UV DISINFECTION OF SOME OF THE FRUIT JUICES

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ABSTRACT

UV DISINFECTION OF SOME OF THE FRUIT JUICES

Although UV-C irradiation is used in the pasteurization of certain liquids, the application to white grape juice is not sited in the literature. Besides there are a few studies about the inactivation of naturally grown microorganisms by using UV-C treatment. The objective of this study was to determine the effect of UV-C treatment on the inactivation of *Escherichia coli* K12 inoculated into white grape juice and naturally grown microorganisms in the fresh squeezed orange juice. Also, the effect of UV light on the colors of the products was determined. The data were evaluated considering the properties of the juices.

Results of microbiological analysis of white grape juice showed that, maximum log reduction was more than 5-log CFU/mL at a UV dose of 75.04 mJ/cm^2 when the inoculation rate was 5.73 (±0.114) log CFU/mL. Although a 5-log reduction requirement of FDA was satisfied with white grape juice, maximum log reduction for the naturally grown microorganisms in orange juice was only 1.76-log CFU/mL even if the sample was subjected to UV dose of 144.36 mJ/cm^2. Color analysis demonstrated that orange juice color was not affected from the treatment. For white grape juice, increased UV dose caused a decrease in a^* value indicating slightly more green region. Also, increased intensity and sample depth had a pronounced effect on b^* value of the juice showing yellow region. As a conclusion, UV-C light can be used as an alternative technique in processing of white grape juice. Combination of UV-C with other techniques is suggested to increase the microbiocidal efficiency in orange juices.

ÖZET

BAZI MEYVE SULARININ UV DEZENFEKSİYONU

UV-C radyasyonu bazı sıvı gıdaların pastörizasyonunda kullanılmasına rağmen, bu teknolojinin beyaz üzüm suyuna uygulaması literatürde yer almamaktadır. Bunun ötesinde, UV-C işlemi kullanılarak doğal olarak gelişmiş mikroorganizmaların inaktivasyonu hakkında çok fazla çalışma bulunmamaktadır. Bu çalışmanın amacı, UV-C uygulamasının beyaz üzüm suyuna aşılanmış *Escherichia coli* K12'nin ve taze sıkılmış portakal suyunda doğal olarak gelişen mikroorganizmaların inaktivasyonu üzerine etkisinin belirlenmesidir. Ayrıca UV ışığın ürünlerin renklerine etkisi de belirlenmiştir. Veriler, meyve sularının özellikleri göz önüne alınarak değerlendirilmiştir.

Beyaz üzüm suyunun mikrobiyolojik analiz sonuçları göstermiştir ki, aşılama oranı 5.73 (± 0.114) log CFU/mL olduğunda en fazla logaritmik azalma 75.04 mJ/cm^2 UV doz değerinde 5-log CFU/mL'den fazladır. FDA'nın 5-log azalma gereksinimi beyaz üzüm suyu çalışmasında sağlanmış olmasına rağmen, portakal suyu örneği 144.36 mJ/cm^2 UV dozuna maruz kaldığında bile doğal olarak gelişen mikroorganizmaların en fazla log azalması sadece 1.76-log CFU/mL olmuştur. Renk analizi, portakal suyunun renginin uygulamadan etkilenmediğini göstermiştir. Beyaz üzüm suyu için ise, artan UV dozu a^* değerinde daha yeşil bölgeyi gösteren bir azalmaya neden olmuştur. Aynı zamanda, artan ışık yoğunluğu ve örnek derinliği b^* değerinde sarı bölgeyi gösteren belirgin bir etkiye sahiptir. Sonuç olarak, UV-C ışığı beyaz üzüm suyu işlemesinde alternatif bir metod olarak kullanılabilir. Portakal suyundaki mikroorganizmaları öldürücü etkiyi arttırmak için UV-C'nin diğer tekniklerle birleştirilmesi önerilir.

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CHAPTER 1

INTRODUCTION

Fruit juices are unfermented but fermentable products obtained from fresh, ripe and healthy fruits (Republic of Turkey Ministry of Agriculture and Rural Affairs General Directorate of Protection and Control 2006). They are also known as very good sources of vitamins and minerals (Kabasakalis, et al. 2000, Bates, et al. 2001). The fruit juice consumption has been increasing during last decades because of its various health benefits (Bates, et al. 2001, Liu 2003, Borenstein, et al. 2005).

However, consumption of unpasteurized fruit juices causes approximately 16000 to 48000 cases of illnesses in a year (Foley, et al. 2002). Previously it was believed that fruit juices are safe due to their low pH values. However, recent outbreaks of *Escherichia coli* O157:H7 and *Salmonella* associated with the consumption of unpasteurized juices show the potential of acidic juices to carry pathogenic microorganisms (Cook, et al. 1998). Conventional heat pasteurization is the best known technique in order to reduce the number of pathogens such as *E. coli* O157:H7, *Salmonella* sp., *Listeria monocytogenes* and *Cryptosporidium parvum* in various types of juices (Tandon, et al. 2003). "Pasteurization" describes a mild heat treatment which is applied at temperatures below 100°C (Silva and Gibbs 2004). The thermal pasteurization criteria for white grape juice are 90- 95 °C for 15-30s (Cemeroğlu 2004). For orange juice temperature and time requirements are 90°C for 1 minute (Graumlich, et al. 1986).

The NACMCF (National Advisory Committee on Microbiological Criteria for Foods) has recommended the use of *E. coli* O157:H7 or *Listeria monocytogenes* as a target microorganism in fruit juices and suggested a 5-log reduction in the target pathogen. Pasteurization is an effective and the most used technology in order to reach safety requirements (FDA 1998). However, cost of the equipment is prohibitive especially for small operations. Because of the adverse effects of thermal pasteurization on the food quality and increased demand for the fresh-like juice products, non-thermal

alternative techniques have been investigated (Tahiri, et al. 2006, Basaran-Akgul, et al. 2009). Some of these alternative methods are addition of microbiocidal agents, high pressure application, pulsed electric field, irradiation, and aseptic packaging (Bates, et al. 2001). However there are some disadvantages of these techniques. Opstal, et al. (2006) reported the loss of ascorbic acid in the peroxidase applied fruit juices. In another study it was shown that PEF treatment causes brightness in the color of orange juice (Min, et al. 2003) and its application to industry is limited due to its high cost (Tahiri, et al. 2006). Also, a slight browning in the color of white grape juice was observed after the high pressure treatment (Daoudi, et al. 2002). Considering these limitations of other techniques, UV-C radiation can be used as an alternative to other preservation techniques. This process does not produce chemical residues (Canitez 2002). Besides, it is a low-cost operation and effective against many microorganisms (Bintsis, et al. 2000). Inactivation mechanism depends on the absorption of UV photons by the genetic materials and subsequently the formation of dimers which inhibit the transcription and replication of the cell (Oguma, et al. 2002, Bolton, et al. 2003, Donahue, et al. 2004, Koutchma 2009).

UV-C light is very well known as its antimicrobial effect and used for the decontamination of hospitals, water effluent, drinking water, water for swimming pools, and surface disinfection of different fruits (Nigro, et al. 1998, Bintsis, et al. 2000, Pan, et al. 2004, Begum, et al. 2009).

In the literature there are a number of studies related to the application of UV-C light irradiation. For example, Hanes, et al. (2002) treated the experimentally contaminated fresh apple cider in a continuous system and they obtained greater than 5-log reduction in the number of *Cyrptosporidium parvum* oocysts after 1.2 to 1.9 s of exposure to 14.32 mJ/cm^2 UV light irradiation. It was reported that maximum 2.2-log reduction was observed in liquid egg white samples spiked with *E. coli* ATCC 8739 after subjected to UV dose of 98 mJ/cm^2 for 20 minutes in a sample depth of 0.153 cm (Unluturk, et al. 2008). According to the study of Taghipour (2004), for a 1-log reduction of *E. coli* in primary and secondary wastewaters UV doses of 3.5 and 6.2 mJ/cm^2 were found to be required, respectively. In another study, after 30 minutes of exposure to UV dose of 45000 mJ/cm^2 a 5.1-log reduction was achieved for 800 ml of apple juice inoculated with *E. coli* K12 (Keyser, et al. 2008).

The objectives of this study were;

- Characterization of juice samples via determination of some physical and chemical properties including pH, turbidity, absorbance, brix values, titratable acidity and color parameters.
- Application of UV-C light in order to determine the efficacy of the system on the reduction of *E. coli* K12 inoculated into white grape juice and investigation of the effectiveness of some factors including sample depth, exposure time, UV intensity and inoculation rate used in the study.
- UV-C treatment of naturally contaminated fresh squeezed orange juice samples using different exposure times.
- Determination of the color changes of juices after the UV-C light exposure.

CHAPTER 2

LITERATURE REVIEW

2.1. Fruit Juice

According to Turkish Food Codex, fruit juice is described as an unfermented but fermentable product obtained from fresh, ripe and healthy fruits. It can be produced using a single type of fruit or mixed fruits. The juice has the characteristics of the fruit which it is made (Republic of Turkey Ministry of Agriculture and Rural Affairs General Directorate of Protection and Control 2006).

2.1.1. Composition of Fruit Juice

The major component of the fruit juice is water. The other most common constituent is carbohydrates which comprise sucrose, fructose, glucose and sorbitol. Also, limited amount of protein and minerals are found in fruit juices. However juice contains no fat or cholesterol. If it is not added, no fiber content can be observed (American Academy of Pediatrics Committee on Nutrition 2001).

Quality of fruit juices is highly affected by the organic acid profile. Major organic acids which compose the 90% or more of the total acidity in grapes are tartaric and malic acids (Soyer, et al. 2003). Besides, citric and malic acids were reported to be the main acids in citrus fruits (Karadeniz 2004). It was reported that acetic acid, lactic, citric and malic acids have antimicrobial effects on *E. coli* O157:H7.

Fruit juices are known as considerable sources of ascorbic acid (vitamin C). Their consumption has been increasing during last years (Kabasakalis, et al. 2000).

Especially citrus fruits and juices are good sources of ascorbic acid, folic acid, vitamin B1, thiamine and potassium. It was noted that a cup of citrus juice (240 mL) provides vitamin C in the quantity of more than daily requirement (Bates, et al. 2001). Vitamin C has several important roles. During phytosynthesis and respiration, reactive species (RS) are formed in plants. These RS cause cellular oxidative damage which is relevant to several human diseases like cancer and atherosclerosis. Ascorbic acid acting as an antioxidant provides a protection against those species. Vitamin C which takes part in cell growth is also associated to the synthesis of anthocyanidins and several secondary metabolites as a co-factor (Dani, et al. 2007).

2.1.2. The Value of Fruit Juices

According to Bates, et al. (2001), the global market for juice and its products was estimated to be about 50 billion liters in the early 1990s. It was declared that production of fruit juices in Turkey increased about 90% between the years of 1994 and 2004 (Republic of Turkey Undersecretariat of the Prime Ministry for Foreign Trade Export Promotion Center 2007). Although the average consumption ratio of fruit juices announced in 2006 is 6.4 L/person/year in Turkey, this value comparatively lower than the ratio obtained in 1997 which is 35 L/person/year in the U.S.A. (Republic of Turkey Undersecretariat of the Prime Ministry for Foreign Trade Export Promotion Center 2007).

The popularity of fruit juices is being arised in terms of their nutritive value and additional health benefits. Especially for very young, elderly and infirm people it is easy to drink fruit juice as an alternative to eating fruits. Also, processing of liquid foods is simpler than that of solid products. Thus, all safety and quality requirements can be readily satisfied (Bates, et al. 2001).

Fruit and vegetables hold various types of antioxidants like phenolics and carotenoids. These phytochemicals prevent oxidative damages and decrease the risk of chronic illnesses. It was speculated that low fruit and vegetable intake increase the risk of cancer by two fold. Fruits were found to have protective effects on the cancers of esophagus, oral cavity and larynx (Liu 2003).

Alzheimer Disease (AD) onset and progression was reported to be related to the accumulation of reactive oxygen species in the brain (Borenstein, et al. 2005). Tchantchou, et al. (2005) indicated that cognitive performance decreased by the oxidative stress. Oxidative stress plays a role in the age related neurodegeneration. Polyphenols found in large amounts in fruit and vegetable juices may help retarding AD onset (Borenstein, et al. 2005).

Grapes are one of the most important sources of phenolic compounds among the other fruits. Phenolic compounds found in red wine and grapes were shown to be effective on the inhibition of low density lipoprotein (LDL) oxidation which is related to the atherosclerosis. Especially catechins and procyanidins extracted from grapes act as radical scavengers (Frankel, et al. 1998). Flavanoid content of fruit juices, especially grape juice products, reduces the risk of coronary artery diseases (CADs) by inhibiting platelet aggregation (Stein, et al. 1999). It was pronounced that platelets involve in myocardial infarction due to coronary thrombosis. Studies about the mechanism of the inhibition of platelet activity mentioned that the flavonoids in grapes inhibit cyclooxygenase and phosphodiesterase enzymes. Difference in the composition of flavonoid compounds in various juice products brings about a different platelet inhibitory effect. Flavonols which are the most important flavonoids in grapes were found to have more potential to protect platelet accumulation than flavanones included in orange and grapefruit juices. It was suggested that purple grape juice may take a part in the prevention of development and progression of CADs (Keevil, et al. 2000). Although red wines and red grape juices are believed to be healthier than white, the only reason for this is the difference in the processing steps. In the most white wine or white grape juice processing, the skins are removed. Hence, the polyphenols found in the skins can not transfer into the resulting wine or juice (Food Navigator 2006). In another study, a moderate amount of red wine consumed daily, was shown to reduce the risk of cardiovascular diseases. Also white wines were found to have beneficial effects on myocardial ischemic injury (Falchi, et al. 2006).

Children by 1 year of age consume fruit juices (American Academy of Pediatrics Committee on Nutrition 2001). Recent studies have shown that 100% white grape juice is the best choice for infants and toddlers. Ease of digestion and absence of sorbitol make the 100% white grape juice first preference. 100% white grape juice can be readily digested because of the equal concentrations of fructose and glucose (Welch's International 2008). If the fructose concentration goes beyond the glucose

amount, malabsorption was demonstrated to be more apparent. Malabsorption may cause diarrhea, flatulence and abdominal pain (American Academy of Pediatrics Committee on Nutrition 2001). Besides, sorbitol may cause gas problems, intestinal discomfort and diarrhea in babies and toddlers (Sea Aloe 2008).

Nicklas and Kleinman (2008) indicated that consumption of 100% fruit juice help better nutrient intake. It was also shown that it does not cause being overweight in children between the ages of 2 to 11.

White grapes have beneficial effects on women who are susceptible to iron deficiencies. Additionally, urinary tract infection which is another prevalent problem among women can be overcome consuming white grape juice. Because it was noted that grapes help kidneys in working properly by decreasing the acid in our urine (Sea Aloe 2008). Also it is known that white grape juice has severeal benefits in the areas of;

- Anti-aging
- Anti-bacterial/ viral
- Anti-inflammatory
- Antioxidant
- Arterial flexibility
- Brain, skin and eye health
- Cardiovascular health
- Gastric health
- Inhibiting prostate cancer (The Perfect Berry 2007).

2.2. Fruit Juice Production

Some of the unit operations that are usually involved in fruit juice production are presented briefly in Table 2.1 (Bates, et al. 2001). Processing steps of white grape juice and orange juice are summarized in the sections of 2.2.1 and 2.2.2, respectively.

Table 2.1. Unit Operations in Fruit Juice Production
(Source: Bates, et al. 2001).

Unit Operation	Result
Mass transfer	Fruit delivered, dry cleaned
Extraction	Washed
Separation	Sized, graded
Separation	Peeled, cored and deseed
Size reduction	Crushed, comminuted
Pressure application	Juice extracted
Separation	Solids screened
Deaeration	Oxygen removed
Centrifugation	Solids separated
Filtration	Clarification
Fluid flow	Juice transferred
Heat transfer	Enzymes inactivated, juice pasteurized and cooled
Concentration/ evaporation	Volume reduction, stability
Mass transfer	Packaging, shipping

2.2.1. White Grape Juice Production

The first step is harvesting of the grapes. Mechanical harvesting is preferable in terms of the quality (Bates, et al. 2001). Collected raw materials are transferred to the processing area and then washed. Stems are not removed in order to improve the pressing ability. However, the juice quality is not affected because hot pressing is not applied to obtain white grape juice. Hence, undesirable extraction of other compounds found in stems is prevented. After washing, cleaned fruits are smashed in the mill. Smashed grapes are filled to the inclined sieved based tanks so as to obtain the fruit juice without pressing. Consequently, 30- 40 % of the fruit juice can be obtained. The remaining part of the juice is obtained from pressing (Cemeroğlu 2004). Cold press is appropriate for grapes which yield a light green to yellow juice to maintain the initial light color (Bates, et al. 2001). Subsequent step is clarification and depectinization. But before clarification, juice is centrifuged and big-sized particles are removed. Heat treatment is applied and then the juice is cooled before enzyme addition. In the depectinization step the fruit juice is filled to the clarification tanks, and pectolitic and amylolitic enzymes are added to the fruit juice. The soluble pectin in the juice has colloidal properties and inhibits the separation of the undissolved cloud particles from

the clear juice. Enzyme treatments are widely used to remove pectinaceous material and clarify the juice. This material, if it is not removed, will clog the filters, reduces production yield, and can result in a haze in the final product. The fractured pectin chains and tannins are removed from the juice with the use of gelatin (fining agents) treatments which can be used in combination with enzyme treatment, bentonite, or by itself. The gelatin cannot precipitate with the colloids in the fruit juices at the temperatures above 40 °C if it is used by itself. So after adding gelatin at these temperatures, kiezelgur has to be added to the juice. The positively charged gelatin at the pH of grape juice (approx. 3.5) will facilitate removal of the negatively charged suspended colloidal material from the juice. After the enzyme treatment, fining and settling process, the juice is clarified by filtration. Before filtration, centrifugation may be used to remove a high percentage of suspended solids. If clarified and filtered grape juice is packed, crystallization will occur and the juice will become cloudy. The reason for this is the production of deposits by the thermoable proteins in the grape. These thermoable proteins can be removed by bentonite treatment. Bentonite is added to the clarified juice. After deposit formation, juice is filtered using Kiezelgur filter. Filtered grape juice is filled to the KZE ("Kurzzeit-erhitzung" means HTST) tanks. In this tank the juice is heat treated at 90- 95 °C for 15-30s. After pasteurization the juice is filled to the packages (Cemeroğlu 2004).

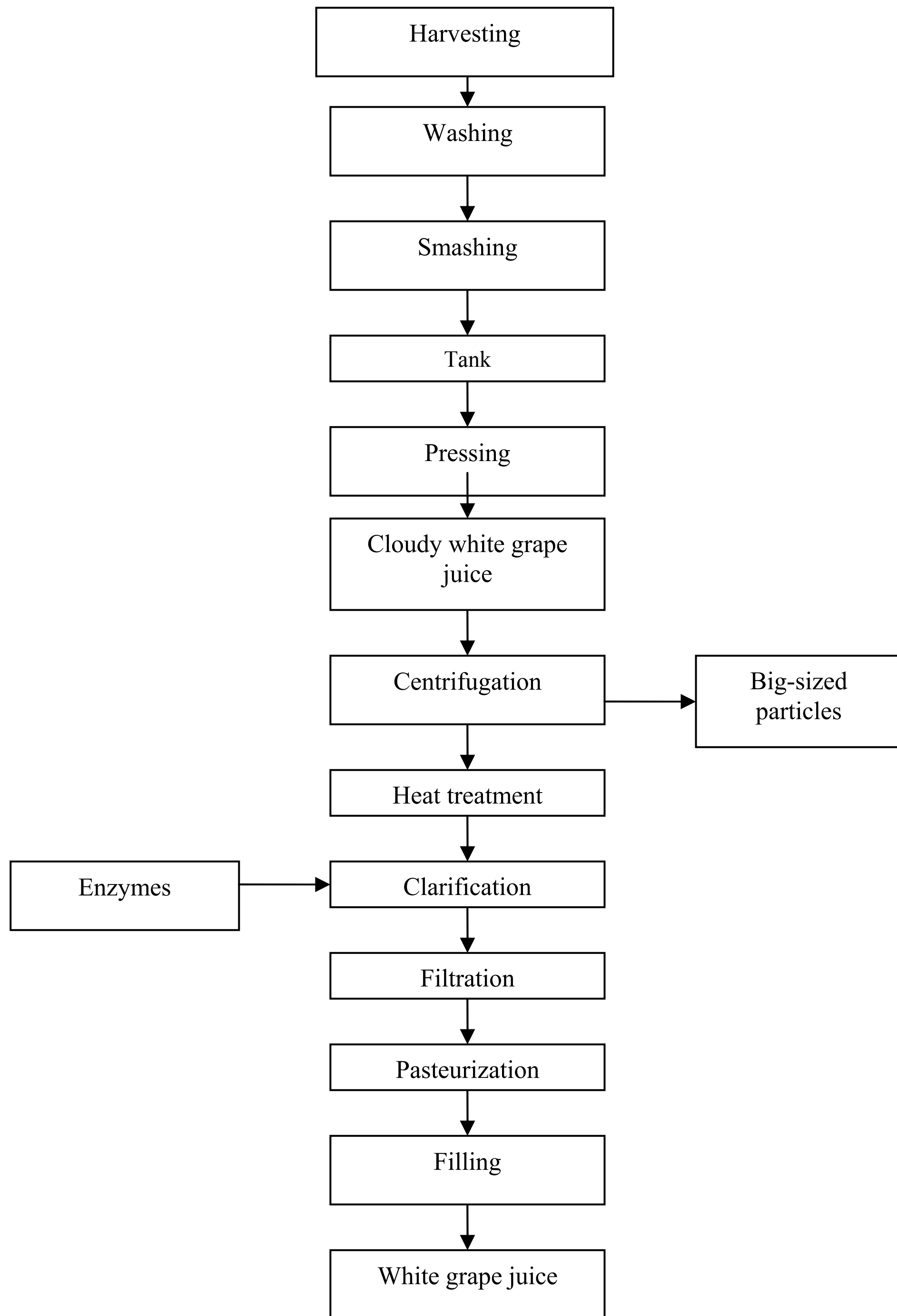
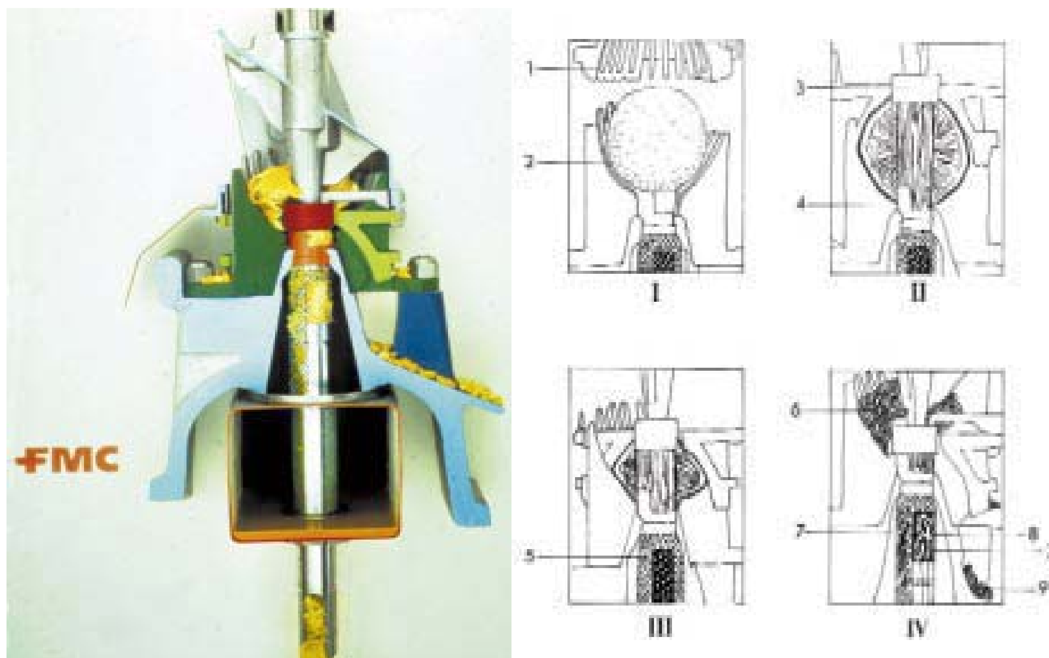


Figure 2.1. Flow diagram for the white grape juice production.
(Source: Cemeroğlu 2004)

2.2.2. Orange Juice Production

Freshly harvested fruits need to be washed after the sorting of stems and leaves. Washing helps to reduce the amount of microorganisms (Bates, et al. 2001). After cleaning and separation, the peel oil is removed in order to prevent oxidation caused by its terpene content. In the citrus juice processing, the equipments which are used to get the juice from the fruit are known as extractors. In these machines, there is a perforated tube and this tube penetrates to the whole fruit. The fruit is pressed and at the same time the juice is gathered in a container and the peel oil is removed from the surface of the peel by washing. These extractors are produced by FMC (Food Machinery Corporation) and they are known as FMC extractors (Cemeroğlu 2004).



I. Fruit is placed in the extractor
II. A plug is cut in the center of the fruit
III. A mechanical hand presses the juice and pulp
IV. The juice exists out the bottom of the FMC Extractor, pulp and the peel is separated from the juice.

Figure 2.2. Citrus Extractor Diagram
(Source: Bates, et al. 2001).

Raw orange juice includes some solid particles. These particles are known as pulp. The pulp extractors are used to remove the solid particles from the fruit juice. The excessive amount of pulp has to be removed in respect of the quality characteristics. 65-90 % (w/w) of the pulp, removed by the pulp extractor. The remaining part of the solid particles after pulp extraction, still have PME enzyme in them and this causes problems if it is not inactivated. In the concentration process, pasteurization of citrus juices is required for two reasons: it inactivates the enzymes which would otherwise cause cloud loss in the juice or gelation of concentrates, and it destroys microorganisms which would otherwise cause fermentation and spoilage in the juice. Since the development of the TASTE (Thermally Accelerated Short Time Evaporator) system in the late 1950s, it has become a standard in the production of FCOJ (frozen concentrated orange juice) in the citrus processing industry. The TASTE system combines pasteurization and high temperature, short time evaporation processes without recirculation to minimize the effect of temperature-time on quality degradation of the product. Although, the TASTE process suffers from the loss of certain natural flavors and the denaturing of other flavor compounds (Cemeroğlu 2004).

Inactivation of PME enzyme is the target for pasteurization because of its resistance to heat. Since the citrus juices have low pH values, even at 75°C the fruit juices become microbiologically sterile (Cemeroğlu 2004). However, it was shown that PME has more thermal resistance than the pathogenic microorganisms which can be found in citrus juices. Thus, inactivation of PME is used as an indicator for pasteurization adequacy (Collet, et al. 2005). The recommended temperature/time requirement for achieving a two log cycle reduction in PME activity and commercial stability is 90°C for 1 minute (Graumlich, et al. 1986). Subsequently pasteurized juice is concentrated by using evaporators. After the evaporation, 65 °Brix concentrate is obtained. In order to obtain a 45 °Brix concentrate of orange juice, fresh orange juice is added to the concentrate and this added juice is known as cut-back orange juice. Cut-back orange juice contains high amounts of pulp. The lost flavor compounds of the concentrate orange juice can be recovered by adding cut-back orange juice. 45°Brix concentrate is then cooled to -4 °C and filled into the containers. The containers are frozen in a freezing tunnel at -45 °C and stored at (-23)-(-26) °C. In the reconstitution, water is added to the concentrate and then the juice is filled to the consumer packages (Cemeroğlu 2004).

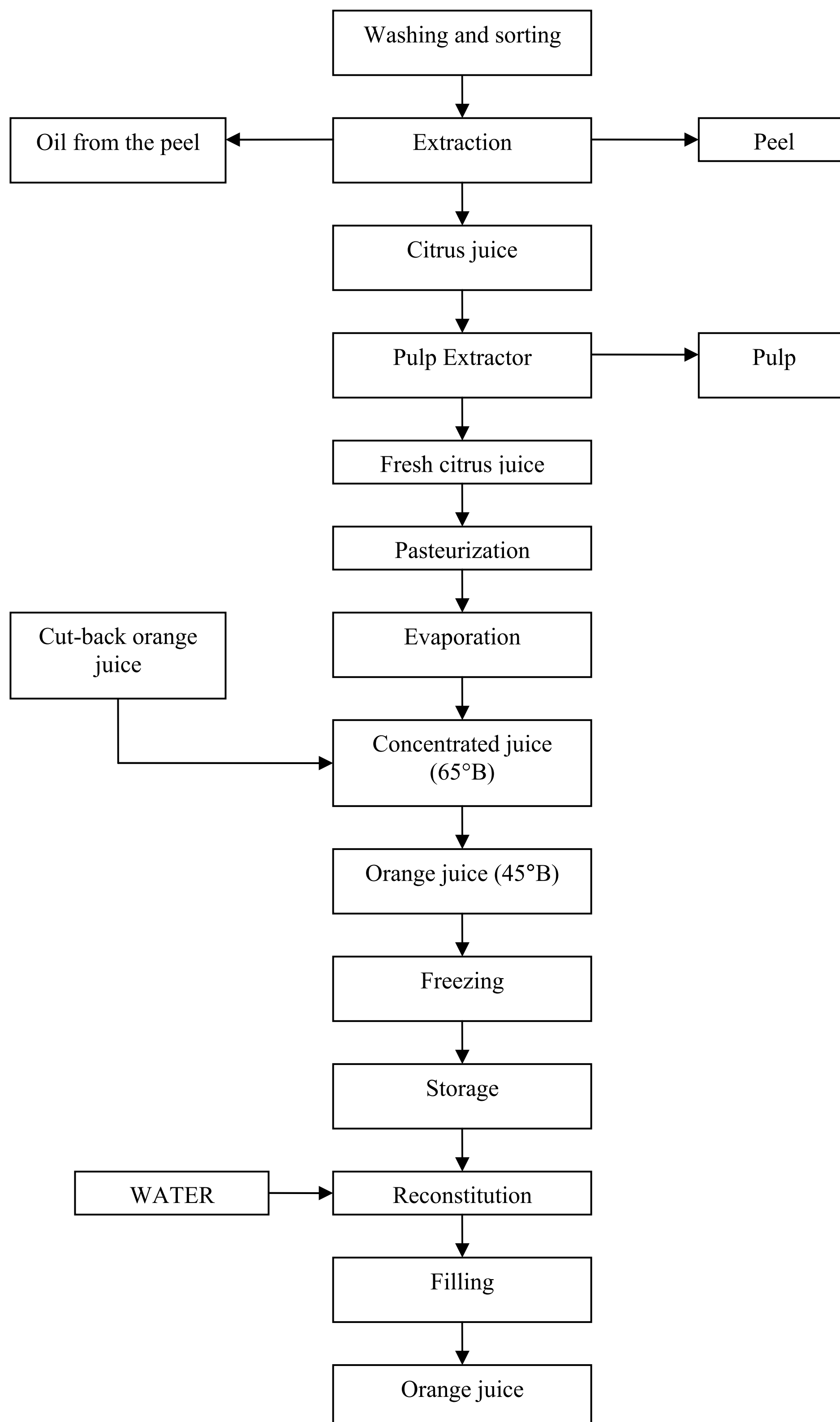


Figure 2.3. Flow diagram of orange juice production.
(Source: Cemeroğlu 2004)

2.3. Juice Spoilage and Deterioration

Microbial, enzymatic, physical and chemical reactions which cause deteriorations in the product, determine the shelf life of the fruit juices (Graumlich, et al. 1986). Natural juices have a short shelf life due to the alterations intensively occurred after the extraction. Even if the juice is stored at refrigerator conditions, undesirable sensorial characteristics can develop and nutritional value reduces (Corrêa de Souza, et al. 2004). Table 2.2 summarizes the main causes of fruit juice deterioration and spoilage.

Table 2.2. Juice Safety and Deterioration Hazards
(Source: Bates, et al. 2001).

Hazard/Deterioration	Result
Microbial contamination	Survival/growth of pathogens
Microbial contamination	Rapid spoilage
Aflatoxins on fruit	Unsafe/illegal product
Pesticide residues	Unsafe/illegal product
Spurious dissolved matter	Unsafe, off-flavor
Enzymatic activity	Browning, consistency/flavor changes
Dissolved oxygen	Browning, nutrient and quality reduced
Metalic cations	Flavor/color/nutrient losses, unsafe
Maillard reactants	Browning, quality loss
Colloidal instability	Sedimentation/precipitation/haze
Extended holding	Quality deterioration

Fresh squeezed orange juice is very popular all over the world (Zook, et al. 1999, Bull, et al. 2004, Elez-Martinez, et al. 2006). However, quality of the fresh orange juice is decreased by microorganisms (Min, et al. 2003). Mainly fermentative yeasts are found to be responsible for the spoilage of the orange juice (Parish 1998, Zook, et al. 1999, El-Hag, et al. 2006, Tahiri, et al. 2006, Campos and Christianini 2007). Hodgins, et al. (2002) reported that acid tolerant bacteria including lactic acid bacteria like *Lactococcus, Lactobacillus* and *Leuconostoc* species can be found in orange juice, also

acetic acid bacteria contribute to the spoilage of fruit juices which have pH values about 4 (Lott and Carr 1964).

2.3.1. Important Enzymes in Fruit Juice Products

Deterioration of fruit and vegetable products is highly correlated with the enzyme activity. The most important enzymes in respect of the quality of fruits and vegetables are peroxidase, catalase, lipoxygenase, catechol oxidase (referred to as polyphenol oxidase) and pectinesterase (referred to as pectinmethylesterase). It was reported that pectinesterase involves in the development or loss of textural characteristics (Vora, et al. 1999). Pectinmethylesterase (PME) is a deesterification enzyme and it is the most important one for the citrus products (Ludikhuyze, et al. 2003, Ingallinera, et al. 2005). It hydrolyzes the methyl ester units giving pectic acid and methanol (Beltran, et al. 2005). After the hydrolysis, low methoxy pectin is occurred and it links up with polyvalent cations like Ca^{2+}. This cross-linking forms insoluble pectate precipitates and causes the cloud loss in turbid juices (Guiavarc'h, et al. 2005). Ingallinera, et al. (2005) found that inactivation of PME is related to the pasteurization temperatures. However, all PME activity can not be inactivated by pasteurization due to the existence of thermostable fraction of the enzyme (Kim and Tadini 1999). Vora, et al. (1999) investigated the conditions in order to completely inactivate the enzyme in Australian carrots. They found that at 70°C PME enzyme was totally deactivated in 10 minutes. The inactivation of PME enzyme and its izoenzymes found in orange juice was reported to be provided by processing the juice at 90°C for 1 minute (Nienaber and Shellhammer 2001).

2.3.2. Microorganisms Effective on Fruit Juice Quality

Many organisms, especially acid-tolerant bacteria and fungi (yeasts and molds) can cause spoilage in the fruit juice using fruit as a substrate. Discoloration, off-flavors,

off-odors may develop in the product. The most common fruit juice spoilers are yeasts (Tournas, et al. 2006). Low pH values of the juices prevent the survival of many types of bacteria and provide a suitable environment for yeasts, molds and a few groups of aciduric bacteria. It was demonstrated that *Bacillus coagulans* is responsible for the flat-sour type spoilage in acidic beverages. It has the ability to grow at pH values as low as pH 4.0 (Chang and Kang 2004). Even though the fruit juices have low pH values and thermal pasteurization can damage spoilage microorganisms, a bacterium called *Alicyclobacillus acidoterrestris* is found to survive under normal pasteurization conditions. It is a non-pathogenic, spore-forming and thermoacidophilic bacterium which is isolated from several commercial pasteurized orange and apple juices (Silva and Gibbs 2001). It can grow in a pH range of 2.5-6.0 at temperatures 25-60°C (Komitopoulou, et al. 1999). The time needed to destroy 90% of this bacterium at 90°C was found as 10.0-20.6 minutes. This much high D-value makes the pasteurization process unsuccessful to destroy the microorganism in orange concentrates (Karagözlü 2004). In recent times, *Alicyclobacilli* are considered as quality control microorganisms in the manufacturing of acidic beverages (Matsubara, et al. 2002). This microorganism causes economic losses and off-flavors in fruit juices (Chang and Kang 2004, Karagözlü 2004).

Although many yeast species can tolerate a broad range of pH, from 1.5 to 10.0, most of them prefer proliferation in a slightly acidic environment ranging from pH 3.5 to 6.0. More than 800 species of yeasts have been identified. However, a small portion of these are considered as spoilage ones (Martorell, et al. 2007). Yeast species commonly isolated from fruit juices are; *Rhodotorula rubra, Candida lambica, Candida sake*, *Kloeckera apis, Hanseniaspora uvarum, Saccharomyces cerevisiae, Zygosaccharomyces bailii, Zygosaccharomyces rouxii, Torulaspora delbrueckii* and *Candida intermedia* (Arias, et al. 2002, Stratford, et al. 2002, Oliveira, et al. 2006, Tournas, et al. 2006, Martorell, et al. 2007). *H. uvarum* is demonstrated to be the only species which was isolated from both pasteurized and unpasteurized orange juice. *Torulaspora delbrueckii, Candida intermedia* and *Saccharomyces cerevisiae* are fermentative yeast species and they are not able to survive after processing of the product (Arias, et al. 2002).

It was reported that pasteurization conditions applied to the fruit juices are adequate to inactivate molds. Though, some species of *Penicillium, Aspergillus* and *Byssochlamys* can release patulin under low pH values (Ünlütürk and Turantaş 2003). If

decayed fruits are used in the production of fruit juices patulin can transfer into the product due to its solubility in water. It is very stable at acidic conditions and conventional heat processing is not able to deactivate this toxin (Acar, et al. 1998). High temperature-short time (HTST) treatments (60, 80, 90°C for 10s) and batch pasteurization (90°C for 10 min) were demonstrated to be effective on the reducing of patulin levels in apple cider but complete damage could not be reached (Kadakal, et al. 2002). Studies showed that patulin has several effects on health. It acts as a carcinogenic, mutagenic and teratogenic agent (Acar, et al. 1998, Morales, et al. 2007). Several European countries and World Health Organization (WHO) recommend a regulatory limit of 50µg/L for patulin in apple juice (Sylos and Rodriguez-Amaya 1999). However, Gökmen and Acar (1998) found that apple juice concentrates produced in Turkey exceed this limit. In another study of Gökmen and Acar (2000), it was demonstrated that preventive treatments help to reduce patulin levels through years.

2.3.3. Health Concerns about Consumption of Unpasteurized Juices

According to Foley, et al. (2002) unpasteurized juices caused 76% of juice contamination cases between 1993 and 1996. Approximately 16000 to 48000 cases of illnesses are associated with the consumption of unpasteurized juices in a year (Foley, et al. 2002).

Pasteurization reduces the initial microbial load and prevents the product from being spiked with pathogens and other heat-sensitive microbes. If the pasteurization is not adequate, microorganisms able to survive can cause spoilage and illnesses. Many reports about the illnesses due to the contaminated juice are dealing with the unpasteurized juice (Tournas, et al. 2006). Contamination of fresh juices with pathogenic microorganisms such as *Escherichia coli* O157:H7 and *Salmonella* was demonstrated to cause many illnesses and fatalities (Table 2.3).

Table 2.3. Fruit juice-associated Food Poisoning Outbreaks
(Source: Bates, et al. 2001).

Juice Product (Year)	Infectious Agent
Sweet cider (1923)	*Salmonella typhi*
Orange juice (1944)	*S.typhi*
Orange juice (1962)	Hepatitis A
Orange juice (1966)	Gastroenteritis agent
Apple cider (1974)	*S. typhimurium*
Apple cider (1980)	*Enterotoxigenic E. coli*
Orange juice (1989)	*S. typhi*
Apple cider (1991)	*E. coli* O157:H7
Orange juice (1992)	*Enterotoxigenic E. coli*
Apple cider (1993)	*E. coli* O157:H7
Apple cider (1993)	*Cyrptosporidium spp*
Carrot juice (1993)	*C. botulinum*
Watermelon juice (1993)	*Salmonella spp*
Orange juice (1994)	Gastroenteritis agent
Orange juice (1995)	*S. hatford, S. gaminera, S. rubislaw*
Apple juice (1996)	*E. coli* O157:H7
Apple juice (1996)	*E. coli* O157:H7
Apple juice (1996)	*Cyrptosporidium parvum*
Apple cider (1997)	*E. coli* O157
Orange juice (1998)	*Salmonella*
Apple cider (1998)	*E. coli* O157:H7
Mamey juice (1999)	*S. typhi*
Orange juice (1999)	*Salmonella enterica*
Orange juice (1999)	*Salmonella muenchen*
Apple cider (1999)	*E. coli* O157:H7
Orange, grapefruit, lemonade (2000)	*Salmonella enterica*

Due to their low pH value, fruit juices were previously thought to be safe. However, recent outbreaks of *Escherichia coli* O157:H7 associated with the consumption of unpasteurized juices show the potential of acidic juices to carry pathogenic microorganisms. Besides, unpasteurized orange juices were reported to harbor *Salmonella.* It was estimated that annually 2 to 4 million cases of salmonellosis appear in the United States (Cook, et al. 1998).

E. coli O157:H7 is a gram-negative *Bacillus*. The O refers to the somatic antigen and the H refers to the flagellar antigen. It belongs to the group of Shiga toxin-producing *E. coli* (STEC) (Besser, et al. 1999). This microbe has the ability to survive under acidic conditions in foods like apple cider. However, it can be easily inactivated by pasteurization (Buzby, et al. 1996). *E. coli* O157:H7 was first identified as an agent responsible for a life threatening gastrointestinal illness called Hemolytic Uremic

Syndrome (HUS) in 1982. The disease was characterized by abdominal cramping, vomiting, and bloody diarrhea, kidney failure and neurological complications (Buzby, et al. 1996). Frenzen, et al. (2005) denoted that the annual cost of the diseases resulted from O157 STEC was $405 million including $370 million for premature deaths, $30 million for medical care and $5 million for lost productivity in 2003 .

2.4. Preservation Method of Fruit Juices

As a result of recent outbreaks related to the juice products, FDA (Food and Drug Administration) suggested new rules to be confident about the safety of fresh and processed fruit and vegetable juices. These rules demand the adaptation of HACCP principles for the prevention of bacterial, chemical and physical contamination (Cook, et al. 1998).

There are a number of preservation techniques to inhibit spoilage. The main idea of the food preservation is to maintain the quality and nutritional characteristics while preventing spoilage. As a general rule, the fresher the juice is, the higher the quality is (Bates, et al. 2001).

2.4.1. Thermal Pasteurization

The word “pasteurization” describes a mild heat treatment which is applied at temperatures below 100°C. It is a very old technique used to preserve food materials. Pasteurization is designed for the inactivation of important enzymes with respect of quality and vegetative forms of microorganisms can be found in the food product. In the designing of a thermal process, heat resistance of the target microorganism for that product and the pH value of the food material should be considered (Silva and Gibbs 2004).

The pasteurization target for high-acid fruit products varies with the product. Generally the most heat resistant or the most common spoilage microorganism or enzyme is selected (Silva and Gibbs 2004).

It is known that conventional heat pasteurization can effectively reduce the number of pathogens such as *E. coli* O157:H7, *Salmonella* sp., *Listeria monocytogenes* and *Cryptosporidium parvum* and suitable for all types of juices (Tandon, et al. 2003). The NACMCF (National Advisory Committee on Microbiological Criteria for Foods) has recommended the use of *E. coli* O157:H7 or *Listeria monocytogenes* as a target microorganism and suggested a 5-log reduction in the target pathogen. These two pathogens are the most resistant microorganisms of public health concern. FDA accepts the ability of thermal pasteurization to meet the NACMCF's criteria. However, there are some disadvantages of this process;

- The technique is cost prohibitive, the equipment is expensive,
- Heating causes off-flavors
- Nutritional value of the product degraded by the applied heat (FDA 1998).

2.4.1.1. Effect of Thermal Pasteurization on the Product Quality

Although heat treatment is necessary to obtain safe foods, it has some undesired effects on the quality of foods. According to the study of Lee and Coates (1999), slight visual differences after thermal pasteurization of red grape juice were reported. The pink to red color of the juice due to lycopene is denoted to be unstable during processing and storage. The juice can develop a muddy, brown color which is unacceptable by the consumers. In their study, most samples were found to show slight increases in L* value after pasteurization which indicated a lightening of juice surface color. A small increase in L* value was associated to partial precipitation of unstable, suspended particles in the juice. After thermal treatment, b* values exhibited more positive values. Changes in a* followed a similar trend. Results suggest that color of the sample got more yellow, more red, slightly lighter and brighter after the pasteurization (Lee and Coates 1999).

Choi and Nielsen (2004) reported that thermal processing can greatly affect the cider color due to the degradation of anthocyanins found in apples. The pasteurized cider was significantly lighter than control and UV treated ciders over 21 day of storage (Choi and Nielsen 2004).

Alper, et al. (2005) found that during heat treatment browning degree increased in pomegranate juice.

It is known that an increase in pH value can cause microbial growth in fruit juices. Acidity in apple juices is an important sensory attribute associated with its characteristic flavor and astringency. HTST was found to affect the pH and the acidity of the juice due to the increase in temperature which causes evaporative effect of organic acids (Aguilar-Rosas, et al. 2007).

In the case of thermal pasteurization of orange juice, shelf life of the product is determined according to the presence of 50% ascorbic acid loss. Results of sensorial analysis suggested that pasteurized orange juice has lower shelf life (Polydera, et al. 2005).

Aroma-flavor authenticity of apple juice depends on odour-active compounds. These compounds are: acetic acid, hexanal, butyl hexanoate, ethyl acetate, ethyl butyrate, methyl butyrate, hexyl acetate, 1-hexanal. In HTST treatment acetic acid was reported to be completely lost and ethyl acetate was highly decreased. Comparing with the non-thermally pasteurized samples, it was observed that the other compounds in the pasteurized sample significantly reduced (Aguilar-Rosas, et al. 2007).

Hernandez, et al. (1997) suggested that enzymatic and thermal treatments applied during juice making may affect phenolic composition. Spanos and Wrolstad (1992) reported that total phenol concentration is reduced up to 50% in the apple juice thermally pasteurized at 80 °C for 15 minutes.

2.4.2. Non-Thermal Alternative Methods

The NACMCF specified the target microorganisms rather than the methods to ensure that the juice does not contain pathogens. Pasteurization is an effective and the most used technology in order to reach safety requirements (FDA 1998). However, for

many producers the cost of the equipment is prohibitive. Increased demand for the fresh-like juice products motivate the researchers to investigate non-thermal alternatives to heat due to the undesirable effects of conventional heat pasteurization on the nutritional and organoleptic properties of the products (Tahiri, et al. 2006, Basaran-Akgul, et al. 2009). Although heat pasteurization is able to destroy microorganisms and inactivate PME in juices, there are other methods which may be equally effective (Tahiri, et al. 2006). Manufacturers are able to use these intervention techniques in order to achieve a 5-log reduction in the target pathogen (FDA 1998). In comparison with the thermal treatment, non-thermal processing methods are reported to preserve nutritional and sensorial characteristics of the product giving a natural taste (Gupta, et al. 2003).

Some of these alternative methods include addition of microbiocidal agents, pulsed electric field, high pressure application, ionizing irradiation, and aseptic packaging. Table 2.4 shows the advantages and disadvantages of these new techniques.

Table 2.4. Advantages and Disadvantages of Minimally Processed Juice
(Source: Bates, et al. 2001)

Advantages	Disadvantages
To consumer: Health image	More expensive
Fresher sensory appeal - flavor, color	Shorter shelf life
Closer to self preparation, but convenient	Quality demands proper storage
"Natural" image	
To marketer: Increased profit	More costly display space
Attractive sales display	Shorter sales life
Promotes fresh produce sales	Handling mistakes costly
High turnover	
To manufacturer: Simplest process	Higher quality fruit required
Adds value to cull fruit	Dictates very careful handling
All juices pass through this step	Safety responsibility high
High seasonal turnover	

2.4.2.1. Microbiocidal Agents Added to Inactivate Microorganisms

Chemical agents used in the preservation of foods are cheap and easy to utilize because of the limited equipment requirements (Moon, et al. 2006). Increased demands

for more natural foods support the use of natural antimicrobials such as peroxidase-based enzyme systems. These enzymes catalyze oxidation reactions yielding short-lived products which have antimicrobial properties. It was reported that low pH values increase the efficiency of peroxidases. Studies suggest the use of lactoperoxidases (LPER) in fruit juices. Although application of peroxidases in the fruit juices caused no color change, significant amount of ascorbic acid loss was observed. This is a disadvantage of these systems (Opstal, et al. 2006). The use of dimethyl dicarbonate (DMDC) in the fruit juices as a microbial control agent is mentioned in the literature. Addition of DMDC into wine and beverages was approved by FDA in 1988. It shows preventive activity primarily against yeasts, molds and bacteria. A 5-log reduction of *E. coli* O157:H7 was observed by adding 250 ppm DMDC into the apple cider. Also SO_2 was shown to have the ability to reduce the number of *E. coli* O157:H7 in apple cider. A 5-log reduction requirement can be achieved by using this agent however longer holding times are indicated to be necessary (Basaran-Akgul, et al. 2009). In addition to these agents, carvacrol and *p*-cymene were demonstrated to be lethal against spoilage yeasts and *E. coli* O157:H7 (Kisko and Roller 2005). Schurman (2001) found that the application of hydrogen peroxide/ organic acid (H_2O_2/ OA) treatment in white grape juice resulted in the highest reduction of *E. coli* O157:H7 than in purple grape juice. Hydrogen peroxide was shown to be a promising agent for *Salmonella* spp. in orange juice, *E. coli* O157:H7 in apple cider and white grape juice (Schurman 2001).

2.4.2.2. Pulsed Electric Field

Pulsed electric field (PEF) technology involves the use of pulses of high electric field (15-80 kV/cm) to liquid or semi-liquid foods positioned between two electrodes at ambient, sub-ambient or slightly above ambient temperature (Gupta, et al. 2003, El-hag, et al. 2006). Gupta, et al. (2003) reported more than 5-log reduction in the number of *E. coli* K12 in apple juice after the application of 40 kV/cm and 100 pulses. In another study a 4.5-log inactivation of *E. coli* O157:H7 in apple juice was observed using a bench scale PEF system (Akdemir Evrendilek, et al. 2000). Charles-Rodri’guez, et al. (2007) indicated 7-log reductions in the number of *E. coli* 8739 after the application of

36 kV/cm. In addition, no definite trend was observed for pH and color following the PEF treatment (Charles-Rodri'guez, et al. 2007). It was also reported that PEF system can effectively preserve the vitamin C content, naturally occurring sugars and the color characteristics in apple juice and cider (Akdemir Evrendilek, et al. 2000, Gupta et al. 2003). On the other hand, it was demonstrated that PEF treatment causes brightness in the color of processed orange juice during storage (Min, et al. 2003). Several factors such as electric field strength, treatment time, pulse width, frequency and polarity were found to be effective on the processing of orange juice by high intensity pulsed electric fields (Elez-Mart'inez, et al. 2006). It was speculated that inactivation of naturally grown microorganisms by PEF treatment is difficult. In order to increase the killing efficiency of the PEF, combination with other methods was suggested (El-Hag, et al. 2006). Wu, et al. (2005) combined the use of nisin and lysozyme with PEF and they obtained a 4.4-log reduction in the naturally grown flora of white grape juice by employing 65 kV/cm and 20 pulses at 50°C. Marselles-Fontanet, et al. (2009) concluded that PEF treatment is not efficient on the inactivation of yeasts. Besides, an industrial scale PEF system has yet to be designed and its application to industry is still a problem from the economical point of view (Tahiri, et al. 2006).

2.4.2.3. High Pressure Technology

High pressure processing, high hydrostatic pressure and ultra-high pressure are all the names for the same process (Morris, et al. 2007). High pressure technology includes the use of pressures of 100-1000 MPa at temperatures ranging from 0-100°C (Bull, et al. 2004, Guerero-Beltran, et al. 2005, Morris, et al. 2007). The inactivation of *Listeria, Salmonella, Escherichia coli* and *Vibrio*, as well as spoilage bacteria, yeasts and molds was reported (Bull, et al. 2004). The first use of high pressure technique for the food processing occurred in Japan in the early 1990s (Fonberg-Broczek, et al. 2005). High pressure processing was demonstrated to be used for inactivation of microorganisms, modification of biopolymers (enzyme activation or inactivation, protein denaturation, gel formation), preservation of quality attributes (flavor and color) and functionality. The mechanism of the system depends on the protein denaturation

and cell injury. The enzymes essential in the cell metabolism are denatured after the pressure treatment. Alternatively, shrinkage may occur in the cell size due to the pressure effect. Consequently, membrane construction can be injured or disrupted causing leakage of the cell content. This brings about cell death (Guerero-Beltran, et al. 2005). Raso, et al. (1998) achieved almost 5-log reductions in the number of *Zygosaccharomyces bailii* after high pressure application at 300 MPa. It was speculated that high research cost of this technique reduces the attention to this new method (Parish 1998). Besides, even if the application of high pressure inactivates yeasts, molds and most bacteria, the elimination of bacterial spores using high pressure is doubtful (Zook, et al. 1999). Also, it was reported that processing of white grape juice samples with high pressure resulted in color changes indicating a slight browning of the samples (Daoudi, et al. 2002). The system was shown to need very high pressures to accomplish a 5-log reduction in viable vegetative cells (Tahiri, et al. 2006). Moreover, it was reported that every 100 MPa cause an increase in the temperature of the product about 3°C, a shift is observed in the pH of foods after the treatment (Morris, et al. 2007).

2.4.2.4. Ionizing Irradiation

Ionizing irradiation is a non-thermal preservation technique which is used to decrease or remove spoilage and pathogenic microorganisms such as *E. coli* O157:H7, *L. monocytogenes* and *Campylobacter jejuni* via fragmenting the genetic material. Irradiation processes can be gamma from radioisotopic sources such as Cobalt60 or Cesium137, electrons or X rays from electron beam accelerators (Morris, et al. 2007).

It was reported that especially protein foods are not appropriate for irradiation process. They may form off-flavor, odor and color. In addition, some fruits may soften and fade after high dosage of irradiation. Unfavorable alterations in the sensorial quality like lipid oxidation, off-flavor and red/pink color formation may take place during the treatment (Morris, et al. 2007). According to the study of Foley, et al. (2002), panelists described the irradiated samples as oily, cooked and medicinal. Although a 5-log reduction in *Salmonella* and *L. monocytogenes* in fresh orange juice was accomplished

by applying 2.65 and 2.4 kGy gamma radiation, the orange juice was reported to be inedible even after the treatment as low as 1.0 kGy (Foley, et al. 2002).

2.4.3. UV-C Radiation

UV-C light treatment is another non-thermal method and has been used to disinfect water systems for many years. This process was reported to be effective on the inactivation of bacteria, protozoa, algae and viruses (Begum, et al. 2009). However, it is not that much effective against yeasts and molds. FDA has approved the use of UV light as a germicidal agent for the disinfection of fruit juices based on the published research data (Bintsis, et al. 2000, Canitez 2002, Tandon, et al. 2003).

2.4.3.1. Description of UV Light

Ultraviolet light is a part of electromagnetic spectrum which ranges between 100-400 nm (Miller, et al. 1999, Bintsis, et al. 2000, Begum, et al. 2009).

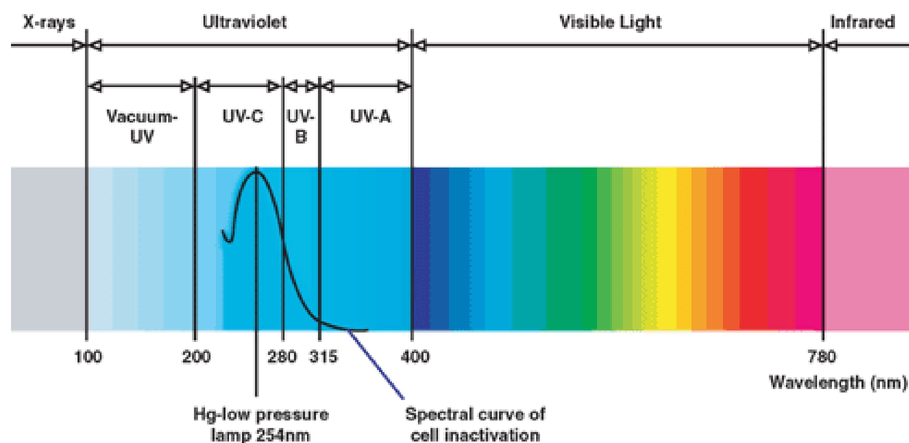


Figure 2.4. The Electromagnetic Spectrum.
(Source: Aqua Best 2007)

The spectrum can be divided into four groups based on wavelengths as it can be seen from Figure 2.4. These subgroups are (Sastry, et al. 2000, Koutchma 2009);

- UV-A (320 to 400 nm) which is responsible for tanning in the human skin,
- UV-B (280 to 315 nm) causes skin burning and further on may lead to skin cancer,
- UV-C (200 to 280 nm) is called as ultraviolet germicidal irradiation (UVGI) range (Beggs, et al. 2000). It destroys effectively bacteria and viruses.
- UV-V (100 to 200 nm) is vacuum UV range. It is absorbed by almost all substances. However, it can only be transmitted in a vacuum.

It was indicated that UV radiation in the range of 250–260 nm is lethal to most microorganisms (Bintsis, et al. 2000, Begum, et al. 2009). The highest germicidal effect of UV light was reported to be observed between 250 and 270 nm. However it tends to decrease with the increase of wavelength (Tran and Farid 2004, and Guerero-Beltran and Canovas 2005). Koutchma (2009) and Oteiza, et al. (2009) declared that the most efficient inactivation can be obtained at 253.7 nm due to the maximum absorption of UV photons by the genetic materials of microorganisms at this specific wavelength.

Mercury lamps are designed to produce energy in the germicidal region (Bintsis, et al. 2000). Low and medium pressure mercury UV lamps were reported to be used for many years in water treatments. Koutchma (2009) stated that FDA allowed the use of low pressure mercury UV lamps for the disinfection of juices.

2.4.3.2. Mechanism of UV-C Radiation

The way of inactivation is related to the absorption of UV photons by DNA or RNA pyrimidine bases (thymine and cytosine in DNA and uracil and cytosine in RNA) (Bolton, et al. 2003, and Koutchma 2009). The incident light causes a dimer formation on the same DNA strand between two adjacent nucleotids. This dimer makes a bulge causing a mutation on the structure of the genetic material. Consequently, transcription and replication are inhibited. This brings about the cell death (Oguma, et al. 2002, Bolton, et al. 2003, Donahue, et al. 2004, and Wang, et al. 2005).

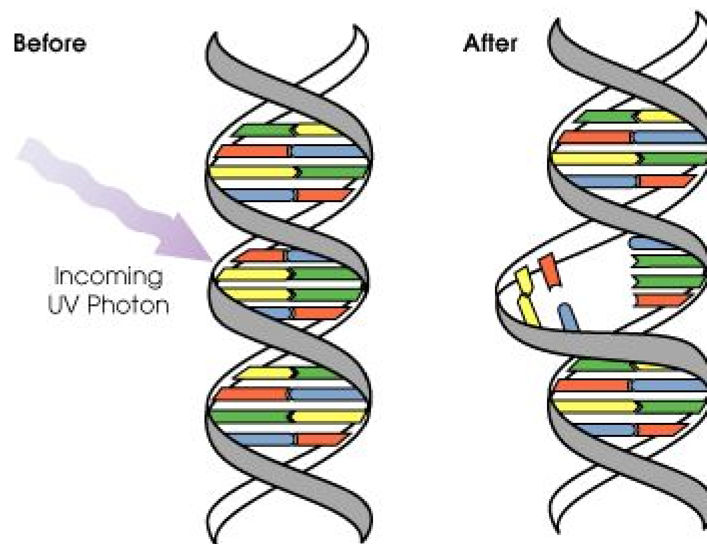


Figure 2.5. Effect of UV-C Light on DNA Structure.
(Source: Infralight 2007)

2.4.3.3. Application Area of UV-C Light Treatment

UV-C light is very well known for its antimicrobial property and used for the decontamination in hospitals, pharmaceutical industry, and public buildings (Begum, et al. 2009). Disinfection of water effluent, drinking water and water for swimming pools by UV-C light was reported. It is also used for the disinfection of the surface of an actual food. Reduced total aerobic and mould counts, inactivation of *Salmonella typhimurium* on the egg shells were reported. It was also suggested that UV-C sterilized packaging materials increase the shelf life of the products (Bintsis, et al. 2000). In strawberry fruit, improved shelf life, reduced decay and softening were observed after the UV-C treatment (Pan, et al. 2004). Nigro, et al. (1998) indicated increased resistance to *Botrytis cineria* in table grapes. Moreover, it is found that exposure to UV light induce the production of resveratrol which is a stilbene type polyphenolic compound in grape berry tissues (Nigro, et al. 1998, LeBlanc 2006). This compound is known as its health benefits. It was denoted that resveratrol reduces the risk of cancer, inhibits the platelet aggregation, shows anti-allergenic properties and acts as an antioxidant and anti mutagen (LeBlanc 2006).

2.4.3.4. Factors Affecting the Performance of UV-C Treatment in Liquid Foods

The efficacy of the system depends on the absorbance of the medium, moisture content, amount of solid particles and suspended materials, flow rate of the fluid, fluid thickness, reactor design, UV intensity which is related to the age of lamps used, exposure time, type of microorganisms and growth phase of the organism, and initial microbial density (Sommer, et al. 1996, Bintsis, et al. 2000, Hassen, et al. 2000, Guerero-Beltran and Barbosa-Canovas 2005, Koutchma, et al. 2006, Caron, et al. 2007, Morris, et al. 2007, and Begum, et al. 2009).

Caron, et al. (2007) suggested that suspended particles and dissolved matters increase the absorbance of the medium. Increased absorbance of the liquid being irradiated reduces the effectiveness of the process because of the raised absorption of incident UV (Murakami, et al. 2006). Also, particulate matters can reduce the efficiency of UV by scattering the incident light (Caron, et al. 2007). In addition to the attenuation of UV light, suspended particles provide a protective shield for the microorganisms (Koutchma 2009). Guerero-Beltran and Barbosa-Canovas (2005) demonstrated that bacteria suspended in air are more sensitive to the light treatment than bacteria suspended in liquids. It was also reported that the order of resistance of microorganisms against UV-C light follows an increasing order starting with bacteria, viruses, fungi, spores and cysts (Hassen, et al. 2000). Since yeasts and molds contain less pyrimidine bases, they are indicated to be more resistant to the UV-C light disinfection process. Furthermore, microorganisms in the lag phase are found to be more resistant than the ones in the log or stationary phase (Koutchma et al. 2006).

According to Koutchma, et al. (2007) the reactor should be designed in order to increase the inactivation efficiency of the system. The use of turbulent flow and extremely thin film is suggested to enhance the effectiveness of UV-C in liquid foods.

2.4.3.5. Advantages and Disadvantages of UV-C Light Processing

One of the most important advantages of the UV-C light processing is the low cost of the operation and ease of use (Bintsis, et al. 2000). The equipment needs less space than the ones used in other methods (EPA 1999). Also, UV light is effective against most type of microorganisms (Bintsis, et al. 2000). It is a physical treatment which does not cause chemical residues. Hence, it is not harmful to humans or aquatic life (EPA 1999, Canitez 2002).

However, lack of penetration in highly absorptive media or in the case of existence of organic matters, and reduced effectiveness due to the presence of the suspended particles are the disadvantages of this technique (EPA 1999).

CHAPTER 3

MATERIALS AND METHODS

3.1. Raw Materials

3.1.1. White Grape Juice Samples

Pasteurized white grape juice samples made from seedless Sultana variety used in this study were purchased from a company in Turkey (Kavaklıdere Winery, Ankara). Pasteurized samples were selected in order to be sure that background microflora did not affect the run of experiments. All samples were from the same batch and stored at 4°C in the refrigerator.

3.1.2. Preparation of Freshly Squeezed Orange Juice Samples

Fresh oranges from Washington variety used to obtain orange juice were purchased from a local market in İzmir and kept in the refrigerator. Before juice extraction washed oranges were cut into half and pressed by using a household table top citrus juice extractor (Arçelik, Robolio, İstanbul).

3.2. Measurement of Physical and Chemical Properties

3.2.1. Density Values of Juice Samples

Density values of the fruit juices were determined by the portable type density meter (Kyoto Electronics DA, Japan) at 20°C.

3.2.2. Determination of pH Values of Samples

Measurement of pH values of the samples were carried out by using a benchtop pH meter (HANNA Instruments, USA) at 20°C.

3.2.3. Determination of Brix Levels

Mettler- Toledo RE40D Bench top Refractometer (AEA Investors Inc., U.S.A.) was used to determine the brix levels of the juice samples. A few drops of the sample were put into the reading cell and the results at 20°C were recorded.

3.2.4. Titratable Acidity

Appropriate amount of sample (ranges between 10 to 25 mL) was poured in a flask and sample was titrated against standardized 0.1N NaOH up to pH 8.1 which is the phenolphthalein end point. The volume of NaOH used was recorded. The acidity of the sample was calculated according to the following formula (3.1). The most common acid

in grape juices is tartaric acid and for citrus juices citric acid is the dominant one. Thus, results were expressed as grams of tartaric or citric acid per 100 mL of fruit juice.

$$TA, \% = (V)\ (f)\ (E)\ (100)\ /\ M \qquad (3.1)$$

V; represents the volume of 0.1N NaOH used up during the titration (mL), f; is the normality factor, E; is the miliequivalent weight of citric or tartaric acid (g), M; is the volume of the sample (mL).

3.3. Measurement of Optical Properties

3.3.1. Absorbance of Juice Samples

Absorbance values of juice samples were determined using Cary 100 UV-Visible Spectrophotometer (Varian, USA) at 254 nm. Different dilution factors were applied (1:10, 1:25, 1:50, 1:100, 1:250, 1:500 and 1:1000). Absorbance coefficient was estimated from the slope of absorbance versus sample concentration plot.

3.3.2. Turbidity of Juice Samples

HACH 2100AN IS Turbidimeter was used to find out the turbidity value of juices. 45-50 mL of the sample was poured into the glass cuvettes of the equipment and measurements were done twice for each measuring cell. The results were given in Nephelometric Turbidity Unit (NTU).

3.3.3. Color Measurements of Juice Samples

Color parameters of juice samples were detected employing Konica Minolta CR 400 chromameter (Konica Inc. Japan). CIE L^*(brightness-darkness), a^* (redness-greenness), and b^* (yellowness-blueness) values were obtained before and after the UV exposure. Also total color difference (ΔE) was calculated according to the following formula (3.2). The data were analyzed by using Analysis of Variance.

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3.2)$$

3.4. Biodosimetric Studies

3.4.1. Background Microflora Analysis of Fruit Juice Samples

In order to determine total yeast and mold count Potato Dextrose Agar (PDA) acidified with 10% tartaric acid was used. Plates were incubated at 25°C for 5 days. Total aerobic count was conducted by using Plate Count Agar (PCA) and plates were incubated at 30°C for 3 days. Violet Red Bile Agar (VRBA) medium was utilized to check whether the test samples included coliform organisms. Plates were incubated at 37°C for 24h.

3.4.2. Target Microorganism and Growth Condition

In the UV-C treatment studies of white grape juice, non-pathogenic strain of *Escherichia coli* K12 (ATCC 25253) was used. Gabriel and Nakano (2009) found that *E. coli* O157:H7 is more heat resistant than *Listeria monocytogenes* and *Salmonella*. Thus the use of *E. coli* O157:H7 as a pasteurization target is more appropriate.

Koutchma, et al. (2004) indicated that *E. coli* K12 showed almost the same UV light sensitivity with *E. coli* O157:H7. It was also reported that *E. coli* K12 is a nonhazardous surrogate microorganism (Gupta, et al. 2003). Considering all of these points; the target for the inactivation processes was selected as *E. coli* K12. Stock cultures were prepared stored in 20% glycerol stocks at -80°C during the study. 10 µL of stock culture was inoculated into a test tube containing 10 mL nutrient broth in order to prepare a subculture. Test tubes were incubated for 18h at 37°C to reach a stationary phase culture at a concentration of 10^8 CFU/mL.

In the UV-C treatment of orange juice samples, inactivation target is the natural flora occurred in freshly squeezed orange juice after the incubation at 22°C for 3 days under continuous shaking at 200 rpm. The obtained juice were poured into the flasks and incubated in order to obtain naturally spoiled juice samples.

3.4.3. Bench Top UV-C Irradiation Equipment

UV biodosimetry studies were performed in order to determine the logarithmic reduction level of the target microorganism by using bench scale collimated beam apparatus which was described by Bolton, et al. (2003). Juice samples were exposed to UV-C light using closed bench top ultraviolet system (Figure 3.1). The system has two identical low pressure mercury vapor UV lamps at 254 nm wavelength (UVP XX-15, UVP Inc., CA, USA) which were mounted on the top. A manually controlled shutter was used to allow or block the incident light to a stage. A hole which has dimensions of a standard Petri dish was placed under the shutter. A platform that is able to move upward or downward by tray system was handled. A cardboard was used in the experiments for the prevention of loss of the incident light. In order to prevent the direct contact of UV light to human skin a cover was closed in front of the system. Before the treatment lamps were switched on about 30 minutes to provide complete activation and the incident light intensity was determined by employing UVX Radiometer (UVP Inc. Upland USA). UVX-25 sensor which is specific for 254 nm wavelength was used to measure the light intensity at the surface of the sample. The sensor was placed below

the light source onto the shaker in order to provide the same distance with the sample. The measurements were given in mW/cm^2 unit.

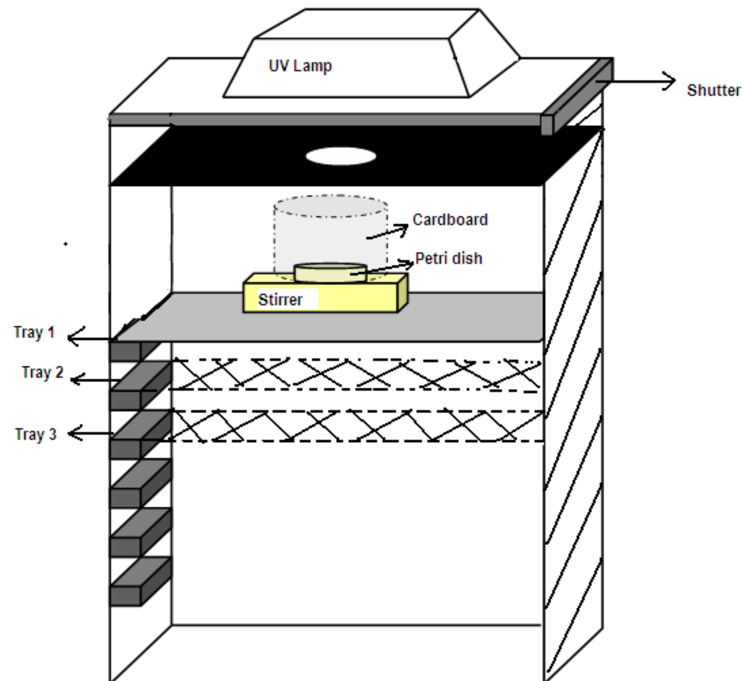


Figure 3.1. Closed Bench Top UV System.
(Source: Atılgan 2007)

3.4.4. Inactivation Studies of *E. coli* K12 in White Grape Juice

Biodosimetric study of *E. coli* K12 was performed using 1.32 mW/cm^2 and 0.38 mW/cm^2 UV incident light intensity levels. Before UV-C treatment, UV intensity (fluence rate) values were determined by using a UVP Radiometer (UVP Inc. Upland USA).

Different amounts of sample volume were added to the standard 50 mm Petri dishes in order to adjust the desired sample depths of 0.153 cm and 0.5 cm. Then, white grape juice samples were spiked with the subculture to obtain a final inoculation rate of 7.81 (±0.09) log CFU/mL or 5.73 (±0.11) log CFU/mL. In order to prevent additional microbial growth after the inoculation of subculture to the sample, the time passed

between the inoculation and UV treatment was kept under control. After the inoculation of pasteurized juice samples, they were exposed to UV radiation of known intensity values (1.32 mW/cm^2 and 0.38 mW/cm^2) for different exposure times. Hence a 2-level factorial design was used and 2 treatment times were selected. These levels were 3 and 10 minutes which were determined according to the preliminary results. Also, there was a control sample which was not treated by UV light. For enumeration, serial dilutions were prepared with sterile 0.1 % buffered peptone water and appropriate dilutions were transferred on to the plates containing tyriptic soy agar (TSA) medium. 0.1 ml of aliquots was spread plated in duplicate. Plates were incubated at 37°C for 24 h and colony counting was performed. All the experiments were conducted between UV dose ranges of 0 - 524.3 mJ/cm^2 and repeated six times. Two parallel sets were performed on the same day and this procedure replicated three times.

Inoculated samples were subjected to different UV dose values. UV dose is the product of exposure time (t = minute) and average UV intensity value ($I_{avg} = mW/cm^2$). Average fluence rate was calculated according to an integration of Beer-Lambert Law (Unluturk, et al. 2008):

$$I_{avg} = I_0 * (1 - \exp(-A_e*L)) / A_e* L \qquad (3.3)$$

In this equation, I_0 represents the incident light intensity (mW/cm^2), A_e value indicates the absorption coefficient (1/cm) and L is the path length (cm).

3.4.5. Inactivation Studies of Natural Microflora in Orange Juice

UV incident light intensity level used in the inactivation study of naturally grown microorganisms in freshly squeezed orange juice and the penetration depth were 1.32 mW/cm^2 and 0.153 cm, respectively. UV intensity level and sample depth were kept at a constant level and were not considered as a factor because of the bad penetration of UV light through the orange juice samples due to their optical properties. Only the exposure time and consequently UV dose received by the sample were

changed during the experiments. 7 different treatment times were studied. Also there was a control sample which was not subjected to UV-C light.

Predetermined amounts of sample were poured into the standard Petri dishes and treated for 3, 6, 9, 12, 15, 18 and 20 minutes. After the light exposure serial dilutions were made with pre-sterilized 0.1% buffered peptone water. Subsequently 0.1 ml of aliquots was spread plated onto acidified PDA medium. Plates were incubated at 25 °C for 5 days and colony counting was performed. During the whole experiments UV dose range of 0 - 144.36 mJ/cm^2 was used. All the experiments were replicated three times.

3.4.6. Statistical Analysis

A 2-level factorial design was used to investigate the effects of sample depth, UV intensity, inoculation rate, and exposure time at 2 different levels of each on the inactivation of *E. coli* K12 in white grape juice. Each experiment week was blocked in order to eliminate the variances caused by the time. There were totally 6 blocks. The response was average log survival number. Totally 48 runs were obtained. Experimental design can be seen in Table 3.1. Analysis of Variance (ANOVA) table was generated by using Design Expert 7.0.0 Trial Version (Stat-Ease Inc., USA).

Table 3.1. Experimental Design Table for White Grape Juice Treatments.

Std	Run	Block	Factor A: Exposure Time (min.)	Factor B: Inoculation rate (log CFU/mL)	Factor C: Intensity Level (mW/cm^2)	Factor D: Sample Depth (cm)
4	4	1.week	10	5.73	1.32	0.153
22	5	1.week	10	7.81	0.38	0.153
43	6	1.week	3	7.81	0.38	0.5
13	7	1.week	3	5.73	0.38	0.153
25	8	1.week	3	5.73	1.32	0.5
16	9	2.week	10	5.73	0.38	0.153
19	10	2.week	3	7.81	0.38	0.153
37	11	2.week	3	5.73	0.38	0.5
28	12	2.week	10	5.73	1.32	0.5
31	13	2.week	3	7.81	1.32	0.5
46	14	2.week	10	7.81	0.38	0.5
1	15	2.week	3	5.73	1.32	0.153
10	16	2.week	10	7.81	1.32	0.153
35	17	3.week	10	7.81	1.32	0.5
5	18	3.week	10	5.73	1.32	0.153
44	19	3.week	3	7.81	0.38	0.5
41	20	3.week	10	5.73	0.38	0.5
26	21	3.week	3	5.73	1.32	0.5
14	22	3.week	3	5.73	0.38	0.153
8	23	3.week	3	7.81	1.32	0.153
23	24	3.week	10	7.81	0.38	0.153
32	25	4.week	3	7.81	1.32	0.5
11	26	4.week	10	7.81	1.32	0.153
38	27	4.week	3	5.73	0.38	0.5
2	28	4.week	3	5.73	1.32	0.153
17	29	4.week	10	5.73	0.38	0.153
47	30	4.week	10	7.81	0.38	0.5
29	31	4.week	10	5.73	1.32	0.5
20	32	4.week	3	7.81	0.38	0.153
27	33	5.week	3	5.73	1.32	0.5
45	34	5.week	3	7.81	0.38	0.5
36	35	5.week	10	7.81	1.32	0.5
9	36	5.week	3	7.81	1.32	0.153
24	37	5.week	10	7.81	0.38	0.153
6	38	5.week	10	5.73	1.32	0.153
42	39	5.week	10	5.73	0.38	0.5
15	40	5.week	3	5.73	0.38	0.153
18	41	6.week	10	5.73	0.38	0.153
30	42	6.week	10	5.73	1.32	0.5
48	43	6.week	10	7.81	0.38	0.5
12	44	6.week	10	7.81	1.32	0.153
3	45	6.week	3	5.73	1.32	0.153
21	46	6.week	3	7.81	0.38	0.153
39	47	6.week	3	5.73	0.38	0.5
33	48	6.week	3	7.81	1.32	0.5

Data analysis for the inactivation of naturally grown microorganisms in freshly squeezed orange juice was performed by Design Expert 7.0.0 Trial Version (Stat-Ease Inc., USA) and Minitab 14.1 (Minitab Inc, US/Canada). One-way ANOVA table was constructed. Also, Tukey's test was applied in order to determine at which level the factor caused significantly different log survival numbers. The only factor of which effect was investigated on the response was exposure time. Thus a one factor design with 8 levels of it was adapted. Totally 24 runs were obtained. The experimental design was shown in Table 3.2.

Table 3.2. Experimental Design Table for Orange Juice Treatments.

Std	Run	Block	Factor A: Exposure Time (min.)
20	1	Block 1	18
19	2	Block 1	18
6	3	Block 1	3
7	4	Block 1	6
17	5	Block 1	15
15	6	Block 1	12
22	7	Block 1	20
9	8	Block 1	6
24	9	Block 1	20
23	10	Block 1	20
21	11	Block 1	18
4	12	Block 1	3
8	13	Block 1	6
14	14	Block 1	12
18	15	Block 1	15
11	16	Block 1	9
1	17	Block 1	0
10	18	Block 1	9
16	19	Block 1	15
5	20	Block 1	3
12	21	Block 1	9
2	22	Block 1	0
3	23	Block 1	0
13	24	Block 1	12

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Physical and Chemical Measurements

Results of physical measurements were shown in Table 4.1. The data were in agreement with the literature (Raso, et al. 1998, Soyer, et al. 2003). Soluble solids (SS) content of white grape juice was found to be 16.59 °Brix at 20°C, however, orange juice SS content was determined as 13.74 °Brix at 20°C. Tahiri, et al. (2006) reported that sugar concentrations (°Brix) can protect the microorganisms from the high pressure inactivation treatments. This may be considered as a limiting factor for the UV irradiation process. Nevertheless, Koutchma, et al. (2004) speculated that individually pH and Brix level did not show an effect on the inactivation of *E. coli*.

Table 4.1. Results of Physical Measurements.

Fruit Juice	pH	Brix (%)	TA (%)	ρ (g/cm^3)
Pasteurized white grape juice	3.22	16.59	0.478 g Tartaric acid/100mL	1.067
Fresh squeezed orange juice	3.67	13.74	1.398 g Citric acid/100mL	1.037

According to Soyer, et al. (2003) tartaric acid content is similar between all types of grape juices (0.407-0.492 g/100mL); however, grape juices obtained from seedless Sultana variety have the maximum tartaric acid content. White grape juice samples which were made from seedless Sultana variety were used in this study and the tartaric acid content was found as 0.478 g/100mL. Also, Karadeniz (2004) reported the citric acid content of sweet orange juices as 1.338 g/100mL. Orange juice samples were

found to have 1.398 g/100mL citric acid. These findings are consistent with the literature.

4.2. Optical Properties

Absorbance coefficients of the juice samples were estimated from the slope of absorbance versus dilution factor curve. As it was shown in Figure 4.1 and Figure 4.2 absorbance coefficients of pasteurized white grape juice and freshly squeezed orange juice were 5.8218 (1/cm) and 71.715 (1/cm), respectively. For orange juice more dilution was needed in order to obtain a reasonable absorbance measurement. Results suggested that orange juice can absorb more UV light.

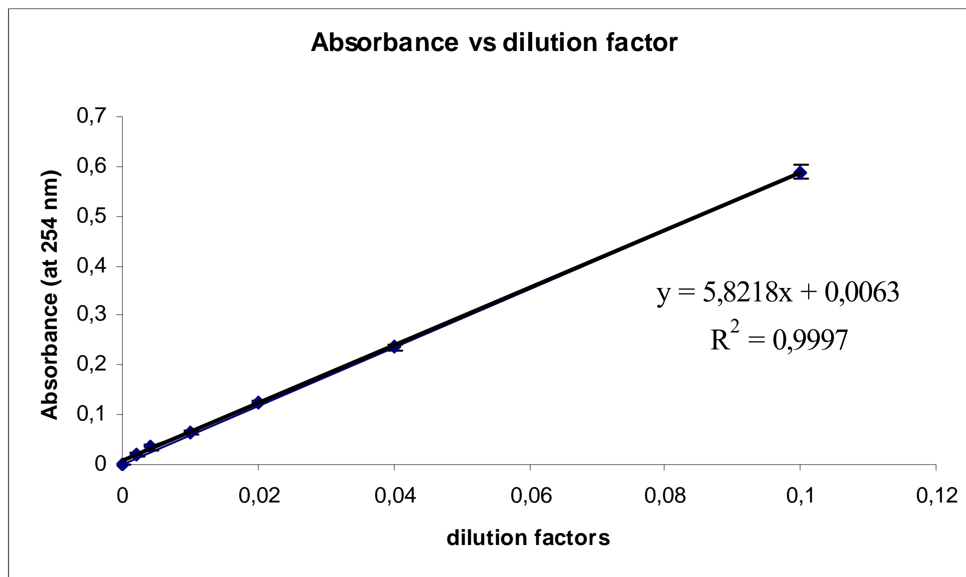


Figure 4.1. Absorbance versus Dilution Factors Plot for White Grape Juice.

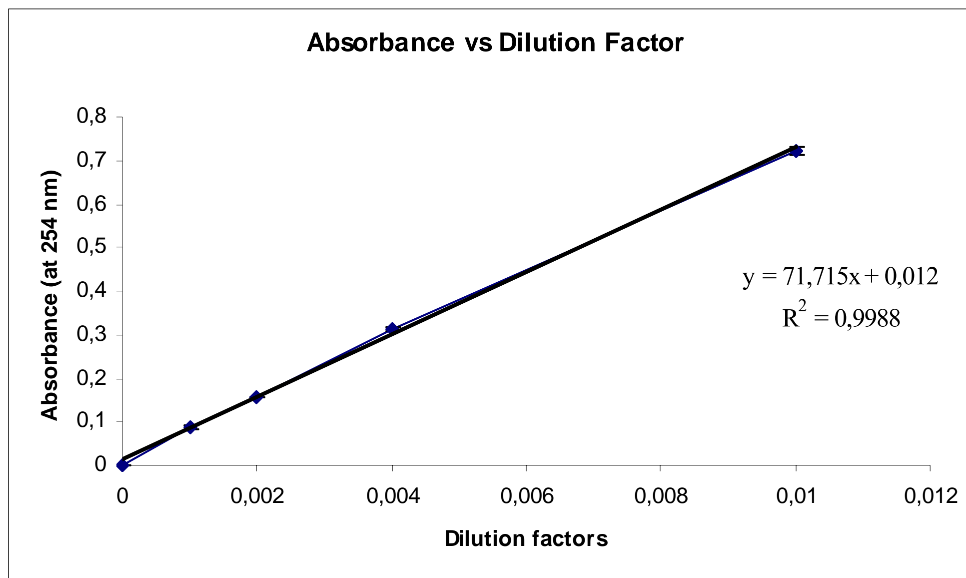


Figure 4.2. Absorbance versus Dilution Factors Plot for Orange Juice.

Turbidity measurements have also indicated that orange juice has significantly higher amounts of suspended particles than white grape juice. This brings about an increase in the absorbance. Begum, et al. (2009) demonstrated that the presence of large suspended particles causes a decrease in the UV penetration by increasing the UV absorptivity. Light scattering caused by the suspended particles was declared to bring about an overestimation of absorbance. It was reported that turbidity values greater than 10 NTU are considered as a reason for the underestimation of the effect of irradiation process (Caron, et al. 2007). Although turbidity value was found as 5.49 NTU for white grape juice, 4061 NTU was obtained for orange juice. This low turbidity value of white grape juice can be attributed to the clarification step of the production. The juice is removed from its suspended particles which make the product cloudy during this operation. Donahue, et al. (2004) demonstrated that turbidity is highly correlated with color. It was reported that the juice color gets darker as the turbidity increases. Lee and Coates (1999) suggested that color differences were more related to changes in b* value, with yellowing of samples. Carotenoid pigments found in the plastids in the juice cells are responsible for the yellow color of orange juice (Ting and Hendrickson 1968, Cortes, et al. 2008). ß-carotene and lycopene are reported as the major pigments which give the visual color of the fresh and pasteurized juices (Lee and Coates 1999). Color parameters of white grape juice and fresh orange juice are shown in Table 4.1.

Table 4.2. Color Measurements for White Grape Juice and Fresh Orange Juice

Sample	L^*	a^*	b^*
White Grape Juice	25.83	1.02	6.72
Fresh Orange Juice	38.81	-2.67	22.72

L^* value represents the luminosity of the sample; a^*, redness-greenness; b^*, yellowness-blueness (Cortes, et al. 2008). In white grape juice, L^* value was found as smaller than L^* value for orange juice. This may be because of the browning reactions occurred during the thermal pasteurization step of the white grape juice. In the literature, it was reported that a decline in lightness (L value) found to be an indication of the browning occurred in fruit juices during thermal treatments (Tiwari, et al. 2008). It was found that lack of polyphenoloxidase enzyme in white grape juice and the low pH value cause browning (Daoudi, et al. 2002). Higher L^* value for the orange juice used in this study can be attributed to being unpasteurized. It was also demonstrated that a^* and b^* values can be used to evaluate nonenzymatic browning. An increase in a^* value towards more positive side is due to the appearance of brown colored melanoidins (Ibarz, et al. 2005). It can be observed that (Table 4.2) b^* value of fresh orange juice was found to be higher than the white grape juice showing more yellowness of the sample. As it was mentioned before, the color differences between juices are more related to the b^* values of the samples and the yellow color of the juices is due to the presence of carotenoids. Higher b^* value means higher content of carotenoids in orange juice. Also, higher b^* value can be considered as an indicator of suspended particles which makes a difference in the visual colors of the juices.

4.3. Microbiological Results of Biodosimetric Studies

4.3.1. Results of the Inactivation of *E. coli* K12 in White Grape Juice

Pasteurized white grape juice samples were cultured on different media to determine the background microflora. According to the results of standard plate count, initial microbial load of the samples were not detectable. Also, no coliform organisms were found in any of the samples.

The best results for the UV inactivation study (5.71-log and 5.75-log CFU/mL) were achieved when minimum inoculation rate (5.73±0.11-log CFU/mL) was used (Table 4.3 and Table 4.4).

Table 4.3. Log Reduction Table (Inoculation Rate: 5.73±0.11 log CFU/mL).

		SAMPLE DEPTH (cm)			
		0.153		**0.5**	
		EXPOSURE TIME (min.)			
		3	**10**	**3**	**10**
UV INTENSITY	**1.32 mW/cm^2**	5.71-log	5.71-log	5.75-log	5.75-log
	0.38 mW/cm^2	2.99-log	5.71-log	2.41-log	5.75-log

Table 4.4. Log Reduction Table (Inoculation Rate: 7.81±0.09 log CFU/mL).

		SAMPLE DEPTH (cm)			
		0.153		0.5	
		EXPOSURE TIME (min.)			
		3	10	3	10
UV INTENSITY	1.32 mW/cm^2	4.1-log	5.07-log	3.86-log	4.77-log
	0.38 mW/cm^2	3.4-log	4.33-log	3.09-log	4.38-log

Minimum UV dose needed to obtain the maximum log reduction was calculated as 75.04 mJ/cm^2 (Table 4.5). The lowest log reduction was obtained as 2.41-log when the lower level of inoculation rate (5.73 ±0.11 log CFU/mL) was used. The juice sample exposed to the minimum UV dose (22.51 mJ/cm^2), which was calculated under the minimum exposure time (3 min.) and minimum intensity level (0.38 mW/cm^2) conditions, at the maximum sample depth (0.5 cm) (Table 4.5).

Table 4.5. UV Dose Values and Log N/N_0 (Inoculation Rate: 5.73±0.11 log CFU/mL).

		UV INTENSITY	EXPOSURE TIME	UV DOSE	Log N/N_0
SAMPLE DEPTH (cm)	0.153	1.32 mW/cm^2	10 min.	524.3 mJ/cm^2	5.71-log
		1.32 mW/cm^2	3 min.	157.3 mJ/cm^2	5.71-log
		0.38 mW/cm^2	10 min.	152.9 mJ/cm^2	5.71-log
		0.38 mW/cm^2	3 min.	45.9 mJ/cm^2	2.98-log
	0.5	1.32 mW/cm^2	10 min.	257.3 mJ/cm^2	5.75-log
		1.32 mW/cm^2	3 min.	77.2 mJ/cm^2	5.75-log
		0.38 mW/cm^2	10 min.	75.04 mJ/cm^2	5.75-log
		0.38 mW/cm^2	3 min.	22.5 mJ/cm^2	2.41-log

The effect of inoculation rate was found to be significant ($p<0.0001$). It was reported that suspended particles prevent the incident UV light and they serve as a protective shield (Koutchma, et al., 2006, Taghipour 2004). Although white grape juice used in this study did not contain high amounts of suspended particles (5.49 NTU), high concentration of the test microorganism inoculated into the juice sample may cause the aggregation of cells and this aggregation forms a shield which can prevent the subjacent cells to be irradiated. Consequently, at the lower amounts of inoculation rate, higher inactivation is expected (Fig. 4.3 and Fig. 4.4).

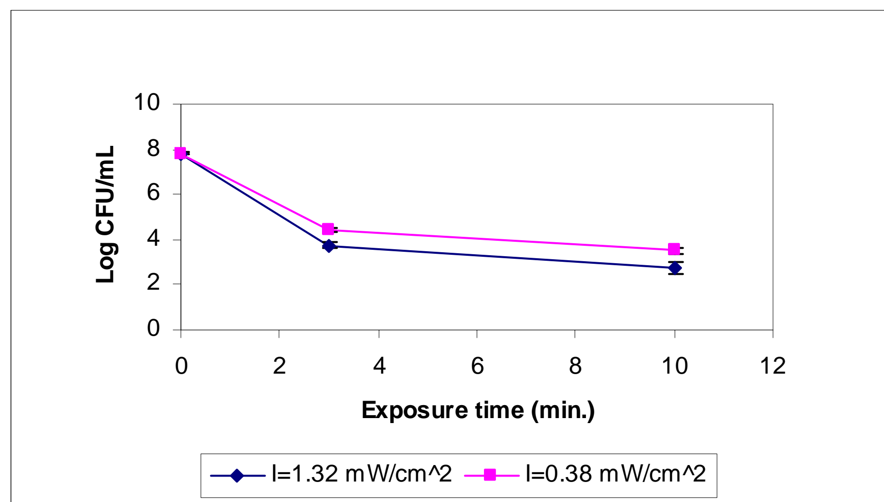


Figure 4.3. Influence of UV-C treatment on *E. coli* K12 inactivation in white grape juice at a sample depth of 0.153 cm and inoculation rate of 7.81-log (±0.09) CFU/mL.

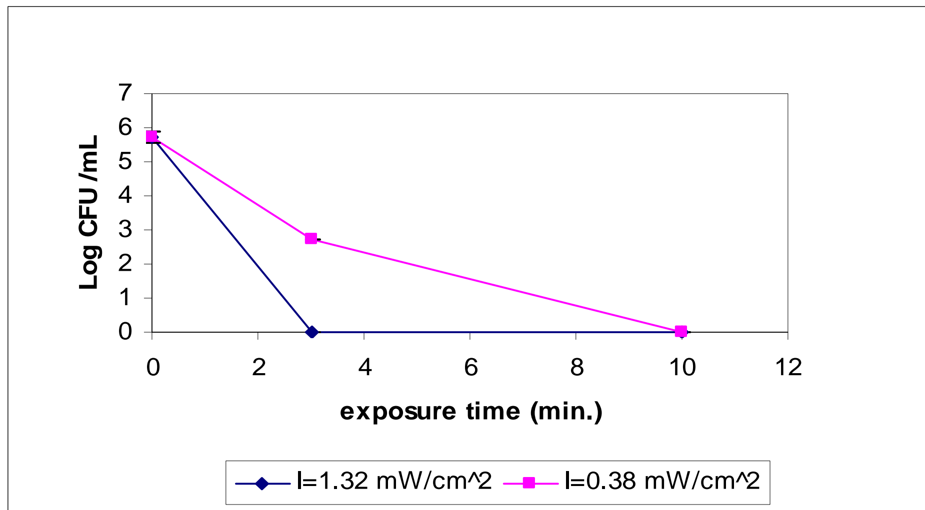


Figure 4.4. Influence of UV-C treatment on *E. coli* K12 inactivation in white grape juice at a sample depth of 0.153 cm and inoculation rate of 5.73-log (±0.11) CFU/mL.

The exposure time significantly ($p<0.0001$) affected the log survival number. However it did not make a difference in the log survival number when the lower concentration (5.73 ±0.11 log CFU/ml) of *E. coli* K12 was used in conjunction with the highest UV intensity value (1.32 mW/cm^2) (Figure 4.5).

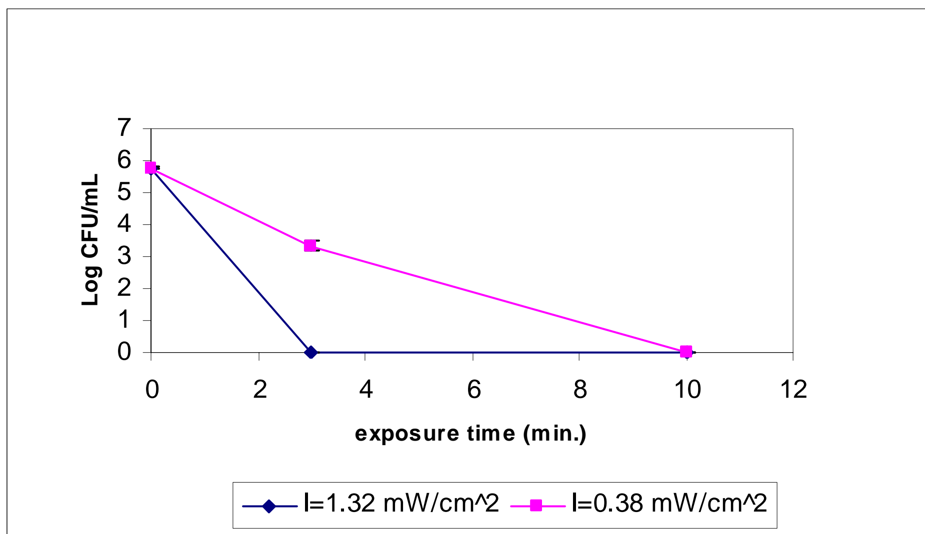


Figure 4.5. Influence of UV-C treatment on *E. coli* K12 inactivation in white grape juice at a sample depth of 0.5 cm and inoculation rate of 5.73-log (±0.11) CFU/mL.

Sample depth was found to be a significantly ($p<0.0001$) effective factor on the response. An increase in the sample depth exhibited a pronounced effect on the log reduction of *E. coli* K12 inoculated at a rate of 7.81 (±0.09) (Fig. 4.3 and Fig.4.6). However, it can be observed from Figure 4.4 and Figure 4.5 that after the exposure to UV light in both penetration depths at the highest UV intensity value and lower inoculation rate, test microorganism was not detectable on the culture medium.

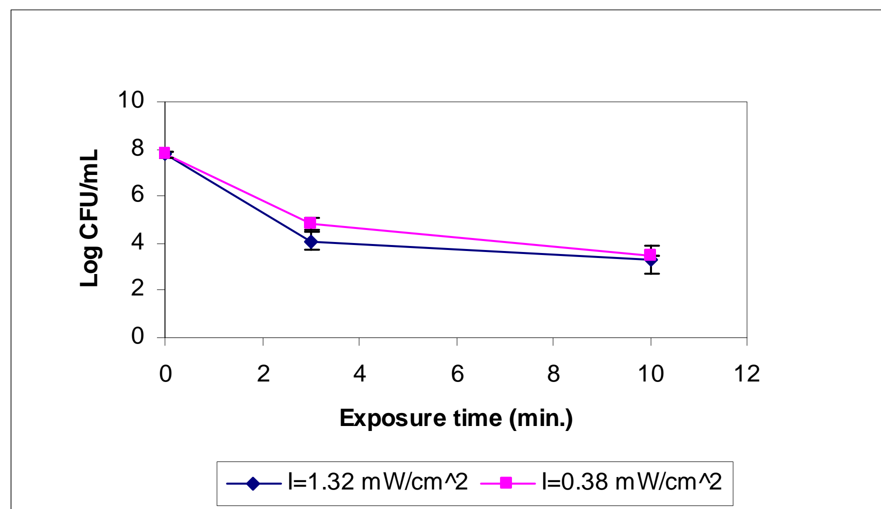


Figure 4.6. Influence of UV-C treatment on *E. coli* K12 inactivation in white grape juice at a sample depth of 0.5 cm and inoculation rate of 7.81-log (±0.09) CFU/mL.

According to the literature, in order to achieve 90% absorption of UV light in juices, typical penetration depth must be 1 mm (Canitez 2002). Results suggested that if higher inoculation rate is used, sample depth should be kept at its minimum level so as to enhance the UV penetration.

In continuous flow system the penetration depth was reported to be increased by generating turbulent flow. Particles can get in a close proximity with UV light under effective mixing conditions (Canitez 2002). On the other hand, Hanes, et al. (2002) obtained a greater than 5-log reduction of *Cyrptosporidium parvum* oocysts inoculated into the apple cider which recieved a UV dose of 14.32 mJ/cm^2 for 1.2 to 1.9 s in a laminar flow UV apparatus (CiderSure 3500A, FPE Inc, Rochester, NY). This equipment provides a sample depth which is less than 1 mm. Tandon, et al. (2003)

reported similar results for the inactivation of *E. coli* in apple cider by using Cider Sure model 3500 UV machine, as well. In a continuous UV treatment system, a turbulent flow can be maintained. Hence, lower exposure times and consequently low UV dose values can be achieved to satisfy the 5-log reduction requirement. Unfortunately, it was not possible to create a turbulent flow in the bench top UV system. Unless continuous stirring at 200 rpm was provided during the treatment, it was not enough for an effective mixing. Furthermore, above 200 rpm spillage of the juice sample was observed. Tandon, et al. (2003) suggested applying an adequate stirring during the treatment in order to ensure equal distribution of UV dose through the sample. In addition to these findings, Bolton, et al. (2003) informed that the stirring which can cause edge effects, should be avoided. They advised the use of small sample volume found near the center of the Petri dish for analysis of the inactivation degree.

Inactivation curves for *E. coli* K12 indicated that when the UV dose increased, a little tailing effect was observed by using both sample depths (Figure 4.7 and Figure 4.8). Taghipour (2004) found that inactivation rate of *E. coli* gradually reduces as the irradiation continues. At the end a plateau region is noticed. There are some reasons for this phenomenon. It is mainly due to the shielding effect of suspended particles and the accumulated cells. Interior microorganisms are protected from the light exposure and require longer treatment times to be inactivated (Taghipour 2004, Ünlütürk, et al. 2008). Formation of protective shields by accumulation of cells can be tried to be averted with continuous stirring. Stirring should be started approximately 10 s before the treatment in order to be confident about a well mixed solution (Bolton, et al. 2003). Another possible reason for the observation of tailing effect is the existence of resistant part of the population (Piyasena, et al. 2003, Ünlütürk, et al. 2008).

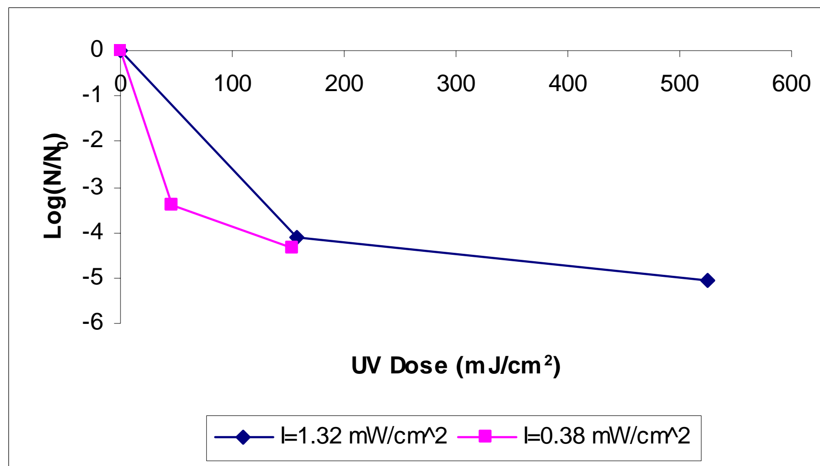


Figure 4.7. Log (N/N_0) vs UV Dose curve for the inactivation of *E. coli* K12 at a sample depth of 0.153 cm and the inoculation rate was 7.81-log (±0.09) CFU/mL.

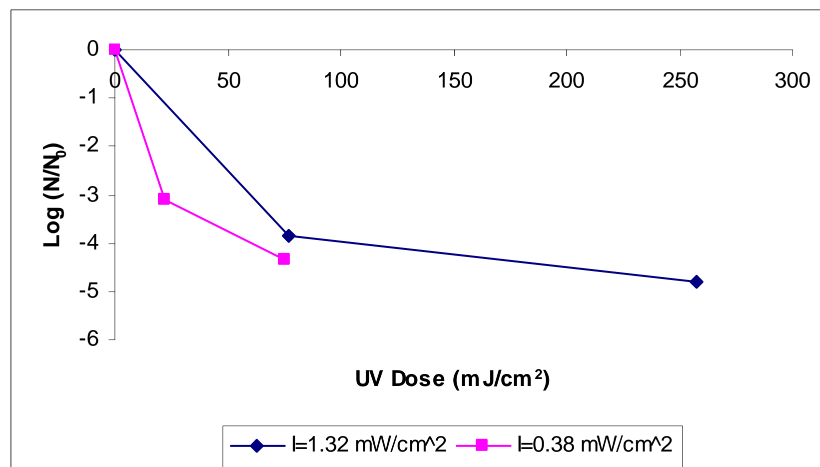


Figure 4.8. Log (N/N_0) vs UV Dose curve for the inactivation of *E. coli* K12 at a sample depth of 0.5 cm and the inoculation rate was 7.81-log (±0.09) CFU/mL.

In the literature there are no available data for the inactivation of microorganisms in white grape juice by UV-C light. It was not possible to compare our results with the literature. Also, the differences between the experimental conditions in different laboratories, different properties of the samples make difficult to evaluate the data. However, Wu, et al. (2005) reported the combined use of nisin and lysozyme with PEF for the inactivation of natural microflora in white grape juice. They applied 20 pulses of 65 kV/cm at 50°C and obtained a 4.4-log reduction.

4.3.2. Inactivation of Natural Flora in Orange Juice

According to the simple staining and microscopic examination of the naturally grown microorganisms in fresh squeezed orange juice, microbial population was found to be composed of mainly yeasts (See in Appendix A). Molinari, et al. (2004) also indicated that yeasts are the major microorganisms found in orange juice.

Fresh orange juice samples were inoculated onto Violet Red Bile Agar (VRBA) and Eosin Methylene Blue (EMB) Agar in order to determine whether they included coliform microorganisms. According to the results, orange juice samples did not contain coliforms. Initial microbial load of the samples were determined before UV treatment by culturing on Potato Dextrose Agar (PDA) medium acidified with pre-sterilized 10% tartaric acid to pH 3.5 which allows the proliferation of acidophilic bacteria and fungi. Initial microbial concentration was found as 6.04-log (±0.38) CFU/mL by incubating orange juices in flasks at 22°C under continuous shaking at 200 rpm for 3 days. Tahiri, et al. (2006) investigated the effect of inoculation rate on the inactivation as a result of high pressure, they concluded that high initial microbial concentrations which are 10^7 CFU/mL or up, decrease the effectiveness of the inactivation. Initial concentrations of 10^6 CFU/mL or less are found to be appropriate to observe a required reduction in the microbial population (Tahiri, et al. 2006).

The maximum log reduction was obtained as 1.76-log CFU/mL after 20 minutes of UV exposure (I= 1.32 mW/cm^2) (Figure 4.9). UV dose calculated to obtain 1.76-log CFU/mL was 144.36 mJ/cm^2. The log reduction of natural flora in orange juice was lower than the reduction of *E. coli* K12 in white grape juice. There may be several explanations for this. It was proved that yeasts and molds are more resistant to ultraviolet light than bacteria (Tran and Farid 2004). Tandon, et al. (2003) found that UV light inactivation is not as effective as pasteurization on the yeast and mold populations. This is due to their big sizes, less pyrimidine base content of their genetic material, differences in their cell wall compositions and thickness. Also rich growth medium was reported to increase the number of ribosomes which can provide a shield for the DNA against UV light (Tran and Farid 2004). On the other hand, it was revealed that inactivation of natural flora is more difficult compared to the inactivation of inoculated microorganisms. The growth phases of cells in two systems are different. In

the naturally contaminated flora, microbial cells are probably in the late exponential phase or early stationary phase of growth in which cells are more resistant to stress (El-Hag, et al. 2006). Tran and Farid (2004) also showed that growth phase is an effective factor on the UV inactivation studies. The microbial population was probably in the late exponential phase in this study. According to the preliminary studies, it was observed that microorganisms reached a stationary phase beginning from the third day of the incubation (See in Appendix B). Besides, the existence of UV absorptive materials like suspended particles and yeasts was demonstrated to reduce the effectiveness of UV radiation. They increase the necessary dose to deactivate target microorganisms (Oteiza, et al. 2009). Orange juice was reported to need higher UV doses in order to reach a satisfactory reduction level while lower doses are sufficient for clear juices (Keyser, et al. 2008). In naturally contaminated juices, combinations of treatments were reported to be essential (El-Hag, et al. 2006). Canitez (2002) suggested the use of ultrasound in order to disperse the suspended particles in fruit juices like apple cider to enhance the UV light irradiation. Also, it was speculated that decreasing the film thickness of any opaque liquid to less than 1.6 mm makes it transparent to UV light (Tran and Farid 2004).

Exposure time was found to be a significantly effective ($p<0.0001$) factor for the inactivation studies of fresh squeezed orange juice. As the time increased log survival number decreased progressively (Figure 4.9).

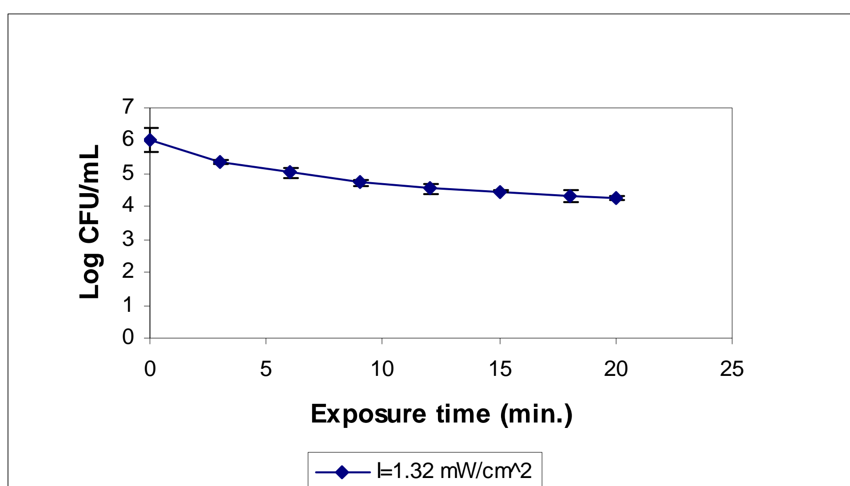


Figure 4.9. Influence of UV-C treatment on the inactivation of natural microflora in fresh squeezed orange juice at a sample depth of 0.153 cm and initial microbial load of 6.04-log (±0.38) CFU/mL.

4.3.3. Statistical Analysis for White Grape Juice Studies

Estimations of the effects of the 2-Level Factorial design for white grape juice studies were shown in Table 4.6.

Table 4.6. Effect Estimation Table.

	Term	Sum of Squares	% Contribution
Model	A- exposure time	19.35	12.54
Model	B- inoculation rate	102.73	66.59
Model	C- light intensity	14.11	9.14
Model	D- sample depth	0.3	0.19
Model	AB	0.72	0.47
Model	AC	7.62	4.94
Model	AD	0.16	0.11
Model	BC	2.23	1.44
Model	BD	7.50E-07	4.86E-07
Model	CD	0.022	0.014
Model	ABC	6.19	4.01
Ignored	ABD		
Model	ACD	0.2	0.13
Model	BCD	0.16	0.1
Aliased	ABCD	Aliased	

According to Table 4.6 all factors and their interactions included in the model except ABD term which had a negligible contribution on the response. ABD term was ignored in order to improve the model. There were some other terms which had very small effects on the response however, because of the hierarchy rule; they had to be included in the model. It was observed that Factor B (inoculation rate), Factor A (exposure time), Factor C (light intensity) had the greatest effects on the response, respectively.

Our response variable was log survival number (CFU/mL). ANOVA table for 2-level factorial design was generated and can be seen on Table 4.7.

Table 4.7. ANOVA Table for 2-Level Factorial Design.

Source	**Sum of Squares**	**df**	**Mean Square**	**F- value**	**p-value Prob>F**	
Block	0.081	5	0.016			
Model	153.78	13	11.83	710.77	<0.0001	significant
A- exposure time	19.35	1	19.35	1162.65	<0.0001	
B- inoculation rate	102.73	1	102.73	6172.42	<0.0001	
C- light intensity	14.11	1	14.11	847.52	<0.0001	
D- sample depth	0.3	1	0.3	17.77	0.0002	
AB	0.72	1	0.72	43.37	<0.0001	
AC	7.62	1	7.62	458.1	<0.0001	
AD	0.16	1	0.16	9.8	0.0041	
BC	2.23	1	2.23	133.89	<0.0001	
BD	7.50E-07	1	7.50E-07	4.506E-05	0.9947	
CD	0.022	1	0.022	1.31	0.2625	
ABC	6.19	1	6.19	371.71	<0.0001	
ACD	0.2	1	0.2	12.03	0.0017	
BCD	0.16	1	0.16	9.4	0.0048	
Residual	0.47	28	0.017			
Cor Total	154.33	46				

ANOVA table showed that terms which had p-values smaller than 0.05 had a significant effect on the response at 5% confidence level ($\alpha = 0.05$).

The R^2 value (0.9970) implies that this model is able to define the variability in the data by 99.70 % of chance. Predicted R^2 (0.9914) shows that this model can define the variability in another set of data by the chance of 99.14 %. Predicted R^2 (0.9914) is in reasonable agreement with the Adjusted R^2 (0.9956). Adjusted R^2 decreased by adding unnecessary terms to the model. But here, this much large value indicates that our model does not contain any unnecessary terms.

Figure 4.10 indicates a significant interaction between factors A and C. According to the figure, if exposure time is used as 3 minutes, intensity should be increased by shifting the tray level to the first stage in order to achieve lower log survival number. The incident light intensity reaches its maximum level (I=1.32 mW/cm^2) as the tray system moves up to the first stage. But if lower intensity (I= 0.38 mW/cm^2) is applied, sample should have longer UV light exposure to decrease the survival number of the microorganisms. Murakami, et al. (2006) found that inactivation of *E. coli* K12 strains is dependent on the UV intensity rather than exposure time. It can be seen from the figure that exposure time did not make a difference on the response if the highest UV light intensity was used.

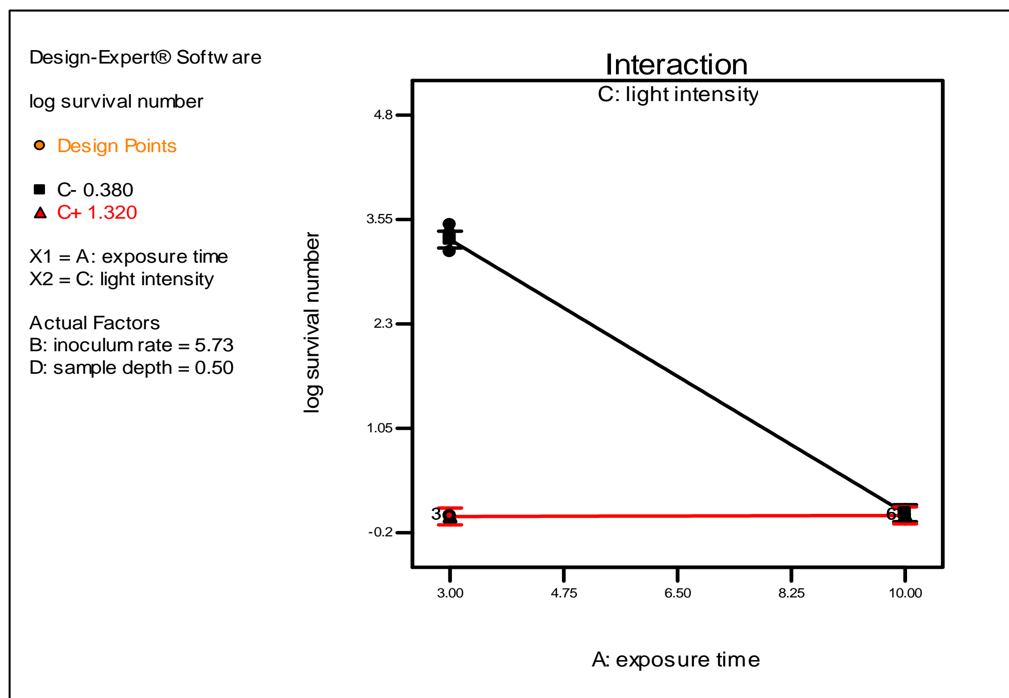


Figure 4.10. Factor A vs. Factor C Interaction Plot.

Significance of the interaction between light intensity level and rate of inoculums can be observed from Figure 4.11 Lower inoculation rate resulted in lower log survival numbers when the sample exposed to the highest UV light intensity. However, ascension in the microbial load reduced the effect of incident light intensity.

As it was mentioned before, this is because of the shielding effect of the accumulated cells.

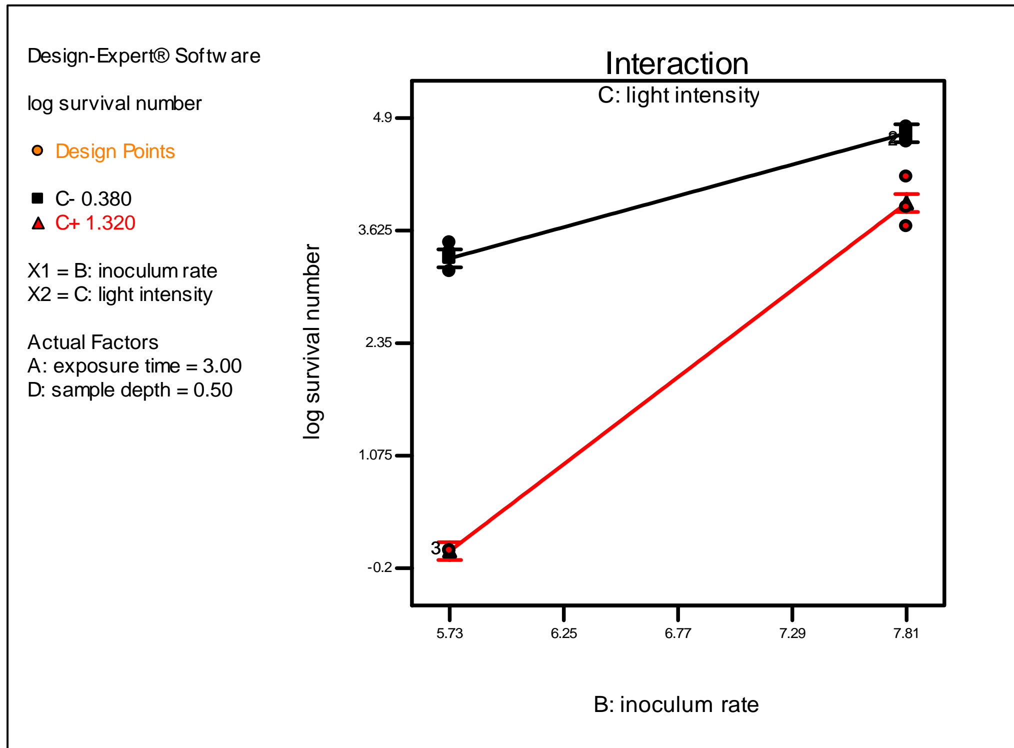


Figure 4.11. Factor B vs. Factor C Interaction Plot.

Interaction plot between exposure time and inoculation rate suggested that as the inoculation rate ascends sample should be irradiated for longer treatment times in order to reduce the microbial load (Figure 4.12). A significant reduction in the log survival number was obtained when the juice sample spiked with the test microorganism at lower concentrations.

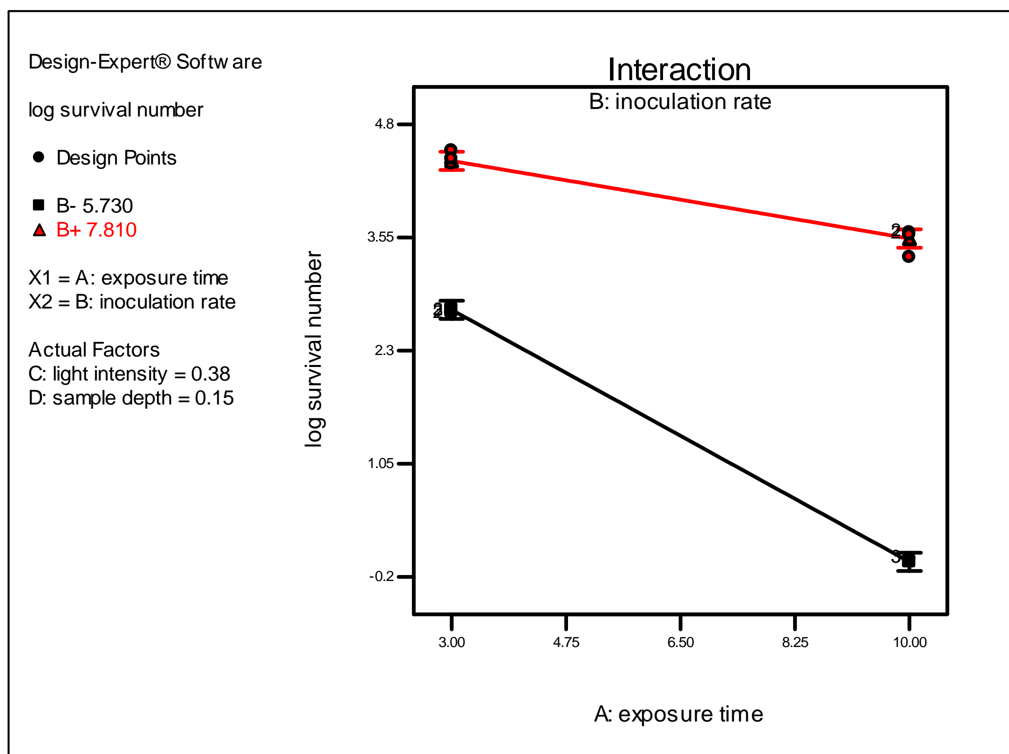


Figure 4.12. Factor A vs. Factor B Interaction Plot.

It can be recognized that almost the same level of microbial survival was obtained for 2 different sample depths when the intensity was applied as its lower level (I= 0.38 mW/cm^2) for longer period of exposure time (10 minutes) for the treatment of the sample inoculated at a rate of 5.73- log (±0.11) CFU/mL (Figure 4.13). Nonetheless, a significant difference in the log survival number can be recognized after exposure to UV light for 3 minutes. Increased sample depth brought about reduced effectiveness of the UV-C light irradiation system. Also, it can be speculated that higher penetration depth requires longer treatment times in order to reach the same reduction level as the sample which has lower penetration depth.

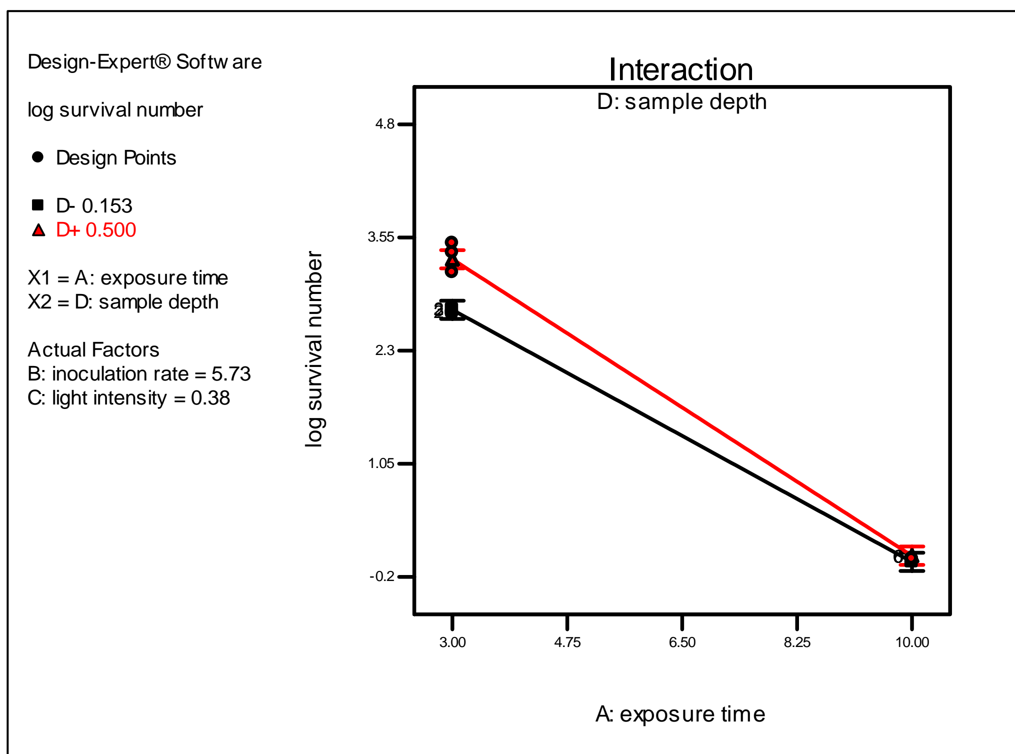


Figure 4.13. Factor A vs. Factor D Interaction Plot.

According to the results, if the samples which were inoculated at higher concentration level exposed to UV light for a short period of time, UV-C treatment resulted in higher survival number. Use of lower inoculation ratio and longer exposure time reduce the number of microorganisms which can survive. It was realized that changing the incident light intensity from its lower level to higher level attenuates the number of survivors for the samples inoculated with higher concentration of microbial suspension.

4.3.4. Statistical Analysis for Orange Juice Studies

Considering the previous results obtained in the white grape juice studies intensity level and sample depth kept constant and their effects on the response were not evaluated. For orange juice studies incident UV light intensity was used as its higher

level (I= 1.32 mW/cm^2) and sample depth was kept at its lower level (0.153 cm) taking the optical properties of the orange juice into consideration. One-way ANOVA was applied and the results were shown on Table 4.8.

Table 4.8. One-way ANOVA Table.

Source	**Sum of Squares**	**df**	**Mean Square**	**F- value**	**p-value Prob > F**	
Model	5.10E-03	7	7.281E-04	38.04	< 0.0001	significant
A-Exposure time	5.10E-03	7	7.281E-04	38.04	< 0.0001	
Pure Error	3.06E-04	16	1.914E-05			
Cor Total	5.40E-03	23				

ANOVA table indicated that exposure time has a significant effect on the response variable ($p<0.0001$). The R^2 value (0.9433) implies that this model is able to define the variability in the data by 94.33 % of probability. Predicted R^2 (0.8725) shows that this model can define the variability in another set of data by the chance of 87.25 %. Predicted R^2 (0.8725) is in reasonable agreement with the Adjusted R^2 (0.9185).

Figure 4.14 shows the effect of exposure time on log survival number. As it can be seen from the figure as the exposure time increases log survival number decreases.

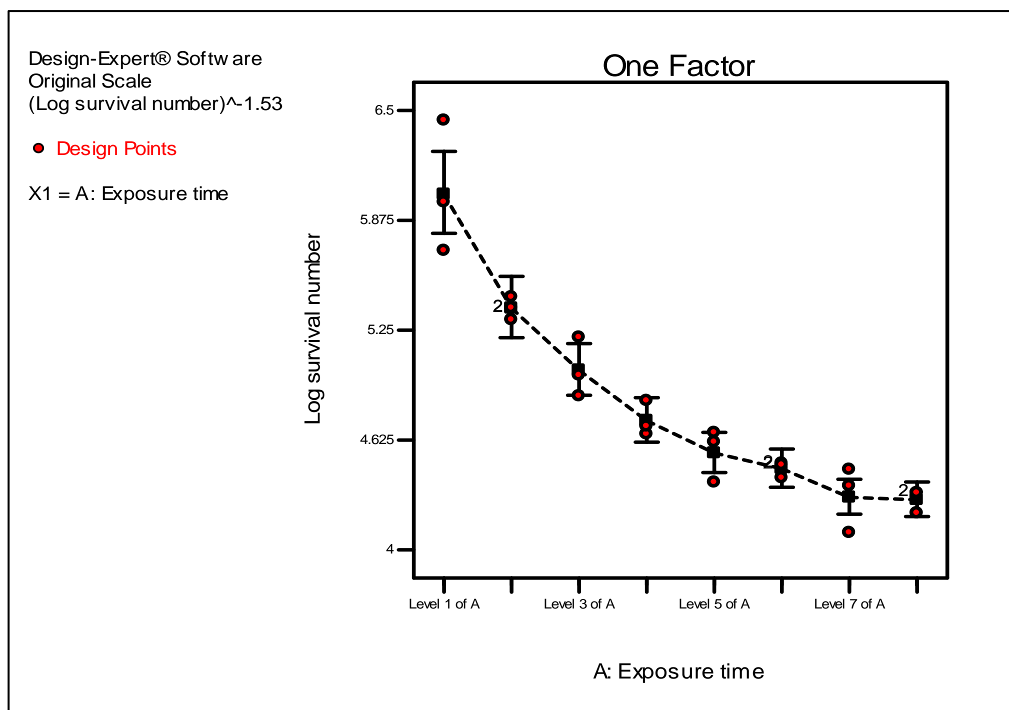


Figure 4.14. Effect of Exposure Time on the Response.

According to Tukey's test, increasing the UV exposure time resulted in significantly lower log survival numbers in comparison to the initial microbial load of the sample (Table 4.9.). The 2nd, 4th, 7th and 8th levels of the factor were found to bring about significant differences in mean values.

Table 4.9. Pairwise Comparison of Treatment Means

	LOG SURVIVAL NUMBER (CFU/mL)							
	Exposure Time (min.)							
Juice Sample	**0**	**3**	**6**	**9**	**12**	**15**	**18**	**20**
Fresh Squeezed Orange Juice	6.04±0.37^{a}	5.38±0.06^{b}	5.03±0.17^{bc}	4.74±0.10^{cd}	4.55±0.15^{de}	4.46±0.05^{de}	4.31±0.19^{e}	4.29±0.07^{e}

$^{a-e}$: Means having different letters within each exposure time denote significant difference at p<0.05
Data are mean values ± S.D. (n=3)

4.4. Effect of UV-C Treatment on the Colors of Juices

4.4.1. Effect of UV on White Grape Juice Color

To determine the effect of the applied UV-C treatment on the color which is one of the very important quality attributes by the consumers, statistical analysis of the color parameters (L^*, brightness; a^*, redness-greenness; b^*, yellowness- blueness) and also, total color difference, ΔE was performed.

2-Level Factorial Design was performed in order to determine the effects of exposure time, light intensity and sample depth on L^*, a^*, and b^* values of the white grape juice. Each factor was evaluated at their 2 levels.

Only the sample depth (Factor C) was found to be the significant factor on L^* value. Other factors were not effective on the response. Although only one factor was indicated to be significantly effective, if the model was reduced by considering only Factor C; R^2, Adj R^2, and Pred R^2 values were very low. So, factors and interactions which have p-values equal or smaller than 0.10 were also included in order to improve the model.

According to ANOVA results of color analysis (See in Appendix C), R^2 values were low for L^*. This may be due to the improper ranges of the factors for this parameter. It can be said that using these levels it was not possible to properly explain the variations in the data for L^* value.

Exposure time (Factor A), intensity level (Factor B), and sample depth (Factor C) were demonstrated to be the effective factors on a^* value at 10% confidence level. This model can explain the variations in the data by 73.89% of chance. Results suggested that as the exposure time increases, a^* value tends to attenuate towards green region. Similarly, an increase in sample depth caused a decrease in a^* which implies more green region. Besides, as the incident light intensity increased, a^* parameter indicated more negative region. Increased sample depth significantly ($p<0.0001$) reduced the a^* value towards greennish tonalities. Ibarz, et al. (2005) observed a similar trend in irradiated fruit juices. They realized that a^* parameter was progressively

decreased to more greenish region due to the increased irradiation time (Ibarz, et al. 2005).

Another response variable was b^* value which ranges between yellow ($+b^*$) and blue ($-b^*$) regions. All the factors were found to be effective on this variable according to ANOVA table (See in Appendix C). Especially, sample depth and intensity level were found to be significantly effective ($p<0.0001$) factors. When longer exposure time was used in combination with maximum sample depth and the light intensity was increased by shifting the tray system upto the first stage, a pronounced change in the b^* value were observed towards positive b^* region which indicates more yellow color.

Lee and Coates (1999) suggested that color changes occurred in juice samples after thermal pasteurization are more related to the b^* value. Similar results supporting these findings have found in this study. All three factors investigated were found to be effective on only b^* parameter for the color changes in white grape juice samples after UV-C treatment. However, the model was not significant for total color difference (ΔE) indicating these factors had no effects on the total color change at their used levels. It can be concluded that UV-C irradiation has no significant effect on the total color of white grape juice.

4.4.2. Effect of UV on Orange Juice Color

A general factorial design with only one factor and 7 levels of it was used in order to observe how UV-C treatment affects the color of fresh squeezed orange juice. The factor was exposure time, levels were 3, 6, 9, 12, 15, 18, and 20 minutes. All the treatments were replicated three times.

ANOVA results (See in Appendix D) showed that there was not any correlation between exposure time and the color parameters (L^*, a^*, b^*) of orange juice. UV-C irradiation did not cause any change on the color parameters and the total color of the juice sample. P-values for the effect of exposure time on each parameter and total color difference (ΔE) were found to be greater than 0.5. This much high p-values can not be caused by random errors.

Orange juice is a highly pigmented product due to its high carotenoid content. In the literature, it was reported that highly pigmented juices are less affected by the processing and storage. High concentrations of color pigments provide a better masking effect on color differences. These type of juices have more acceptible color after the processing (Lee and Coates 1999). However, it was also reported that orange juice has less red, more yellow color and slightly increased L^* value after the pasteurization, indicating lighter and more saturated product color (Lee and Coates 2003).

CHAPTER 5

CONCLUSION

Bench top collimated beam apparatus was used throughout the study to disinfect white grape juice samples inoculated with *Escherichia coli* K12 and naturally contaminated fresh squeezed orange juice samples. Sample depth, inoculation rate, incident light intensity and exposure time were evaluated as factors effective on the performance of UV-C treatment of white grape juice. Maximum log reduction (5.75-log CFU/mL) was obtained using the lowest sample depth, lowest inoculation rate, and highest UV intensity value. At these conditions exposure time did not make a difference between the log reductions. Considering the limiting factors of the UV-C light processing and best levels of the factors for the inactivation of *E. coli* K12 in white grape juice, minimum sample depth and the maximum intensity value were chosen for the disinfection of orange juice samples. Although more than 5-log reduction was observed for white grape juice exposed to UV dose of 75.04 mJ/cm^2, the log reduction was relatively small for naturally contaminated orange juice samples even if the highest exposure time (20 minutes) was used (UV dose of 144.36 mJ/cm^2). The difference between the effects of UV-C treatment on the samples was attributed to the optical properties of two different juices. Turbidity values and UV light absorptivities of the juices were discovered as very important parameters for the UV-C light processing. Another reason for the discrepancy between the log reductions of two different juice samples was the difficulty of the inactivation of naturally grown microorganisms than the inactivation of inoculated ones.

According to the results, UV-C light treatment was found as a compromising method for the disinfection of white grape juices. FDA requirement of 5-log reduction in the target microorganism was satisfied for white grape juice samples. But color parameters of a^* and b^* were found to be affected by the process. This is probably due to the low color pigment content of white grape juices. High concentrations of the color pigments were reported to be able to mask the color changes occurred in juices.

This system was not able to successfully inactivate naturally grown microorganisms in a highly absorptive medium such as orange juice. On the other hand, no color changes were observed for the orange juice samples treated with UV-C light. This brings about the use of UV-C light as a pretreatment of the juices or combined use of the technique with other preservation methods to protect quality attributes and obtain a safe product.

Low cost of the equipment and ease of use are the advantages of this technique. Also, FDA has approved the use of UV-C light in the processing of fruit juices to reduce the microbial load. However, more research is needed to prove this system's ability to inactivate the important enzymes in fruit juices which reduce the quality of the juices. Besides, yeasts and molds are known to be more resistant to UV light inactivation than bacteria. Complementary work for disinfection of juices contaminated with resistant forms of microorganisms is necessary. Especially, inactivation of naturally grown microorganisms using UV-C light should be further investigated.

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APPENDIX A

MICROSCOPIC APPEARANCE OF NATURAL FLORA IN ORANGE JUICE

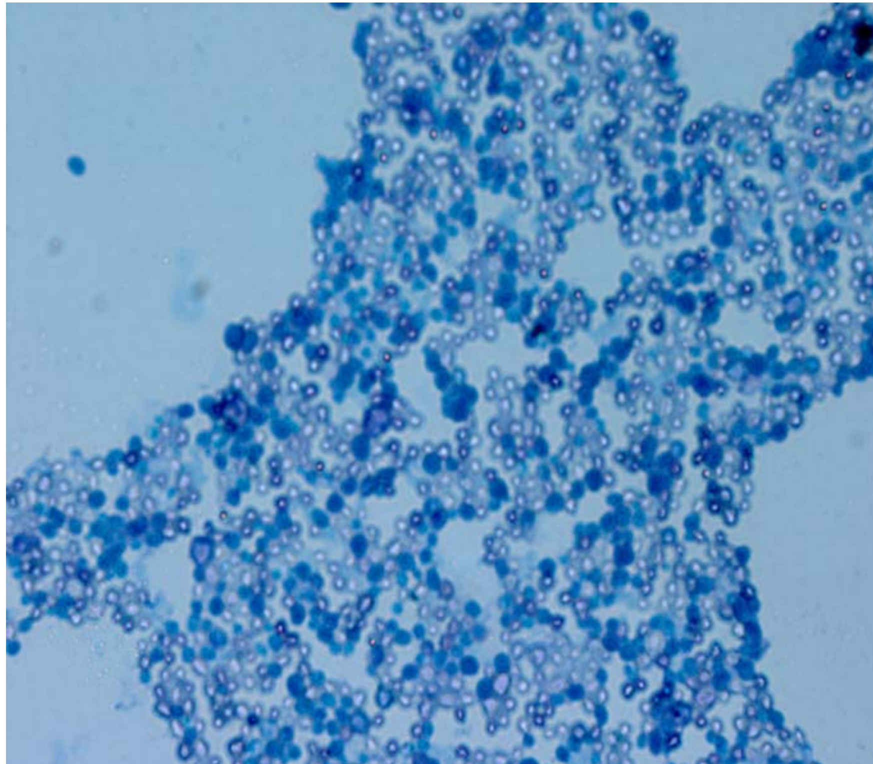

Figure A.1. Microscopic appearance of the cells in orange juice samples.

APPENDIX B

GROWTH CURVE FOR NATURAL FLORA IN FRESH SQUEEZED ORANGE JUICE

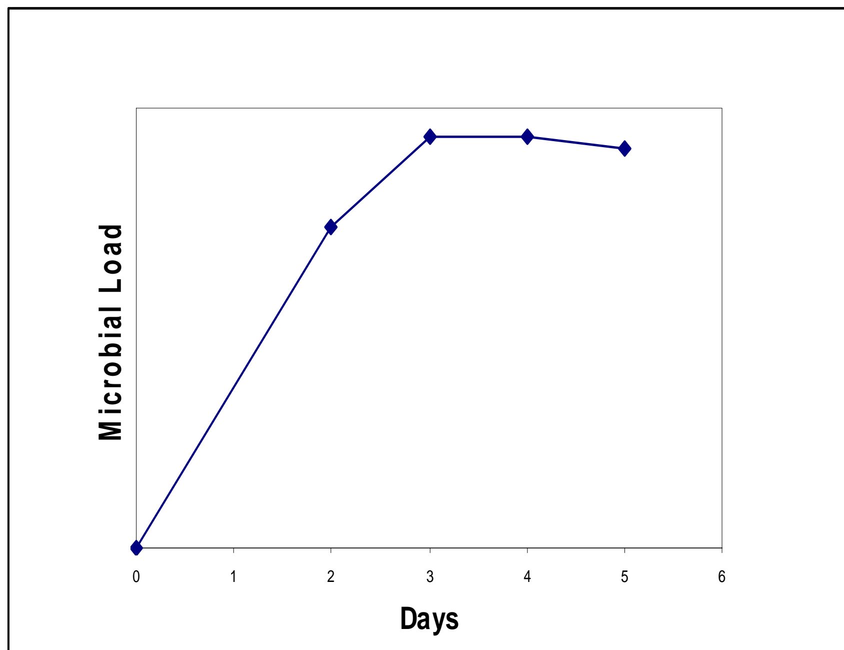

Figure A.2. Growth curve for natural flora in fresh squeezed orange juice.

APPENDIX C

ANOVA TABLE FOR COLOR RESULTS OF WHITE GRAPE JUICE

Table B.1. ANOVA Table for L^* value

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2.33	5	0.466	7.36	0.0001	significant
A-Exposure time	0.02	1	0.023	0.36	0.5524	
B-Intensity Level	6.67E-05	1	6.67E-05	1.054E-03	0.9743	
C-Sample depth	1.87	1	1.870	29.56	< 0.0001	
AC	0.19	1	0.187	2.96	0.0950	
ABC	0.25	1	0.248	3.92	0.0564	
Residual	2.02	32	0.063			
Lack of Fit	1.01	16	0.063	1.00	0.5000	not significant
Pure Error	1.01	16	0.063			
Cor Total	4.35	37				

Table B.2. R^2 Values for the Statistical Analysis of L^* Parameter.

R-Squared Values	
R-Squared	0.5349
Adj R-Squared	0.4622
Pred R-Squared	0.5247

Table B.3. ANOVA Table for a^* Value.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.20	3	0.068	30.18	< 0.0001	significant
A-Exposure time	7.704E-03	1	7.704E-03	3.44	0.0729	
B-Intensity level	0.012	1	0.012	5.23	0.0290	
C-Sample depth	0.18	1	0.18	81.88	< 0.0001	
Residual	0.072	32	2.240E-03			
Lack of Fit	0.036	16	2.240E-03	1.00	0.5000	not significant
Pure Error	0.036	16	2.240E-03			
Cor Total	0.27	35				

Table B.4. R^2 Values for the Statistical Analysis of a^* Parameter.

R-Squared Values	
R-Squared	0.7389
Adj R-Squared	0.7144
Pred R-Squared	0.7466

Table B.5. ANOVA Table for b^* Value.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	66.432	4	16.608	3796.13	< 0.0001	significant
A-Exposure time	0.029	1	0.029	6.56	0.0153	
B-Intensity Level	0.088	1	0.088	20.02	< 0.0001	
C-Sample depth	66.301	1	66.301	15154.40	< 0.0001	
BC	0.016	1	0.016	3.54	0.0689	
Residual	0.140	32	4.375E-03			
Lack of Fit	0.070	16	4.375E-03	1.00	0.5000	not significant
Pure Error	0.070	16	4.375E-03			
Cor Total	66.572	36				

Table B.6. R^2 Values for the Statistical Analysis of b^* Parameter.

R-Squared Values	
R-Squared	0.9979
Adj R-Squared	0.9976
Pred R-Squared	0.9983

APPENDIX D

ANOVA TABLE FOR COLOR RESULTS OF FRESH SQUEEZED ORANGE JUICE

Table C.1. ANOVA Table for L^* Value.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.21	6	0.035	0.68	0.6660	not significant
A-Exposure time	0.21	6	0.035	0.68	0.6660	
Pure Error	0.72	14	0.052			
Cor Total	0.93	20				

Table C.2. R^2 Values for the Statistical Analysis of L^* Parameter.

R-Squared Values	
R-Squared	0.2266
Adj R-Squared	-0.1048
Pred R-Squared	-0.7401

Table C.3. ANOVA Table for a^* Value.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.35	6	0.058	0.87	0.5421	not significant
A-Exposure time	0.35	6	0.058	0.87	0.5421	
Pure Error	0.94	14	0.067			
Cor Total	1.28	20				

Table C.4. R^2 Values for the Statistical Analysis of a^* Parameter.

R-Squared Values	
R-Squared	0.2709
Adj R-Squared	-0.0415
Pred R-Squared	-0.6404

Table C.5. ANOVA Table for b^* Value.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	3.77	6	0.628	0.68	0.6703	not significant
A-Exposure time	3.77	6	0.628	0.68	0.6703	
Pure Error	12.97	14	0.926			
Cor Total	16.73	20				

Table C.6. R^2 Values for the Statistical Analysis of b^* Parameter.

R-Squared Values	
R-Squared	0.2251
Adj R-Squared	-0.1071
Pred R-Squared	-0.7436

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