetables, dairy products, spices, oils, sugary products. Each product has different characteristics. For example, a / b ratio is often an important criterion in determining the color of tomato paste, and when this ratio is above 1.9, the tomato paste is considered to be of good quality in color. In addition, the L value is a measure of brightness and, in a good paste, it is desirable that the brightness be as high as possible. In this way, the characteristics of each food are different and different standards can be set for each food.

The Hunter Lab colorimetry instrument is first calibrated. The white balance is adjusted using the black calibration plate and the white balance using the white calibration plate.

3.6 Statistical Analysis

Response surface methodology (Stat-Ease, Design-Expert software, version 6.0.1) was used for experimental data, for generation of response surface mathematical models and plots. It was used for statistical analysis of experimental data, determination of the regression coefficients, modelling and optimization, and three dimensional graphs. The level of significance for all tests was set at 95 % confidence level.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Initial Properties of Raw Chopped Pistachio

Pistacia vera L. (pistachio) was obtained from Gaziantep (Turkey). In the form of chopped was used for this study. The properties of chopped pistachio before microbial preparation and before microwave (MW) treatment are given as in Table 4.1. According to the results of analysis, moisture content of raw pistachio was found to be 4.49 %.

No peroxide was found before and after the MW treatment. Pistachio color was determined using a Hunter Lab colorimeter. The individual lightness L^* , a^* and b^* parameters were recorded and results were reported as the mean $\pm$ S.D (n=3) for each MW treatment duration. Color values of raw chopped pistachio in this study are given in Table 4.1, $L^*=56.9$, $a^*=3.85$ and $b^*=34.1$. Differences in color values could be caused by differences in measurement procedures.

The main purpose of this study was to reduce the microbial load, which was one of the main problems of pistachio processing, to a permissible level by MW treatment. According to microbiological analysis of raw pistachio, total bacteria count (log cfu/g) is 4.47, mold and yeast count (log cfu/g) is 4.54.

Table 4.1Initial properties of raw pistachio

Parameter	Raw pistachio
Moisture content (%)	4.49± 0.04
Peroxide value (meq g/kg)	ND
L*	56.9
a*	3.85
b*	34.1
Total bacteria count (log cfu/g)	4.47
Mold and yeast count (log cfu/g)	4.08

ND: Not determined

4.2 Physical and Chemical Properties of Microwave Applied Pistachio

In this study, effects of MW treatment on quality and safety of attributes of pistachio were investigated. Pistachio quality was evaluated based on chemical properties such as moisture content, peroxide value etc., and other physical properties.

In this study, changes in moisture content and color values of chopped pistachio with microwave duration at different power (90-330 watt) and time intervals (0-40 min) were investigated and results are given below. Experimental design for investigating the effect of microwave on moisture content and color of microwave applied pistachios was determined by RSM. Totally five different microwave power (90, 150, 210, 270, 330 watt) and three different treatment time (3, 20, 38 min) was applied according to RSM.

The physical and chemical properties of microwave applied pistachio are given as in Table 4.2.

Table 4.2 Physical and chemical properties of microwave applied pistachio

Run	MW power (watt)	MW treatment time (min)	Moisture content (%)	color		
				L*	a*	b*
1	210	20	1.96	57.91	3.73	34.63
2	210	20	1.97	57.49	3.46	34.91
3	330	20	1.69	57.35	4.17	33.08
4	150	3	2.54	56.38	3.95	33.1
5	270	38	1.82	57.97	3.9	35.68
6	210	20	2.25	56.97	3.58	34.16
7	150	38	2.41	56.65	3.75	34.77
8	210	20	1.99	58.85	3.53	37.36
9	90	20	2.24	57.99	3.52	35.57
10	270	3	2.17	57.86	3.79	34.08

4.2.1 Evaluation of Moisture Content Analysis

Before microwave processing, initial moisture content of pistachio was found 4.49 %. According to the literature should be between 4-8 % (Souci et al., 1981/1982; Watt and Merrill, 1975).

The results after microwave treatment are showed in Table 4.2. As shown in the Table moisture content values of MW treated pistachios were changed between 1.69 and 2.54 % at different microwave power for different periods of time. As can be seen from the results, the highest moisture content was obtained in Run 4 (150 W-3 min) as 2.54 %, and difference between in this run and initial value was found as 1.95 %. Whereas the lowest was in Run 3 (330 W-20 min) as 1.69 %. and difference between in this run and initial value was found as 2.5 %. When the results are examined, the moisture content of the pistachio with high microwave power was lower. As seen in Table 4.2. at Run 8 (210 watt-20 min) and Run 9 (90 watt-20 min) different microwave power was applied to the pistachio for equal periods. However, moisture content of pistachio was lower, 1.99 % at 210 watt-20 min. At Run 4 (150 watt-3 min) and Run 7 (150 watt-38 min) equal microwave power was applied to pistachios for different periods. At these time intervals moisture content of pistachio was close to each other.

All data were statistically analyzed by RSM (Stat-Ease, Design-Expert software, version 6.0.1) linear model was found to be significant ($p < 0.05$) and suggested to explain moisture content of MW-treated pistachio. Effects of microwave power (A), and treatment time (B) had negative, and only microwave power significant ($p < 0.05$) effects on moisture content (Equation 4.1 and Table 4.3).

Table 4.3 Analysis of variance table for effect of the MW on moisture content of inoculated pistachio

Source	Sum of squares	DF	Mean square	F value	Prob > F	
Model	0.41	2	0.20	6.37	0.0265	S
A	0.35	1	0.35	11.05	0.0127	S
B	0.054	1	0.054	1.69	0.2349	
Residual	0.22	7	0.032			
Lack of fit	0.17	4	0.042	2.15	0.2775	NS
Pure error	0.058	3	0.019			
Cor total	0.63	9				

S: significant; NS: not significant

$$R^2 = 0.6454$$

Final equation in terms of actual factors:

$$\text{Moisture content} = 2.83896 - 2.86111E * A \quad (4.1)$$

A is microwave power (watt), B is microwave treatment time (min).

As the power of the microwave increases, the temperature of the pistachio also increases. The moisture content of the pistachio decreased as the temperature rised. In fact, the temperature of the pistachios in each microwave power increases with time but not, so not significant. Such a conclusion was reached when comparing the moisture content of the pistachio before and after the microwave treatment and the values after the microwave. Also, effect of MW power and treatment time on the moisture content of inoculated pistachio is showed in Figure 4.1.

Arena et al. (2007), in a study, the moisture content of pistachio fruits from different countries were examined, the results are shown in Table 4.4. The average moisture content of Turkish varieties was found to be 5.7% and the lowest moisture content was 4.2%. For ‘Agrigento’ type moisture was determined to be the highest with 6.9%.

Table 4.4 Moisture content of varieties from different countries (Arena et al., 2007)

Samples	Moisture content (%)
Italy (Bronte)	6.0
Italy (Agrigento)	6.9
Turkey	4.9
Greece	5.2
Iran	6.3
Average value	5.7

In a study conducted by Çağlarırnak and Batkan (2005) on the moisture content of peeled and roasted pistachios, hazelnut and peanut fruits. Moisture content of pistachio fruit was 1.97-2.46%, and moisture content of hazelnut fruit was 3.26-3.99 % and peanut. Moisture content of fruit was determined between 1.93-5.11%.

Moisture loss is achieved because the microwave heats the pistachio from inside to outside completely and homogeneously. According to the literature, foods containing low moisture (as low water activity < 0.6) are more reliable and quality also storable. Microorganisms generally do not grow but can activate in food at this level. If there are microorganisms in low moist food products, it results food-borne illness as contaminated from nuts and nut products (Cavallaro et al., 2011; CDCP, 2014, 2016; Chang et al., 2013; Keady, 2004; Wittenberger and Dohlman, 2010). Foodborne pathogens such as *Salmonella*, *E. coli* O157:H7, and *Staphylococcus aureus* can be determined in low moisture foods (Beuchat et al., 2011, 2013).

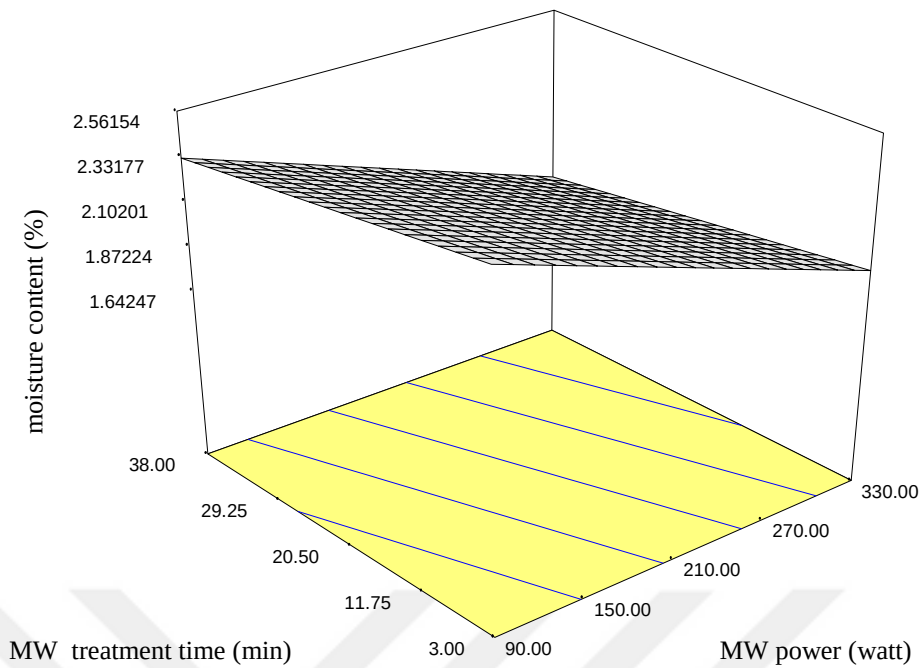


Figure 4.1 Effect of MW power and treatment time on the moisture content of inoculated pistachio

Consequently, the microbial safety in low water activity products has emerged as a high priority challenge across the food industry. As a major kind of low moisture foods, in-shell tree nuts have been a main carrier of pathogens as reflected in many outbreaks and recalls (CDCP, 2011, 2016; Palumbo et al., 2016). Some studies have reported that the pathogens on in-shell nuts can survive for extended periods and migrate to the kernels through hulls and shells in wet conditions (Danyluk et al., 2008; Blessington et al., 2014; Frelka et al., 2016; Uesugi and Harris, 2006). Except for the recalls of walnuts, several surveys have also isolated *Salmonella* and *E. coli* from walnuts (Little et al., 2010; CFIA, 2012; Davidson et al., 2015). In addition, it has been reported that *Enterobacteriaceae* is the dominant family on in-shell walnut surface. *Staphylococcus aureus*, as an opportunistic pathogen, can also be isolated on the surface of in-shell walnuts (Zhang and Wang, 2017). Moreover, the microorganisms are mainly attached to the shells of walnuts because they can provide some levels of protection for kernels from contamination (Frelka et al., 2012; Frelka, 2013; Frelka and Harris, 2015). All these observations show that it is necessary to eliminate the risk of pathogens on in-shell walnuts.

4.2.2 Evaluation of Peroxide Value Analysis

No peroxide was found before and after the MW treatment. In a study, the effects of roasting time on some quality characteristics were investigated in the production of roasted pistachios by K ro lu et al. (2000). Peroxide value of unroasted pistachios was found to be 8.12. However, roasting and roasting time showed further effect of pistachio oil quality on peroxide value. While the number of peroxide in unroasted pistachio fruits was 8.12 and it reached to 15.00 for 5 minutes roasted fruits and 25.71 for 12 minutes roasted fruits. This could be due to that the quality of pistachio oil have negative effect if the roasting time is prolonged (Labavitch, J.M., et al., 1982).

However, the peroxide was not found before and after the microwave process in the samples we studied. This may be because the samples were brought to the laboratory in a vacuum manner. Thus, the contact of the samples with air was stopped. Another reason may be because no heat treatment is applied without microwaving.

4.2.3 Evaluation of Color Analysis

In this study, changes in color values of chopped pistachio with microwave processing were determined and discussed. Before microwave processing, initial color values of pistachio was found as $L^*=56.9$, $a^*=3.85$, $b^*=34.1$. The results after microwave treatment are showed in the Table 4.2. Changes in the color of MW treated pistachios were evaluated by measuring lightness (L^*), redness (a^*), blueness (b^*) after MW treatment. The MW treatment did not perform any significant ($p>0.05$) color changes at specified time intervals.

After microwave treatment, it was found that a^* and b^* values of pistachios were close to those of fresh samples and also the microwave treatment caused to increase the L^* value in some experiments (Table 4.2). For example, at Run 8 (210 watt-20 min), L^* value was found 58.85. In this experiment, the lightness of pistachios seems to increase slightly. As a result of the experiments, no visible darkening was observed. It is seen that microwave process is a kind of heating process without any harm to food. However, according to a study conducted in 2009 by Ba aran and

Akhan, changes in the color of MW-treated hazelnuts were investigated by measuring lightness (L^*), hue angle and chroma shortly after MW treatment. The MW treatment not exert any significant color changes up to 100 s was observed in their study. However, after 120 s nuts showed dark brown burning color. In contrast to our study, L^* values decreased with increasing treatment time. The initial L^* value of the sample was 66.03 and decreased to 61.06 after 120 seconds of microwave application (Başaran, and Akhan, 2009).

Statistical analysis for determining effect of the MW on color of inoculated pistachio was discussed according to analysis of variance (ANOVA). L^* , a^* and b^* values were entered for each run. Then, interpretation was done according to model and model graphs. Looking at the table, there are no significant model terms for L^* values. However, for a^* values quadratic model was suggested to explain color of MW-treated pistachio, but found as not significant ($p>0.05$). Effects of microwave treatment time (B) had negative, microwave power (A), and interaction of microwave power and microwave treatment time (AxB) had positive effect. On the other hand, for b^* values linear model was suggested to explain color of MW-treated pistachio, but found as not significant ($p>0.05$). Microwave power had a negative effect, while microwave treatment time had a positive effect on color of MW-treated pistachios.

In a study conducted by Polat (2016), the effect of drying on some properties of terebinth (*pistacia terebinthus*), betoum (*pistacia khinjuk*) and pistachio (*pistacia vera*) fruits was investigated. Color values of pistachio fruits in color tests; in fresh samples (before conventional drying), L value was measured as 37.06, a value 7.36, b value 9.66. In dry samples, L value was 41.15, a value was 7.08, b value was 8.52. In roasted samples, L value was 34.44, a value was 7.40 and b value was 7.11. It was observed that drying process caused to increase in L , decrease in a and b values in pistachio samples.

4.3 Effects of Microwave Heating on Microbial Contamination of Inoculated Pistachio

Microbial contamination is the major problem encountered during production, storage and marketing of pistachios. Increasing interest in the pistachio producer is to develop economically feasible and environmentally safe non-chemical processes to control microbial contamination in pistachios while retaining product quality.

In order to determine the effects of microwaves on microorganisms, numerous studies have been conducted. Studies on this subject started to be carried out after 1950 and gained weight in recent years. The effects of microwave heating on microorganisms in various foodstuffs such as meat, dairy eggs, cereals, fruit and vegetable products were generally examined in comparison with conventional heating methods (Ercan et al., 1989).

Researches in this field have been made in various foods on some bacteria such as *Bacillus cereus*, *Bacillus subtilis*, *Clostridium perfringens*, *Escherichia coli*, *Lactobacillus plantarium*, *Leuconostoc mesenteroides*, *Proteus sp.*, *Pseudomonas putrefaciens*, *Salmonella senftenberg*, *Salmonella typhimurium*, *Salmonella typhi*, *Serratia marcescens*, *Streptococcus faecalis*, *Streptococcus faecium*, *Staphylococcus aureus* and some molds *Aspergillus niger*, *Penicillium sp.*, *Rhizopus nigricans* (Ercan et al., 1989).

In these processes, microbiological changes in pistachio were examined. Different microwave treatment time and power intervals were tried with the help of the design created by the surface method to respond to pistachios. The experiments were carried out for the most suitable time for the chopped pistachio and the re-validation attempts at the microwave power.

4.3.1 Evaluation of *Staphylococcus aureus* Count

Because of the investigating the effects of microwave heating treatment on pistachios that inoculated with *Staphylococcus aureus*. The number of inoculated *S.aureus* determined in commercial pistachio samples is reported by the company as 6.0 log

cfu/g. Results of MW treatment for inactivation of *S. aureus* for various durations are shown in Table 4.5. The maximum reduction in the number of microorganisms was obtained from Run 5 (270 watt – 38 min). Nearly four log cfu reduction in *S. aureus* count was observed after 38 min exposure time, when the microwave power was 270 watt.

Table 4.5 Experimental design and results for the two variables studied on *S.aureus* inoculated pistachio

Run	MW power (watt)	MW treatment time (min)	<i>S.aureus</i> (log cfu/g)
1	210	20	4.14
2	210	20	4.51
3	330	20	3.36
4	150	3	5.08
5	270	38	2.30
6	210	20	4.58
7	150	38	2.78
8	210	20	4.54
9	90	20	4.83
10	270	3	4.82

According to the results of statistical analysis made by RSM (Stat-Ease, Design-Expert software, version 6.0.1) linear model was found to be significant ($p < 0.05$) and suggested to explain effect of microwave on inoculated pistachio with *S. aureus*. Effects of microwave power (A), and treatment time (B) had negative, and both of microwave power and microwave treatment time significant ($p < 0.05$) effects on reduction of *S.aureus* (Equation 4.2 and Table 4.6). Compared to inoculated pistachio, there is a reduction in all microwave power. Microbial contamination decreased as the pistachio prolonged exposure to microwaves.

Similar situation in the literature, by Hong et al. (2004) is reported that nearly 6-log reduction of coliform in sludge after 120 s applying microwave heating at frequency of 2.45 GHz. Firstly, dentures were inoculated with *Candida albicans* after, sterilization is done by microwave heating at 650 W for 6 min (Oh et al., 2006).

Table 4.6. Analysis of variance (ANOVA) table for effect of the MW on *S. aureus* inoculated pistachio

Source	Sum of squares	DF	Mean square	F value	Prob > F	
Model	7.03	2	3.52	22.27	0.0009	S
A	1.13	1	1.13	7.15	0.0318	
B	5.90	1	5.90	37.39	0.0005	S
Residual	1.10	7	0.16			
Lack of fit	0.98	4	0.25	5.91	0.0882	NS
Pure error	0.12	3	0.014			
Cor total	8.14	9				

S: significant; NS: not significant

$$R^2 = 0.8642$$

Final equation in terms of actual factors:

$$S. aureus = 6.56907 - 5.11111E - 0.03 * A - 0.069393 * B \quad (4.2)$$

A is microwave power (watt), B is microwave treatment time (min).

Thermal destruction of microorganisms has been proven to be the most effective way of decontaminating food to ensure food safety. This process is a reduction in the number of viable pathogens so that the food products are unlikely to cause disease. Conventional heating techniques can impart undesirable changes in product quality, as it takes longer to achieve the desired thermal treatment needed to inactivate pathogens deeper within the food.

White and Hobbs (1963) *B. subtilis*, *S. senftenberg*, *S. aureus*, and *C. perfringens* in the microwave heating of the inoculated meat, the number of these microorganisms were found to decrease significantly.

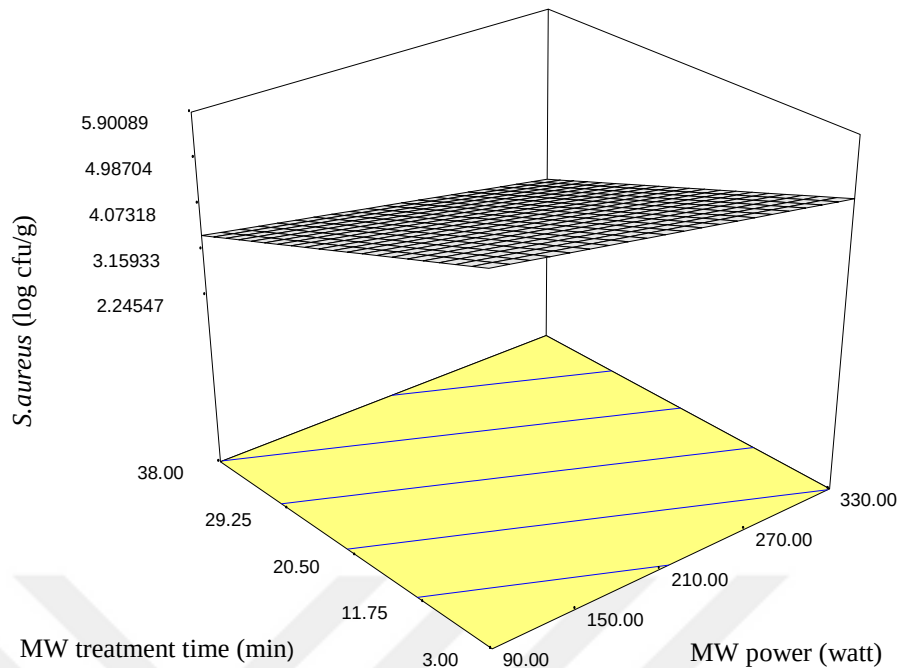


Figure 4.2 Effect of MW power and treatment time on *S. aureus* inoculated pistachio

4.3.2 Evaluation of *Escherichia coli* Count

Escherichia coli (*E. coli*) as G (-) bacteria was inoculated to pistachio groups. The number of *E. coli* detected in commercial pistachio samples is reported by the company as 6.0 log cfu/g. Results of MW treatment for inactivation of *E. coli* for various durations are shown in Table 4.6.

Effects of microwave power (A), treatment time (B) had positive, interaction between the microwave power and treatment time (AxB) has negative effects on reduction of *E. coli*. Both of microwave power and treatment time showed significant ($p < 0.05$) effects on reduction of *E. coli* reduction (Equation 4.3 and Table 4.7

Table 4.7 Experimental design and results for two variables studied on *E.coli* inoculated pistachio

Run	MW power (watt)	MW treatment time (min)	<i>E.coli</i> (log cfu/g)
1	210	20	2.65
2	150	3	3.42
3	210	20	2.84
4	330	20	0.00
5	270	3	2.70
6	270	38	0.00
7	90	20	2.94
8	210	20	2.39
9	150	38	3.54
10	210	20	2.69

According to the results of statistical analyses made by RSM quadratic model was found to be significant ($p < 0.05$) and suggested to explain effect of microwave on inoculated pistachio with *E.coli*. As seen in the Table 4.6, maximum decrease was found in Run 4-6 (330 watt-20 min and 270 watt-38 min). The minimum decrease was seen in Run 9 (150 watt-38 min). All microorganisms (*E.coli*) completely disappeared after 270 watt-38 min and 330 watt-20 min.

Table 4.8 ANOVA for effect of the MW on *E.coli* inoculated pistachio

Source	Sum of squares	DF	Mean square	F value	Prob > F	
Model	14.15	5	2.83	31.36	0.0026	S
A	8.43	1	8.43	93.41	0.0006	S
B	1.67	1	1.67	18.55	0.0126	S
A ²	1.83	1	1.83	20.31	0.0108	S
B ²	0.017	1	0.017	0.19	0.6889	
AB	2.02	1	2.02	22.41	0.0091	S
Residual	0.36	4	0.090			
Lack of fit	0.26	1	0.26	7.31	0.0736	
Pure error	0.11	3	0.035			
Cor total	14.51	9				

S: significant; NS: not significant

$$R^2 = 0.9751$$

Final equation in terms of actual factors:

$$E. coli \text{ count} = -5.56270E - 0.03 + 0.033768 * A + 0.094061 * B - 8.14236E - 0.05 * A^2 - 6.77047E - 0.04 * Ax B \quad (4.3)$$

As shown in the table, the effect of the MW treatment time and power on *E.coli* inoculated pistachios was significant ($p<0.05$). Also, effect of MW power and treatment time on the *E.coli* inoculated pistachio is shown with Figure (4.3).

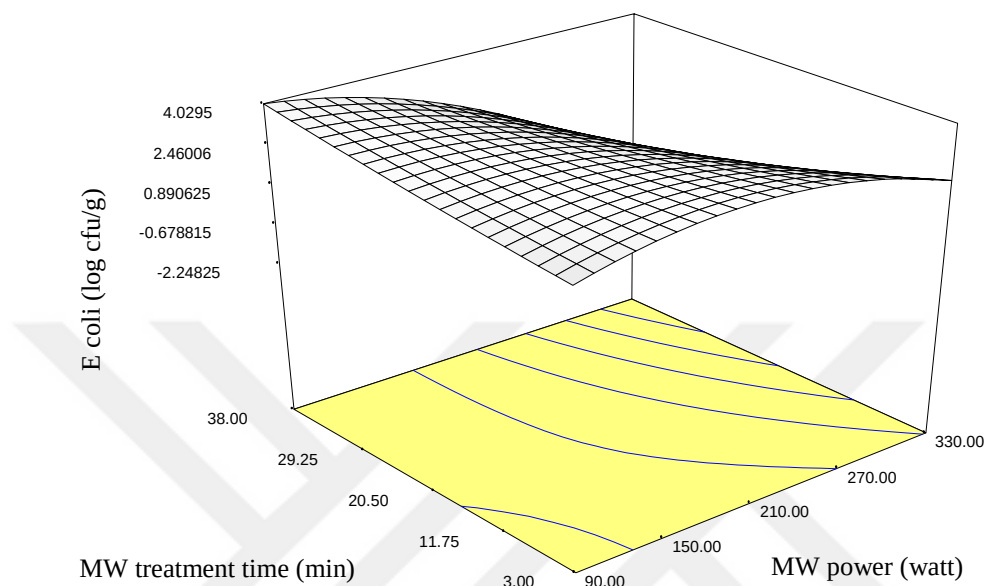


Figure 4.3 Effect of MW power and treatment time on the *E.coli* inoculated pistachio

In a study, Culkin and Fung (1975) reported that the killing of *E.coli* and *Salmonella typhirium* in microwave-cooked soups was not directly related to the ambient temperature reached after irradiation. The researchers heated tomato soup, vegetable soup and beef broth at 915 MHz. In the application of the process for a certain period of time, it was found that the temperature in the middle regions of the soups was highest, the medium at the base and the lowest at the upper part. However, it was determined that the microorganisms in the upper part of the soups died more quickly than those in the center and at the bottom. In this case, the researchers reported that the only factor in killing microorganisms in microwave application can not be the temperature reached in the environment.

4.3.3 Evaluation of Mold and Yeast Count of *E.coli* Inoculated Pistachio

The effect of microwave treatment time and microwave power on number of mold and yeast of *E.coli* inoculated pistachio was investigated. As can be seen from the Table 4.9 the number of mold and yeast decreased with increasing microwave power.

Table 4.9 Experimental design and results for two variables studied on mold and yeast count of pistachio

Run	MW power (watt)	MW treatment time (min)	Mold and yeast (log cfu/g)
1	210	20	3.18
2	150	3	4.24
3	210	20	3.20
4	330	20	2.47
5	270	3	2.30
6	270	38	1.69
7	90	20	3.16
8	210	20	3.21
9	150	38	3.76
10	210	20	4.00

Linear model was found to be not significant ($p>0.05$) and suggested to explain effect of MW on mold and yeast count of *E.coli* inoculated pistachio.

At first, 4.47 log cfu/g mold and yeast were found in inoculated pistachio that contaminated with *E.coli*. The maximum reduction in the number of microorganisms was Run 6 (270 watt – 38 min). Nearly 3-log cfu/g reduction in mold and yeast was observed after 38 min exposure time, when the microwave power was 270 watt. As shown in the Table 4.10, the effect of the MW on inoculated pistachios with *E.coli* is not significant ($p<0.05$). Power of the microwave is significant ($p<0.05$) in reducing the microbial contamination.

Table 4.10 ANOVA table for effect of the MW on the mold and yeast count of *E.coli* inoculated pistachio

Source	Sum of squares	DF	Mean square	F value	Prob>F	
Model	2.73	2	1.36	3.32	0.0968	NS
A	2.42	1	2.42	5.90	0.0455	S
B	0.30	1	0.30	0.74	0.4174	
Residual	2.87	7	0.41			
Lack of fit	2.39	4	0.60	3.70	0.1557	NS
Pure error	0.48	3	0.16			
Cor total	5.60	9				

S: significant; NS: not significant

$$R^2 = 0.4869,$$

Final equation in terms of actual factors:

$$\text{Mold and yeast count} = 5.01154 - 7.48611E - 0.03 * A \quad (4.4)$$

Effect of MW power and treatment time on the mold and yeast count of *E.coli* inoculated pistachio was showed below (Figure 4.4).

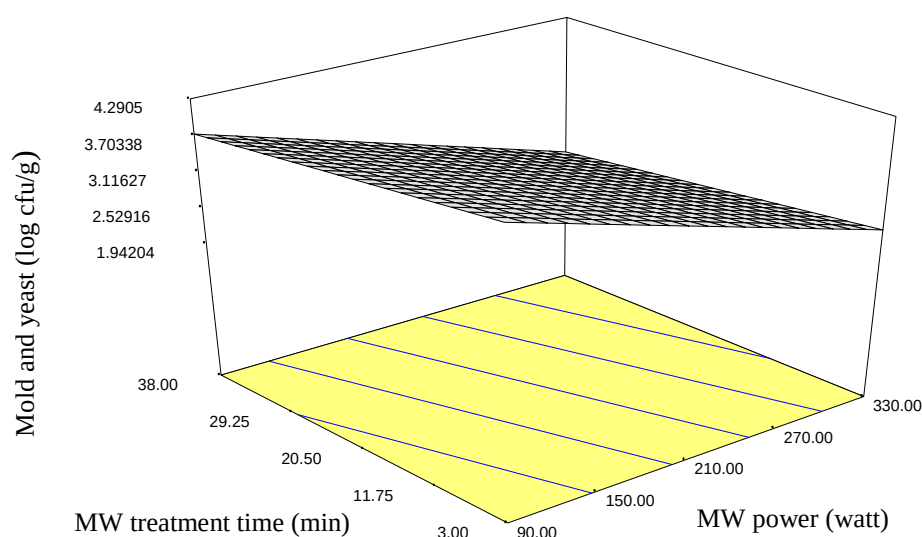


Figure 4.4 Effect of MW power and treatment time on the mold and yeast count of *E.coli* inoculated pistachio

In a study, removing of mold spores on bread had benefited from microwaves by Olsen (1965). The researcher reported that this process inhibited the growth of *A. niger*, *R. nigricans* and some *Penicillium* species more than 1% added sodium propionate.

The following findings are obtained from the researches (Fung and Cunnigham, 1969):

- Microwave heating of food is more dependent on the structure and composition of food than conventional heating.
- The microwave processing time required for cooking or heating certain foods may not be sufficient to kill the microorganisms if there are too many microorganisms in the medium.
- Using microwaves in combination with conventional methods enables food to be heated and reduced microorganism load more effectively.
- The heat generated by the microwaves is sufficient to remove natural flora and contaminants in the environment when the type, size, and foodstuff of the food are carefully correlated with the microwave application time.
- Microwaves have different lethal effects on different types of microorganisms.

4.4 Optimization for Microwave Heating on Quality Parameters

The optimum conditions for microwave heating on quality parameters of *S.aureus* inoculated pistachio were based on the minimum levels of responses. The optimum MW power and MW treatment time for, *S.aureus* count and moisture content were estimated as 323.76 W, 37.90 min for, 2.28 log cfu/g and 1.66 % respectively. It was found that the most important variable was MW power for the desirability levels of quality parameters. Desirability value of microwave power and treatment time on the *S.aureus* inoculated pistachio was found 1.000 by RSM created optimization numerical graphs as shown in the graph below (Figure 4.5).

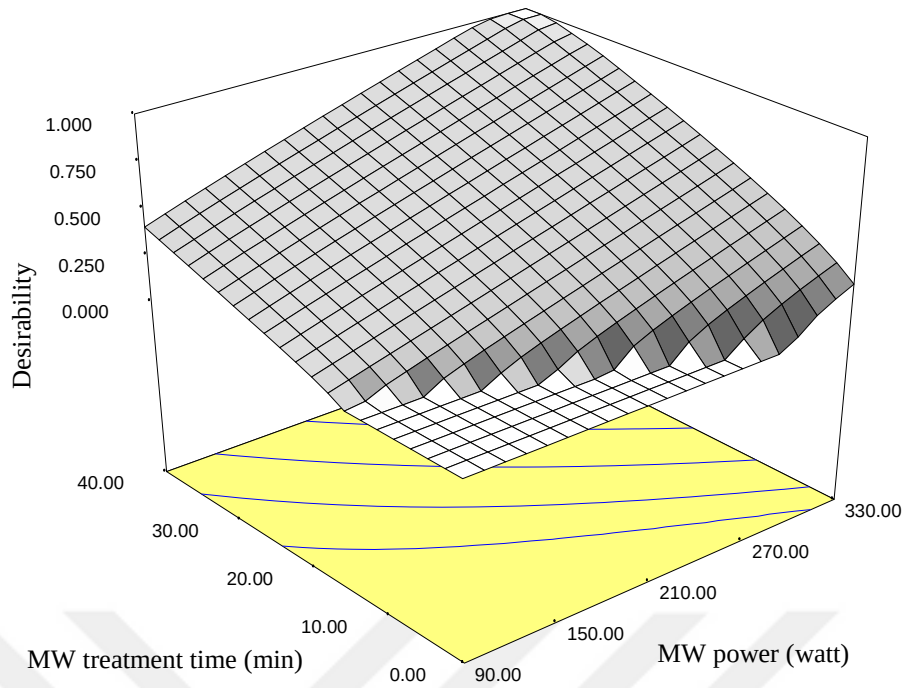


Figure 4.5 Desirability of MW power and treatment time on the *S.aureus* inoculated pistachios

The optimum conditions for microwave heating on quality parameters of *E.coli* inoculated pistachios were based on the minimum levels of responses. The optimum MW power and MW treatment time for, *E.coli*, mold and yeast, moisture content were estimated as 330.00 W, 40.00 min, -2.46 log cfu/g, 1.91 log cfu/g and 1.65 %, respectively. It was found that the most important variable was MW power for the desirability levels of quality parameters like *S.aureus* inoculation. Desirability value of microwave power and treatment time on the *E.coli* inoculated pistachio was found 0.970 by RSM created optimization numerical graphs as shown in the graph below (Figure 4.6).

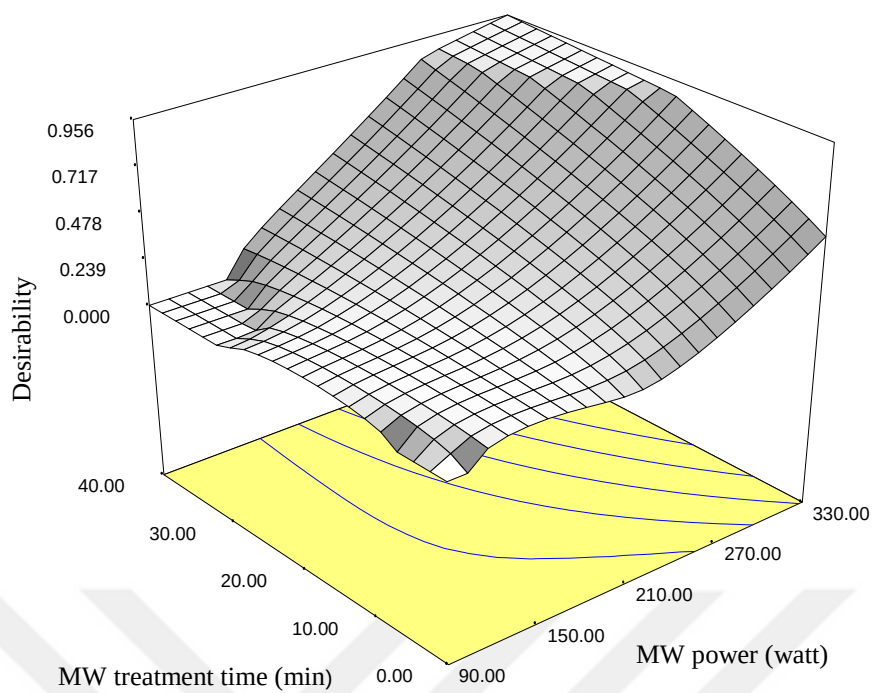


Figure 4.6 Desirability of MW power and treatment time on the *E.coli* inoculated pistachios

CHAPTER 5

CONCLUSION

The effects of microwave treatment on pistachios artificially contaminated with *S.aureus* and *E.coli* were evaluated by use of response surface methodology to optimize the conditions of the reduce the number of microorganisms (*S.aureus* and *E.coli*). The physical quality attributes (moisture content, peroxide value and sensory attributes) of microwave treated pistachios were also evaluated.

In microwave heating of pistachios microwave power showed significant ($p<0.05$) effects on moisture content of pistachios. Microwave time showed significant ($p<0.05$) effects on count of *S.aureus*. A significant ($p<0.05$) reduction in *E.coli* and mold and yeast count (in inoculated pistachios with *E.coli*) contamination was observed by applying different microwave power. Increased microwave power was the most effective variable on pistachios.

The optimum conditions obtained from microwave heating system for *S.aureus*; 323.76 watt microwave power, 37.90 min microwave treatment time. At this condition moisture content was 1.66 % and count of *S.aureus* was 2.28 log cfu/g.

For *E.coli*; 330.00 watt microwave power, 40 min microwave treatment time was the optimum condition as, 1.65 % moisture content and count of *E. coli* and mold and yeast were 2.46 log cfu/g and 1.91 log cfu/g, respectively.

Therefore, it can be concluded that reduce the number of microorganisms by use of microwave heating system could be an effective way to produce reliable, high quality pistachio products.

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