DETECTION OF GAMMA IRRADIATED SPICES WITH OSL METHOD AND ITS RELIABILITY

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DETECTION OF GAMMA IRRADIATED SPICES WITH OSL METHOD AND ITS RELIABILITY

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ABSTRACT

DETECTION OF GAMMA IRRADIATED SPICES WITH OSL METHOD AND ITS RELIABILITY

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The aim of this current work is to analyze the behavior of OSL (Optically Stimulated Luminescence) signals of irradiated spices with respect to time, temperature, origin and the type. Throughout the experiments, 3 different type spices from four different origins were stored at 4°C and 25°C for six months after irradiation.

During experiments, unirradiated red pepper, thyme and cumin samples were analyzed by using OSL technique in order to determine the background OSL signal values of samples.

Samples were irradiated 10 kGy by Cobalt 60 gamma source in TAEK (Turkey Atomic Energy Association).

After irradiation process, OSL signal values of different samples were analyzed according to the given parameters. In order to determine the effect of temperature

on OSL signal loss, temperature (4°C- 25°C) was set as storage temperature. The analyses were made monthly.

According to the statistical analyses (ANOVA- General Linear Model), origin and type of samples were detected as significant parameters of design experiment. Time and temperature effect on OSL signal loss changed with respect to origin and type of samples.

After six months storage period, OSL signal was lost for most of the origin and sample type. At the end of sixth month, an ESR analysis was performed to detect the accuracy of the OSL technique. With respect to the results of these experiments, it was seen that, due to optical fading, most of the samples was observed as unirradiated by OSL technique, however ESR analyze the samples as irradiated at the end of sixth month.

Irradiation had a detrimental effect on the microbiological load of the samples and resulted 6 log reduction on the microbial population. After irradiation, no colony formation was observed in total bacteria and yeast- mold count. During six month period, no injury recovery was observed.

Key words: Spices, Gamma irradiation, Optically Stimulated Luminescence (OSL)

ÖZ

IŞINLANMIŞ BAHARATLARIN OSL TEKNİĞİ İLE SAPTANMASI VE YÖNTEMİN GÜVENİLİRLİĞİNİN TEST EDİLMESİ

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Bu çalışmanın amacı, ışınlanmış baharatların OSL (Optik Uyarmalı Luminesans) sinyallerinin zamana, sıcaklığa, baharat türüne ve baharatın kökenine göre davranışlarını analiz etmektir. Deneyler süresince, 4 farklı kökenden, 3 baharat türü ışınlandıktan sonra 4°C ve 25°C' de 6 ay boyunca depolandı.

Deneyler sırasında, ışınlanmamış kırmızı biber, kekik ve kimyon numunelerinin taban OSL sinyal değerlerini bulmak için OSL tekniği kullanıldı. Numuneler, TAEK' te bulunan Kobalt- 60 gama kaynağı ile 10 kGy ışınlandı.

Işınlama işleminden sonra, numunelerin belirlenen parametrelere göre OSL ölçümleri alındı. Sıcaklığın OSL sinyal değerlerinin kaybı üzerindeki etkisini analiz edebilmek için, depolama sıcaklıkları 4°C ve 25°C olarak sabitlendi. Analizler aylık olarak devam ettirildi.

İstatistiksel analizlere göre (ANOVA), köken ve tür, deney düzeneği için parametre olduğu saptanmış ancak zaman ve sıcaklığın OSL sinyal kaybı üzerine etkisi köken ve baharat türüne göre değiştiği görülmüştür.

6 aylık depolama periyodu sonunda, pek çok köken ve baharat türü için OSL sinyali kaybedildi. Altıncı ayın sonunda, OSL tekniğinin kesinliğini analiz etmek amacıyla ESR analizi uygulandı. Deney sonuçlarına göre, optik solma nedeniyle OSL tekniği ile pek çok numune ışınlanmamış olarak analiz edilmektedir ancak ESR tekniği numunelerin 6. ayın sonunda da ışınlanmış olduğunu saptamaktadır.

Işınlama,mikrobiyal yük üzerinde yaklaşık 6 log azalma sağlamıştır. Işınlama işleminden sonra, toplam bakteri, küf- maya oluşumu saklama süresi boyunca gözlenmemiştir.

***Anahtar Kelimeler**: Baharat, Gama Işınlaması, Optik Uyarmalı Luminesans*

Dedicated to my mother...

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INTRODUCTION

1.1 Food Irradiation

Through more than 50 years of research, irradiation has received approval for use in different food types and food industries and has been admitted as an effective and promising food safety method. In food industry, the most important concept is safety. Approximately; 25 % of all food products are lost after harvesting due to insects, vemin and microbial spoilage (Smith and Pillai, 2004). Currently, a significant number of chemicals are used on food products for preserving food and preventing insect losses. In addition to that, some pasteurization and sterilization methods are considered in order to achieve desirable parameters in food safety. So, irradiation has the potential to significantly reduce both food production losses and food borne illnesses.

Food irradiation is applied as controlled amount of ionizing radiation (having sufficient energy to create positive and negative charges) which includes gamma rays from radioactive isotopes Cobalt-60 (Smith and Pillai, 2004). The amount of ionizing radiation (radiation energy) absorbed per unit mass is termed radiation absorbed dose and is measured in units of Grays. The level of microbial reduction is dependent on the dose absorbed by the target food.

1.1.1 Application and Aim of Food Irradiation Treatment

The purposes of irradiation process in food industry are (Eurofins, 2001);

- prevention of germination and sprouting of potatoes and onions
- killing of insects in grains or dried foods
- retardation of the ripening and aging of fruits
- prolongation of shelf life of meat and poultry
- killing of microorganism in herbs and spices

Irradiation applications are effectively done for total inactivation of microorganism or reduce the number of microorganism. In the food; during these applications, ionizing radiation directly affects the microbial DNA and cause irreversible inactivation. However; if ionizing radiation does not affect DNA directly, it can affect only enzymes or other compounds which may cause reversible damage on cell (V.C.H. Wu, 2008). The reduction of microorganism and inactivation rate depends on (Levanduski and Jaczynski, 2007):

- radiation absorbed dose (energy given to the system per unit mass)
- duration of application
- type of microorganism

1.2 The Main Concerns about Irradiation Treatments

1.2.1 Irradiation Detection

The main concern of irradiation process for the market is the conformation of irradiation for the applied foods and also loss of detection of irradiation through storage time. The level of irradiation in different food systems is approved by Food and Drug Administration (FDA) and for pasteurization; it is in the range of 1-10 kGy and for sterilization, it is above 10 kGy (Smith, Pillai, 2004). There is a list of foods that approved for irradiation process by FDA. These are wheat, wheat flour, white potatoes, pork, enzyme, fruits, vegetables, herbs, spices, poultry, meat and animal feeds (Sommers and Fan, 2006).

When food is irradiated, a dosimeter is inserted with the food to measure the amount of irradiation absorbed (Rosenthal, 1993). In addition, there are several independent irradiation detection methods being investigated for post process measurements of the irradiation application. Detection methods measure the levels of DNA damage of microorganisms or the radiation absorbance of minerals in the food materials (Chauhan et al., 2009). These applied methods are chemical, physical and biological methods (DNA Comet Assay) and the physical ones (Thermoluminescence (TL), chemiluminescence, Electron Spin Resonance (ESR), Photo Stimulated Luminescence (PSL) and gas chromatography (GC)) are preferred mostly in the food industry.

1.2.2 Marketing

Worldwide; irradiation applications has significant concerns and different point of views can be seen for different countries. United Kingdom (UK) and international experts have accepted irradiation as a safe food process and UK legislation has been placed for more than 10 years to regulate the applications and trade parameters. The Food Standards Agency, cooperate with a number of UK Local Authorities, has performed an enforcement exercise, which emphasize on the illegally irradiated foods in the UK markets. Under UK and EU law, only licensed or approved irradiation facilities may treat specific food products, for a specific purpose and within defined irradiation dose limits (Food Standards Agency, 2006). Also, food labeling regulations are required that the food on sale is labeled as "irradiated" or "treated with ionizing radiation". So by the need of this; FDA labeling requirements call for inclusion of the **RADURA** (Figure1.1), which is the symbol developed to signify a food having been irradiated (Smith and Pillai, 2004).

Figure1.1: RADURA (Symbol of Irradiated Foods)

Despite its effectiveness, irradiation is still not a major concept in today's world food processing and sterilization applications. In the past years, irradiated fruits and

vegetables and fresh and frozen uncooked poultry accounted for only 0.002% and spices and botanicals was 9.5% of annual US consumption (Smith and Pillai, 2004).

1.2.3 Consumer Acceptance

Another important problem in the irradiation process is the consumer acceptance. Over the years, acceptability rates are ranged from 45% to more than 90%, depending on the food type and method of preservation (Fox, 2002). According to the recent reports; consumers would purchase irradiated foods if the awareness and enough background information is supplied (Hayes et al., 2002).

1.3 Irradiation Treatment in Turkey

According to the Food Irradiation Regulations renewed in 2003, in Turkey, the researches on food irradiation and irradiation process are carried by Turkish Atomic Energy Authority (TAEK). The main area of this government agency is to regulate, license, apply and control the import, export, transportation and storage of irradiated products. In order to achieve this, Radiation Safety Constitution and Food Irradiation Regulations are applied.

1.3.1 Turkish Food Codex about Irradiation Application

In Turkey, the government accepts and encourages the irradiation processes by the Codex about irradiated foods and its applications. The first publishing date of this

codex is 1999, but it was renewed 2 times and the last renewal was in 2003. According to this last Codex; the principals of food irradiation are:

- The food irradiation process is applied in order to reduce the microbial load, biochemical reactions that cause the spoilage of foods, so; the shelf life of the product is increased.
- The process applications are done in the suitable technological and hygienic background.
- During irradiation applications, chemical preservations methods can not be applied to food.
- No spoiled foods can be irradiated in order to be served to the consumers.
- The dosage of the irradiation is determined by the volume of the foods by using internationally accepted dosimetric methods.
- The foods such as cereals, spices, dried foods which have low moisture content can be irradiated once more after the first irradiation if there is a case of insect infection.
- The irradiation process can be applied by Co- 60, Cs-137, X- rays and electrons.
- The irradiation institution regulations are controlled and settled by TAEK.
- All the labeling, storage and licensing conditions are regulated by TAEK.
- The necessary conditions of irradiation process are; the technological background, no health danger, suitable for the consumers.

1.3.2 Irradiated Food Ingredients in Turkey

In Turkey, the main irradiated food ingredients are spices. Their radiation dose is regulated by the "Turkish Food Codex". The upper value of radiation application dose is 10 kGy for spices. On the other hand, all spice types can not be irradiated. The leafy ones and powdered ones can be irradiated but the particulate ones are not suitable for irradiation application.

1.4 Commercial Irradiation Application

Gamma rays are the specific energies that normally come from the spontaneous decay of radionuclide. They are man- made radionuclide and unstable. The radionuclide used for the irradiation of food by gamma rays is mainly Cobalt-60. It is man- made and produced by neutron bombardment in a nuclear reactor of the metal cobalt-59, and then doubly encapsulated in stainless steel "pencils" to prevent any leakage during its use in a radiation plant. When not in use, the gamma "source" is stored in a pool of water (Sommers and Fan, 2006). In order to irradiate food or some other product, the source is pulled out of the water into a chamber with massive concrete walls. Medical products or foods to be irradiated are brought into the chamber, and are exposed to the rays for a defined period of time. After it is used, the source is returned to the water tank. The irradiation treatment is done in an irradiation room in a typical plant. The radiation source is fixed on the elevator system and when it is not used the source is localized in a water tank. When irradiation is going to be applied, the samples are put on a conveyor, and move across the source (Figure 1.2). The required time is calculated according to the power of the source and the desired absorbed dose (retrieved from web page of TAEK).

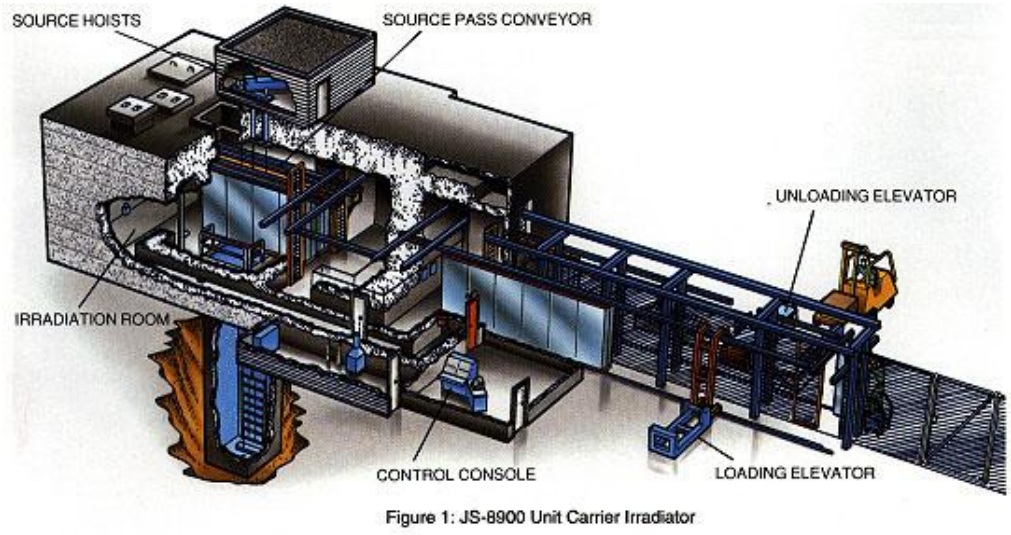

Figure1.2: Irradiation Plant Design (http://uw-food-irradiation.engr.wisc.edu/Process.html)

1.5 Detection of Irradiated Foods

There are several methods, which are valid and accurate, and these methods are used all over the world for determination of irradiated foods. The irradiation process, when applied at usual doses as equal or less than 10 kGy, involves few chemical changes on food than other treatments such as heating or freezing. So mostly, the effect of irradiation on DNA is detected by used methods (Sadecka, 2007).

The most important irradiation detection methods are electron spin resonance spectroscopy (ESR), thermoluminescence (TL), Photo Stimulated Luminescence (PSL) and DNA Comet Assay.

1.5.1 Electron Spin Resonance (ESR) Spectroscopy

ESR principals based on quantum theory and it detects the long-lived paramagnetic (presence of unpaired electrons) active sites of the free radicals produced during irradiation process in the organic and inorganic samples possessing a transition metal ion (Weil et al, 2001). Unpaired electrons are trapped at different defects (vacancies and interstitials) of the crystal lattice in free radicals and in other paramagnetic species. Anions of the crystal-forming anionic radicals with unpaired paramagnetic electrons trap the other electrons. The negative charges on the electron are spinning and constitute a circular electric current. These electrons exist in their natural state and they can change their spin either their magnetic moment is parallel or antiparallel to the magnetic field if it exists. In ESR, an external magnetic field was applied to the system in order to split the spins of electrons. The splitting spins have an energy difference of $\Delta E = g.\beta.H_0$ (H_0 is the external magnetic field,g and β are Lande Factor and Bohr Magneton respectively). If an electromagnetic energy (in microwave region) applied, an electron may change its spin state. If the energy of the microwave photon energy becomes equal to the energy difference, the system is come to the resonance and ESR signal was observed in that stage (Chauhan et al, 2009). The main advantages of this process are that; it is rapid and it has no destructive effect during repeated measurements. However its disadvantages are; decay of signals with storage time, depending on moisture and sensitivity depends on type and amount of crystalline structure (Bayram and Delince, 2003). This process has a wide range of food applicability containing bones, crystalline sugar or cellulose, herbs, spices, nut, shells and fruits.

1.5.2 Luminescence Techniques

The luminescence techniques are other methods for irradiation detection. Luminescence can arise from the thermal or optical stimulation of minerals that have been previously exposed to ionizing radiation. Irradiation creates free charges in the solid which may be captured by lattice defects acting as traps. If the matter has a crystalline structure, the excited charge carriers can remain trapped in the crystalline lattice defects. When heat or light is applied to the sample, the stored charges are released and recombined resulting in a light emission (Chauhan et al., 2009). If heat is applied to the system, mechanism is called as thermoluminescence (TL), and light is applied to the system, it is called as photo stimulated luminescence (PSL). The recorded luminescence intensity is proportional to the absorbed radiation dose.

Food is contaminated by very small quantities of silicate minerals such as quartz and feldspar for most of the time and separation of these minerals from the food materials can be important for a reliable dose measurement. For both TL and PSL techniques, signal source is the minerals in the sample. In TL measurements, due to the high temperature, organic materials in samples are burned and can cause failure of detection and also the machine. So, it is reasonable to separate inorganic minerals and organic compounds and analyze only inorganic compounds. However in PSL, there is nearly no need for separation of inorganic minerals and organic compounds because, during measurement, no heating is required, so there is no possibility of denaturing of organic compounds.

1.5.2.1 Photo stimulated luminescence (PSL)

PSL, also named as (Optically Stimulated Luminescence) OSL, is specified as a method for the detection of irradiated foods by European Standards and based on optical stimulation of mineral debris, typically silicates, bioinorganic materials such as calcite, feldspar or hydroxyapatite. The only difference between OSL and PSL is the output representation of the signal (Lee et al., 2008). Irradiation of food causes such minerals to store energy in charge carriers, when stimulated with optical energy, release the energy trapped in the charge carriers as luminescence (Sanderson, 1991).

In this technique, trapped electrons are excited with light of appropriate wavelength and intensity, and luminescence is monitored as a function of stimulation time. Observed luminescence is due to recombination of electrons with holes trapped at hole traps which act as recombination centers (Figure 1.3).

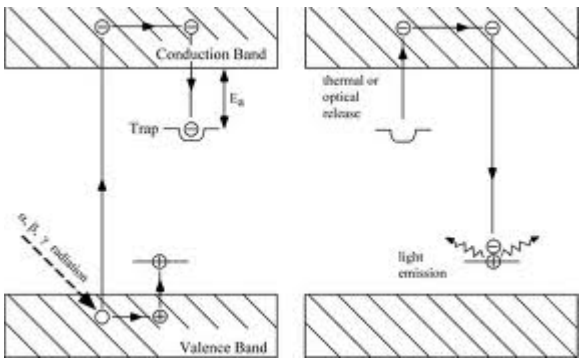

Figure1.3: Simplified Band Model for Describing Luminescence Mechansim (http://rses.anu.edu.au**)**

The amount of light detected during photo stimulation, is compared with two thresholds, which have been obtained from collaborative trials. If the food is irradiated, the signal is strong, if the signal is weak; the food is non- irradiated. If the signal is intermediate, this means that; the sample can be a mixture of irradiated and non- irradiated foods or the sample has a low sensitivity. In other words; irradiated materials with low sensitivity can give lower signal than the lower threshold (Chauhan et al., 2009). At this point, OSL sensitivity of the product becomes important. Sensitivity depends on quantity and type of minerals in the sample (Figure 1.4).

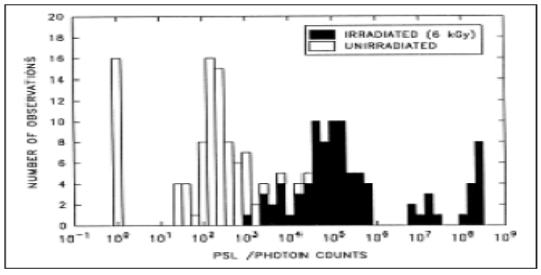

Figure1.4: Output of PSL (Sanderson et al, 1996)

In OSL detection, if irradiated samples are irradiated once more, samples show only a small increase in OSL; however unirradiated samples show a substantial increase in OSL after the first irradiation. This is due to the energy store in the carriers. In the case of irradiated foods, charged carriers are transferred into upper energy levels. When they are irradiated once more, only left electrons are transferred to the upper level, so they show little increase in OSL signal (Alberti et al., 2007).

The advantages of this system can be summarized as; it is rapid, cost effective and sample can be used more than once. However the disadvantages are; there is a risk of inaccuracy and there are decays of signals with storage time and on repeated measurements (Bortolin et al., 2007). In order to avoid false negative results for clean spices, calibrated OSL measurement should be used. In the case of spices, optimum results are obtained from unblended products.

1.5.2.2 Thermoluminescence (TL)

TL is based on the principle that light energy can be released from the trapped charge carriers present in the silicate mineral contaminants of irradiated food when the sample is heated. In TL measurements the sample is linearly heated and luminescence is recorded as a function of temperature. The plot of luminescence intensity as a function of temperature is called TL glow curve and may contain peak(s). The applied dose is related with the area under the curve (Figure 1.5). When the applied dose is increased, the area under the curve is also increased (Chauhan et al., 2009). The most important advantages of this system are that it is very specific and sensitive and no decay of signals even after years. However, during analysis, it requires isolation of silicates and the sensitivity depends on the type of silicates (Boniglia et al., 2009).

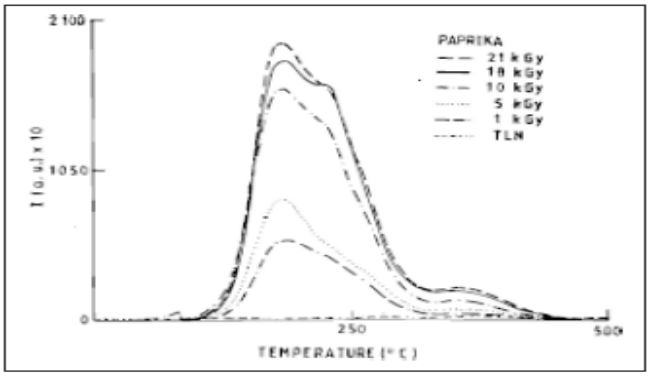

Figure1.5: TL glow curves of irradiated paprika (Correcher et al., 1998)

1.5.3 DNA Comet Assay

The basic target of radiation in the cells is the DNA. The radiation-induced DNA damage caused inactivation of microorganisms and inhibition of growth. By this way, it is reasonable to use DNA damage and its amount in the cell as an irradiation detection technique. This technique facilitates analysis of DNA leakage extracted from single cell of food materials or others and it is analyzed in agarose gel (gel electrophoresis) in order to observe tail formation (Figure 1.6). In an irradiated sample, fragmented DNA will leak out from nuclei and form a tail in the direction of anode during electrophoresis (Chauhan et al., 2009). Cells from non-irradiated samples appear as nuclei with no or only slight tails. This is an alternative method but it can be only applied to the fresh foods.

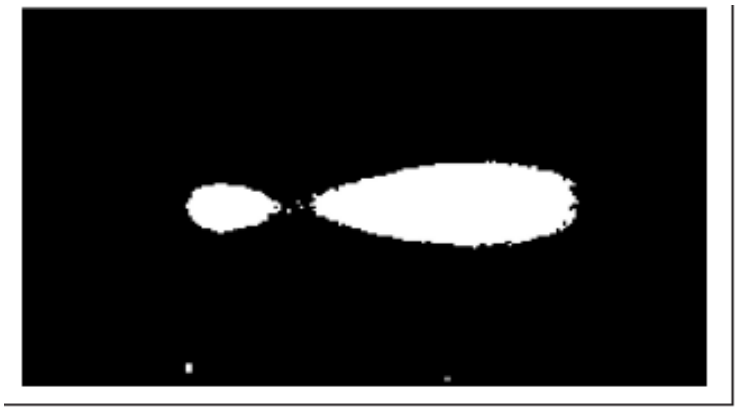

Figure1.6: Output of DNA Comed Assay (Cerda et al., 1998)

1.6 Effect of Irradiation on Microorganisms

1.6.1 Microbial Effects:

The parameters that effect the microbial reduction due to irradiation process are given below:

- Size of the microorganisms
- Age of the microorganisms
- Radiation absorbed dose
- Type of microorganisms
- Absence or presence of oxygen
- Time of exposure

Irradiation lethal dose is different for different microorganism (Table 1.1).

Table1.1: Table for effects of irradiation on different microorganisms (Sommers and Fan, 2006)

Microorganism type	Irradiation lethal dose (kGy)	Medium
B. cereus	0.14- 0.19	Beef
C. jejuni	0.18	Beef
E. coli 0157:H7	0.25	Beef
L. monocytogenes	0.51- 0.59	Beef
Salmonella	0.38- 0.5	Chicken
S. aureus	0.42	Chicken
C. Botulinum (spore)	3.56	Chicken

Spices, dried vegetables and herbs may not be suitable substrates for growth or long survival of *Salmonella* or other pathogenic microorganisms. However, if the product is held warm, 30- 50°C, pathogens may grow and cause illness. At this point, irradiation is an alternative method because of its toxicological and microbial safety and effectiveness (Wu, 2008).

The main principle of microbial reduction during irradiation process is the DNA damage. During application; DNA is broken by gamma rays or electron crush. The DNA damage can be direct or indirect. Direct damage is the hitting of ionizing radiation to the DNA and destabilizing the hydrogen bonds between molecules. The indirect damage is performed by the reaction of DNA with other adjacent molecules that are formed during irradiation procedure (Boer et al., 1983). Most of the time, DNA damage is lethal for microorganisms. However, sometimes, repair mechanism of the damage is possible. Mainly, during irradiation, gamma rays can

also degrade enzymes or structural proteins of the organisms. These may result injury after the irradiation.

1.6.2 Injury Recovery Detection

Injury is induced by sublethal heat, freezing, freeze- drying, drying, irradiation, high hydrostatic pressure (HHP), antibiotics, heavy metals, sanitizing compounds and chemicals. After those treatments;

- microorganism may be killed (non- viable)
- microorganism may survive (can grow on selective media)
- microorganism may sublethally injured (can not grow on selective media but can grow on non- selective media)

The recovered microorganism is the type of sublethally injured ones. It is important to detect injured and non- injured microorganism and to distinguish between live and dead cells to prevent false positive or false negative results.

Injuries in microorganisms occur mainly in 2 types as I1 (injury type 1) and I2 (injury type 2). I1 type injury is the structural injury. This type of injury is caused by the sublethal damage of cell wall, cytoplasmic membrane, ribosomal RNA and mostly enzymes. I2 type injury is the metabolic injury and caused by the damage of synthesis of ATP, RNA, DNA and mycopeptides (Bozoğlu et al., 2003).

Supplementing the medium with specific nutrients allows the injured cells to regain the ability to multiply. In this period, injured cells have extended lag- phase for repairing damage and synthesize proteins and nucleic acids. Generally, most injured cells repair within 48 hour at a suitable incubation temperature in a nutritionally rich non- selective medium. Also their recovery time vary with the

type of stress, microorganism species, the composition and consistency of the food and storage condition (Levanduski and Jaczynski, 2008).

1.7 Spice Samples

Spices are mostly used for importing aroma, color and taste to food products. They are also used to mask undesirable odors and flavors (Schweiggert et al., 2007). The main part of spices that import the taste is volatile oil. These volatile oils are also used in pharmaceutical industry and have antioxidant properties and some health benefits. In food industry, spices used as a preservative agent. Most of the spices are in the form of seed, fruit, leaf, stem and buds. General examples for spices used mainly in food industry are black pepper, cardamom, ginger, cinnamon, clove, cumin, paprika, thyme, vanilla and fennel.

The materials that used in this thesis project are cumin, red pepper and thyme.

Cumin: is made from the fruit of the same plant. Its origin is India, Iran, Lebanon and Turkey mainly and they are also very popular in North African, Middle Eastern, China and India. It is mostly used in foods, beverages, medicines and perfumery. As a plant, it grows in mild climates. Due to this property; in Turkey, it can be cultivated only from may to august. Its essential oil has strong antimicrobial activity against *E. coli*, *S. aureus* and *L. monocytogenes*. It has also fungicidal and larvicidal activities (Zach et al., 2008). The result of X- ray fluorescence assay of cumin show that, Aluminum is 105 mg/kg and Silica is 396 mg/kg. These are the basic properties which effect the detection of irradiation. Also its humidity in dry basis is nearly 8% (Parthasarathy et al., 2008).

Paprika and Chili: are mainly named as red pepper. They are sweet, dry and red powders and produced from any type of *Capsicum annuum*. Red peppers are

mostly non- pungent but in Hungary, Spain and Turkey, there are also some pungent types. The most important quality parameters of red peppers are humidity and color. Before drying the water content of red pepper is nearly 70% and after drying, for paprika it is nearly 6.64% and for chili, it is 5.62% (Zach et al., 2008).

Carotenoids are the most important color parameter and they give the characteristic color to the red pepper. Its color range is from yellowish red to dark red. According to the Turkish Food Codex, the important texture parameter in red peppers is granulation. Any granulation ranging between 300-500µ is accepted.

Thyme: it is originated from Mediterranean. It is mostly used in fatty cheese production and flavoring the alcoholic beverages. It has mild pungent taste and distinct odor. If they are kept too moist, leaves become blacken and lose their flavor in refrigerator over a week. Good quality dried thyme is gray green in color (Schweiggert et al., 2007). In order to protect its color and flavor, most important procedure is the packaging. The package should be airtight pack and protect from extreme heat, light and humidity.

CHAPTER 2

MATERIALS AND METHOD

Spice samples are obtained from, Acity Mısır Carsısı, Bağdat Baharat Co. and spices coming from Polatlı and Maraş. The untreated samples are stored at 25℃. The treated samples are stored at 4℃ and 25℃ in dark.

2.1 Sample Preparation for Irradiation:

The spice samples are put into small bags and covered with aluminum foil and placed in opaque boxes which are not transparent to light. The boxes are sent to the TAEK. The samples are irradiated in Sarayköy Nükleer Araştırma ve Eğitim Merkezi (SANAEM).

2.2 Irradiation treatment:

In agency; the boxes are placed in 45*45*90 cm size irradiation boxes. The boxes are loaded on horizontal conveyor and transported to the irradiation room as shown in Figure 2.1.

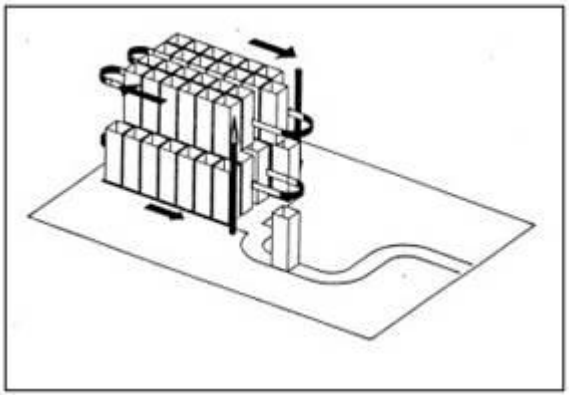

Figure2.1: Irradiation system used in TAEK

The irradiation treatment takes place in this room and samples are passing across the gamma source and absorbed the radiation. The process is continued till reaching the wanted absorbed dose for samples. After process ends, gamma source is placed back in the water.

2.3 Detection with Optically Stimulated Luminescence (OSL) Technique

2.3.1 Sample Preparation in OSL Detection:

The irradiated spice samples were kept in dark for constant period of times (1 month). For detection experiments, the samples were prepared in a red lighten room on 10 mm aluminum disks. Disks were initially covered with silicon oil in order to paste spices. A thin layer of spice was put on the disks and packed.

After samples were prepared, they were loaded into OSL equipment. Each sample was measured for 200 seconds in order to obtain reliable signals from the samples.

2.3.2 OSL Detection:

OSL detection system is composed of a stimulation light source and a sensitive photo detector. According to the type of mineral in the sample, Infra red (IR) (~ 880 nm) and blue light (~470 nm) can be used as the light source. During the analysis, spice samples were kept in a light sealed closed system which did not expose any interfering light except light from the system source.

Luminescence is detected in photon counting mode using a photomultiplier tube with a bialkali photocathode (Electron Tubes, 9532 Q) with a UV band pass filter (Hoya U-340) transmitting wavelengths between 280-380 nm. Stimulation was done with a blue light source employing a cluster of 24 blue light emitting diodes. Power density on the sample was 30 mW/cm^2.

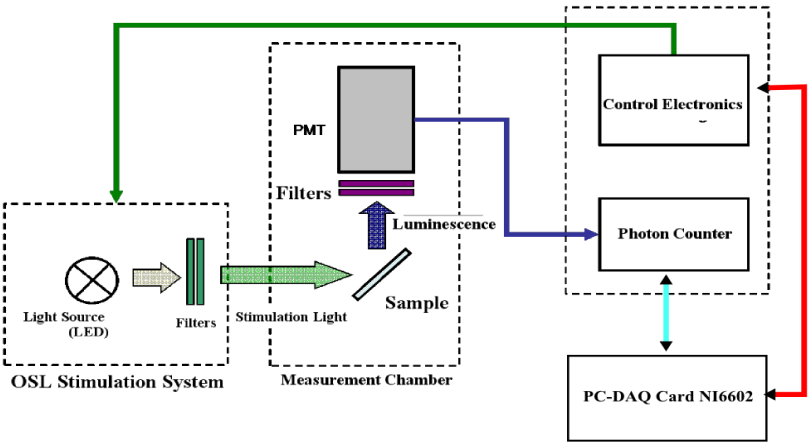

Figure 2.2: System of OSL Detector

For the stimulation in the samples, generally light emitting diodes (LED) are used. They are mostly chosen because of their long life and easier availability. In this technique, photo multiplier tubes (PMT) are used as photo detectors. The signals obtained from PMT are in the form of pulses and pulses are counted by a computer connected to the equipment with USB cable.

2.4 Analyses on Spice Samples

2.4.1 Microbiological Analysis

For the microbiological analyses of spice, yeast, mold and total bacteria count were done. Yeast and mold count were determined on Potato Dextrose Agar (PDA).

Total bacteria count were made using Plate Count Agar (PCA). For microbiological analyses of spice samples, spread plate technique was used and 1% peptone water solution was used for serial dilutions. All plates were made in duplicate for each sample and incubated at 37°C for 24 hours for total bacteria and 48 hours for yeast and molds.

2.4.2 Irradiation Detection Analysis

For the irradiation detection analysis, optically stimulated luminescence technique was used. For OSL measurement, the suitable measurement was chosen as 200 seconds (with one second intervals) in order to completely delete radiation induced signals until a background value is reached. OSL measurements are made in triplicates for each origin and type of spice samples studied.

The experiment parameters were defined as time of storage, storage temperature and sample type. The analyses were repeated monthly. The total time for long time detection was selected as 6 months due to shelf-life of samples on markets.

Effect of storage temperature on detection of the irradiation is determined by storing samples at 4°C (refrigeration temperature) and 25°C (room temperature).

During calculations of data, average values were obtained for every sample. These average values were the mean value for unirradiated samples because they have no signal formation. However for irradiated samples, intensity values were calculated by subtracted last 20 steady intervals from first 20 peak intervals which were obtained from the 200 seconds signal curves of the samples.

In order to define if there was electron transfer mechanism in the samples, UV-light application was done. Samples were exposed to UV-light for 1, 2, 4, 8, 10, 20, 40, 80 minutes and checked if there was an increase in the signal during UV exposure.

2.4.3 Electron Spin Resonance Applications (ESR)

ESR method was used in order to prevent false positive results due to non-homogenous amount of dust (inorganic materials) on the spices. In ESR analyses; 200 mg samples were weighted and put into quartz tubes. Prepared samples were analyzed in ESR spectrometer (Bruker EMX 106) which was set for central intensity field of 300 mT (millitelsa). The measurement was done in the range of 20 mT in order to see the cellulose peaks (60 Gauss (6 mT)) intensity difference accurately. During measurements, 0.791 mW microwave power with frequency of 9.804 GHz was used.

2.4.4 Humidity Analysis

Humidity analyses were performed at 100°C oven and samples were weighted for 2 hours interval until the constant weight was achieved.

2.5 Statistical Analyses of Results

Results of the experiments were analyzed by ANOVA (Analyses of Variance). Effects of spice type, origin, time and temperature on irradiation detection were the parameters. For microbiological analyses, ANOVA was also used for the effect of

origin, time and type of spices on total aerobic microorganism, yeast and mold. Significance differences between means and ANOVA testing were done by Minitab 15.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Physical Experiments

3.1.1 Effect of Origin and Type on Background OSL Values of Samples

Three different samples (red pepper, cumin, thyme) from four different origins for each sample were selected for experiments. The aim of different origin selection was to obtain a reliable data about the behavior of OSL signals after irradiation and minimize the error that may come from origin as a parameter. For this reason; background OSL signals of the unirradiated samples were analyzed before irradiation. Background OSL value is important in order to detect if the sample is irradiated or not. Mostly, background value is the lowest value of OSL and if the samples have lost their signals during storage or optical fading, the counts observed during experiments are decreased till the background value. Background value is mostly affected by origin and type of samples. Different originated samples may have different background value due to different dust type and amount during cultivation. In irradiated Acity red pepper samples, there is a signal starting from 450000 c/s, on the other hand, unirradiated samples do not show any signal (Figure 3.1). Graphs related to unirradiated and irradiated samples from all types and all origins are given in Appendices A and B.

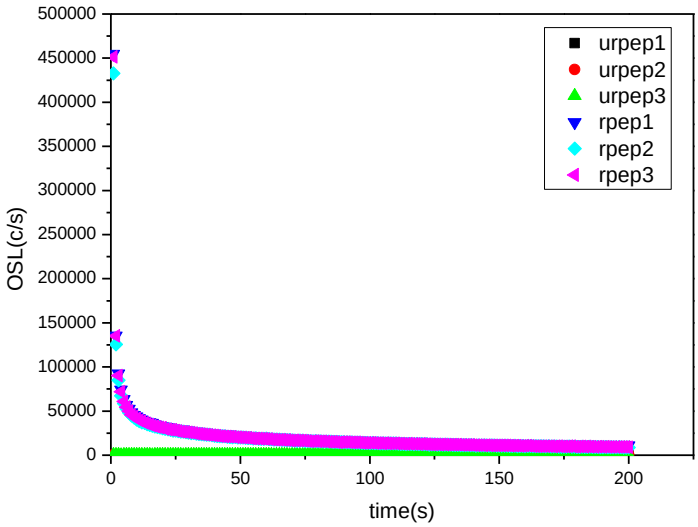

Figure 3.1 Signals obtained for irradiated and unirradiated Acity Red Pepper (urpep: unirradiated samples, rpep: irradiated samples**)**

The results of average background values for all unirradiated samples and origins are presented in Figures 3.2, 3.3 and 3.4. There were background deviations of results between all origins. Deviations in red pepper samples were higher than other samples. This shows that type of sample and origin has a significant effect on background OSL values of the samples.

The reasons for such kind of a deviation may be explained as:

- non-homogenous nature of spices
- different humidity values of different samples
- type and amount of dust in the nature of spices

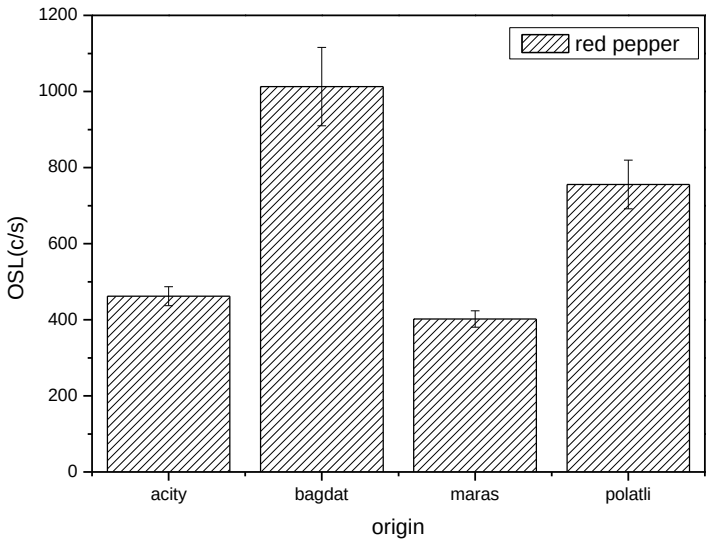

Figure3.2 Background OSL values of Red Pepper Samples for all origins

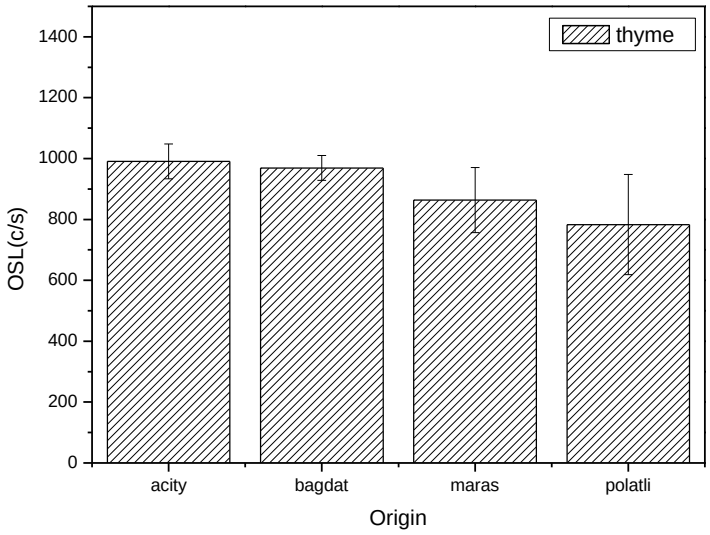

Figure3.3 Background OSL values of Thyme Samples for all origins

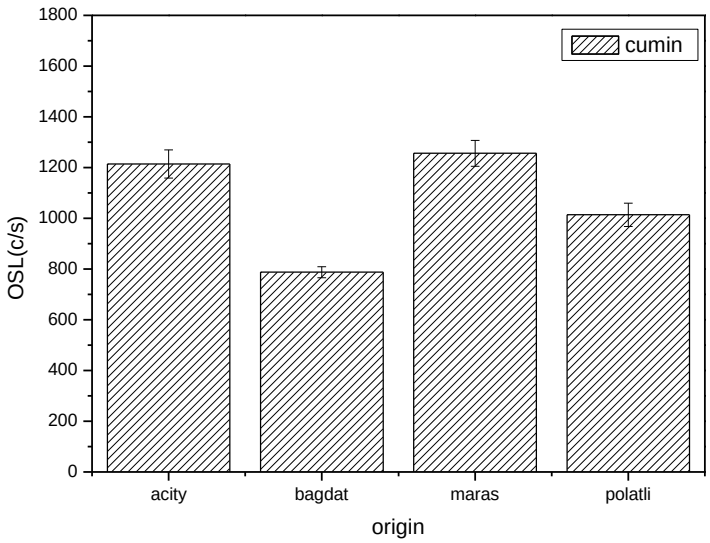

Figure 3.4 Background OSL values of Cumin Samples for all origins

Table 3.1 Average and Standard Deviation Values of OSL for all Origin and Sample Types

	Bagdat Spices	Acity Samples	Maras Sample	Polatlı Sample	Average	Standard Deviation	Interval
Red pepper	1013	462	402	755	658	282	376-940
Thyme	969	991	864	783	902	97	805-999
Cumin	788	1214	1257	1014	1068	215	853-1283

3.1.2 Effect of Humidity

Humidity is an important factor for irradiation detection due to hydrolization of water into H^+ and OH^- ions and forming of free radicals. During irradiation application, electrons are transferred between conduction and valence bands and trapped in the defects of mineral debris of the sample. During detection, these electrons trapped in the defects are monitored. If the sample has higher humidity values than normal, hydrolyzed OH^- ions can also bind instead of electrons during irradiation and during detection, by the energy given to the system as light or thermal, trapped electrons also changes their position. This phenomenon will cause a decrease in the observed levels of irradiation (Kitai and Furuta, 2009). For these reason initial humidity levels of the samples are determined (Table 3.2).

Table 3.2 Humidity values for all origins and sample types before and after irradiation

Origin	Sample Type	Before	After
Acity	Red pepper	10.0± 0.6	8.2± 0.4
	Thyme	8.0± 1.2	6.7± 0.8
	Cumin	8.0± 1.8	4.8± 0.5
Bagdat	Red pepper	10± 1.1	8.2± 1.1
	Thyme	8.8± 3.8	8.6± 0.9
	Cumin	6.7± 0.6	5.2± 0.9
Maras	Red pepper	6.8± 1.5	4.7± 0.6
	Thyme	9.9± 0.4	5.7± 0.3
	Cumin	3.0± 0.2	5.0± 1.4

Table 3.2 Continued

Polatlı	Red pepper	8.7± 1.8	8.5± 0.6
	Thyme	6.9± 2.0	10.2± 0.9
	Cumin	4.0± 0.1	8.3± 1.1

All the spice samples are subjected to the humidity limitations (Turkish Food Codex). Maximum humidity values according to the codex are 11 for red pepper, 12 for thyme and 10 for cumin.

From the statistical analysis, humidity values of spices from Maras are out of the confidence interval (Table 3.2). This change may be due to hydrolization of water into H^+ and OH^- ions. Gamma irradiation may also weaken the intermolecular bonds between water and other molecules in the spice and thereby enhancing increase in water uptake (Sharif and Farkas, 2009). These changes may affect the consistency of the irradiation detection data. If there is a deviation in the results of OSL detection, humidity can be one of the reasons for such kind of a situation.

Table 3.3 Statistical analyses for humidity values of samples from all origins before and after Irradiation

	Before		After	
	Average	Standard Deviation	Average	Standard Deviation
Red pepper	8.8	1.5	7.4	1.8
Thyme	8.5	1.3	7.8	2.1
Cumin	5.5	2.3	5.8	1.7

3.1.3 OSL Values Measurements after Irradiation

After irradiation (10 kGy), the samples were analyzed for OSL value detection. In order to prevent the optical fading, this means losing OSL signals due to light, samples were stored in dark room (Alvarez et al., 1999). Before starting experiments, it was expected to observe a sharp increase on OSL intensities of samples after irradiation. However, just after irradiation, samples did not give the expected OSL values. Only red peppers taken from Acity showed significant difference than its background values.

For irradiated red pepper samples, the highest OSL value is observed for samples taken from Acity. Also samples taken from Maraş and Bağdat Spices showed slight increase of OSL signal values (Figure 3.5).

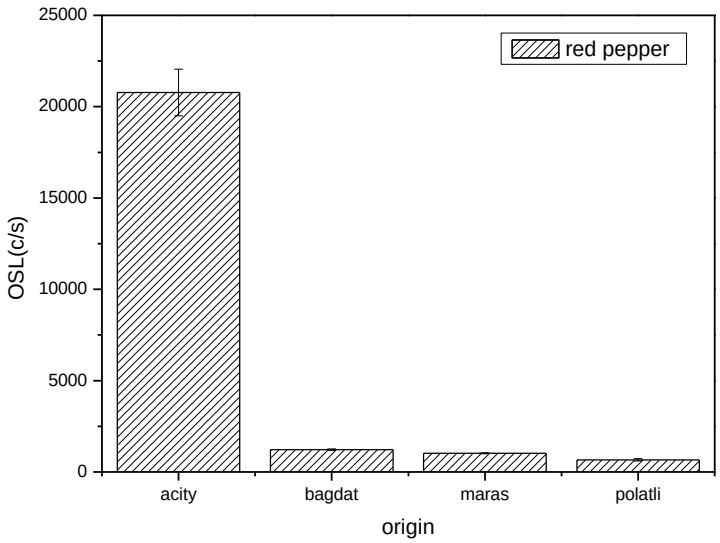

Figure 3.5 Average OSL Values for Irradiated Red Pepper Samples from All Origins

The reasons of high OSL values of Acity samples after irradiation can be due to the amount of dust in the sample and also the amount of traps in the mineral debris. This situation can cause high amount of electron transfer and as a result, cause high luminescence formation. After irradiation process, there was a decrease in the value of OSL signal of other three red pepper samples. The reason for such observations may be due to the presence of shallow traps (not deep traps). The captured electrons in such traps can be lost at room temperature with little optical effect (Alberti et. al, 2007).

The increase of OSL levels for all samples of irradiated thymes were approximately in the same range (Figure 3.6).

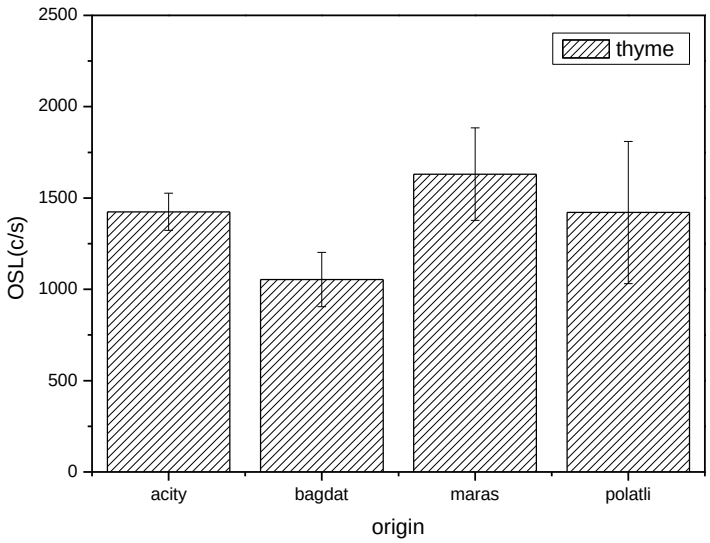

Figure 3.6 Average OSL Values for Irradiated Thyme Samples from All Origins

Cumin from the Acity is the only sample that resulted OSL signal for the irradiation. OSL levels of other cumin samples are from background signals. (Figure 3.7).

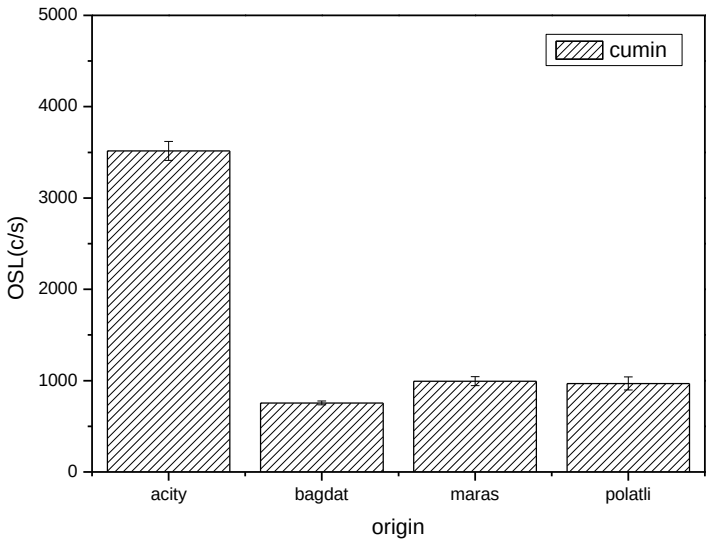

Figure 3.7 Average OSL Values for Irradiated Cumin Samples from All Origins

3.1.4 OSL Value Change with respect to Time and Temperature

Different spice types and origins show different responses to the irradiation process, so same amount of increase or decrease in the OSL values can not be observed after irradiation application . This shows that spice type and origin are the key parameters for irradiation detection. In order to determine whether time is a parameter for OSL detection level, monthly analyses were done for each origin and sample type. In addition to time dependency, in order to analyze the effect of storage temperature on OSL detection, samples were stored at 4°C as refrigeration temperature and 25°C as room temperature. With this approach the presence of shallow traps were also analyzed (if there are shallow traps in samples, electrons in these traps may be lost at room temperature with time) (Alberti et. al, 2007).

Irradiated spice samples were stored at 4°C and 25°C and analyzed monthly in order to see the OSL value change with respect to time and temperature. The OSL data was obtained for four different origins and three different sample types.

In the case of red pepper samples of Acity, the first month of analyses of 4°C samples show an increase of OSL signals compared to the just irradiated signals. This behaviour may be due to the expected electron transfer mechanism. This means that, electrons in deep traps, transfer to the shallow traps during storage period and give luminescence signal during analyses. In 25°C samples, there is no significant change observed for first month data for the same sample. After the first month, OSL data is set into equilibrium. At the end of sixth month, the observed OSL signal is still higher than background (unirradiated) OSL value, which means that samples are already detectable by OSL method **(**Figure 3.8).

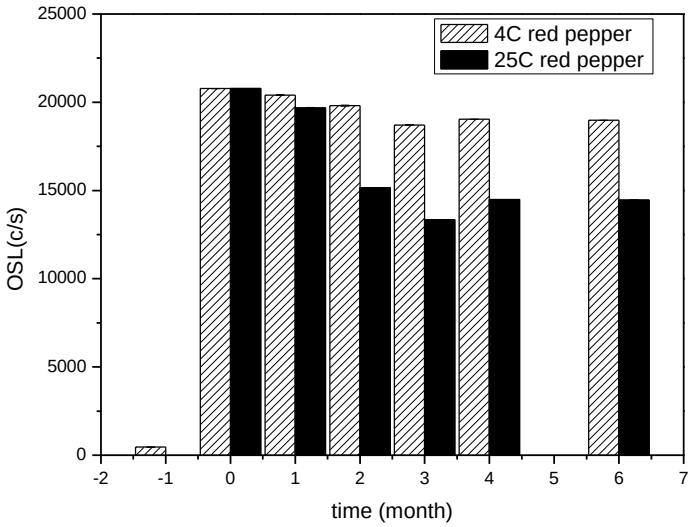

Figure 3.8 OSL Value Analyses of Acity Red Pepper Between Background and Six Month Storage (-1 refers to unirradiated samples, 0 as just irradiated samples and 1, 2, 3, 4 and 6 refer to the analysed months)

OSL signals of Acity thyme were more conserved at 4^{o}C when compared to the samples kept at 25^{o}C during the six month of storage. For 25^{o}C the detection of OSL is statistically lost after the 3th month of storage (Figure 3.9).

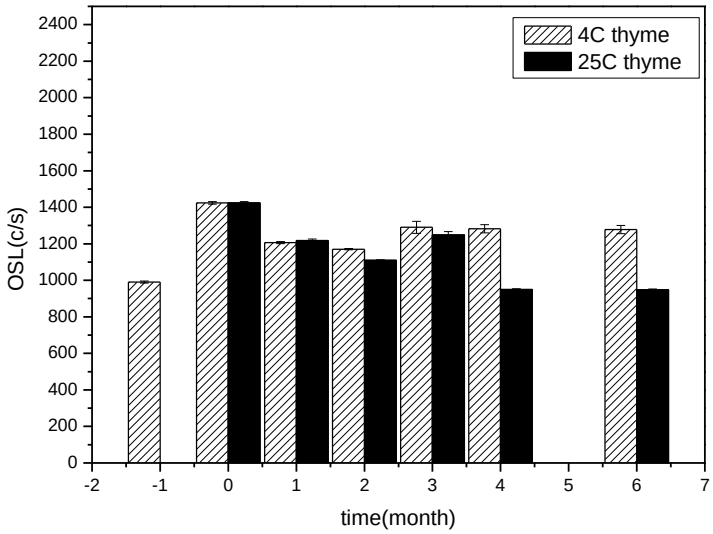

Figure 3.9 OSL Value Analyses of Acity Thyme Between Background and Six Month Interval -1 refers to unirradiated samples, 0 as just irradiated samples and 1, 2, 3, 4 and 6 refer to the analysed months

Although there were some deviations in measured OSL signal intensities for Acity cumin during storage at both temperatures, the OSL signals were strong even to the end of the six mounth storage (Figure 3.10).

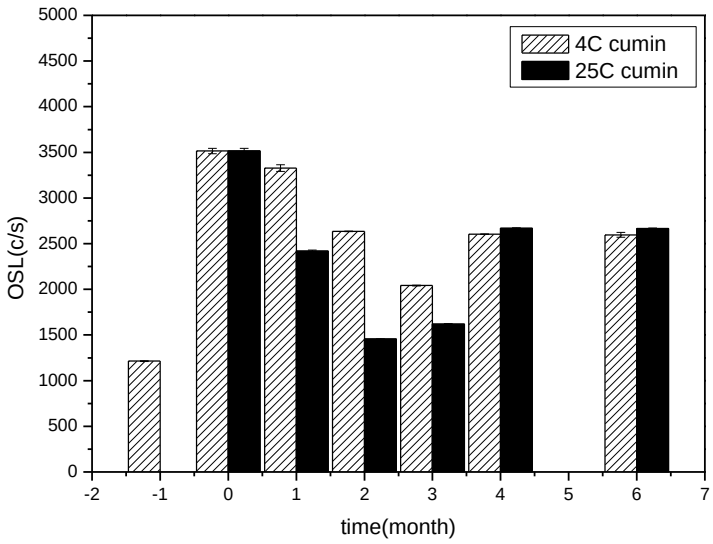

Figure 3.10 OSL Value Analyses of Acity Cumin Between Background and Six Month Interval -1 refers to unirradiated samples, 0 as just irradiated samples and 1, 2, 3, 4 and 6 refer to the analysed months

For red peppers from Bağdat the OSL signals fall below the ground level after the first month for both storage temperatures resulting impractable measurements for the detection of irradiation after these mounths. (Figure 3.11).

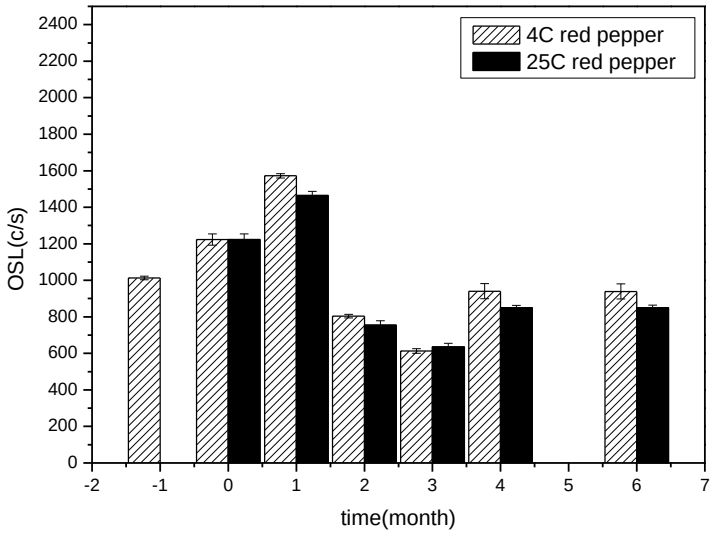

Figure 3.11 OSL Value Analyses of Bağdat Red Pepper Between Background and Six Month Interval -1 refers to unirradiated samples, 0 as just irradiated samples and 1, 2, 3, 4 and 6 refer to the analysed months

In the case of thyme samples from Bağdat Spices; observed OSL signal fall below the background value after the first month for 25°C stored samples, however samples stored at 4°C lost their signals just after irradiation application. For both storage temperatures the detection of irradiation after these mounths were impractical (Figure 3.12).

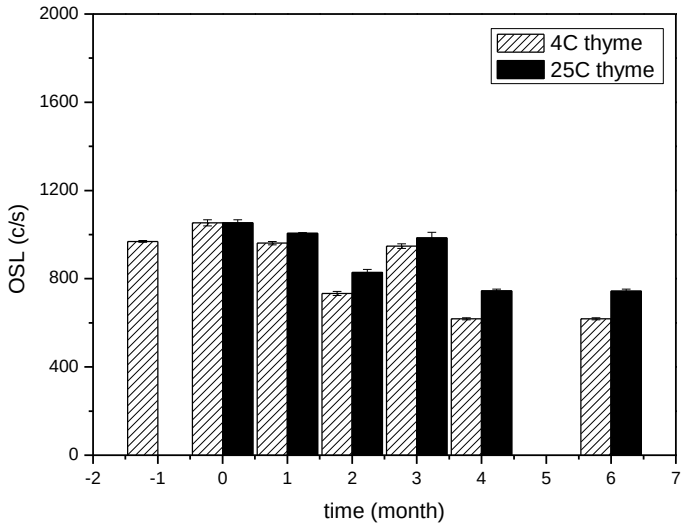

Figure 3.12 OSL Value Analyses of Bağdat Thyme Between Background and Six Month Interval -1 refers to unirradiated samples, 0 as just irradiated samples and 1, 2, 3, 4 and 6 refer to the analysed months

For cumin from Bağdat Spices, no significant difference on OSL signal before and after irradiation application were detected. Therefore temperature and shelflife studies were not carried for this sample.

For Maraş red pepper samples, there is a significant increase on the OSL signal upon irradiation. Samples stored at 4°C have higher detectable signals than the samples stored at 25°C. At the end of six month, samples still had observable signals for both temperature (Figure 3.13).

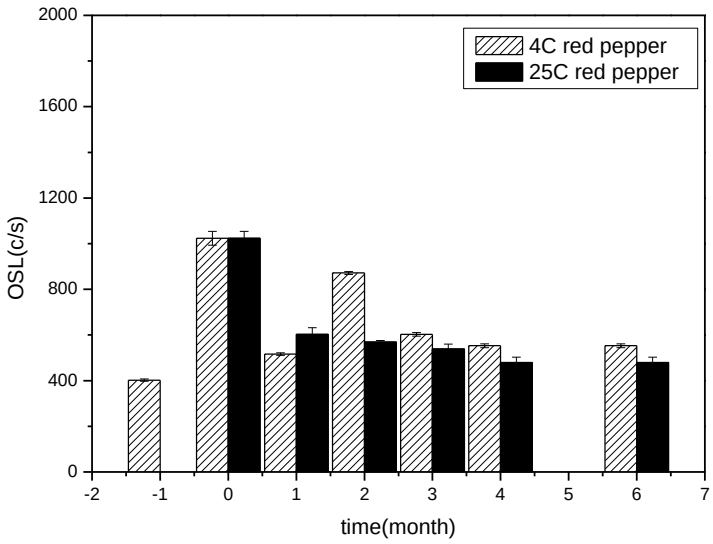

Figure 3.13 OSL Value Analyses of Maraş Red Pepper Between Background and Six Month Interval -1 refers to unirradiated samples, 0 as just irradiated samples and 1, 2, 3, 4 and 6 refer to the analysed months

For thyme samples of Maraş the OSL signals were detectable for both storage temperatures during the six month storage (Figure 3.14).

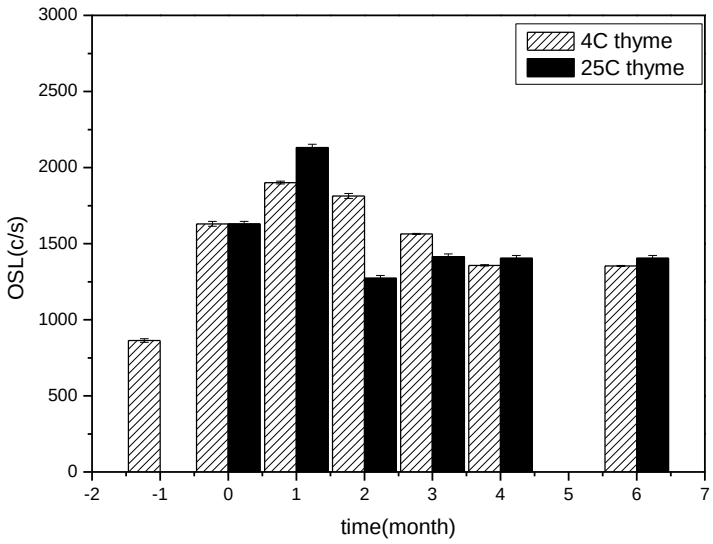

Figure 3.14 OSL Value Analyses of Maraş Thyme Between Background and Six Month Interval -1 refers to unirradiated samples, 0 as just irradiated samples and 1, 2, 3, 4 and 6 refer to the analysed months

Since no significant difference for OSL signals for the irradiated and unirradiated samples of cumin from Maraş was observed, studies for the storage temperature and time were omitted for this sample.

For the thyme samples from Polatlı, though OSL signal showed a steady decrease, signals were detectable until the end of the forth mounth but droped back to the ground level intensity at the end of the sixth month (Figure 3.15).

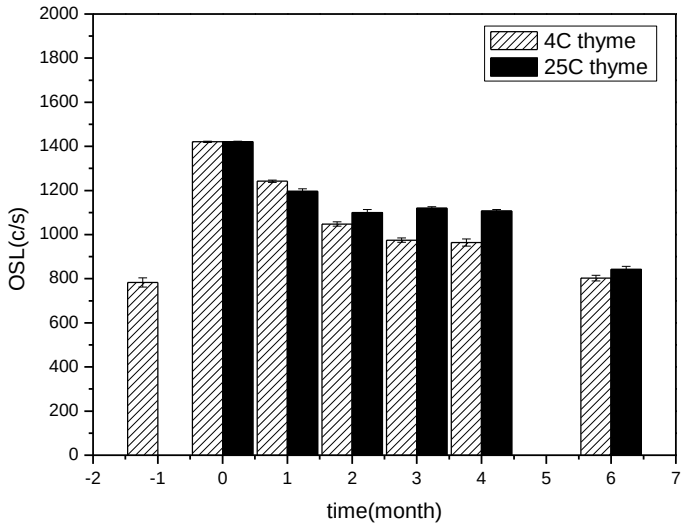

Figure 3.15 OSL Value Analyses of Polatlı Thyme Between Background and Six Month Interval -1 refers to unirradiated samples, 0 as just irradiated samples and 1, 2, 3, 4 and 6 refer to the analysed months

Since cumin and red pepper from Polatlı did not result significant difference for OSL signal after irradiation application compared to those unirratiated samples, temperature effect and storage time experiments are not done for those samples.

3.1.5 Comparison of ESR and OSL Results

ESR detection was done for only red pepper samples of Acity, Bagdat Spices, Maras and Polatlı at the sixth month in order to compare the obtained OSL results with ESR results at the end of the experimental duration. Also the reason of making ESR for only sixth month is that, no detection observation for Bagdat Spices,

Maras and Polatlı at the end of sixth month. By this way, significant difference between Acity samples and the others can be explained clearly.

In ESR analyses, all the samples show a similar cellulose peak (Figure 3.16). This means that, they were irradiated homogeniously with the same amount of Gamma Ray. The cellulose peak is clearly observed and this means that, detection with ESR is more appropriate after six month. At this step, it can be easily understood that, there is enormous effect of light, so optical fading, on irradiation detection by using OSL system.

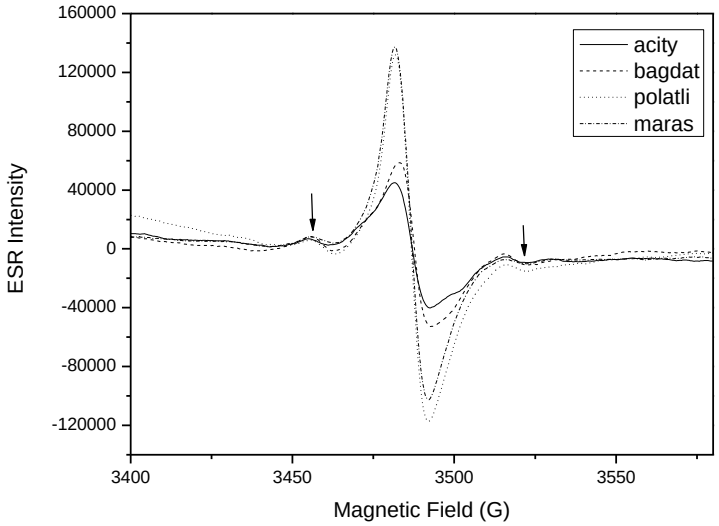

Figure 3.16 Results of ESR measurements at the end of six month for red peppers from all origins (Arrows represent radiation induced cellulose peaks and magnetic field difference between peeaks is nearly 60 Gauss)

In addition to these, the significant difference on OSL signals of Acity samples from others can be explained as the difference of amount of dust and also the structural difference of dust.

In order to see the effect of irradiation on cellulose peak, unirradiated Acity samples were irradiated at 5 kGy. They were compared and the signal difference between cellulose peaks is determined. In unirradiated samples, no cellulose peak formation was observed however, in irradiated samples, there was a peak formation in the 60 Gauss range as mentioned before (Figure 3.17).

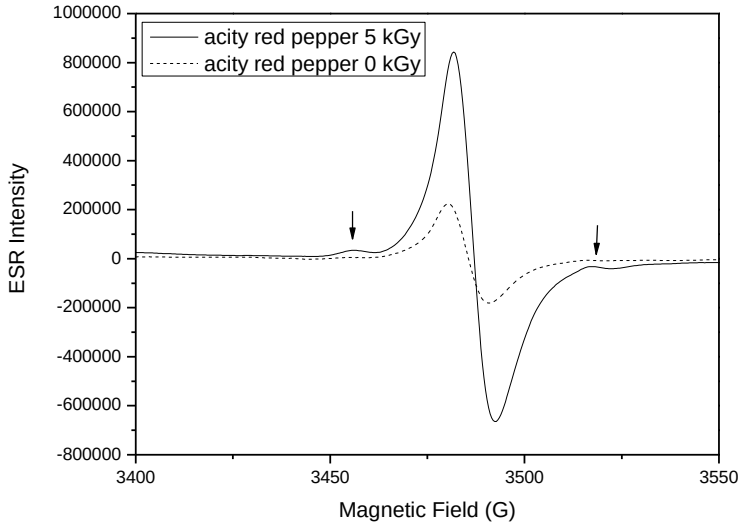

Figure 3.17 Results of ESR measurements for Acity red peppers unirradiated and irradiated 5kGy (Arrows represent radiation induced cellulose peaks and magnetic field difference between peeaks is nearly 60 Gauss)

3.1.6 Existence of Electron Transfer Mechanism

According to the results of the experiments, it has expected to observe electron transfer mechanism because during measurements, some samples show increase of OSL signals with respect to time. Electron transfer mechanism can be explained as transfer of electrons from deep traps to the shallow traps during storage and give luminescence signal during analyses.

In order to see this effect, samples were exposed to UV- light. The aim of this experiment is to accelerate the electron transfer mechanism, by this way, this mechanism can be seen easily.

In order to see the effect of UV- light on irradiation detection of samples, UV light was used. Time interval was selected as 1, 2, 4, 10, 20, 40, 80 minutes in order to observe long term behavior of OSL signal.

It was expected to see an increase of OSL signal in this experimental duration because it was assumed as there may be electron transfer mechanism. However, the increase was so rapid that we can not observe. The signal was decreased sharply after 1 minute UV exposure, then after 20 minutes, it settled to the steady state and did not decrease any more (Figure 3.18).

This shows that there is an electron transfer based on the phototransfer of electrons from deep traps to traps responsible from OSL signal. The decrease is due to emptying of deep traps. In unirradiated sample no OSL signal upon UV exposure was observed. Such a mechanism can be used as a second trial for detecting irradiation. Accidental exposures to visible light may delete the OSL signal, however signal may be regained by exposing the sample to UV light.

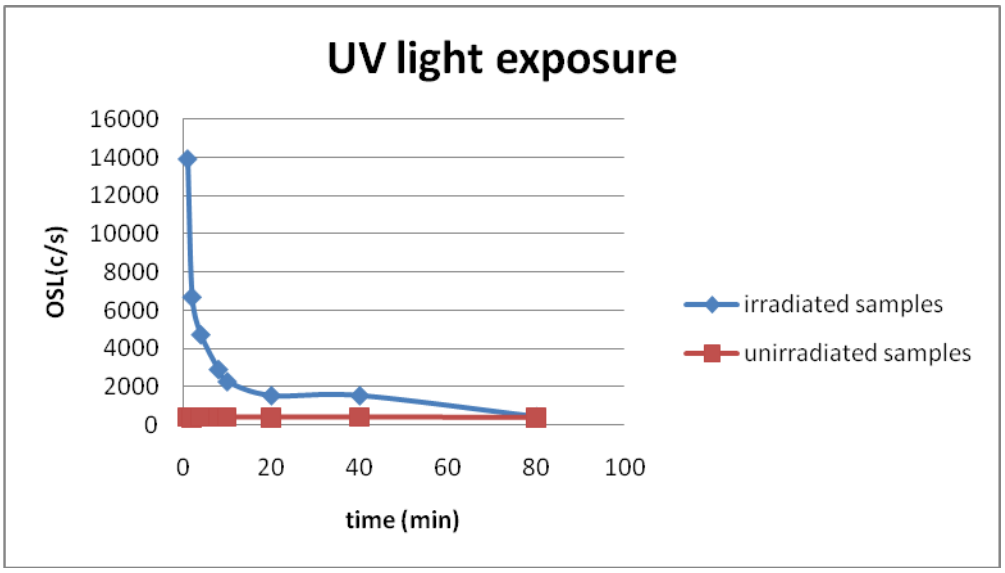

Figure 3.18 Behavior of Irradiated Acity Red Pepper Samples with UV- light Exposure

3.2 Microbiological Experiments

During experiments only total bacteria (aerobic mesophilic), total yeast and mold counts were done. Irradiation had a detrimental effect on the microbiological load of the samples. The dose given has resulted at least 6 log reduction on the microbial population and this inhibition has not resulted any growth during the storage period indicating no repairable injury under these conditions for all contaminating microflora for all spices used in the experiments.

Results of the inhibition and possible repair studies for the spices from Acity are presented in the Figures 3.19- 3.20- 3.21. Graphical results for other spices are given in Appendix C.

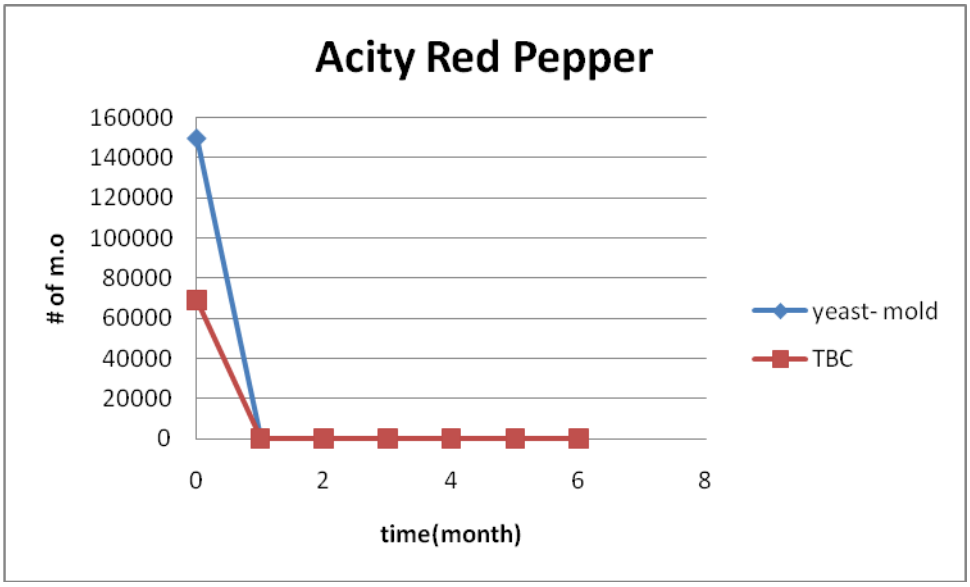

Figure 3.19 Microbial Analyses of Acity Red Pepper Samples

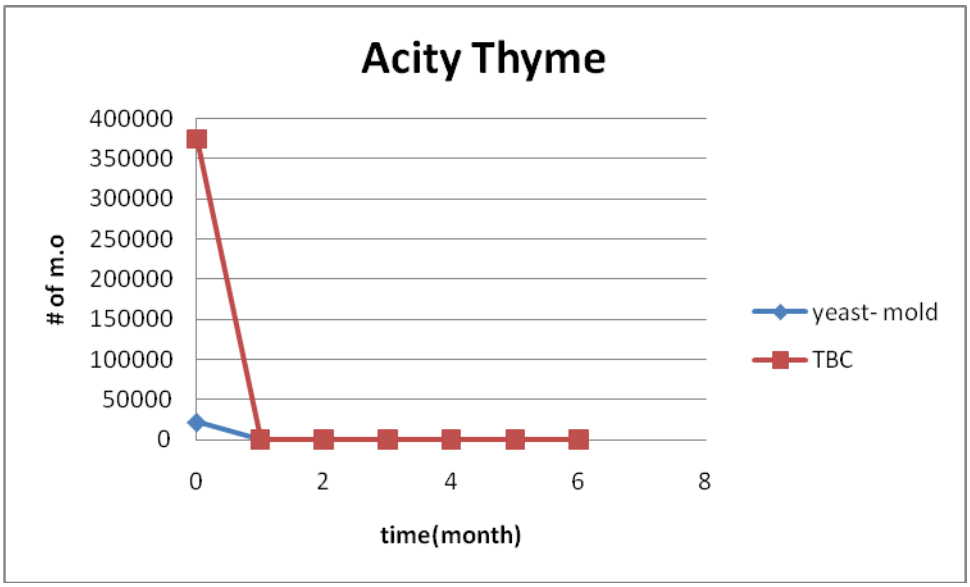

Figure 3.20 Microbial Analyses of Acity Thyme Samples

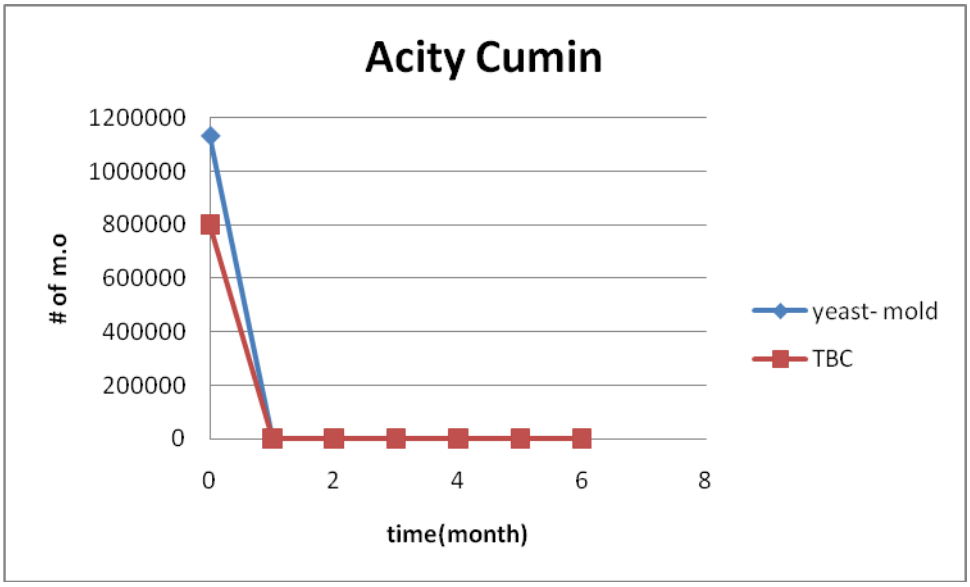

Figure 3.21 Microbial Analyses of Acity Cumin Samples

3.3 Statistical Analysis

At the beginning of the analyses, OSL was chosen as the response parameter in order to analyze the detection loss. The parameters that has effect on OSL were also chosen as time, temperature, origin of spices and type of spices.

In order to determine the effect of time, monthly OSL analyses was performed. The experiments was continued for 6 month beause according to the market survey from Bagdat Coop. Spices, the possible time for spices that stay on shelfs is 4 month.

In order to detect the effect of temperature, storage temperatures were set as 4°C and 25°C.

At the end of the experiments, 4 way ANOVA (General Linear Model) was performed in order to analyze the parameters. Data was obtained as OSL versus spice types, origin, time and temperature.

Spice types and origin can be taken as parameters of OSL detection. However, the selected time interval and selected temperatures can not be seen as parameters of OSL detection. These results can be understood from p- values (Table 3.4). The confidence interval was selected as 95%, so p values lower than 0.05, can be considered as significantly different and can be selected as true parameters of the system.

Table 3.4 P- values Obtained from ANOVA

Parameters	**P- values**
Spice type	0.000
Origin	0.000
Time	0.718
Temperature	0.335

According to the results of the Tukeys Test (95%) given in Appendix D, red pepper is significantly different than cumin and thyme as OSL response. This can be due to the structure of the red pepper. It can have more free electrons than other samples which can cause higher OSL signals after irradiation applications.

In origin point of view, Acity samples are significantly different from Bagdat Spices, Maras and Polatlı samples. This may be most probably due to the amount and type of dust on the samples.

According to the Tukeys' Test, time and temperature were not parameters of OSL detection. Because their p- values are much higher than 0.05 (Appendix D).

CHAPTER 4

CONCLUSION

The aim of this current work is to analyze the behavior of OSL signals of irradiated spices with respect to time, temperature, origin and the type. Throughout the experiments, 3 different type spices from four different origins were stored at 4°C and 25°C for six months after irradiation. During this period OSL signals were observed for all samples (Table 4.1). In the given table, (+) represents observable and (-) represents not observable.

The results of the studies show that OSL can be used as an alternative technique in the laboratories or customs for determination of the irradiation application of spices because it is rapid, cost effective and since samples are not affected by the system they can be stored and analyzed again without loosing their OSL levels. Main drawback experienced in these studies is the inconvenience of the system to be applicable to all type of spices.

At the end of experimental duration, ESR and OSL results were compared for the sixth month. According to the results of ESR, red pepper samples from all origins show a typical curve formation and they can be considered as irradiated at the end of experimental duration. As a result of this comparison, it was clearly observed that optical fading was an important parameter for OSL results; however ESR results were only affected by storage time and humidity mainly.

Irradiation is a safe and confidential method for food pasteurization. After irradiation, no injury recovery in other words, no growth of microorganism had been observed in a six month period for all spices studied as expected. Since it is a nonthermal technique, while rendering the product safe it also decreases the loss of

vitamins and other quality parameters (color, flavor, taste) due to thermal treatment.

In statistical analysis, origin and spice types were determined as significant parameters of OSL detection of irradiated samples. However time and temperature were not significant on OSL signal detection during storage.

Table 4.1 **Results of Time Dependent Experiments**

Origin	Sample Type	Time (month)											
		0		1		2		3		4		6	
		4°C	25°C	4°C	25°C	4°C	25°C	4°C	25°C	4°C	25°C	4°C	25°C
Acity	R. Pep.	+	+	+	+	+	+	+	+	+	+	+	+
	Thyme	+	+	+	+	+	+	+	+	+	+	+	-
	Cumin	+	+	+	+	+	+	+	+	+	+	+	+
Bagdat	R. Pep.	+	+	+	+	-	-	-	-	-	-	-	-
	Thyme	+	+	-	+	-	-	-	-	-	-	-	-
	Cumin	-	-	-	-	-	-	-	-	-	-	-	-
Maras	R. Pep.	+	+	+	+	+	+	+	+	+	+	+	+
	Thyme	+	+	+	+	+	+	+	+	+	+	+	+
	Cumin	-	-	-	-	-	-	-	-	-	-	-	-
Polatlı	R. Pep.	-	-	-	-	-	-	-	-	-	-	-	-
	Thyme	+	+	+	+	+	+	+	+	+	+	-	-
	Cumin	-	-	-	-	-	-	-	-	-	-	-	-

CHAPTER 5

RECOMMENDATION

The results indicate that detection of OSL solely depends on the dust content (inorganic compound) and dust type that spices contain. It is advisable to concentrate on the type of the inorganic compounds that highly relects the application of irradiation that would be easily detected by OSL. Since the development of simple OSL systems, it can be easily used in customs and laboratories in detection of irradiated samples.

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APPENDIX A

RESULTS OF THE UNIRRADIATED SAMPLES

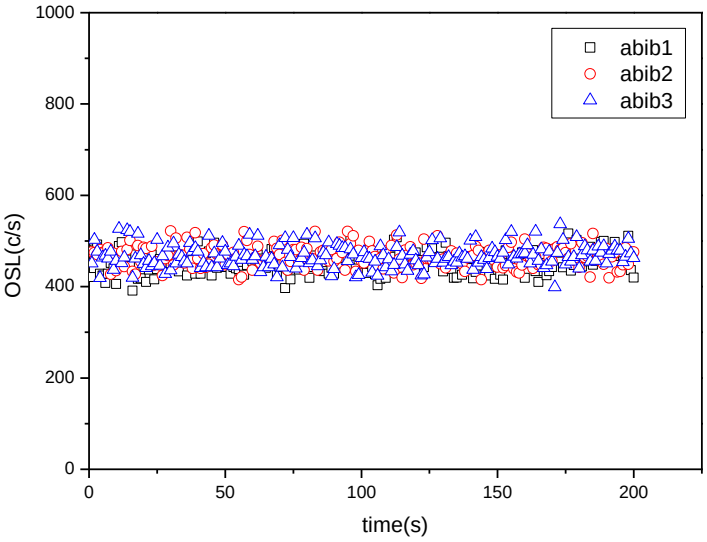


Figure A1: Unirradiated Acity red pepper

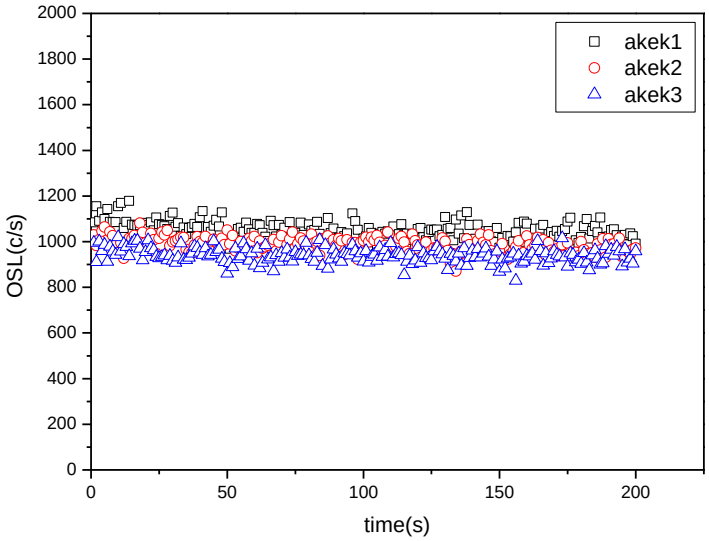


Figure A2: Unirradiated Acity thyme

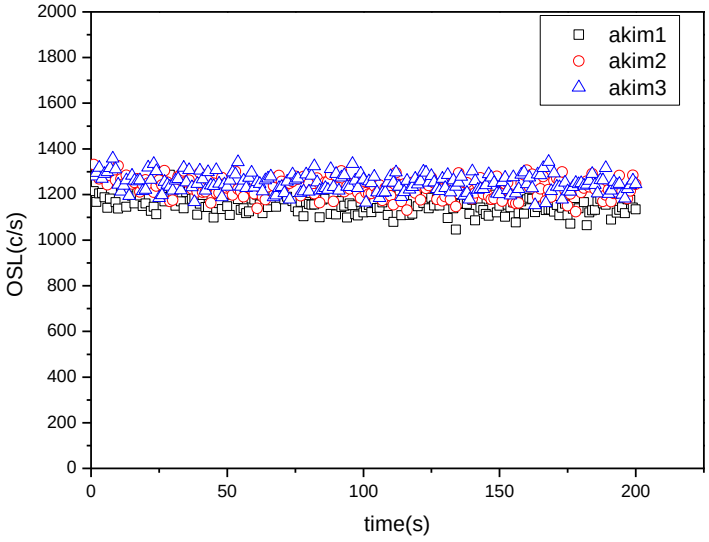


Figure A3: Unirradiated Acity cumin

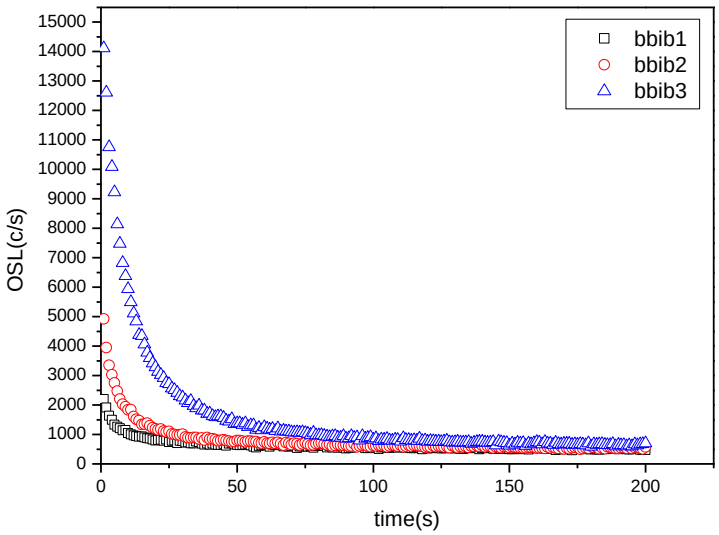


Figure A4: Unirradiated Bagdat red pepper

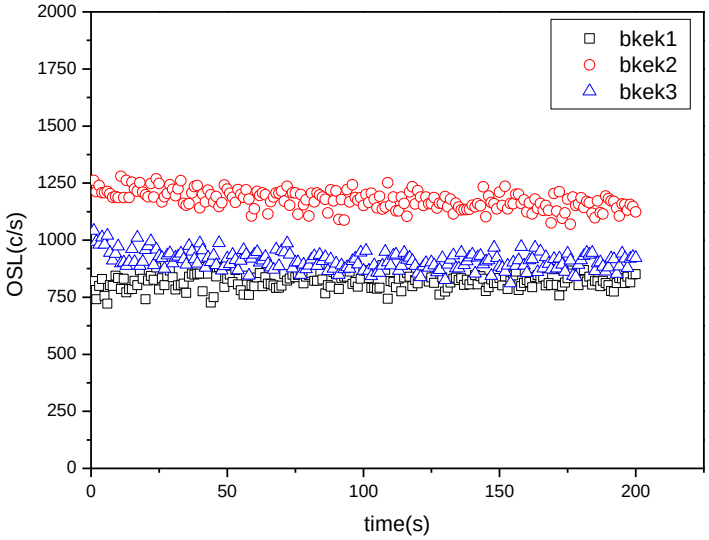


Figure A5: Unirradiated Bagdat thyme

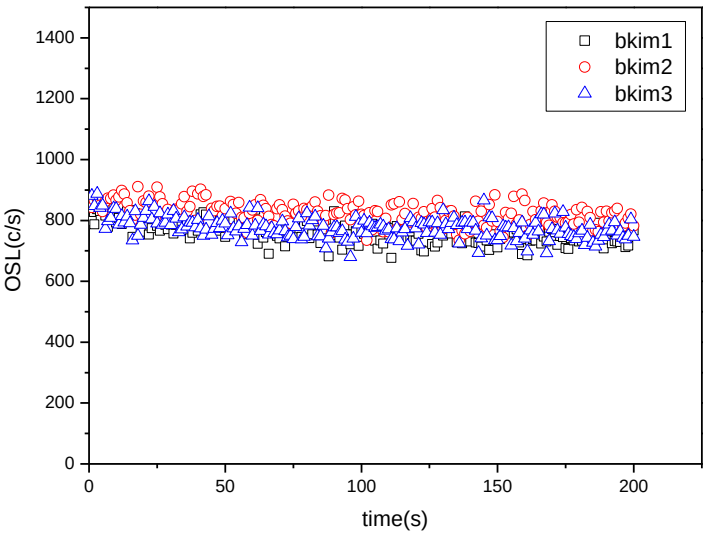


Figure A6: Unirradiated Bagdat cumin

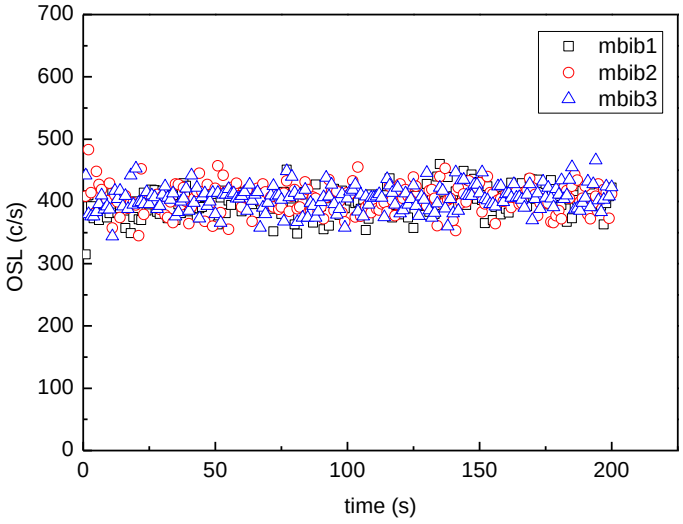


Figure A7: Unirradiated Maras red pepper

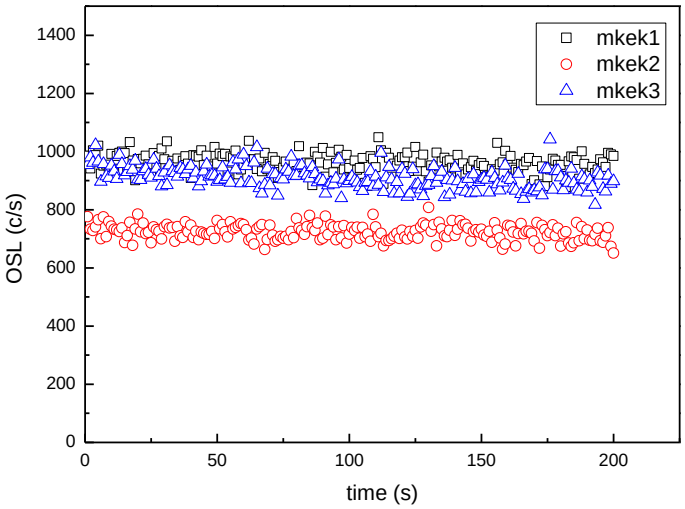
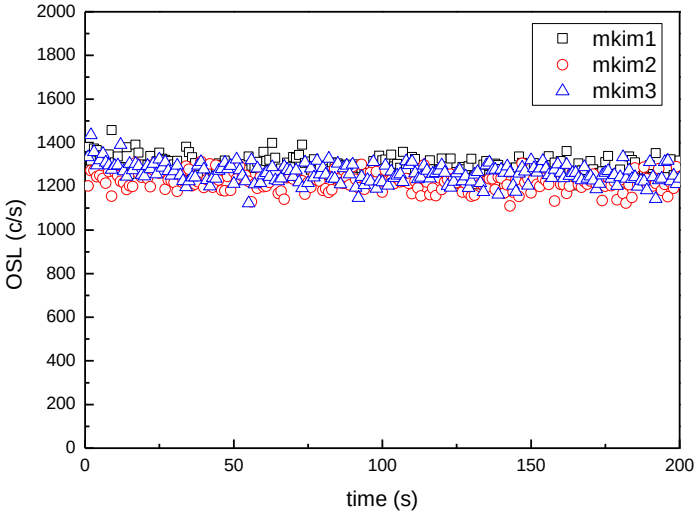


Figure A8: Unirradiated Maras thyme



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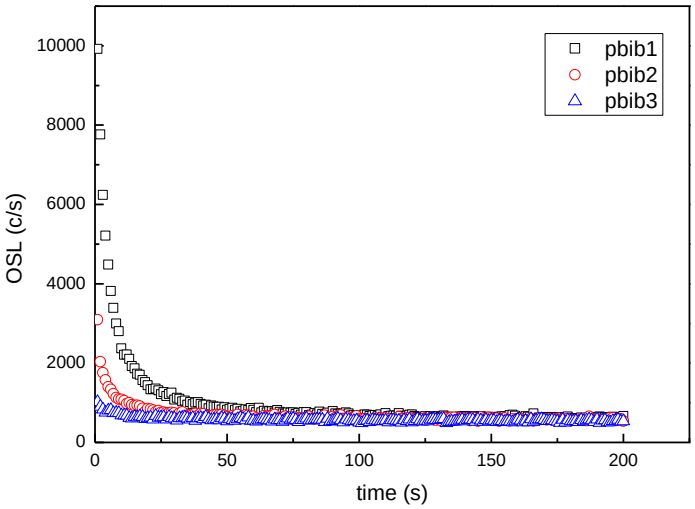


Figure A10: Unirradiated Polatlı red pepper

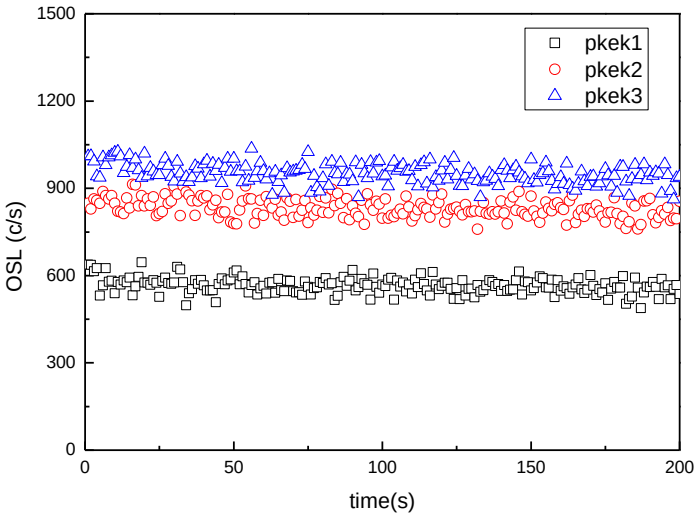


Figure A11: Unirradiated Polatlı thyme

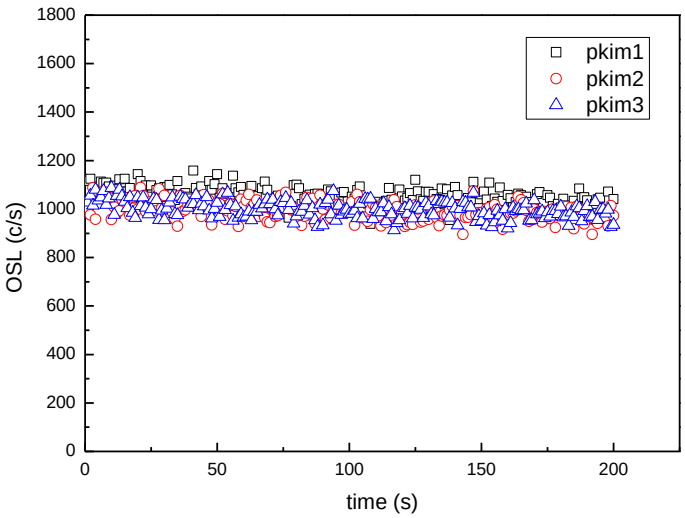


Figure A12: Unirradiated Polatlı cumin

APPENDIX B

RESULTS OF IRRADIATED SAMPLES

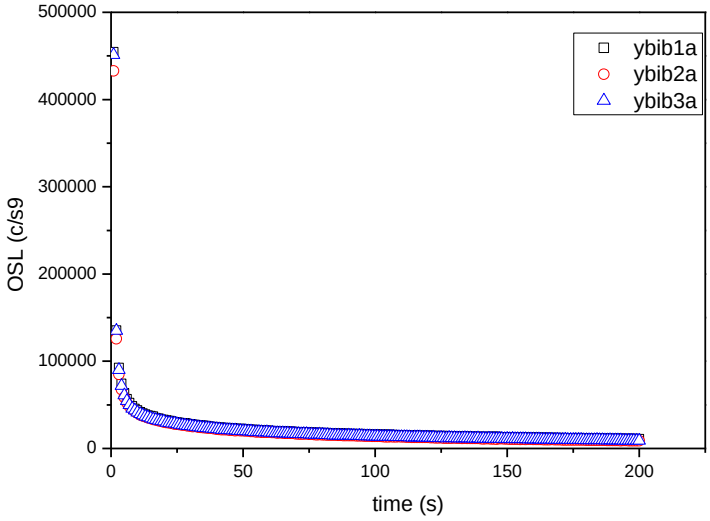
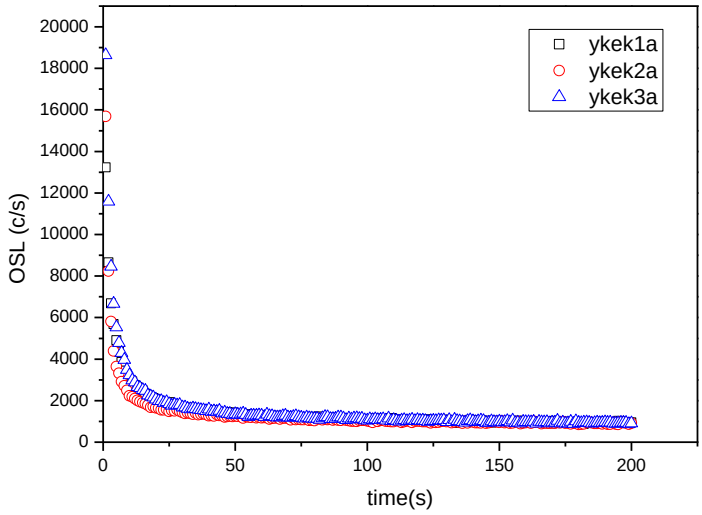


Figure B1: Irradiated Acity red pepper



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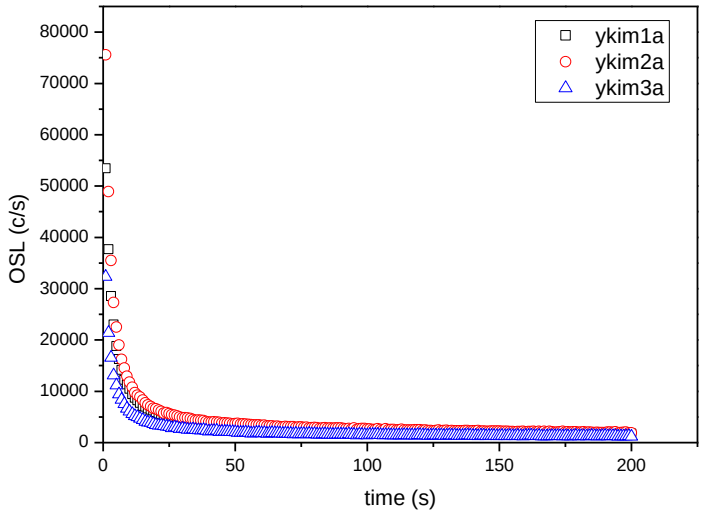

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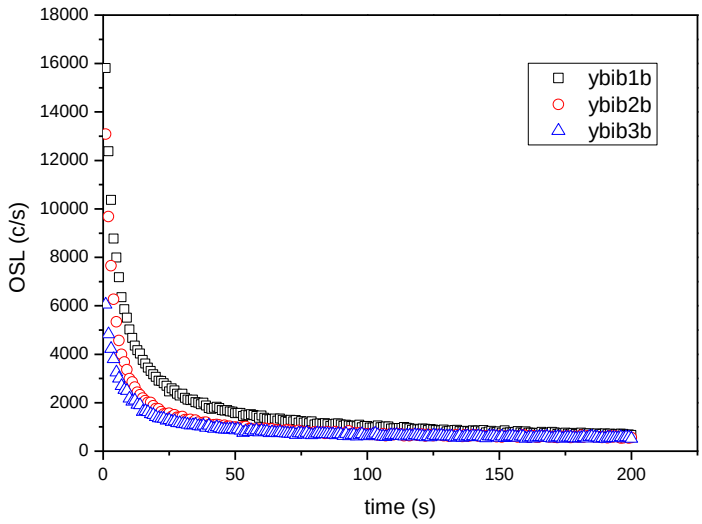


Figure B4: Irradiated Bagdat red pepper

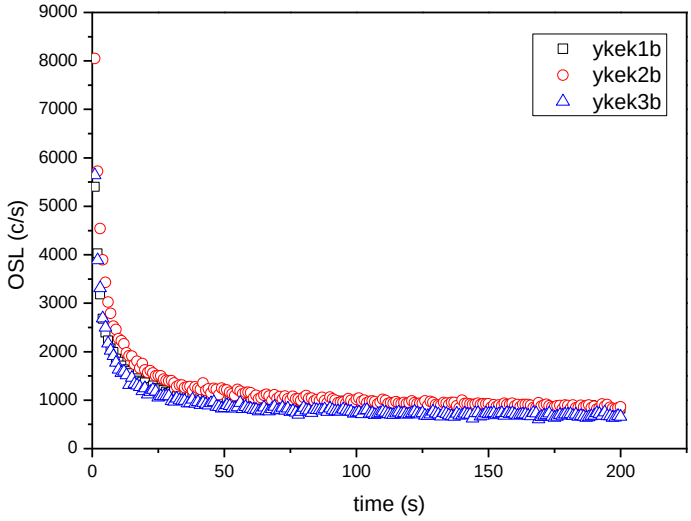


Figure B5: Irradiated Bagdat thyme

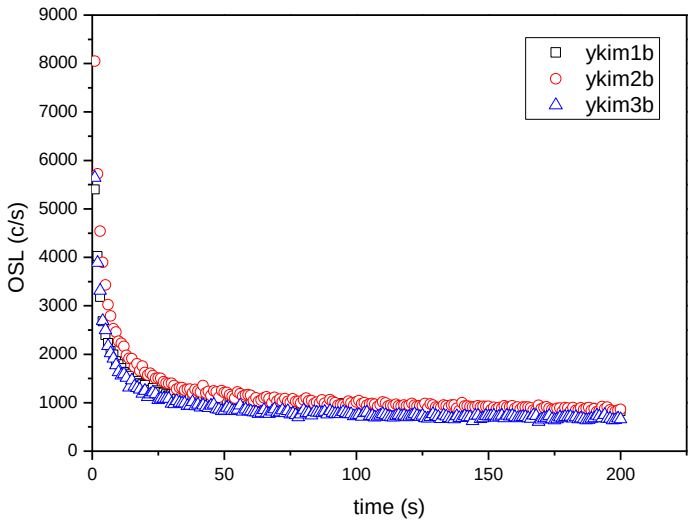


Figure B6: Irradiated Bagdat cumin

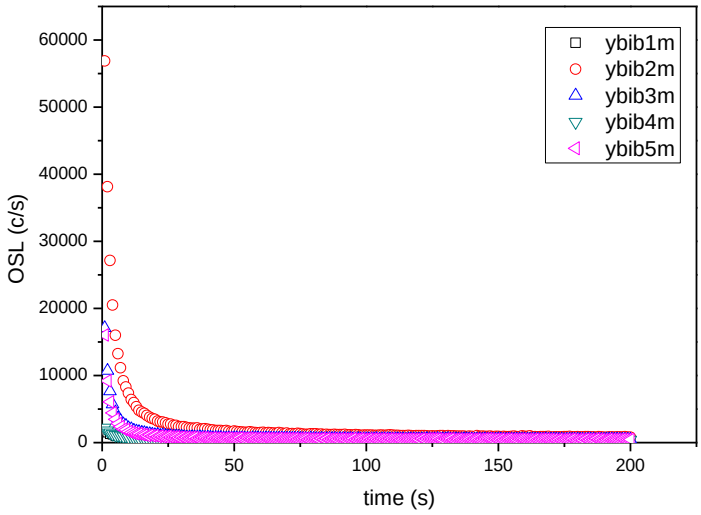
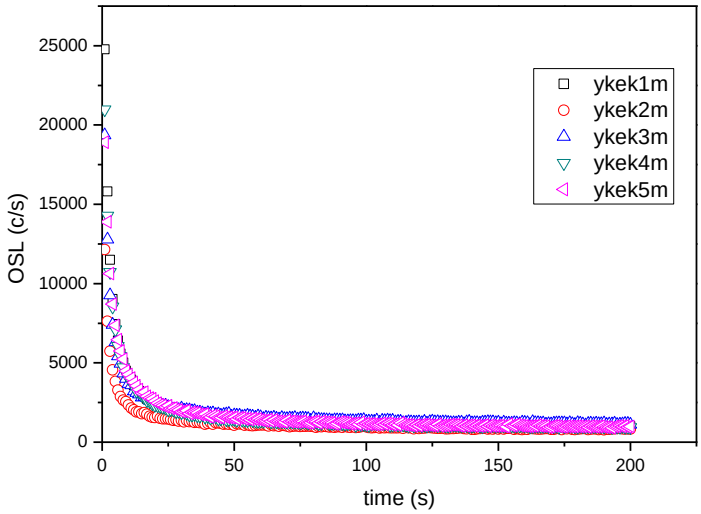


Figure B7: Irradiated Maras red pepper



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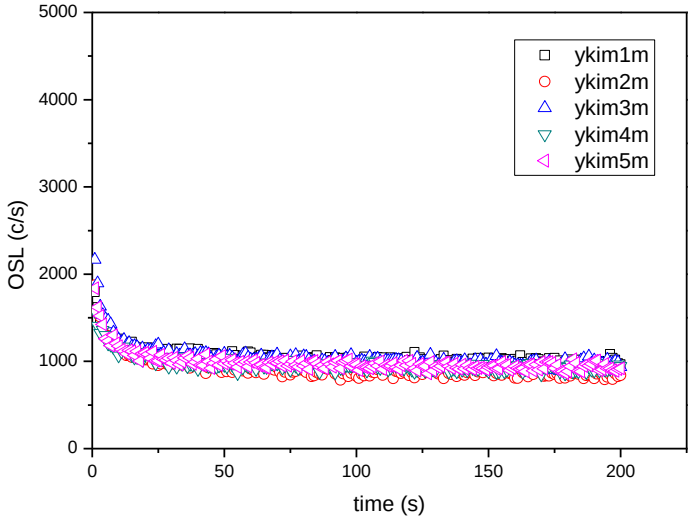

Figure B9: Irradiated Maras cumin

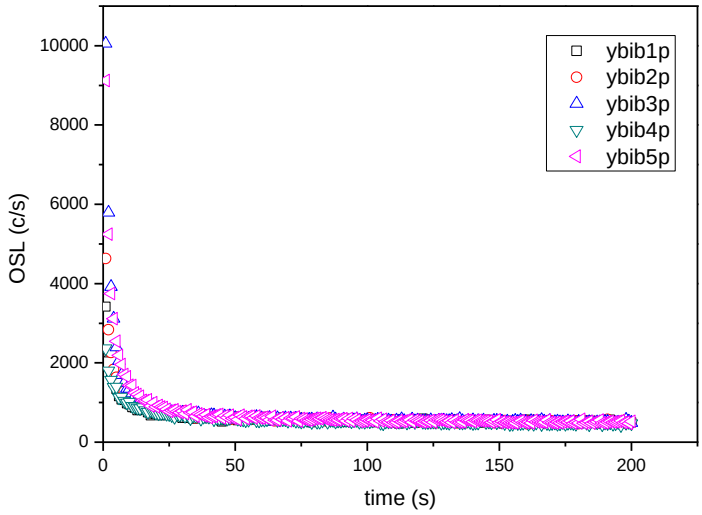

Figure B10: Irradiated Polatlı red pepper

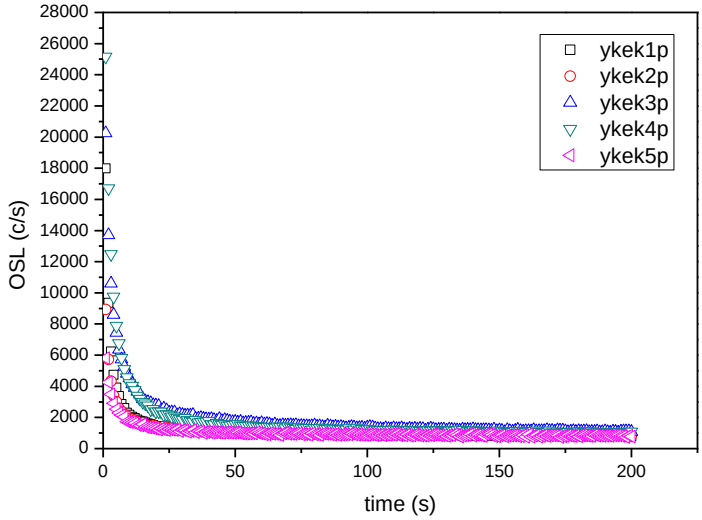

Figure B11: Irradiated Polatlı thyme

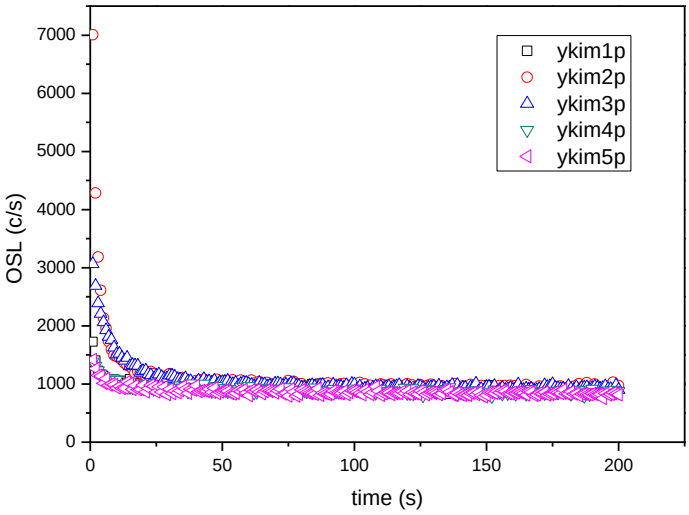

Figure B12: Irradiated Polatlı cumin

APPENDIX C

RESULTS OF MICROBIOLOGICAL ANALYSIS

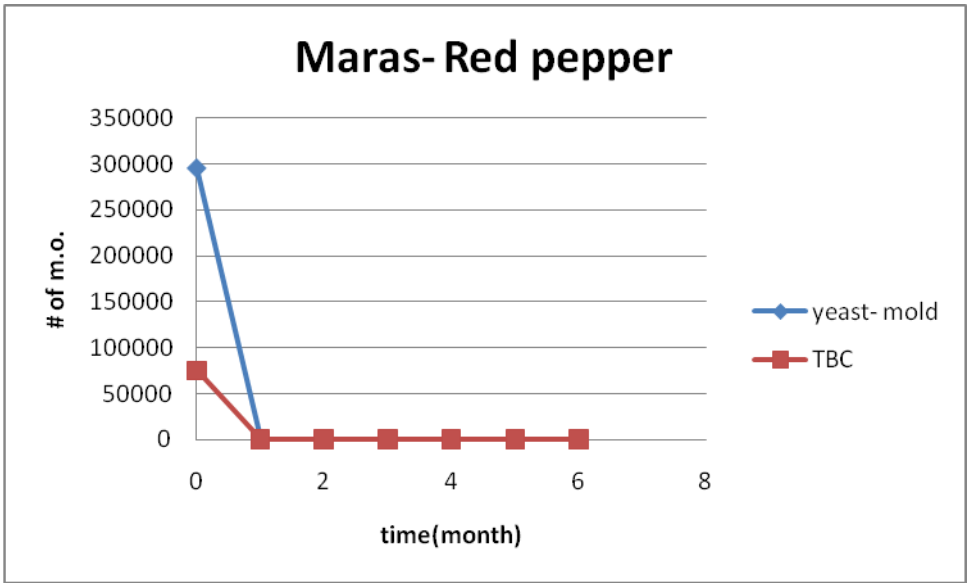

Figure C1: Microbiological analysis of Maras Red Pepper

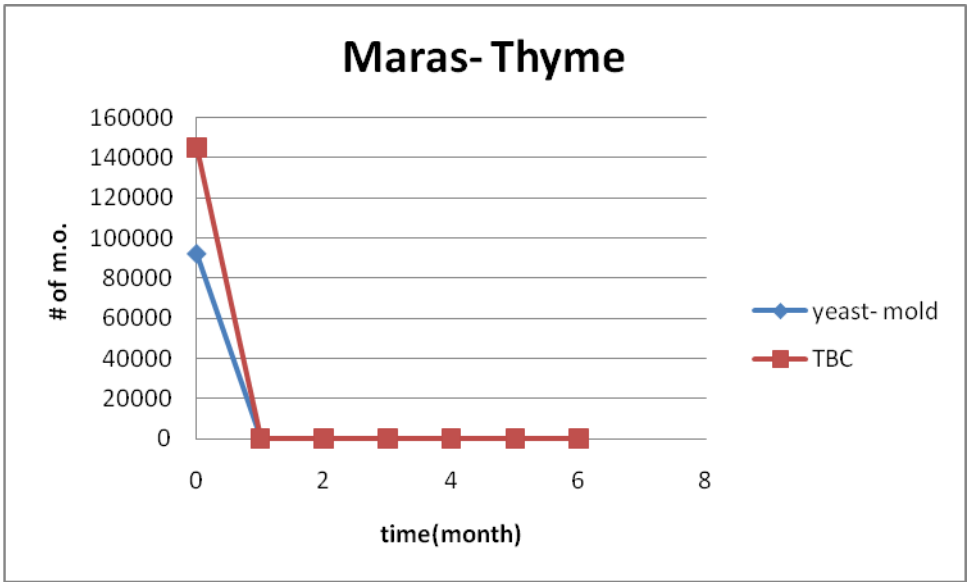

Figure C2: Microbiological analysis of Maras Thyme

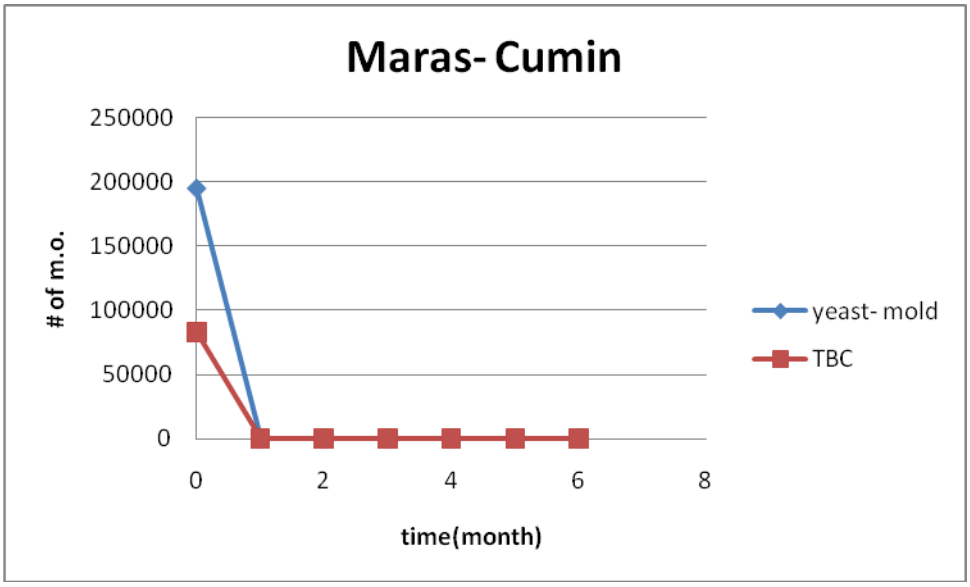

Figure C3: Microbiological analysis of Maras Cumin

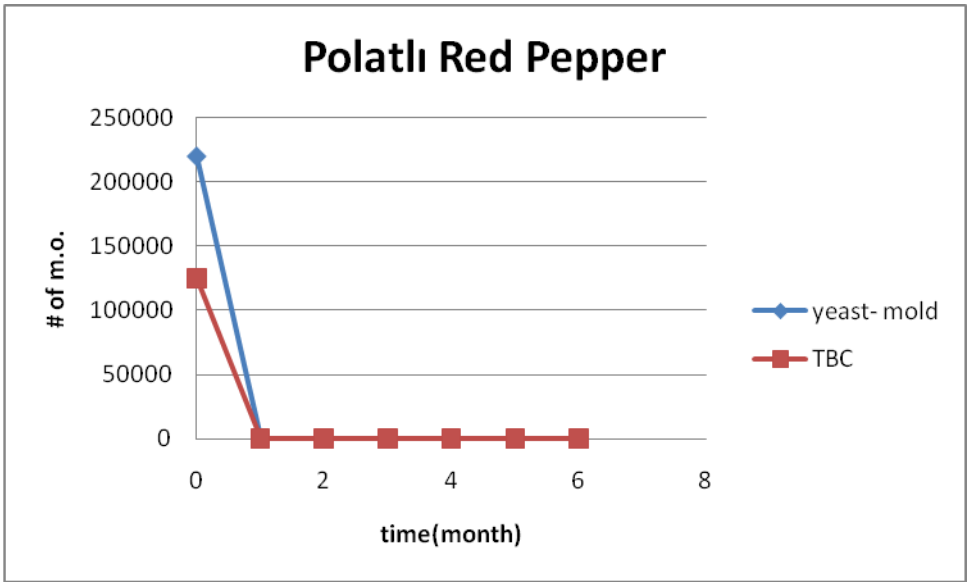

Figure C4: Microbiological analysis of Polatlı Red Pepper

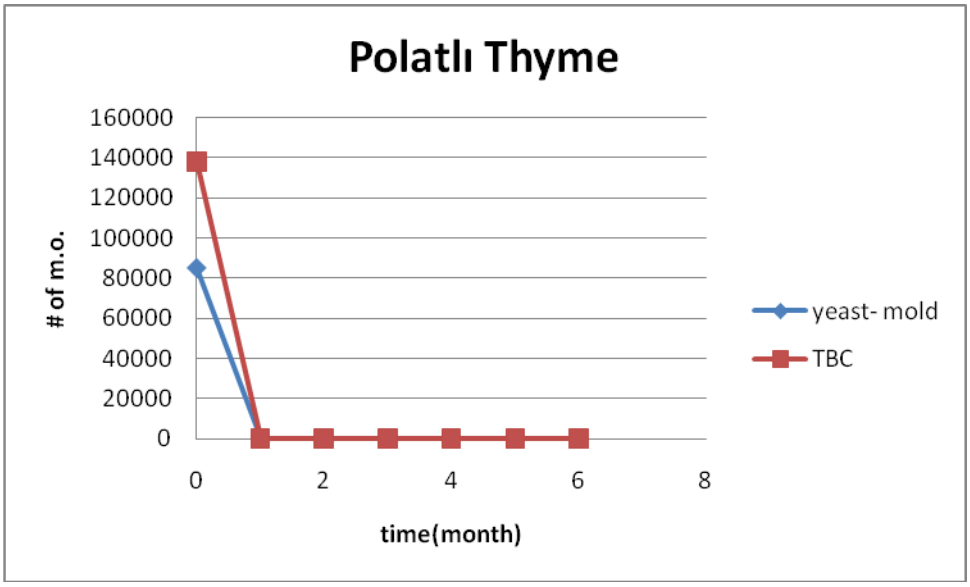

Figure C5: Microbiological analysis of Polatlı Thyme

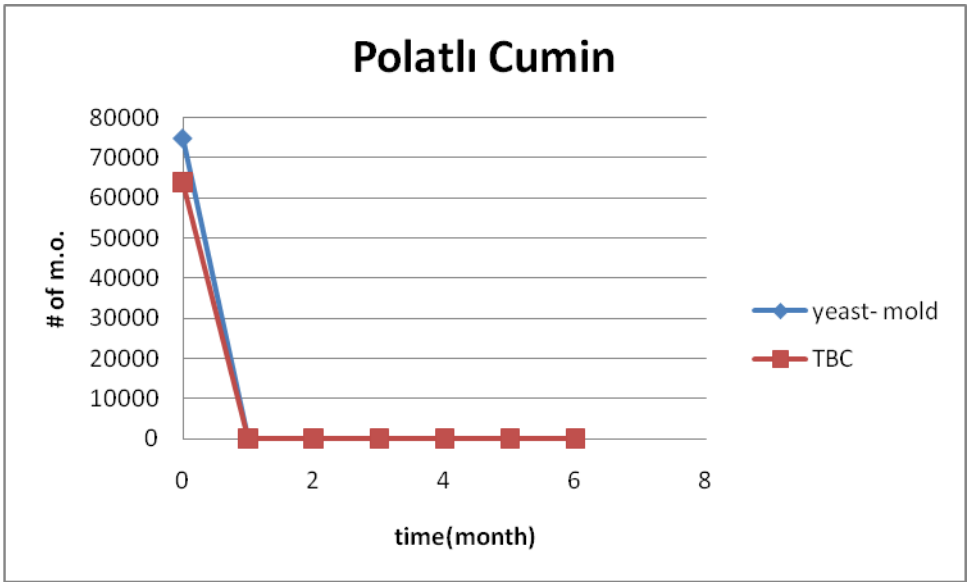

Figure C6: Microbiological analysis of Polatlı Cumin

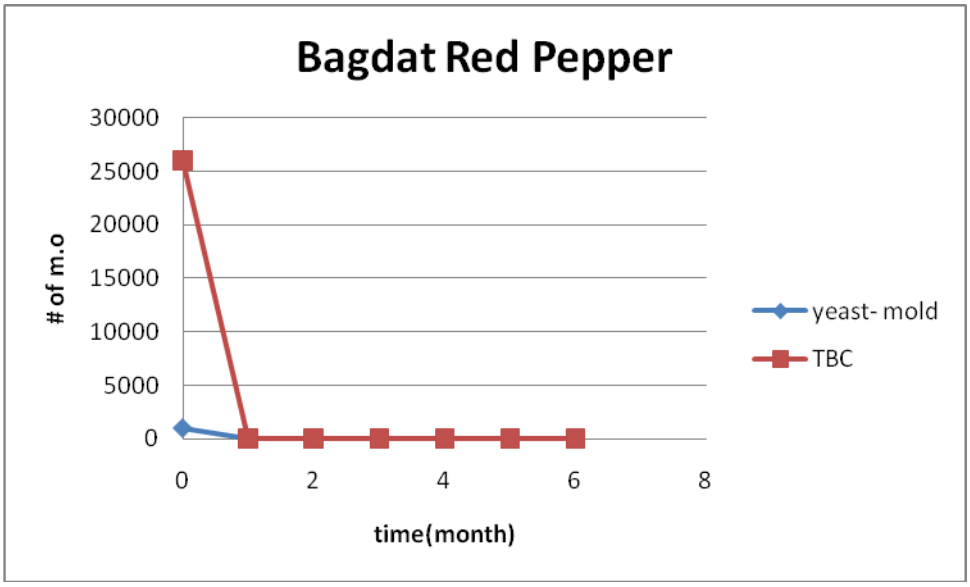

Figure C7: Microbiological analysis of Bagdat Red Pepper

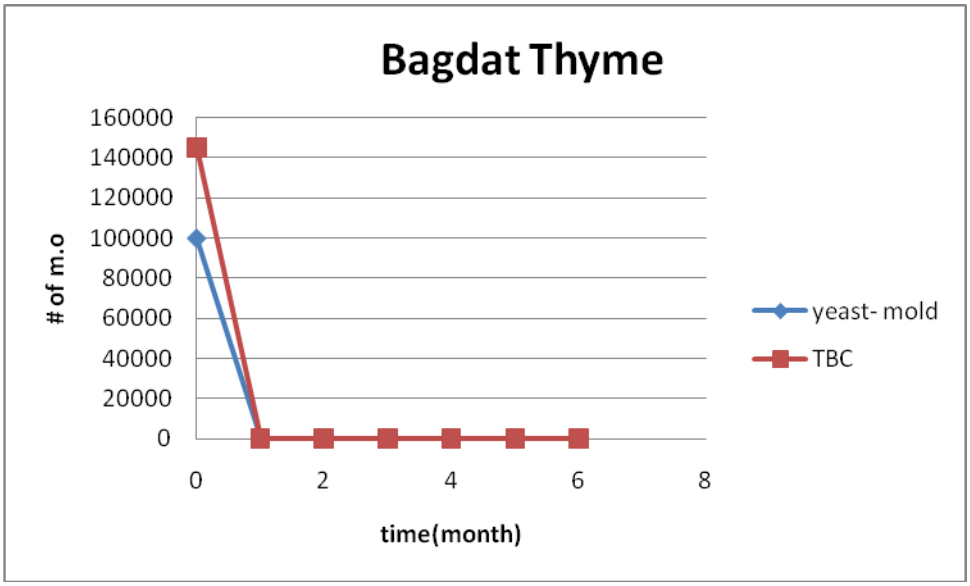

Figure C8: Microbiological analysis of Bagdat Thyme

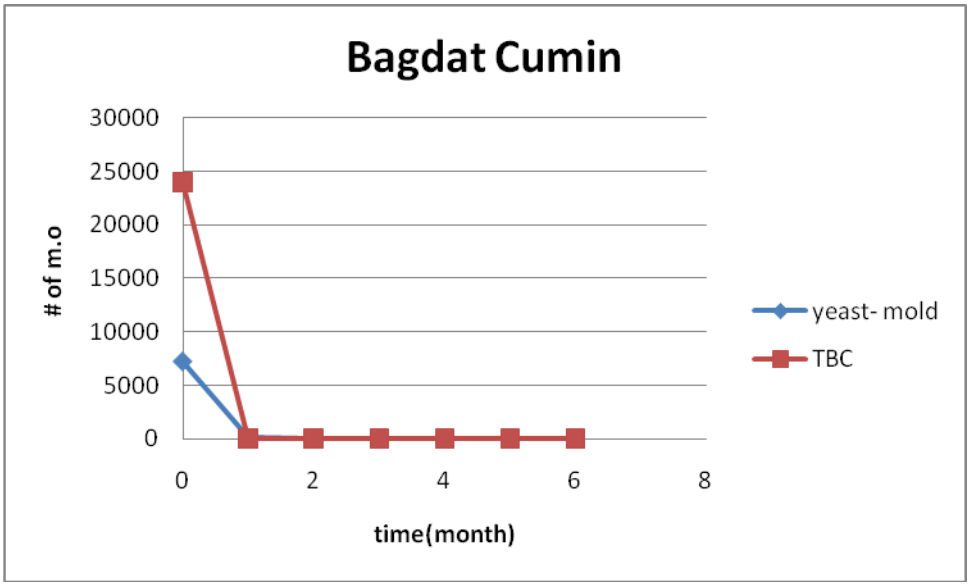

Figure C9: Microbiological analysis of Bagdat Cumin

APPENDIX D

ANOVA RESULTS

```
Welcome to Minitab, press F1 for help.
Retrieving project from file: 'E:\ANOVA\17.03.2010\GENEL 6 AY EN SON.MPJ'
```

General Linear Model: osl versus baharat cesi; origin; time; temperature

```
Factor          Type   Levels  Values
baharat cesidi  fixed       3  k.biber; kekik; kimyon
origin          fixed       4  acity; bagdat; maras; polatlı
time            fixed       6  0; 1; 2; 3; 4; 6
temperature     fixed       2  25; 4


Analysis of Variance for osl, using Adjusted SS for Tests

Source           DF       Seq SS      Adj SS      Adj MS      F      P
baharat cesidi    2   1479302975  1479302975   739651487  52,53  0,000
origin            3   3346027739  3346027739  1115342580  79,22  0,000
time              5     40602292    40602292     8120458   0,58  0,718
temperature       1     13143433    13143433    13143433   0,93  0,335
Error           420   5913403942  5913403942    14079533
Total           431  10792480381


S = 3752,27   R-Sq = 45,21%   R-Sq(adj) = 43,77%


Unusual Observations for osl

Obs      osl      Fit  SE Fit  Residual  St Resid
1  22242,6  10563,3   625,4   11679,3      3,16 R
2  19862,0  10563,3   625,4    9298,7      2,51 R
3  20228,8  10563,3   625,4    9665,5      2,61 R
4  22242,6  10214,4   625,4   12028,2      3,25 R
5  19862,0  10214,4   625,4    9647,5      2,61 R
6  20228,8  10214,4   625,4   10014,4      2,71 R
7  29962,0  10699,2   625,4   19262,8      5,21 R
8  28328,9  10699,2   625,4   17629,7      4,77 R
9  20938,6  10699,2   625,4   10239,4      2,77 R
10  22471,2  10350,4   625,4   12120,8      3,28 R
11  21353,7  10350,4   625,4   11003,3      2,97 R
13  18516,4  10160,7   625,4    8355,7      2,26 R
15  25038,6  10160,7   625,4   14877,9      4,02 R
16  17219,0   9811,8   625,4    7407,2      2,00 R
19  18036,5   9895,8   625,4    8140,6      2,20 R
20  22823,5   9895,8   625,4   12927,7      3,49 R
25  21040,4   9994,3   625,4   11046,1      2,99 R
26  17958,0   9994,3   625,4    7963,6      2,15 R
27  18110,9   9994,3   625,4    8116,6      2,19 R
31  20980,4   9981,2   625,4   10999,2      2,97 R
32  18045,9   9981,2   625,4    8064,7      2,18 R
33  17902,0   9981,2   625,4    7920,8      2,14 R
```

```
376  11717,5   -561,4   625,4   12279,0      3,32 R

R denotes an observation with a large standardized residual.


Tukey 95,0% Simultaneous Confidence Intervals
Response Variable osl
All Pairwise Comparisons among Levels of baharat cesidi
baharat cesidi = k.biber  subtracted from:

baharat
cesidi   Lower  Center  Upper  -----+---------+---------+---------+-
kekik    -4995   -3960  -2925  (----*----)
kimyon   -4925   -3890  -2855  (-----*----)
-----+---------+---------+---------+-
-4000     -2000         0      2000


baharat cesidi = kekik  subtracted from:

baharat
cesidi    Lower  Center  Upper  -----+---------+---------+---------+-
kimyon   -965,5   69,46   1104                     (----*-----)
-----+---------+---------+---------+-
-4000     -2000         0      2000


Tukey Simultaneous Tests
Response Variable osl
All Pairwise Comparisons among Levels of baharat cesidi
baharat cesidi = k.biber  subtracted from:

baharat  Difference       SE of           Adjusted
cesidi     of Means  Difference  T-Value   P-Value
kekik         -3960       442,2   -8,954    0,0000
kimyon        -3890       442,2   -8,797    0,0000


baharat cesidi = kekik  subtracted from:

baharat  Difference       SE of           Adjusted
cesidi     of Means  Difference  T-Value   P-Value
kimyon        69,46       442,2   0,1571    0,9865


Tukey 95,0% Simultaneous Confidence Intervals
Response Variable osl
All Pairwise Comparisons among Levels of origin
origin = acity  subtracted from:

origin   Lower  Center  Upper  ------+---------+---------+---------+
bagdat   -7867   -6556  -5246  (---*----)
maras    -7612   -6302  -4991   (---*---)
polatlı  -7724   -6413  -5103  (----*---)
------+---------+---------+---------+
-6000     -3000         0      3000


origin = bagdat  subtracted from:

origin   Lower  Center  Upper  ------+---------+---------+---------+
maras    -1056   254,6   1565                       (----*---)
```

```
polatlı  -1168   142,8   1453                          (---*----)
------+---------+---------+---------+
-6000     -3000         0      3000


origin = maras  subtracted from:

origin   Lower  Center  Upper  ------+---------+---------+---------+
polatlı  -1422  -111,8   1199                       (----*---)
------+---------+---------+---------+
-6000     -3000         0      3000


Tukey Simultaneous Tests
Response Variable osl
All Pairwise Comparisons among Levels of origin
origin = acity  subtracted from:

Difference       SE of           Adjusted
origin     of Means  Difference  T-Value   P-Value
bagdat        -6556       510,6   -12,84    0,0000
maras         -6302       510,6   -12,34    0,0000
polatlı       -6413       510,6   -12,56    0,0000


origin = bagdat  subtracted from:

Difference       SE of           Adjusted
origin     of Means  Difference  T-Value   P-Value
maras         254,6       510,6   0,4986    0,9594
polatlı       142,8       510,6   0,2797    0,9924


origin = maras  subtracted from:

Difference       SE of           Adjusted
origin     of Means  Difference  T-Value   P-Value
polatlı     -111,8       510,6  -0,2189    0,9963


Tukey 95,0% Simultaneous Confidence Intervals
Response Variable osl
All Pairwise Comparisons among Levels of time
time = 0  subtracted from:

time  Lower  Center  Upper  -------+---------+---------+---------
1     -1646   136,0   1918         (-----------*-----------)
2     -2185  -402,6   1380     (-----------*-----------)
3     -2450  -667,4   1115   (-----------*----------)
4     -2351  -568,9   1213   (-----------*-----------)
6     -2364  -582,1   1200   (-----------*-----------)
-------+---------+---------+---------
-1500         0      1500


time = 1  subtracted from:

time  Lower  Center   Upper  -------+---------+---------+---------
2     -2321  -538,5  1243,6     (----------*-----------)
3     -2585  -803,4   978,7  (-----------*-----------)
4     -2487  -704,9  1077,2  (-----------*-----------)
6     -2500  -718,0  1064,1  (-----------*-----------)
```

```
-------+---------+---------+---------
-1500         0      1500


time = 2  subtracted from:

time  Lower  Center  Upper  -------+---------+---------+---------
3     -2047  -264,8   1517     (-----------*-----------)
4     -1948  -166,3   1616      (-----------*-----------)
6     -1962  -179,5   1603      (-----------*-----------)
-------+---------+---------+---------
-1500         0      1500


time = 3  subtracted from:

time  Lower  Center  Upper  -------+---------+---------+---------
4     -1684   98,51   1881        (-----------*-----------)
6     -1697   85,38   1867        (-----------*----------)
-------+---------+---------+---------
-1500         0      1500


time = 4  subtracted from:

time  Lower  Center  Upper  -------+---------+---------+---------
6     -1795  -13,14   1769       (-----------*-----------)
-------+---------+---------+---------
-1500         0      1500


Tukey Simultaneous Tests
Response Variable osl
All Pairwise Comparisons among Levels of time
time = 0  subtracted from:

Difference       SE of           Adjusted
time    of Means  Difference  T-Value   P-Value
1          136,0       625,4    0,217    0,9999
2         -402,6       625,4   -0,644    0,9877
3         -667,4       625,4   -1,067    0,8944
4         -568,9       625,4   -0,910    0,9442
6         -582,1       625,4   -0,931    0,9387


time = 1  subtracted from:

Difference       SE of           Adjusted
time    of Means  Difference  T-Value   P-Value
2         -538,5       625,4   -0,861    0,9556
3         -803,4       625,4   -1,285    0,7936
4         -704,9       625,4   -1,127    0,8703
6         -718,0       625,4   -1,148    0,8611


time = 2  subtracted from:

Difference       SE of           Adjusted
time    of Means  Difference  T-Value   P-Value
3         -264,8       625,4  -0,4235    0,9983
4         -166,3       625,4  -0,2660    0,9998
6         -179,5       625,4  -0,2870    0,9997
```

time = 3 subtracted from:

time	Difference of Means	SE of Difference	Adjusted T-Value	P-Value
4	98,51	625,4	0,1575	1,000
6	85,38	625,4	0,1365	1,000

time = 4 subtracted from:

time	Difference of Means	SE of Difference	Adjusted T-Value	P-Value
6	-13,14	625,4	-0,02101	1,000

Tukey 95,0% Simultaneous Confidence Intervals
Response Variable osl
All Pairwise Comparisons among Levels of temperature
temperature = 25 subtracted from:

temperature	Lower	Center	Upper	---------+---------+---------+-------
4	-360,9	348,9	1059	(-----------------*----------------)

---------+---------+---------+-------
0   400   800

Tukey Simultaneous Tests
Response Variable osl
All Pairwise Comparisons among Levels of temperature
temperature = 25 subtracted from:

temperature	Difference of Means	SE of Difference	Adjusted T-Value	P-Value
4	348,9	361,1	0,9662	0,3340